UNIVERSITY OF NAIROBI

PHYTOCHEMICAL ANALYSIS OF SELECTED *TEPHROSIA* SPECIES FOR ANTI-INFLAMMATORY PRINCIPLES

BY

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2021

DECLARATION

I declare that this Ph.D. thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work has been used, this has properly been acknowledged and referenced as per the University of Nairobi's requirements.

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DEDICATION

This worked is dedicated to my devoted wife Sophie Moreen Byantaka and my loving children (Annastashia Rashina Atono, Shadrick Oriko Owor and Richlene Oriko Siem)

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My profound gratitude goes to my supervisors: Dr. Albert Ndakala, Dr. Solomon Derese and Dr. George O. Ong'amo for their invaluable expertise, guidance and encouragement throughout my research. I extend special thanks to Prof. Abiy Yenesew, who although not my supervisor but has always been open to discussion and has offered me a lot of guidance throughout my studies. I extend my gratitude and appreciation to Prof. Dr. Dr. Michael Spiteller, INFU, TU Dortmund and his entire group particularly Dr. Kibrom Gebreheiwot Bedane and Dr. Sebastian Zühlke for hosting me in his laboratory for a nine-month research visit and guiding me in structure characterization and biological assay.

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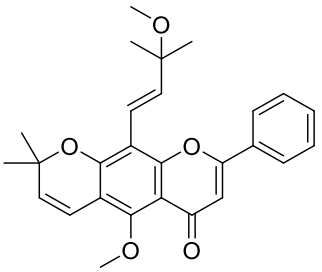
Lastly, I am indebted to my family members … words cannot appropriately express how your persistent prayers and encouragement have helped me to surmount the difficult moments. Above all, to the Almighty God for strength and wisdom.

ABSTRACT

Inflammation is a vital immune process in the restoration of inflamed tissues and it is regulated by mediators such as cytokines. However, dysregulation of inflammation is associated with the onset and development of chronic respiratory diseases and cancer. The management of inflammation and associated diseases using currently available anti-inflammatory drugs such as ibuprofen has been linked with adverse side effects. Thus, there is a need to develop alternative anti-inflammatory drugs. Plants of the genus *Tephrosia* Pers. (Fabaceae) have been used widely in ethnomedicine in the management of various ailments and they elaborate diversity of flavonoids. Although flavonoids are reported to have anti-inflammatory effects, little has been reported on the anti-inflammatory properties of flavonoids from the genus *Tephrosia*. Therefore, in this study selected *Tephrosia* species (*T. linearis*, *T. hildebrandtii*, *T. vogelii*, *T. elata*, and *T. rhodesica*) were phytochemically analyzed with the aims of identifying flavonoids with anti-inflammatory effects. The methanol-dichloromethane (1:1) crude extracts of these plants were fractionated on silica gel and purified using Sephadex LH-20, chromatotron and preparative HPLC. HRESIMS, ECD and NMR data were used to characterize the compounds. The anti-inflammatory activities of the isolated compounds as well as the crude extracts were evaluated for inhibition of cytokine production [interleukins (IL-1β, IL-2, IL-6), interferon-*gamma* (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-*alpha* (TNF-α)] from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs). Ibuprofen was used as a standard drug for anti-inflammation. Overall, fifty-six (56) compounds were identified from the selected *Tephrosia* species, eleven of which were new compounds and one derivative was prepared. From the aerial parts of *T. linearis*, seven new flavonoids [lineaflavone A-D (**1-4**), 6-methoxygeraldone (**5**), acetylobovatin (**6**) and 5-hydroxy-7-methoxysaniculamin A (**7**)] and seventeen known compounds (**8-24**) were isolated. From the aerial parts of *T. hildebrandtii*, a new flavone, hildeflavone (**25**) and ten known compounds (**22**, **26-34**) were isolated. *T. vogelii* seedpods yielded two new isoflavones [vogelisoflavone A (**35**) and vogelisoflavone B (**36**)] and ten known compounds (**34**, **37-43**). From the stem of *T. elata* one new isoflavone, elatisoflavone (**44**) and three known isoflavones (**45-47**) were isolated. Eleven known compounds (**21**, **23** and **48-56**) were isolated from the extract of stem of *T. rhodesica* stem. Pyrazoisopongaflavone (**57**), a pyrazole derivative of isopongaflavone (**38**) was prepared. Lineaflavone B (**2**), luteolin (**13**), patuletin-3-*O*-rhamnoside (**16**), pisatin (**30**), vogelisoflavone B (**36**), isopongaflavone (**38**) and genistein (**55**) exhibited stronger anti-inflammatory activities

compared to the standard drug, ibuprofen. In synergetic studies, combinations of flavonoids showed superior anti-inflammatory activities over the individual flavonoids. The findings of this study imply that flavonoids from the genus *Tephrosia* could be used as templates for anti-inflammatory drug discovery.



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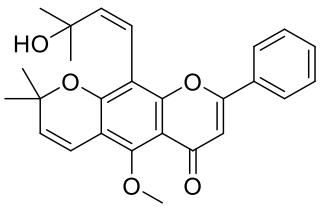

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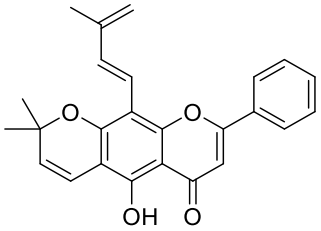

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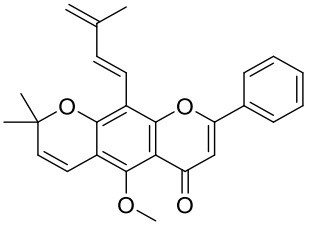

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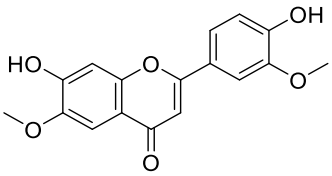

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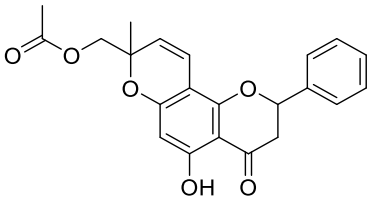

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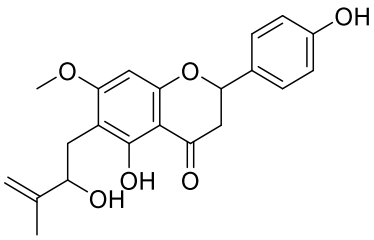

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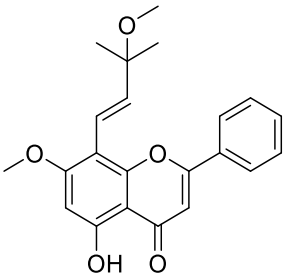

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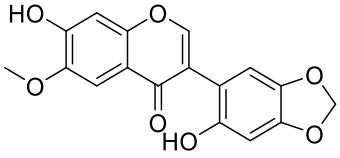

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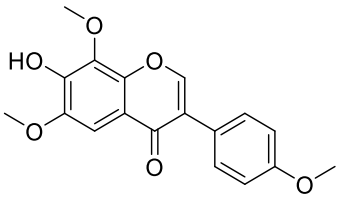

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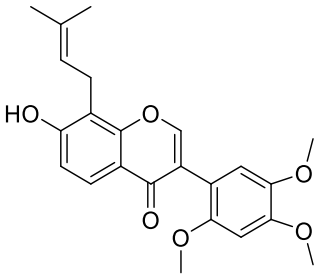

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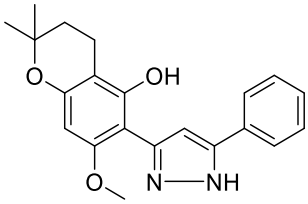

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LIST OF PUBLICATIONS FROM THIS THESIS

1. **Owor, R. O**., Bedane, K. G., Openda, Y. I., Zühlke, S., Derese, S., Ong'amo, G., Ndakala, A., and Spiteller, M. (2020). Synergistic anti-inflammatory activities of a new flavone and other flavonoids from *Tephrosia hildebrandtii* vatke. *Natural Product Research*, 1-8.
2. **Owor, R. O.,** Bedane, K. G., Zühlke, S., Derese, S., Ong'amo, G. O., Ndakala, A., and Spiteller, M. (2020). Anti-inflammatory Flavanones and Flavones from *Tephrosia linearis*. *Journal of Natural Products, 83*(4), 996-1004.
3. **Owor, R. O.,** Derese, S., Bedane, K. G., Zühlke, S., Ndakala, A., and Spiteller, M. (2020). Isoflavones from the seedpods of *Tephrosia vogelii* and pyrazoisopongaflavone with anti-inflammatory effects. *Fitoterapia, 146,* 104695.
4. **Owor, R. O**., Derese, S., Bedane, K. G., Zühlke, S., Ndakala, A., and Spiteller, M. (2021). Anti-inflammatory and synergistic activities of flavonoids from two *Tephrosia* species. *Drafted manuscript, xxxxxxx.*
5. **Owor, R. O**., Derese, S., Bedane, K. G., Zühlke, S., Ndakala, A., and Spiteller, M. (2021). Anti-inflammatory isoflavones from *Tephrosia elata*. *Drafted manuscript, xxxxxxx*

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LISTS OF ABBREVIATIONS/ACRONYMS AND SYMBOLS

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
1D NMR	One-Dimensional Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
2D NMR	Two-Dimensional Nuclear Magnetic Resonance
AP-1	Activator Protein-1
CID	Collision-Induced Dissociation
COSY	Correlation Spectroscopy
COX	Cyclooxygenase
DMSO	dimethyl sulfoxide
ECD	Electronic Circular Dichroism
EtOAc	Ethyl acetate
EtOH	Ethanol
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High performance liquid chromatography
HRESIMS	High resolution Electron Spray Ionization Mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IFN-γ	Interferon gamma
IL	Interleukin
*i*NOS	inducible nitric oxide synthase
LC-MS	Liquid Chromatography Mass Spectrometry
LPS	Lipopolysaccharide
MeOH	Methanol
MS	Mass Spectrometry

NF-kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells.
NMR	Nuclear Magnetic Resonance
NO	Nitric oxide
NOESY	Nuclear Overhauser and Exchange Spectroscopy
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
PG	Prostaglandin
PMBCs	Peripheral blood mononuclear cells
Prep.-HPLC	Preparative High-performance liquid chromatography
RP	Reverse Phase
TLC	Thin Layer Chromatography
TNF-α	Tumor necrosis factor alpha
UV	Ultra Violet
WHO	World Health Organization
*μ*g	Microgramme
*μ*L	Microlitre
d	doublet
dd	doublet of doublet
g	Gramme
Hz	Hertz
J	Coupling constant
kg	Kilogramme
m	Multiplet
m/z	Mass to charge ratio
*m*g	Milligramme
MHz	Mega Hertz

min	Minute
*m*L	Millilitre
ppm	Parts per million
s	Singlet
t	Triplet
δ	Chemical shift

CHAPTER 1: INTRODUCTION

1.1: Background

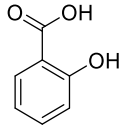
Medicinal plants have played a vital role since antiquity in the management and prevention of different illnesses (Hafidh *et al.*, 2009; Biljana, 2012; Sofowora *et al.*, 2013) as well as to relieve pain (Sayhan *et al.*, 2017). Medicinal plants play a crucial role in rural communities in developing countries where the healthcare systems are poorly developed (Strasser, 2003; WHO, 2008; Auditeau *et al.*, 2019; Mintah *et al.*, 2019). Thus, they are still of great value and part of the customs of many communities (Kokwaro, 2009; Mahomoodally, 2013) where they are administered in forms of decoctions and concoctions for oral administration or as a paste for skin surface applications (Tabuti *et al.*, 2003; Malik *et al.*, 2019).

Plants biosynthesize a large variety of compounds which are often referred to as natural products or sometimes as phytochemicals (Dewick, 2002; Yang *et al.*, 2018). These phytochemicals belong to different classes of compounds mainly; phenylpropanoids (flavonoids, coumarins, and lignans), alkaloids, terpenoids, and polyketides (Wink, 2015; Che *et al.*, 2017; Thirumurugan *et al.*, 2018). These compounds are used by the plants for defensive purposes, as attractants to pollinators and seed-dispersing agents. They also act as allelochemicals to suppress competing neighbouring plants (Dewick, 2002; Osbourn and Lanzotti, 2009). Throughout history, some phytochemicals have been used to make valuable products such as flavouring agents, fragrances, preservatives, repellents and drugs (Beattie, 2009; Osbourn and Lanzotti, 2009).

Compounds isolated from medicinal plants have either served as a source of new drugs or as templates for the development of new drugs against several diseases including inflammation and inflammatory-related diseases (Harvey, 2008; Cragg and Newman, 2013). For example, salicylic acid (**58**), obtained from the bark of *Salix alba* (Jones, 2011; Desborough and Keeling,

2017), served as a template for aspirin (**59**) which was the first non-steroidal anti-inflammatory drug (NSAID) (Rainsford, 1984; Mahdi *et al.*, 2006).

Inflammation is a two-phased (acute and chronic) reaction involving a variety of inflammatory cells that secrete mediators (vasoactive amines, eicosanoids and cytokines) (Abdulkhaleq *et al.*, 2018; Poluha and Grossmann, 2018). Acute inflammation is triggered by the presence of harmful stimuli or injury (Chen *et al.*, 2017). This phase involves recruitment of blood plasma and leukocytes (neutrophils and macrophages) into the inflamed tissue (de Oliveira *et al.*, 2016). Generally, acute inflammation proceeds to chronic inflammation if the response is inefficient in clearing the stimuli or healing the damaged tissue (Beattie, 2009). However, chronic inflammation always happens in cohorts with chronic diseases (Yiu *et al.*, 2018). Current anti-inflammatory drugs used to manage inflammation and related diseases target enzymes involved in the biosynthesis of eicosanoids particularly phospholipase A_2 and cyclooxygenase in the arachidonic acid metabolism (Dhikav *et al.*, 2003; Dinarello, 2010). However, these drugs are associated with adverse effects like gastrointestinal bleeding and peptic ulcer (Lichtenstein *et al.*, 1995; Drini, 2017; Wong, 2019). Anti-inflammatory drugs targeting the suppression of cytokine have been pursued as alternatives in the treatment of inflammation and its associated diseases (Aggarwal *et al.*, 2009; Leyva-López *et al.*, 2016). Therefore, there is an effort to discover anti-inflammatory drugs which target cytokine production.



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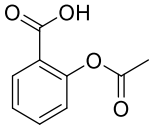

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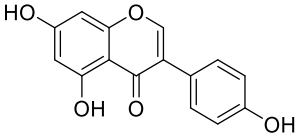

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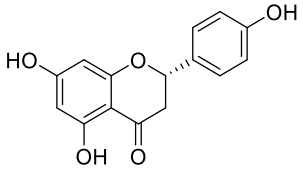

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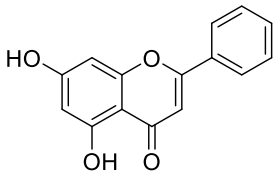
Flavonoids, secondary metabolites that occur in a wide range of plants have exhibited anti-inflammatory activities (Abdallah *et al.*, 2015; Leyva-López *et al.*, 2016). For instance, the isoflavone genistein (**55**) reduces pro-inflammatory cytokine over-activation (Valsecchi *et al.*, 2008) while the flavanone naringenin (**52**) decreases the release of cytokines (Tsai *et al.*, 2012). Chrysin (**60**), kaempferol (**61**) and quercetin (**62**) exhibit anti-inflammatory properties by decreasing the production of inflammatory mediators (Ginwala *et al.*, 2019). There is an increasing amount of effort focused on flavonoids as potential anti-inflammatory agents targeting cytokines.



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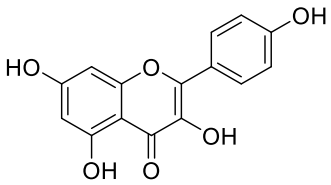

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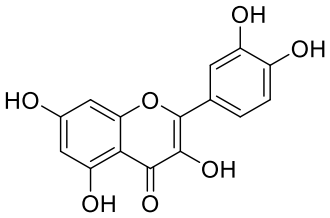

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Since plants from the genus *Tephrosia* Pers. (Fabaceae) elaborate flavonoids (Touqeer *et al.*, 2013; Chen *et al.*, 2014), in this study, five *Tephrosia* species (*T. linearis*, *T. hildebrandtii*, *T. rhodesica*, *T. vogelii,* and *T. elata*) were phytochemically studied to identify flavonoids with anti-inflammatory properties.

1.2: Statement of the Problem

Prolonged use of the current anti-inflammatory drugs such as aspirin and ibuprofen for management of inflammation and associated chronic diseases (Dinarello, 2010; Durgaprasad *et al.*, 2013) has been linked to severe side effects most commonly gastrointestinal bleeding and peptic ulcer (Lichtenstein *et al.*, 1995; Dhikav *et al.*, 2003; Zarghi and Arfaei, 2011; Goldstein and Cryer, 2015; Wong, 2019). Therefore, there is a need to develop new anti-inflammatory drugs that are safe and effective. Plant secondary metabolites are increasingly being recognized as effective and safer alternatives to the current available anti-inflammatory

drugs. In this study, the potential of flavonoids from *Tephrosia* species was assessed for their anti-inflammatory effects.

1.3: Objectives

1.3.1: General Objective

The main objective of this study was to identify anti-inflammatory principles from *Tephrosia* species.

1.3.2: Specific Objectives

The study was structured based on the following specific objectives:

i. To characterize isolated compounds from *T. linearis, T. hildebrandtii, T. rhodesica, T. vogelii* and *T. elata*.

ii. To determine the anti-inflammatory effects of the crude plant extracts, the isolated compounds and their derivatives.

iii. To determine the anti-inflammatory synergetic effects of the isolated compounds.

1.4: Justification and Significance

Although inflammation is a vital immune process in the restoration of tissue homeostasis, its dysregulation is linked to the pathogenesis of chronic diseases including cancer, respiratory and neurological diseases (Chen *et al.*, 2017; Yiu *et al.*, 2018). Sufficient evidence exists for the use of anti-inflammatory drugs for the treatment of chronic diseases but these drugs have adverse side effects (Lichtenstein *et al.*, 1995; Drini, 2017). Flavonoids have been attributed to possess anti-inflammatory properties with diverse modes of action (García-Lafuente *et al.*, 2009; Funakoshi-Tago *et al.*, 2011; Abdallah *et al.*, 2015; Leyva-López *et al.*, 2016; Chen *et al.*, 2019). Among these include the regulation of pro-inflammatory cytokines which have become an attractive therapeutic target for the management of inflammation (Leyva-López *et*

al., 2016). Plants from the genus *Tephrosia* Pers. (Fabaceae) are known to elaborate several classes of flavonoids (Chen *et al.*, 2014; Muiva-Mutisya *et al.*, 2014; Atilaw *et al.*, 2017a; Atilaw *et al.*, 2017b; Muiva-Mutisya *et al.*, 2018). Moreover, crude extract of some *Tephrosia* species (*T. purpurea*, *T. maxima*, *T. vogelii* and *T. sinapou*) have shown anti-inflammatory activities (Adaudi *et al.*, 2009; Sandhya *et al.*, 2010; Valli *et al.*, 2011). But the phytochemicals responsible for the activities are yet to be identified. Therefore, in this study, the crude extracts, as well as flavonoids isolated from selected Tephrosia species, were evaluated to determine their anti-inflammatory activity and their potential for the development of anti-inflammatory drugs.

CHAPTER 2: LITERATURE REVIEW

2.1: Inflammation

Inflammation is an immune process triggered by the presence of pathogens in the body or tissue damage (Beattie, 2009; Chen *et al.*, 2017; Pahwa and Jialal, 2019). The inflammatory response involves the recruitment of blood plasma, platelets and leukocytes (neutrophils, mast cells and macrophages) into the inflamed tissue (Raghavendra *et al.*, 2015). In the acute phase, this process is manifested in form of swelling, pain, heat and loss of organ function (Sherwood and Toliver-Kinsky, 2004; Chen *et al.*, 2017). The inflammatory cells in this phase secrete shorted-lived mediators like histamine and prostaglandins (Abdulkhaleq *et al.*, 2018). However, if inflammation lingers for a long time, it becomes chronic (Pahwa and Jialal, 2019). Macrophages, neutrophils and lymphocytes in chronic inflammation are capable of sustaining the production of eicosanoids and cytokines for a long period (Selders *et al.*, 2017; Qu *et al.*, 2018).

The eicosanoids and cytokines initiate and regulate the inflammatory response and also contribute to its pathological manifestation (Voronov *et al.*, 1999; Sugimoto *et al.*, 2016). Their secretion from inflammatory cells (lymphocytes, neutrophils, mast cells, and macrophages) are triggered by pathogens, damaged tissue or biochemical imbalances (Parisi *et al.*, 2018; Yiu *et al.*, 2018). Eicosanoids are biosynthesized through the arachidonic acid pathway while cytokines through the nuclear factor κB signaling pathway (Noverr *et al.*, 2003; Traish *et al.*, 2018).

2.1.1: Biosynthesis of Eicosanoids

Eicosanoids are oxylipins metabolized from arachidonic acid (Serhan *et al.*, 2010) and they are subdivided into prostanoids and leukotrienes based on the enzymes that mediate their biosynthesis (Goodman, 1996). The prostanoids, which are principally prostaglandins and

thromboxane are biosynthesized by cyclooxygenase (COX) while leukotrienes are products of lipoxygenase (LOX) (Ding *et al.*, 2003; Noverr *et al.*, 2003; Smyth and FitzGerald, 2010; Hanna and Hafez, 2018). Eicosanoids are only biosynthesized when required and their production depends on the availability of arachidonic acid (Goodman, 1996). When there is an infection or injury, arachidonic is released from phospholipids by phospholipase A_2 (PLA2) and metabolized to eicosanoids (Katayama and Lee, 2003; Norris and Carr, 2013; Weinberger *et al.*, 2015).

Prostaglandins (E_2, I_2, D_2 and F_2) are the most important mediators and they are biosynthesized from the oxidation of arachidonic acid by the action of cyclooxygenase enzymes (COX-1 and COX-2) (Willoughby *et al.*, 2000; Ding *et al.*, 2003; Botting and Ayoub, 2005; Monitto *et al.*, 2011). COX-1 is present in the majority of cells and is involved in the normal homeostatic system while COX-2 is expressed only during inflammation (Lone and Taskén, 2013). Consequently, COX-2 is used as a therapeutic target for the management of inflammation (Samad *et al.*, 2002; Turman and Marnett, 2010; Norris and Carr, 2013). Prostaglandins act on many cells and are responsible for the manifestation of hyperalgesia during inflammation (Pettipher, 1998; Ricciotti and FitzGerald, 2011). Incidentally, elevated levels of prostaglandins have been found in patients with asthma, arthritis and cancer (Steele *et al.*, 1999; Ricciotti and FitzGerald, 2011; Gomes *et al.*, 2018; Jo-Watanabe *et al.*, 2019; Rittchen and Heinemann, 2019).

2.1.2: Biosynthesis of Cytokines

Cytokines are proteins that mediate inflammation and the most important ones are tumor necrosis factor-*alpha* (TNF-α), interferons *gamma* (IFN-γ), interleukins (IL) and colony-stimulating factors (CSF) (Chen *et al.*, 2017; Ferreira *et al.*, 2018). They are secreted by a variety of cells (macrophages, lymphocytes, mast cells and endothelial cells) (Thèze, 1998;

Duque and Descoteaux, 2014; Turner *et al.*, 2014). Their biosynthesis during inflammation is initiated when normal tissue homeostasis is disturbed by either infection, injury or imbalance in biochemical composition in the body (Traish *et al.*, 2018). When such happen, the macrophages and lymphocytes detect the pathogens or tissue damage through pattern-recognition receptors for instance toll-like receptors (TLRs) present on their surface (Mogensen, 2009; Jang *et al.*, 2015; Kim *et al.*, 2016).

As illustrated in Figure 2.1, the TLRs recognize the pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) of Gram-negative bacteria (Mogensen, 2009). Stimulated TLRs lead to downstream activation of transcription factor NF-κB through several adaptor molecules (Agbeko and Peters, 2011; Oviedo-Boyso *et al.*, 2014). The activated NF-κB translocates to the nucleus and binds with DNA to stimulate the production of the cytokines (Liu *et al.*, 2017). Further, pro-inflammatory cytokines have been shown to induce the production of cyclooxygenase enzyme and therefore, elevate the production of prostaglandins through "crosstalk" (LaPointe and Isenović, 1999; Yao and Narumiya, 2019). Likewise, prostaglandins influence cytokine secretion via prostaglandin E receptors (EP) which triggers downstream expression of cytokines.

Generally, cytokines upregulate inflammatory response intensifying widespread pain as well as prompting a sickness syndrome such as fever, anorexia and altered mood (Luheshi and Rothwell, 1996; Conti *et al.*, 2004; Zhang and An, 2007). For instance, the upregulation of pro-inflammatory cytokines during menstruation account for dysmenorrhea (menstrual cramps) and endometriosis (Ma *et al.*, 2013). Besides, the intensive release of cytokines has been associated with the pathogenesis of many diseases (Torres *et al.*, 2019) as is the case in coronavirus disease 2019 (COVID-19) patients who experience a cytokine storm (Tang *et al.*, 2020).

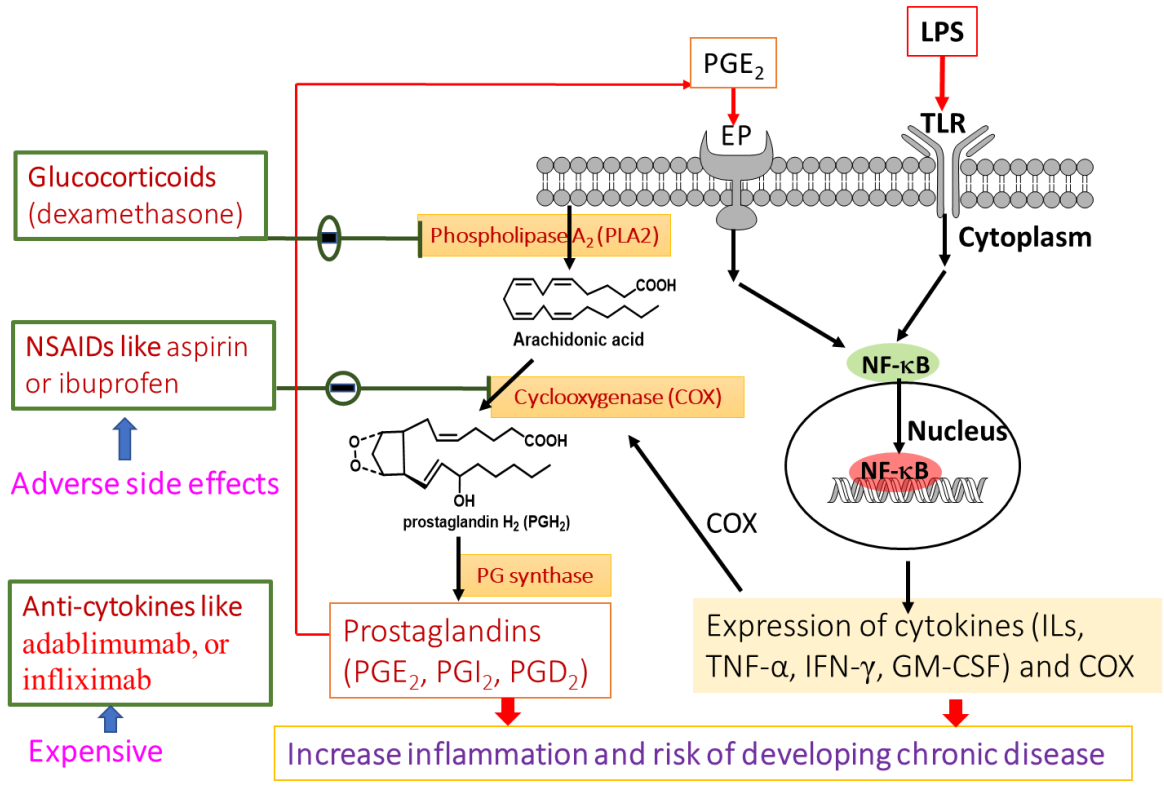


Figure 2.1: Pathways for Biosynthesis of Prostaglandins and Cytokines

2.2: Anti-inflammatory Drugs

Anti-inflammatory drugs are essentially used to decrease inflammatory response compared to analgesics such as opioids that block pain signals to the brain (Maroon *et al.*, 2010; Slater *et al.*, 2010). The anti-inflammatory drugs target either the biosynthesis of eicosanoids or cytokines (Ricciotti and FitzGerald, 2011). Drugs that target arachidonic acid metabolism are either steroidal or nonsteroidal. The anti-cytokines are mainly monoclonal antibodies.

2.2.1: Steroidal Anti-inflammatory Drugs

These drugs inhibit the arachidonic acid release from phospholipids which is the initial step in prostaglandin biosynthesis (Malcher-Lopes *et al.*, 2008; Szefel *et al.*, 2015). They are steroids and always are referred to as corticosteroids (or glucocorticoids) (Goppelt-Struebe *et al.*, 1989; Buttgereit *et al.*, 2013; de Kloet *et al.*, 2017). Prednisone (**63**), cortisone (**64**), hydrocortisone (**65**), dexamethasone (**66**) and methylprednisolone (**67**) are some of the typical examples

(Goppelt-Struebe *et al.*, 1989; Rainsford, 2007; Serhan *et al.*, 2010). Besides their use as anti-inflammatory drugs, they are also used to manage inflammatory-related diseases such as rheumatoid arthritis and asthma (Barnes, 1998). However, their drawbacks are in their side effects such as high blood pressure, ulcers, cataracts, menstrual irregularity and obesity (Bond, 1977; Schäcke *et al.*, 2002; Brown, 2009; Saag and Furst, 2013; Oray *et al.*, 2016).

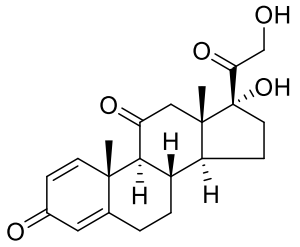
2.2.2: Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

These drugs exhibit their therapeutic anti-inflammatory effects by inhibition of COX (Drini, 2017). They include aspirin (**59**), ibuprofen (**68**), diclofenac (**69**), naproxen (**70**) and Indocin (**71**) (Serhan *et al.*, 2010).

Prolonged use of NSAIDs has been associated with gastrointestinal injury and the toxicities are related to their inhibition of the COX-1 (Goldstein and Cryer, 2015). COX-2 selective drugs such as celecoxib (**72**) and rofecoxib (**73**) have been developed (Zarghi and Arfaei, 2011). However, they have been withdrawn because of their associated health risks such as stroke and thrombosis (Grosser *et al.*, 2006).

2.2.3: Anti-cytokine Drugs

Some drugs that prevent the expression and secretion of pro-inflammatory cytokines have been developed (Leyva-López *et al.*, 2016). They are mainly monoclonal antibodies such as adalimumab, etanercept and infliximab which are TNF-α inhibitors (Lis *et al.*, 2014; Menegatti *et al.*, 2019) and tocilizumab which is an IL-6 inhibitor (Venkiteshwaran, 2009). These drugs are also used to treat inflammatory-related diseases such as arthritis, Crohn's disease and depression (Oldfield *et al.*, 2009). There is an effort to discover anti-inflammatory drugs that target cytokines especially from natural products like flavonoids that have shown some anti-inflammatory properties (Aggarwal *et al.*, 2009; Leyva-López *et al.*, 2016).



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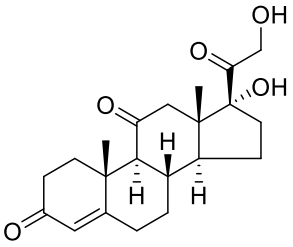

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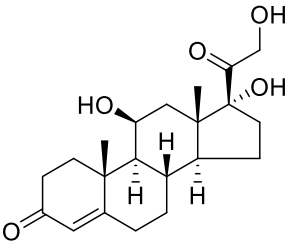

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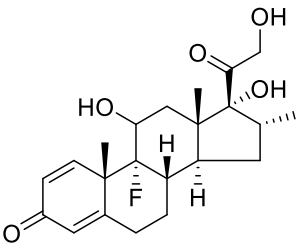

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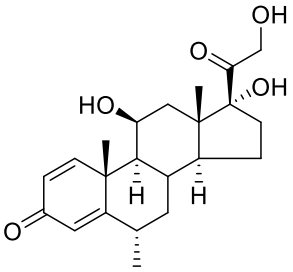

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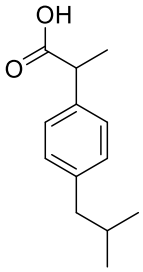

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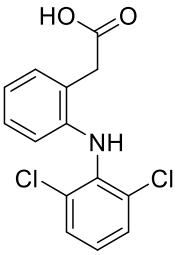

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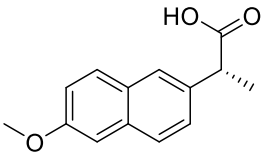

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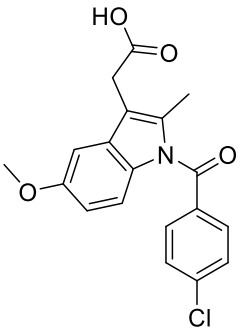

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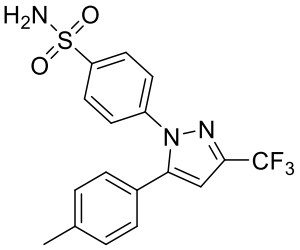

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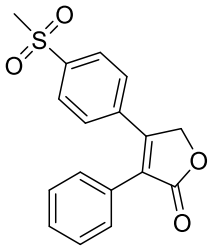

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2.3: Anti-inflammatory Flavonoids

Flavonoids are common in many plants especially as prominent constituents in fruits, vegetables, flowers and legumes (García-Lafuente *et al.*, 2009; Kumar and Pandey, 2013b; Panche *et al.*, 2016). Flavonoids are classified as chalcones, flavanones, flavanols, flavones,

isoflavones, pterocarpans and rotenoids (Agrawal, 1989). Several biological activities have been ascribed to flavonoids including anti-inflammatory properties (Kumar and Pandey, 2013a; Panche *et al.*, 2016; Ruiz-Cruz *et al.*, 2017). Some flavonoids have been shown to exhibit their anti-inflammatory effects by attenuating the cytokines release in either *in vitro* or *in vivo* in animal models as shown in Table 2.1 (Attiq *et al.*, 2018; Tungmunnithum *et al.*, 2018).

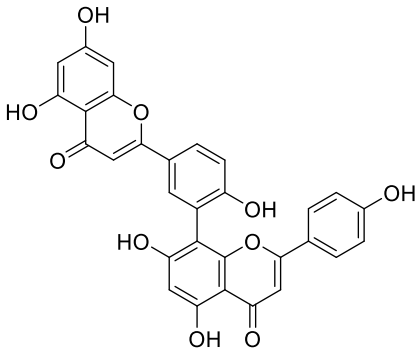
Specifically, a biflavonoid isolated from *Sellaginella tamariscina* suppressed the NF-kB signalling pathway and downregulated the COX-2 production in TNF-α-activated A549 cells (Banerjee *et al.*, 2002). Flavonoids from *Chrozophora tinctoria* showed significant suppression of IL-1β and IL-6 release from the phytohemagglutinin (PHA)-stimulated PMBCs (Abdallah *et al.*, 2015). *Broussonetia* flavonoids also showed anti-inflammatory properties by reducing the production of TNF-α and IL-6 from LPS-induced RAW 264.7 cells (Ryu *et al.*, 2019). Similarly, glycoside flavonoids from *Smilax glabra* exhibit anti-inflammatory effects by supressing NF-kB production (Shu *et al.*, 2018), while C-Geranyl flavonoids from *Palownia fortunei* showed anti-inflammatory activities by decreasing IL-6 and TNF-α release from LPS-treated cardiomyocytes (Zhang *et al.*, 2019a).

Although, little is known about the anti-inflammatory properties of the genus *Tephrosia*, some of the flavonoids such as apigenin (**12**) (Plioukas *et al.*, 2016), rutin (**76**) (Jain *et al.*, 2009) and quercetin (**76**) (Gómez-Garibay *et al.*, 2002) that have been isolated in the genus show anti-inflammatory activities.

Table 2.1: Anti-inflammatory Effects of flavonoids

No.	Flavonoid	Molecular target	Anti-inflammatory Effect	Reference
74	Amentoflavone	TNF-α-activated A549 cells.	Reduced COX-2 release by inhibiting NF-κB.	(Banerjee *et al.*, 2002)

No.	Flavonoid	Molecular target	Anti-inflammatory Effect	Reference
		Phytohemagglutinin-stimulated PBMCs.	Suppressed the release of IL-1β, IL-6, and PGE_2.	(Abdallah *et al.*, 2015)
75	Apigenin-7-*O*-β-D-glucopyranoside	LPS-RAW264.7 cells.	Decreased release of NO, *i*NOS, TNF-α, and IL-6.	(Ryu *et al.*, 2019)
76	Rutin			
77	Ochnaflavone	Rat platelets.	Inhibited phospholipase A_2 (PLA2).	(Chang *et al.*, 1994)
12	Apigenin	LPS-stimulated RAW 264.5 cells.	Inhibited the production of NO and *i*NOS.	(Hu *et al.*, 2017)
13	Luteolin			
78	Quercetin			
79	Acacetin			
80	Diosmetin			
81	Bonanzin			
82	Artmetin			
83	Broussochalcone C	LPS-stimulated RAW 264.7 cells	Inhibited the release of TNF-α and IL-6.	(Ryu *et al.*, 2019)
84	Broussoflavanonol A			
85	Broussoflavonol B			
86	5-hydroxy-6,8-dimethoxyflavonone-7-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside	TNF-α stimulated J-170 cells	Inhibited NF-*κ*B production	(Shu *et al.*, 2018)
87	5-Hydroxy-3,8-dimethoxyflavone-7-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside	TNF-α stimulated J-170 cells	Inhibited NF-*κ*B production	(Shu *et al.*, 2018)
88	3,7-Dihydroxy-8-methoxyflavone-6-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside	TNF-α stimulated J-170 cells	Inhibited NF-*κ*B production	(Shu *et al.*, 2018)
89	Paulownione D	LPS treated cardiomyocytes	Suppressed the production of IL-6 and TNF-α	(Zhang *et al.*, 2019a).
90	Paulownione E			
91	Paulownione F			
92	Paulownione G			



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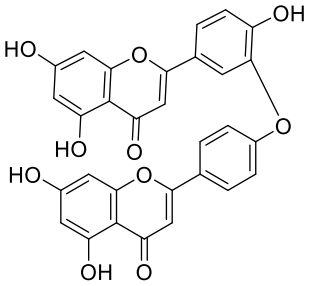

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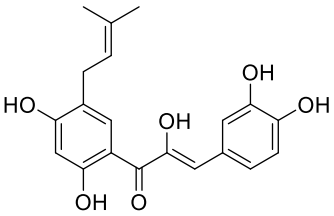

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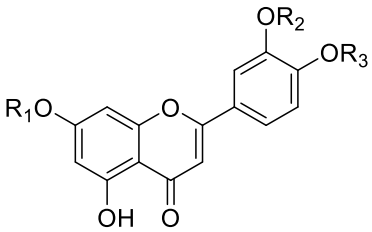
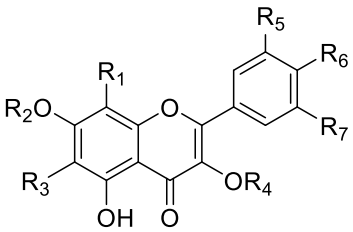
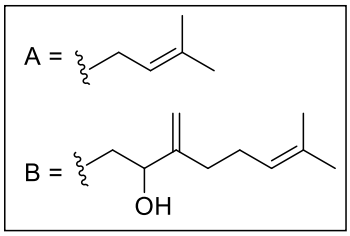
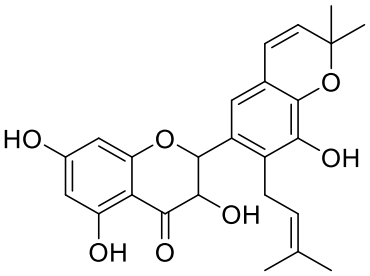


75 R_1 = Glc; R_2 = H; R_3 = H
12 R_1 = H; R_2 = H; R_3 = H
79 R_1 = H; R_2 = H; R_3 = Me
13 R_1 = H; R_2 = OH; R_3 = H
80 R_1 = H; R_2 = OH; R_3 = Me





76 R_1 = R_2 = R_3 = R_7 = H, R_4 = $(Glc)_2$, R_5 = R_6 = OH
78 R_1 = R_2 = R_3 = R_4 = R_7 = H, R_5 = R_6 = OH
81 R_1 = R_2 = R_7 = H, R_3 = R_5 = R_6 = OMe, R_4 = Me
82 R_1 = R_7 = H, R_2 = R_4 = Me, R_3 = R_5 = R_6 = OMe
85 R_1 = R_3 = A, R_2 = R_7 = H, R_4 = Me, R_5 = R_6 = OH
87 R_1 = OMe, R_2 = $(Glc)_2$, R_3 = R_5 = R_6 = R_7 = H, R_4 = Me
88 R_1 = R_3 = H, R_2 = Glc, R_4 = Me, R_5 = R_7 = OMe, R_6 = OH
90 R_1 = R_2 = R_4 = R_7 = H, R_3 = B, R_5 = R_6 = OH



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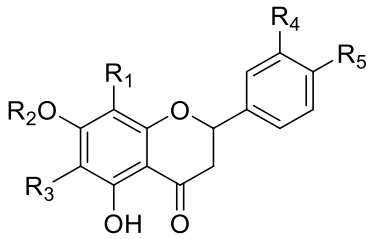
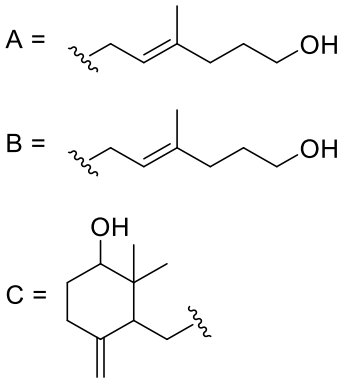




86 R_1 = R_3 = R_4 = R_5 = H, R_2 = $(Glc)_2$
89 R_1 = A, R_2 = R_3 = R_4 = H, R_5 = OH
91 R_1 = R_2 = H, R_3 = B, R_4 = OMe, R_5 = OH
92 R_1 = R_2 = H, R_3 = C, R_4 = R_5 = OH

2.4: Botany of the Genus *Tephrosia*

The genus *Tephrosia* Pers. belongs to the subfamily Papilionoideae that is distinguished from other subfamilies in the family Fabaceae by the zygomorphic papilionoid (butterfly-like) flower (Taylor *et al.*, 2009). The genus is mainly distributed in the tropics and subtropics (Pedley, 2014). Plants in this genus are either annual/perennial herbs or soft woody shrubs (Agnew, 2013; Al-Ghamdi, 2013). It contains over 353 species all over the world with over 30 species occurring in Kenya (Atilaw *et al.*, 2017a). *T. linearis*, *T. hildebrandtii*, *T. vogelii, T. elata,* and *T. rhodesica* are the species under investigation and found in Kenya.

2.4.1: Tephrosia linearis

Tephrosia linearis (Willd.) Pers. (Figure 2.2) is a short-lived perennial plant that is commonly found growing in grassland and rocky bushy slopes mainly in areas with higher rainfall (Agnew, 2013).

Figure 2.2: Photograph of *Tephrosia linearis*

T. linearis is an erect herb that grows up to 130 cm tall with a densely pubescent stem. Its leaves are pinnated with 5-15 leaflets, the flowers are either pink or orange in a terminal and axillary stiff pedunculated pseudo-raceme of 6-10 nodes. The pods are pubescent and about 5 cm long

x 3 mm wide with 9-12 seeds (Gillett *et al.*, 1971). The plant is native to tropical and austral Africa and Madagascar. In Kenya, it is found in the highlands, western, central and coast regions (Agnew, 2013).

2.4.2: Tephrosia hildebrandtii

Tephrosia hildebrandtii Vatke Pers. (Figure 2.3) is a short-lived perennial plant that is commonly found growing in upland grassland and semi-evergreen bushland at altitudes of 1100-1900 m (Agnew, 2013).

Figure 2.3: Photograph of *Tephrosia hildebrandtii*

T. hildebrandtii is an erect herb that can grow up to a height of 100 cm (Lwande *et al.*, 1986b). Its stem is rather sparsely pubescent, appressed or spreading while the leaves are unifoliolate. The flowers are reddish-purple born at the upper leaf axils and also terminal pseudo-racemes. Its pods are about 6 cm long x 4 mm wide with approximately 8 seeds (Gillett *et al.*, 1971). It

is native to Ethiopia, Kenya and Tanzania. In Kenya, it is found in the highland areas like Rift valley, Machakos, Nairobi and Kajiado (Agnew, 2013).

2.4.3: Tephrosia vogelii

Tephrosia vogelii Hook. f. Pers. (Figure 2.4) commonly called fish bean or fish-poison bean is native to tropical Africa and grows in a variety of habitats including savanna-like vegetation, grasslands, forest margins, wasteland and formerly cultivated fields (Mwaura *et al.*, 2013). It is a soft woody perennial plant with dense foliage and can grow up to 4 m tall (Agnew, 2013). Its stem is hairy while the leaves are arranged spirally with lateral leaflets (12-29) tomentose above and more densely beneath (Mwaura *et al.*, 2013). The flowers are white or pale violet borne at the terminal or axillary pseudo-racemes and fragrant when fresh. Its pods are about 110 x 13 x 4 mm with approximately 12-16 seeds (Gillett *et al.*, 1971). In Kenya, it is found in areas like Mt. Elgon, Trans Nzoia, Nakuru-Kisumu region, Kitale and Embu (Agnew, 2013).

Figure 2.4: Photograph of *Tephrosia vogelii*

2.4.4: Tephrosia elata

Tephrosia elata Deflers Pers. (Figure 2.5) is a short-lived bushy perennial plant that is commonly found growing in grasslands, formerly cultivated land fields, and thicket margins (Agnew, 2013). It is either an erect herb or soft woody shrub that can grow to the height of 150 cm. The stems are usually ridged and appressed strigose (Gillett *et al.*, 1971). Its leaves are pinnated with 15-21 leaflets, the flowers are either pink or purple in rather dense terminal racemes that are longer than their stalks (Gillett *et al.*, 1971). The pods are about 55x5mm which may be erect or bent upwards (Agnew, 2013). The plant is native to Ethiopia, Sudan, Kenya, Tanzania, Uganda, Zimbabwe, and the Arabian Peninsula. In Kenya, it is cosmopolitan and can be found in the western, central and coast regions (Agnew, 2013).

Figure 2.5: Photograph of *Tephrosia elata*

2.4.5: Tephrosia rhodesica

Tephrosia rhodesica Baker f. Pers. (Figure 2.6) is a short-lived perennial plant that is commonly found growing in grassland and rocky bushy slopes mainly in areas with higher rainfall (Agnew, 2013). The plant is a branching erect shrub and can grow to the height of 150 cm. The leaves are pinnate with 6-9 leaflets and the flowers are purple in terminal racemes. Its pods are

flat and bend upwards (Gillett *et al.*, 1971). The plant is native to tropical Africa including East Africa, Malawi, Zambia and South Africa. In Kenya, it is found in the Coast, Machakos, Magadi and Narok (Agnew, 2013).

Figure 2.6: Photograph of *Tephrosia rhodesica*

2.5: Traditional Uses of the Genus *Tephrosia*

Many *Tephrosia* species have been used traditionally in disease management and agricultural practices (Gachene and Wortmann, 2004). Their use varies from country to country and species to species. Notably, *T. linearis* leaf juice is traditionally used in Kenya to manage a broad spectrum of ailments in infants (Kokwaro, 1976; Kokwaro, 2009). In Uganda, the plant is used for treating swollen body parts (Oryema *et al.*, 2010) and managing premature ejaculation (Tabuti *et al.*, 2003). In Tanzania, *T. linearis* is used for treating cardiac palpitations (Chhabra and Mahunnah, 1994).

T. vogelii has multiple applications in ethnomedicine across East Africa. Its aqueous leaf extract is used to control ticks, lice and worms in livestock (Mwaura *et al.*, 2013; Dharani *et al.*, 2015).

The water extract of the plant is also used as an insecticide to control insect pests, mites and a rodenticide for mole-rats (Mwine *et al.*, 2011; Kisangau and Amri, 2012; Mkindi *et al.*, 2019). A hot water decoction of the leaf, stem bark and unripe fruit of *T. vogelii* is used to induce abortion (Dzenda *et al.*, 2007), while its macerated leaf is used as a purgative and emetic (Dafam *et al.*, 2014). The leaves of *T. vogelii* are also used as a fish poison for fishing (Tabuti *et al.*, 2003; Kerebba *et al.*, 2019).

T. purpurea has diverse applications in ethnomedicine. It is used as a fish poison for fishing and an antidote for snakebite (Heuzé *et al.*, 2018). It is also traditionally used for wound and ulcer treatment (Chinniah *et al.*, 2009). It is used as a purgative and medicine for stomach pains (Kokwaro, 2009)

The other *Tephrosia* species of relevant ethnomedical uses include *T. nana* which is used for the treatment of tuberculosis (Hamill *et al.*, 2003), *T. uniflora* used to manage snake bites (Abreu and Luis, 1996), *T. aequilata* is used to relieve abdominal pain, *T. bracteolata* used to treat syphilis in pregnant women (Williams, 2012), *T. noctiflora* used as a cough remedy and *T. villosa* used for liver and spleen pain management (Kokwaro, 2009).

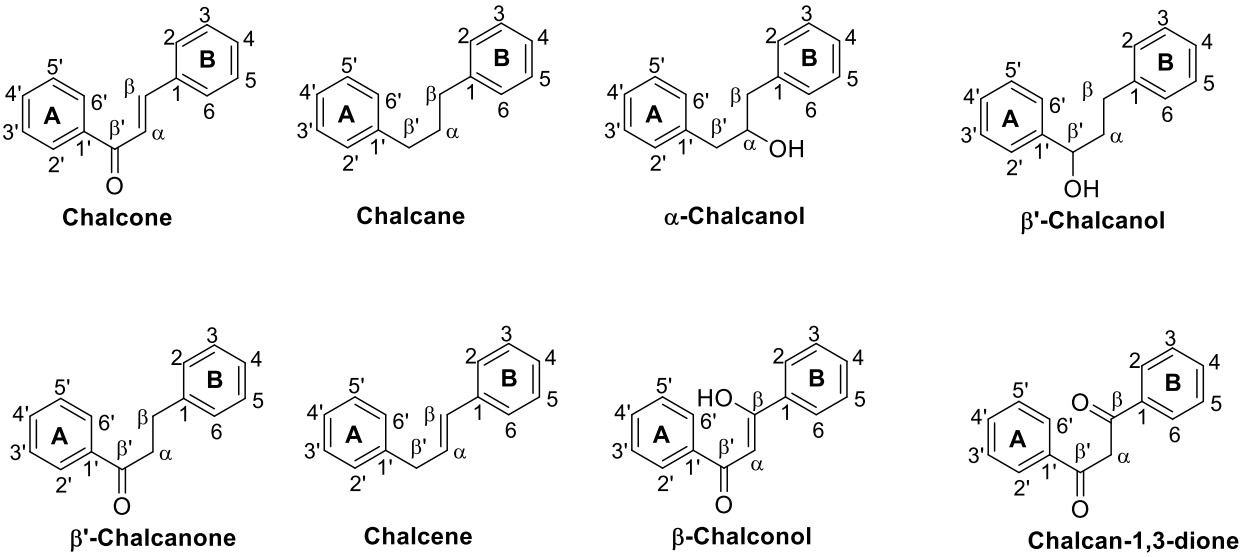
2.6: Phytochemistry of the Genus *Tephrosia*

Over the years, efforts have been made to isolate and identify bioactive compounds of the plants of this genus. Several compounds have been isolated and characterized from the genus. The compounds are mainly flavonoids in the sub-classes: chalcononoids, flavans, flavanones, flavones, flavonols and isoflavonoids (isoflavones, pterocarpans, coumestans, and rotenoids) (Touqeer *et al.*, 2013; Chen *et al.*, 2014).

2.6.1: Chalconoids from the Genus Tephrosia

A chalconoid is a flavonoid with a 1,3-diphenylpropane skeleton that may have an olefinic bond, keto, or hydroxyl group (Agrawal, 1989; Dewick, 2002; Rauter *et al.*, 2018). They are

subclassified as chalcanes, α-chalcanols, β-chalcanols, chalcenes, β-chalcanones (dihydrochalcones), α-chalcanones, chalcones, β-chalconols and chalcan-1,3-diones as shown in Scheme 2.1 depending on the substitution pattern in the propane moiety.



Scheme 2.1: Basic Skeletons of Subclasses of Chalconoids

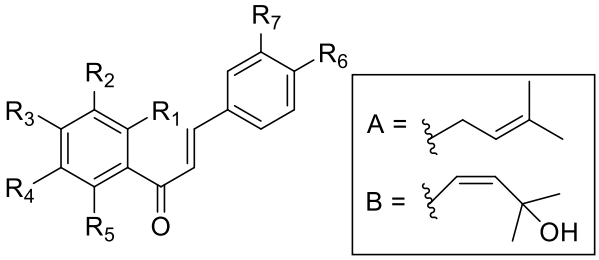
As shown in Table 2.2, over twenty chalconoids in the subclasses of chalcones, β-chalcanones, and β-chalconols have been reported in the genus *Tephrosia*.

Table 2.2: Chalconoids from the Genus *Tephrosia*

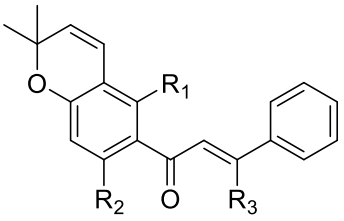
No.	Chalconoid	Plant species (part)	References
93	Isoliquiritigenin	*T. toxicaria* (ST)	(Jang *et al.*, 2003)
94	Tephrone	*T. candida* (SD)	(Chibber and Dutt, 1982)
95	Ovalichacone	*T. candida* (SD)	(Roy *et al.*, 1986)
96	Spinochalcone A	*T. spinosa* (RT)	(Rao and Prasad, 1992b)
97	Candidachalcone	*T. candida* (AP)	(Hegazy *et al.*, 2011)
98	(+)-Tephrosone	*T. purpurea* (AP)	(Chang *et al.*, 2000)
99	(+)-Tepropurpurin	*T. purpurea* (AP)	(Chang *et al.*, 1997)
100	Obovatachalcone	*T. obovata* (WP)	(Chen *et al.*, 1978)
101	Spinochalcone C	*T. spinosa* (RT)	(Rao and Prasad, 1992a)
102	Spinochalcone B	*T. spinosa* (RT)	(Rao and Prasad, 1992b)
103	2',6'-Dihydroxy -3'-prenyl-4'-methoxy-β-chalcone	*T. major* (RT, AP)	(Gómez-Garibay *et al.*, 2002)
104	Purpurenone	*T. purpurea* (RT)	(Rao and Raju, 1984)
105	Praecansone A	*T. praecans* (SD)	(Camele *et al.*, 1980)

No.	Chalconoid	Plant species (part)	References
106	Praecansone B	*T. praecans* (SD)	(Camele *et al.*, 1980)
107	Demethylpraecansone B	*T. aequilata* (RT)	(Tarus *et al.*, 2002)
108	Aequichalcone C	*T. aequilata* (RT)	(Atilaw *et al.*, 2017a)
109	Pongamol	*T. purpurea* (SD)	(Chang *et al.*, 1997)
110	*O*-Methylpongamol	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
111	Elatadihydrochalcone	*T. elata* (SD)	(Muiva *et al.*, 2009)
112	Aequichalcone A	*T. aequilata* (RT)	(Atilaw *et al.*, 2017a)
113	Aequichalcone B	*T. aequilata* (RT)	(Atilaw *et al.*, 2017a)
114	Tunicatachalcone	*T. tunicata* (RT)	(Andrei *et al.*, 2000)
234	2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone	*T. pulcherrima* (RT)	(Ganapaty *et al.*, 2008b)
273	Tephrone	*T. candida* (SD)	(Chibber and Dutt, 1982)

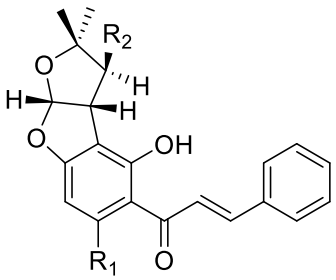
Key: RT – roots, SD – seeds/seedpods, FL – flowers, AP – aerial parts, WP – whole plant



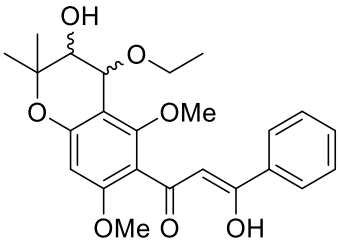
93 $R_1 = R_2 = R_4 = R_7 = H$, $R_3 = R_5 = R_6 = OH$
94 $R_1 = OH$, $R_2 = R_4 = H$, $R_3 = R_5 = OMe$, $R_6 = R_7 = OCH_2O$
95 $R_1 = OH$, $R_2 = A$, $R_3 = R_5 = OMe$, $R_4 = R_6 = R_7 = H$
96 $R_1 = R_6 = R_7 = H$, $R_2 = R_4 = A$, $R_3 = R_5 = OH$
97 $R_1 = OMe$, $R_2 = R_7 = OH$, $R_3 = R_5 = R_6 = OH$, $R_4 = B$
273 $R_1 = OH$, $R_2 = R_4 = H$, $R_3 = R_5 = OMe$, $R_6 = R_7 = OCH_2O$



100 $R_1 = OH$, $R_2 = OMe$, $R_3 = H$
101 $R_1 = OH$, $R_2 = R_3 = H$
104 $R_1 = OMe$, $R_2 = R_3 = OH$
105 $R_1 = R_2 = OMe$, $R_3 = OMe$
106 $R_1 = R_2 = OMe$, $R_3 = OH$
107 $R_1 = OH$, $R_2 = OMe$, $R_3 = OH$
234 $R_1 = R_2 = OMe$, $R_3 = H$



98 $R_1 = H$, $R_2 = OH$
99 $R_1 = OMe$, $R_2 = OAc$



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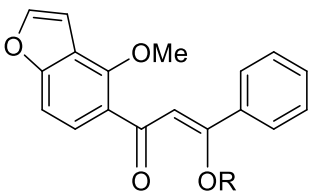
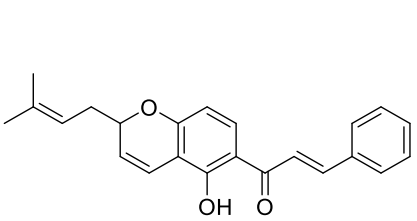


109 R = H
110 R = Me



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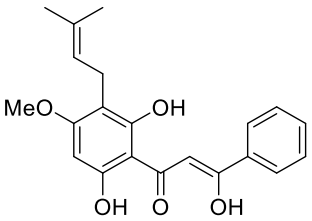

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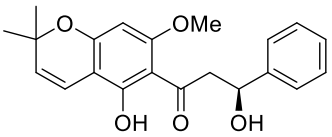

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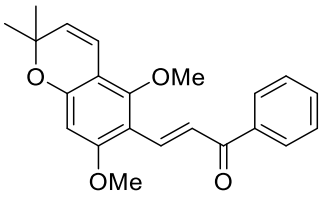

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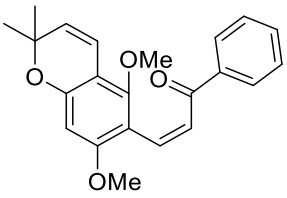

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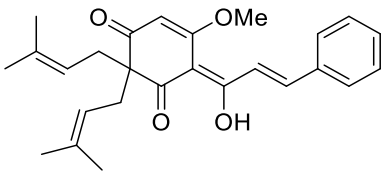
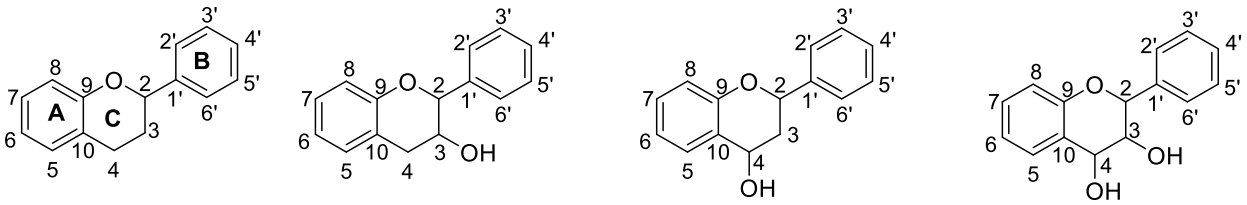


114

2.6.2: Flavans of the Genus Tephrosia

Flavans are 2-phenylchromane derivatives and they are subdivided into flavans, flavan-3-ols, flavan-4-ols and flavan-3,4-diols as shown in Scheme 2.2 (Agrawal, 1989; Dewick, 2002; Rauter *et al.*, 2018). A few of these classes of flavonoids have been reported in the genus *Tephrosia* as listed in Table 2.3.



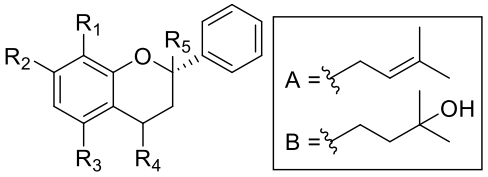
Flavan **Flavan-3-ol** **Flavan-4-ol** **Flavan-3,4-ol**

Scheme 2.2: Basic Skeletons of Subclasses of Flavans

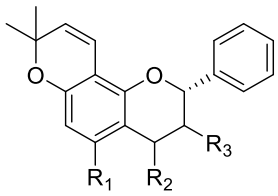
Table 2.3: Flavans of the Genus *Tephrosia*

No.	Flavan	Plant source (part)	Reference
115	Tephrowatsin E	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
116	5,7-Dimethoxy-8-prenylflavan	*T. madrensis* (LF & FL)	(Gómez *et al.*, 1983)
117	5-Hydroxy-7-methoxy-8-prenyflavan	*T. madrensis* (LF & FL)	(Gómez *et al.*, 1983)
118	Tephrowatsin D	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
119	Tephrowatsin A	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
120	Quercetol B	*T. quercetorum* (RT)	(Gomez-Garibay *et al.*, 1988)
121	Nitenin	*T. nitens*	(Gomez *et al.*, 1984)
122	Methylhildgardtol A	*T. hildbrandtii* (RT)	(Monache *et al.*, 1986)
123	Hildgardtol A	*T. hildbrandtii* (RT)	(Monache *et al.*, 1986)
124	Hildgardtol B	*T. hildbrandtii* (RT)	(Monache *et al.*, 1986)
125	Methylhildgardtol B	*T. hildbrandtii* (RT)	(Monache *et al.*, 1986)
126	Quercetol A	*T. quercetorum* (RT)	(Gomez-Garibay *et al.*, 1988)
127	Tephrowatsin B	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
128	Hildgardtene	*T. hildbrandtii* (RT)	(Monache *et al.*, 1986)
129	Tepicanol A	*T. tepicana* (RT & AP)	(Gómez-Garibay *et al.*, 1997)
271	Rhodiflavan A	*T. rhodesica* (RT)	(Atilaw *et al.*, 2020)
272	Rhodiflavan B	*T. rhodesica* (RT)	(Atilaw *et al.*, 2020)

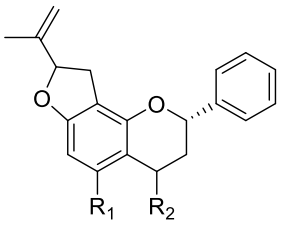
Key: RT – roots, SD – seeds or seedpods, FL–flowers, AP – aerial parts, WP – whole plant



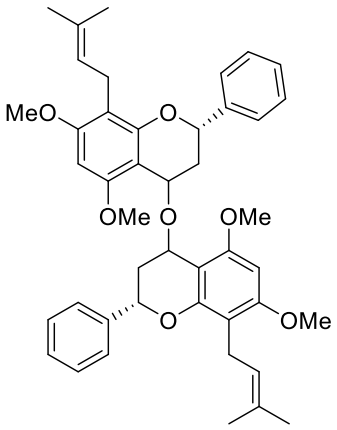
115 $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = OMe$
116 $R_1 = A$, $R_2 = R_3 = OMe$, $R_4 = R_5 = H$
117 $R_1 = A$, $R_2 = OMe$, $R_3 = OH$, $R_4 = R_5 = H$
118 $R_1 = A$, $R_2 = R_3 = R_5 = OMe$, $R_4 = H$
119 $R_1 = A$, $R_2 = R_3 = OMe$, $R_4 = OH$, $R_5 = H$
120 $R_1 = A$, $R_2 = R_2 = R_3 = R_4 = OMe$, $R_5 = H$
121 $R_1 = B$, $R_2 = R_3 = OMe$, $R_4 = R_5 = H$



124 $R_1 = OMe = R_2 = OH$, $R_3 = H$
125 $R_1 = R_2 = OMe$, $R_3 = H$
126 $R_1 = OMe$, $R_2 = R_3 = OH$



122 $R_1 = R_2 = OMe$
123 $R_1 = OMe$, $R_2 = OH$



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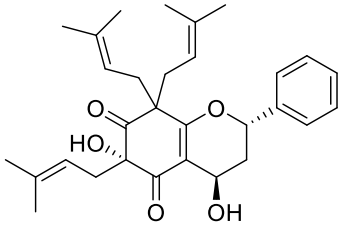

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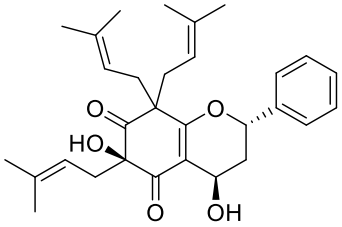
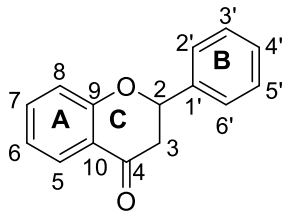


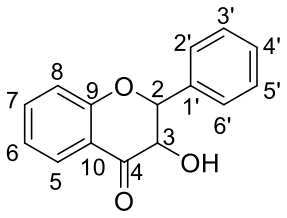
271

2.6.3: Flavanones

Flavanones have a 2-phenylchroman-4-one skeleton and some may possess a 3-hydroxy group (Agrawal, 1989; Dewick, 2002; Rauter *et al.*, 2018) as shown in Scheme 2.3. There are a number of this group of flavonoids that have been reported in *Tephrosia* species as listed in Table 2.4. The majority of these compounds have no substitution in ring B except a few like lupinifolin (**148**), lupinifolinol (**150**), tephrocandidins B (**140**) and 5-methyl ether citflavanone (**157**). Almost all flavanones reported in the genus are prenylated with prenylation occurring at either C-6 or C-8.



Flavanone



Flavanonol

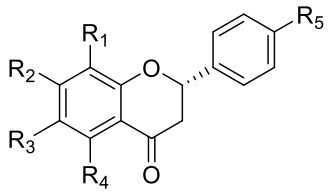
Scheme 2.3: Basic Skeleton of Subclasses of flavanones

Table 2.4: Flavanones of the Genus *Tephrosia*

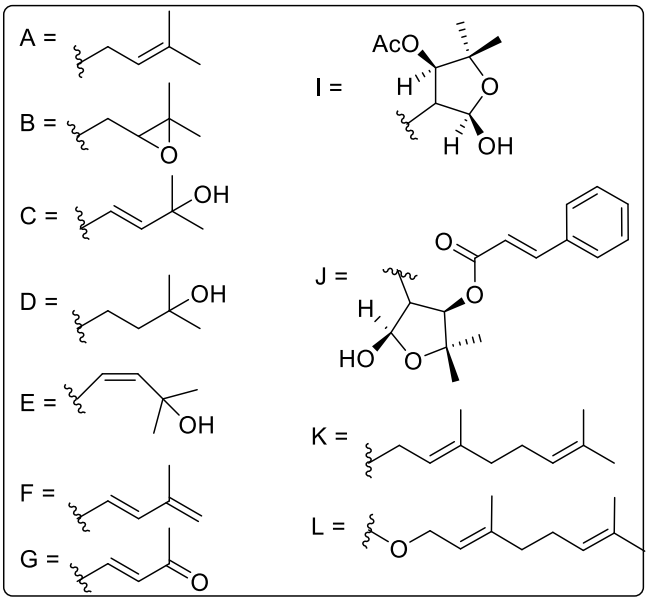
NO	Flavanone	Plant source (part)	Reference
49	Glabranin	*T. major* (RT & AP)	(Gómez-Garibay *et al.*, 2002)
50	7-Methylglabranin	*T. villosa* (RT)	(Jayaraman *et al.*, 1980)
130	Candidone	*T. candida* (ST, LF)	(Roy *et al.*, 1986)
131	7-Hydroxy-5-methoxy-8-prenylflavanone	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
132	Epoxycandidone	*T. hamiltonii* (WP)	(Falak and Shoeb, 1987)
133	Tephroleocarpin A	*T. leiocarpa* (RT)	(Go´mez-Garibay *et al.*, 1991)
134	Falciformin	*T. falciformis* (SD)	(Khan *et al.*, 1986)
135	Tephrowatsin C	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
136	Quercetol C	*T. quercetorum* (RT)	(Gomez-Garibay *et al.*, 1988)
137	*Z*-Quercetol C	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
138	Tephrocandidins A	*T. candida* (AP)	(Hegazy *et al.*, 2011)
139	Tephrocandidins B	*T. candida* (AP)	(Hegazy *et al.*, 2011)
140	Tephroleocarpin B	*T. leiocarpa* (RT)	(Go´mez-Garibay *et al.*, 1991)
141	5-Methyl ether tephroleocarpin B	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
142	Dehydroisoderricin	*T. purpurea* (RT)	(Rao and Raju, 1984)
143	5-Hydroxy-7-methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone	*T. toxicaria* (ST)	(Jang *et al.*, 2003)
144	Spinoflavanone B	*T. spinosa* (RT)	(Rao and Prasad, 1992a)
145	Tephrorins A	*T. purpurea* (AP)	(Chang *et al.*, 2000)
146	Tephrorins B	*T. purpurea* (AP)	(Chang *et al.*, 2000)
147	Fulvinervin A	*T. fulvinervis* (SD)	(Venkata Rao *et al.*, 1985)
148	Lupinifolin	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1974)
149	Mundulinol	*T. subtriflora* (AP)	(Muiva-Mutisya *et al.*, 2018)
150	Lupinifolinol	*T. lupinifolia* (RT)	(Smalberger *et al.*, 1974)
151	Fulvinervin B	*T. fulvinervis* (SD)	(Venkata Rao *et al.*, 1985)
152	Isolonchocarpin	*T. purpurea* (SD)	(Gupta *et al.*, 1980)
153	5-Methoxyisolonchocarpin	*T. emoroides* (RT)	(Machocho *et al.*, 1995)
154	Spinoflavanone A	*T. spinosa* (RT)	(Rao and Prasad, 1992a)
155	Obovatin	*T. obovata* (WP)	(Chen *et al.*, 1978)
156	Obovatin methylesther	*T. obovata* (WP)	(Chen *et al.*, 1978)
157	5-Methyl ether citflavanone	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
158	3-Hydroxy-5-methoxy-6",6"-dimethylpyrano-[2",3":7,8]-flavanone	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
159	Lanceolatin B	*T. purpurea* (SD)	(Gupta *et al.*, 1980)
160	Rhodimer	*T. rhodesica* (RT)	(Atilaw *et al.*, 2020)

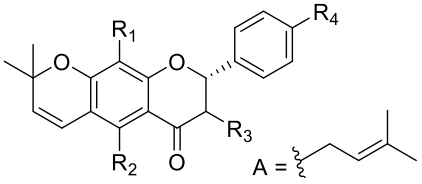
NO	Flavanone	Plant source (part)	Reference
161	5-Methoxy-6",6"-dimethyl-4",5"-dihydrocyclopropa-furano-[2",3":7,8]-flavanone	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
162	Subtruflavanonol	*T. subtriflora* (AP)	(Muiva-Mutisya *et al.*, 2018)
163	MS-II	*T. subtriflora* (AP)	(Muiva-Mutisya *et al.*, 2018)
164	(-)-Purpurin	*T. purpurea* (SD)	(Gupta *et al.*, 1980)
165	(+)-Purpurin	*T. purpurea* (RT)	(Rao and Raju, 1984)
166	Maximaflavanone A	*T. maxima* (RT)	(Venkata Rao *et al.*, 1994)
167	Isoglabratephrin B	*T. purpurea* (ST)	(Chen *et al.*, 2015)
168	5,4'-Dihydroxy7-O-[(*E*)-3,7-dimethyl-2,6-octadienyl]-flavanone	*T. villosa* (RT)	(Madhusudhana *et al.*, 2010)
169	5,4'-Dihydroxy-7-O-[(*E*)-3,7-dimethyl-2,6-octadienyl]-8-C-[(E)-3,7-dimethyl-2,6-octadienyl]-flavanone	*T. villosa* (RT)	(Madhusudhana *et al.*, 2010)

Key: RT – roots, SD – seeds/seedpods, FL – flowers, AP – aerial parts, WP – whole plant

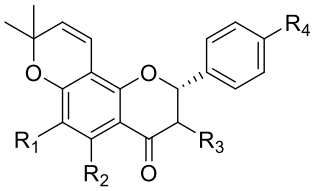


49 R_1 = A, R_2 = R_4 = OH, R_3 = R_5 = H
50 R_1 = A, R_2 = OMe, R_3 = R_5 = H, R_4 = OH
130 R_1 = A, R_2 = R_4 = OMe, R_3 = R_5 = H
131 R_1 = A, R_2 = OH, R_3 = R_5 = H, R_4 = OMe
132 R_1 = B, R_2 = R_4 = OMe, R_3 = R_5 = H
133 R_1 = C, R_2 = OMe, R_4= OH, R_3 = R_5 = H
134 R_1 = C, R_2 = OMe, R_3 = R_4 = R_5 = H
135 R_1 = D, R_2 = R_4 = OMe, R_3 = R_5 = H
136 R_1 = C, R_2 = R_4 = OMe, R_3 = R_5 = H
137 R_1 = E, R_2 = R_4 = OMe, R_3 = R_5 = H
138 R_1 = E, R_2 = OH, R_4 = OMe, R_3 = R_5 = H
139 R_1 = E, R_2 = R_5 = OH, R_3 = H, R_4 = OMe
140 R_1 = F, R_2 = OMe, R_4 = OH, R_3 = R_5 = H
141 R_1 = F, R_2 = R_4 = OMe, R_3 = R_5 = H
142 R_1 = F, R_2 = OMe, R_3 = R_4 = R_5 = H
143 R_1 = G, R_2 = OMe, R_4 = OH, R_3 = R_5 = H
144 R_1 = R_3 = A, R_2 = R_4 = OH, R_5 = H
145 R_1 = I, R_2 = OMe, R_3 = R_4 = R_5 = H
146 R_1 = J, R_2 = OH, R_3 = R_4 = R_5 = H
168 R_1 = R_3 = H, R_2 = L, R_4 = R_5 = OH
169 R_1 = K, R_2 = L, R_3 = H, R_4 = R_5 =OH

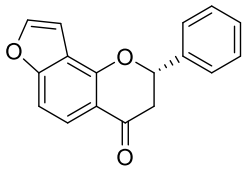




147 R_1 = A, R_2 = OH, R_3 = R_4 = H
148 R_1 = A, R_2 = R_4 = OH, R_3 = H
149 R_1 = A, R_2 = R_3 = OH, R_4 = H
150 R_1 = A, R_2 = R_3 = R_4 = OH



151 R_1 = A, R_2 = OH, R_3 = R_4 = H
152 R_1 = R_2 = R_3 = R_4 = H
153 R_1 = R_3 = R_4 = H, R_2 = R_3 = OMe
154 R_1 = A, R_2 = OH, R_3 = R_4 = H
155 R_1 = H, R_2 = OH, R_3 = R_4 = H
156 R_1 = H, R_2 = OMe, R_3 = R_4 = H
157 R_1 = H, R_2 = OMe, R_3 = OH, R_4 = H
158 R_1 = H, R_2 = OMe, R_3 = H, R_4 = OH
166 R_1 = A, R_2 = R_3 = R_4 = H



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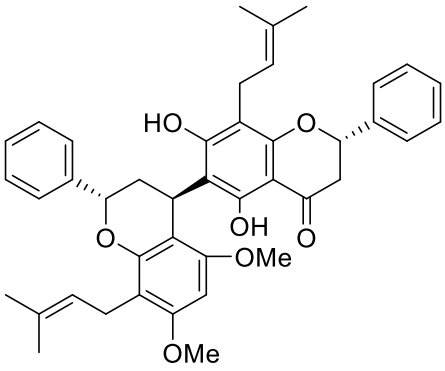

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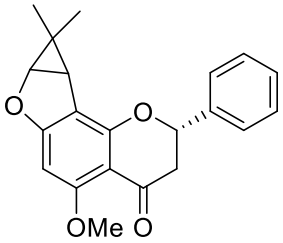

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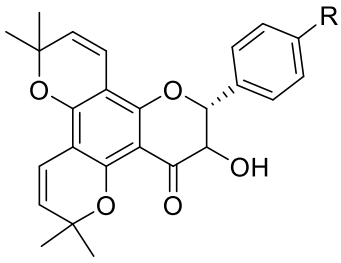
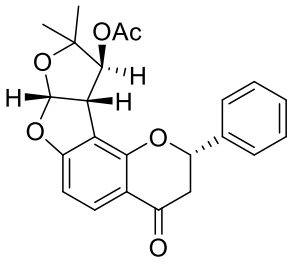


162 R = OH
163 R = H



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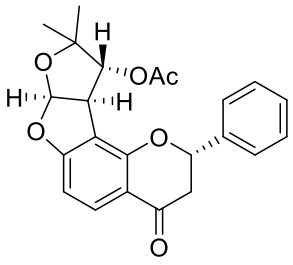

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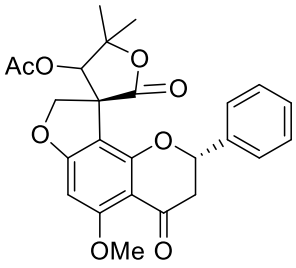
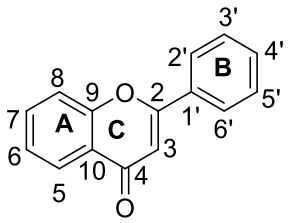


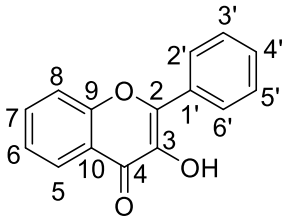
167

2.6.4: Flavones and Flavonols of the Genus Tephrosia

Flavones are 2-phenylchromen-4-one derivatives while flavonols are 3-hydroxyflavones (Agrawal, 1989; Dewick, 2002; Rauter *et al.*, 2018) as shown in Scheme 2.4. Many flavones and few flavonols that have been reported from the genus *Tephrosia* are listed in Table 2.5. Substitution in ring B is very rare except oxygenation at C-3 and C-4 in very few compounds like quercetin (**170**), methyl quercetin (**171**), chrysoeriol (**172**) and glucosides (**186** – **189**).



Flavone



Flavonol

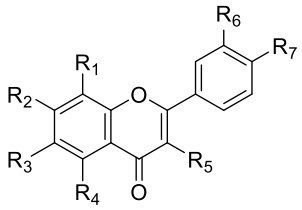
Scheme 2.4: Basic Skeletons of Flavones and Flavonols

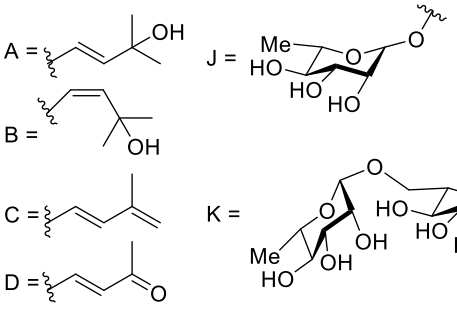
Table 2.5: Flavones and Flavonols of the Genus *Tephrosia*

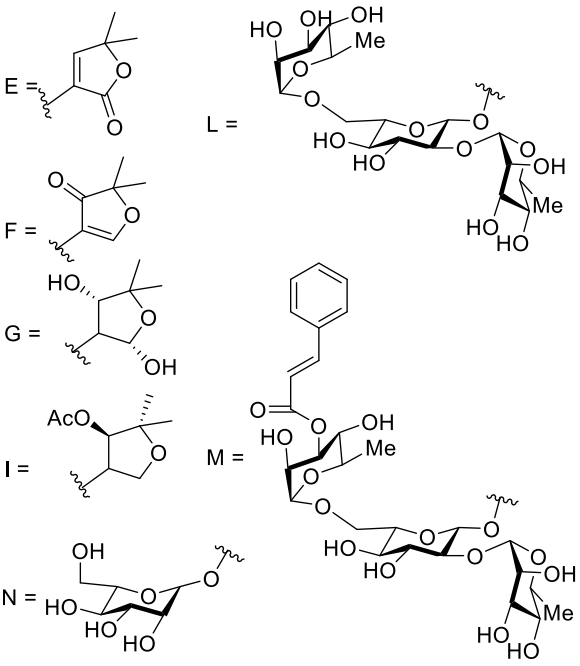
No.	Flavone	Plant source (part)	Reference
38	Isopongaflavone	*T. bracteolata* (SD)	(Khalid and Waterman, 1981)
170	Kaempferitrin	*T. purpurea* (ST)	(Gómez-Garibay *et al.*, 2002; Atilaw *et al.*, 2017b)
171	Methyl quercetin	*T. watsoniana* (ST)	(Gómez *et al.*, 1985b)
172	Chrysoeriol	*T. toxicaria* (ST)	(Jang *et al.*, 2003)
173	*trans*-Tephrostachin	*T. bracteolata* (SD)	(Khalid and Waterman, 1981)
174	Emoroidone	*T. emoroides* (RT)	(Machocho *et al.*, 1995)
175	(*E*)-5-Hydroxytephrostachin	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)
176	Lanceolatin A	*T. apollinea* (AP)	(Nenaah, 2014)
177	*Z*-Tephrostachin	*T. vogelii* (LF)	(Stevenson *et al.*, 2012)
178	(*E*)-5-Hydroxyanhydrotephrostachin	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)
179	*trans*-Anhydrotephrostachin	*T. bracteolata* (SD)	(Khalid and Waterman, 1981)
180	Purleptone	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)
185	Terpurinflavone	*T. purpurea* (ST)	(Juma *et al.*, 2011)
181	Apollinine	*T. apollinea* (SD)	(Waterman and Khalid, 1980)
182	Tephroglabrin	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
183	Hookerianin	*T. hookeriana* (SD)	(Prabhakar *et al.*, 1996)
184	Tepurindiol	*T. purpurea* (RT)	(Pelter *et al.*, 1981)
186	6-Hydroxykaempferol-6-methyl ether-3-*O*-α-rhamnopyranosyl(1→2)[α-rhamnopyranosyl(1→6)]-β-galactopyranoside-7-*O*-α-rhamnopyranoside - 27	*T. candida* (LF)	(Stevenson *et al.*, 2012)
187	6-Hydroxykaempferol-6-methyl ether-3-*O*-α-rhamnopyranosyl(1→6)-β-galactopyranoside-7-O-α-rhamnopyranoside -28	*T. candida* (LF)	(Stevenson *et al.*, 2012)

No.	Flavone	Plant source (part)	Reference
188	6-Hydroxykaempferol-6-methyl ether 3-*O*-α-rhamnopyranosyl(1→2)[α-rhamnopyranosyl(1→6)]-β-galactopyranoside -33	*T. candida* (LF)	(Stevenson *et al.*, 2012)
189	6-Hydroxykaempferol-6-methyl ether-3-*O*-α-rhamnopyranosyl(1→2)](3-*O*-*E*-feruloyl)-α-rhamnopyranosyl(1→6)]-β-galactopyranoside -60	*T. candida* (LF)	(Stevenson *et al.*, 2012)
190	Fulvinervin B	*T. fulvinervis* (SD)	(Venkata Rao *et al.*, 1985)
191	Fulvinervin C	*T. fulvinervis* (SD)	(Venkataratnam *et al.*, 1986)
192	Pseudosemiglabrin	*T. apollinea* (WP)	(Ahmad, 1986)
193	Pseudosemiglabrinol	*T. apollinea* (WP)	(Ahmad, 1986)
194	(-)-Semiglabrin	*T. semiglabrin* (AP, RT)	(Smalberger *et al.*, 1973)
195	(-)-Semiglabrinol	*T. semiglabrin* (AP, RT)	(Smalberger *et al.*, 1973)
196	Glabratephrin	*T. semiglabrin* (AP)	(Vleggaar *et al.*, 1978)
197	Isoglabratephrin	*T. purpurea* (AP)	(Hegazy *et al.*, 2009)
198	Glabratephrinol	*T. semiglabrin* (SD)	(Vleggaar *et al.*, 1978)
199	Kanjone	*T. purpurea* (SD)	(Gupta *et al.*, 1980)
200	Tephropurpulin	*T. purpurea* (AP)	(Hegazy *et al.*, 2009)
201	Tephrodin	*T. purpurea* (ST)	(Muiva-Mutisya *et al.*, 2014)
235	Kaempferol-3-*O*-β-D-glucopyranoside	*T. calophylla* (RT)	(Kishore *et al.*, 2003)
268	Terpurlepflavone	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)
269	Tachrosin	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)

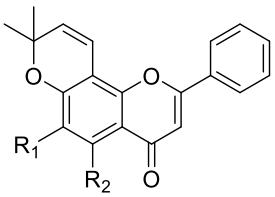
Key: RT–roots, SD–seeds/seedpods, FL-flowers, AP–aerial parts, ST– the stem, WP–whole plant



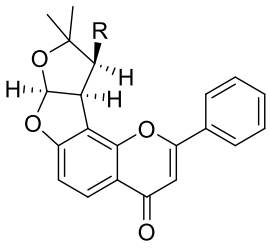




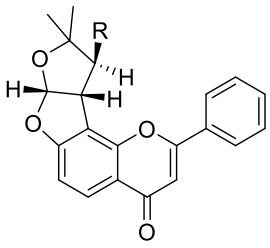
170 $R_1 = R_3 = R_6 = H$, $R_2 = R_5 = J$, $R_4 = R_7 = OH$
171 $R_1 = R_3 = H$, $R_2 = R_5 = R_6 = R_7 = OH$, $R_4 = OMe$
172 $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = R_5 = R_7 = OH$, $R_6 = OMe$
173 $R_1 = A$, $R_2 = R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$
174 $R_1 = A$, $R_2 = OH$, $R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$
175 $R_1 = A$, $R_2 = OMe$, $R_4 = OH$, $R_3 = R_5 = R_6 = R_7 = H$
176 $R_1 = A$, $R_2 = OMe$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$
177 $R_1 = B$, $R_2 = OH$, $R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$
178 $R_1 = C$, $R_2 = OMe$, $R_4 = OH$, $R_3 = R_5 = R_6 = R_7 = H$
179 $R_1 = C$, $R_2 = R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$
180 $R_1 = D$, $R_2 = OMe$, $R_4 = OH$, $R_3 = R_5 = R_6 = R_7 = H$
181 $R_1 = E$, $R_2 = OMe$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$
182 $R_1 = F$, $R_2 = OMe$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$
183 $R_1 = E$, $R_2 = R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$
184 $R_1 = G$, $R_2 = OMe$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$
185 $R_1 = I$, $R_2 = OAc$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$
186 $R_1 = R_6 = H$, $R_2 = J$, $R_3 = OMe$, $R_4 = R_7 = OH = R_5 = L$
187 $R_1 = R_6 = H$, $R_2 = J$, $R_3 = OMe$, $R_4 = R_7 = OH = R_5 = K$
188 $R_1 = R_6 = H$, $R_2 = R_4 = R_7 = OH$, $R_3 = OMe$, $R_5 = L$
189 $R_1 = R_6 = H$, $R_2 = R_4 = R_7 = OH$, $R_3 = OMe$, $R_5 = M$
235 $R_1 = R_3 = R_6 = H$, $R_2 = R_4 = R_7 = OH$, $R_5 = N$
269 $R_1 = F$, $R_2 = R_4 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$



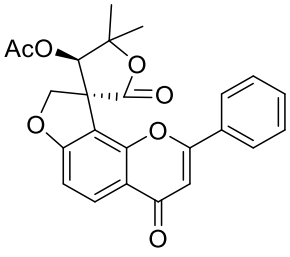
38 $R_1 = H$, $R_2 = OMe$
190 $R_1 = C$, $R_2 = OH$
191 $R_1 = A$, $R_2 = OH$



192 R = OAc
193 R = OH



194 R = OAc
195 R = OH



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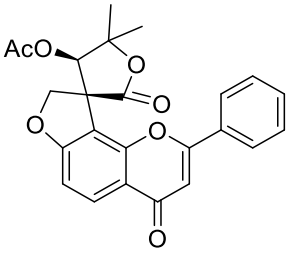

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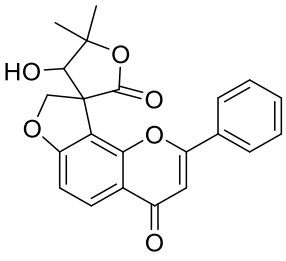

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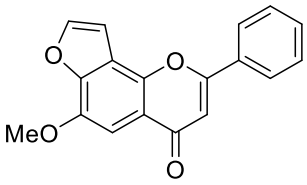

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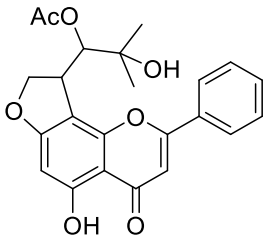

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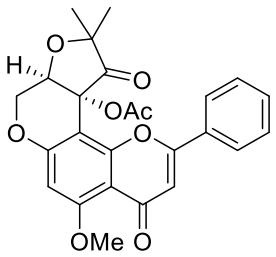

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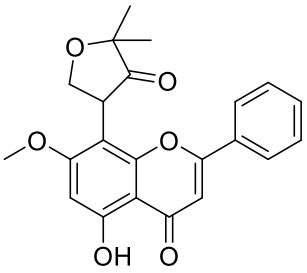

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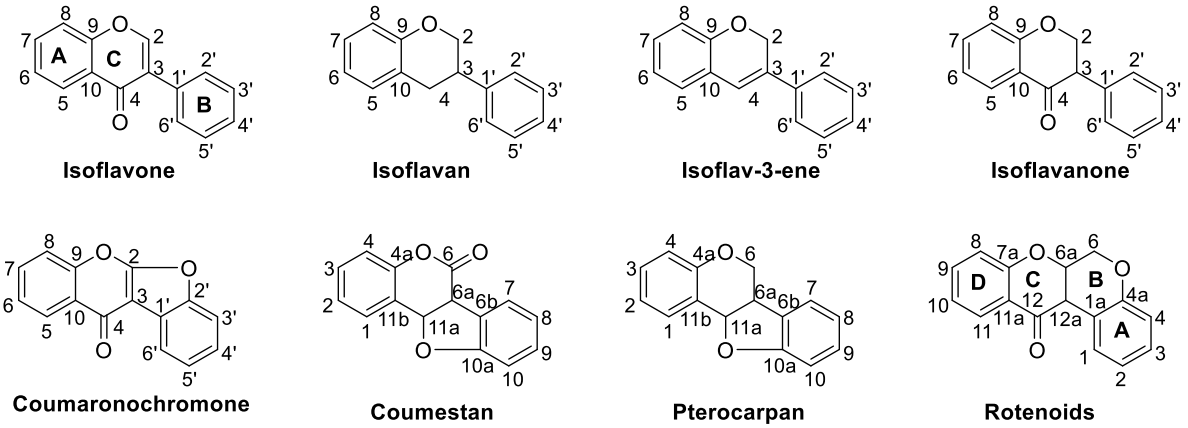
2.6.5: Isoflavonoids of the Genus Tephrosia

Isoflavonoids are commonly subclassified into isoflavones, isoflavans, isoflav-3-enes, coumestans, coumaronochromones, pterocarpans and rotenoids based on their structural skeleton as illustrated in Scheme 2.5 (Whitten *et al.*, 1997; Rauter *et al.*, 2018). The isoflavones that have been reported in several species of *Tephrosia* are listed in Table 2.6. The majority of the reported isoflavones lack C-5 oxygenation but *O*-prenylation is common. Very few coumestans have been reported from the genus as listed in Table 2.7. Pterocarpans especially those having a 6a,11a-dihydrobenzofurobenzopyran ring are common in the genus *Tephrosia* as listed in Table 2.8.

Rotenoids are isoflavonoids having an extra carbon in form of a methylene which bridges C-2-C-2' of an isoflavone skeleton via an epoxy residue (Abidi, 1987; Agrawal, 1989; Whitten *et al.*, 1997). Rotenoids are considered as 2-methylene-2'-epoxyisoflavonoids with a *cis*-configuration at the fusion between *B*/*C* rings (Abidi, 1987). Several rotenoids have been reported in the genus as listed in Table 2.9.



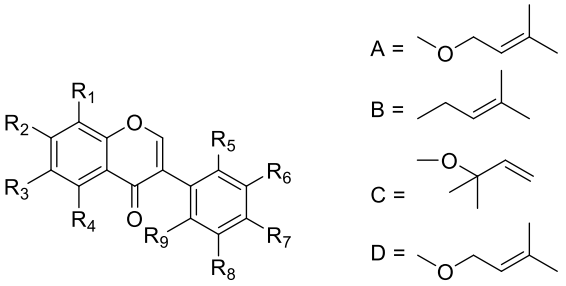
Scheme 2.5: Basic Skeletons of Isoflavonoids

Table 2.6: Isoflavones Reported from the *Tephrosia* species

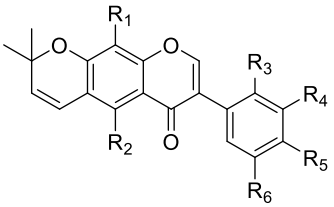
No.	Isoflavone	Plant species (part)	References
46	Calopogoniumisoflavone B	*T. maxima* (RT)	(Murthy and Rao, 1985)
55	Genistein	*T. toxicaria* (ST)	(Jang *et al.*, 2003)
202	Maximaisoflavone J	*T. maxima* (RT)	(Murthy and Rao, 1985)
203	Viridiflorin	*T. viridiflora* (RT & AP)	(Gómez *et al.*, 1985a)
204	Maximaisoflavone H	*T. maxima* (RT)	(Rao and Murthy, 1985)
205	Maximaisoflavone C	*T. maxima* (AP)	(Rao *et al.*, 1984)
206	Maximaisoflavone E	*T. maxima* (AP, RT)	(Rao *et al.*, 1984)
207	Maximaisoflavone D	*T. maxima* (AP, RT)	(Rao *et al.*, 1984)
208	Maximaisoflavone F	*T. maxima* (AP, RT)	(Rao *et al.*, 1984)
209	7,8,6'-Trimethoxy-3',4'-methylenedioxyisoflavone	*T. maxima* (AP)	(Rao *et al.*, 1984)
210	Maximaisoflavone G	*T. maxima* (AP, RT)	(Rao *et al.*, 1984)
211	6-Methoxy-7-hydroxy-3',4'-methylenedioxyisoflavone	*T. maxima* (AP)	(Rao *et al.*, 1984)
212	7,6'-Trimethoxy-3',4'-methylenedioxyisoflavone	*T. maxima* (AP)	(Rao *et al.*, 1984)
213	Maximaisoflavone B	*T. maxima* (RT)	(Rao and Murthy, 1985)
214	Maximaisoflavone A	*T. maxima* (AP)	(Rao *et al.*, 1984)
215	Pumilaisoflavone C	*T. pumila* (SD)	(Yenesew *et al.*, 1989)
216	Pumilaisoflavone B	*T. pumila* (SD)	(Dagne *et al.*, 1988)
217	Derrone	*T. purpurea* (ST)	(Atilaw *et al.*, 2017b)
218	5,7-Di-*O*-prenylbiochanin A	*T. tinctoria* (ST)	(Khalivulla *et al.*, 2008)
219	Pumilaisoflavone A	*T. pumila* (SD)	(Dagne *et al.*, 1988)
220	Warangalone	*T. elata* (RT)	(Lwande *et al.*, 1985a)
221	Pumilaisoflavone D	*T. pumila* (SD)	(Yenesew *et al.*, 1989)
222	Elongatin	*T. viridiflora* (RT & AP)	(Smalberger *et al.*, 1975)
223	Auriculatin	*T. calophylla* (RT)	(Ganapaty *et al.*, 2014)
224	Auriculasin	*T. calophylla* (RT)	(Ganapaty *et al.*, 2014)
225	Isoauriculatin	*T. calophylla* (RT)	(Ganapaty *et al.*, 2014)
226	Isoauriculasin	*T. calophylla* (RT)	(Ganapaty *et al.*, 2014)
227	Pumilanol	*T. pumila* (RT)	(Ganapaty *et al.*, 2008a)

No.	Isoflavone	Plant species (part)	References
237	7,4'-Dihydroxy-3',5'-dimethoxyisoflavone	*T. purpurea* (WP)	(Chang *et al.*, 2000)
238	3'-Methoxydaidzein	*T. purpurea* (WP)	(Chang *et al.*, 2000)

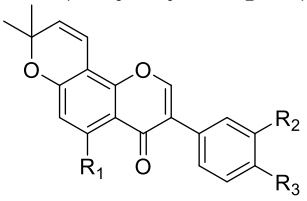
Key: RT – roots, SD – seeds/seedpods, AP – aerial parts, ST – stem



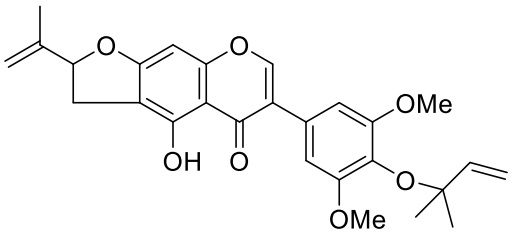
55 $R_1 = R_3 = R_5 = R_6 = R_8 = R_9 = H$, $R_2 = R_4 = R_7 = OH$
202 $R_2 = A$, $R_1 = R_3 = R_4 = R_5 = R_6 = R_8 = R_9 = H$, $R_7 = OMe$
203 $R_1 = R_6 = R_9 = H$, $R_2 = R_4 = R_7 = OH$, $R_3 = B$, $R_5 = R_8 = OMe$
204 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = R_5 = R_6 = R_8 = R_9 = H$, $R_7 = OMe$
205 $R_1 = R_3 = R_4 = R_6 = R_9 = H$, $R_2 = A$, $R_7 = R_8 = OCH_2O$
206 $R_1 = OMe$, $R_2 = OH$, $R_3 = R_4 = R_5 = R_8 = R_9 = H$, $R_6 = R_7 = OCH_2O$
207 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = R_5 = R_8 = R_9 = H$, $R_6 = R_7 = OMe$
208 $R_1 = R_9 = OMe$, $R_2 = OH$, $R_3 = R_4 = R_5 = R_8 = H$, $R_6 = R_7 = OCH_2O$
209 $R_1 = R_2 = R_9 = OMe$, $R_3 = R_4 = R_5 = R_8 = H$, $R_6 = R_7 = OCH_2O$
210 $R_1 = R_3 = R_4 = R_5 = R_8 = H$, $R_3 = OH$, $R_6 = R_7 = OCH_2O$, $R_9 = OMe$
211 $R_1 = R_4 = R_5 = R_8 = R_9 = H$, $R_2 = OH$, $R_3 = OMe$, $R_6 = R_7 = OCH_2O$
212 $R_1 = R_3 = R_4 = R_5 = R_8 = H$, $R_2 = R_9$ OMe, $R_6 = R_7 = OCH_2O$
213 $R_2 = A$, $R_1 = R_3 = R_4 = R_5 = R_8 = R_9 = H$, $R_6 = R_7 = OCH_2O$
214 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = R_5 = R_8 = R_9 = H$, $R_6 = R_7 = OCH_2O$
215 $R_1 = R_9 = H$, $R_2 = R_4 = R_7 = OH$, $R_3 = R_5 = B$, $R_6 = R_8 = OMe$
237 $R_1 = R_3 = R_4 = R_5 = R_9 = H$, $R_2 = R_7 = OH$, $R_6 = R_8 = OMe$
238 $R_1 = R_3 = R_4 = R_5 = R_8 = R_9 = H$, $R_2 = R_7 = OH$, $R_6 = OMe$



219 $R_1 = R_3 = H$, $R_2 = OH$, $R_4 = R_6 = OMe$, $R_5 = C$
220 $R_1 = B$, $R_2 = R_5 = OH$, $R_3 = R_4 = R_5 = R_6 = H$
221 $R_1 = R_3 = H$, $R_2 = R_5 = OH$, $R_4 = R_6 = OMe$
222 $R_1 = R_4 = H$, $R_2 = R_5 = OH$, $R_3 = R_6 = OMe$
223 $R_1 = B$, $R_2 = R_3 = R_5 = OH$, $R_4 = R_6 = H$
224 $R_1 = B$, $R_2 = R_4 = R_5 = OH$, $R_3 = R_6 = H$
225 $R_1 = R_4 = R_6 = H$, $R_2 = R_3 = OH$, $R_5 = D$
226 $R_1 = R_3 = R_6 = H$, $R_2 = R_4 = OH$, $R_5 = D$



46 $R_1 = H$, $R_2 = R_3 = OCH_2O$
217 $R_1 = R_3 = OH$, $R_2 = H$



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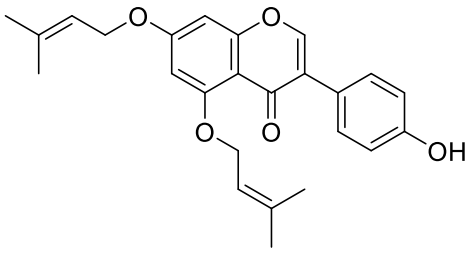
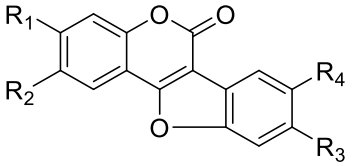


218

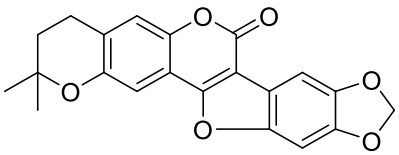
Table 2.7: Coumestans Reported from the Genus *Tephrosia*

No.	Coumestan	Plant species (part)	References
227	2-Methoxy-3,9-dihydroxycoumestone	*T. hamiltonii* (RT)	(Rajani and Sarma, 1988)
228	Flemichaparin C	*T. hamiltonii* (RT)	(Rajani and Sarma, 1988)
229	Tephrosol	*T. villosa* (RT)	(Rao and Srimannarayana, 1980)
230	Tephcalostan D	*T. calophylla* (RT)	(Ganapaty *et al.*, 2009)
231	Tephcalostan C	*T. calophylla* (RT)	(Ganapaty *et al.*, 2009)
232	Tephcalostan B	*T. calophylla* (RT)	(Ganapaty *et al.*, 2009)
232	Tephcalostan	*T. calophylla* (WP)	(Kishore *et al.*, 2003)
239	3,9-Dihydroxy-8-methoxycoumestan	*T. purpurea* (WP)	(Chang *et al.*, 1997)

Key: RT – Roots, WP – whole plant



227 $R_1 = R_3 = OH$, $R_2 = OMe$, $R_4 = H$
228 $R_1 = OMe$, $R_2 = H$, $R_3 = R_4 = OCH_2O$
229 $R_1 = OH$, $R_2 = OMe$, $R_3 = R_4 = OCH_2O$
239 $R_1 = R_3 = OH$, $R_2 = H$, $R_4 = OMe$



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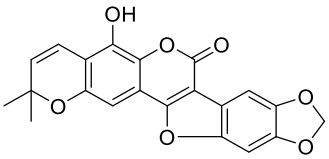

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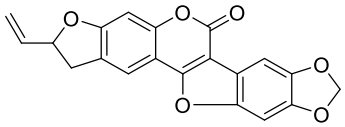

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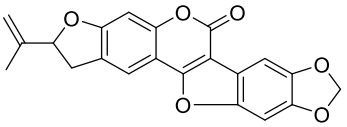

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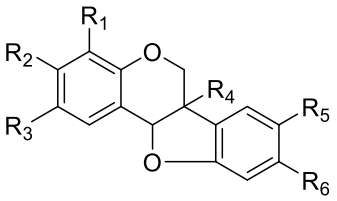
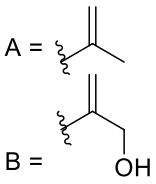
Table 2.8: Pterocarpans Reported from the Genus *Tephrosia*

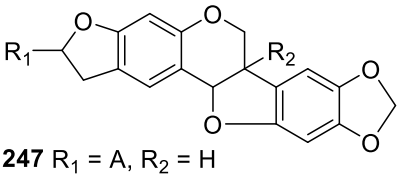
No.	Pterocarpan	Plant species (Part)	Reference
24	Maackiain	*T. purpurea* (WP)	(Chang *et al.*, 1997)
30	Pisatin	*T. bidwilli* (SD)	(Ingham and Markham, 1980)
240	3-Hydroxy-4-methoxy-8,9-methylenedioxypterocarpan	*T. purpurea* (WP)	(Chang *et al.*, 1997)
241	Medicarpin	*T. purpurea* (WP)	(Chang *et al.*, 1997)
242	Methoxymaackiain	*T. bidwilli* (SD)	(Ingham and Markham, 1980)
243	Tephrocarpin	*T. bidwilli* (SD)	(Ingham and Markham, 1980)
244	Acanthocarpan	*T. bidwilli* (SD)	(Ingham and Markham, 1980)
245	3,4-Dimethylenedioxy-pterocarpan	*T. aequilata* (RT)	(Tarus *et al.*, 2002)
246	Hildecarpin	*T. hildebrandtii* (RT)	(Lwande *et al.*, 1986a)
247	Emoroidocarpan	*T. emoroides* (RT)	(Machocho *et al.*, 1995)

No.	Pterocarpan	Plant species (Part)	Reference
248	4'-Hydroxyemeroroidocarpan	*T. purpurea* (ST)	(Li *et al.*, 2011)
249	Hildecarpidin	*T. hildebrandtii* (RT)	(Lwande *et al.*, 1987)
250	Flemichaparin B	*T. hamiltonii* (RT)	(Rajani and Sarma, 1988)
251	3,4:8,9-Dimethylenedioxy-6a,11a-pterocarpene	*T. aequilata* (RT)	(Atilaw *et al.*, 2017a)
262	Rhodacarpin	*T. rhodesica* (RT)	(Atilaw *et al.*, 2020)

KEY: ST – stems, WP – whole plant, SD – seeds/seedpods, RT - roots

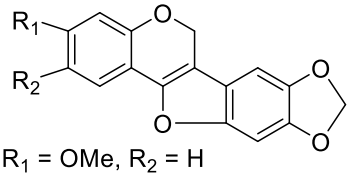






247 R_1 = A, R_2 = H
248 R_1 = B, R_2 = H
249 R_1 = B, R_2 = OH

24 $R_1 = R_3 = R_4 = H$, $R_2 = OH$, $R_5 = R_6 = OCH_2O$
30 $R_1 = R_3 = H$, $R_2 = OMe$, $R_4 = OH$, $R_5 = R_6 = OCH_2O$
240 $R_1 = OMe$, $R_2 = OH$, $R_3 = R_4 = H$, $R_5 = R_6 = OCH_2O$
241 $R_1 = R_3 = R_4 = H$, $R_2 = R_5 = OH$, $R_6 = OMe$
242 $R_1 = OMe$, $R_2 = OH$, $R_3 = R_4 = H$, $R_5 = R_6 = OCH_2O$
243 $R_1 = OMe$, $R_2 = R_4 = OH$, $R_3 = H$, $R_5 = R_6 = OCH_2O$
244 $R_1 = R_2 = OCH_2O$, $R_3 = H$, $R_4 = OH$, $R_5 = R_6 = OCH_2O$
245 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = H$, $R_5 = R_6 = OCH_2O$
246 $R_1 = H$, $R_2 = R_4 = OH$, $R_3 = OMe$, $R_5 = R_6 = OCH_2O$



250 $R_1 = OMe$, $R_2 = H$
251 $R_1 = R_2 = OCH_2O$

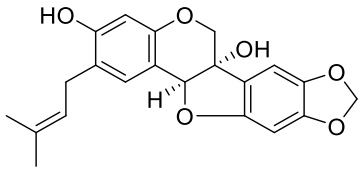


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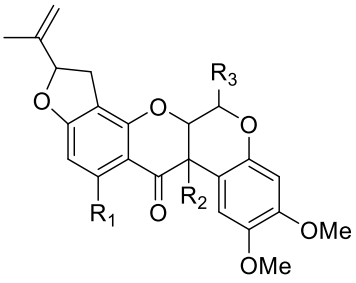
Table 2.9: Rotenoids Reported in the Genus *Tephrosia*.

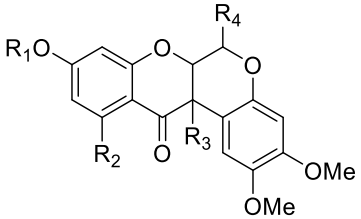
No.	Rotenoid	Plant source (part)	Reference
18	Munduserone	*T. fulvinervis* (RT) *T. pentaphylla* (LV)	(Dagne *et al.*, 1989)
20	Rotenone	*T. pentaphylla* (RT)	(Dagne *et al.*, 1989)
22	Deguelin	*T. fulvinervis* (RT)	(Dagne *et al.*, 1989)
23	Tephrosin	*T. obovata* (WP)	(Chen *et al.*, 1958)
19	*cis*-12a-Hydroxymunduserone	*T. fulvinervis* (RT)	(Dagne *et al.*, 1989)
42	Dehydrorotenone	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
252	Sumatrol	*T. pentaphylla* (RT)	(Dagne *et al.*, 1989)
21	*cis*-12a-Hydroxyrotenone	*T. pentaphylla* (RT)	(Dagne *et al.*, 1989)

No.	Rotenoid	Plant source (part)	Reference
254	6a-Hydroxyrotenone	*T. pentaphylla* (RT)	(Dagne *et al.*, 1989)
255	Villol	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
256	Villosin	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
257	6-Acetoxydihydrostemonal	*T. pentaphylla* (RT, LV)	(Dagne *et al.*, 1989)
258	Dihydrostemonal	*T. pentaphylla* (RT, LV)	(Dagne *et al.*, 1989)
259	9-Demethyldihydrostemonal	*T. pentaphylla* (SD)	(Dagne *et al.*, 1989)
260	6-Hydroxy-α-toxicarol	*T. villosa* (RT)	(Muiva-Mutisya *et al.*, 2014)
261	4',5'-Dihydo-5',11-dihydroxy-4'-methoxytephrosin	*T. toxicaria* (ST)	(Jang *et al.*, 2003)
262	α-toxicarol	*T. pentaphylla* (LV) *T. fulvinervis* (RT)	(Dagne *et al.*, 1989)
263	Villosol	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
264	Villosinol	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
265	Villinol	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
266	Villosone	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)
267	Rotenonone	*T. villosa* (SD)	(Krupadanam *et al.*, 1977a)

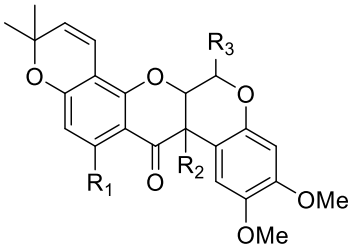
Key: RT – roots, SD – seeds/seedpods, LV – leaves, ST – the stem, WP – whole plant



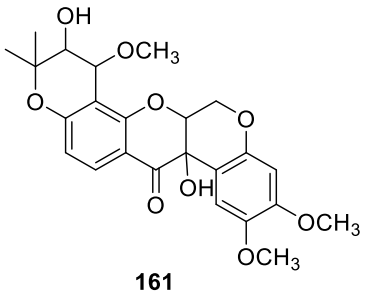
20 $R_1 = R_2 = R_3 = H$
252 $R_1 = OH, R_2 = R_3 = H$
253 $R_1 = R_3 = H, R_2 = OH$
254 $R_1 = R_2 = H, R_3 = OH$
255 $R_1 = R_2 = R_3 = OH$
256 $R_1 = R_3 = OH, R_2 = H$



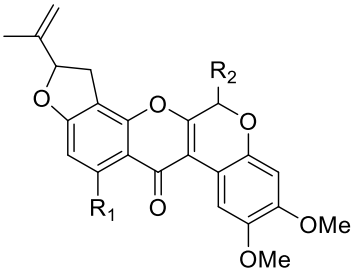
18 $R_1 = Me, R_2 = R_3 = R_4 = H$
19 $R_1 = Me, R_2 = R_4 = H, R_3 = OH$
257 $R_1 = Me, R_2 = R_3 = H, R_4 = OH$
258 $R_1 = Me, R_2 = R_4 = OH, R_3 = H$
259 $R_1 = R_3 = H, R_2 = R_4 = OH$



22 $R_1 = R_2 = R_3 = H$
23 $R_1 = R_3 = H, R_2 = OH$
260 $R_1 = R_3 = OH, R_2 = H$
262 $R_1 = OH, R_2 = R_3 = H$



161



42 $R_1 = R_2 = H$
263 $R_1 = OH, R_2 = H$
264 $R_1 = R_2 = OH$
265 $R_1 = OH, R_2 = OMe$
266 $R_1 = OH, R_2 = O$
267 $R_1 = H, R_2 = O$

2.7: Biological Activities of Compounds Isolated from the Genus *Tephrosia*

Numerous flavonoids have been reported from the genus *Tephrosia* (Touqeer *et al.*, 2013; Chen *et al.*, 2014; Samuel *et al.*, 2019). However, just a few of the isolated compounds have been evaluated for their biological activities including anticancer, anti-plasmodial, anti-protozoal, anti-microbial and insecticidal (Table 2.10). Despite the reports that some of the crude extracts exhibited anti-inflammatory activities (Adaudi *et al.*, 2009; Sandhya *et al.*, 2010; Valli *et al.*, 2011), compounds isolated from this genus have not been assessed for their anti-inflammatory effects. However, compounds such as genistein (**55**) (Hämäläinen *et al.*, 2007), isoliquiritigenin (**93**) (Lee *et al.*, 2009), and chrysoeriol (**172**) (Wu *et al.*, 2020) isolated from other sources have shown significant anti-inflammatory activities.

Table 2.10: Biological Activities of Compounds Isolated from the Genus *Tephrosia*

No	Compound	Plant source	Activity	Reference
20	Rotenone	*T. elata*	antifeedant	(Bentley *et al.*, 1987)
22	Deguelin	*T. elata*	Antiplasmodial	(Muiva *et al.*, 2009)
23	Tephrosin	*T. elata*	Antifeedant	(Bentley *et al.*, 1987)
24	Maackiain	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
29	Hildecarpin	*T. hildebrandtii*	Antifeedant Antifungal	(Lwande *et al.*, 1986a)
31	Pongachin	*T. pulcherrima*	Antimicrobial	(Ganapaty *et al.*, 2008b)

No	Compound	Plant source	Activity	Reference
32	Emoroidenone	*T. emoroides*	Antifeedant	(Machocho *et al.*, 1995)
38	Isopongaflavone	*T. elata*	Antifeedant,	(Bentley *et al.*, 1987)
		T. aquilata	Antiplasmodial	(Atilaw *et al.*, 2017a)
53	Rhodiflavan C	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
55	Genistein	*T. toxicaria*	Cancer chemopreventive	(Jang *et al.*, 2003)
93	Isoliquiritigenin	*T. toxicaria*	Cancer chemopreventive	(Jang *et al.*, 2003)
97	Candidachalcone	*T. candida*	Estrogenic	(Hegazy *et al.*, 2011)
98	Tephrosone	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
99	Tephropurpurin	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
100	Obovatachalcone	*T. aquilata*	Antiplasmodial	(Atilaw *et al.*, 2017a)
105	Praecansone A	*T. praecans*	Antibacterial	(Tarus *et al.*, 2002)
		T. aquilata	Antiplasmodial	(Atilaw *et al.*, 2017a)
106	Praecansone B	*T. praecans,*	Antibacterial	(Tarus *et al.*, 2002)
		T. aquilata	Antiplasmodial	(Atilaw *et al.*, 2017a)
107	Demethylpraecansone B	*T. aequilata*	Antibacterial	(Tarus *et al.*, 2002)
108	Aequichalcone C	*T. aequilata*	Antiplasmodial	(Atilaw *et al.*, 2017a)
109	Pongamol	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
111	Elatadihydrochalcone	*T. elata*	Antiplasmodial	(Muiva *et al.*, 2009)

No	Compound	Plant source	Activity	Reference
112	Aequichalcone A	*T. aequilata*	Antiplasmodial	(Atilaw *et al.*, 2017a)
113	Aequichalcone B	*T. aequilata*	Antiplasmodial	(Atilaw *et al.*, 2017a)
119	Tephrowatsin A	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
120	Quercetol B (9)	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
128	Hildgardtene	*T. emoriodes*	antifeedant	(Machocho *et al.*, 1995)
138	Tephrocandidins A	*T. candida*	Estrogenic	(Hegazy *et al.*, 2011)
139	Tephrocandidins B	*T. candida*	Estrogenic	(Hegazy *et al.*, 2011)
144	Spinoflavanone B	*T. subtriflora*	Antiplasmodial	(Muiva-Mutisya *et al.*, 2018)
149	Mundulinol	*T. subtriflora*	Antiplasmodial	(Muiva-Mutisya *et al.*, 2018)
156	Obovatin methyl ether	*T. elata*	Antiplasmodial Piscicidal	(Muiva *et al.*, 2009) (Chen *et al.*, 1978)
159	Lanceolatin B	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
162	Subtruflavanonol	*T. subtriflora*	Antiplasmodial	(Muiva-Mutisya *et al.*, 2018)
163	MS-II	*T. subtriflora*	Antiplasmodial	(Muiva-Mutisya *et al.*, 2018)
168	Purpurin	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
172	Chrysoeriol	*T. toxicaria*	Cancer chemopreventive	(Jang *et al.*, 2003)
174	Emoroidone	*T. emoroides*	Antifeedant	(Machocho *et al.*, 1995)
180	Purleptone	*T. purpurea*	Cytotoxicity	Atilaw et al., 2017
185	Terpurinflavone	*T. purpurea*	Antiplasmodial	Juma et al., 2011

No	Compound	Plant source	Activity	Reference
201	Tephrodin	*T. purpurea*	Antiplasmodial	(Muiva-Mutisya et al., 2014)
227	Pumilanol	*T. pumila*	Antiprotozoal	(Ganapaty *et al.*, 2008a)
237	7,4'-Dihydroxy-3',5'-dimethoxyisoflavone	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
240	(-)-3-Hydroxy-4-methoxy-8,9-methylenedioxypterocarpan	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
241	Medicarpin	*T. purpurea*	Cancer chemopreventive	(Chang *et al.*, 2000)
245	3,4-Dimethylenedioxy-pterocarpan	*T. aequilata*	Antifeedant	(Tarus et al., 2002)
247	Emoroidocarpan	*T. emoroides*	Antifeedant	(Machocho et al., 1995)
251	3,4:8,9-Dimethylenedioxy-6a,11a-pterocarpene	*T. aequilata*	Antiplasmodial	(Atilaw et al., 2017a)
260	6-Hydroxyrotenone	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
262	α-Toxicarol	*T. toxicaria*	Larvicidal	(Vasconcelos *et al.*, 2009)
268	Terpurlepflavone	*T. purpurea*	Antiplasmodial Cytotoxicity	(Atilaw *et al.*, 2017b)
269	Tachrosin	*T. purpurea*	Antiplasmodial Cytotoxicity	(Atilaw *et al.*, 2017b)
270	Rhodiflavan A	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
271	Rhodiflavan B	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)
272	Rhodacarpin	*T. rhodesica*	Antiplasmodial	(Atilaw *et al.*, 2020)

CHAPTER 3: MATERIALS AND METHODS

3.1 Column Chromatography

Column chromatography was performed using Merck silica gel 60 (70-230 mesh) as a stationary phase and *n*-hexane or cyclohexane/ethyl acetate as a mobile phase while gel filtration chromatography was done using Sephadex LH-20 with dichloromethane/methanol (1:1) as mobile phase. TLC was carried out on pre-coated silica gel 60 $PF_{254+365}$ plates. Chromatotron 7924T (USA) was used for purification. Chromatographic spots were detected under a UV lamp (254 and 365 nm) or sprayed with 5% sulphuric acid in methanol.

3.2 Preparative High-Performance Liquid Chromatography

Preparative HPLC was performed on a Shimadzu LC-20AP system equipped with a DGU-20A5R degassing unit, SPD-M20A detector and SIL-20ACHT autosampler. A C18 column (Nucleodur Polartec 5μm, 10 x 125 mm or Phenomenex 10 μm, 10 x 250 mm) was used for reverse-phase separation. Methanol/water in 0.1% formic acid was used as the mobile phase. The fractions were loaded in the range of 50-200 μL as injection volume with a flow rate of 4 mL/min and the temperature was maintained at 25^{o}C. The HPLC was operated using the LabSolution software system. The fractions were concentrated under reduced pressure on a rotary evaporator and appropriately combined after verification by LC-MS.

3.3 High-Resolution Electron Spray Ionization Mass Spectrometry

The HPLC–$HRMS^n$ experiments were carried out on an LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. The spectrometer was operated in positive mode with a nominal mass resolving power of 60,000 at m/z 400 with a scan rate of 1 Hz under the following parameters: spray voltage 6 kV, capillary temperature 300°C and tube lens 100 V. Argon served as collision gas and nitrogen was used as sheath gas (66 arbitrary units) and auxiliary gas (8 arbitrary units). All MS^n experiments were performed with collision-induced dissociation at 35 eV. The spectrometer was equipped with an Agilent 1200 HPLC system

(Santa Clara, USA) including a pump, PDA detector, column oven (30 °C) and auto-sampler (injection volume: 5 μL for Full scan, 7 μL for MS^n). The HPLC analyses were performed with a Luna C18 column (60 × 3 mm, 3 μm particle size) from Phenomenex (Torrance, USA) with a water (+0.1% formic acid) (A) and methanol (+0.1% formic acid) (B) gradient (flow rate 360 µL/min). The gradient was set as follows: linear gradient from 95% A to 100% B over 24 min, 100% B isocratic for 5 min, the system returned within 1 min to initial conditions of 95% A and was equilibrated for 5 min. All the samples were dissolved in methanol.

3.4 Nuclear Magnetic Resonance Spectroscopy

NMR spectra were recorded on a Bruker Advance III 600 MHz spectrometer equipped with a cryoprobe unit using standard pulse sequences and referenced with the TMS. The chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). COSY, NOESY, HSQC and HMBC experiments were acquired using the standard Bruker programs. All the experiments were performed in deuterated solvents and chemical shifts were calibrated relative to the solvent peaks.

3.5 Optical Rotation and Circular Dichroism

Optical rotation was determined using an Autopol IV automatic polarimeter. CD measurements were done on a Jasco J-715 spectrometer.

3.6: Plant Materials

Detailed information regarding the collection of *Tephrosia* species used in this study is presented in Table 3.1. The plants were authenticated by Mr. Patrick Mutiso of the University Herbarium, School of Biological Sciences, the University of Nairobi, where voucher specimens were deposited.

Table 3.1: Plant collection and voucher details

Tephrosia species	Plant part	Locality	Collection date	Voucher number
T. vogelii	Seedpods	Tororo, Uganda N0°39'37.2" E034°12'5.6"	04.08.2016	ORO-2016/04
T. hildebrandtii	Aerial parts	Thika, Kenya S01°03'19.2" E037°14'10.4"	23.02.2017	ORO-2017/07
T. elata	Aerial parts	Thika, Kenya S01°03'20.5" E037°14'12.4"	23.02.2017	ORO-2017/08
T. rhodesica	Stems	Dzombo hills, Kwale county, Kenya S04°29'28.9" E039°15'16.3"	27.03.2018	ORO-2018/09
T. linearis	Aerial parts	Gongoni, Kwale county, Kenya S04°23'57.3" E039°27'17.4"	28.03.2018	ORO-2018/11

3.7: Extraction and Isolation

3.7.1: The Aerial Parts of Tephrosia linearis

The powder of *T. linearis* aerial parts (890 g) was extracted using dichloromethane/methanol (1:1). The concentrated extract (72.5 g) was partitioned between water and *n*-hexane to remove fats. The aqueous layer was further partitioned in ethyl acetate (EtOAc). The ethyl acetate portion was concentrated to provide a brown paste (18.4 g) that was then fractionated on silica gel using cyclohexane/ethyl acetate (EtOAc) in increasing polarity. The fraction that eluted at 5% EtOAc in cyclohexane was purified on Sephadex LH-20 using dichloromethane/methanol (1:1) followed by preparative HPLC (20:80, MeOH/H_2O-100% MeOH gradient elution for 50 min at a flow rate of 4 mL/min) yielding lineaflavone C (**3**) (1.1 mg). Similarly, the 10% EtOAc in cyclohexane fraction was purified using Sephadex LH-20 followed by preparative HPLC to give acetylobovatin (**6**) (1.0 mg). The 20% EtOAc in cyclohexane fraction was purified in a similar way to give maackiain (**24**) (11.5 mg), tephrosin (**23**) (5.3 mg), rotenone (**20**) (3.7 mg), deguelin (**22**) (6.3 mg) and 6-*C*-prenylapigenin (**10**) (18.2 mg). Fractions from 30% EtOAc in

cyclohexane were also purified on Sephadex LH-20 followed by preparative HPLC to give lineaflavone A (**1**) (24.3 mg), lineaflavone B (**2**) (5.3 mg), lineaflavone D (**4**) (1.9 mg), 6-methoxygeraldone (**5**) (1.0 mg), 5-hydroxy-7-methoxysaniculamin A (**7**) (1.0 mg), 7-*O*-methyl-6-prenylnaringenin (**8**) (2.3 mg), erylivingstone I (**9**) (1.5 mg), 5,7,4',2''-tetrahydroxy-6-[3''-methyl-3''-butenyl]-flavone (**11**) (8.8 mg), apigenin (**12**) (1.2 mg), atalantoflavone (**15**) (4.9 mg), geraldone (**14**) (1.0 mg), munduserone (**18**) (1.0 mg), *cis*-12a-hydroxymunduserone (**19**) (1.0 mg) and 12a-hydroxyrotenone (**21**) (3.3 mg). Purification of the 100% EtOAc fractions by preparative HPLC gave luteolin (**13**) (3.3 mg), patuletin 3-*O*-rhamnoside (**16**) (1.0 mg) and eupatolitin 3-*O*-rhamnoside (**17**) (16.5 mg).

3.7.2: The Aerial Parts of Tephrosia hildebrandtii

The powder of *T. hildebrandtii* aerial part (1.0 kg) was extracted using dichloromethane-methanol (1:1) to provide a dark brown paste (82.4 g). The crude extract (42.9 g) was fractionated on silica gel using cyclohexane-EtOAc in increasing polarity. The fraction that eluted with 30% EtOAc in cyclohexane was purified on Sephadex LH-20 using dichloromethane-methanol, (1:1) followed by Prep.-HPLC (20:80, MeOH/H_2O-100% MeOH gradient elution for 50 min with flow rate of 4 mL/min) to give 5,7,3'-trihydroxy-4-methoxy-8-prenylisoflavone (**26**) (1.0 mg), 5,3'-dihdroxy-4'-methoxy-2'',2''-dimethylpyrano[5'',6'':8,7]isoflavone (**27**) (1.1 mg), 4-hydroxyemoroidocarpan (**28**) (1.0 mg), pongachin (**31**) (1.6 mg), emoroidenone (**32**) (2.0 mg) and tephrosin (**22**) (1.8 mg). Similarly, the 50% EtOAc in cyclohexane fraction was purified using Sephadex LH-20 followed by Prep.-HPLC to give hildaflavone (**25**) (1.0 mg), desmoxyphyllin A (**33**) (1.0 mg), hildecarpin (**29**) (1.7 mg), pisatin (**30**) (1.6 mg) and pinoresinol (**34**) (1.2 mg).

3.7.3: The Seedpods of Tephrosia vogelii

The seedpods powder of *T. vogelii* (4.0 kg) was extracted using dichloromethane/methanol (1:1) to provide a dark brown paste (427.1 g). The concentrated extract was partitioned between water and *n*-hexane to remove fats. The aqueous layer was further partitioned in ethyl acetate (EtOAc). The ethyl acetate portion was concentrated to provide a brown paste (122.5 g) that was then fractionated on silica gel using *n*-hexane/EtOAc in increasing polarity. The fractions that eluted with 5-10% EtOAc in *n*-hexane were combined together and purified on Sephadex LH-20 using dichloromethane/methanol (1:1) followed by chromatotron (20:80, EtOAc/n-hexane-100% EtOAc gradient elution) to give isopongaflavone (**38**) (54.7 mg), 6a,12a-dehydro-α-toxicarol (**43**) (2.4 mg) and dehydrorotenone (**42**) (20.1 mg). 15% EtOAc in *n*-hexane fraction was purified on Prep.-HPLC (20:80, MeOH/H_2O-100% MeOH gradient elution for 35 min) to give vogelisoflavone A (**35**) (3.3 mg), onogenin (**37**) (1.7 mg) and tephrosin (**23**) (1.5 mg). The fraction eluted at 20% was purified on Prep.-HPLC (75:25, MeOH/H_2O isocratic elution for 20 min) to give luteolin (**13**) (1.3 mg). 70% EtOAc in *n*-hexane fraction was purified on Prep.-HPLC (5:80, MeOH/H_2O-100% MeOH gradient elution for 50 min) to give vogelisoflavone B (**36**) (2.3 mg), 4',7-dihydroxy-3'-methoxyflavanone (**39**) (1.2 mg), *trans-p*-hydroxycinnamic acid (**40**) (2.6 mg), 2-methoxygliricidol (**41**) (1.1 mg) and pinoresinol (**34**) (1.2 mg).

3.7.4: The Stems of Tephrosia elata

The powdered stem of *T. elata* (1.5 kg) was extracted using dichloromethane/methanol (1:1) to provide a dark brown paste (63.5 g). A portion (25.0 g) of the concentrated extract was partitioned between water and *n*-hexane to remove fats. The aqueous layer was further partitioned in EtOAc. The ethyl acetate portion was concentrated to provide a brown paste (10.1 g). It was then fractionated on silica gel using *n*-hexane/EtOAc/MeOH (6:3:1) and two major fractions were obtained. The second fraction was purified on Prep.-HPLC (35:80,

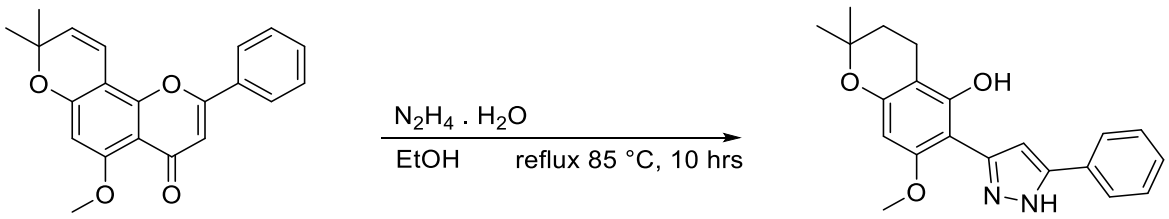
MeOH/H_2O-100% MeOH gradient elution for 50 min) to give elatisoflavone (**44**) (1.0 mg), barbigerone (**45**) (3.2 mg), calopogoniumisoflavone B (**46**) (1.0 mg) and jamaicin (**47**) (1.0 mg).

3.7.5: The Stems of Tephrosia rhodesica

The powdered stems of *T. rhodesica* (889 g) was extracted using dichloromethane/fractionated on silica gel using cyclohexane/EtOAc in increasing polarity. The fractions that eluted with 10% EtOAc in cyclohexane was purified on Sephadex LH-20 using dichloromethane/methanol (1:1) followed by Prep.-HPLC (20:80, MeOH/H_2O-100% MeOH gradient elution for 45 min) to give glabranin (**49**) (1.0 mg) and edunol (**56**) (2.8 mg). The 20% EtOAc in cyclohexane fraction was purified on Prep.-HPLC (20:80, MeOH/H_2O-100% MeOH gradient elution for 35 min) to give 3-methoxycoumestrol (**48**) (1.0 mg). The fraction eluted at 30% EtOAc in cyclohexane was purified on Prep.-HPLC (20:85, MeOH/H_2O -100% MeOH gradient elution for 50 min) to give rhodiflavan C (**53**) (1.0 mg), liquiritigenin (**51**) (1.0 mg), naringenin (**52**) (1.0 mg), 3'-*O*-methylorobol (**54**) (1.1 mg), genistein (**55**) (1.0 mg), 7-*O*-methylglabranin (**50**) (1.0 mg), tephrosin (**23**) (1.0 mg) and 12a-hydroxyrotenone (**21**) (1.2 mg).

3.8: Synthesis of the Pyrazoline Derivative of Isopongaflavone

Isopongaflavone, **38** (10 mg) was dissolved in ethanol (5 mL) and hydrazine monohydrate (0.5 mL) was added dropwise. The mixture was refluxed at 85°C for 10 hours with the progress of the reaction being monitored using LC-MS. The reaction mixture was concentrated *in vacuo*, diluted with water (100 mL) and extracted with dichloromethane (3 x 15 mL). The dichloromethane extract was purified using prep.-HPLC (20:85, MeOH/H_2O-100% MeOH gradient elution for 45 min) to give a pyrazole isopongaflavone derivative, pyrazoisopongaflavone (**57**) (6.98 mg, 67% yield).



3.9: Anti-inflammatory Assay

The anti-inflammatory assays were done in Pharmacelsus, Saarbrücken, Germany. They were conducted using peripheral blood mononuclear cells obtained from Immunospot (ePBMC®-Uncharacterized Cryopreserved Human PBMC) purchased from Cellular Technologies Limited [C.T.L., Ohio, USA, Appendix B2, (http://www.immunospot.com/CatalogueRetrieve.aspx?ProductID=10537096&A=SearchResult&SearchID=10324581&ObjectID=10537096&ObjectType=27)]. The pure compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 20 *m*M stock solutions while the crude plant extract was made as a 20 *m*g/mL stock solution. Ibuprofen was prepared as a stock solution of 20 *m*M in DMSO and was used as a positive control. Lipopolysaccharide (LPS) was dissolved in a cell culture medium at a concentration of 1 *m*g/mL. Pure compounds were tested at a concentration of 100 μM, while the crude plant extract was used at a concentration of 100 μg/mL. The final DMSO concentration was 0.5% in all the samples. The positive control, ibuprofen, was also used at 100 μM and all samples were co-incubated with 10 μg/mL LPS. PBMCs were the main source of cytokines [interleukins (IL-1β, IL-2, IL-6), interferon-*gamma* (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-*alpha* (TNF-α)] within the circulating blood. Due to the small amounts of cytokines released by PBMCs into the supernatant, a bead-based assay (ProcartaPlex, Luminex) was used to quantify the six cytokines in parallel within a 50 μL sample using appropriate calibration standards. Human cryopreserved PBMCs were thawed according to the manufacturer's instructions. Three vials of cells from different donors were pooled. Cells were

washed, resuspended in RPMI 1640 containing 10% FBS, plated in 96-well round-bottom plates at 100,000 PBMCs/well and exposed to the test items at the concentrations stated above. Therefore, subsequent dilutions of stock solutions of the test items were prepared in a 96-well plate and transferred to the PBMCs containing wells. The cells were incubated for 24 hours at 37°C and 5% CO_2. The plates were then centrifuged for 3 min at 350 gyrations and the cell-free supernatant was collected and subjected to cytokine bead-array assay. The cytokine bead-array assay was conducted according to the manufacturer's instructions and read on a MagPix reader. For the dose-response relationship, absolute concentrations were calculated by the MagPix software using two separate calibration series as provided by the manufacturer. For negative control, cells were incubated only with a cell culture medium. As a positive control for inflammation, cells were incubated with 10 μg/mL LPS, while as a positive control for anti-inflammation, cells were co-incubated with 10 μg/mL LPS and 100 μM ibuprofen.

CHAPTER 4: RESULTS AND DISCUSSION

In this study, five *Tephrosia* species (*T. linearis*, *T. hildebrandtii*, *T. vogelii, T. elata* and *T. rhodesica*) were phytochemically studied to identify secondary metabolites with anti-inflammatory properties. Their crude extracts were subjected to chromatographic separations leading to the identification of fifty-six compounds. Their characterizations were based on UV, NMR and MS spectroscopic data. The structural elucidation and anti-inflammatory activities of the compounds from these five *Tephrosia* species are discussed in the following sections.

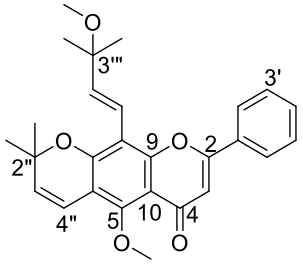
4.1: Characterization of Compounds Isolated from *Tephrosia linearis*

The following compounds were isolated from the aerial parts of *T. linearis*: seven new compounds; lineaflavone A (**1**), lineaflavone B (**2**), lineaflavone C (**3**), lineaflavone D (**4**), 6-methoxygeraldone (**5**), acetylobovatin (**6**) and 5-hydroxy-7-methoxysaniculamin A (**7**), together with eighteen other known compounds identified as 7-*O*-methyl-6-prenylnaringenin (**8**), erylivingstone I (**9**), 6-*C*-prenylapigenin (**10**), 5,7,4',2''-tetrahydroxy-6-[3''-methyl-3''-butenyl]-flavone (**11**), apigenin (**12**), luteolin (**13**), geraldone (**14**), atalantoflavone (**15**), patuletin 3-*O*-rhamnoside (**16**), eupatolitin 3-*O*-rhamnoside (**17**), munduserone (**18**), *cis*-12a-hydroxymunduserone (**19**), rotenone (**20**), 12a-hydroxyrotenone (**21**), deguelin (**22**), tephrosin (**23**) and (-)-maackianin (**24**).

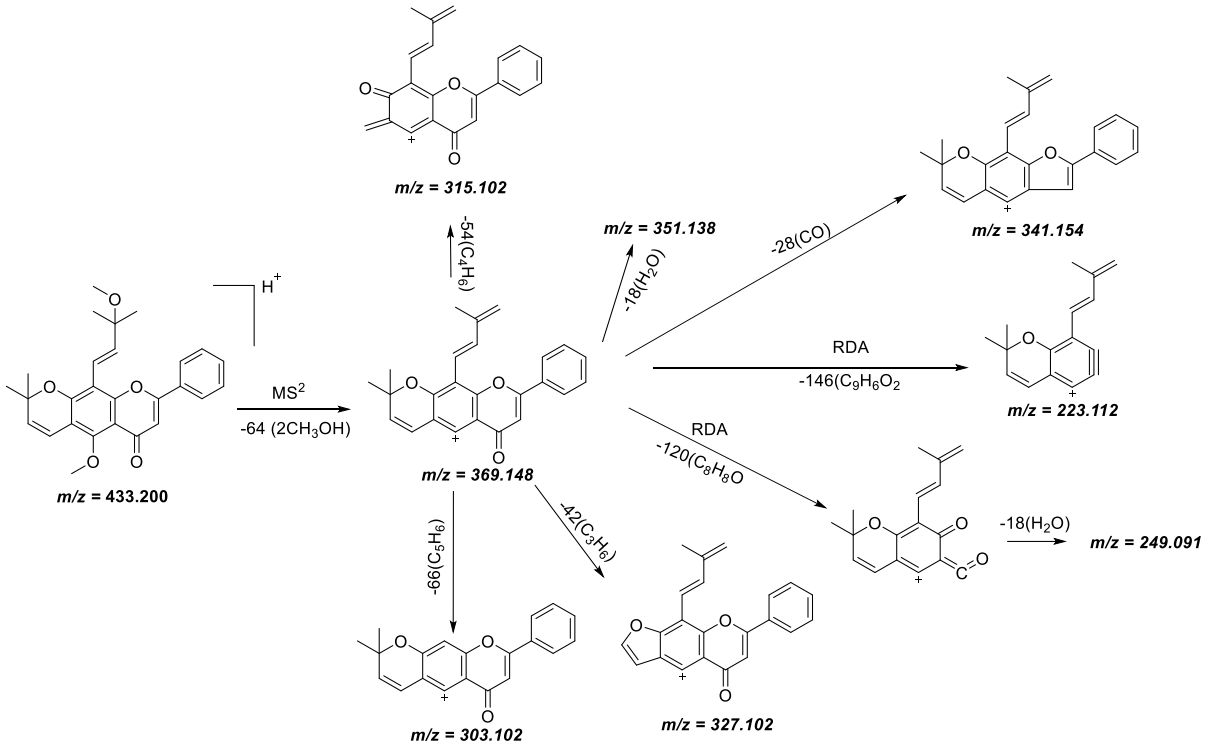
4.1.1: Lineaflavone A (1)

Compound **1** was isolated as a pale-yellow paste. Its molecular formula was deduced as $C_{27}H_{28}O_5$ based on the HRESIMS molecular ion $[M+H]^+$ at *m/z* 433.2011 (calcd for $C_{27}H_{29}O_5$, 433.2010) and $[M+Na]^+$ at *m/z* 455.1828 (calcd for $C_{27}H_{28}O_5Na$ 455.1829) together with ^{13}C NMR data (Table 4.1 and Appendix A1). A flavone skeleton was evident from the UV (λ_{max} 252, 300, and 336 nm) and NMR data [δ_H 6.69 (H-3); δ_C 161.4 (C-2), 109.0 (C-3), and 176.6 (C-4)] (Table 4.1 and Appendix A1) (Mabry *et al.*, 1970; Agrawal, 1989). The ^1H NMR data

also exhibited signals for a methoxy group (δ_H 3.87, δ_C 62.9), a dimethylpyran ring [(*cis*-olefinic protons at δ_H 5.93, 6.77 (d, J = 10.1 Hz) and methyl protons at δ_H 1.42 (s, 6H)] and a *trans*-olefinic 3-methoxy-3-methylbut-1-enyl group [olefinic protons at δ_H 6.86, 6.63 (d, J = 16.7 Hz)] (Atilaw *et al.*, 2017b)]. The ^{1}H NMR further revealed signals for sets of mutually coupled protons resonating at δ_H 8.05 (2H, m), 7.60 (2H, m) and 7.61 (1H, m) assigned to ring B (Prabhakar *et al.*, 1996; Atilaw *et al.*, 2017b). The MS data (Scheme 4.1 and Appendix A1) of **1** showed fragments of 64 Da (2xCH_3OH) in the positive-ion mode confirming the occurrence of two methoxy groups in the compound. Further, loss of 42 Da (C_3H_6), 54 Da (C_4H_6), and 66 Da (C_5H_6) from $[M+H-64]^+$ ion in the MS^3 spectrum was ascribed to the prenyl and dimethylpyran moieties (Xu *et al.*, 2012). The absence of substitution in ring B was further evident from the retro-Diels Alder MS fragmentations of the C-ring (Ma *et al.*, 1997) yielding *m/z* 249 indicating a loss of C_8H_6 and also *m/z* 233 for loss of $C_9H_6O_2$. This implied that ring A was completely substituted. The HMBC correlations of the proton at δ_H 6.77 (H-4") with C-5 and C-7 and δ_H 5.93 (H-5") with C-6 allowed the placement of the 2,2-dimethylpyran ring at C-6/7. HMBC correlations of δ_H 6.86 (H-1'") with C-7 and C-9 supported the placement of the *trans*-olefinic 3-methoxy-3-methylbut-1-enyl substituent at C-8. The placement of the methoxy group (δ_H 3.87) at C-5 was established based on its HMBC correlation. Therefore, compound **1** was characterized as 5-methoxy-2",2"-dimethylpyrano[5",6":6,7]-8-(*E*-3-methoxy-3-methylbut-1-enyl)flavone. This is a new compound and was given the trivial name lineaflavone A.



1

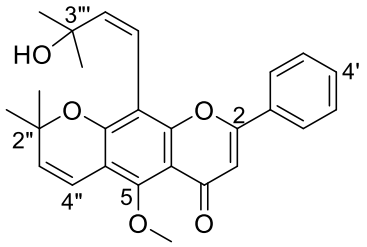


Scheme 4.1: Collision-Induced Dissociation (CID) Mass Fragments of Compound 1

4.1.2: Lineaflavone B (2)

This compound was isolated as a yellow paste. It had a molecular formula of $C_{26}H_{26}O_5$ from HRESIMS data [molecular ion $[M+H]^+$, *m/z* 419.1853 (calcd for $C_{26}H_{27}O_5$, 419.1853) and the $[M+Na]^+$ ion at *m/z* 441.1673 (calcd for $C_{26}H_{26}O_5Na$, 441.1672)] and ^{13}C NMR data (Table 4.1 and Appendix A2). The NMR data of **2** showed similarity with those of **1**. The only discrepancy was in the nature of the prenyl substituent at C-8. The prenyl substituent in **2** has a hydroxy group at C-3''' instead of a methoxy group and a *cis*-olefinic double bond (*J* = 12.1 Hz) rather than *trans* as in **1** (Hegazy *et al.*, 2011; Stevenson *et al.*, 2012). The NOESY correlation between H-1''' and H-2''' confirmed the *cis*-configuration. Consistent with this, its MS showed neutral losses of 18 Da (H_2O) and 32 Da (CH_3OH) confirming the presence of the hydroxy group and one methoxy group, instead of two methoxy groups as in compound **1**. Therefore, compound **2** was characterized as 5-methoxy-2",2"-dimethylpyrano[5",6":6,7]-8-(*Z*-3-

hydroxy-3-methylbut-1-enyl)flavone. This new compound was given the trivial name lineaflavone B.



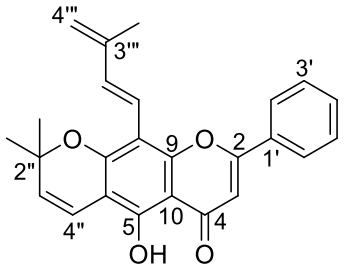
2

Table 4.1: NMR Data for Compounds 1 and 2 in Acetone-d_6 (600 MHz)

	1			2		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	161.4			161.2		
3	109.0	6.69, s	C-2, C-4, C-10, C-1'	100.4	6.67, s	C- 2, C-4, C-10, C-1'
4	176.6			176.9		
5	154.7			154.6		
6	113.7			113.0		
7	156.1			155.0		
8	111.4			113.2		
9	156.4			156.3		
10	113.3			112.9		
1'	132.8			132.6		
2'/6'	127.0	8.05, m	C-2, C-2'/6', C-4'	127.0	8.06, m	C-2'/6', C-4', C-2
3'/5'	130.0	7.60, m	C-1', C-3'/5'	129.8	7.56, m	C-1', C-3'/5'
4'	132.2	7.61, m	C-2'/6'	132.1	7.57, m	C-2'/6'
2"	78.8			78.7		
3"	131.7	5.93, d (10.1)	C-6, C-2", C-2"-Me_2	131.6	5.87, d (10.1)	C-6, C-2", C-2"-Me_2
4"	116.9	6.77, d (10.1)	C-5, C-6, C-7, C-2"	116.9	6.75, d (10.1)	C-5, C-6, C-7, C-2"
2"-Me_2	28.4	1.53, s	C-2", C-3", C-2"-Me	28.7	1.49, s	C-2", C-3", C-2"-Me
1"'	118.1	6.86, d (16.7)	C-8, C-7, C-9, C-2"', C-3"'	116.0	6.13, d (12.1)	C-8, C-7, C-9, C-2"', C-3"'
2"'	142.1	6.63, d (16.7)	C-8, C-3"', C-3"'-Me_2	144.6	6.09, d (12.1)	C-8, C-3"', C-3"'-Me_2
3"'	76.0			71.8		
3"'-Me_2	26.4	1.42, s	C-2"', C-3"', C-3"'-Me	30.2	1.21, s	C-2"', C-3"', C-3"'-Me
5-OMe	62.9	3.87, s	C-5	62.8	3.86, s	C-5
3"'-OMe	50.6	3.26, s	C-3"'			

4.1.3: Lineaflavone C (3)

Compound **3** was obtained as a yellow paste. Its molecular formula, $C_{25}H_{22}O_4$, was established from its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 387.1591 (calcd for $C_{25}H_{23}O_4$, 387.1591) and the $[M+Na]^+$ peak at *m/z* 409.1408 (calcd for $C_{25}H_{22}O_4Na$ 409.1410)] and NMR data (Table 4.2 and Appendix 3). A 5-hydroxyflavone derivative was evident from the UV (λ_{max} 230 and 280 nm) and NMR [δ_H 6.89 (H-3) and 13.55 (5-OH); δ_C 164.1 (C-2), 106.4 (C-3) and 183.8 (C-4)] (Mabry *et al.*, 1970). The NMR data of **3** showed close relation with those of **1**. The notable differences were the occurrence of a hydroxy group at C-5 in **3** rather than a methoxy group in **1** and the nature of the prenyl residue at C-8. The prenyl group in compound **3** had olefinic methylene (δ_H 5.13, 5.14 (brs), δ_C 117.3) instead of a methoxy group at C-3''' in **1**. The MS^2 of the compound in the positive-ion mode was dominated by neutral losses of 42 Da (C_3H_6), 54 Da (C_4H_6) and 70 Da (C_3H_6+CO). This confirmed the presence of prenyl and dimethylpyran substituents (Xu *et al.*, 2012). Thus, compound **3** was characterized as 5-hydroxy-2",2"-dimethylpyrano[5",6":6,7]-8-(*E*-3-methylbuta-1,3-dienyl)flavone. This is a new compound that was given the trivial name lineaflavone C.

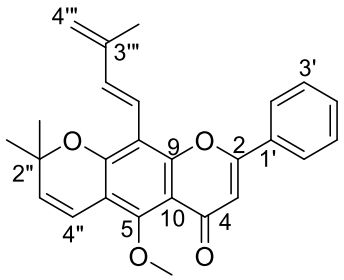


3

4.1.4: Lineaflavone D (4)

Compound **4** was obtained as a yellow paste. The HRESIMS [molecular ion $[M+H]^+$ at *m/z* 401.1747 (calcd. for $C_{26}H_{25}O_4$, 401.1747) and $[M+Na]^+$ at *m/z* 423.1567 (cacld. $C_{26}H_{24}O_4Na$, 423.1567)] was consistent with a molecular formula of $C_{26}H_{24}O_4$. The UV (λ_{max} at 232, 284

and 342 nm) and NMR data (Table 4.2 and Appendix A4) showed that the compound is a flavone derivative (Agrawal, 1989). The ^{1}H, ^{13}C NMR and MS data of **4** are closely related to compound **3**; the difference was in the presence of a methoxy group (δ_H 3.88 and δ_C 62.9) at C-5 in this compound rather than the hydroxy group in **3**. Its MS^2 in positive ion mode was dominated by neutral losses of 32 Da (CH_3OH) confirming the presence of a methoxy group. Therefore, compound **4**, was characterized as 5-methoxy-2",2"-dimethylpyrano[5",6":6,7]-8-(*E*-3-methylbuta-1,3-dienyl)flavone, a new compound given the trivial name lineaflavone D.



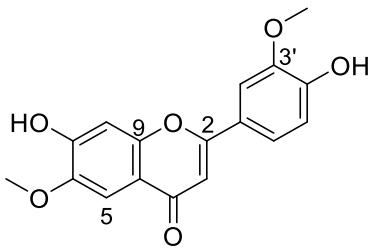
4

Table 4.2: NMR Data for Compounds 3 and 4 in Acetone-d_6 (600 MHz)

	3			4		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	164.1			161.5		
3	106.4	6.89, s	C-2, C-4, C-10, C-1'	109.0	6.70, s	C-2, C-4, C-10, C-1'
4	183.8			176.7		
5	156.3			154.7		
6	106.2			113.7		
7	158.0			156.3		
8	106.3			111.5		
9	155.1			156.5		
10	106.1			113.4		
1'	132.4			132.7		
2'/6'	127.4	8.11, m	C-2, C-2'/6', C-4'	127.0	8.05, m	C-2, C-2'/6', C-4'
3'/5'	130.1	7.65, m	C-1', C-3'/5'	130.0	7.61, m	C-1', C-3'/5'
4'	132.9	7.64, m	C-2'/6'	132.3	7.60, m	C-2'/6'
2"	79.5			79.0		
3"	129.3	5.84, d (10.1)	C-6, C-2", C-2"-Me_2	131.7	5.94, d (10.1)	C-6, C-2", C-2"-Me_2
4"	115.9	6.72, d (10.0)	C-5, C-6, C-7, C-2"	116.8	6.77, d (10.1)	C-5, C-6, C-7, C-2"
2"-Me_2	28.5	1.56, s	C-2", C-3", C-2"-Me	28.4	1.55, s	C-2", C-3", C-2"-Me
1"'	118.2	6.93, d (16.5)	C-7, C-8, C-9, C-2"', C-3"'	118.5	6.99, d (16.5)	C-7, C-8, C-9, C-2"', C-3"'
2"'	136.0	7.45, d (16.5)	C-8, C-3"', C-4"', C-5"'	136.8	7.49, d (16.5)	C-8, C-3"', C-4"', C-5"'
3"'	143.9			143.9		
4"'	117.3	5.14, br s	C-2"', C-5"'	118.0	5.17, br s	C-2"', C-5"'
5"'	18.3	2.07, s	C-2"', C-3"', C-4"'	18.3	2.07	C-2"', C-3"', C-4"'
5-OH		13.55, s	C-5, C-6, C-10			
5-OMe				62.9	3.88, s	C-5

4.1.5: 6-Methoxygeraldone (5)

This was isolated as a yellow paste. Its molecular formula was deduced as $C_{17}H_{14}O_6$, based on the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 315.0863 (calcd for $C_{17}H_{15}O_6$, 315.0863) and the $[M+Na]^+$ peak at *m/z* 337.0684 (calcd for $C_{[7}H_{14}O_6Na$, 337.0683)] and NMR data (Table 4.3 and Appendix A5). The UV data (λ_{max} 218, 224 and 342 nm) and NMR data [δ_H 6.66 (s, H-3); δ_C 163.4 (C-2), 105.6 (C-3) and 177.0 (C-4)] were typical of a flavone (Table 4.3 and Appendix A5) (Mabry *et al.*, 1970; Agrawal, 1989). Further, the NMR data exhibited the presence of two methoxy groups (δ_H 3.97, δ_C 56.5 and δ_H 4.00, δ_C 56.5). The MS^3 spectrum of the compound was dominated by losses of 15 Da (CH_3), thus supporting the presence of methoxy groups. The ^1H NMR disclosed signals for two aromatic singlets at δ_H 7.46 and 7.11 and a set of ABX coupled protons [δ_H 7.00 (d, *J* = 8.3 Hz), 7.58 (dd, *J* = 8.3, 2.1 Hz and 7.62 (d, *J* = 2.1 Hz)]. HMBC correlations of the proton at δ_H 7.46 with C-7, C-9 and C-4 placed it at C-5. Further, this proton (δ_H 7.46) showed a NOESY correlation with the methoxy proton at δ_H 3.97 that allowed the placement of the methoxy group at C-6. This implied the other singlet proton at δ_H 7.11 could only be at C-8 and the ABX system was in ring B. Two ABX protons at δ_H 7.58 (dd, *J* = 8.3, 2.1 Hz) and 7.62 (d, *J* = 2.1 Hz) showed HMBC correlation with C-2 which placed them at C-6' and C-2', respectively, and their coupling partner (δ_H 7.00) at C-5'. The hydroxy group proton at δ_H 8.38 showed HMBC correlation with C-5', C-4' and C-3' which allowed for the placement of the hydroxy group at C-4' and the methoxy group (δ_H 4.00) at C-3'. This was supported by the NOESY correlation of H-2' (δ_H 7.62) with the methoxy protons ((δ_H 4.00). Thus, compound **5** was characterized as 7,4'-dihydroxy-6,3'-dimethoxyflavone. This is a new compound and was given the trivial name 6-methoxygeraldone by comparison with geraldone (**14**) (Lopes *et al.*, 1979).



5

Table 4.3: NMR Data for Compound 5 in Acetone-d_6 (600 MHz)

	5		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	163.4		
3	105.6	6.66, s	C-2, C-4, C-10, C-1'
4	177.0		
5	105.3	7.46, s	C-4, C-6, C-7, C-9
6	147.3		
7	153.1		
8	104.1	7.11, s	C-6, C-7, C-10
9	153.3		
10	117.7		
1'	124.4		
2'	110.2	7.62, d (2.1)	C-2, C-4', C-3', C-6'
3'	148.8		
4'	150.8		
5'	116.3	7.00, d (8.3)	C-1', C-3'
6'	120.8	7.58, dd (8.3, 2.1)	C-2, C-2', C-4'
6-OMe	56.5	3.97, s	C-6
3'-OMe	56.5	4.00, s	C-3'
4'-OH		8.38	C-3', C-4', C-5'

4.1.6: Acetylobovatin (6)

Compound **6** was isolated as an off-white paste. The molecular formula was deduced as $C_{22}H_{20}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 381.1333 (calcd for $C_{22}H_{21}O_6$, 381.1333) and a$[M+Na]^+$ peak at *m/z* 403.1161 (calcd for $C_{22}H_{20}O_6Na$, 403.1152)] and ^{13}C NMR data (Table 4.4 and Appendix A6). The UV (λ_{max} 224, 272, 296 and 358 nm) and NMR data [δ_H 5.68 (dd, *J* = 12.7, 3.1 Hz, H-2), 2.90 (dd, *J* = 17.1, 3.2 Hz, H-3_{eq}), 3.22 (dd, *J* = 17.1, 12.7 Hz, H-3_{ax}) and 12.24 (OH-5); δ_C 80.2 (C-2), 43.4 (C-3) and 197.4 (C-4)] showed the presence of a 5-hydroxyflavanone skeleton (Agrawal, 1989). A modified 2,2-dimethylpyran ring was evident in the NMR spectra [*cis*-olefinic protons at δ_H 6.68, 5.58 (d, *J* = 10.2 Hz, 1H),

a methyl group δ_H 1.45 (s, 3H), oxymethylene protons δ_H 4.09, 4.25 (d, J = 11.7 Hz, 2H) and an acetyl group [δ_H 1.97 (s, 3H), δ_C 20.6, 170.6 (C=O)]. HMBC correlation of the oxymethylene protons at δ_H 4.09 with C-2", C-3", 2"-CH_3 and C=O allowed the placement of the acetyl group at 2"-CH_2. The presence of the acetyl group and the pyran ring was further supported by MS^3 data that showed fragments of 18 Da (H_2O), 28 Da, (CO), 60 Da (CH_3COOH), 70 Da (C_3H_6, CO) and 104 Da (C_8H_8) (Xu *et al.*, 2012). The NMR data showed signals for an unsubstituted ring B [(δ_H 7.60 (H-2'/6'), 7.47 (H-3'/5') and 7.42 (H-4'))] and a singlet at δ_H 5.91 that was assigned to ring A. HMBC correlation between the hydroxy proton at δ_H 12.24 (OH-5) with δ_C 97.6 (C-6, δ_H 5.91) allowed the placement of the modified 2",2"-dimethylpyran ring at C-7/8. A (2*S*)-configuration was established from the ECD spectrum of **6** (Figure 4.1) that showed positive and negative Cotton effects at 317 and 295 nm (Stevenson *et al.*, 2012). However, the configuration at 2"-CH_2 remains undetermined. Therefore, compound **6** was characterized as (2*S*)-5-hydroxy-2"-methyl-2"-acetoxymethylpyrano[5",6":7,8]flavanone. It is a new compound that was given the trivial name acetylobovatin because of its close similarity with obovatin (Andrei *et al.*, 2000).

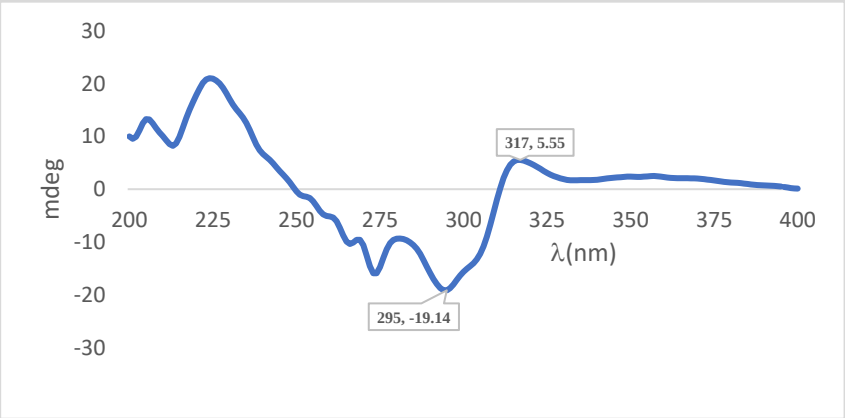
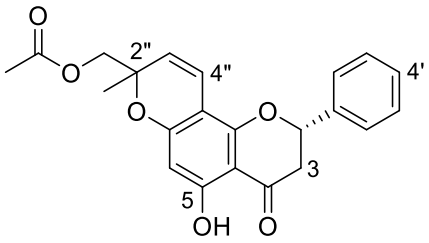


Figure 4.1: ECD spectrum of compound 6



6

4.1.7: 5-Hydroxy-7-methoxysaniculamin A (7)

Compound **7** was isolated as an off-white paste. Its molecular formula was deduced as $C_{21}H_{22}O_6$ based on the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 371.1488 (calcd for $C_{21}H_{23}O_6$, 371.1489) and the $[M+Na]^+$ peak at *m/z* 393.1309 (calcd for $C_{21}H_{22}O_6Na$, 393.1309)] and NMR (Table 4.4 and Appendix A7). In the NMR, the AMX spin system at δ_H 5.47 (dd, *J* = 13.2, 2.5 Hz, H-2), 2.73 (m, H-3_{eq}) and signals at 3.22 (dd, *J* = 17.2, 13.0 Hz, H-3_{ax}), 12.35 (OH-5); δ_C 79.3 (C-2), 42.7 (C-3) and 196.9 (C-4)] (Xu *et al.*, 2016) and UV (λ_{max} 224, 230, 292 and 336 nm) data (Mabry *et al.*, 1970) indicated that compound **7** has a 5-hydroxyflavanone skeleton. Further, the NMR spectra exhibited signals for a methoxy group (δ_H 3.88, δ_C 55.5) and a 2"-hydroxy-3"-methylbut-3"-enyl [δ_H 2.86, 2.77 (m) for H-1", 4.29 (dd, *J* = 7.0, 3.8 Hz) for H-2", 4.69 and 4.63 (brs) for H-4" and 1.79 (s) for H-5"] group. The MS data was dominated by losses of 72 Da (C_4H_8O) and 18 Da (H_2O), supporting the existence of a hydroxy-3-methylbut-3-enyl group (Xu *et al.*, 2012). The ^{1}H NMR spectrum further showed an AA'XX' spin system at δ_H 7.41 and 6.91 (*J* = 8.5 Hz, d, 2H) assigned to a 4'-substituted ring B. HMBC correlations of H-1" (δ_H 4.25, 4.09) with C-7 (δ_C 166.1) and C-5 (δ_C 160.9) allowed placement of the 2-hydroxy-3-methylbut-3-enyl group at C-6 (δ_C 106.8). A (2*S*)-configuration was established from the ECD spectrum of **7** (Figure 4.2) that showed positive and negative Cotton effects at 335 and 291 nm (Bedane *et al.*, 2016). However, the configuration at 2" remains undetermined. Therefore, compound **7** was characterized as (2*S*)-5,4',dihydroxy-7-methoxy-6-(2-hydroxy-3-

methylbut-3-enyl)flavanone, it is a new compound given the trivial name 5-hydroxy-7-methoxysaniculamin A, by comparison with saniculamin A (Xu *et al.*, 2016).

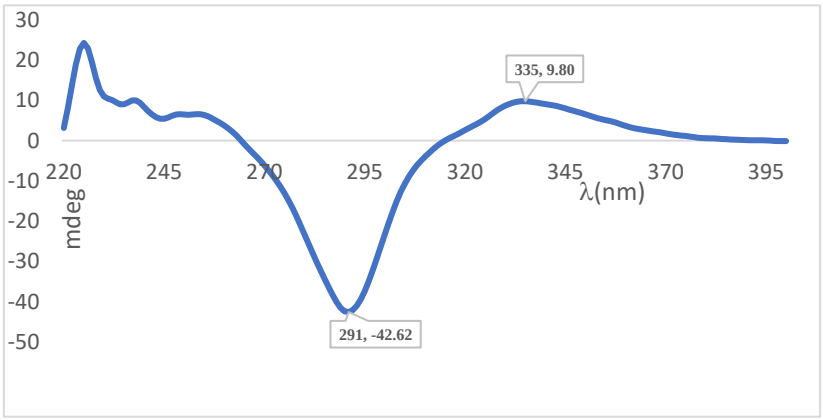
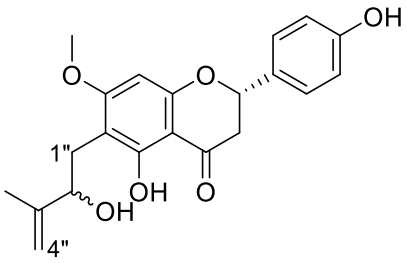


Figure 4.2: ECD spectrum of compound 7



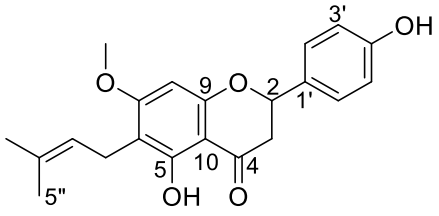
7

Table 4.4: NMR Data for Compounds 6 and 7 in Acetone-d_6 (600 MHz)

	6			7		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	80.2	5.68, dd (12.7, 3.1)	C-4, C-1', C-2'/6'	79.3	5.47, dd (13.2, 2.5)	C-4, C-1', C-2'/6'
3	43.4	2.90, dd (17.1, 3.2 H_{eq})	C-2, C-4, C-10, C-1'	42.7	2.73, m (H_{eq})	C-2, C-4, C-10, C-1'
		3.22, dd (17.1, 12.7 H_{ax})			3.22, dd (17.2, 13.0 H_{ax})	
4	197.4			196.9		
5	164.8			160.9		
6	97.6	5.91, s	C-5, C-7, C-8, C-10	106.8		
7	162.4			166.1		
8	102.3			90.9	6.15, s	C-6, C-7, C-9, C-10
9	157.9			162.1		
10	103.8			102.5		
1'	139.8			129.9		
2'/6'	127.2	7.60, m	C-2, C-2'/6', C-4'	128.1	7.41, d (8.5)	C-2, C-2'/6', C-4'
3'/5'	129.5	7.47, m	C-1', C-4', C-3'/5'	115.2	6.91, d (8.5)	C-1', C-3'/5', C-4'
4'	129.6	7.42, m	C-2'/6', C-3'/5'	157.9		
1"				28.6	2.76, m	C-5, C-6, C-7, C-2"
					2.86, m	
2"	80.0			74.5	4.29, m	C-1", C-4", C-5"
3"	123.1	5.58, d (10.1)	C-8, C-4", 2"-CH_3, 2"-CH_2	148.4		
4"	118.5	6.68, d (10.1)	C-7, C-8, C-9, C-2"	109.3	4.69 and 4.63, s(broad)	C-2", C-3", C-5"
5"				16.6	1.66, s	C-2", C-3", C-4"
2"-CH_2	68.9	4.09, d (11.8)	C-2", C-3", 2"-CH_3, C=O			
		4.25, d (11.7)				
2"-CH_3	23.9	1.45, s	C-2", C-3", 2"-CH_2			
7-OMe				55.5	3.75, s	C-7
5-OH		12.24	C-5, C-6, C-10,		12.35	C-5, C-6, C-10
$\underline{C}(O)CH_3$	170.6					
$C(O)\underline{CH_3}$	20.6	1.97	C=O			

4.1.8: 7-O-Methyl-6-prenylnaringenin (8)

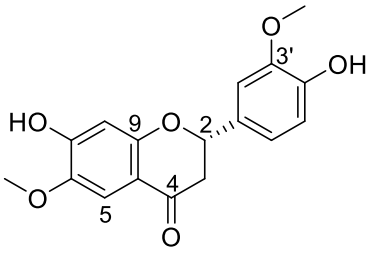
Compound **8** was isolated as an off-white paste. Its molecular formula was deduced as $C_{21}H_{22}O_5$ from its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 355.1537 (calcd for $C_{21}H_{23}O_5$, 355.1540)] and ^{13}C NMR data (Table 4.5 and Appendix A8). A 5-hydroxyflavanone was evident from the UV (λ_{max} 236 and 292 nm) and NMR [AMX system; δ_H 5.39 (1H, dd, *J* = 13.2, 2.9 Hz) for H-2, 3.04 (1H, dd, *J* = 16.8, 13.2 Hz) for H-3ax, 2.75 (1H, dd, *J* = 16.8, 2.9 Hz) for H-3eq and 12.27 for 5-OH] (Table 4.5 and Appendix A8) (Mabry *et al.*, 1970; Agrawal, 1989). Further, the NMR spectra exhibited signals for a methoxy group [δ_H 3.89 (δ_C 56.5)], prenyl group [δ_H 3.22, 2H (δ_C 21.6) for methylene group; 5.16, t, *J* = 7.3 Hz, 1H (δ_C 123.4) for olefinic protons, δ_C 131.4 for a quaternary carbon and δ_H 1.63 (s, 3H) and δ_H 1.74 (s, 3H) for methyl groups]. The NMR data also showed peaks for AA'XX'-coupled aromatic protons [δ_H 7.42 (δ_C 129.0) and 6.91 (δ_C 116.2) (2H, d, *J* = 8.7 Hz) to H-2'/6' and H-3'/5', respectively, assigned to ring B] and a singlet aromatic proton at δ_H 6.15. The attachment of the prenyl group to C-6 also followed HMBC correlations of H-1'' (δ_H 3.22) with C-6, C-5 and C-7, while the methoxy group was placed at C-7 based on the HMBC correlation of its protons with C-7. Thus, compound **8** was identified as 7-*O*-methyl-6-prenylnaringenin (Intekhab and Aslam, 2009; Zhang *et al.*, 2019b). 7-*O*-Methyl-6-prenylnaringenin (**8**) was previously isolated from *Feronia limonia* (Intekhab and Aslam, 2009) and *Mallotus conspurcatus* Croizat (Zhang *et al.*, 2019b). This is its first report in the genus *Tephrosia.*



8

4.1.9: Erylivingstone I (9)

Compound **9** was obtained as an off-white solid. The molecular formula of $C_{17}H_{16}O_6$ was established from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 317.1021 (calcd for $C_{17}H_{17}O_6$, 317.1020)]. Compound **9** was established as a flavanone from UV (λ_{max} 236 and 292 nm) and ^{1}H NMR data (Table 4.5 and Appendix A9) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data exhibited signals of two methoxy groups [δ_H 3.85 (δ_C 56.5) and δ_H 3.87 (δ_C 56.3)]. Further, the NMR data revealed signals for an ABX spin system [δ_H 7.18 (1H, d, *J* = 2.0 Hz); 6.86 (1H, d, *J* = 8.1 Hz) and 6.99 (1H, dd, *J* = 8.1, 2.0 Hz)] and two aromatic singlet protons (δ_H 7.26 and 6.47). Placement of the singlet at δ_H 7.26 at C-5 followed its HMBC correlations with C-4, C-7and C-9. The other singlet proton at δ_H 6.47 could only be at C-8 as evident from its HMBC correlations with C-6, C-7, C-9and C-10. The HMBC correlation of the methoxy protons at δ_H 3.85 with C-6 placed the substituent at C-6. Two ABX protons at δ_H 7.18 (1H, d, *J* = 2.0 Hz) and 6.99 (1H, dd, *J* = 8.1, 2.0 Hz) showed HMBC correlations with C-2 that placed them at C-2' and C-6', respectively, and their coupling partner [6.86 (1H, d, *J* = 8.1 Hz)] at C-5'. HMBC correlations of H-5' and methoxy protons (δ_H 3.87) with C-3' place the methoxy group at C-3'. These spectral data of **9** were comparable to those described in the literature and thus compound **9** was identified as 7,4′dihydroxy-6, 3′-dimethoxyflavanone (erylivingstone I) (Bedane *et al.*, 2016). Erylivingstone I (**9**) was previously isolated from *Erythrina livingstoniana* (Bedane *et al.*, 2016) and this is its first report in the genus *Tephrosia*.



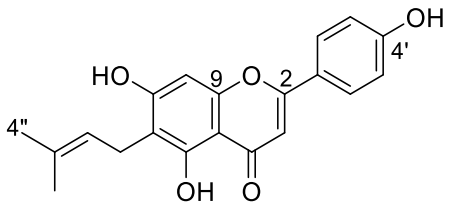
9

Table 4.5: NMR Data for Compounds 8 and 9 in Acetone-d_6 (600 MHz

	8			9		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	80.1	5.46, dd (13.0, 3.0)	C-2'/6', C-1', C-4	80.9	5.39, dd (13.2, 2.9)	C-2', C-6', C-1', C-4
3	43.6	2.75, dd (17.1, 3.1 H_{eq}) 3.19, m (H_{ax})	C-2, C-4, C-1', C-10	44.7	2.66, dd (16.8, 2.9 H_{eq}) 3.04, dd (16.8, 13.2 H_{ax})	C-4, C-1', C-10
4	197.7			190.5		
5	160.9			107.9	7.26, s	C-10, C-6, C- 9, C-7, C-4
6	109.9			144.2		
7	166.3			159.0		
8	91.8	6.15, s	C-7, C-9, C-6, C-10	104.3	6.47, s	C-7, C-9, C-6, C-10
9	162.7			155.2		
10	103.5			113.9		
1'	130.8			131.9		
2'	129.0	7.42, d (8.5)	C-2, C-2'/6', C-4'	111.1	7.18, d (2.0)	C-2, C-6', C-4'
3'	116.2	6.91, d (8.5)	C-3'/5', C-1'	148.3		
4'	158.7			147.7		
5'	116.2	6.91, d (8.5)	C-3'/5', C-1'	115.6	6.86, d (8.1)	C-3', C-1'
6'	129.0	7.42, d (8.5)	C-2, C-2'/6', C-4'	120.4	6.99, dd (8.1, 2.0)	C-2, C-2', C-4'
1"	21.6	3.22, m	C-6, C-2", C-5, C-7			
2"	123.4	5.16, t (7.3)	C-4", C-5", C-1"			
3"	131.3					
4"	25.9	1.63, s	C-5", C-2", C-3"			
5"	17.8	1.74, S	C-4", C-2", C-3"			
5-OH		12.27, s				
6-OMe				56.5	3.85, s	C-6
7-OMe	56.5	3.89, s	C-7			
3'-OMe				56.3	3.87, s	C-3'

4.1.10: 6-C-Prenylapigenin (10)

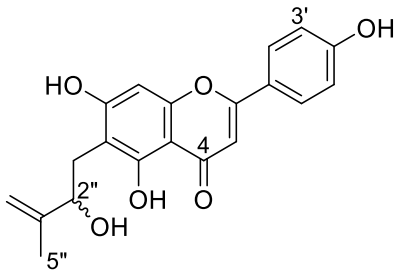
Compound **10** was isolated as a yellow paste. Its molecular formula was deduced as $C_{20}H_{18}O_5$ from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 339.1228 (calcd $C_{20}H_{19}O_5$, 339.1227) and ^{13}C NMR data (Table 4.6 and Appendix A10). The presence of a 5-hydroxyflavone skeleton was evident from the UV (λ_{max} 230, 274 and 332 nm) and NMR data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data exhibited signals for a prenyl group [δ_H 3.25, d, *J* = 7.2 Hz, 2H (δ_C 22.0) for a methylene group; δ_H 5.28, t, *J* = 7.5 Hz, 1H (δ_C 123.2) for an olefinic proton, δ_C 131.6 for a quaternary carbon and δ_H 1.65, s, 3H (δ_C 25.9) and δ_H 1.78, s, 3H (δ_C 17.9) for methyl groups] and downfield signals at δ_H 9.62 and 9.20 attributed to two hydroxy groups. Further, the NMR spectra exhibited signals for a singlet in the aromatic region [δ_H 6.61 (δ_C 94.1)] and AA'XX' coupled aromatic protons [δ_H 7.93 and 6.91 (2H, d, *J* = 8.8 Hz)] assigned to ring B. 5-Hydroxy proton (δ_H 13.30) and H-1" (δ_H 3.25) showed HMBC correlations with C-6 that allowed for placement on the prenyl group at C-6. The HMBC correlations of the hydroxy proton at δ_H 9.20 with C-3'/5' and C-4' allowed for the placement of this group at C-4'. Thus, compound **10** was identified as 5,7,4'-trihydroxy-6-(3-methylbut-2-enyl)flavone (6-C-prenylapigenin) (Monache *et al.*, 1994; Chang *et al.*, 1995; Abegaz *et al.*, 1998). 6-*C*-Prenylapigenin was previously isolated from *Polygonum* and *Dorstenia* species (Abegaz *et al.*, 1998; Dzoyem *et al.*, 2017) and *Cudrania cochinchinensis* (Chang *et al.*, 1995). It is the first time it is being reported in the genus *Tephrosia*.



10

4.1.11: 5,7,4',2''-Tetrahydroxy-6-[3''-methylbut-3''-enyl]flavone (11)

Compound **11** was isolated as a yellow paste. Its molecular formula was deduced as $C_{20}H_{18}O_6$ from its HRESIMS molecular ion $[M+H]^+$ at *m/z* 355.1176 (calcd for $C_{20}H_{19}O_6$, 355.1176) and ^{13}C NMR data (Table 4.6 and Appendix A11). The presence of a 5-hydroxyflavone skeleton was evident from the UV (λ_{max} 224, 274 and 334 nm) and NMR data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed very close similarity with compound **10**. The only difference was in the nature of the prenyl group. The prenyl substituent in **11** had a hydroxy group at C-2" [δ_H 4.43 (dd, J = 7.9, 3.5 Hz), δ_C 76.5 for an oxymethine residue]. Based on this spectral data, compound **11** was identified as 5,7,4',2"-tetrahydroxy-6-[3"-methylbut-3"-enyl]flavone (Lee *et al.*, 1998) but C-2" absolute configuration remains undetermined. This compound was previously isolated from *Maclura pomifera* (Lee *et al.*, 1998). This is its first report in the genus *Tephrosia*.



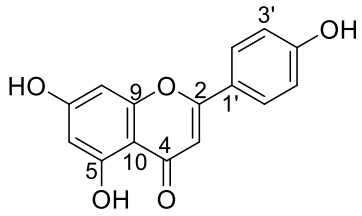
11

Table 4.6: NMR Data for Compounds 10 and 11 in Acetone-d_6 (600 MHz)

	10			11		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	164.8			165.0		
3	104.1	6.63, s	C-2, C-4, C-10, C-1'	104.0	6.64, s	C-2, C-4, C-10, C-1'
4	183.2			183.2		
5	160.2			160.7		
6	112.3			110.2		
7	162.4			164.1		
8	94.1	6.61, s	C-6, C-7, C-9, C-10	95.3	6.56, s	C-6, C-7, C-9, C-10
9	156.6			157.1		
10	105.2			105.0		
1'	123.4			123.4		
2'/6'	129.2	7.93, d (8.8)	C-2, C-2'/6', C-4'	129.3	7.95, d (8.8)	C-2, C-2'/6', C-4'
3'/5'	116.9	7.02, d (8.8)	C-1', C-3'/5', C-4'	116.8	7.03, d (8.8)	C-1', C-3'/5', C-4'
4'	161.8			161.8		
1"	22.0	3.35, d (7.2)	C-5, C-6, C-7, C-2", C-3"	30.3	2.92, dd (14.5, 7.9) 3.07, dd (14.5, 3.5)	C-5, C-7, C-2"
2"	123.2	5.28, t (7.5)	C-1", C-4", C-5"	76.5	4.43, dd (7.9, 3.5)	C-1", C-3", C-4"
3"	131.6			148.2		
4"	25.9	1.65, s	C-2", C-3", C-5"	110.4	4.77, br s 4.94, br s	C-2", C-5"
5"	17.9	1.78, s	C-2", C-3", C-4"	18.4	1.84, s	C-3, C-2", C-4"
5-OH		13.30, s	C-5, C-6, C-10		13.51	C-5, C-6, C-10
7-OH		9.62, s	C-6, C-7			
4'-OH		9.20	C-3'/5', C-4'			

4.1.12: Apigenin (12)

Compound **12** was isolated as a yellow paste. Its molecular formula was deduced as $C_{15}H_{10}O_5$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 271.0601 (calcd for $C_{15}H_{11}O_5$, 271.0601)] and ^{13}C NMR data (Table 4.7 and Appendix A12). The presence of a 5-hydroxyflavone skeleton was evident from UV (λ_{max} 230, 266 and 336 nm) and NMR data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra showed the presence of two *meta*-coupled aromatic protons at δ_H 6.26, (d, J = 2.1 Hz) and 6.54, (d, J = 2.2 Hz) and an AA'XX' spin system [δ_H 7.95 and 7.03 (d, J = 8.8 Hz, 2H) assigned to ring B]. The HMBC correlations of the proton at δ_H 6.26 with C-8, C-5, C-10 and C-7 allowed its placement at C-6, while the proton at δ_H 6.54 was assigned to H-8 due to its HMBC correlations with C-6, C-9, C-10 and C-7. Thus, compound **12** was identified as 5,7,4'-trihydroxyflavone (apigenin). Apigenin was previously reported from *Tamarix dioica* (Parmar *et al.*, 1994) and *T. elata* (Atilaw, 2018) but this is its first report in the plant.

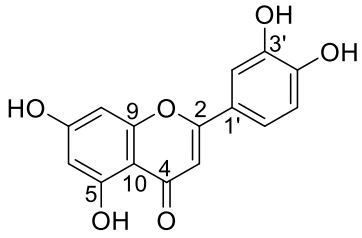


12

4.1.13: Luteolin (13)

Compound **13** was isolated as a yellow paste. Its molecular formula was deduced as $C_{15}H_{10}O_6$ on the basis of its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 287.0551 (calcd for $C_{15}H_{11}O_6$, 287.0550)] and ^{13}C NMR data (Table 4.7 and Appendix A13). The presence of a 5-hydroxyflavone skeleton was evident from the UV (λ_{max} 264 and 350 nm) and NMR data (Table 4.7 and Appendix A13) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra exhibited

signals for *meta*-coupled aromatic protons [δ_H 6.25 and 6.52 (1H, d, J = 2.0 Hz) assigned to ring A with biogenetically expected oxygenation at C-7] and ABX-coupled aromatic protons [δ_H 7.50 (1H, d, J = 2.2 Hz), 7.47 (1H, dd, J = 8.3, 2.2 Hz) and 7.00 (1H, d, J = 8.3 Hz)] assigned to ring B. HMBC correlations of C-2 with the protons at δ_H 7.50 and 7.47 allowed the placement of an OH at C-3'. Based on these spectral data and comparison with literature (Lin *et al.*, 2015), compound **13** was identified as 5,7,3',4'-tetrahydroxyflavone (luteolin). Luteolin was previously isolated from *Dendranthema morifolium* (Lin *et al.*, 2015), but this is its first report in the genus *Tephrosia*.



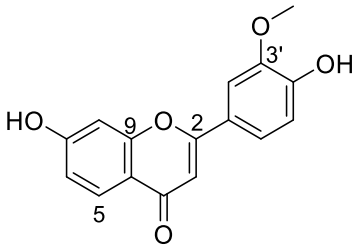
13

Table 4.7: NMR Data for Compounds 12 and 13 in Acetone-d_6 (600 MHz)

	12			13		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	165.1			165.2		
3	104.1	6.64, s	C-2, C-4, C-10, C-1'	104.2	6.58, s	C-2, C-4, C-10, C-1'
4	183.1			183.0		
5	163.4			163.4		
6	99.7	6.26, d (2.1)	C-5, C-7, C-8, C-10	99.7	6.25, d (2.0)	C-5, C-7, C-8, C-10
7	164.8			164.9		
8	94.7	6.54, d (2.2)	C-6, C-7, C-9, C-10	94.7	6.52, d (2.0)	C-6, C-9, C-7, C-10
9	158.8			158.8		
10	105.4			105.3		
1'	123.3			123.6		
2'	129.3	7.95, d (8.8)	C-2, C-2'/6', C-4'	114.1	7.50, d (2.2)	C-2, C-6', C-4'
3'	116.9	7.03, d (8.8)	C-1', C-3'/5', C-4'	146.6		
4'	161.9			150.3		
5'	116.9	7.03, d (8.8)	C-1', C-3'/5', C-4'	116.7	7.00, d (8.3)	C-1', C-3', C-4'
6'	129.3	7.95, d (8.8)	C-2, C-2'/6', C-4'	120.1	7.47, d (8.3, 2.2)	C-2, C-2', C-4'
5-OH		13.02	C-5, C-6, C-10		13.04, s	C-5, C-6, C-10

4.1.14: Geraldone (14)

Compound **14** was obtained as a yellow paste. The ^{13}C NMR data and HRESIMS molecular ion $[M+H]^+$ at *m/z* 285.0758 (calcd for $C_{16}H_{13}O_5$, 285.0757) were consistent with the molecular formula $C_{16}H_{12}O_5$ (Table 4.8 and Appendix A14). The UV (λ_{max} 238 and 340 nm) and NMR data indicated that this compound is a flavone (Mabry *et al.*, 1970; Agrawal, 1989). Its NMR data showed the presence of a methoxy group (δ_H 4.00, δ_C 56.6). Further, the NMR spectra showed signals for two ABX spin systems [δ_H 7.96 (1H, d, *J* = 8.7 Hz), 7.97 (1H, dd, *J* = 8.7, 2.3 Hz) and 7.84 (1H, d, *J* = 2.2 Hz)] and [δ_H 7.62 (1H, d, *J* = 2.1 Hz), 7.59 (1H, dd, *J* = 8.3, 2.1 Hz) and 7.00 (1H, d, *J* = 8.3 Hz)]. The ABX-coupled aromatic proton at δ_H 7.96 showed HMBC correlations with C-9 (δ_C 163.2), C-7 (δ_C 158.8) and C-4 (δ_C 177.2) allowing for its placement at C-5 in ring A and thus, the other ABX system was assigned to ring B. The substitution pattern of ring B of **14** and **13** are identical. HMBC correlation of the *meta* proton (H-2') with C-3' which in turn with methoxy protons allowed for the placement of the methoxy group at C-3'. Thus, compound **14** was identified as 3'-methoxy-7,4'-dihydroxyflavone (geraldone) (Jung *et al*., 2004). Geraldone (**14)** was previously isolated from *Sahertia concallariodora* (Lopes *et al.*, 1979) and *Albizzia julibrissin* (Jung *et al*., 2004). This is its first report in the genus *Tephrosia*.



14

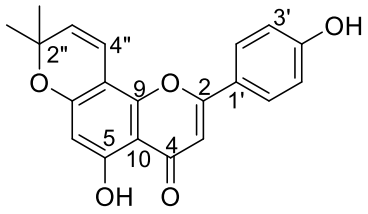
Table 4.8: NMR Data for Compound 14 in Acetone-d_6 (600 MHz)

	14		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	163.6		
3	106.1	6.66, s	C-2, C-4, C-10, C-1'
4	177.2		
5	127.7	7.96, d (8.7)	C-4, C-7, C-9,
6	115.3	6.97, dd (8.7, 2.3)	C-8, C-10
7	158.8		
8	103.6	7.84, d (2.2)	C-6, C-7, C-10, C-9
9	163.2		
10	118.0		
1'	124.3		
2'	110.3	7.62, d (2.1)	C-2, C-3', C-4', C-6'
3'	148.8		
4'	150.9		
5'	116.3	7.00, d (8.3)	C-1', C-3', C- 4'
6'	120.9	7.59, dd (8.3, 2.1)	C-2, C-4', C-3'
6-OMe			
3'-OMe	56.6	4.00, s	C-3'
4'-OH			

4.1.15: Atalantoflavone (15)

Compound **15** was isolated as a yellow paste. Its molecular formula was deduced as $C_{20}H_{16}O_5$ from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 337.1071 (calcd for $C_{20}H_{17}O_5$, 337.1071) and ^{13}C NMR data (Table 4.9 and Appendix A15). The presence of a 5-hydroxyflavone skeleton was evident from UV (λ_{max} 242, 306 and 354 nm) and NMR data (Table 4.9 and Appendix A15) (Mabry *et al.*, 1970; Agrawal, 1989). The ^1H and ^{13}C NMR spectra exhibited signals for a 2,2-dimethylchromene moiety [δ_H 1.47, (s, 6H) for methyl groups and δ_H 5.76 (*J* = 10.0 Hz, 1H) for H-3" and 6.66 (*J* = 10.0 Hz, 1H) for H-4"]. Further, the NMR revealed the presence of an AA'XX' spin system [δ_H 7.96 (d, *J* = 8.9 Hz, 2H) for H-2'/6' and δ_H 7.03 (d, *J* = 8.8 Hz, 2H) for H-3'/5'] assigned to a *p*-substituted ring B and a singlet proton at δ_H 6.49 for a ring A proton. The placement of the proton at δ_H 6.49 to H-6 was based on its HMBC correlations with C-8, C-5, C-10 and C-7. The HMBC correlation of H-4" (δ_H 6.66) with C-8, C-7, C-9 and C-2" placed the 2,2-dimethylchromene moiety at C-7/8. The above spectral data of **15** were identical to those described in the literature (Banerji *et al.*, 1988; Chang, 1990) for 5,4'-dihydroxy-2",2"-

dimethylpyrano[5",6":7,8]flavone (atalantoflavone or limomianin). Atalantoflavone (**15**) was previously reported from *Atalantza racemosa* (Banerji *et al.*, 1988) and *Citrus limonia* (Chang, 1990). This is its first report in the genus *Tephrosia*.



15

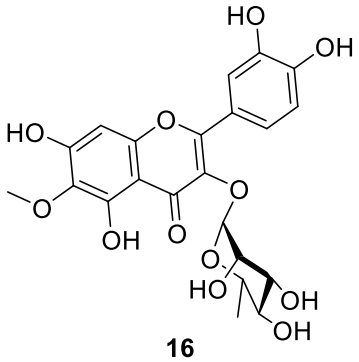
Table 4.9: NMR Data for Compound 15 in Acetone-d_6 (600 MHz)

	15		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	165.2		
3	104.1	6.67 s	C-2, C-4, C-10, C-1'
4	183.3		
5	157.9		
6	95.7	6.49, s	C-5, C-7, C-8, C-10
7	160.1		
8	106.1		C-6, C-7, C-9, C-10
9	157.3		
10	105.9		
1'	123.2		
2'	129.3	7.96, d (8.9)	C-2, C-2'/6', C-4'
3'	116.9	7.03, d (8.8)	C-1', C-3'/5', C-4'
4'	162.0		
5'	116.9	7.03, d (8.8)	C-1', C-3'/5', C-4'
6'	129.3	7.96, d (8.9)	C-2, C-2'/6', C-4'
2"	78.8		
3"	129.3	5.76, d (10.0)	C-8, C-2", 2"-Me_2
4"	115.8	6.66, d (10.0)	C-7, C-8, C-9, C-2"
2"-Me_2	28.4	1.47, s	
5-OH		13.31, s	C-6, C-10, C-5

4.1.16: Patuletin-3-O-rhamnoside (16)

Compound **16** was isolated as a yellow solid. Its molecular formula was deduced as $C_{22}H_{22}O_{12}$ on the basis of the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 479.1177 (calcd for $C_{22}H_{23}O_{12}$, 479.1184) with its sodiated ion $[M+Na]^+$ at *m/z* 501.0996 (calcd for $C_{22}H_{22}O_{12}Na$, 501.1003)] and ^{13}C NMR data (Table 4.10 and Appendix A16). The presence of a 5-hydroxyflavonol

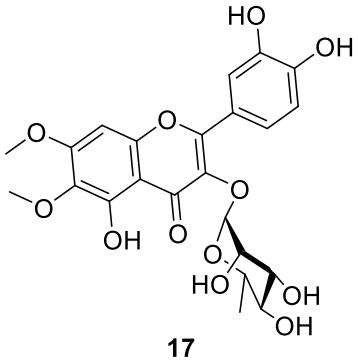
skeleton was evident from the UV absorption (λ_{max} 260 and 344 nm) and NMR data [δ_H 12.93 (5-OH); δ_C 158.5 (C-2), 135.4 (C-3) and 179.7 (C-4)] (Mabry *et al.*, 1970; Agrawal, 1989). The ^1H and ^{13}C NMR spectra exhibited signals for a methoxy [δ_H 3.88, 3H (δ_C 60.7)] and rhamnose [δ_H 5.53, 1H for an anomeric proton, H-2" (δ_C 102.7) and methyl protons for 6"-Me at δ_H 0.91 (d, J = 6.1 Hz) (δ_C 17.8)] (Nawwar *et al.*, 1984) moieties. Further, the NMR spectra revealed signals for an aromatic singlet proton at δ_H 6.55 and an ABX spin system [δ_H 6.99, d, J = 8.3 Hz, 1H for H-5'; δ_H 7.40, dd, J = 8.3, 2.1 Hz, 1H for H-6'; and 7.51 d, J = 2.1 Hz for H-2']. The HMBC correlations of the proton at δ_H 6.55 with C-6, C-9, C-10 and C-7 allowed for its placement at C-8 and thus, the ABX system was assigned to ring B. The HMBC correlations of the anomeric proton, H-2" (δ_H 5.53) with C-3 allowed the placement of the rhamnose moiety at C-3. HMBC correlations of the 5-OH with C-5 and C-6 which in turn correlates with the methoxy protons allowed for the placement of the methoxy group at C-6. The HMBC correlations of C-2 with the aromatic protons at δ_H 7.40 and 7.51 placed the hydroxy substituents at C-3' and C-4'. Thus, compound **16** was identified as patuletin 3-*O*-*α*-*L*-rhamnopyranoside (patuletin-3-*O*-rhamnoside) (Costa *et al.*, 1994). Patuletin-3-*O*-rhamnoside (**16**) was previously isolated from *Kalanchoe gracilis* (Liu *et al.*, 1989) and *Kalanchoe brasiliensis* (Costa *et al.*, 1994). This is its first report in the genus *Tephrosia.*



16

4.1.17: Eupatolitin-3-O-rhamnoside (17)

Compound **17** was isolated as a yellow solid. Its molecular formula was deduced as $C_{23}H_{24}O_{12}$ from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 493.1333 (calcd for $C_{23}H_{25}O_{12}$, 493.1341) with its sodiated ion $[M+Na]^+$ at *m/z* 515.1153 (calcd for $C_{23}H_{24}O_{12}Na$, 515.1160) and NMR data (Table 4.10 and Appendix A17). The presence of a 5-hydroxyflavonol skeleton was evident from UV absorption (λ_{max} 266 and 348 nm) and NMR data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed very close similarity with compound **16**. The only difference between the two compounds was the presence of a methoxy group (δ_H 3.80, δ_C 60.6) in this compound at C-7 instead of a hydroxy group. Thus, compound **17** was identified as eupatolitin 3-*O*-α-*L*-rhamnopyranoside (eupatolitin-3-*O*-rhamnoside) (Quijano *et al.*, 1970). Eupatolitin-3-*O*-rhamnoside (**17**) was previously isolated from *Eupatorium ligustrinum* (Quijano *et al.*, 1970) and this is its first report in the genus *Tephrosia*.



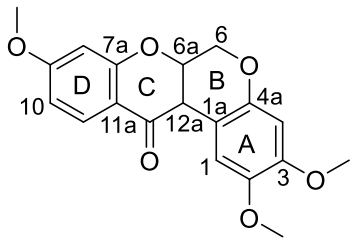
17

Table 4.10: NMR Data for Compounds 16 and 17 in Acetone-d_6 (600 MHz)

	16			17		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	158.5			158.6		
3	135.4			135.7		
4	179.7			179.6		
5	153.7			153.6		
6	132.0			133.2		
7	153.2			160.2		
8	94.5	6.55, s	C-6, C-7, C-9, C-10	91.7	6.77, s	C-6, C-7, C-9, C-10
9	157.8			153.3		
10	106.2			106.9		
1'	122.9			122.9		
2'	116.8	7.51, d (2.1)	C-2, C-4', C-6'	116.7	7.52, d (2.1)	C-2, C-4', C-6'
3'	145.9			145.9		
4'	149.1			149.0		
5'	116.2	6.99, d (8.3)	C-1', C-3'	116.1	7.00, d (8.4)	C-1', C-3'
6'	122.6	7.40, dd (8.3, 2.1)	C-2, C-2', C-4'	122.6	7.42, dd (8.3, 2.1)	C-2, C-2', C-4'
2"	102.7	5.53, d (1.6)	C-3, C-3", C-4", C-6"	102.7	5.54, d (1.4)	C-3, C-3", C-4", C-6"
3"	71.5	4.21, dd (3.5, 1.6)	C-4", C-5"	71.4	4.21, dd (3.5, 1.6)	C-2", C-4", C-5"
4"	72.1	3.73, dd (9.3, 3.4)	C-3", C-5", C-6"	72.1	3.71, dd (9.2, 3.4)	C-3". C-5", C-6"
5"	73.0	3.34, m	C-4", 6"-Me	73.0	3.34, dd (9.4, 9.4)	C-4", 6"-Me
6"	71.4	3.41, m	C-4"	71.4	3.40, m	C-4", 2"-Me
6"-Me	17.8	0.91, d (6.1)	C-5", C-6"	17.8	0.91, d (6.1)	C-3", C-5"
6-OMe	60.7	3.88, s	C-6	56.8	3.98, s	C-6
7-OMe				60.6	3.80, s	C-7
5-OH		12.93, s	C-5, C-6, C-10		12.65	C-5, C-6, C-10

4.1.18: Munduserone (18)

Compound **18** was obtained as a white paste. Its molecular formula was deduced as $C_{19}H_{18}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 343.1177 (calcd $C_{19}H_{19}O_6$, 343.1176), $[M+Na]^+$ at *m/z* 365.0998 (calcd $C_{19}H_{18}O_6Na$, 365.0996)] and NMR data (Table 4.11 and Appendix A18). A rotenoid skeleton was evident from the UV (λ_{max} 230 and 280 nm) and NMR data [δ_H 4.61, dd, *J* = 12.2, 2.9 Hz, and 4.30, dd, *J* = 12.2, 1.3 Hz for H-6 (δ_C 66.1); δ_H 5.12, ddd, *J* = 3.9, 3.0, 1.0 Hz for H-6a (δ_C 72.3); δ_H 3.91, d, *J* = 4.0 Hz for H-12a (δ_C 44.1) and δ_C 188.8 for C-12)] (Mabry *et al.*, 1970; Agrawal, 1989) (Table 4.11 and Appendix A18). The NMR data of **18** displayed signals for three methoxy groups [δ_H 3.65 (δ_C 56.0), 3.76 (δ_C 55.1) and 3.86 (δ_C 55.3]. Further, ^{1}H NMR revealed signals for two singlet protons [δ_H 6.72 (δ_C 112.6) and 6.47 (δ_C 102.3)], and an ABX spin system [δ_H 7.82, d, *J* = 8.9 Hz (δ_C 128.8), 6.62, dd, *J* = 8.8, 2.3 Hz (δ_C 110.2) and 6.46, d, *J* = 2.4 Hz (δ_C 100.4)]. HMBC correlations of one of the ABX protons at δ_H 7.82 with C-7a, C-12 and C-9 allowed for its placement at C-11. This placed the singlet aromatic protons in ring B at H-1 and H-4. HMBC correlation of the proton at δ_H 6.47 with C-1a, C-2, C-4a and C-3 allowed for its placement at C-4, whereas the correlation of the other singlet proton at δ_H 6.72 with C-12a, C-1a, C-2, C-3 and C-4a allowed for its placement at C-1. The chemical shift value of H-1 can be used to determine whether the stereochemistry of the B/C ring junction is *cis* (δ 6.4-6.8) or *trans* (δ 7.7-8.3) (Yenesew *et al.*, 1998). Since H-1 resonated at δ_H 6.72, the stereochemistry of the B/C ring junction was determined to be *cis* in this compound. Thus, compound **18** was identified as munduserone (Dagne *et al.*, 1989). Munduserone (**18**) was previously isolated from *Tephrosia fulvinervis* (Dagne *et al.*, 1989). This is its first report in this species.



18

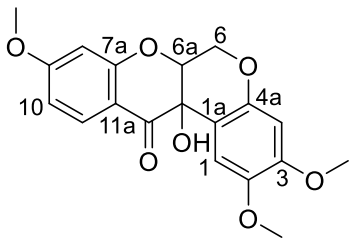
Table 4.11: NMR Data for Compound 18 in Acetone-d_6 (600 MHz)

	18		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	111.6	6.72, s	C-1a, C-2, C-3, C-4a, C-12a
1a	105.2		
2	143.9		
3	150.2		
4	101.3	6.47, s	C-1a, C-2, C-3, C-4a
4a	148.1		
6	66.1	4.61, dd (12.2, 2.9) 4.30, dd (12.2, 1.3)	C-4a, C-6a, C-12, C-12a
6a	72.3	5.14, ddd (3.9, 3.0, 1.0)	C-4a, C-6a, C-12, C-12a
7a	162.9		
8	100.4	6.46, d (2.4)	C-7a, C-10, C-11a
9	166.4		
10	110.2	6.62, dd (8.8, 2.3)	C-8, C-11a
11	128.8	7.82, d (8.9)	C-7a, C-9, C-12
11a	112.8		
12	188.8		
12a	44.1	3.91, d (4.0)	C-1, C-1a, C-4a, C-12
2-OMe	56.0	3.65, s	C-2
3-OMe	55.1	3.76, s	C-3
9-OMe	55.3	3.86, s	C-9

4.1.19: cis-12a-Hydroxymunduserone (19)

Compound **19** was isolated as a white paste. The molecular formula was deduced as $C_{23}H_{22}O_7$ from its NMR data (Table 4.13 and Appendix A19). A 12a-hydroxyrotenoid skeleton was evident from the NMR data [δ_H 4.58, dd, *J* = 12.2, 2.5 Hz, and 4.47, dd, *J* = 12.1, 1.1 Hz for H-6 (δ_C 64.7); δ_H 4.69, dd, *J* = 2.5, 1.1 Hz for H-6a (δ_C 77.1); δ_C 68.5 for C-12a and δ_C 191.7 for C-12] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **19** was very similar to that of **18**. The key difference was the presence of the hydroxyl group at C-12a (δ_C 68.5) in

compound **19**. This was evident from the HMBC correlation of H-6 (δ_H 4.58 and 4.47 with C-12a and also the correlation of H-1 (δ_H 6.62) with C-12a. The NMR data was found to be similar to that of *cis*-12a-hydroxymunduserone (Dagne *et al.*, 1989). This compound was previously isolated from *T. fulvinervis* (Dagne *et al.*, 1989). This is the first report in this species.



19

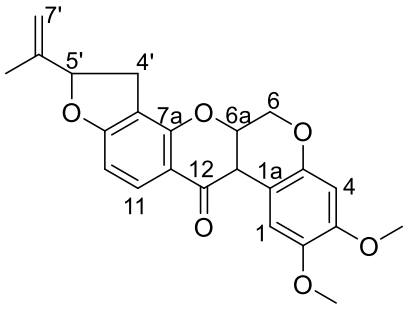
Table 4.12: NMR Data for Compound 19 in Acetone-d_6 (600 MHz)

		19	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	112.3	6.62, s	C-1a, C-2, C-3, C-4a, C-12a
1a	109.9		
2	144.7		
3	152.5		
4	102.0	6.49, s	C-1a, C-2, C-3, C-4a
4a	149.8		
6	64.7	4.58, dd (12.2, 2.5) 4.47, dd (12.1, 1.1)	C-4a, C-6a, C-12, C-12a
6a	77.1	4.69, dd (2.5, 1.1)	C-4a, C-6a, C-12, C-12a
7a	163.1		
8	101.3	6.42, d (2.3)	C-7a, C-10, C-11a
9	167.6		
10	111.5	6.62, dd (8.8, 2.3)	C-8, C-11a
11	129.7	7.79, d (8.8)	C-7a, C-9, C-12
11a	112.8		
12	191.7		
12a	68.5		
2-OMe	56.8	3.60, s	C-2
3-OMe	56.0	3.76, s	C-3
9-OMe	56.3	3.84, s	C-9

4.1.20: Rotenone (20)

Compound **20** was isolated as a white paste. The molecular formula was established as $C_{23}H_{22}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 395.1486 (calcd for $C_{23}H_{23}O_6$, 395.1489), $[M+Na]^+$ at *m/z* 417.1305 (calcd for $C_{23}H_{22}O_6Na$, 417.1309) and NMR data (Table

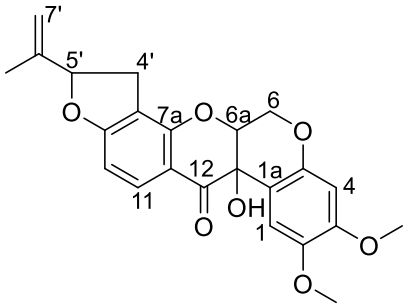
4.13 and Appendix A20). A rotenoid skeleton was evident from the UV (λ_{max} 222 and 296 nm) and NMR data (Table 4.13 and Appendix A20) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **20** displayed signals for two methoxy groups [δ_H 3.65 (δ_C 56.9) and 3.77 (δ_C 56.0)] and 2-isopropenyltetrahydrofuran residues [δ_H 2.95 (1H, dd, *J* = 15.7, 8.1 Hz) and 3.30 (1H, dd, *J* = 15.7, 9.8 Hz) for methylene protons (δ_C 31.9), 5.35 (1H, t, *J* = 8.9 Hz) for methine protons (δ_C 88.9), 5.08, broad-s for olefinic methylene protons (δ_C 112.4), 1.77 (3H, s) for methyl protons (δ_C 17.2) and δ_C 144.4 for quaternary carbon]. Further, ^{1}H NMR revealed signals for two singlet protons [δ_H 6.72 and 6.46] and *ortho*-coupled aromatic protons [δ_H 6.51 (1H, d, *J* = 8.6 Hz) and 7.79 (1H, d, *J* = 8.5 Hz)]. The deshielded aromatic proton at δ_H 7.79 showed HMBC correlations with C-7a, C-12 and C-9 which allowed for its placement at C-11 and its coupling partner at C-10. HMBC correlations of the aromatic singlet protons at δ_H 6.46 and 6.72 with C-2 and C-3 placed the methoxy groups at C-2 and C-3. HMBC correlations of H-4' with C-8, C-7a and C-9 allowed for the placement of the 2-isopropenyltetrahydrofuran moiety at C-8/C-9. In the ^{1}H NMR, H-1 resonated at a shielded resonance of δ_H 6.72 indicating the *B*/*C* ring junction is *cis*. Thus, compound **20** was identified as rotenone (Carlson *et al.*, 1973). Rotenone (**20**) has been reported in several *Tephrosia* species including the root of this plant (Were *et al.*, 1990) but this is its first report from the aerial part.



20

4.1.21: 12a-Hydroxyrotenone (21)

Compound **21** was isolated as a white paste. The molecular formula was established as $C_{23}H_{22}O_7$ from its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 411.1438 (calcd for $C_{23}H_{23}O_7$, 411.1438), $[M+Na]^+$ at *m/z* 433.1257 (calcd for $C_{23}H_{22}O_7Na$, 433.1258) and NMR data (Table 4.14 and Appendix A21). A 12a-hydroxyrotenoid skeleton was evident from (λ_{max} 226 and 298 nm) and NMR data (Table 4.14 and Appendix A21) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **21** displayed very close similarity with those of compound **20**. The notable difference was the presence of the hydroxyl group at C-12a (δ_C 68.5) in compound **21**. It was evident from HMBC correlations of H-6 (δ_H 4.59 and 4.48 with C-12a and also H-1 (δ_H 6.64) with C-12a. In the ^{1}H NMR, H-1 resonated at a shielded resonance of δ_H 6.64 indicating that the *B*/*C* ring junction is *cis*. These spectral data were identical to those described in the literature for 12a-hydroxyrotenone (Carlson *et al.*, 1973; Oberholzer *et al.*, 1974). 12a-Hydroxyrotenone (**21**) was previously reported in the roots of this plant (Were *et al.*, 1990) but this is its first report from the aerial part.



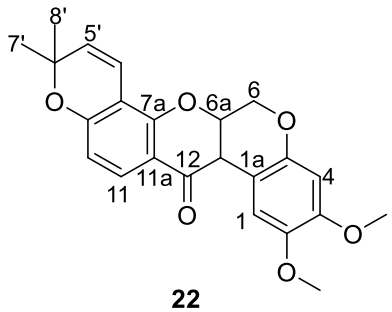
21

Table 4.13: NMR Data for Compounds 20 and 21 in Acetone-d_6 (600 MHz)

	20			21		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	112.6	6.72, s	C-1a, C-2, C-3, C-4a, C-12a	112.5	6.64, s	C-1a, C-2, C-3, C-4a, C-12a
1a	106.2			109.2		
2	144.9			144.7		
3	151.1			152.5		
4	102.3	6.46, s	C-1a, C-2, C-3, C-4a	102.0	6.49, s	C-1a, C-2, C-3, C-4a
4a	149.0			149.8		
6	67.0	4.60, dd (12.3, 2.9) 4.29, dd (12.3, 1.4)	C-4a, C-6a, C-12, C-12a	64.6	4.59, dd (12.2, 2.5) 4.48, dd (12.2, 1.2)	C-4a, C-6a, C-12a
6a	73.2	5.13, ddd (4.0, 2.9, 1.1)	C-1a, C-6, C-7a, C-12	77.1	4.70, dd (2.4, 1.1)	C-1a, C-6, C-12
7a	158.9			168.2		
8	113.8			113.9		
9	167.9			158.3		
10	105.1	6.51, d (8.6)	C-8, C-9, C-11a	105.5	6.54, d (8.6)	C-8, C-9, C-11a
11	130.3	7.79, d (8.5)	C-7a, C-9, C-12	130.4	7.77, d (8.5)	C-7a, C-9, C-12
11a	114.4			113.6		
12	189.4			191.5		
12a	45.1	3.90, d (4.0)	C-1, C-1a, C-4a, C-11a, C-12	68.5		
4'	31.9	2.95, dd (15.7, 8.1) 3.30, dd (15.7, 9.8)	C-7a, C-8, C-9, C-5', C-6'	31.7	2.93, dd (15.8, 8.1) 3.27, dd (15.8, 9.8)	C-7a, C-8, C-9, C-5', C-6'
5'	88.4	5.35, t (8.9)	C- 4', C-7'	88.5	5.34, m	C-7', C- 4', C-6', C- 8'
6'	144.4			144.5		
7'	112.4	5.08, s 4.93, s	C- 5', C-6', C-8'	112.4	5.07, s 4.93, s	C- 5', C-6', C-8'
8'	17.2	1.77, s	C- 5', C-6', C-7'	17.2	1.76, s	C- 5', C-6', C-7'
2-OMe	56.9	3.65, s	2	56.9	3.61, s	2
3-OMe	56.0	3.77, s	3	56.0	3.77, s	3

4.1.22: Deguelin (22)

Compound **22** was isolated as a white paste. The molecular formula was established as $C_{23}H_{22}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 395.1495 (calcd for $C_{23}H_{23}O_6$, 395.1489), $[M+Na]^+$ at *m/z* 417.1304 (calcd for $C_{23}H_{22}O_6Na$, 417.1309) and NMR data (Table 4.14 and Appendix A22). A rotenoid skeleton was evident from the UV absorption (λ_{max} 234, 268 and 298 nm) and NMR data (Table 4.14 and Appendix A22) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **22** displayed very close similarity with those of rotenone (**20**). The notable difference was the presence of a 2,2-dimethylchromene moiety at C-8/C-9 in **22** rather than a 2-isopropenyltetrahydrofuran group in **20**. These spectral data were identical to those described in the literature for deguelin (Luyengi *et al.*, 1994). This compound was previously reported in the roots of this plant (Were *et al.*, 1990) but this is its first report from the aerial part.

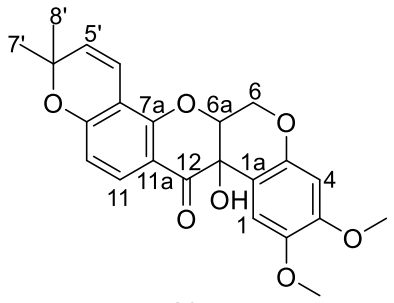


22

4.1.23: Tephrosin (23)

Compound **23** was isolated as a white paste. The molecular formula was established as $C_{23}H_{22}O_7$ from its HRESIMS data [molecular ion $[M+H]^+$ at *m/z* 411.1430 (calcd for $C_{23}H_{23}O_7$, 411.1438), $[M+Na]^+$ at *m/z* 433.1238 (calcd for $C_{23}H_{22}O_7Na$, 433.1258) and NMR data (Table 4.14 and Appendix A23). A 12a-hydroxyrotenoid skeleton was evident from the UV absorption (λ_{max} 234, 270 and 316 nm) and NMR data (Table 4.14 and Appendix A23) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **23** displayed very close similarity with those of

compound **22**. The notable difference was the presence of the hydroxy group at C-12a (δ_C 77.1) in compound **23**. This was evident from HMBC correlation of H-6 (δ_H 4.65 and 4.49 with C-12a and also H-1 (δ_H 6.65) with C-12a. These spectral data were identical to those described in the literature for tephrosin (Luyengi *et al.*, 1994). This compound was previously reported in the roots of this plant (Were *et al.*, 1990) but this is its first report from the aerial part.



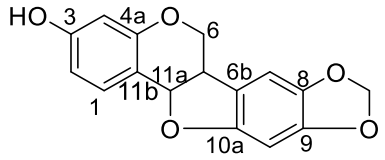
23

Table 4.14: NMR Data for Compounds 22 and 23 in Acetone-d_6 (600 MHz)

	22			23		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	112.5	6.73, s	C-2, C-3, C-4a, C-12a	112.3	6.65, s	C-2, C-3, C-4a, C-12a
1a	106.0			108.9		
2	144.8			144.7		
3	151.1			152.5		
4	102.3	6.45, s	C-1a, C-2, C-3, C-4a	102.0	6.46, s	C-1a, C-2, C-4a, C-3
4a	149.0			149.8		
6	67.0	4.30, m 4.66, dd (12.2, 2.9)	C-4a, C-6a, C-12, C-12a	64.6	4.49, dd (12.1, 1.1) 4.65, dd (12.1, 2.5)	C-4a, C-6a
6a	73.5	5.14, ddd (4.0, 2.9, 1.1)	C-1a, C-6, C-12, C-12a	77.3	4.71, dd (2.5, 1.1)	C-1a, C-6, C-12, C-12a
7a	160.4			157.1		
8	109.9			108.9		
9	157.8			160.7		
10	111.7	6.45, d (8.0)	C-8, C-11a	112.1	6.47, d (8.0)	C-8, C-11a
11	129.0	7.70, d (8.7)	C-7a, C-9, C-12	129.0	7.69, d (8.7)	C-7a, C-9, C-12
11a	113.8			112.5		
12	189.6			191.8		
12a	44.9	3.91, d (4.0)	C-1, C-1a, C-4a, C-11a, C-12	77.1		
4'	116.1	6.65, d (10.1)	C-7a, C-8, C-9, C-6'	115.9	6.61, d (10.1)	C-7a, C-8, C-9, C-6'
5'	130.0	5.73, d (10.1)	C-8, C-6', C-7', C-8'	130.0	5.73, d (10.1)	C-8, C-6', C-7', C-8'
6'	78.4			78.6		
7'	28.2*	1.36, s	C-5', C-6', C-8'	28.2*	1.35, s	C-5', C-6', C-8'
8'	28.6*	1.44, s	C-5', C-6', C-7'	28.6*	1.44, s	C-5', C-6', C-7'
2-OMe	56.8	3.64, s	2	56.8	3.61, s	2
3-OMe	56.0	3.74, s	3	56.0	3.76, s	3
	*interchangeable positions			*interchangeable positions		

4.1.24: Maackiain (24)

Compound **24** was isolated as a white paste. The molecular formula was established as $C_{16}H_{12}O_5$ from its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 285.0758 (calcd for $C_{16}H_{13}O_5$, 285.0757) and NMR data (Table 4.15 and Appendix A24). A pterocarpan skeleton was evident from the UV (λ_{max} 228 and 310 nm) and NMR [δ_H 3.61, dd, *J* = 10.6, 10.2 Hz, and 4.27, dd, *J* = 10.7, 4.6 Hz for H-6 (δ_C 67.0); 3.56, ddd, *J* = 10.1, 7.0, 4.7 Hz for H-6a (δ_C 41.0); and 5.49, d, *J* = 7.0 Hz for H-11a (δ_C 79.4)] (Table 4.15 and Appendix A24) data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra displayed signals for a methylenedioxy group [two singlets at δ_H 5.93 and 5.90, 2H (δ_C 102.1)]. Further, the NMR data revealed signals for two singlet protons [δ_H 6.89 and 6.40] and an AMX spin system [δ_H 7.30, d, *J* = 8.3 Hz (δ_C 133.0), 6.55, dd, *J* = 8.4, 2.4 Hz (δ_C 110.4) and 6.35, d, *J* = 2.4 Hz (δ_C 103.9)]. HMBC correlation of one of the ABX system protons at δ_H 7.30 with C-11a, C-4a, C-3 and C-4 allowed for its placement at C-1 with its coupling partners at δ_H 6.55 (dd, *J* = 8.4, 2.4 Hz) and 6.35 (d, *J* = 2.4 Hz) at C-2 and C-4, respectively. HMBC correlation of the singlet proton at δ_H 6.89 with C-6a, C- 9, C-10a, C-10 and C-8 allowed for its placement at C-7, whereas the correlation of the proton at δ_H 6.40 with C-6b, C-8, C-9 and C-10a allowed for its placement at C-10. The placement of the methylenedioxy group at C-8/C-9 was based on the HMBC correlation of its protons with C-8 and C-9. Therefore, compound **24** was identified as 3-hydroxy-8,9-methylenedioxypterocarpan (maackiain) (Abdel-Kader, 2001). Maackiain has previously been reported in several plants including *Ononis vaginalis* (Abdel-Kader, 2001) and *T. elata* (Atilaw, 2018), but this is its first report in this plant.



24

Table 4.15: NMR Data for Compound 24 in Acetone-d_6 (600 MHz)

		24	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	133.0	7.30, d (8.3)	C-3, C-4, C-4a, C-11a
2	110.4	6.55, dd (8.4, 2.4)	C-3, C-4, C-11b
3	159.7		
4	103.9	6.35, d (2.4)	C-2, C-3, C-4a, C-11b
4a	157.7		
6	67.0	3.61, dd (10.6, 10.2) 4.27, dd (10.7, 4.6)	C-4a, C-6a, C-6b, C-11a
6a	41.0	3.56, ddd (10.1, 7.0, 4.7)	C-6, C-6b, C-10a
6b	119.5		
7	105.9	6.89, s	C-6a, C-8, C- 9, C-10, C-10a
8	142.4		
9	148.9		
10	94.0	6.40, s	C-6b, C-8, C-9, C-10a
10a	155.3		
11a	79.4	5.49, d (7.0)	C-1, C- 4a, C-6, C-6a, C-6b, C-11b
11b	112.8		
OCH_2O	102.1	5.93, s 5.90, s	C-8, C-9

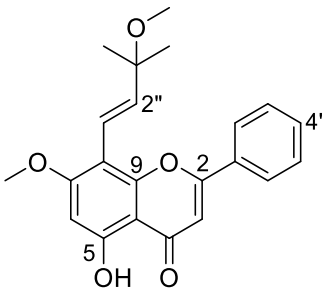
4.2: Characterization of Compounds Isolated from *Tephrosia hildebrandtii*

An extract of the aerial parts of *T. hildebrandtii* was subjected through sets of chromatographic techniques to give a new flavone named hildeflavone (**25**) together with other ten known compounds identified as 5,7,3'-trihydroxy-4'-methoxy-8-prenylisoflavone (**26**), 5,3'-dihdroxy-4'-methoxy-2",2"-dimethylpyrano[5",6":8,7]isoflavone (**27**), 4'-hydroxyemoroidocarpan (**28**), hildecarpin (**29**), pisatin (**30**), pongachin (**31**), emoroidenone (**32**), desmoxyphyllin A (**33**), pinoresinol (**34**) and tephrosin (**22**).

4.2.1: Hildeflavone (25)

Compound **25** was isolated as a yellow paste. The molecular formula was established as $C_{22}H_{22}O_5$ from the HRESIMS molecular ion $[M+H]^+$ at m/z 367.1540 (calcd for $C_{22}H_{23}O_5$, 367.1540), $[M+Na]^+$ at m/z 389.1361 (calcd for $C_{22}H_{22}O_5Na$, 389.1359) and ^{13}C NMR data (Table 4.16 and Appendix A25). A 5-hydroxyflavone skeleton was evident from the UV (λ_{max} 228 and 268 nm) and NMR data [δ_H 6.86 for H-3 and 13.26 for 5-OH; δ_C 165.2 (C-2), 105.6 (C-3), 183.8 (C-4)] (Table 4.16 and Appendix A25) (Mabry *et al.*, 1970; Agrawal, 1989). Further, the NMR spectra exhibited signals for a methoxy [δ_H 4.02 (δ_C 56.9)] and a *trans*-oriented-3"-methoxy-3"-methylbut-1"-enyl [δ_H 1.40, s, 6H (δ_C 26.5) for methyl groups, 6.80, d, 1H, J = 16.7 Hz (δ_C 118.1) for H-1", 6.54, d, 1H, J = 16.7 Hz (δ_C 140.0) for H-2" and 3.23, s, 3H (δ_C 50.52) for a methoxy substituent] groups (Khalid and Waterman, 1981; Atilaw *et al.*, 2017b). The NMR also exhibited signals for three sets of mutually coupled protons [δ_H 8.11, m, 2H (δ_C 127.5), 7.63, m, 2H (δ_C 130.0 and 7.65, m, 1H (δ_C 132.9] typical of an unsubstituted flavone ring B and a singlet aromatic proton at δ_H 6.55 assigned to ring A. HMBC correlations of the proton at δ_H 6.55 with C-8, C-5, C-10 and C-7 allowed for its placement at C-6. The methoxy protons at δ_H 4.02 showed HMBC correlation with C-7 (δ_C 164.3) which allowed for the placement of this substituent at C-7. Placement of the prenyl group at C-8 was based on HMBC correlation of H-1" with C-9 (δ_C 155.0), C-8 (δ_C 106.3) and C-7 (δ_C 164.3). Thus,

compound **25** was identified as 5-hydroxy-7-methoxy-8-(*E*-3-methoxy-3-methylbut-1-enyl)flavone. This is a new compound and was given the trivial name hildeflavone.



25

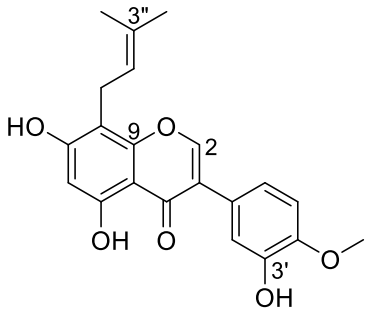
Table 4.16: NMR Data for Compound 25 in Acetone-d_6 (600 MHz)

	25		
Position	δ_C	δ_H, **m (*J* in Hz)**	**HMBC**
2	165.2		
3	106.2	6.86, *s*	C-10, C-4, C-2, C-1'
4	183.8		
5	162.5		
6	96.2	6.55, *s*	C-5, C-8, C-7, C-10
7	164.3		
8	106.3		
9	155.0		
10	105.8		
1'	132.5		
2'/6'	127.5	8.11, *m*	C-2, C-2'/6', C-4'
3'/5'	130.0	7.63, *m*	C-3'/5', C-1'
4'	132.9	7.65, *m*	
1"	118.1	6.80, *d* (16.7)	C-3", C-2", C-7, C-9
2"	140.0	6.54, *d* (16.7)	C-3"-Me_2, C-8
3"	76.0		
3"-Me_2	26.5	1.40, *s*	C-3"-Me, C-3", C-2"
3"-OMe	50.5	3.23, *s*	C-3"
7-OMe	56.9	4.02, *s*	C-7
5-OH		13.26, *s*	

4.2.2: 5,7,3'-Trihydroxy-4'-methoxy-8-prenylisoflavone (26)

Compound **26** was isolated as a pale yellow paste. The molecular formula was established as $C_{21}H_{20}O_6$ from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 369.1349 (calcd for $C_{21}H_{21}O_6$, 369.1333) together with NMR data (Table 4.17 and Appendix A26). A 5-hydroxyisoflavone

skeleton was evident from the UV (λ_{max} 230, 274 and 332 nm) and NMR data [δ_H 8.30 for H-2 and 12.99 for 5-OH; δ_C 154.6 for C-2, 123.7 for C-3 and 181.9 for C-4] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data exhibited signals for a prenyl and a methoxy group. Further, the NMR spectra showed signals for a singlet aromatic proton at δ_H 6.37 and an ABX spin system [δ_H 6.89, d, J = 8.1 Hz (δ_C 115.6), 7.10, dd, J = 8.2, 2.0 Hz (δ_C 122.8) and 7.27, d, J = 2.0 Hz (δ_C 113.7)]. The proton at δ_H 6.37 was placed at C-6 based on its HMBC correlation with C-5 and C-8. Two of the AXY system protons (δ_H 6.81 and 7.10) showed HMBC correlation with C-4'. Further correlation with the methoxy protons allowed the placement of this substituent at C-4'. HMBC correlation of H-1" (δ_H 3.45) with C-8, C-9 and C-7 placed the prenyl at C-8. Thus, compound **26** was identified as 5,7,3'-trihydroxy-4'-methoxy-8-prenylisoflavone (Souza *et al.*, 2017). This compound was previously reported in *Vatairea guianensis* by Souza *et al.*, (2017). However, this is its first report in this plant.

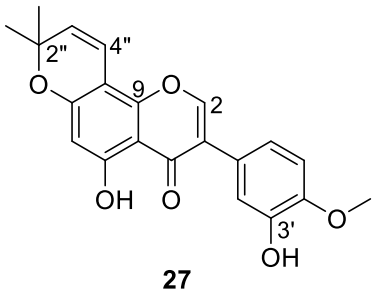


26

4.2.3: 5,3'-Dihydroxy-4'-methoxy-2",2"-dimethylpyrano[5",6":8,7]isoflavone (27)

Compound **27** was isolated as a pale yellow paste. The molecular formula was deduced as $C_{21}H_{18}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 367.1198 (calcd for $C_{21}H_{19}O_6$, 367.1176)] and NMR data (Table 4.17 and Appendix A27). Both the 1D- and 2D-NMR data of **27** showed very close similarity with those of compound **26**. The only notable difference was the presence of a 2,2-dimethylchromene moiety in compound **27** instead of a 3-methylbut-2-enyl group in **26**. Therefore, compound **27** was identified as 5,3'-dihydroxy-4'-methoxy-2",2"-

dimethylpyrano[5",6":8,7]isoflavone (Souza *et al.*, 2013). This compound was previously reported in *Vatairea guianensis* by Souza *et al.*, (2013), but this is its first report in the genus *Tephrosia*.



27

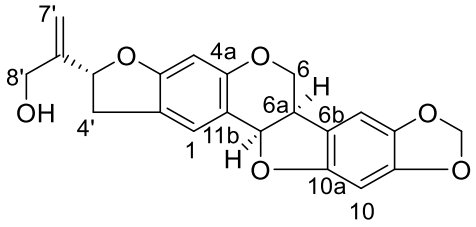
Table 4.17: NMR Data for Compounds 26 and 27 in Acetone-d_6 (600 MHz)

	26			27		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	154.6	8.30, s	C-3, C-4, C-9	154.6	8.30, s	C-3, C-4, C-9
3	123.7			123.5		
4	181.9			182.6		
5	161.5			160.2		
6	99.5	6.37, s	C-5, C-8	100.4	6.21, s	C-5, C-8
7	161.2			163.3		
8	107.2			100.9		
9	156.2			152.9		
10	107.2			100.4		
1'	123.6			123.3		
2'	113.7	7.27, d (2.0)	C-3, C-4', C-6'	113.7	7.26, d (2.0)	C-3, C-4', C-6'
3'	147.6			147.7		
4'	148.0			148.0		
5'	115.6	6.89, d (8.2)	C-1', C-3', C-4'	115.7	6.90, d (8.1)	C-1', C-3', C-4'
6'	122.8	7.10 dd (8.1, 2.0)	C-3, C-1', C-2', C-4'	122.8	7.09 dd (8.1, 2.0)	C-2', C-4'
1"	22.0	3.45, d (7.3)	C-8, C-9, C-3"			
2"	123.1	5.25, t (7.2)		79.0		
3"	132.0			128.7	5.76, d (10.0)	C-2", 2"-Me_2
4"	17.9*	1.81	C-2", C-3", C-5"	115.0	6.73, d (9.9)	C-7, C-2"
5"	25.9*	1.66	C-2", C-3", C-4"			
6"						
2"-Me_2				28.3	1.48, s	C-2", C-3", 2"-Me
5-OH		12.99, s			13.14 s	
4'-OMe	56.4	3.89, s	C-4'	56.4	3.89, s	C-4'

*interchangeable

4.2.4: 4'-Hydroxyemoroidocarpan (28)

Compound **28** was isolated as a white paste. The molecular formula was established as $C_{21}H_{18}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 367.1193 (calcd for $C_{21}H_{19}O_6$, 367.1176)] and NMR data (Table 4.18 and Appendix A28). A pterocarpan skeleton was evident from NMR data [δ_H 3.62, dd, *J* = 11.7, 10.1 Hz, and 5.28, dd, *J* = 10.5, 4.4 Hz for H-6 (δ_C 67.2); 3.57, ddd, *J* = 9.9, 7.1, 4.5 Hz for H-6a (δ_C 41.0) and 5.50, d, *J* = 7.1 Hz for H-11a (δC 79.7)] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra displayed signals for a methylenedioxy [δ_H 5.93 and 5.91, 2H (δ_C 102.1)] and 2-(3-hydroxy-isopropenyl)-tetrahydrofuran [δ_H 3.10, dd, *J* = 15.3, 7.9 Hz and 3.39, dd, *J* = 15.5, 9.7 Hz for H-4' (δ_C 35.0); 5.35, t, *J* = 8.6 Hz for H-5' (δ_C 85.0); 4.18, s for H-8' (δ_C 62.3); 5.18 and 5.20, broad-s for H-7' (δ_C 109.9); and δ_C 150.0 for C-6'] groups. Further, the NMR data revealed the presence of four singlet aromatic protons [δ_H 7.25, 6.28, 6.90 and 6.39]. HMBC correlation of the aromatic singlet proton at δ_H 7.25 with C- 11a, C-4a and C-3 allowed for its placement at C-1, whereas the correlation of the proton at δ_H 6.28 with C-2 and C-11b allowed for its placement at C-4. HMBC correlation of the proton at δ_H 6.90 with C-6a, C-8, C-9 and C-10a allowed for its placement at C-7. HMBC correlation of methylenedioxy protons with C-8/C-9 placed this group at these carbons. The placement of the 2-(3-hydroxy-isopropenyl)-tetrahydrofuran group at C-2/C-3 was from the HMBC correlations of H-4' (δ_H 3.10 and 3.39) with C-5', C-1, C-6' and C-3. Thus, compound **28** was identified as 4'-hydroxyemoroidocarpan (Harinantenaina *et al.*, 2010). This compound was previously reported from the roots of the endemic Malagasy *Pongamiopsis pervilleana* by Harinantenaina *et al.*, (2010), but it is the first report in this plant.



28

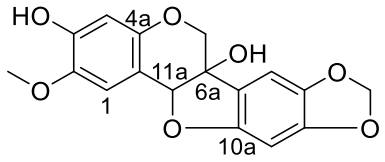
Table 4.18: NMR Data for Compound 28 in Acetone-d_6 (600 MHz)

	28		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	127.8	7.25, s	C-3, C-4a, C-11a, C-4'
2	113.5		
3	161.8		
4	98.4	6.28, s	C-2, C-3, C-4a, C-11b
4a	157.1		
6	67.2	3.62, dd (11.7, 10.1) 5.28, dd (10.5, 4.4)	C-4a, C-6a, C-6b, C-5'
6a	41.0	3.57, dd (9.9, 7.1, 4.5)	C-6, C-6b, C-10a
6b	119.5		
7	105.9	6.90, s	C-6a, C-8, C-9, C-10a
8	142.5		
9	148.9		
10	93.9	6.39, s	C-6b, C-8, C-9, C-10a
10a	155.3		
11a	79.7	5.50, d (7.1)	C-1, C-2, C-4a, C-6
11b	121.6		
4'	35.0	3.10, dd (15.3, 7.9) 3.39, dd (15.5, 9.7)	C-1, C-3, C-5', C-6'
5'	85.0	5.35, t (8.6)	C-4', C-6', C-7'
6'	150.0		
7'	109.9	5.18, s 5.20, s	C-5', C-6', C-8'
8'	62.3	4.18, s	
OCH_2O	102.1	5.91, s 5.93, s	C-8, C-9

4.2.5: Hildecarpin (29)

Compound **29** was isolated as a white paste. Its molecular formula was deduced as $C_{17}H_{14}O_7$ from HRESIMS molecular ion peak $[M+H]^+$ at *m/z* 381.0814 (calcd for $C_{17}H_{15}O_7$, 381.0812) and NMR data (Table 4.19 and Appendix A29). A 6a-hydroxypterocarpan skeleton was evident from the NMR data [δ_H 4.00, d, *J* = 11.3 Hz, 4.08, d, *J* = 11.4 Hz for H-6; 5.25, s for H-11a and 4.98, s for 6a-OH] (Table 4.19 and Appendix A29) (Mabry *et al.*, 1970; Agrawal, 1989). The

NMR spectra showed signals for a methylenedioxy and methoxy group. Additionally, the NMR data exhibited signals for four singlet aromatic protons (δ_H 6.99, 6.89, 6.35 and 6.36). A hydroxy proton at δ_H 7.86 showed HMBC correlation with C-2, C-3 and C-4 allowing for its placement at C-3 and methoxy group at C-2, while the singlet aromatic proton (δ_H 6.89) was placed at C-4. HMBC correlation of the methylenedioxy protons (δ_H 5.92 and 5.95) with C-8 and C-9 supported its placement at C-8/C-9. Thus, compound **29** was identified as hildecarpin (Lwande *et al.*, 1985b). Hildecarpin was previously reported from the root of this plant (Lwande *et al.*, 1985b). But this is the first report of hildecarpin in the aerial parts of *T. hildebrandtii*.

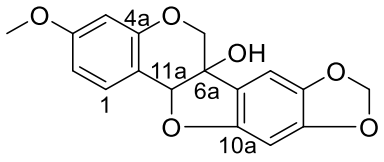


29

4.2.6: Pisatin (30)

Compound **30** was isolated as a white paste. The molecular formula was deduced as $C_{17}H_{14}O_6$ from its HRESIMS $[M\text{-}H_2O]^+$ at *m/z* 297.0756 (calcd for $C_{17}H_{15}O_6$, 315.0863) and NMR data **(**Table 4.19 and Appendix A30). A 6a-hydroxypterocarpan skeleton was evident from the NMR data [δ_H 4.10, d, J = 11.4 Hz, 4.14, d, J = 11.4 Hz for H-6; 5.29, s for H-11a and 5.03, s for 6a-OH] (Table 4.19 and Appendix A30) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra showed signals for a methylenedioxy and a methoxy substituent (Table 4.19 and Appendix A30). Further, the NMR exhibited signals for two singlet aromatic protons (δ_H 6.90 and δ_H 6.36) and an ABX spin system [δ_H 7.37, d, J = 8.5 Hz, 6.63, dd, J = 8.6, 2.6 Hz and 6.40, d, J = 2.5 Hz]. HMBC correlations of the proton at δ_H 7.37 with C-11a, C-4a and C-3 allowed for its placement at C-1 with its coupling partners δ_H 6.63 and 6.40 at C-2 and C-4, respectively. Placement of the methylenedioxy group at C-8/9 was based on the HMBC correlations of its

protons with C-8 and C-9. HMBC correlation of the methoxy protons with C-3 allowed for its placement at C-3. Thus, compound **30** was identified as pisatin (Ingham and Markham, 1980). Pisatin was previously isolated from the seeds of *Tephrosia bidwilli* by Ingham and Markham, (1980) but it is the first report in this plant.



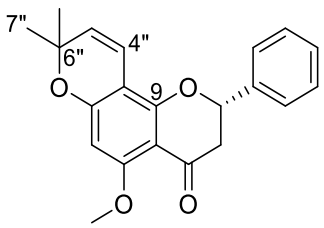
30

Table 4.19: NMR data for Compounds 29 and 30 in Acetone-d_6 (600 MHz)

	29			30		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	113.3	6.99, s	C-2, C-3, C-4a, C-11a, C-11b	132.1	7.37, d (8.5)	C-3, C-4a, C-11a
2	148.2			109.0	6.63, dd (8.6, 2.6)	C-3, C-4, C-11b
3	143.1			161.0		
4	103.4	6.89, s	C-2, C-3, C-4a, C-11b	101.1	6.40, d (2.5)	C-2, C-3, C-4a, C-11b
4a	149.7			156.2		
6	69.6	4.00, d (11.3) 4.08, d (11.4)	C-4a, C-6a, C-6b, C-11a,	69.4	4.10, d (11.4) 4.14, d (11.4)	C-4a, C-6a, C-6b, C-11a
6a	76.5			76.4		
6b	120.8			120.6		
7	103.5	6.35, s	C-6a, C-8, C-9, C-10, C-10a	103.5	6.90, s	C-6a, C-8, C-9, C-10, C-10a
8	142.0			142.0		
9	149.3			149.4		
10	93.2	6.36, s	C-6b, C-8, C-9, C-10a	93.3	6.36, s	C-6b, C-7, C-8, C-9, C-10a
10a	154.6			154.6		
11a	85.4	5.25, s	C-1, C-4a, C-6a, C-10a, C-11b	85.0	5.29, s	C-1, C-4a, C-6, C-6a, C-6b, C-11b
11b	111.2			113.3		
2-OMe	55.9	3.84, s	C-2			
3-OMe				54.7	3.77, s	C-3
3-OH		7.86, s	C-2, C-3, C-4			
6a-OH		4.98, s	C-6, C-6a, C-6b, C-11b		5.03, s	C-6, C-6a, C-6b, C-11b
OCH_2O	101.4	5.92, s 5.95, s	C-8, C-9	101.4	5.92, s 5.96, s	C-8, C-9

4.2.7: Pongachin (31)

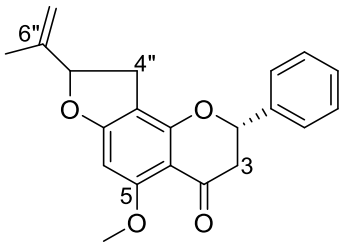
Compound **31** was obtained as an off-white solid. The molecular formula was established as $C_{21}H_{20}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 337.1427 (calcd for $C_{21}H_{21}O_4$, 337.1431, $[M+Na]^+$ at *m/z* 359.1243 (calcd for $C_{21}H_{20}O_4Na$, 359.1254)] and ^{13}C NMR data (Table 4.20 and Appendix A31). A flavanone skeleton was evident from the UV (λ_{max} 268 and 293 nm) and NMR data [AMX spin system of δ_H 5.55,dd, *J* = 12.7, 3.1 Hz for H-2 (δ_C 79.8), 2.71, dd, *J* = 16.3, 3.0 Hz for H-3eq and 2.97, dd, *J* = 16.3, 12.7 Hz for H-3ax (δ_C 46.2); δ_C 187.8 for C-4] (Table 4.20 and Appendix A31) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR showed signals of a methoxy and 2,2-dimethylchromene moiety [δ_H 6.58 (1H, d, *J* = 10.0 Hz) for H-4", 5.79 (1H, d, *J* = 10.0 Hz) for H-5", 1.42 (3H, s) for H-7" and 1.44 (3H, s) for H-8"] groups. The NMR spectra revealed the presence of three sets of mutually coupled protons [δ_H 7.57, m, 2H (δ_C 127.0), 7.45, m, 2H (δ_C 129.5 and 7.39, m, 1H (δ_C 129.2)] assigned to an unsubstituted ring B and a singlet proton at δ_H 6.11 (δ_C 94.5) assigned to ring A. HMBC correlations of the singlet proton at δ_H 6.11 with C-8, C-5, C-10 and C-7 allowed for its placement at C-6. The methoxy group at δ_H 3.82 (δ_C 56.2) showed HMBC correlations with C-5 allowing its placement at this position. HMBC correlations of H-4" (δ_H 6.58) with C-8, C-9 and C-7 placed the 2,2-dimethylchromene moiety at C-7/8. Thus, compound **31** was identified as 5-methoxy-6",6"-dimethylpyrano[2",3":6,7]flavanone (pongachin) (Andrei *et al.*, 2000). Pongachin was previously reported from the roots of *Tephrosia tunicata* (Andrei *et al.*, 2000) but, this is its first report from this plant.



31

4.2.8: Emoroidenone (32)

Compound **32** was obtained as an off-white solid. Its molecular formula was deduced as $C_{21}H_{20}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 337.1433 (calcd for $C_{21}H_{21}O_4$, 337.1431, $[M+Na]^+$ at *m/z* 359.1251 (calcd for $C_{21}H_{20}O_4Na$, 359.1254)] and ^{13}C NMR data (Table 4.20 and Appendix A32). A flavanone skeleton was apparent from the UV (λ_{max} 215 and 290 nm) and NMR data [AMX spin system of δ_H 5.54 (1H, dd, *J* = 12.5, 3.0 Hz for H-2), 2.70 (1H, dd, *J* = 16.2, 3.1 Hz for H-3eq) and 2.95 (1H, dd, *J* = 16.2, 12.5 Hz, H-3ax) and δ_C 186.4 for C-4] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **32** had close similarity to those of compound **31**. The difference was the presence of an 2-isopropenyltetrahydrofuryl group [δ_H 2.90 (dd, *J* = 15.1, 7.8 Hz) and 3.34, m for H-4" (δ_C 31.1); 5.35, m for H-5" (δ_C 87.4); 1.76, s for H-8' (δ_C 16.4); 4.91 and 5.08, broad-s for H-7' (δ_C 111.4); and δ_C 143.9 for C-6"] in compound **32** instead of a 2,2-dimethylchromene moiety in **31**. Based on these spectral data, compound **32** was identified as 4",5"-dihydro-5-methoxy-5"-isopropenylfurano-(2",3":7,8)-flavanone (emoroidenone). Emoroidenone was previously reported from the roots of *Tephrosia emoroides* (Machocho *et al.*, 1995) but this is its first report in this plant.



32

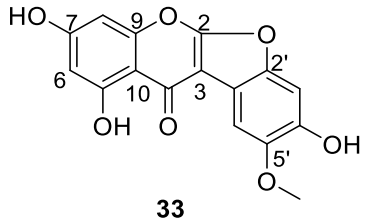
Table 4.20: NMR Data for Compounds 31 and 32 in Acetone-d_6 (600 MHz)

	31			32		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	79.8	5.55, dd (12.7, 3.1)	C-2'/6', C-1', C-4	78.7	5.54, dd, (12.5, 3.0)	C-2'/6', C-1', C-4
3	46.2	2.71, dd (16.3, 3.0) 2.97, dd (16.3, 12.7)	C-2, C-10, C-1, C-4	45.6	2.70, dd (16.2, 3.1) 2.95, dd (16.2, 12.5)	C-2, C-10, C-1, C-4
4	187.8			186.4		
5	159.4			163.3		
6	94.5	6.11, s	C-8, C-10, C-7, C-9, C-4	87.4	6.21, s	C-8, C-10, C-7, C-9, C-4
7	160.3			166.2		
8	103.4			104.8		
9	163.0			159.1		
10	106.5			105.7		
1'	140.5			139.7		
2'/6´	127.0	7.57, m	C-2, C-2'/6', C-4'	126.2	7.56, m	C-2, C-2'/6', C-4'
3'/5'	129.5	7.45, m	C-3'/5', C-1'	128.5	7.44, m	C-3'/5', C-1'
4'	129.2	7.39, m	C-2'/6'	128.3	7.38, m	C-2'/6'
4"	116.6	6.58, d (10.0)	C-6", C-8, C-9, C-7	31.1	2.90, dd (15.1, 7.9) 3.34, m	C-6", C-8, C-9, C-7
5"	127.3	5.59, d (10.0)	C-7", C-8", C-6", C-8	87.8	5.35, m	C-7", C-8", C-6", C-8
6"	78.5			143.9		
7"	28.3*	1.42, s	C-8", C-6", C-5"	111.2	4.91, s 5.08, s	C-8", C-6", C-5"
8"	28.6*	1.44, s	C-7", C-6", C-5"	16.4	1.76, s	C-7", C-6", C-5"
5-OMe	56.2	3.82, s	C-5	55.5	3.82, s	C-5

*interchangeable

4.2.9: Desmoxyphyllin A (33)

Compound **33** was isolated as a yellow paste. Its molecular formula was deduced as $C_{21}H_{20}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 315.0499 (calcd for $C_{21}H_{21}O_4$, 315.0499)] and ^{13}C NMR data (Table 4.21 and Appendix A33). The presence of a 5-hydroxycoumaronochromone skeleton was evident from the UV (λ_{max} 258, 284 and 336 nm) and NMR data [δ_H 13.00 for 5-OH; δ_C 165.5 (C-2), δ_C 98.7 (C-3) and δ_C 179.6 (C-4)] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra showed a signal for a methoxy group [δ_H 4.00, s, 3H (δ_C 56.9)]. Further, the NMR exhibited a set of *meta*-coupled protons [δ_H 6.39 and 6.62 (1H, d, J = 2.2 Hz)] and two singlet aromatic protons (δ_H 7.20 and 7.51). HMBC correlations of the proton at δ_H 6.39 with C-7, C-8 and C-10 allowed for its placement at C-6 while its coupling partner at C-8. ^3J HMBC correlations of the proton at δ_H 7.20 with C-5' which in turn correlates with the methoxy protons allowed for the placement of this substituent at C-5'. Thus, compound (**33**) was identified as 7,4'-dihydroxy-5'-methoxycoumaronochromone (desmoxyphyllin A) (Mizuno *et al.*, 1992). Desmoxyphyllin A was previously isolated from the leaves of *Desmodium oxyphyllum* (Mizuno *et al.*, 1992). This is its first report in the genus *Tephrosia*.

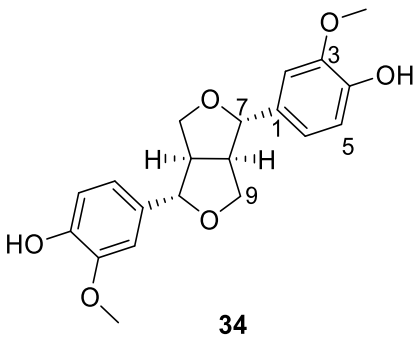


33

4.2.10: Pinoresinol (34)

Compound **34** was isolated as a white paste. Its molecular formula was deduced as $C_{20}H_{22}O_6$ from the HRESIMS [molecular ion $[M_2+H]^+$ at *m/z* 717.2900 (calcd for $C_{20}H_{23}O_6$, 315.0499)for a dimer (Banoub *et al.*, 2015)] and ^{13}C NMR data (Table 4.21 and Appendix A34**)** representing an index of hydrogen deficiency of 10. The NMR showed signals for a methoxy

group [δ_H 3.86, (δ_C 56.4)]. The ^{1}H NMR spectra showed signals for an ABX spin system [δ_H 6.77 (1H, d, *J* = 8.1 Hz), 6.82 (1H, dd, *J* = 8.1, 2.0 Hz) and 6.95 (1H, d, *J* = 1.9 Hz) attributed to a trisubstituted benzene ring]. The NMR further displayed resonance for four mutually coupled aliphatic protons [δ_H 4.77 (1H, d, *J* = 4.3 Hz, δ_C 87.5) for oxymethine, 3.15 (1H, dd, *J* = 6.4, 4.9 Hz, δ_C 55.4) for methine and 4.24 (H, dd, *J* = 9.1, 6.9 Hz)/3.84 (1H, dd, *J* = 8.7, 3.9 Hz, δ_C 72.6) for oxymethylene]. These signals only represent a methoxy, a phenyl and three aliphatic carbons corresponding to $C_{10}H_{11}O_3$ which is half of the molecular formula for this compound. Therefore, compound **34** is a symmetrical dimer consistent with a lignan skeleton. HMBC correlation of the oxymethine proton (δ_H 4.71) with C-6 (δ_C 120.1) and C-2 (δ_C 111.0) indicated that the ABX system is 1,3,4-trisubstituted with oxygenation at C-3 and C-4. HMBC correlations of the protons at δ_H 6.95 (H-1, d, *J* = 1.9 Hz) and 6.77 (H-5, d, *J* = 8.1 Hz) with C-3 (δ_C 147.3) which in turn correlated with methoxy protons allowed for the placement of this substituent at C-3. Thus, compound **34** was identified as pinoresinol (Ouyang *et al.*, 2007). Pinoresinol was previously isolated from the root of *Rhus javanica* (Ouyang *et al.*, 2007) and the leaves of *Calotropis gigantea* (Nguyen *et al.*, 2017). This is its first report in the genus *Tephrosia*.



34

Table 4.21: NMR Data for Compounds 33 and 34 in Acetone-d_6 (600 MHz)

	33			34		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1				133.8		
2	165.5			111.0	6.95, d, (1.9)	C-7, C-6, C-1, C-4
3	98.7			149.1		
4	179.6			147.3		
5	164.0			116.1	6.77, d, (8.1)	C-1, C-3
6	100.7	6.39, d, (2.2)	C-7, C-8, C-10	120.1	6.82, dd, (8.1, 2.0)	C-7, C-2, C-4
7	164.6			87.5	4.71, d, (4.3)	C-8, C-9, C-6, C-1, C-2
8	95.6	6.62, d, (2.2)	C-6, C-10, C-9, C-7	55.4	3.15, dd, (6.4, 4.9)	C-8, C-7, C-1
9	156.1			72.6	4.24, dd, (9.1, 6.9)	C-8, C-7
					3.84, dd, (8.9, 3.9)	
10	104.3					
1'	114.3					
2'	144.9					
3'	99.7	7.20, s	C-1', C-2', C-4', C-5'			
4'	146.9					
5'	147.4					
6'	103.8	7.51, s	C-3, C-2', C-4', C-1'			
3-OMe				56.4	3.86, s	C-3
5'-OMe	56.9	4.00, s	5′			
5-OH		13.00, s				

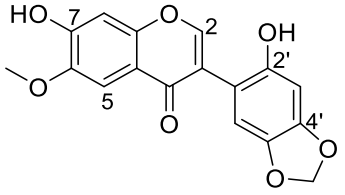
4.3: Characterization of Compounds Isolated from *Tephrosia vogelii*

Chromatographic fractionation of an extract of the seedpods of *Tephrosia vogelii* led to the isolation and characterization of twelve compounds. These compounds were vogelisoflavone A (**35**), vogelisoflavone B (**36**), onogenin (**37**), isopongaflavone (**38**), luteolin (**13**), 4',7-dihydroxy-3'-methoxyflavanone (**39**), *trans-p*-hydroxycinnamic acid (**40**), tephrosin (**23**), 2-methoxygliricidol (**41**), dehydrorotenone (**42**), 6a,12a-dehydro-α-toxicarol (**43**) and pinoresinol (**34**). Compounds **35** and **36** are reported here as new compounds.

4.3.1: Vogelisoflavone A (35)

Compound **35** was obtained as a white paste. The molecular formula of **35** was established as $C_{17}H_{12}O_7$ from HRESIMS [molecular ion peak $[M+H]^+$ at *m/z* 329.0654 (calcd for $C_{17}H_{13}O_7$, 329.0656) and $[M+Na]^+$ at *m/z* 351.0475 (calcd for $C_{17}H_{12}O_7Na$, 351.0475)] and NMR data (Table 4.22 and Appendix A35). The UV (λ_{max} 232, 268 and 312 nm) and NMR data [δ_H 8.37 for H-2; δ_C 156.6 (C-2), 123.9 (C-3) and 178.0 (C-4)] showed characteristic signals for an isoflavone skeleton (Table 4.22 and Appendix A35) (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra displayed signals of a methylenedioxy [δ_H 5.97 (δ_C 102.3)] and a methoxy [δ_H 4.01, (δ_C 56.7)] group. The NMR spectra also showed signals for four singlet protons (δ_H 7.60, 7.07, 6.84 and 6.52). HMBC correlations of the proton at δ_H 7.60 with C-7, C-4 and C-9 allowed for its placement at C-5, whereas correlations of the proton at δ_H 7.07 with C-10, C-6 and C-7 allowed for its placement at C-8. HMBC correlations of the protons at δ_H 6.52 with C-1', C-4', C-5' and C-2' allowed for its placement at C-3', whereas correlations of δ_H 6.84 with C-3, C-4', C-5' and C-2' placed it at C-6'. Placement of the methoxy group at C-6 was based on HMBC correlations of its protons (δ_H 4.01) with C-6. The methylenedioxy protons showed an HMBC correlation with C-4'/C-5' which supported its placement at these carbons. Based on these spectral data, compound **35** was characterized as 2',7-dihydroxy-6-methoxy-4',5'-

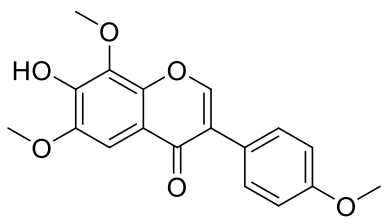
methylenedioxyisoflavone, a new compound that was given the trivial name vogelisoflavone A.



35

4.3.2: Vogelisoflavone B (36)

Compound **36** was obtained as a white paste. The molecular formula of **36** was deduced as $C_{18}H_{16}O_6$ from its HRESIMS [molecular ion peak $[M+H]^+$ at *m/z* 329.1021 (calcd for $C_{18}H_{17}O_6$, 329.1020)] and NMR data (Table 4.22 and Appendix A36). An isoflavone skeleton was evident from the UV (λ_{max} 262 and 312 nm) and NMR [δ_H 8.25 for H-2; δ_C 153.0 (C-2,), 124.3 (C-3) and 175.3 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra showed the presence of three methoxy groups [δ_H 3.84 (δ_C 55.6), 3.96 (δ_C 56.6) and 3.99 (δ_C 61.5)]. Further, the NMR data exhibited signals for an AA'XX' set of aromatic coupled protons [δ_H 7.58 and 6.99 (2H, d, J = 8.7 Hz)] and a singlet aromatic (δ_H 7.36) proton that was assigned to ring B and A, respectively. HMBC correlation of the singlet proton at δ_H 7.36 with C-4, C-7 and C-9 placed this proton at C-5. Thus, compound **36** was characterized as 7-hydroxy-4',6,8-trimethoxyisoflavone and it is a new compound. Vogelisoflavone B has been suggested as its trivial name.



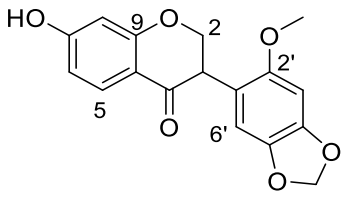
36

Table 4.22: NMR Data for Compounds 35 and 36 in Acetone-d_6 (600 MHz)

	35			36		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	156.6	8.37, s	C-3, C-1', C-9, C-4	153.0	8.25, s	C-3, C-1', C-9, C-4
3	123.9			124.3		
4	178.0			175.3		
5	105.3	7.60, s	C-9, C-4, C-7	100.4	7.36, s	C-9, C-4, C-7
6	148.2			147.9		
7	154.6			146.2		
8	103.5	7.07, s	C-10, C-6, C-7	136.3		
9	153.5			147.2		
10	117.1			117.6		
1'	113.0			125.7		
2'	152.9			131.0	7.58, d (8.7)	C-3, C-6', C-4'
3'	100.9	6.52, s	C-1', C-4', C-5', C-2'	114.4	6.99, d (8.7)	C-5', C-1', C- 4'
4'	150.0			160.4		
5'	142.3			114.4	6.99, d (8.7)	C-3', C-1', C-4'
6'	109.9	6.84, s	C-3, C-4', C-5', C-2'	131.0	7.58, d (8.7)	C-3, C-2', C-4'
6-OMe	56.7	4.01, s	C-6	56.6	3.96, s	C-6
8-OMe				61.5	3.99, s	C-8
4'-OMe				55.6	3.84, s	C-4'
4',5'-OCH_2O	102.3	5.97, s	C-4', C-5'			

4.3.3: Onogenin (37)

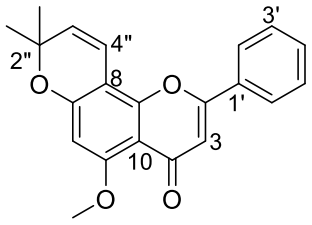
Compound **37** was obtained as an off-white paste. Its molecular formula was deduced as $C_{17}H_{14}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 315.0862 (calcd for $C_{17}H_{14}O_6$, 315.0863)] and NMR data (Table 4.23 and Appendix A37). The presence of an isoflavanone skeleton was evident from UV (λ_{max} 234, 276 and 302 nm) and NMR data [AMX spin system of δ_H 4.55 (dd, *J* = 11.2, 10.7 Hz) for H-2ax, 4.46 (dd, *J* = 10.9, 5.4 Hz) for H-2eq and 4.22 (dd, *J* = 11.6, 5.4 Hz) for H-3; δ_C 61.9 (C-2), 38.3 (C-3) and 190.9 (C-4)]. The NMR spectra showed signals for methylenedioxy [δ_H 5.94 (δ_C 102.2)] and methoxy [δ_H 3.75, (δ_C 47.1)] groups. Further, NMR spectra revealed signals for two aromatic singlets (δ_H 6.72 and 6.67) and an AMX spin system [δ_H 7.77 (d, *J* = 8.6 Hz), 6.59 (dd, *J* = 8.6, 2.3 Hz) and 6.41 (d, *J* = 2.3 Hz)]. HMBC correlations of the proton at δ_H 7.77 with C-4, C-7 and C-9 allowed for its placement at C-5. This placed the singlet aromatic protons in ring B. HMBC correlations of the singlet aromatic proton at δ_H 6.67 with C-3, C-4', C-5' and C-2' allowed for its placement at C-6'. The HMBC correlation of H-6' with C-2' which in turn correlated with the methoxy protons placed the methoxy group at C-2'. HMBC correlations of the methylenedioxy protons with C-4' and C-5' allowed for the placement of the methylenedioxy group at C-4'/C-5'. Based on these spectral data, compound **37** was identified as 7-hydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone (onogenin). Onogenin was previously isolated from the roots of *Ononis spinosa* (Kovalev *et al.*, 1975) and *Ononis arvensis* (Gampe *et al.*, 2016). This is its first report in the genus *Tephrosia*.



37

4.3.4: Isopongaflavone (38)

Compound **38** was isolated as a yellow solid. Its molecular formula was deduced as $C_{21}H_{18}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 335.1279 (calcd for $C_{21}H_{19}O_4$, 335.1278)] and NMR data (Table 4.23 and Appendix A38). The presence of a flavone skeleton was evident from the UV (λ_{max} 272, 300 and 346 nm) and NMR [δ_H 6.70, s for H-2; δ_C 160.2 for C-2, 108.9 for C-3 and 177.7 for C-4] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed signals for a methoxy [δ_H 3.98 (δ_C 56.5)] and a 2,2-dimethylchromene [δ_H 1.53 (6H, s, δ_C 28.2), 5.64 (1H, d, J = 10.0 Hz, δ_C 127.6) and 6.88 (1H, d, J = 10.0 Hz δ_C 115.3)] moiety. Further, the NMR data showed signals for three sets of mutually coupled protons [δ_H 7.88 (m, 2H), 7.53 (m, 2H) and 7.54 (m, 1H)] assigned to an unsubstituted flavone ring B and a singlet aromatic proton δ_H 6.36 assigned to ring A. HMBC correlations of the singlet proton at δ_H 6.36 with C-8, C-5 and C-10 allowed for its placement at C-6. This placed the methoxy group at C-5 and the 2,2-dimethylchromene moiety at C-7/8. The placement of the 2,2-dimethylchromene moiety at C-7/8 was further established from the HMBC correlations of H-4" (δ_H 6.88) with C-8, C-9 and C-7. Based on these spectral data, compound **38** was identified as 5-methoxy-2",2"-dimethylpyrano[5",6":7,8]flavone (isopongaflavone). Isopongaflavone was previously reported from the roots of *Tephrosia elata* (Bentley *et al.*, 1987). This is its first report in this plant.



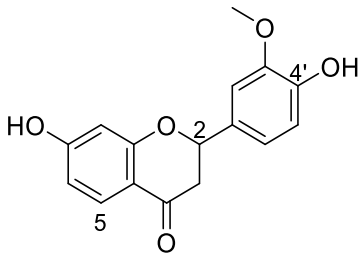
38

Table 4.23: NMR Data for Compounds 37 and 38 in Acetone-d_6 (600 MHz)

	37			38		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	71.7	4.55, dd (11.2, 10.7) 4.46, dd (10.9, 5.4)	C-3, C-4, C-9, C-1'	160.2		
3	48.2	4.22, dd (11.6, 5.4)	C-2, C-4, C-1', C-2', C-6'	108.9	6.70, s	C-2, C-4, C-10, C-1'
4	190.9			177.7		
5	130.0	7.77, d (8.6)	C-4, C-7, C-9	160.7		
6	111.2	6.59, dd (8.6, 2.3)	C-10	96.7	6.36, s	C-5, C-8, C-10
7	165.0			154.0		
8	103.5	6.41, d (2.3)		102.7		
9	164.6			158.0		
10	115.8			108.9		
1'	117.1			131.8		
2'	153.9			125.9	7.88, m	C-2, C-2'/6', C-4'
3'	96.2	6.72, s	C-1', C-2', C-4', C-5'	129.0	7.53, m	C-1', C-3'/5'
4'	142.0			131.2	7.54, m	C-2'/6'
5'	148.6			129.0	7.53, m	C-1', C-3'/5'
6'	110.5	6.67, s	C-3, C-2', C-4', C-5'	125.9	7.88, m	C-2, C-2'/6', C-4'
2"				78.1		
3"				127.6	5.64, d (10.0)	C-8, C-2", C-2"-Me_2
4"				115.3	6.88, d (10.0)	C-7, C-8, C-9, C-3", C-2"
2"-Me_2				28.2	1.53, s	C-2", C-3", C-2"-Me,
2'-OMe	57.1	3.75, s	C-2'			
5-OMe				56.5	3.98, s	C-5
4',5'-OCH_2O	102.2	5.94, s (broad)	C-4', C-5'			

4.3.5: 4',7-Dihydroxy-3'-methoxyflavanone (39)

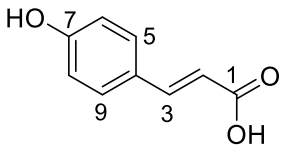
Compound **39** was obtained as an off-white paste. Its molecular formula was established as $C_{16}H_{14}O_5$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 287.0915 (calcd for $C_{16}H_{15}O_4$, 287.0914)] and NMR data (Table 4.24 and Appendix A39). A flavanone skeleton was evident from the UV (λ_{max} 258, 278 and 312 nm) and NMR [AMX spin system of [δ_H 5.44 (1H, dd, *J* = 13.2, 2.9 Hz for H-2), 2.67 (1H, dd, *J* = 16.7, 2.8 Hz for H-3eq), 3.08 (1H, dd, *J* = 16.7, 13.1 Hz for H-3ax) and δ_C 190.5 for C-4] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra exhibited signals for a methoxy group at δ_H 3.89 (δ_C 56.3)]. Further, NMR data showed two sets of ABX spin systems [δ_H 7.73 (1H, d, *J* = 8.6 Hz, δ_C 129.5), 6.58 (1H, dd, *J* = 8.7, 2.3 Hz, δ_C 111.2) and 6.43 (1H, d, *J* = 2.3 Hz, δ_C 103.7)] and [δ_H 6.87 (1H, d, *J* = 8.1 Hz, δ_C 115.7), 7.01 (1H, dd, *J* = 8.1, 2.1 Hz, δ_C 120.5) and 7.20 (1H, d, *J* = 2.0 Hz, δ_C 111.2)]. HMBC correlations of the deshielded proton at δ_H 7.73 with C-4, C-7 and C-9 allowed for its placement at C-5 and its coupling partner protons δ_H 6.58 (dd, *J* = 8.67, 2.3 Hz) and 6.43 (d, *J* = 2.3 Hz) at C-6 and C-8, respectively. HMBC correlations of the protons at δ_H 7.20 (1H, d, *J* = 2.0 Hz) and 7.01 (1H, dd, *J* = 8.1, 2.1 Hz) with C-2 allowed for their placement at C-2' and C-6', respectively, indicating that ring B is substituted at C-3' and C-4'. HMBC correlation of H-5' (δ_H 6.87) with C-3' which in turn correlated with the methoxy protons allowed for the placement of the methoxy group at C-3'. Compound **39** was identified as 4',7-dihydroxy-3'-methoxyflavanone (Recourt *et al.*, 1991). This compound was previously reported in the roots of *Vicia sativa* (Recourt *et al.*, 1991) but this is its first report in the genus *Tephrosia*.



39

4.3.6: Trans-p-hydroxycinnamic Acid (40)

Compound **40** was obtained as an off-white paste. Its molecular formula $C_9H_8O_3$ was deduced from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 165.0544 (calcd for $C_9H_9O_3$, 165.0546) and NMR data (Table 4.24 and Appendix A40). The UV (λ_{max} 230 and 310 nm) and NMR data indicated compound **40** was a hydroxycinnamic acid derivative (Harbaum *et al.*, 2007). The ^1H NMR exhibited *ortho*-coupled aromatic protons [δ_H 7.54 and 6.89 (2H, d, J = 8.6 Hz) assigned to a disubstituted benzene ring]. Further, the NMR data showed signals for *trans*-oriented olefinic protons [δ_H 7.59 and 6.33 (1H, d, J = 16.0 Hz)] and carboxylic carbon at δ_C 168.9. HMBC correlation of one of the *trans*-oriented olefinic proton (δ_H 7.59) with C-2, C-5/9, C-4 and C-1 allowed for its placement at C-3. Based on these spectral data, compound **40** was identified as *trans-p*-hydroxycinnamic acid (Ming *et al.*, 2005). *trans-p*-Hydroxycinnamic acid was previously isolated from *Tephrosia elata* (Atilaw, 2018) but this is its first report in this plant.



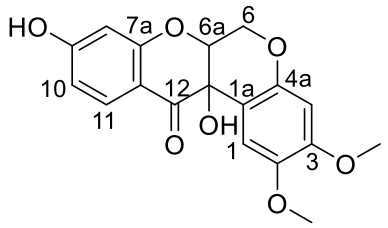
40

Table 4.24: NMR Data for Compounds 39 and 40 in Acetone-d_6 (600 MHz)

	39			40		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1				168.9		
2	80.8	5.44, dd (13.2, 2.9)	C-2', C-6', C-1', C-4	115.9	6.33, d (15.9)	C-4, C-3, C-1
3	44.8	2.67, dd (16.7, 2.8) 3.08, dd (16.7, 13.1)	C-2, C-10, C-1', C-4	145.5	7.59, d (16.0)	C-2, C-5/9, C-4, C-1
4	190.5			127.0		
5	129.5	7.73, d (8.6)	C-9, C-7, C-4	116.7	6.89, d (8.6)	C-4, C-7, C-5/9
6	111.2	6.58, dd (8.7, 2.3)	C-8, C-10	130.8	7.54, d (8.6)	C-6/8, C-7, C-4
7	165.3			160.5		
8	103.7	6.43, d (2.3)	C-6, C-10, C-9, C-7	130.8	7.54, d (8.6)	C-6/8, C-7, C-4
9	164.5			116.7	6.89, d (8.6)	C-4, C-7, C-5/9
10	115.2					
1'	131.8					
2'	111.2	7.20, d (2.0)	C-6', C-2, C-4'			
3'	148.4					
4'	147.8					
5'	115.7	6.87, d (8.1)	C-1', C-3'			
6'	120.5	7.01, dd (8.1, 2.1)	C-2, C-2', C-5', C-4'			
3'-OMe	56.3	3.89, s	C-3'			

*4.3.7: 2-Methoxygliricidol (***41***)*

Compound **41** was isolated as a white paste. The molecular formula was deduced as $C_{18}H_{16}O_7$ from its HRESIMS data $[M-H_2O]^+$ at *m/z* 327.0869 (calcd $C_{18}H_{17}O_7$, 345.0969) and NMR data (Table 4.25 and Appendix A41). A rotenoid skeleton was evident from the UV (λ_{max} 238 and 294 nm) and NMR [δ_H 4.56 (1H, dd, *J* = 12.1, 2.5 Hz)/4.45 (1H, dd, *J* = 12.2, 1.1 Hz) for H-6, δ_H 4.65 (1H, dd, *J* = 2.5, 1.1 Hz) for H-6a indicating that C-12a (δ_C 68.5) is oxygenated] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed that compound **41** was very similar to compound **19**. The only notable difference was that compound **41** has a hydroxy group at C-9 instead of a methoxy group as in **19**. Therefore, compound **41** was identified as 2-methoxygliricidol (Rastrelli *et al.*, 1999). 2-Methoxygliricidol was previously isolated from *Gliricidia sepium* bark by Rastrelli *et al.*, (1999) but this is its first report in the genus *Tephrosia*.



41

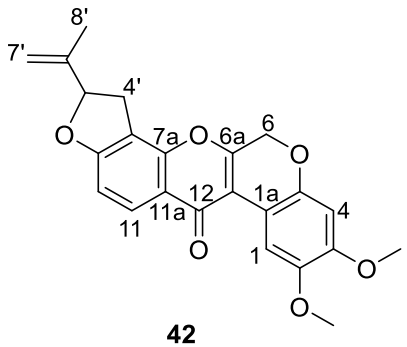
Table 4.25: NMR Data for Compound 41 in Acetone-d_6 (600 MHz)

	41		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	112.4	6.64, s	C-12a, C-1a, C-2, C-3, C-4a
1a	110.1		
2	144.6		
3	152.5		
4	102.0	6.47, s	C-1a, C-2, C-4a, C-3
4a	149.8		
6	64.7	4.56, dd (12.1, 2.5) 4.45, dd (12.2, 1.1)	C-12, C-12a, C-6a, C-4a
6a	77.0	4.65, dd (2.5, 1.1)	C-6a, C-4a, C-12, C-12a
7a	163.2		
8	103.3	6.31, d (2.2)	C-10, C-11a, C-7a
9	166.2		
10	112.0	6.64, dd (8.7, 2.2)	C-8, C-11a
11	130.2	7.75, d (8.7)	C-7a, C-12, C-9
11a	112.2		
12	191.6		
12a	68.5		
2-OMe	56.8	3.60, s	C-2
3-OMe	56.0	3.76, s	C-3

4.3.8: Dehydrorotenone (42)

Compound **42** was isolated as a yellow paste. The molecular formula was deduced as $C_{23}H_{20}O_6$ from its HRESIMS [molecular ion $[M+H]^+$ at *m/z* 393.1330 (calcd $C_{23}H_{21}O_6$, 393.1330)] and NMR data (Table 4.26 and Appendix A42). A 6a,12a-dehydrorotenoid skeleton was evident from the UV (λ_{max} 240, 278 and 310 nm) and NMR data [δ_H 5.00 (2H, s) for H-6; δ_C 64.6 for C-6, 156.2 for C-6a and 111.9 for C-12a] (Agrawal, 1989). The NMR data of **42** displayed signals for two methoxy [δ_H 3.96 (δ_C 56.4) and 3.87 (δ_C 56.1)] and 2-isopropenyltetrahydrofuran [δ_H 3.20 (1H, dd, *J* = 15.7, 8.1 Hz/3.53 (1H, dd, *J* = 15.7, 9.9 Hz) for H-4', 5.41 (1H, dd, *J* = 10.0, 7.7 Hz) for H-5', 1.88 (3H, s, for H-8'), 4.98/5.14 (broad-s) for H-7'] groups. Further, the NMR data showed signals for two singlet aromatic protons [δ_H 8.45 and 6.55 and *ortho*-coupled protons [δ_H 8.13, d, *J* = 8.7 Hz and 6.92, d, *J* = 8.6Hz]. The deshielded aromatic proton at δ_H 8.13 showed HMBC correlations with C-7a, C-12 and C-9 which allowed for its placement at C-11 and its coupling partner at C-10. HMBC correlations

of the aromatic singlet protons at δ_H 8.45 and 6.55 with C-2 and C-3 placed the methoxy groups at C-2 and C-3. The 2-isopropenyltetrahydrofuran moiety was placed at C-8/C-9 because H-4' had HMBC correlations with C-8 (δ_C 113.1), C-7a (δ_C 152.4), and C-9 (δ_C 165.0). Thus, compound **42** was identified as dehydrorotenone (Carlson *et al.*, 1973; Krupadanam *et al.*, 1977b). Dehydrorotenone was previously isolated from the pods of *Tephrosia villosa* (Krupadanam *et al.*, 1977b) and also from the stems and leaves of *Tephrosia candida* (Roy *et al.*, 1986). This is its first report in this plant.

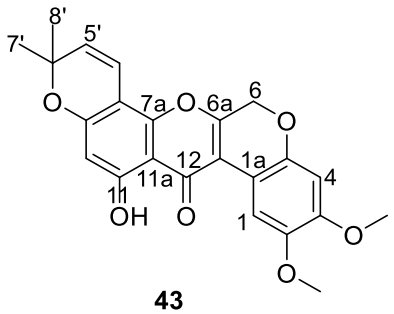


42

4.3.9: 6a,12a-Dehydro-α-toxicarol (43)

Compound **43** was obtained as a yellow solid. The molecular formula was established as $C_{18}H_{16}O_7$ from its NMR data (Table 4.26 and Appendix A43). An 11-hydroxy-6a,12a-dehydrorotenoid skeleton was evident from the NMR data [δ_H 4.99 (3H, s) for H-6 and 13.01 for 11-OH; δ_C 67.0 for C-6, 156.9 for C-6a and 110.8 for C-12a]. The NMR data showed the presence of a 2,2-dimethylchromene moiety and two methoxy groups. Further, the NMR data showed signals for three singlet aromatic protons (δ_H 8.27, 6.56 and 6.29). The singlet aromatic proton at δ_H 6.29 showed HMBC correlations with C-8 and C-11a which allowed for its placement at C-10. HMBC correlations of the aromatic singlet protons at δ_H 8.27 and 6.56 with C-2 and C-3 allowed the placement of the methoxy groups at C-2 and C-3. The 2,2-dimethylchromene moiety was placed at C-8/C-9 because H-4' had HMBC correlations with C-8 (δ_C 101.1), C-7a (δ_C 159.2), and C-9 (δ_C 159.7). Thus, compound **43** was identified as

6a,12a-dehydro-α-toxicarol (Lin and Kuo, 1993). This compound was previously reported from *Derris oblonga* (Lin and Kuo, 1993) and *Amorpha fruticose* (Reisch *et al.*, 1976). This is its first report in this plant.



43

Table 4.26: NMR Data for Compounds 42 and 43 in Acetone-d_6 (600 MHz)

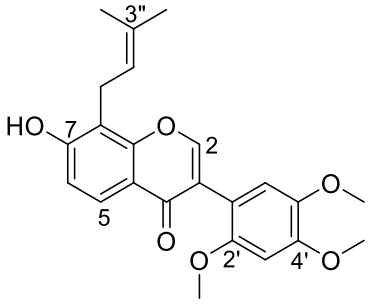
	42			43		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	110.1	8.45, s	C-2, C-3, C-4a, C-12a	110.0	8.27, s	C-2, C-3, C-4a, C-12a
1a	110.7			109.9		
2	144.2			144.3		
3	149.0			149.2		
4	100.5	6.55, s	C-1a, C-2, C-4a	100.7	6.56, s	C-1a, C-2, C-3, C-4a
4a	149.8			149.0		
6	64.6	5.00, s	C-4a, C-6a, C-12a	67.0	4.99, s	C-4a, C-6a, C-12, C-12a
6a	156.2			156.9		
7a	152.4			159.2		
8	113.1			101.1		
9	165.0			159.7		
10	108.9	6.92, d (8.6)	C-8, C-11a	1008	6.29, s	C-8, C-11a
11	128.0	8.13, d (8.7)	C-7a, C-9, C-12	162.5		
11a	119.1			105.4		
12	174.5			172.5		
12a	111.9			110.8		
4'	31.6	3.20, dd (15.7, 8.1) 3.53, dd (15.7, 9.9)	C-7a, C-8, C-9, C-5', C-6'	114.5	6.64, d (10.1)	C-7a, C-8, C-9, C-6'
5'	130.0	5.41, dd (10.0, 7.7)	C-8, C-6', C-7', C-8'	127.9	5.60, d (10.1)	C-8, C-6', C-7', C-8'
6'	142.9			78.3		
7'	113.2	5.14, s (broad) 4.98, s (broad)	C-5', C-6', C-8'	28.3	1.47, s	C-5', C-6', C-8'
8'	17.2	1.80, s	C-5', C-6', C-7'	28.3	1.47, s	C-5', C-6', C-7'
2-OMe	56.4	3.96, s	2	56.5	3.95, s	2
3-OMe	56.1	3.87, s	3	56.1	3.88, s	3
11-OH					13.01, s	

4.4: Characterization of Compounds Isolated from *Tephrosia elata*

Chromatographic fractionation of the crude extract of the stems of *Tephrosia elata* led to isolation of four compounds; elatisoflavone (**44**), barbigerone (**45**), calopogoniumisoflavone B (**46**) and jamaicin (**47**).

*4.4.1: Elatisoflavone (***44***)*

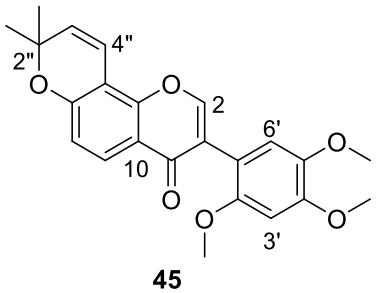
Compound **44** was obtained as a white paste. Its molecular formula was deduced as $C_{23}H_{24}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 397.1642 (calcd for $C_{23}H_{25}O_6$, 397.1646) and NMR data (Table 4.27 and Appendix A44). The isoflavone skeleton was evident from the UV (λ_{max} 238, 264 and 306 nm) and NMR [δ_H 8.12 (1H, s) for H-2; δ_C 154.9 for C-2, 122.3 for C-3 and 175.8 for C-4] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed signals for three methoxy [δ_H 3.95 (δ_C 56.3), δ_H 3.80 (δ_C 57.1) and δ_H 3.87 (δ_C 56.7)] and a 3-methylbut-2-enyl [δ_H 3.57 (2H, d, J = 7.3 Hz) for methylene, 5.25 (1H, t, J = 7.3 Hz) for olefinic methine, 1.67 (3H, s) for methyl and δ_H 1.83 (H, s) for methyl] groups. Further, the NMR data exhibited signals for two singlet aromatic protons [δ_H 6.98 and 6.78] and *ortho*-coupled protons [δ_H 7.89 and 7.06 (1H, d, J = 8.7 Hz)]. HMBC correlations of the deshielded proton at δ_H 7.89 with C-4 (δ_C 175.8), C-7 (δ_C 160.4) and C-9 (δ_C 156.6) allowed for its placement at C-5 and its coupling partner at C-6. HMBC correlations of the singlet protons at δ_H 6.98 (H-3') and 6.78 (H-6') with C-2', C-4' and C-5' placed the three methoxy groups in ring B at C-2', C-4' and C-5'. The placement of the 3-methylbut-2-enyl group at C-8 was consistent with HMBC correlations of H-1" (δ_H 3.57) with C-7, C-8 and C-9. Thus, this compound was identified as 7-hydroxy-2',4',5'-trimethoxy-8-(3-methylbut-2-enyl)isoflavone. It is a new compound for which the trivial name elatisoflavone was suggested.



44

4.4.2: Barbigerone (45)

Compound **45** was isolated as a white paste. Its molecular formula was deduced as $C_{23}H_{22}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 395.1487 (calcd for $C_{23}H_{25}O_6$, 395.1489)] and NMR data (Table 4.27 and Appendix A45). The isoflavone skeleton was evident from the UV (λ_{max} 232, 264 and 294 nm) and NMR [δ_H 8.12 (1H, s) for H-2; δ_C 154.9 for C-2, 122.7 for C-3 and 175.4 for C-4] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of this compound were related to that of **44**. The difference was the presence of a 2,2-dimethylchromene moiety [δ_H 1.50, 6H (δ_C 28.2), 5.92, d, J = 10.0 Hz (δ_C 131.7) and 6.86, d, J = 10.0 Hz (δ_C 115.4)] at C-7/8 in compound **45** instead of the 3-methylbut-2-enyl group in **44**. Compound **45** was, therefore, identified as 2',4',5'-trimethoxy-2",2"-dimethylpyrano(5",6":7,8)isoflavone (barbigerone). Barbigerone was first isolated from *Tephrosia barbigera* (Vilain, 1980) but this is its first report in this plant.



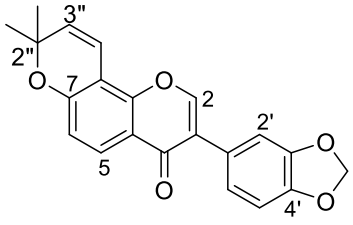
45

Table 4.27: NMR Data for Compounds 44 and 45 in Acetone-d_6 (600 MHz)

	44			45		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	154.9	8.12, s	C-3, C-4, C-9, C-1'	154.9	8.12, s	C-4, C-1', C-9, C-3
3	122.3			122.7		
4	175.8			175.4		
5	125.2	7.89, d (8.7)	C-4, C-7, C-9	127.0	7.94, d (8.8)	C-4, C-7, C-9
6	114.8	7.06, d (8.7)	C-7, C-8, C-10	115.6	6.89, d (8.7)	C-7, C-8, C-10
7	160.4			157.8		
8	116.3			110.3		
9	156.6			153.2		
10	118.7			119.3		
1'	113.8			113.4		
2'	151.1			153.0		
3'	117.7	6.98, s	C-1', C-2', C-4', C-5'	117.7	6.98, s	C-1', C-4', C-5'
4'	144.0			151.3		
5'	153.2			144.0		
6'	99.6	6.78, s	C-3, C-2', C-4', C-5'	99.6	6.79, s	C-2', C-3, C-4'
1"	22.7	3.57, d (7.3)	C-7, C-8, C-9, C-2", C-3"			
2"	122.7	5.29, t (7.3)	C-1", C-4", C-5"	78.5		
3"	132.4			131.7	5.92, d (10.0)	C-8, C-2", C-4"
4"	25.9	1.67, s	C-2", C-3", C-5"	115.4	6.86, d (10.0)	C-7, C-8, C-9, C-2", C-3"
5"	18.0	1.83, s	C-2", C-3", C-4"			
2"-Me_2				28.2	1.50, s	C-2", C-3", C-2"-Me
2'-OMe	56.3	3.95, s	C-2'	56.4	3.88, s	C-2'
4'-OMe	57.1	3.80, s	C-4'	57.1	3.76, s	C-4'
5'-OMe	56.7	3.87, s	C-5'	56.9	3.77, s	C-5'

*4.4.3: Calopogoniumisoflavone B (***46***)*

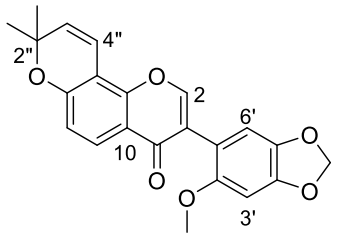
Compound **46** was isolated as a white paste. Its molecular formula was deduced as $C_{21}H_{16}O_5$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 349.1068 (calcd for $C_{21}H_{17}O_5$, 349.1071)] and NMR data (Table 4.28 and Appendix A46). The isoflavone skeleton was evident from the UV (λ_{max} 230, 264 and 294 nm) and NMR [δ_H 8.28 (s, H-2); δ_C 153.6 (C-2), 125.0 (C-3) and 175.5 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of this compound showed the presence of a 2,2-dimethylpyrano ring and methylenedioxy residue. Further, the NMR spectra exhibited signals of *ortho*-coupled protons [δ_H 7.99 and 6.91 (1H, d, J = 8.0 Hz)] and an ABX spin system [δ_H 7.19 (1H, d, J = 1.8 Hz), 7.11, (1H, dd, J = 8.0, 1.8 Hz) and 6.93 (1H, d, J = 8.5 Hz)]. The *ortho*-coupled proton at δ_H 7.99 showed HMBC correlation with C-4, C-7 and C-9 allowing for its placement at C-5 and its coupling partner at C-6. This implied that the ABX spin system was in ring B. HMBC correlations of the protons at δ_H 7.19 (1H, d, J = 1.8 Hz) and 7.11 (1H, dd, J = 8.0, 1.8 Hz) with C-3 allowed for the placement of the methylenedioxy group at C-3'/4'. Placement of the 2,2-dimethylpyrano ring at C-7/8 was supported by the HMBC correlation of H-4" with C-7 and C-9. Thus, compound **46** was identified as 4',5'-methylenedioxy-2",2"-dimethylpyrano(5",6":7,8)isoflavone (calopogoniumisoflavone B) (Rao and Murthy, 1985; Sree and Venkata, 1985). Calopogoniumisoflavone B was previously isolated from the roots of *Tephrosia maxima* by Sree and Venkata, (1985) and grains of *Millettia pachyloba* (Mai *et al.*, 2010). This is its first report in this plant.



46

*4.4.4: Jamaicin (***47***)*

Compound **47** was isolated as a white paste. Its molecular formula was deduced as $C_{22}H_{18}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 379.1177 (calcd for $C_{22}H_{19}O_6$, 379.1177)] and NMR data (Table 4.28 and Appendix A47). The isoflavone skeleton was evident from the UV (λ_{max} 230 and 264) and NMR [δ_H 8.11 (1H, s) for H-2; δ_C 155.0 (C-2), 122.8 (C-3) and 175.3 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The 1D- and 2D-NMR of this compound (Table 4.28) is closely related with that of **45**. The only difference was the presence of the methylenedioxy group at C-4/5 in compound **47** instead of the methoxy groups at the same positions in **45**. Compound **47** was, therefore, identified as 2'-methoxy-4',5'-methylenedioxy-2",2"-dimethylpyrano(5",6":7,8)isoflavone (jamaicin). Jamaicin was previously isolated from the root of the Jamaican Dogwood *Piscidia erythrina* (Falshaw *et al.*, 1966) and also from the grains of *Millettia pachyloba* (Mai *et al.*, 2010). This is its first report in *Tephrosia*.



47

Table 4.28: NMR Data for Compounds 46 and 47 in Acetone-d_6 (600 MHz),

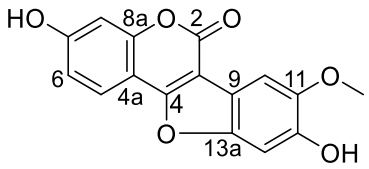
	46			47		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	153.6	8.28, s	C-3, C-4, C-9	155.0	8.11, s	C-3, C-4, C-9, C-1'
3	125.0			122.8		
4	175.5			175.3		
5	127.0	7.99, d (8.0)	C-4, C-7, C-9	127.0	7.93, d (8.7)	C-4, C-7, C-9
6	115.8	6.91, d (8.2)	C-7, C-8, C-10	115.6	6.89, d (8.6)	C-7, C-8, C-10
7	158.0			157.9		
8	110.2			110.3		
9	153.0			153.0		
10				119.3		
1'	119.2			114.1		
2'	110.5	7.19, d (1.8)	C-3, C-3'	154.1		
3'	148.4			112.0	6.85, s	C-1', C-3', C-4', C-5'
4'				141.8		
5'	108.8	6.93, d (8.5)		149.2		
6'	123.3	7.11, dd (8.0, 1.8)	C-2'	96.1	6.77, s	C-3, C-2', C-4', C-5'
2"	78.6			78.5		
3"	131.8	5.94, d (10.0)	C-8, C-2"	131.8	5.92, d (10.0)	C-8, C-2", C-2"-Me
4"	115.3	6.87, d (10.0)	C-7, C-2"	115.4	6.86, d (10.1)	C-7, C-9, C-2"
2"-Me_2	28.2	1.52, s	C-2", C-3", C-2"-Me	28.2	1.50, s	C-2", C-3", C-2"-Me
2'-OMe				57.1	3.73, s	C-2'
OCH_2O	102.1	6.06, s	C-4', C-5'	102.3	5.99, s	C-4', C-5'

4.5: Characterization of Compounds Isolated from *Tephrosia rhodesica*

Chromatographic separation of the crude extract from the stem of *Tephrosia rhodesica* led to the isolation and characterization of eleven compounds; 3-methoxycoumestrol (**48**), glabranin (**49**), 7-*O*-methylglabranin (**50**), liquiritigenin (**51**), naringenin (**52**), rhodiflavan (**53**), 3'-*O*-methylorobol (**54**), genistein (**55**), edunol (**56**), 12a-hydroxyrotenone (**21**) and tephrosin (**23**).

*4.5.1: 3-Methoxycoumestrol (***48***)*

Compound **48** was isolated as a yellow paste. Its molecular formula was deduced as $C_{16}H_{10}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 299.0549 (calcd for $C_{16}H_{11}O_6$, 299.0550)] and NMR data (Table 4.29 and Appendix A48). A coumestan skeleton was evident from the UV (λ_{max} 248, 308 and 348 nm) and NMR [δ_C 158.6 (C-2), 104.0 (C-3) and 156.0 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra exhibited a signal for a methoxy group [δ_H 4.00 (δ_C 56.9)]. Further, the ^{1}H NMR spectra displayed signals for a set of ABX spin system [δ_H 7.89 (1H, d, J = 8.6 Hz), 7.02 (1H, dd, J = 8.6, 2.2 Hz) and 6.95 (1H, d, J = 2.2 Hz)] and two singlet protons (δ_H 7.45 and 7.25). HMBC correlations of the proton at δ_H 7.89 with C-4, C-7 and C-8a allowed for its placement at C-5 and its coupling partners δ_H 7.02 (1H, dd, J = 8.6, 2.2 Hz) and 6.95 (1H, d, J = 2.2 Hz) at C-6 and C-8, respectively. The singlet proton at δ_H 7.45 was placed at C-10 as it had HMBC correlations with C-3, C-11, C-12 and C-13a. ^{3}J HMBC correlations of the proton at δ_H 7.25 with C-11, which in turn correlated with the methoxy protons, placed this substituent at C-11. Based on these spectral data, this compound was identified as 3-methoxycoumestrol. 3-Methoxycoumestrol was previously isolated from alfalfa (*Medicago sativa*) (Bickoff *et al.*, 1966) and the stem of *Vigna angularis* (Guo *et al.*, 2019). This is its first report in this genus.



48

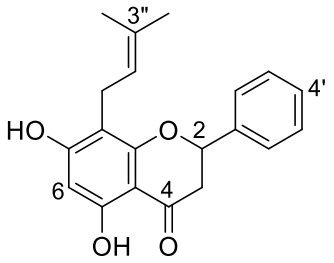
Table 4.29: NMR Data for Compound 48 in Acetone-d_6 (600 MHz),

		48	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	158.6		
3	104.0		
4	156.0		
4a	106.2		
5	123.5	7.89, d (8.6)	C-4, C-7, C-8a
6	114.3	7.02, dd (8.6, 2.2)	
7	160.5		
8	104.1	6.95, d (2.2)	
8a	161.8		
9	115.8		
10	102.8	7.45, s	C-3, C-11, C-12, C-13a
11	147.5		
12	151.1		
13	99.5	7.25, s	C-9, C-11, C-12, C-13a
13a	147.6		
11-OCH_3	56.9	4.00, s	C-11

*4.5.2: Glabranin (***49***)*

Compound **49** was isolated as a white paste. Its molecular formula was deduced as $C_{20}H_{20}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 325.1433 (calcd for $C_{20}H_{20}O_4$, 325.1433)] together with the NMR data (Table 4.30 and Appendix A49). A 5-hydroxyflavanone skeleton was evident from the UV (λ_{max} 238 and 298 nm) and NMR [AMX spin system: δ_H 5.58 (1H, dd, *J* = 12.6, 3.2 Hz for H-2), 2.85 (1H, m for H-3eq), 3.13 (1H, dd, *J* = 17.0, 12.6 Hz for H-3ax) and 12.12 (1H, s for 5-OH); δ_C 79.7 (C-2), 43.6 (C-3) and 197.2 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra exhibited signals for a prenyl group [δ_H 3.25 (2H, d, *J* = 7.2 Hz, δ_C 22.3), 5.21 (1H, t, *J* = 7.3 Hz, δ_C 123.7), 1.63(3H, s, δ_C 25.9) and 1.61 (3H, s, δ_C 17.9)]. Further, the NMR data showed signals for three sets of mutually coupled protons [δ_H 7.59 (2H, m, δ_C 127.1), 7.46 (2H, m, δ_C 129.3) and 7.39 (1H, m, δ_C 129.2)] assigned to

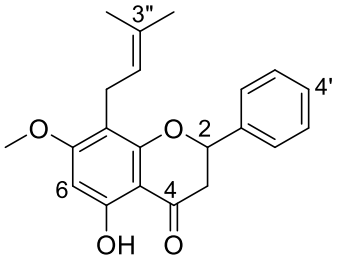
unsubstituted ring B and a singlet proton δ_H 6.05 (1H, s) assigned to ring A. HMBC correlations of the singlet proton at δ_H 6.05 with C-5 and C-10 allowed for its placement at C-6. The placement of the prenyl group at C-8 was based on the HMBC correlations of H-1" with C-7, C-8 and C-9. Thus, this compound was identified as 5,7-dihydroxy-8-(3"-methylbut-2"-enyl)flavanone (glabranin). Glabranin was previously isolated from *Glycyrrhiza glabra* (Yuldashev *et al.*, 2000; Biondi *et al.*, 2003, 2005) and also from *Tephrosia purpurea* (Atilaw *et al.*, 2017b). This is, however, its first report in this plant.



49

*4.5.3: 7-O-Methylglabranin (***50***)*

Compound **50** was isolated as a white paste. Its molecular formula was deduced as $C_{21}H_{24}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 341.1749 (calcd for $C_{21}H_{25}O_4$, 341.1747)] and NMR data (Table 4.30 and Appendix A50). A 5-hydroxyflavanone skeleton was evident from the NMR data [AMX spin system: δ_H 5.41 (1H, dd, J = 12.8, 3.1 Hz for H-2), 2.85 (1H, dd, J = 17.1, 3.1 Hz for H-3eq), 3.05 (1H, dd, J = 17.1, 12.1 Hz for H-3ax); δ_C 78.8 (C-2), δ_C 43.6 (C-3), and δ_C 196.4 (C-4)] (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data of **50** was closely related to that of **49**. The difference was the presence of a methoxy group [δ_H 3.88, 3H (δ_C 56.0)] at C-7 in compound **50** rather than a hydroxy group in **49**. Thus, compound **50** was identified as 7-*O*-methylglabranin (Jayaraman *et al.*, 1980). This compound was previously reported from the roots of *Tephrosia villosa* (Jayaraman *et al.*, 1980) but this is its first report in this plant.



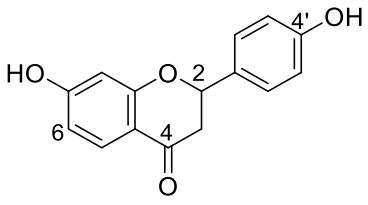
50

Table 4.30: NMR Data for Compounds 49 and 50 in Acetone-d_6 (600 MHz),

	49			50		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	79.7	5.58, dd (12.6, 3.2)	C-4, C-1', C-2'/6'	78.8	5.41, dd (12.8, 3.1)	C-1', C-2'/6', C-4
3	43.6	2.85, m 3.13, dd (17.0, 12.6)	C-2, C-4, C-10	43.6	2.85, dd (17.1, 3.1) 3.05, dd (17.1, 12.1)	C-2, C-4, C-10
4	197.2			196.4		
5	162.7			162.8		
6	96.5	6.05, s	C-5, C-7, C-8, C-10	95.7	6.10, s	C-5, C-7, C-8, C-10
7	165.0			165.9		
8	108.3			109.2		
9	160.9			158.9		
10	103.3			103.1		
1'	140,4			139.1		
2'/6'	127.1	7.59, m	C-2, C-2'/6', C-4'	126.1	7.46, m	C- C-2, 2'/6', C-4'
3'/5'	129.4	7.46, m	C-1', C-3'/5'	128.9	7.39, m	C-1', C-3'/5'
4'	129.3	7.39, m	C-2'/6'	129.3	7.42, m	C-2'/6'
1"	22.3	3.25, d (7.2)	C-8, C-7, C-9, C-2"	21.8	3.24, d (8.2)	C-7, C-8, C-9, C-2"
2"	123.7	5.21, t (7.3)	C-4", C-5"	122.6	5.14, t (7.3)	C-4", C-5"
3"	131.3			131.3		
4"	25.9*	1.63, s	C-2", C-3", C-5"	25.9*	1.67, s	C-2", C-3", C-5"
5"	17.9*	1.61, s	C-2", C-3", C-4"	17.9*	1.62, s	C-2", C-3", C-4"
5-OH		12.12, s			12.13, s	
7-OMe				56.0	3.88, s	C-7
	*Interchangeable			*Interchangeable		

4.5.4: Liquiritigenin (51)

Compound **51** was isolated as an off-white paste. Its molecular formula was deduced as $C_{15}H_{12}O_4$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 257.0807 (calcd for $C_{15}H_{13}O_4$, 257.0808)] and NMR data (Table 4.31 and Appendix A51). A flavanone skeleton was evident from the UV (λ_{max} 234, 276 and 312 nm) and NMR [AMX spin system: δ_H 5.45 (1H, dd, *J* = 13.0, 2.9 Hz for H-2), 2.67 (1H, dd, *J* = 16.7, 2.9 Hz for H-3eq) and 3.04 (1H, dd, *J* = 16.7, 13.0 Hz for H-3ax); δ_C 70.5 (C-2), δ_C 44.7 (C-3), δ_C 190.5 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). Further, the NMR data showed signals for AA'XX' set of coupled aromatic protons [δ_H 7.40 and 6.90 (2H, d, *J* = 8.6 Hz)] assigned to ring B and a set of ABX spin systems [δ_H 7.72 (1H, d, *J* = 8.7 Hz), 6.58 (1H, dd, *J* = 8.6, 2.3 Hz) and 6.42 (1H, d, *J* = 2.2 Hz) assigned to ring A]. Thus, compound **51** was identified as 7,4'-dihydroxyflavanone (liquiritigenin) (Recourt *et al.*, 1991). Liquiritigenin has previously been reported from several plants including *Spatholobus suberectus* (Liu *et al.*, 2019) and *Millettia speciosa* (Fu *et al.*, 2016) but this is its first report in the genus Tephrosia.

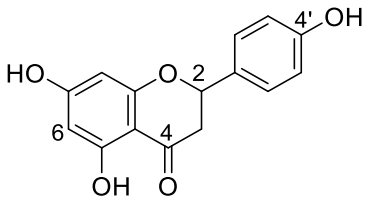


51

4.5.5: Naringenin (52)

Compound **52** was isolated as an off-white paste. Its molecular formula was deduced as $C_{15}H_{12}O_5$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 273.0758 (calcd for $C_{15}H_{12}O_5$, 273.0757)] and NMR data (Table 4.31 and Appendix A52). The presence of a 5-hydroxyflavanone skeleton was evident from the UV (λ_{max} 232 and 288 nm) and NMR [δ_H 5.43 (1H, dd, *J* = 12.9, 3.1 Hz for H-2), 2.71 (1H, dd, *J* = 17.0, 3.1 Hz for H-3eq) and 3.15 (1H,

dd, J = 17.0, 12.9 Hz for H-3ax); δ_C 79.9, (C-2), 43.5 (C-3) and 196.8 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR of this compound showed signals for a set of AA'XX' coupled aromatic protons [δ_H 7.41 and 6.89 (2H, d, J = 8.5 Hz)] assigned to ring B and two *meta*-coupled aromatic protons [δ_H 5.93 and 5.94 (1H, d J = 2.2 Hz)] assigned to ring A. Thus, compound **52** was identified as 5,7,4'-trihydroxyflavanone (naringenin). Naringenin was previously isolated from *Citrus junos* (Heo *et al.*, 2004), *Glycyrrhiza glabra* leaves (Biondi *et al.*, 2005) and the flowers of *Nymphaea mexicana* (Hsu *et al.*, 2013). However, this is its first report in *Tephrosia*.



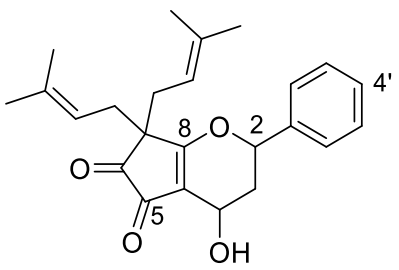
52

Table 4.31: NMR data for Compounds 51 and 52 in Acetone-d_6 (600 MHz),

	51			52		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	70.5	5.45, dd (13.0, 2.9)	C-4, C-1', C-2'/6'	79.9	5.43, dd (12.9, 3.1)	C-1', C-2'/6'
3	44.7	2.67, dd (16.7, 2.9) 3.04, dd (16.7, 13.0)	C-2, C-4, C-10	43.5	2.71, dd (17.0, 3.1) 3.15, dd (17.0, 12.9)	C-2, C-4, C-1'
4	190.5			196.8		
5	129.5	7.72, d (8.7)	C-4, C-7, C-9			
6	111.2	6.58, dd (8.6, 2.3)	C-10	97.0	5.93, d (2.2)	C-7, C-8, C-10
7	164.5			164.3		
8	103.7	6.42, d (2.2)	C-6, C-7	96.1	5.94, d (1.8)	C-6, C-7, C-10
9	165.3			165.3		
10	115.2			102.8		
1'	131.3			130.9		
2'/6'	129.0	7.40, d (8.5)	C-2, C-2'/6', C-4'	129.0	7.41, d (8.5)	C-2, C-2'/6', C-4'
3'/5'	116.1	6.90, d (8.6)	C-1', C-3'/5'	116.1	6.89, d (8.5)	C-1', C-3'/5'
4'	158.6			158.6		C-2'/6'
5-OH					12.20, s	

4.5.6: Rhodiflavan C (53)

Compound **53** was obtained as an oily paste. Its molecular formula was deduced as $C_{24}H_{28}O_4$ from the HRESIMS molecular ion $[M+H]^+$ at *m/z* 381.2060 (calcd for $C_{24}H_{29}O_4$, 381.2060) together with NMR data (Table 4.32 and Appendix A53). The presence of a modified flavan-4-ol skeleton was evident from the UV (λ_{max} 230 and 296 nm) and NMR [δ_H 5.55 (1H, dd, *J* = 12.9, 2.3 Hz for H-2), 2.10 (1H, m for H-3eq) and 2.24 (1H, m for H-3ax), 4.69 (1H, t, *J* = 2.6 Hz for H-4); δ_C 79.3 (C-2), 38.8 (C-3), 55.8 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data revealed the presence of two prenyl groups (Table 4.32). Further, the NMR data revealed three sets of mutually coupled protons [δ_H 7.54 (2H, m, δ_C 127.5), 7.50 (2H, m, δ_C 129.7) and 7.44 (1H, m, δ_C 129.6] assigned to an unsubstituted ring B, two carbonyl carbons (δ_C 184.3 and 204.6) and a quaternary carbon (δ_C 53.1). Since ring B and C were completely assigned, the prenyl groups, the carbonyl carbons and the quaternary carbon could only be in ring A which is a fully substituted five-membered non-aromatic ring with two carbonyl carbons (Atilaw *et al.*, 2020). This was supported by the HMBC correlations of H-4 (δ_H 4.69) with C-2 (δ_C 79.3), C-8 (δ_C 187.1) and C-9 (δ_C 127.4). The prenyl methylene protons at δ_H 2.45 (H-1" and H-1"') of both prenyl groups showed HMBC correlations with C-6 (δ_C 204.6), C-7 (δ_C 53.1) and C-8 (δ_C 187.1) placed the two prenyl groups at C-7. Thus, compound **53** was identified as rhodiflavan C (Atilaw *et al.*, 2020). Rhodiflavan C was previously reported from the roots of this plant (Atilaw *et al.*, 2020) but this is its first report from the stem.



53

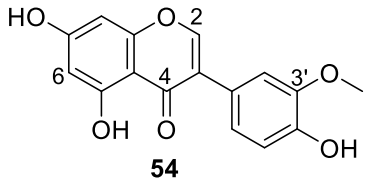
Table 4.32: NMR Data for Compound 53 in Acetone-d_6 (600 MHz)

		53	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	79.3	5.55, dd (12.4, 2.3)	C-3, C-4, C-1', C-2'/6'
3	38.8	2.10, m	C-2, C-4, C-9, C-1'
		2.24, m	
4	55.8	4.69, t (2.6)	C-2, C-8, C-9
5	184.3		
6	204.6		
7	53.1		
8	187.1		
9	127.4		
1'	139.8		
2'/6'	127.5	7.54, m	C-2, C-2'/6', C-4'
3'/5'	129.7	7.50, m	C-3'/5', C-1'
4'	129.6	7.44, m	C-2'/6'
1"	33.3	2.45, m	C-6, C-7, C-8, C-2", C-3", C-1"'
2"	118.4	4.95, t (7.8)	C-6, C-1", C-4", C-5"
3"	136.5		
4"	25.9	1.61, s	C-2", C-3", C-5"
5"	17.8	1.57, s	C-2", C-3", C-4"
1"'	33.8	2.45, m	C-6, C-7, C-8, C-1", C-2"', C-3"'
2"'	118.4	4.95, t (7.8)	C-7, C-1"', C-4"', C-5"'
3"'	136.5		
4"'	25.9	1.61, s	C-2"', C-3"', C-5"'
5"'	17.9	1.57, s	C-2", C-3", C-4"

*4.5.7: 3'-O-Methylorobol (***54***)*

Compound **54** was isolated as a white paste. Its molecular formula was deduced as $C_{16}H_{12}O_6$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 301.0706 (calcd for $C_{16}H_{13}O_6$, 301.0707)] and NMR data (Table 4.33 and Appendix A54). A 5-hydroxyisoflavone skeleton was evident from the UV (λ_{max} 228 and 262) and NMR [δ_H 8.20 (1H, s for H-2) and 13.04 (1H, s for 5-OH); δ_C 154.5 (C-2), 124.1 (C-3) and 181.6 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR spectra showed signals for a methoxy group [δ_H 3.88 (δ_C 56.4)]. Further, the ^{1}H NMR showed signals for *meta*-coupled aromatic protons [δ_H 6.29 and 6.42 (1H, d, *J* = 2.0 Hz)] and an ABX spin system [δ_H 7.25 (1H, d, *J* = 2.0 Hz), 7.07 (1H, dd, *J* = 8.1, 2.0 Hz) and 6.89 (1H, d, *J* = 8.1 Hz)]. Two of the ABX system protons (δ_H 7.25 and 7.07) showed HMBC correlation with C-3 (δ_C 124.1) and C-4' (δ_C 147.7) placing these protons at C-2' and C-6', respectively, and their coupling partner (δ_H 6.89) at C-5'. C-3' showed HMBC correlation with H-5' and methoxy

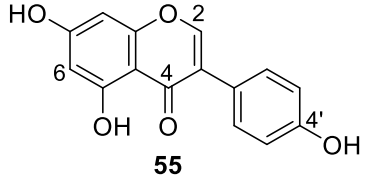
protons allowing for the placement of the methoxy substituent at C-3' (δ_C 148.0). Based on these spectral data, compound **54** was identified as 5,7,4'-trihydroxy-3'-methoxyisoflavone (3'-*O*-methylorobol). 3'-*O*-Methylorobol was previously reported from *Thermopsis montuna* (Dement and Mabry, 1972). This is its first report in the genus Tephrosia.



54

*4.5.8: Genistein (***55***)*

Compound **55** was isolated as a white paste. Its molecular formula was deduced as $C_{15}H_{10}O_5$ from the HRESIMS [molecular ion $[M+H]^+$ at *m/z* 271.0600 (calcd for $C_{15}H_{10}O_5$, 271.0601)] and NMR data (Table 4.33 and Appendix A55). A 5-hydroxyisoflavone skeleton was evident from the UV (λ_{max} 218 and 262) and NMR [δ_H 8.11 (1H, s for H-2) and 13.01 (1H, s for 5-OH); δ_C 153.9 (C-2), 123.9 (C-3) and 181.4 (C-4)] data (Mabry *et al.*, 1970; Agrawal, 1989; Wang *et al.*, 2007). Further, the NMR exhibited signals for an AA'XX' spin system [(δ_H 6. 74 and 6.90 (2H, d, *J* = 8.6 Hz] assigned to ring B and *meta*-coupled protons at δ_H 6.26 and 6.38 (1H, d, *J* = 1.8 Hz) assigned to ring A. Thus, compound **55** was identified as 5,7,4'-trihydroxyisoflavone (genistein). Genistein has previously been isolated in many plant species including *Ginkgo biloba* (Wang *et al.*, 2007), *Hericium erinaceum* mycelium (He *et al.*, 2018) and *Tephrosia purpurea* (Atilaw *et al.*, 2017b). This is the first report of genistein in this plant.



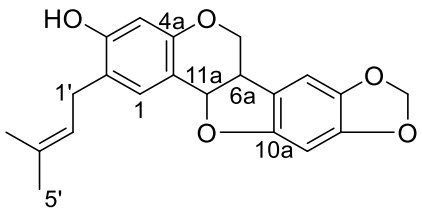
55

Table 4.33: NMR Data for Compounds 54 and 55 in Acetone-d_6 (600 MHz)

	54			55		
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2	154.5	8.20, s	C-3, C-4, C-9	153.9	8.11, s	C-3, C-4, C-9
3	124.1			123.9		
4	181.6			181.4		
5	163.9			163.6		
6	99.9	6.29, d (2.0)	C-7, C-8, C-10	100.3	6.26, d (1.8)	C-8, C-10
7	165.3			163.8		
8	94.5	6.42, d (2.1)	C-6, C-7, C-9, C-10	94.8	6.38, d (1.9)	C-6, C-9, C-10
9	159.0			159.2		
10	106.1			105,4		
1'	123.4			123.2		
2'	113.7	7.25, d (2.0)	C-3, C-1', C-4', C-3', C-6'	131.2	7.45, d (8.6)	C-3, C-2'/6', C-4'
3'	148.0			115.9	6.90, d (8.6)	C-1', C-3'/5'
4'	147.7			158.3		
5'	115.7	6.89, d (8.1)	C-1', C-3', C-4'	115.9	6.90, d (8.6)	C-1', C-3'/5'
6'	122.8	7.07, dd (8.2, 2.0)	C-3, C-2', C-4'	131.2	7.45, d (8.6)	C-3, C-2'/6', C-4'
5-OH		13.04, s			13.01, s	
3'-OMe	56.4	3.88, s	C-2'			

*4.5.9: Edunol (***56***)*

Compound **56** was isolated as a white paste. Its molecular formula was deduced as $C_{21}H_{20}O_5$ from its HRESIMS [molecular ion peak $[M+H]^+$ at *m/z* 353.1382 (calcd for $C_{21}H_{21}O_5$, 353.1382)] and NMR data (Table 4.34 and Appendix A56). A pterocarpan skeleton was evident from the UV (λ_{max} 234 and 310) and NMR [δ_H 3.55 (1H, m)/4.24 (1H, dd, *J* = 10.0, 4.0 Hz) for H-6, 3.52 (1H, m for H-6a) and 5.46 (1H, d, *J* = 6.7 Hz for H-11a); δ_C 67.0 (C-6), δ_C 41.1 (C-6a) and δ_C 79.5; (C-11a)] data (Mabry *et al.*, 1970; Agrawal, 1989). The NMR data showed the presence of a prenyl [δ_H 3.29 (2H, d, *J* = 7.4 Hz, δ_C 28.4); 5.35 (1H, t, *J* = 7.4 Hz, δ_C 123.9), 1.73 (3H, s, δ_C 25.9) and 1.73 (3H, s, δ_C 17.8] and a methylenedioxy (δ_H 5.91, δ_C 102.1) substituent. Further, the NMR data showed signals for four aromatic singlets (δ_H 7.16, 6.88, 6.40 and 6.39). HMBC correlations of the singlet proton at δ_H 7.16 (H-1) with C-1', C-11a, C-11b, C-4a, C-3 and C-2 allowed for its placement at C-1, whereas the proton at δ_H 6.39 showed correlations with C-11b, C-3, C-2 and C-4a allowing for its placement at C-4. HMBC correlation of the protons at δ_H 6.88 and 6.40 with C-8 and C-9 placed the methylenedioxy group at C-8/9. Placement of the prenyl to C-2 was based on the HMBC correlations of H-1' (δ_H 3.29) with C-1 and C-3. Based on these spectral data, this compound was identified as 3-hydroxy-2-prenyl-8,9-methylenedioxypterocarpan (edunol). Edunol was previously reported from *Brongniartia podalyrioides* and *Tephrosia purpurea* (Reyes-Chilpa *et al.*, 1994; Li *et al.*, 2011). This is its first report in this plant.



56

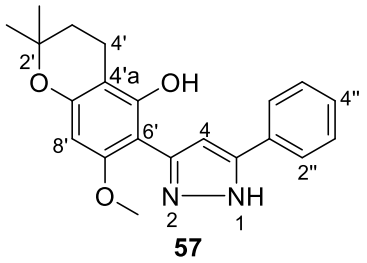
Table 4.34: NMR Data for Compound 57 in Acetone-d_6 (600 MHz)

		56	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
1	132.4	7.16, s	C-2, C-3, C-4a, C-11a, C-11b, C-1'
2	112.5		
3	157.0		
4	103.5	6.39, s	C-2, C-3, C-4a, C-11b
4a	155.6		
6	67.0	3.55, m 4.24, dd (10.0, 4.0)	C-4a, C-6a, C-6b, C-11a
6a	41.1	3.52, m	C-6, C-6b, C-10a
6b	119.6		
7	105.9	6.88, s	C-6a, C-8, C-9, C-10, C-10a
8	142.4		
9	148.9		
10	94.0	6.40, s	C-6b, C-8, C-9, C-10a
10a	155.3		
11a	79.5	5.46, d (6.7)	C-1, C-4a, C-6a, C-10a, C-11b
11b	103.5		
1'	28.4	3.29, d (7.4)	C-1, C-2'
2'	123.9	5.35, t (7.4)	C-4', C-5'
3'	132.3		
4'	25.9	1.73, s	C-2', C-3', C-5'
5'	17.8	1.73, s	C-2', C-3', C-4',
OCH_2O	102.1	5.91, s	C-8, C-9

4.6: Synthesis of Pyrazoisopongaflavone (57)

Pyrazoisopongaflavone (**57**), a pyrazole derivative of isopongaflavone (**38**), was prepared by using hydrazine monohydrate. It was obtained as an off-white paste. Its molecular formula $C_{21}H_{22}O_3N_2$ was established from the HRESIMS molecular ion peak $[M+H]^+$ at *m/z* 351.1696 (calcd for $C_{21}H_{23}O_3N_2$, 351.1703) and $[M+Na]^+$ at *m/z* 373.1516 (calcd for $C_{21}H_{22}O_3N_2Na$ 373.1523). The NMR data confirmed the presence of a methoxy group at δ_H 3.91 (δ_C 55.9), an unsubstituted benzene ring [δ_H 7.85 (126.6 for C-2"/6"), 7.50 (130.1 for C-3"/5") and 7.41 (129.5 for C-4")] and 2',2'-dimethylchromane ring [δ_H 1.80 (δ_C 33.2 for C-3'), 2.60 (δ_C 17.7 for C-4'), 1.32 (δ_C 27.1 for C-2'-Me_2) and 6.02 (δ_C 92.3 for C-8'); δ_C 75.0 (C-2'), 102.4 (C-4'a), 157.4 (C-5'), 99.8 (C-6'), 158.3 (C-7') and 155.7 (C-8'a)] (Table 4.35 and Appendix A57). The pyrazole ring was evident from the ^{13}C NMR signals at δ_C 151.6 (C-3), 104.2 (C-4) and 143.4

(C-5) (He *et al.*, 2009). Based on these spectroscopic characteristics, compound **57** was elucidated as 3-(5-hydroxy-7-methoxy-2,2-dimethylchromanyl)-5-phenyl-pyrazole.



57

Table 4.35: NMR Data for Compound 57 in Acetone-d_6 (600 MHz)

		57	
Position	**δ_C**	**δ_H, mult. (*J* in Hz)**	**HMBC**
2			
3	151.6		
4	104.2	7.34, s	C-3, C-5
5	143.4		
2'	75.0		
3'	33.2	1.80, t (6.8, 6.8)	C-2', C-4', C-4'a, C-2'-Me_2
4'	17.7	2.64, t (6.8, 6.8)	C-2', C-3', C-4'a, C-5, C-8'a
4'a	102.4		
5'	157.4		
6'	99.8		
7'	158.3		
8'	92.3	6.02, s	
8'a	155.7		
1"	130.5		
2"/6"	126.6	7.85, m	C-2"/6", C-4"
3"/5"	130.1	7.50, m	C-1", C-3"/5"
4"	129.5	7.41, m	C-2"/6"
2'-Me_2	27.1	1.32, s	C-2', C-3', C-2'-Me
7'-OMe	55.9	3.91, s	C-7

4.7: Anti-inflammatory Activity

An assay was conducted to assess the anti-inflammatory activities of the crude extracts as well as the compounds isolated from the aerial parts of *T. linearis,* aerial parts of *T. hildebrandtii,* seedpods of *T. vogelii*, *the* stem of *T. elata* and stem of *T. rhodesica*. Further, the synergistic anti-inflammatory activities of the different classes of flavonoids were also investigated. This assay was done by measuring the levels of cytokines (IL-1β, IL-2, IFN-γ, GM-CSF and TNF-α) released from LPS-stimulated PBMCs. The tests relied on the evidence that during inflammation, cytokines are secreted from the PBMCs as part of the inflammatory reaction (Davis *et al.*, 2010). This can be assessed by incubating PBMCs with bacterial LPS (O'Bryan *et al.*, 2000). Therefore, the cytokine release from LPS stimulated PBMC was quantified with or without tested compounds in the supernatant by Luminex. The results of the assays are discussed below.

4.7.1: Activity of Compounds Isolated from Tephrosia linearis

Figure 4.3 shows the results for controls and it is clear that in the presence of LPS, the PBMCs were induced to secrete the cytokines as compared to the medium., It also observed that ibuprofen, the standard drug suppressed the release of the cytokines to 12.2–80.3% of the LPS control except for IL-1β secretion which was increased to 199.3**% (Table 4.36**).

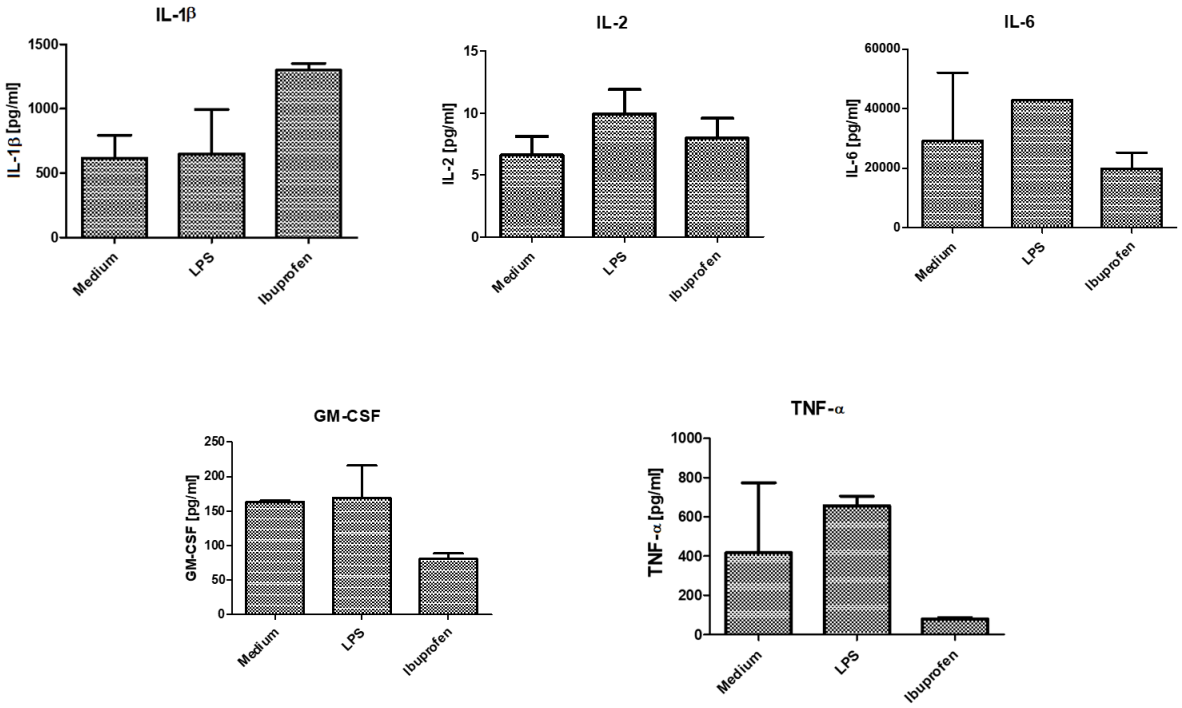


Figure 4.3: The LPS stimulated the secretion of the cytokines in comparison to untreated control cells and ibuprofen (mean ± SD, n=3 for medium and n = 4 for LPS and ibuprofen)[1]

As shown in (Figure 4.4 and Table 4.36), the crude extract reduced the production of all cytokines except IL-1β that slightly increased to about 107.67% in comparison to the LPS control. All the compounds evaluated resulted in decreased release of IL-2, GM-CSF and TNF-α. Compounds **1**, **2**, **4**, **7**, **10**, **13**, **15** and **16** decreased the production of IL-1β with a strong inhibitory effect occurring in the presence of compound **13**. Except for compounds **3** and **11**, which provoked the production of IL-6, the rest of the compounds reduced their production.

[1] The data used to generate the graphs is presented in Appendix B1, Table B1.

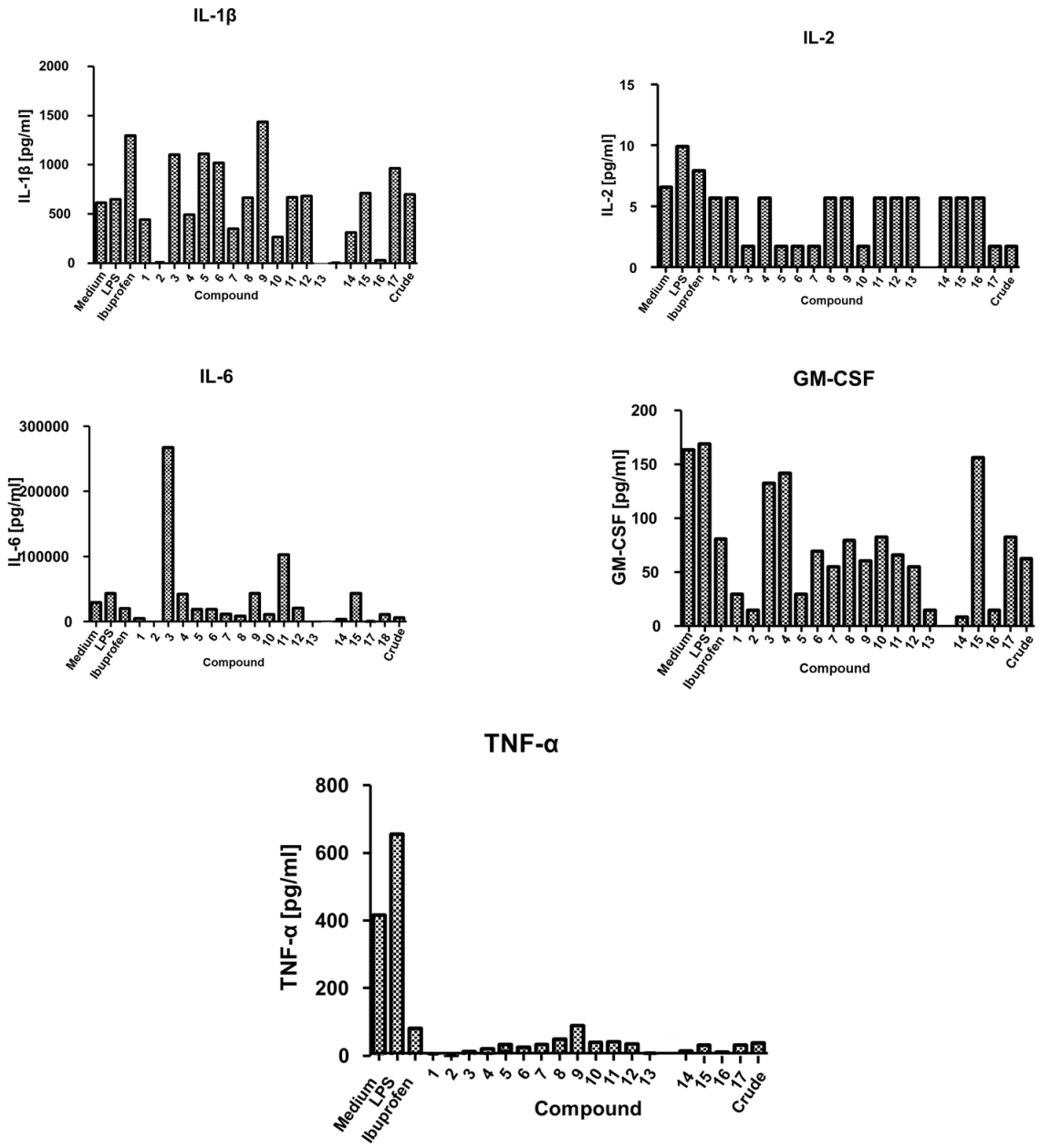


Figure 4.4: The cytokine release after LPS stimulated PMBCs incubated with the crude extracts and isolated compounds from *Tephrosia linearis*[2]

[2] The data used to generate the graphs are presented in Appendix B1, Table B2

Table 4.36: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with Compounds from *Tephrosia linearis* [3]

	Percentage cytokine Release				
Samples	**IL-1β**	**IL-2**	**IL-6**	**GM-CSF**	**TNF-α**
Ibuprofen	199.29	80.33	46.46	47.76	12.23
Crude Extract	107.67	17.72	13.91	36.83	4.61
1	68.27	57.60	10.89	17.39	0.92
2	1.18	57.60	0.00	8.63	0.17
3	169.84	17.72	622.32	78.39	1.89
4	75.85	57.60	97.43	83.73	3.15
5	170.51	17.72	43.11	17.39	4.03
6	157.09	17.72	43.39	40.97	2.66
7	53.77	17.72	25.79	32.47	4.03
8	102.34	57.64	19.57	46.89	6.38
9	220.43	57.60	100.00	35.76	12.55
10	40.83	17.72	24.72	48.79	5.01
11	103.28	57.64	238.54	38.92	5.20
12	105.17	57.60	48.33	32.47	4.22
13	0.67	57.60	0.04	8.63	0.17
14	0.80	57.60	0.09	8.63	0.92
15	48.35	57.64	7.23	4.87	1.12
16	109.22	57.64	100.00	92.58	3.64
17	4.81	57.60	0.64	8.63	0.54
18	148.44	17.72	24.94	48.79	3.83

4.7.2: Activity of Compounds Isolated from Tephrosia hildebrandtii

The anti-inflammatory activities of the crude extract of *T. hildebrandtii*, as well as the isolated compounds (**25**-**32**), were assessed for their anti-inflammatory activities. The crude extract decreased the production of IFN-γ, GM-CSF, and TNF-α. However, the crude extract did not affect IL-6 but provoked the production of IL-1β and IL-2 to 139 % and 106%, respectively, as compared to the LPS control (Table 4.37 and Figure 4.5). Almost, all the compounds showed superior suppression of the cytokines compared to ibuprofen. The strongest inhibition for IL-6 release was observed for compound **27**, an isoflavone that attenuated the production to 0.6% compared to LPS. Similarly, compound **27** attenuated the production of TNF-α to 0.4% compared to LPS. The pterocarpans (compounds **29** and **30**) exhibited the highest reduction in the production of IFN-γ to 2.7% and GM-CSF to 11.0%, respectively, as compared to LPS.

[3] The data used for calculation of the percentage cytokine release is presented in the Appendix B1, Table B2

While compounds **25**, **27**, **28**, **30** and **32** suppressed the production of IL-1β, compounds **26**, **29** and **31** stimulated its production. The strongest inhibition was exhibited by compound **30** which decreased IL-1β production to 20.2% in comparison to LPS control.

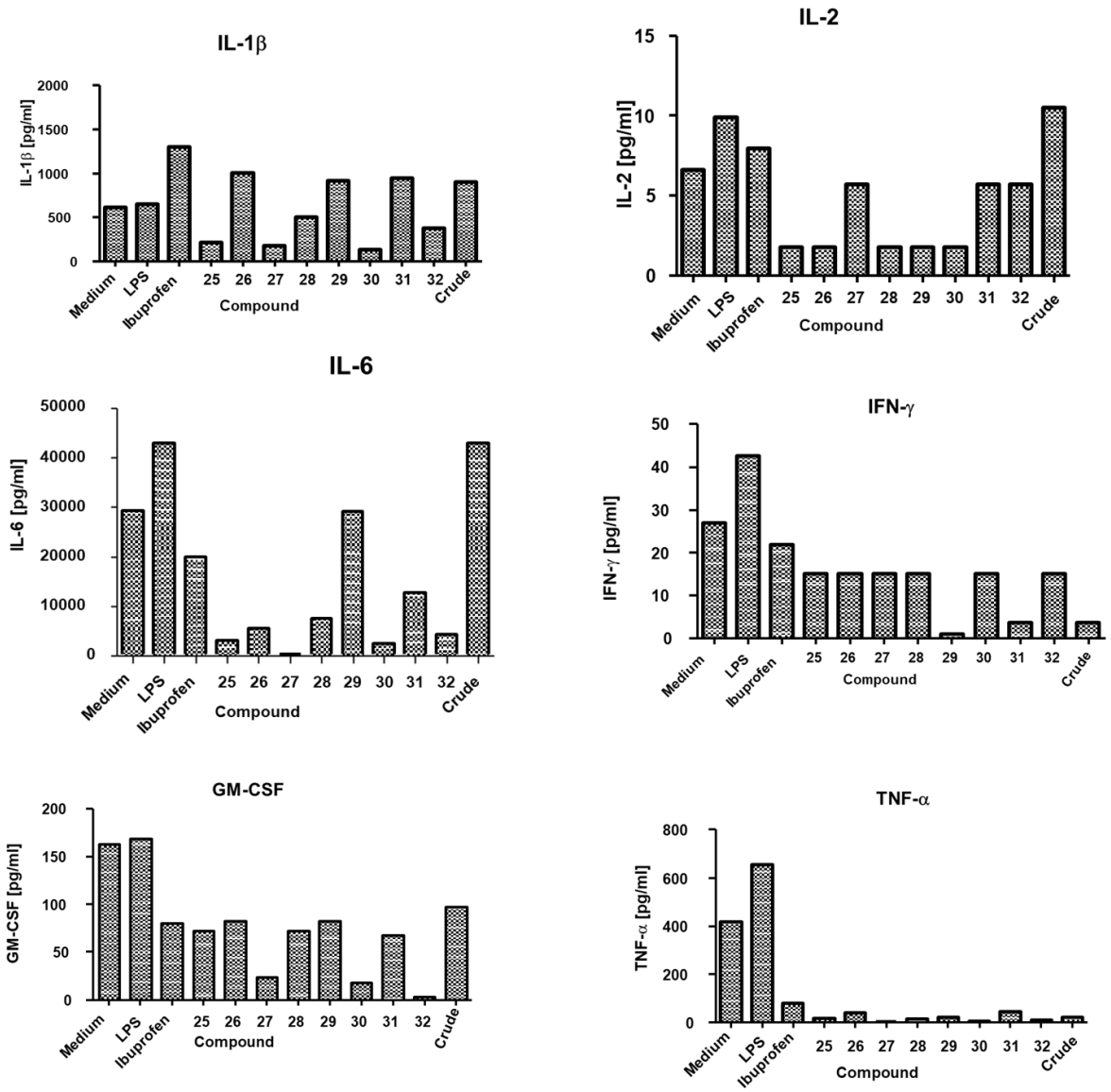


Figure 4.5: The cytokine release after LPS stimulated PMBCs incubated with the crude extracts and isolated compounds from *Tephrosia hildebrandtii*[4]

[4] The data used to generate the graphs are presented in Appendix B1, Table B2

Table 4.37: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with Compounds from *Tephrosia hildebrandtii*[5]

	Percentage Cytokine Release					
Sample	**IL-1ß**	**IL-2**	**IL-6**	**IFN-γ**	**GM-CSF**	**TNF-α**
Ibuprofen	199.3	80.3	46.5	51.2	47.8	12.2
THC	139.0	106.1	100.0	8.6	57.9	3.3
25	32.5	17.7	7.4	35.5	43.0	2.9
26	153.9	17.7	13.0	35.5	48.8	6.4
27	27.1	57.6	0.6	35.5	14.4	0.4
28	77.0	17.7	17.5	35.5	43.0	2.3
29	140.3	17.7	67.8	2.7	48.8	3.1
30	20.2	17.7	5.8	35.5	11.0	1.1
31	146.1	57.6	29.4	8.6	40.0	6.6
32	57.6	57.6	10.0	35.5	1.9	1.5

4.7.3: Activity of Compounds Isolated from Tephrosia vogelii

The crude extract, as well as three compounds (35, 36 and 38) obtained from T. vogelii seedpods, were assessed for their anti-inflammatory activities. As shown in Figure 4.6 and Table 4.36, all the tested compounds and the crude extract showed a decreased IL-1β release between 57.9 and 75.2% compared to the LPS control. In comparison, ibuprofen resulted in a decreased IL-1β release of 25.5% compared to the LPS control. IFN-γ release was decreased between 0.5% and 10.1% when treated with the compounds as well as the crude extract. Likewise, ibuprofen led to a decreased IFN-γ production of 16.8%. All the tested compounds and crude extract were able to decrease the GM-CSF release from the PBMCs. In comparison, the GM-CSF release was reduced to 24.2% in the presence of ibuprofen. The suppression of the release of TNF-α by all the compounds and the crude was more significant than that of ibuprofen.

[5] The data used for calculation of the percentage cytokine release is presented in the Appendix B1, Table B2

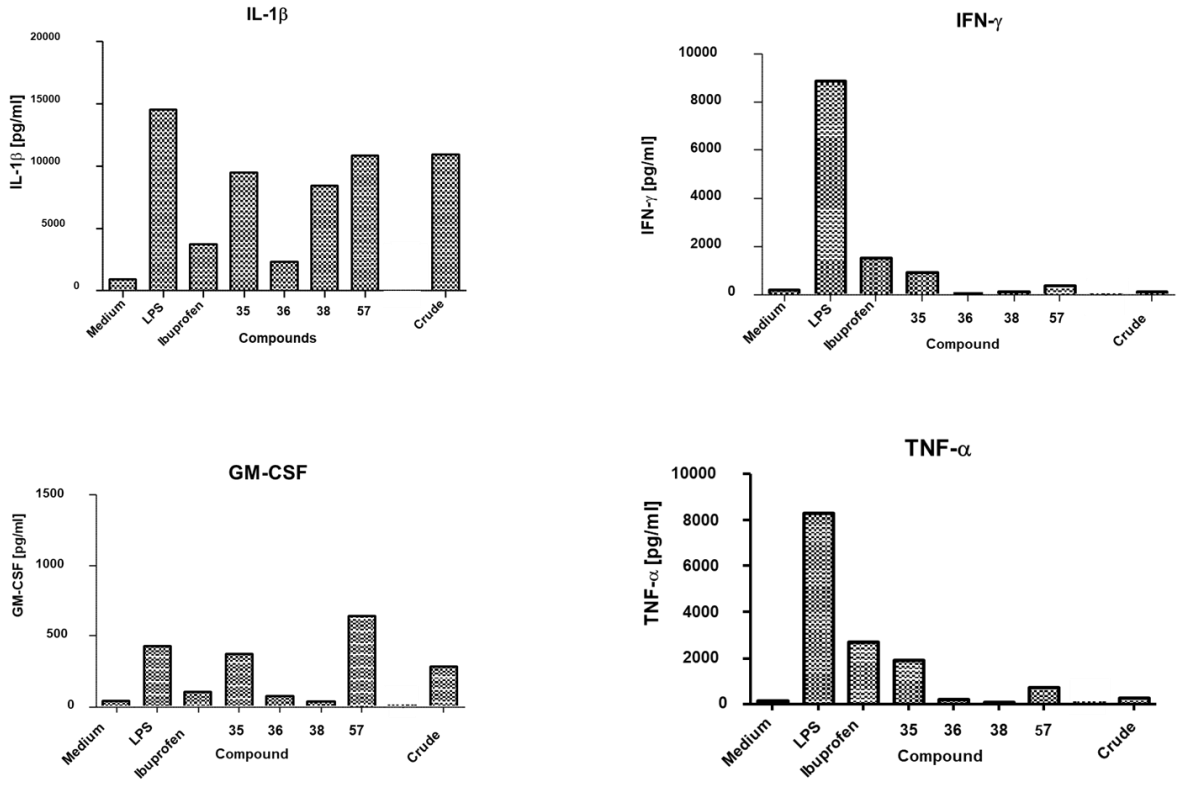


Figure 4.6: The cytokine release after LPS stimulated PMBCs incubated with the crude extracts and isolated compounds from *Tephrosia vogelii*[6]

Table 4.38: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with Compounds from *Tephrosia vogelii*[7]

	Percentage Cytokine Release			
Sample	**IL-1β (%)**	**IFN-γ (%)**	**GM-CSF (%)**	**TNF-α (%)**
Ibuprofen	25.5	16.8	24.2	32.2
35	65.1	10.1	86.7	22.7
36	16.0	0.5	17.6	2.2
38	57.9	1.4	7.1	0.7
57	74.6	3.9	148.8	8.5
Crude extract	75.2	1.1	66.1	3.2

4.7.4: Activity of Compounds Isolated from Tephrosia elata

All the compounds (**44**-**47**) tested decreased the production of IL-β, IFN-γ, GM-CSF, and TNF-α though, the activity of ibuprofen was better than most of the compounds. Compound **45**

[6] The data used to generate the graphs are presented in Appendix B1, Table B3 and B4

[7] The data used for calculation of the percentage cytokine release is presented in the Appendix B1, Table B3 and B4

exhibited relatively strong inhibition by decreasing the production of IFN-γ to 0.3 and TNF-α to 3.4% compared to LPS.

Table 4.39: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with Compounds from *Tephrosia elata*[8]

	Percentage Cytokine Release			
Sample	**IL-1β**	**IFN-γ**	**GM-CSF**	**TNF-α**
44	65.6	11.9	59.2	28.5
45	30.0	0.3	29.3	3.4
46	46.9	3.5	49.7	16.6
47	91.2	49.2	43.9	56.7
Ibuprofen	25.5	16.8	24.2	32.2

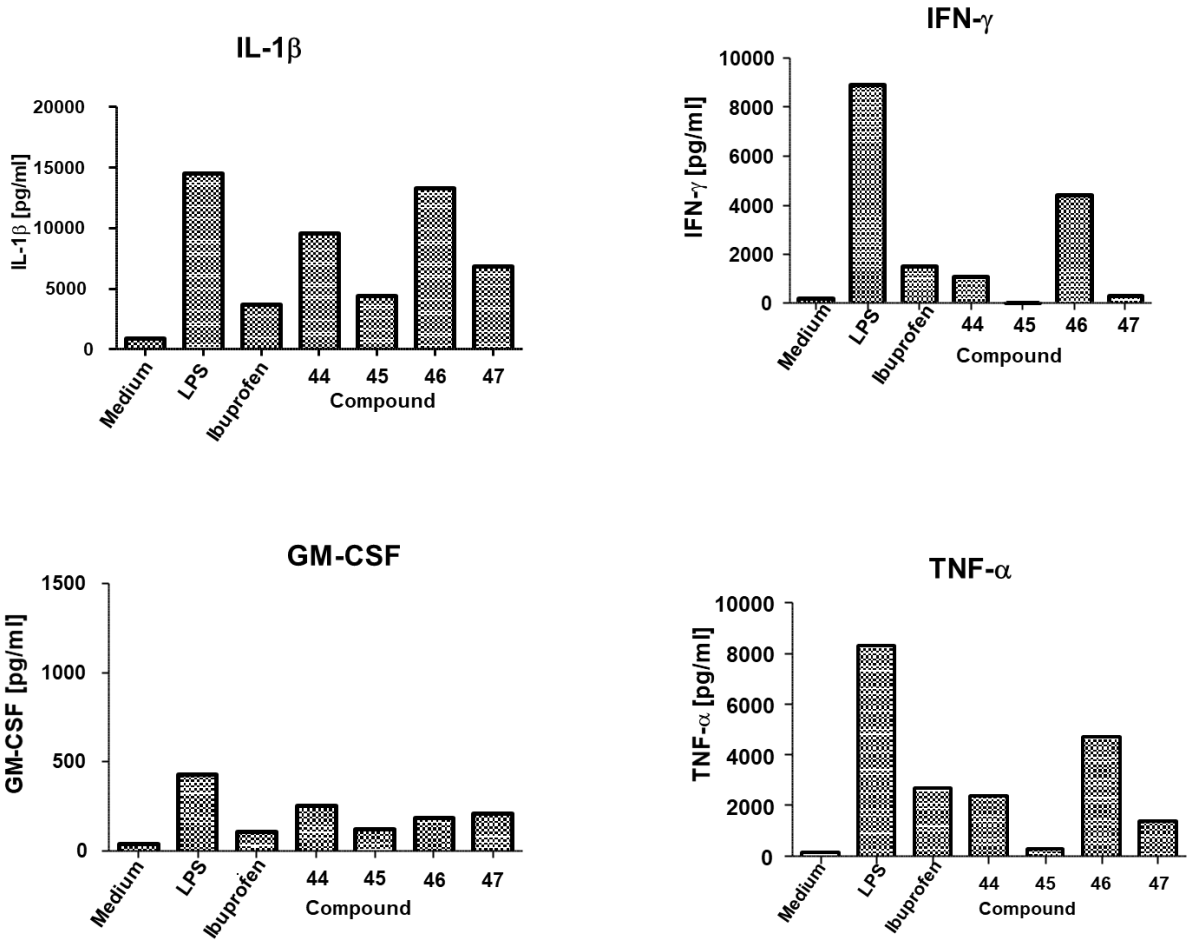


Figure 4.7: The cytokine release after LPS stimulated PMBCs incubated with the crude extracts and isolated compounds from *Tephrosia elata*[9]

[8] The data used for calculation of the percentage cytokine release is presented in the Appendix B1, Table B3 and B4

[9] The data used to generate the graphs are presented in Appendix B1, Table B3 and B4

4.7.5: Activity of Compounds Isolated from Tephrosia rhodesica

The crude extract decreased the production of IL-1β, IFN-γ and TNF-α except for GM-CSF which was increased to 188% as compared to the LPS control (Table 4.40 and Figure 4.8). All the compounds (**48**, **52**-**56**) decreased the production of IL-1β, IFN-γ except compound **56** which stimulated the production of GM-CSF to about 248.1% compared to ibuprofen (**Figure 4.8** and **Table 4.40)**. Compounds **53**-**55** exhibited a strong reduction of IFN-γ release compared to LPS. Similarly, compound **55** attenuated the production of TNF-α to 5.1% compared to LPS.

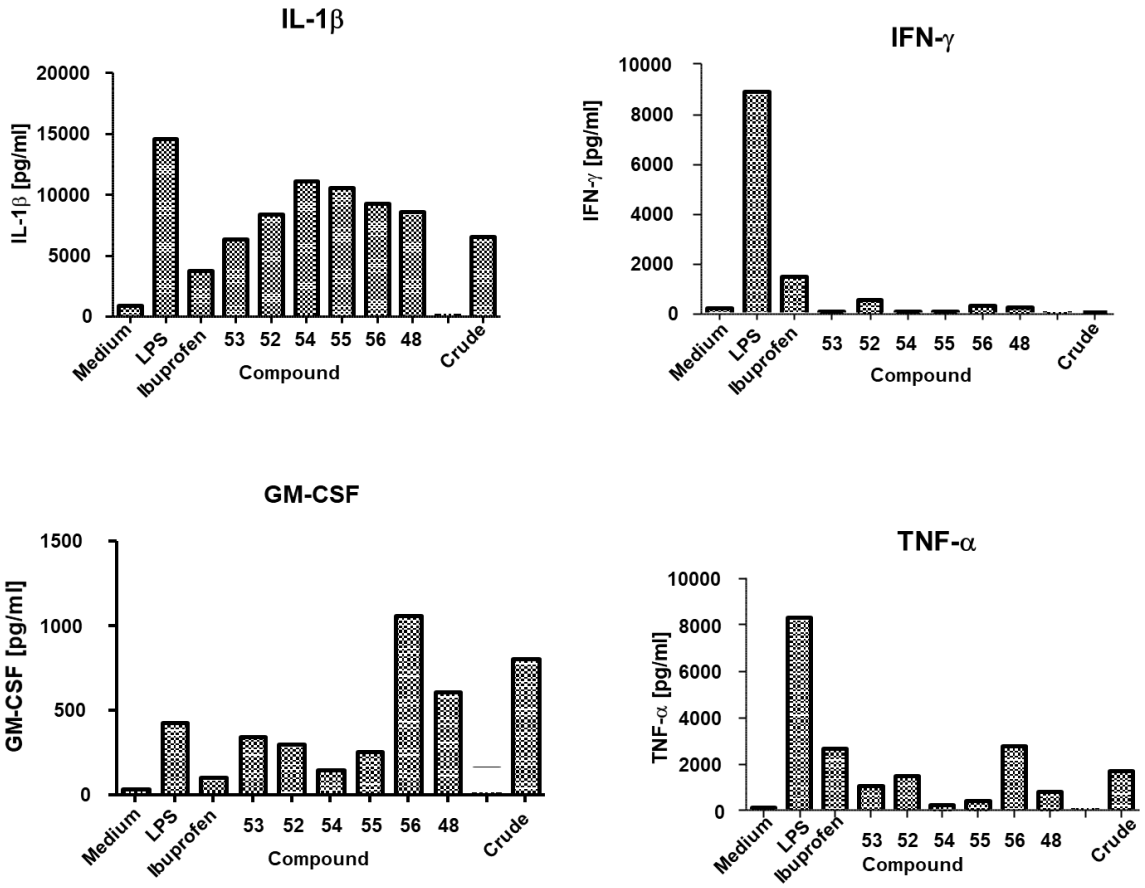


Figure 4.8: IL-1β, IFN-γ, GM-CSF and TNF-α release after LPS stimulated PMBCs incubated with the crude extracts and isolated compounds from *Tephrosia rhodesica*[10]

[10] The data used to generate the graphs are presented in Appendix B1, Table B3 and B4

Table 4.40: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with Compounds from *Tephrosia rhodesica* [11]

	Percentage Cytokine Release			
Sample	**IL-1β**	**IFN-γ**	**GM-CSF**	**TNF-α**
Ibuprofen	25.5	16.8	24.2	32.2
Crude extract	45.0	0.5	188.1	20.6
48	58.7	2.5	142.5	9.9
52	57.6	5.8	70.2	17.8
53	43.6	1.0	79.9	12.8
54	76.1	0.8	34.7	2.8
55	72.5	0.9	60.6	5.1
56	63.4	3.6	248.1	33.4
57	74.6	3.9	148.8	8.5

4.7.6: Anti-inflammatory Activity of Pyrazoisopongaflavone (57)

Pyrazoisopongaflavone (**57**) which was structurally modified from isopongaflavone (**38**) was evaluated for anti-inflammatory activity. Pyrazoisopongaflavone (**57**) decreased the production of IL-β, IFN-γ, and TNF-α to 74.6, 3.9 and 8.5%, respectively, compared to isopongaflavone (**38**) which decreased the production of IL-β, IFN-γ and TNF-α to 57.9, 1.4 and 0.7%. Pyrazoisopongaflavone stimulated the production of GM-CSF to about 148% but isopongaflavone reduced its production to 7.1%. It is worth noting that the conversion of some natural products such as curcumin into their pyrazole derivatives enhanced their anti-inflammatory activity (Somchit *et al.*, 2018; Fernández-Moriano *et al.*, 2019). Also, pyrazole derivatives have been found to have potent anti-inflammatory properties and some, such as celecoxib, have been developed into anti-inflammatory drugs (Ismail *et al.*, 2009; McCormack, 2011; Karrouchi *et al.*, 2018). However, in this study, isopongaflavone was found to be more active than its pyrazole derivative, pyrazoisopongaflavone.

[11] The data used for calculation of the percentage cytokine release is presented in the Appendix B1, Table B3 and B4

4.7.7: Synergistic Anti-inflammatory Activities of the Isolated compounds

The synergistic anti-inflammatory activities of combinations of flavonoids isolated in this study were evaluated. The combinations were as follows: group 1 (compounds **1**-**2**, **8**, and **9**), group 2 (compounds **12–17**, **5**), group 3 (compounds **6**, **7**, **10** and **11**), group 4 (compounds **25**, **31** and **32**), group 5 (compounds **26**, **27**) and group 6 (compounds **28 - 30**). As shown in Figure 4.9 and Table 4.41, all the combinations showed a reduction of IL-1β, IFN-γ, GM-CSF and TNF-α production except group 5 that stimulated the production of GM-CSF to about 184% in comparison to the LPS control. Groups 1-3 reduced the secretion of IL-6, IFN-γ and TNF-α to levels that were not expressively different from the untreated control (medium). Generally, the combinations of flavonoids showed stronger activities than the individual compounds. For instance, lineaflavone B (**2**) was the most active compound among the compounds in group 1 decreasing the production of IL-1β, IFN-γ, GM-CSF and TNF-α to 1.2, 35.6, 17.4 and 0.2% as compared to LPS, respectively (Table 4.36). However, group 1 decreased the production of IL-1β, IFN-γ, GM-CSF to 0.3, 0.2, 3.4 and 0.0%, respectively and a similar trend was observed in all other groups. Thus, combining the flavonoids showed synergism.

Generally, the results for anti-inflammatory activities of the isolated compounds and combinations supplement early reports on anti-inflammatory properties of flavonoids (Tordera *et al.*, 1994; Hyun Pyo *et al.*, 2004; Ko *et al.*, 2004; Ibrahim *et al.*, 2007; Huang *et al.*, 2009; Feng *et al.*, 2012; Hu *et al.*, 2017; Ryu *et al.*, 2019). For instance, luteolin (**13**) was earlier found to suppress the NF-κB pathway and inhibit the release of pro-inflammatory cytokines (Ueda *et al.*, 2002; Seelinger *et al.*, 2008) and in this study, luteolin showed anti-inflammatory effect by reducing the production of IL-1β, IL-2, IL-6, GM-CSF and TNF-α (Table 4.36).

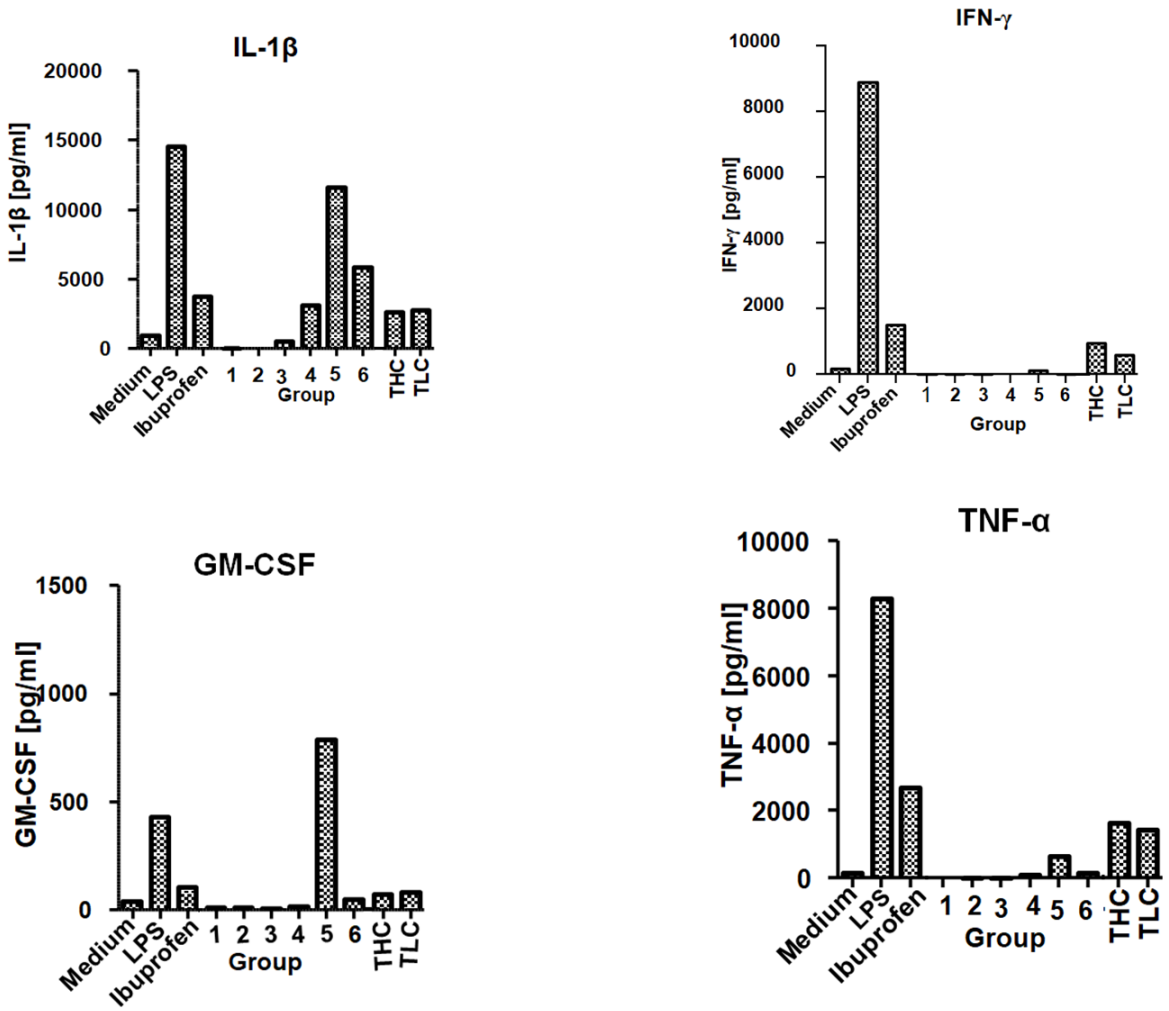


Figure 4.9: IL-1β, IFN-γ, GM-CSF and TNF-α release after LPS stimulated PMBCs incubated with the crude extracts and combinations of isolated compounds[12]

Table 4.41: The Percentage of Cytokine Release in Comparison to Lipopolysaccharide Control after Incubation with combinations of isolated compounds[13]

Sample	Percentage Cytokine Release in Comparison LPS Control			
	IL-1β	IFN-γ	GM-CSF	TNF-α
Ibuprofen	25.5	16.8	24.2	32.2
Group 1	0.3	0.2	3.4	0.0
Group 2	0.1	0.2	3.4	0.1
Group 3	3.4	0.2	2.1	0.1
Group 4	21.1	0.1	3.7	1.0
Group 5	79.4	1.2	184.3	7.9
Group 6	40.0	0.3	12.1	1.7

[12] The data used to generate the graphs are presented in Appendix B1, Table B5. THC = crude extract of *Tephrosia hildebrandtii* and TLC = crude extract of *Tephrosia linearis*.

[13] The data used to generate the table are presented in Appendix B1, Table B5.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1: Conclusions

In this study, five plants in the genus *Tephrosia* Pers. (Fabaceae) namely; *Tephrosia linearis*, *Tephrosia hildebrandtii*, *Tephrosia vogelii*, *Tephrosia elata* and *Tephrosia rhodesica* were phytochemically investigated and a total of fifty-six compounds were isolated and characterized. The compounds were evaluated for their anti-inflammatory activities and their synergistic effects.

The specific conclusions drawn from this study are outlined herein.

i. Phytochemical investigation of the selected plants led to isolation and characterization of eleven new compounds (**1-7, 25, 35, 36** and **44**) and forty-five known compounds (**8-24, 26-34, 37-43** and **45-56** as summarised in Table 5.1. It is worthy to note that isopongaflavone (**38**) was structurally modified to the pyrazole derivative pyrazoisopongaflavone (**57**).

Table 5.1: Summary of the Compounds Characterized from the five *Tephrosia* species

Plant species (part)	New compounds	Known compounds
T. linearis (AP)	Seven (**1-7**)	Sixteen (**8-24**)
T. hildebrandtii (AP)	One (**25**)	Ten (**22**, **26-34**)
T. vogelii (SD)	Two (**35** and **36**)	Ten (**34**, **37-43**)
T. elata (ST)	One (**44**)	Three (**45-47**)
T. rhodesica (ST)	-	Eleven (**21**, **23** and **48-56**)

Key: AP-aerial parts, SD -seedpods and ST-stems

ii. The treatment of LPS-stimulated PBMCs with the isolated compounds (at 100 μM) and extracts (100 μg/mL) showed anti-inflammatory activities by suppressing the production of the pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IFN-γ, GM-CSF and

TNF-α). Among the compounds that were tested, lineaflavone B (**2**), luteolin (**13**), patuletin-3-*O*-rhamnoside (**16**) and pisatin (**30**) showed the strongest activity.

iii. The synergistic anti-inflammatory effects of the isolated compounds were also investigated. All the combinations of flavonoids (groups 1-6) showed superior anti-inflammatory activities than the individual flavonoids isolated in this study suggesting anti-inflammatory synergetic effects.

5.2: Recommendations

Based on the results of this study, the recommendations are made as follows:

1. Since the plants investigated led to the isolation of novel compounds and structurally diverse flavonoids with the potential to serve as anti-inflammatory agents, further phytochemical studies should be carried on other parts of these plants.
2. Diverse analogues of the most active compounds should be prepared to optimize their anti-inflammatory effects.
3. The cytotoxicity of the most active compounds should be evaluated to determine their safety.

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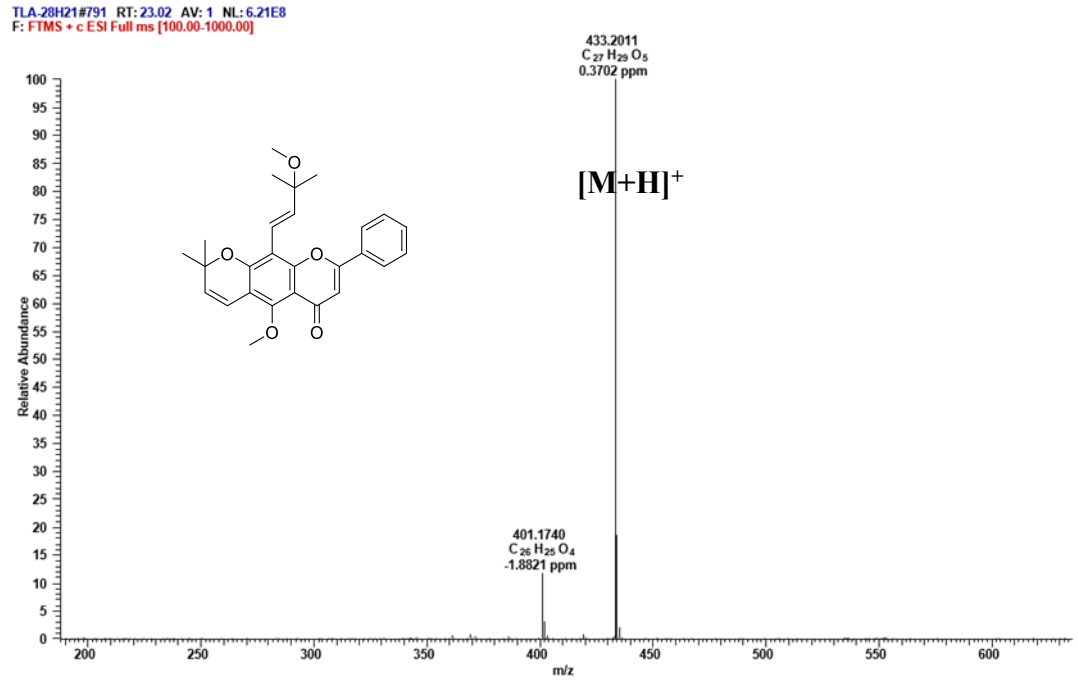
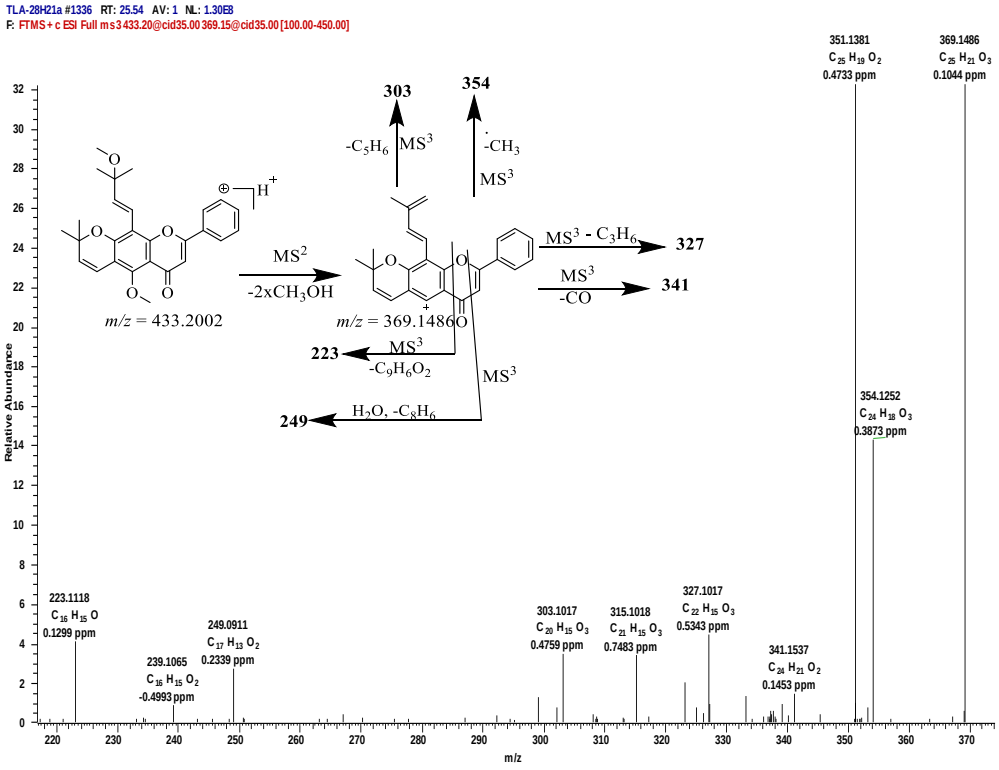
APPENDIX A: Spectra for the Isolated Compounds

Appendix A1: Spectra for compound 1

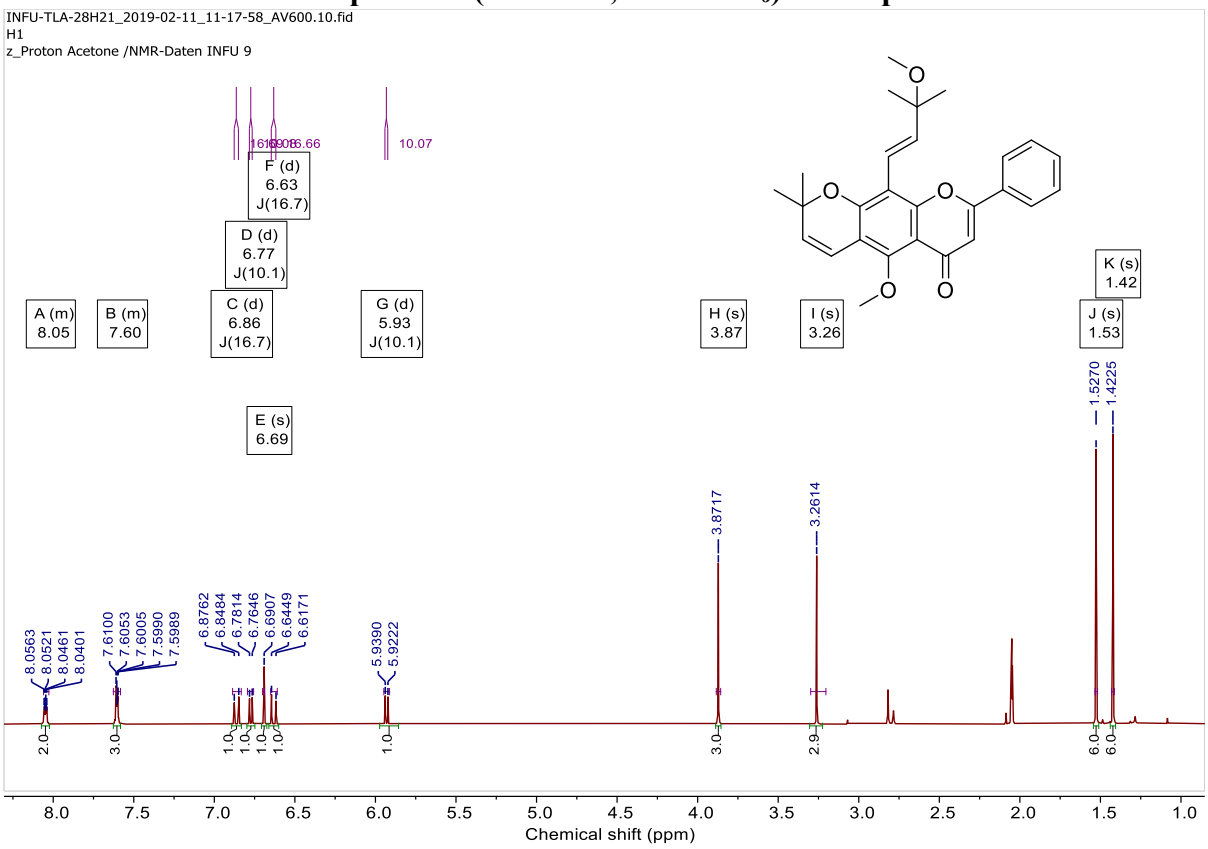
HRESIMS spectrum of compound 1



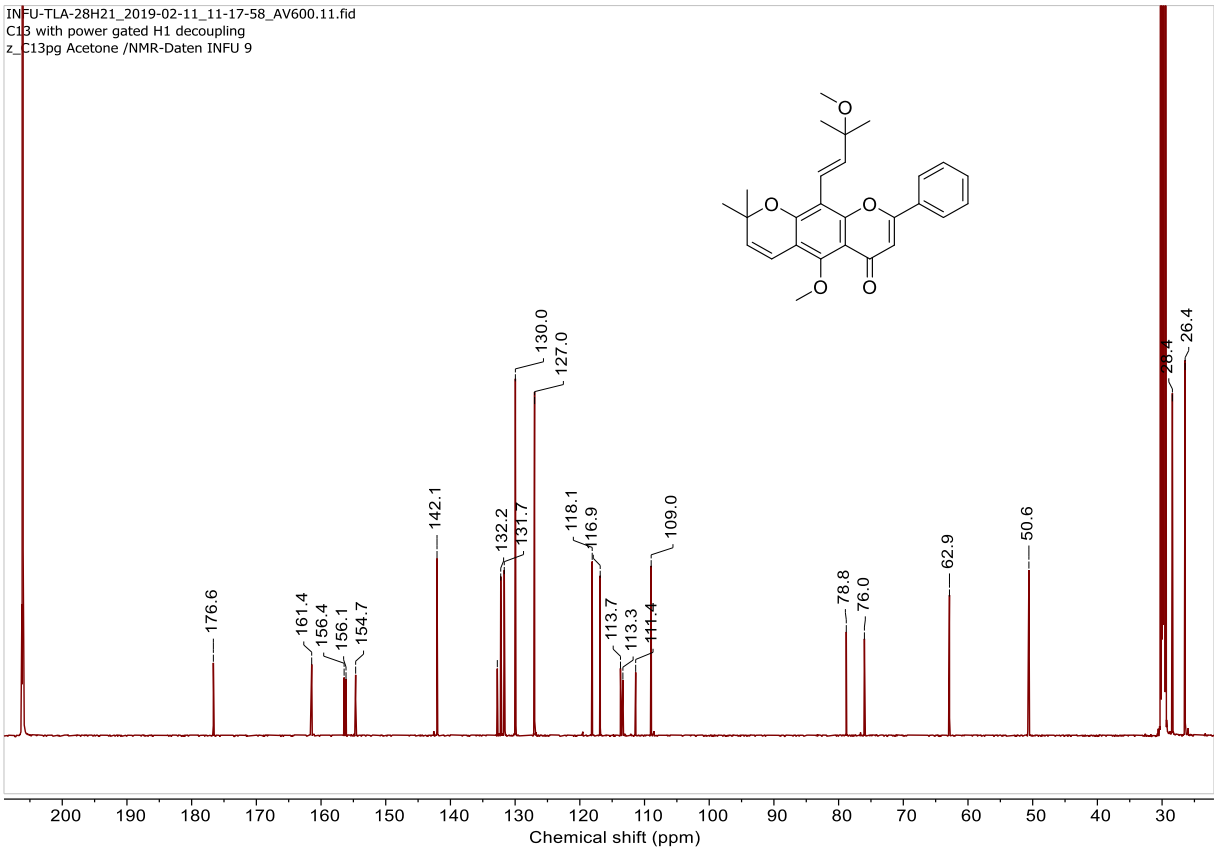
HRESIMS^2 spectrum of compound 1



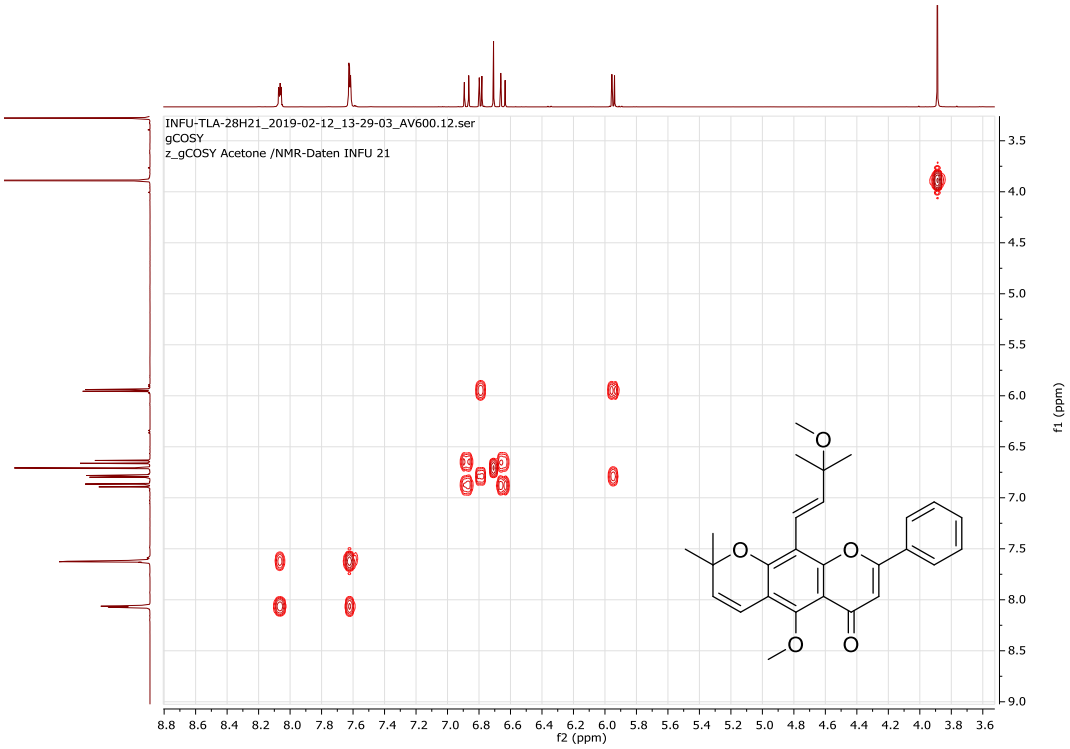
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 1



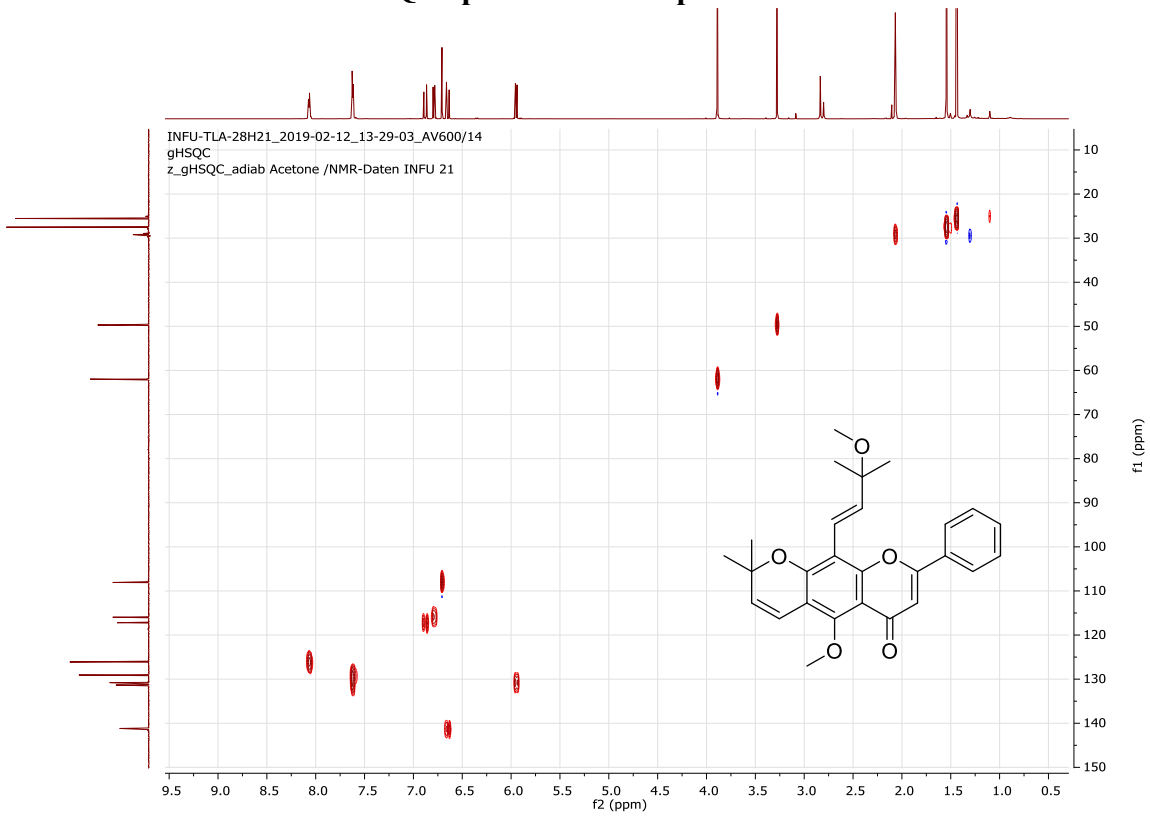
^{13}C NMR spectrum (150 MHz, Acetone-d6) of compound 1



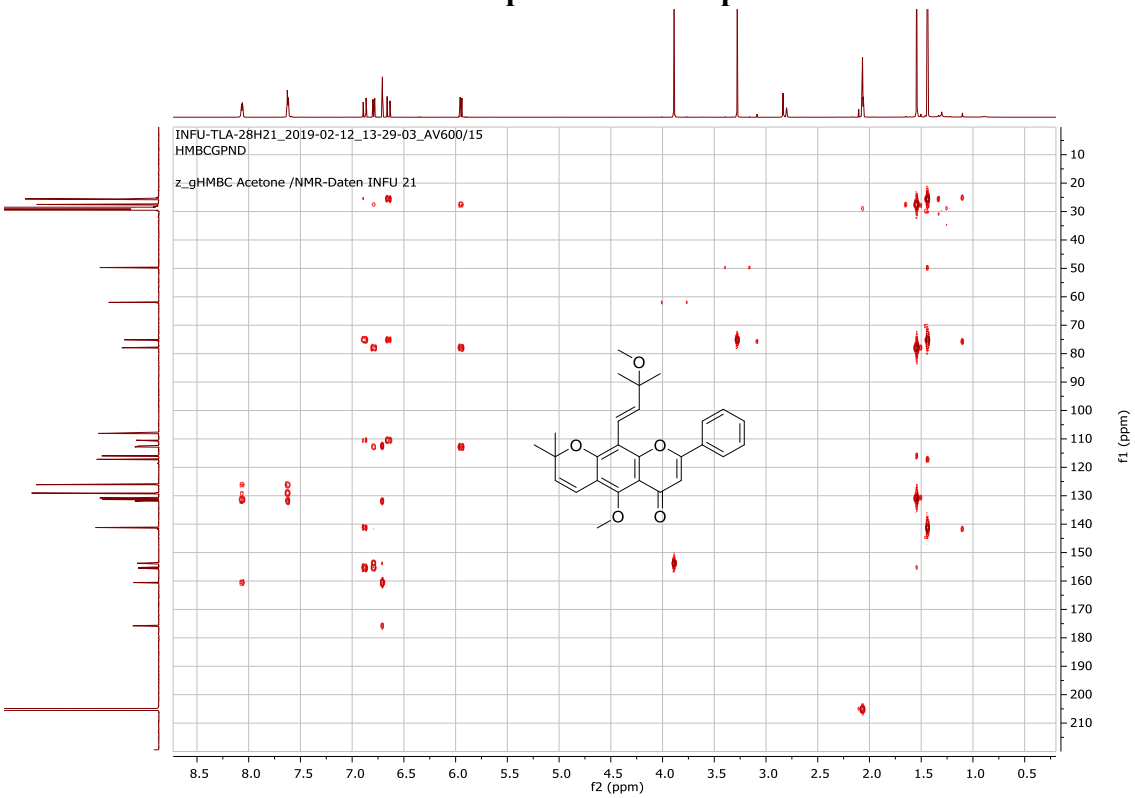
^{1}H-^{1}H COSY spectrum of compound 1



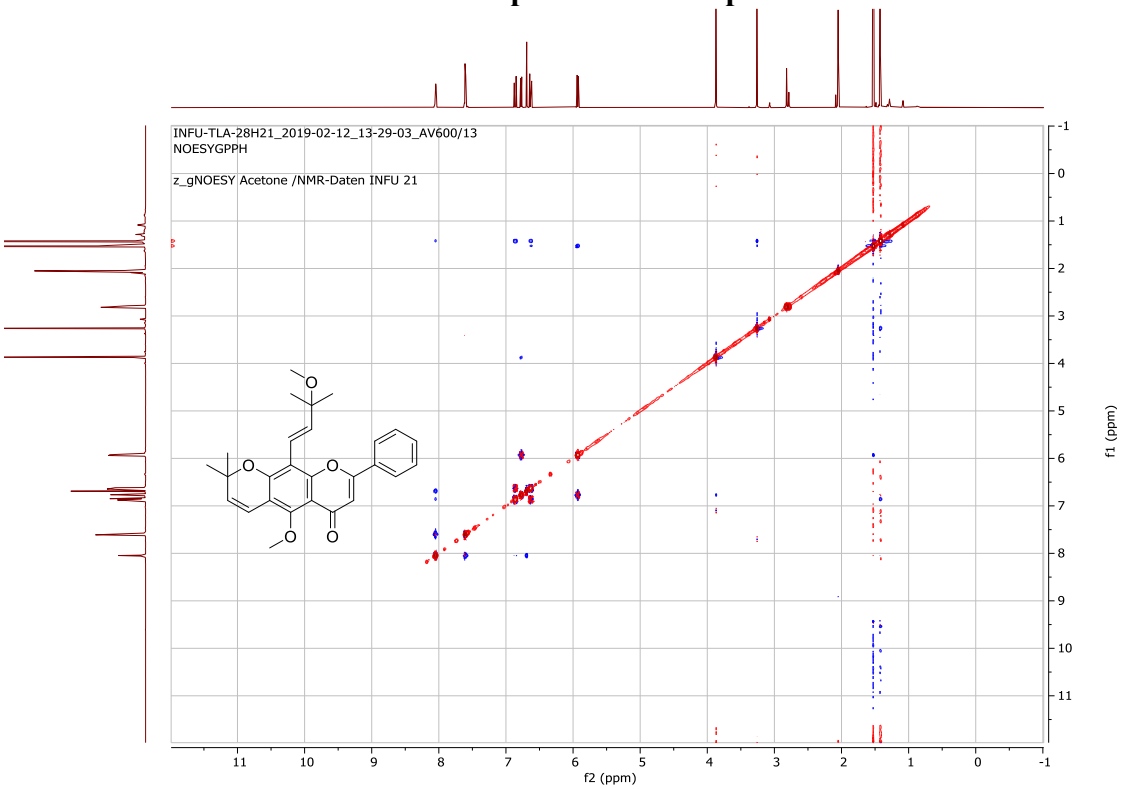
HSQC spectrum of compound 1



HMBC spectrum of compound 1

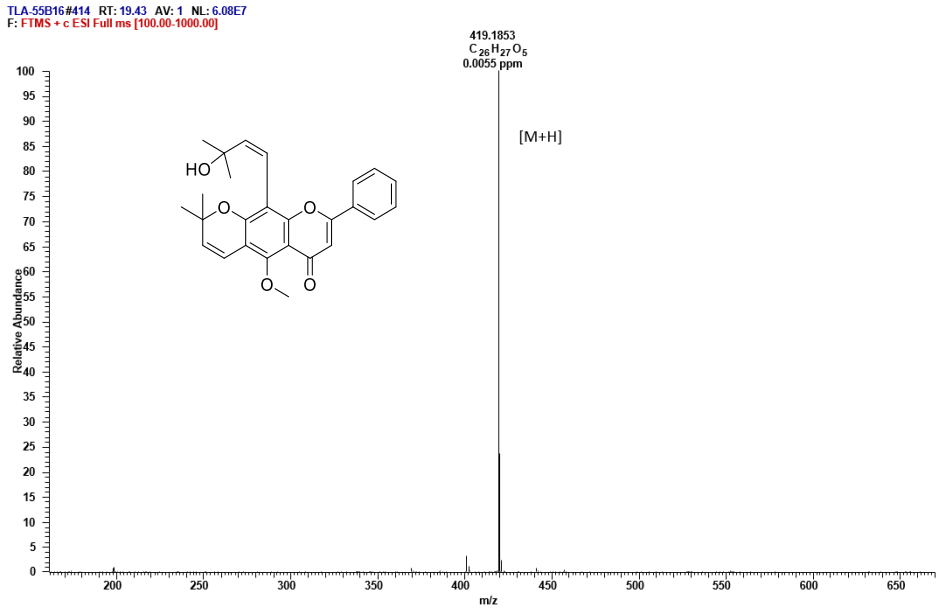


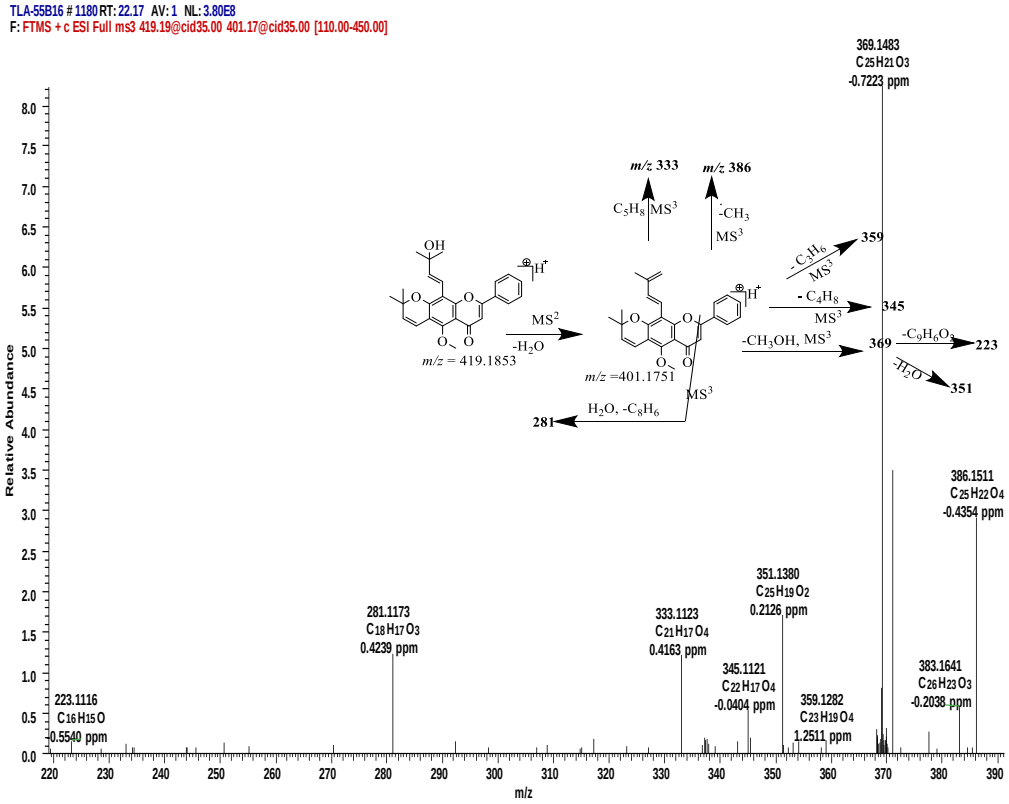
NOESY spectrum of compound 1



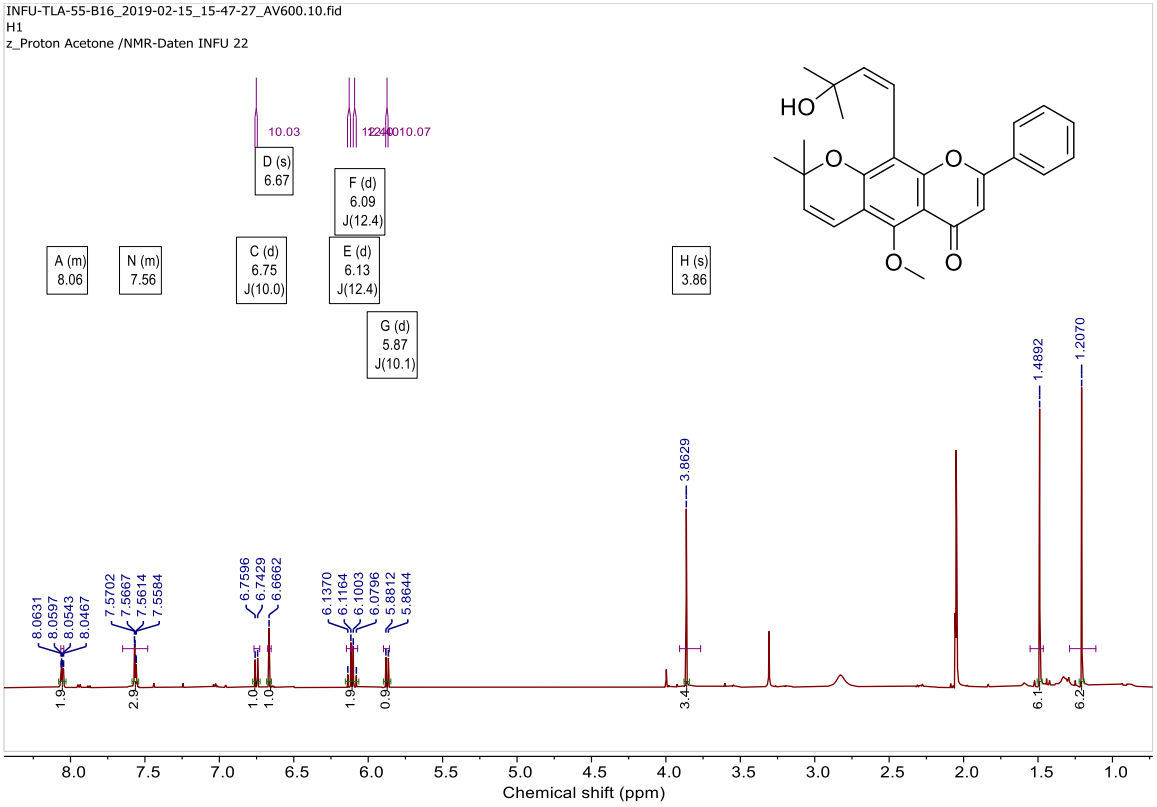
Appendix A2: Spectra for compound 2

HRESIMS spectrum of compound 2

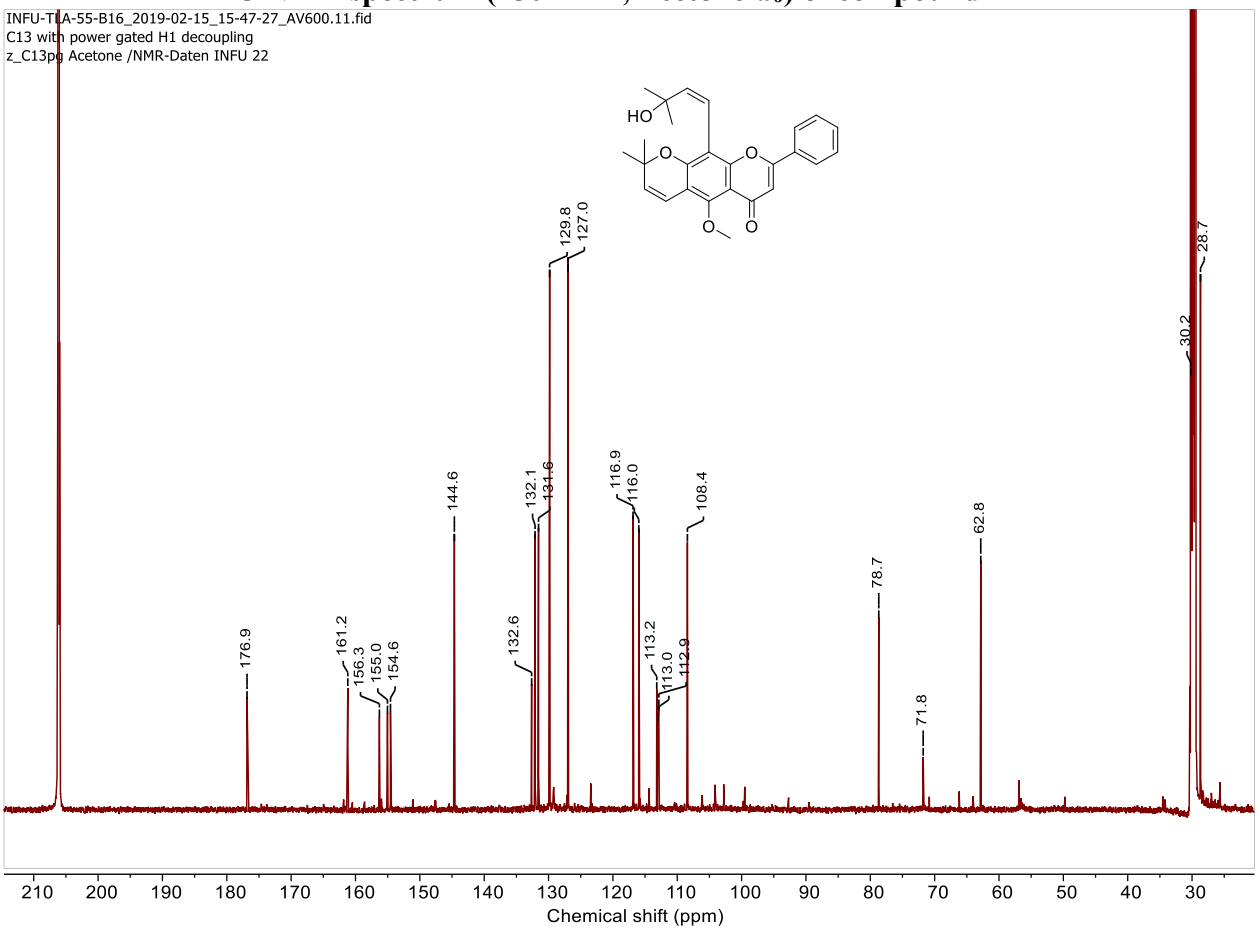




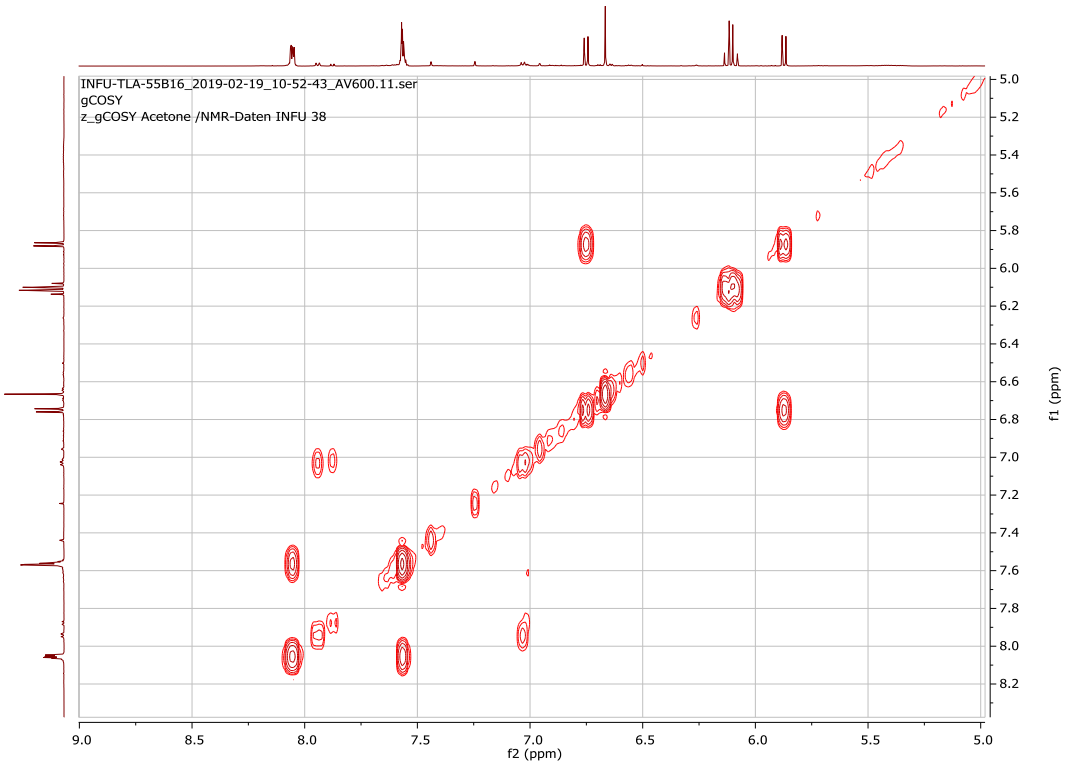
NMR spectrum (600 MHz, Acetone-d_6) of compound 2



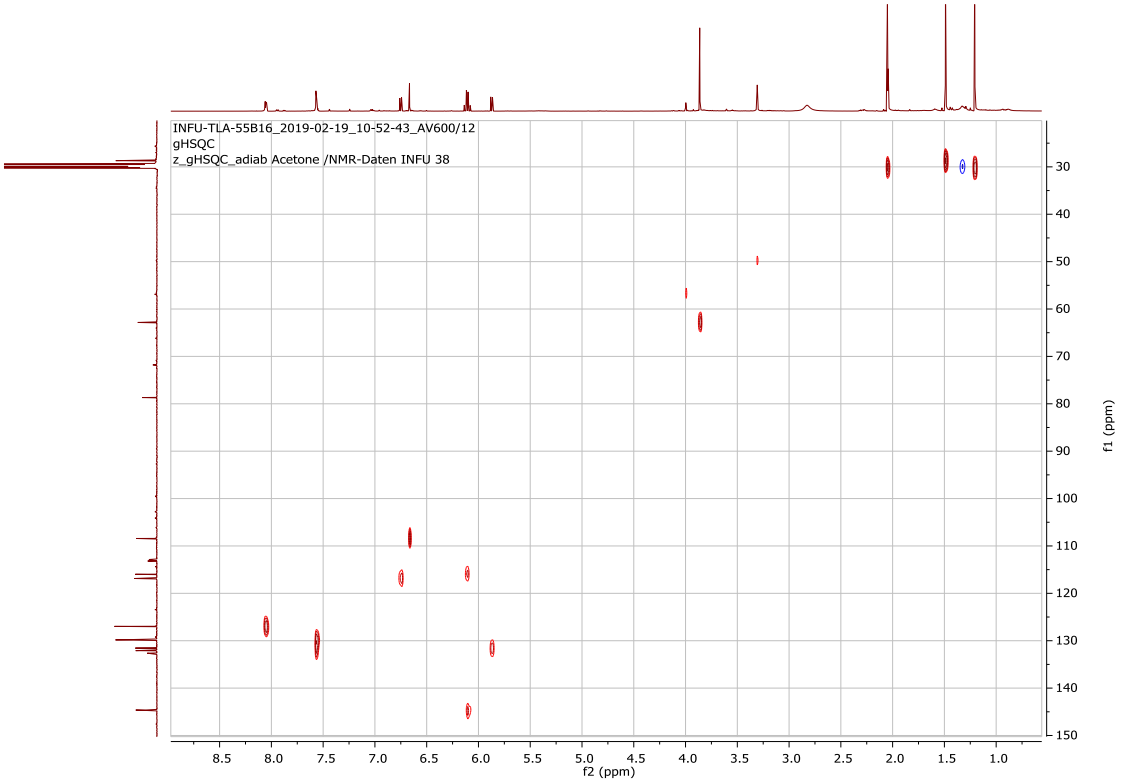
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 2



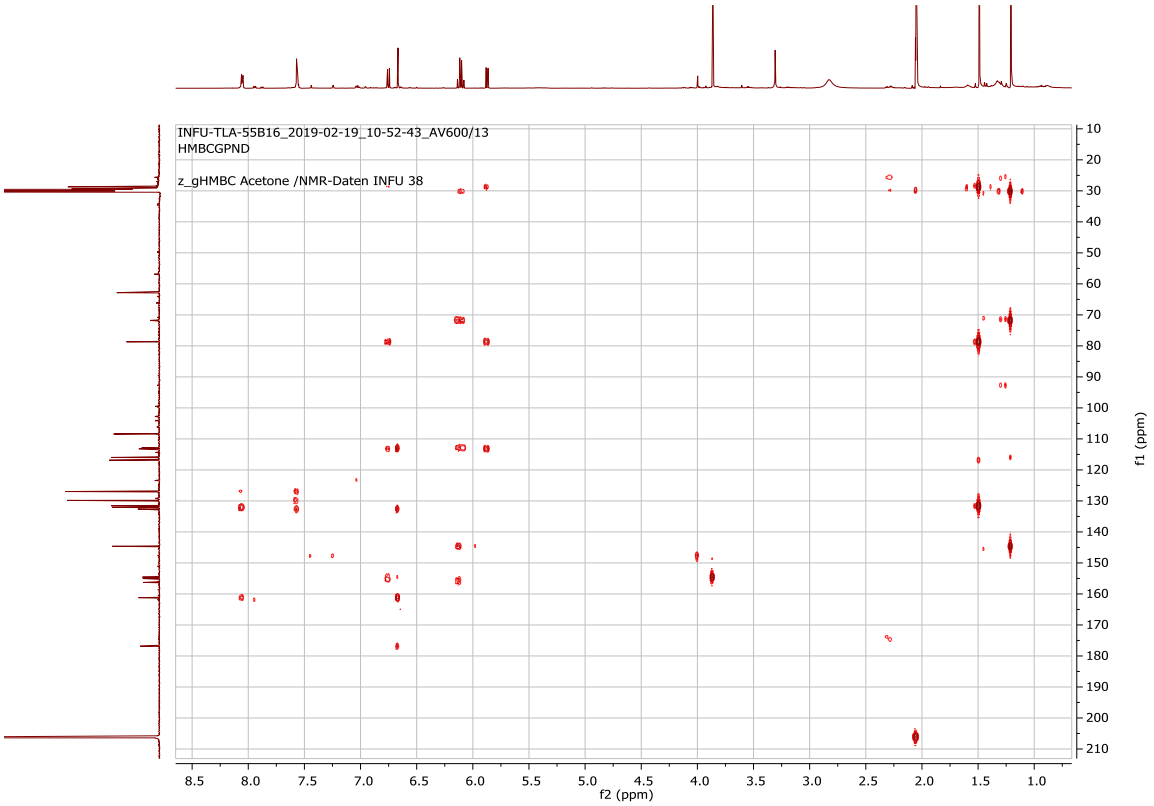
^{1}H-^{1}H COSY spectrum of compound 2



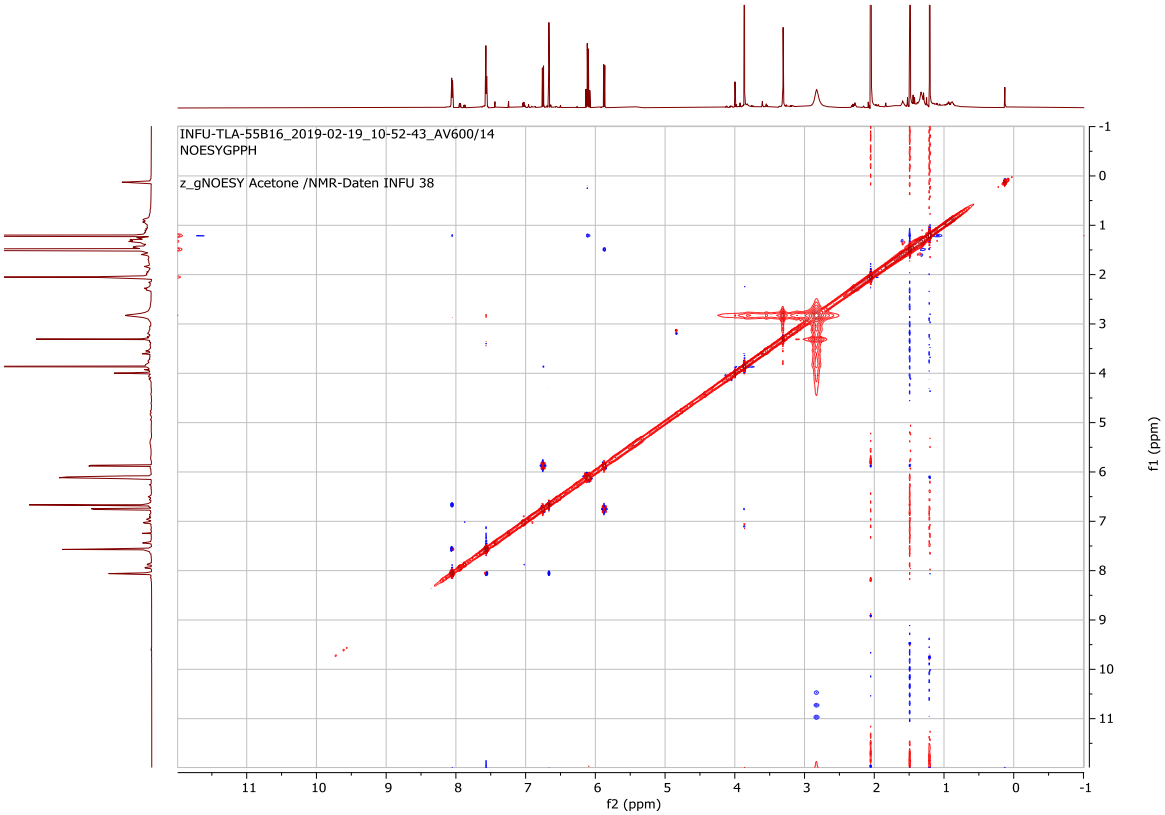
HSQC spectrum of compound 2



HMBC spectrum of compound 2

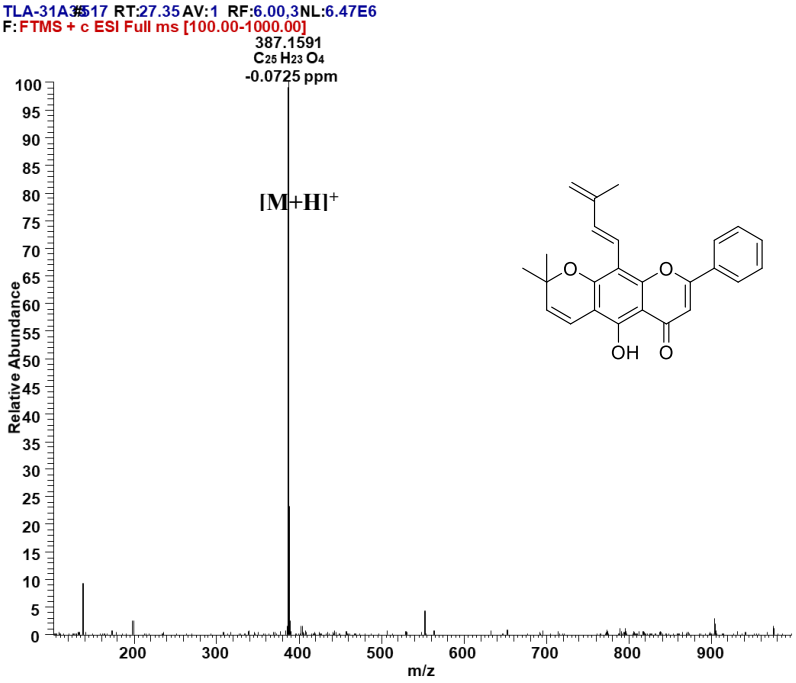


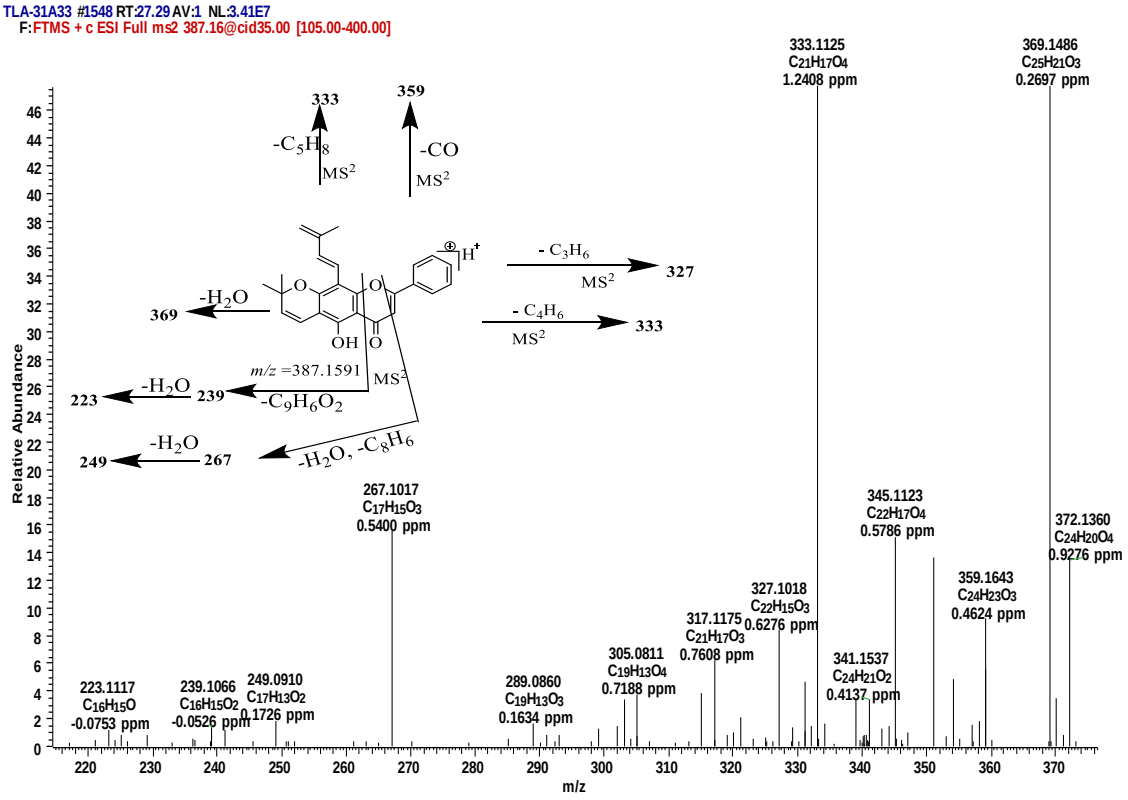
NOESY spectrum of compound 2



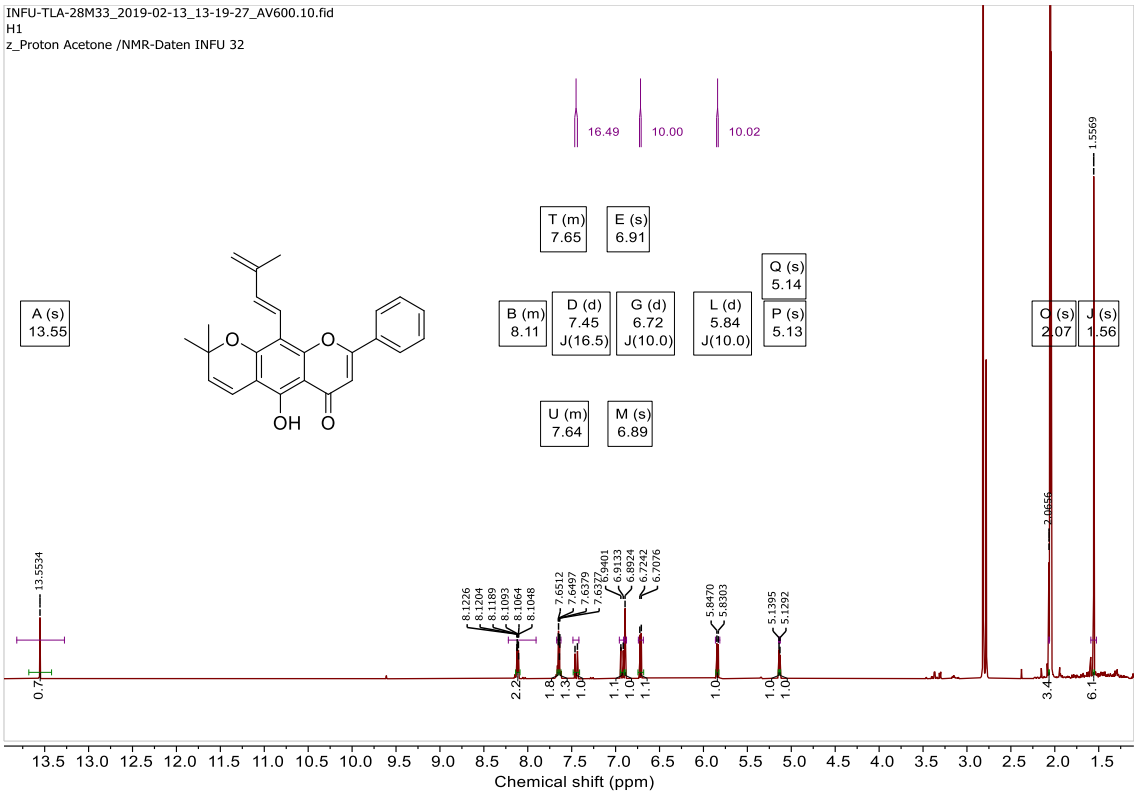
Appendix A3: Spectra for compound 3

HRESIMS spectrum of compound 3

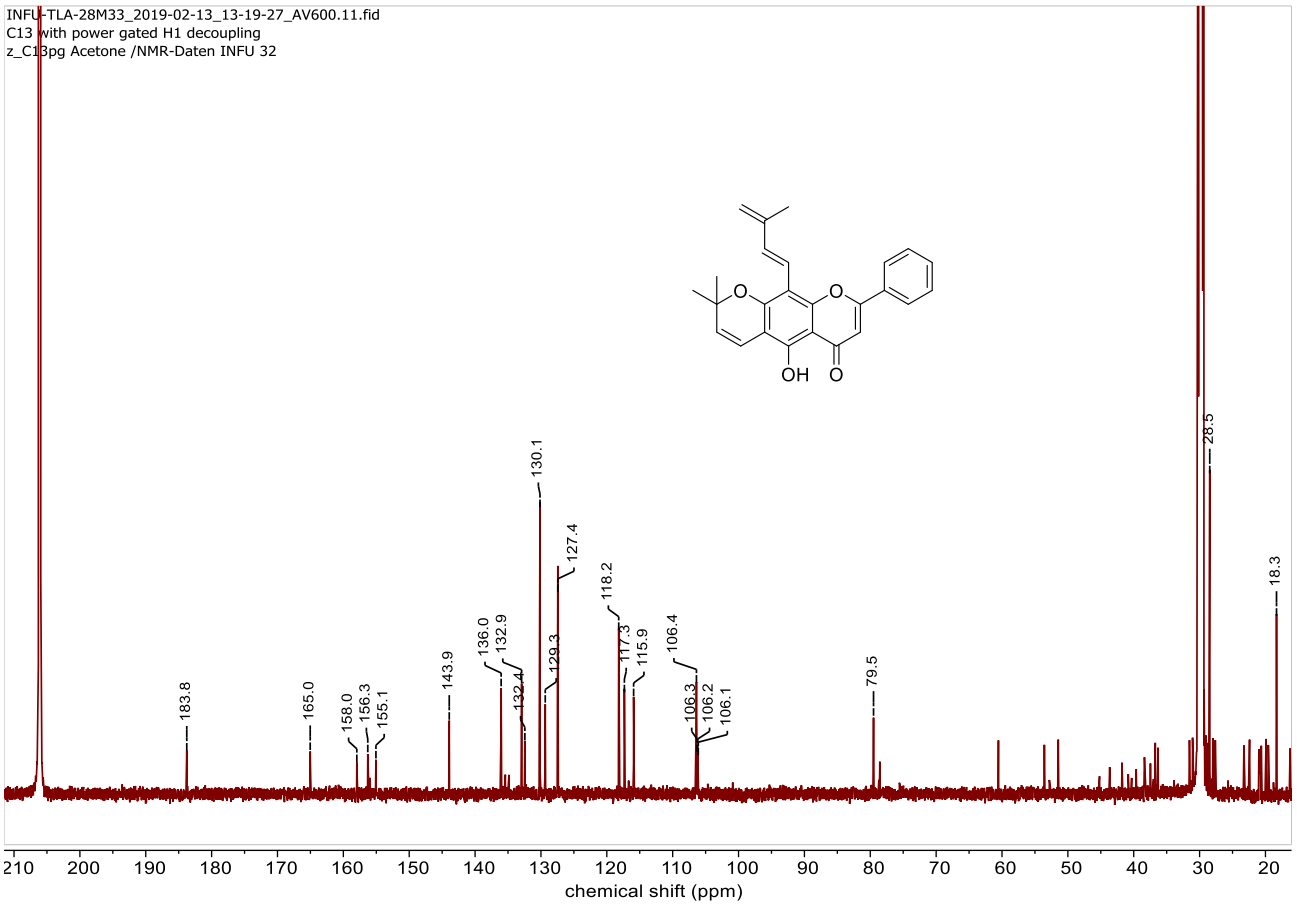




^{1}H NMR spectrum (600 MHz, Acetone-d6) of compound 3

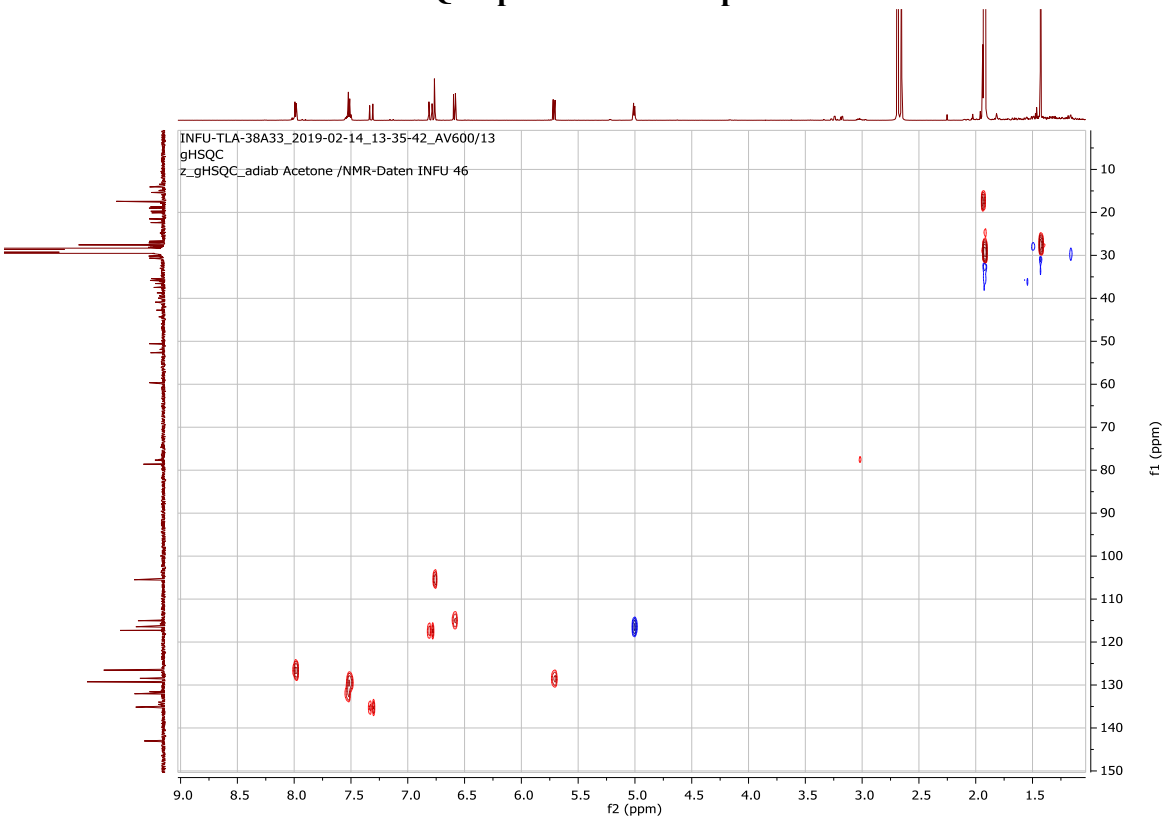


^{13}C NMR spectrum (150 MHz, Acetone-d6) of compound 3

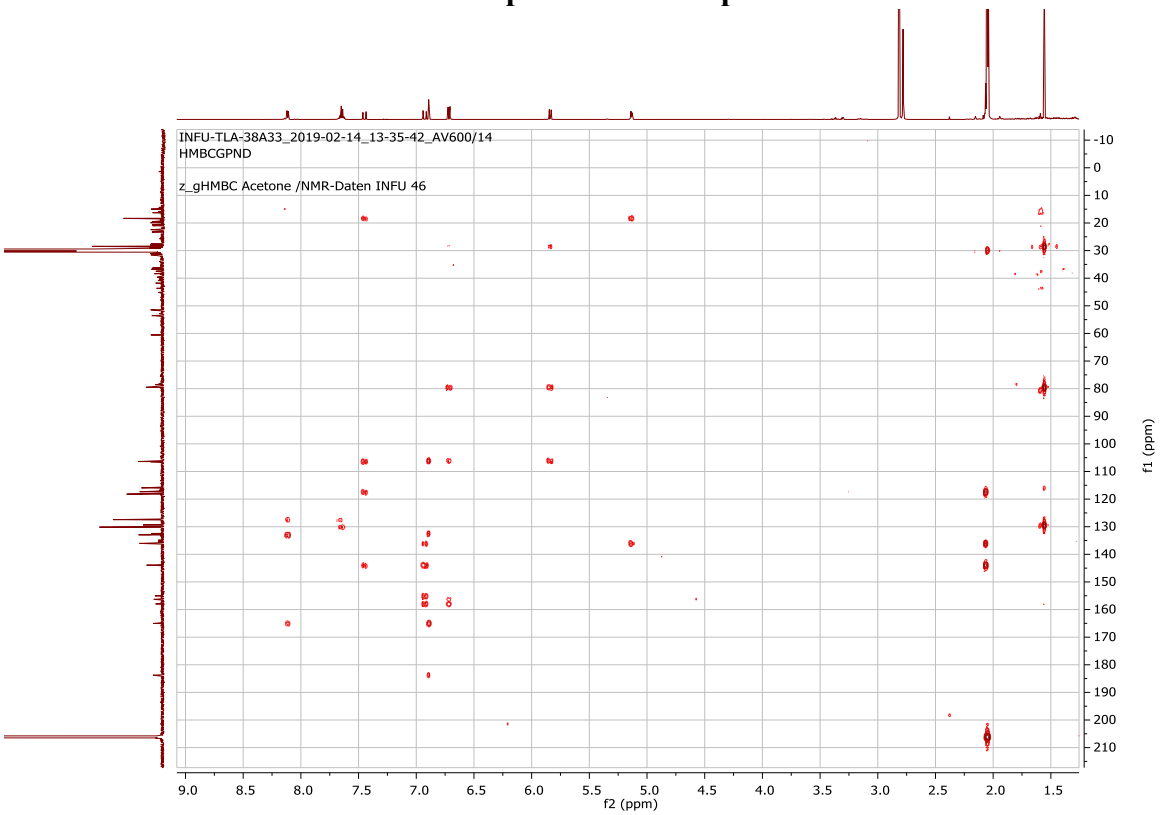


^{1}H-^{1}H COSY spectrum of compound 3

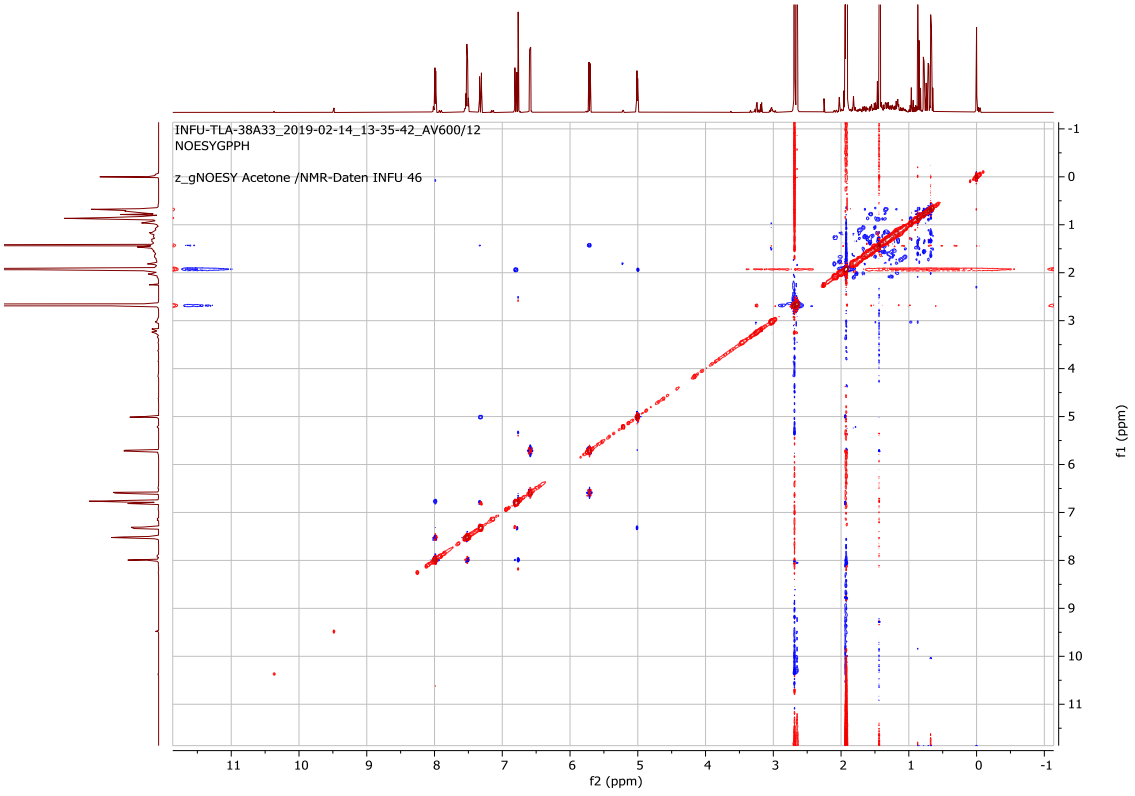
HSQC spectrum of Compound 3



HMBC spectrum of compound 3

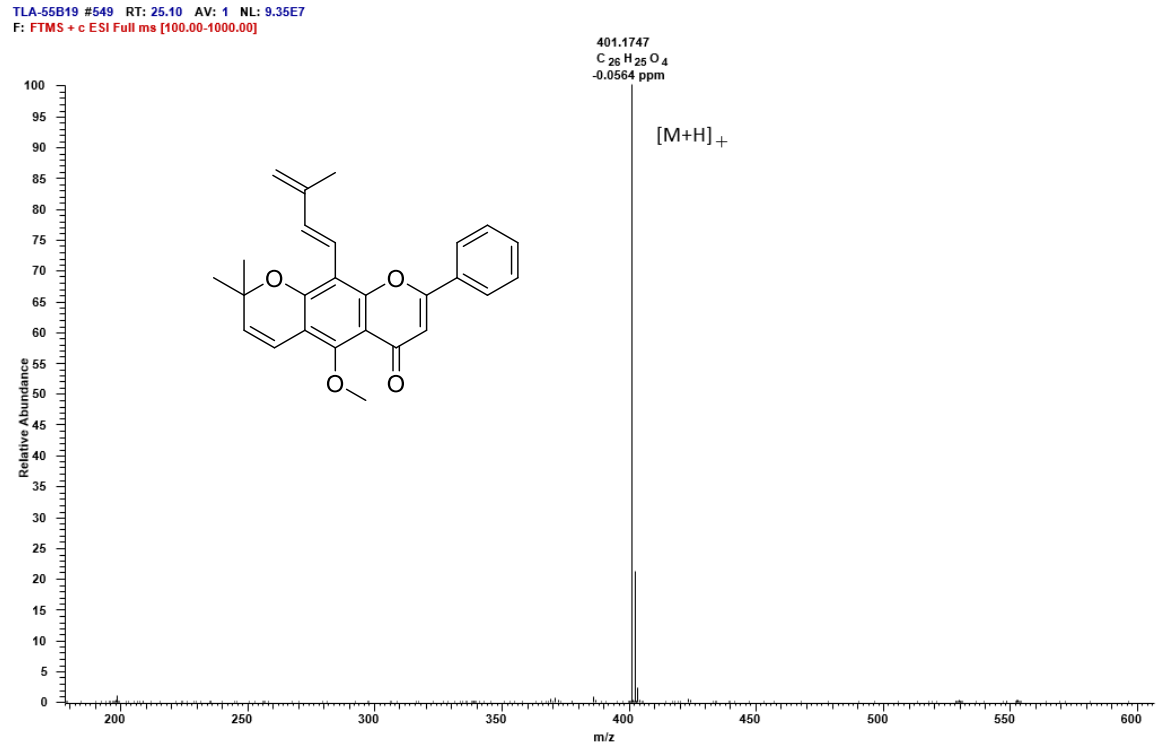


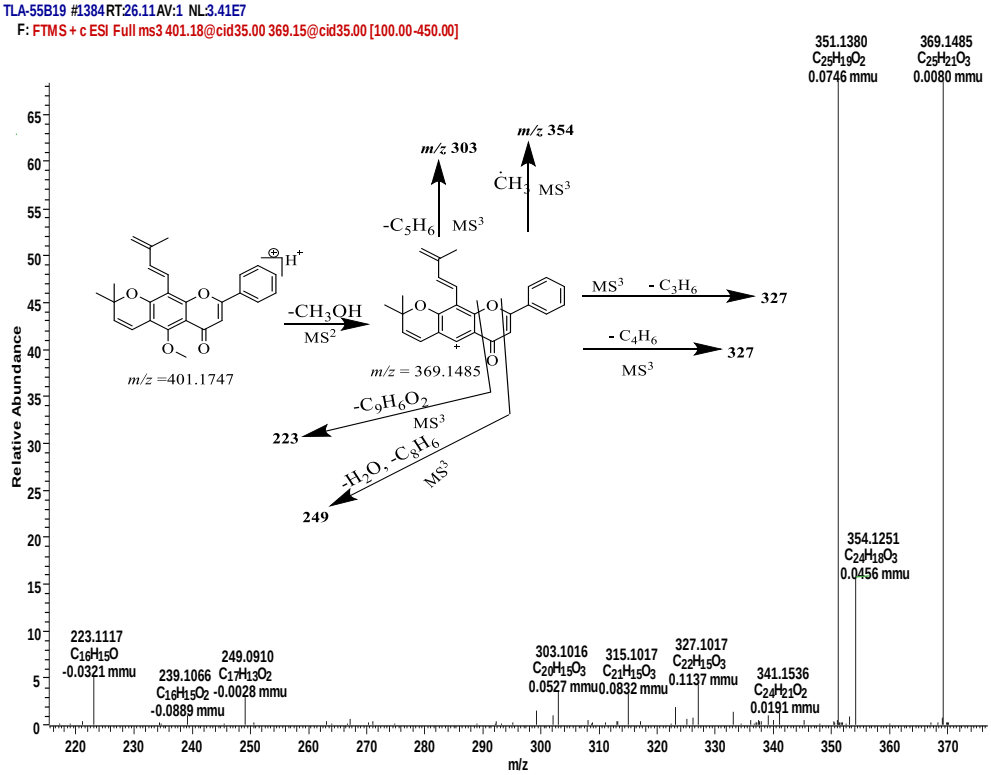
NOESY spectrum of compound 3



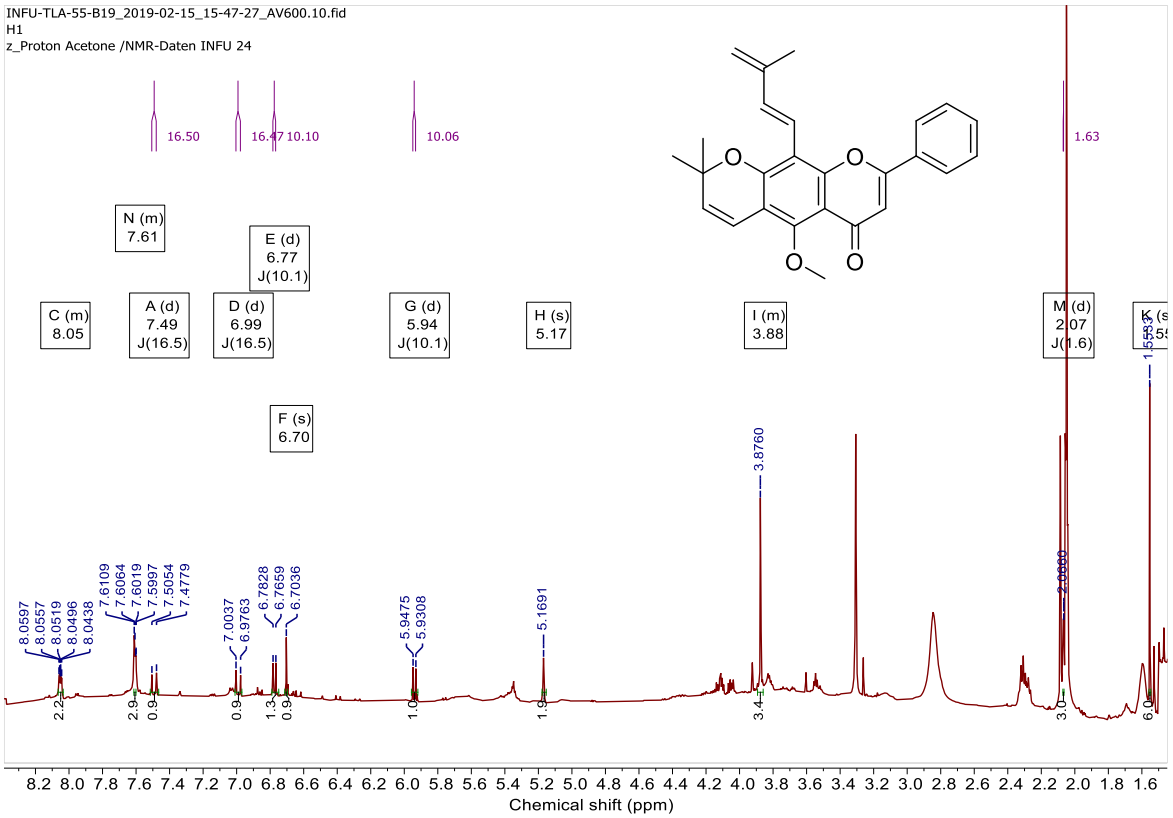
Appendix A4: Spectra for compound 4

HRESIMS spectrum of compound 4

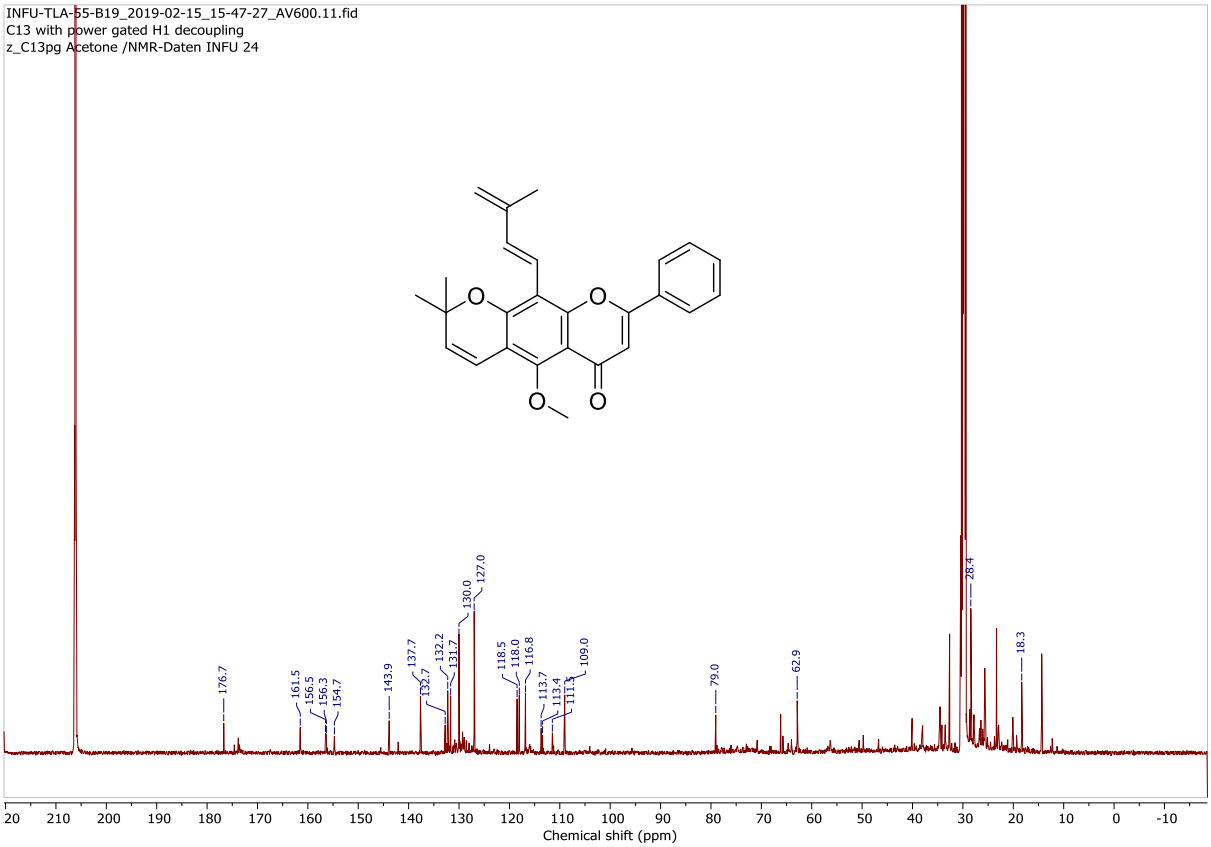




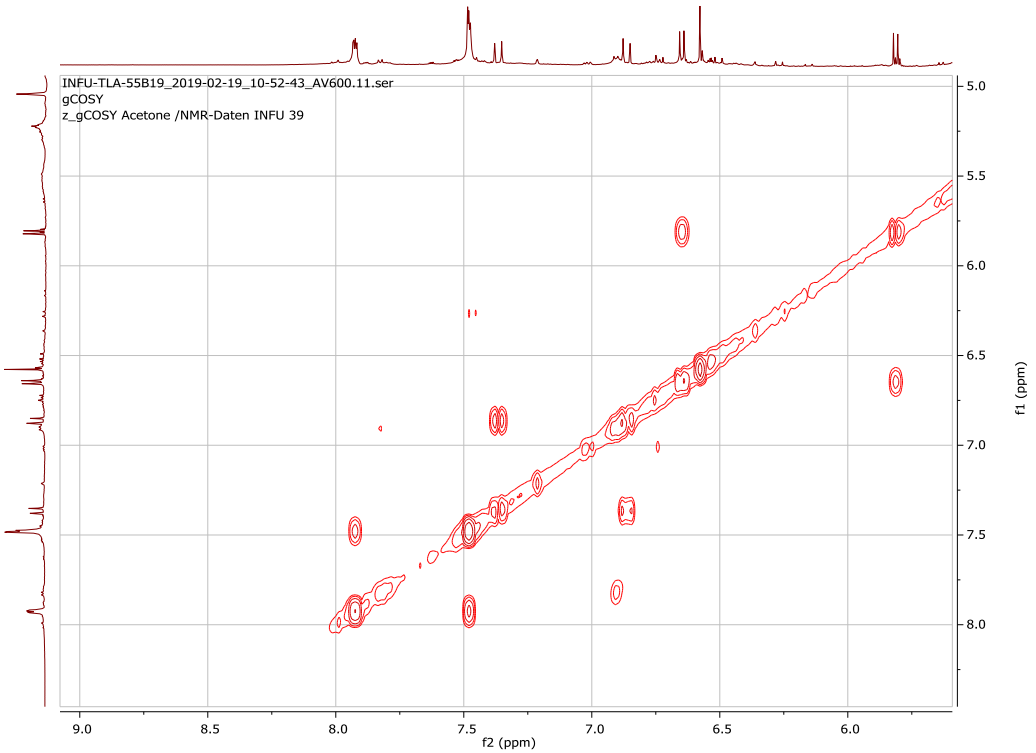
^{1}H NMR spectrum (600 MHz, Acetone-d6) of compound 4



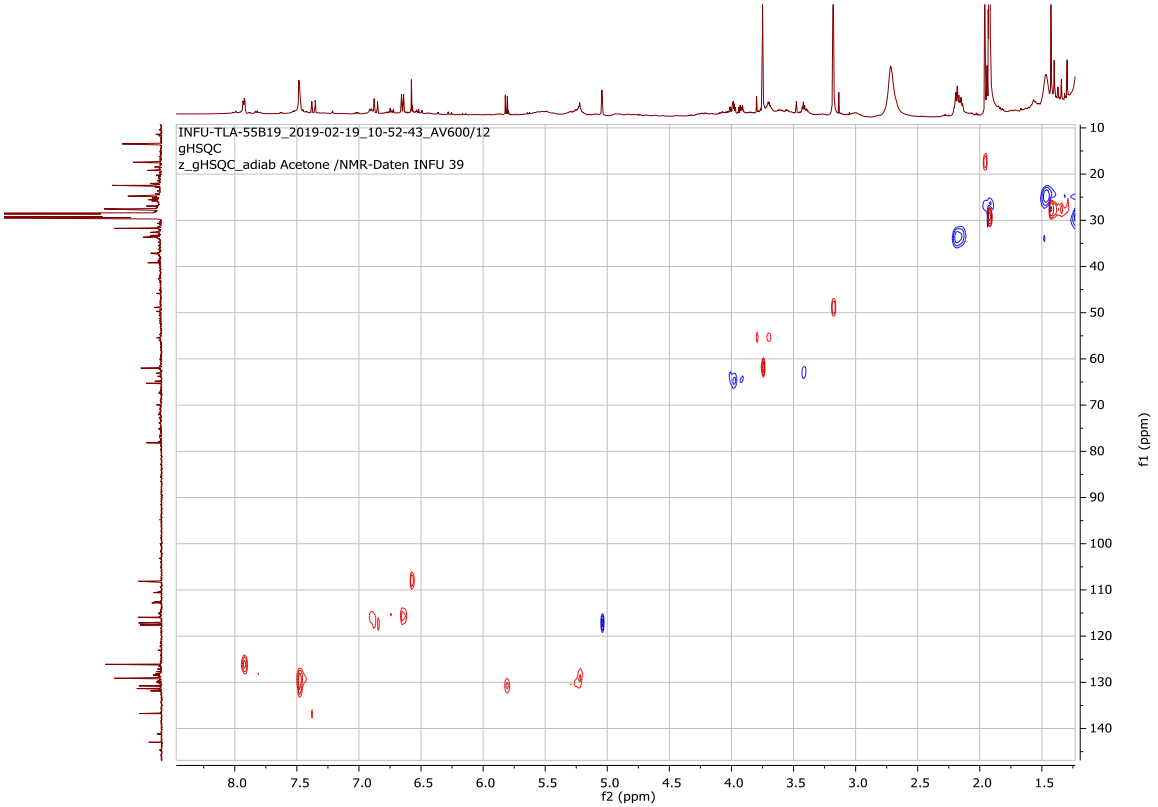
^{13}C NMR spectrum (150 MHz, Acetone-d6) of compound 4



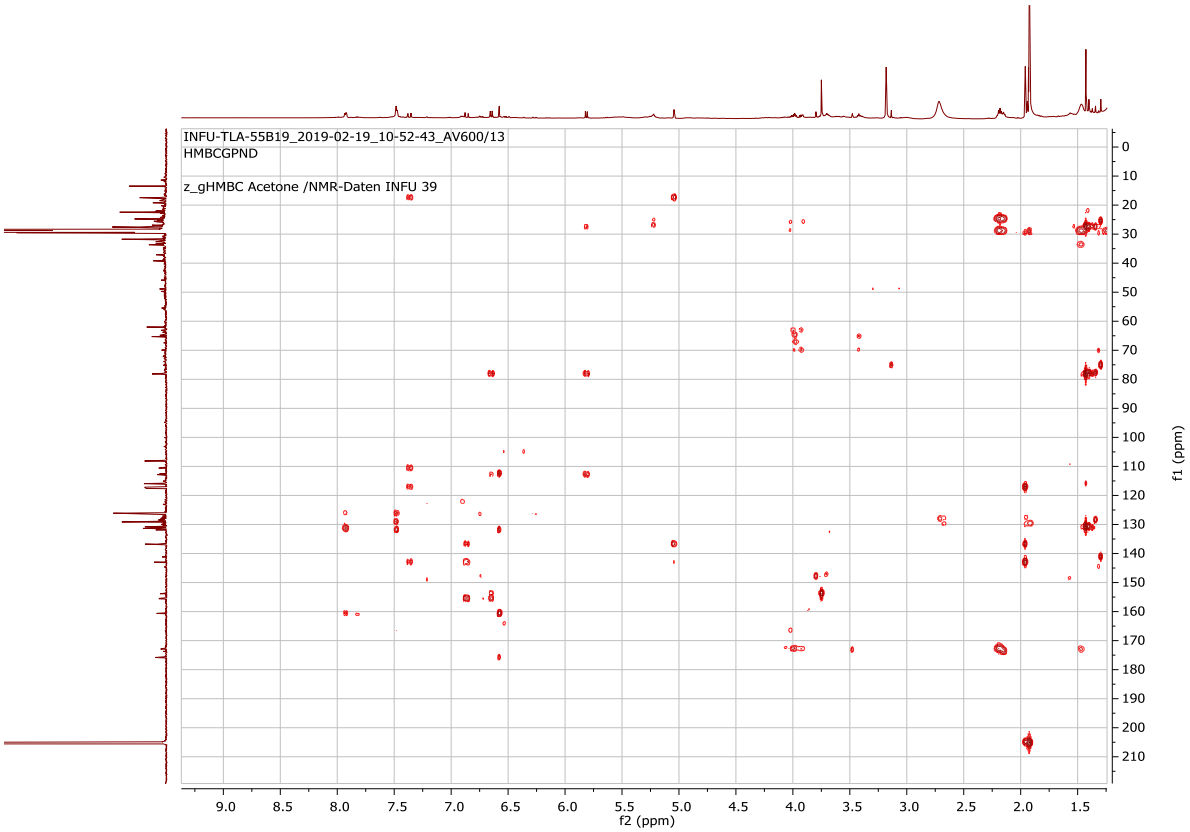
^{1}H-^{1}H-COSY Spectrum of Compound 4



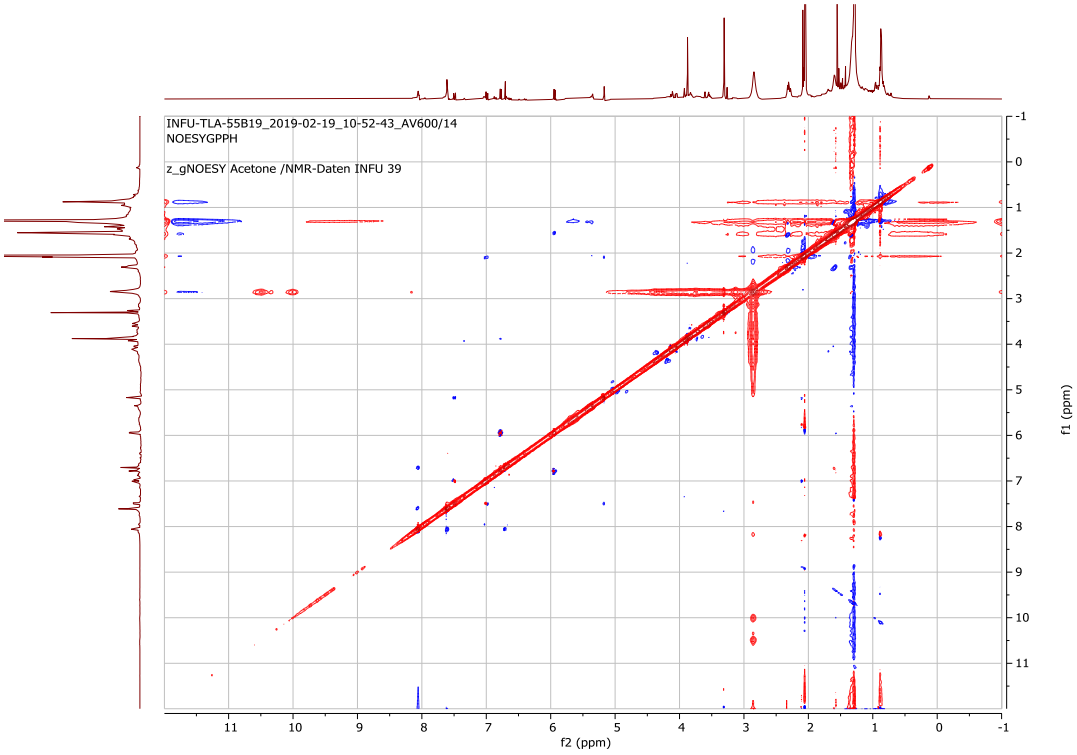
HSQC spectrum of compound 4



HMBC spectrum of compound 4

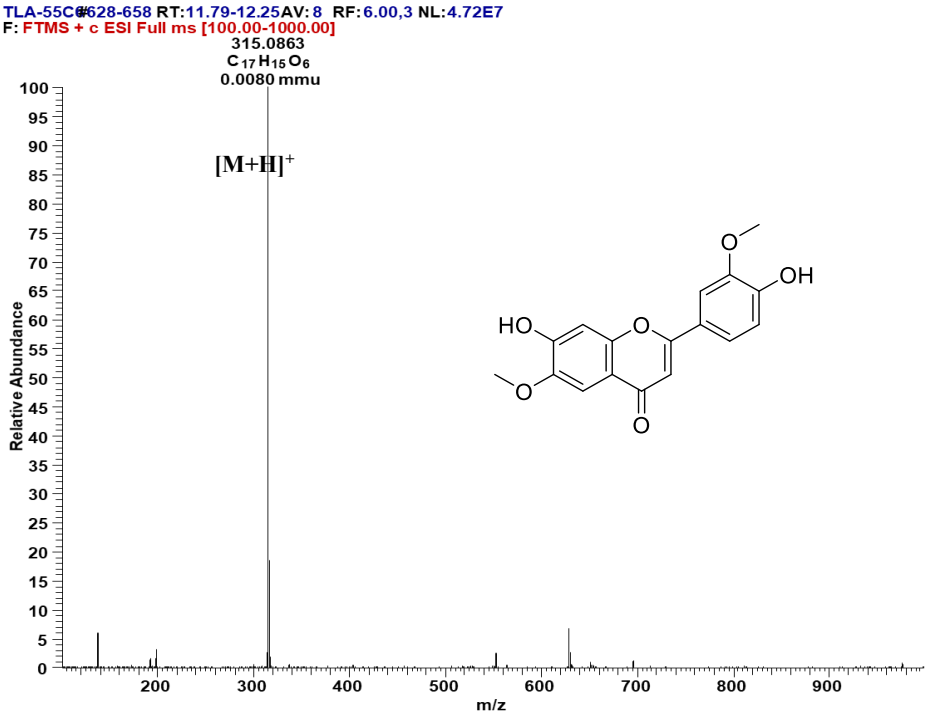


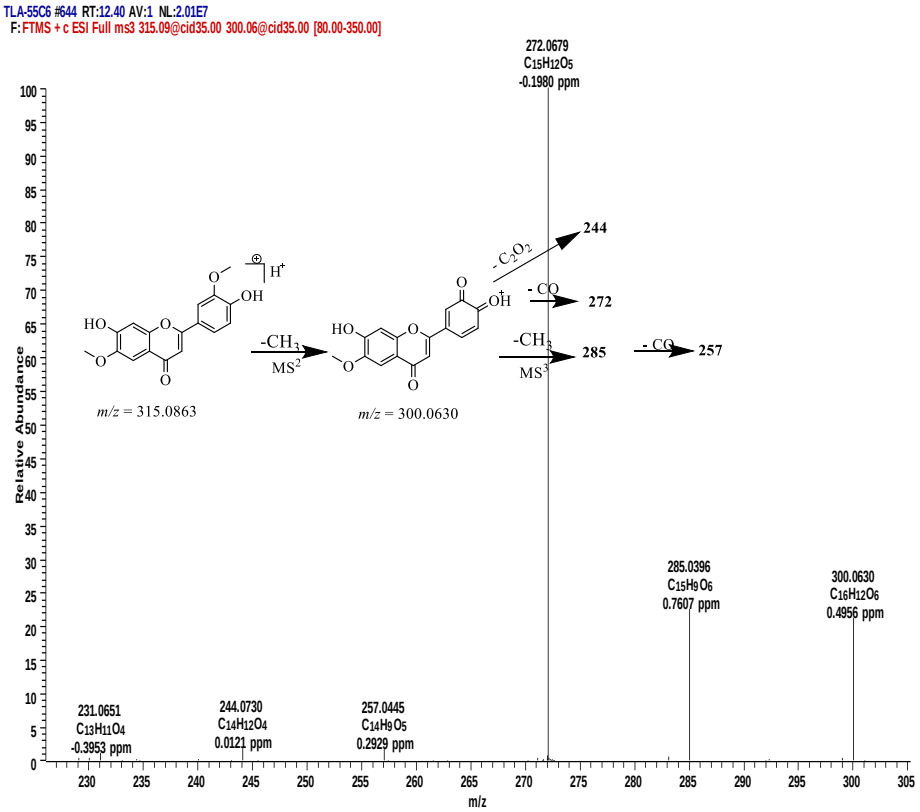
NOESY spectrum of compound 4



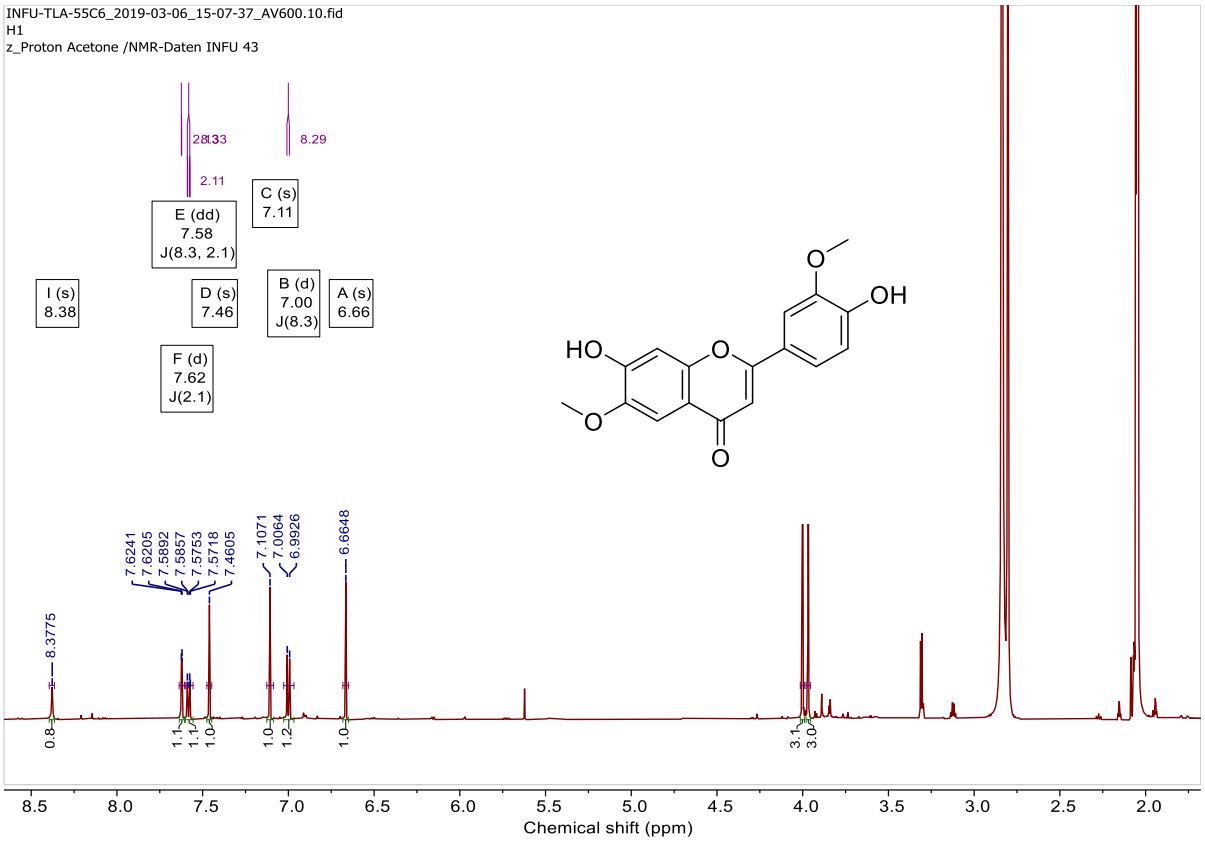
Appendix A5: Spectra for compound 5

HRESIMS spectrum of compound 5

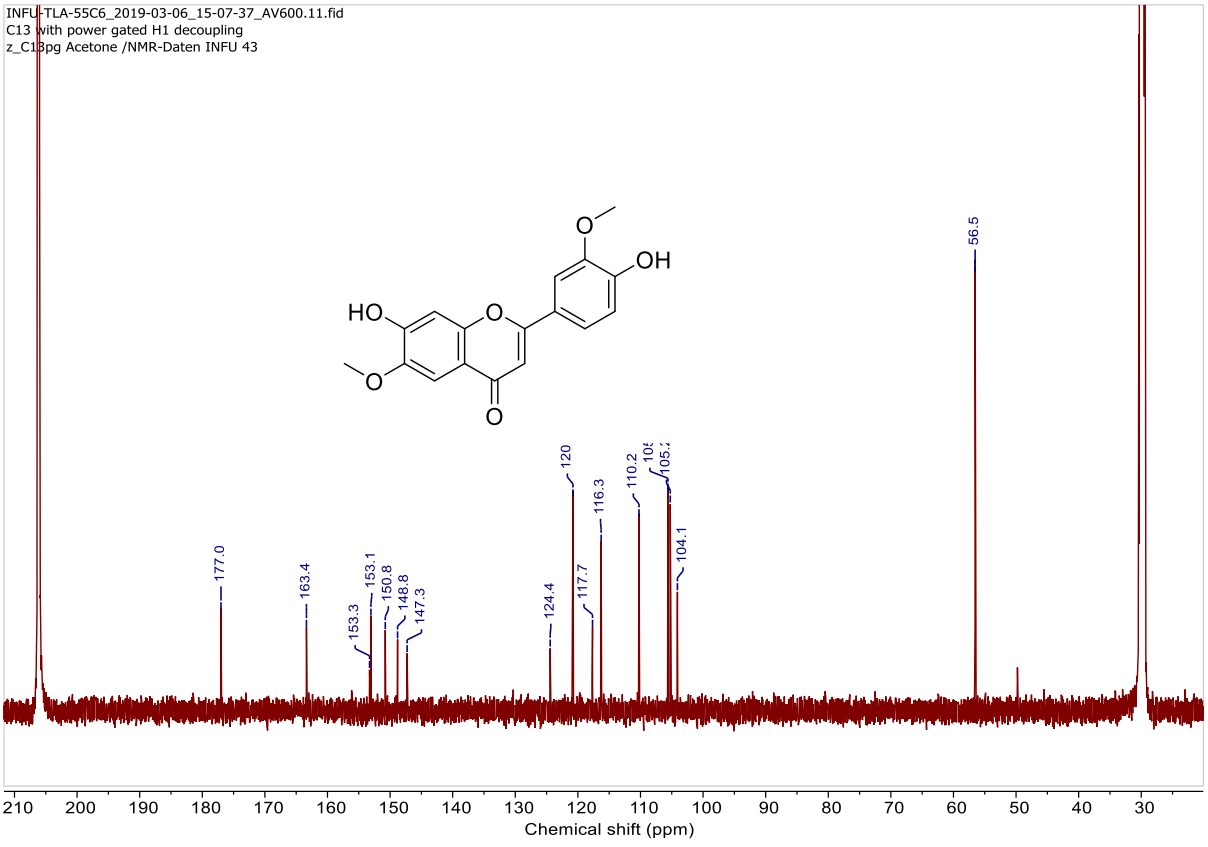




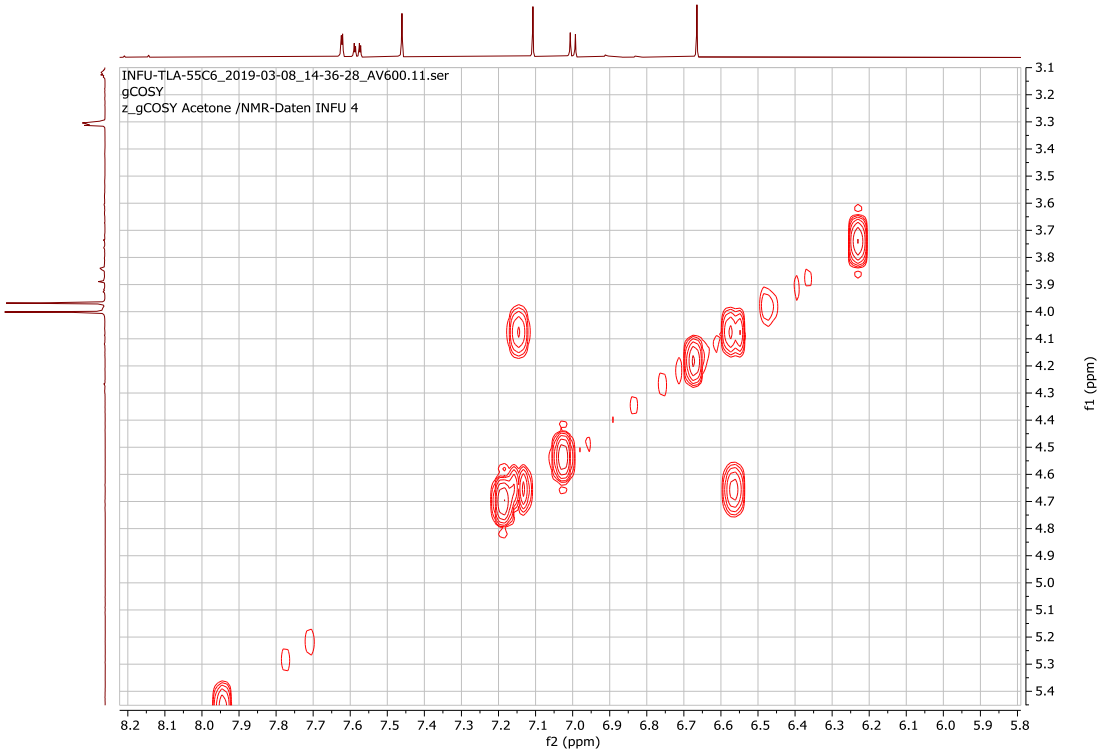
^{1}H NMR spectrum (600 MHz, Acetone-d6) of compound 5



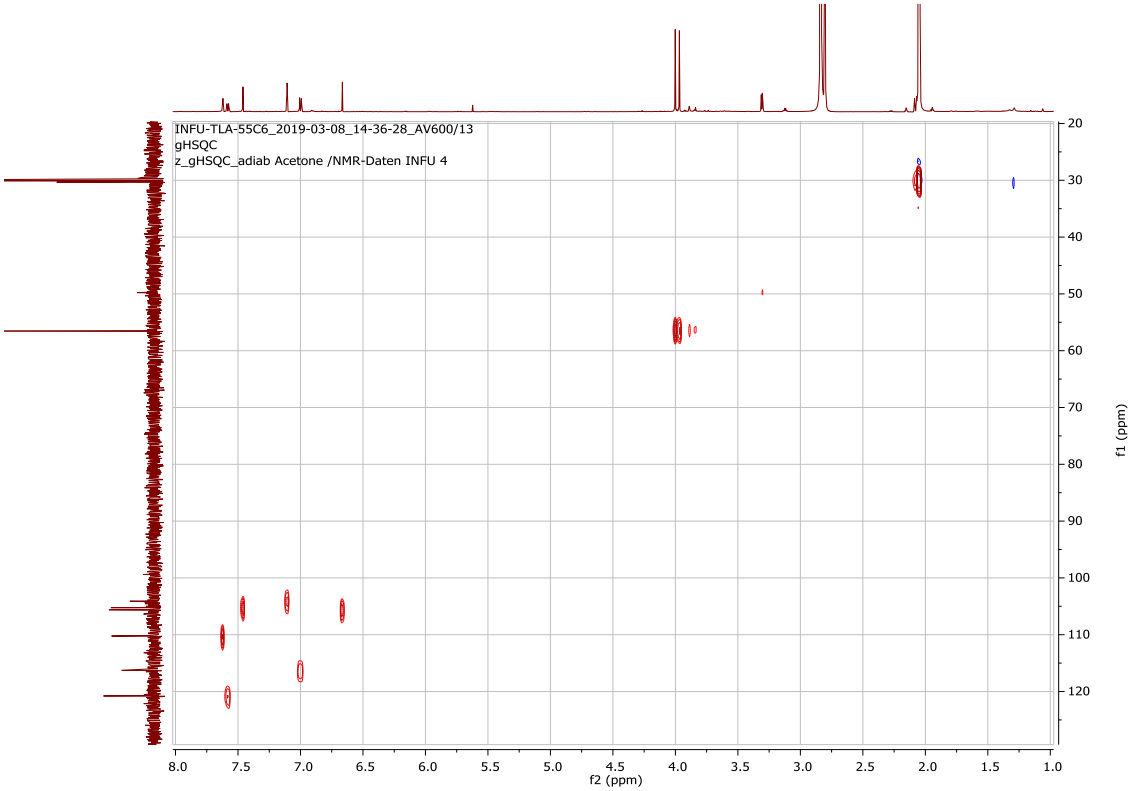
^{13}C NMR spectrum (150 MHz, Acetone-d6) of compound 5



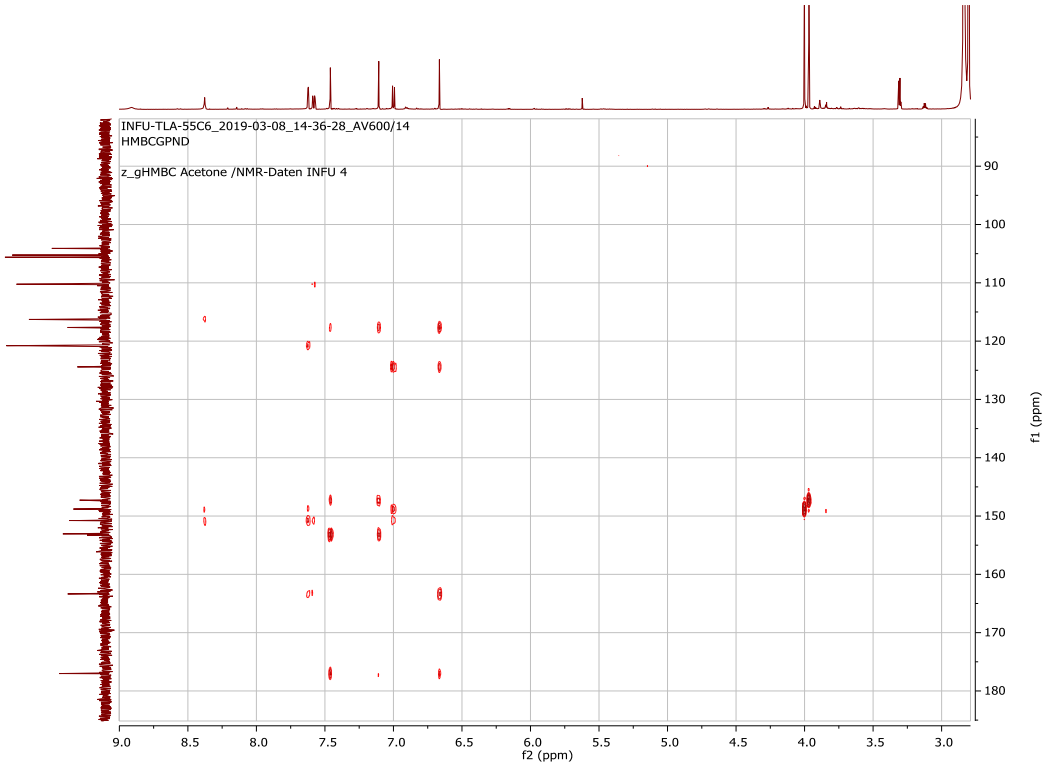
^{1}H-^{1}H-COSY spectrum of compound 5



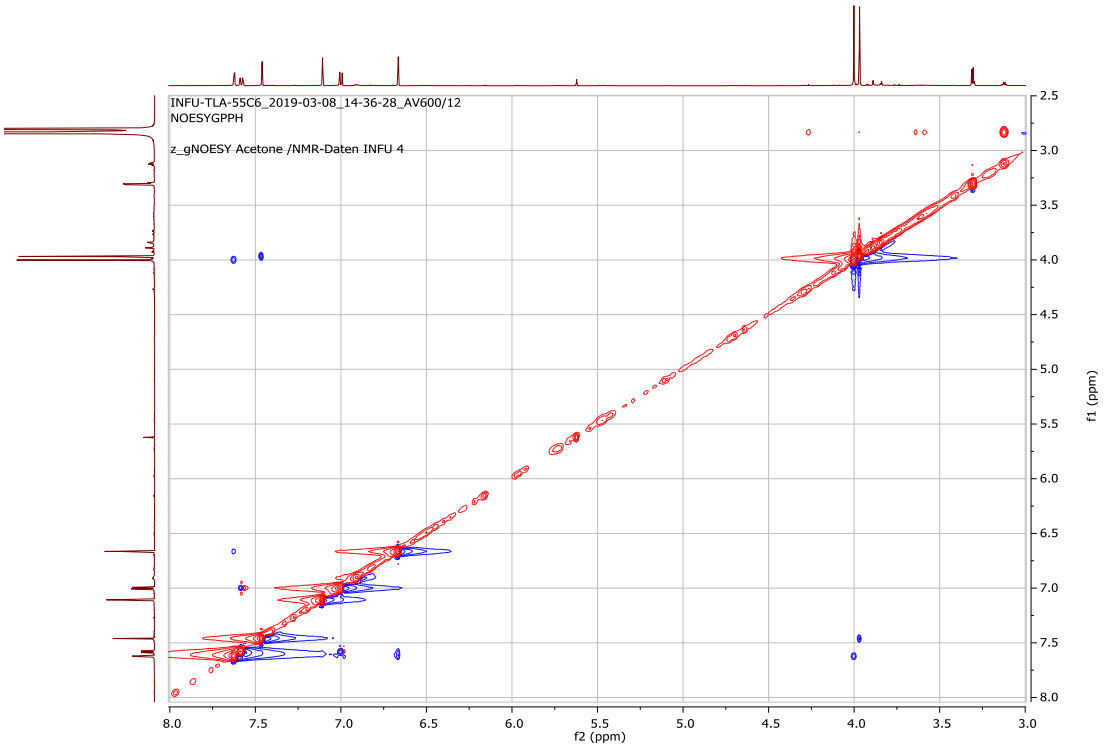
HSQC spectrum of compound 5



HMBC spectrum of compound 5

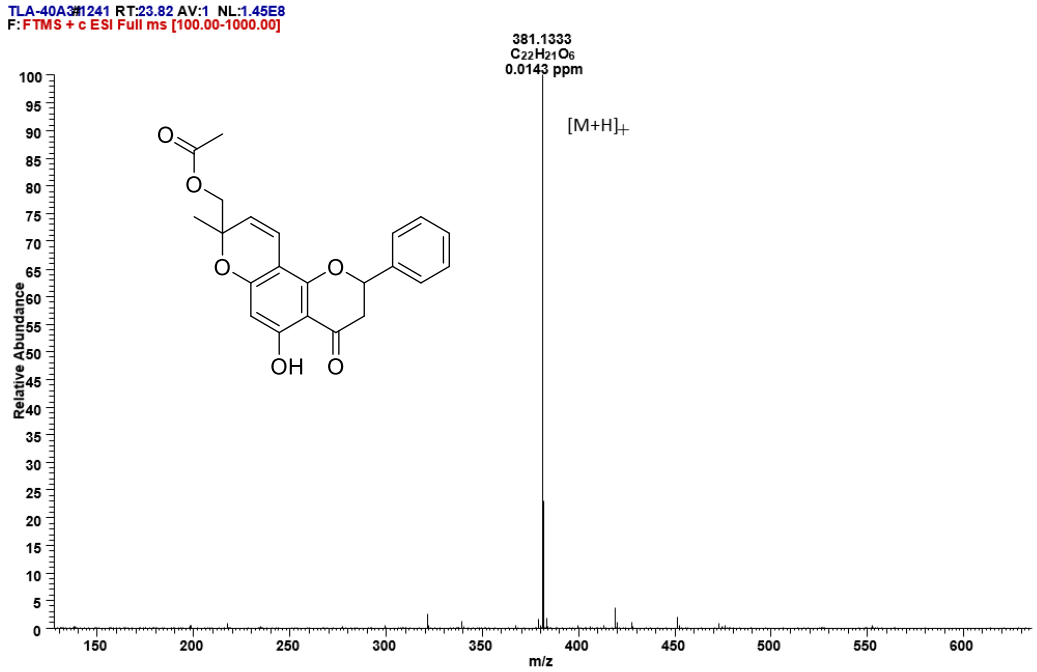


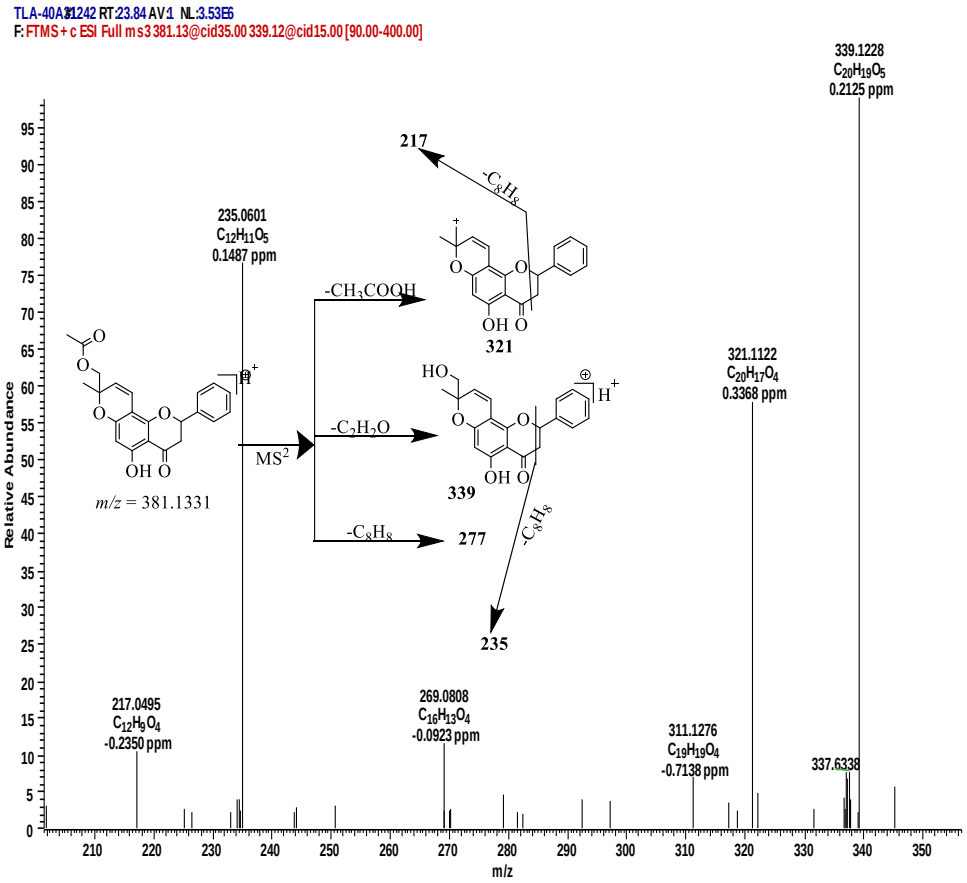
NOESY spectrum of compound 5



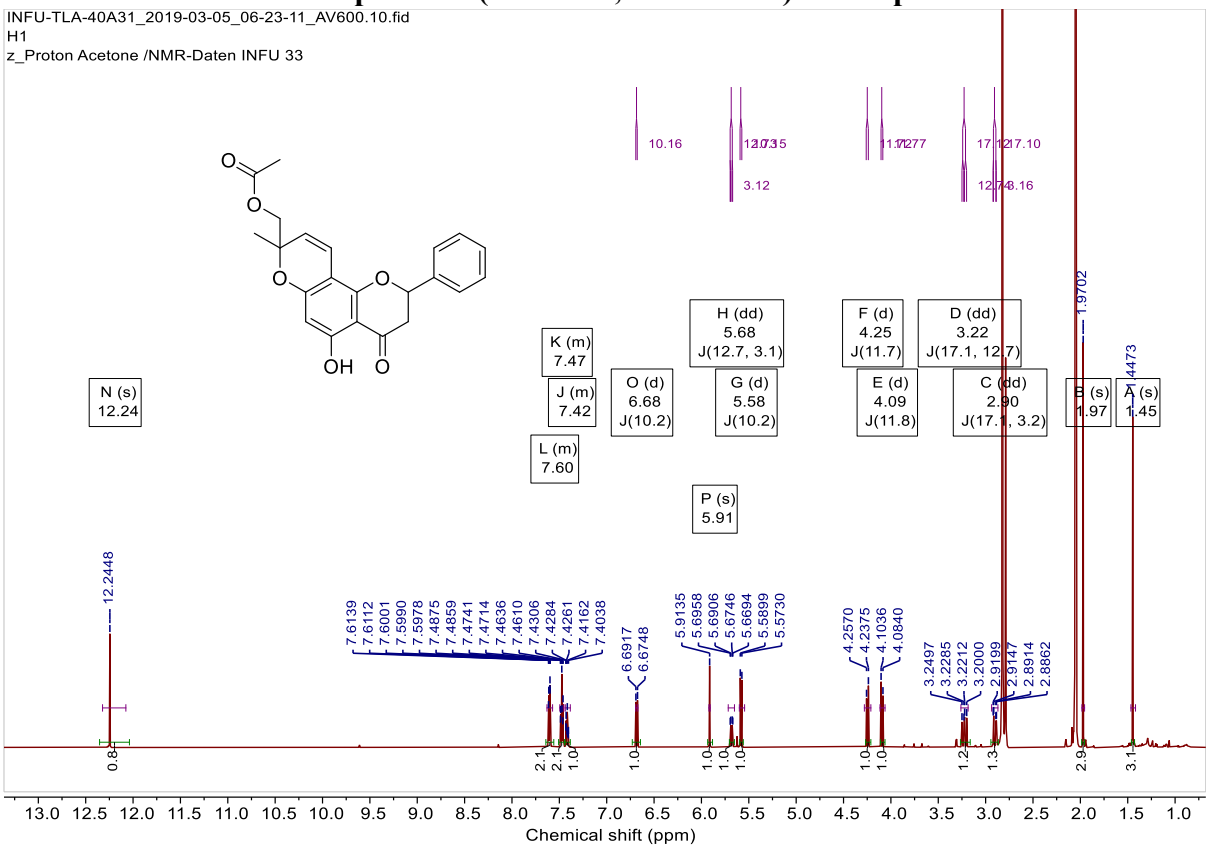
Appendix A6: Spectra for compound 6

HRESIMS spectrum of compound 6

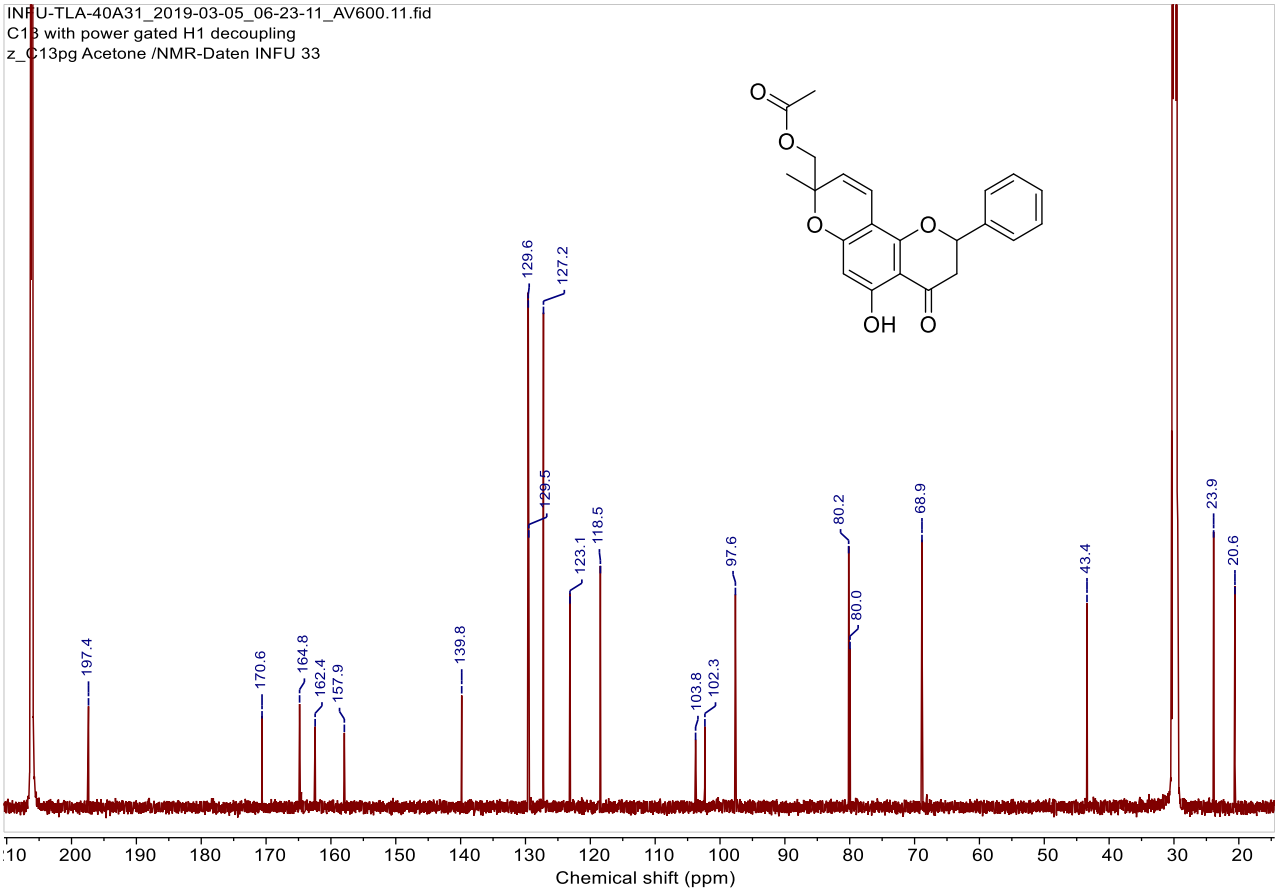




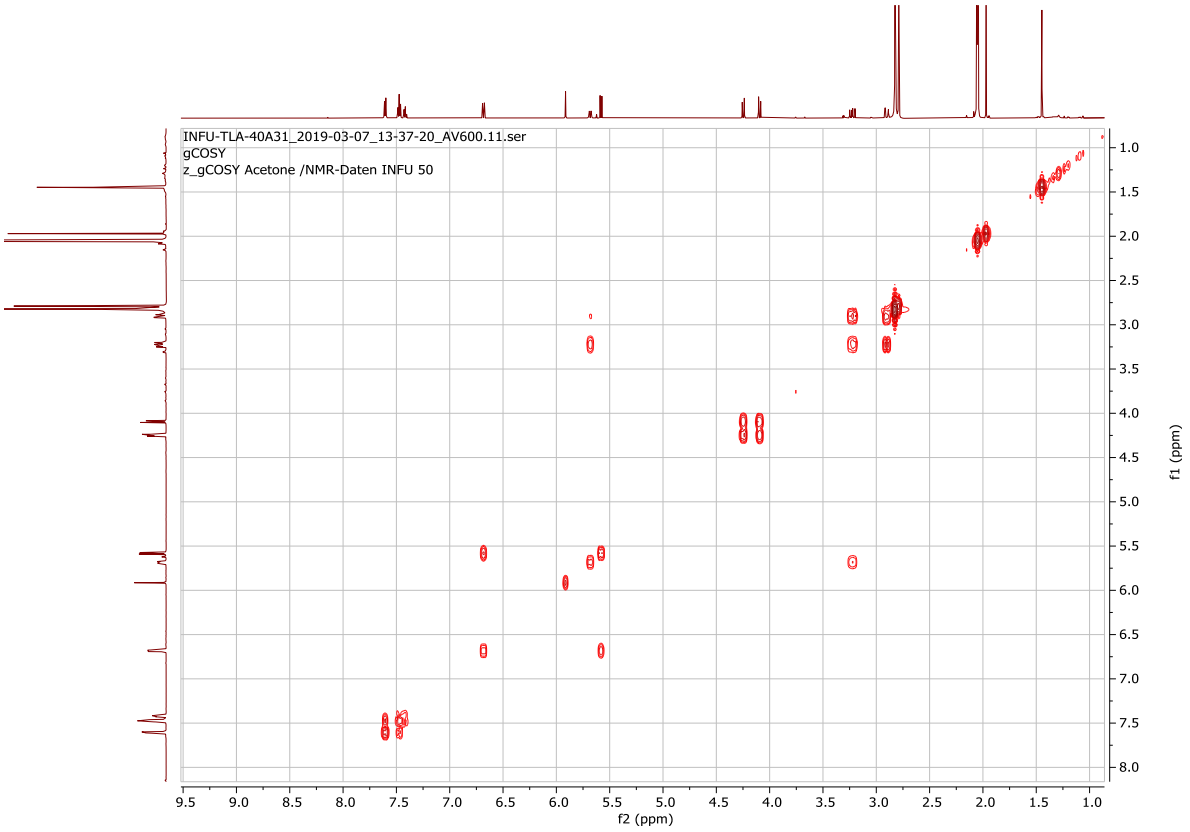
^{1}H NMR spectrum (600 MHz, Acetone-d6) of compound 6



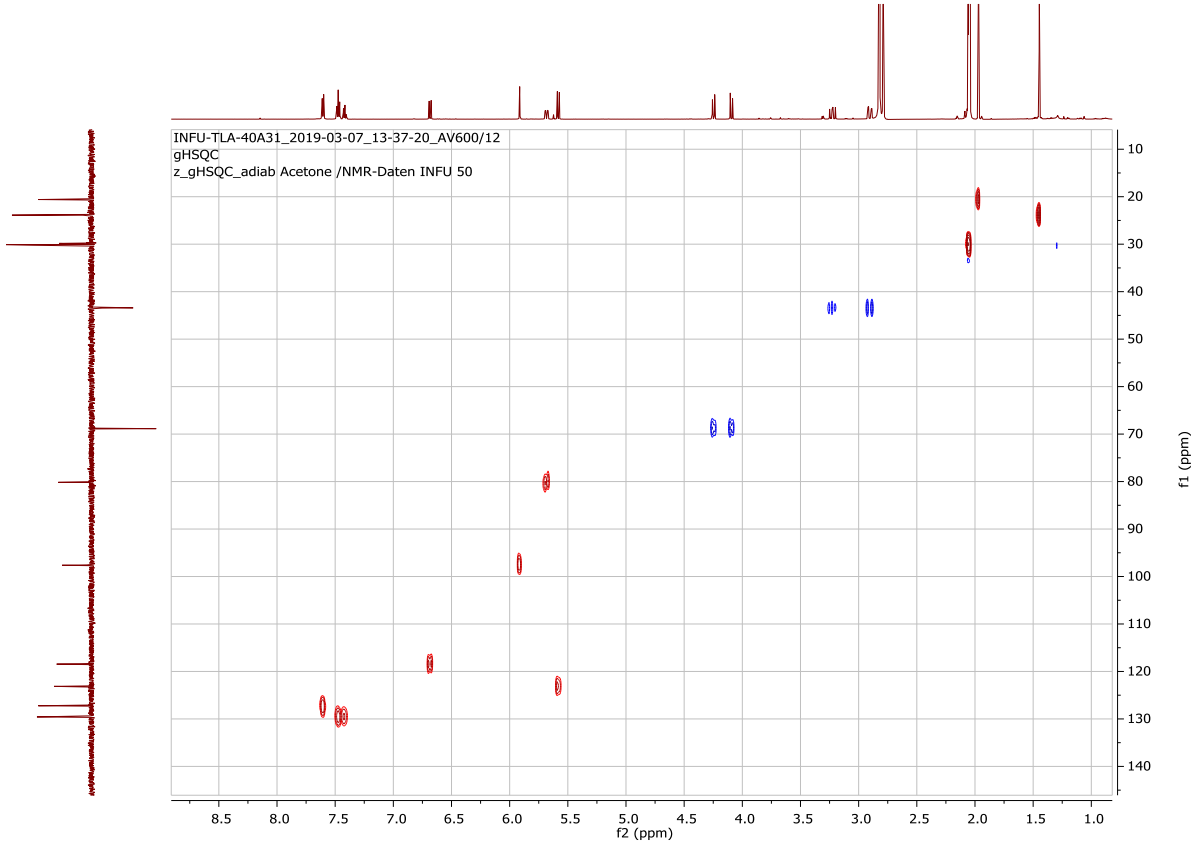
^{13}C NMR spectrum (150 MHz, Acetone-d6) of compound 6



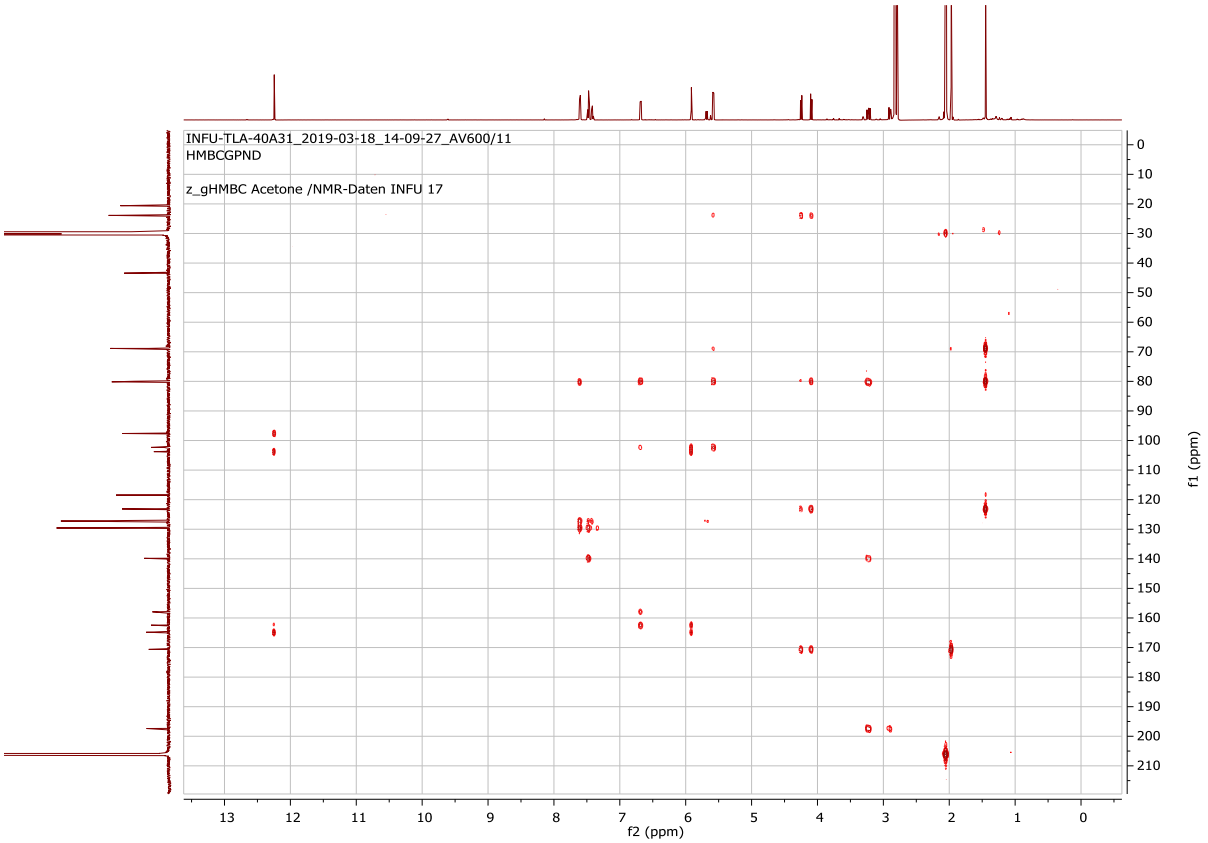
^{1}H-^{1}H-COSY spectrum of compound 6



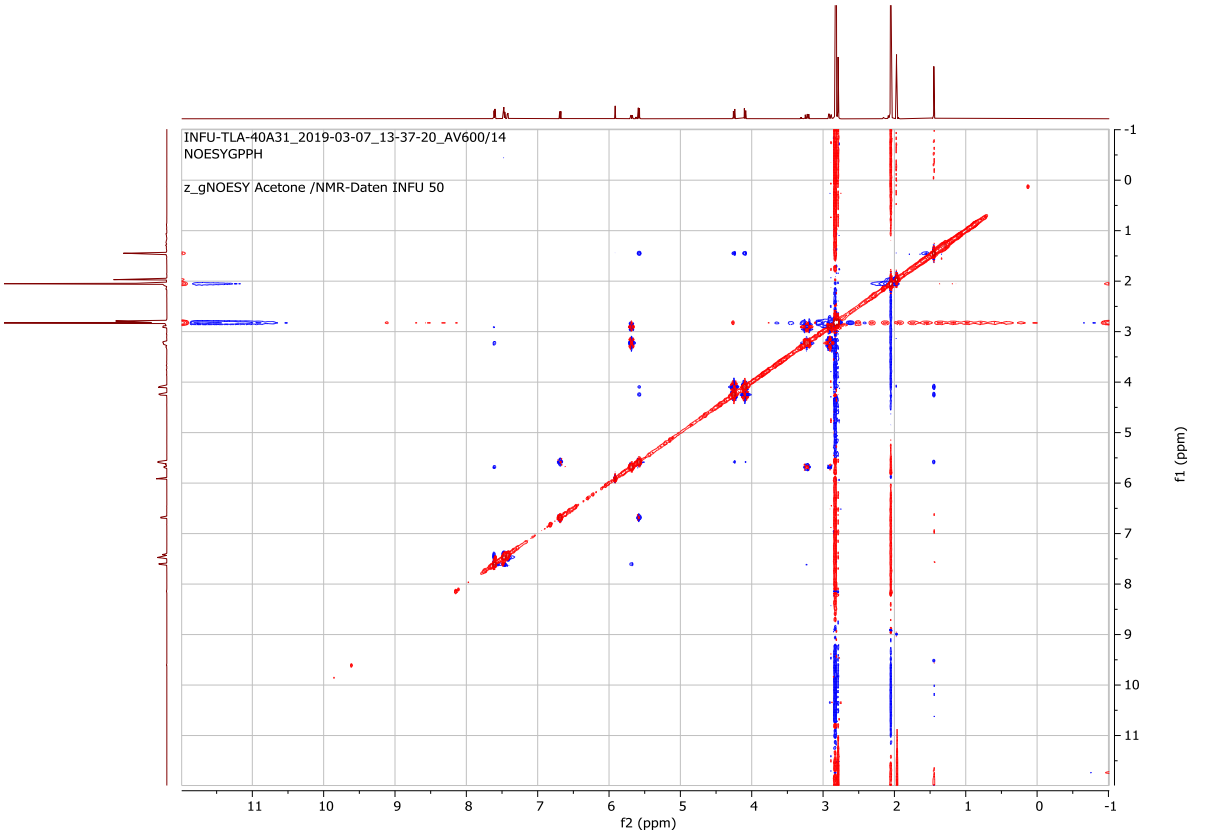
HSQC spectrum of compound 6



HMBC spectrum of compound 6

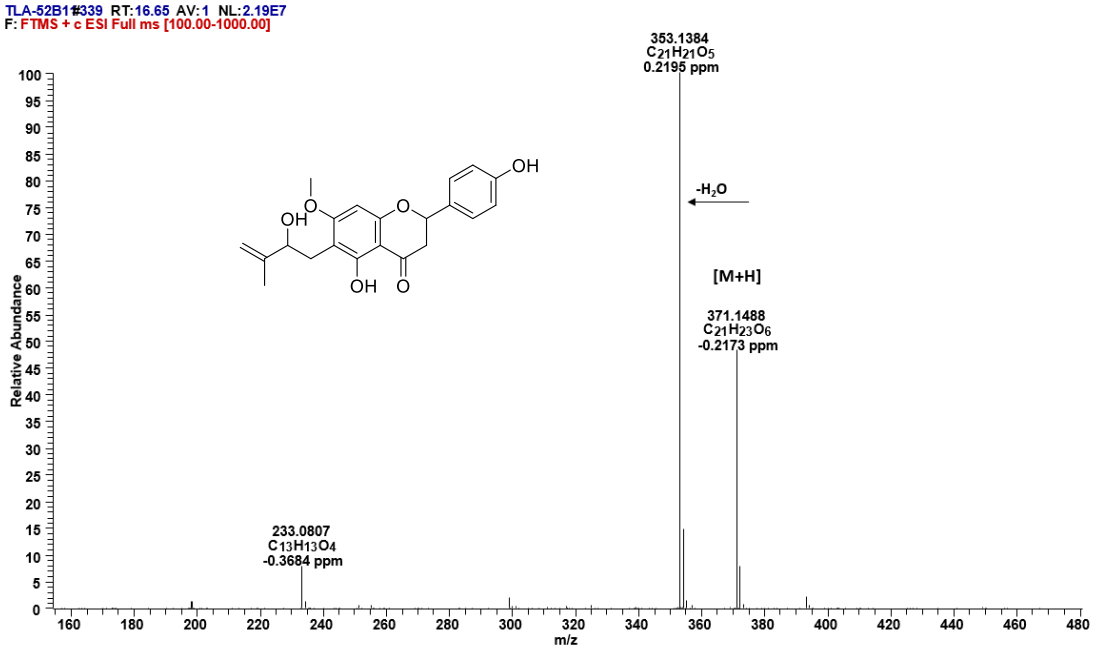


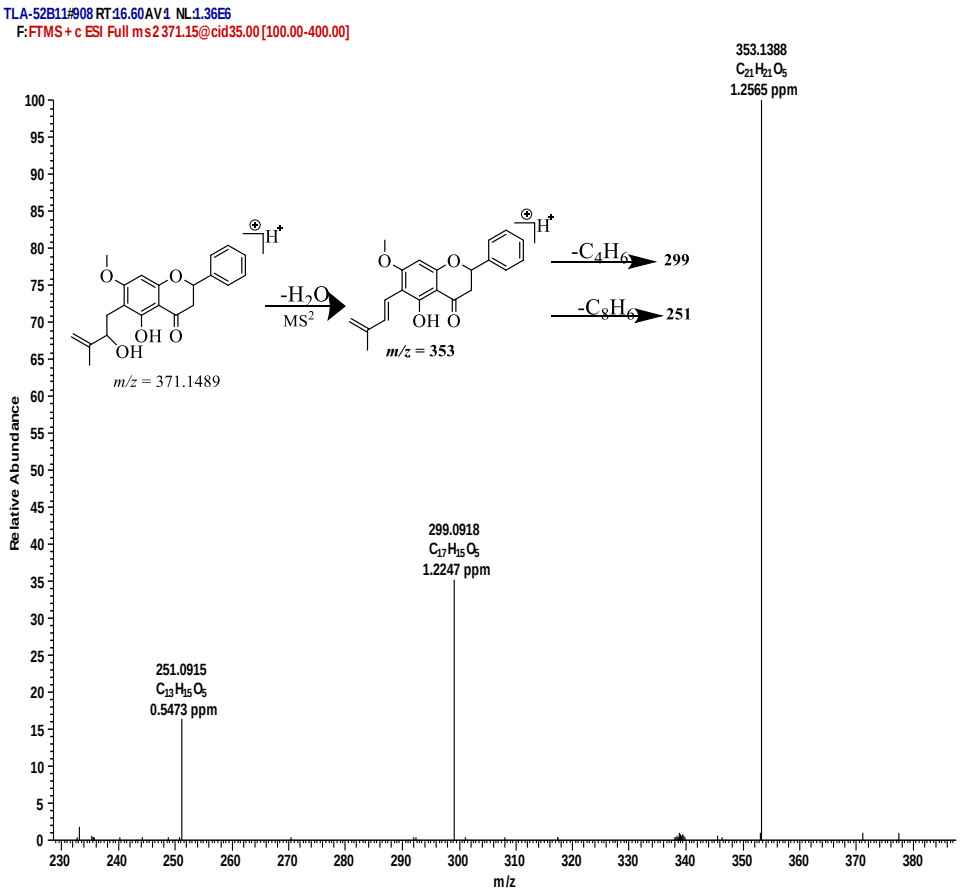
NOESY spectrum of compound 6



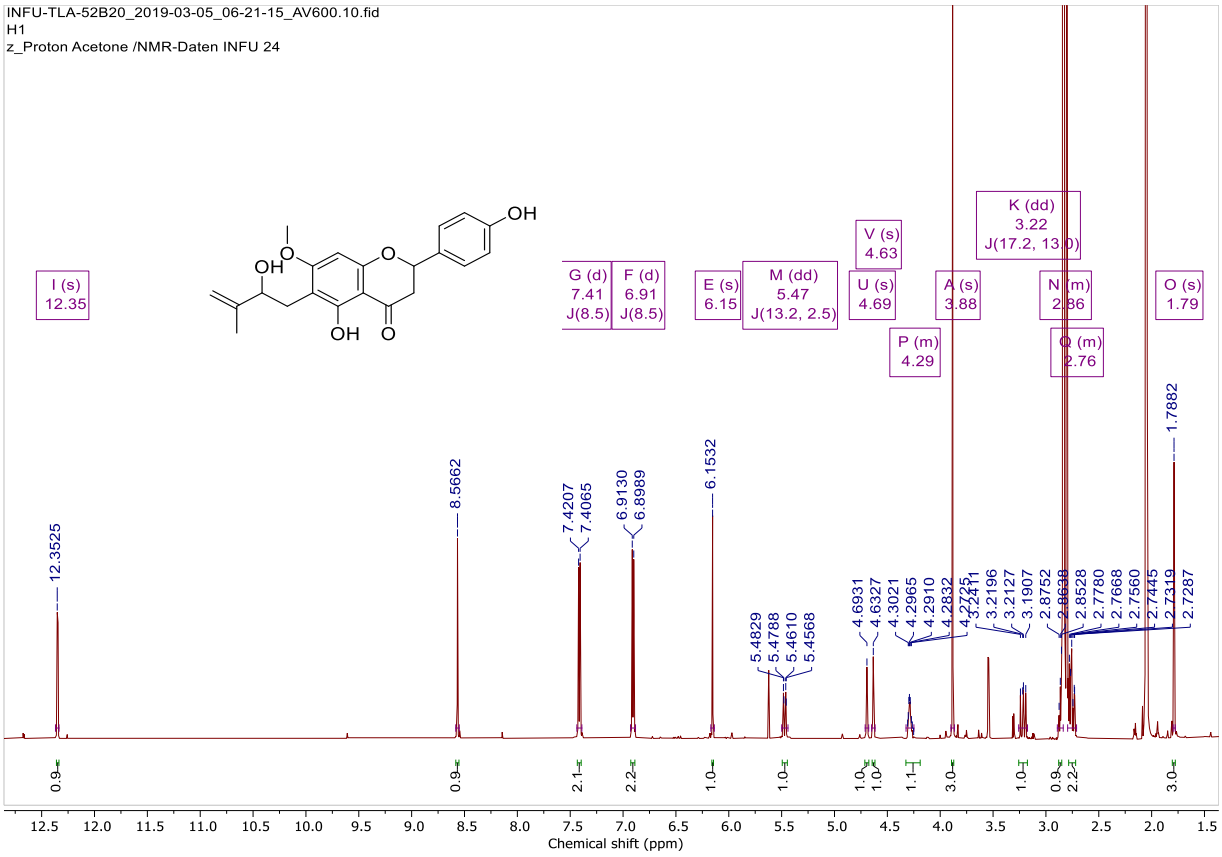
Appendix A7: Spectra for compound 7

HRESIMS spectrum of compound 7

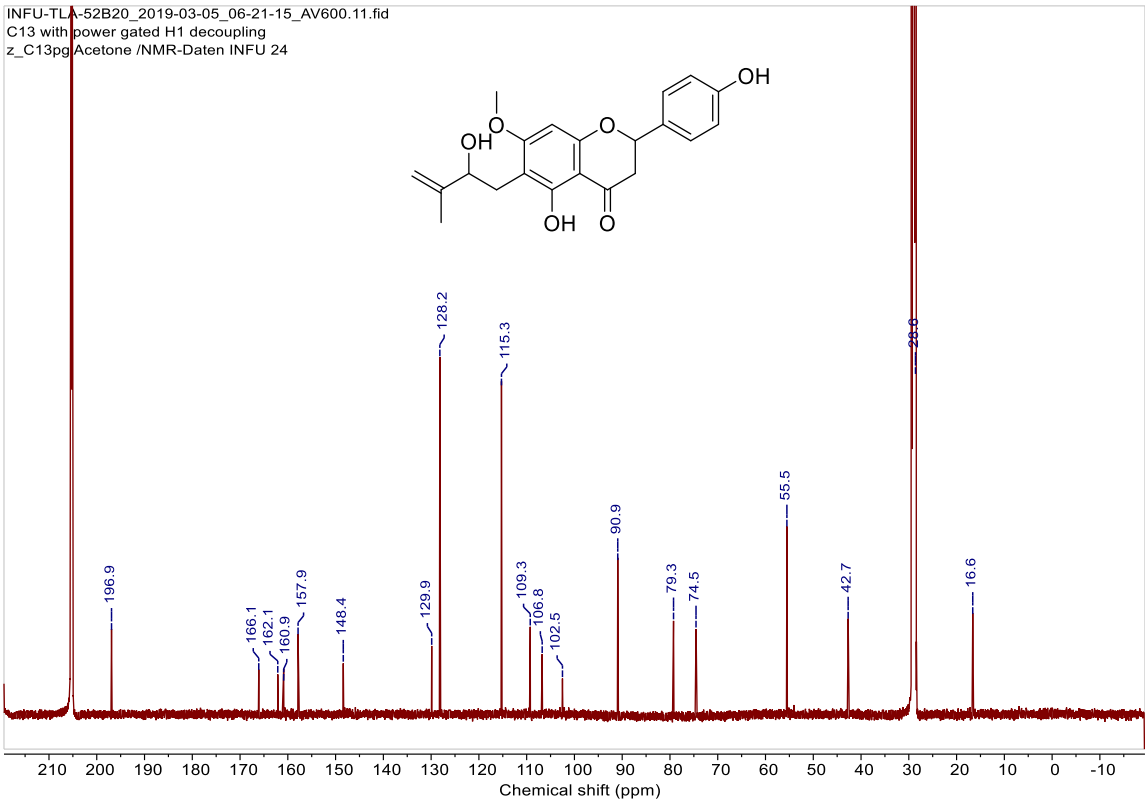




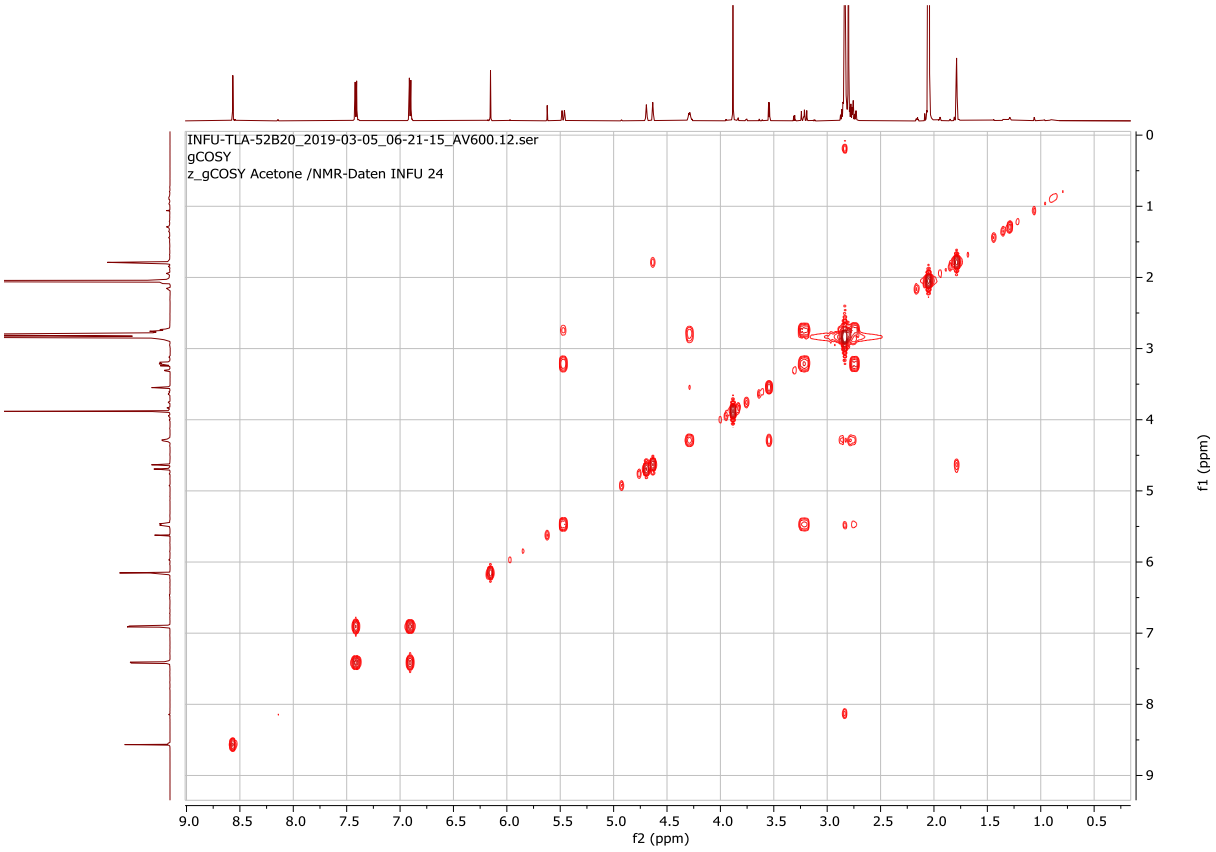
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 7



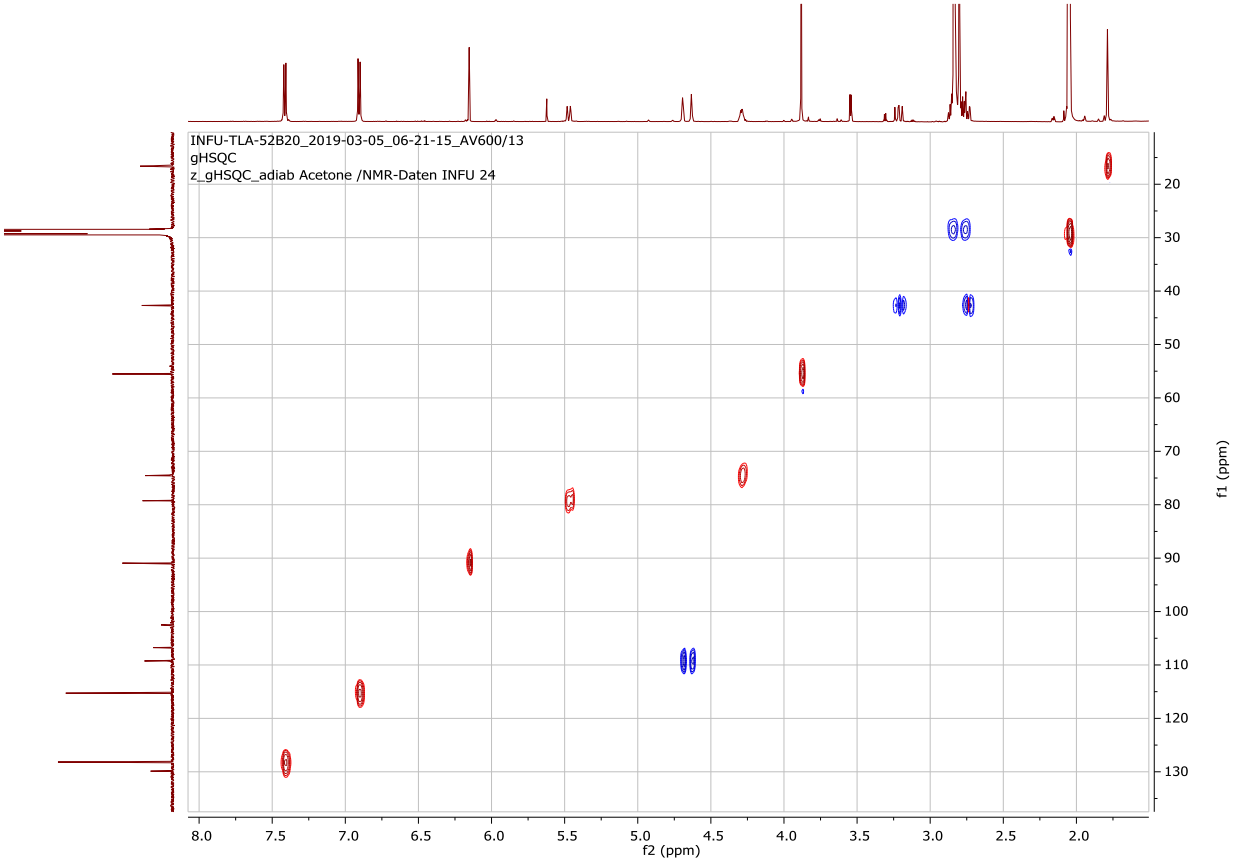
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 7



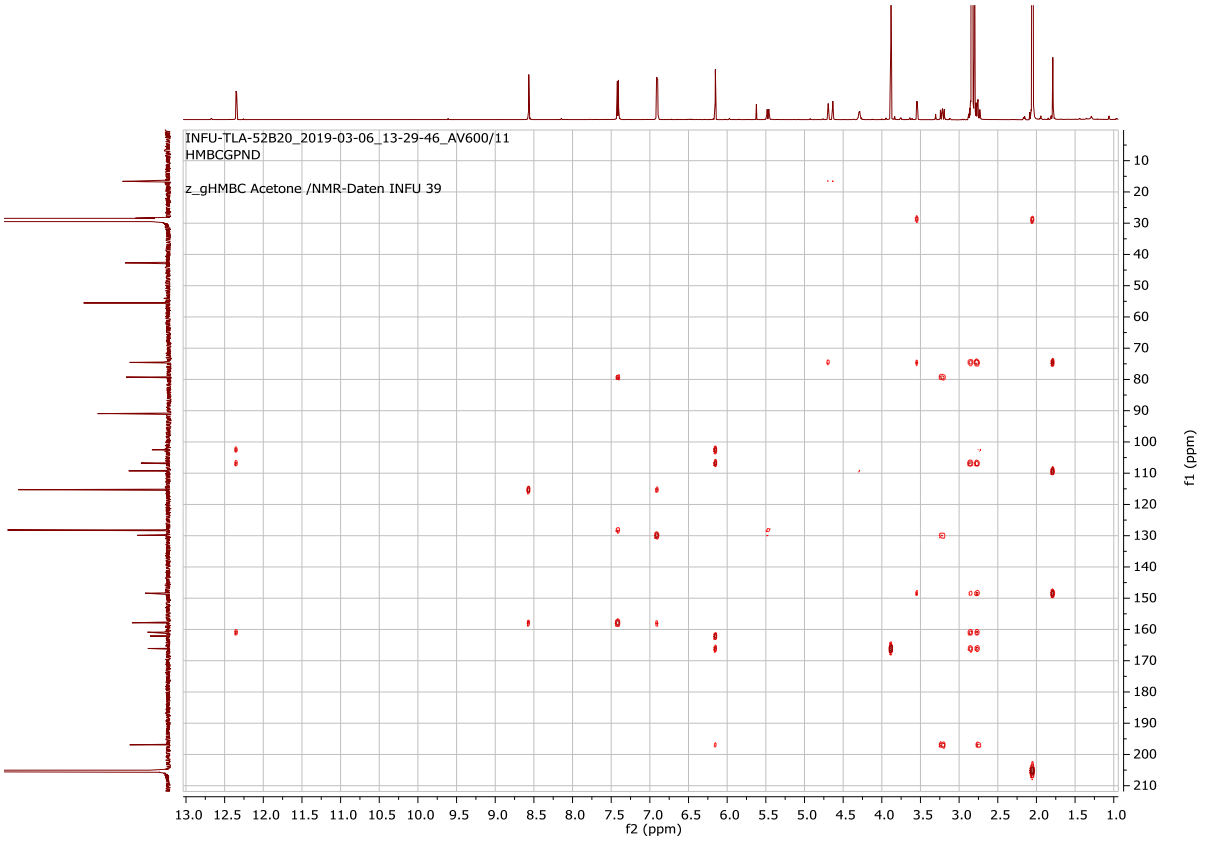
^{1}H-^{1}H-COSY spectrum of compound 7



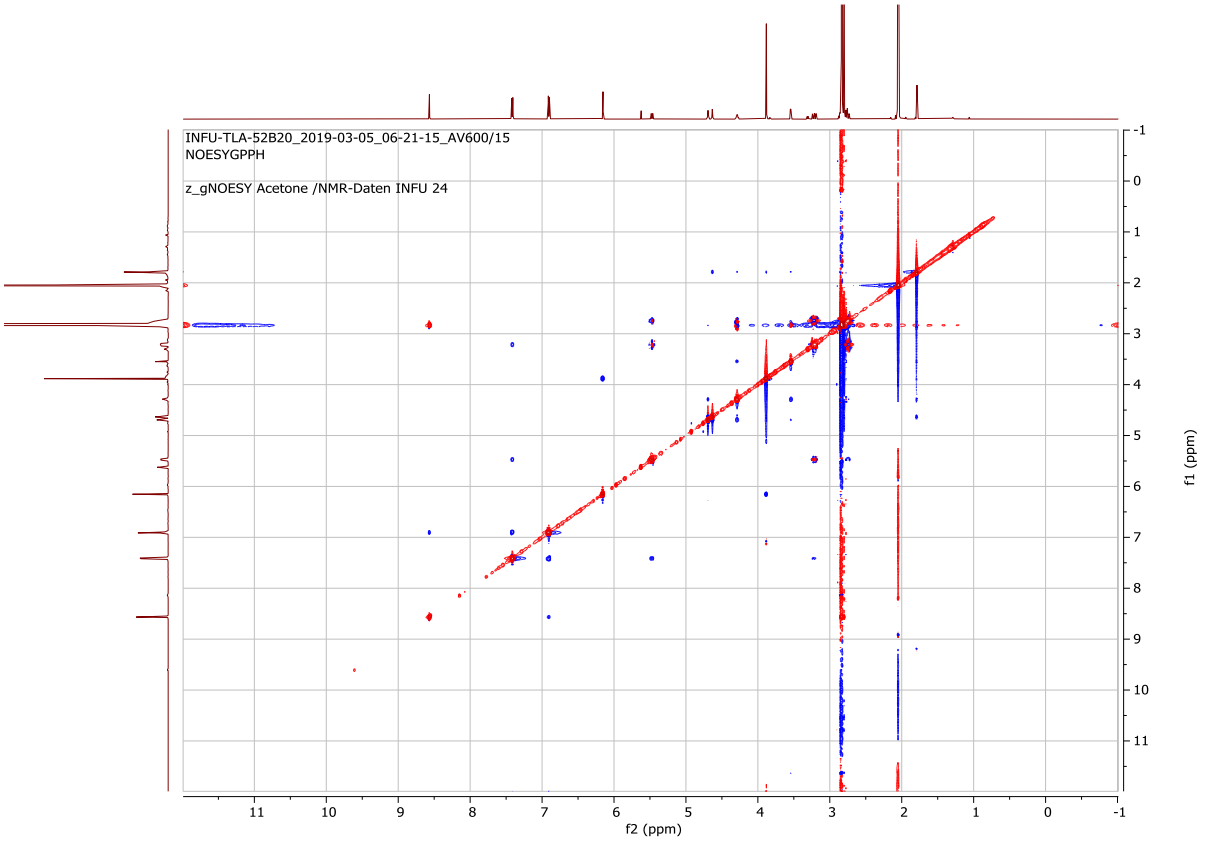
HSQC spectrum of compound 7



HMBC spectrum of compound 7

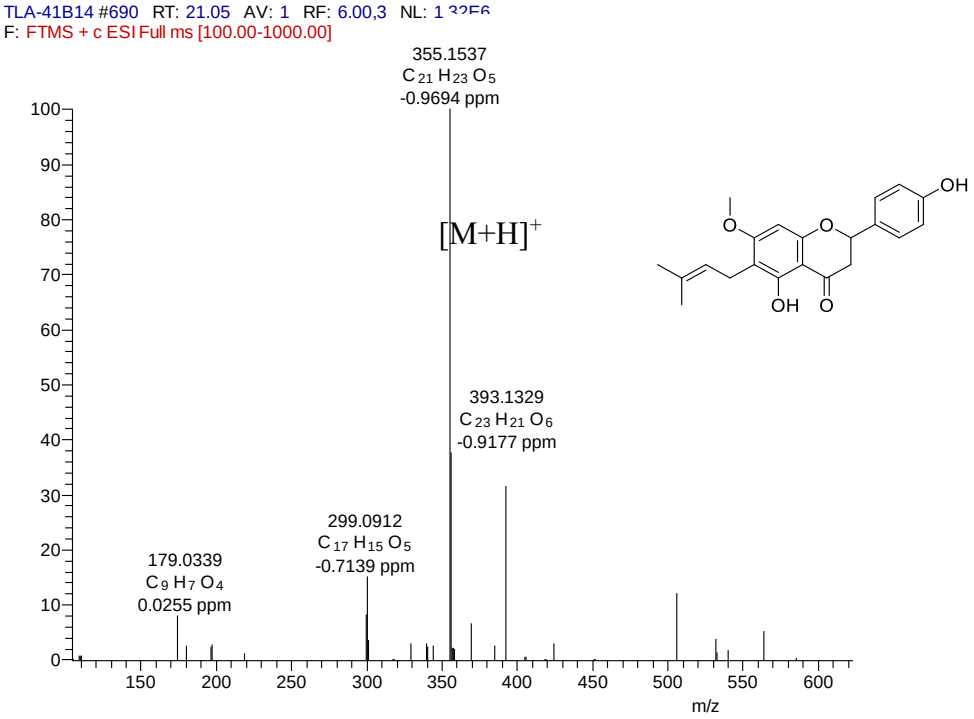


NOESY spectrum of compound 7

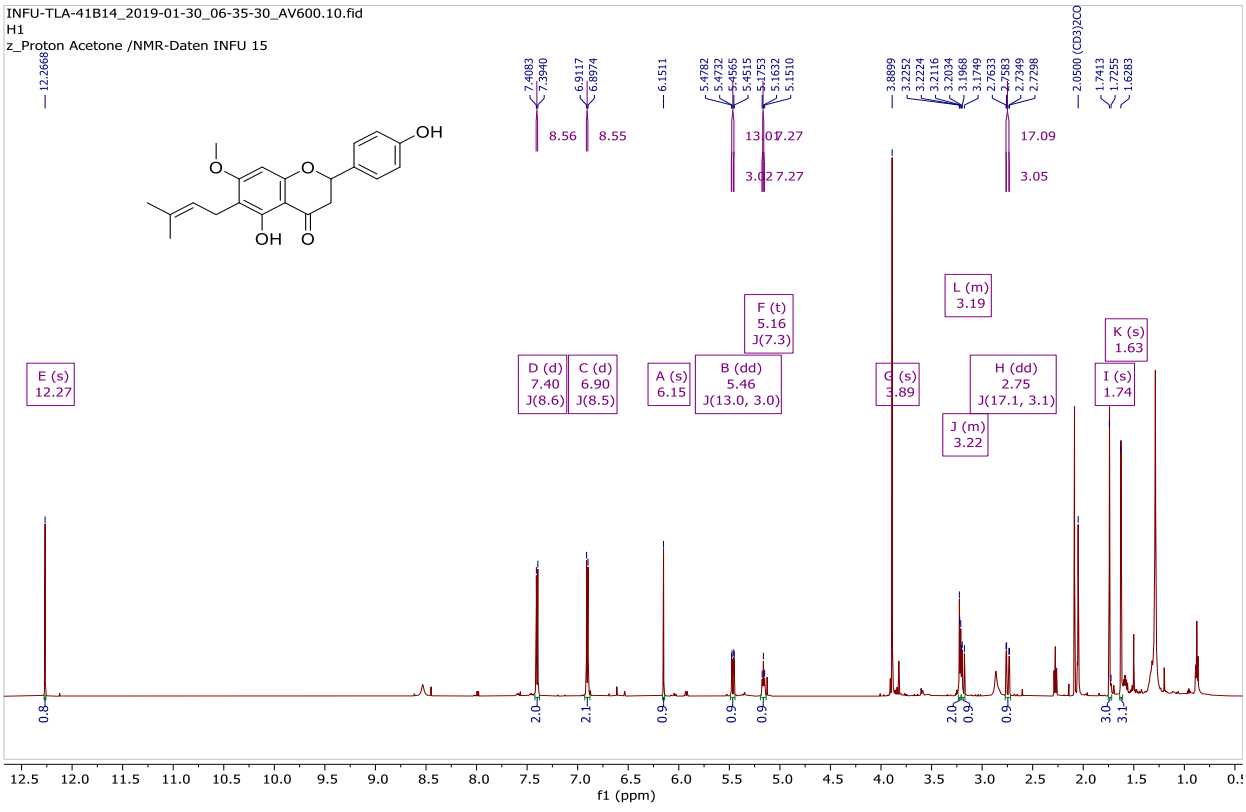


Appendix A8: Spectra for compound 8

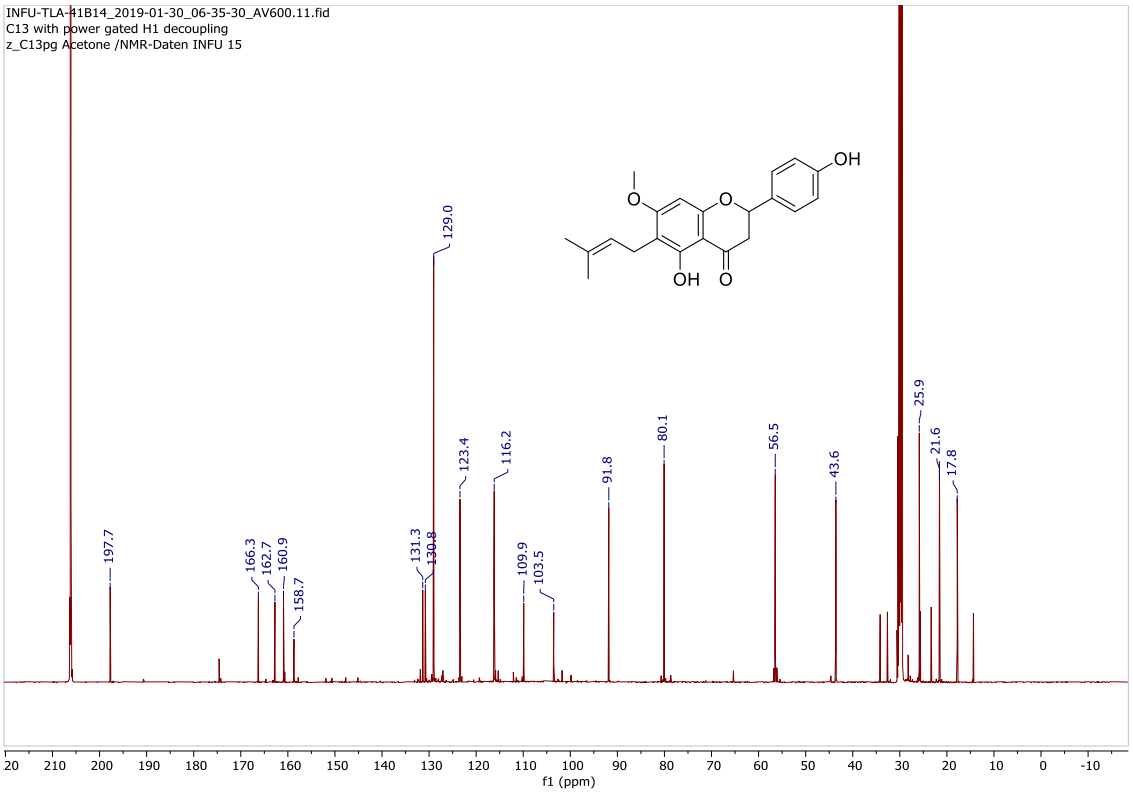
HRESIMS spectrum of compound 8



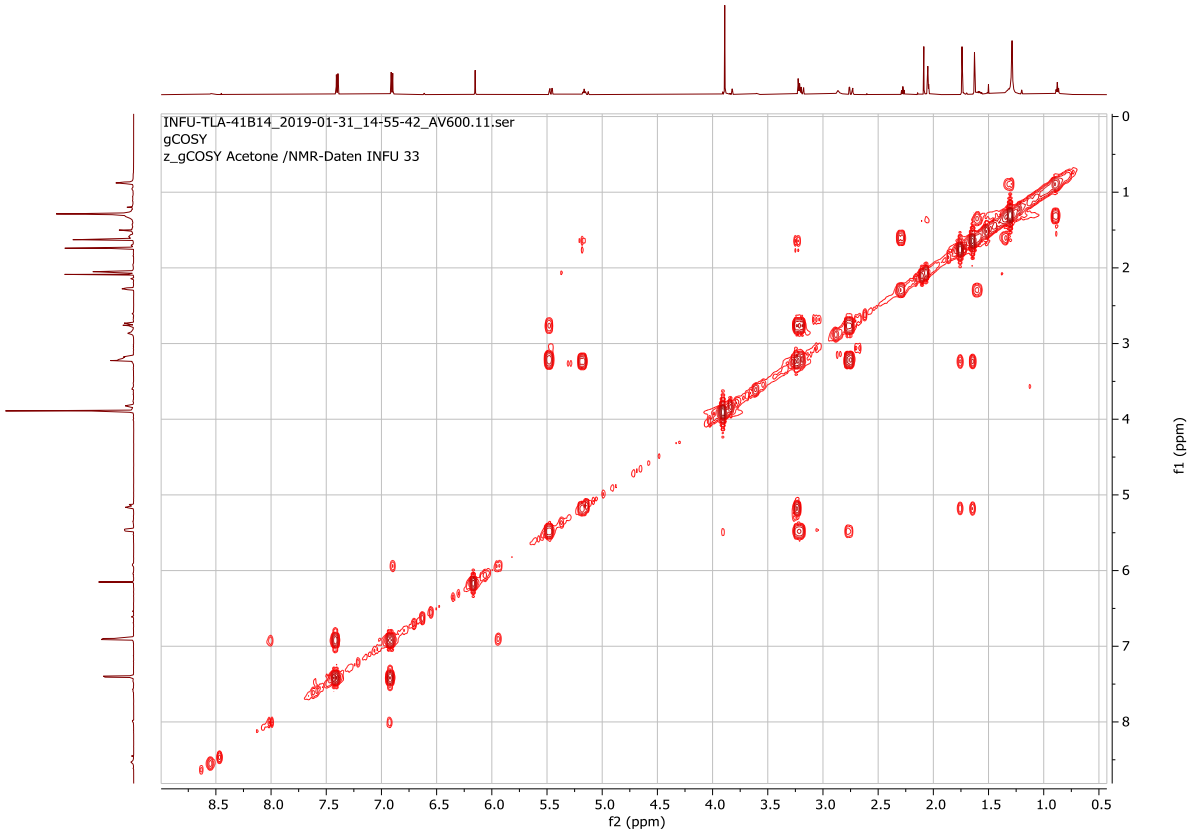
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 8



^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 8

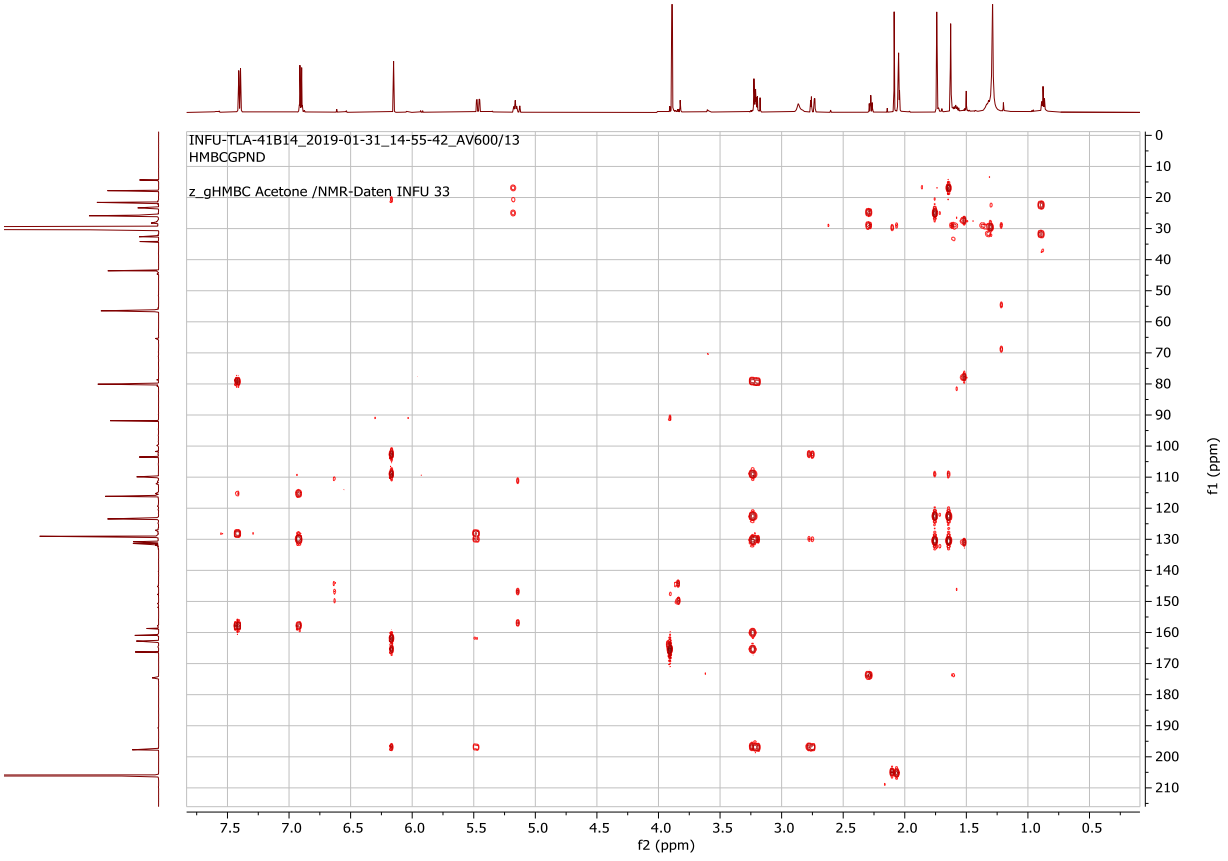


^{1}H-^{1}H-COSY spectrum of compound 8



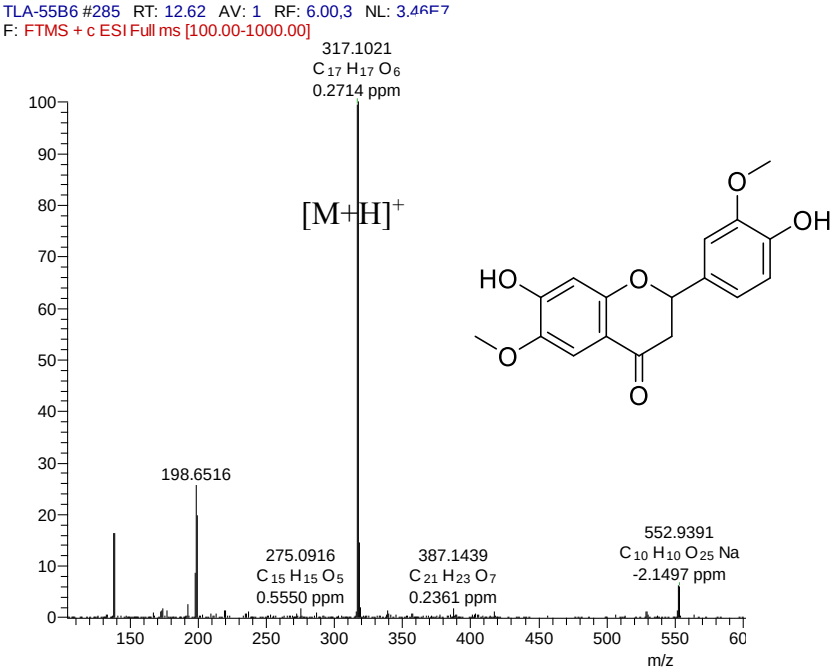
HSQC spectrum of compound 8

HMBC spectrum of compound 8

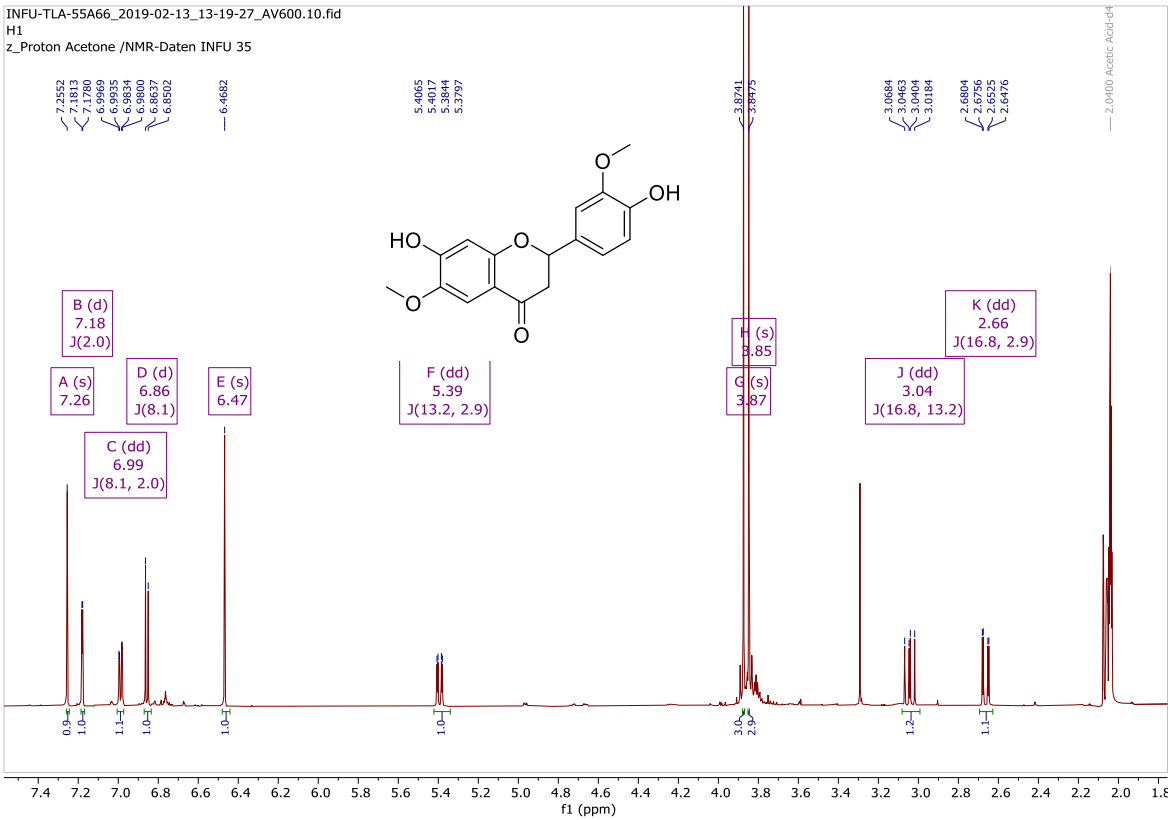


Appendix A9: Spectra for compound 9

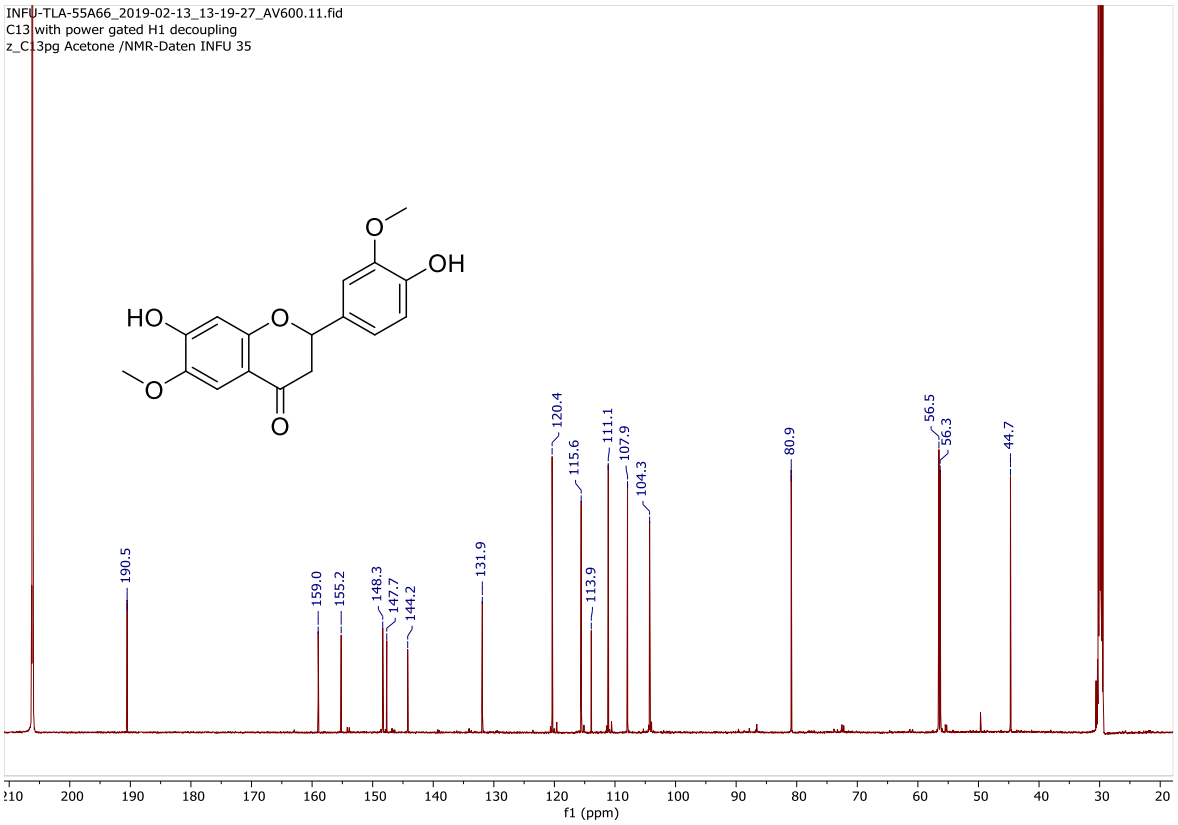
HRESIMS spectrum of compound 9



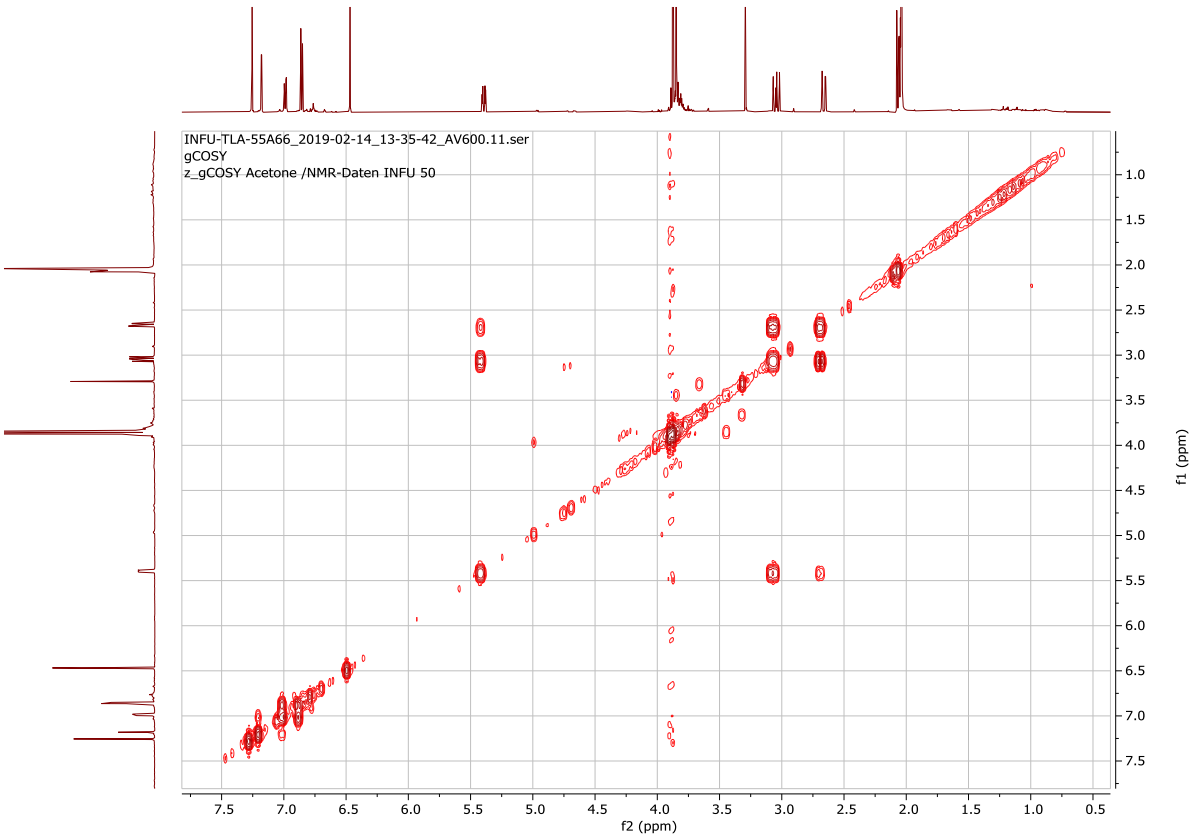
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 9



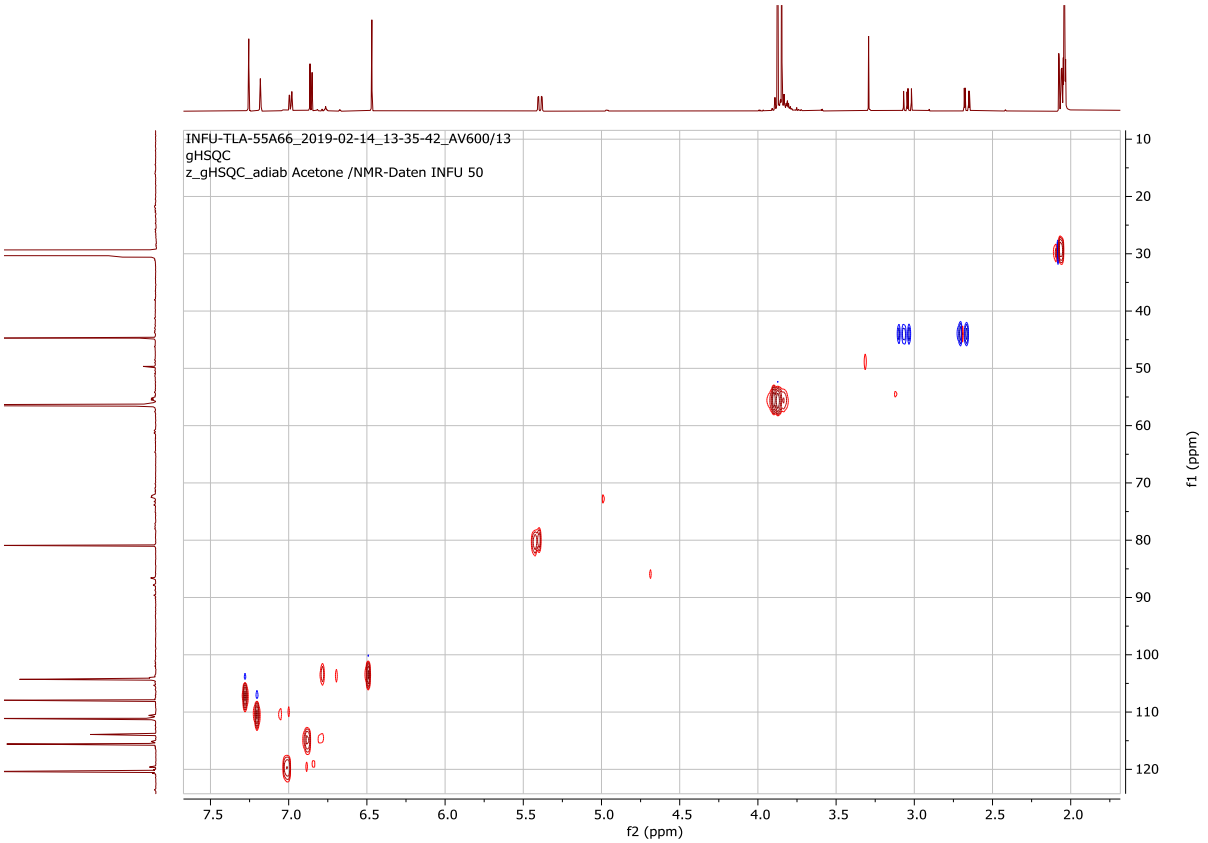
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 9



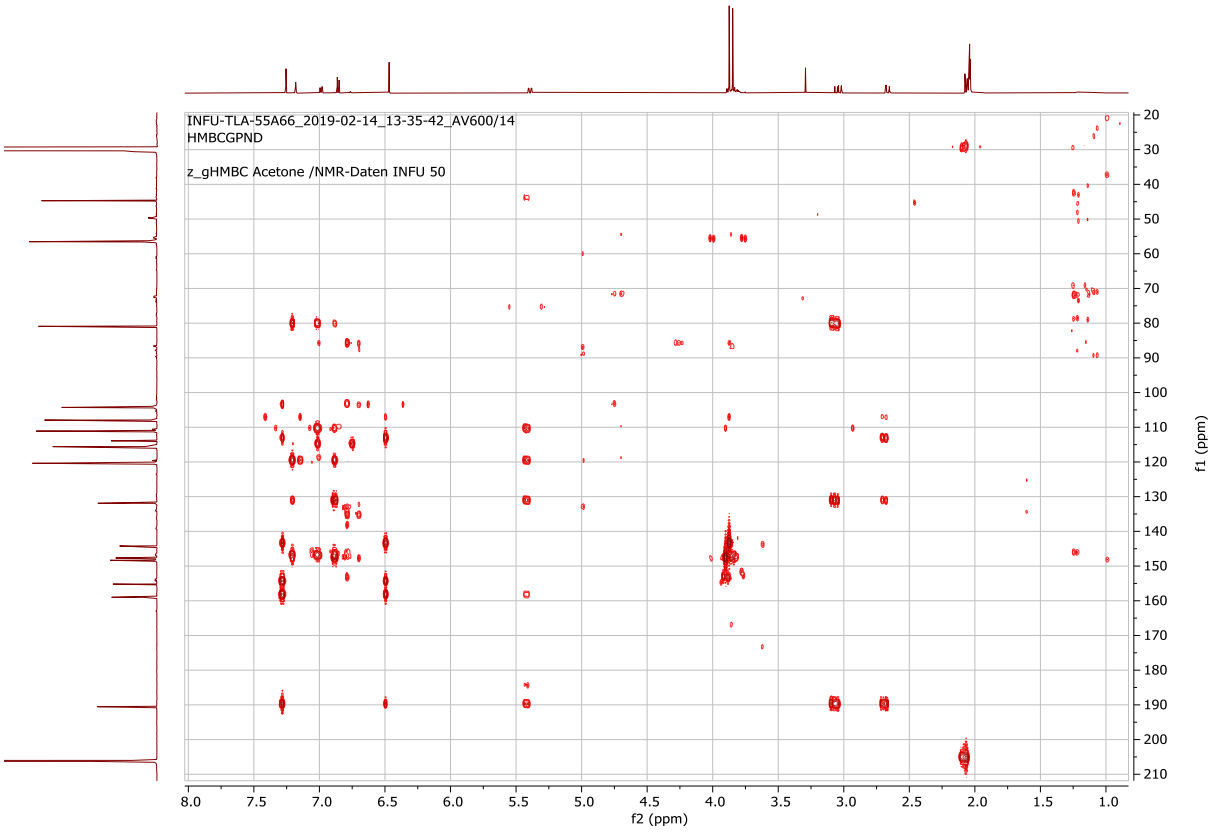
^{1}H-^{1}H-COSY spectrum of compound 9



HSQC spectrum of compound 9

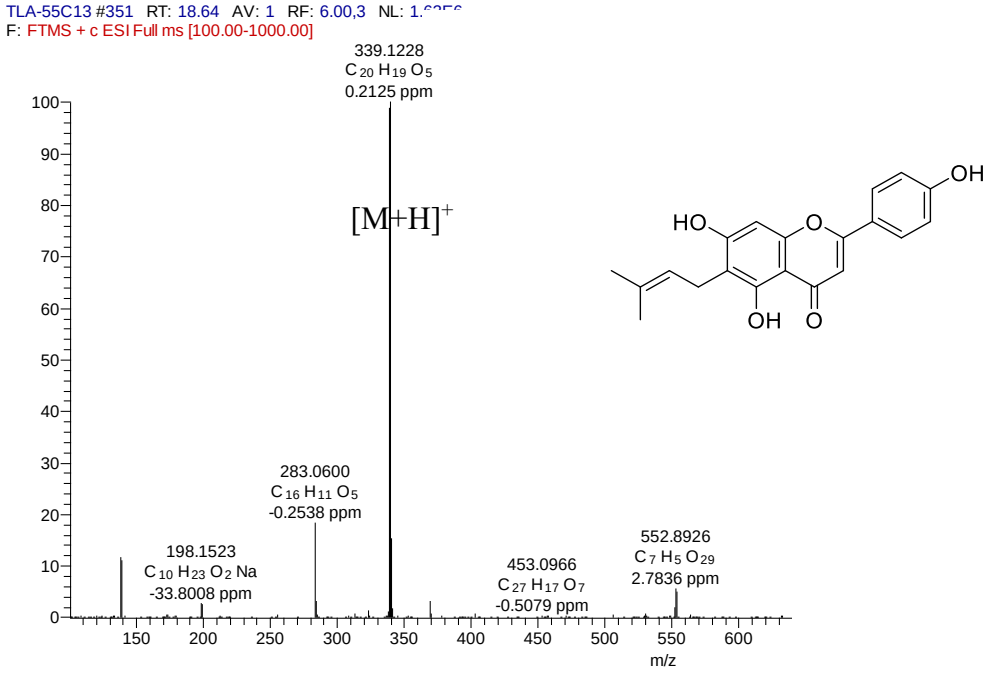


HMBC spectrum of compound 9

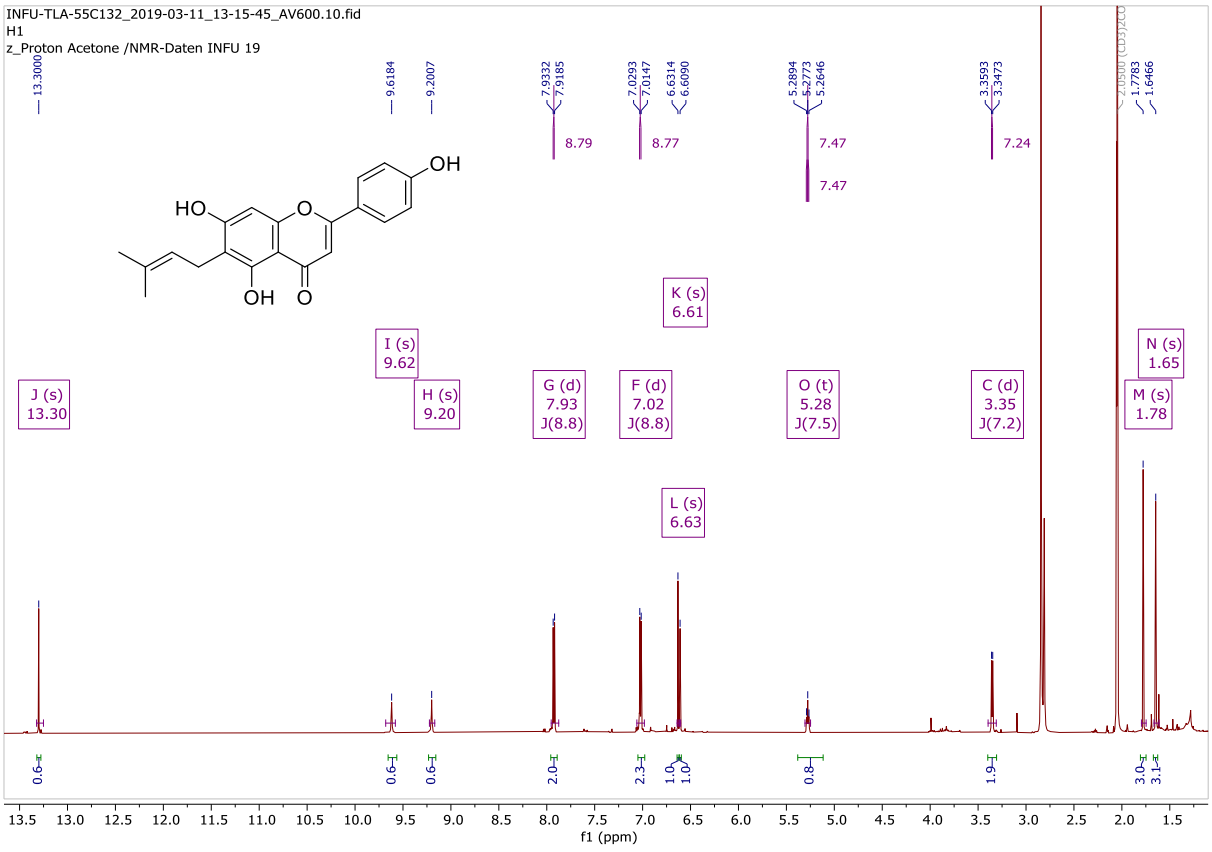


Appendix A10: Spectra for compound 10

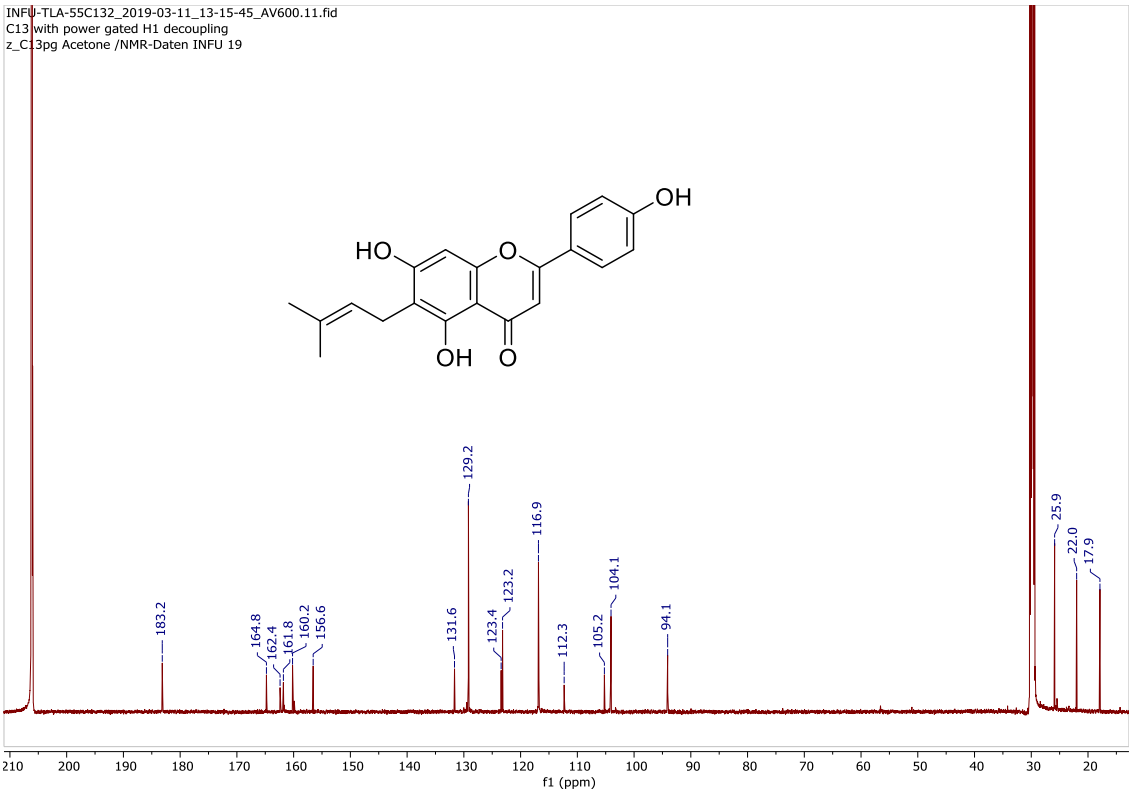
HRESIMS spectrum of compound 10



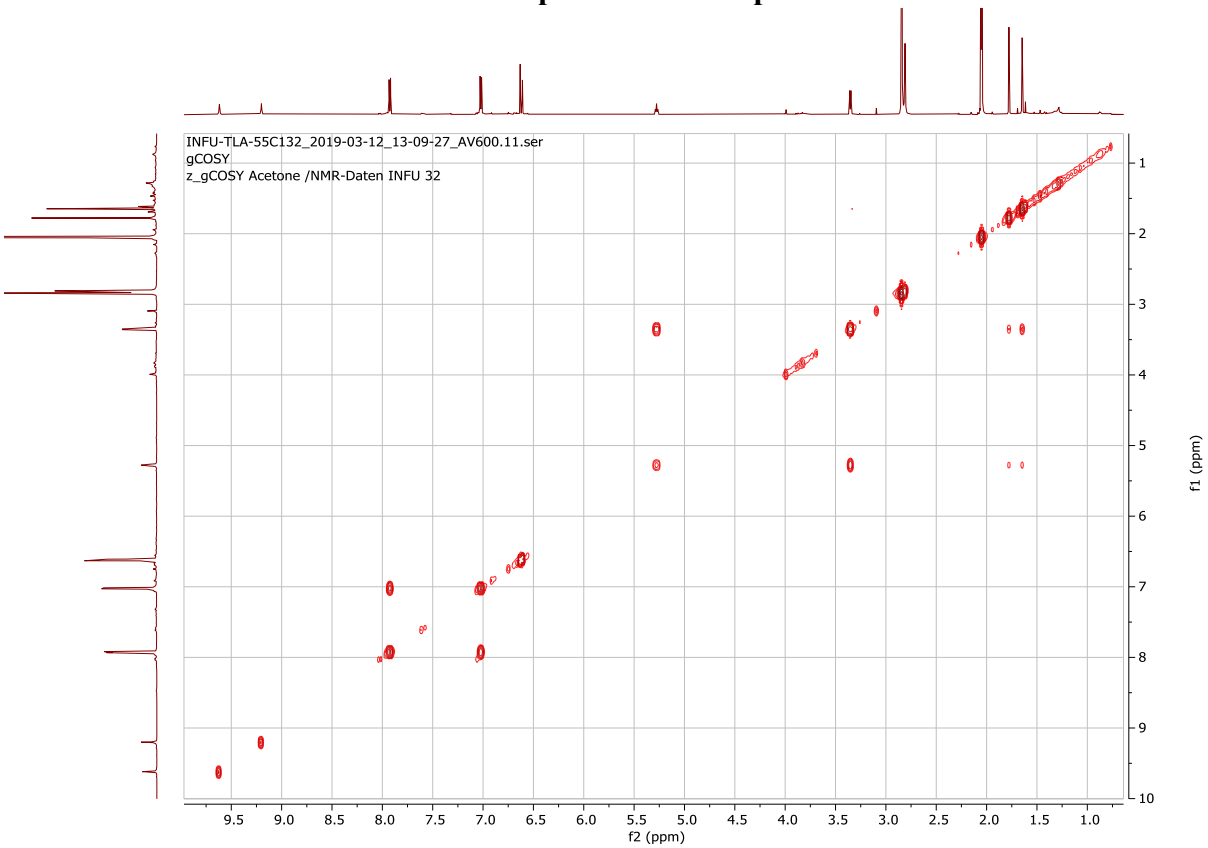
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 10



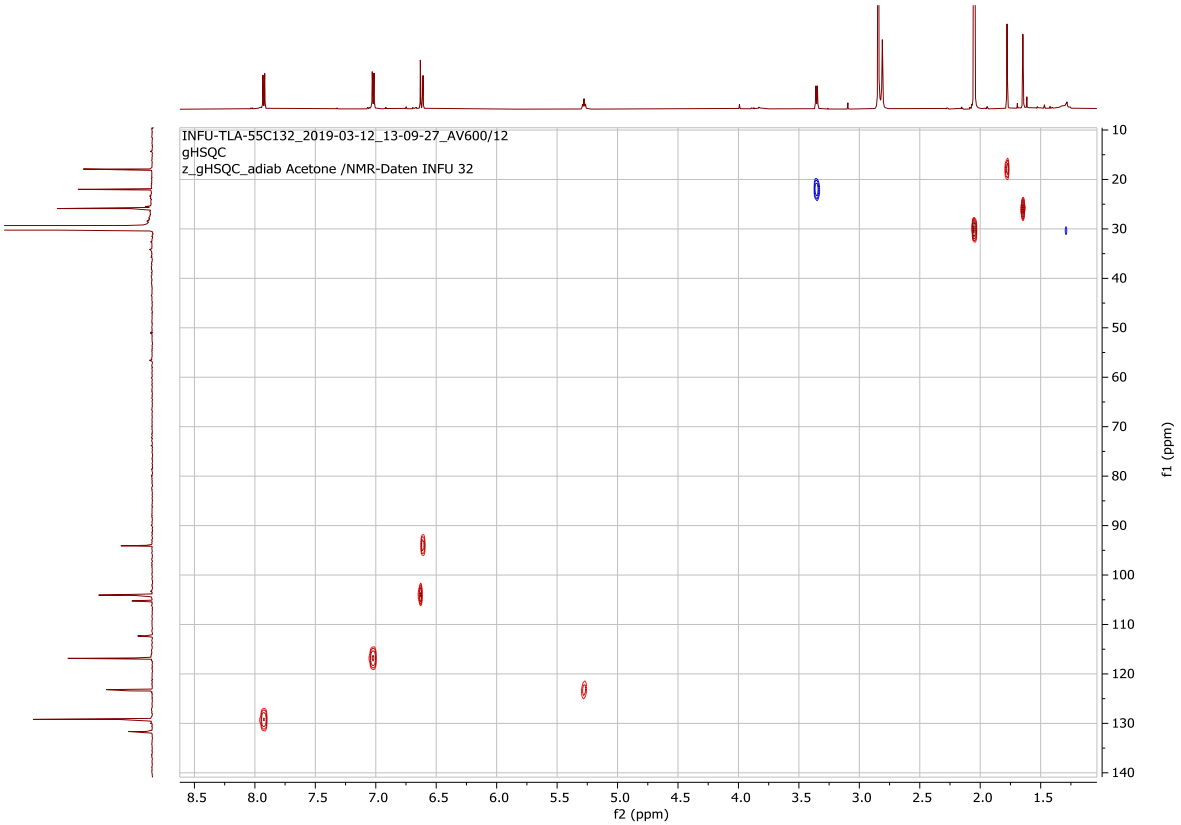
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 10



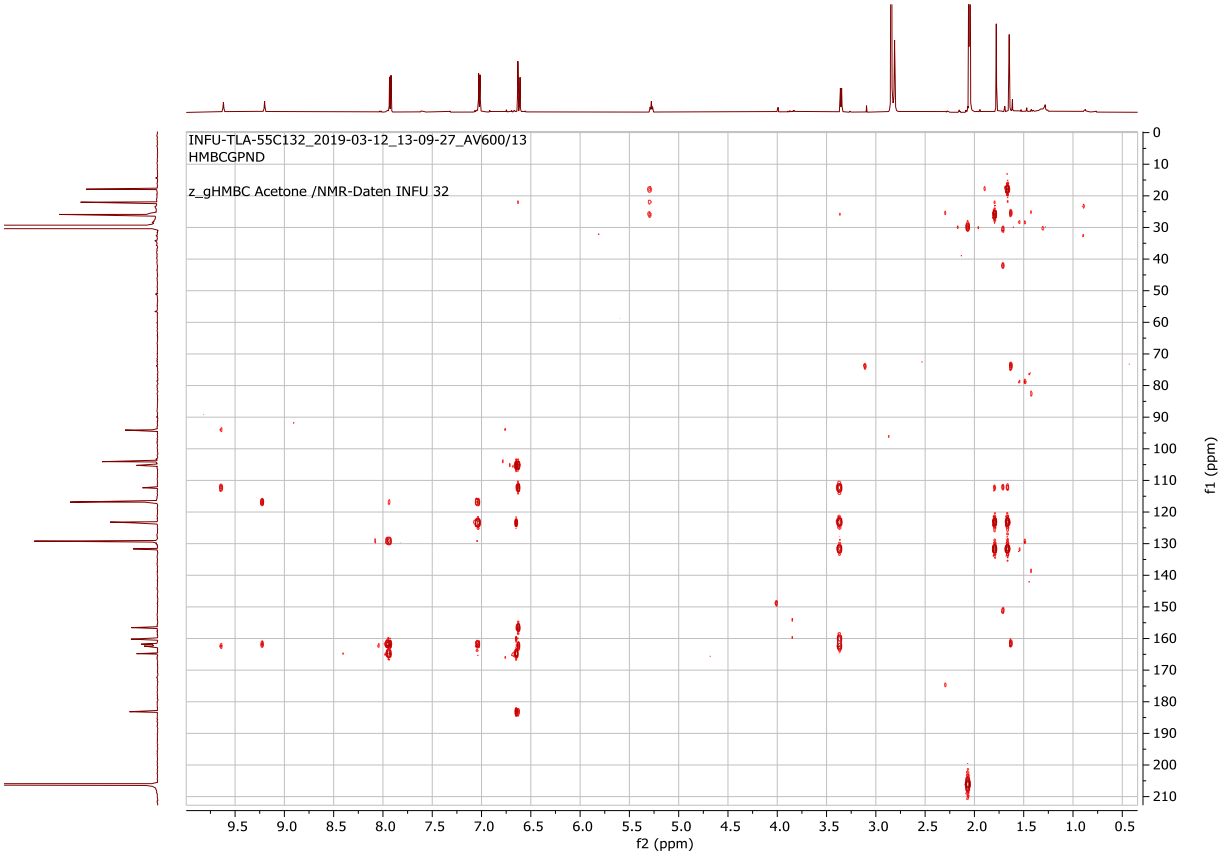
^{1}H-^{1}H-COSY spectrum of compound 10



HSQC spectrum of compound 10

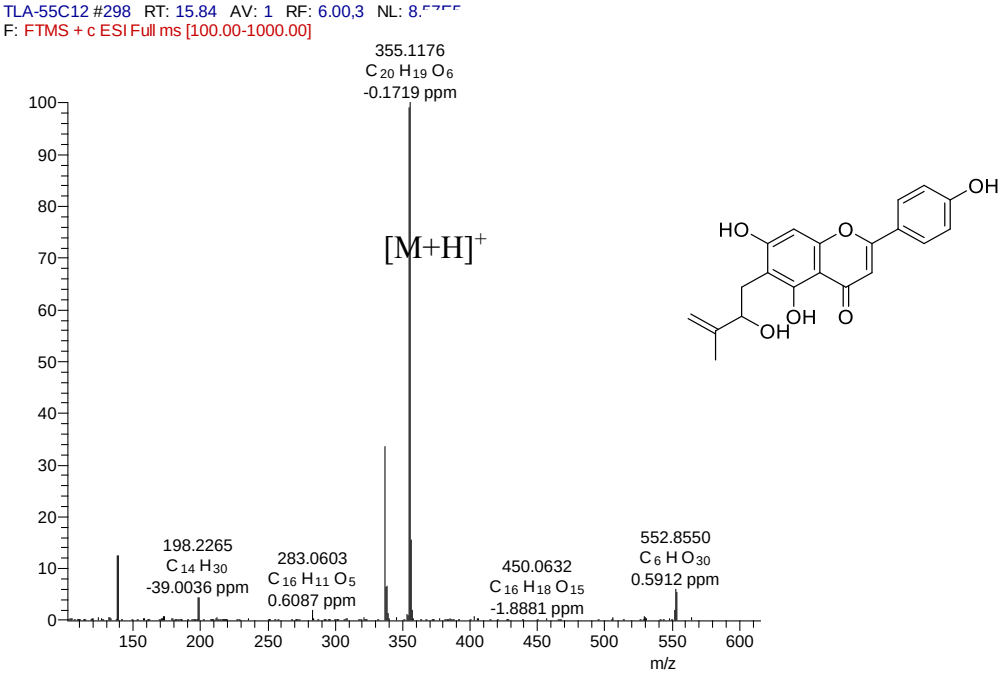


HMBC spectrum of compound 10

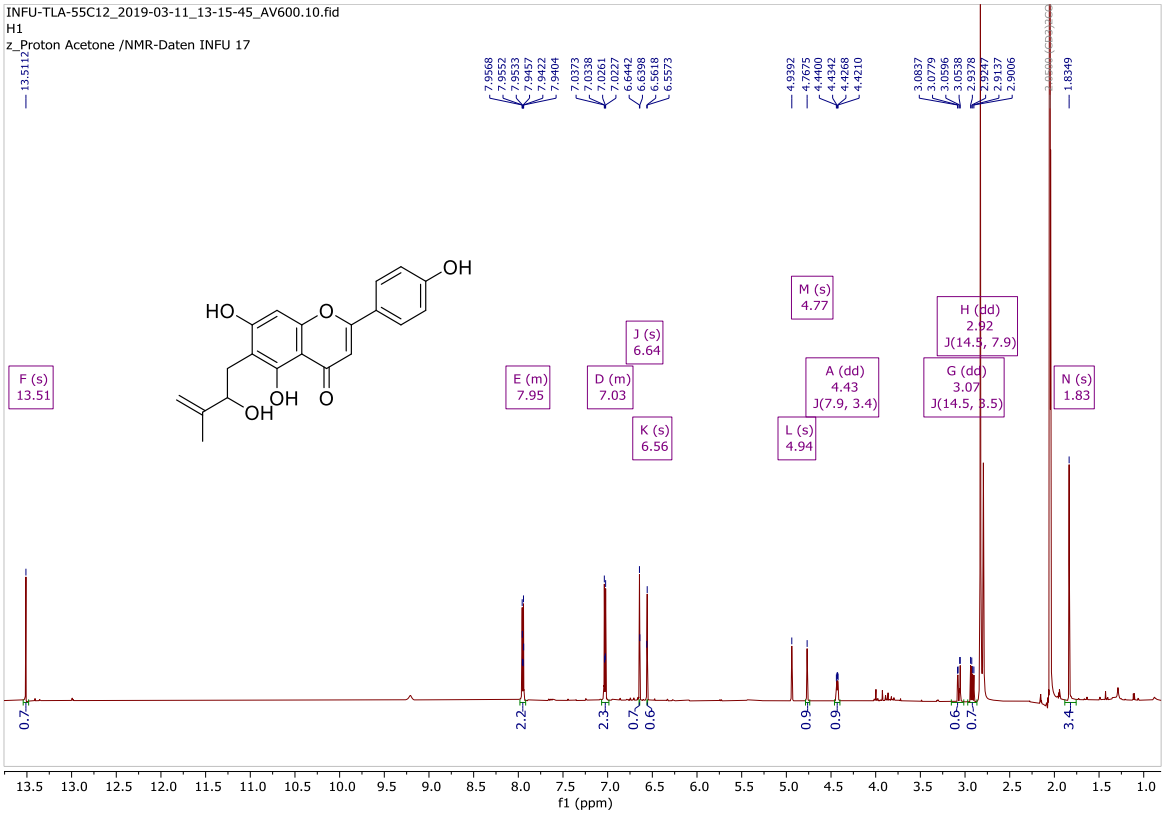


Appendix A11: Spectra for compound 11

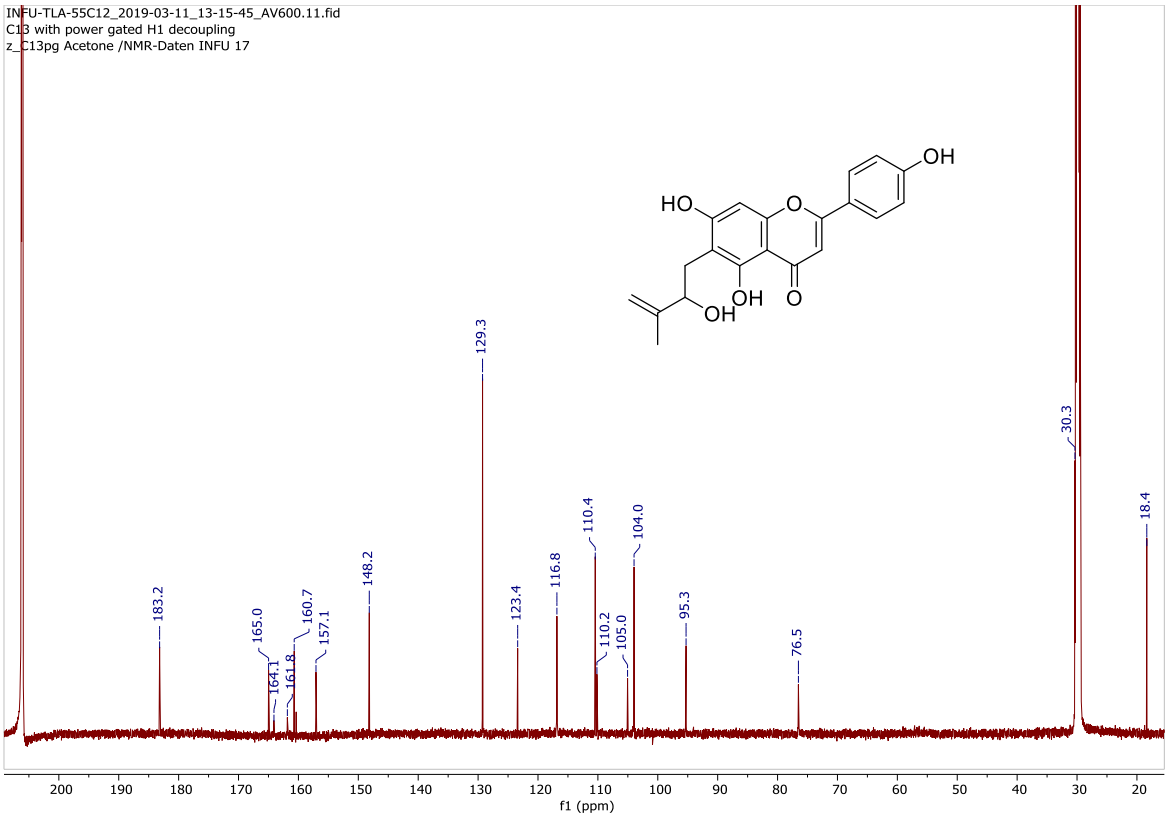
HRESIMS spectrum of compound 11



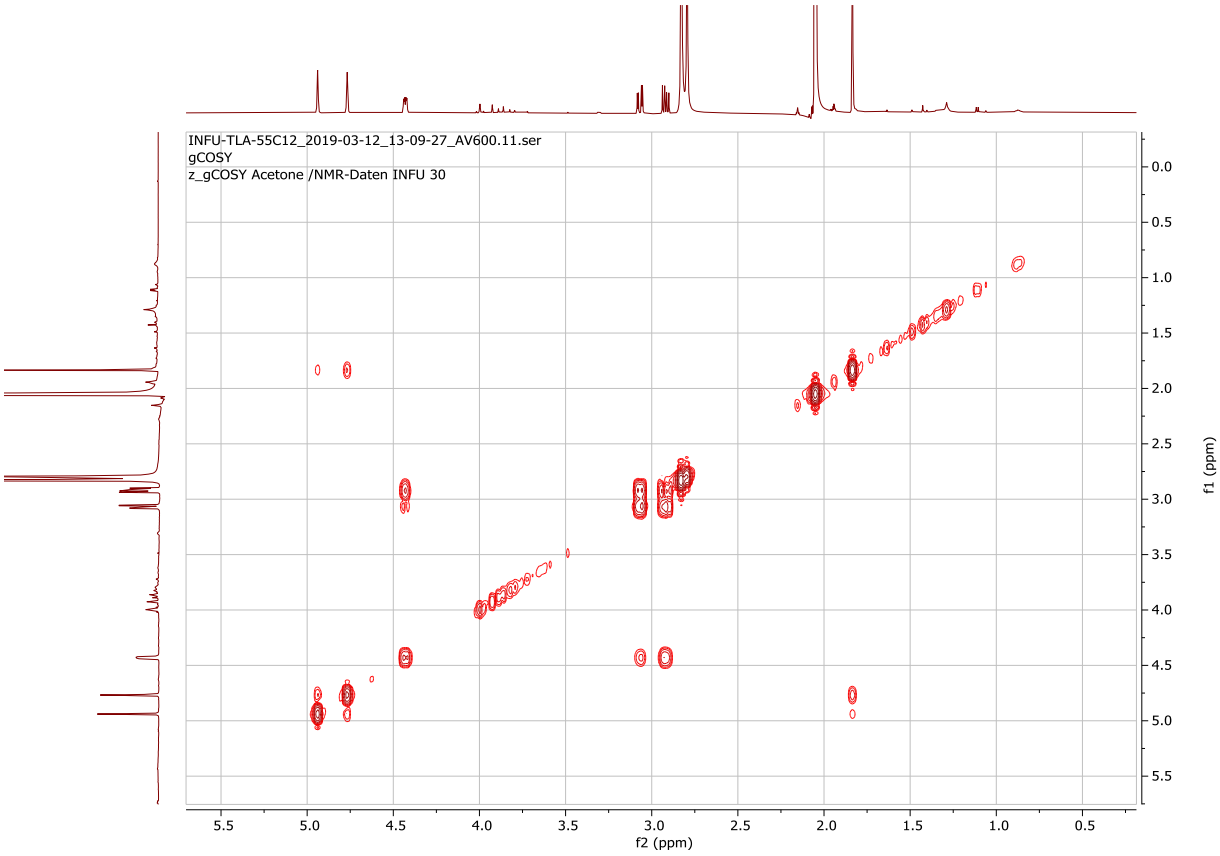
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 11



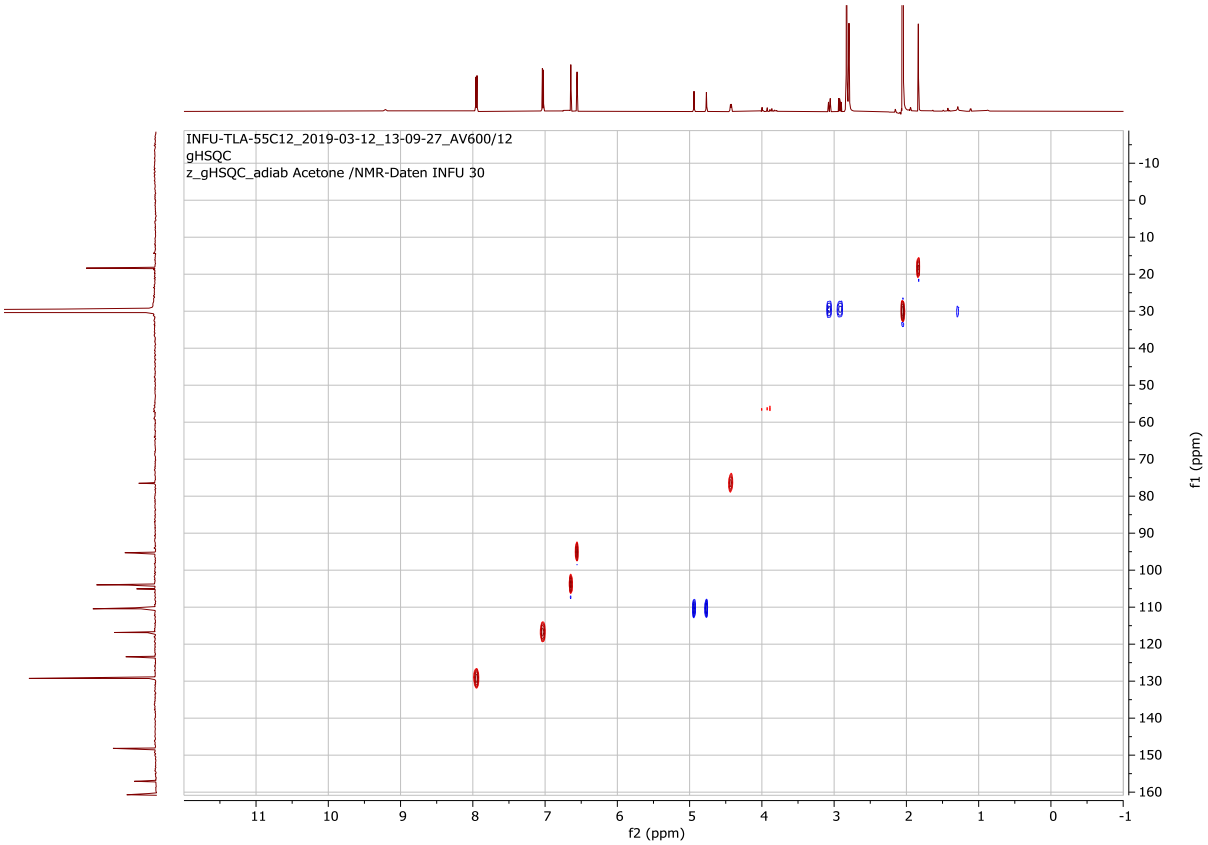
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 11



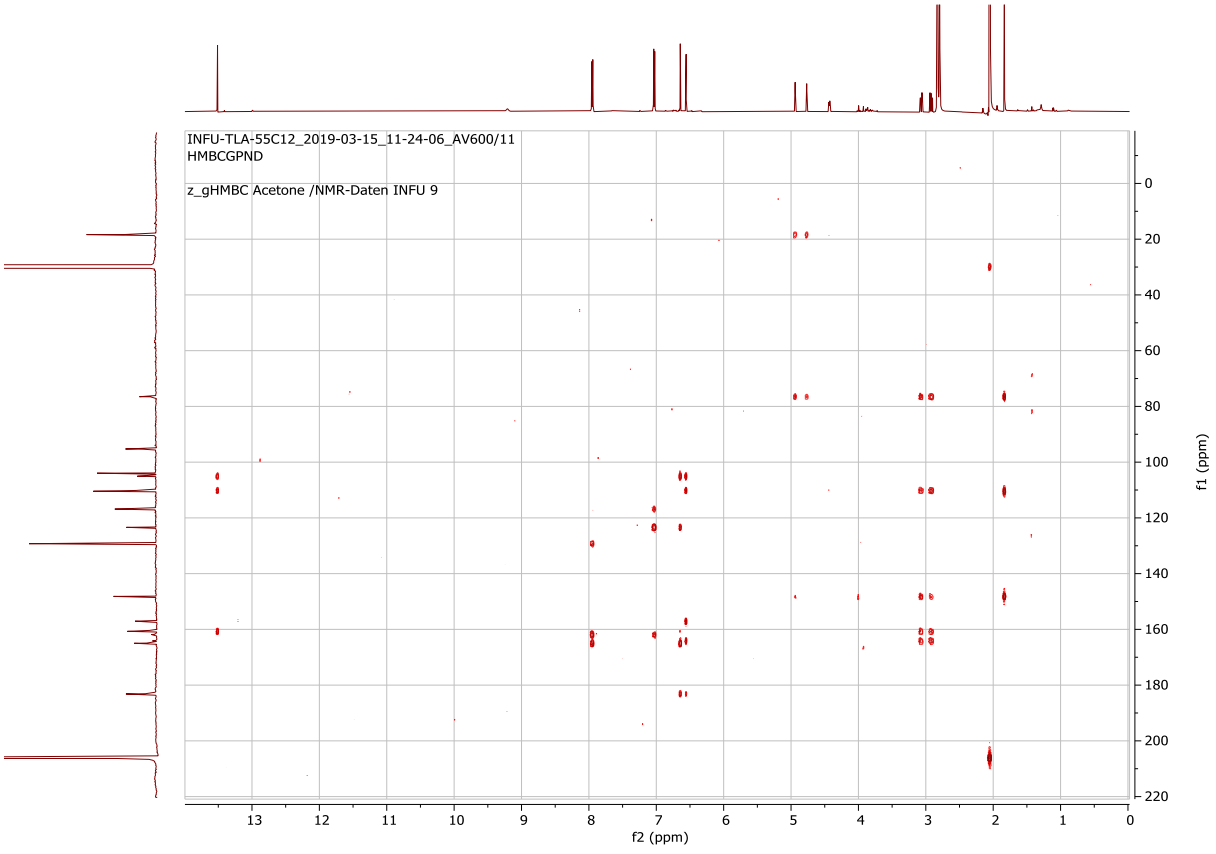
^{1}H-^{1}H-COSY spectrum of compound 11



HSQC spectrum of compound 11

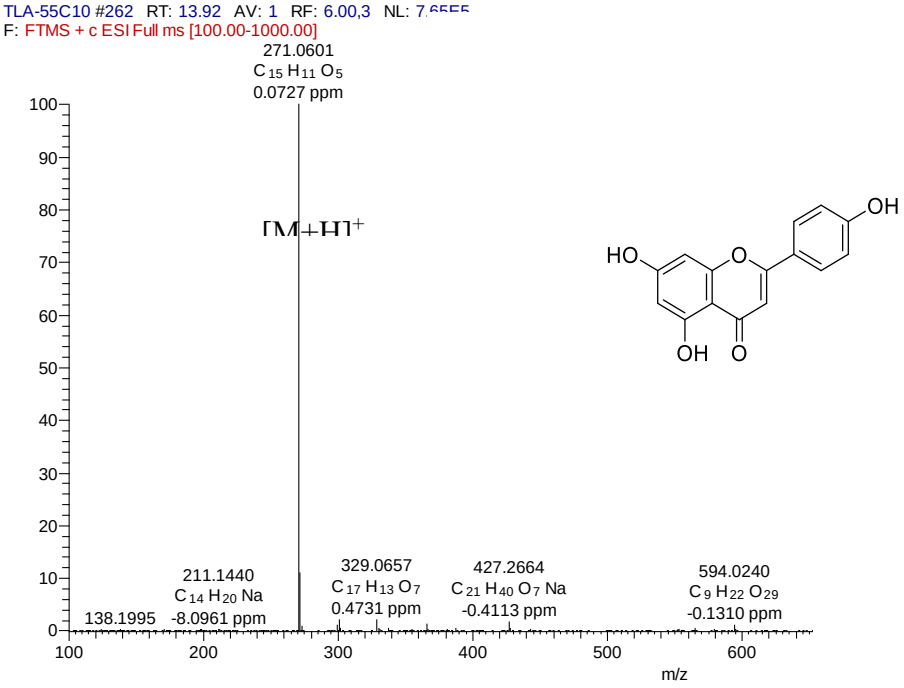


HMBC spectrum of compound 11

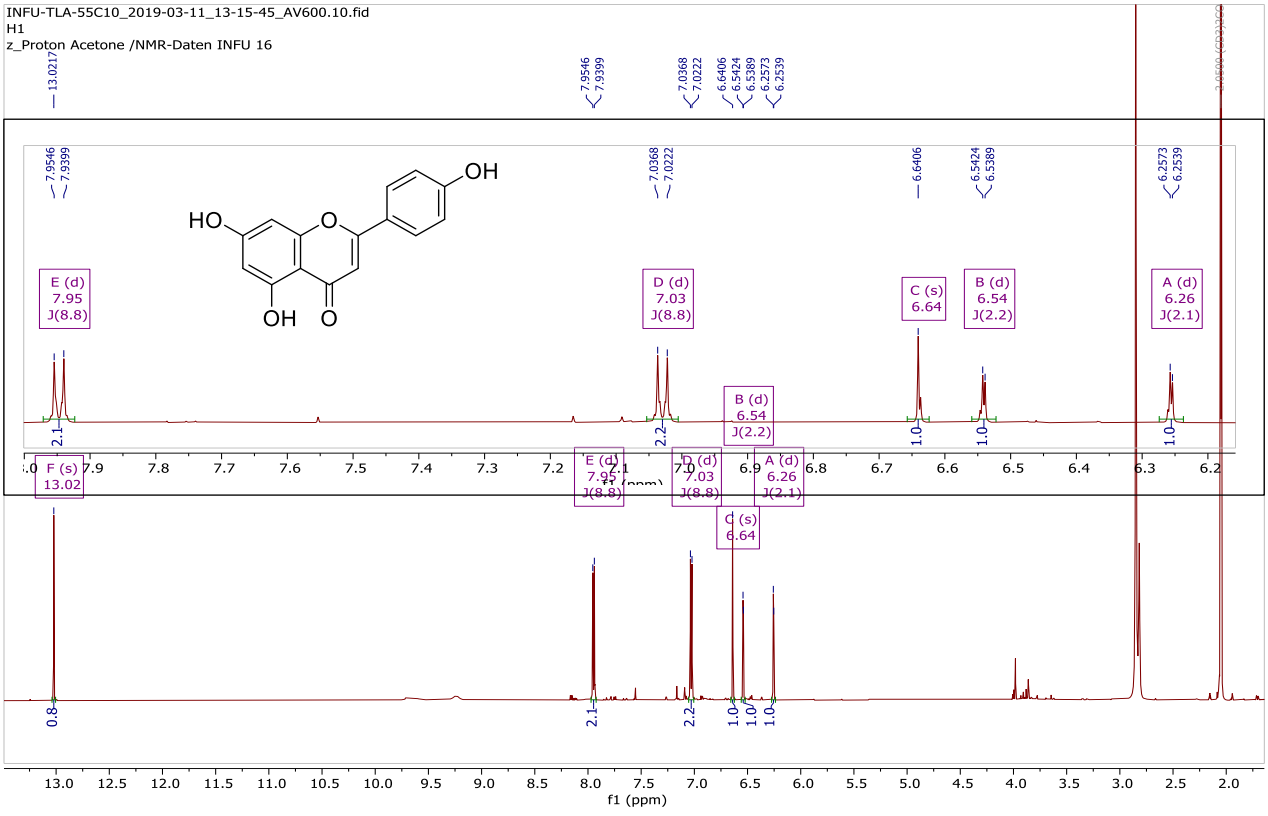


Appendix A12: Spectra for compound 12

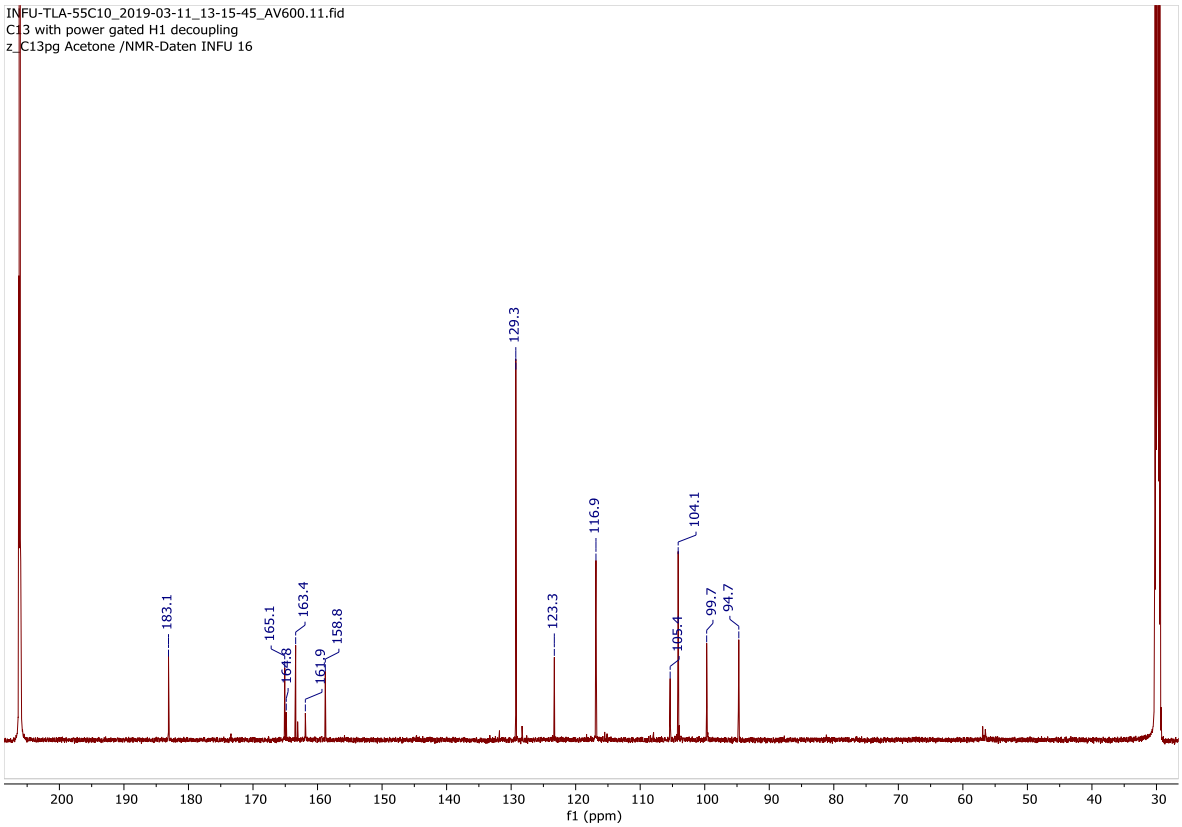
HRESIMS spectrum of compound 12



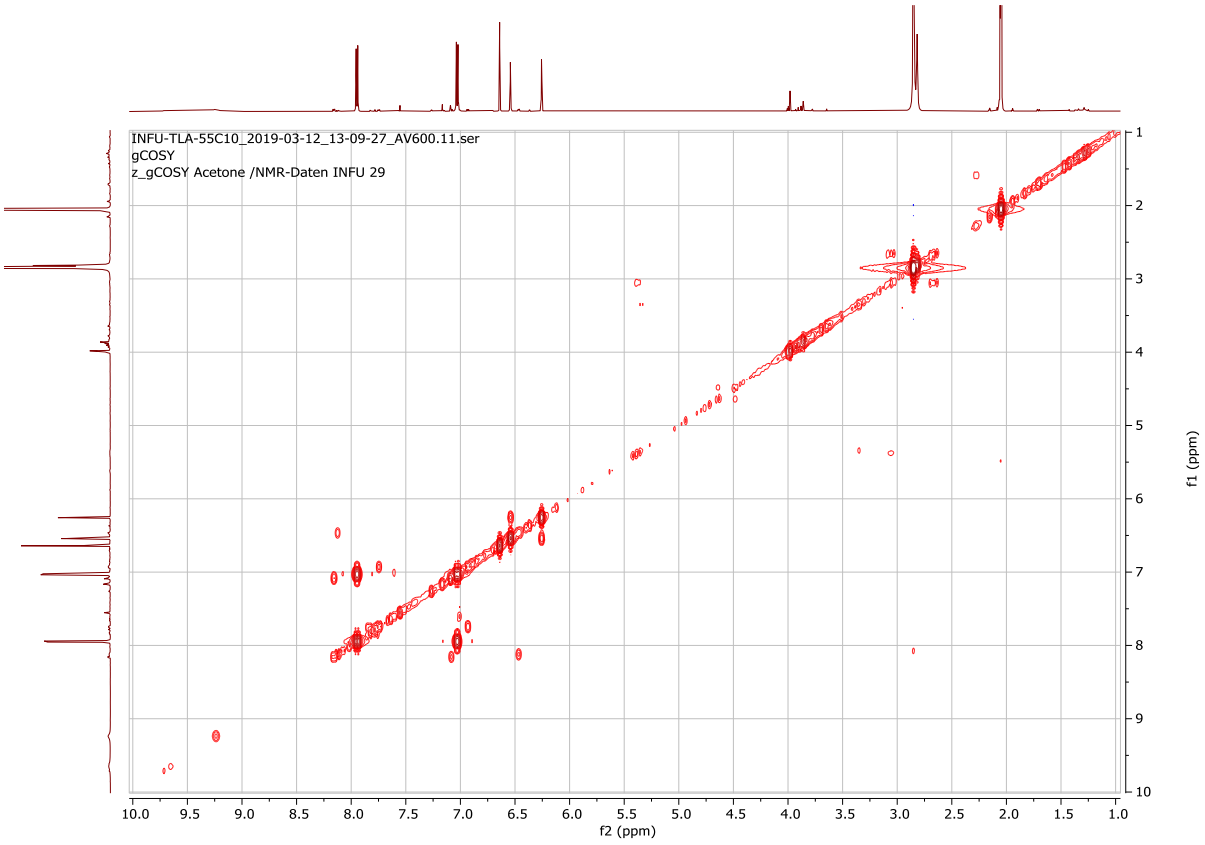
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 12



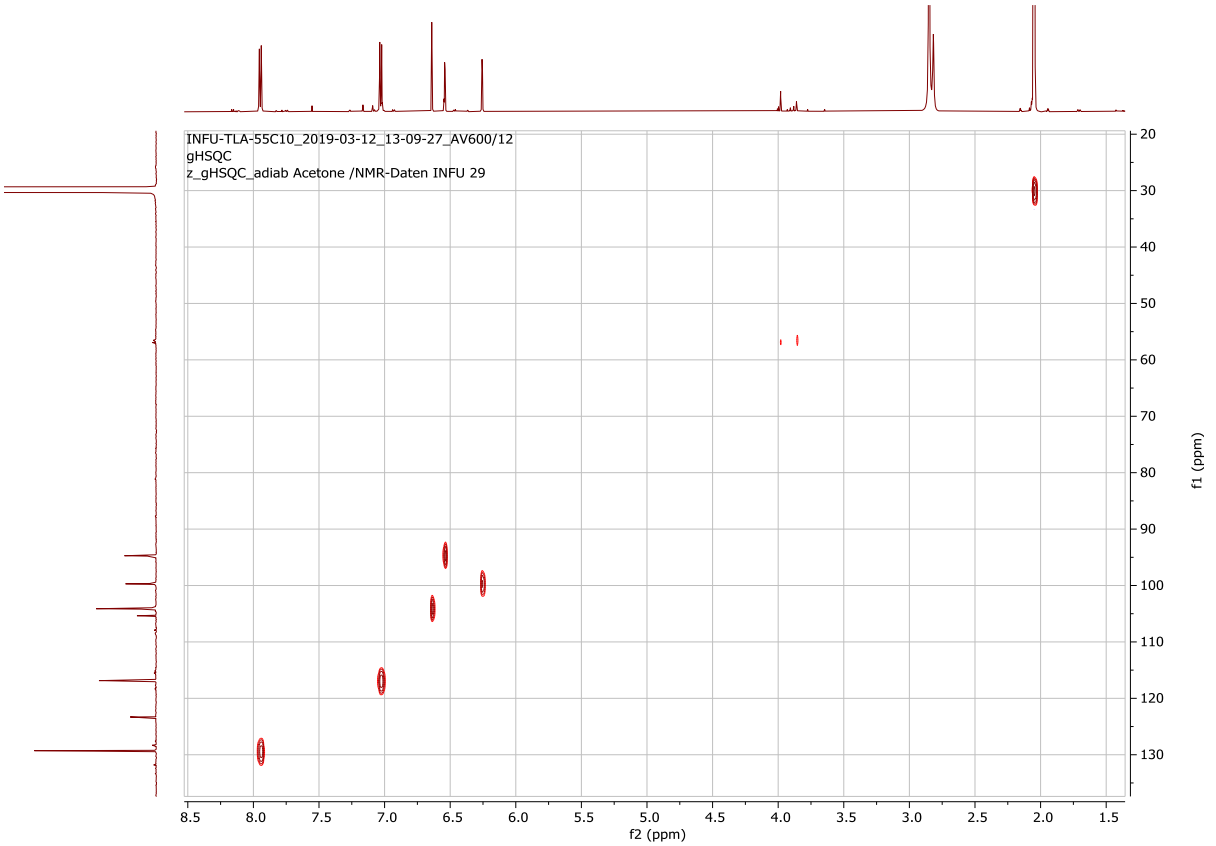
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 12



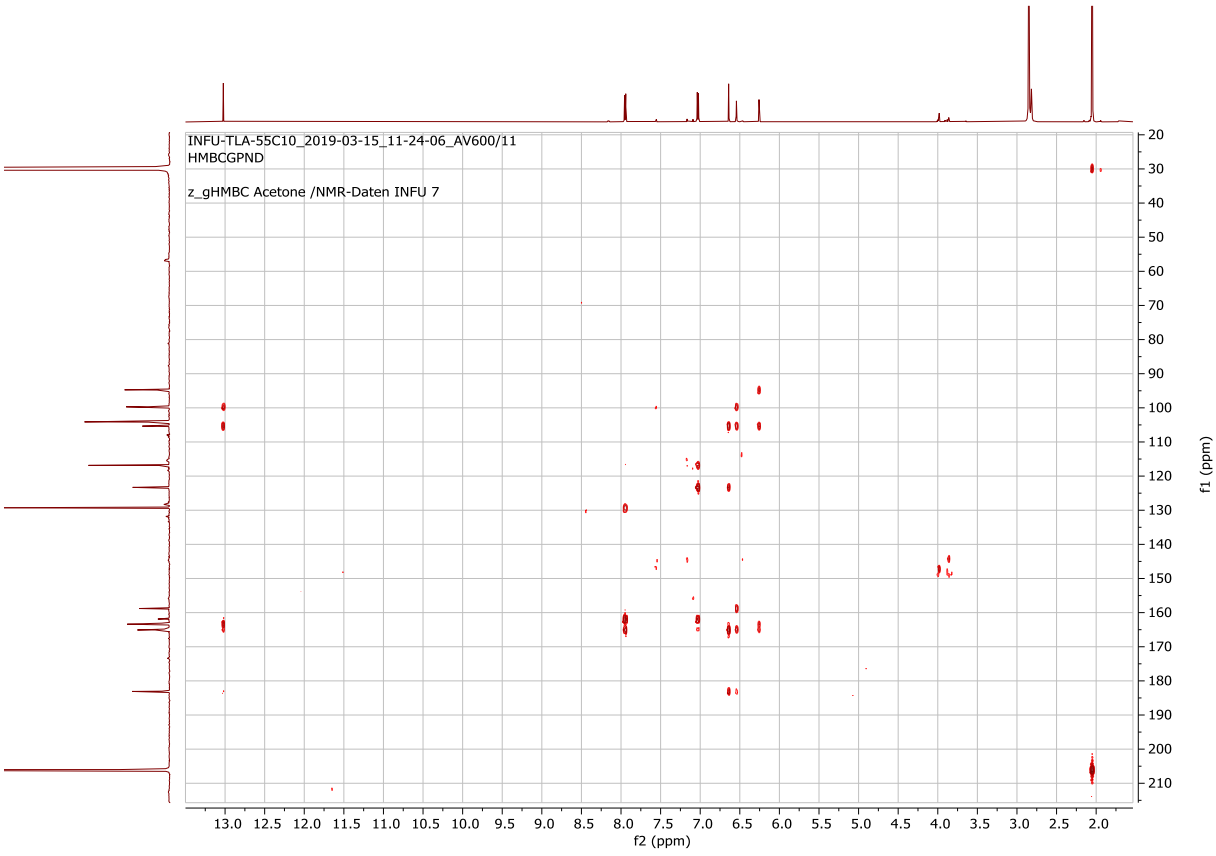
^{1}H-^{1}H-COSY spectrum of compound 12



HSQC spectrum of compound 12

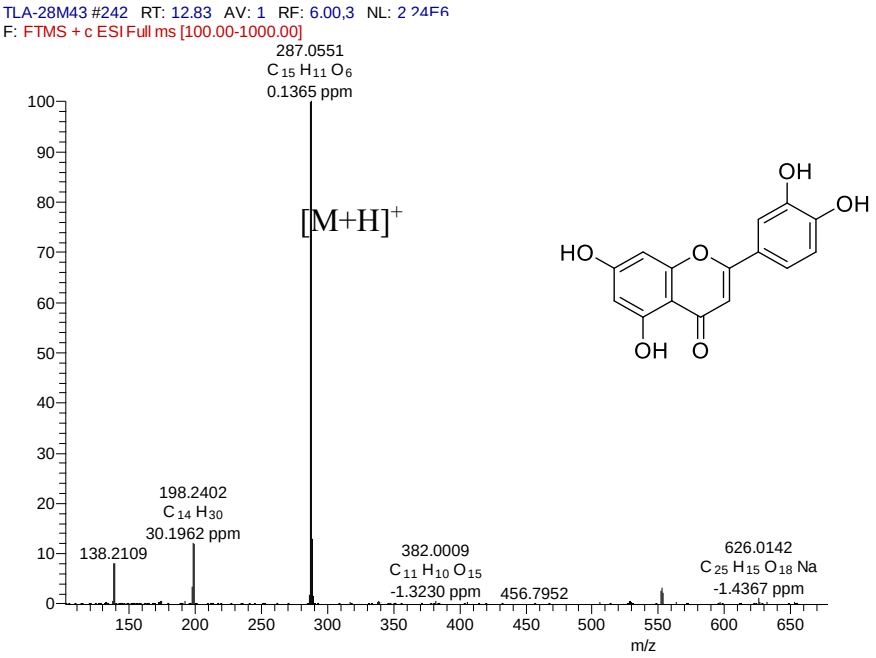


HMBC spectrum of compound 12

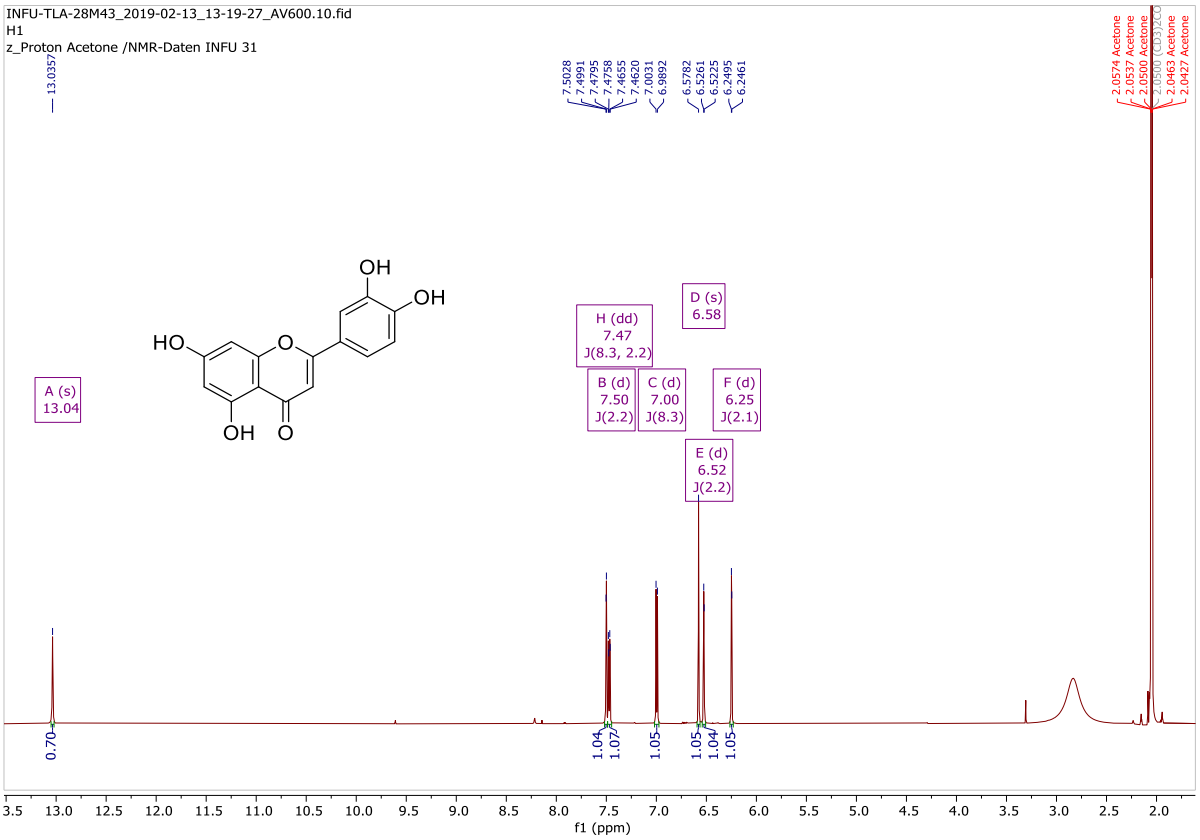


Appendix A13: Spectra for compound 13

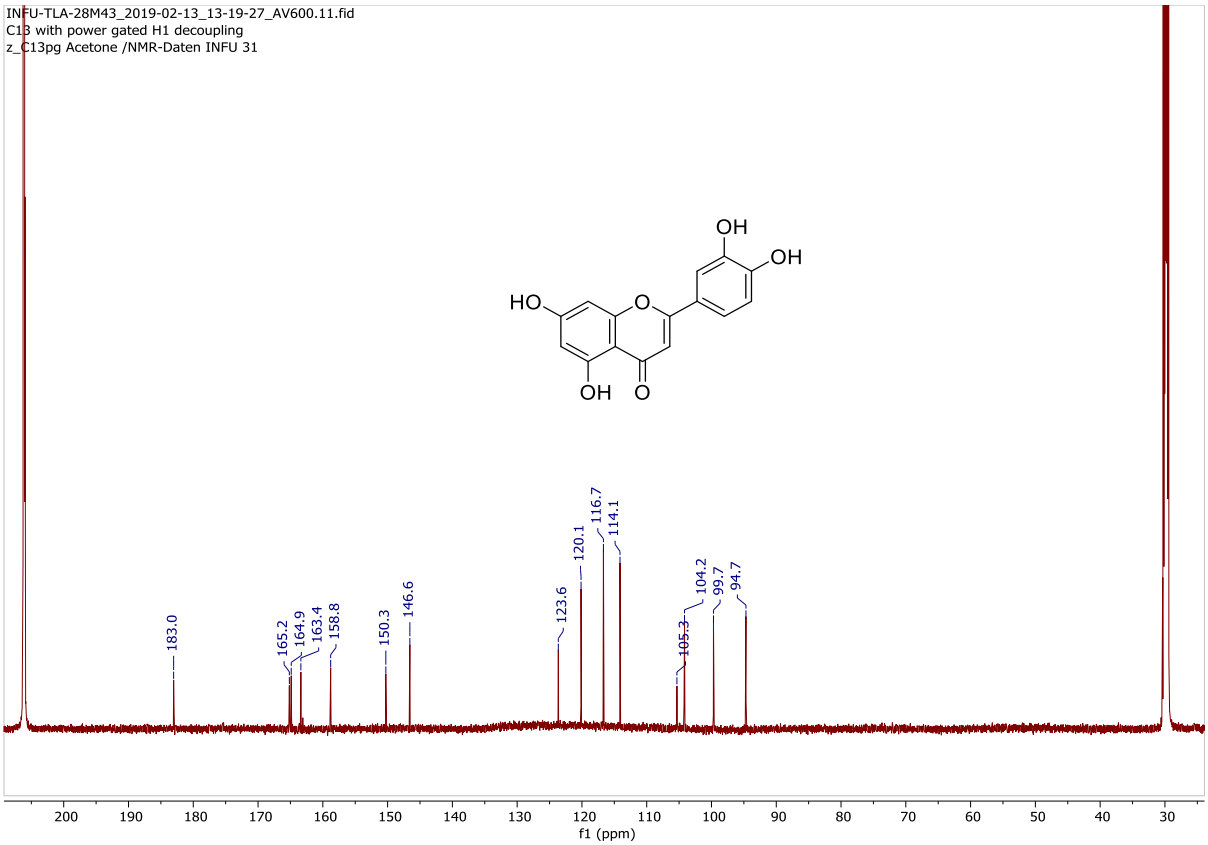
HRESIMS spectrum of compound 13



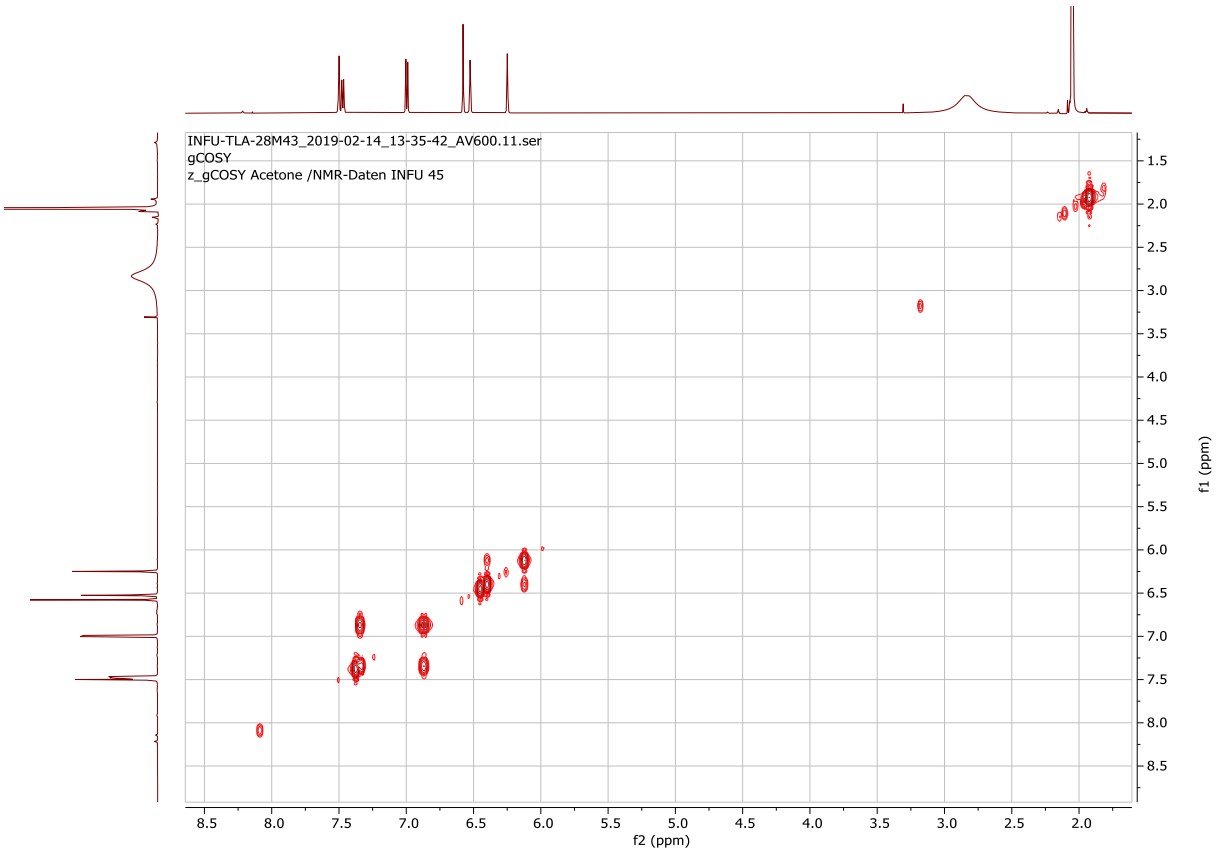
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 13



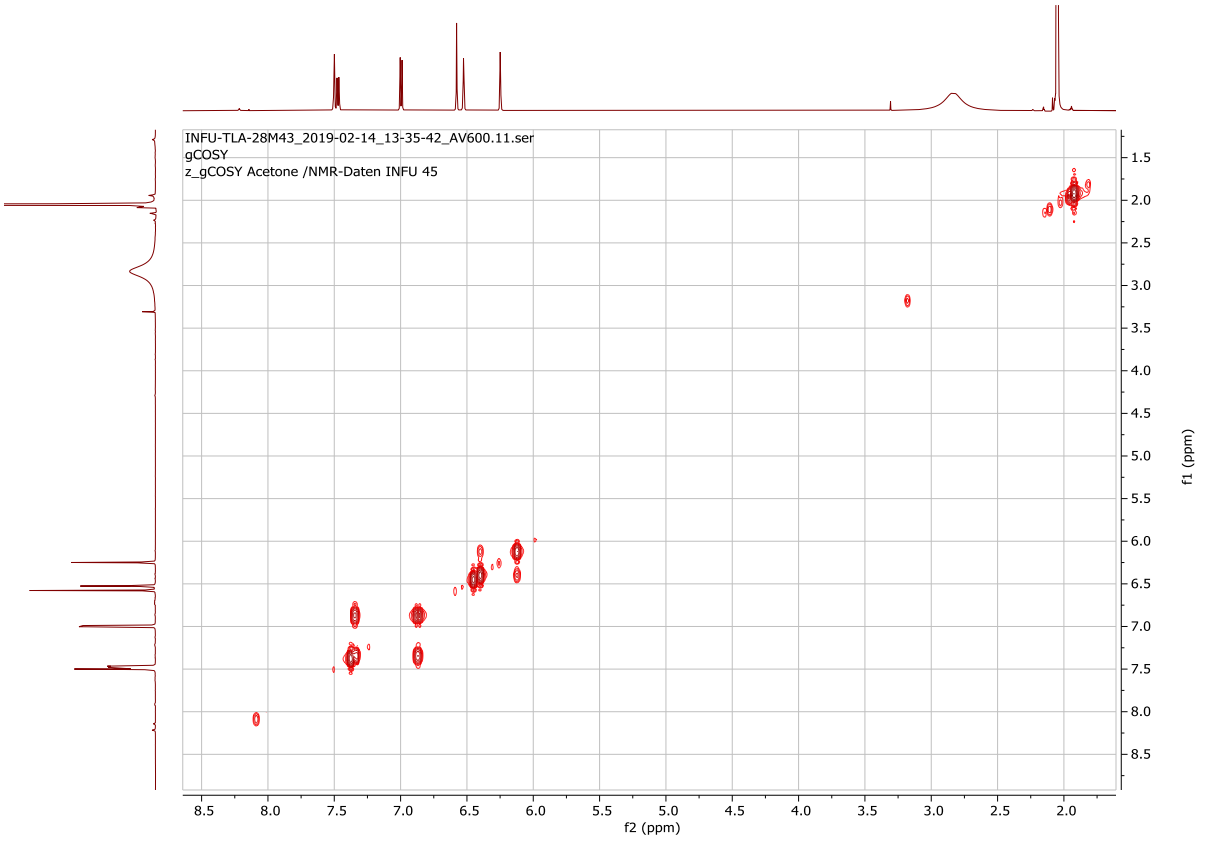
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 13



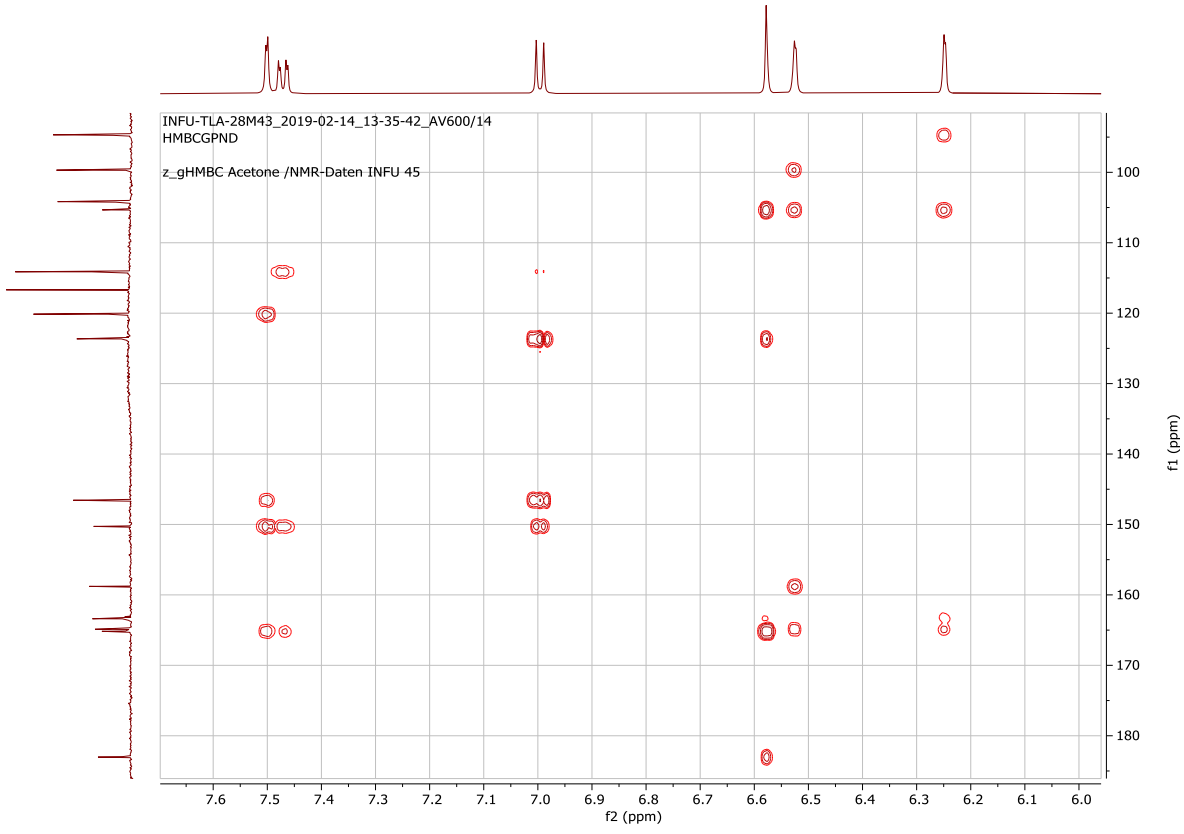
^{1}H-^{1}H-COSY spectrum of compound 13



HSQC spectrum of compound 13

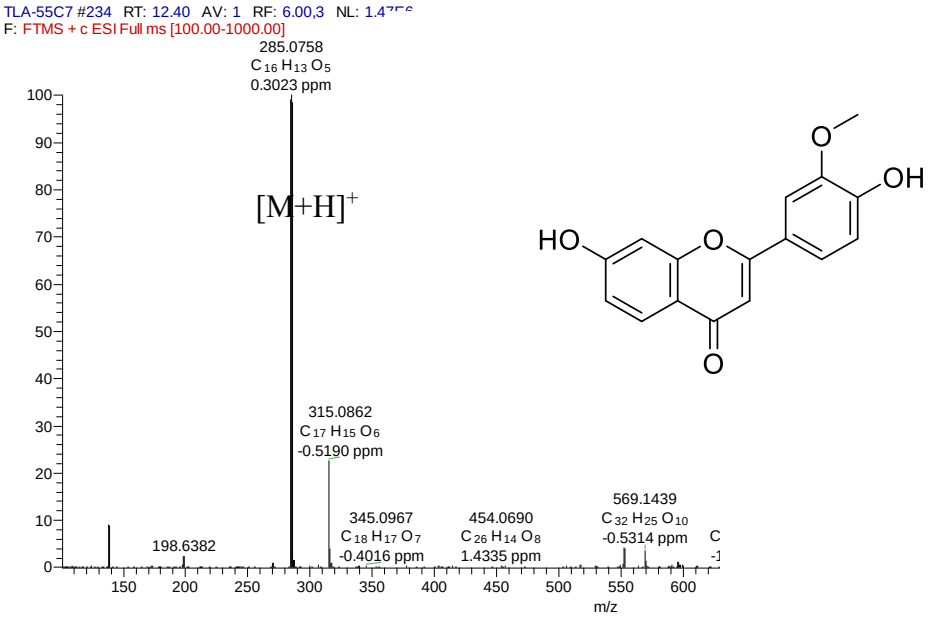


HMBC spectrum of compound 13

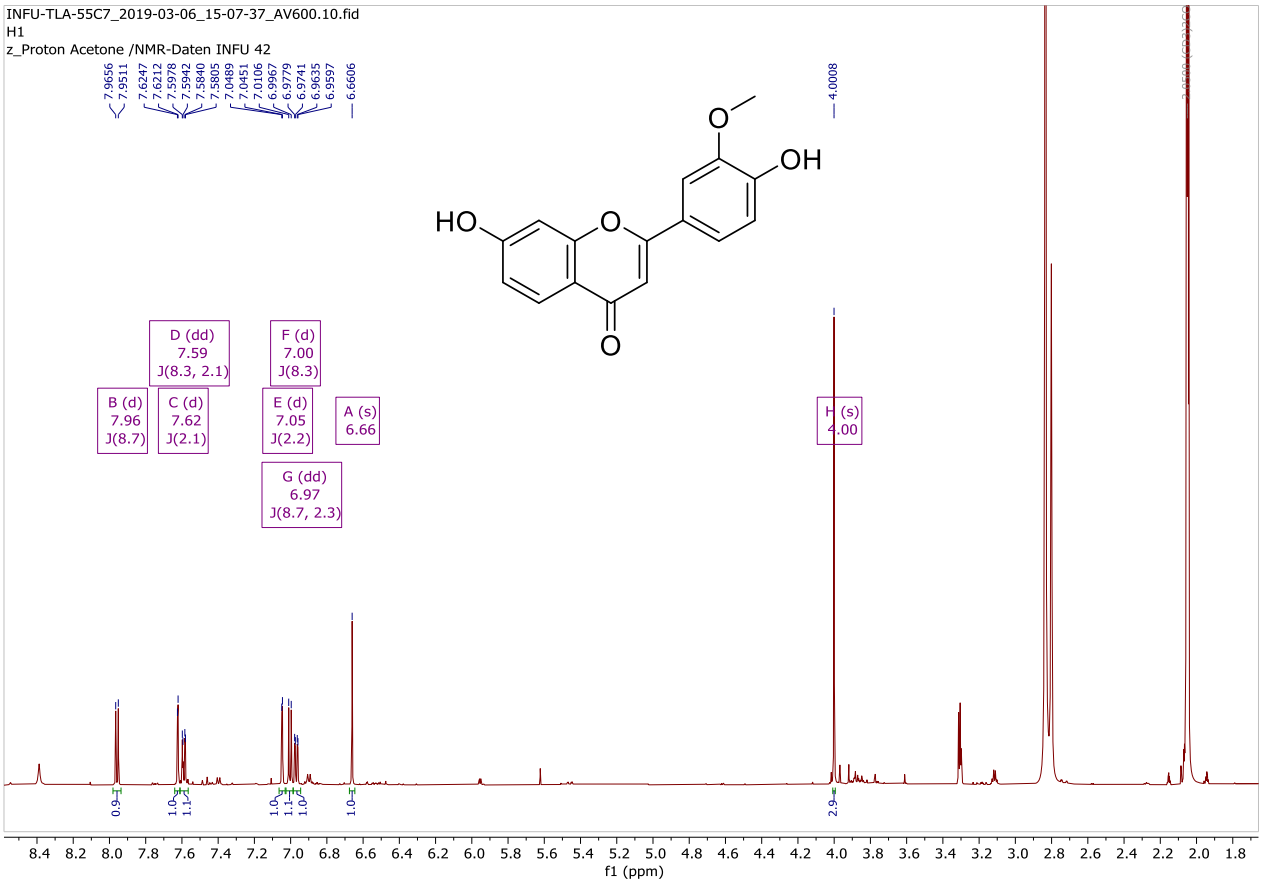


Appendix A14: Spectra for compound 14

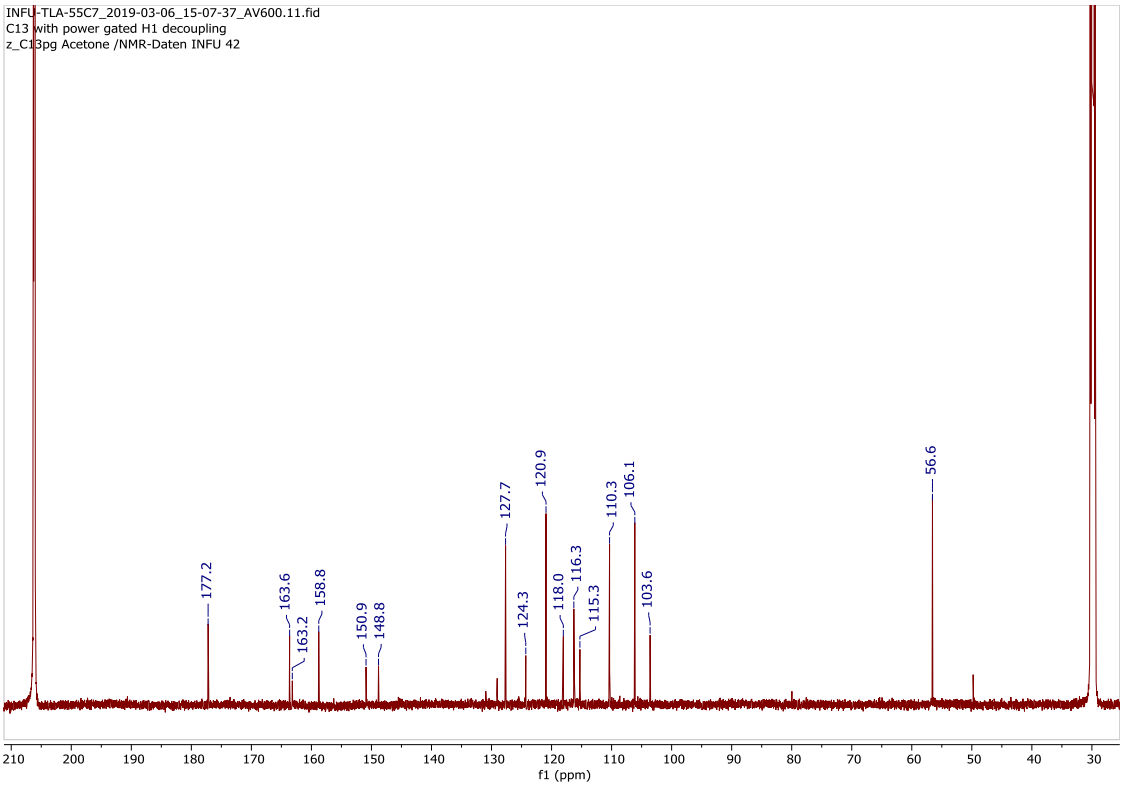
HRESIMS spectrum of compound 14



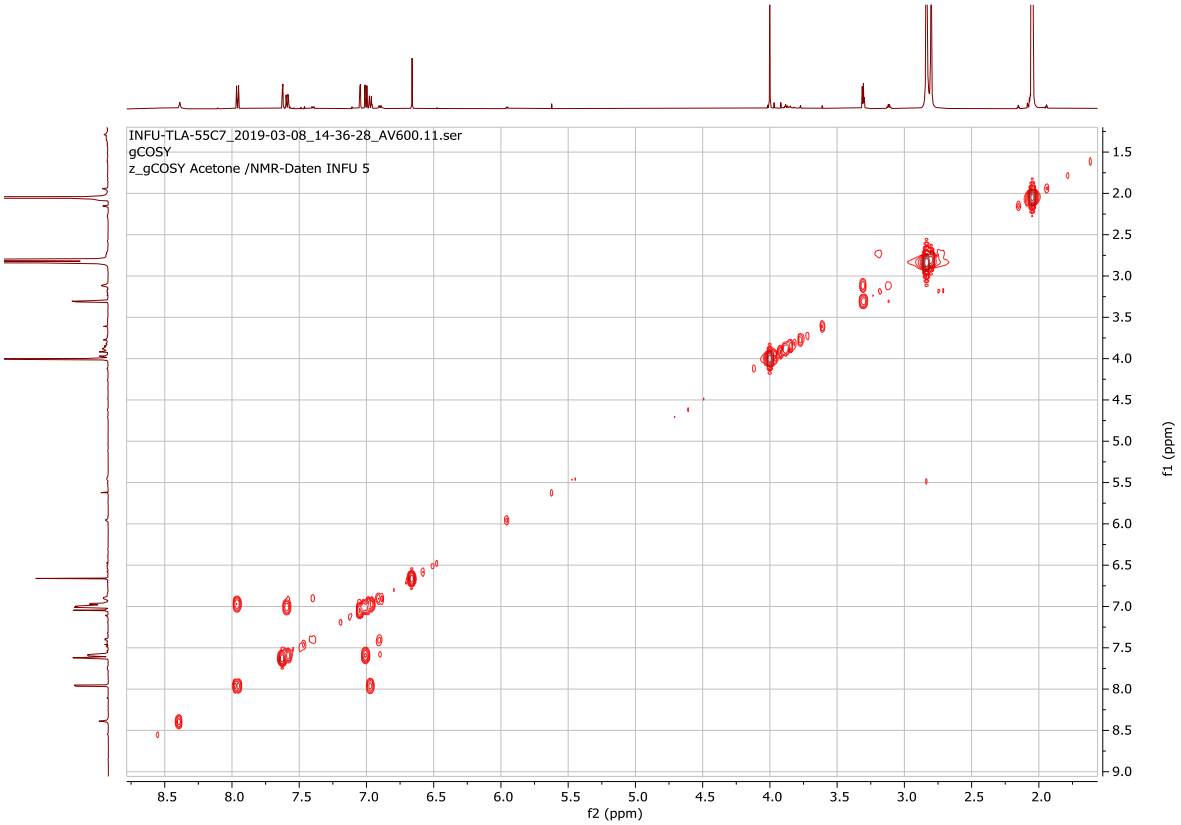
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 14



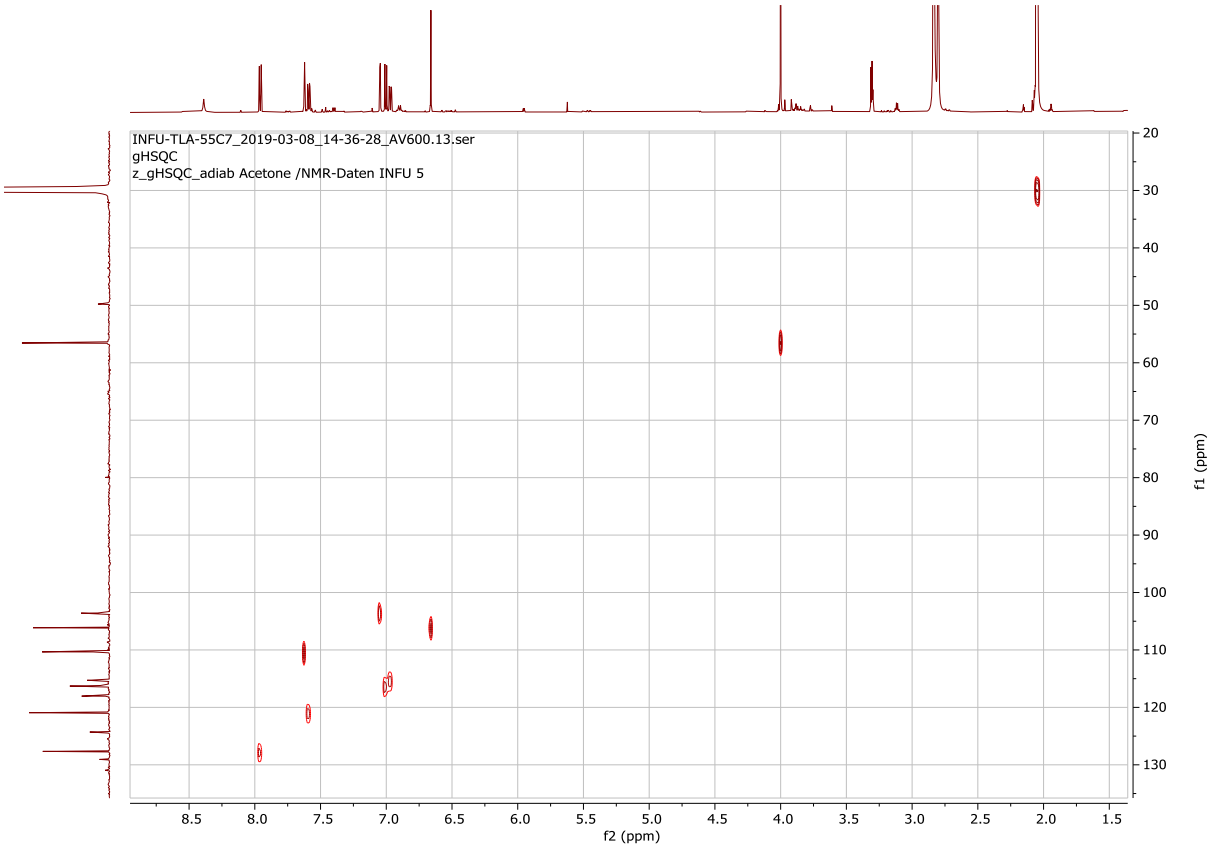
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 14



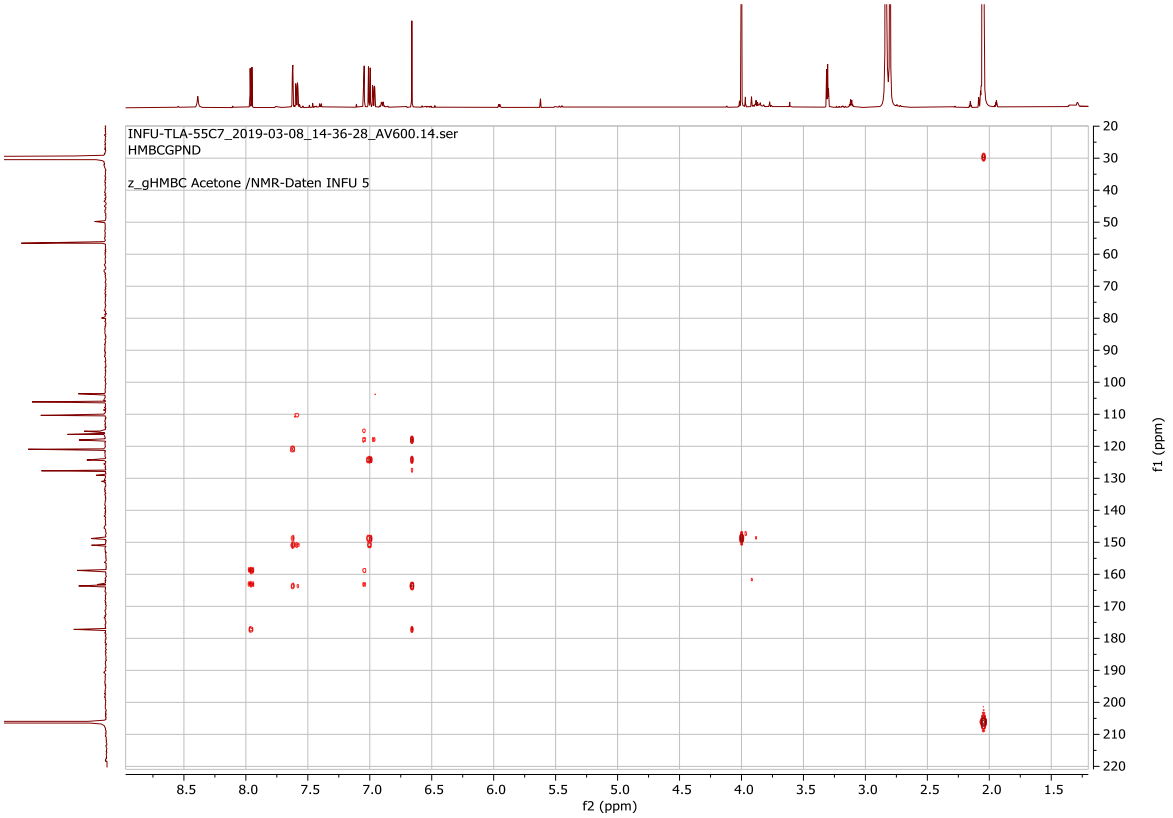
^{1}H-^{1}H-COSY spectrum of compound 14



HSQC spectrum of compound 14

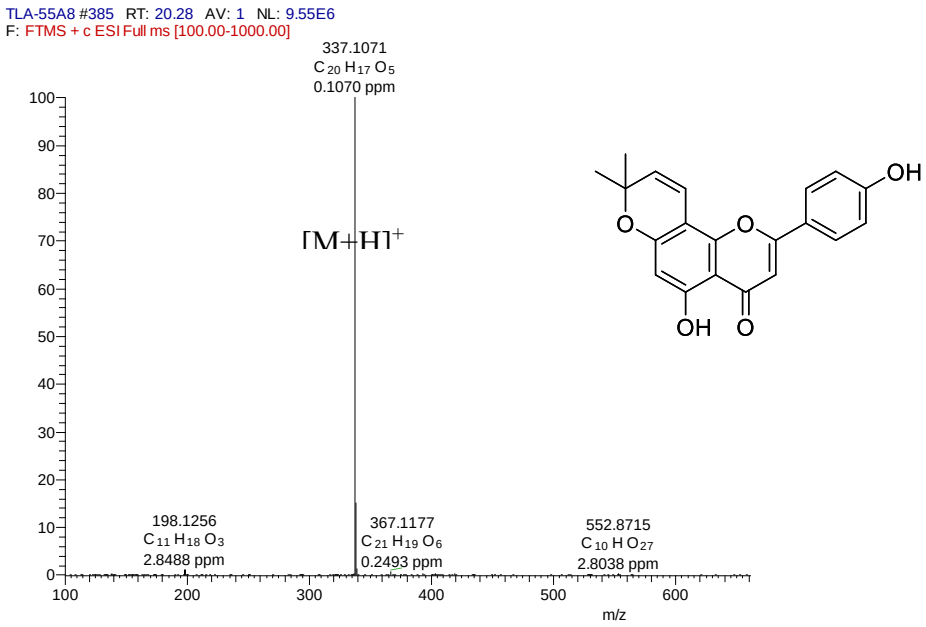


HMBC spectrum of compound 14

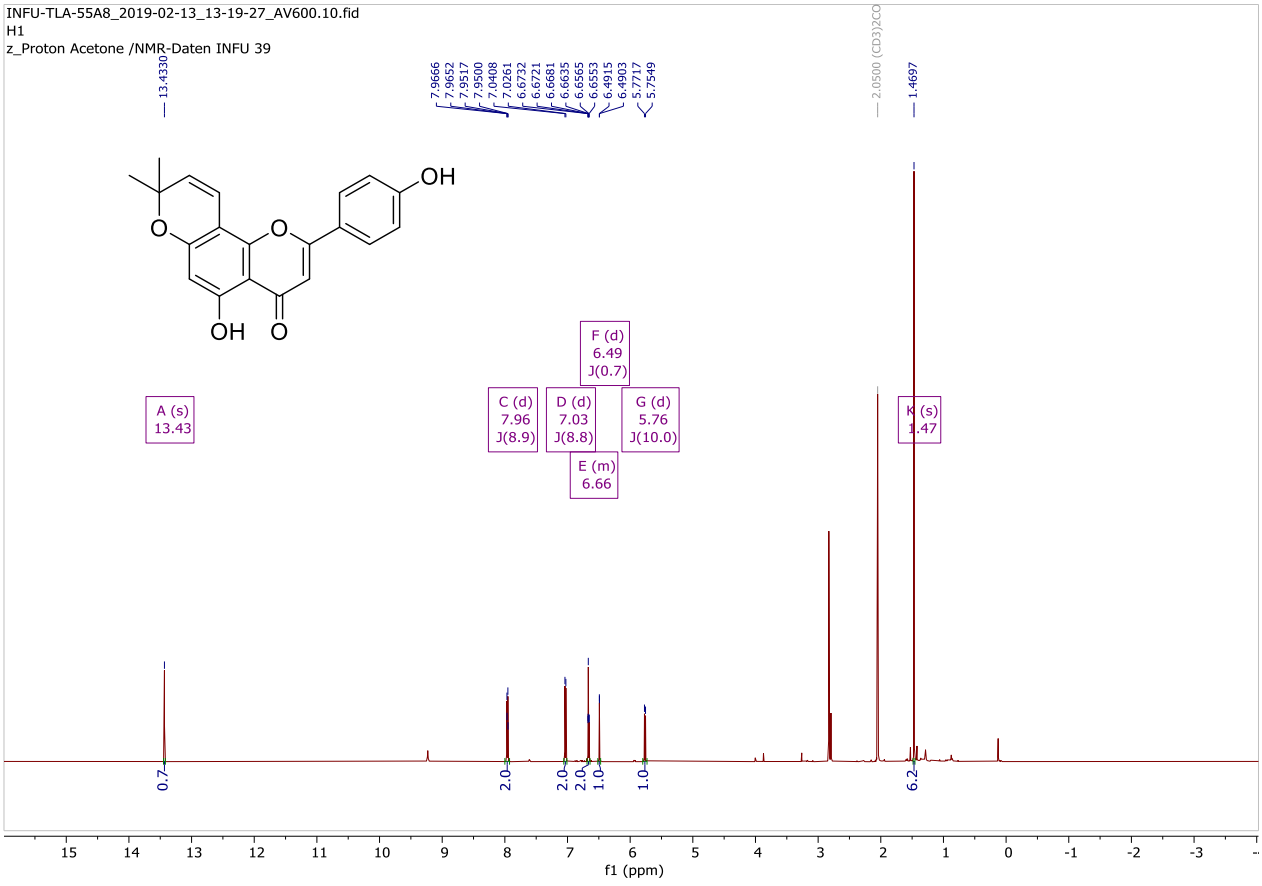


Appendix A15: Spectra for compound 15

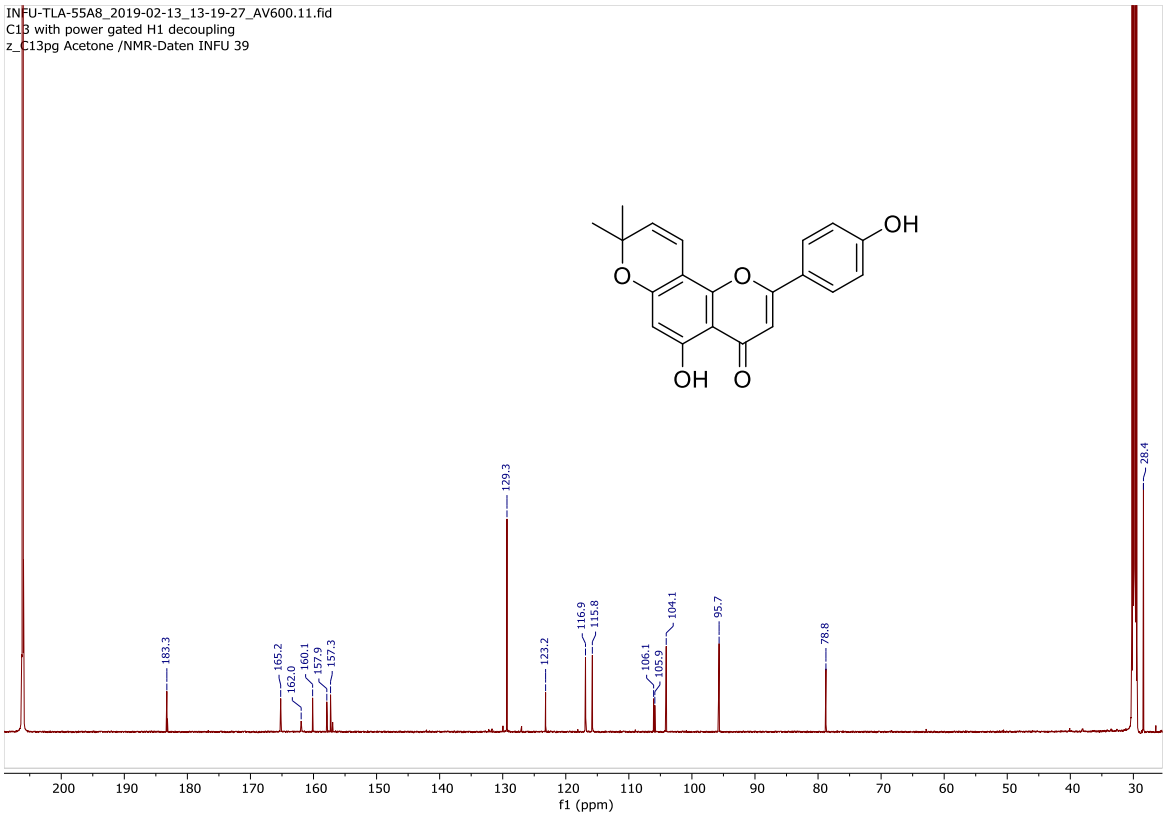
HRESIMS spectrum of compound 15



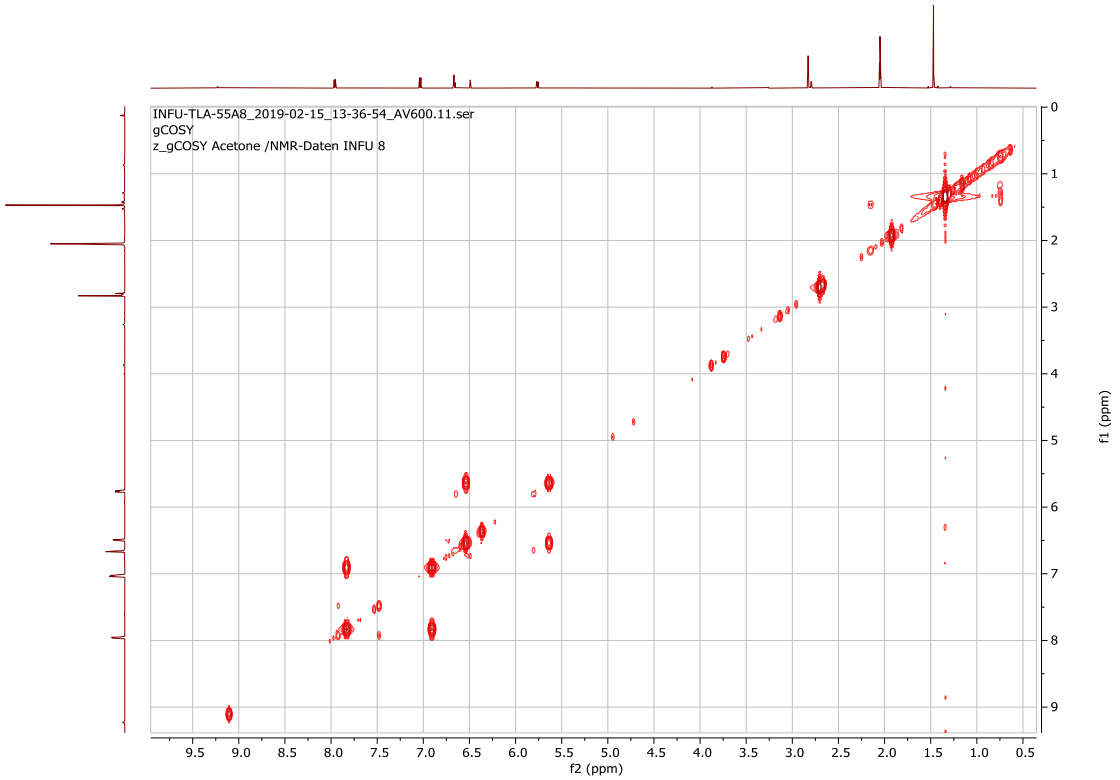
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 15



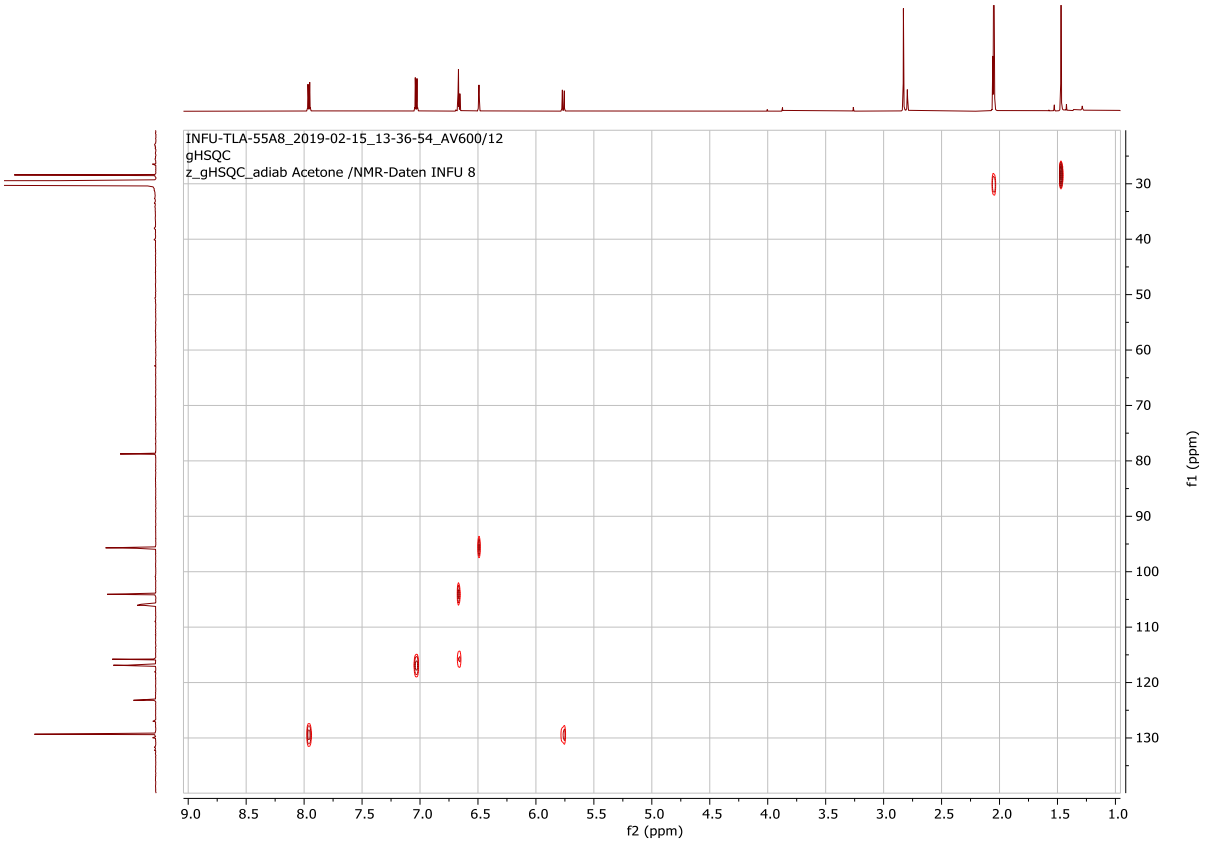
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 15



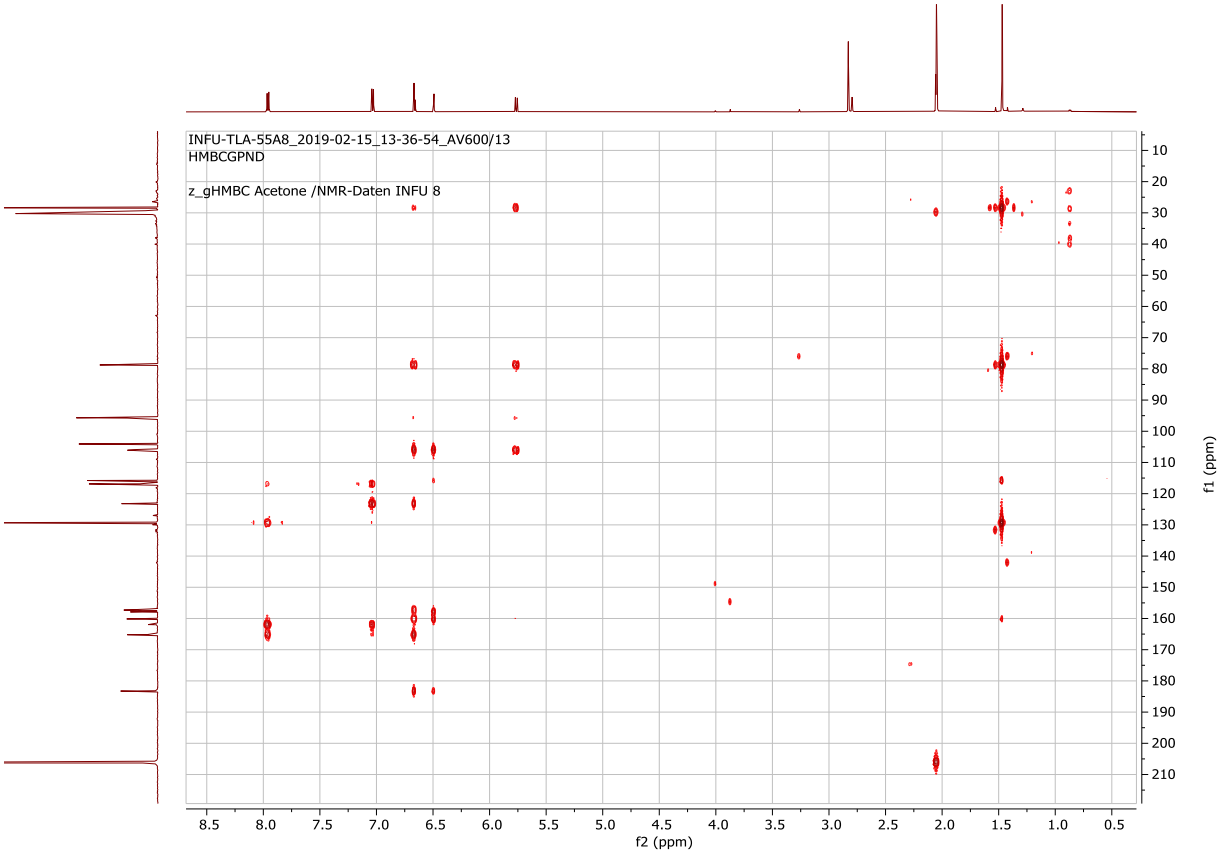
^{1}H-^{1}H-COSY spectrum of compound 15



HSQC spectrum of compound 15

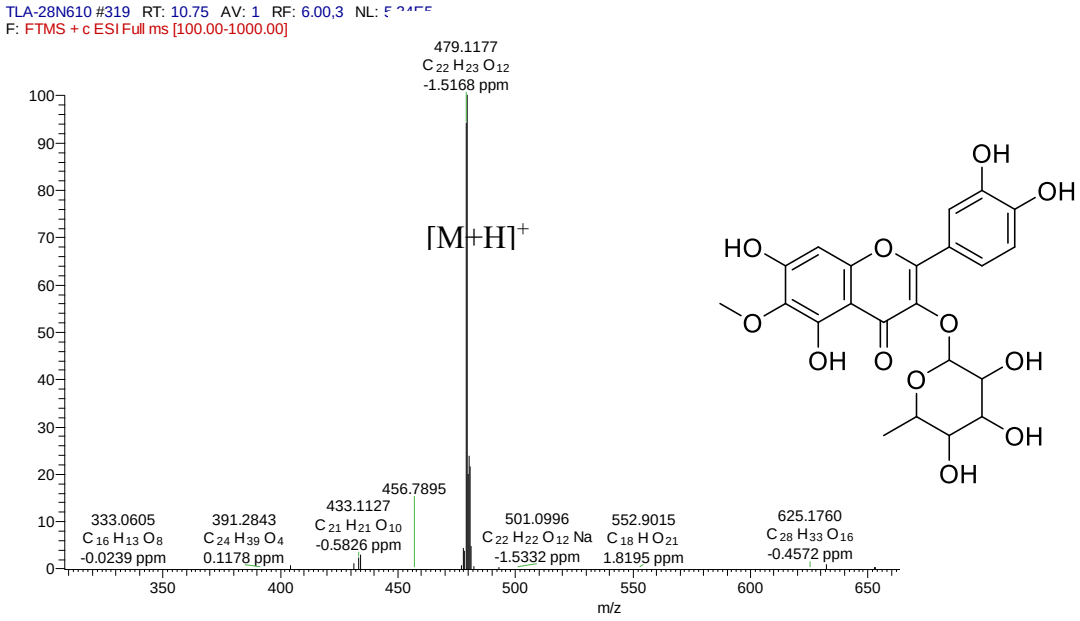


HMBC spectrum of compound 15

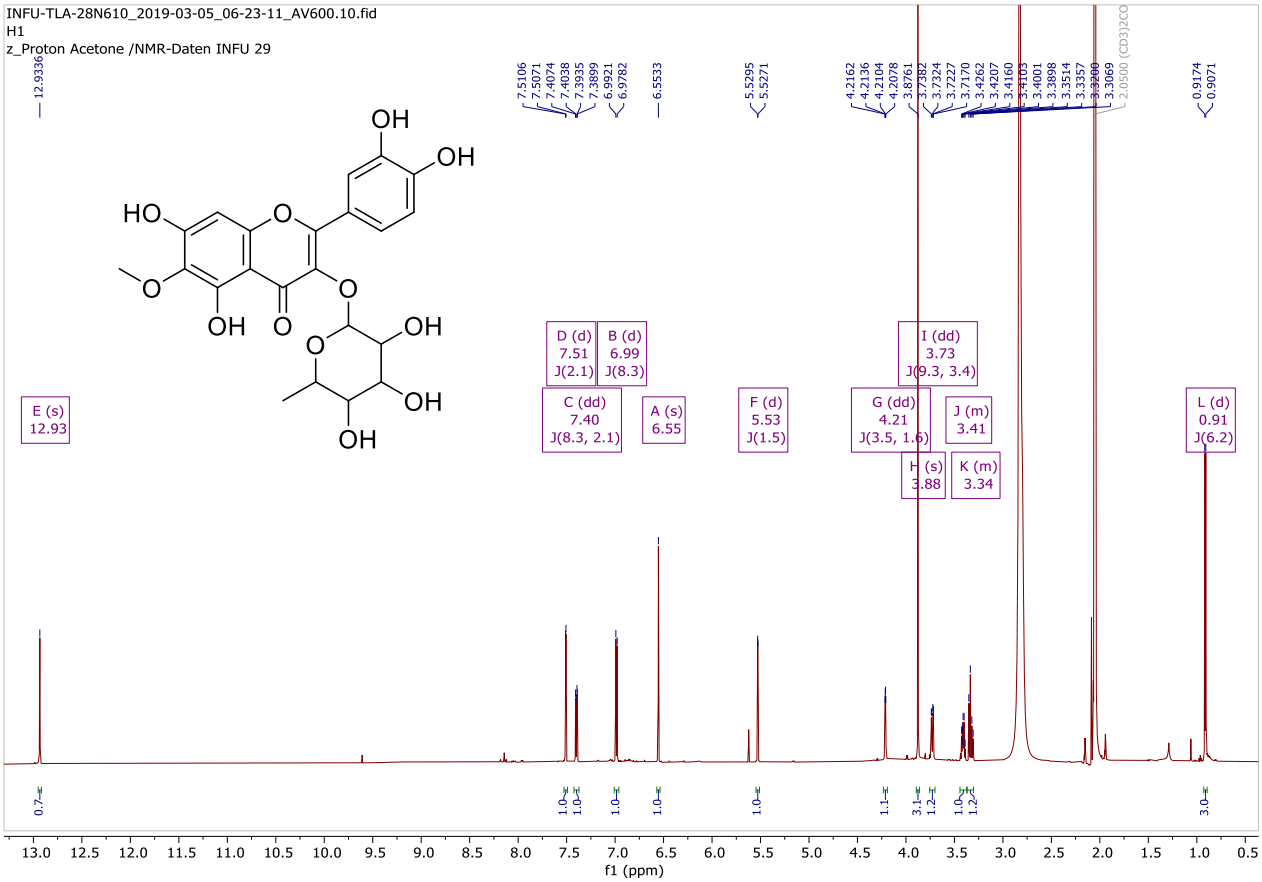


Appendix A16: Spectra for compound 16

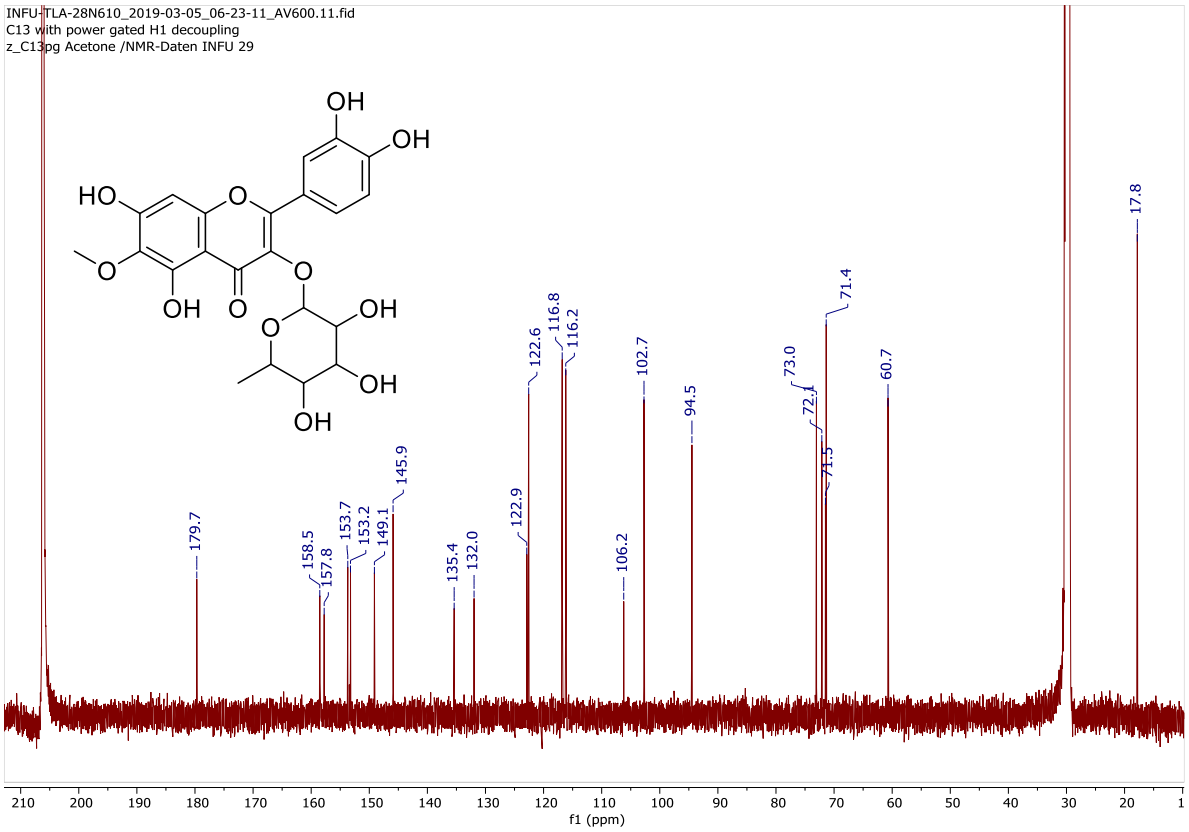
HRESIMS spectrum of compound 16



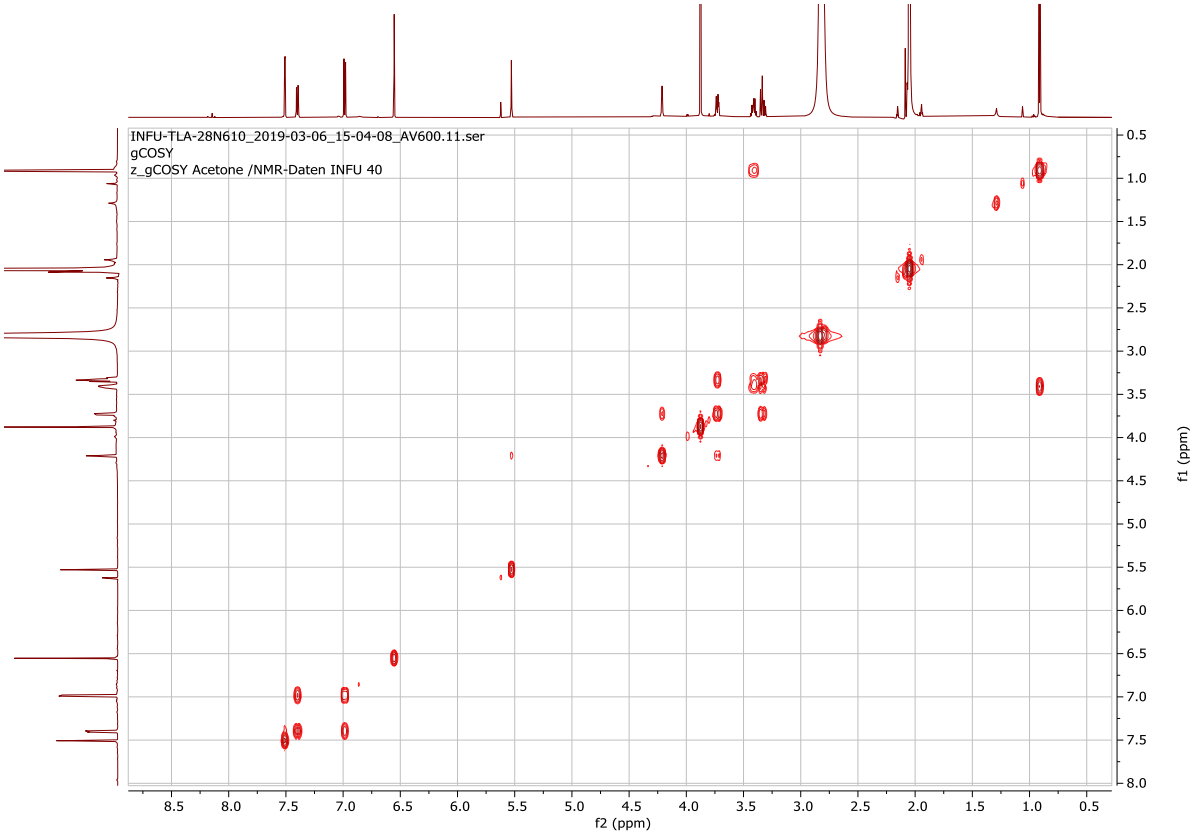
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 16



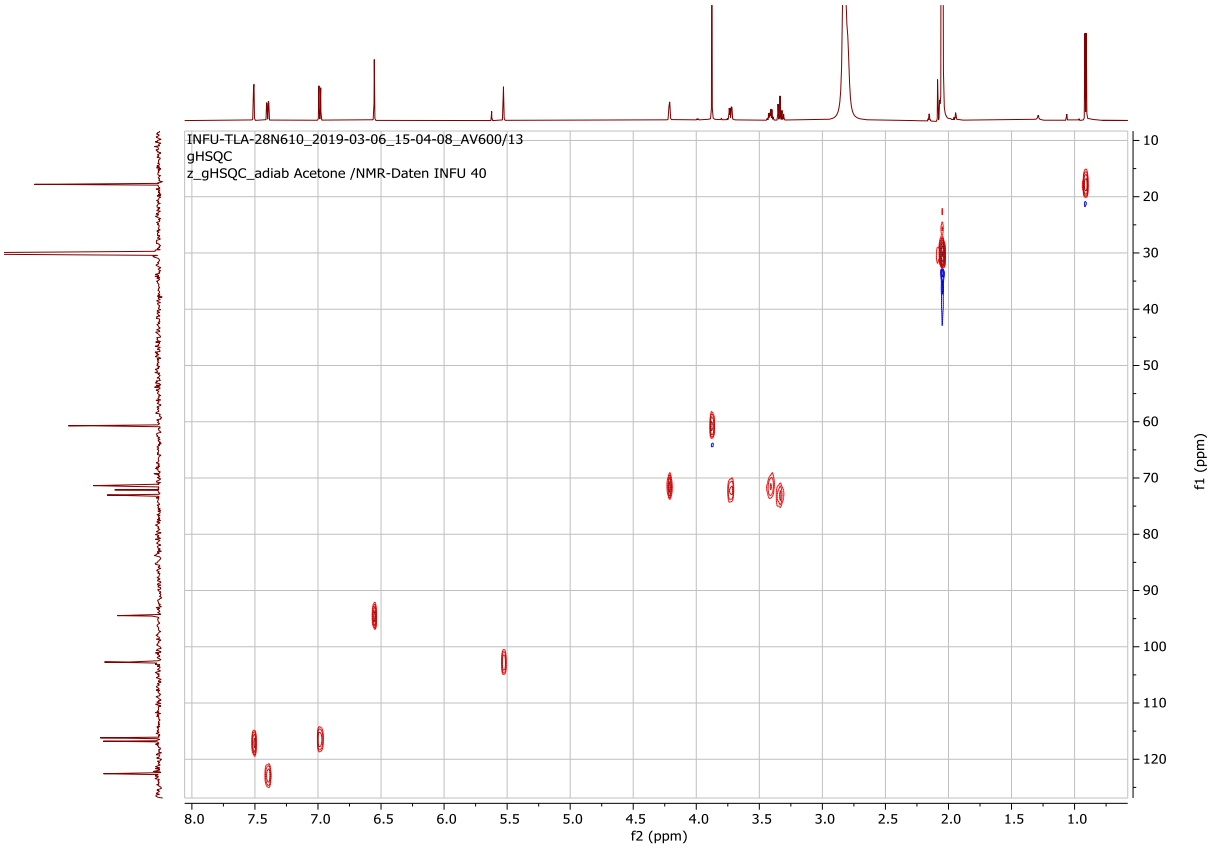
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 16



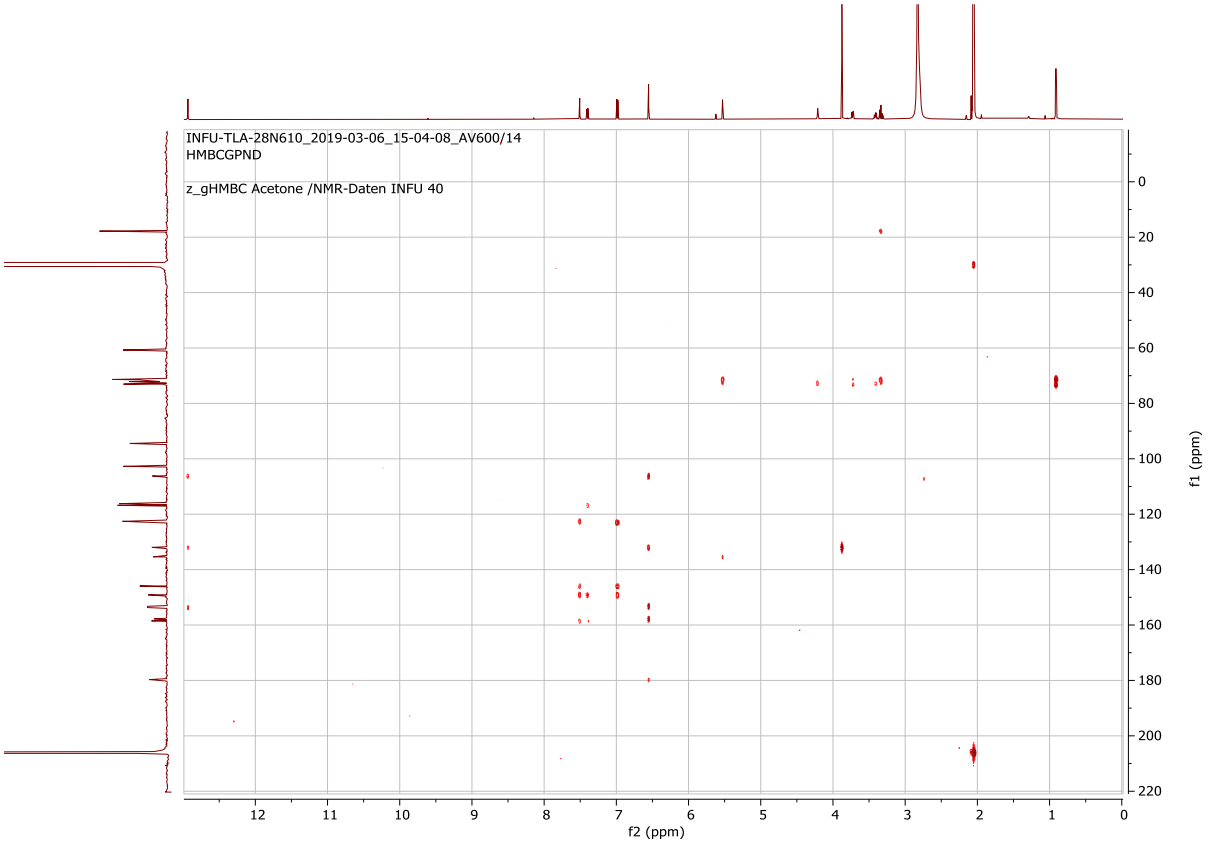
^{1}H-^{1}H-COSY spectrum of compound 16



HSQC spectrum of compound 16

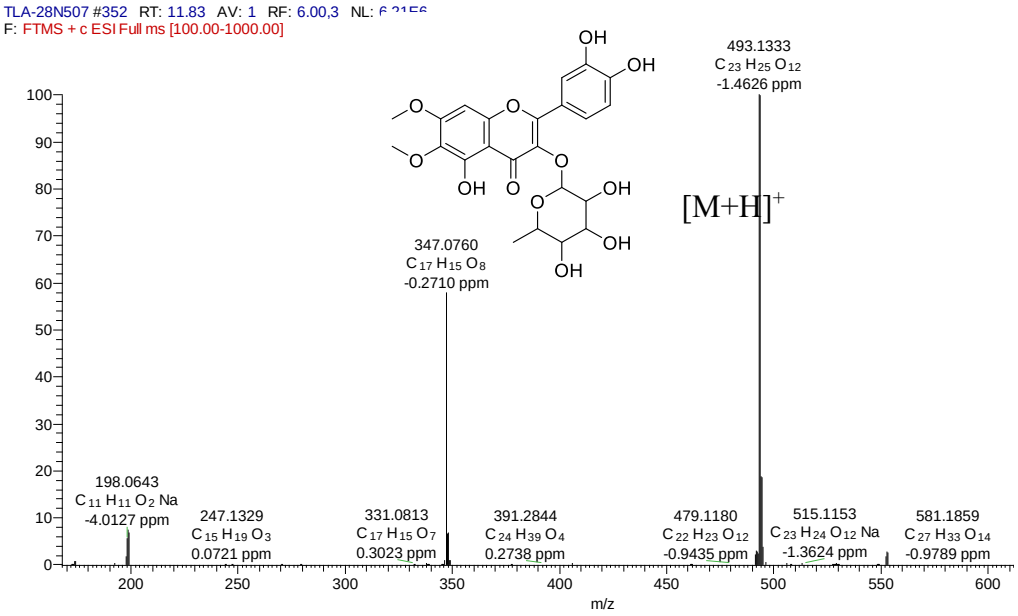


HMBC spectrum of compound 16

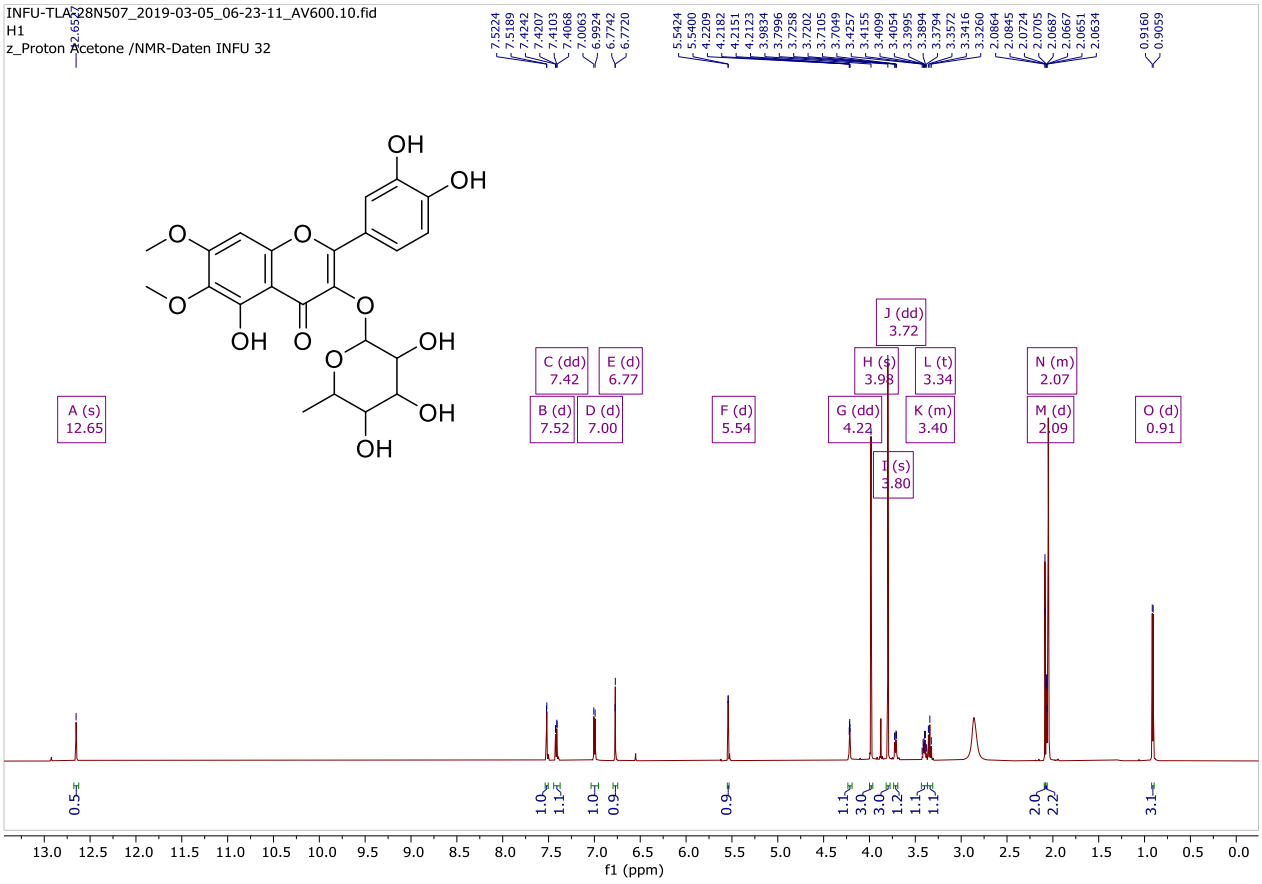


Appendix A17: Spectra for compound 17

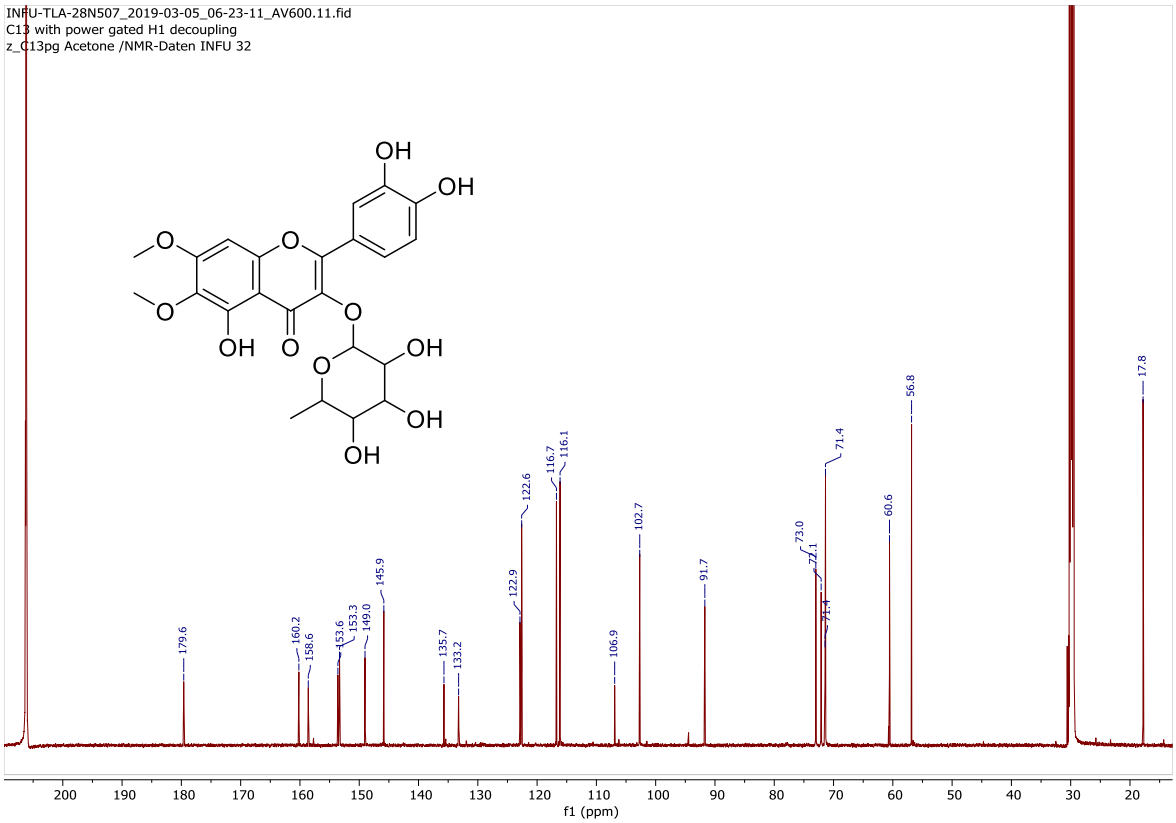
HRESIMS spectrum of compound 17



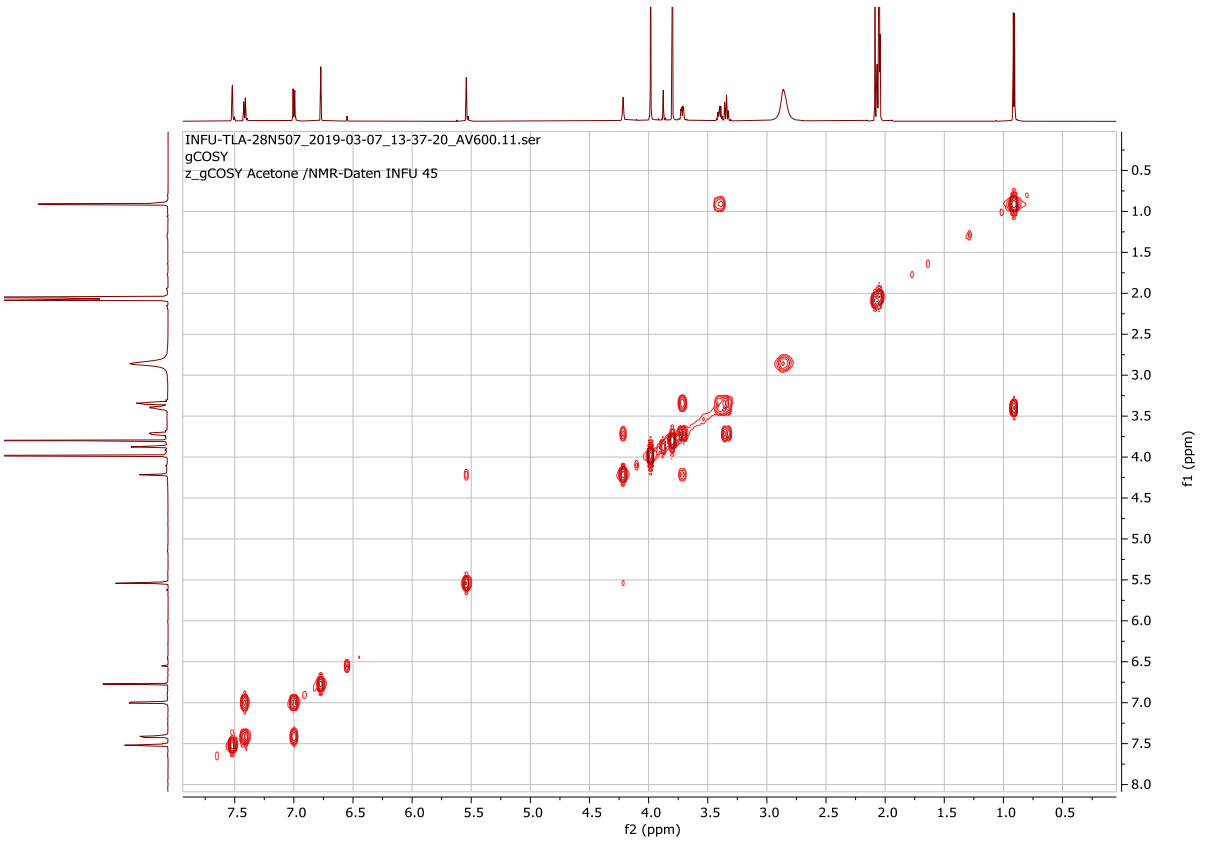
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 17



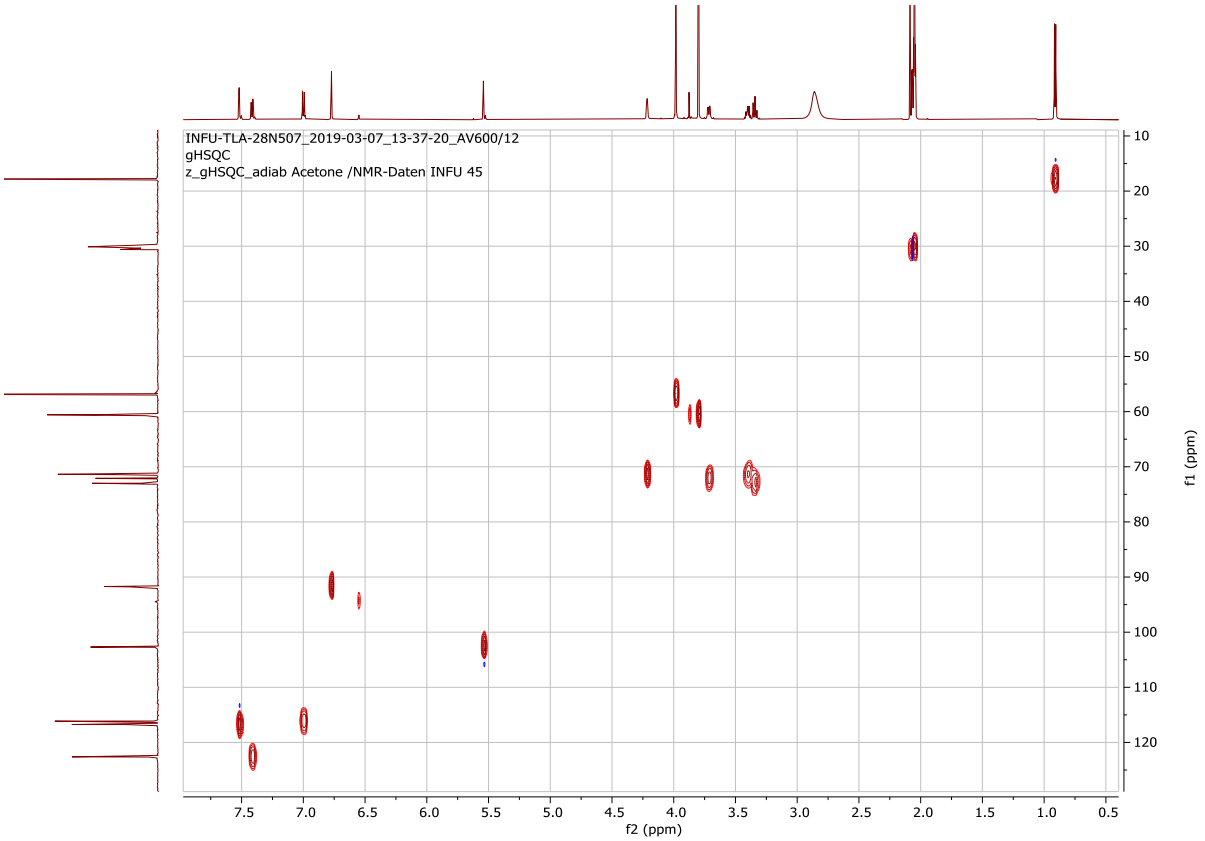
^{13}C NMR spectrum (150 MHz, Acetone-d$_6$) of compound 17



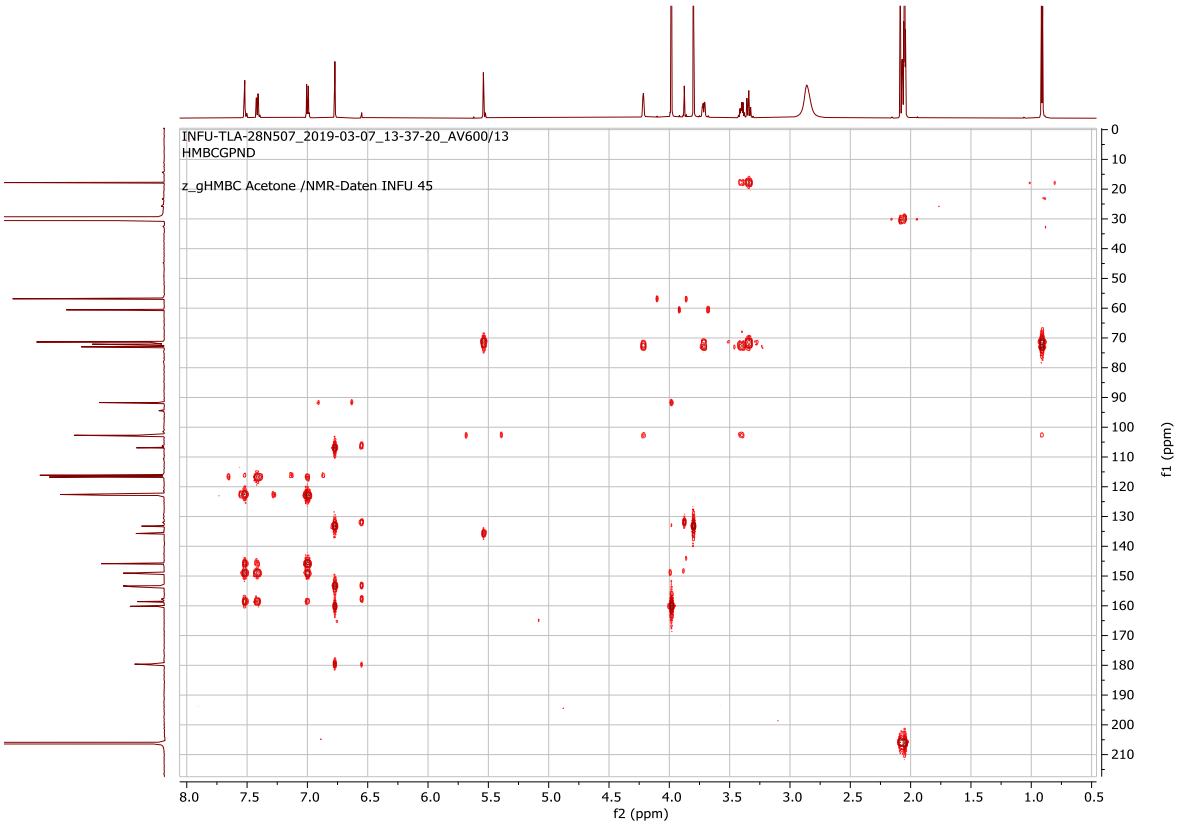
^{1}H-^{1}H-COSY spectrum of compound 17



HSQC spectrum of compound 17

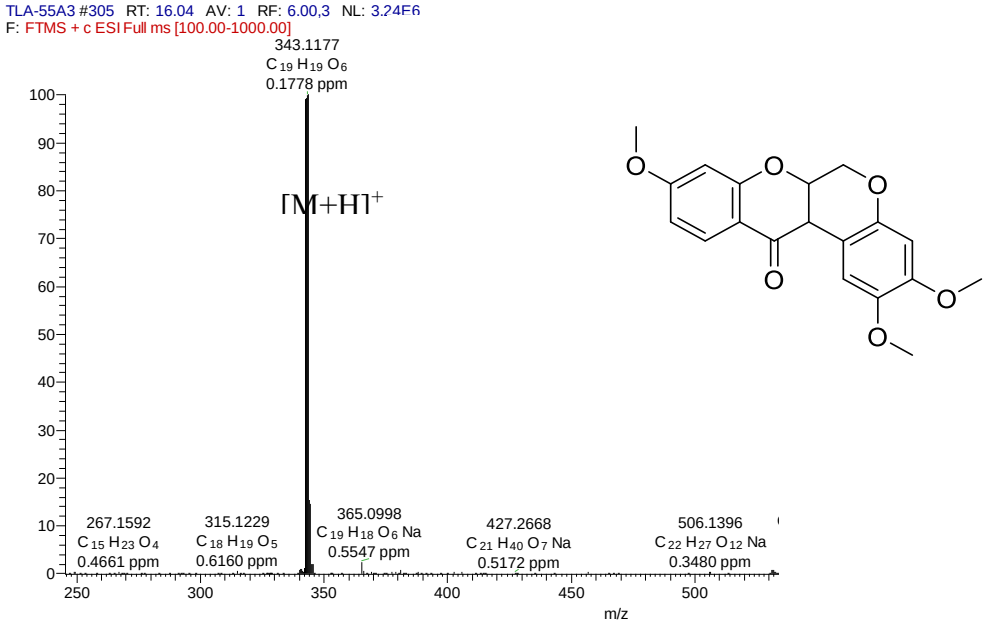


HMBC spectrum of compound 17

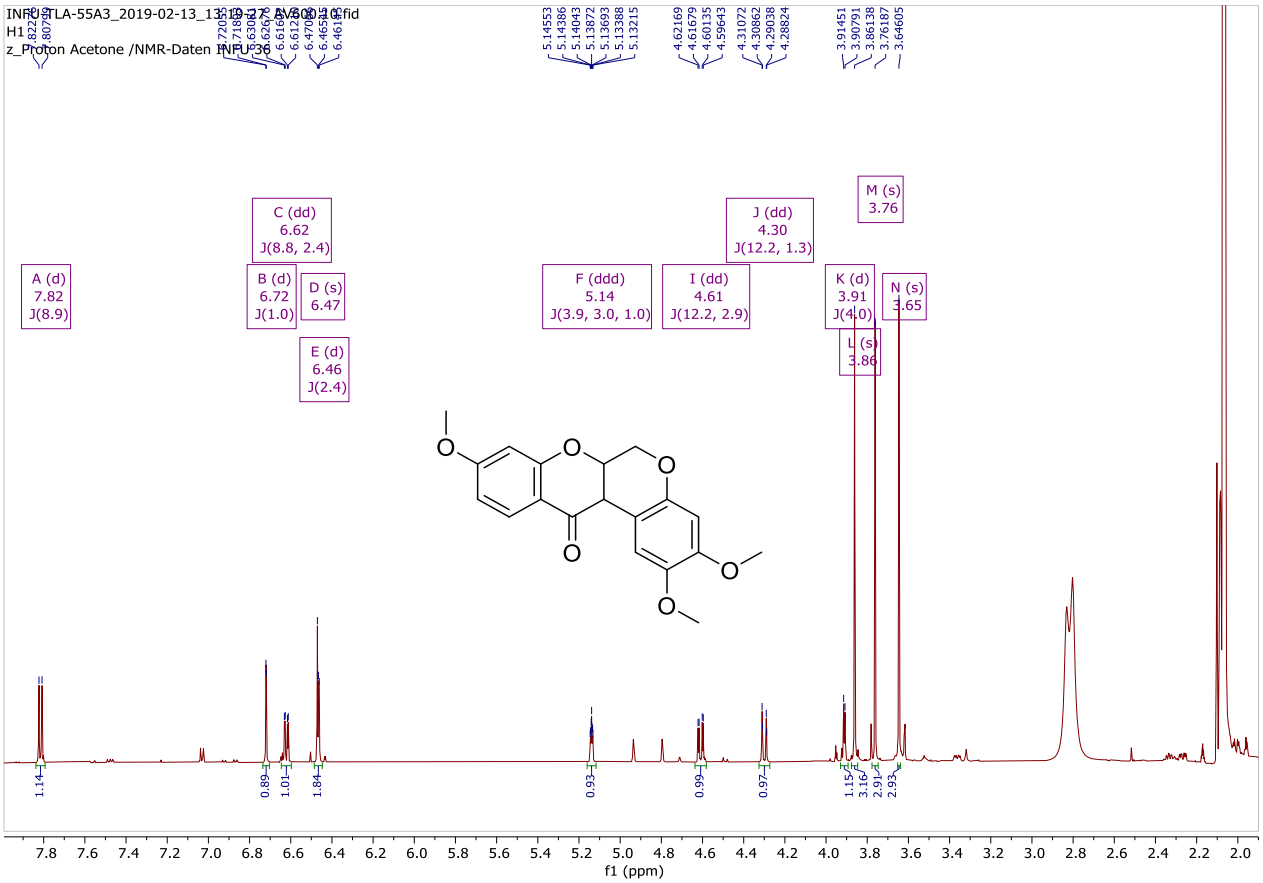


Appendix A18: Spectra for compound 18

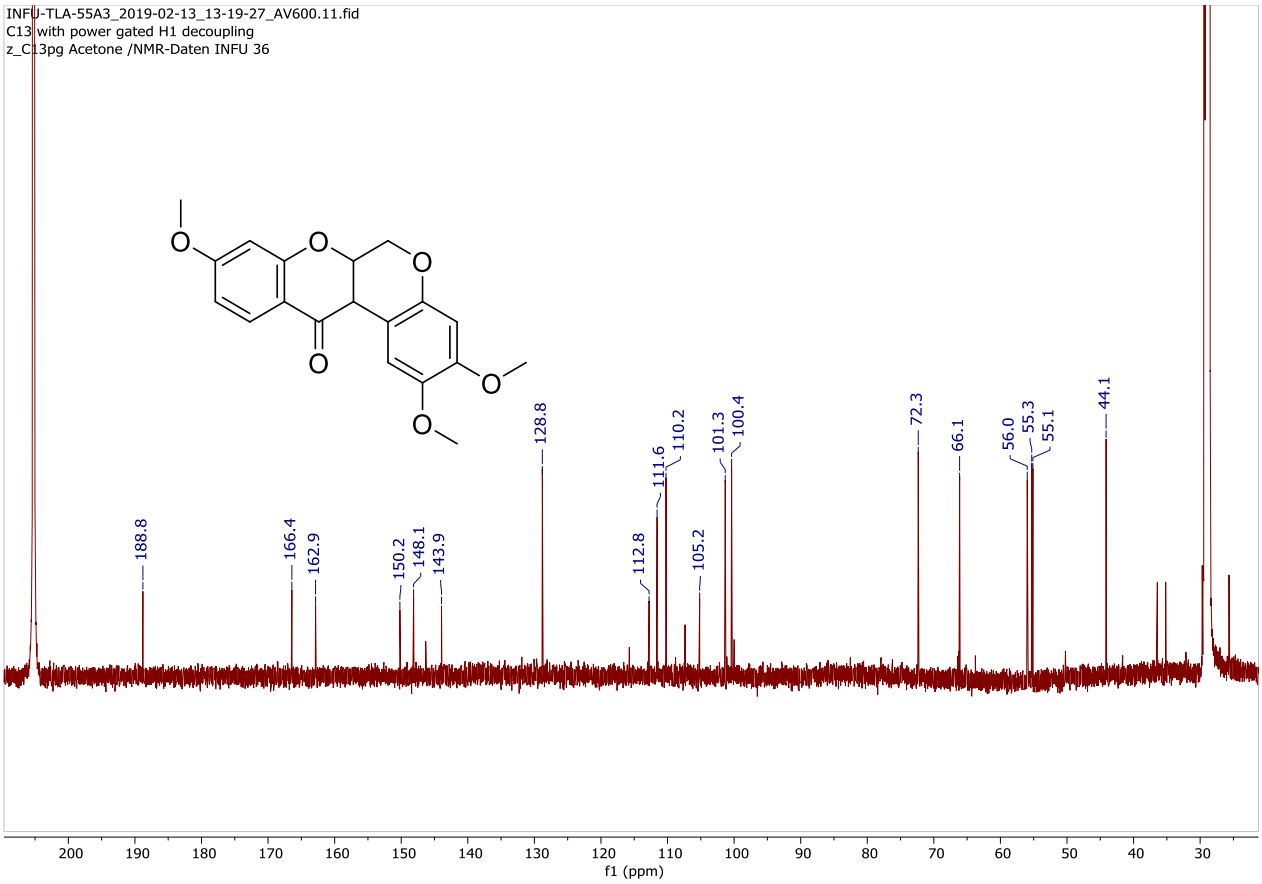
HRESIMS spectrum of compound 18



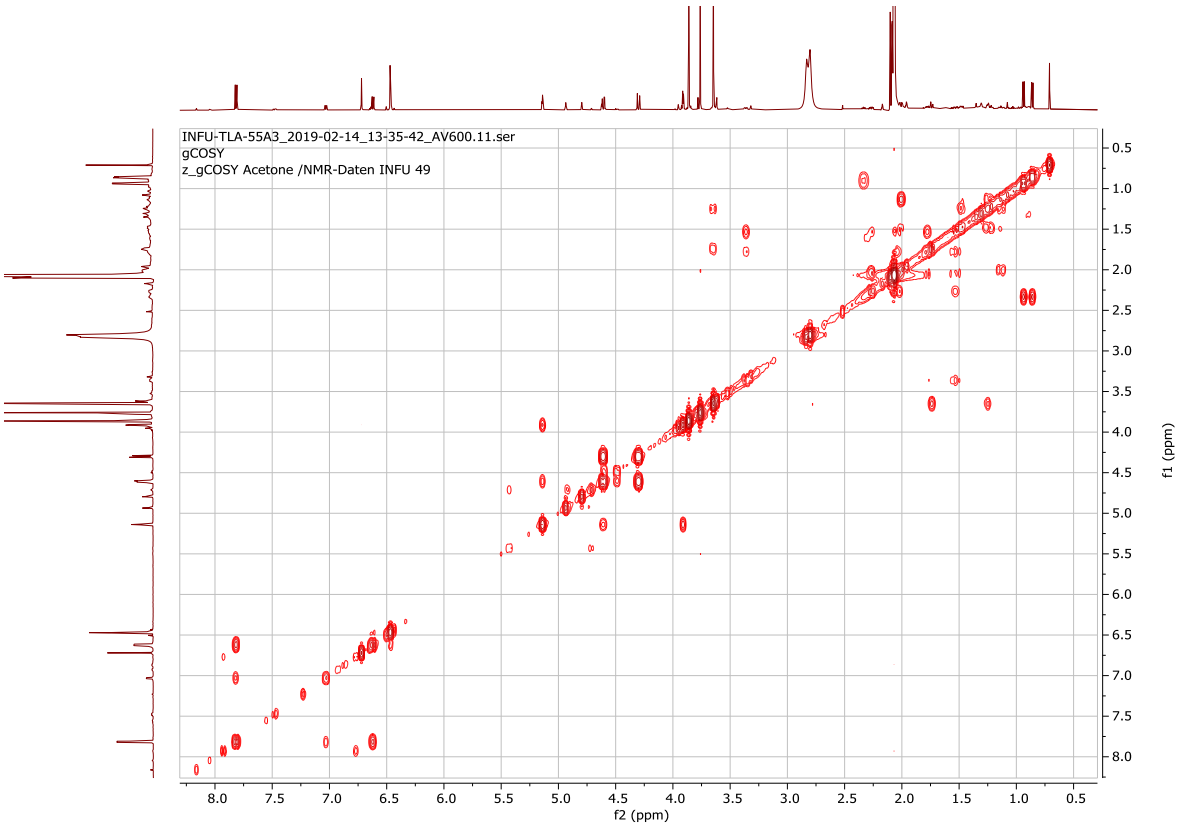
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 18



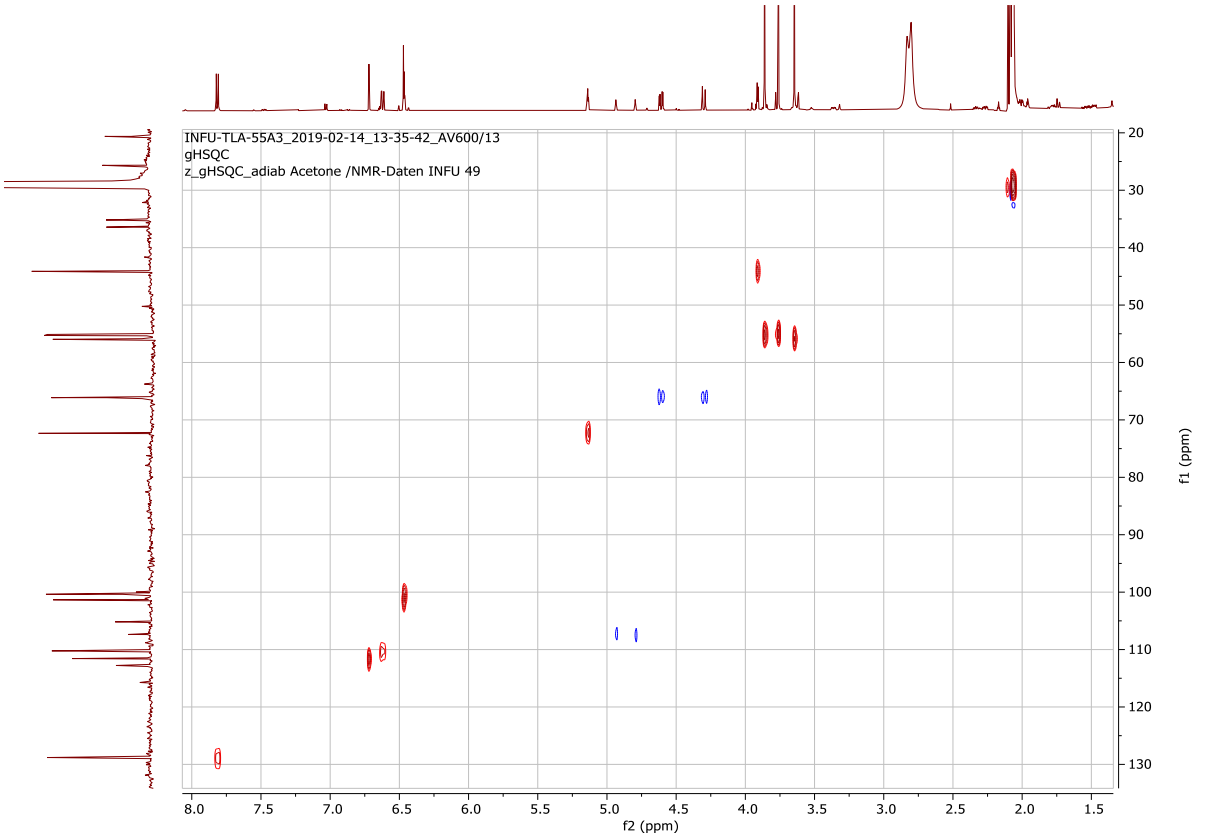
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 18



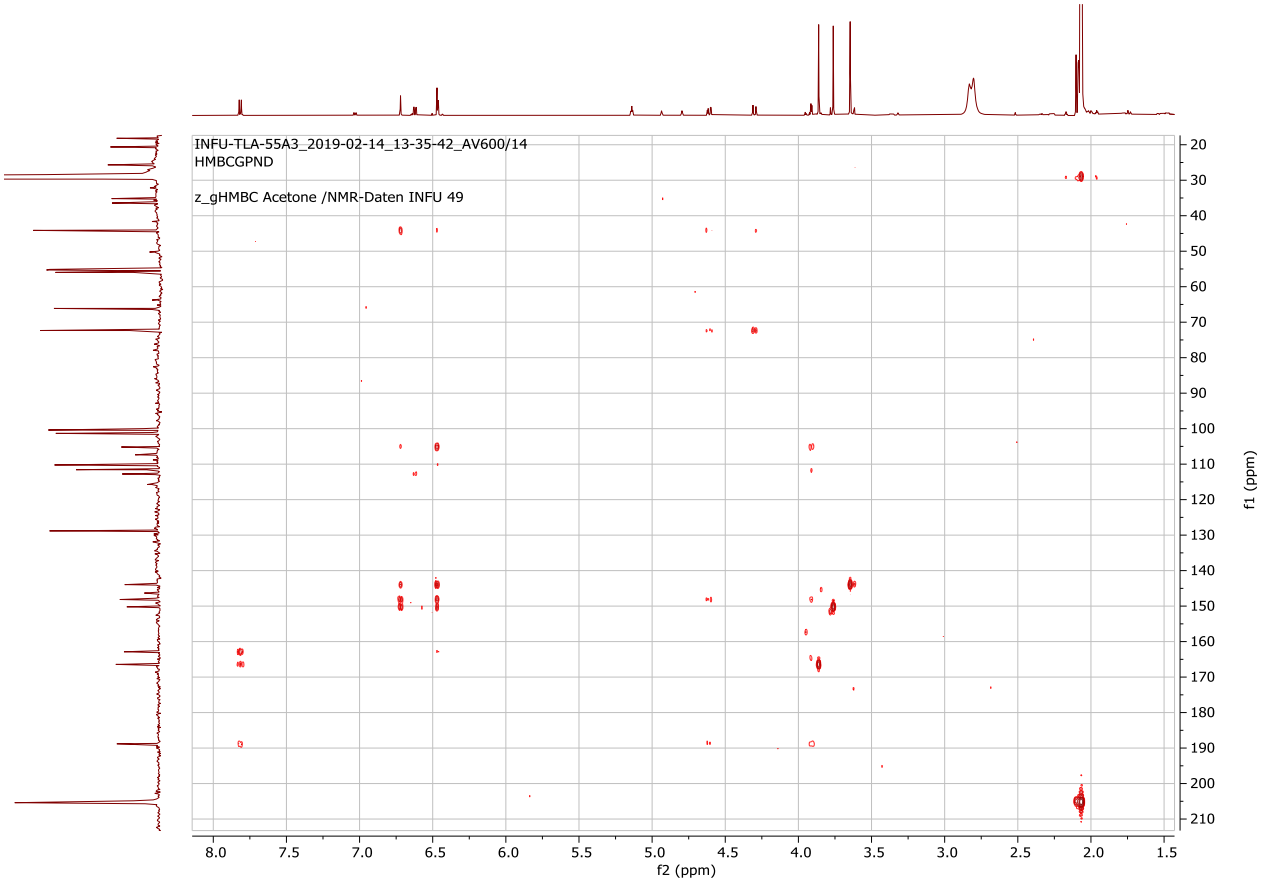
^{1}H-^{1}H-COSY spectrum of compound 18



HSQC spectrum of compound 18

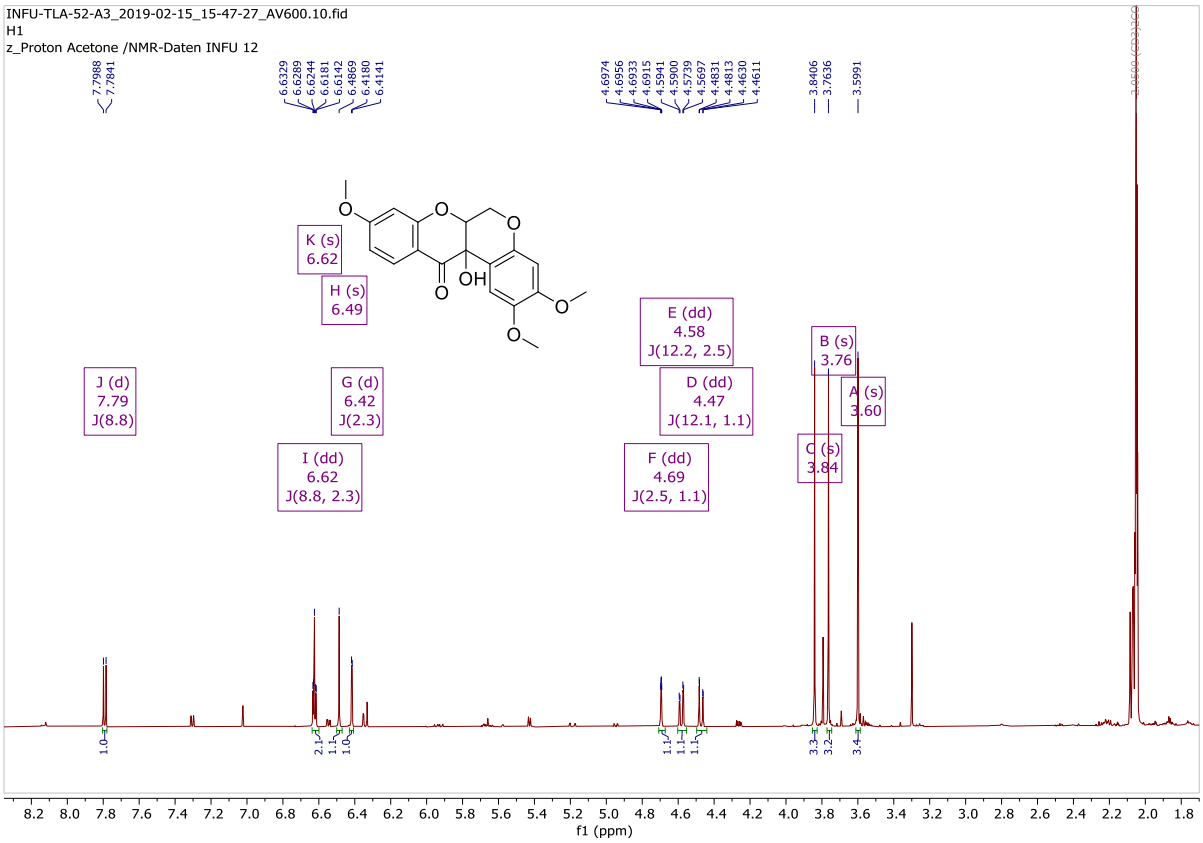


HMBC spectrum of compound 18

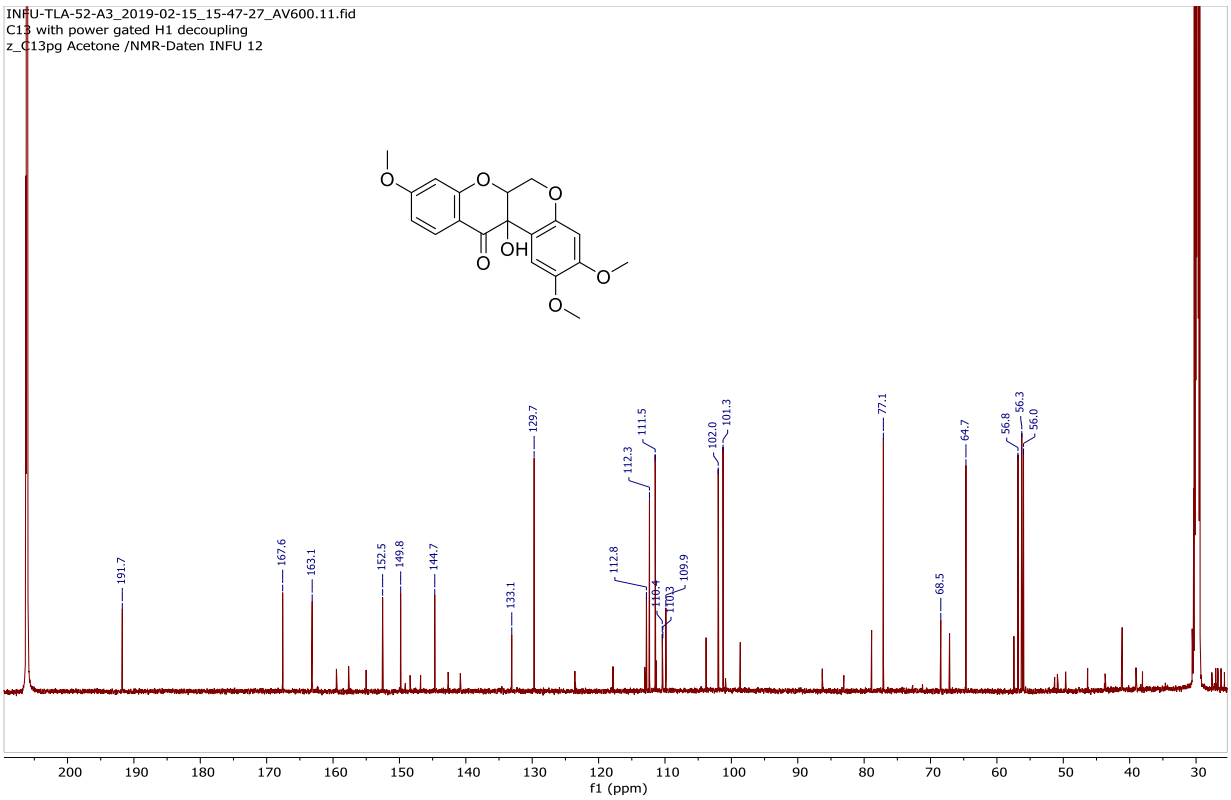


Appendix A19: Spectra for compound 19

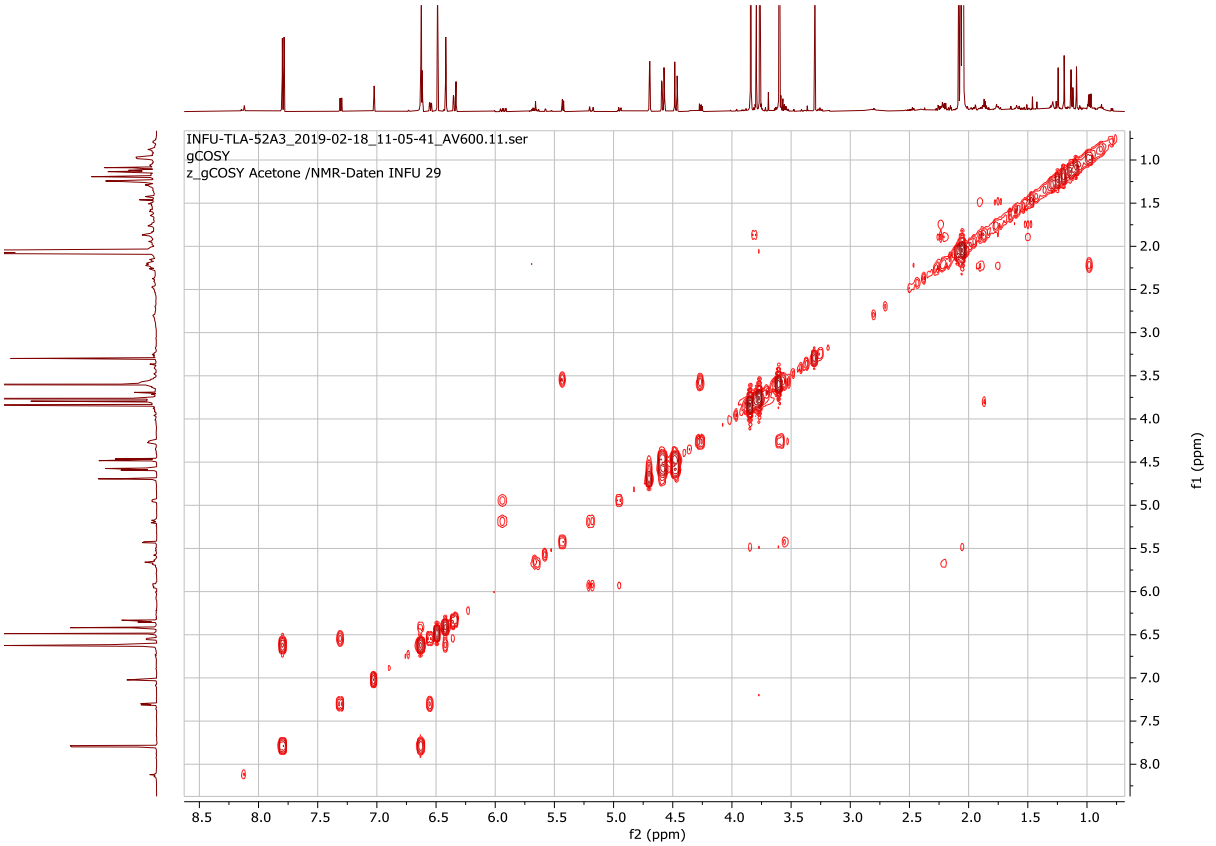
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 19



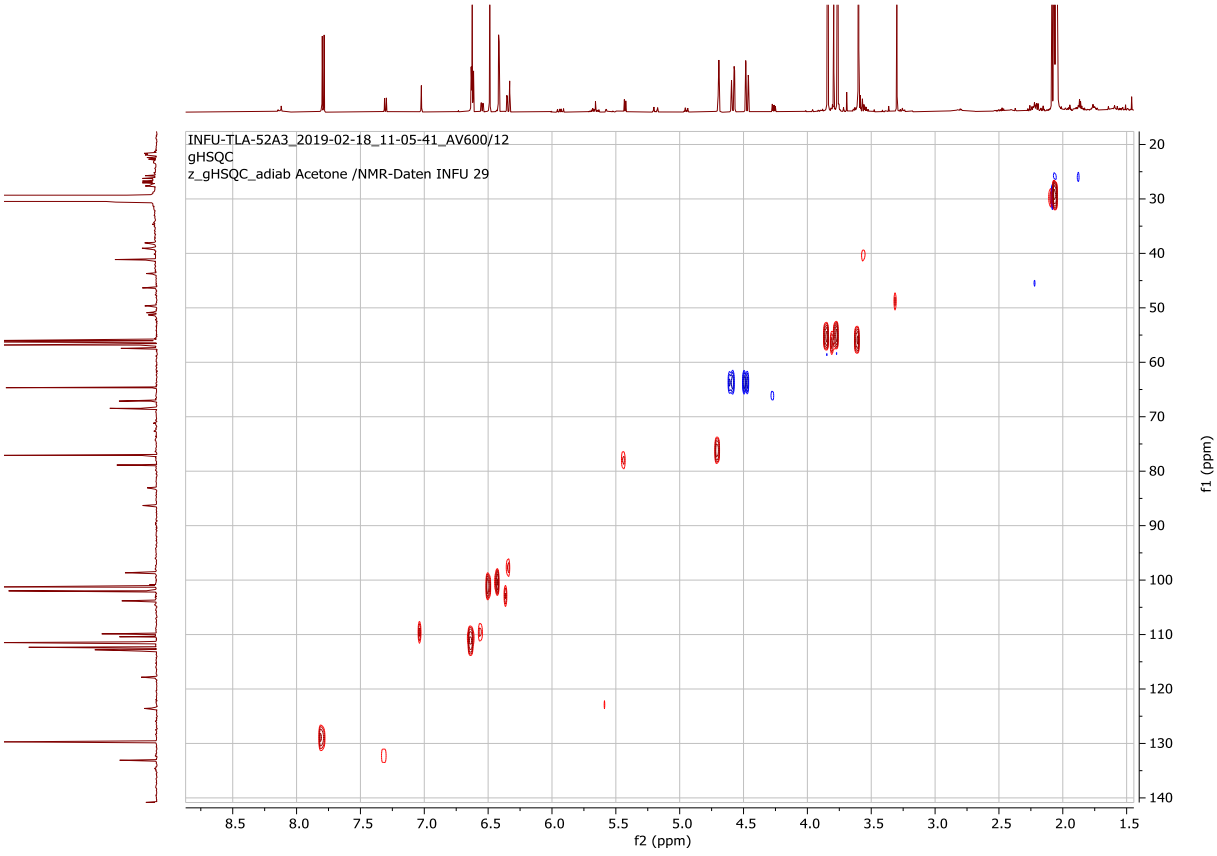
^{13}C NMR spectrum (150 MHz, Acetone-d$_6$) of compound 19



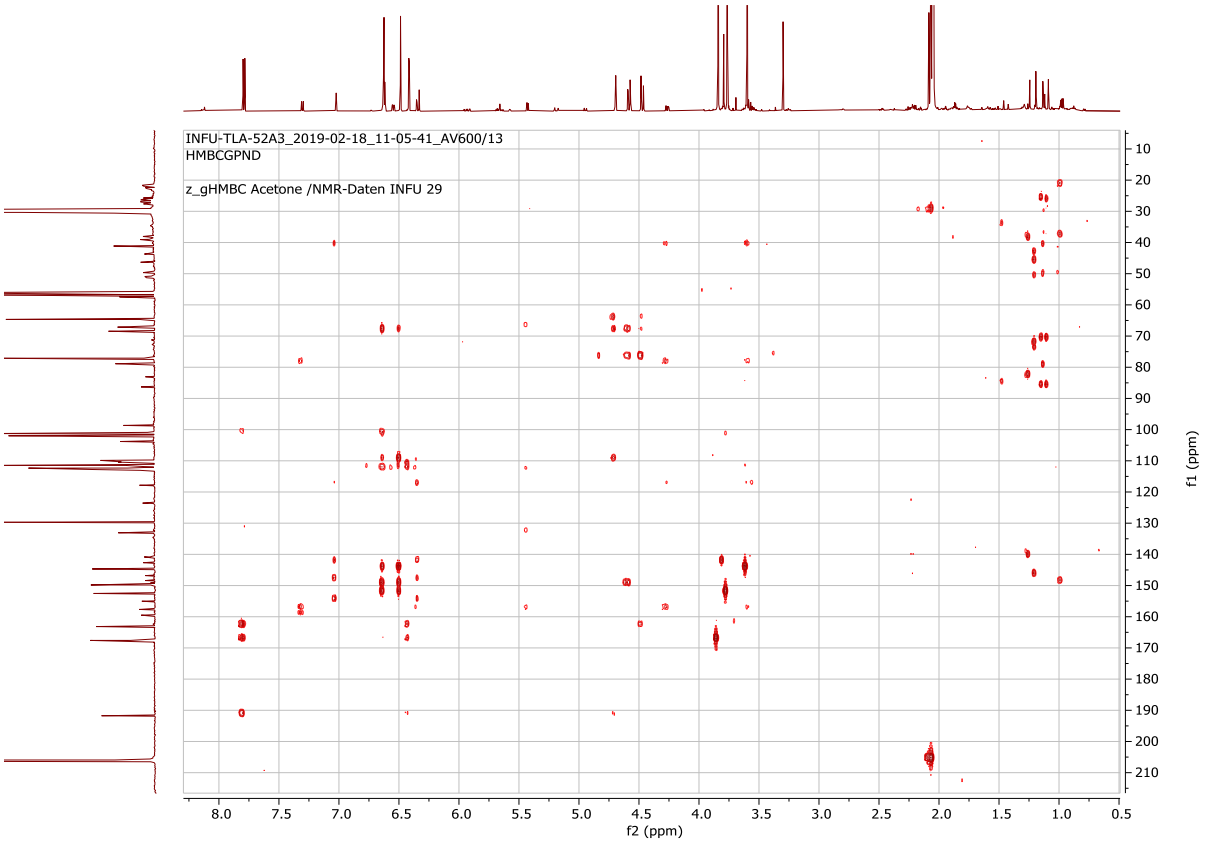
^{1}H-^{1}H-COSY spectrum of compound 19



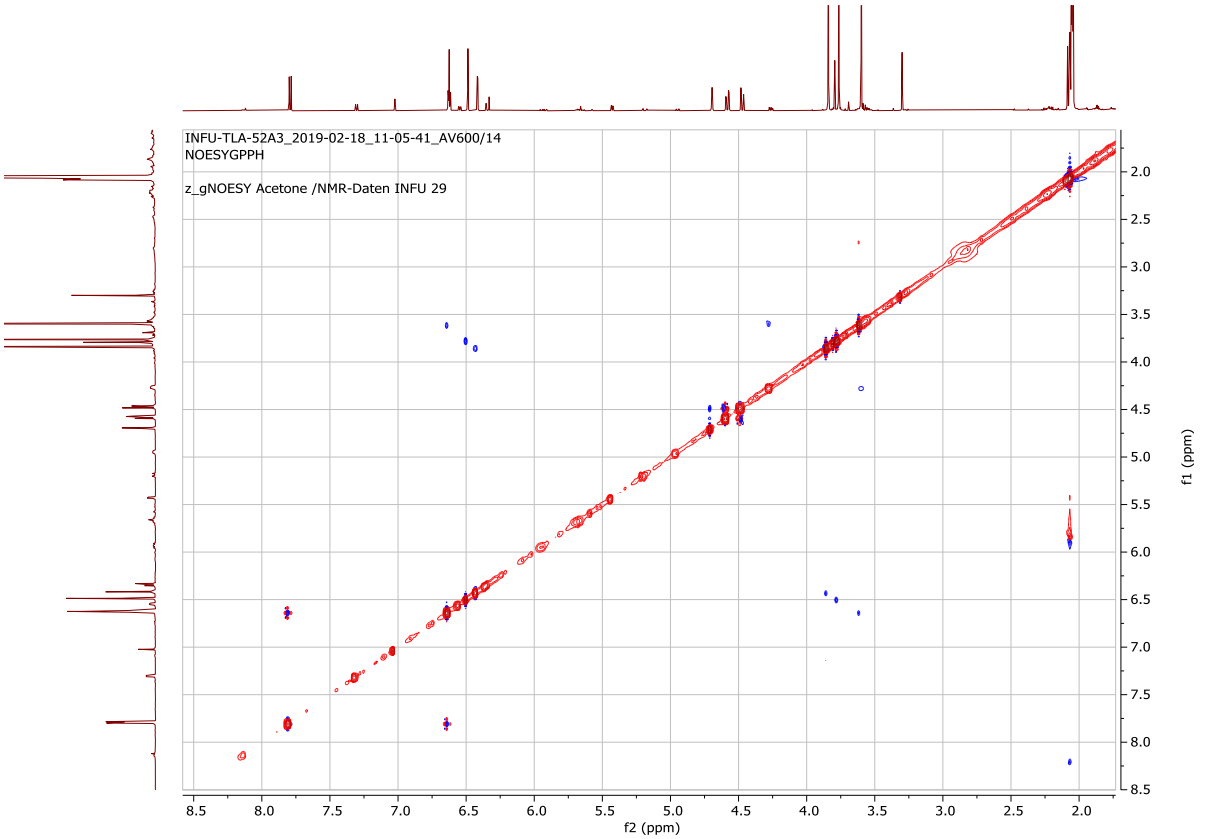
HSQC spectrum of compound 19



HMBC spectrum of compound 19

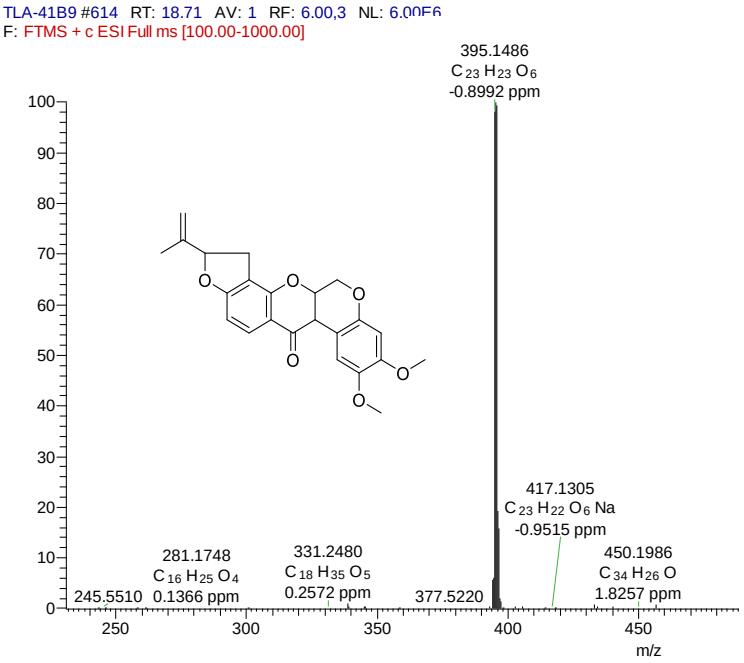


NOESY spectrum of compound 19

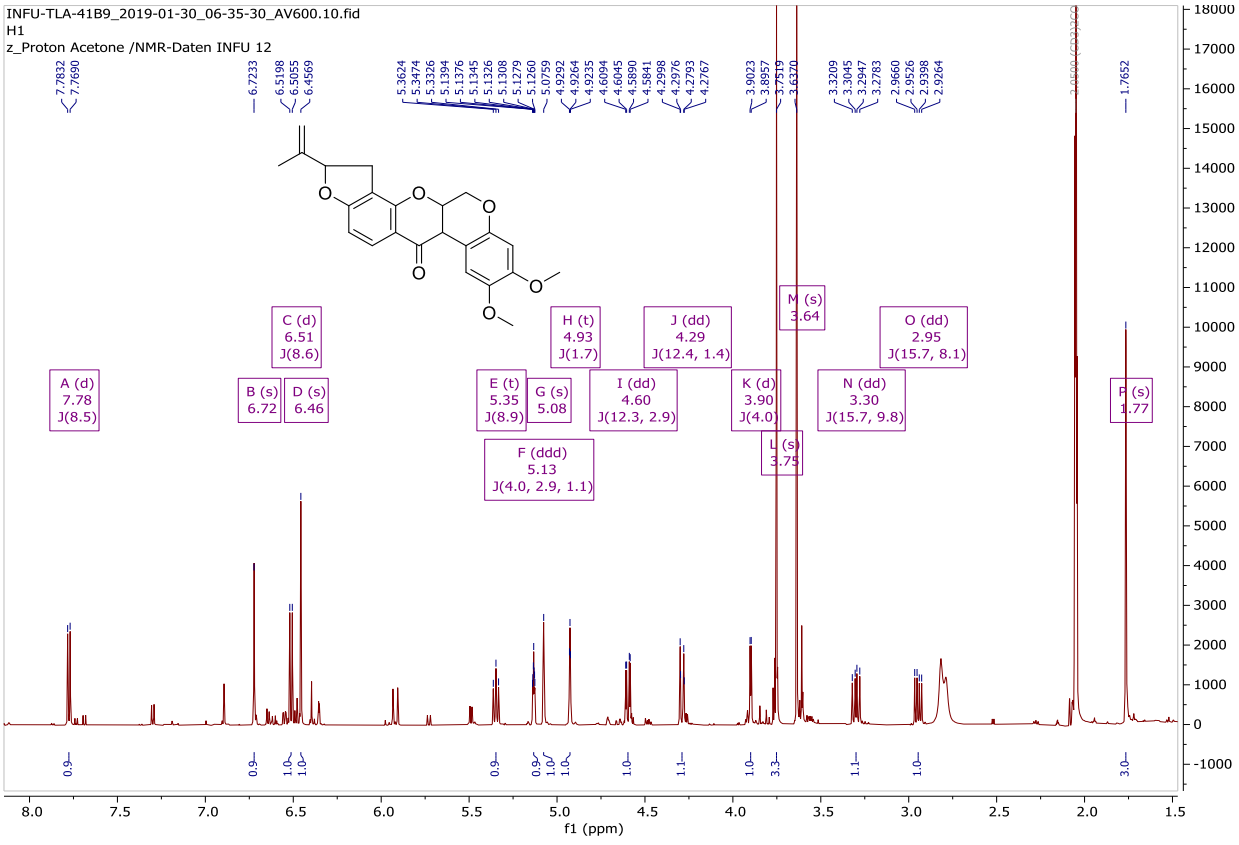


Appendix A20: Spectra for compound 20

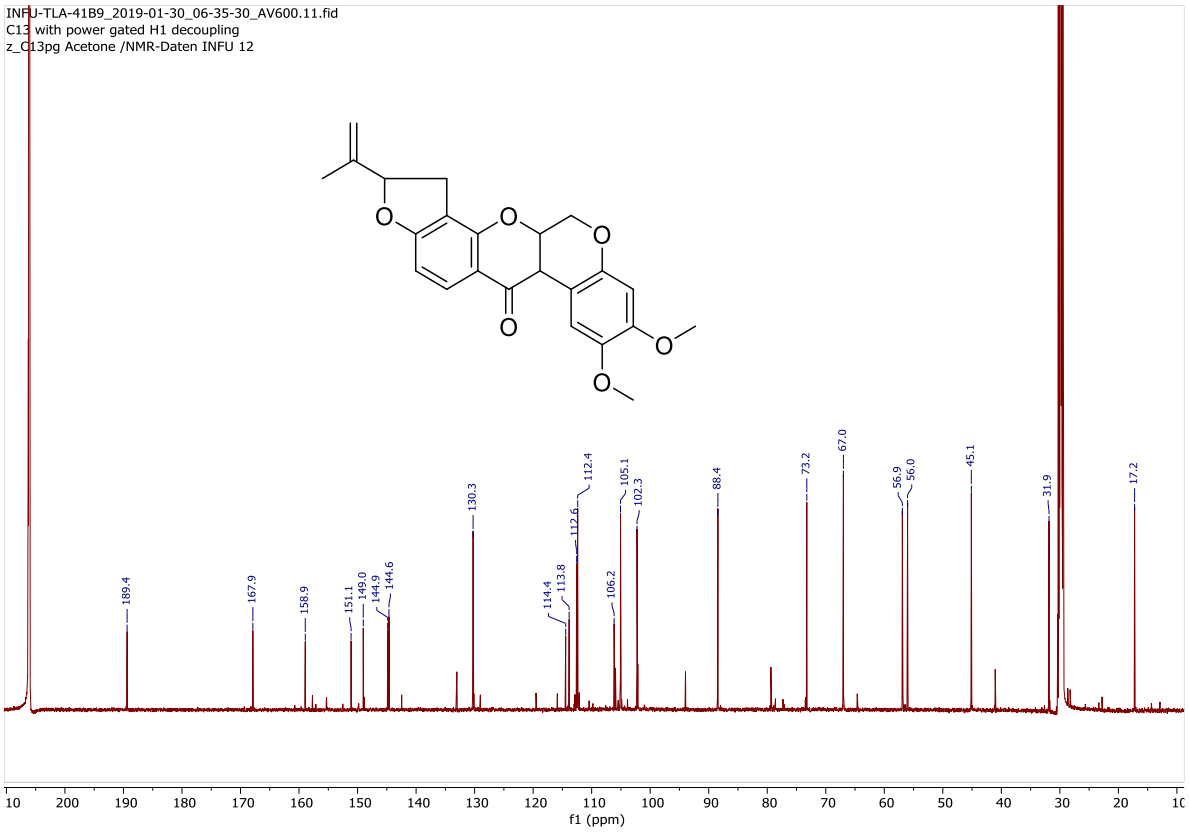
HRESIMS spectrum of compound 20



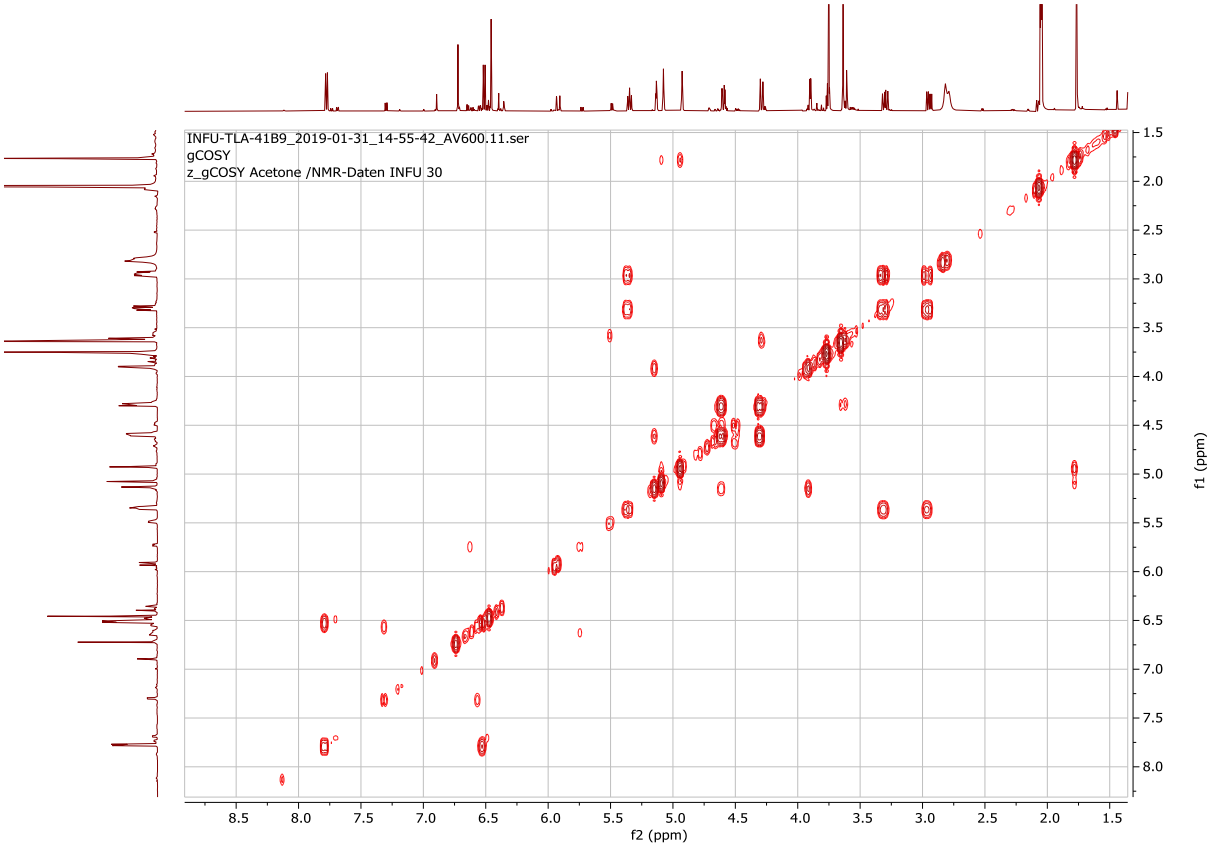
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 20



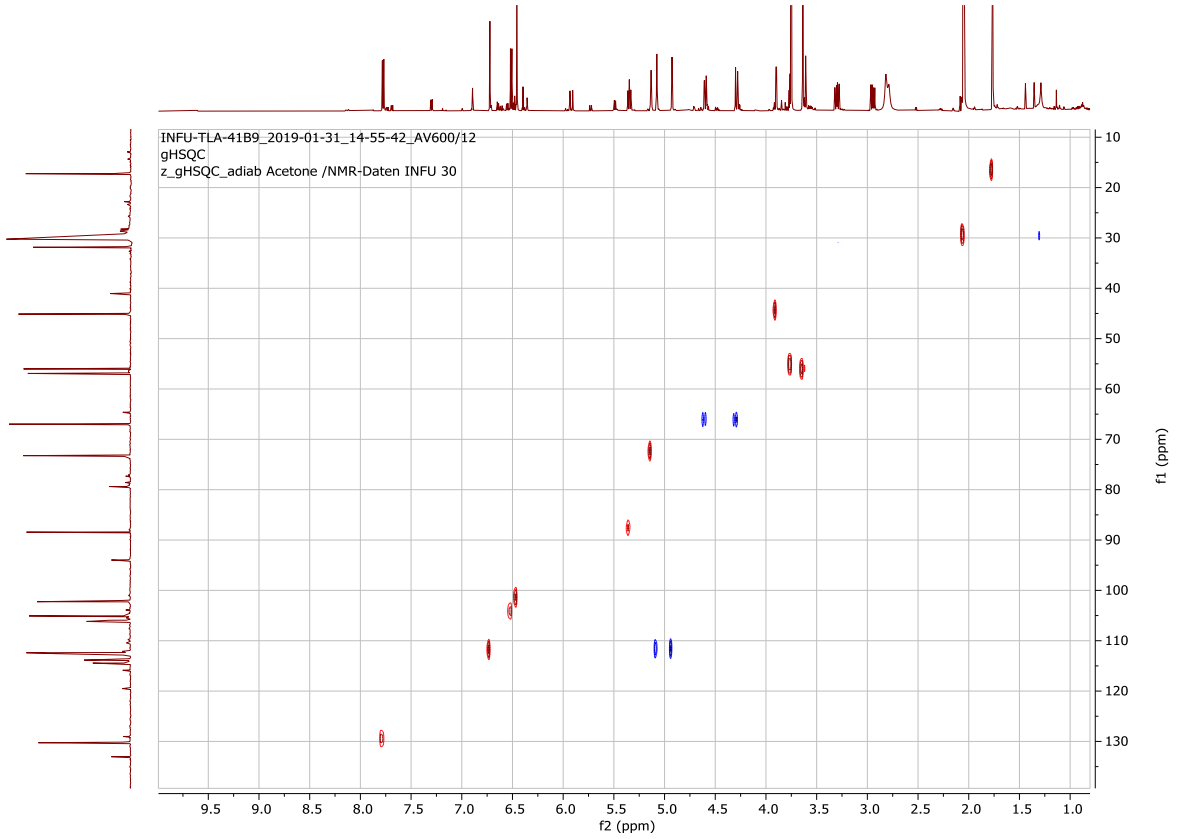
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 20



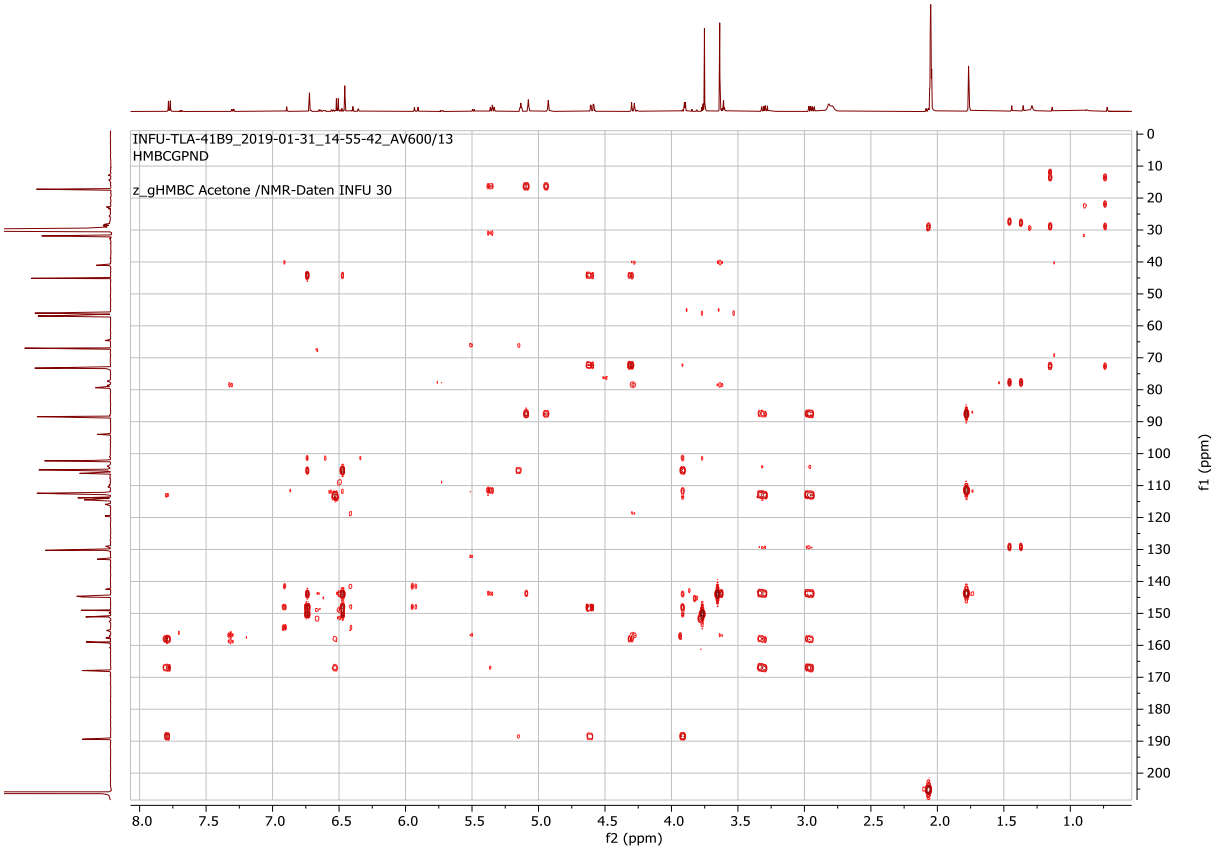
^{1}H-^{1}H-COSY spectrum of compound 20



HSQC spectrum of compound 20

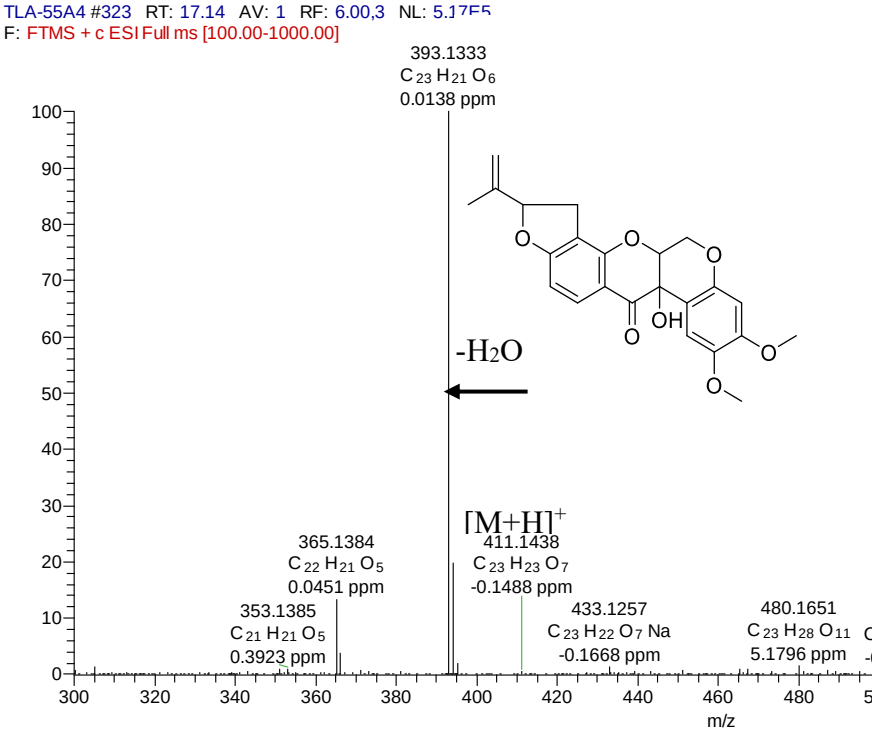


HMBC spectrum of compound 20

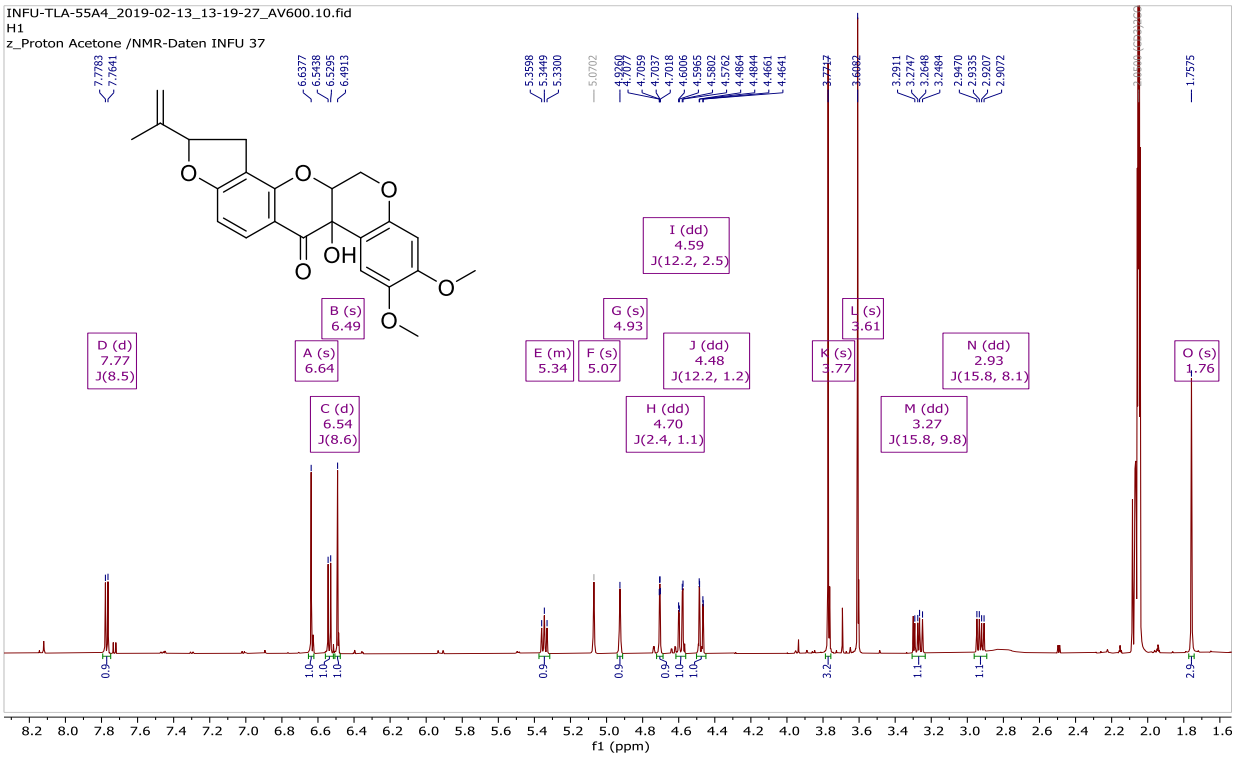


Appendix A21: Spectra for compound 21

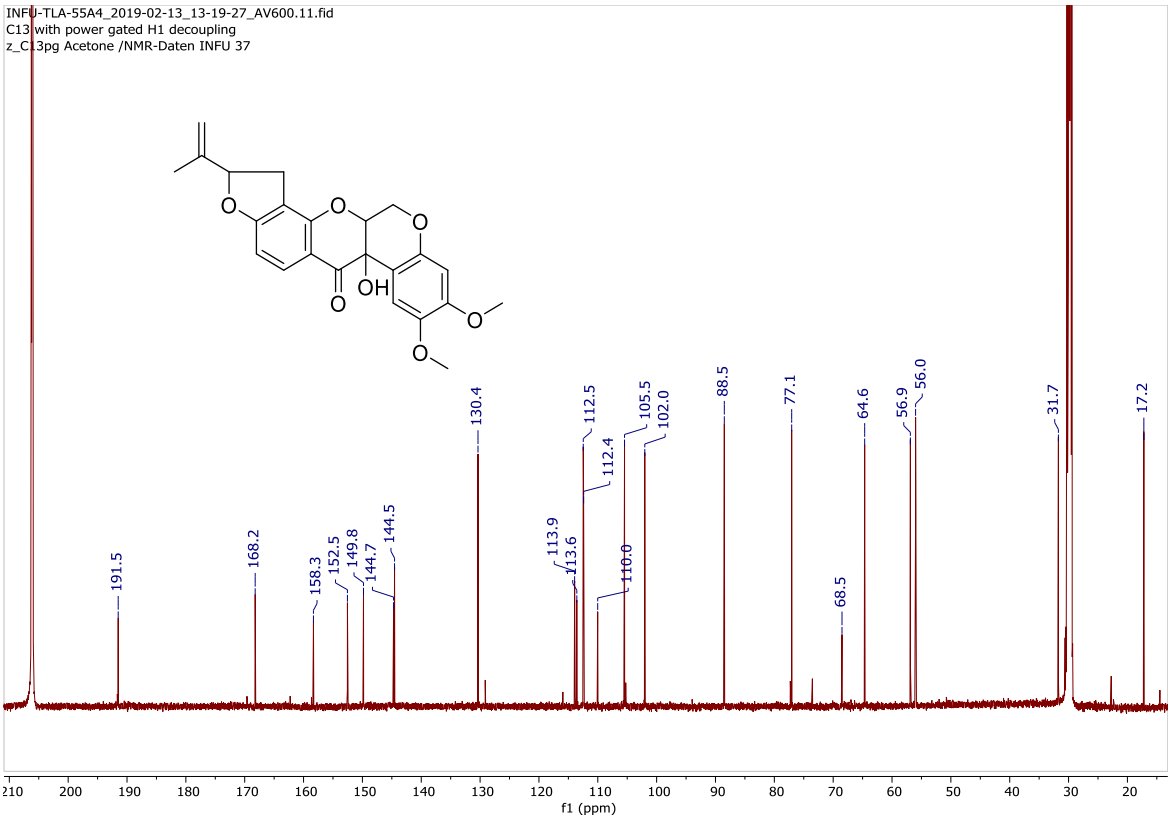
HRESIMS spectrum of compound 21



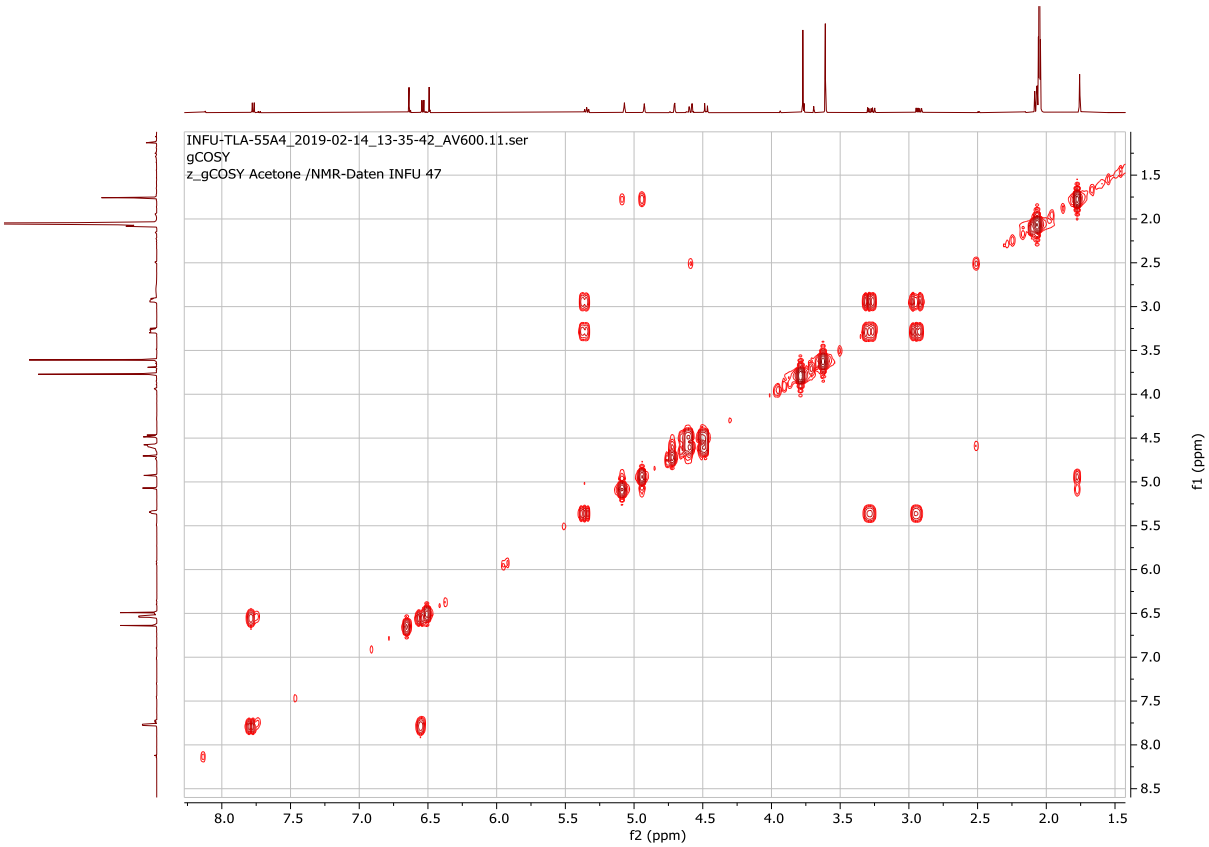
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 21



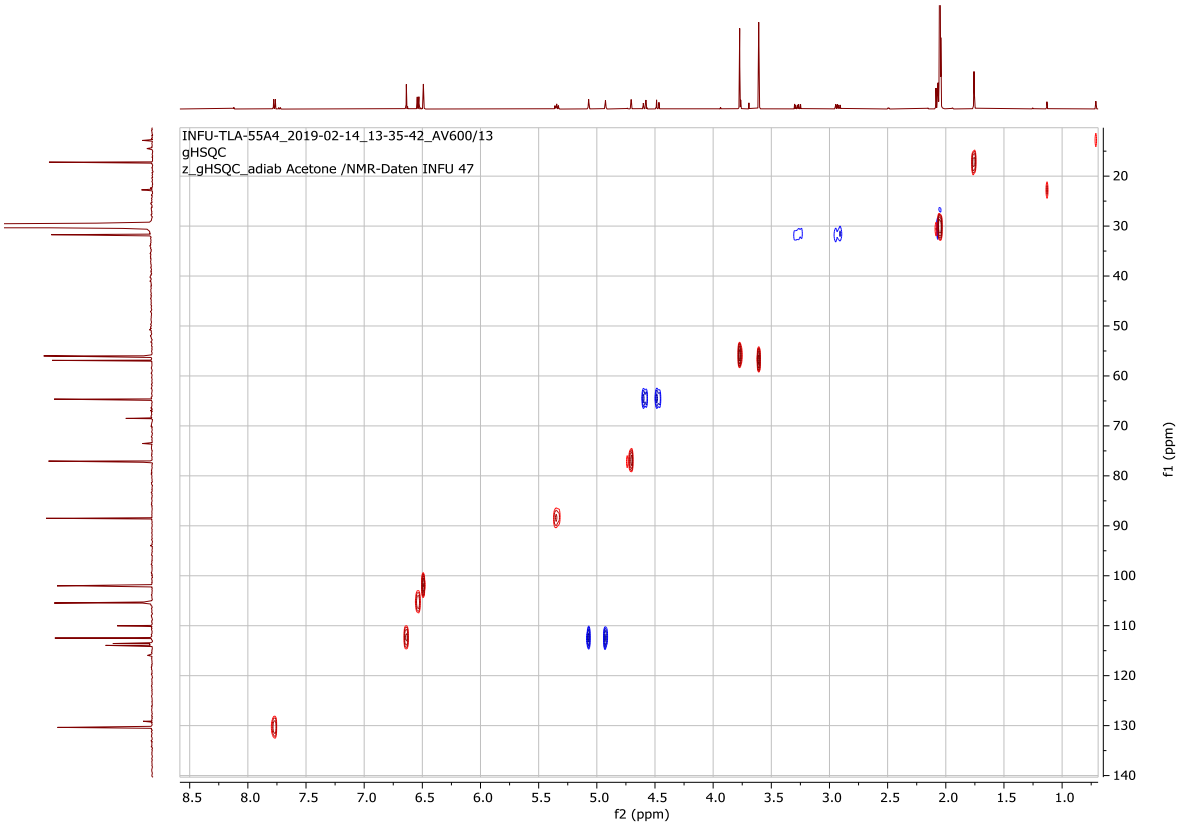
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 21



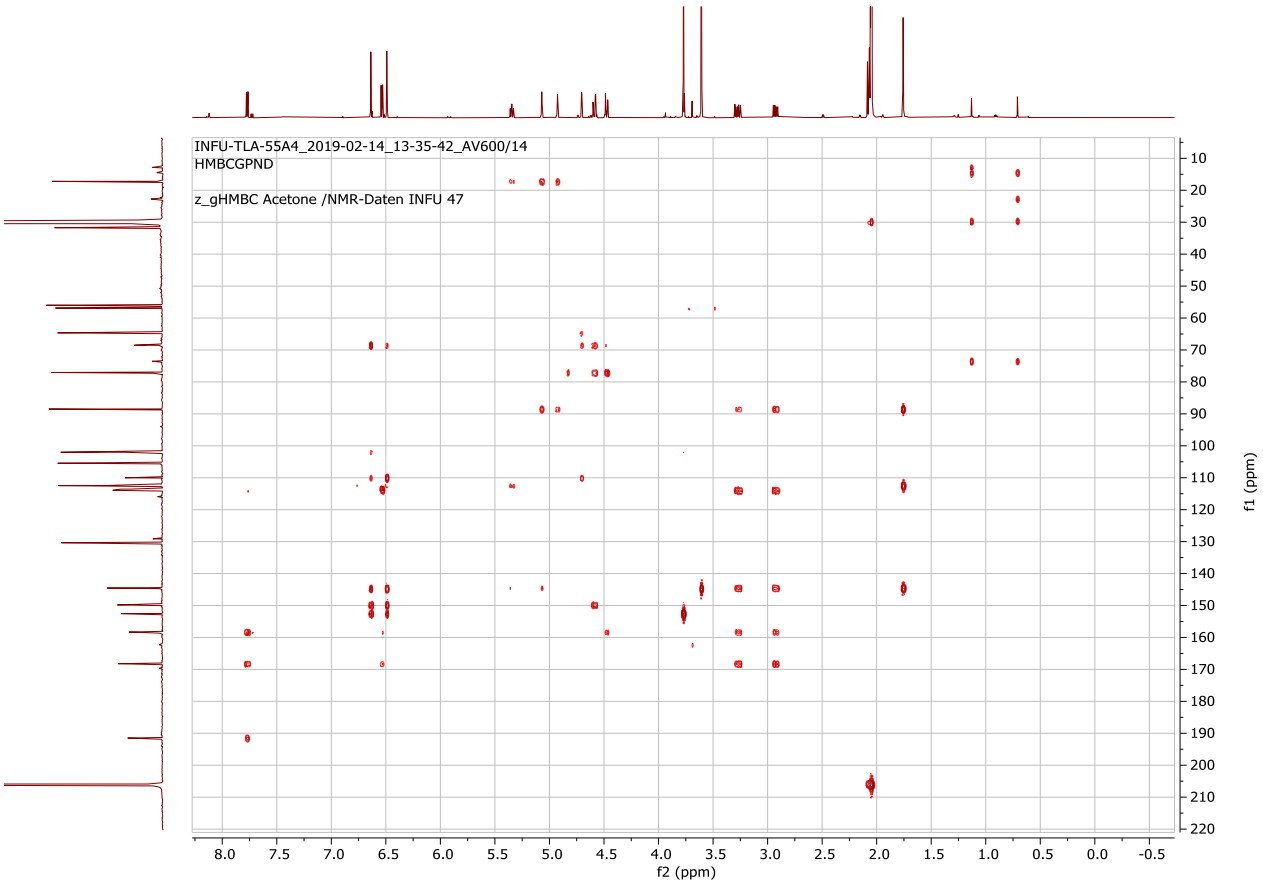
^{1}H-^{1}H-COSY spectrum of compound 21



HSQC spectrum of compound 21

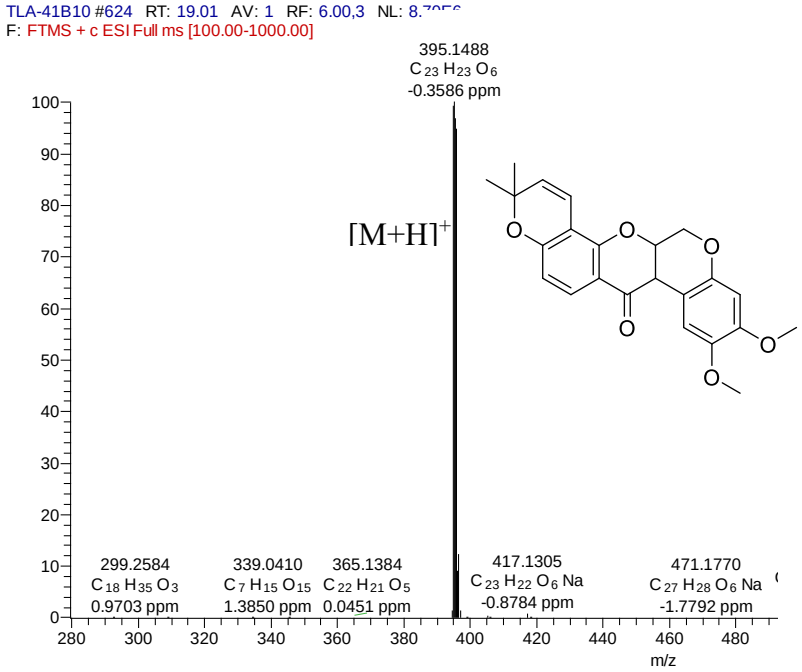


HMBC spectrum of compound 21

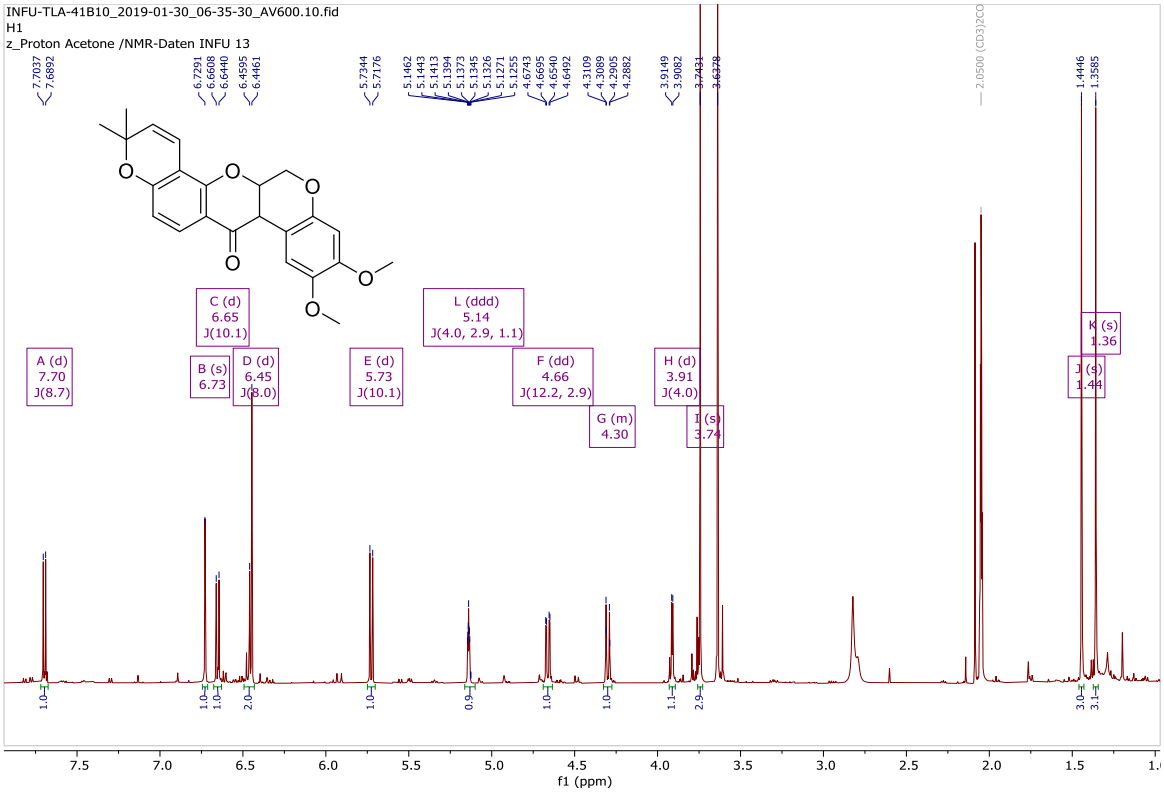


Appendix A22: Spectra for compound 22

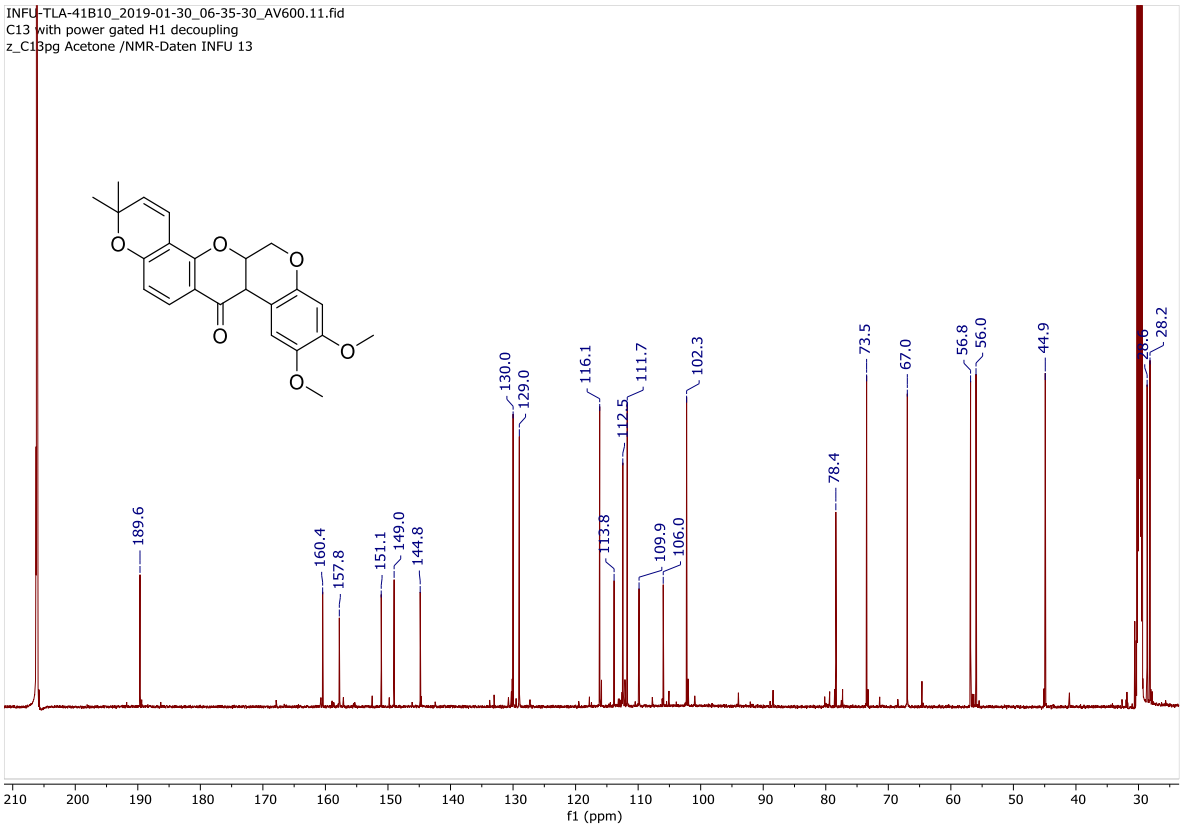
HRESIMS spectrum of compound 22



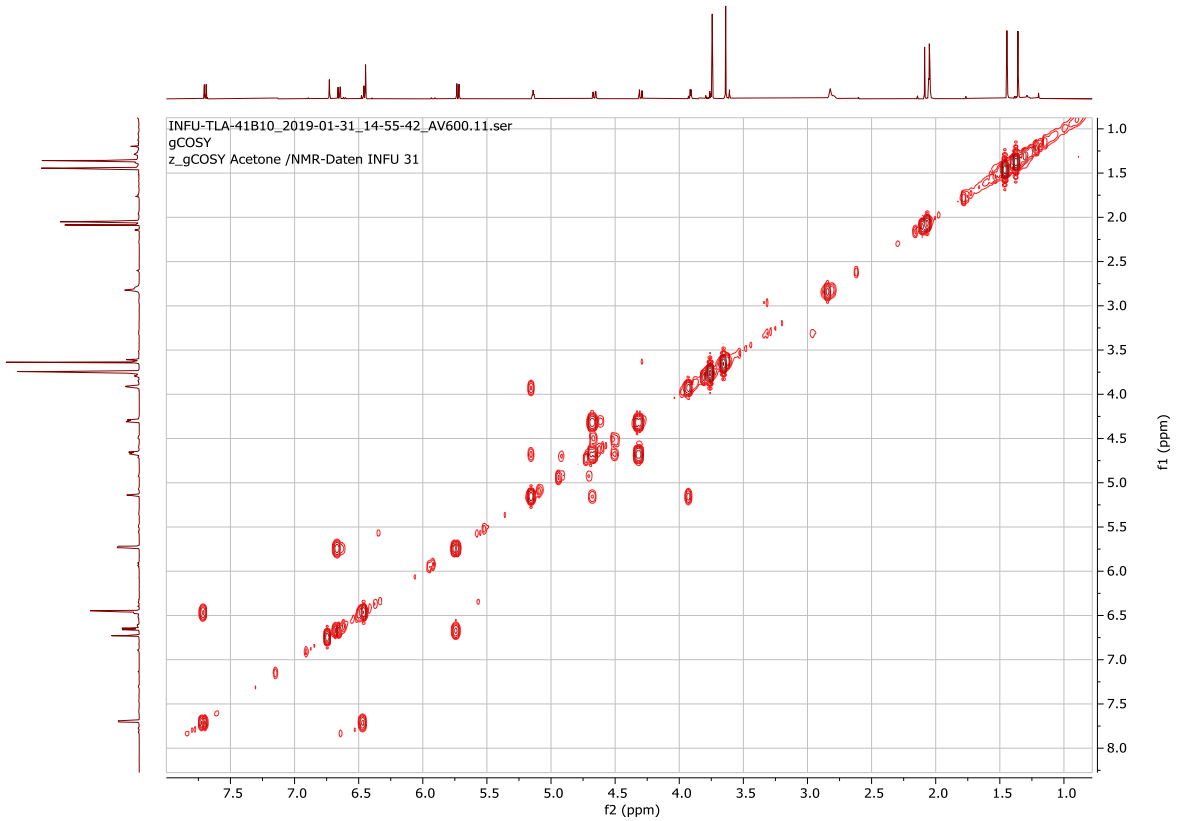
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 22



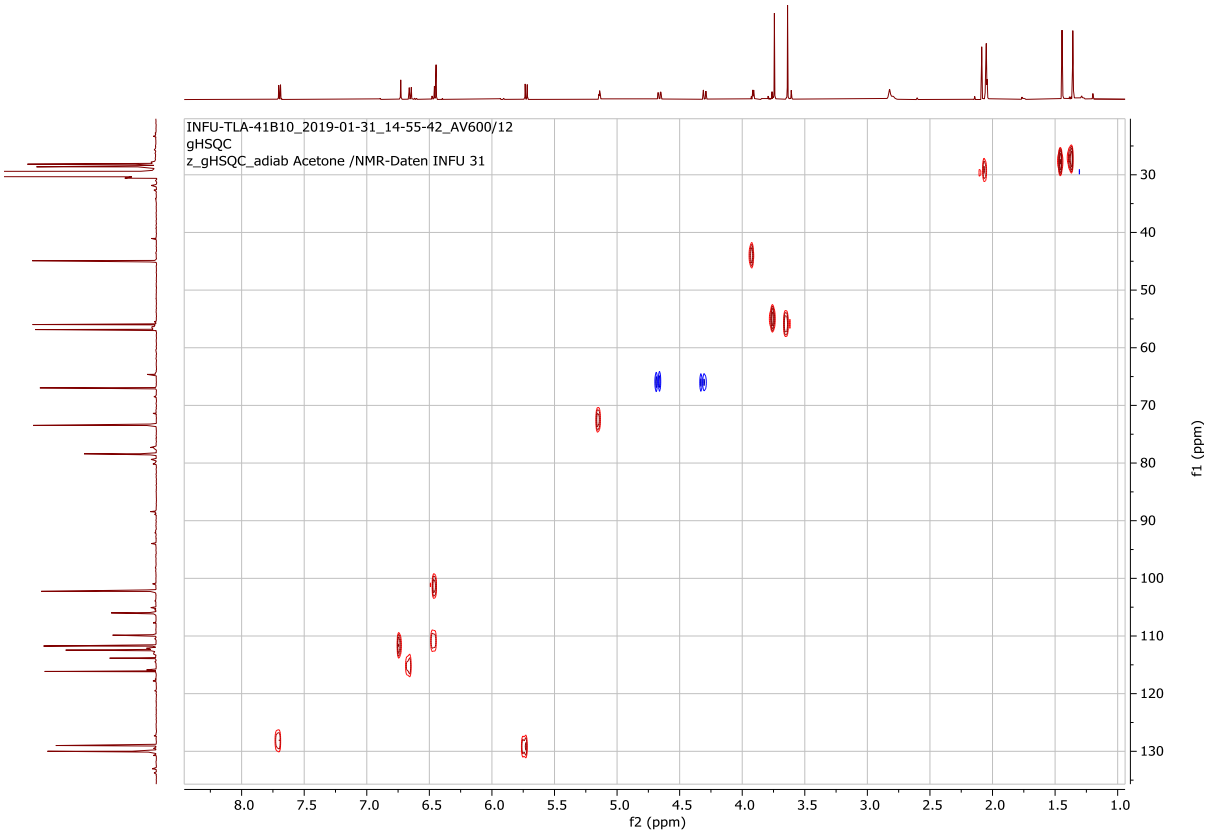
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 22



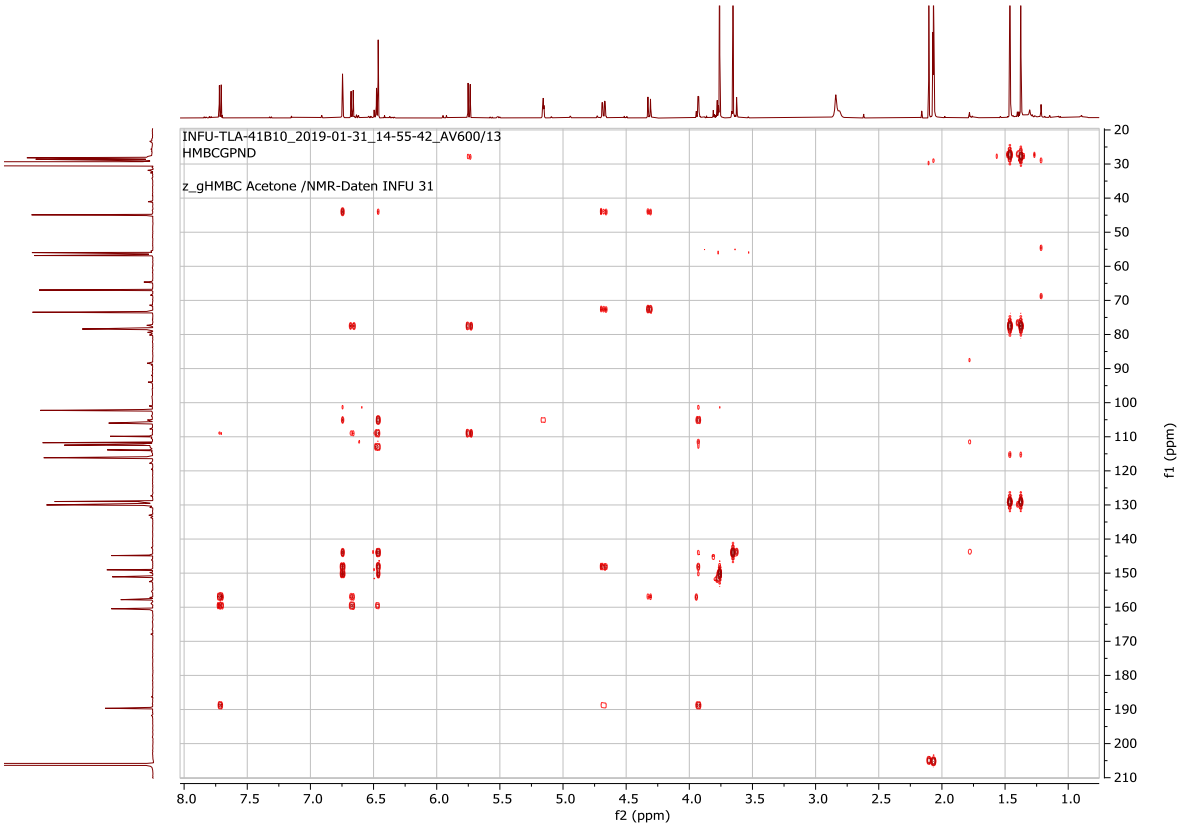
^{1}H-^{1}H-COSY spectrum of compound 22



HSQC spectrum of compound 22

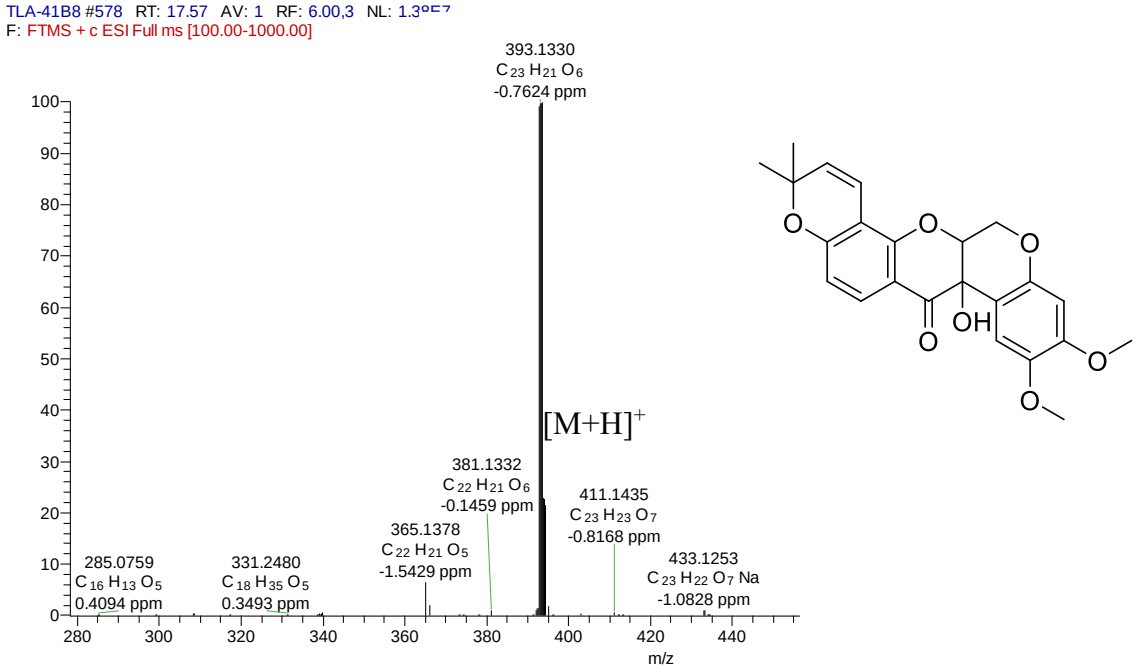


HMBC spectrum of compound 22

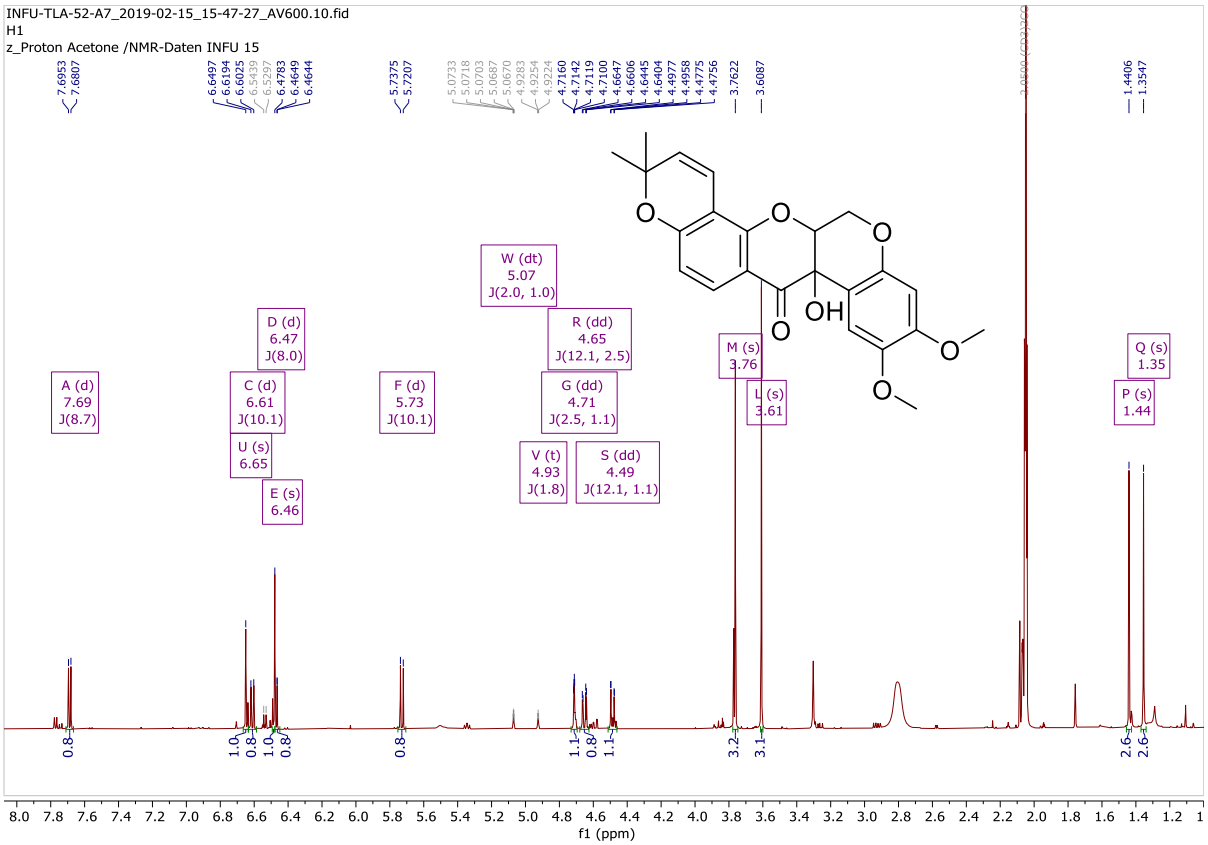


Appendix A23: Spectra for compound 23

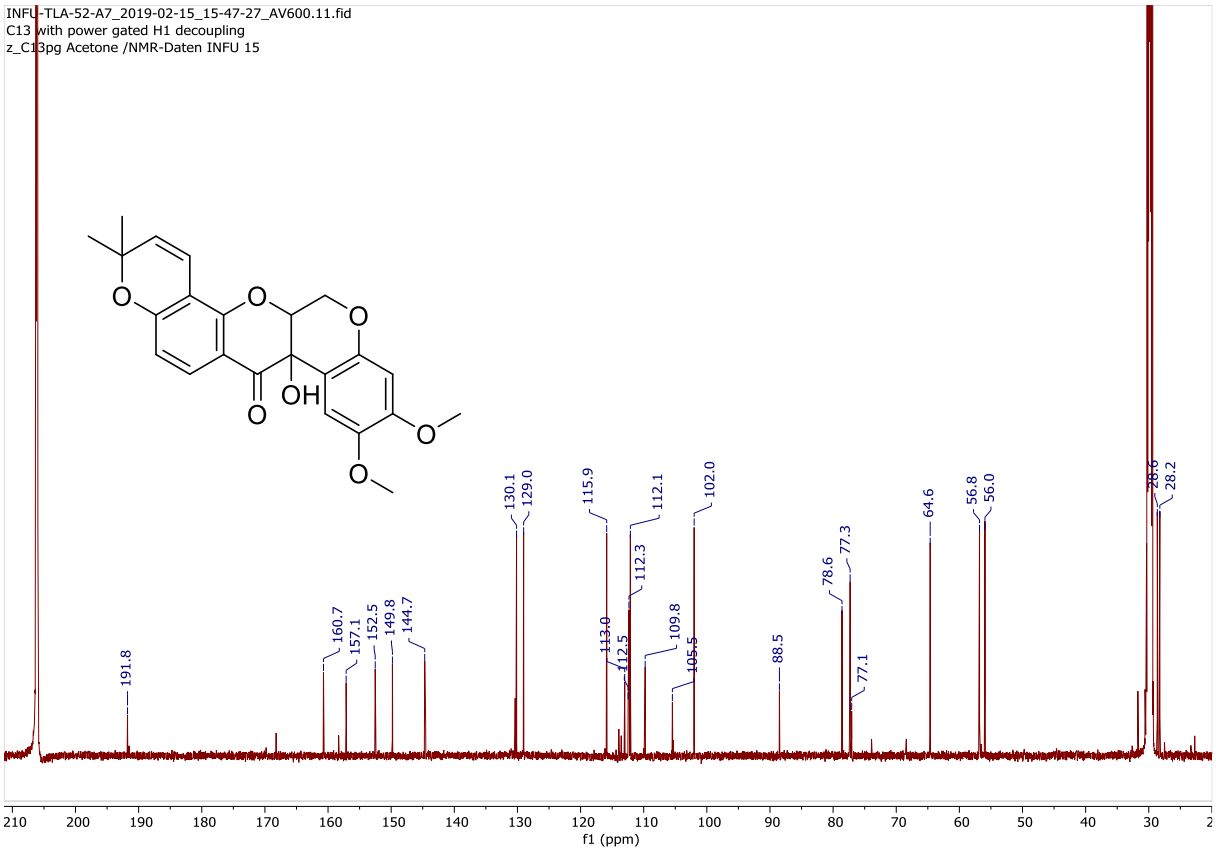
HRESIMS spectrum of compound 23



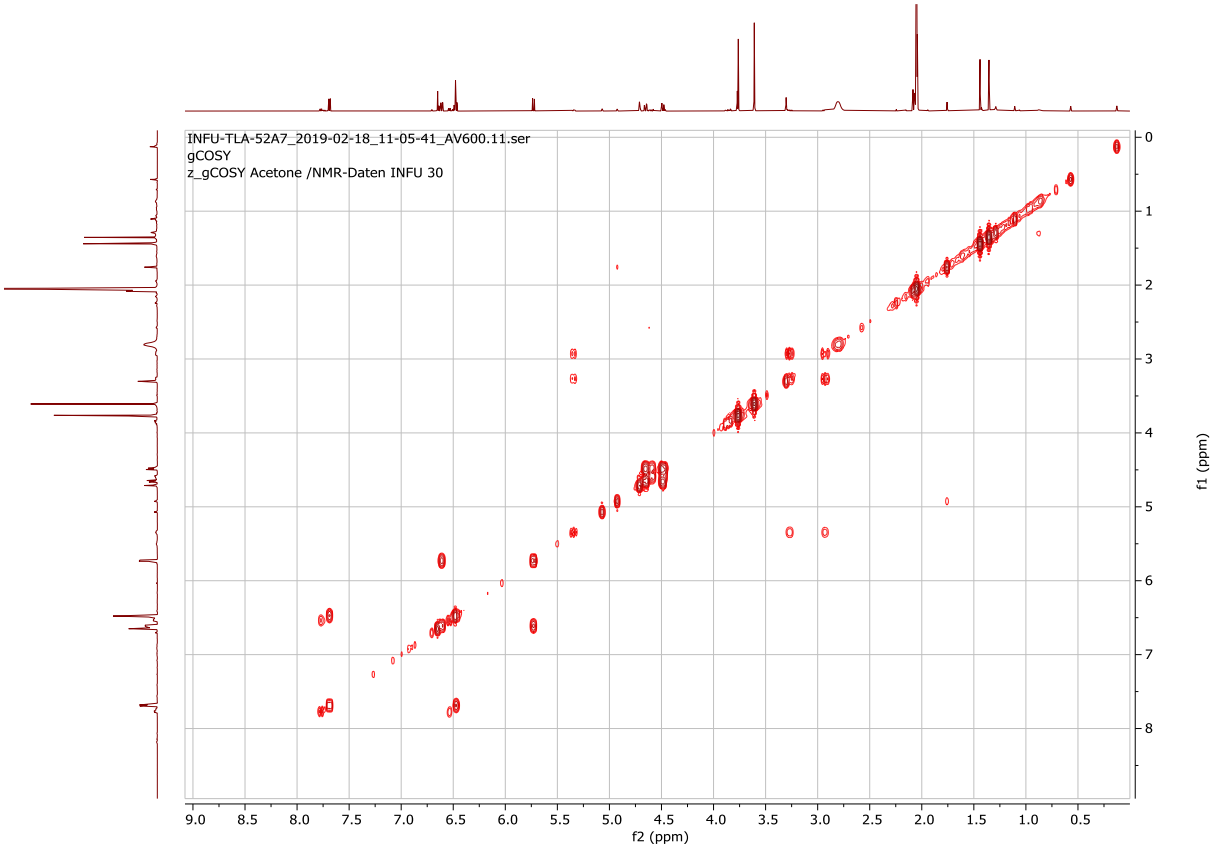
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 23



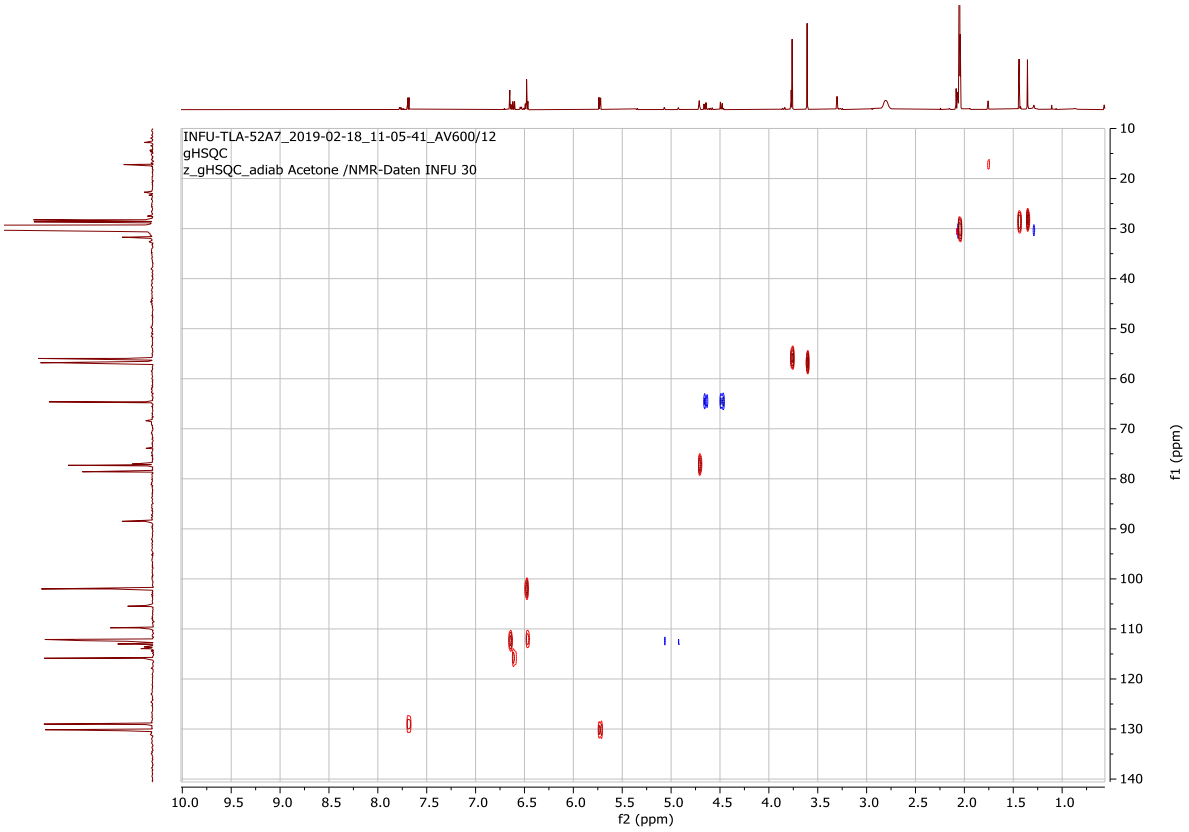
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 23



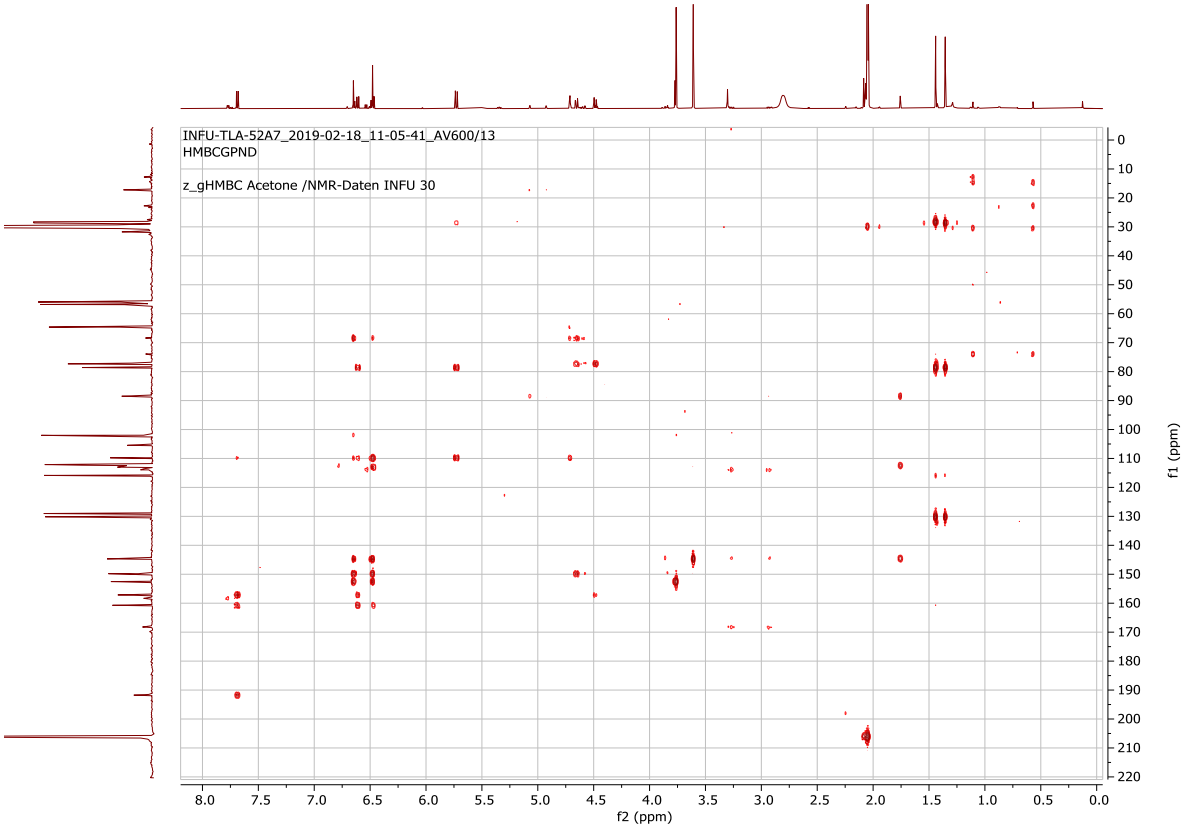
^{1}H-^{1}H-COSY spectrum of compound 23



HSQC spectrum of compound 23

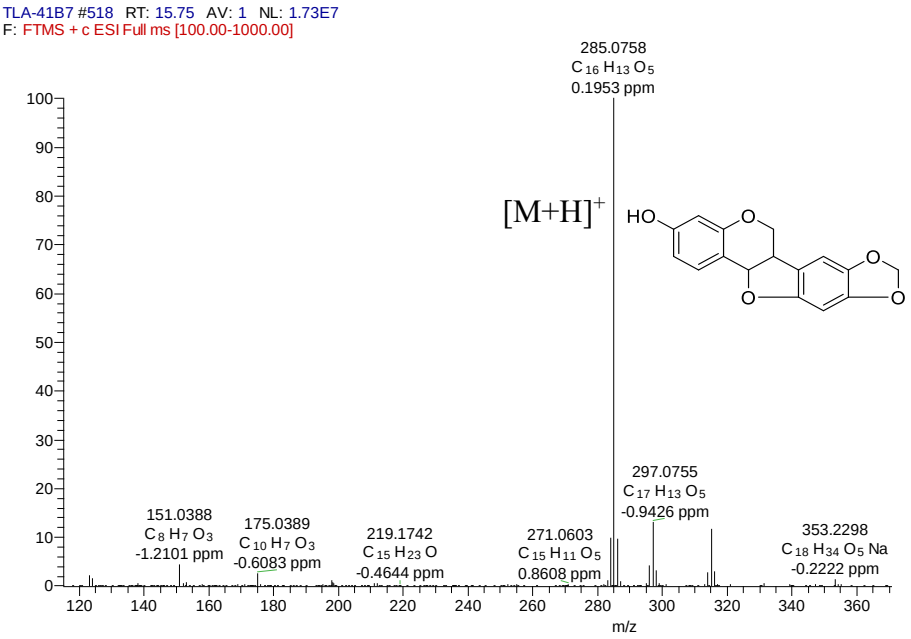


HMBC spectrum of compound 23

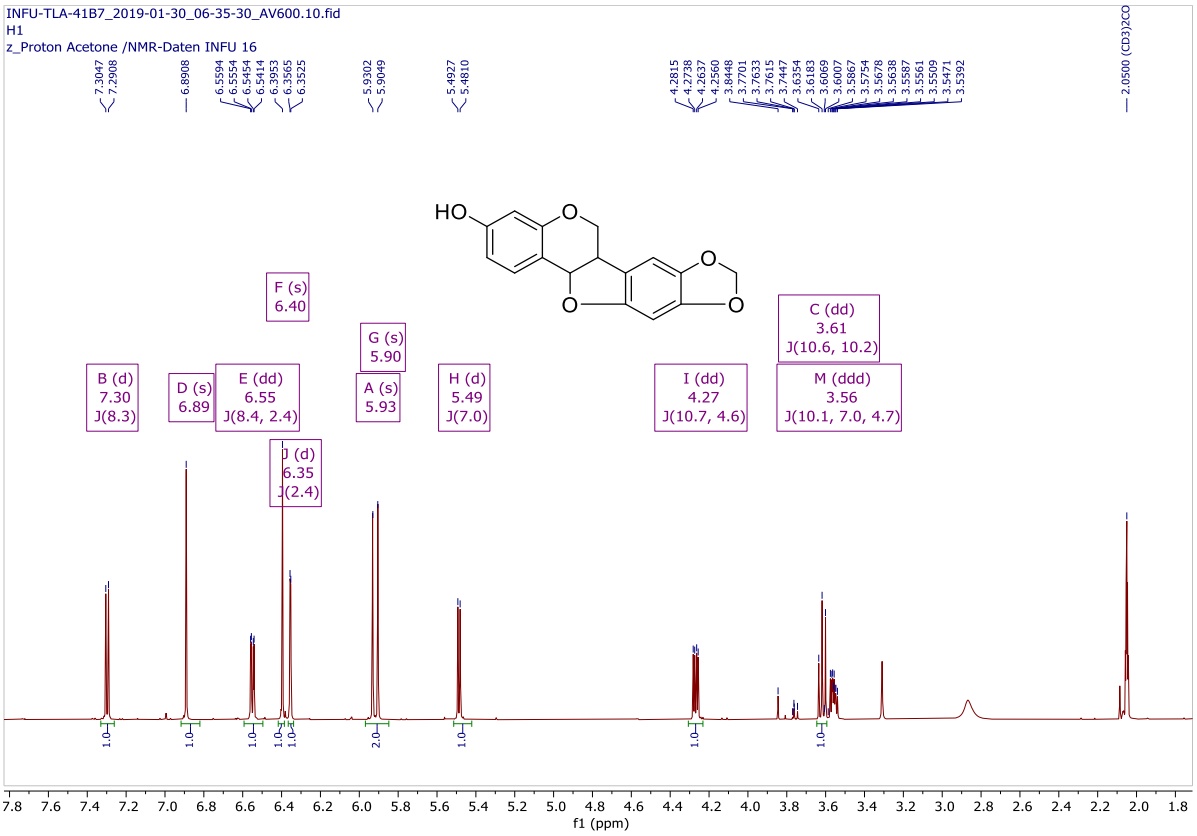


Appendix A24: Spectra for compound 24

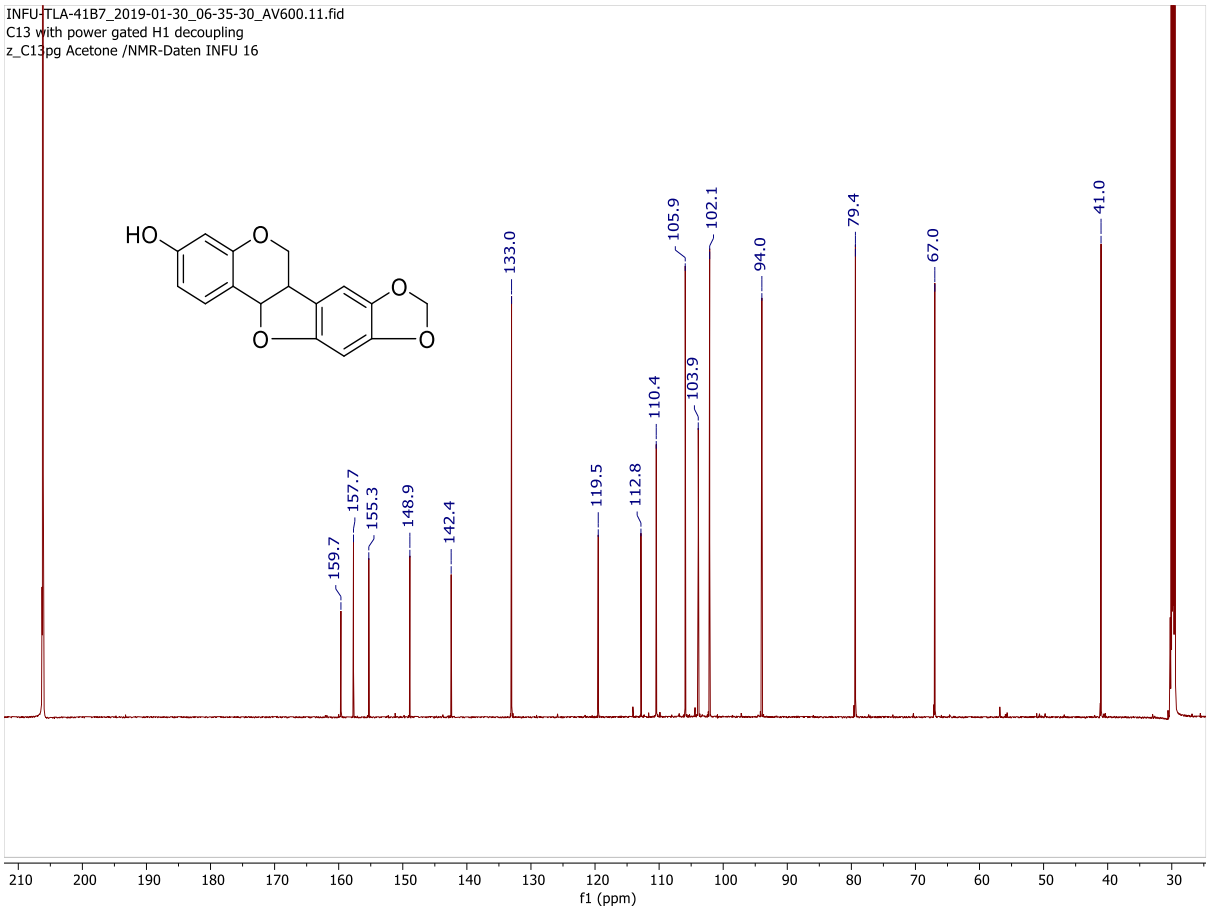
HRESIMS spectrum of compound 24



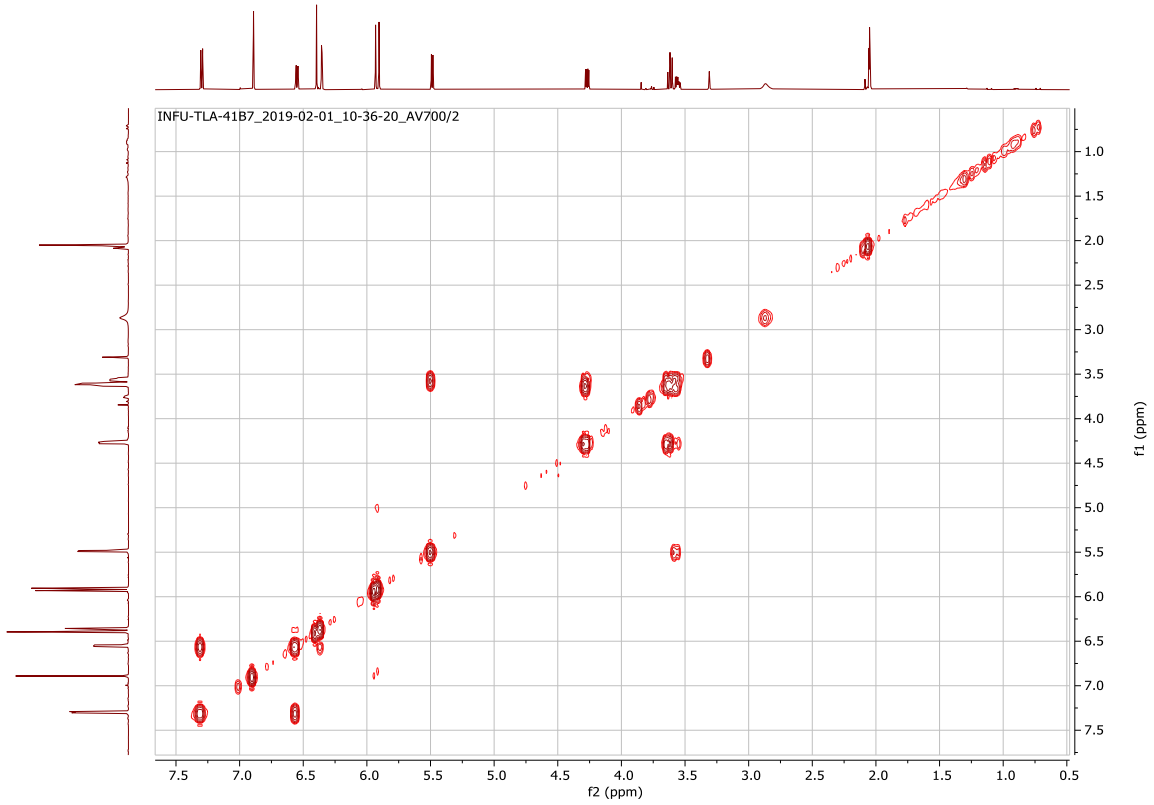
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 24



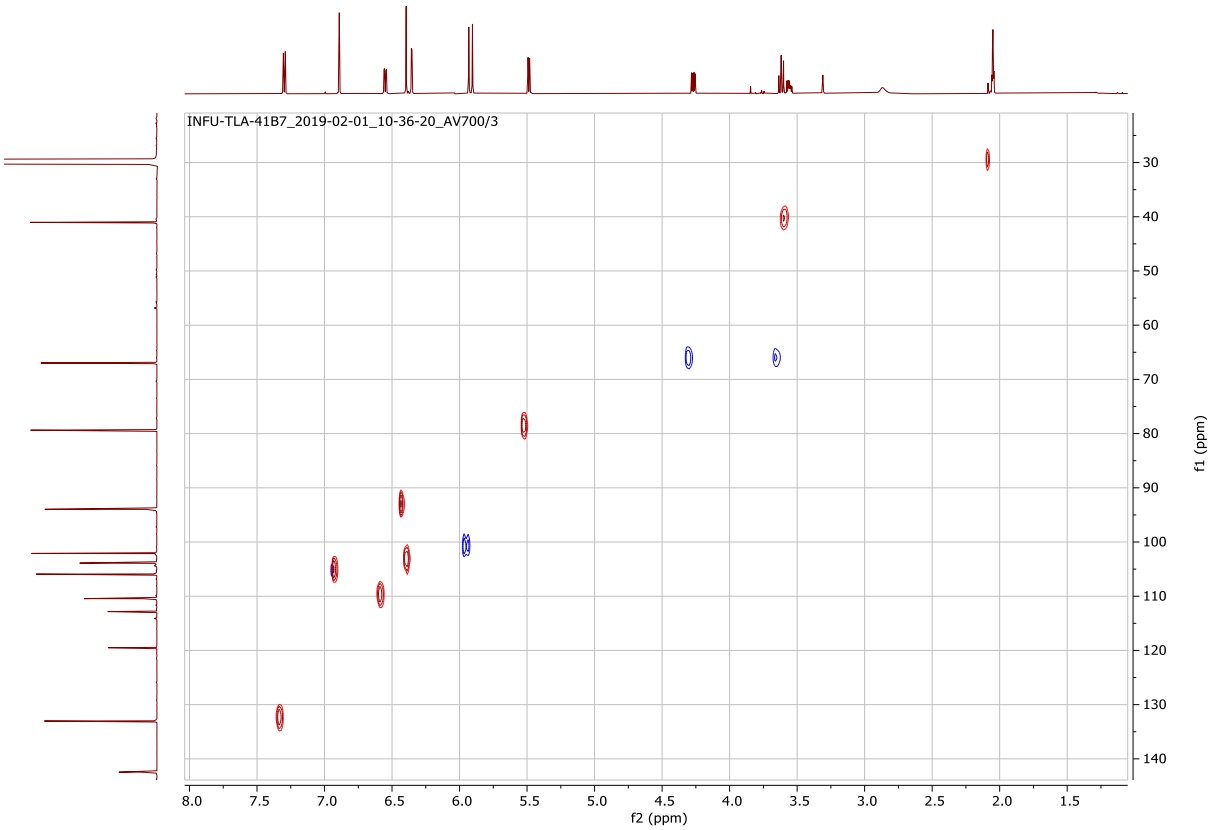
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 24



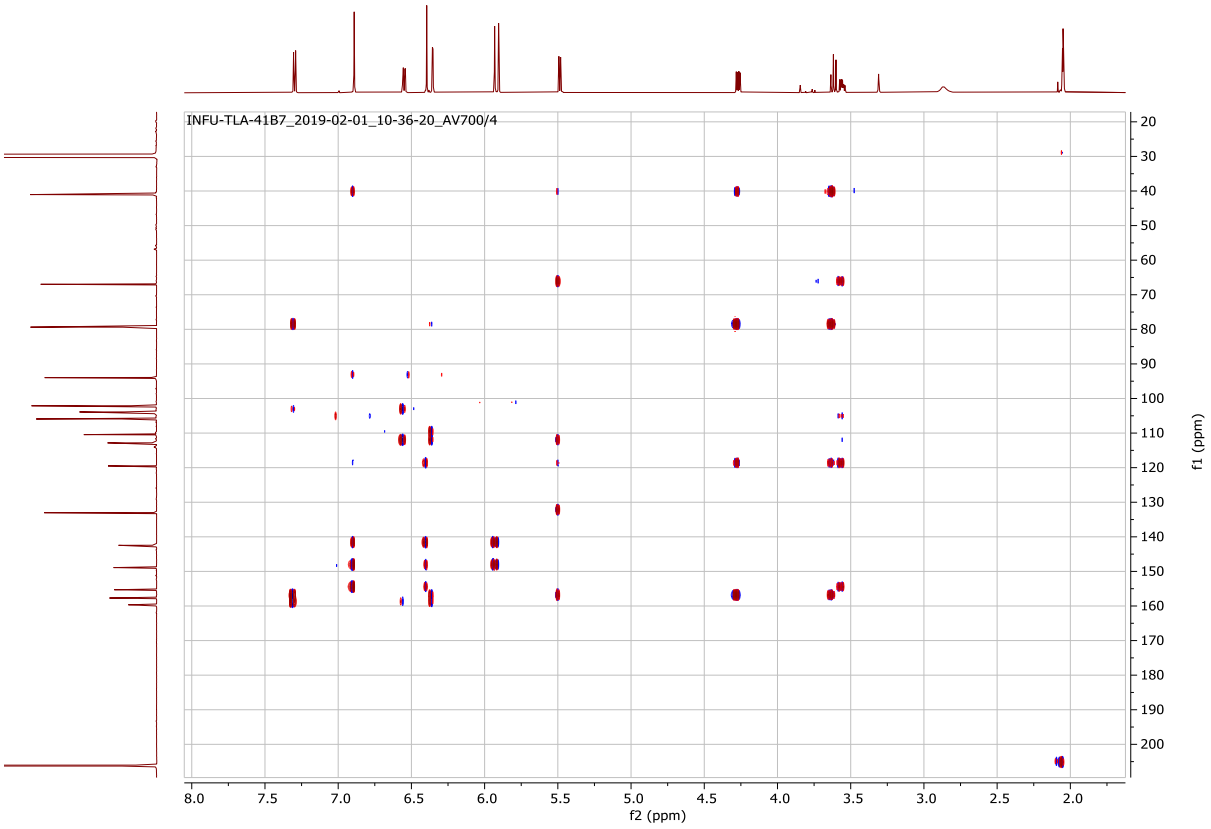
^{1}H-^{1}H-COSY spectrum of compound 24



HSQC spectrum of compound 24

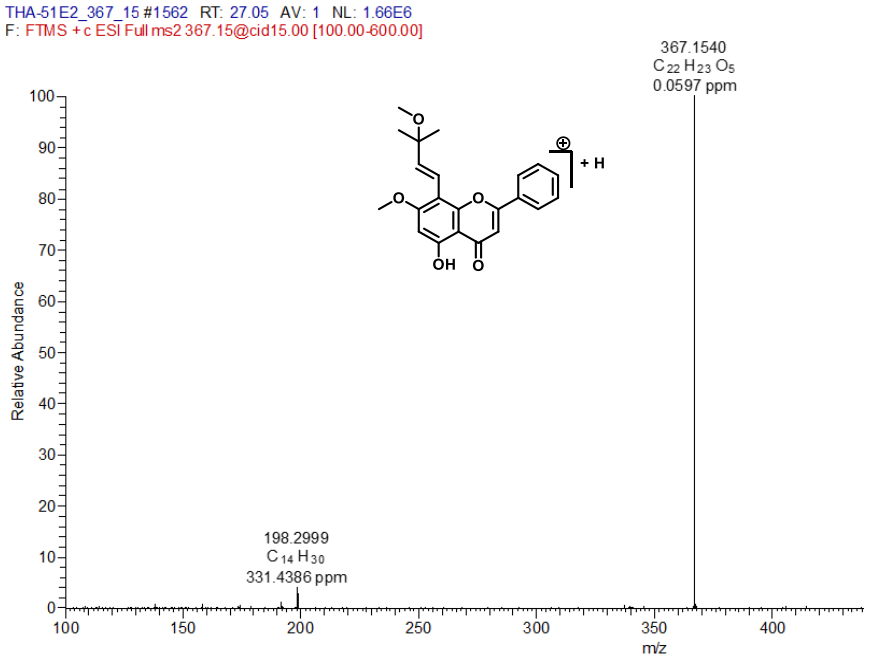


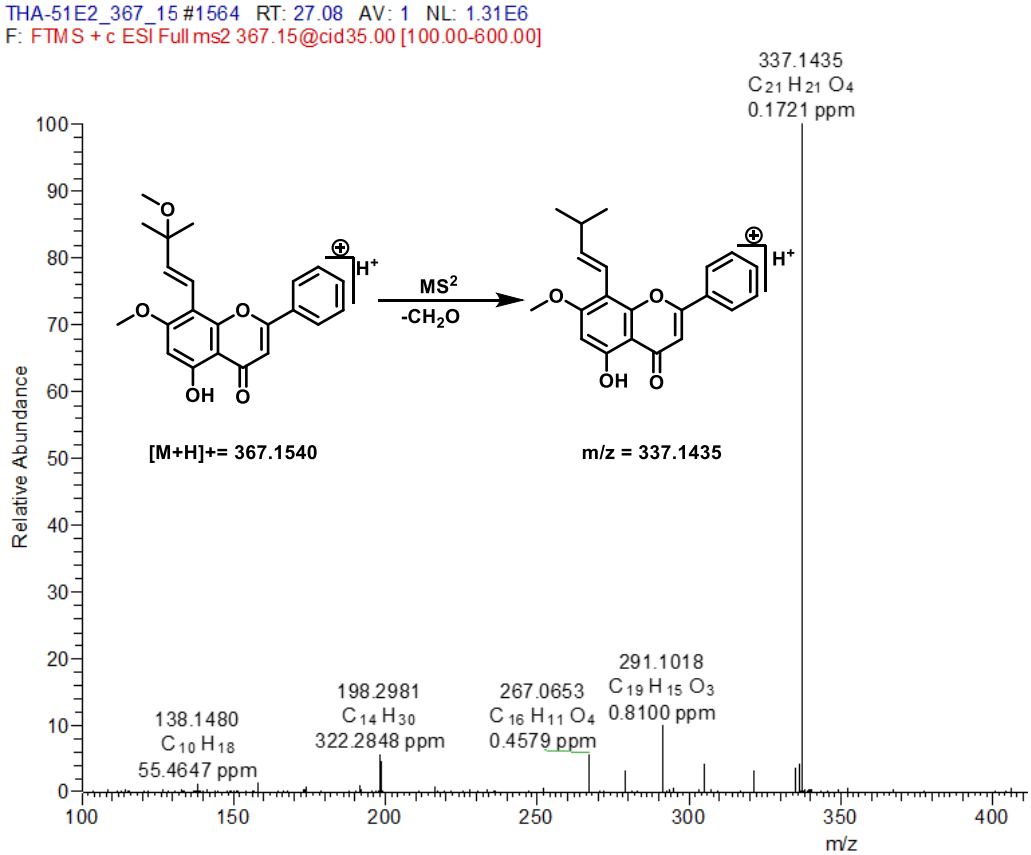
HMBC spectrum of compound 24



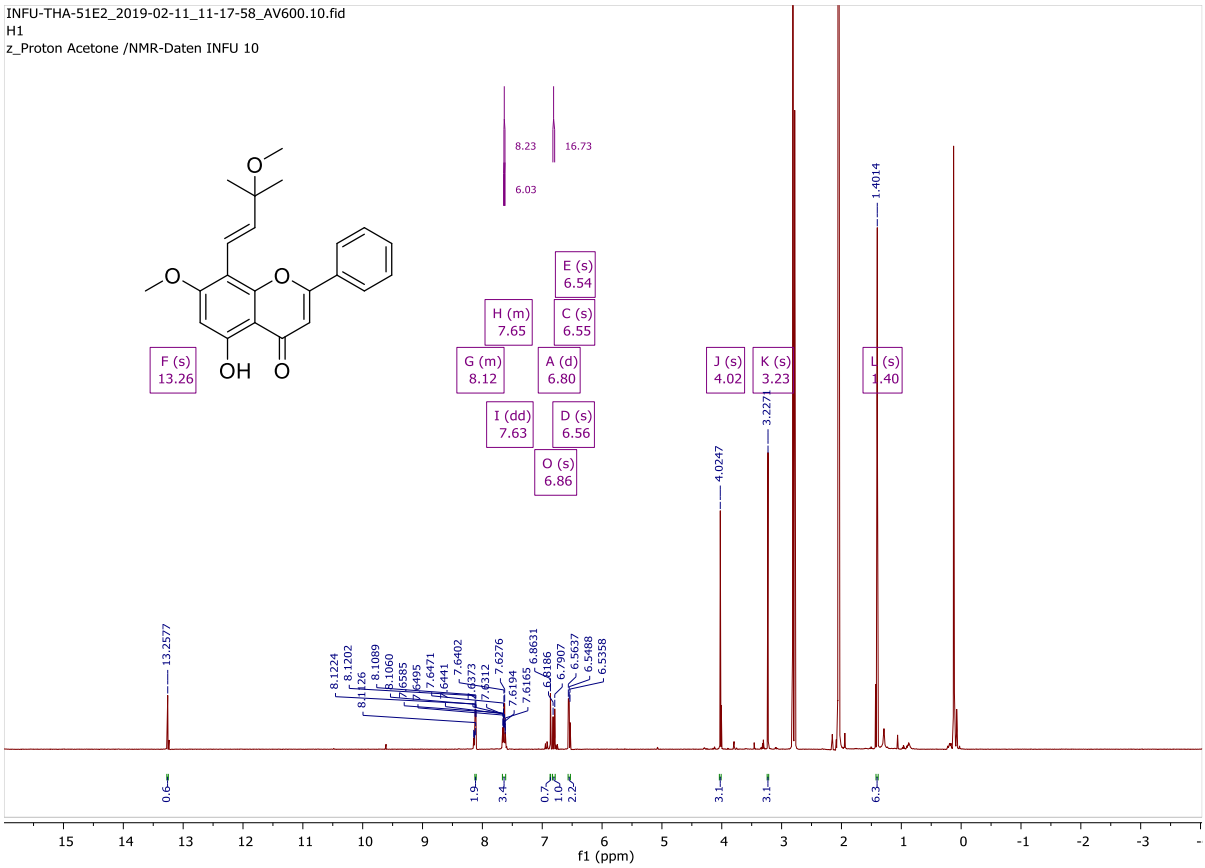
Appendix A25: Spectra for compound 25

HRESIMS spectrum of compound 25

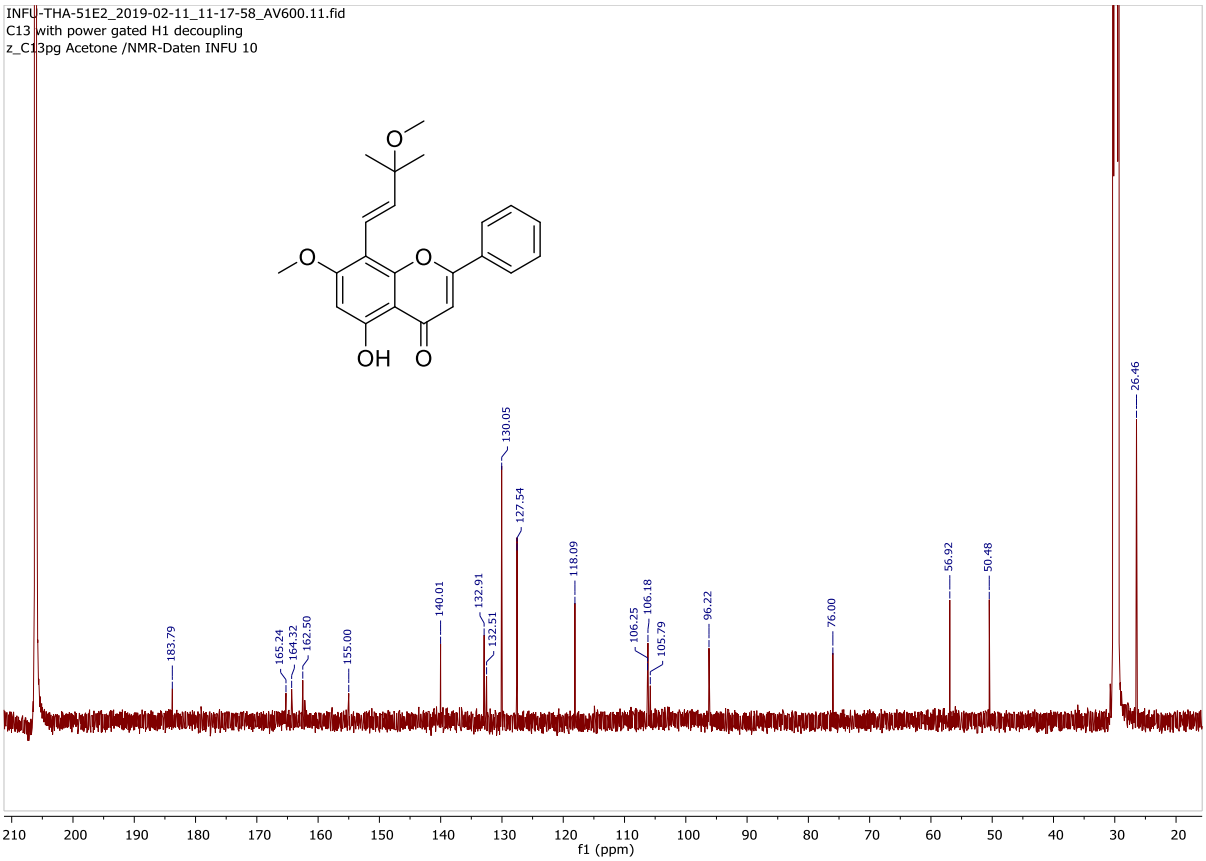




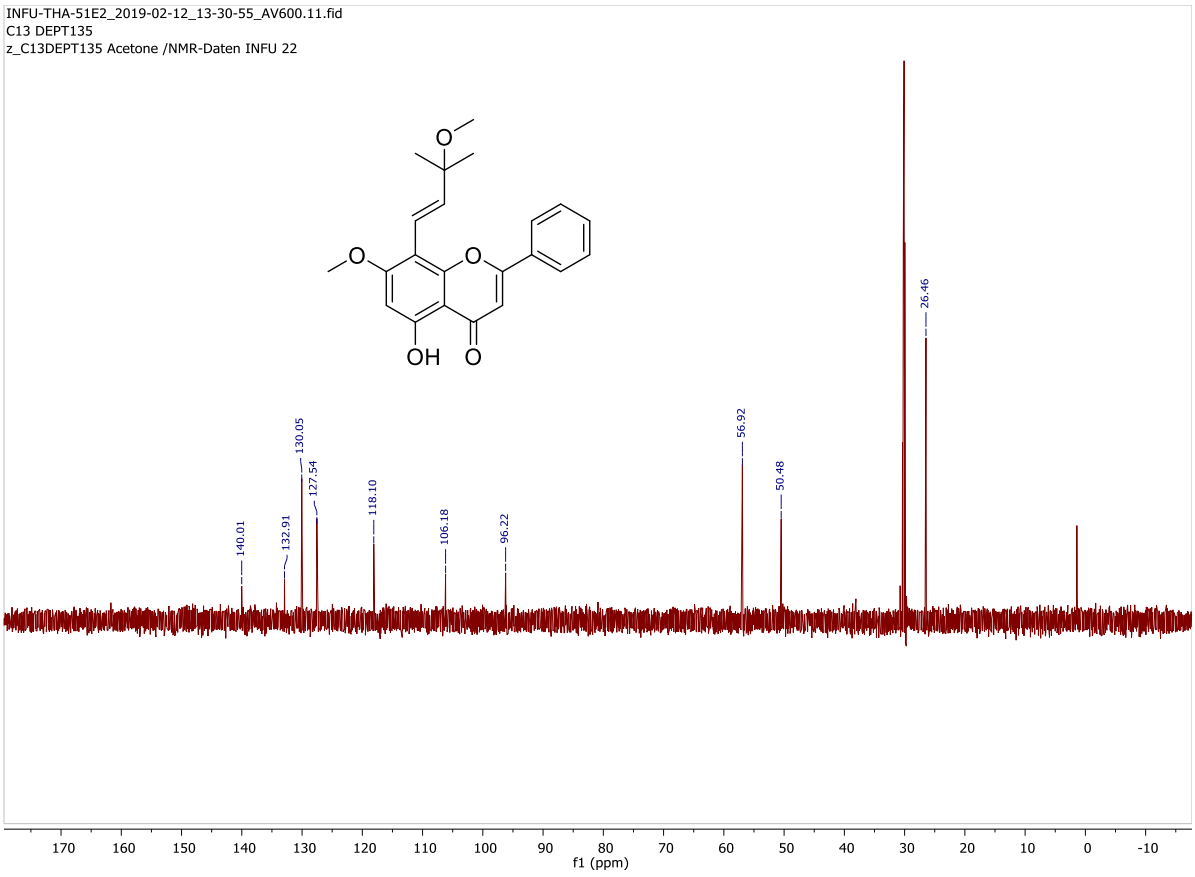
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 25



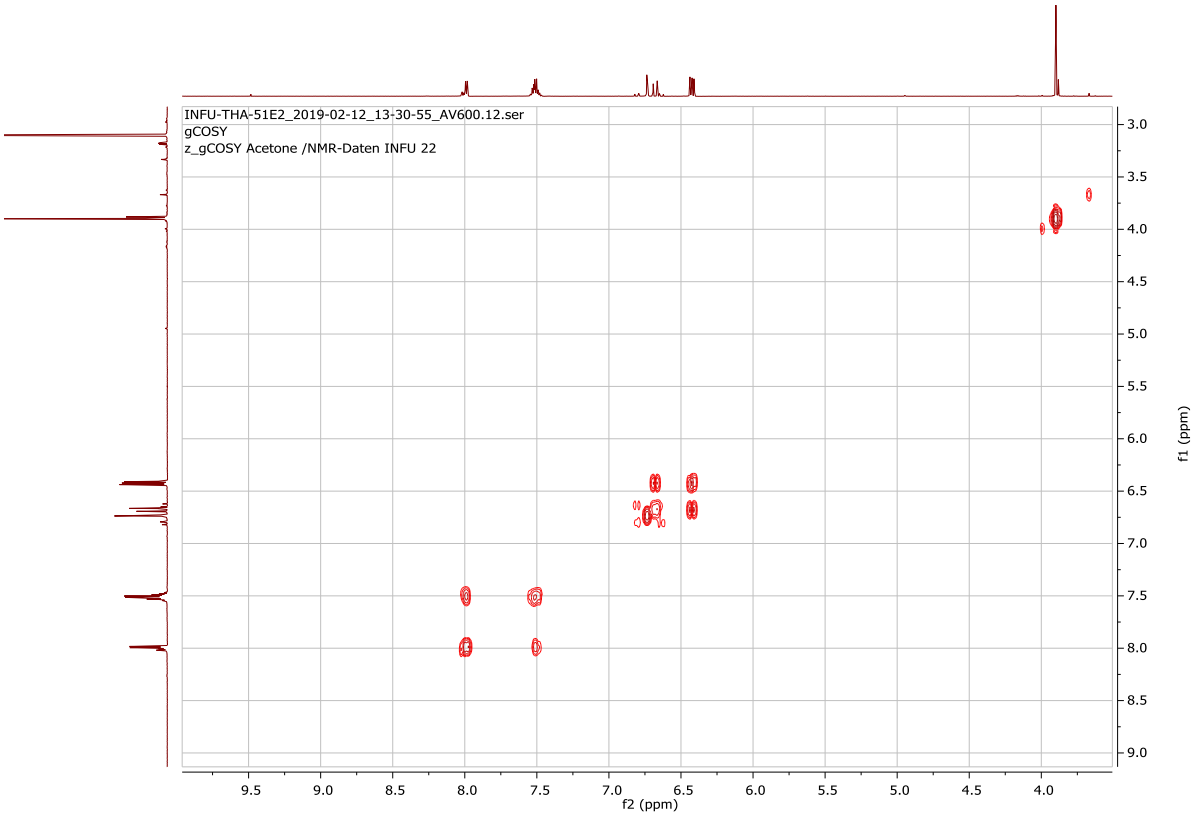
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 25



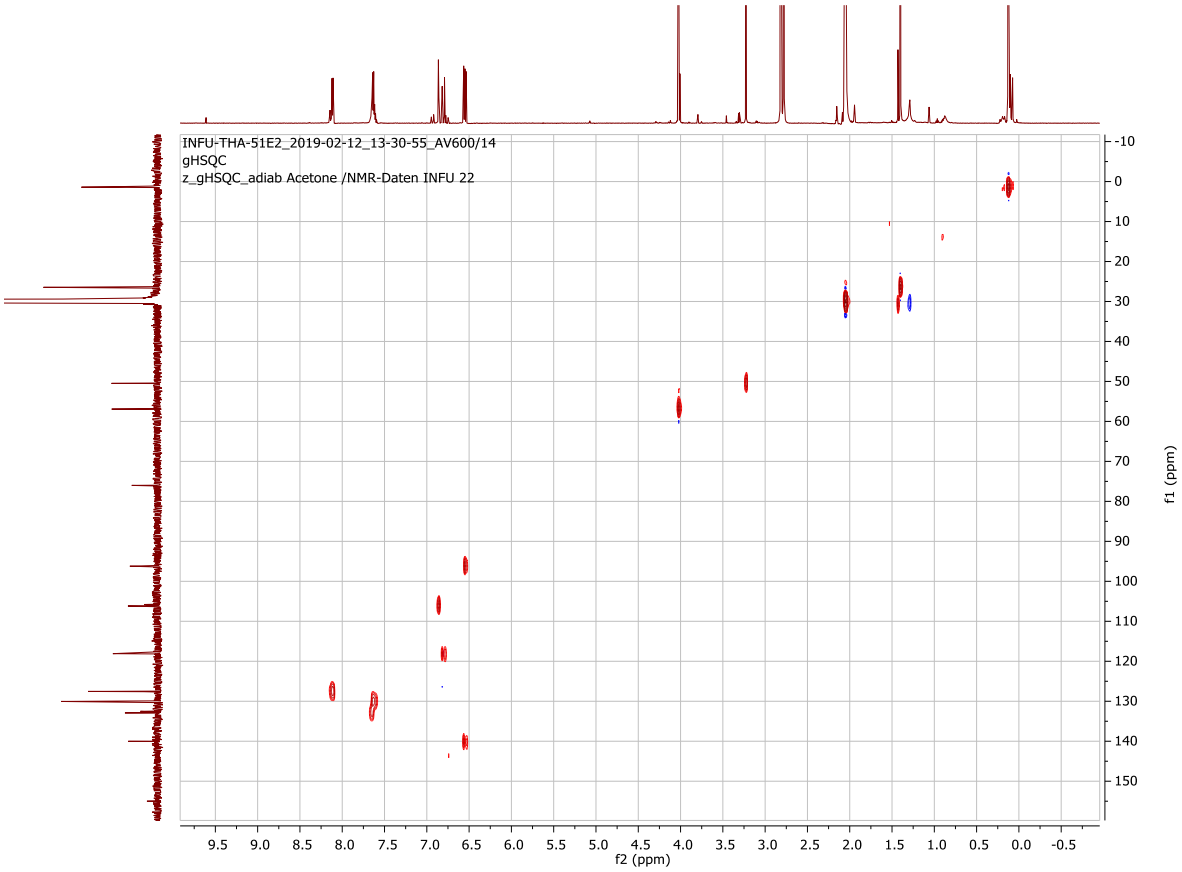
^{13}C DEPT134 NMR spectrum (150 MHz, Acetone-d_6) of compound 25



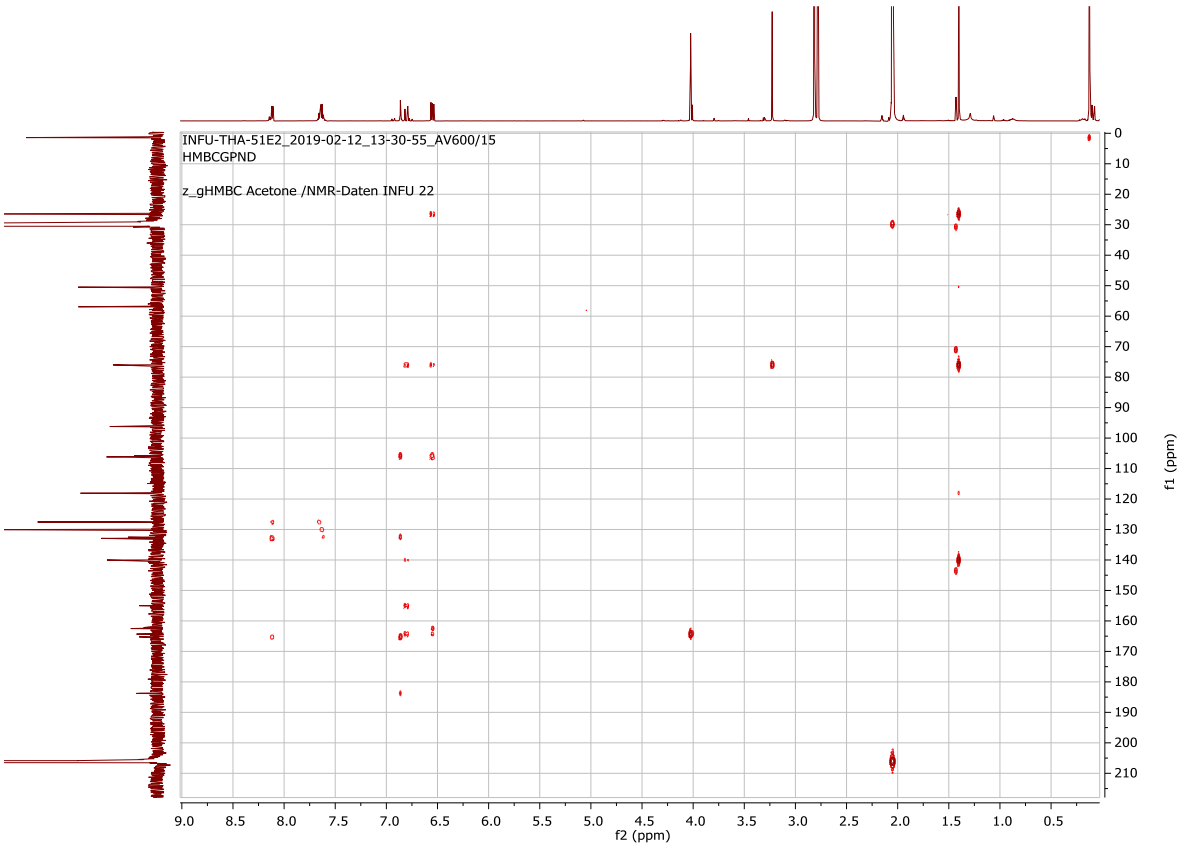
^{1}H-^{1}H-COSY spectrum of compound 25



HSQC spectrum of compound 25

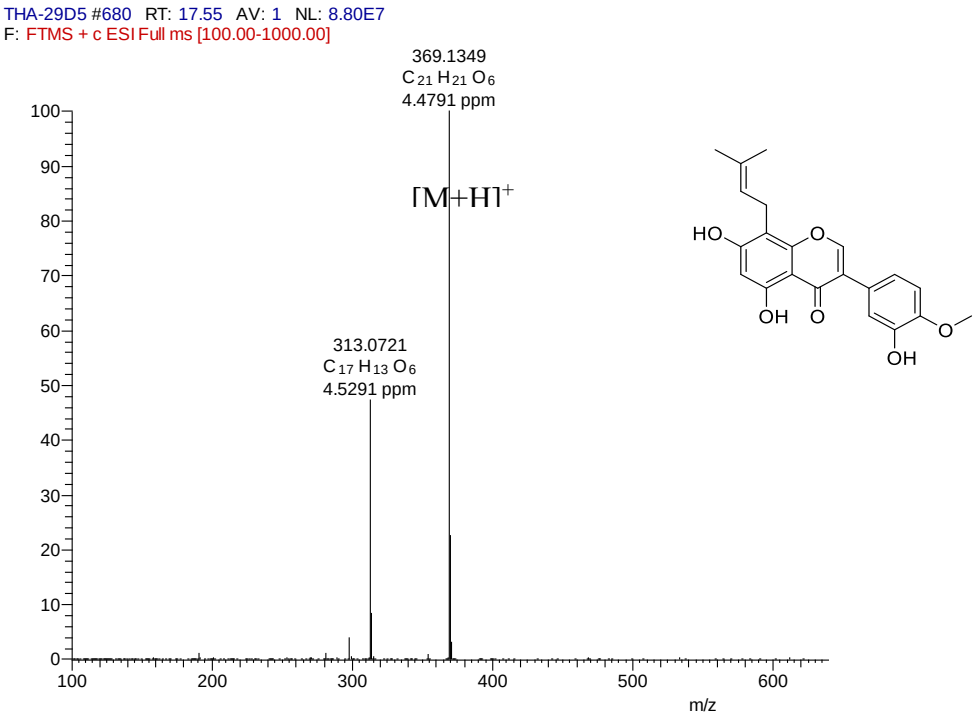


HMBC spectrum of compound 25

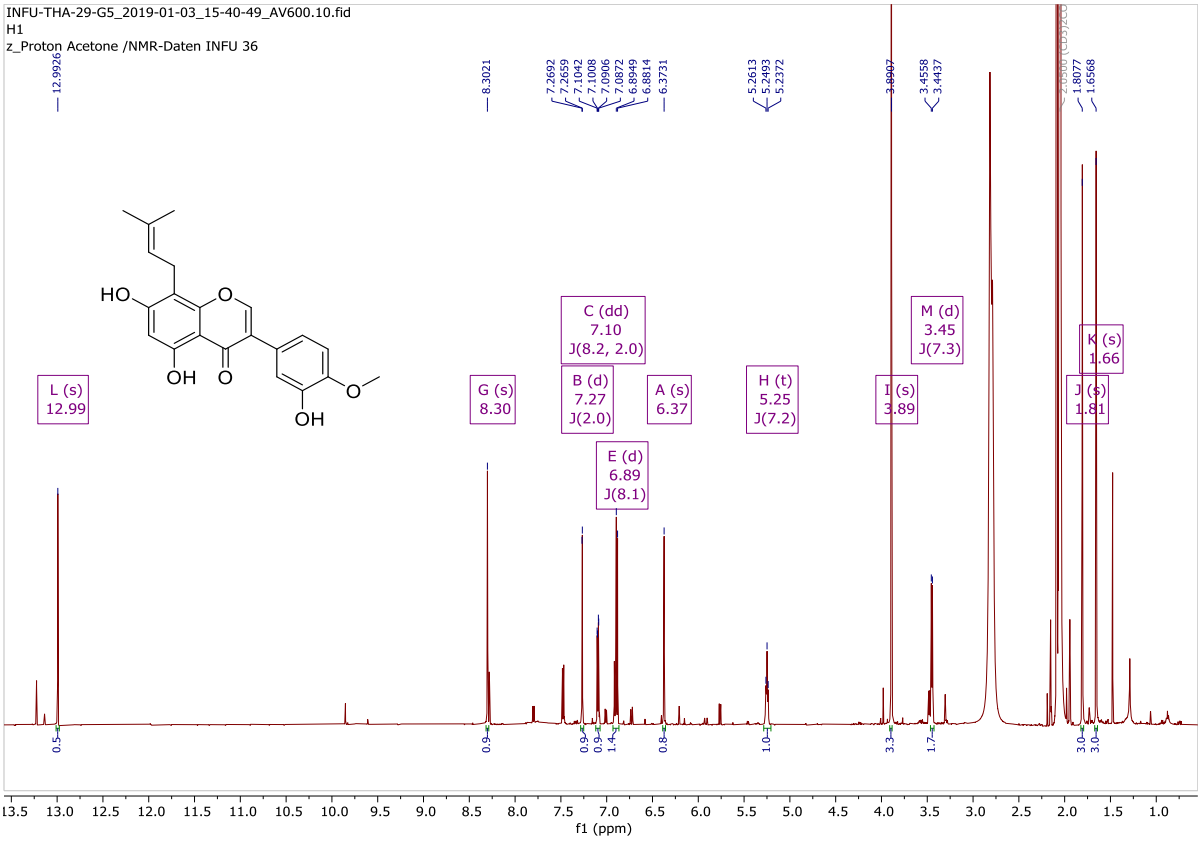


Appendix A26: Spectra for compound 26

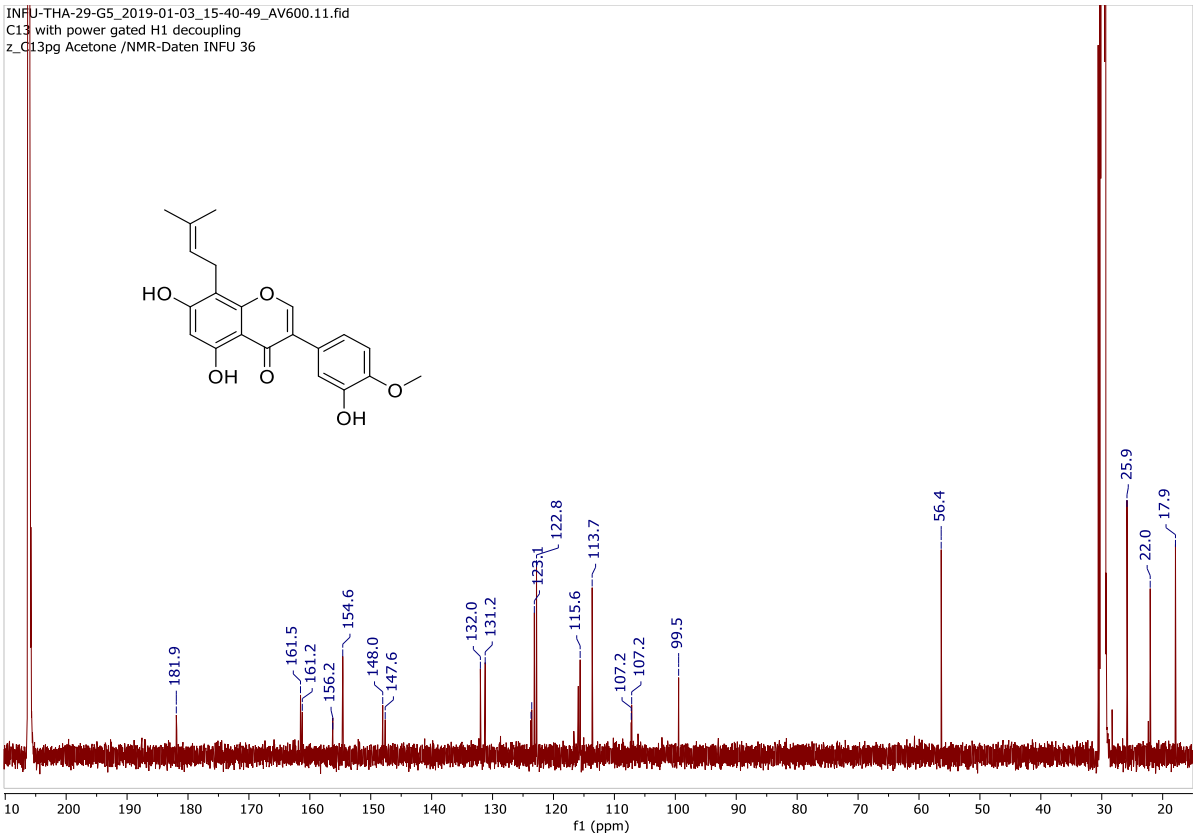
HRESIMS spectrum of compound 26



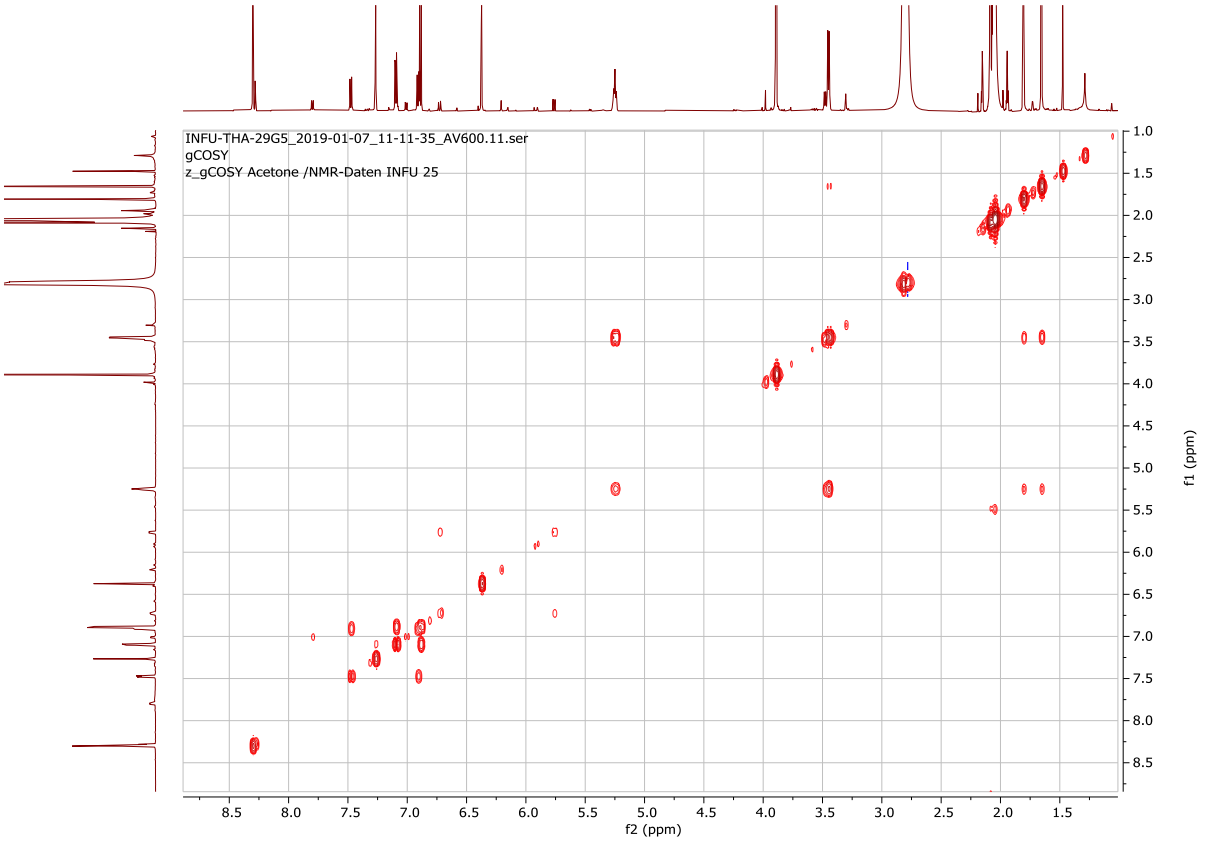
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 26



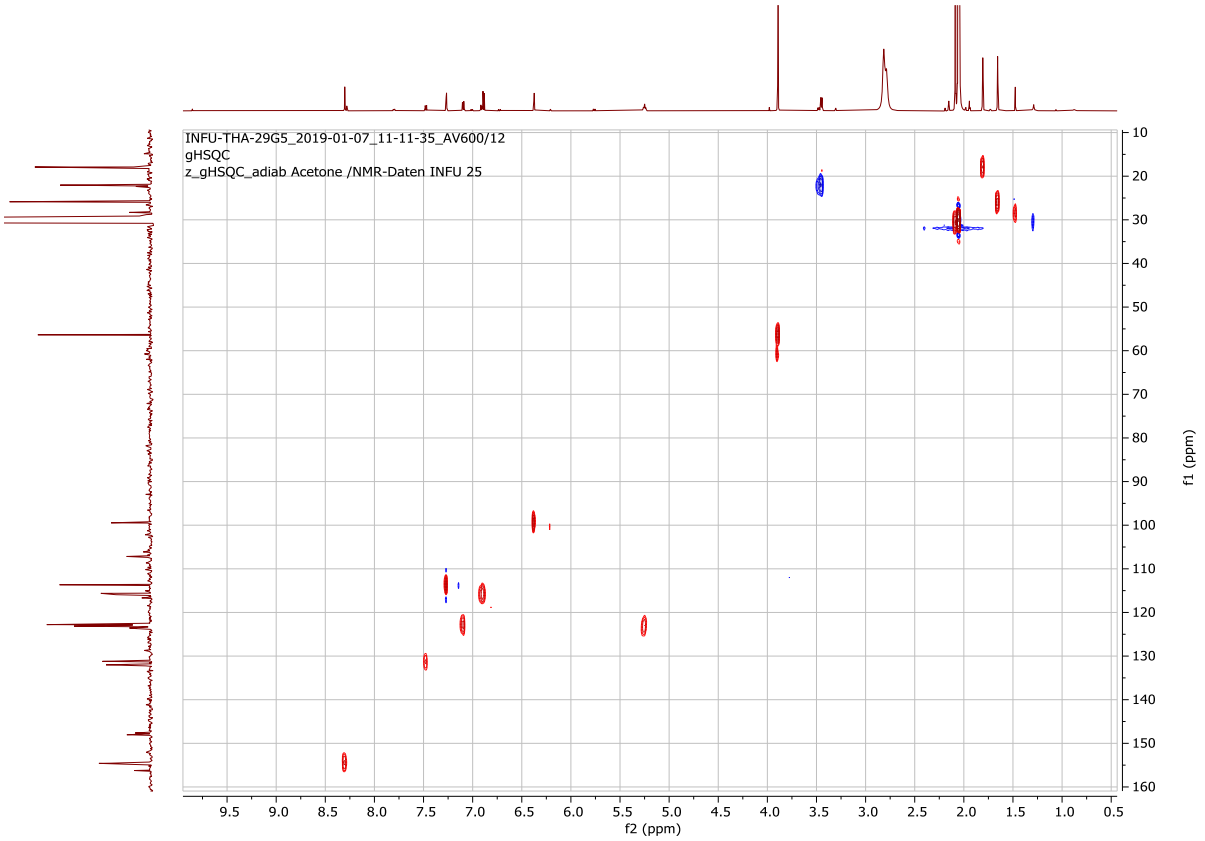
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 26



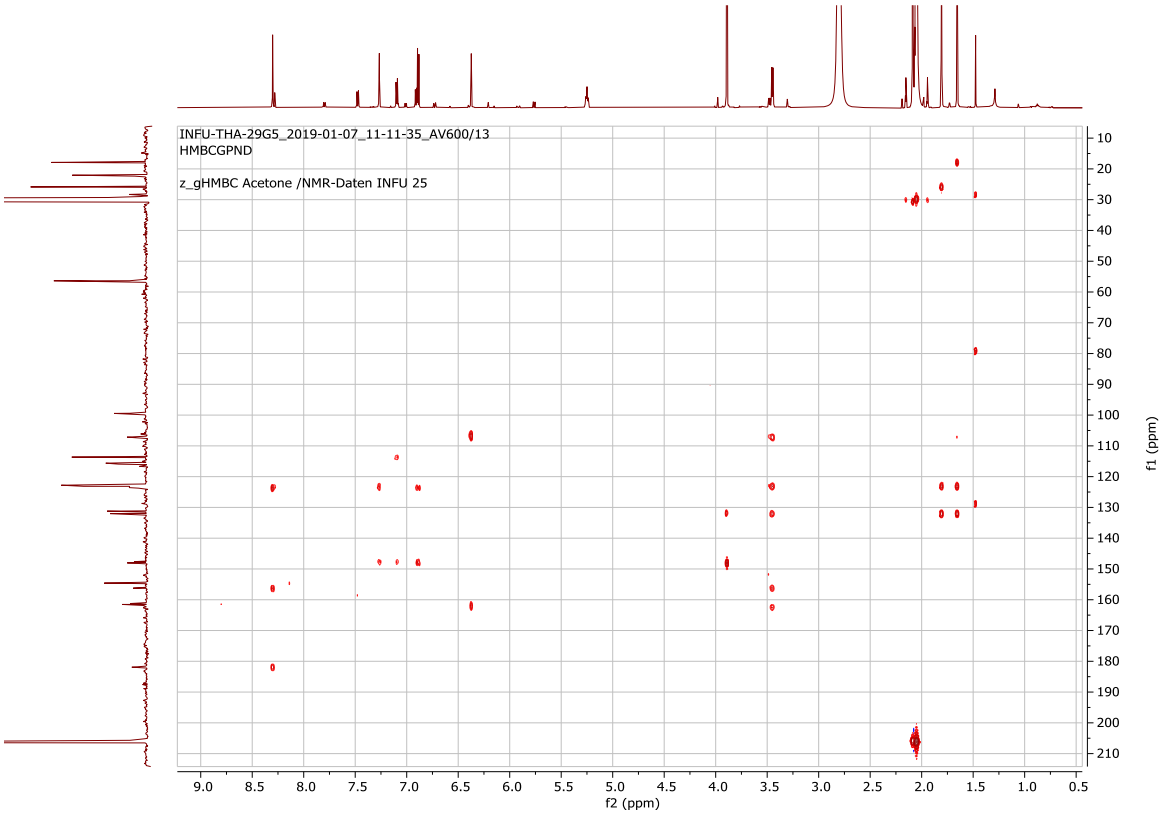
^{1}H-^{1}H-COSY spectrum of compound 26



HSQC spectrum of compound 26

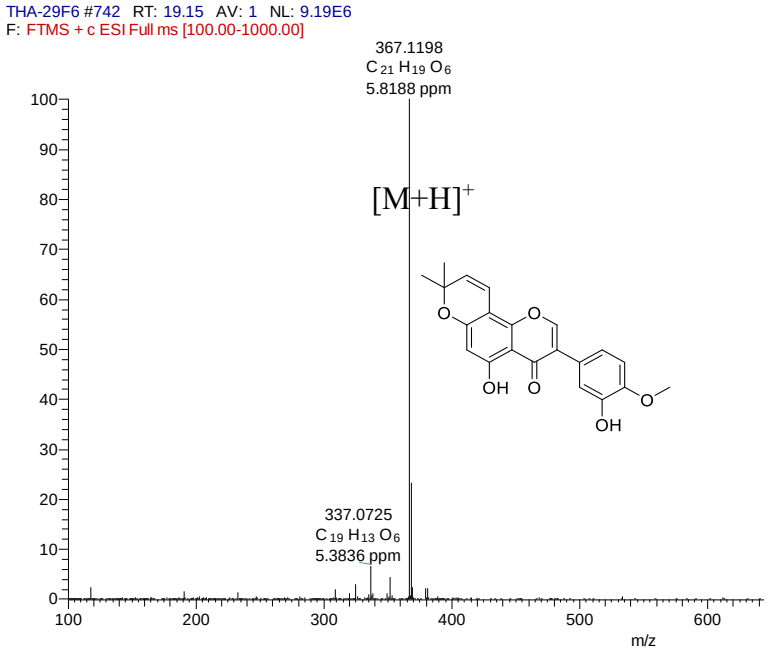


HMBC spectrum of compound 26

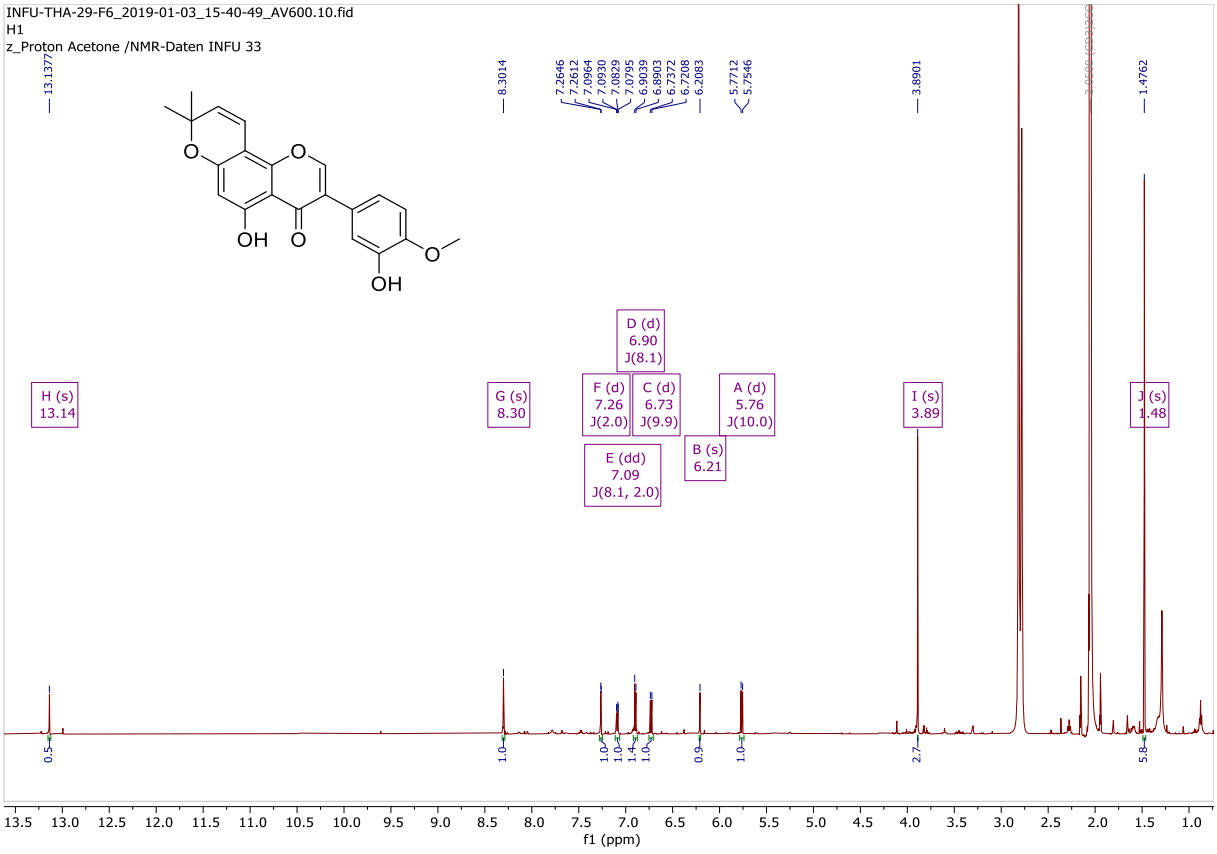


Appendix A27: Spectra for compound 27

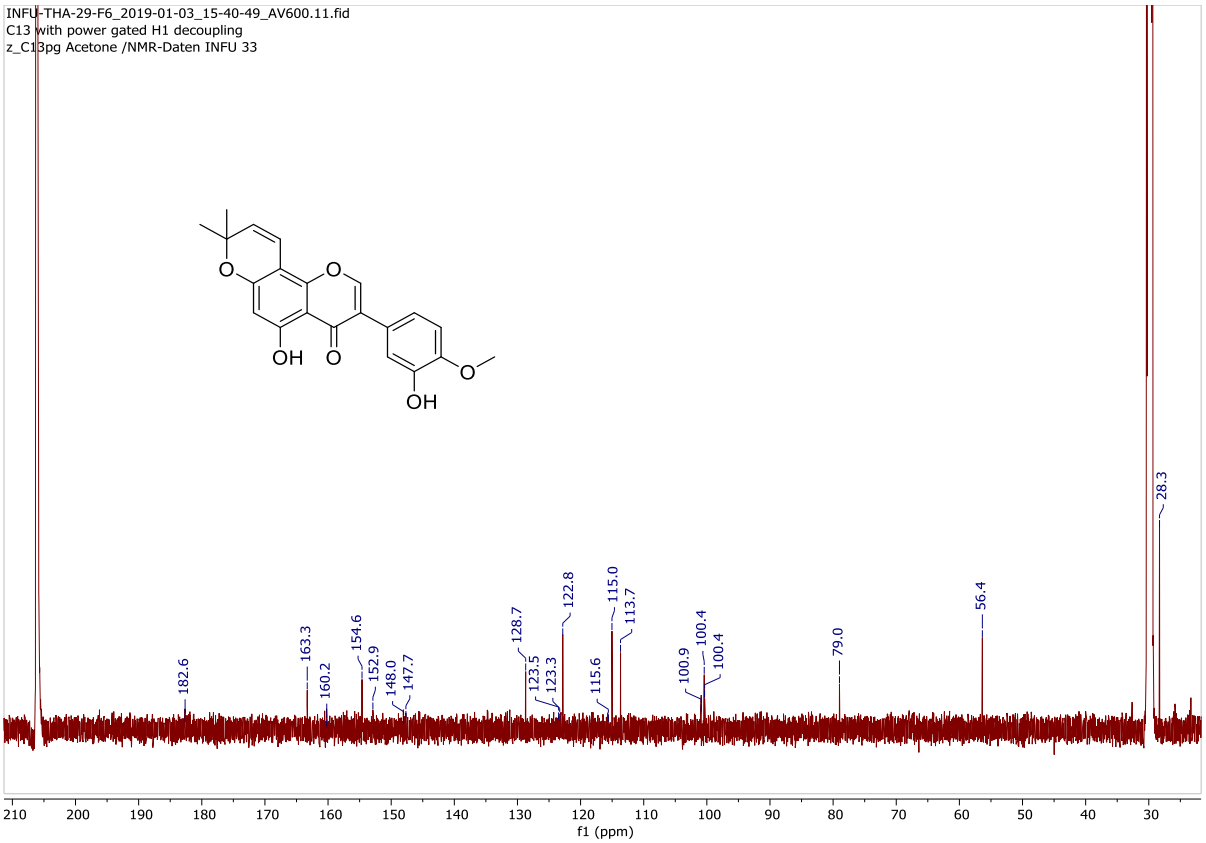
HRESIMS spectrum of compound 27



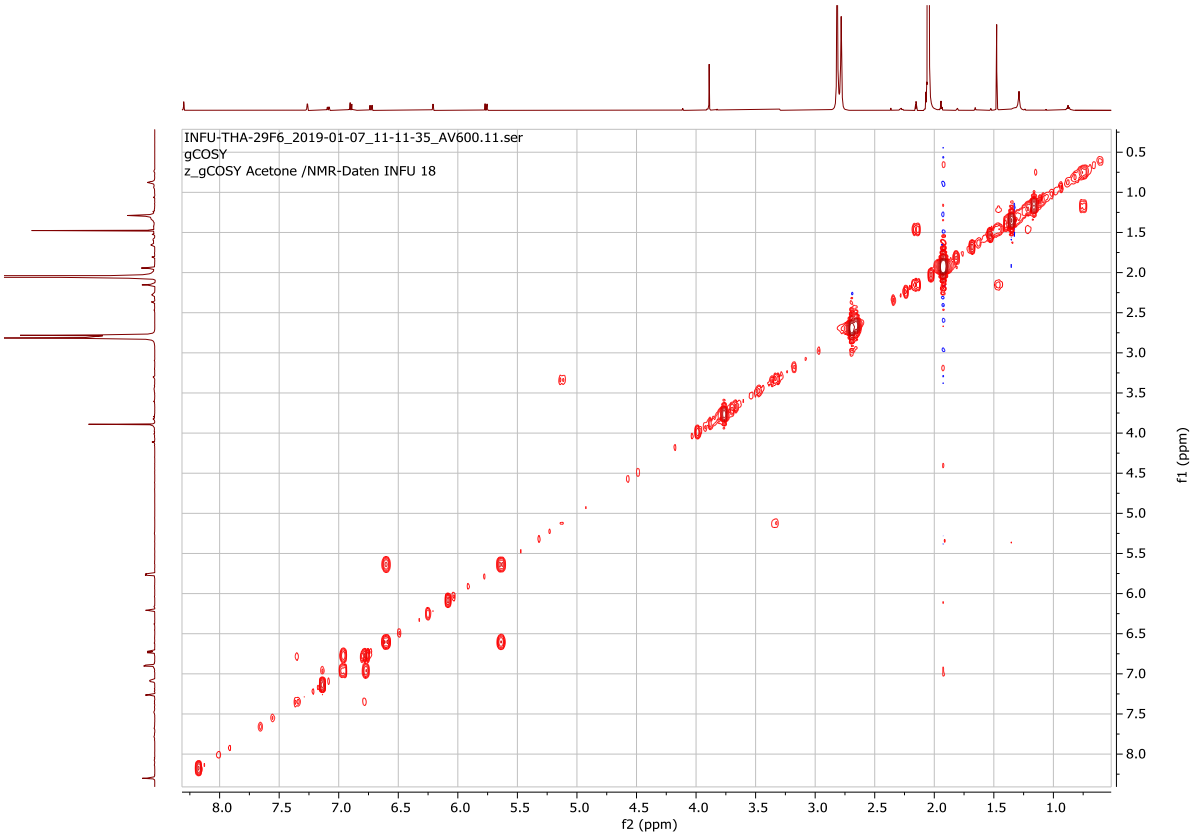
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 27



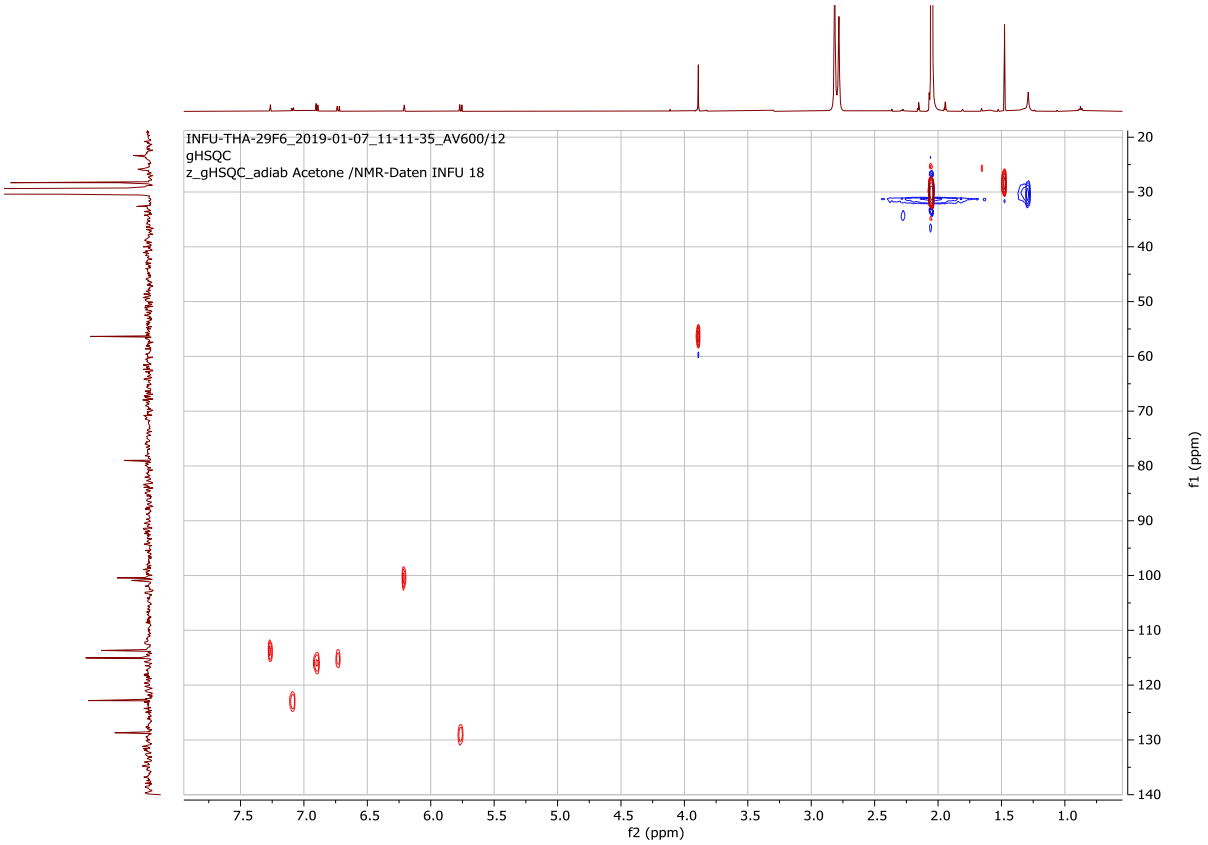
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 27



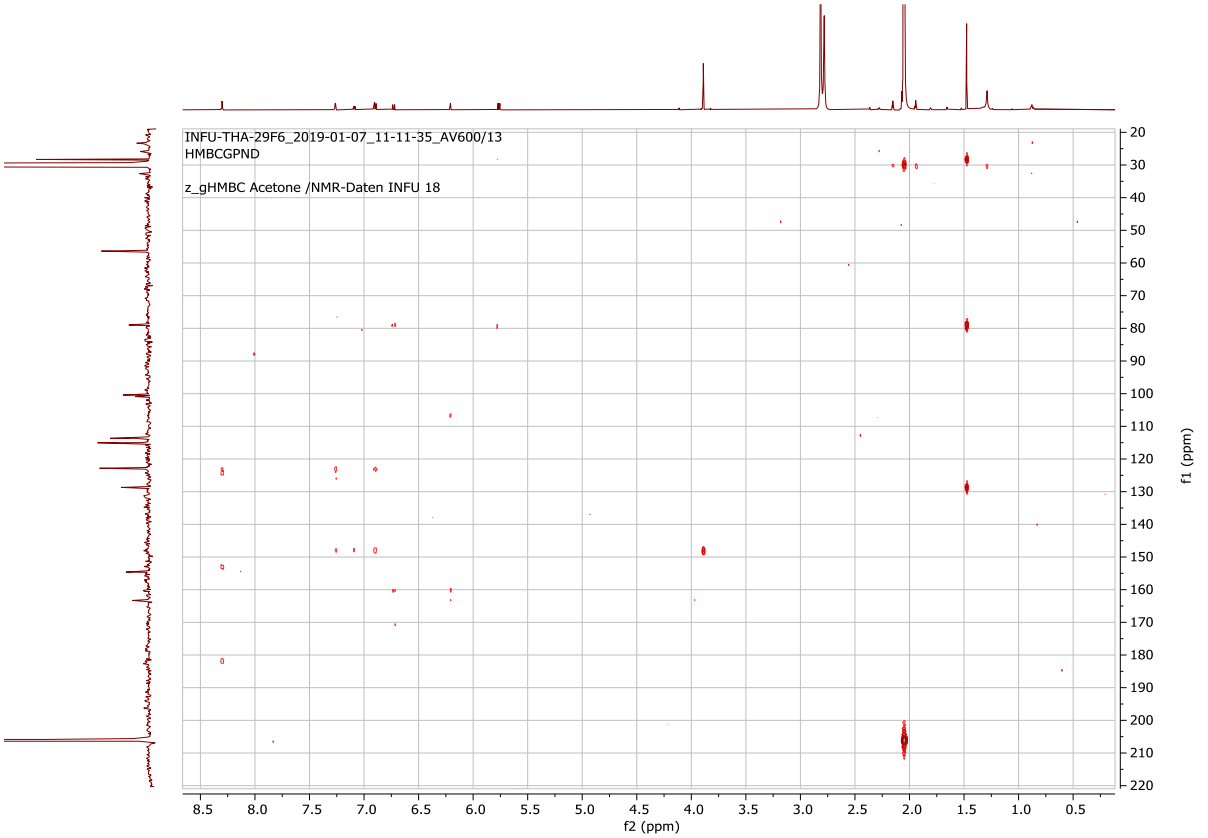
^{1}H-^{1}H-COSY spectrum of compound 27



HSQC spectrum of compound 27

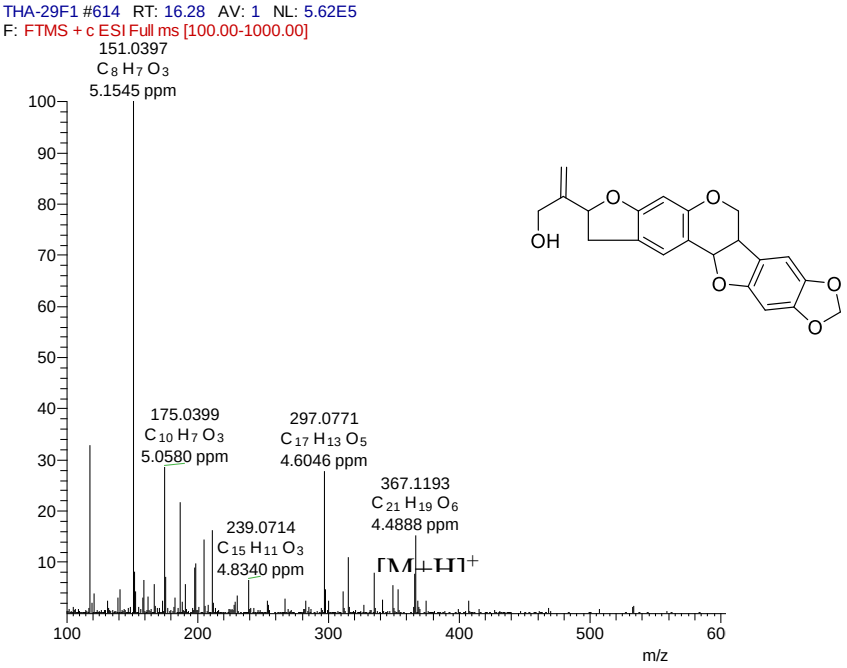


HMBC spectrum of compound 27

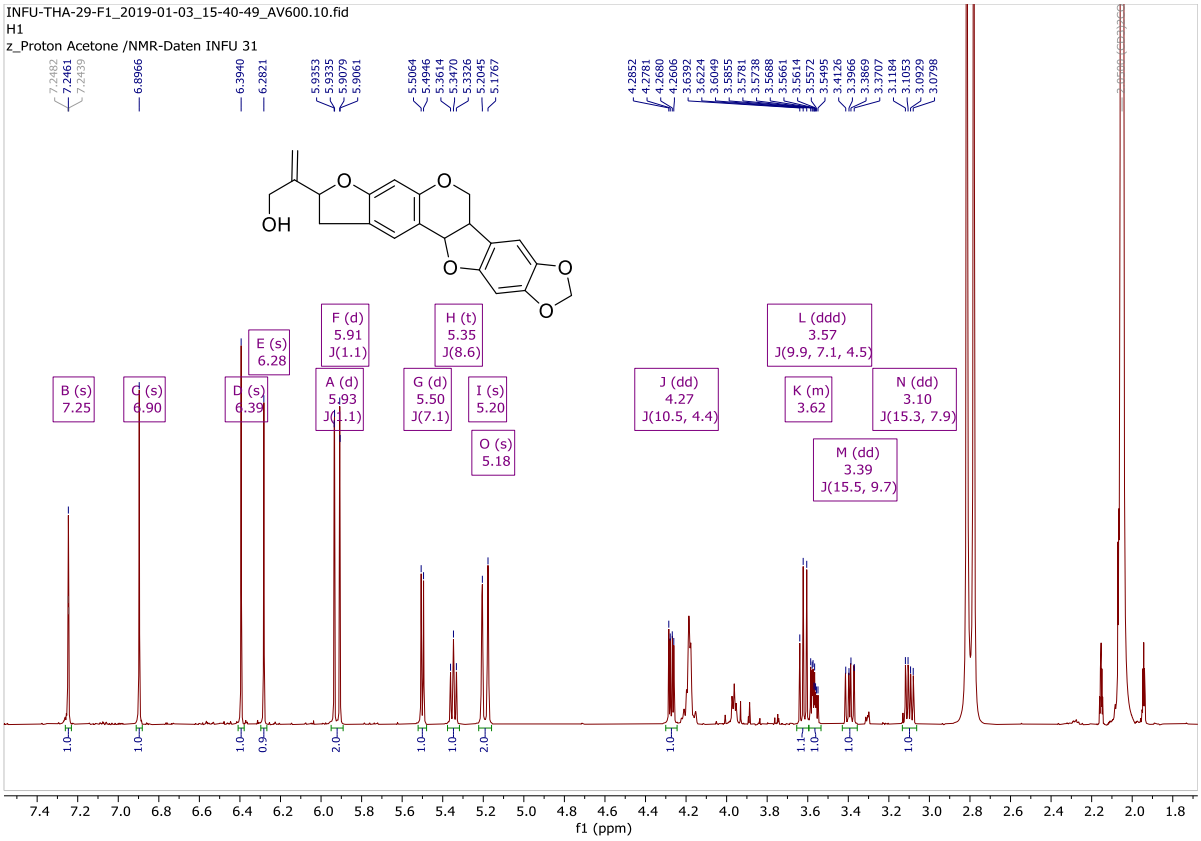


Appendix A28: Spectra for compound 28

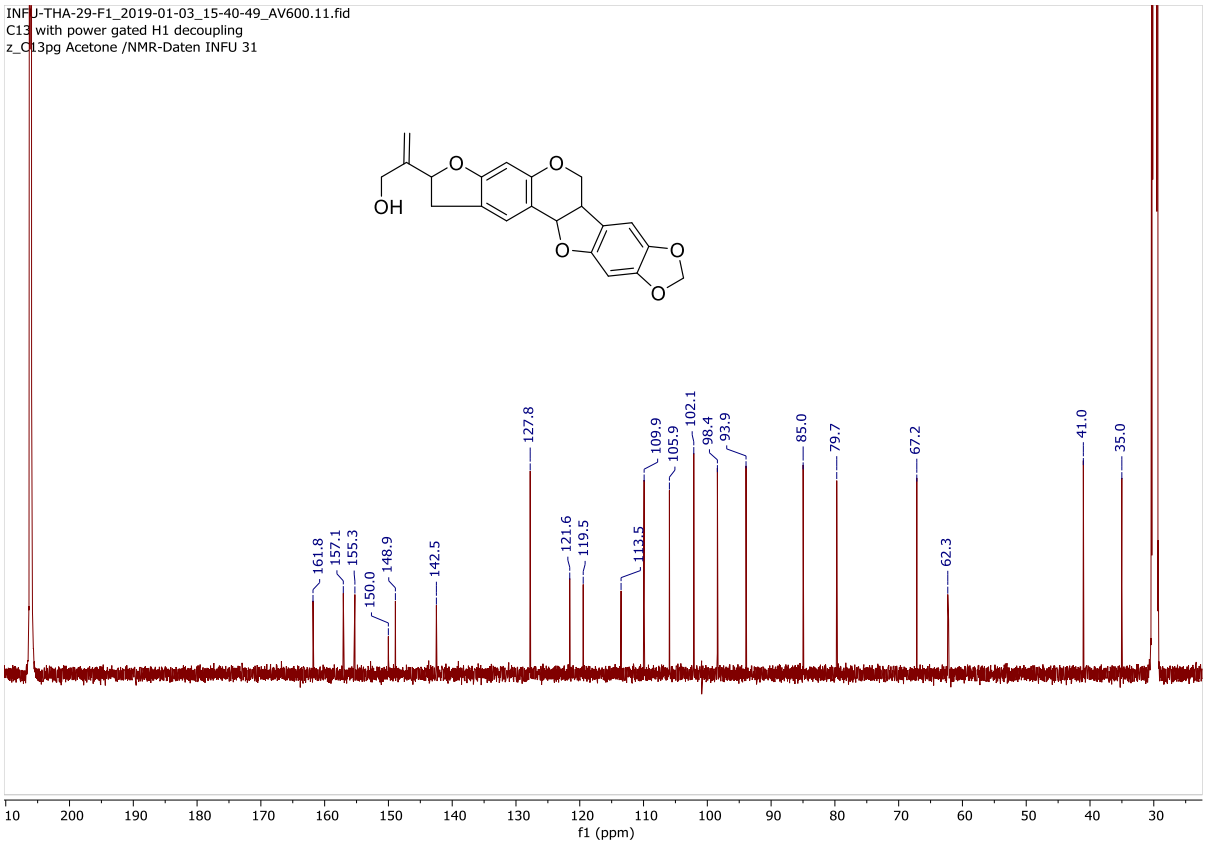
HRESIMS spectrum of compound 28



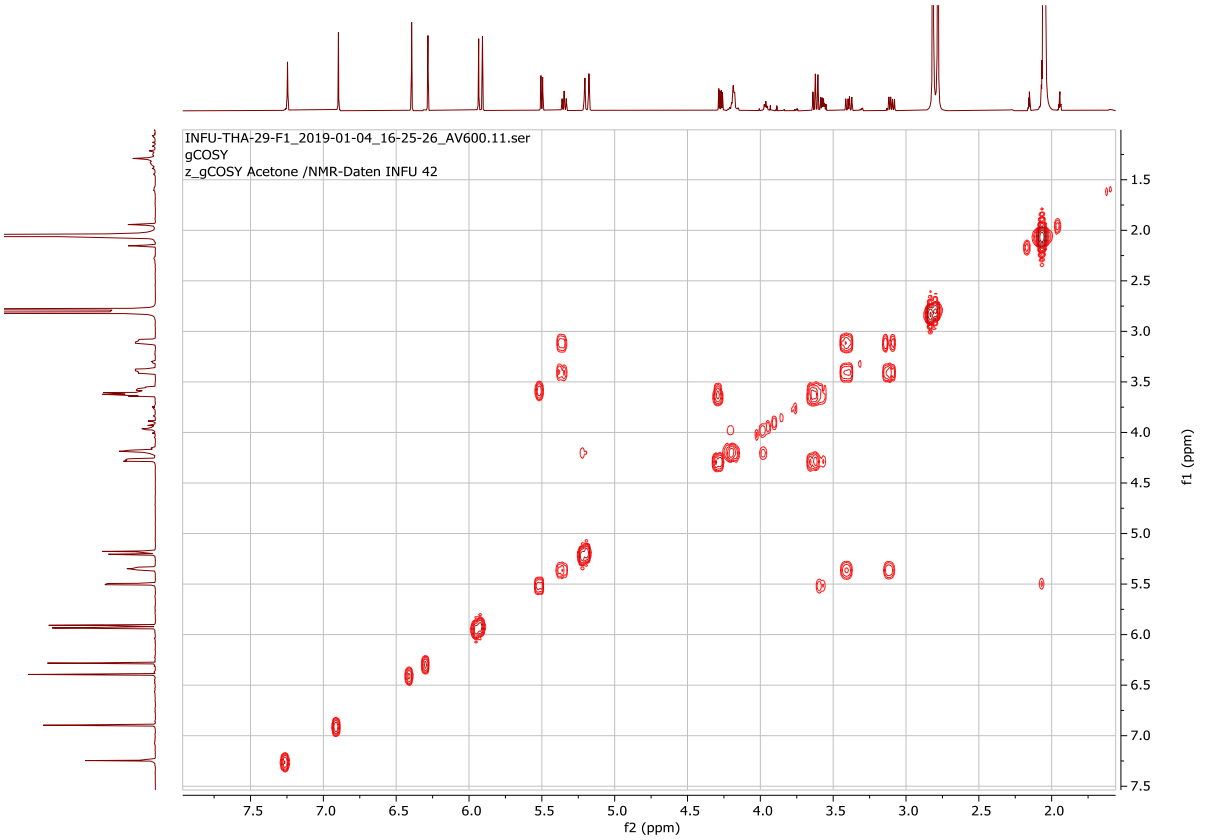
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 28



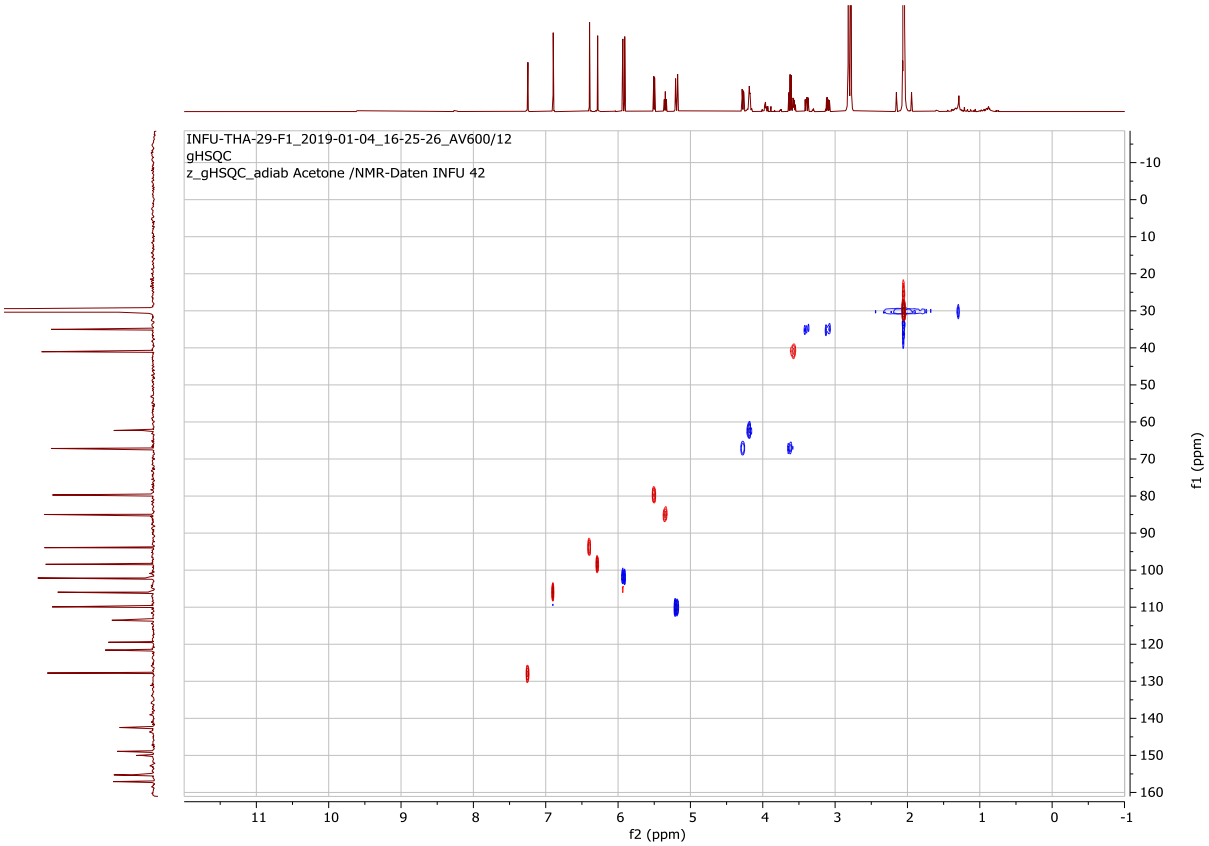
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 28



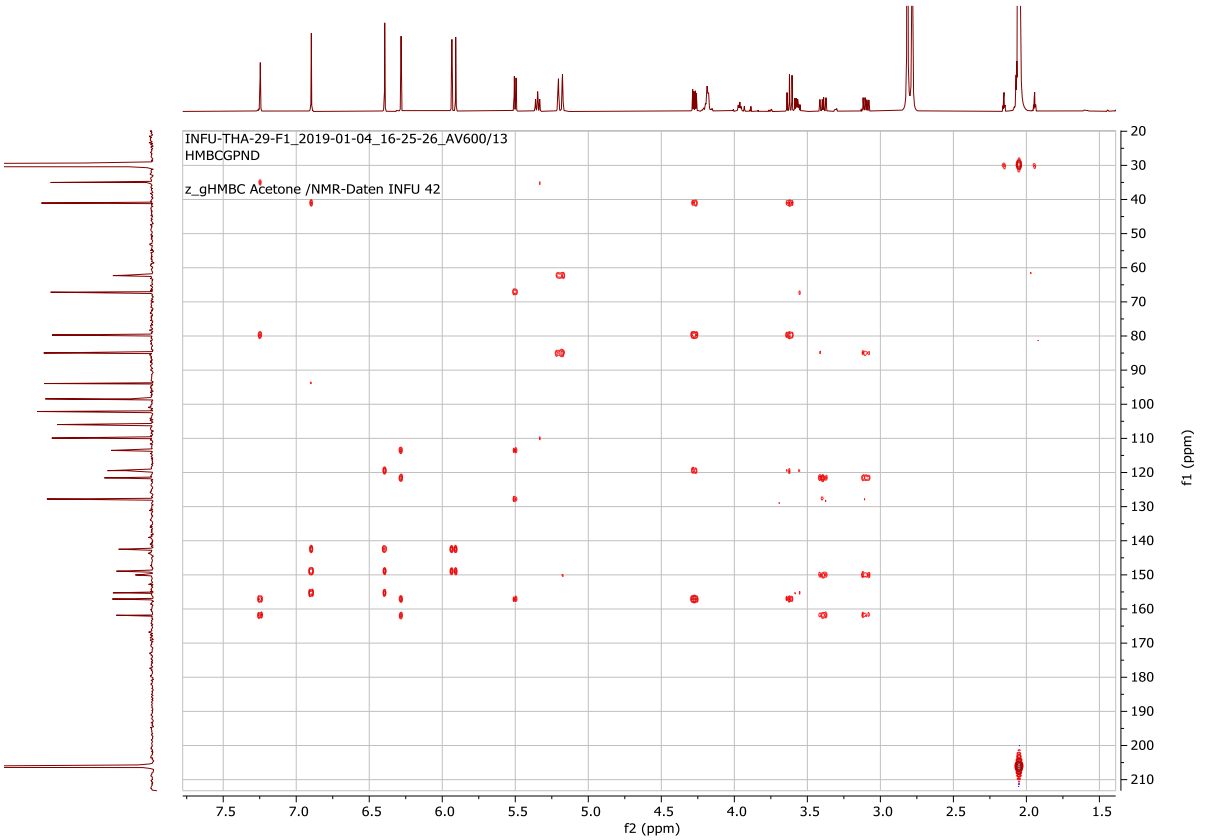
^{1}H-^{1}H-COSY spectrum of compound 28



HSQC spectrum of compound 28

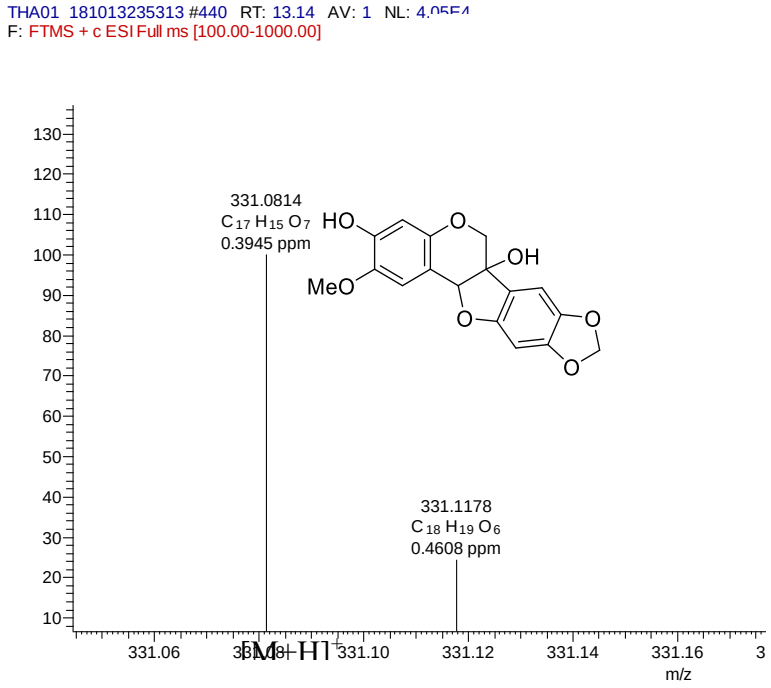


HMBC spectrum of compound 28

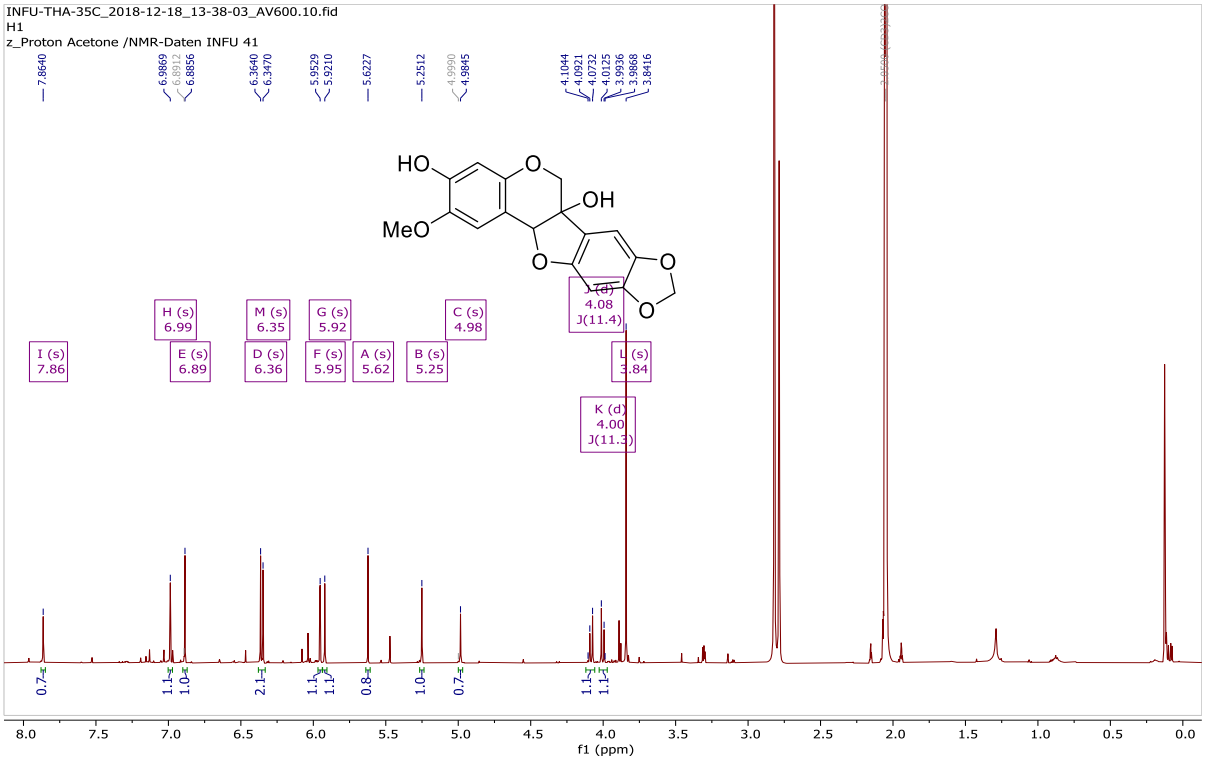


Appendix A29: Spectra for compound 29

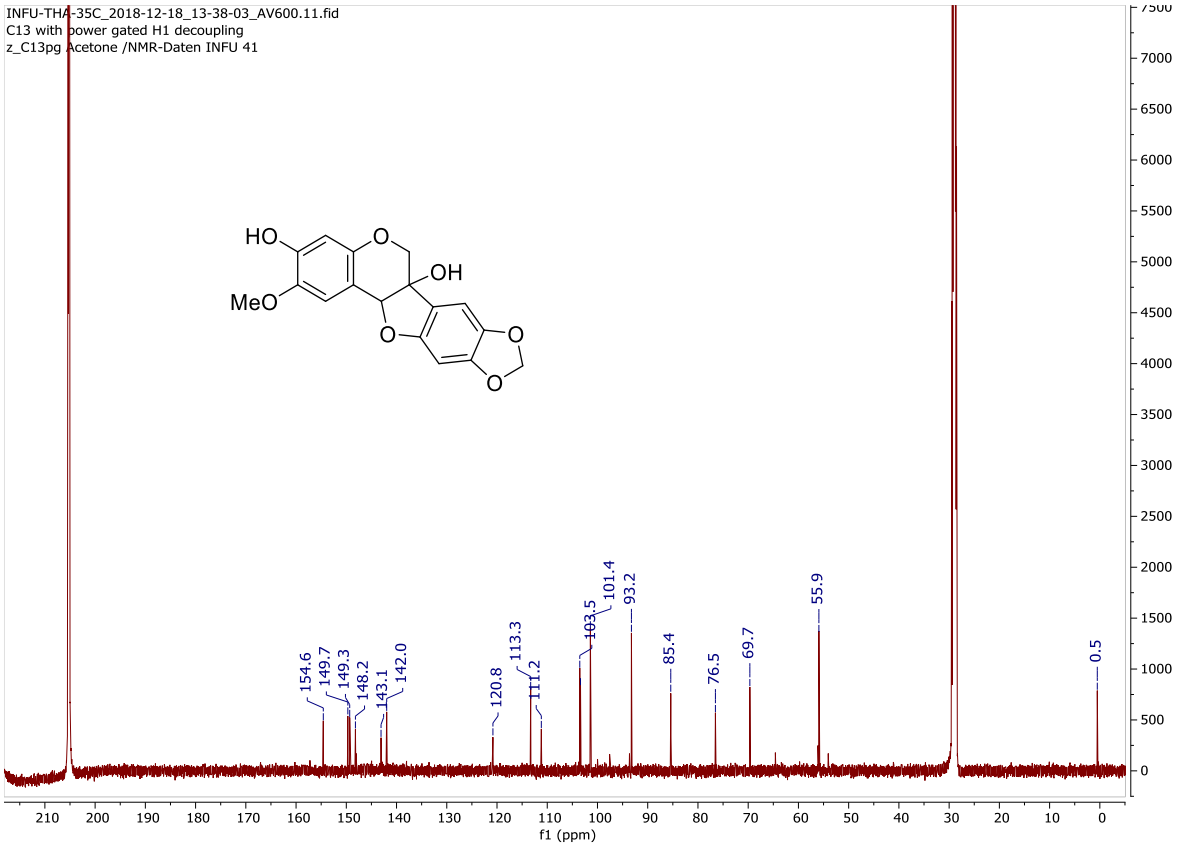
HRESIMS spectrum of compound 29



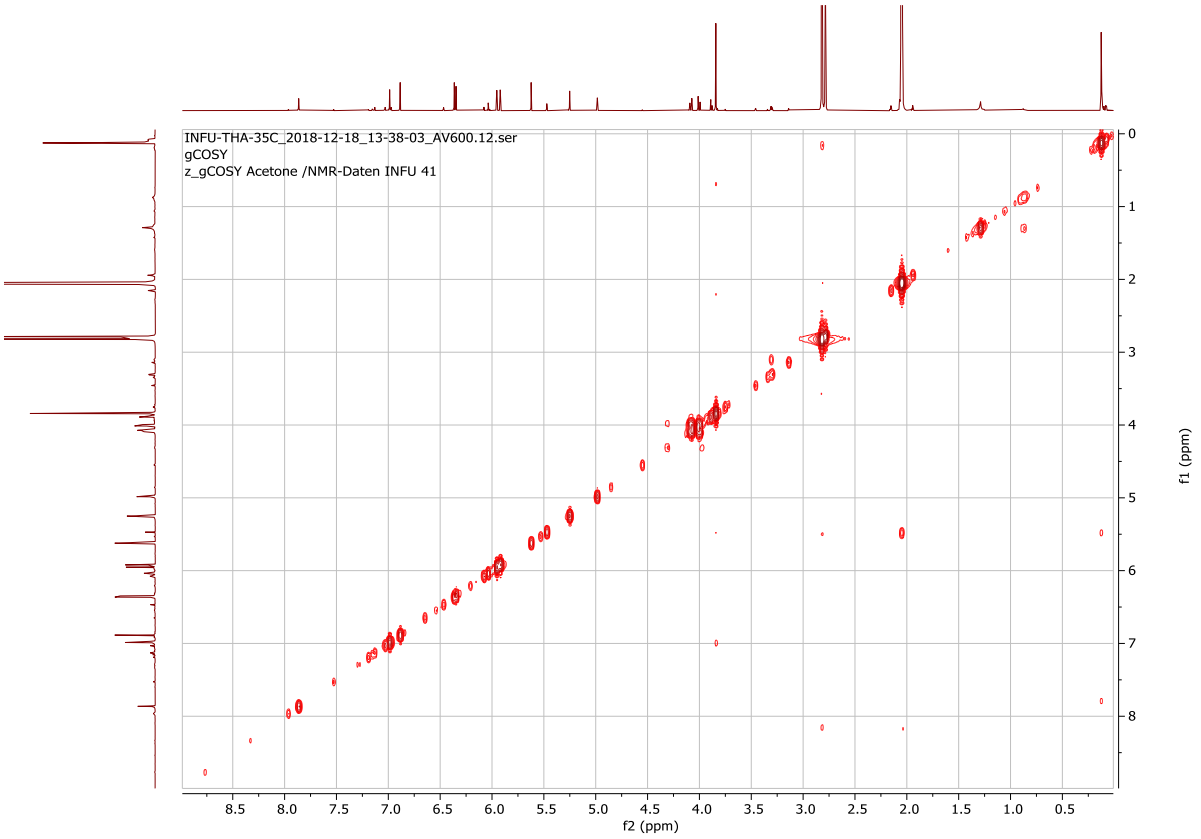
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 29



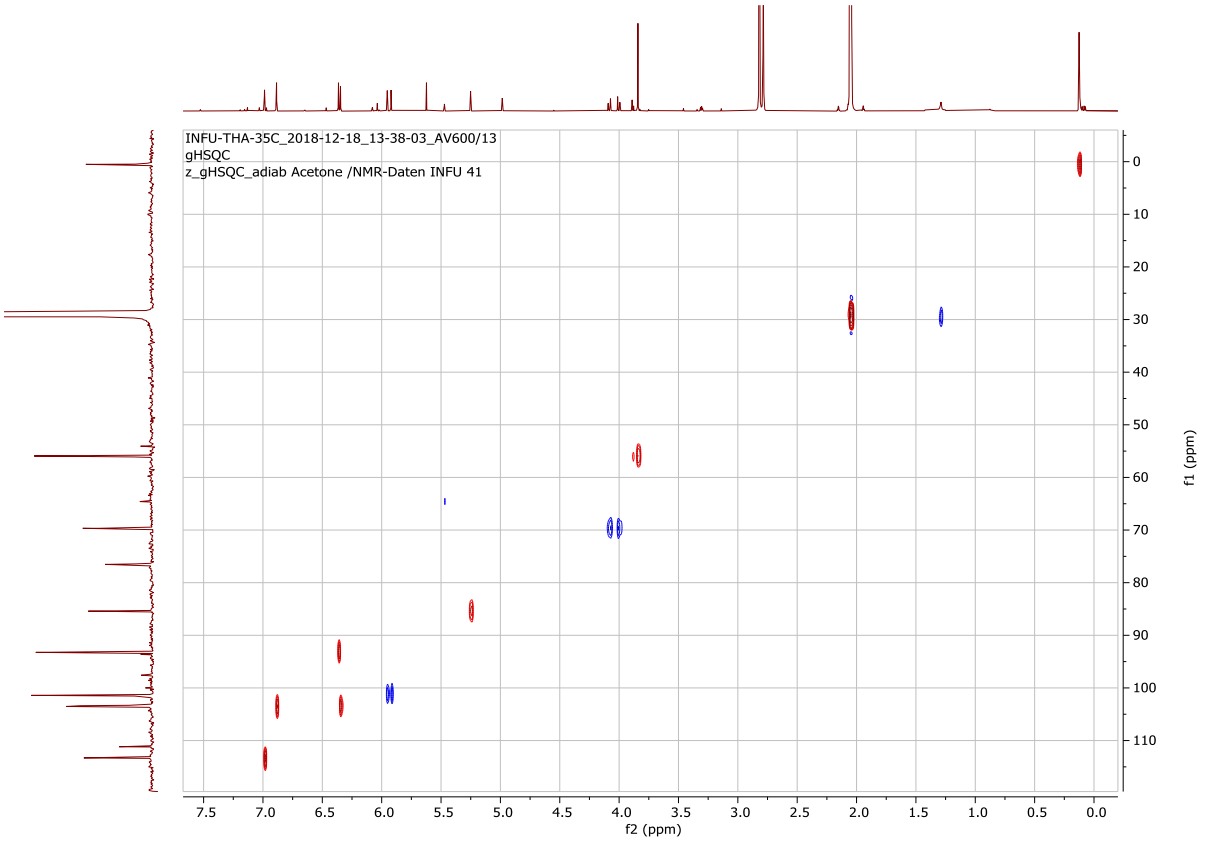
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 29



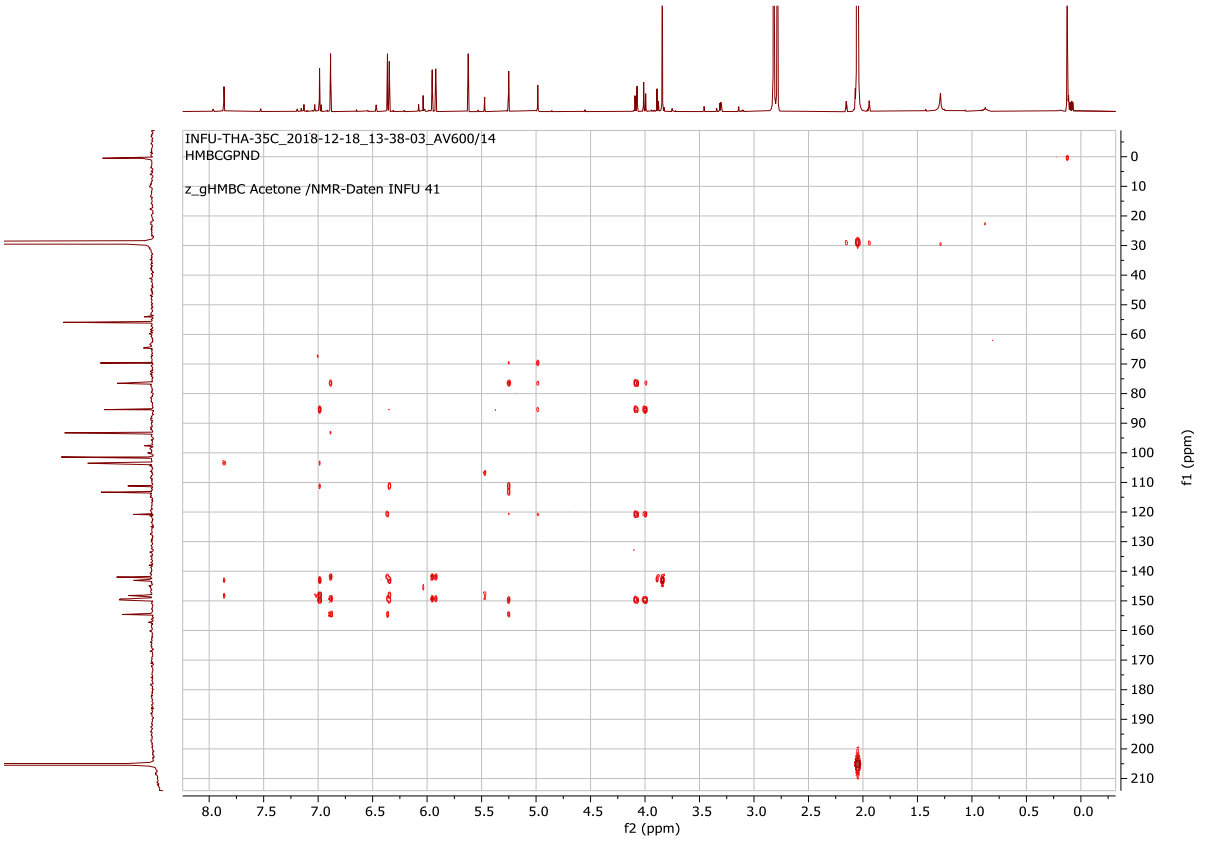
^{1}H-^{1}H-COSY spectrum of compound 29



HSQC spectrum of compound 29

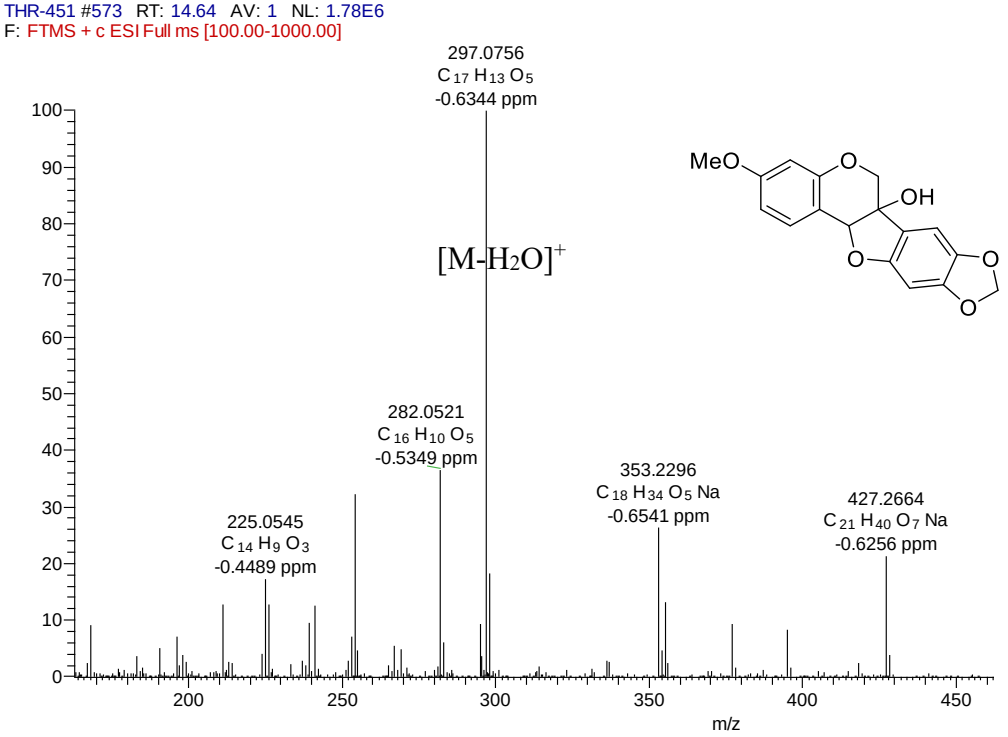


HMBC spectrum of compound 29

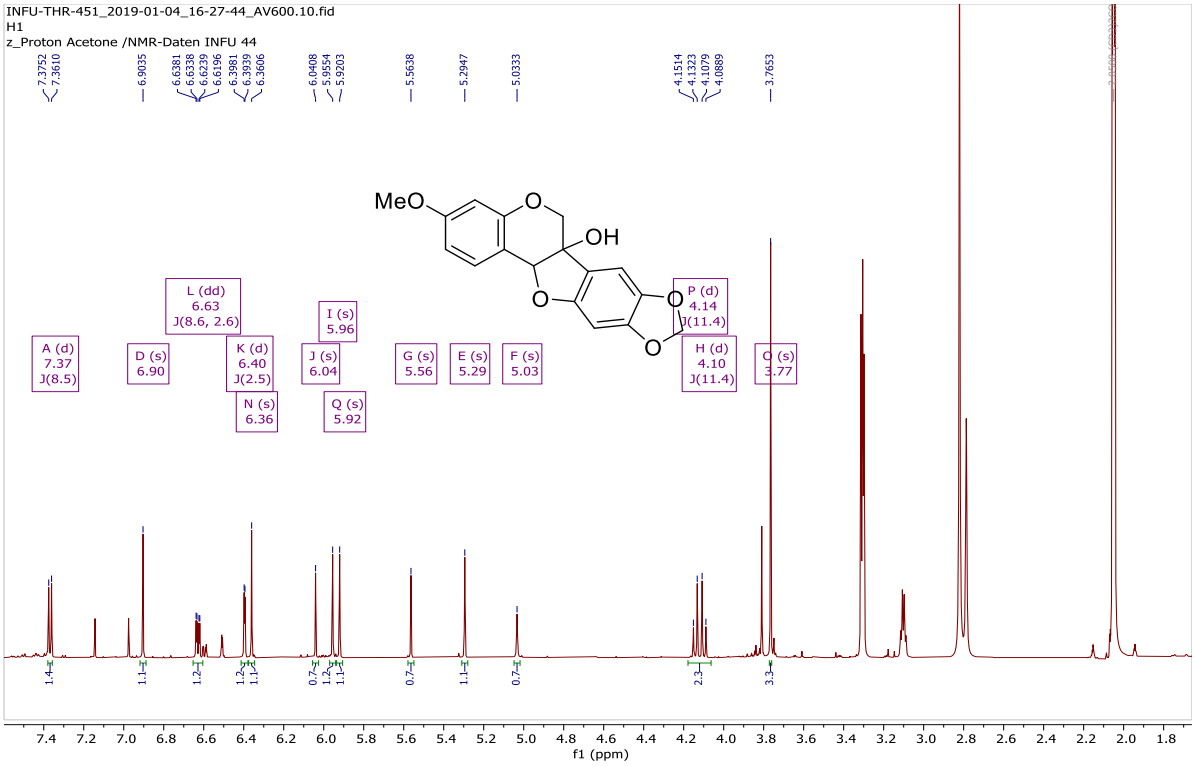


Appendix A30: Spectra for compound 30

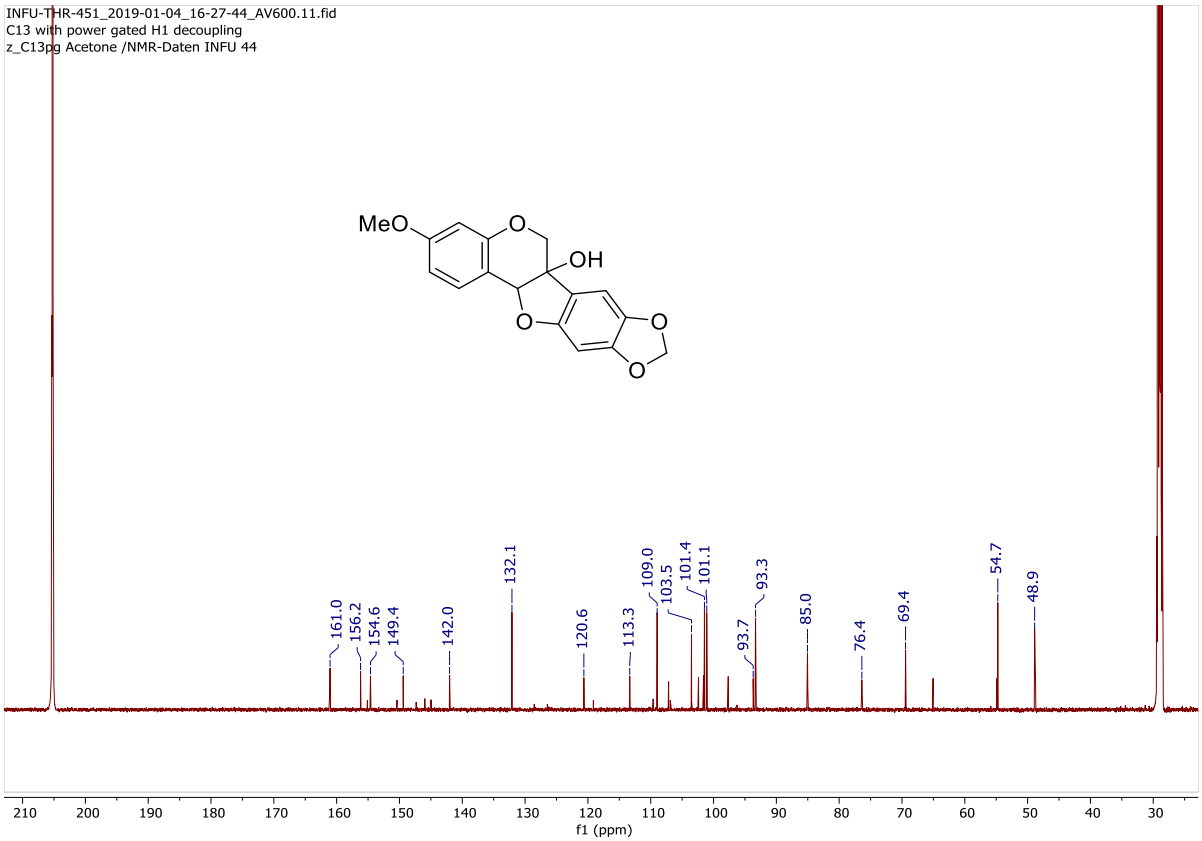
HRESIMS spectrum of compound 30



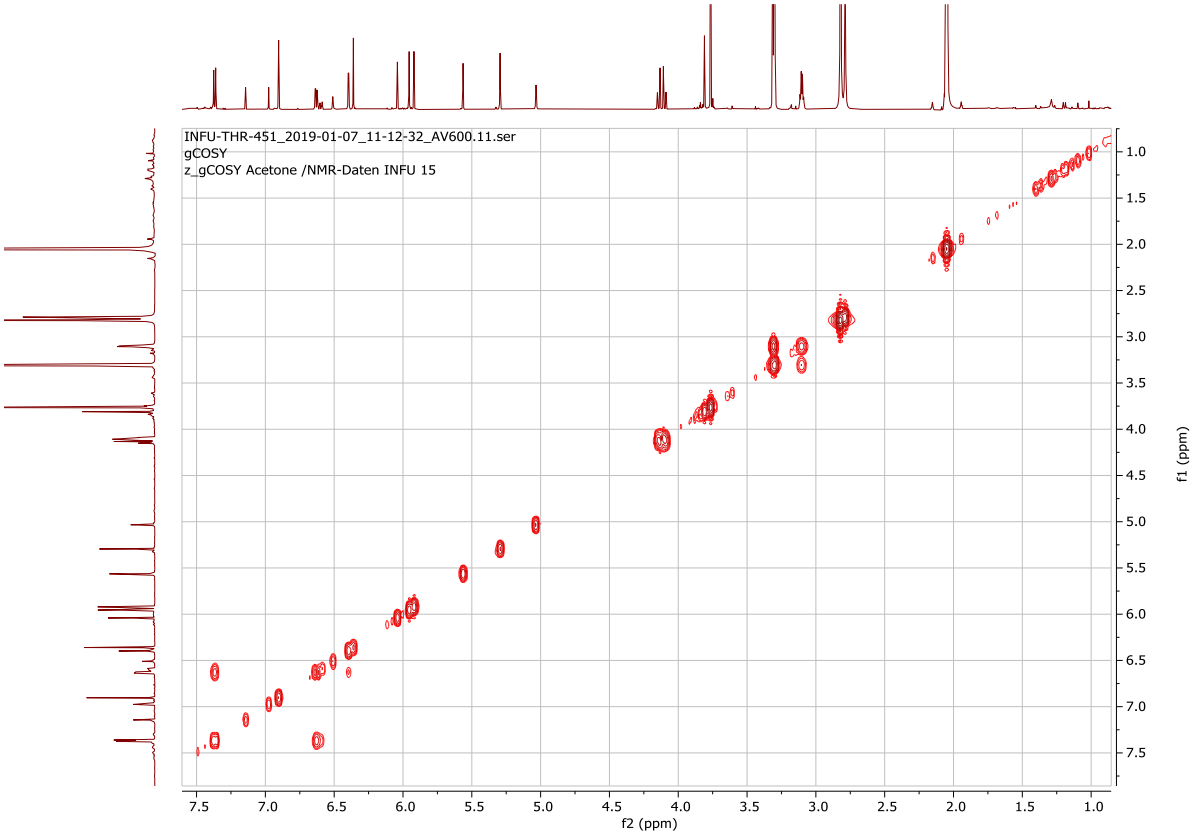
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 30



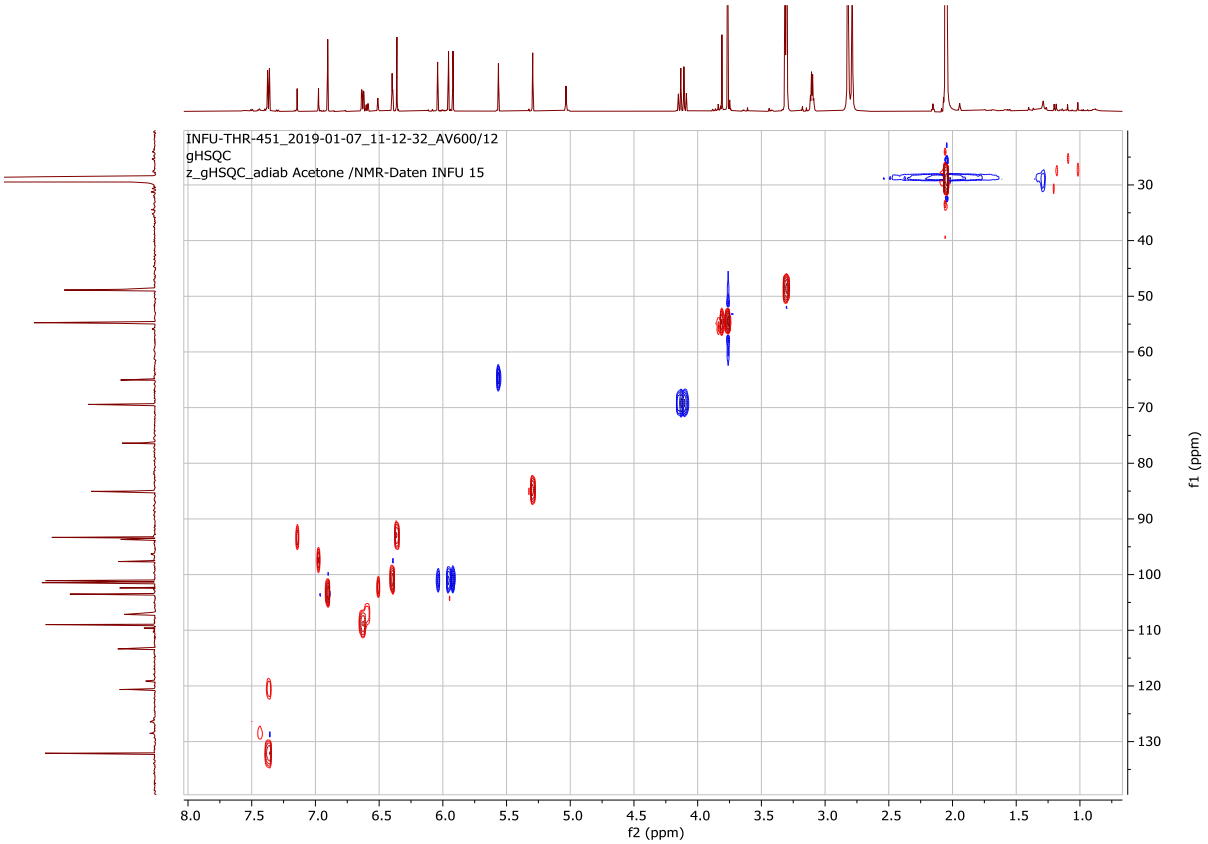
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 30



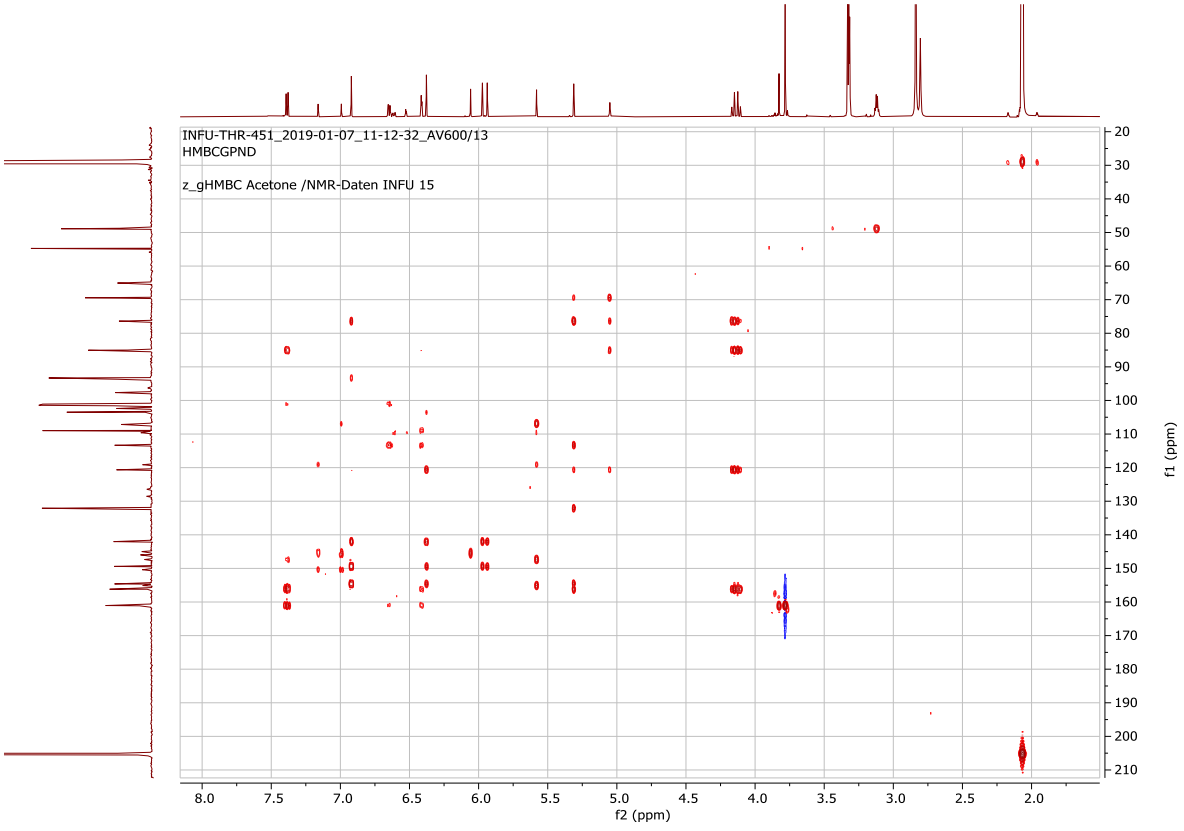
^{1}H-^{1}H-COSY spectrum of compound 30



HSQC spectrum of compound 30

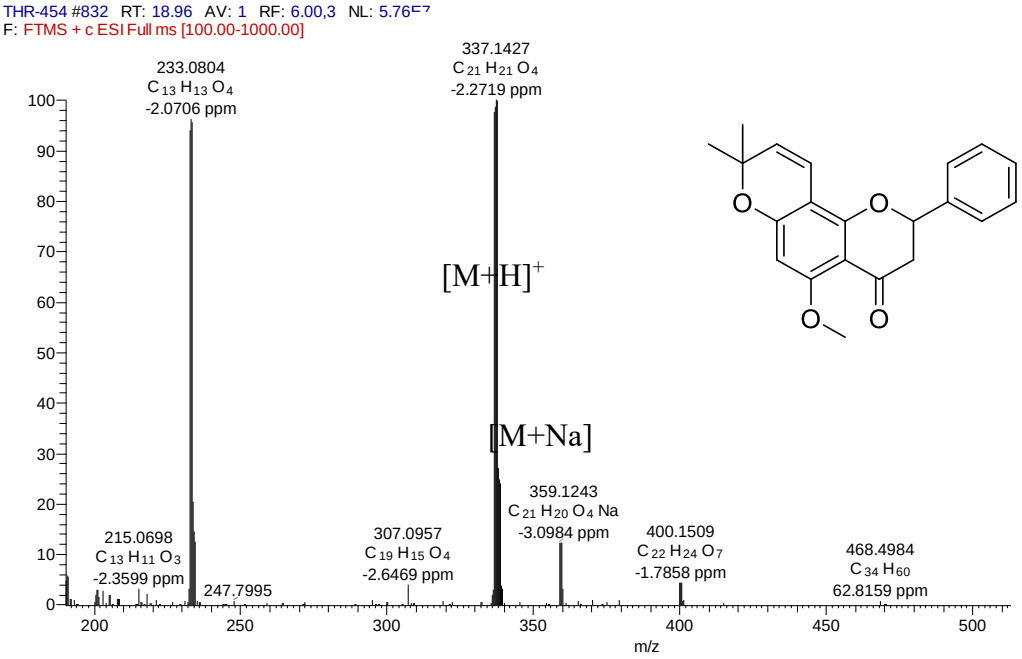


HMBC spectrum of compound 30

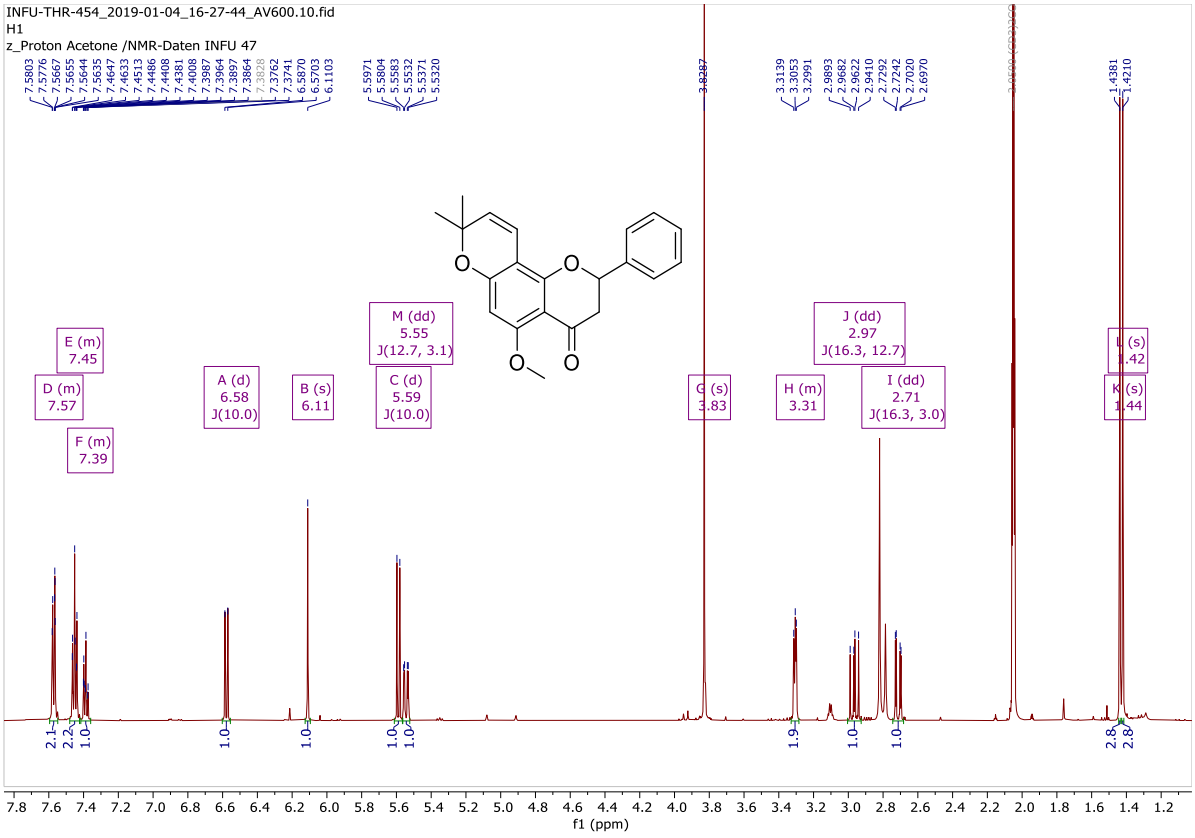


Appendix A31: Spectra for compound 31

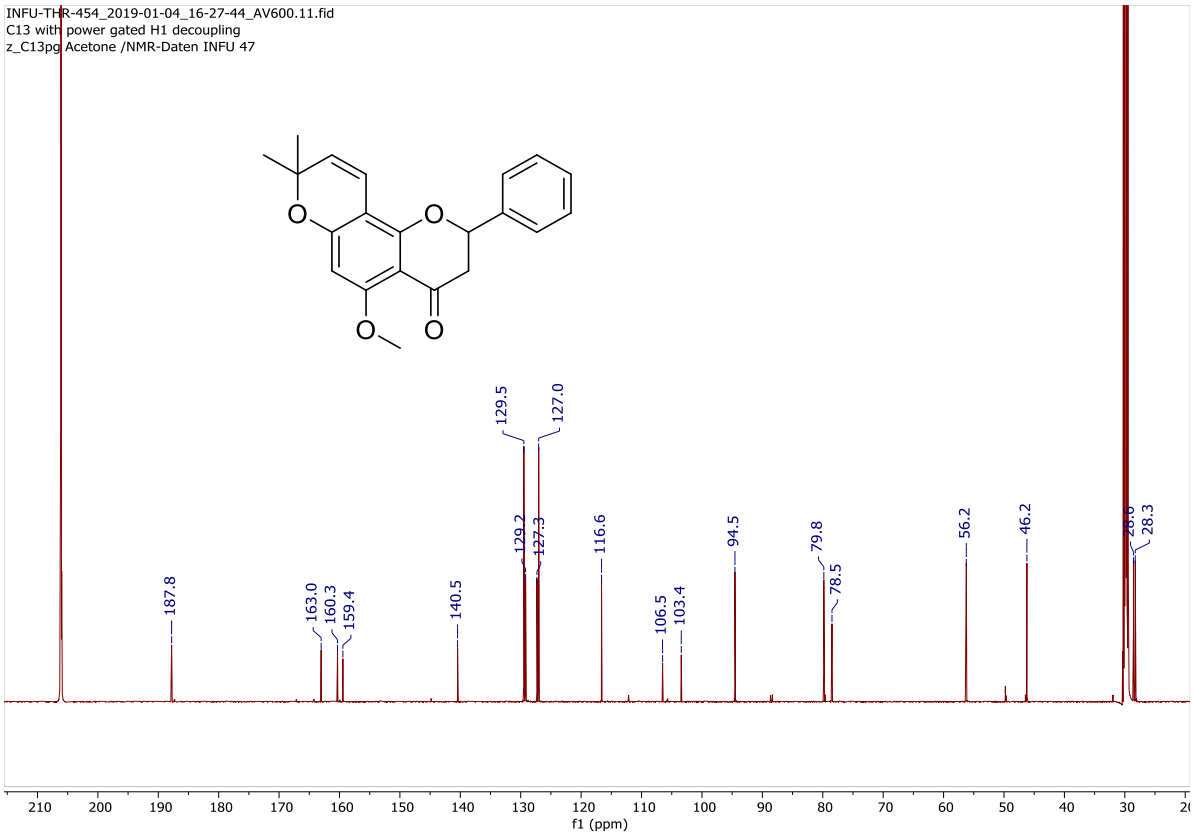
HRESIMS spectrum of compound 31



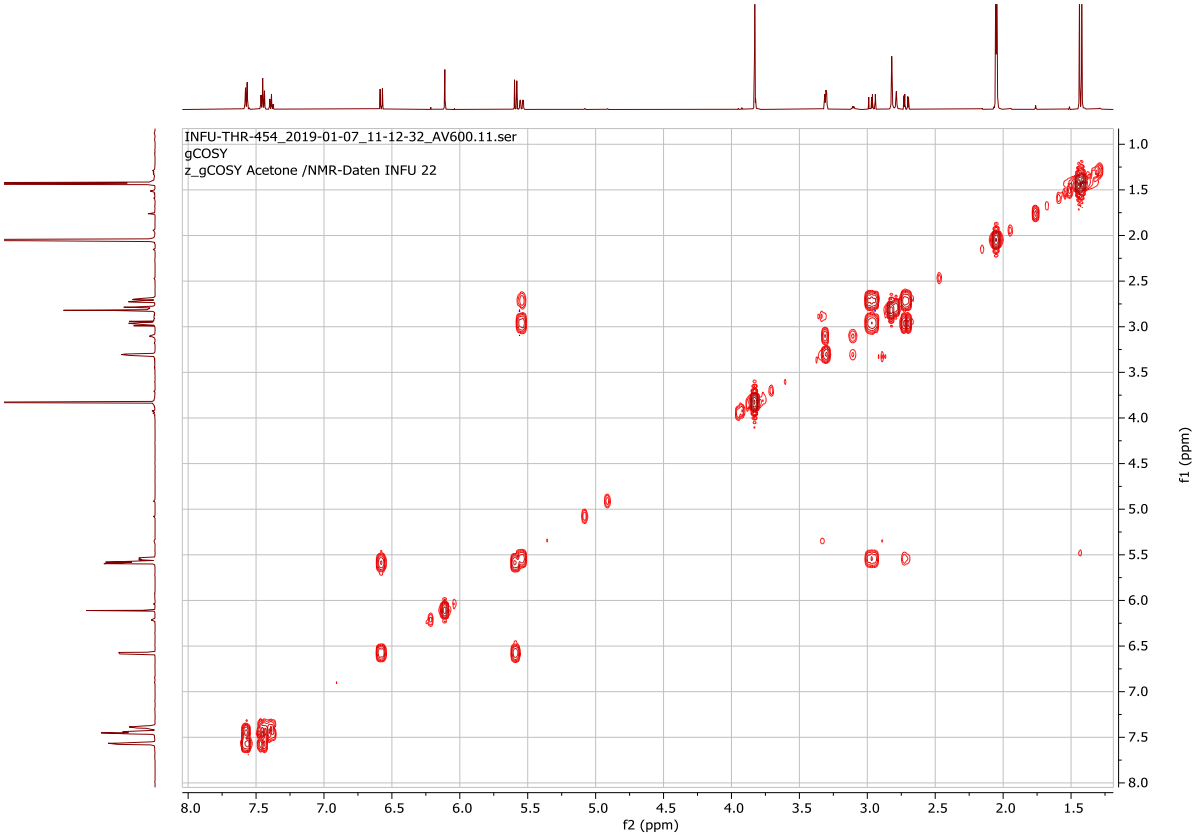
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 31



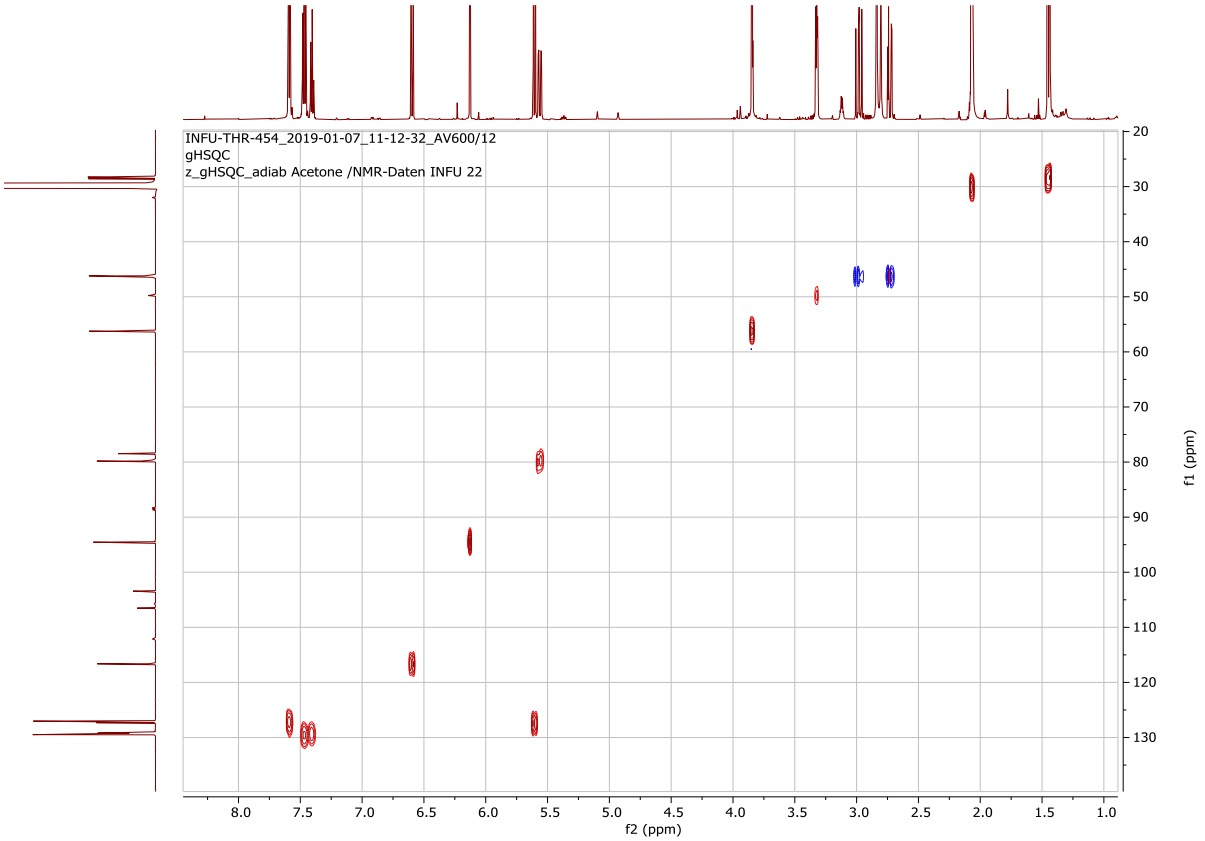
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 31



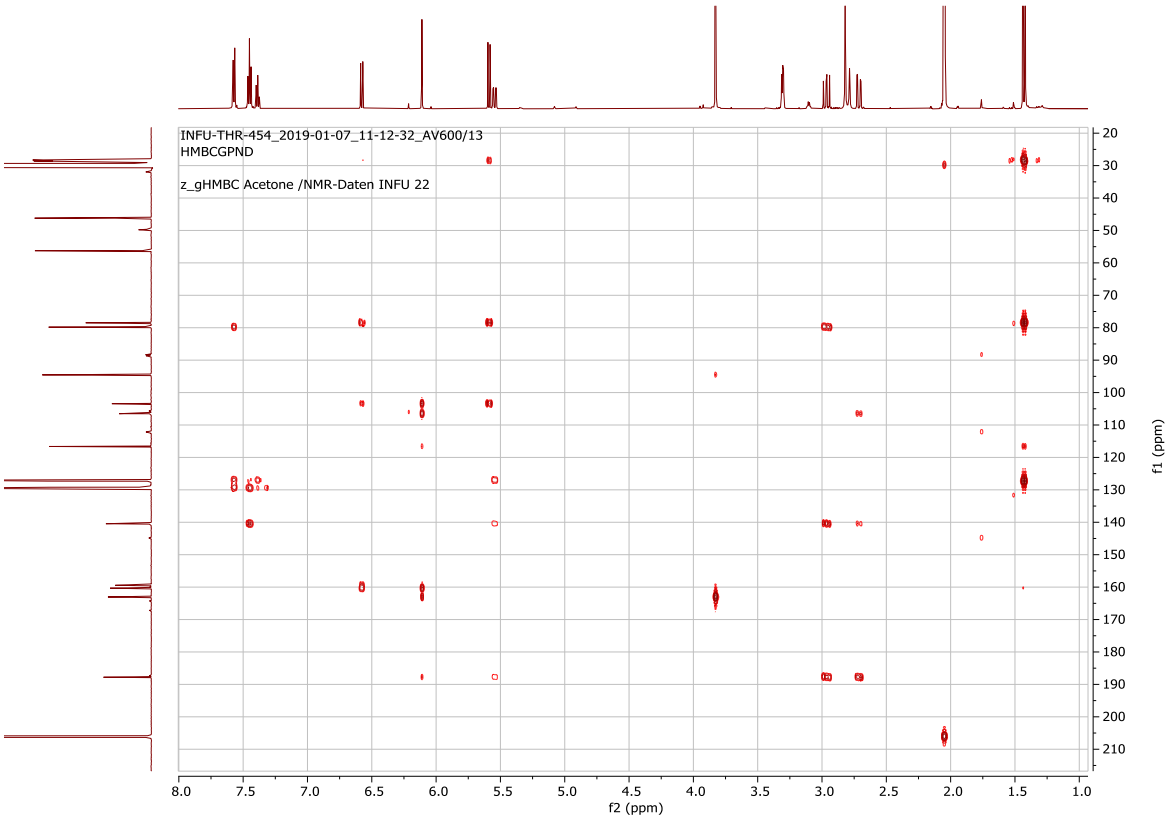
^{1}H-^{1}H-COSY spectrum of compound 31



HSQC spectrum of compound 31

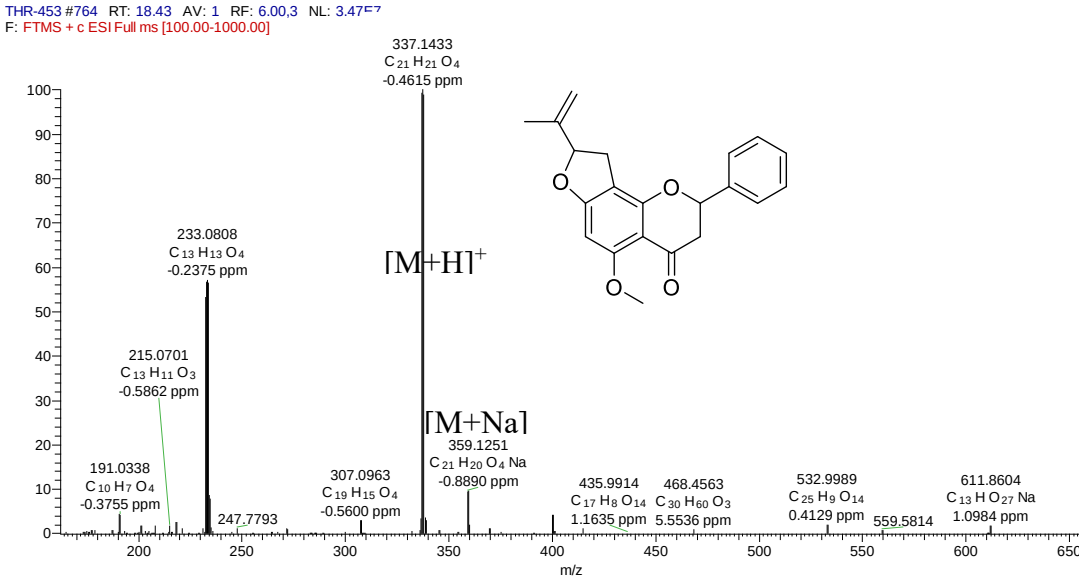


HMBC spectrum of compound 31

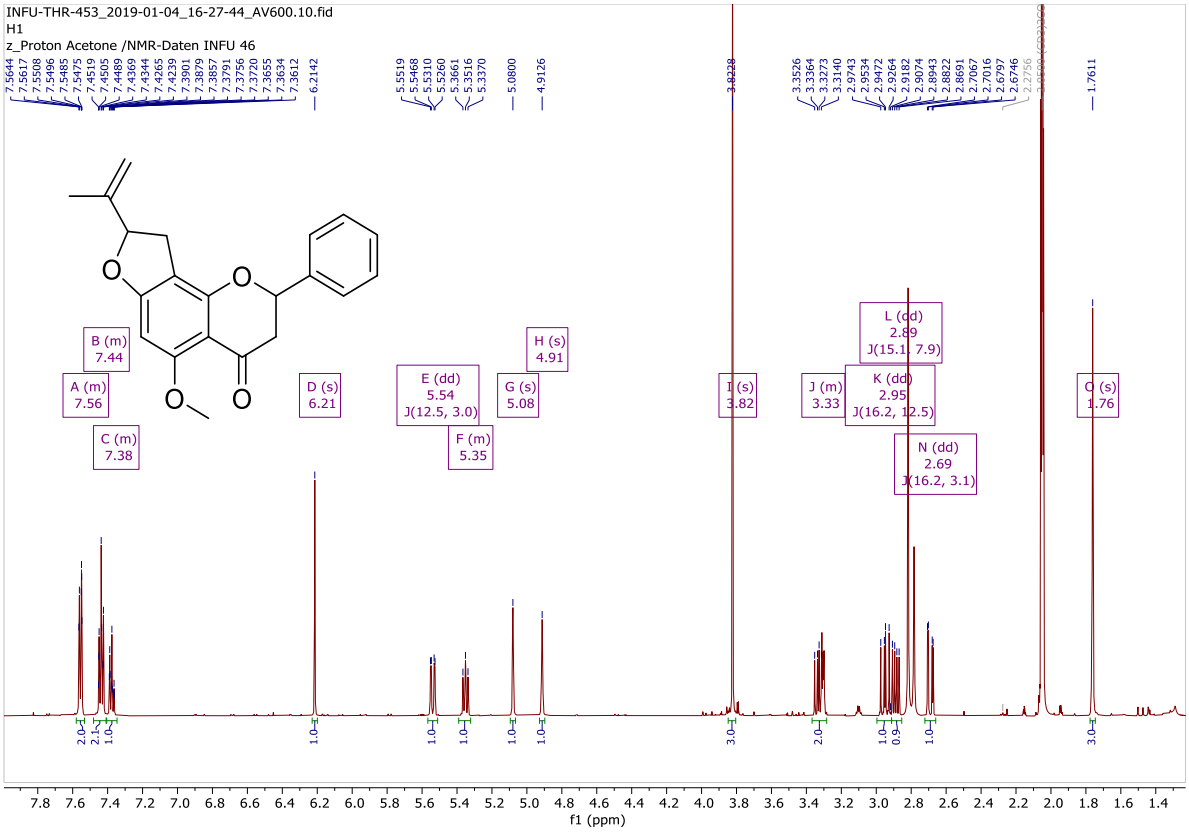


Appendix A32: Spectra for compound 32

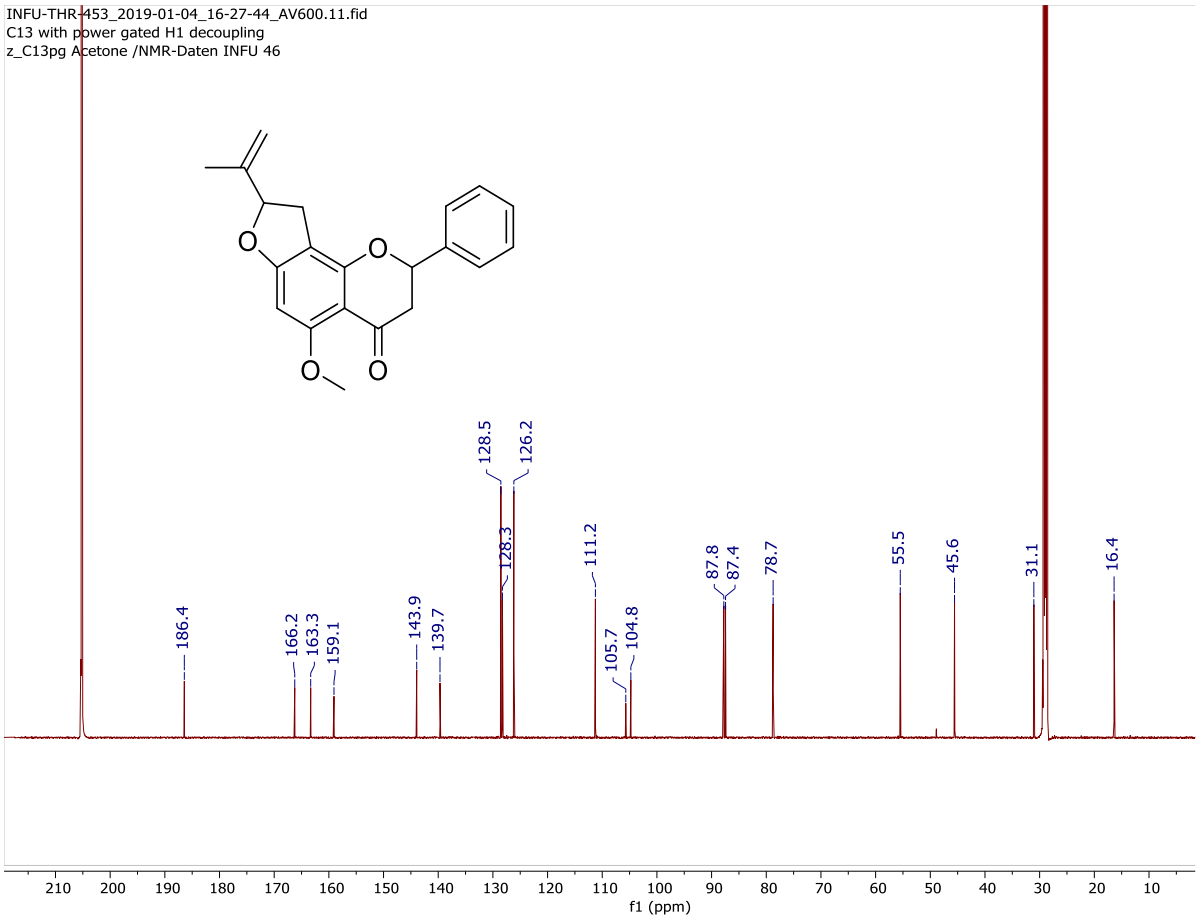
HRESIMS spectrum of compound 32



^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 32

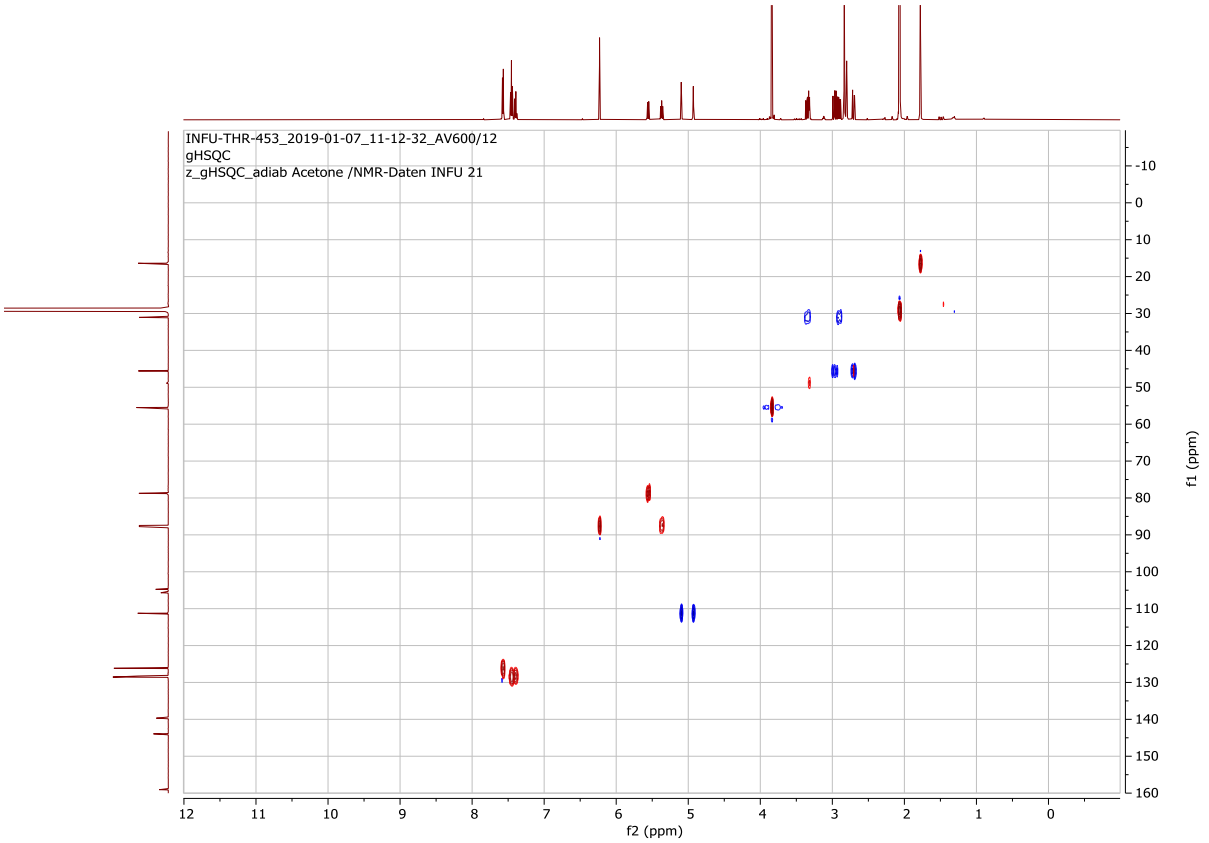


^{13}C NMR spectrum (150 MHz, Acetone-d$_6$) of compound 32

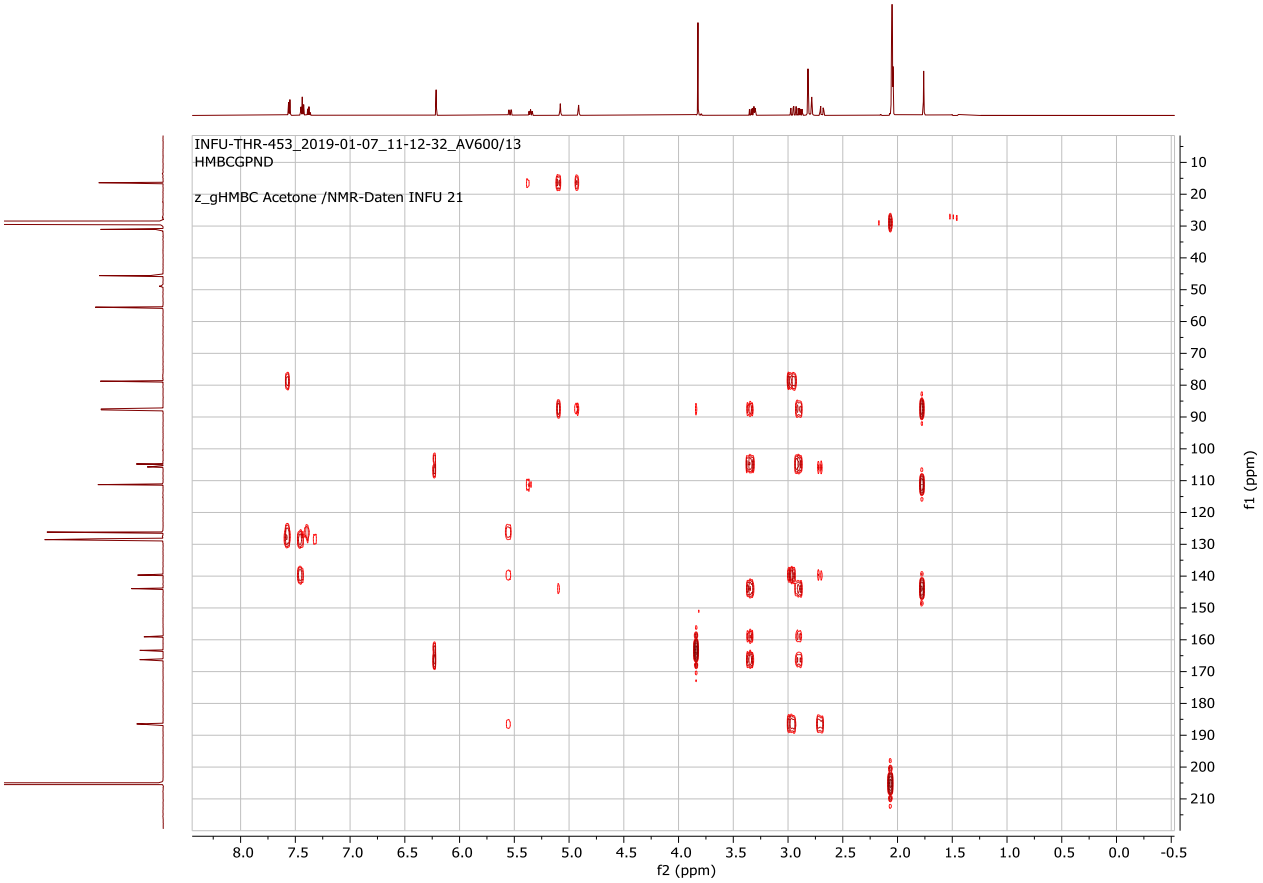


^{1}H-^{1}H-COSY spectrum of compound 32

HSQC spectrum of compound 32

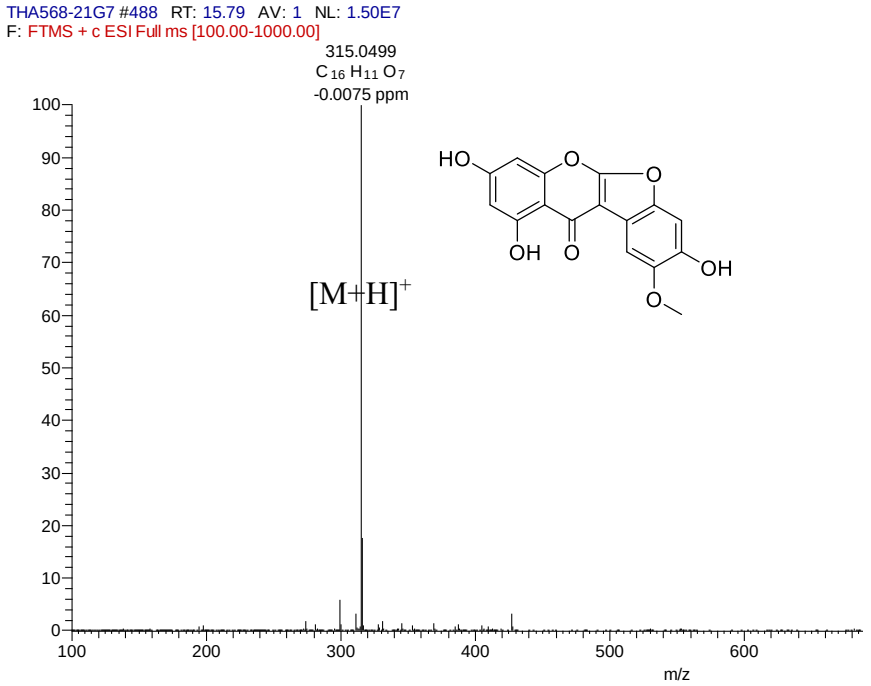


HMBC spectrum of compound 32

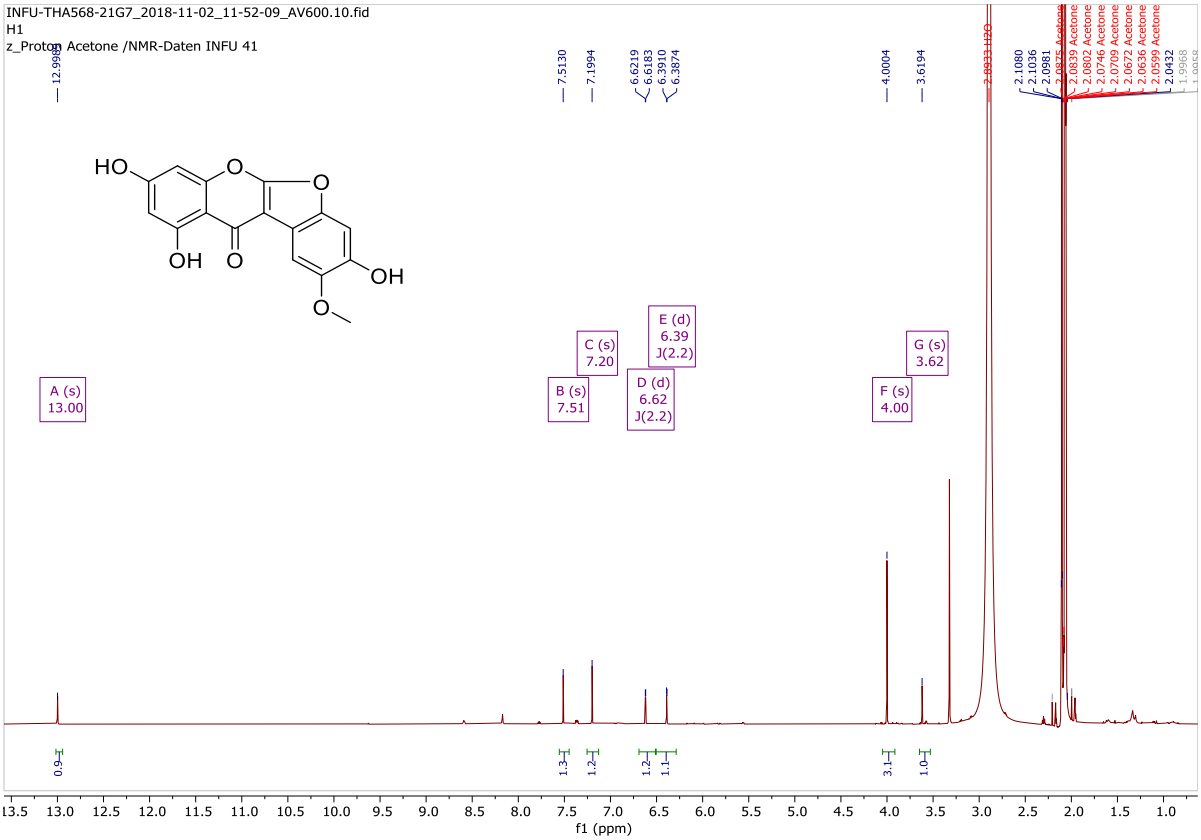


Appendix A33: Spectra for compound 33

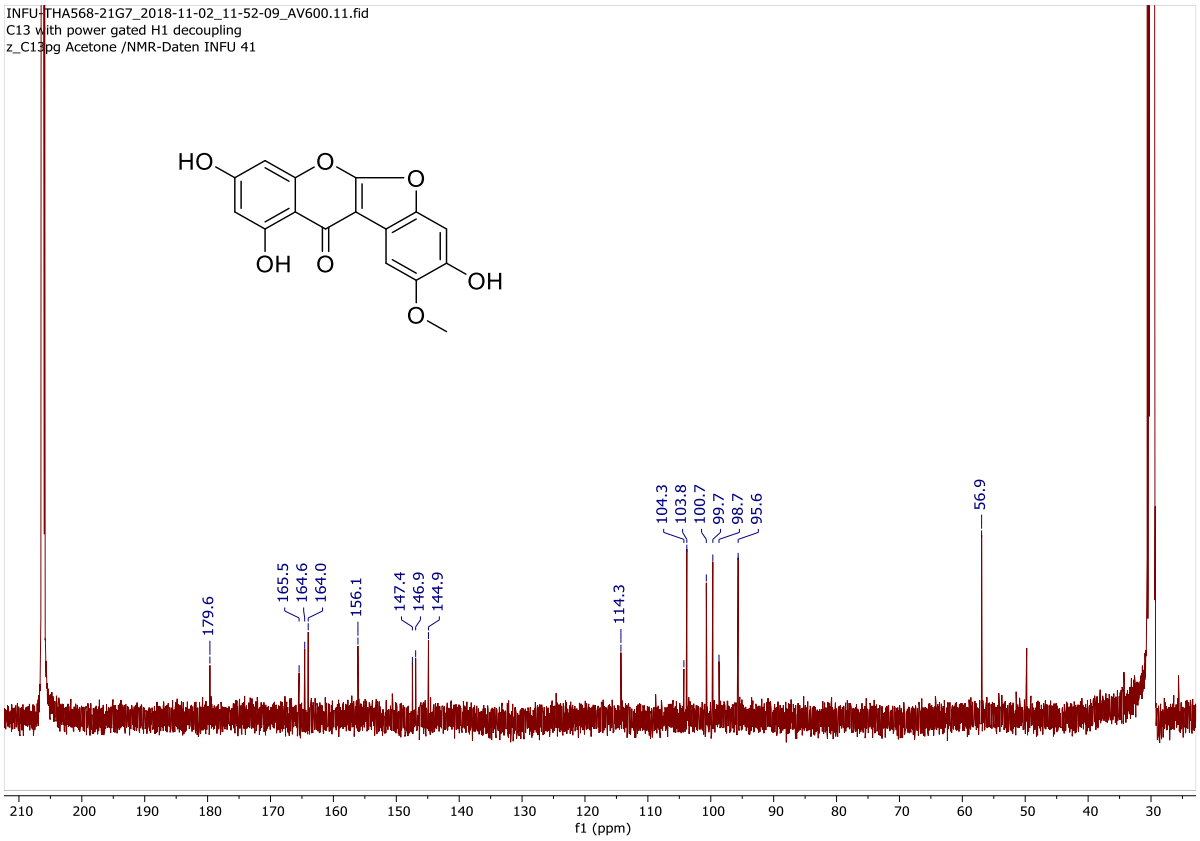
HRESIMS spectrum of compound 33



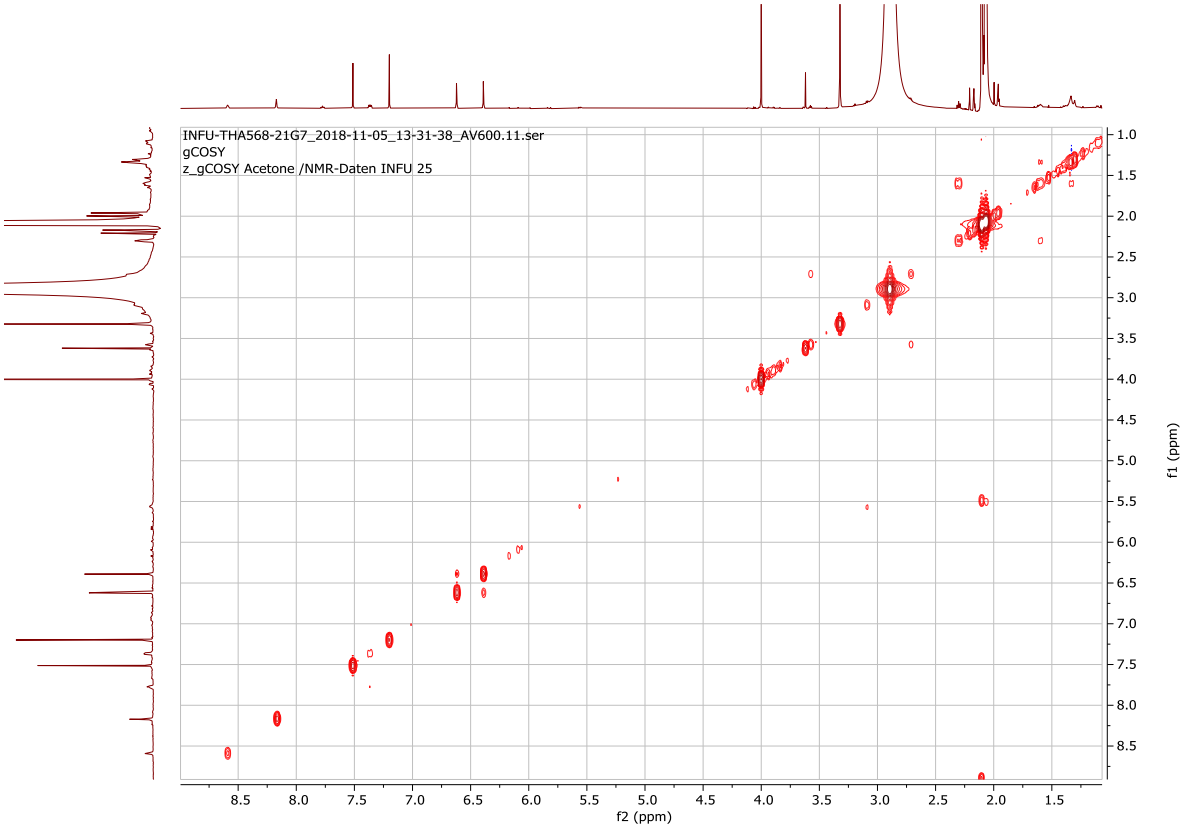
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 33



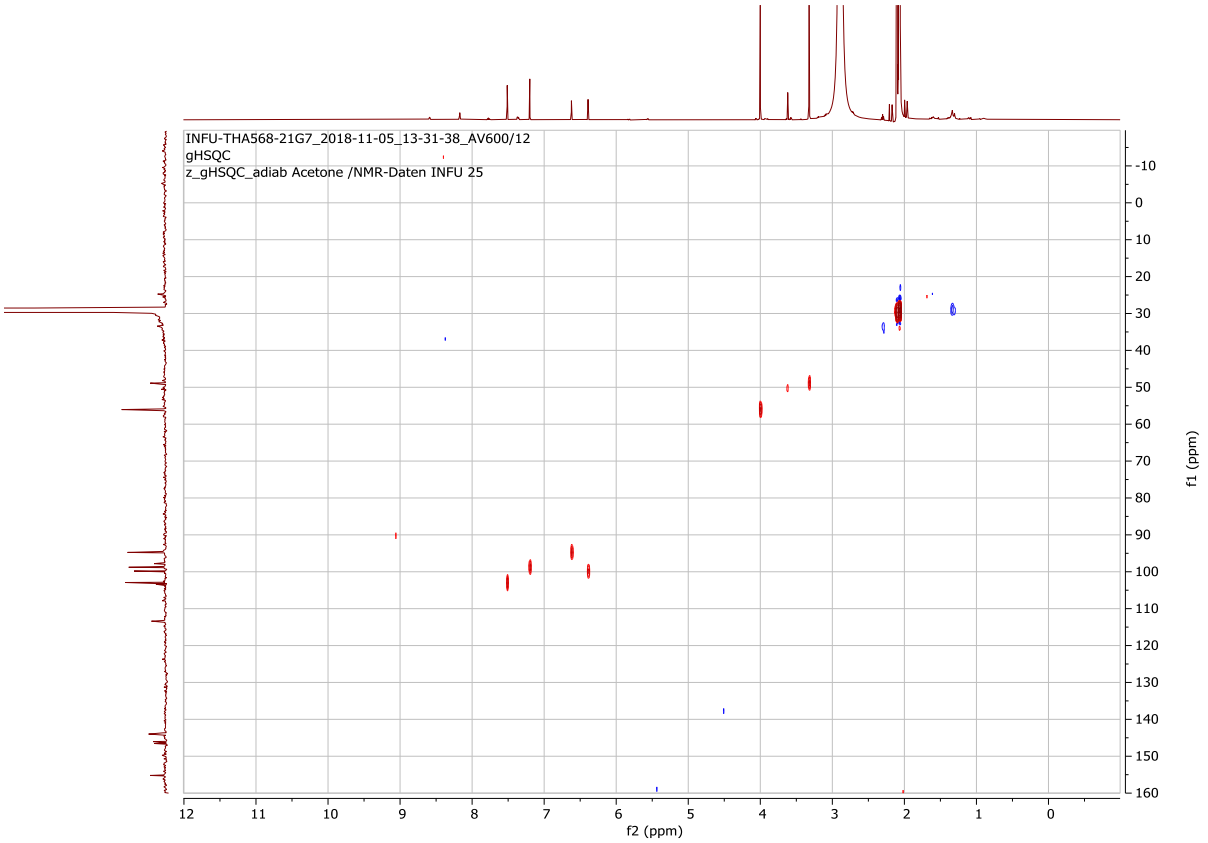
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 33



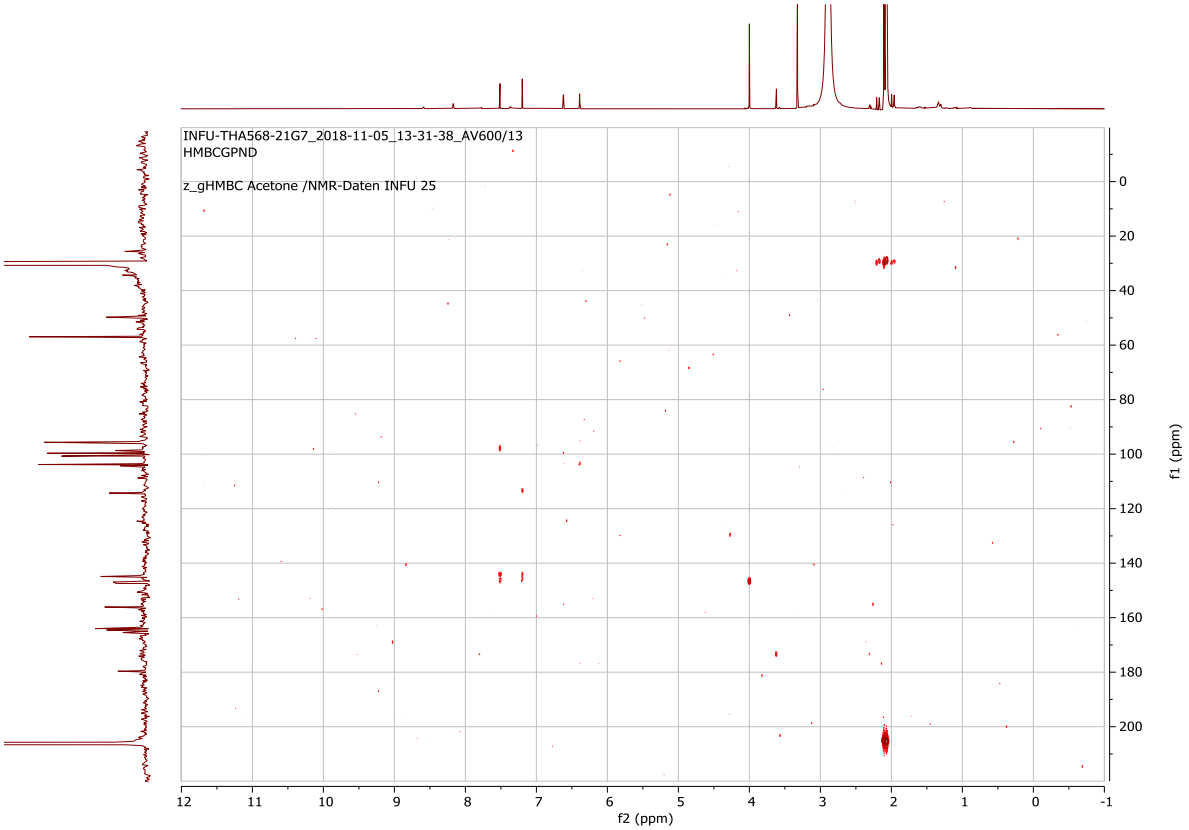
^{1}H-^{1}H-COSY spectrum of compound 33



HSQC spectrum of compound 33

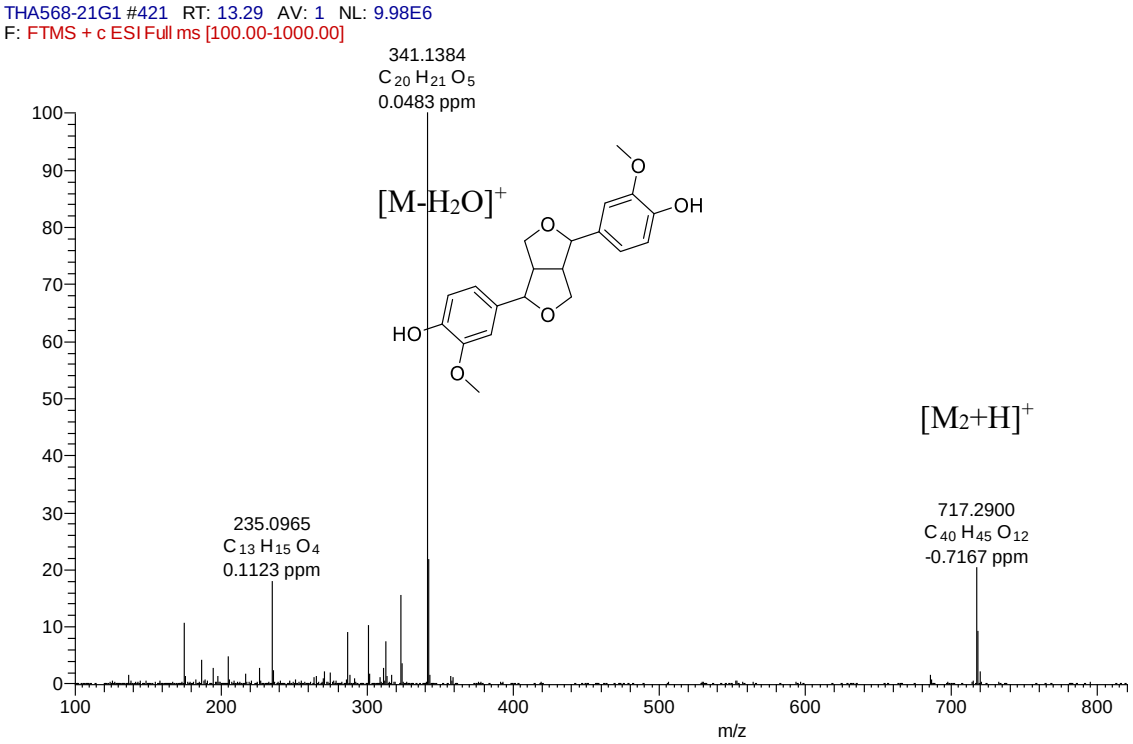


HMBC spectrum of compound 33

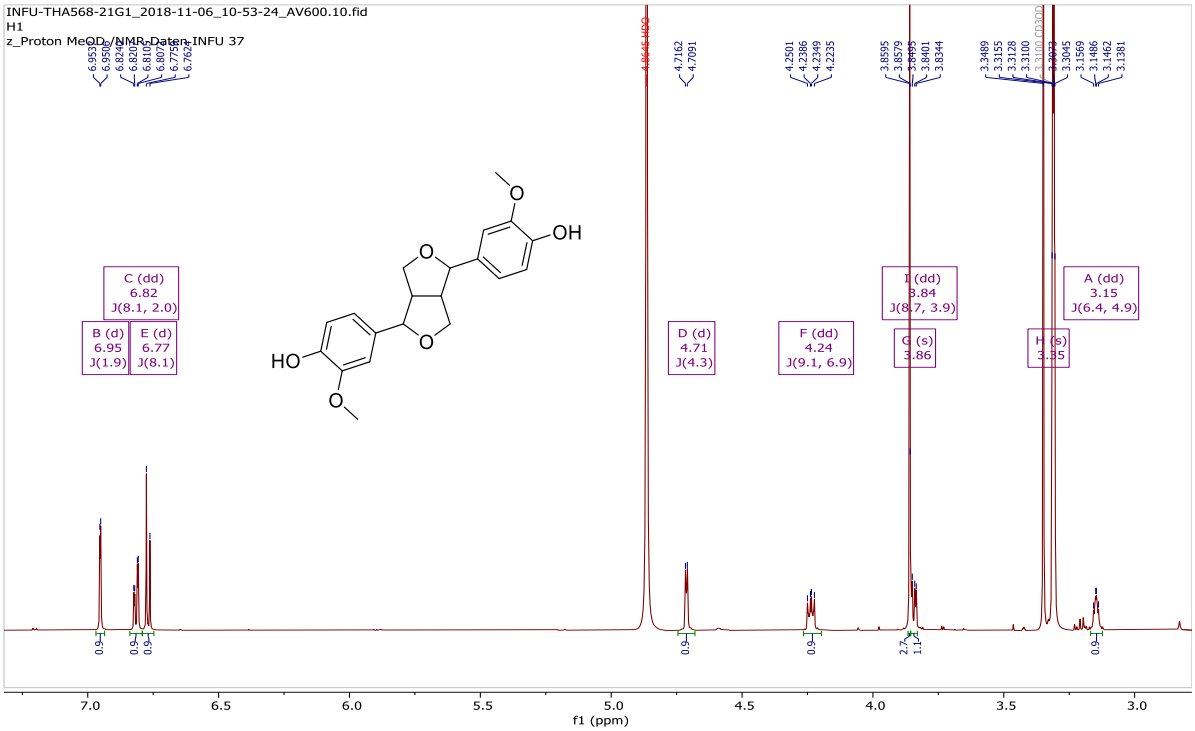


Appendix A34: Spectra for compound 34

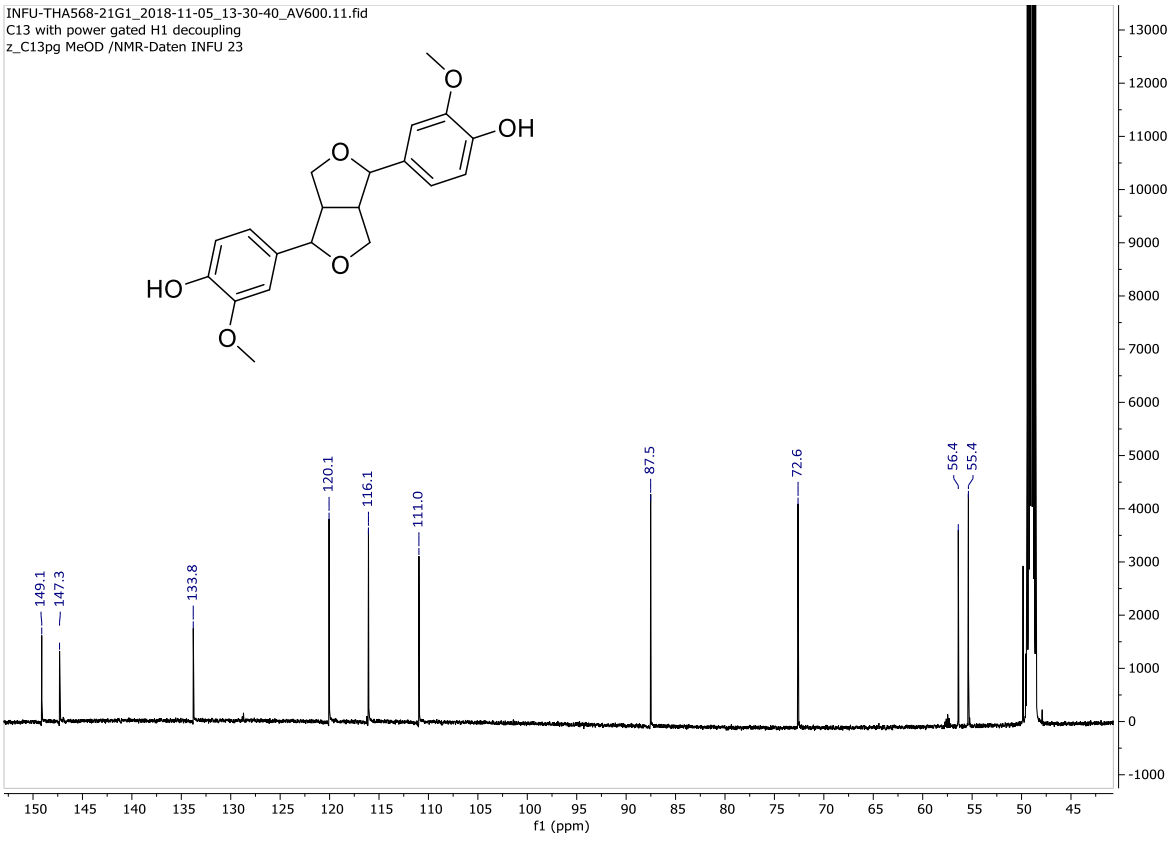
HRESIMS spectrum of compound 34



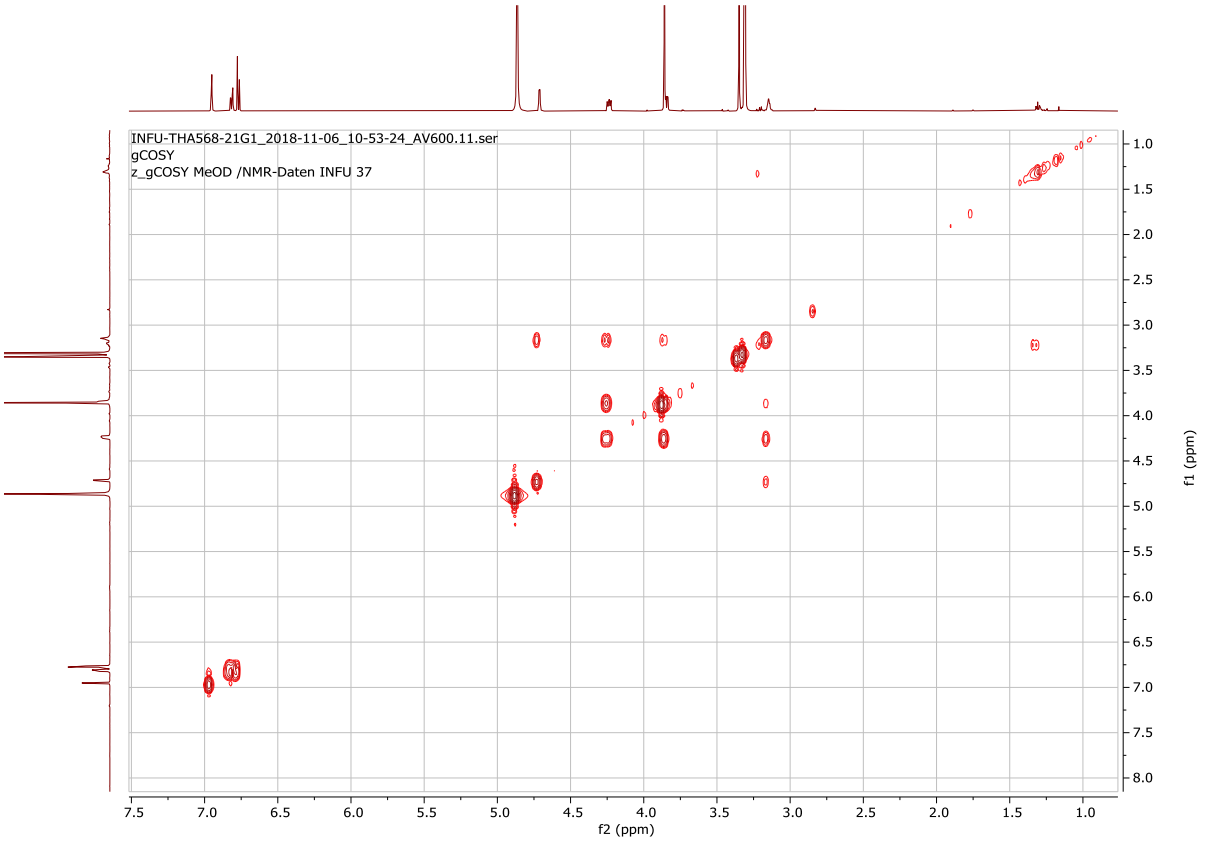
^{1}H NMR spectrum (600 MHz) of compound 34



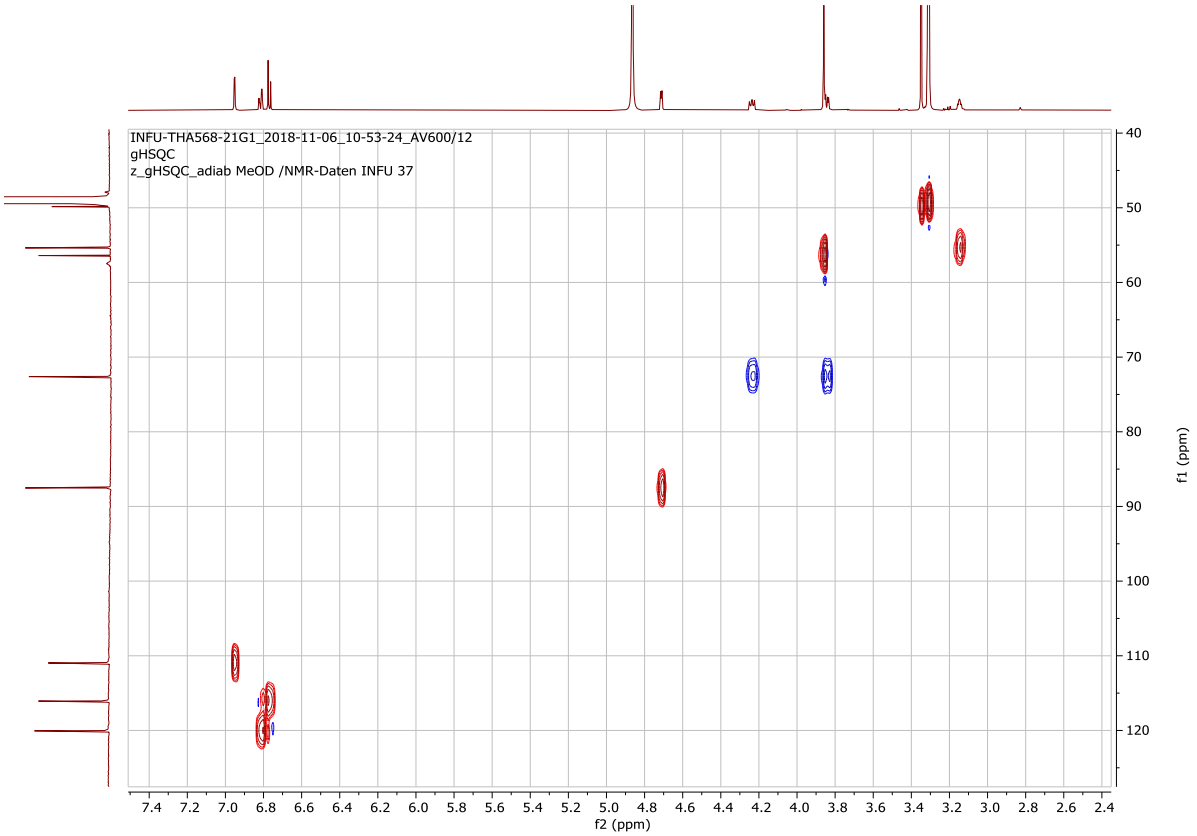
^{13}C NMR spectrum (150 MHz, CD_3OD) of compound 34



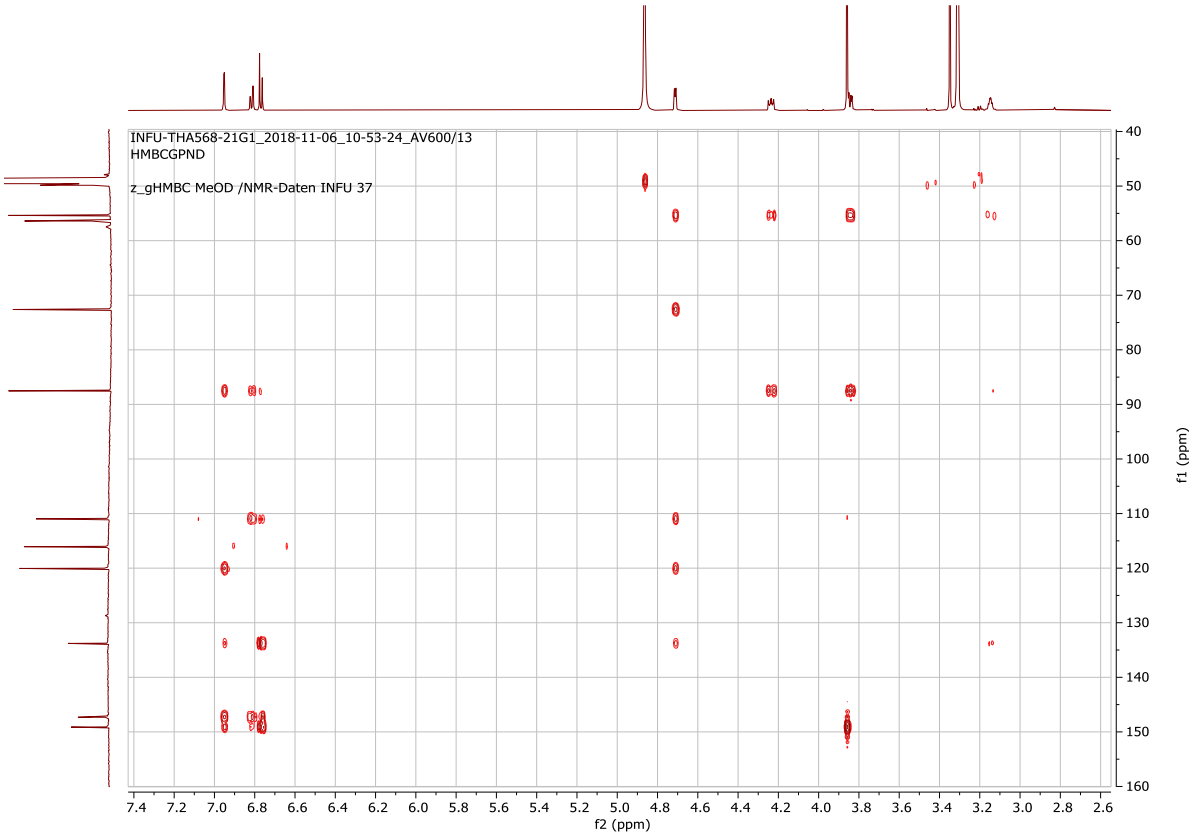
^{1}H-^{1}H-COSY spectrum of compound 34



HSQC spectrum of compound 34

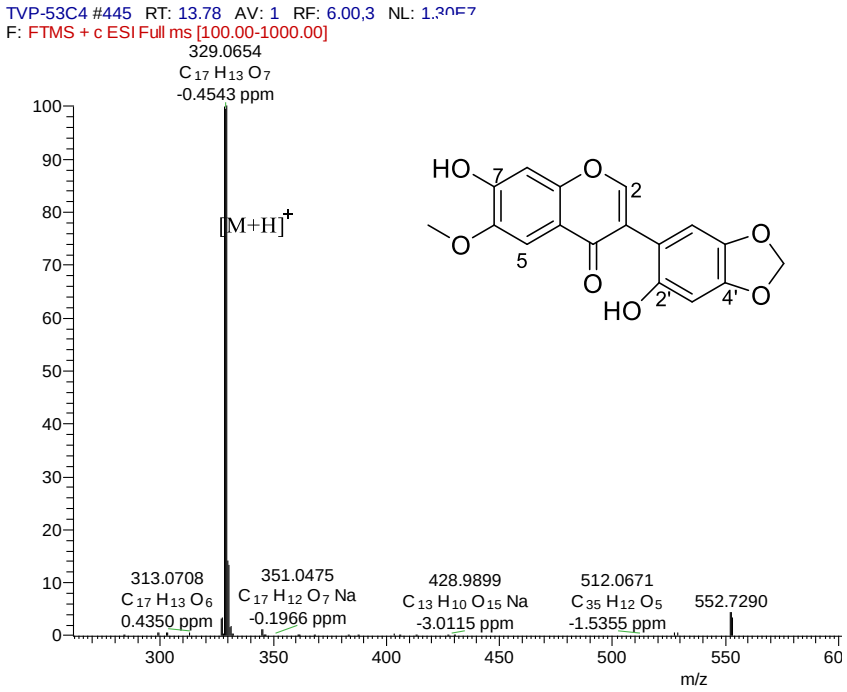


HMBC spectrum of compound 34

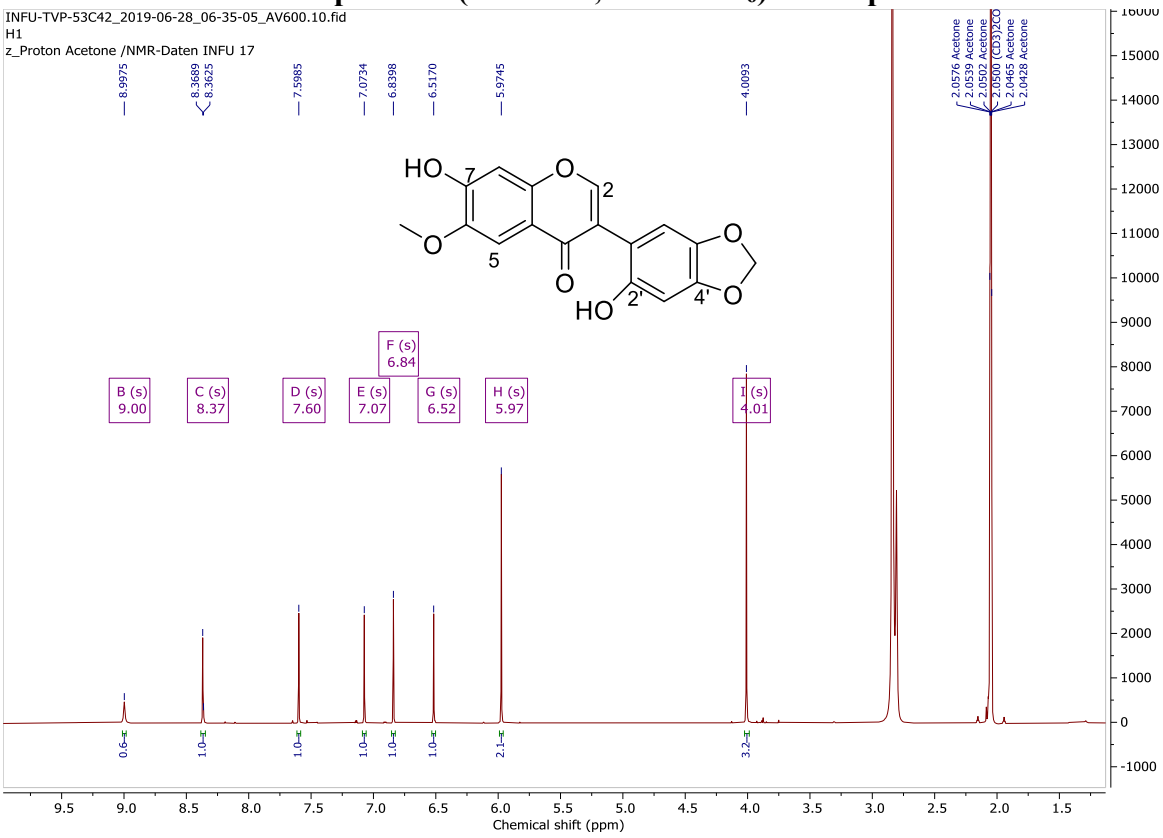


Appendix A35: Spectra for compound 35

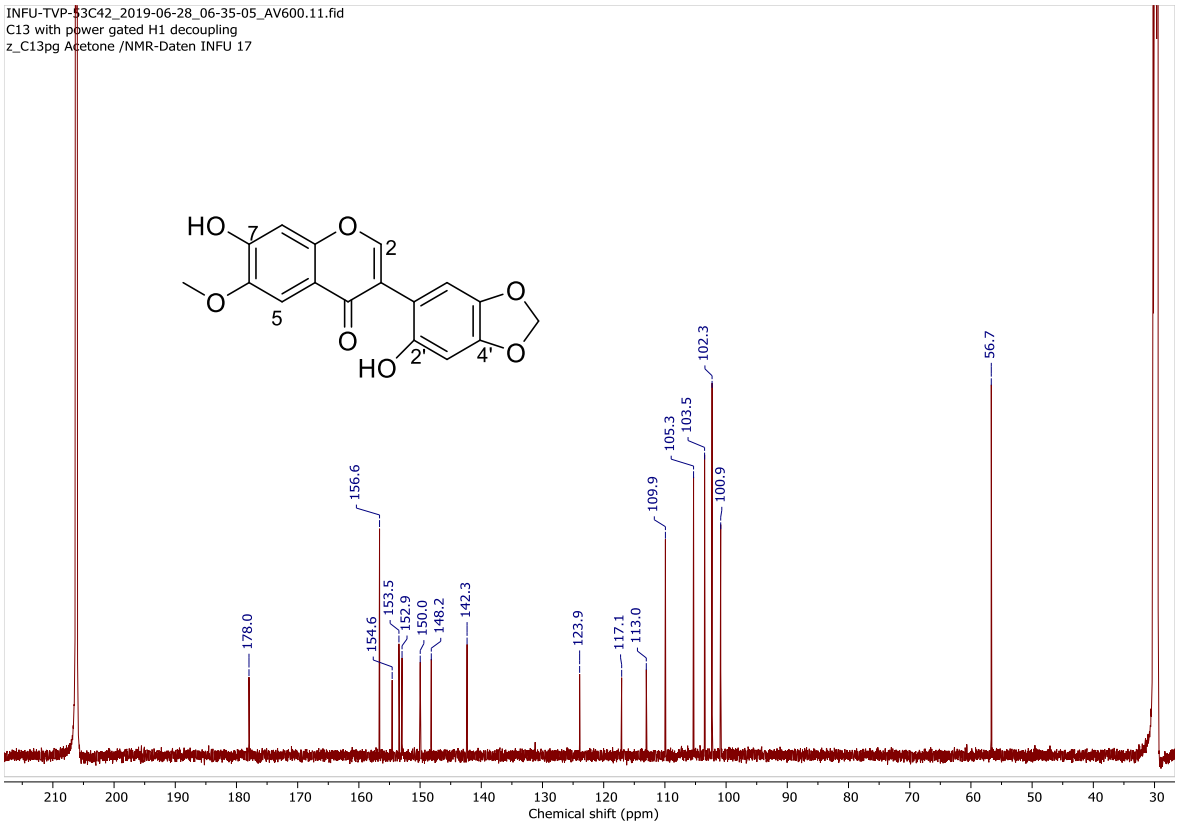
HRESIMS spectrum of compound 35



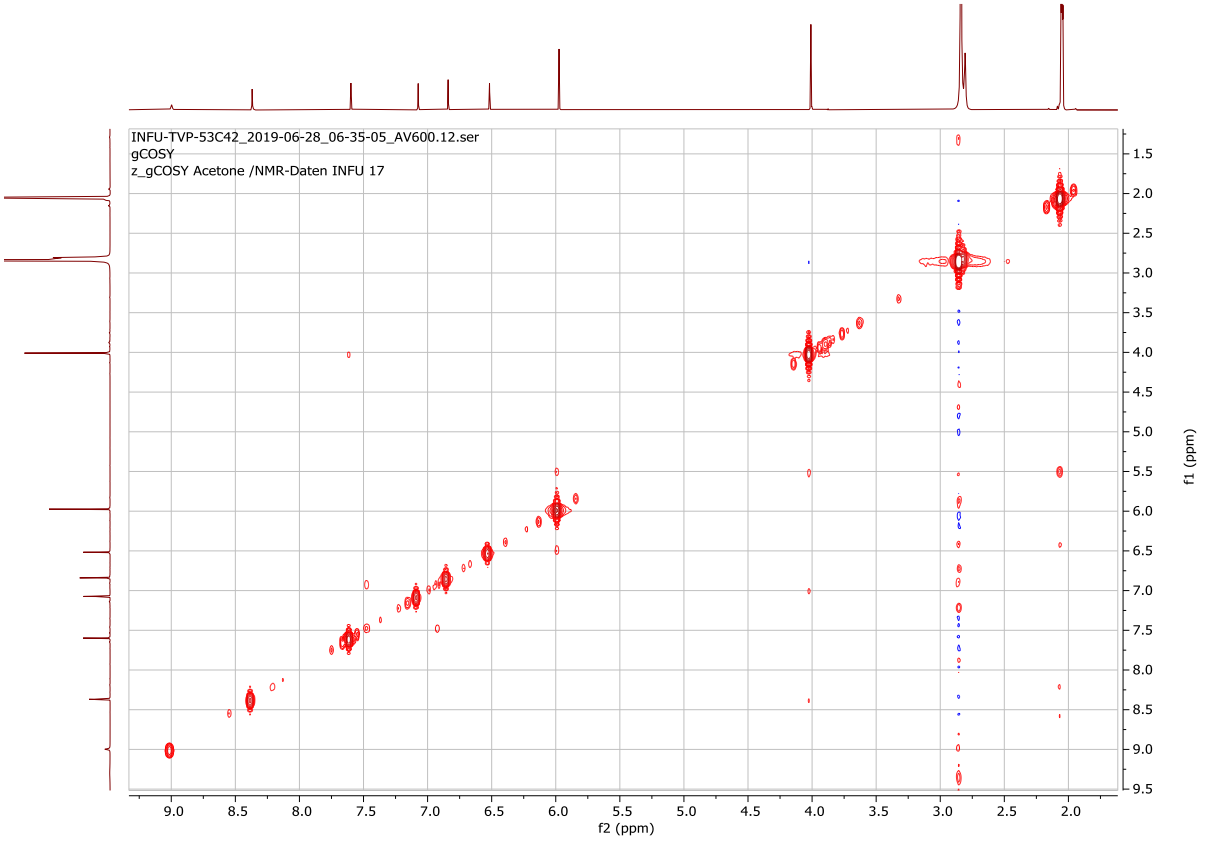
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 35



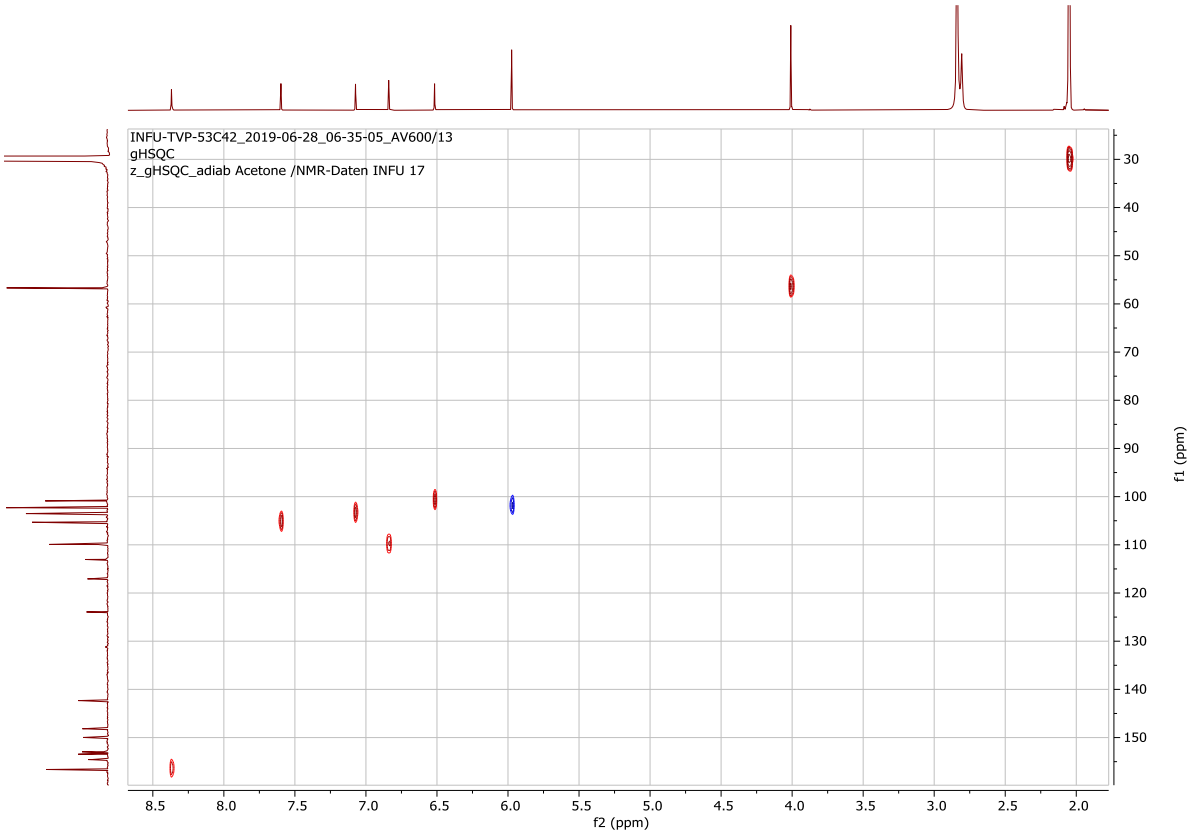
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 35



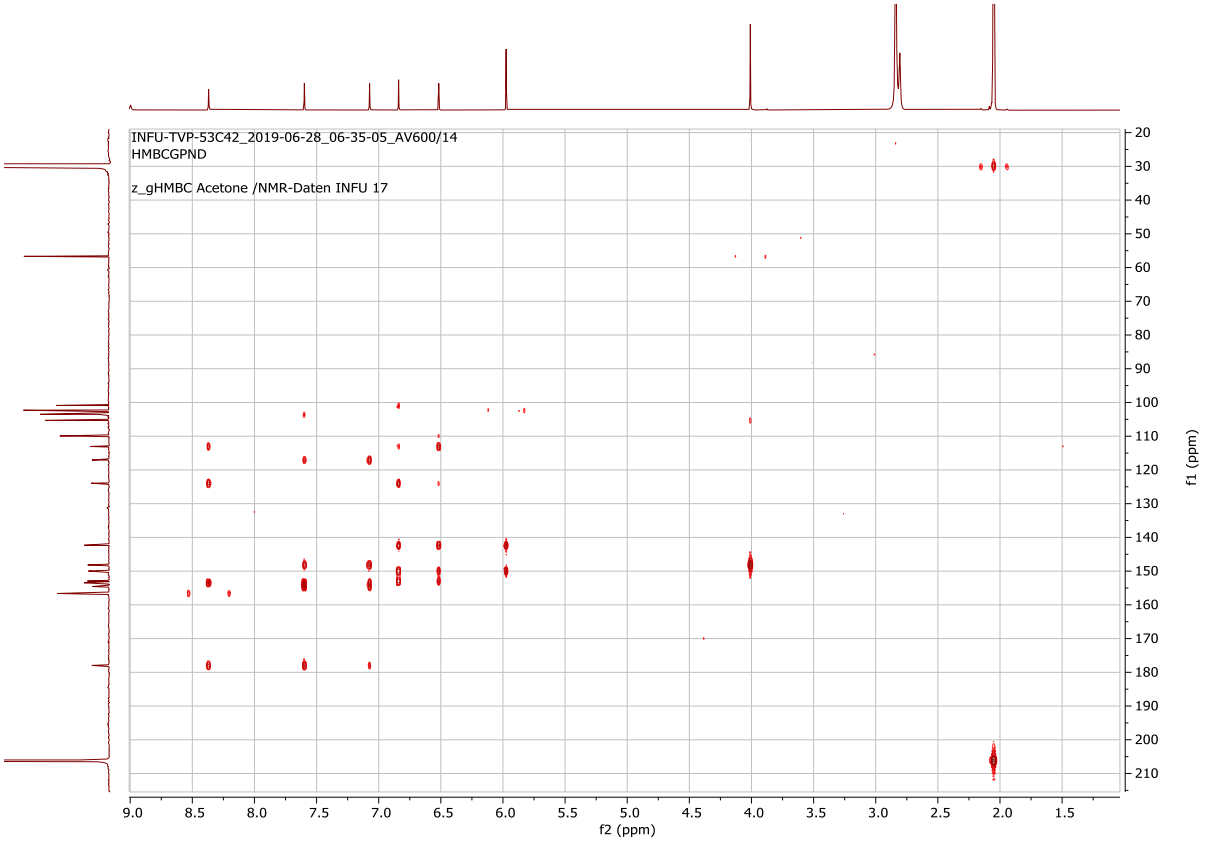
^{1}H-^{1}H-COSY spectrum of compound 35



HSQC spectrum of compound 35

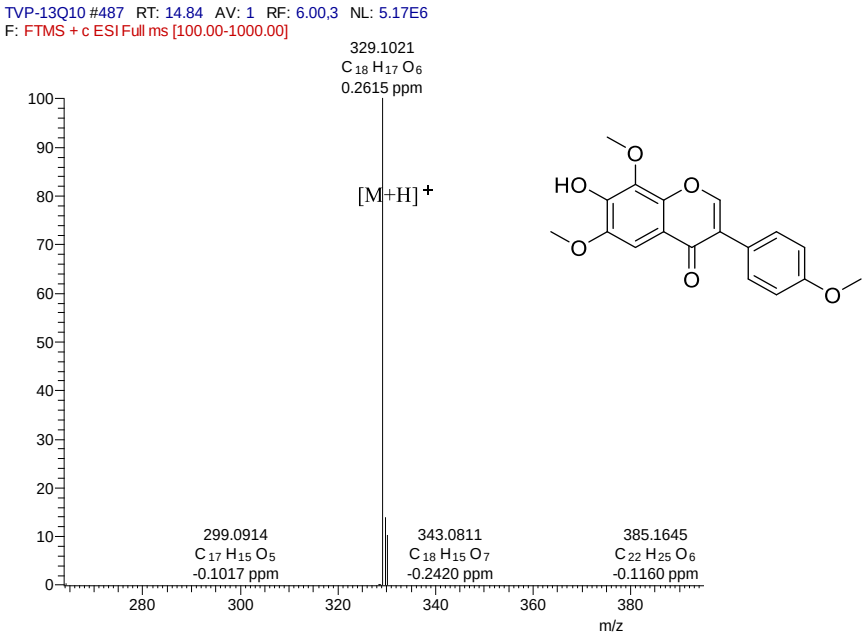


HMBC spectrum of compound 35

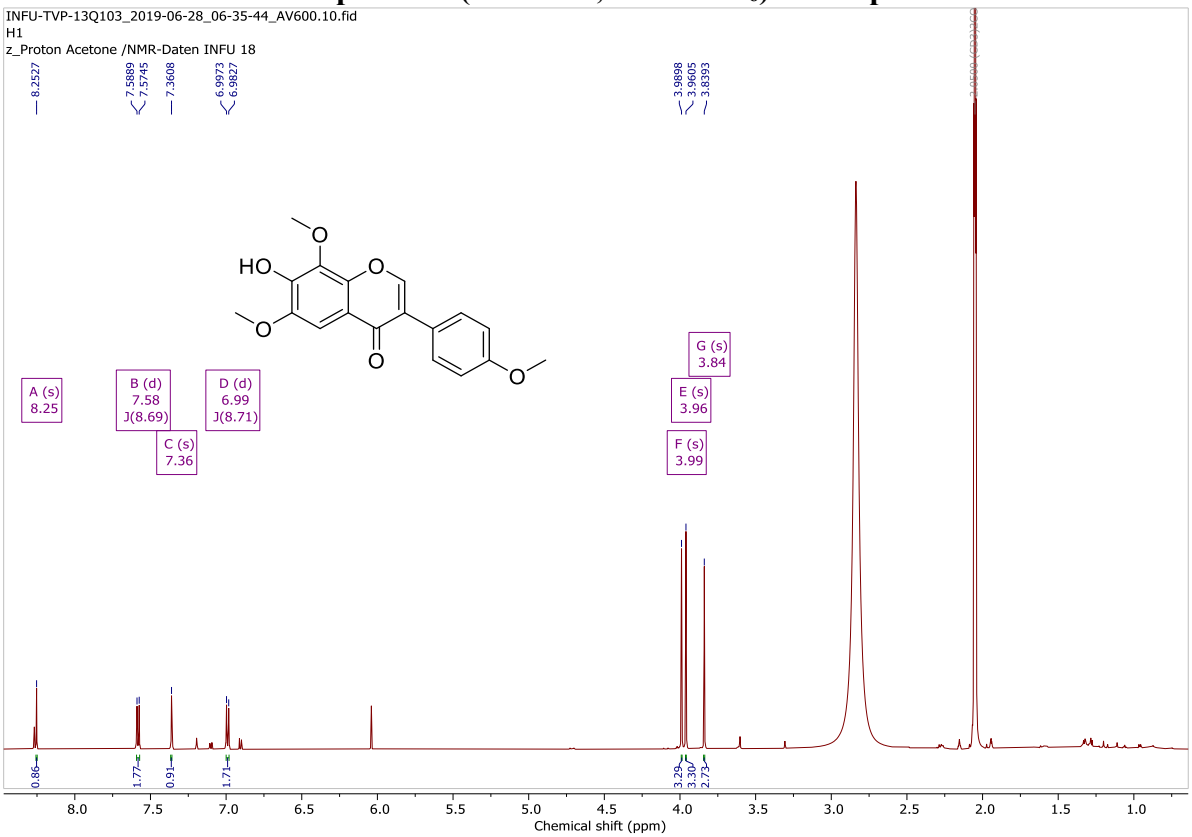


Appendix A36: Spectra for compound 36

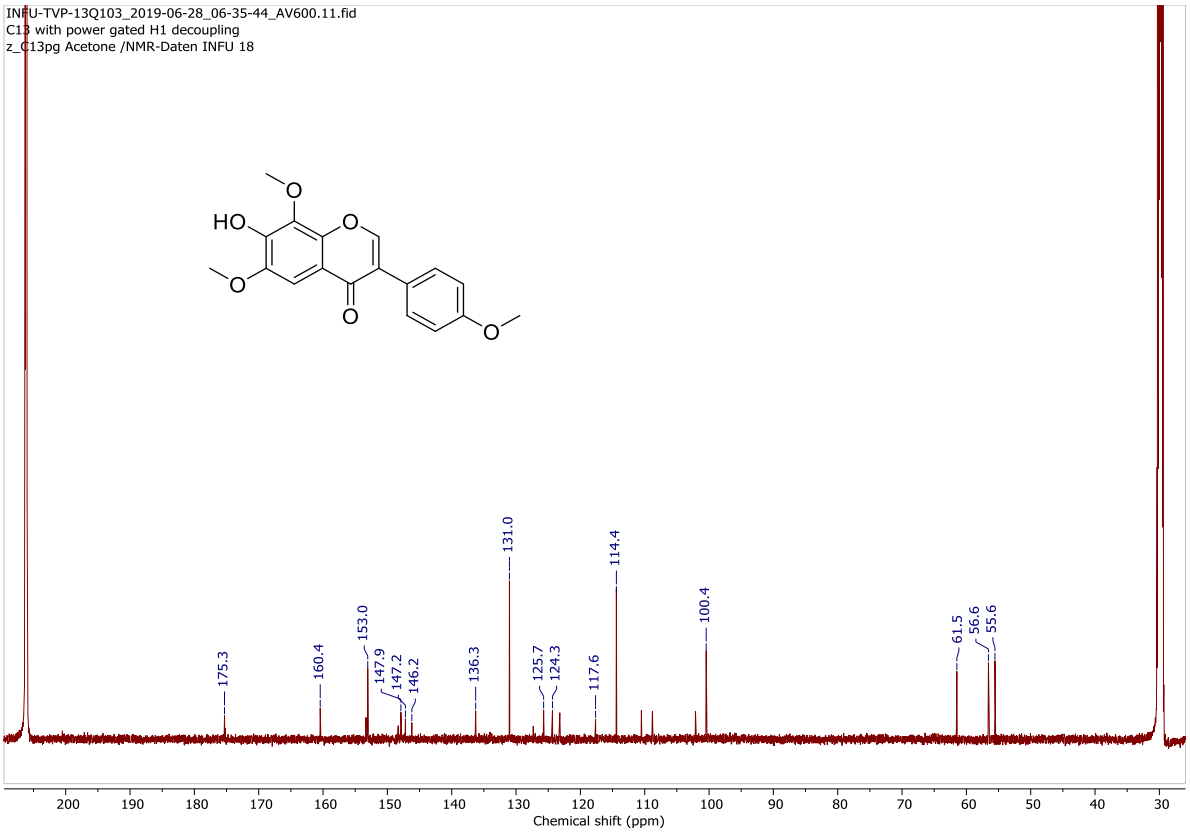
HRESIMS spectrum of compound 36



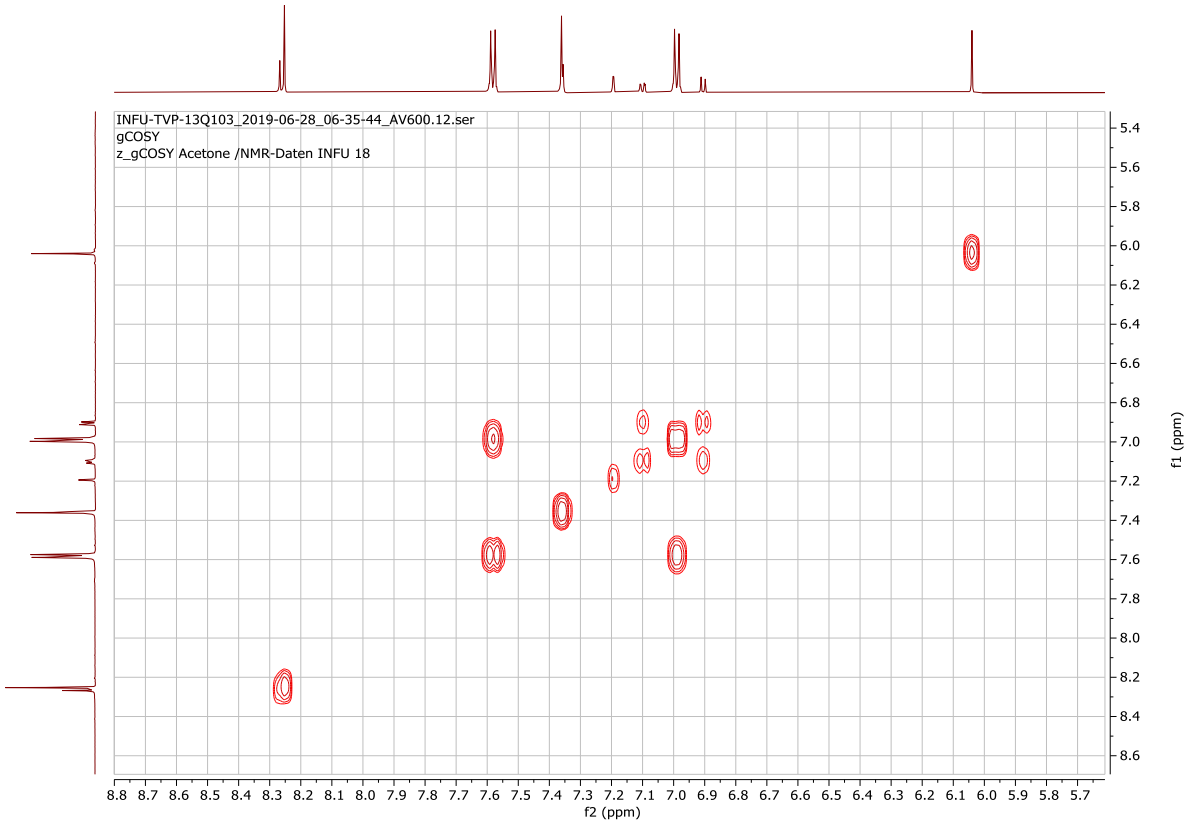
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 36



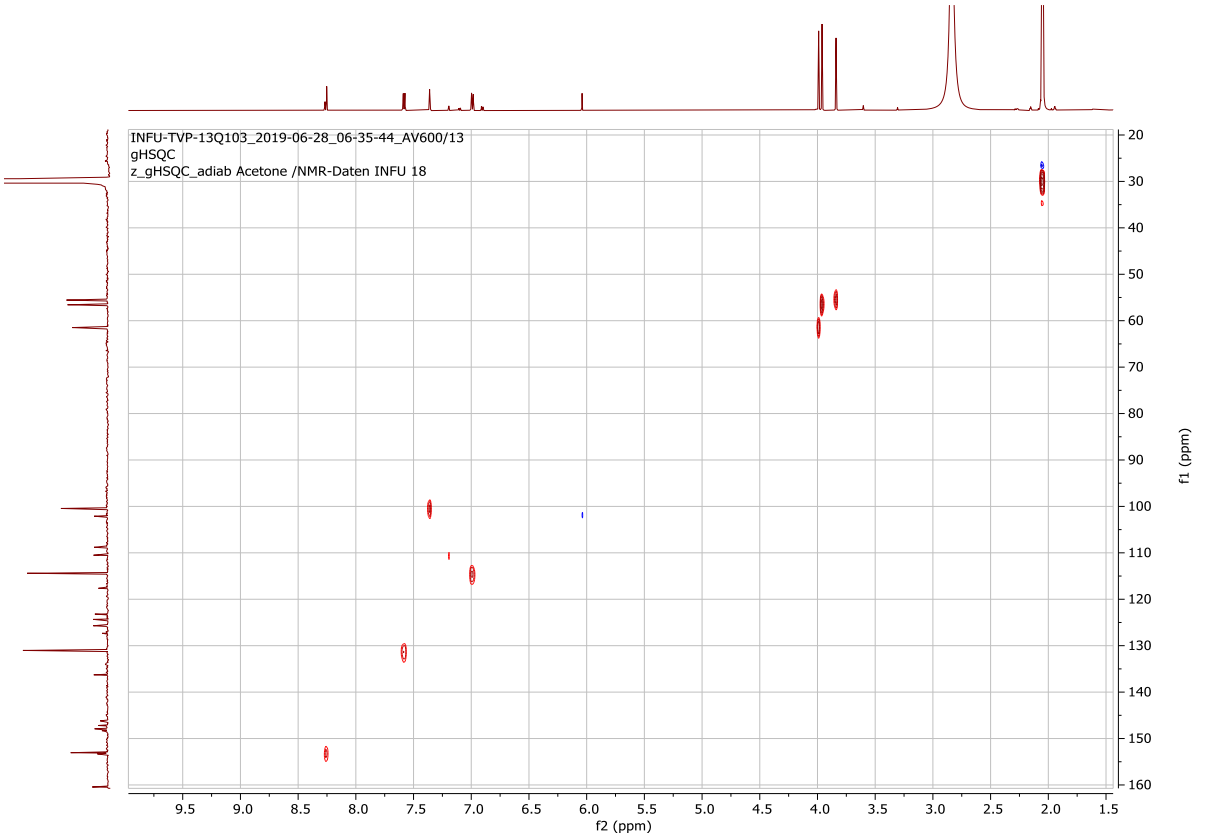
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 36



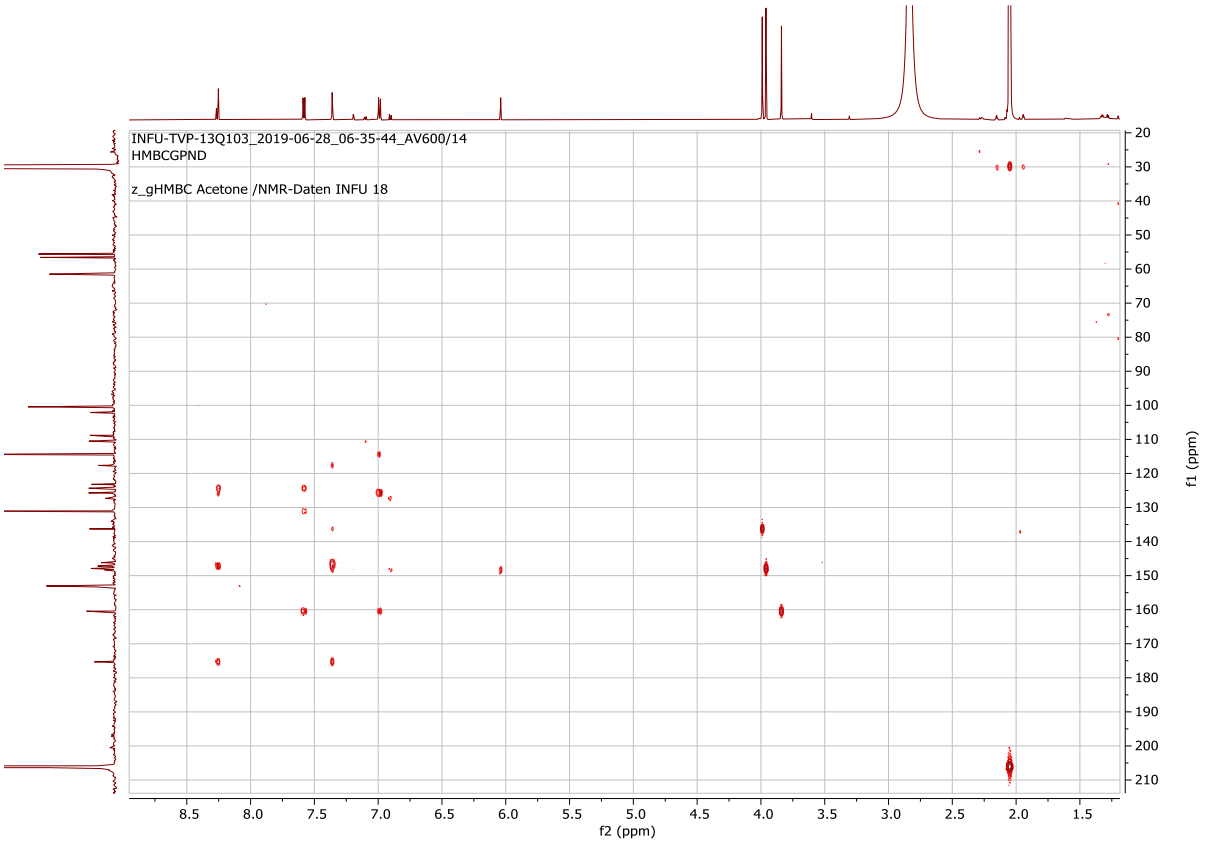
^{1}H-^{1}H-COSY spectrum of compound 36



HSQC spectrum of compound 36

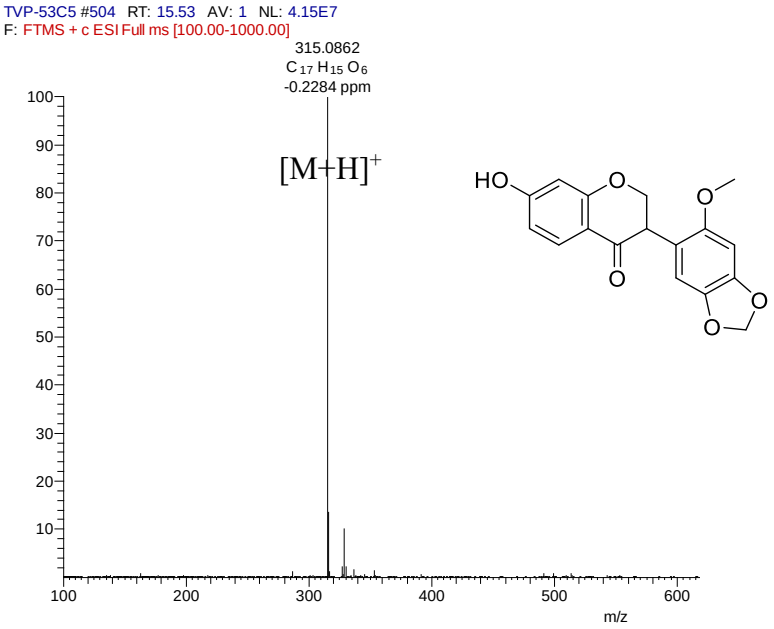


HMBC spectrum of compound 36

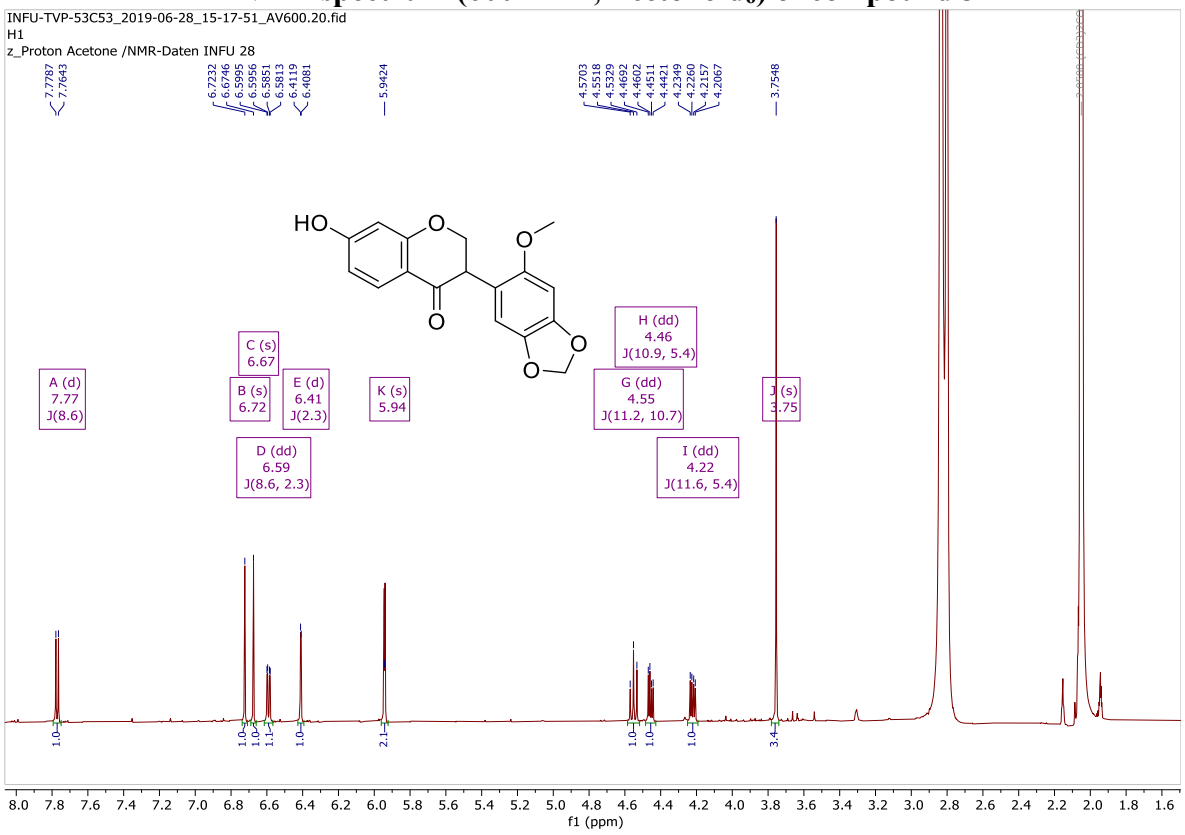


Appendix A37: Spectra for compound 37

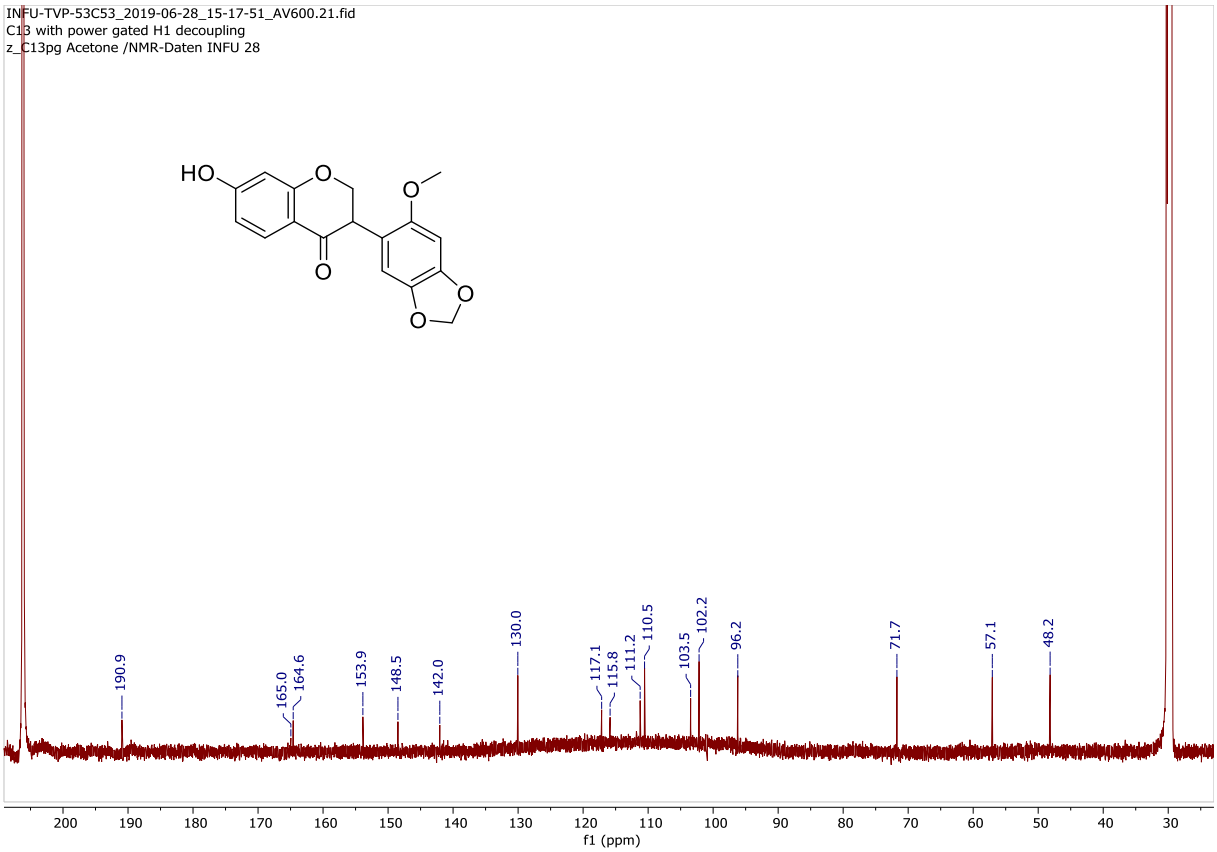
HRESIMS spectrum of compound 37



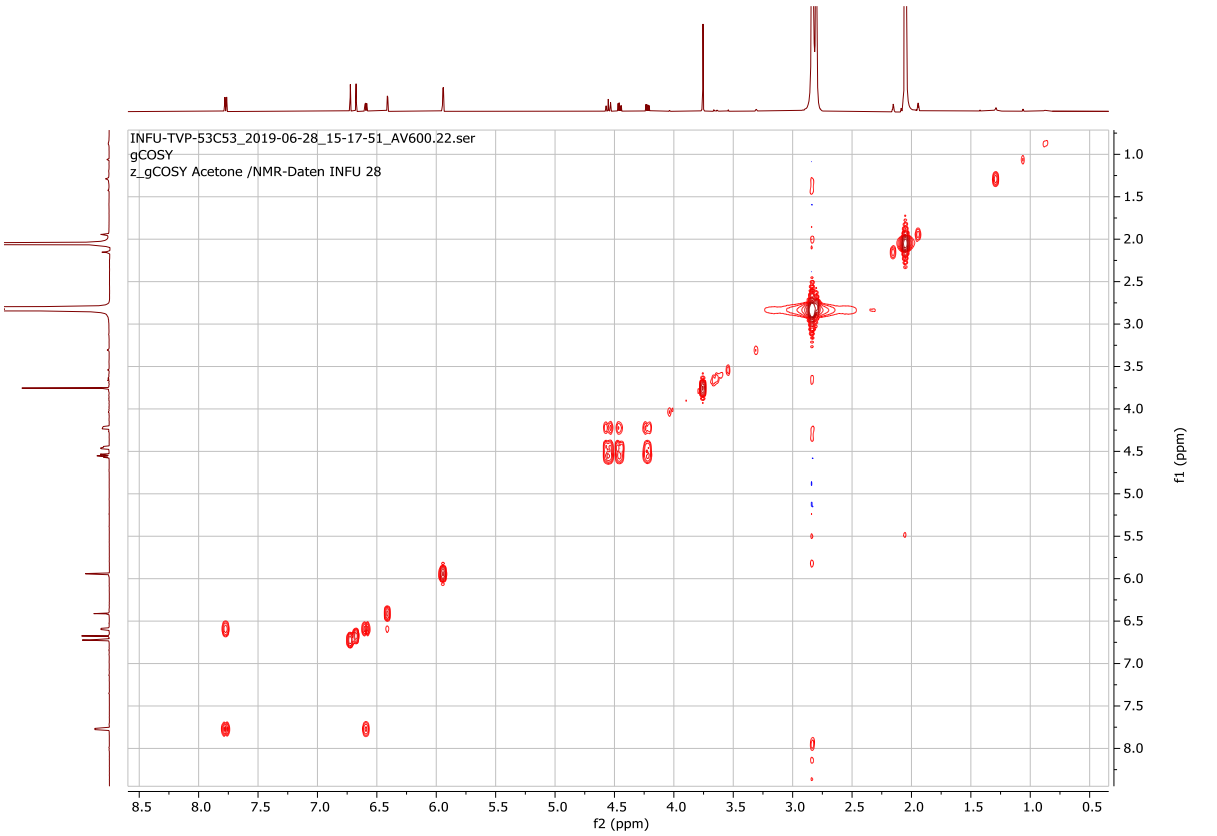
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 37



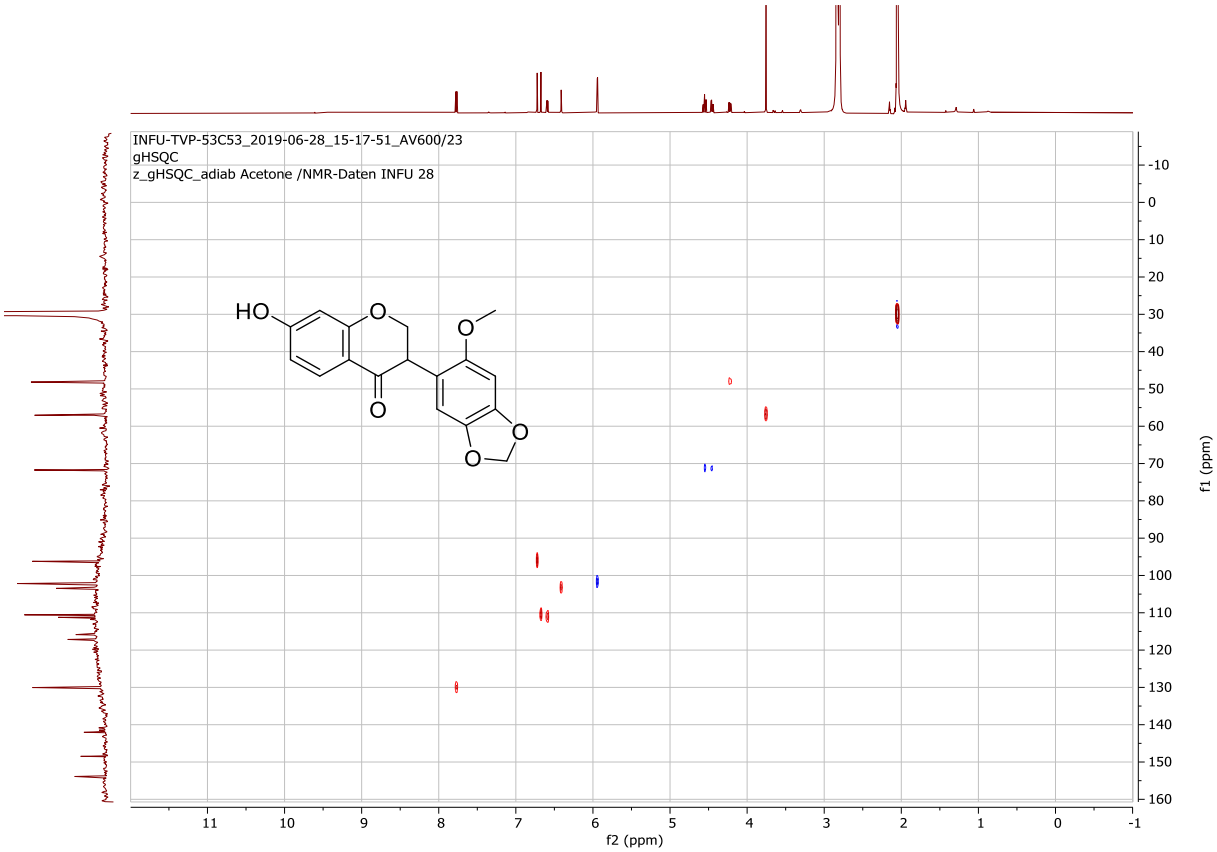
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 37



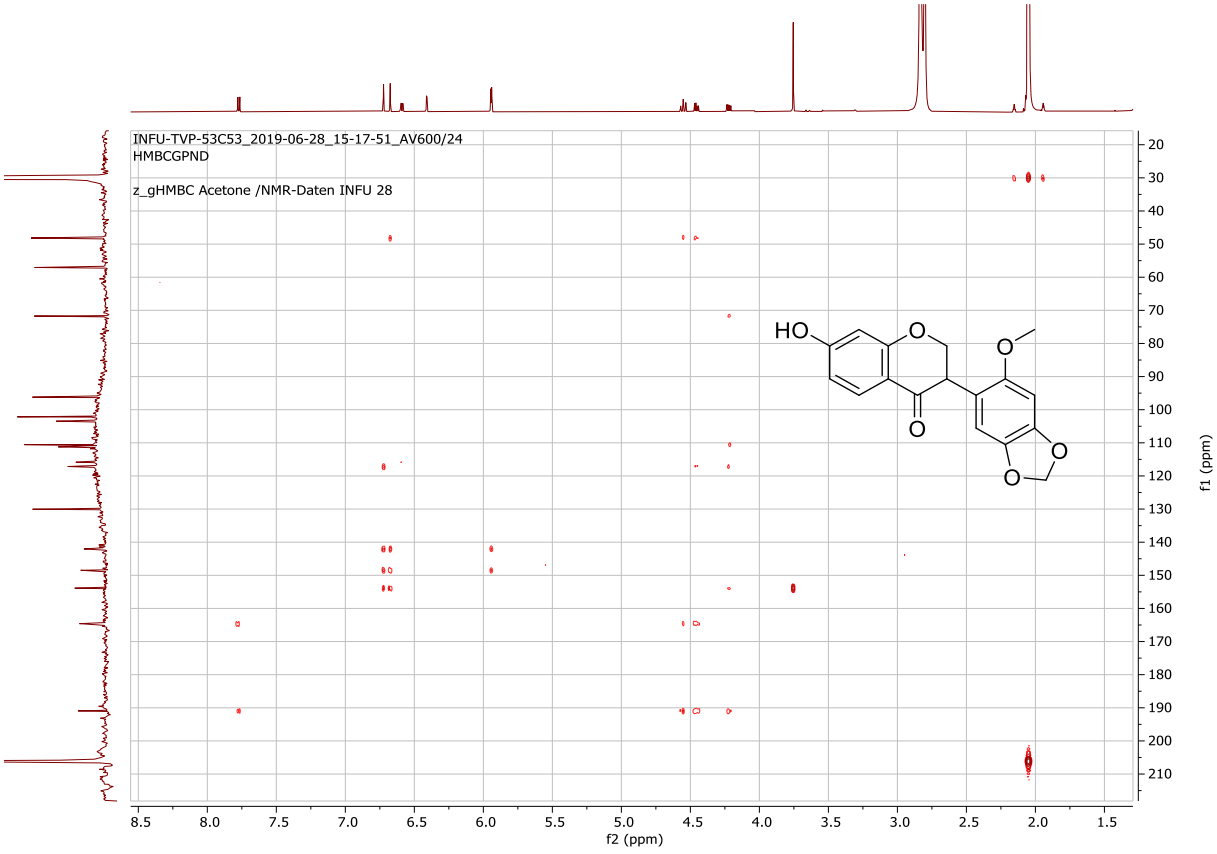
^{1}H-^{1}H-COSY spectrum of compound 37



HSQC spectrum of compound 37

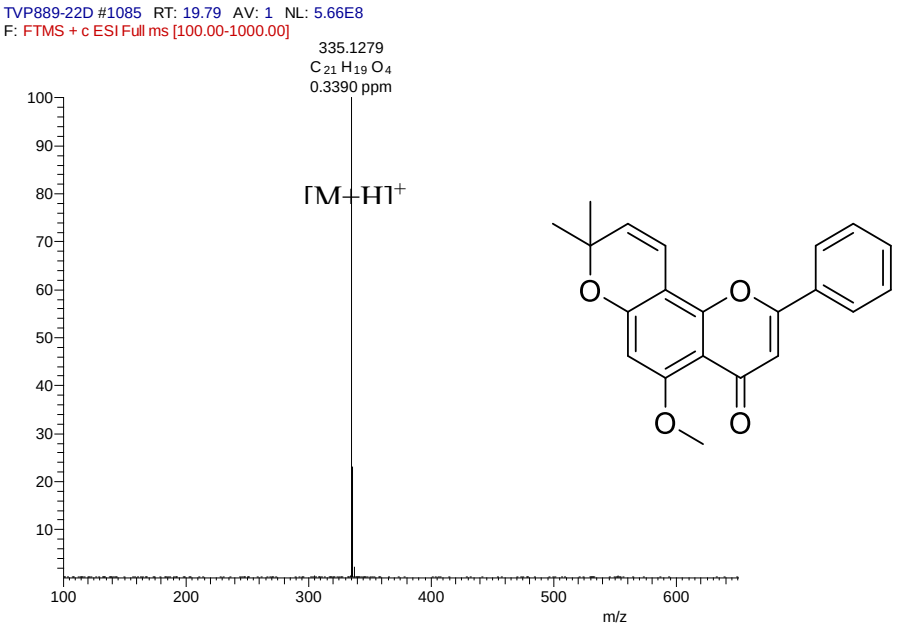


HMBC spectrum of compound 37

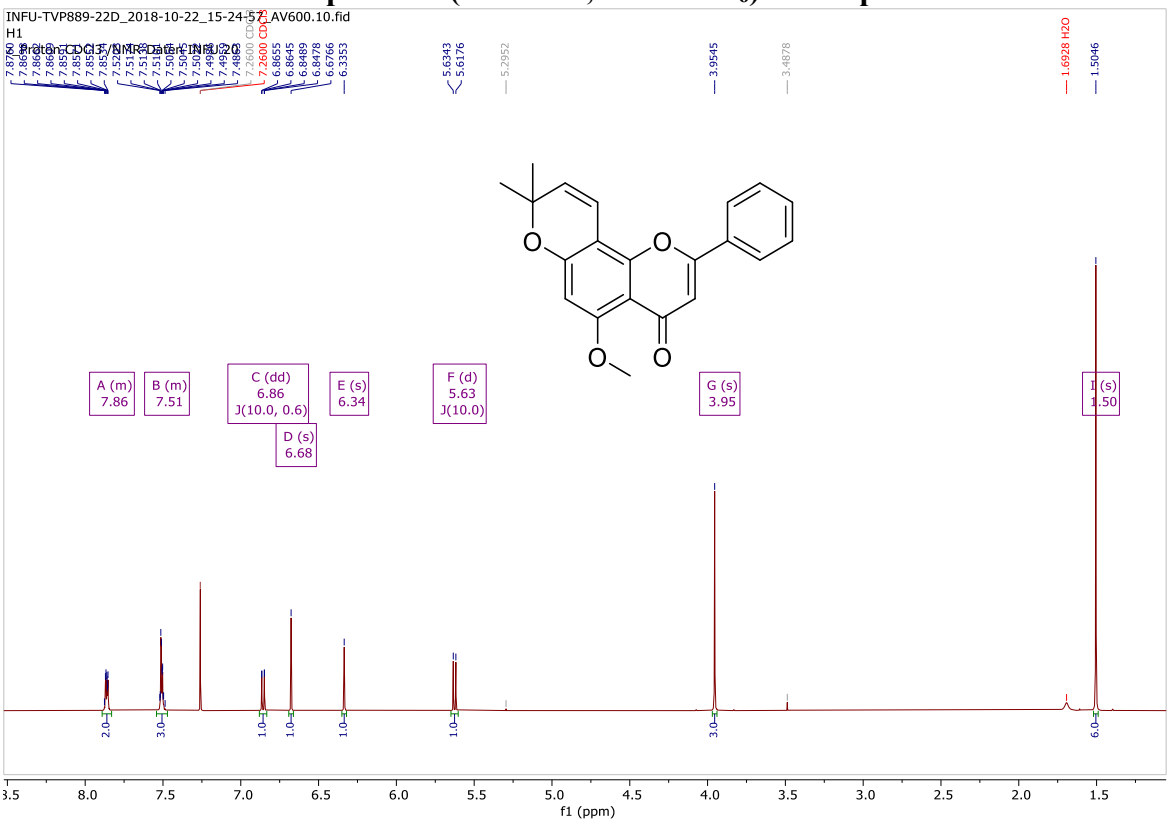


Appendix A38: Spectra for compound 38

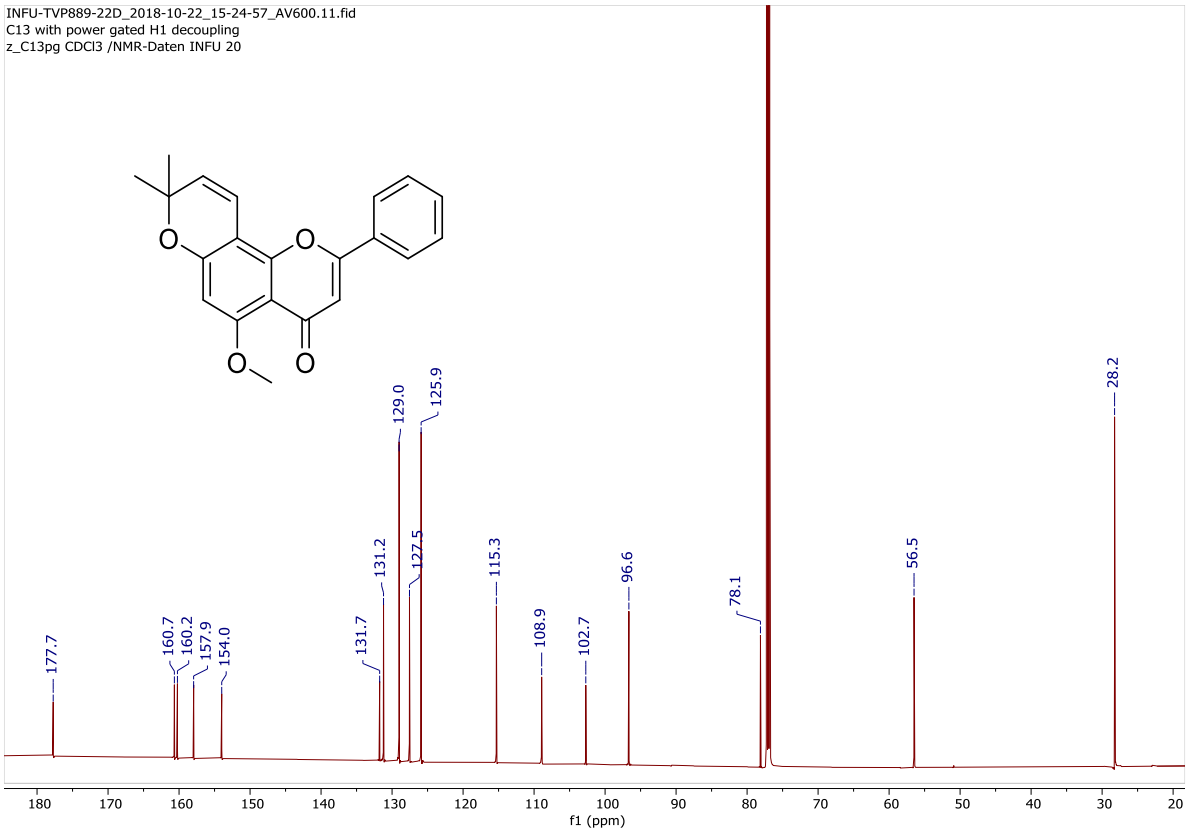
HRESIMS spectrum of compound 38



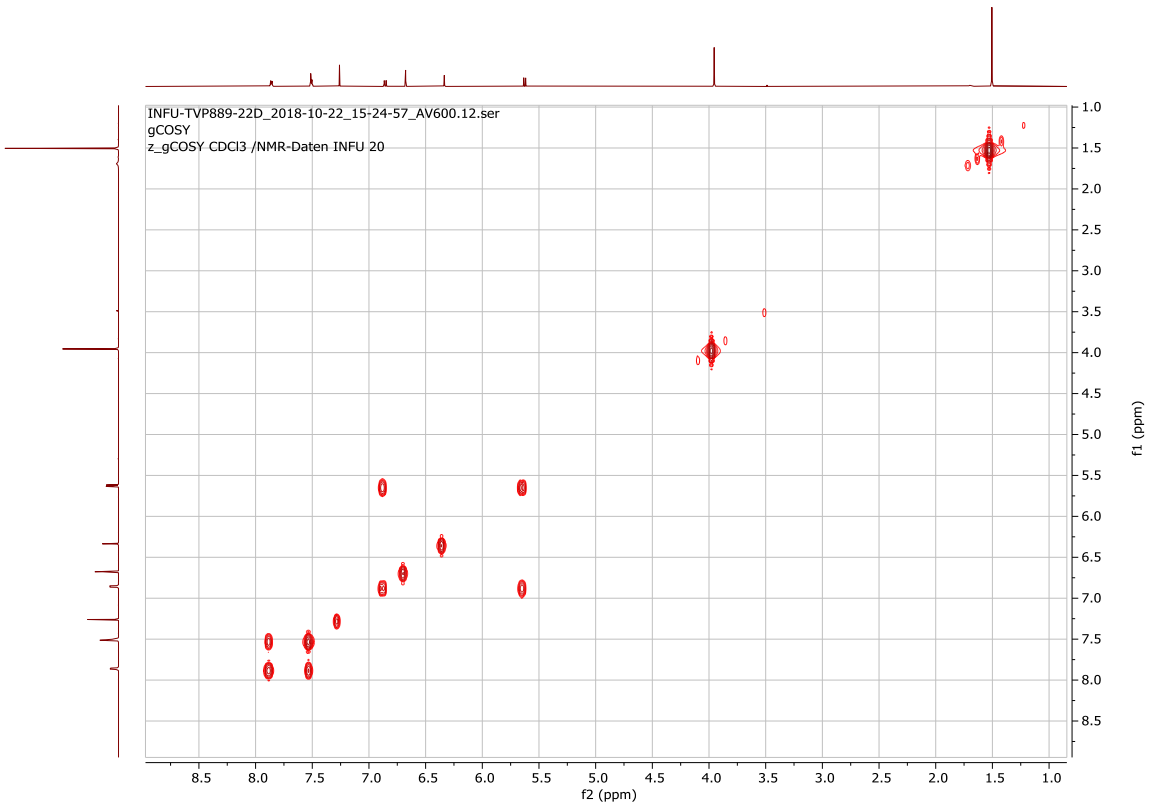
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 38



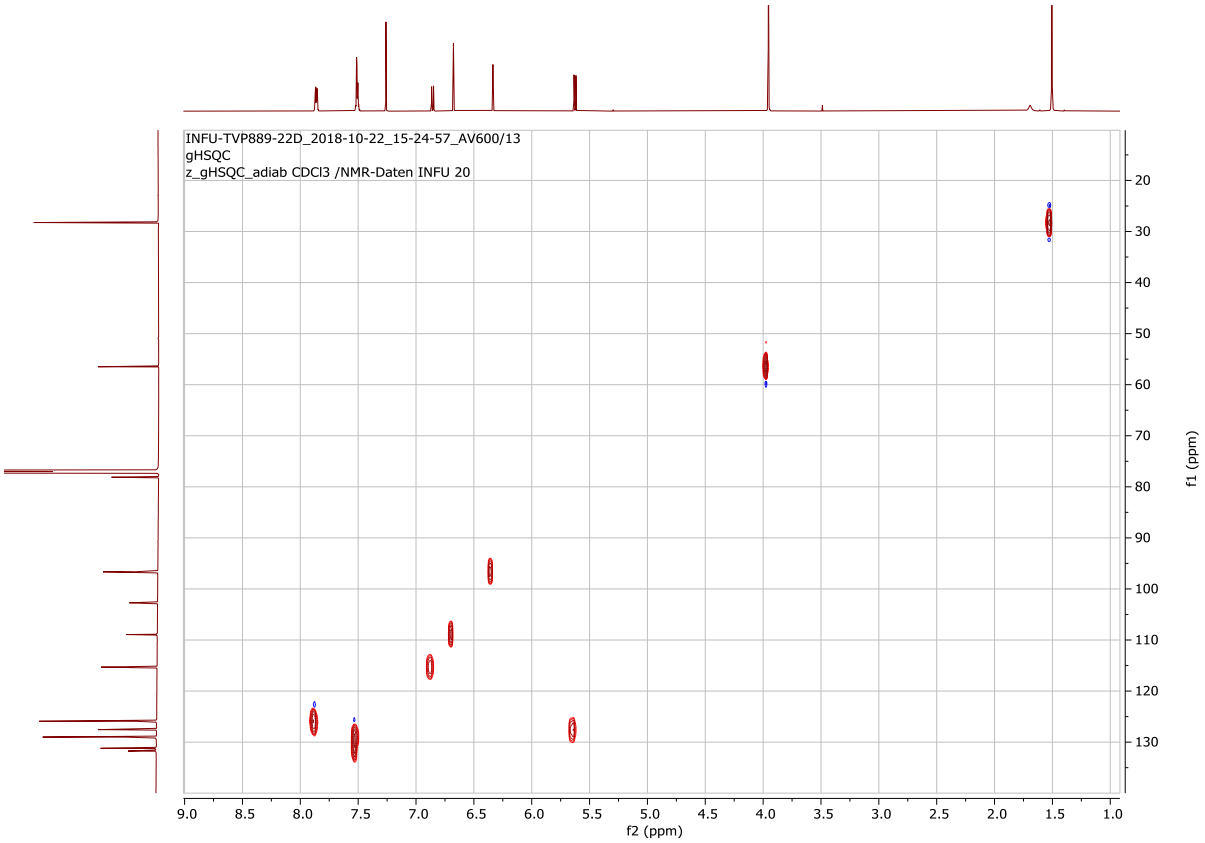
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 38



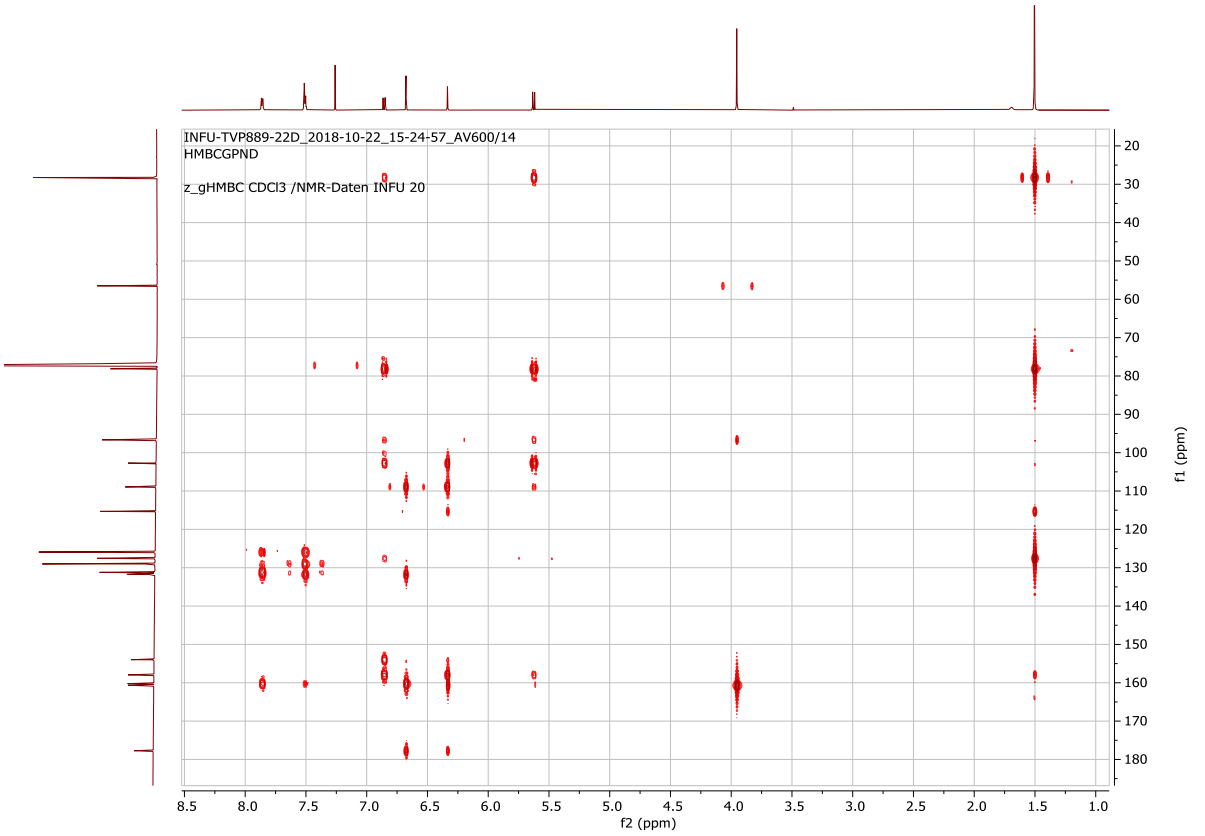
^{1}H-^{1}H-COSY spectrum of compound 38



HSQC spectrum of compound 38

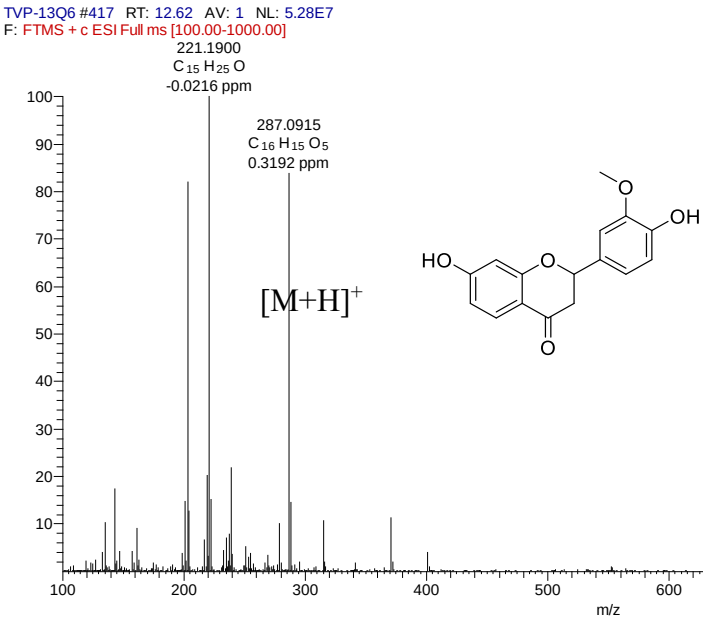


HMBC spectrum of compound 38

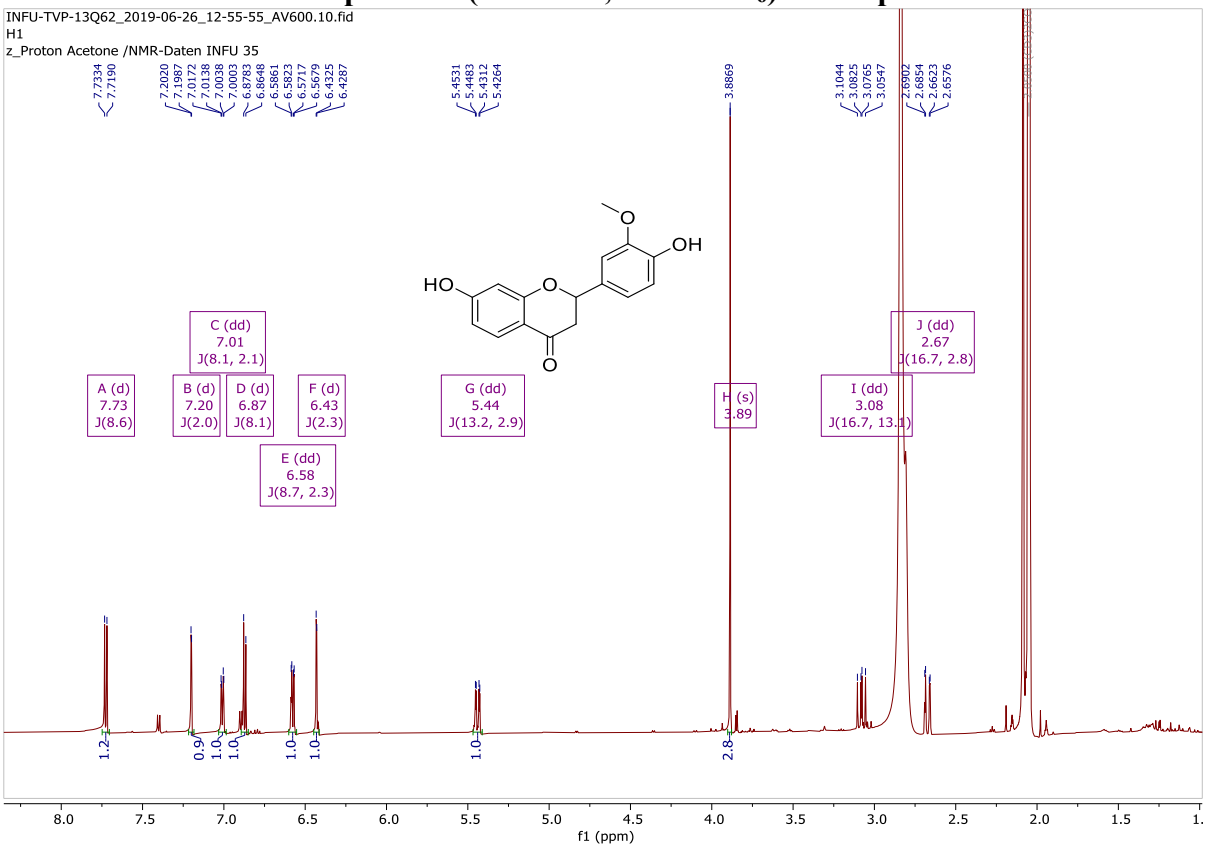


Appendix A39: Spectra for compound 39

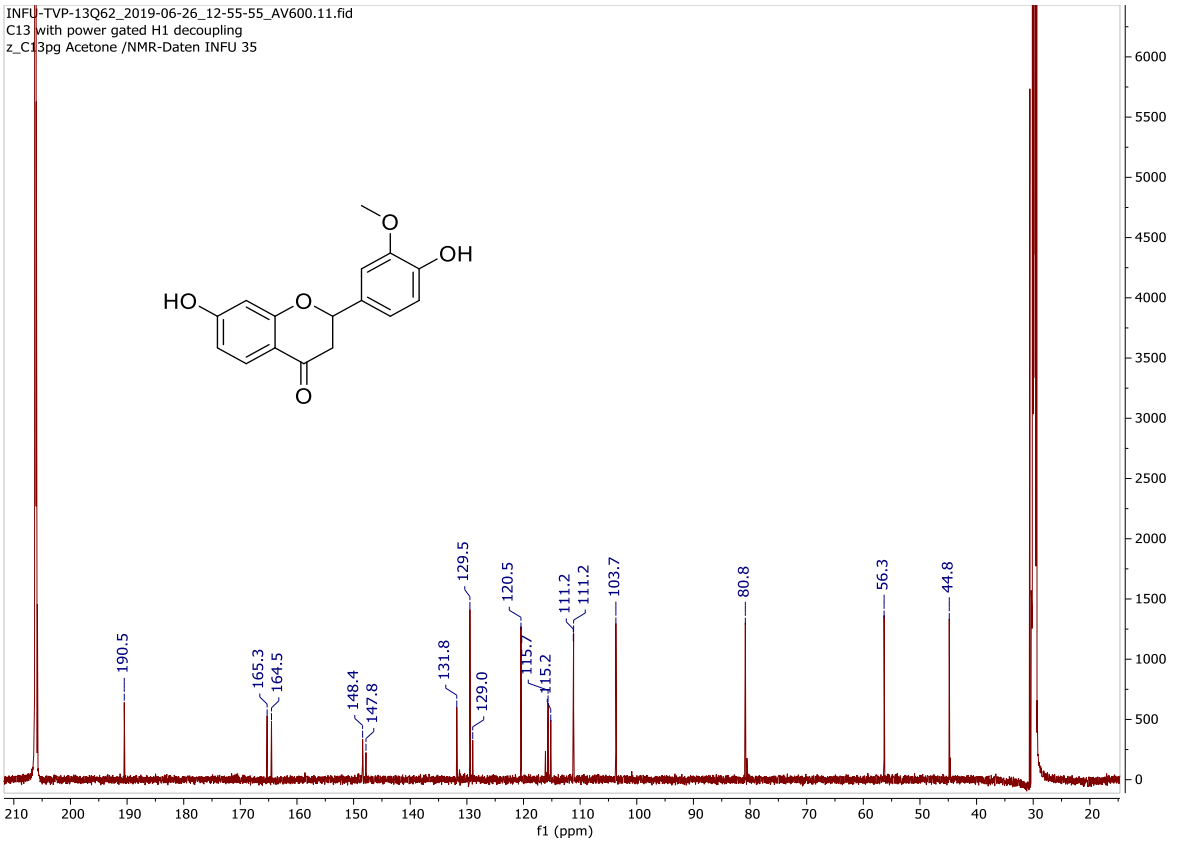
HRESIMS spectrum of compound 39



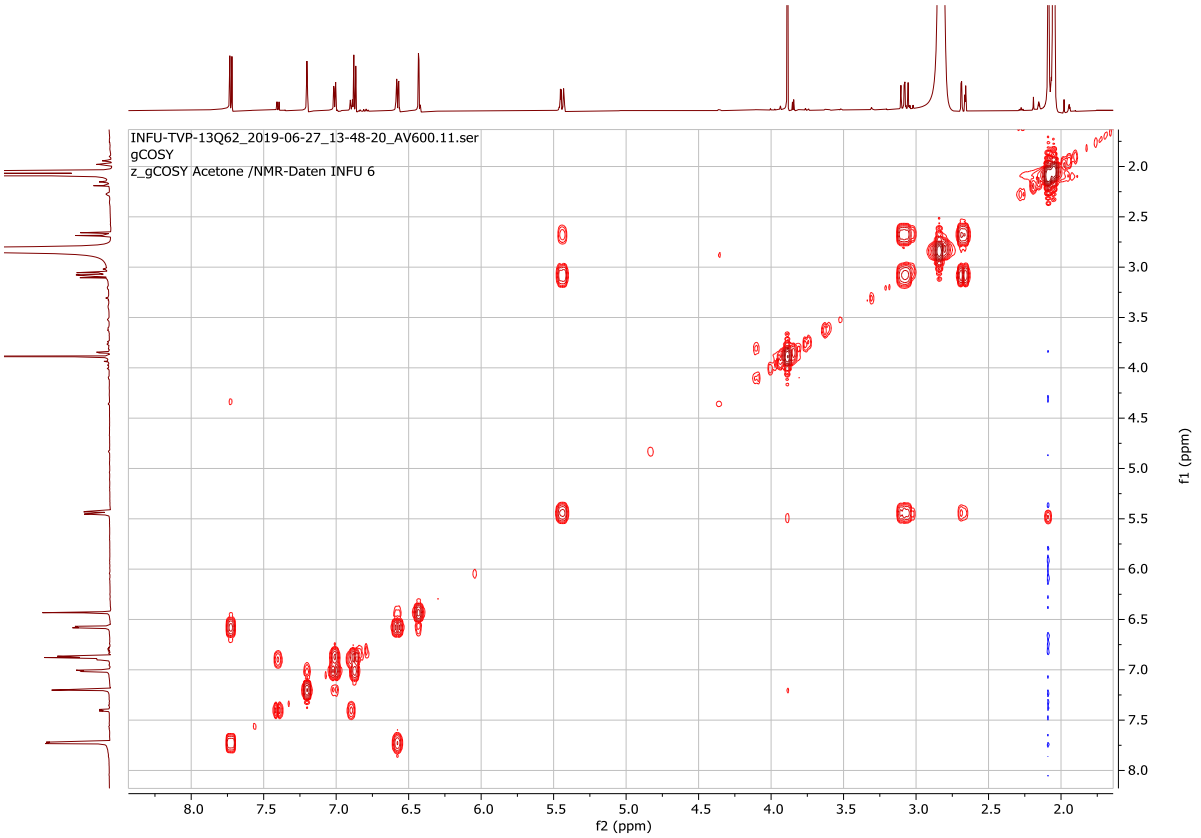
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 39



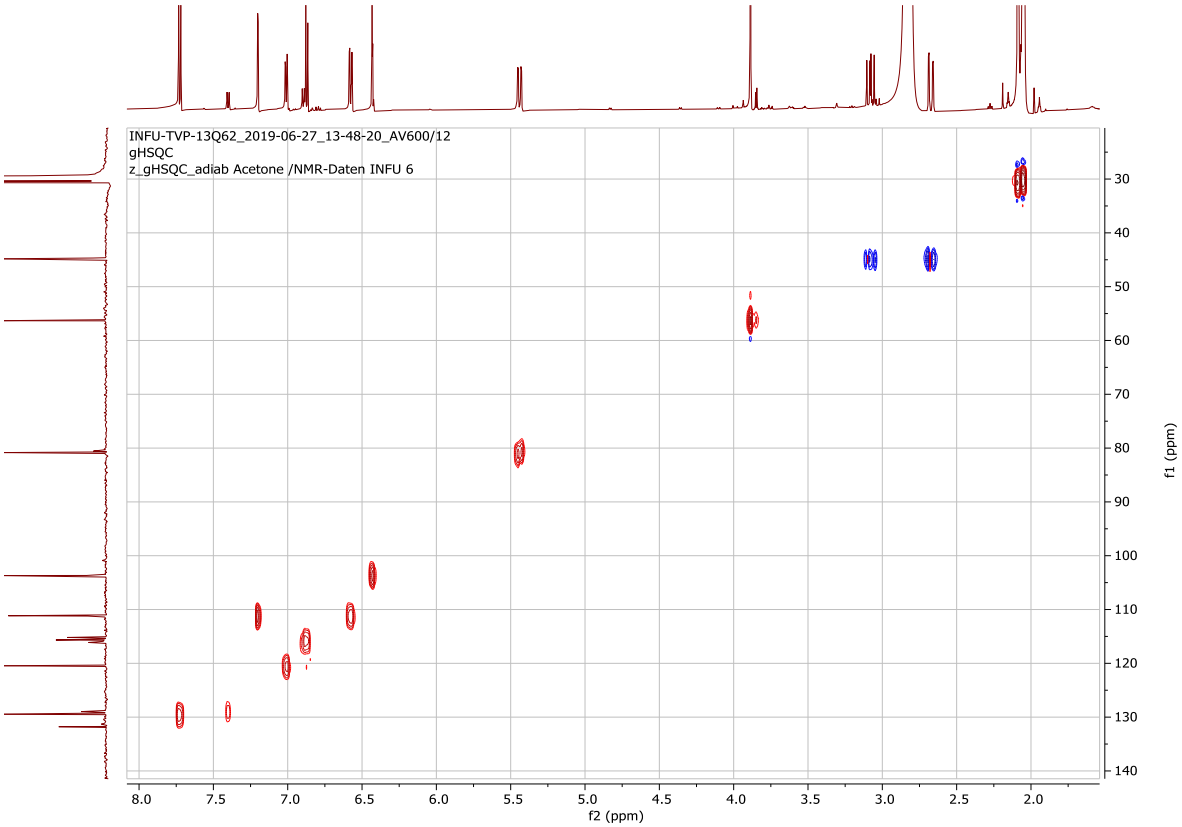
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 39



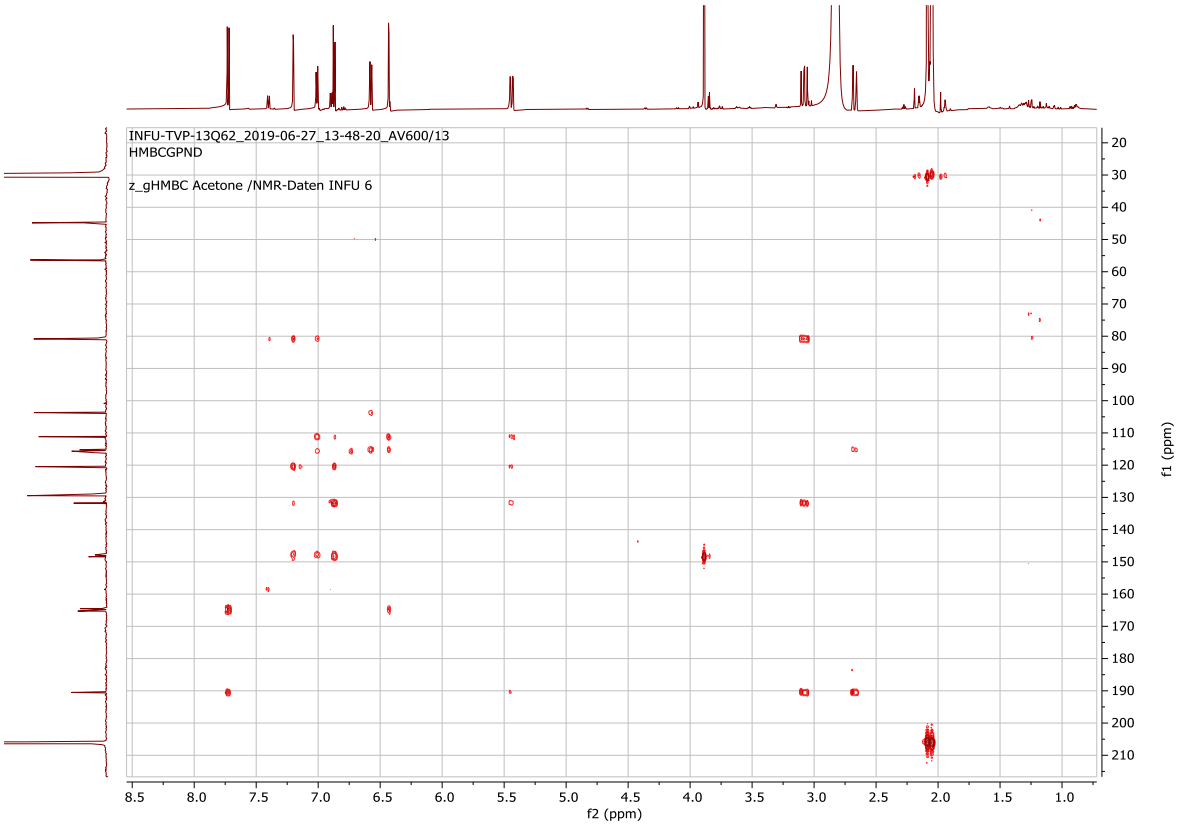
^{1}H-^{1}H-COSY spectrum of compound 39



HSQC spectrum of compound 39

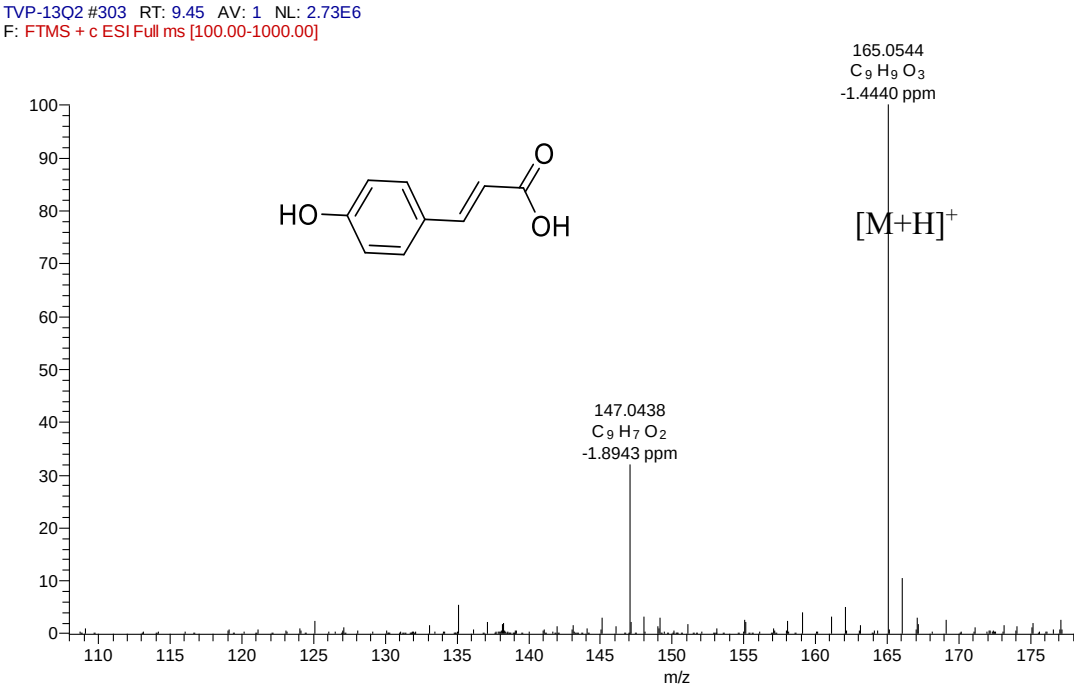


HMBC spectrum of compound 39

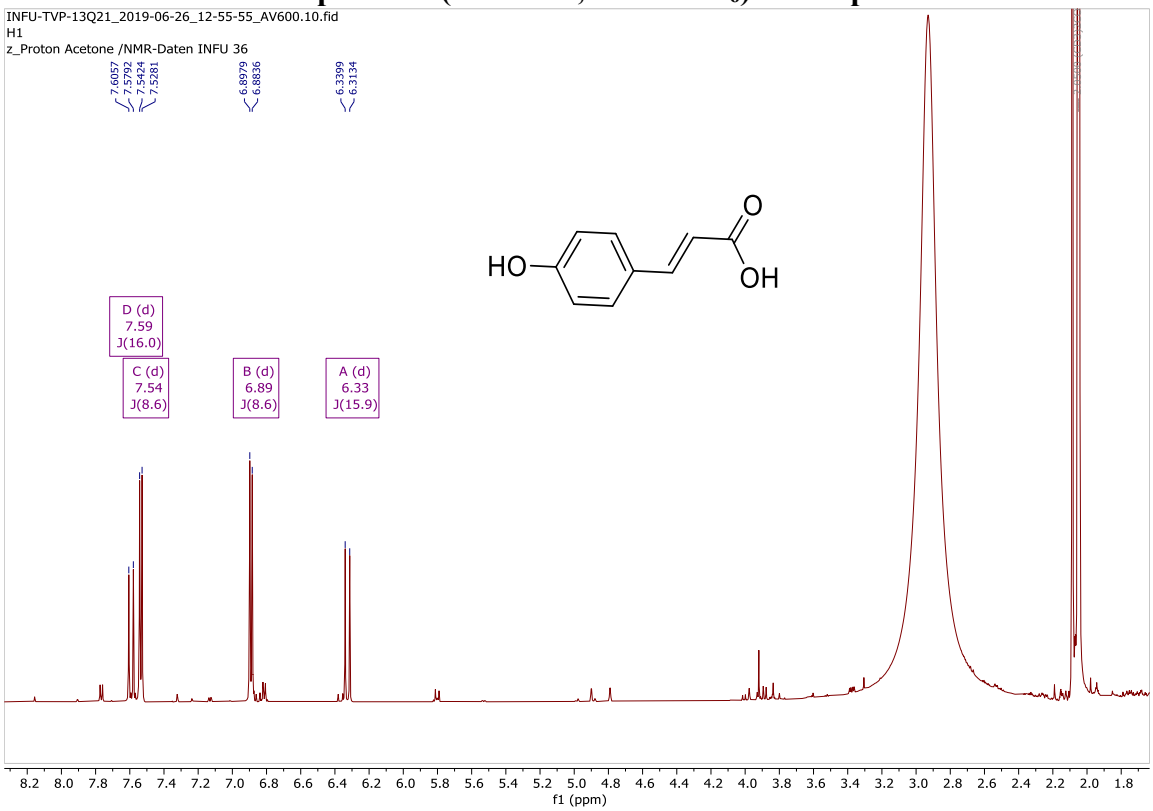


Appendix A40: Spectra for compound 40

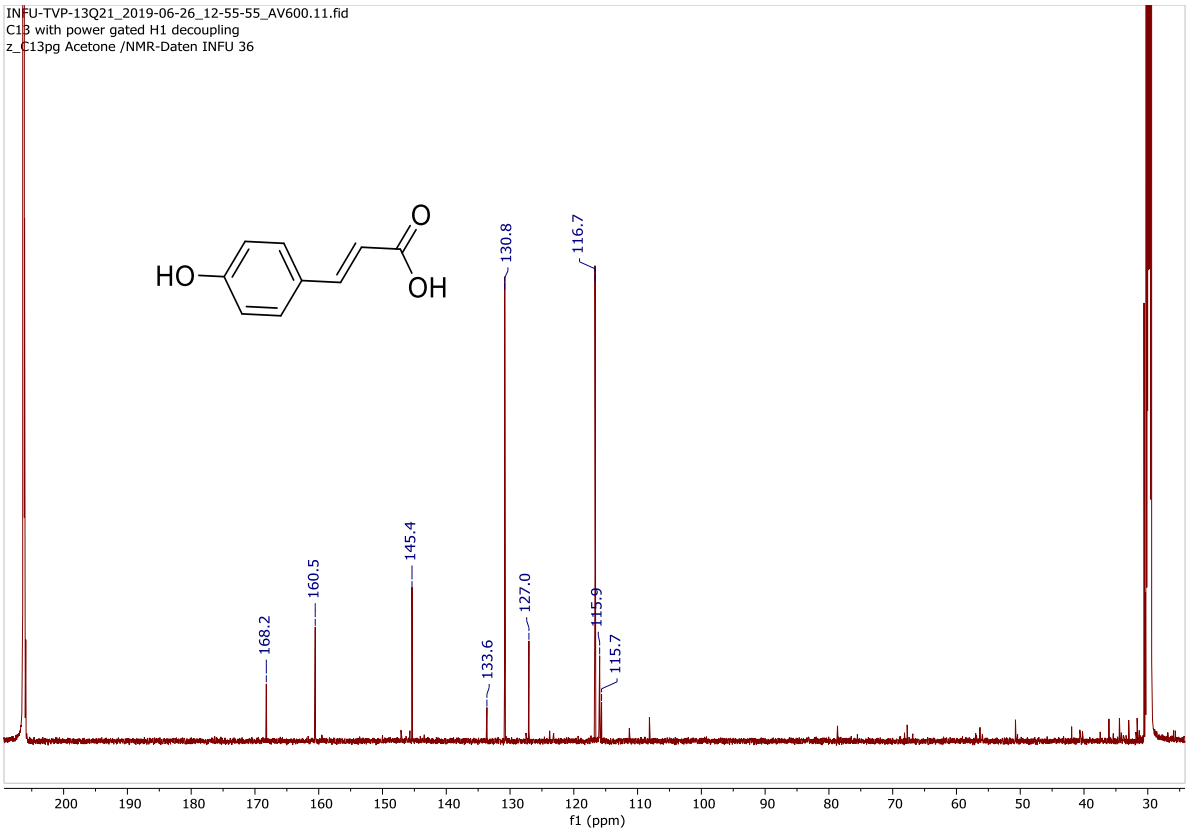
HRESIMS spectrum of compound 40



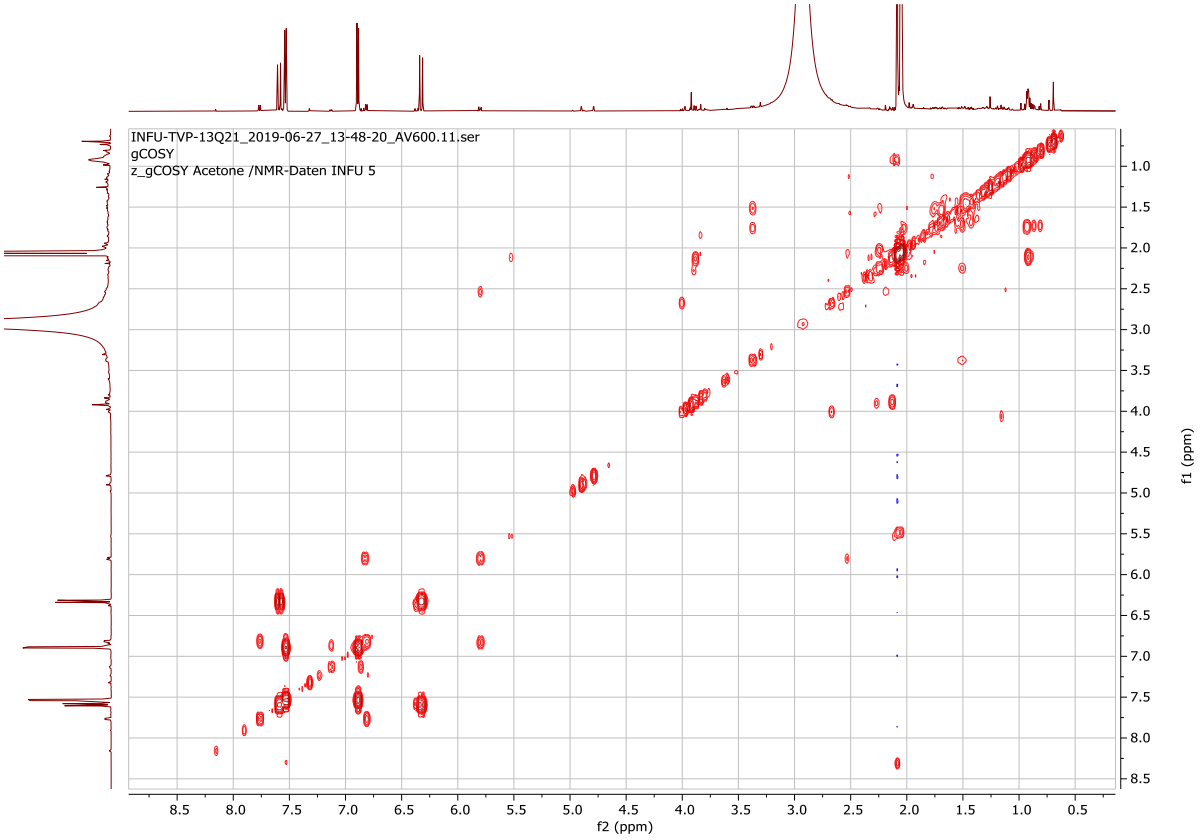
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 40



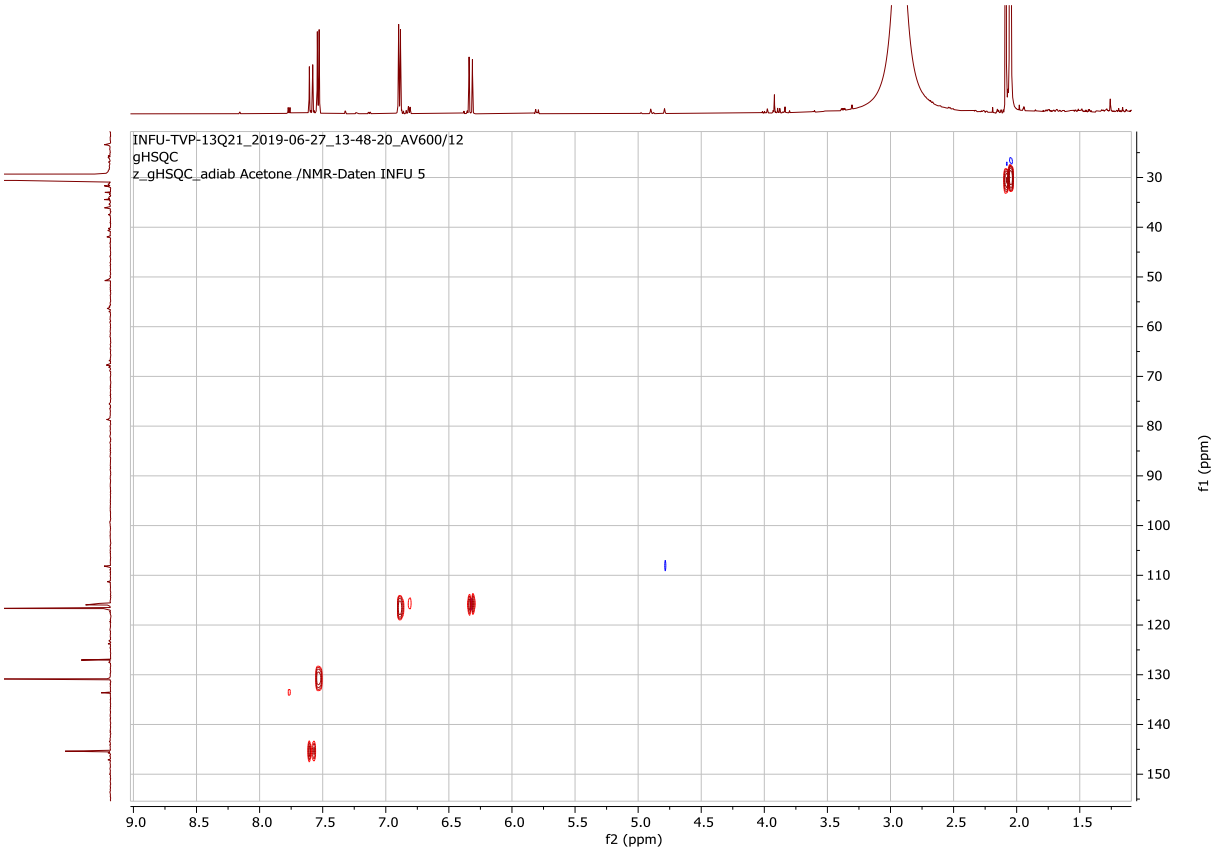
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 40



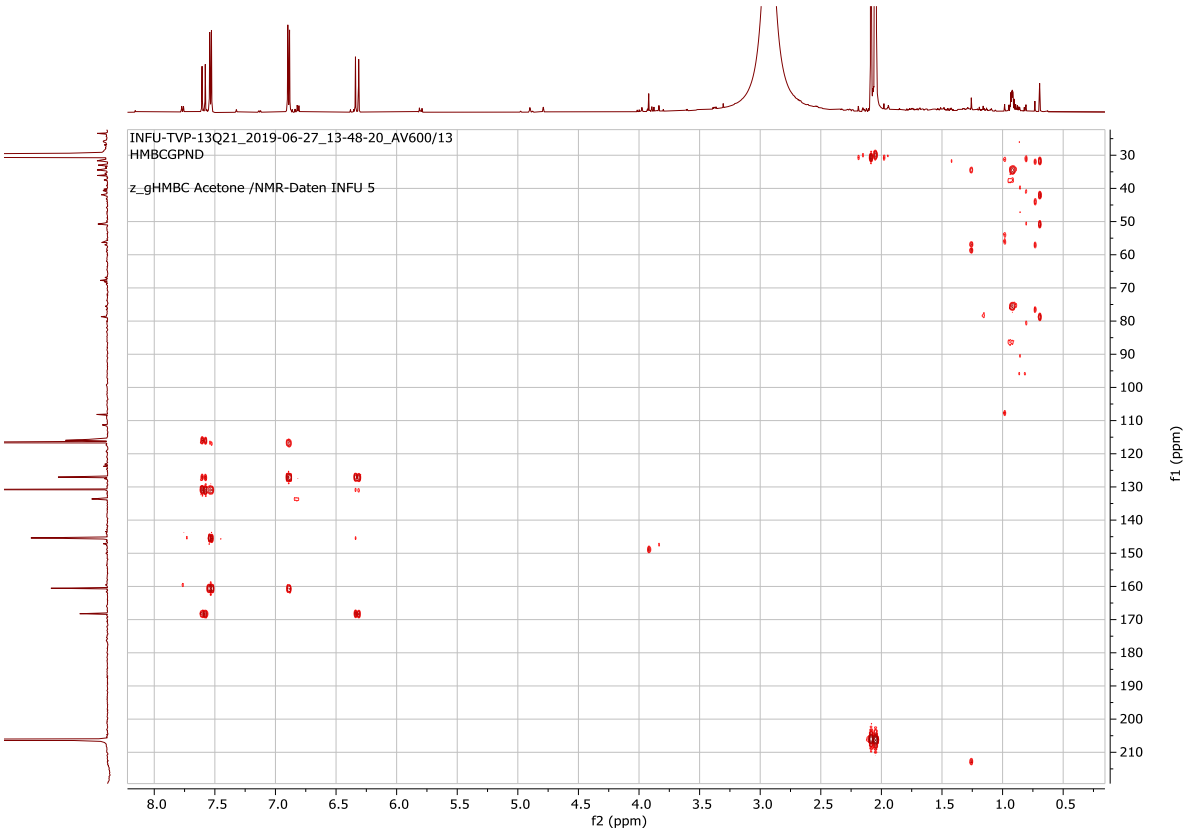
^{1}H-^{1}H-COSY spectrum of compound 40



HSQC spectrum of compound 40

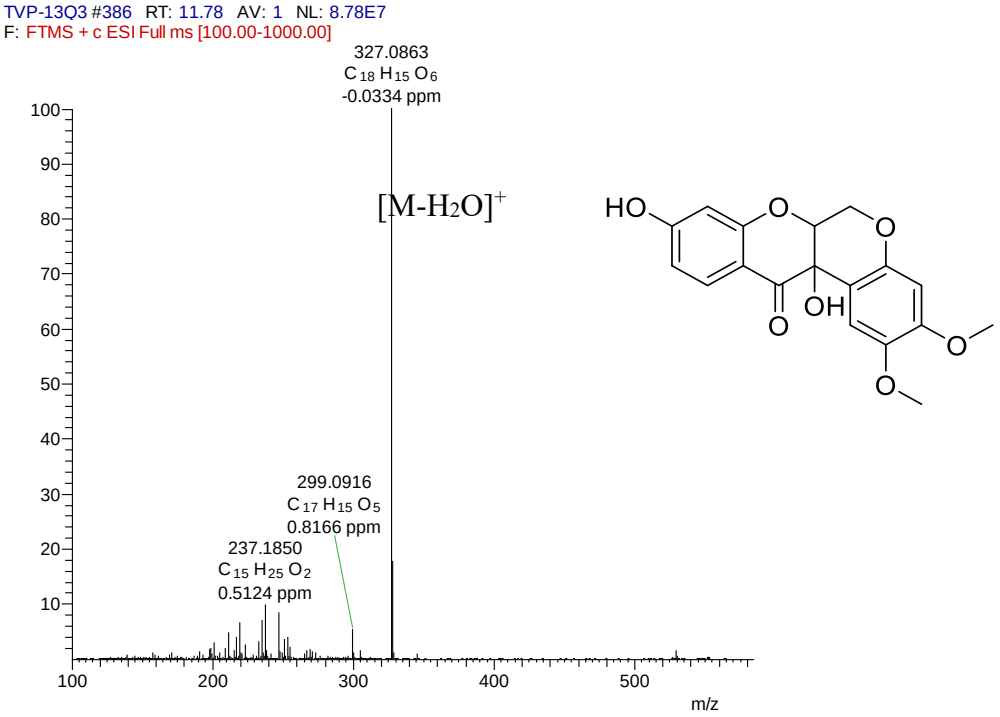


HMBC spectrum of compound 40

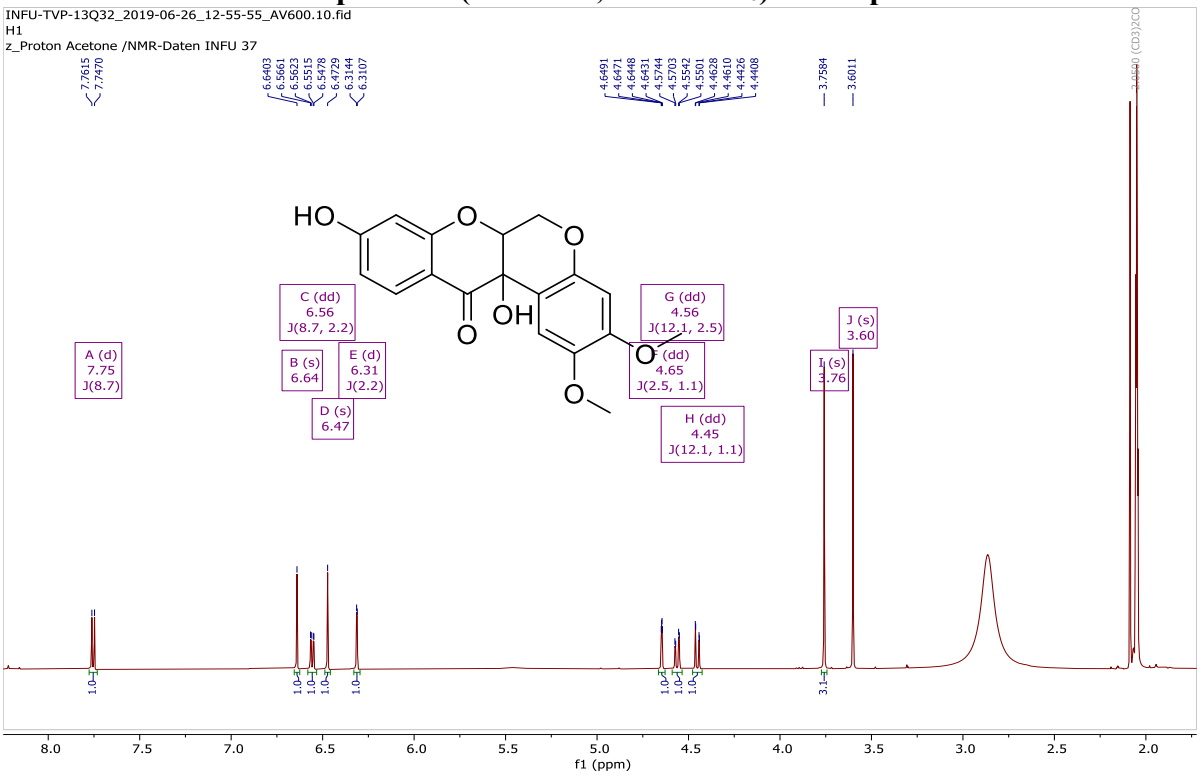


Appendix A41: Spectra for compound 41

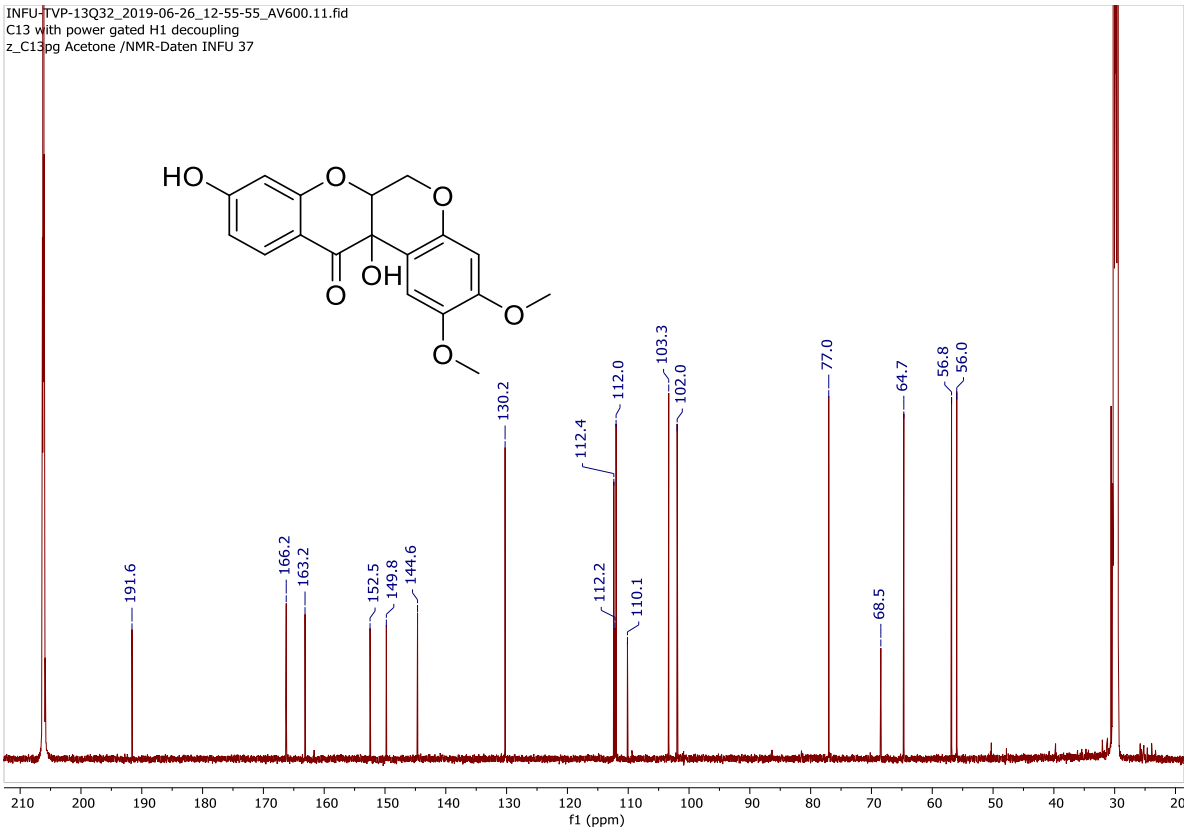
HRESIMS spectrum of compound 41



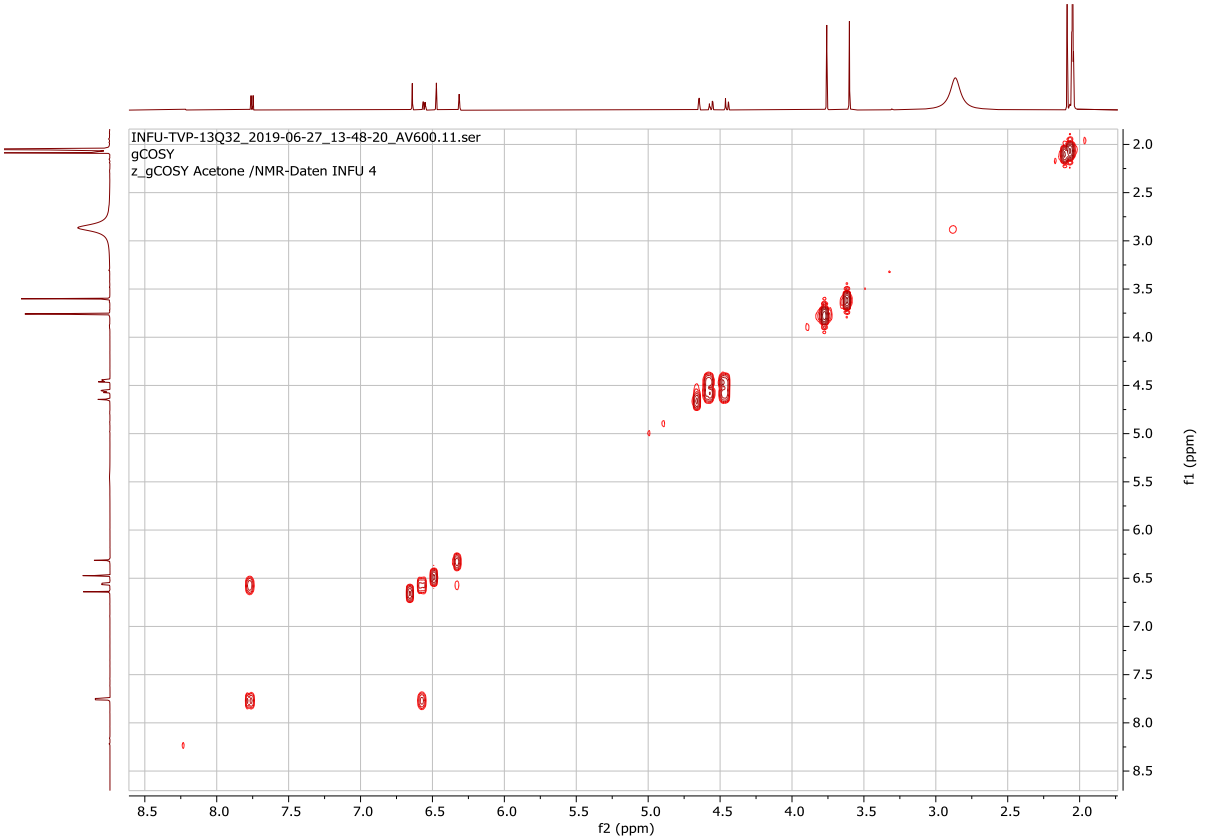
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 41



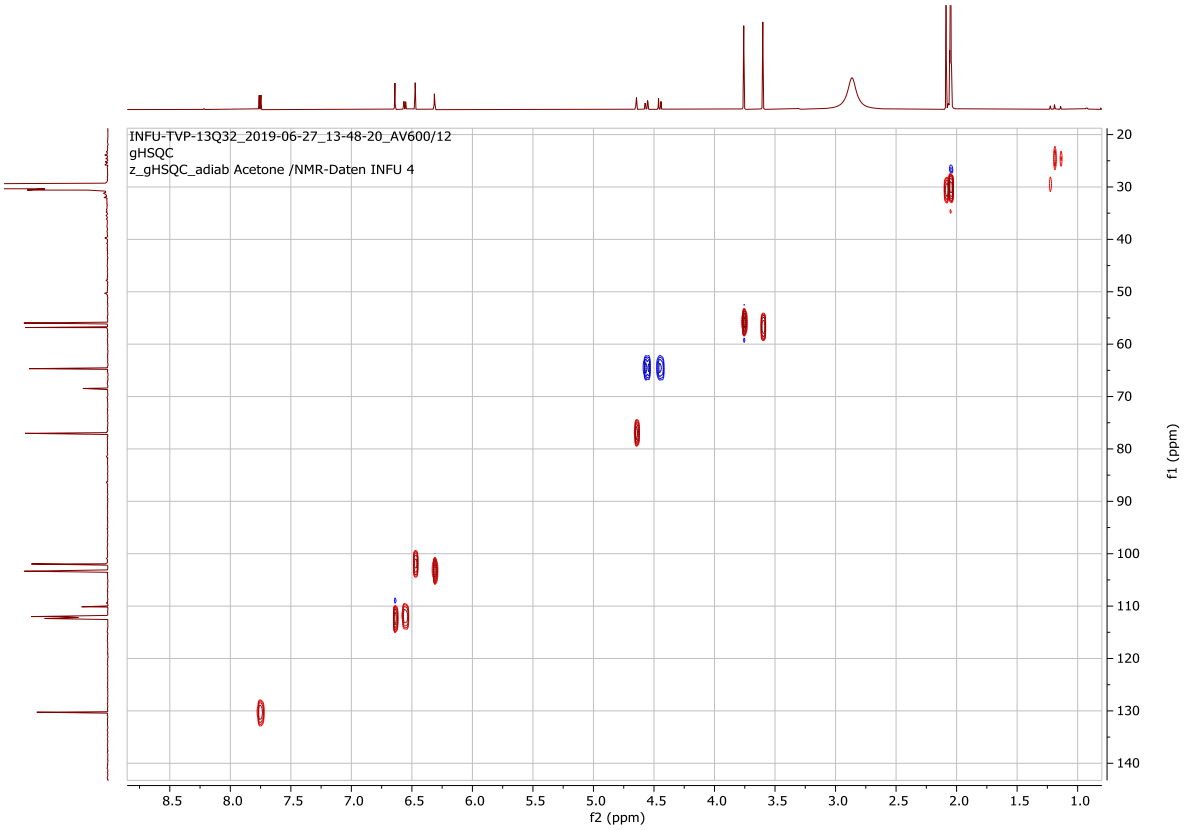
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 41



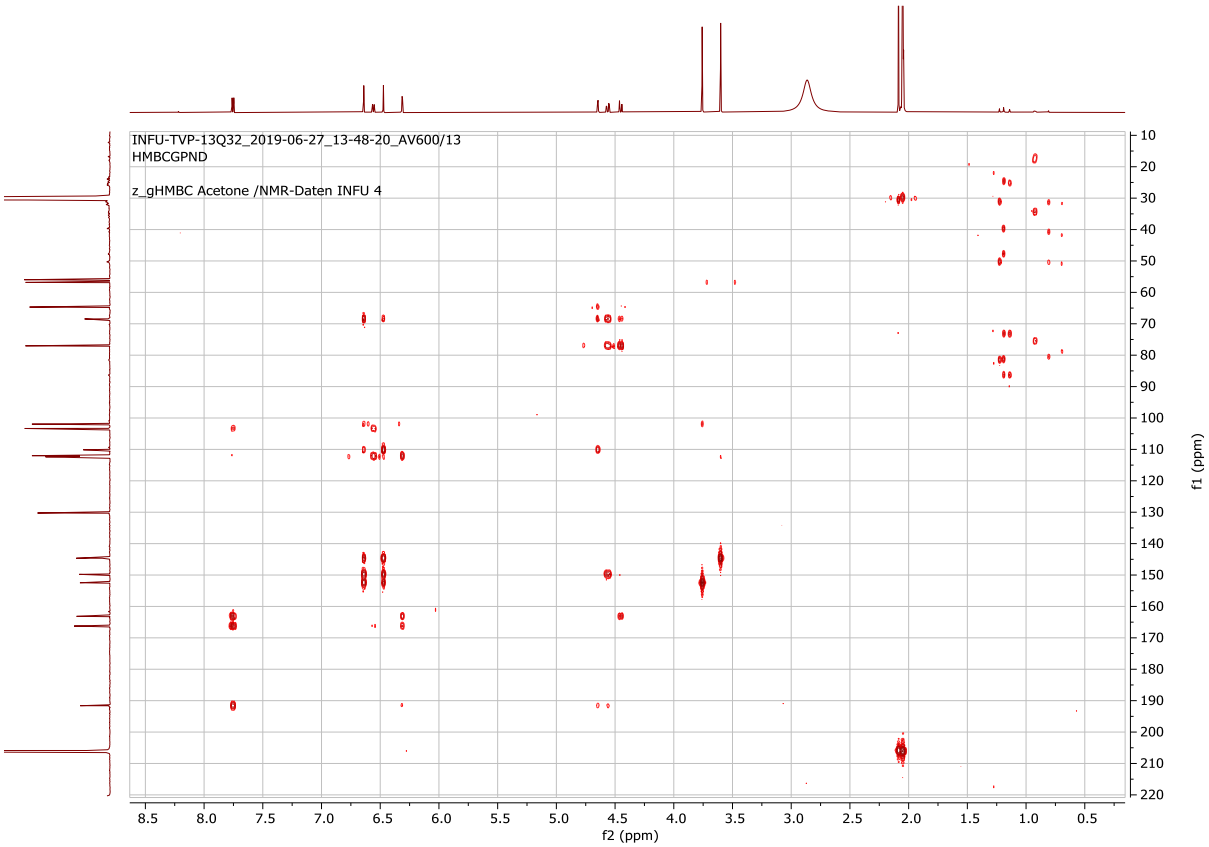
^{1}H-^{1}H-COSY spectrum of compound 41



HSQC spectrum of compound 41

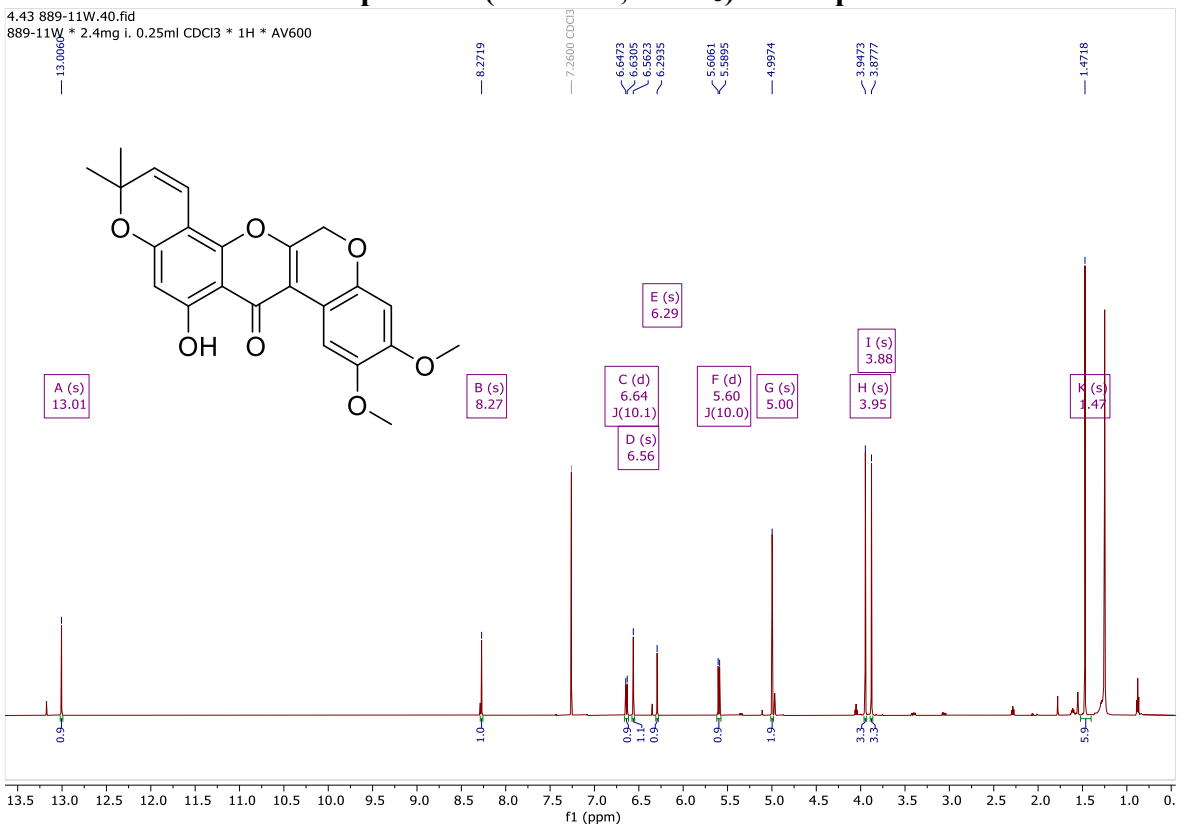


HMBC spectrum of compound 41

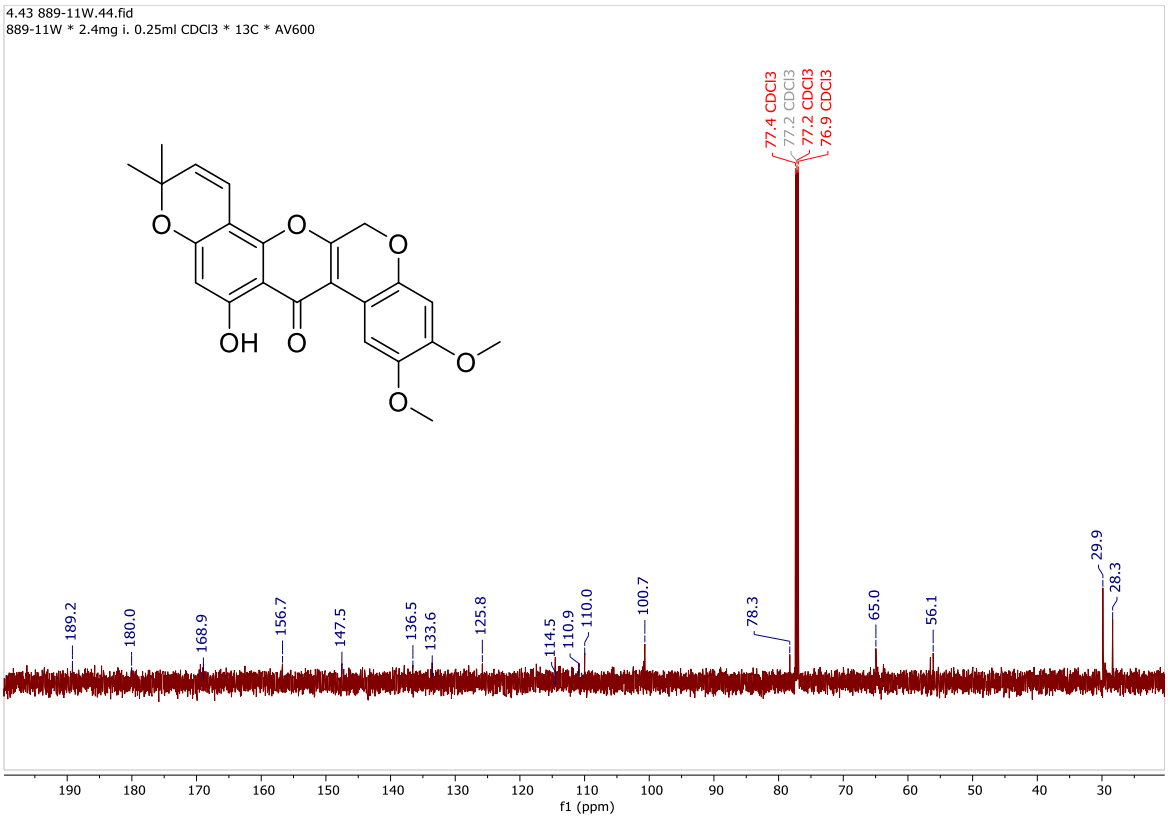


Appendix A42: Spectra for compound 42

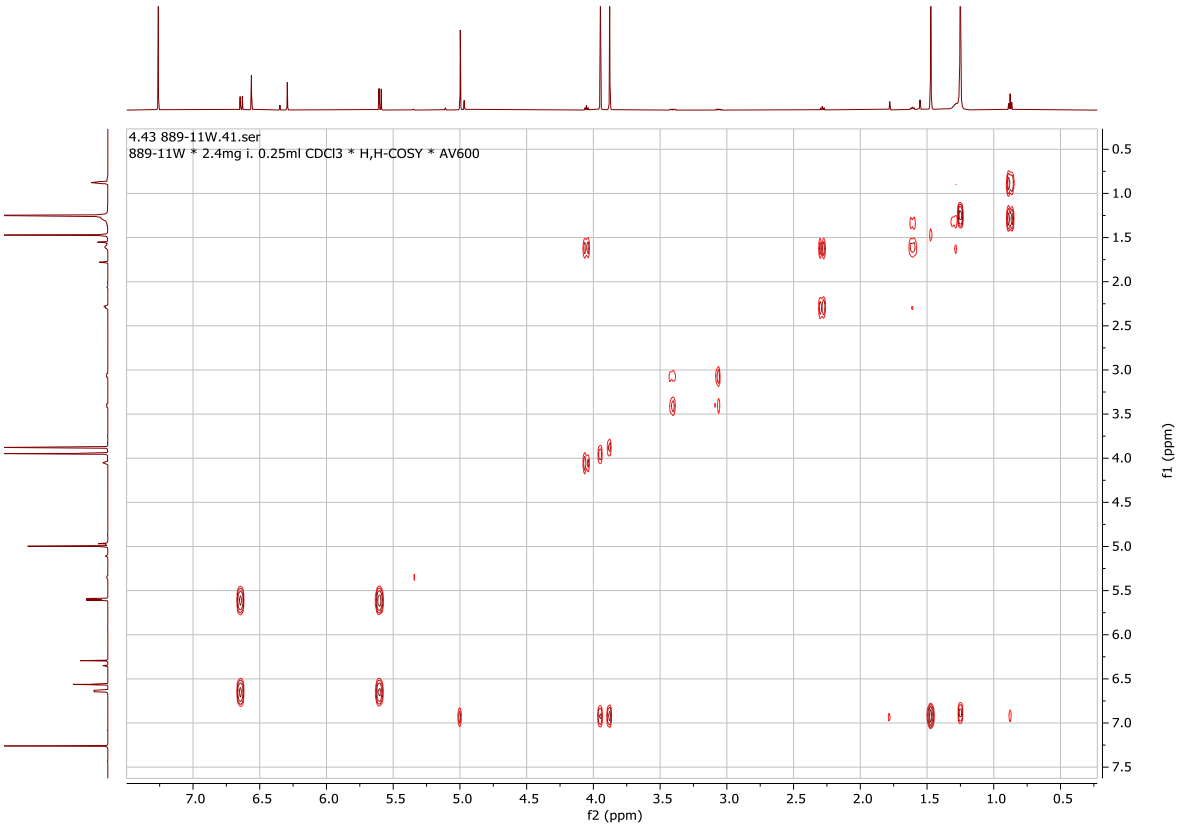
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound 42



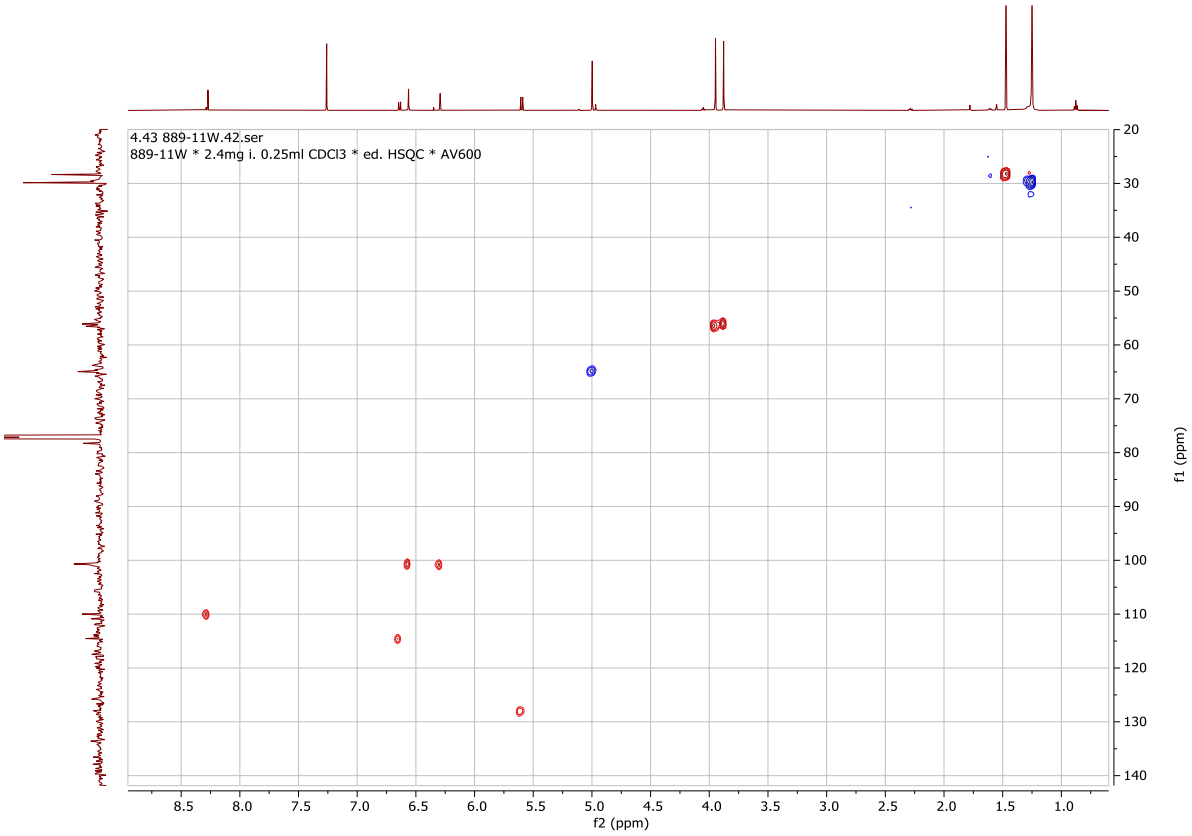
^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound 42



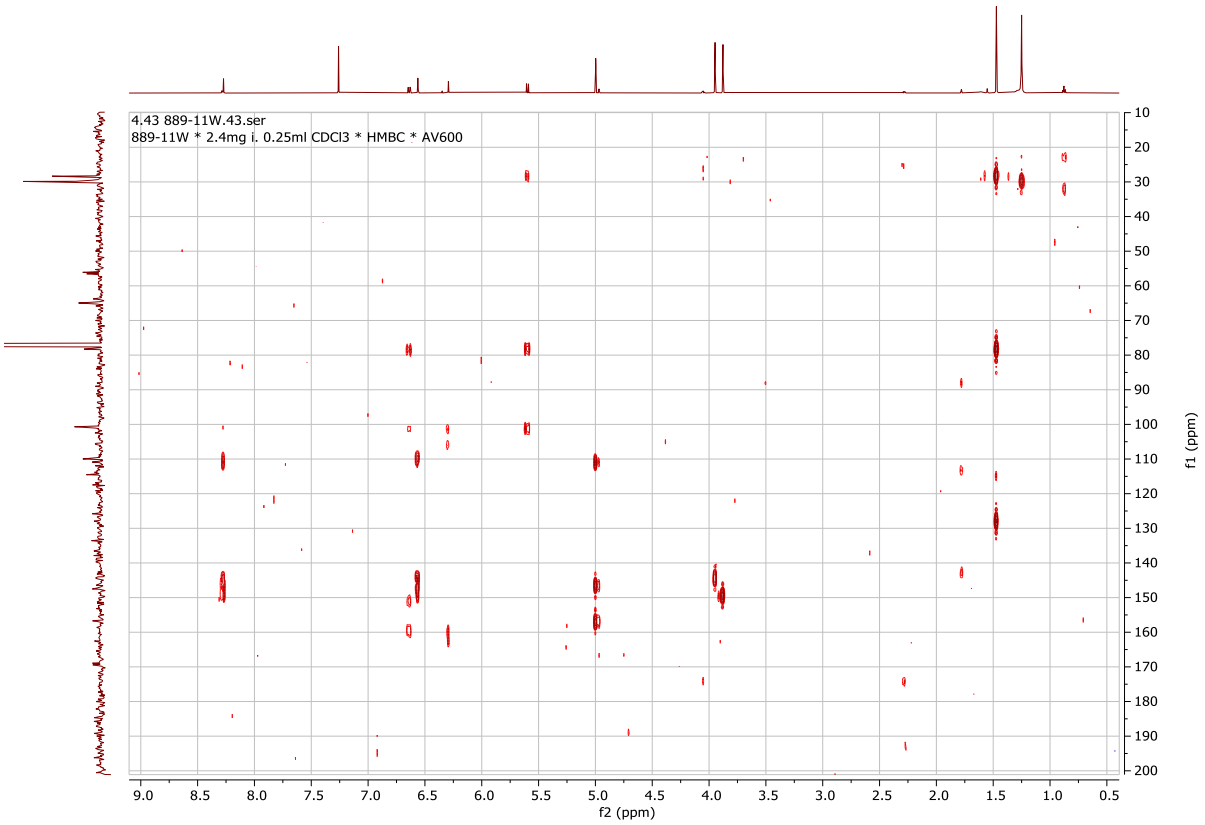
^{1}H-^{1}H-COSY spectrum of compound 42



HSQC spectrum of compound 42

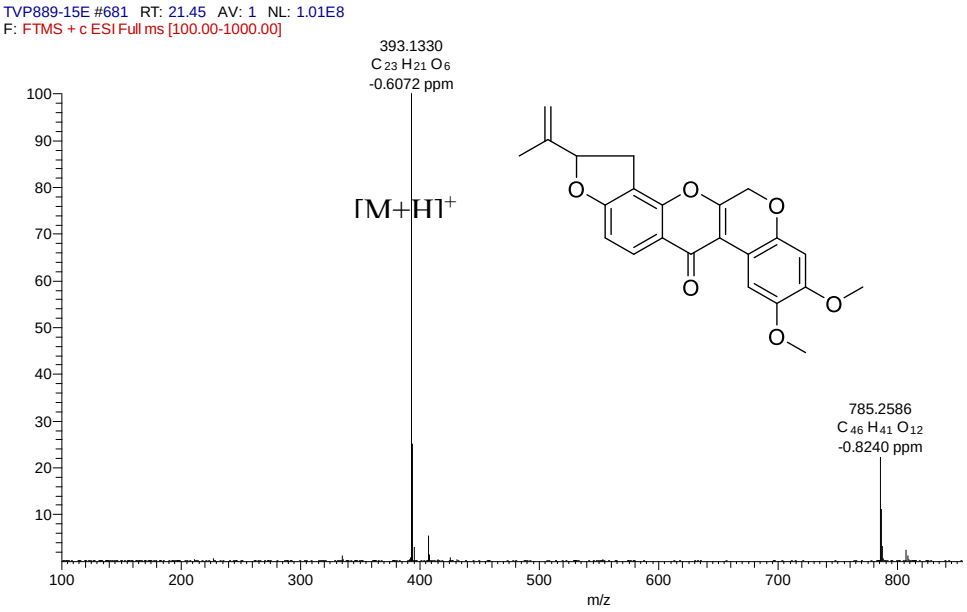


HMBC spectrum of compound 42

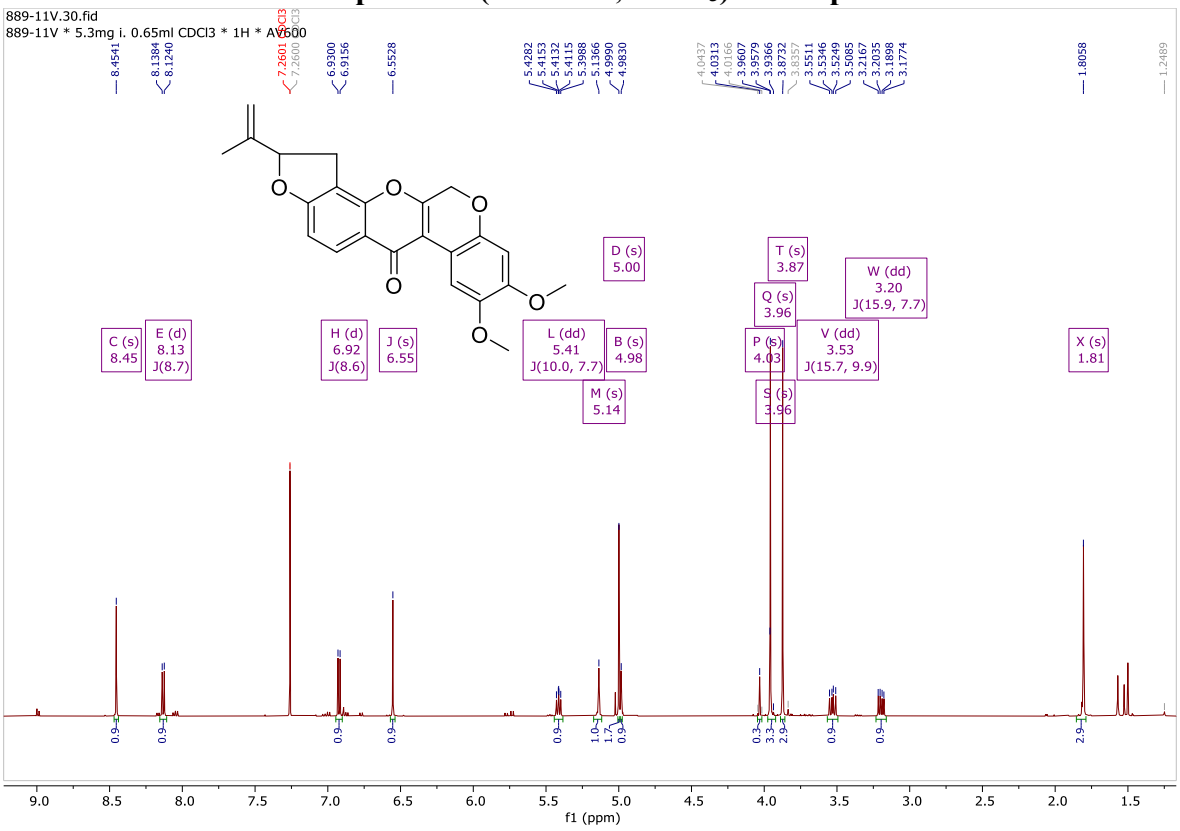


Appendix A43: Spectra for compound 43

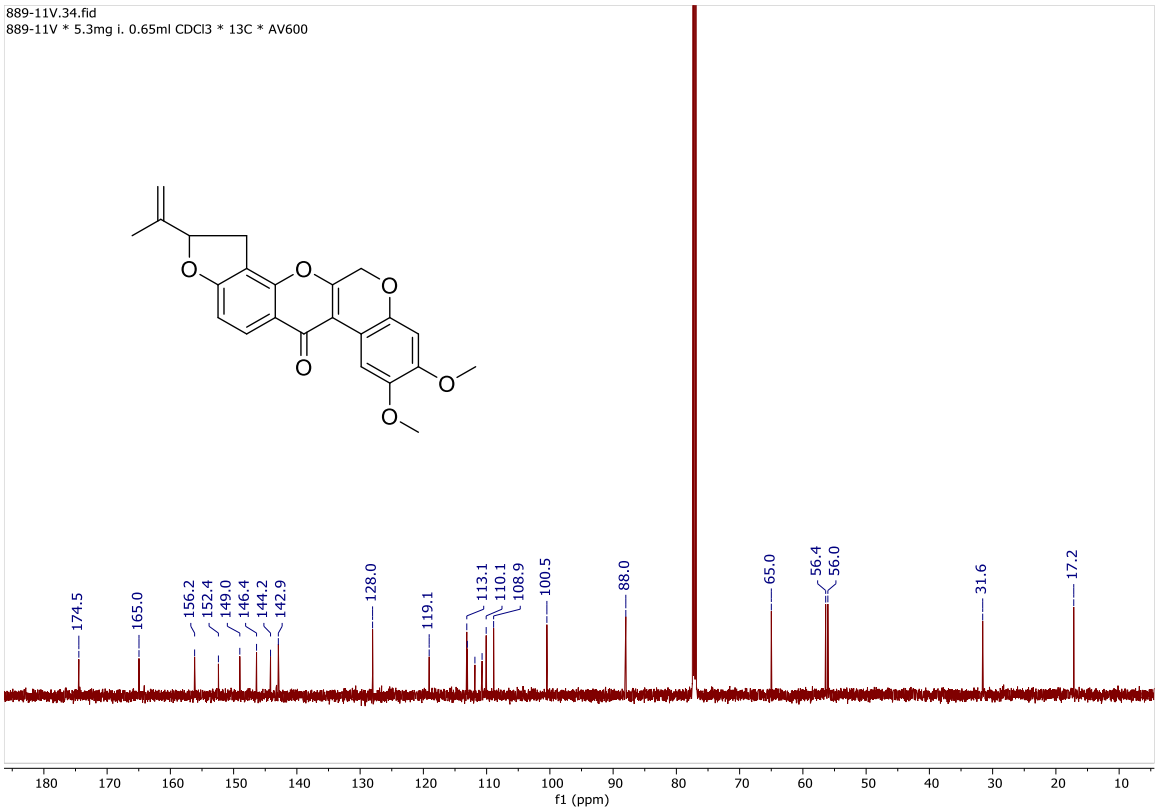
HRESIMS spectrum of compound 43



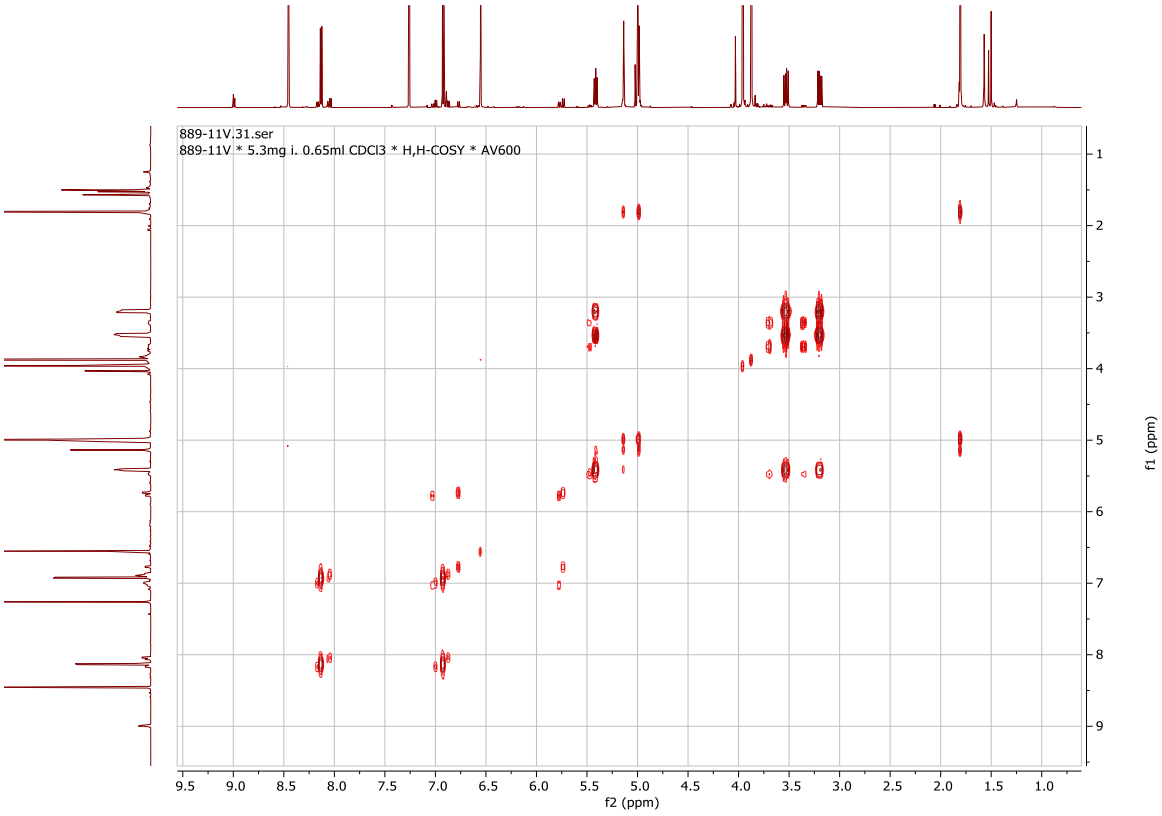
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound 43



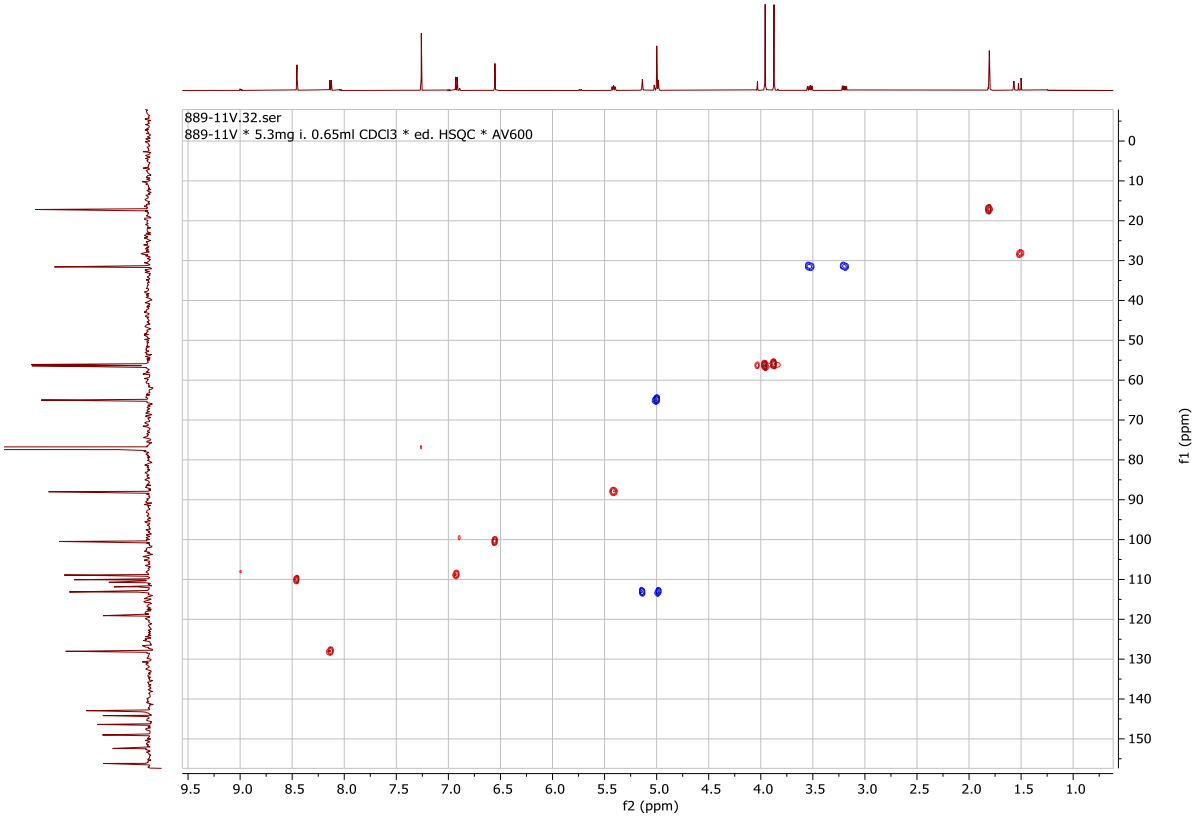
^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound 43



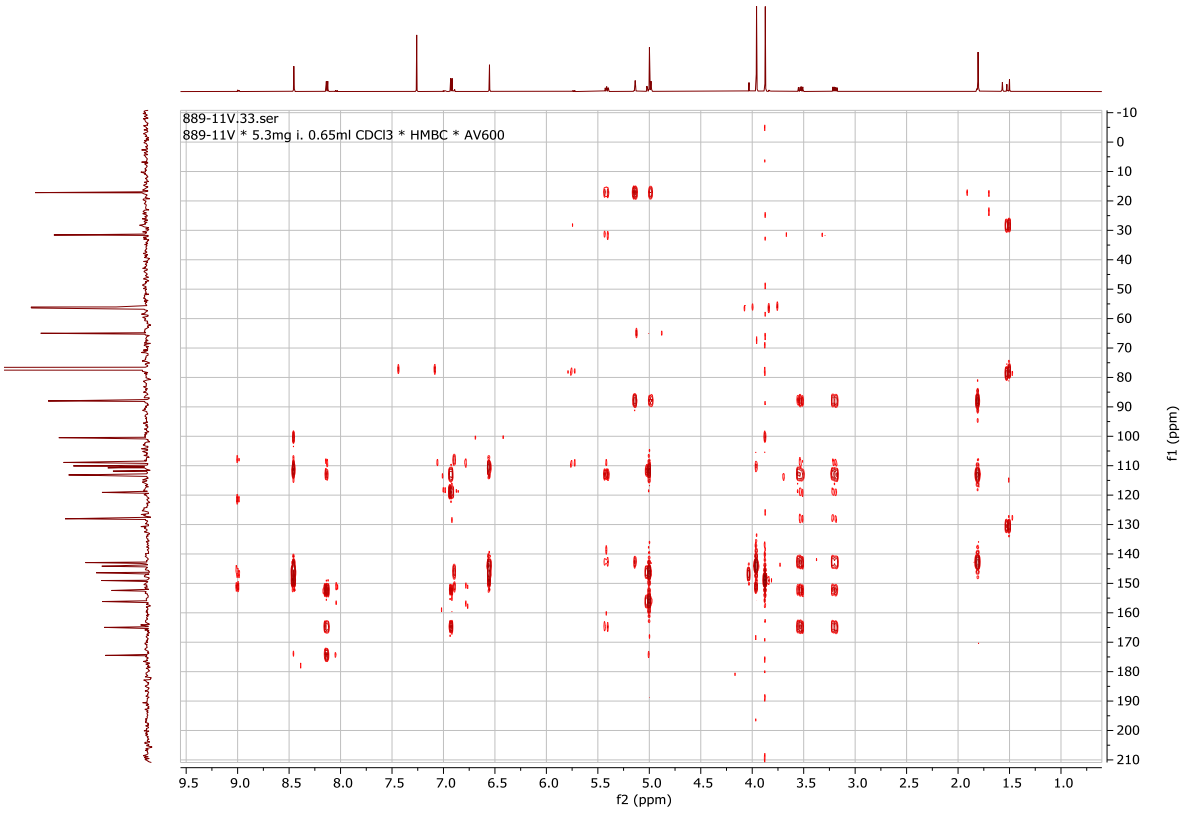
^{1}H-^{1}H-COSY spectrum of compound 43



HSQC spectrum of compound 43

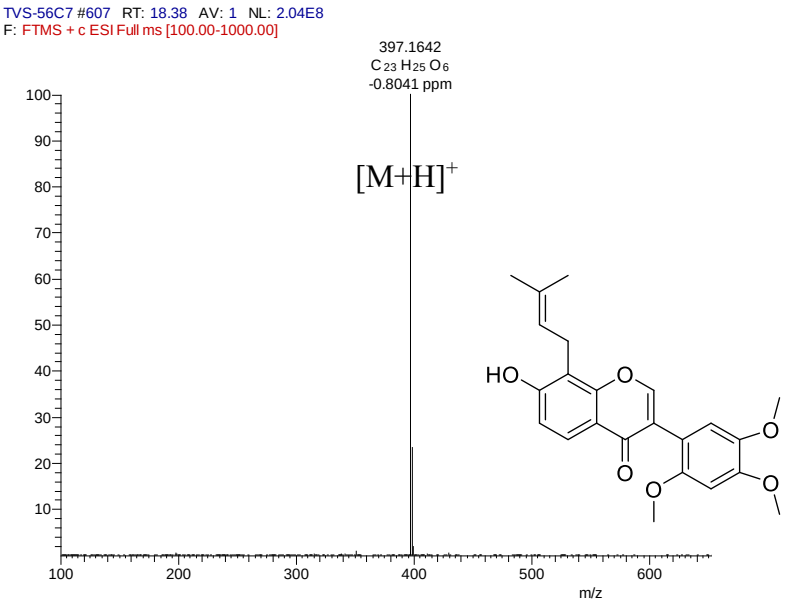


HMBC spectrum of compound 43

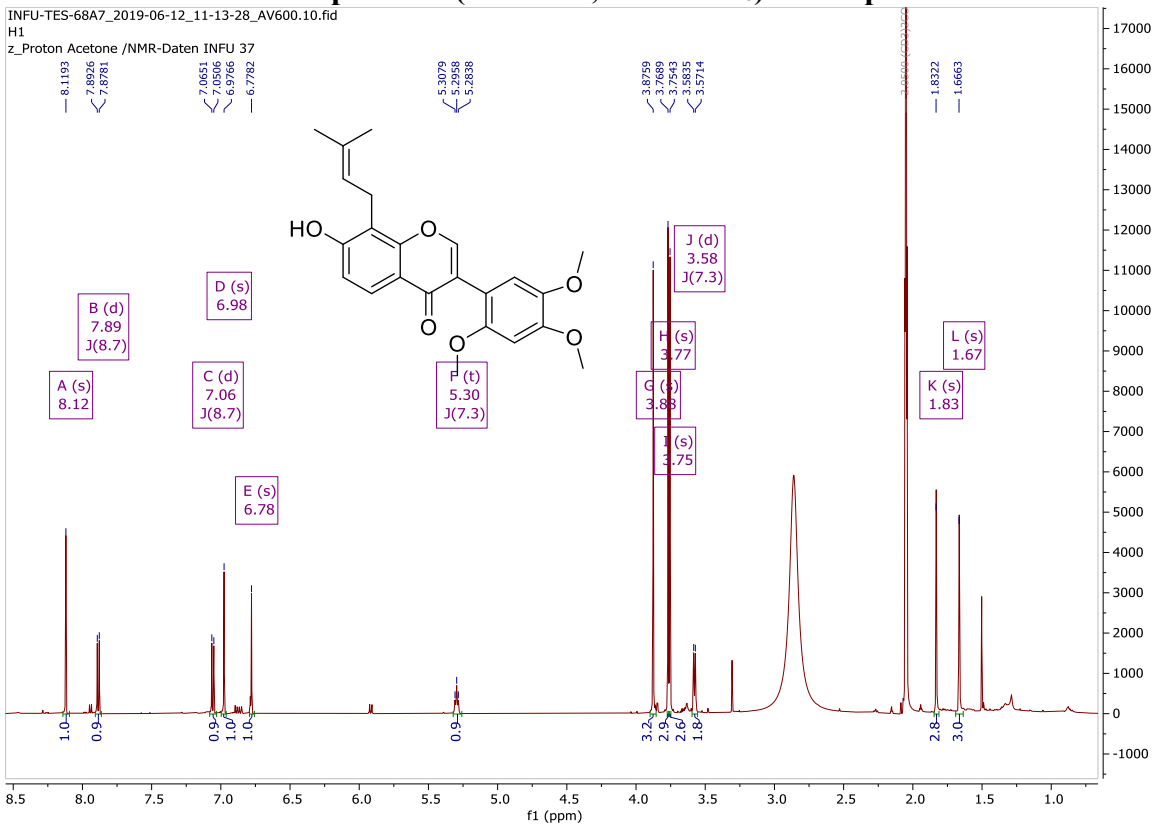


Appendix A44: Spectra for compound 44

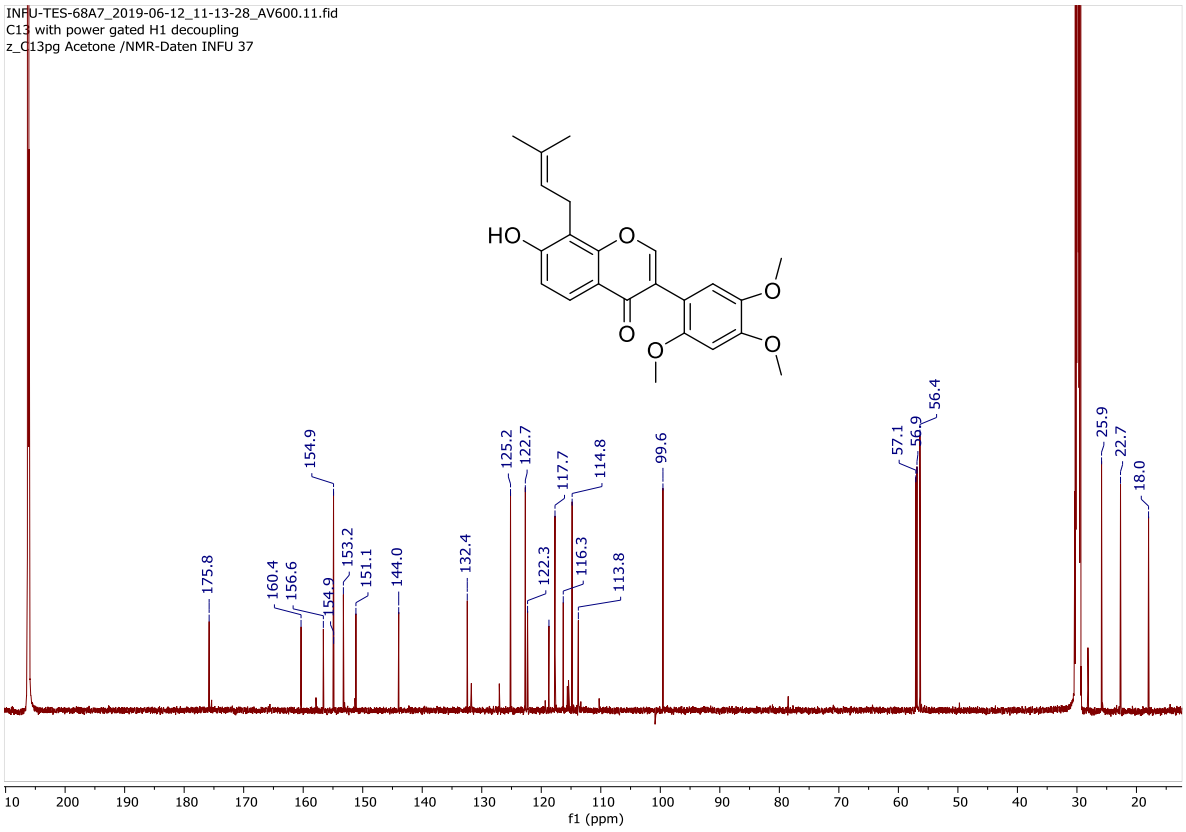
HRESIMS spectrum of compound 44



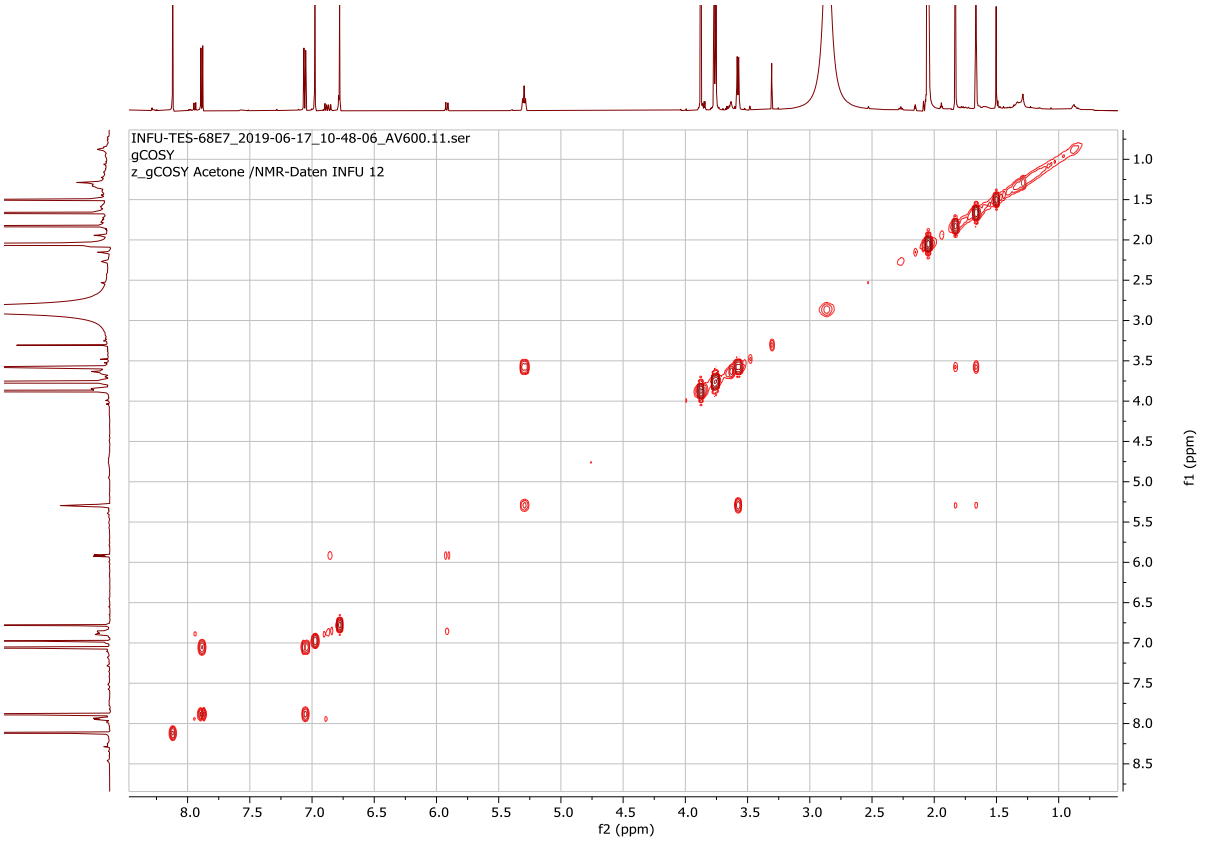
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 44



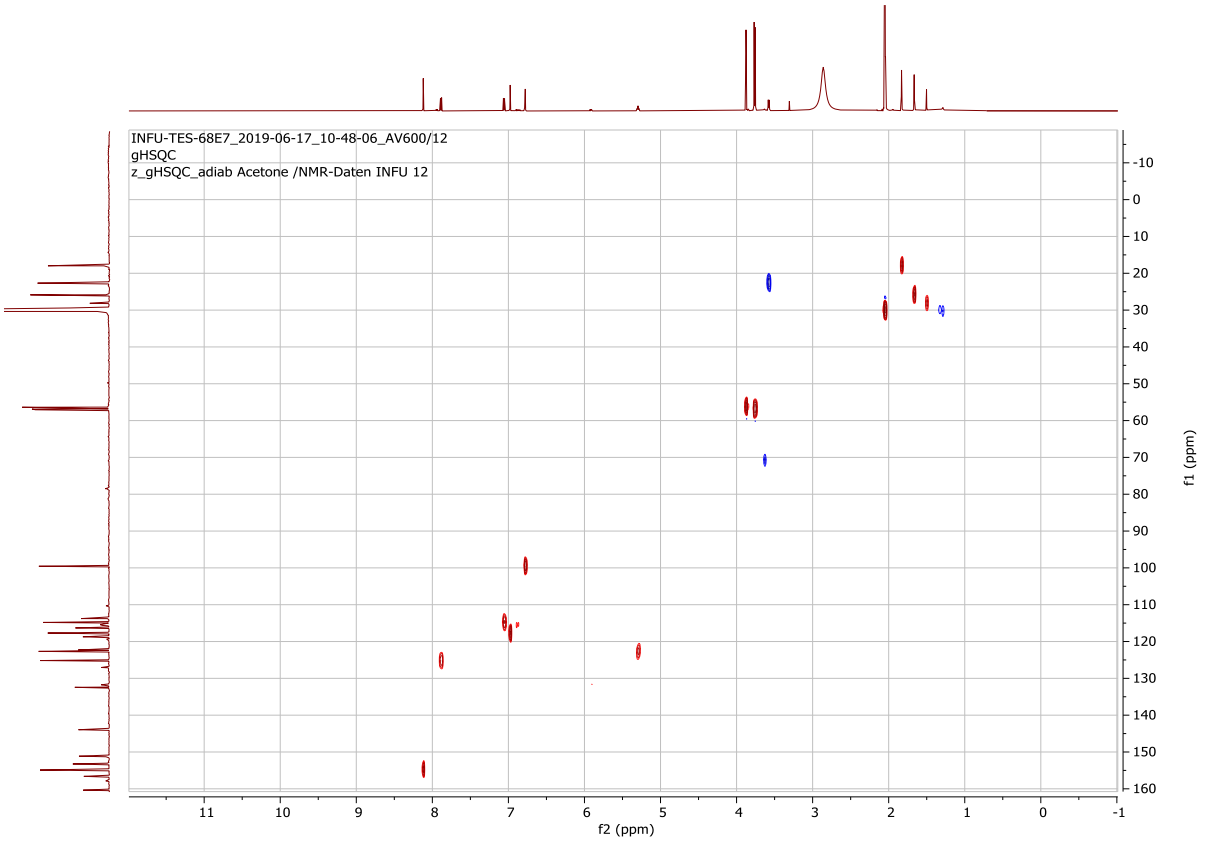
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 44



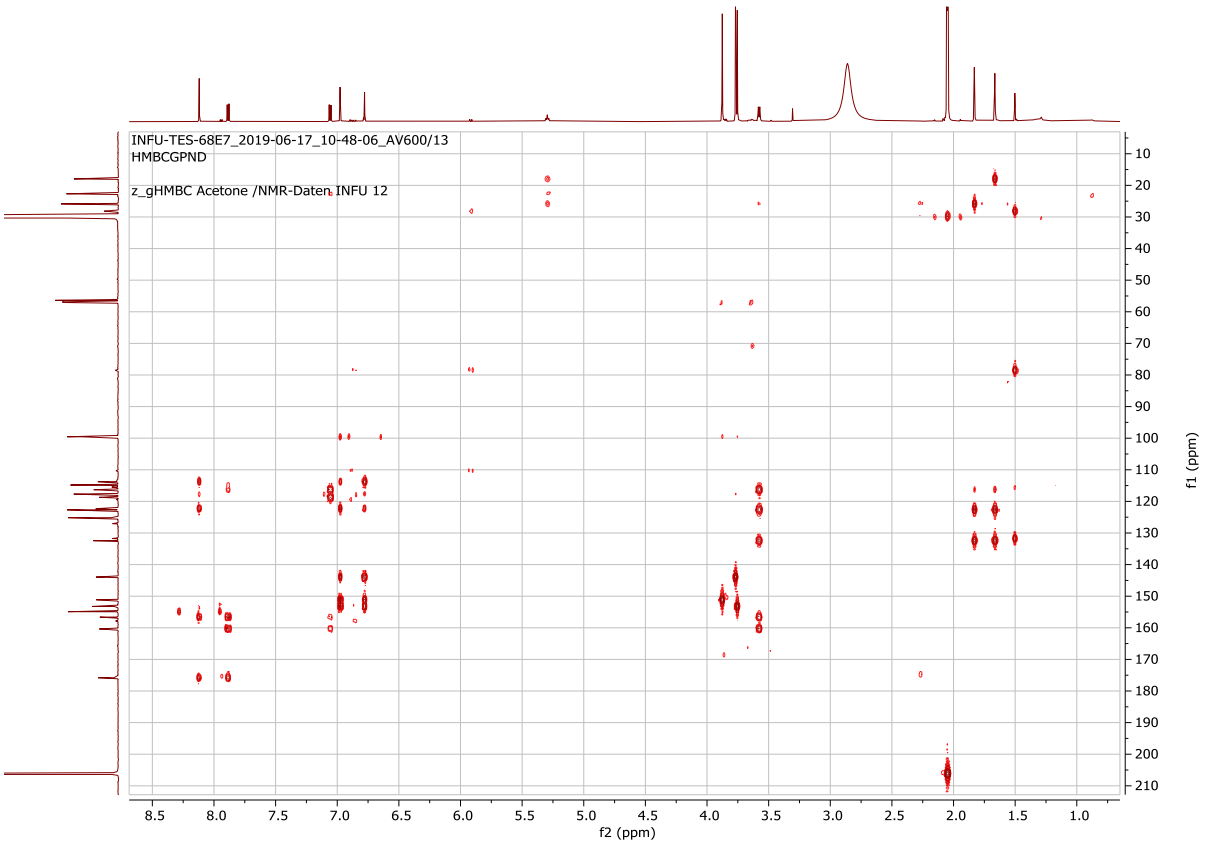
^{1}H-^{1}H-COSY spectrum of compound 44



HSQC spectrum of compound 44

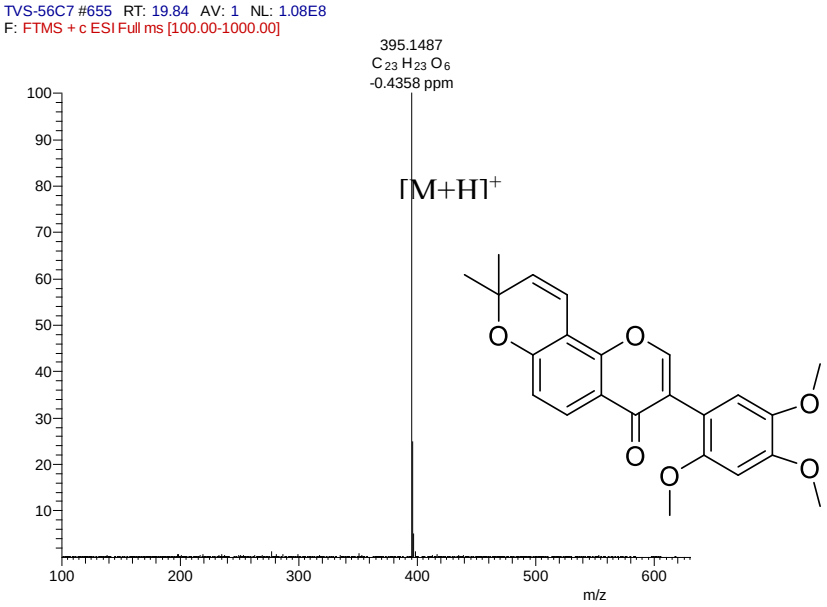


HMBC spectrum of compound 44

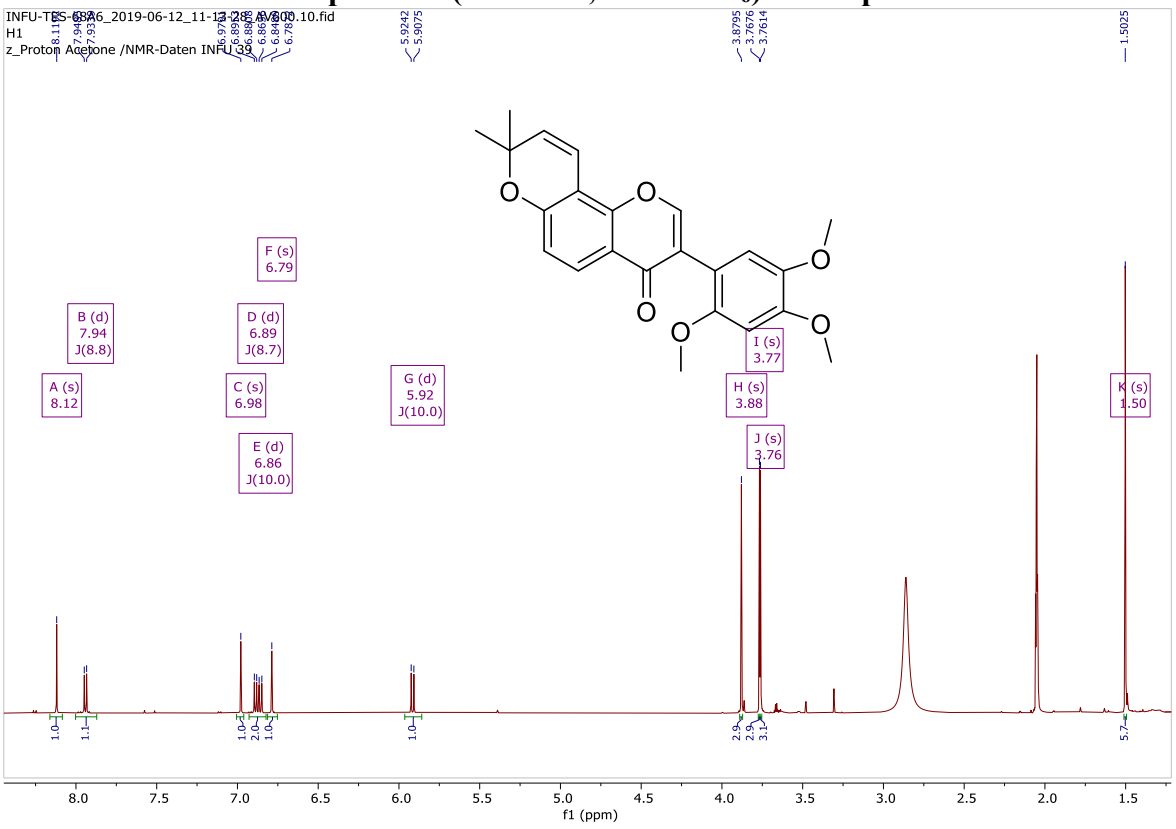


Appendix A45: Spectra for compound 45

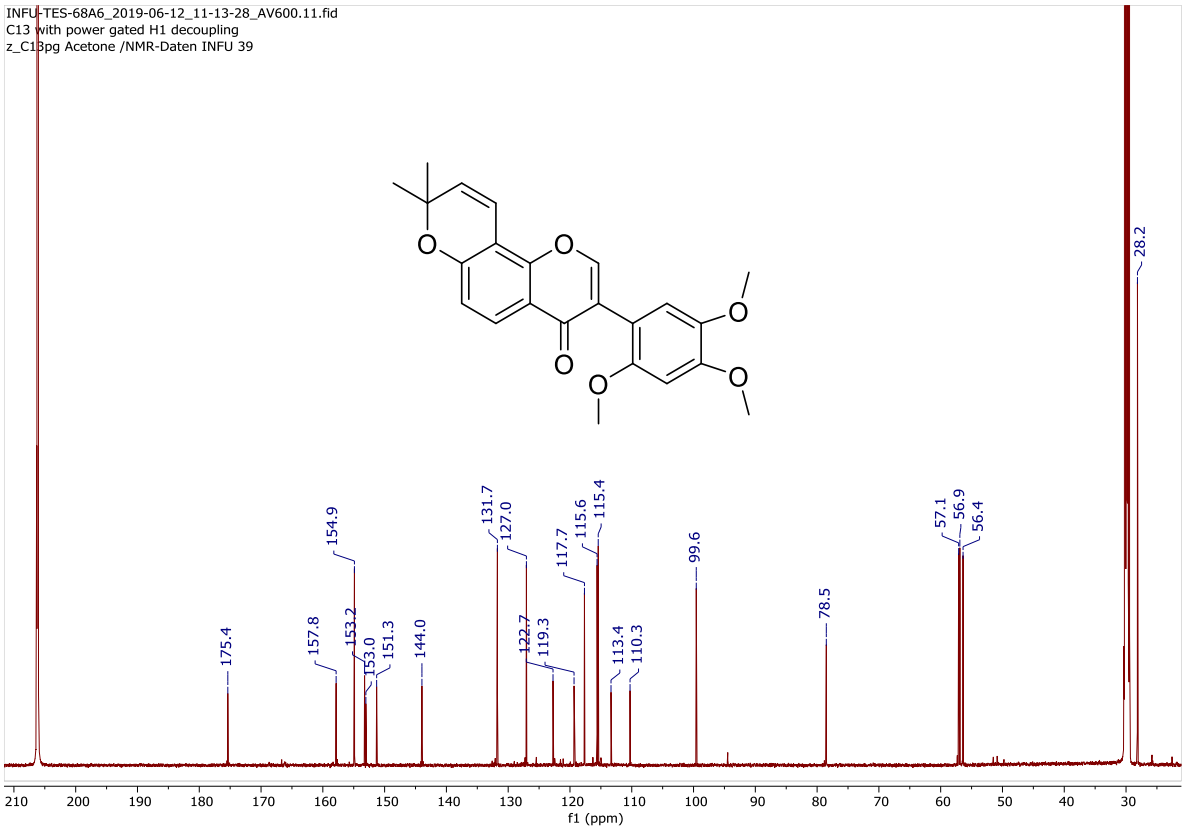
HRESIMS spectrum of compound 45



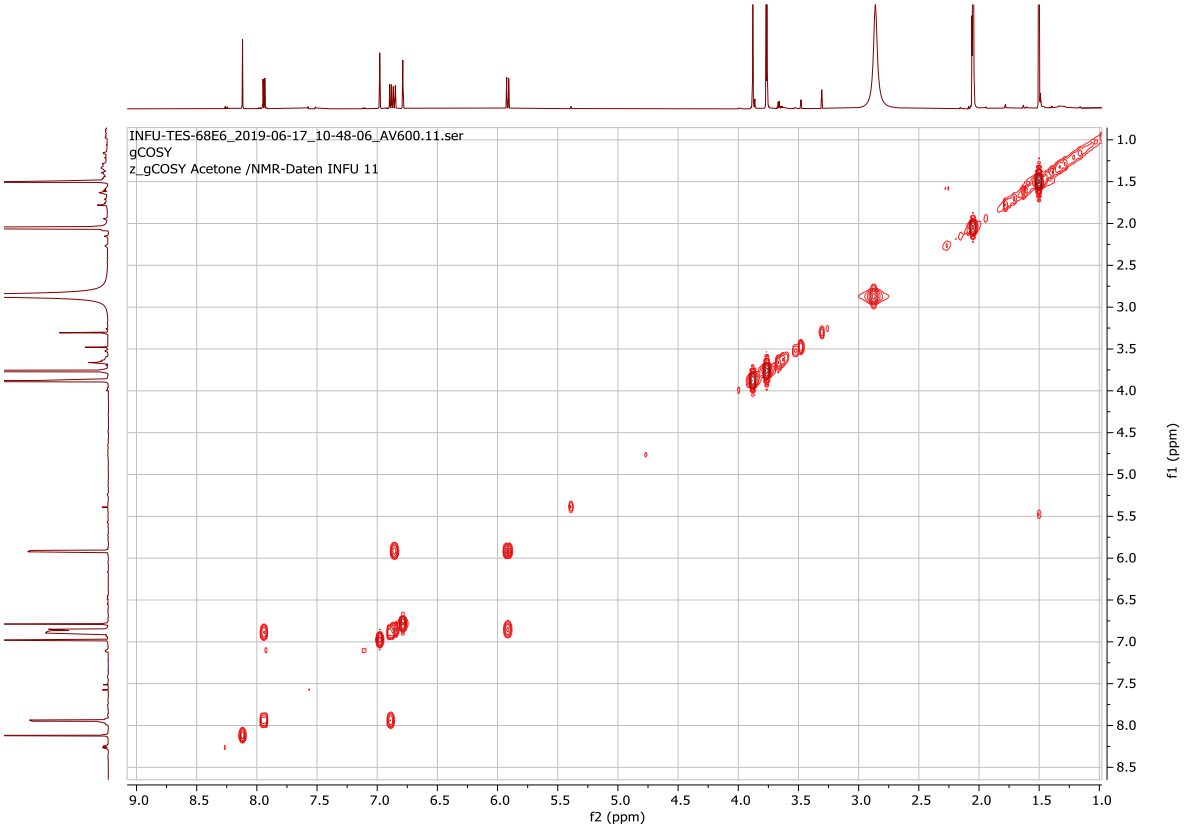
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 45



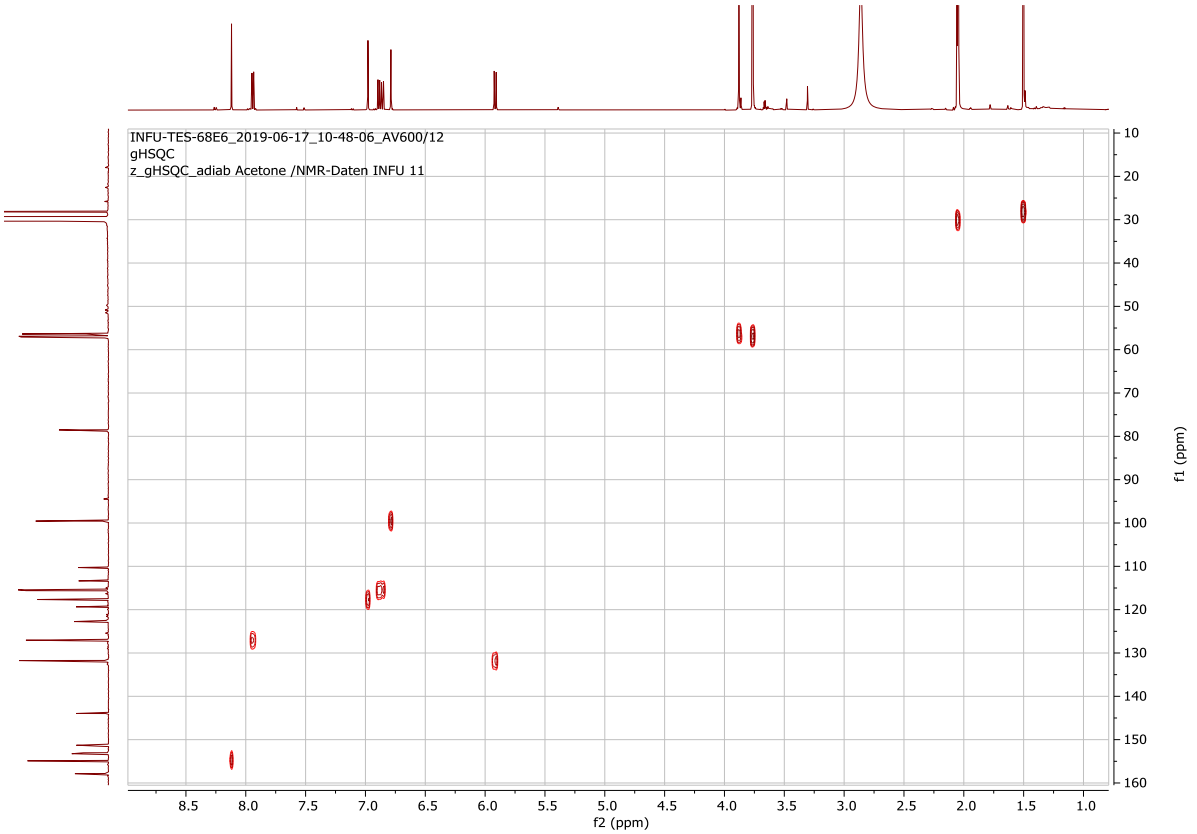
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 45



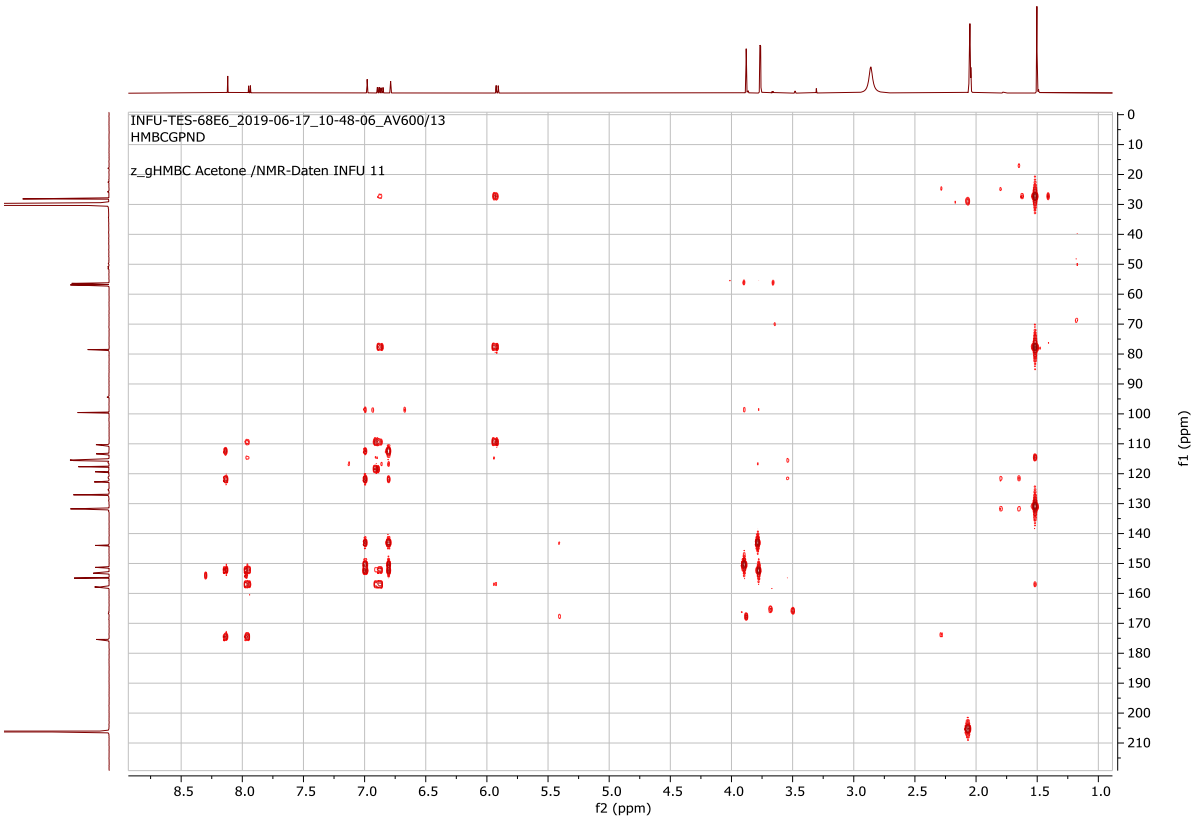
^{1}H-^{1}H-COSY spectrum of compound 45



HSQC spectrum of compound 45

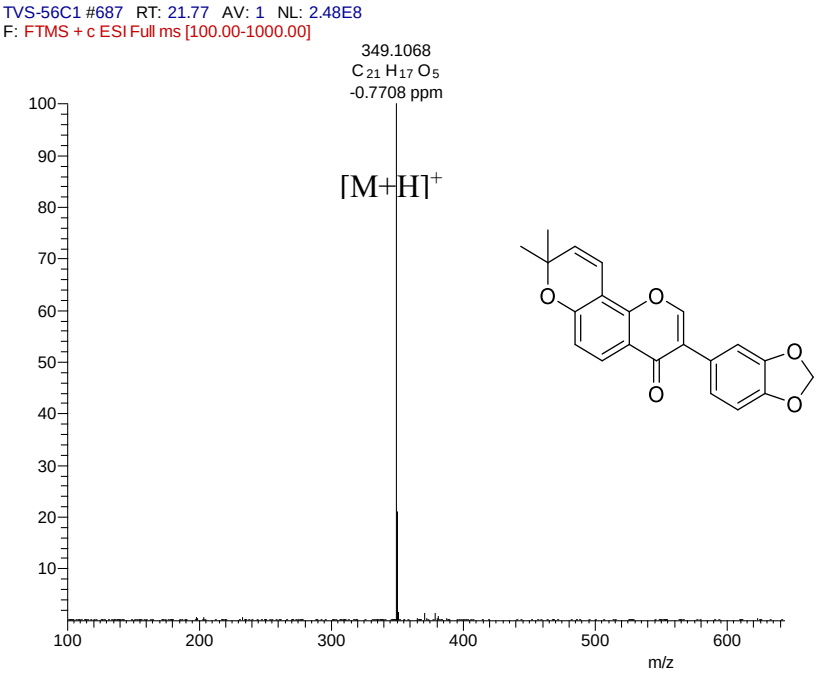


HMBC spectrum of compound 45

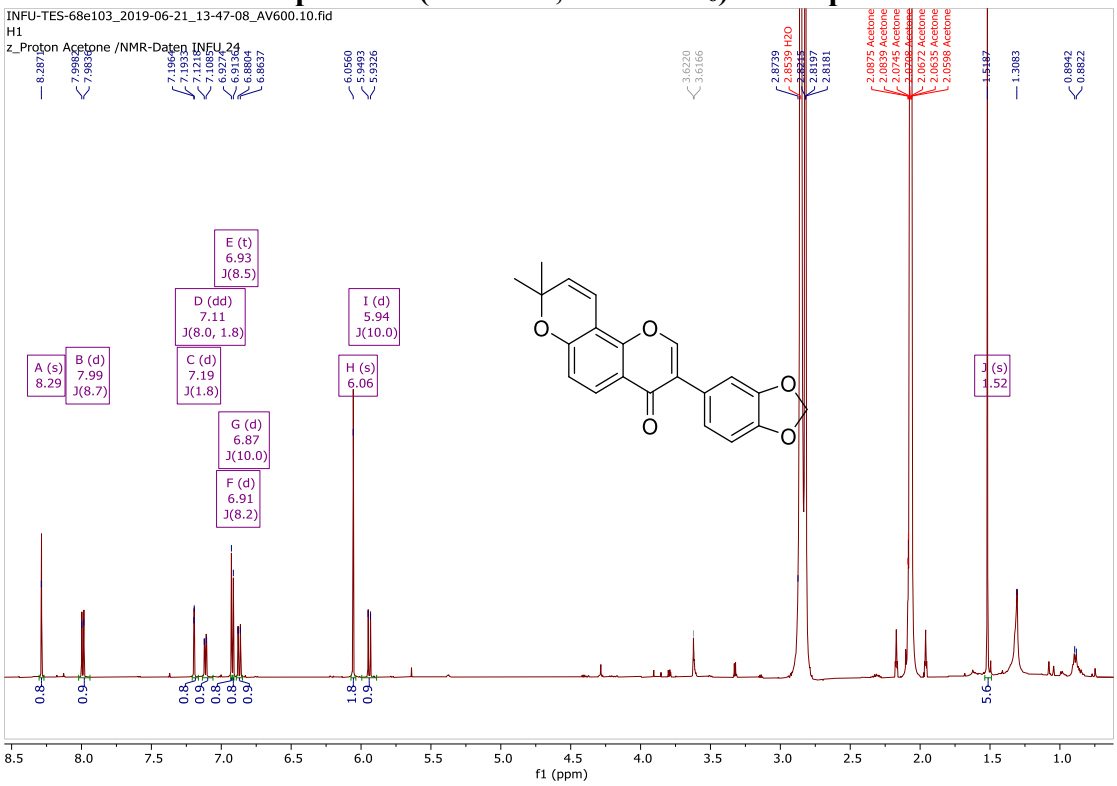


Appendix A46: Spectra for compound 46

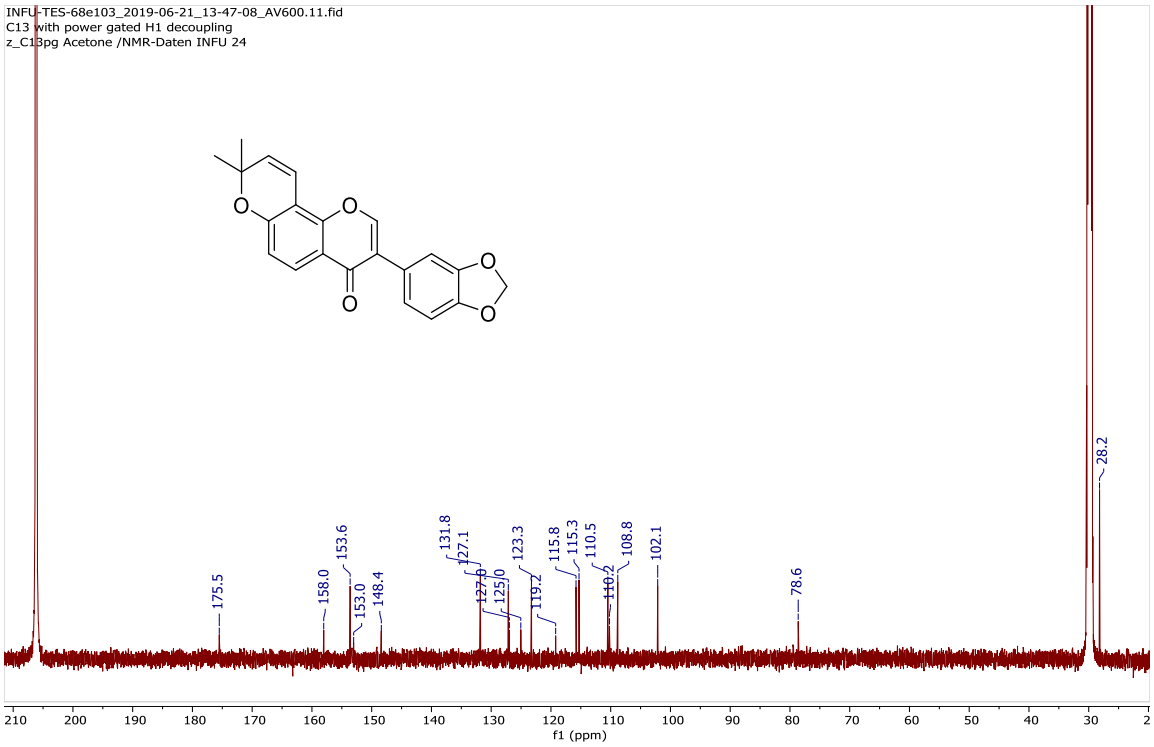
HRESIMS spectrum of compound 46



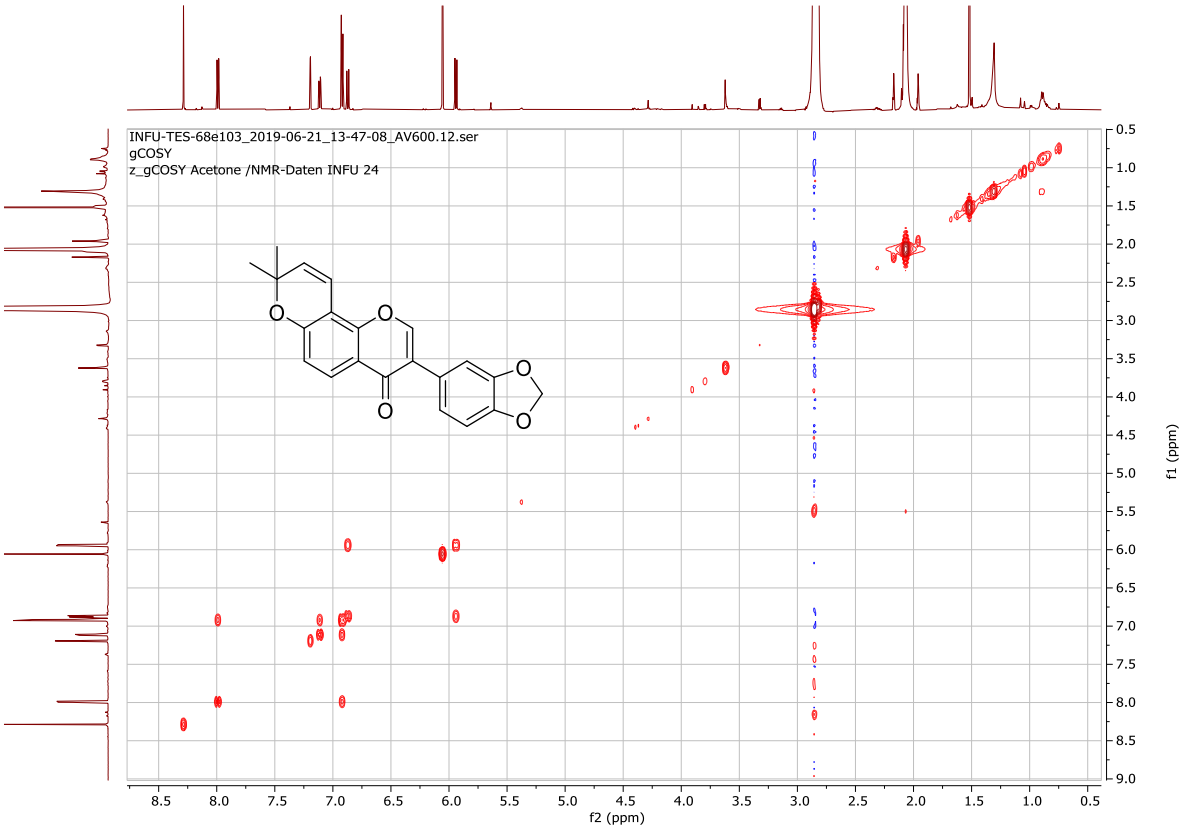
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 46



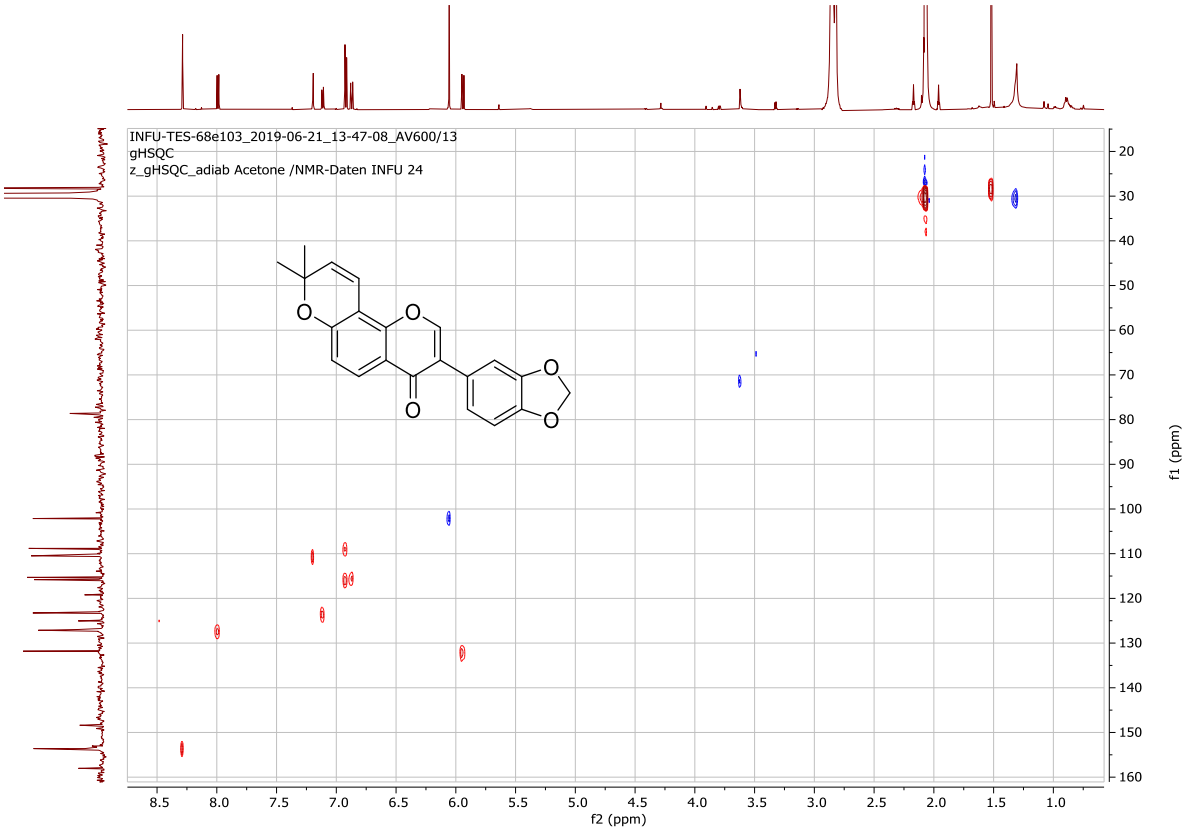
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 46



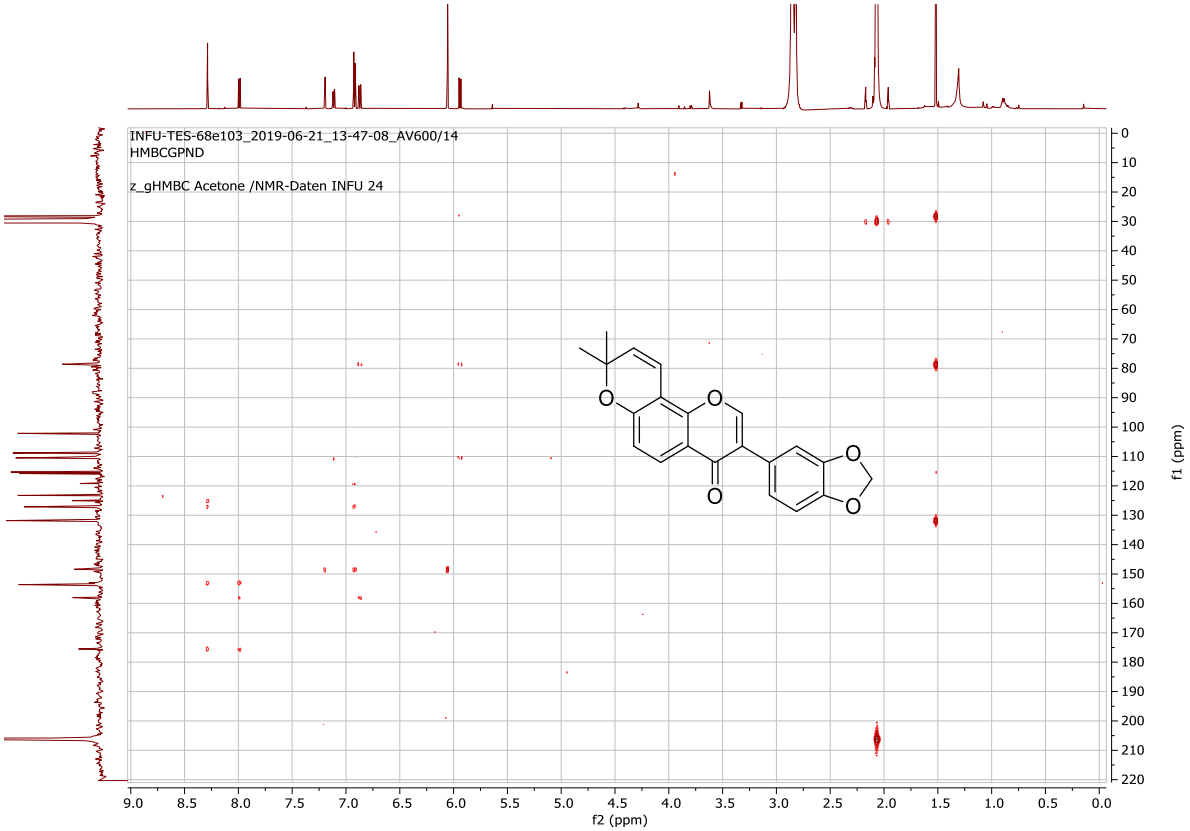
^{1}H-^{1}H-COSY spectrum of compound 46



HSQC spectrum of compound 46

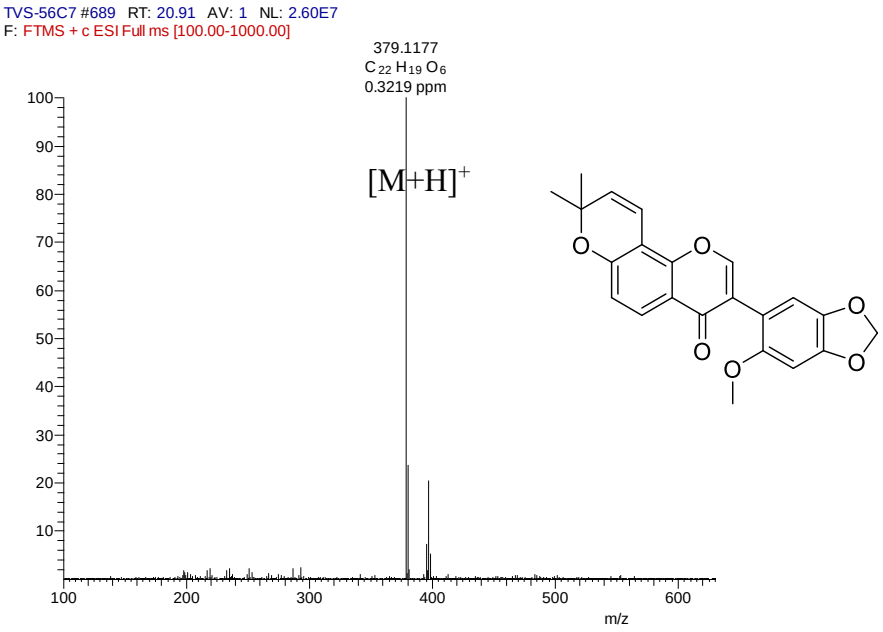


HMBC spectrum of compound 46

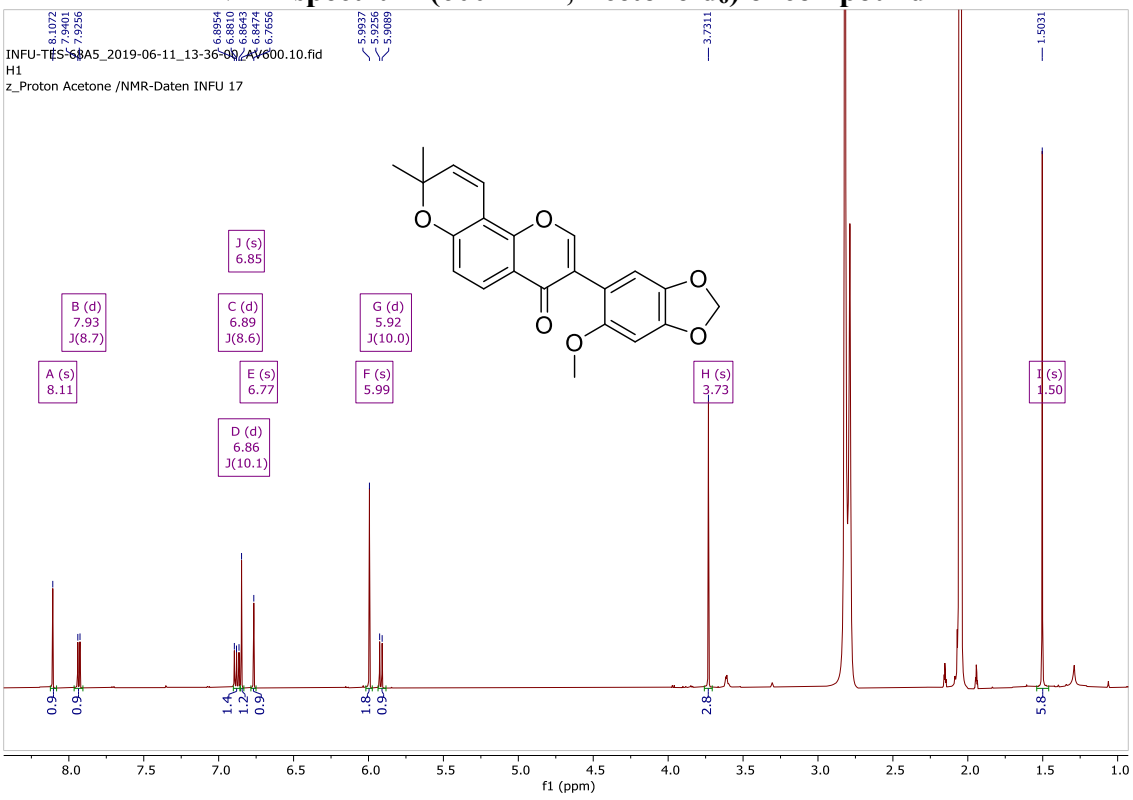


Appendix A47: Spectra for compound 47

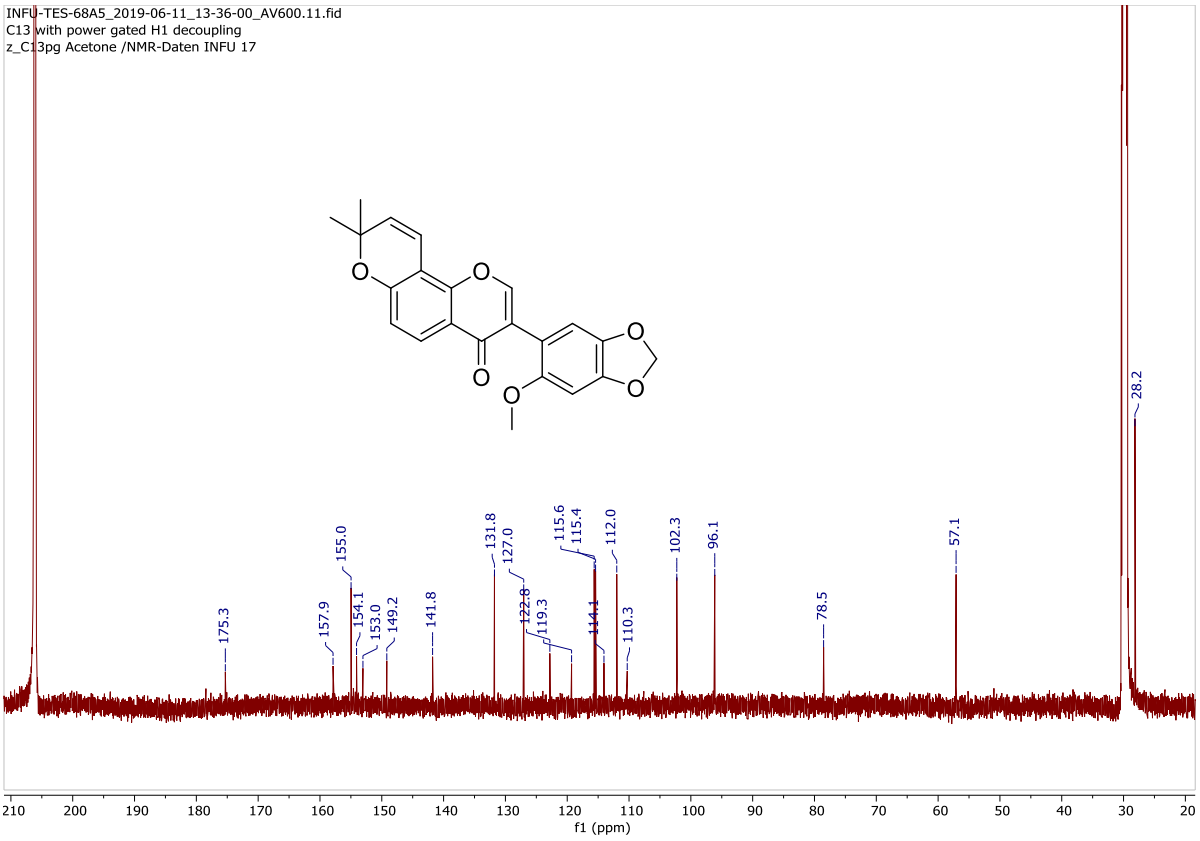
HRESIMS spectrum of compound 47



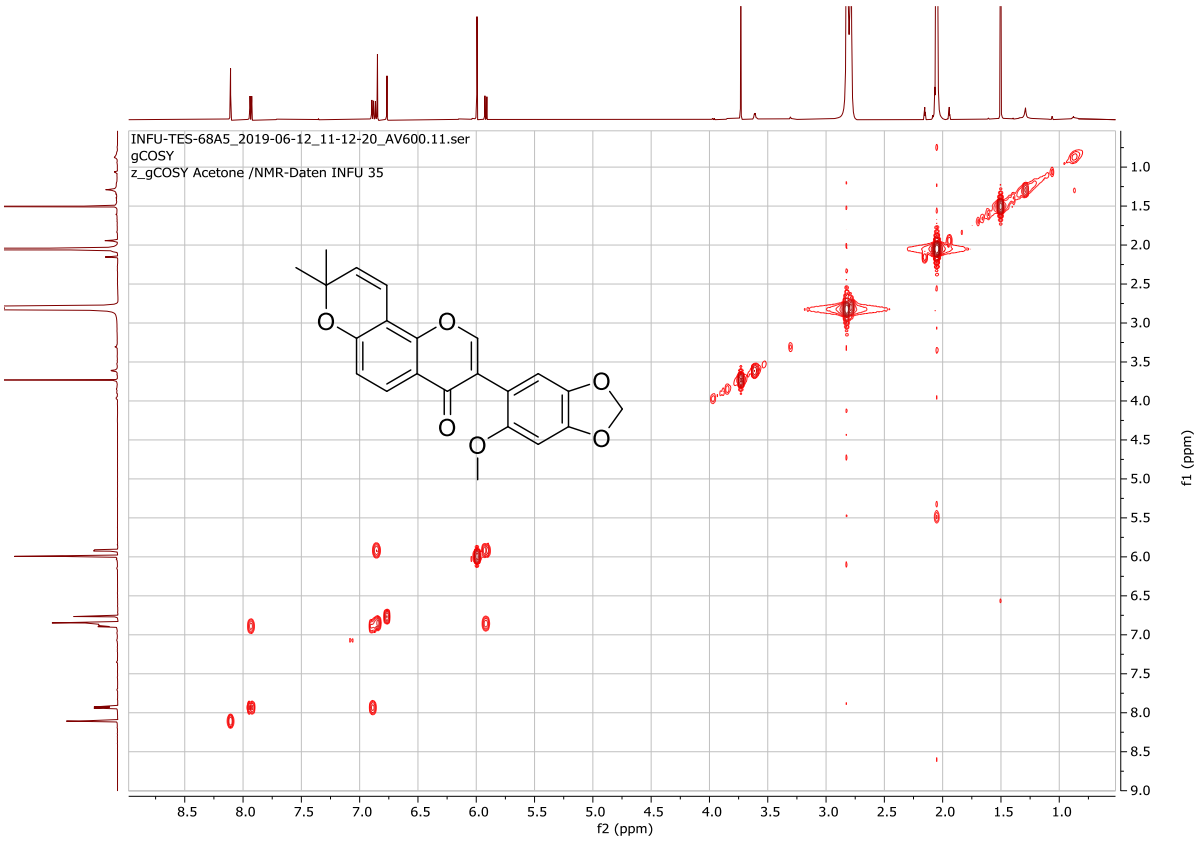
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 47



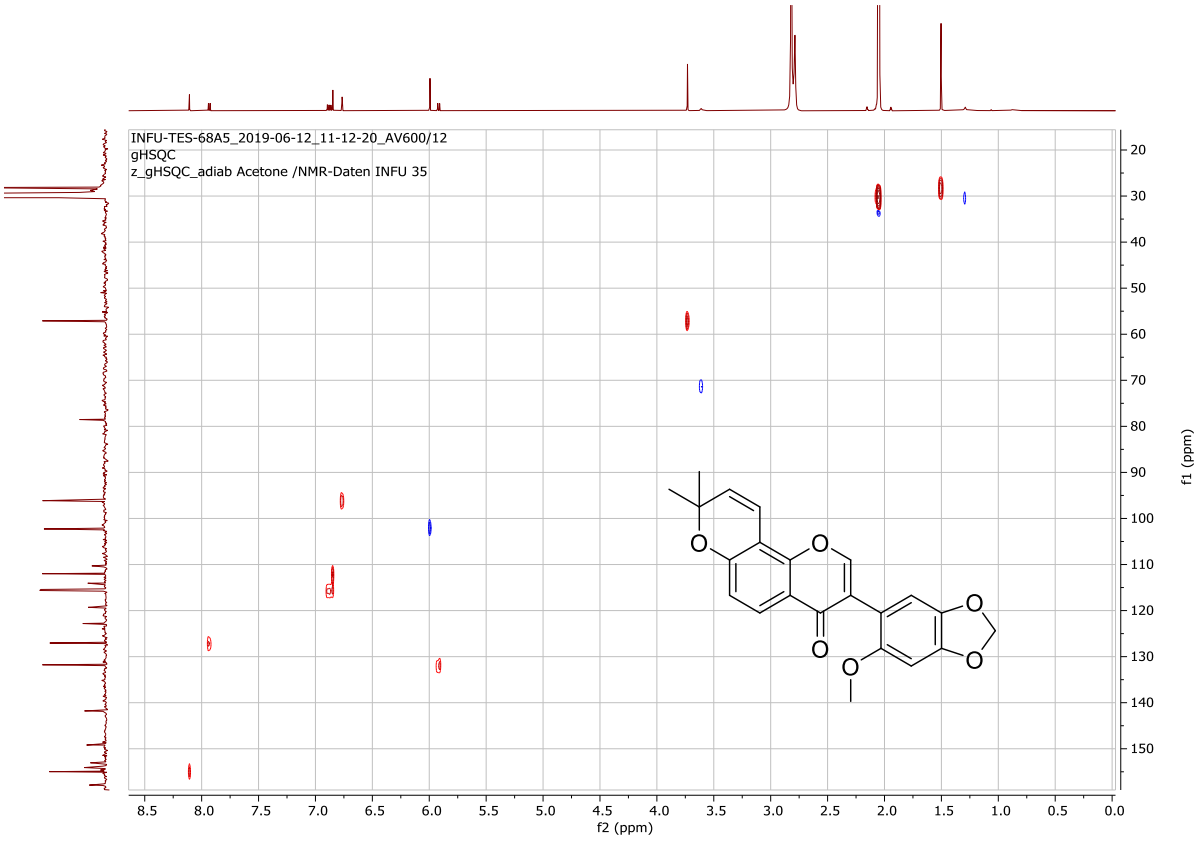
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 47



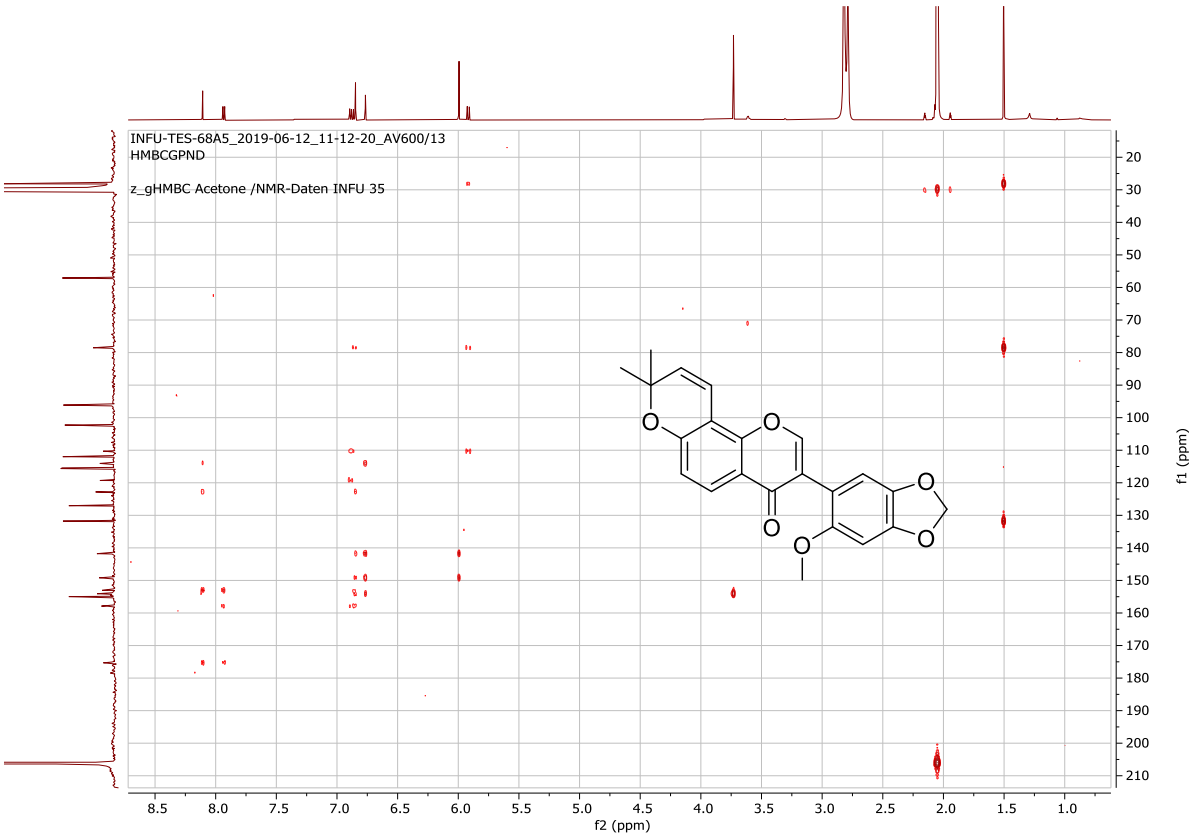
^{1}H-^{1}H-COSY spectrum of compound 47



HSQC spectrum of compound 47

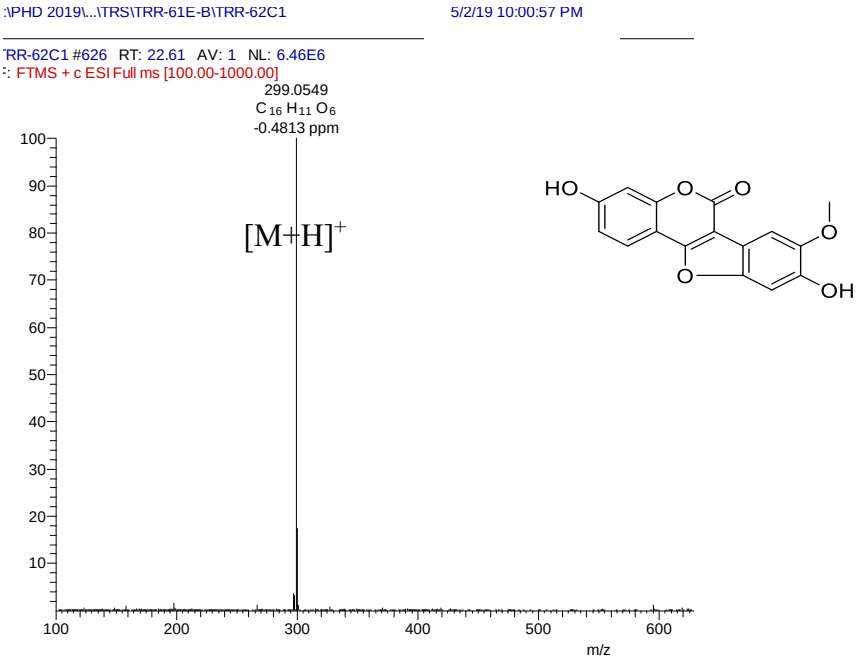


HMBC spectrum of compound 47

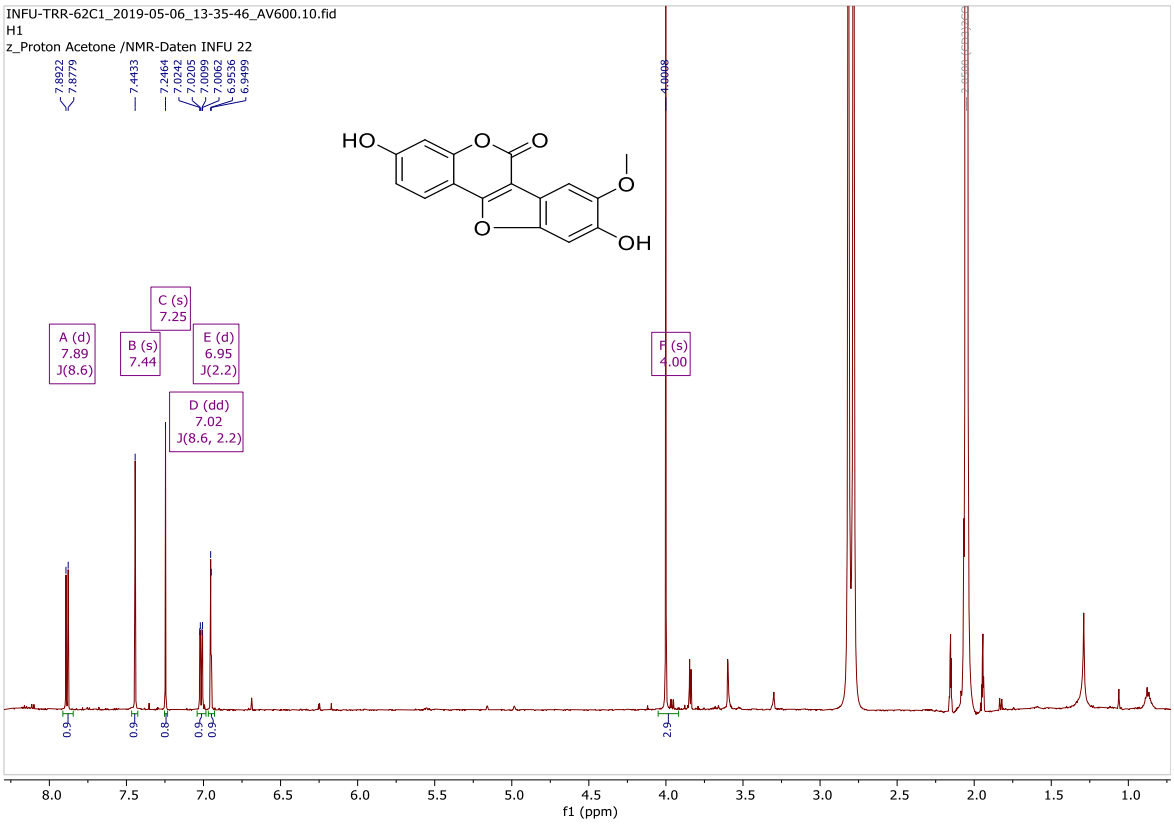


Appendix A48: Spectra for compound 48

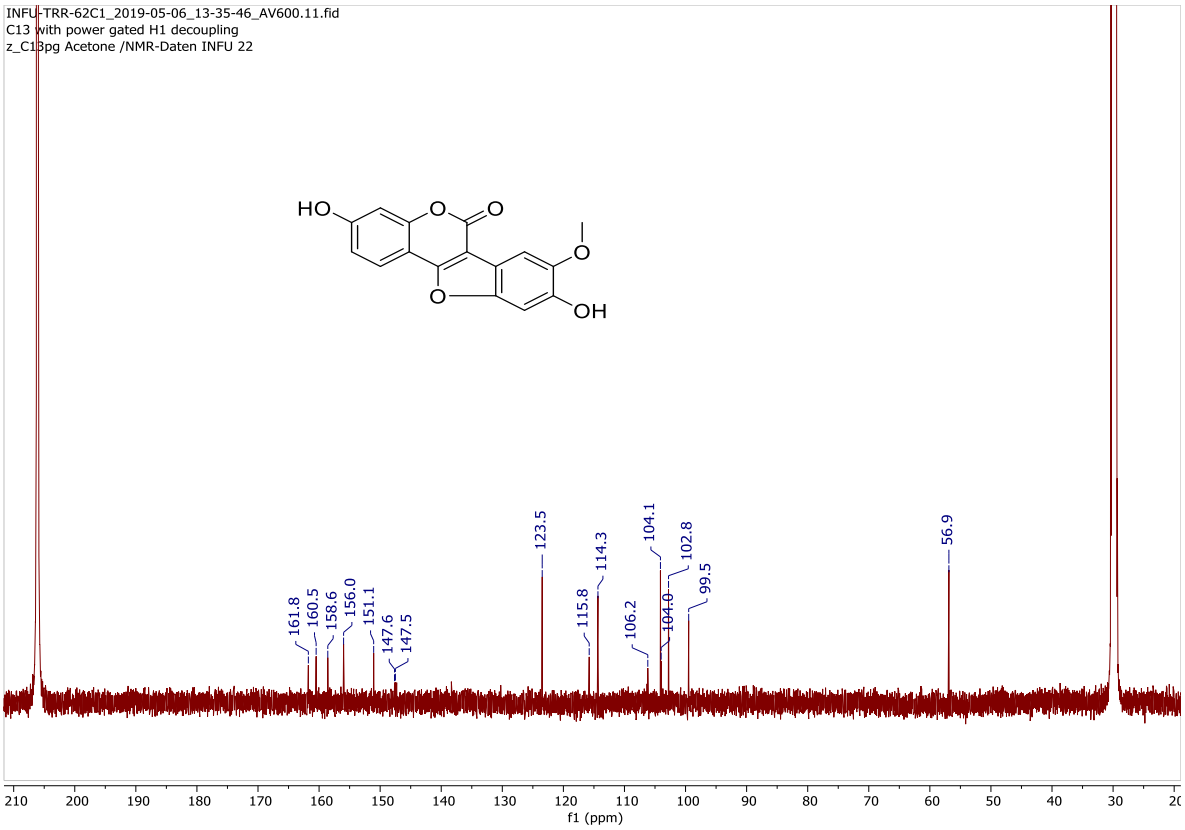
HRESIMS spectrum of compound 48



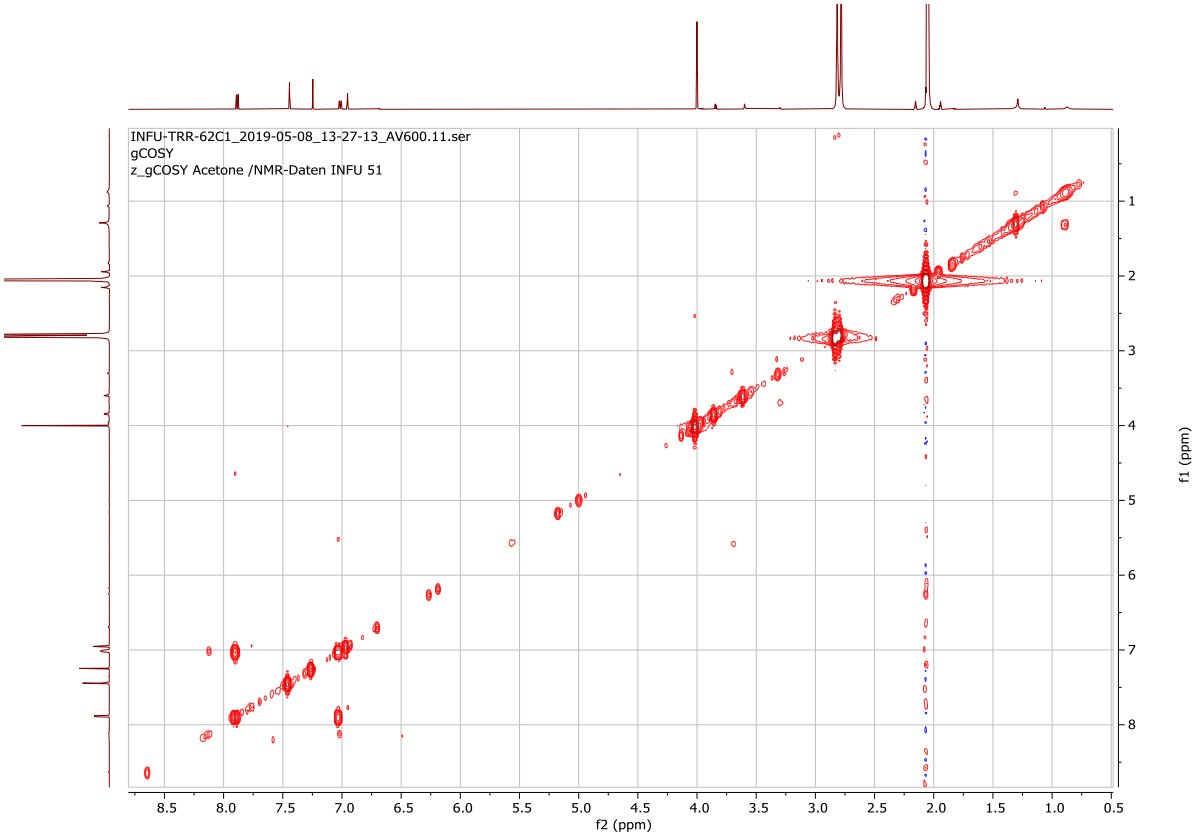
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 48



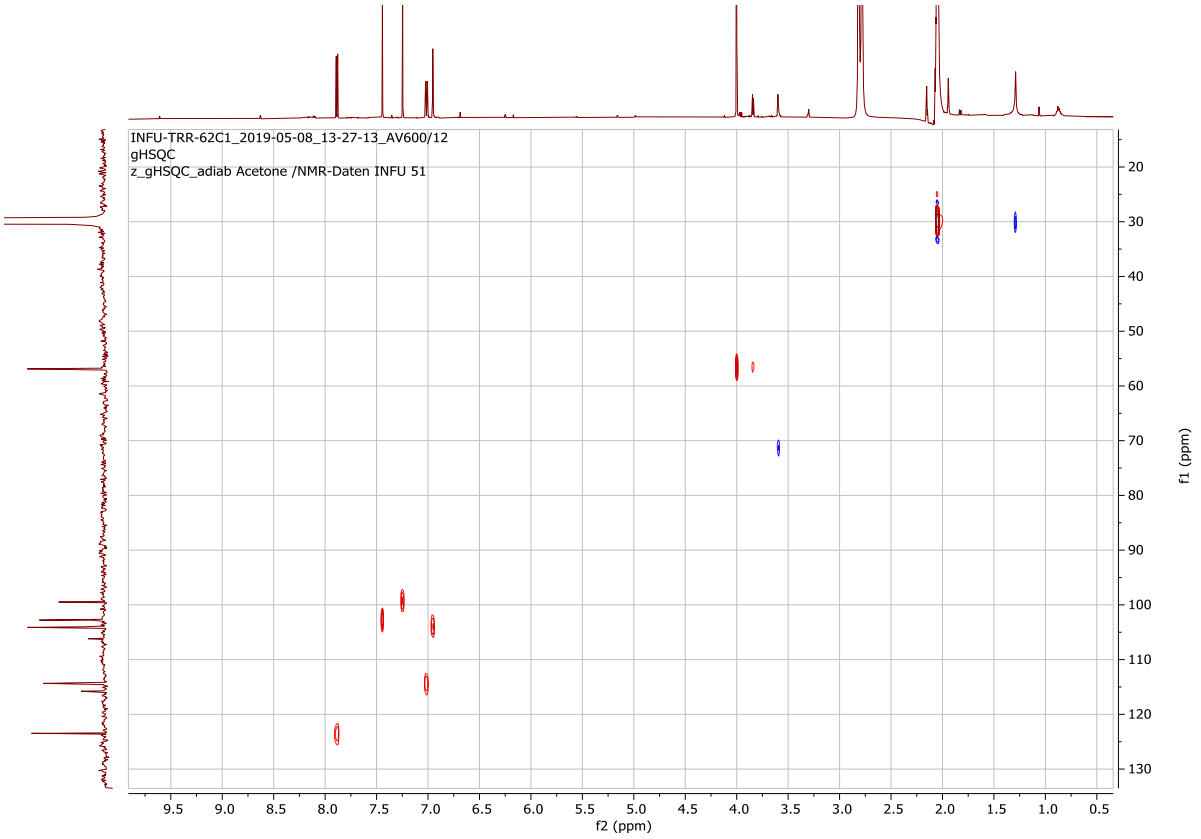
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 48



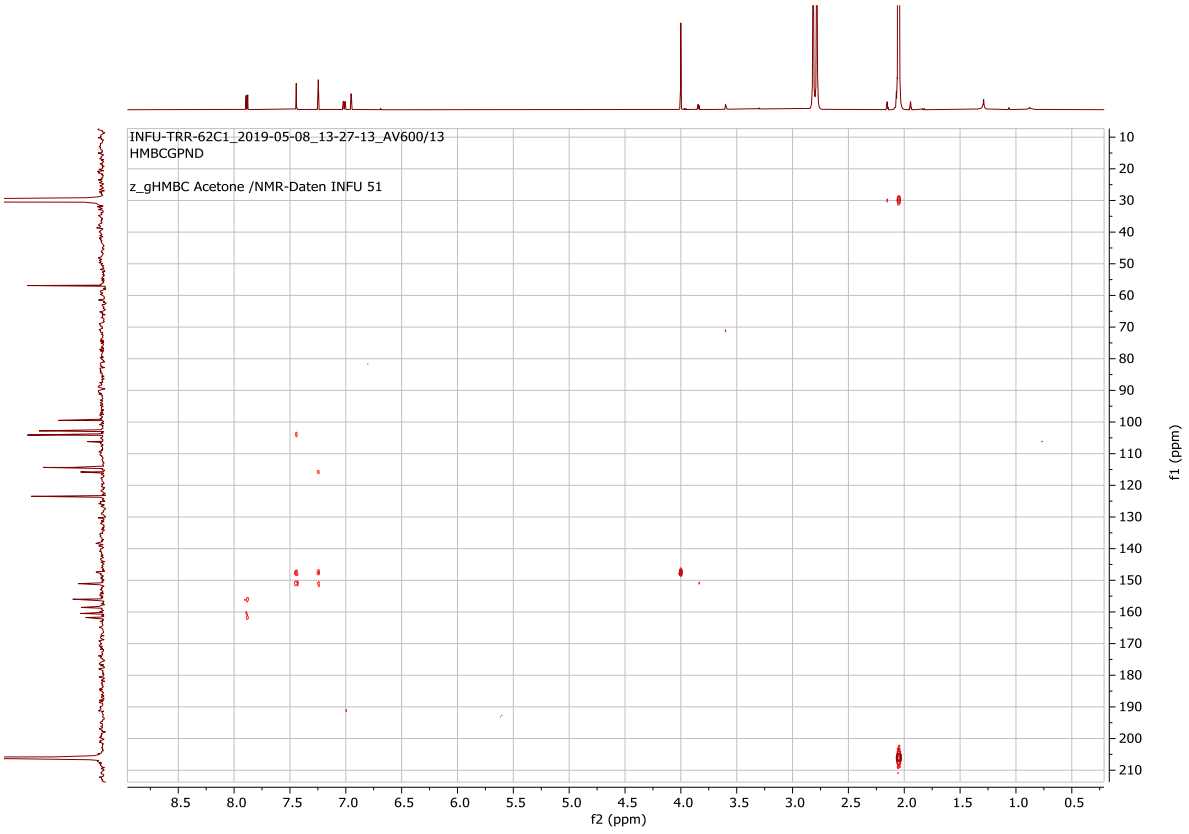
^{1}H-^{1}H-COSY spectrum of compound 48



HSQC spectrum of compound 48

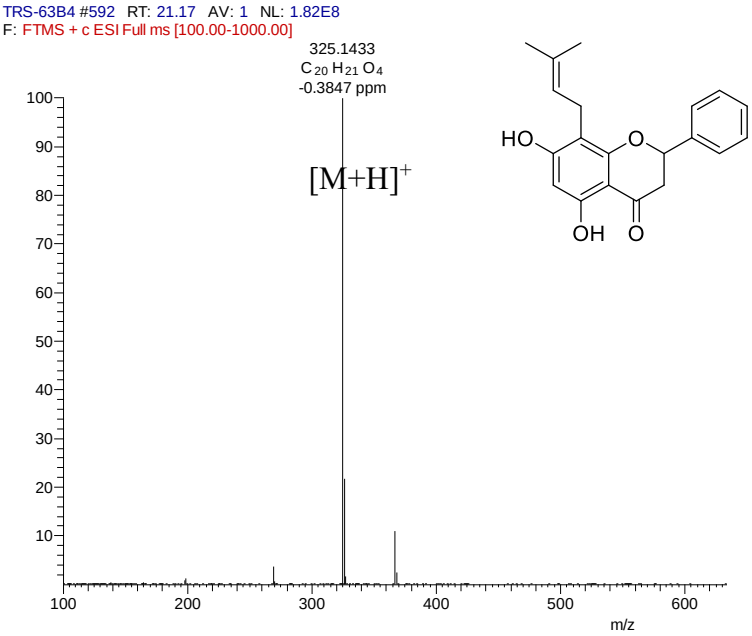


HMBC spectrum of compound 48

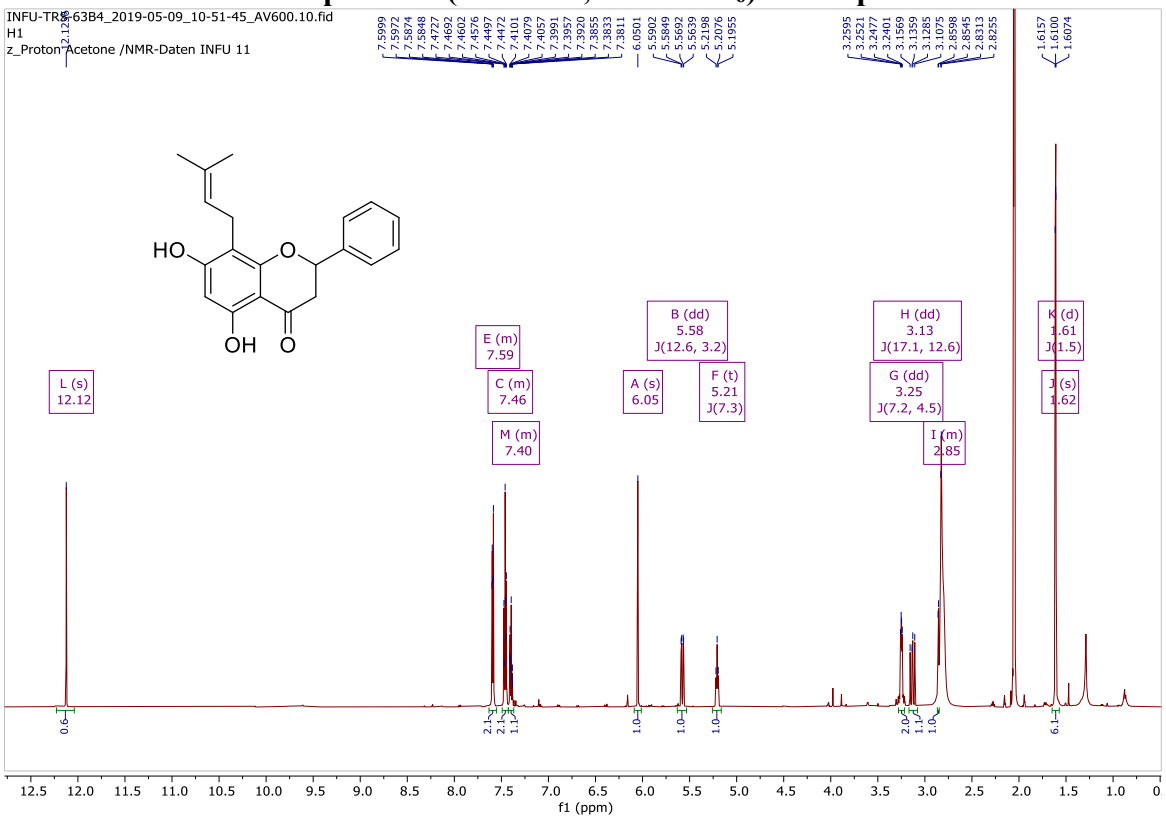


Appendix A49: Spectra for compound 49

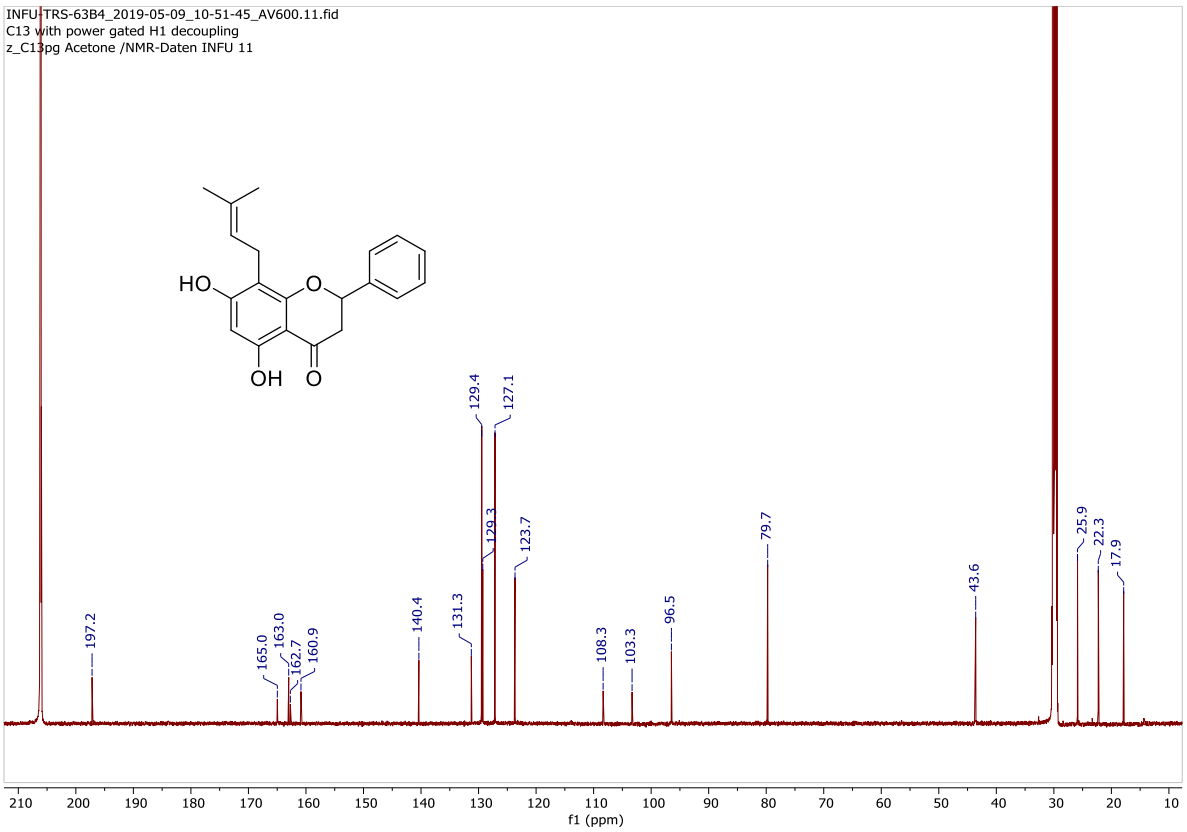
HRESIMS spectrum of compound 49



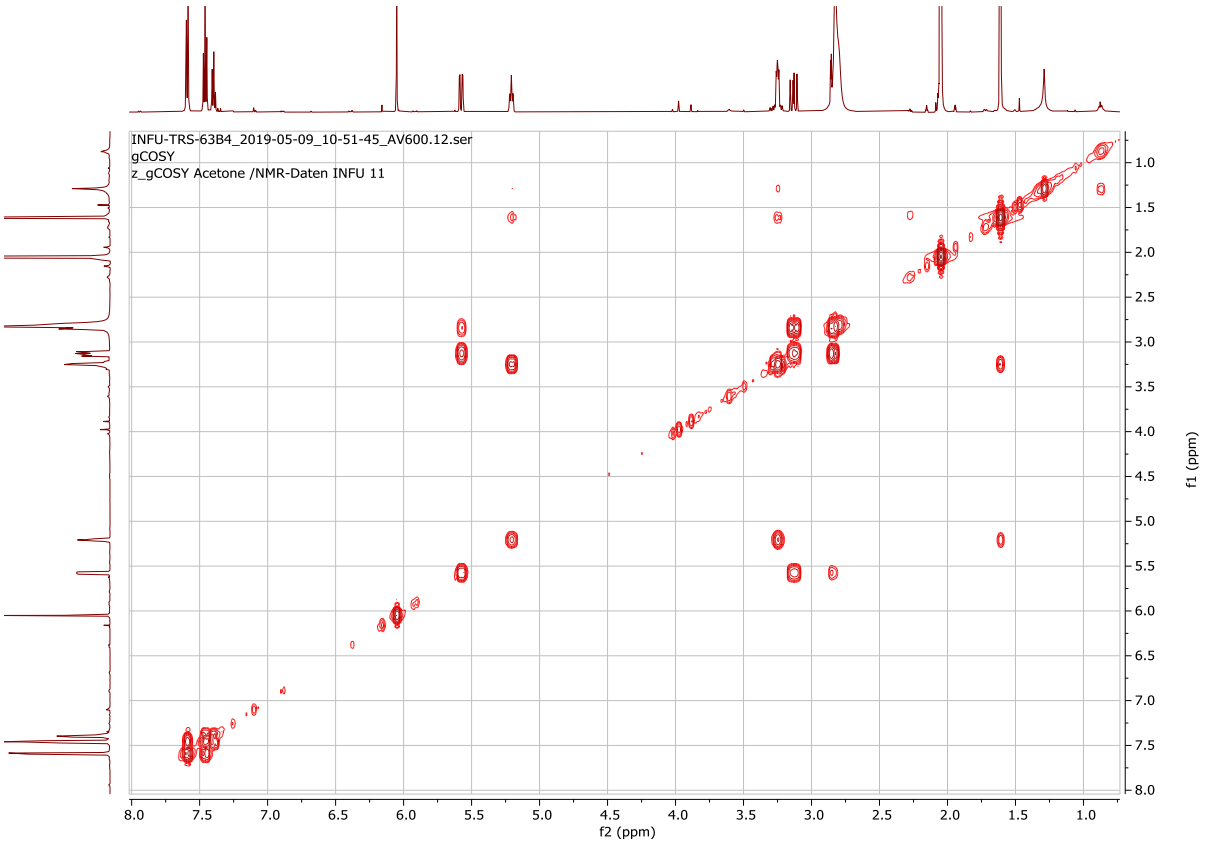
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 49



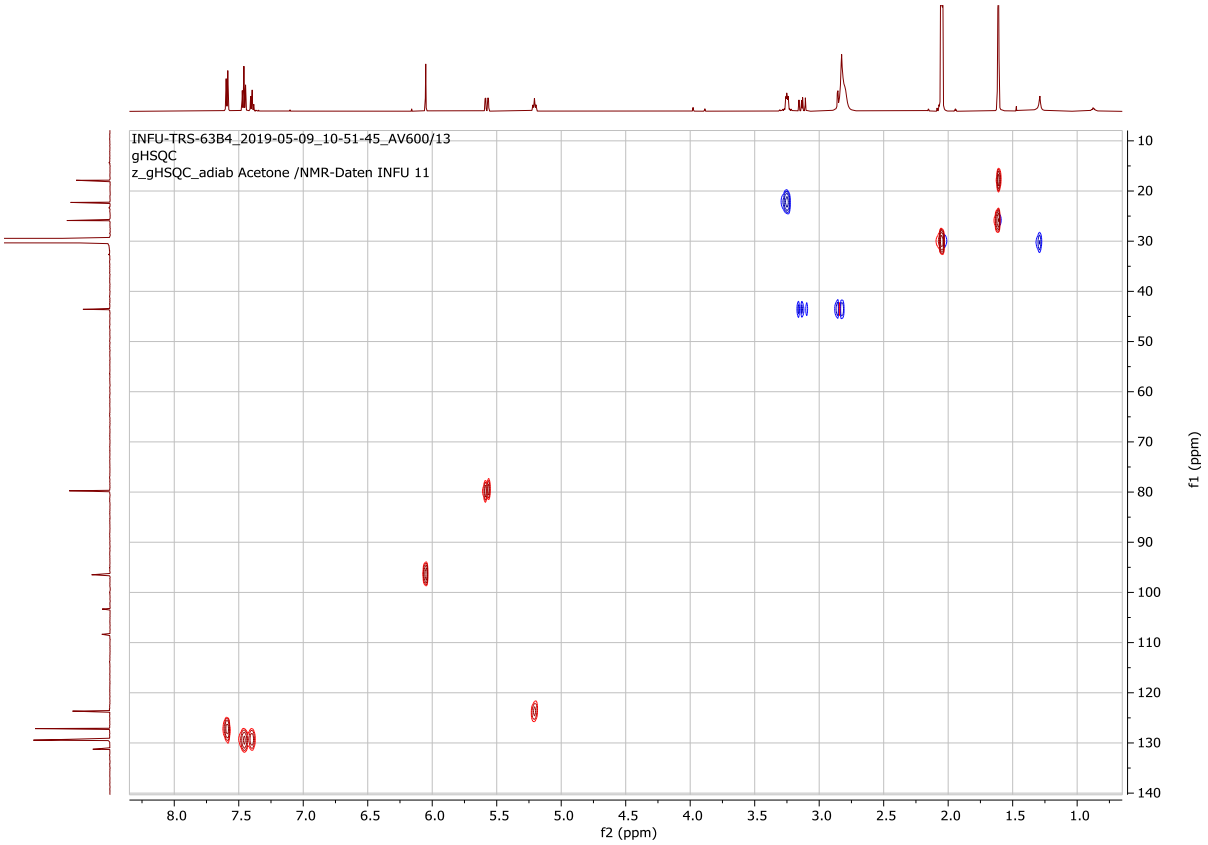
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 49



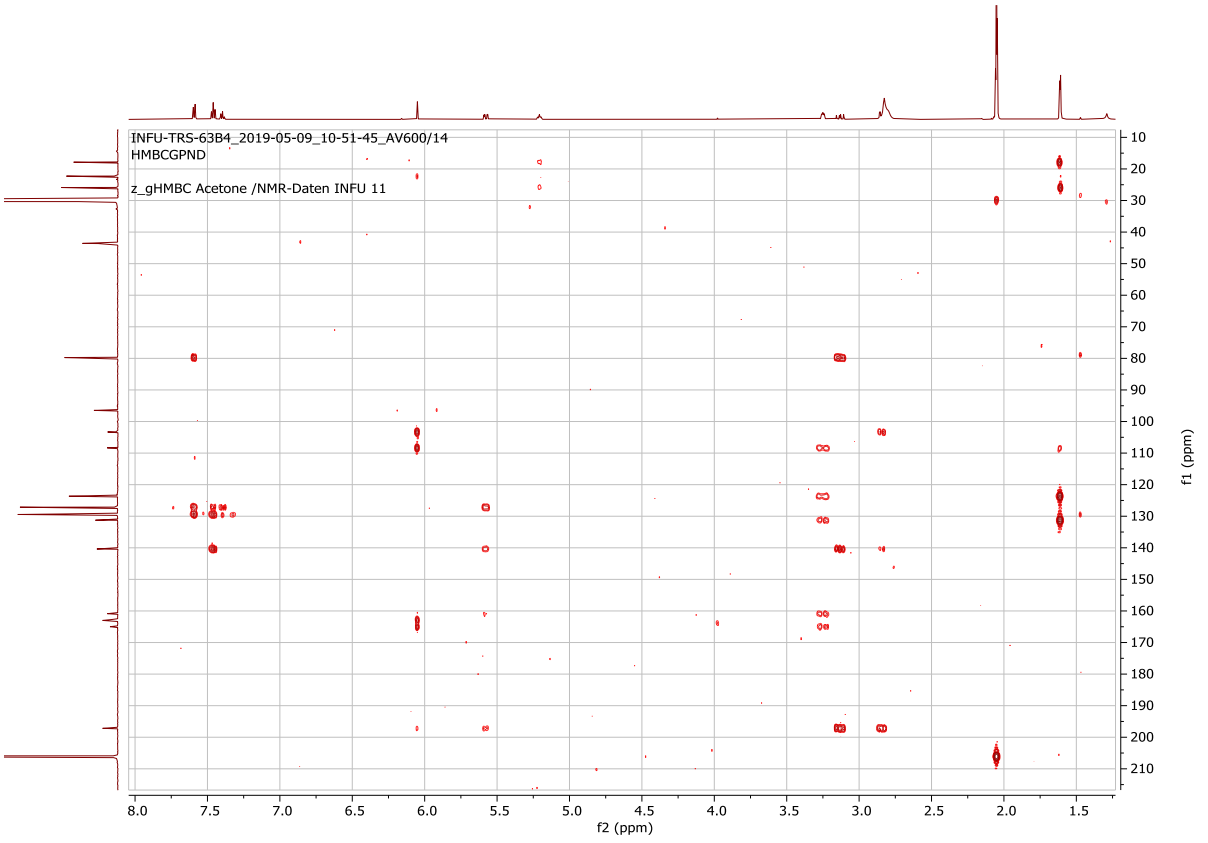
^{1}H-^{1}H-COSY spectrum of compound 49



HSQC spectrum of compound 49

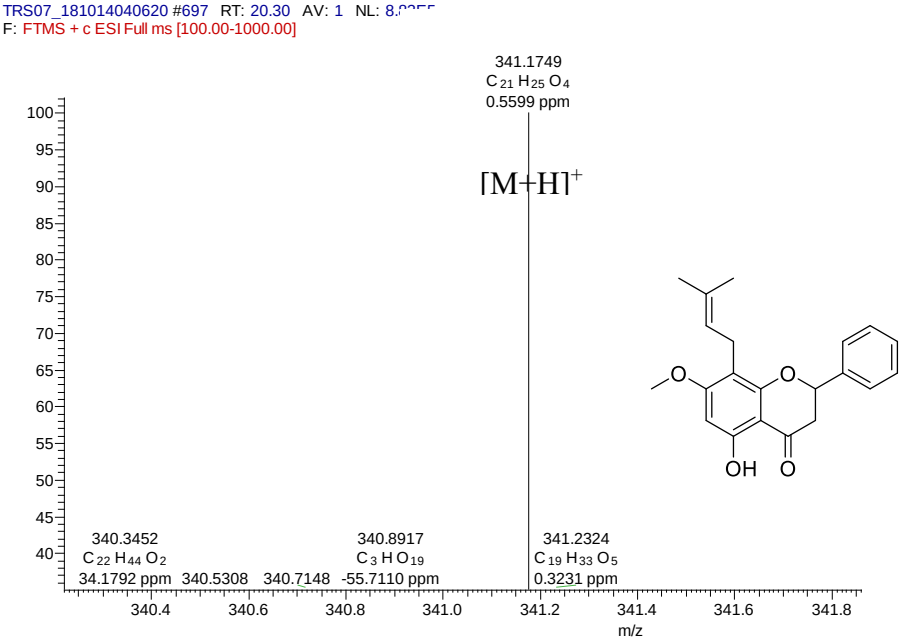


HMBC spectrum of compound 49

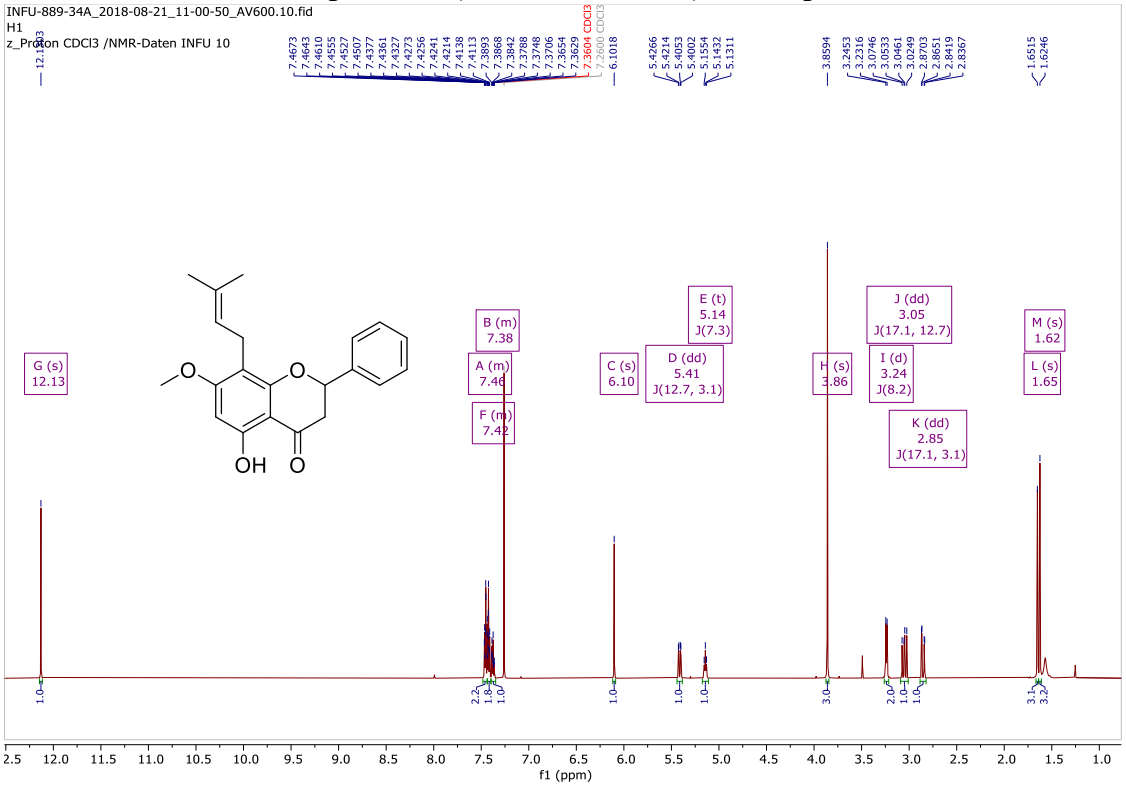


Appendix A50: Spectra for compound 50

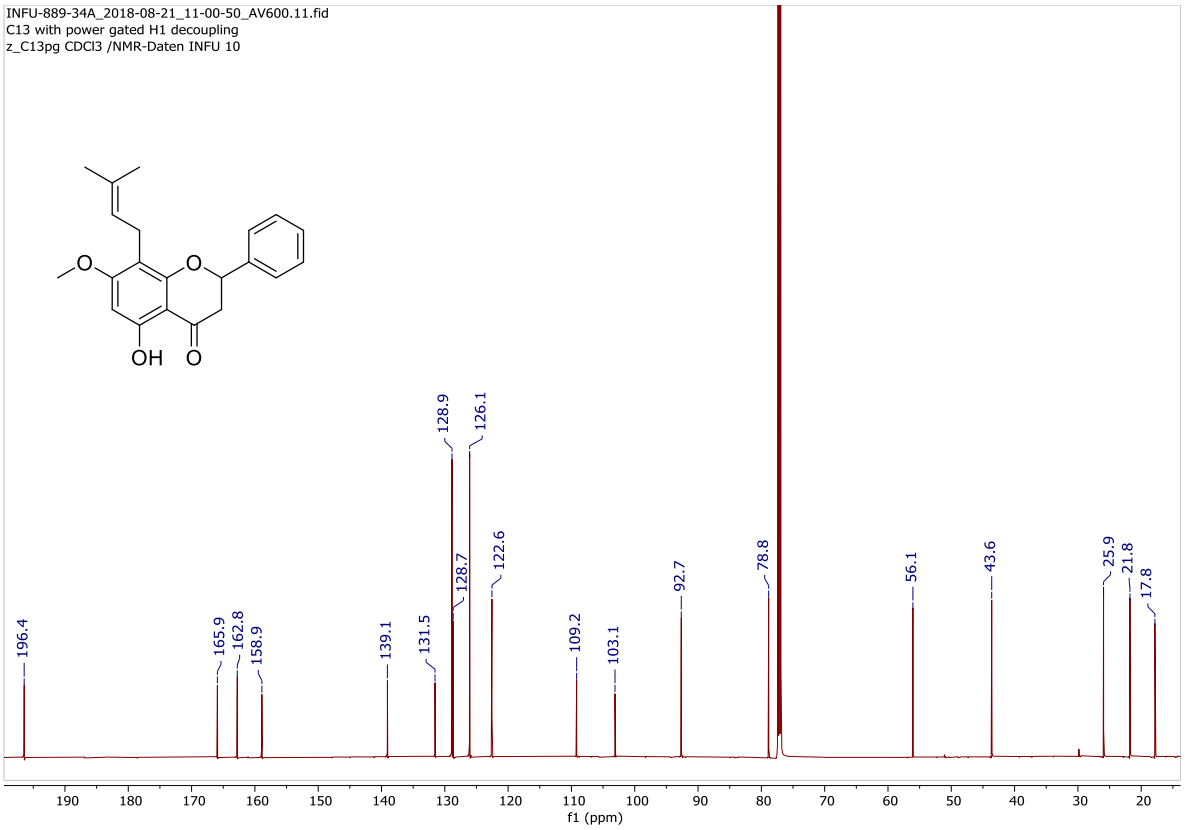
HRESIMS spectrum of compound 50



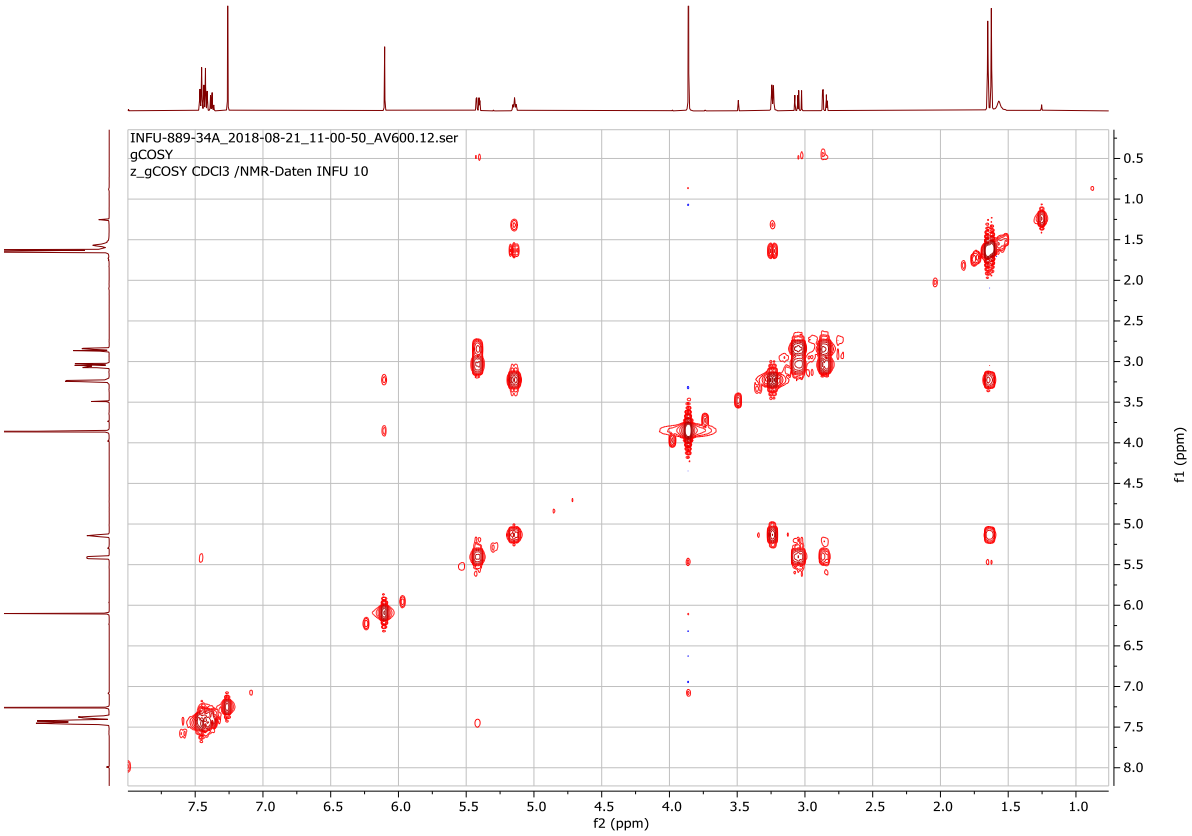
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound 50



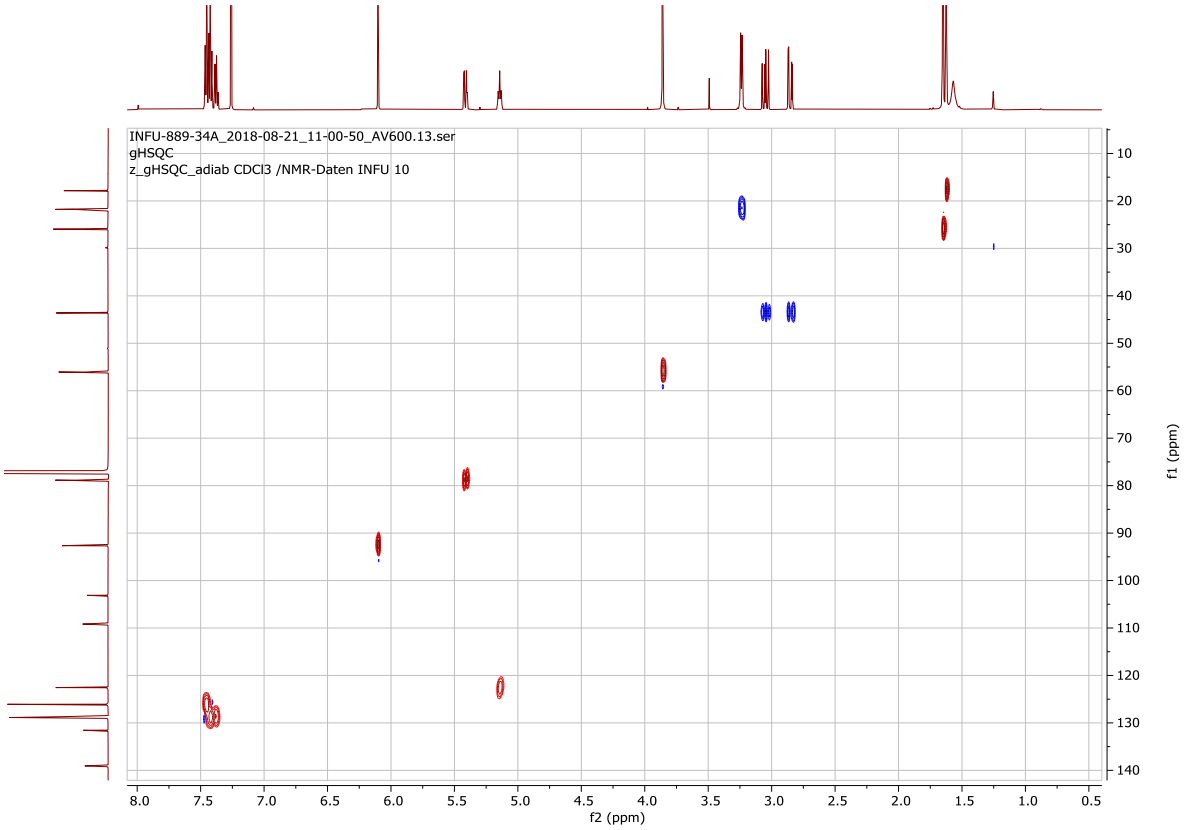
^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound 50



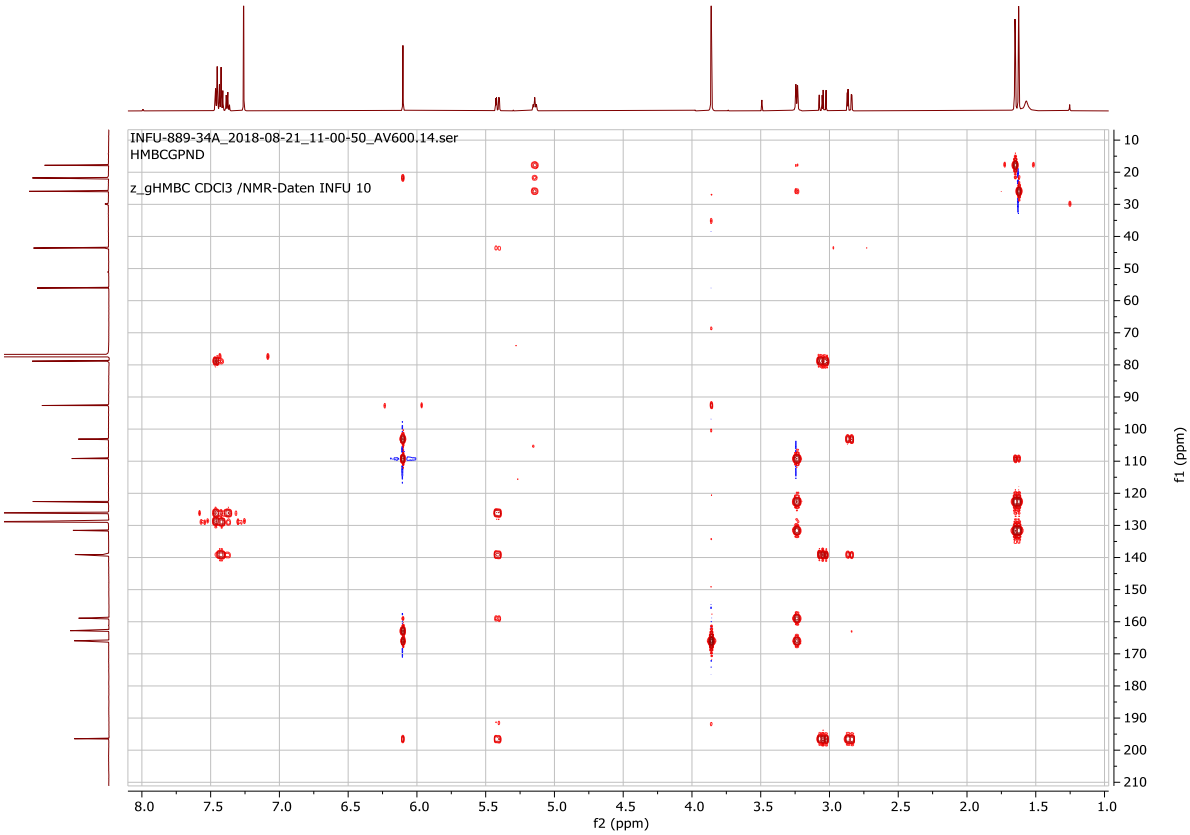
^{1}H-^{1}H-COSY spectrum of compound 50



HSQC spectrum of compound 50

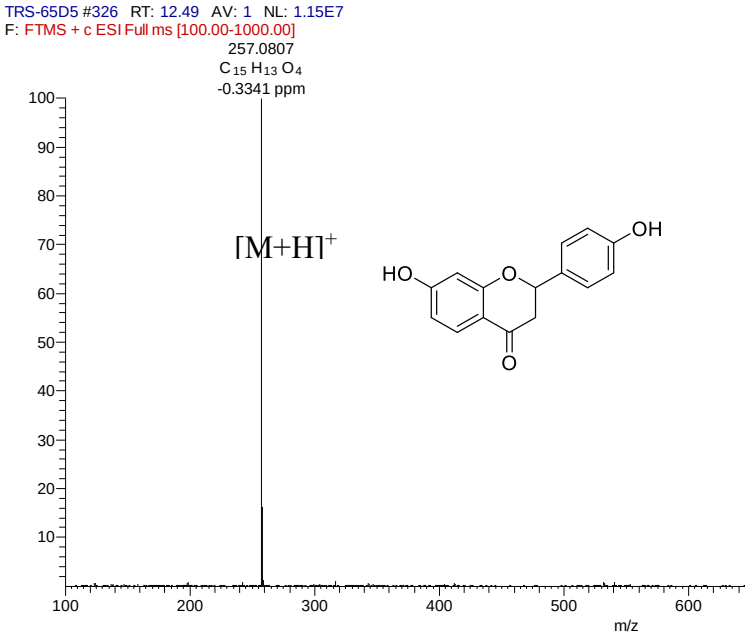


HMBC spectrum of compound 50

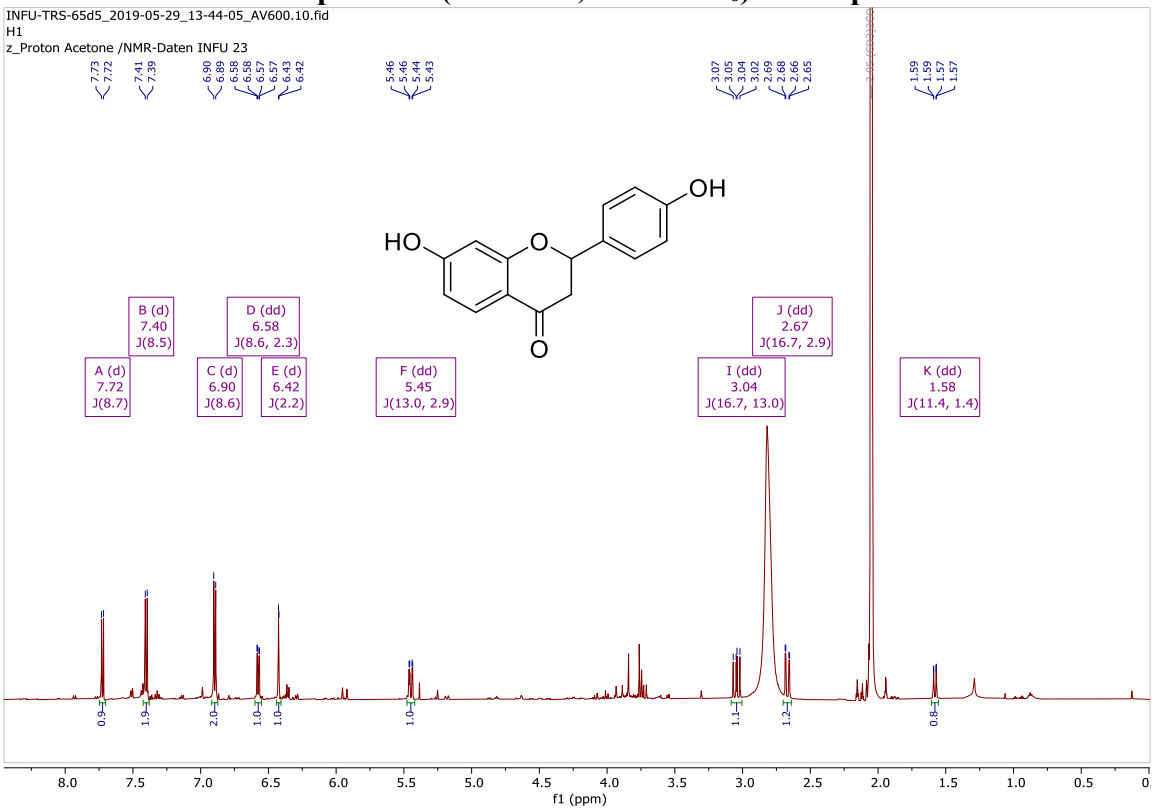


Appendix A51: Spectra for compound 51

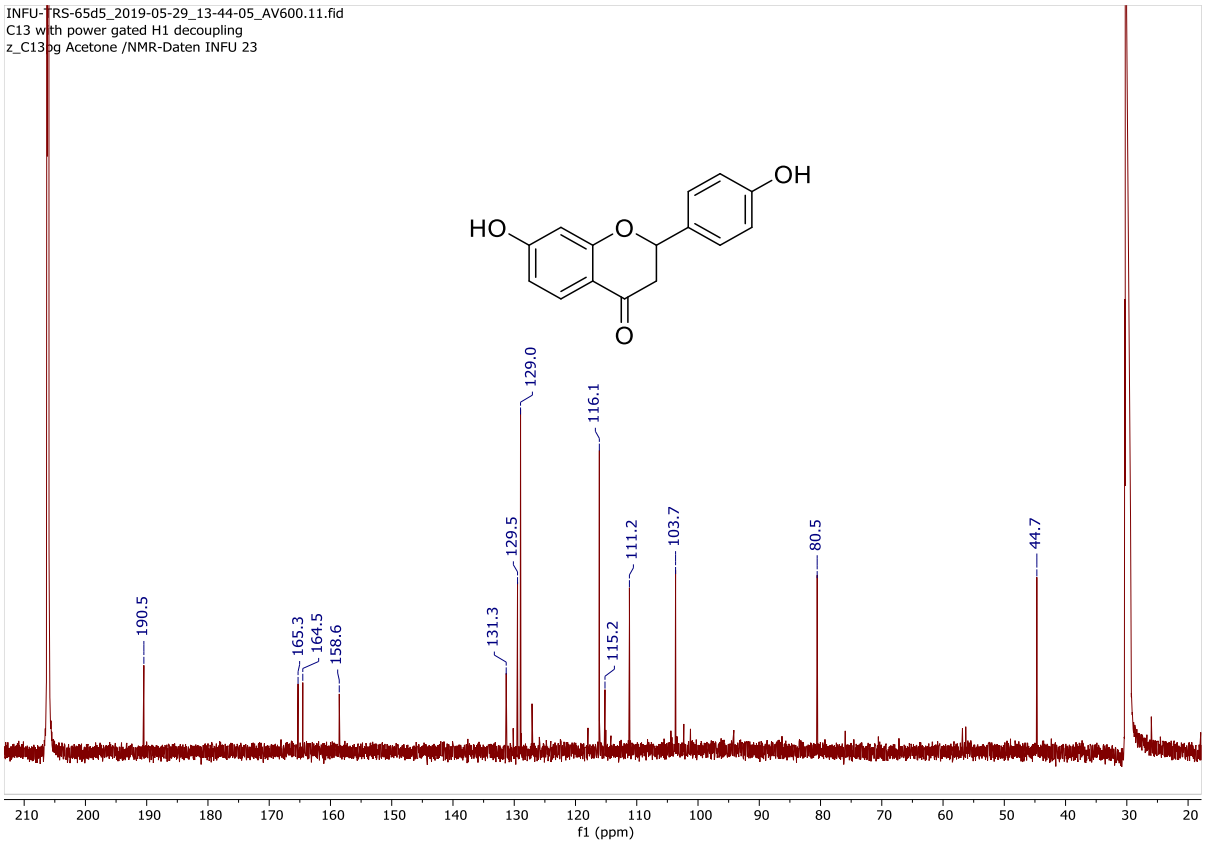
HRESIMS spectrum of compound 51



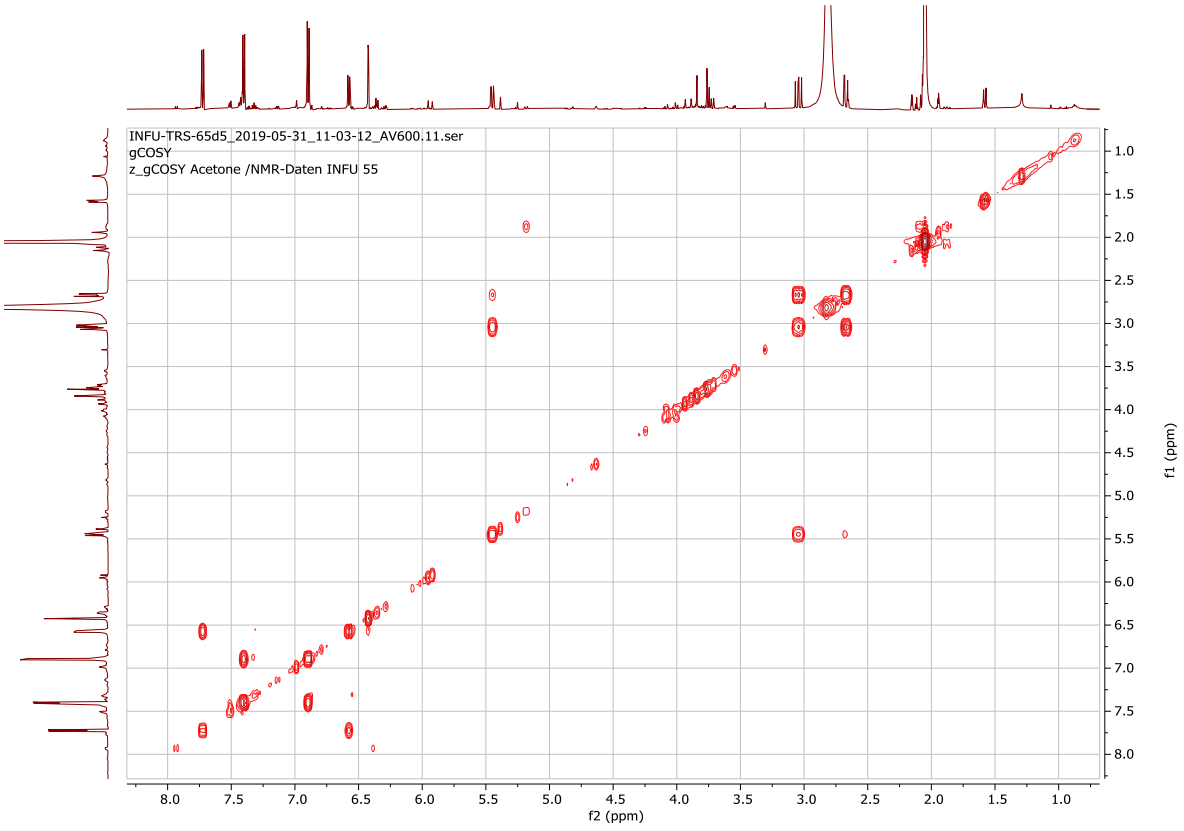
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 51



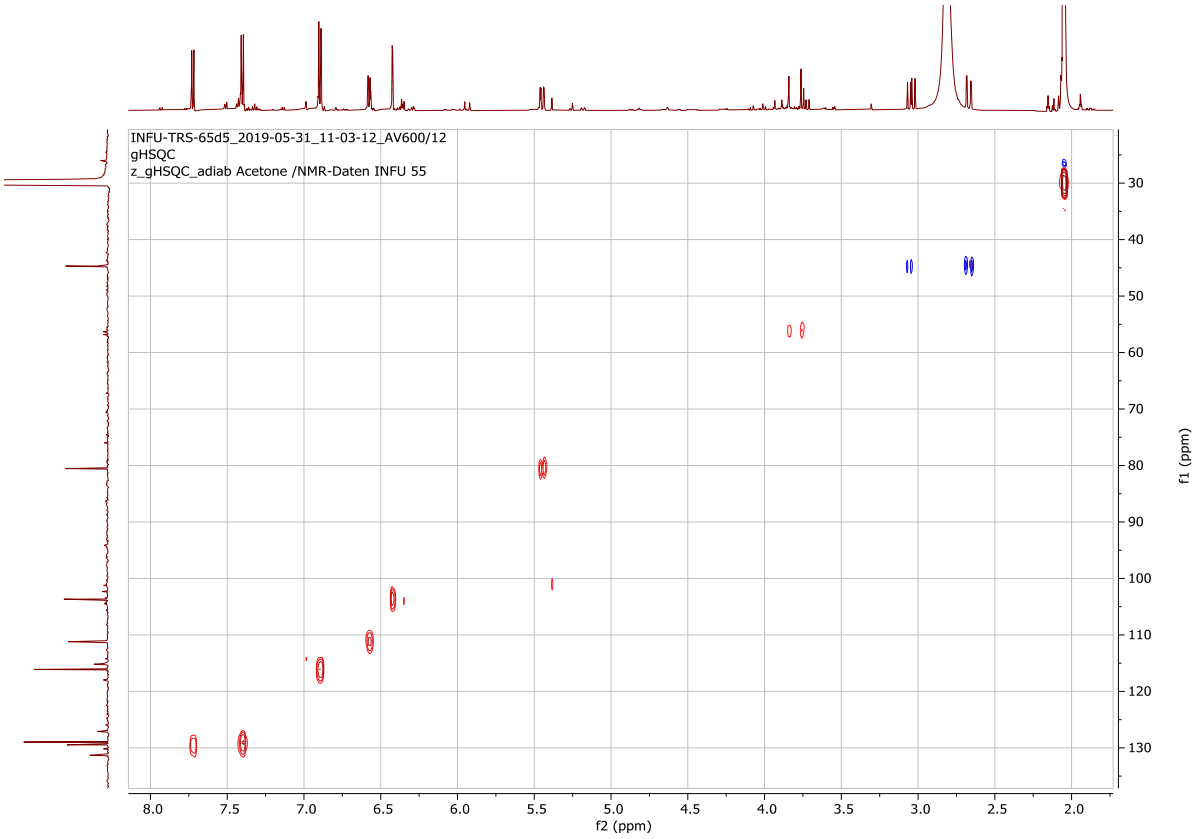
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 51



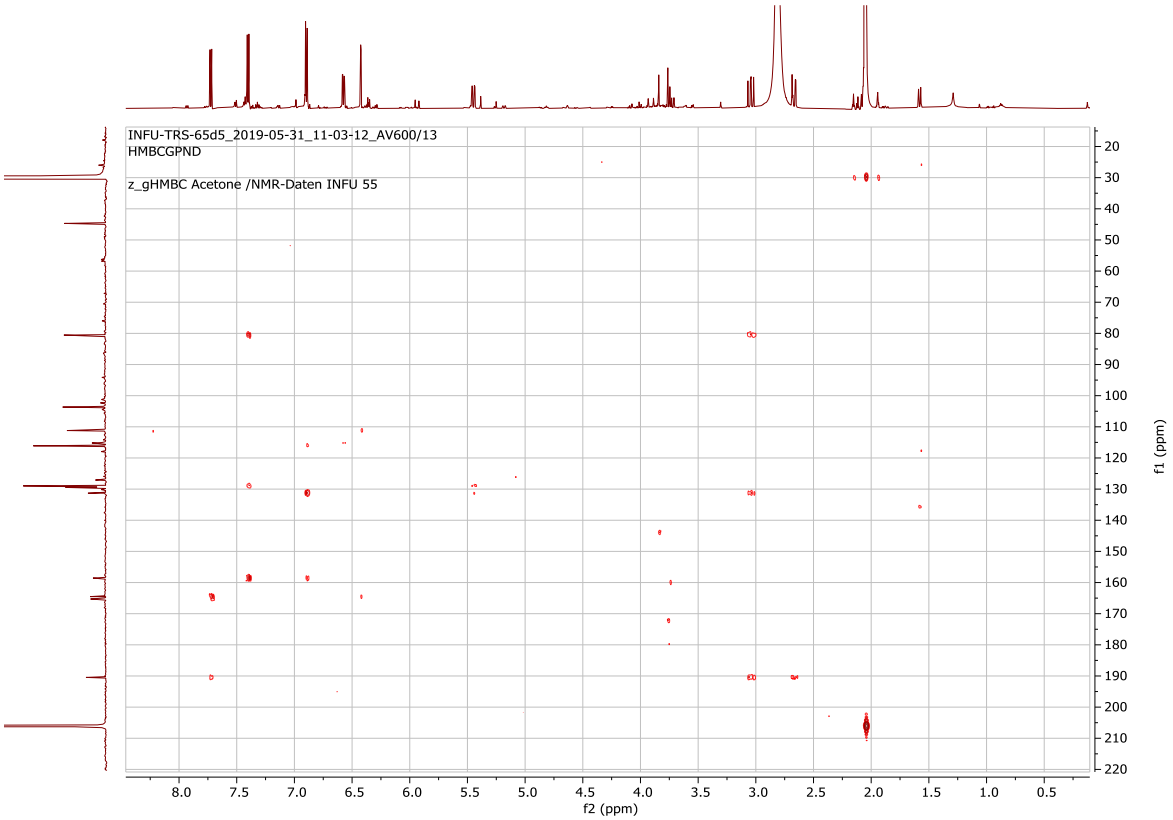
^{1}H-^{1}H-COSY spectrum of compound 51



HSQC spectrum of compound 51

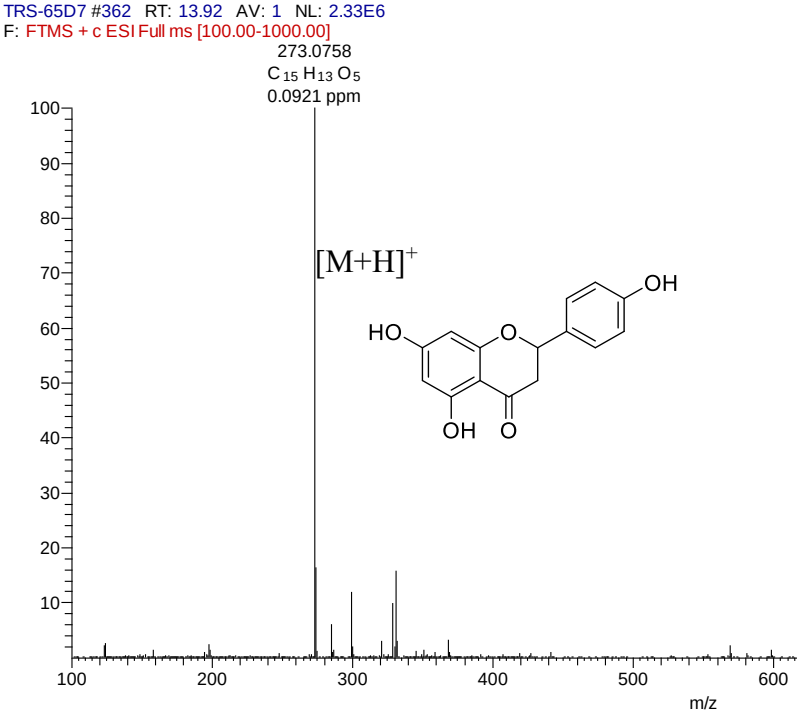


HMBC spectrum of compound 51

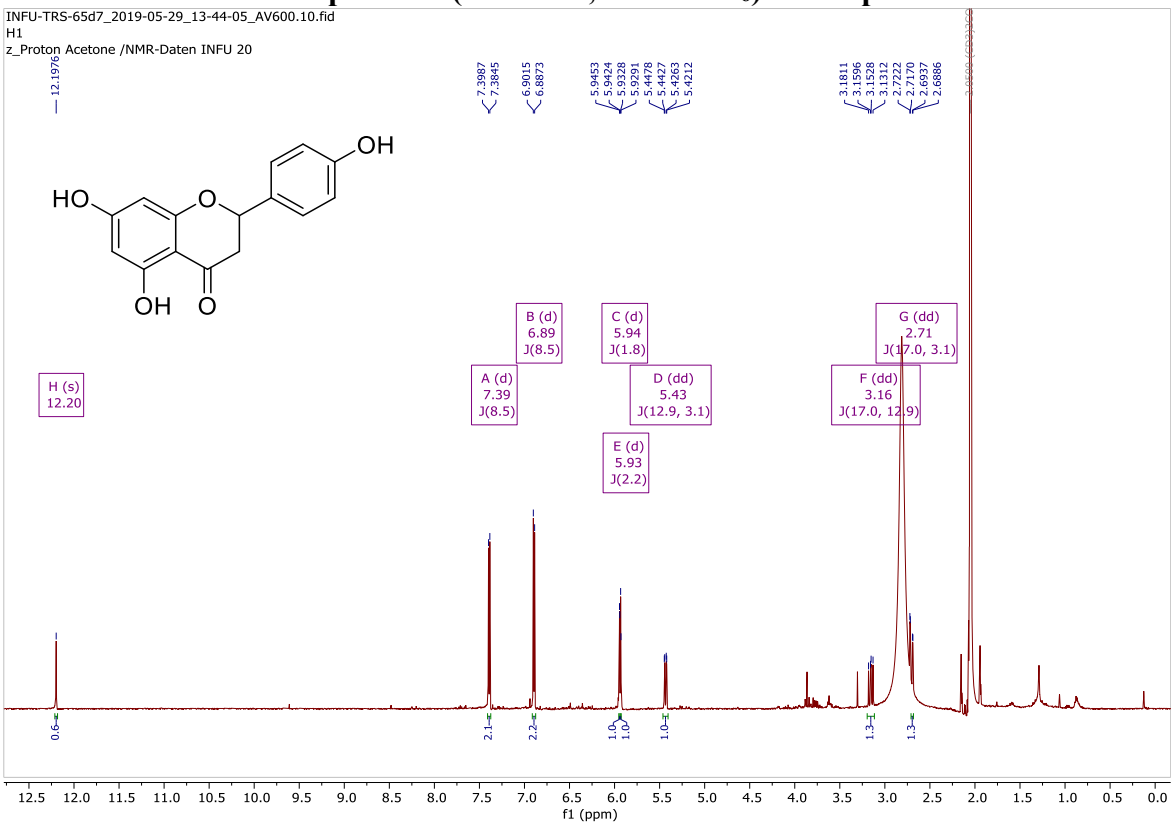


Appendix A52: Spectra for compound 52

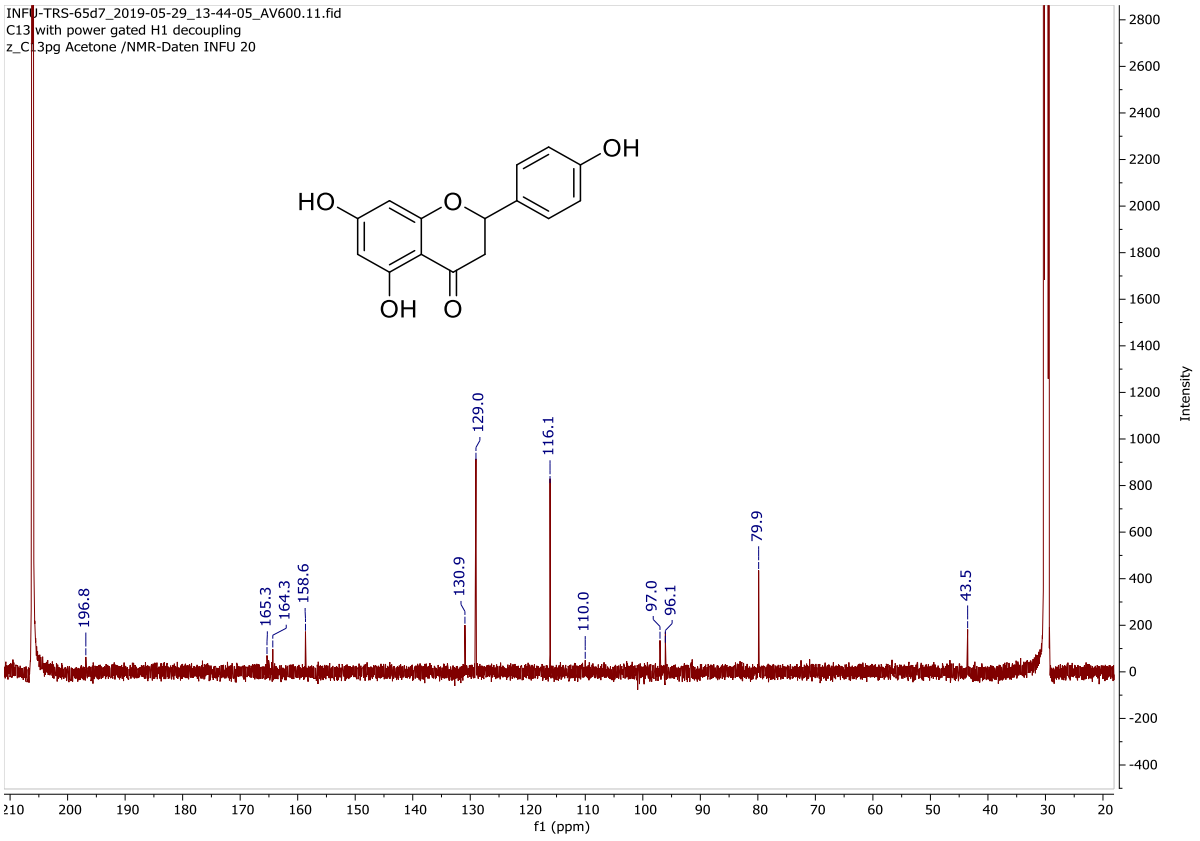
HRESIMS spectrum of compound 52



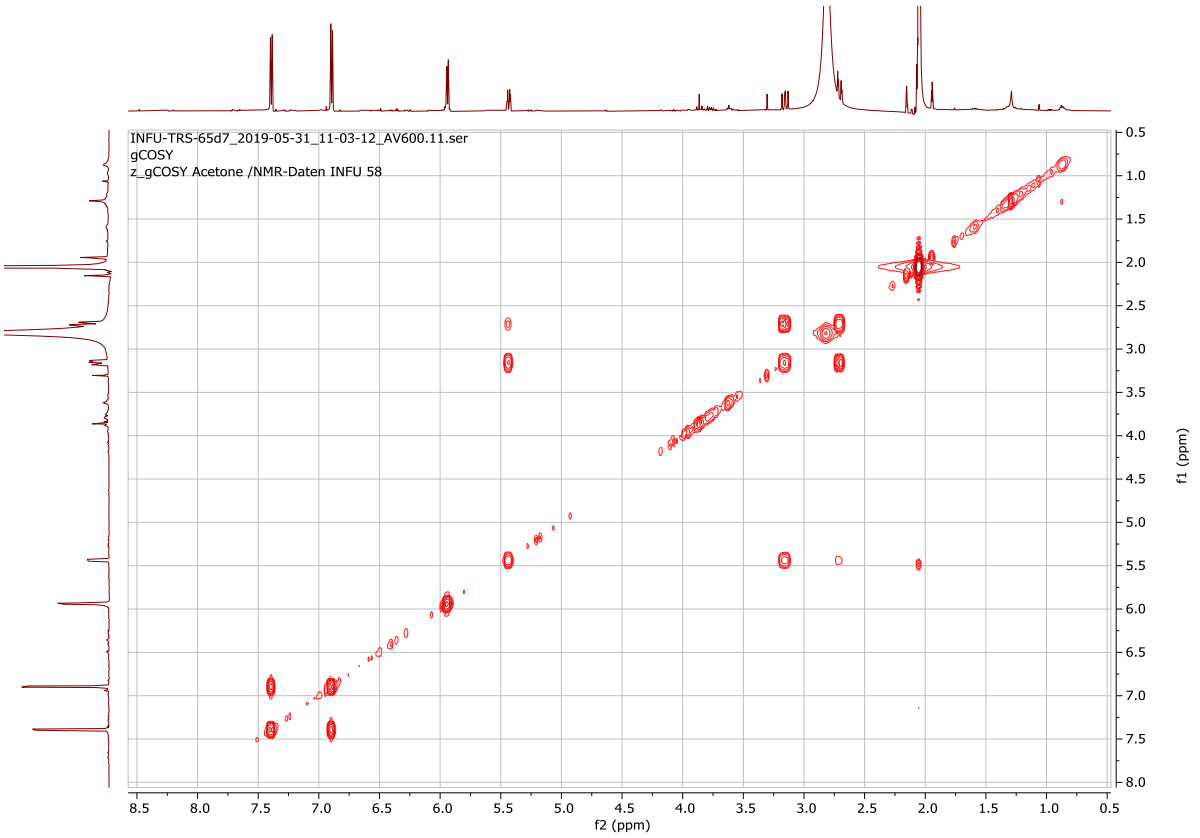
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 52



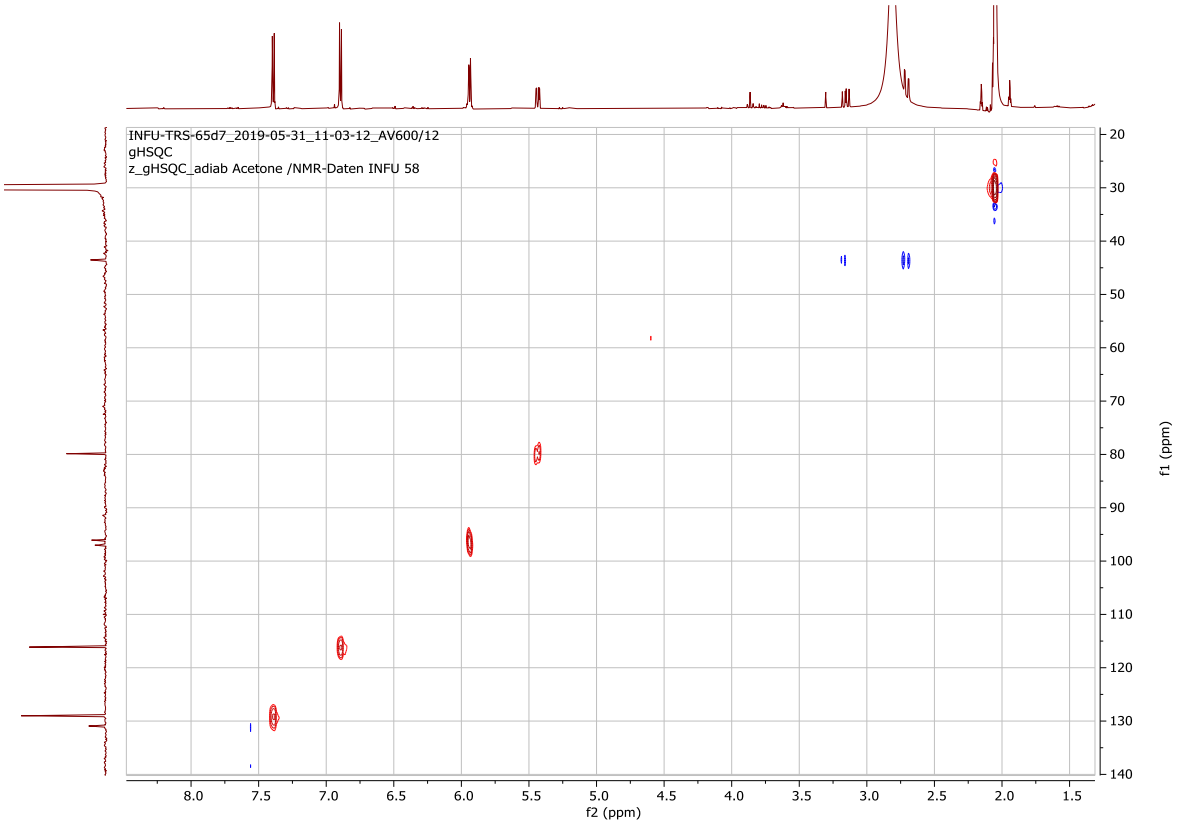
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 52



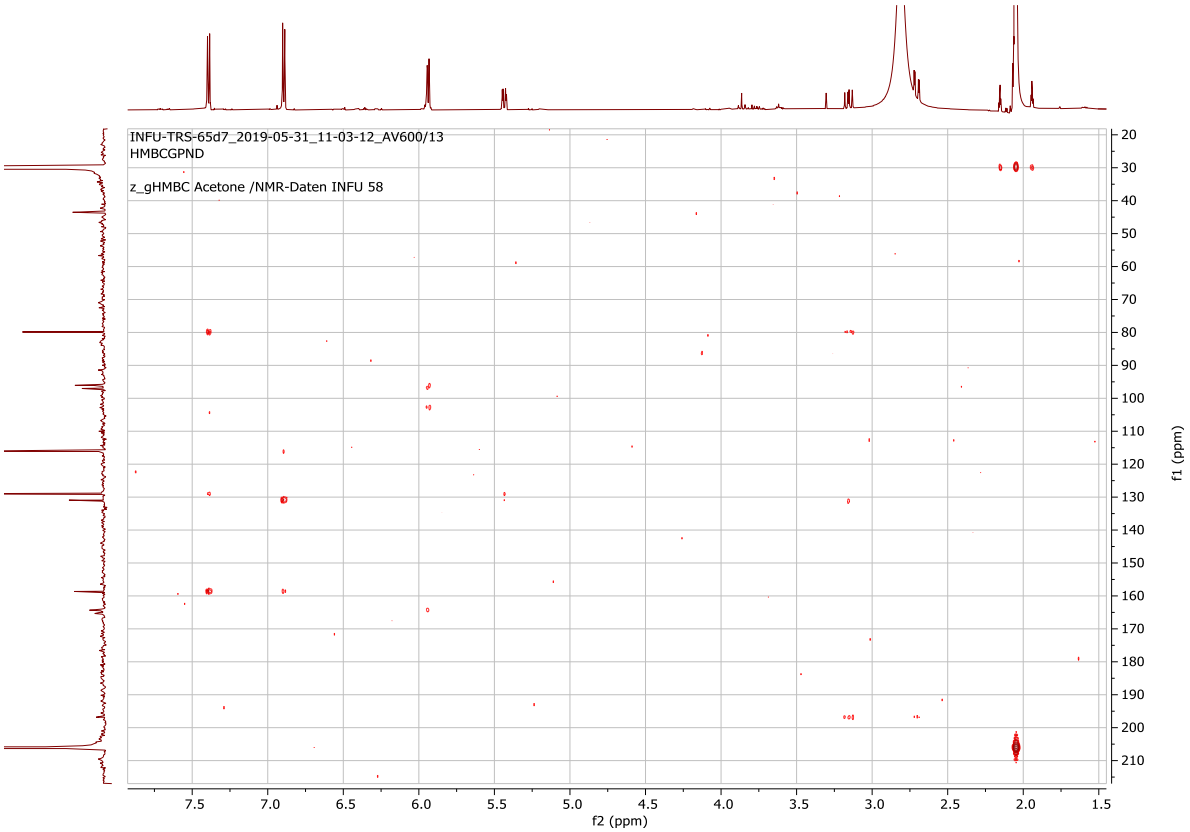
^{1}H-^{1}H-COSY spectrum of compound 52



HSQC spectrum of compound 52

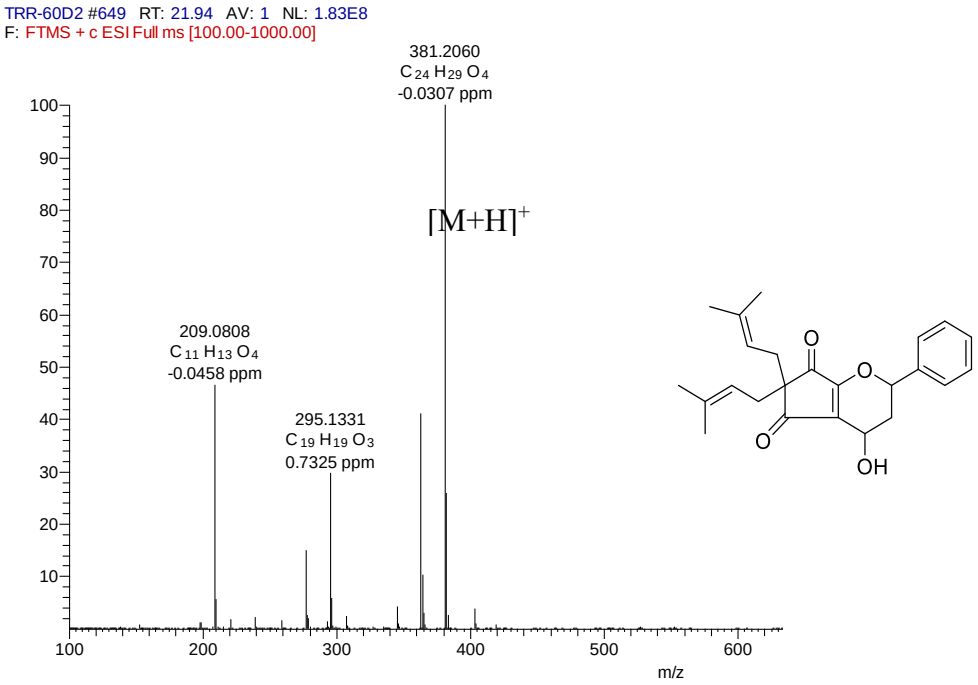


HMBC spectrum of compound 52

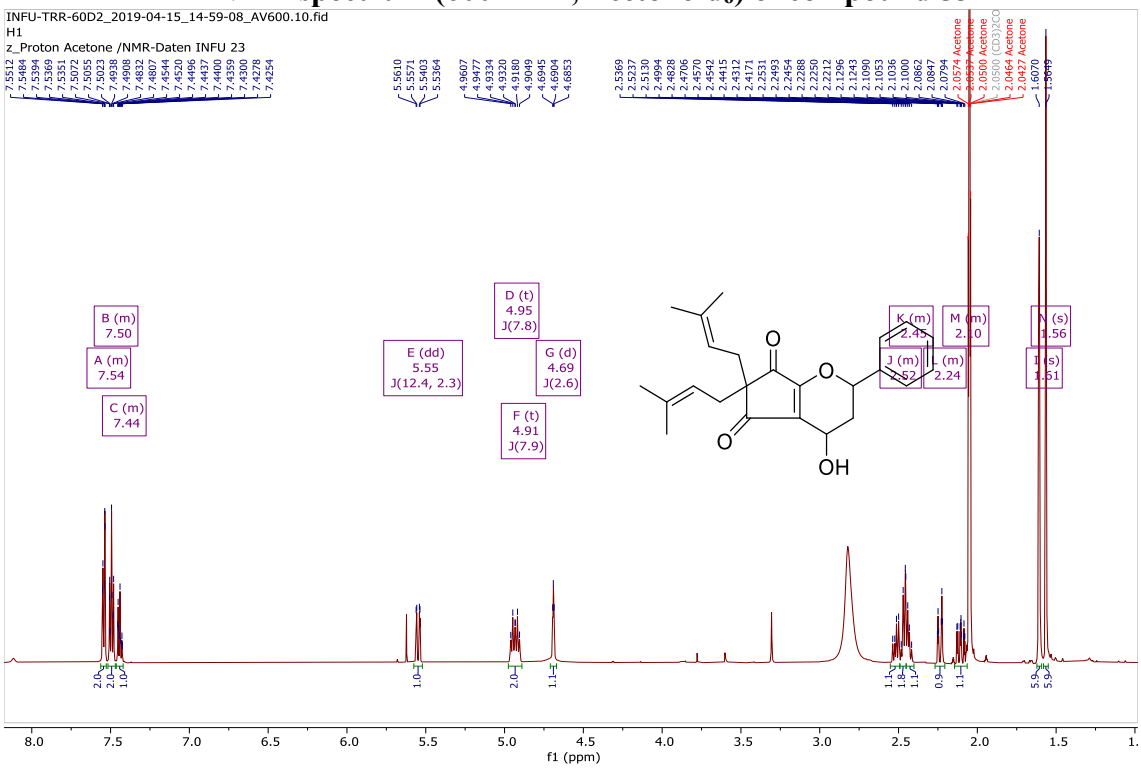


Appendix A53: Spectra for compound 53

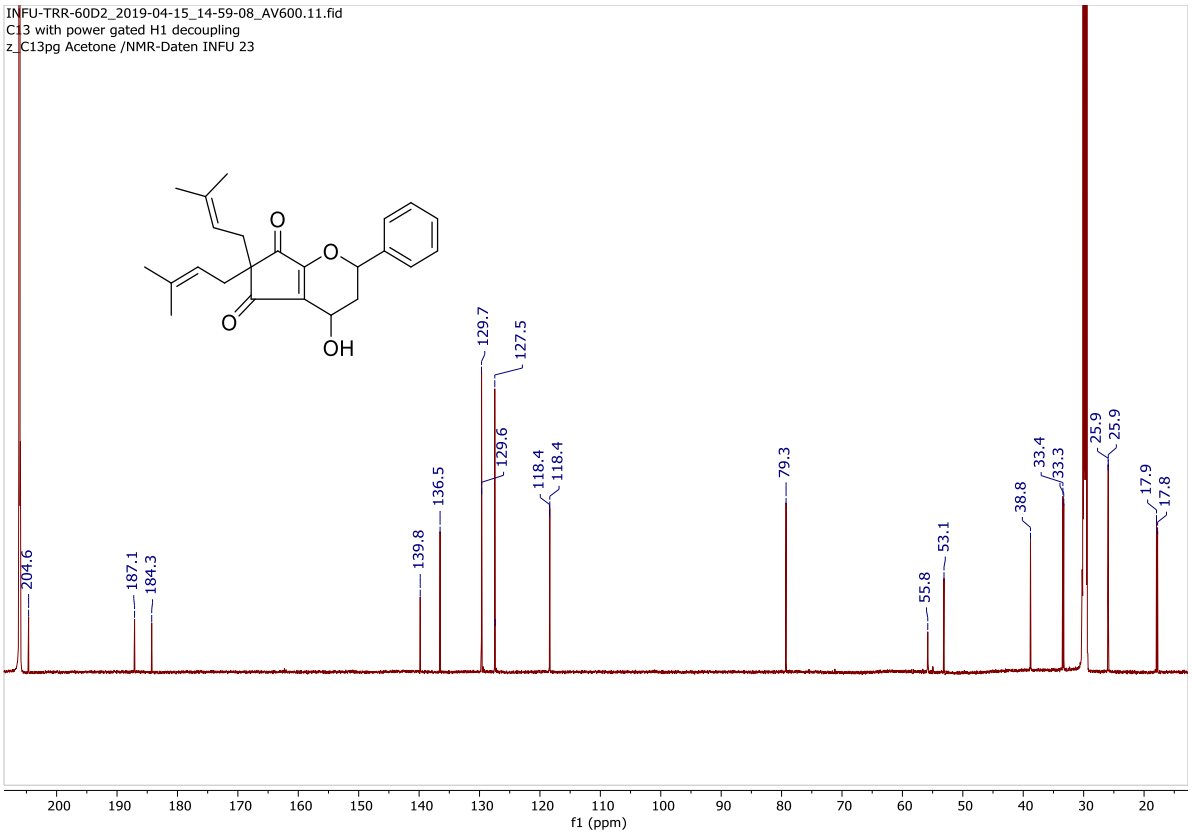
HRESIMS spectrum of compound 53



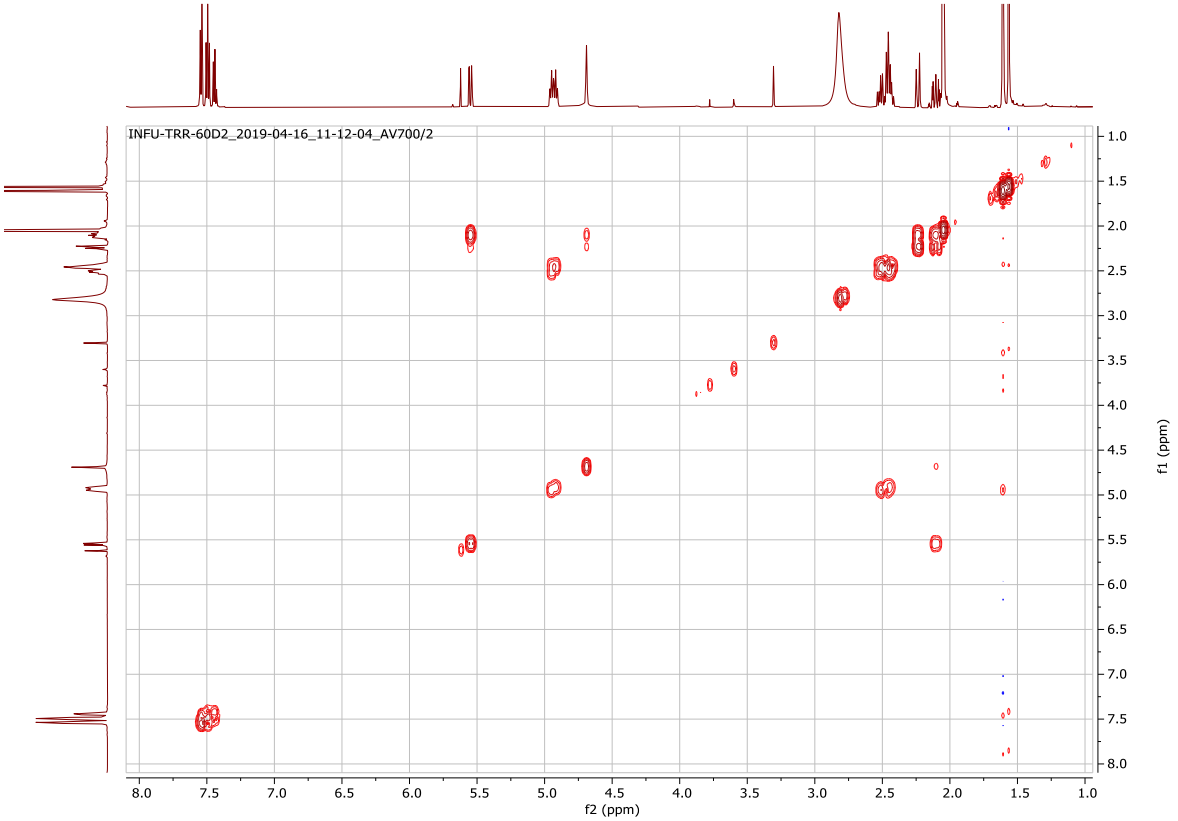
^{1}H NMR spectrum (600 MHz, Acetone-d$_6$) of compound 53



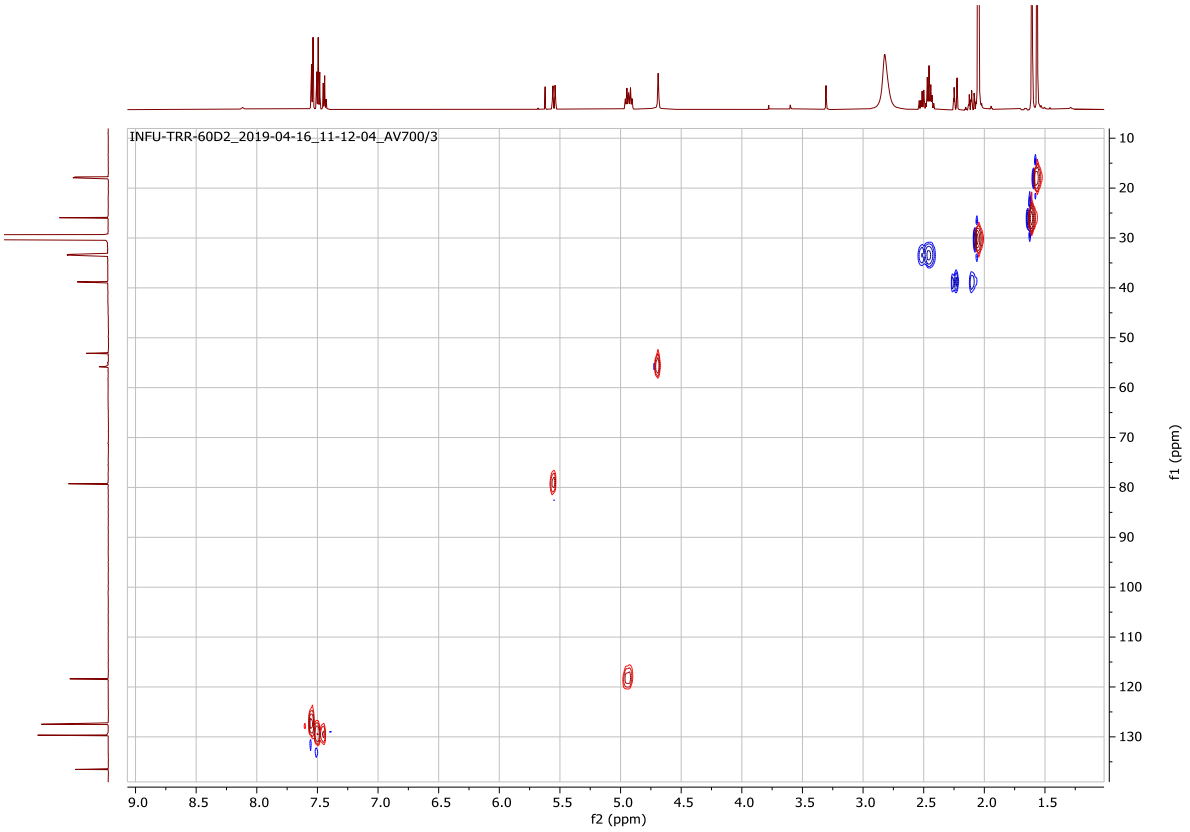
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 53



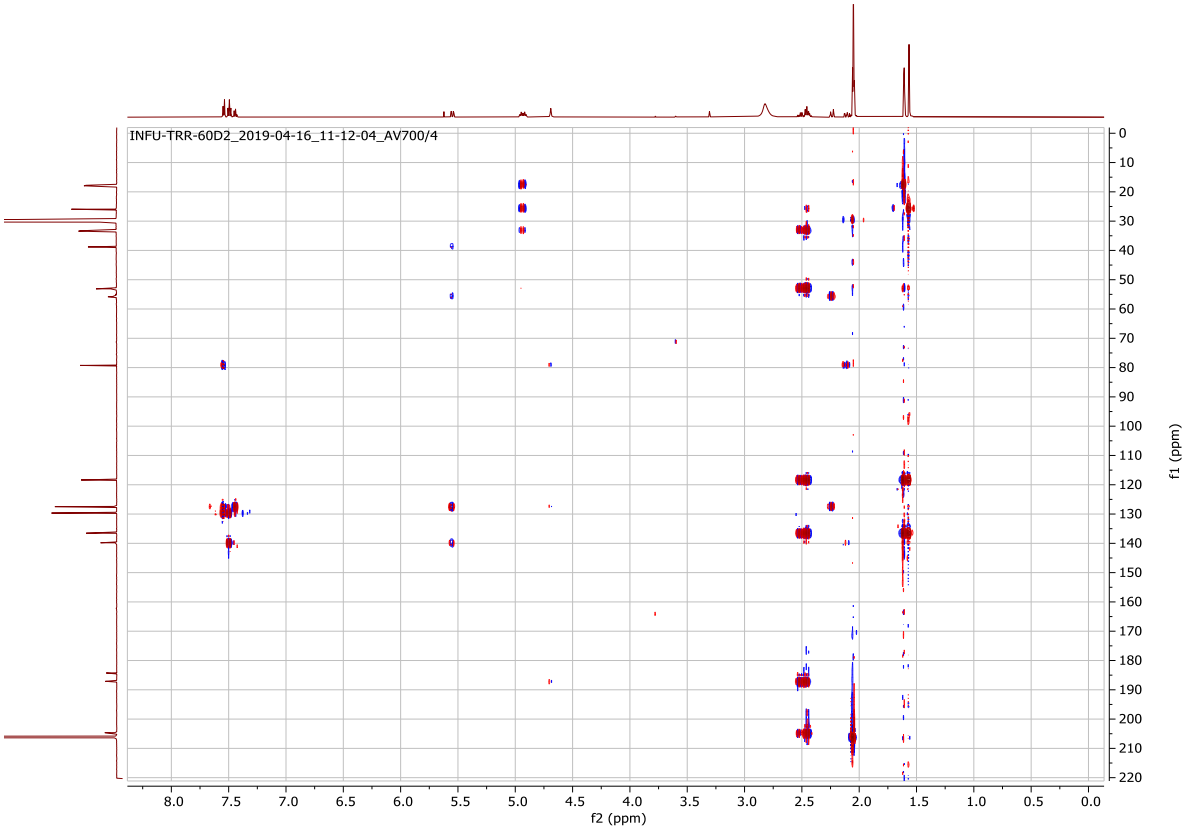
^{1}H-^{1}H-COSY spectrum of compound 53



HSQC spectrum of compound 53

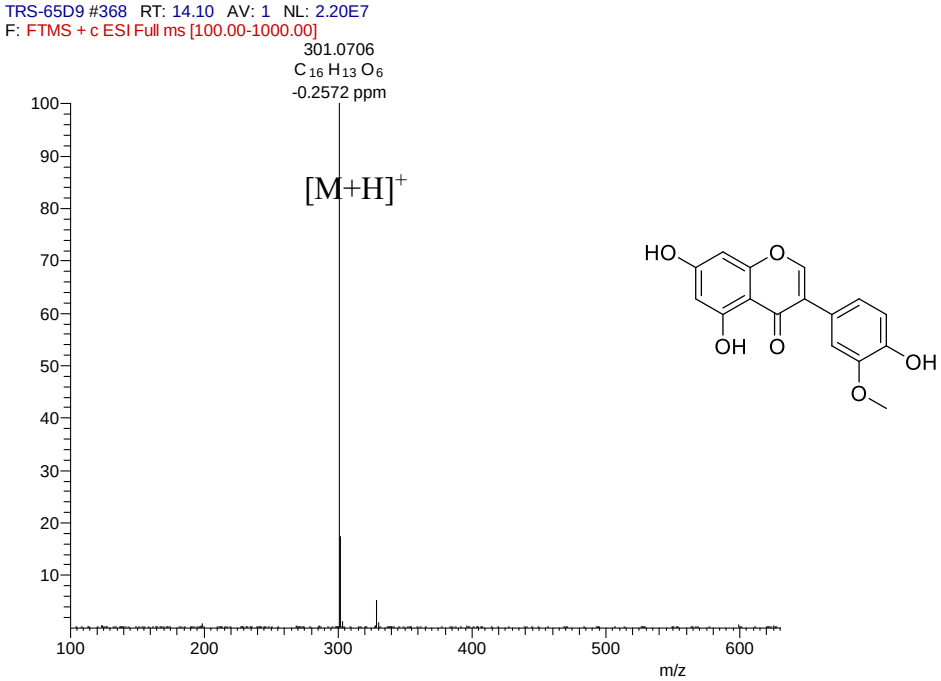


HMBC spectrum of compound 53

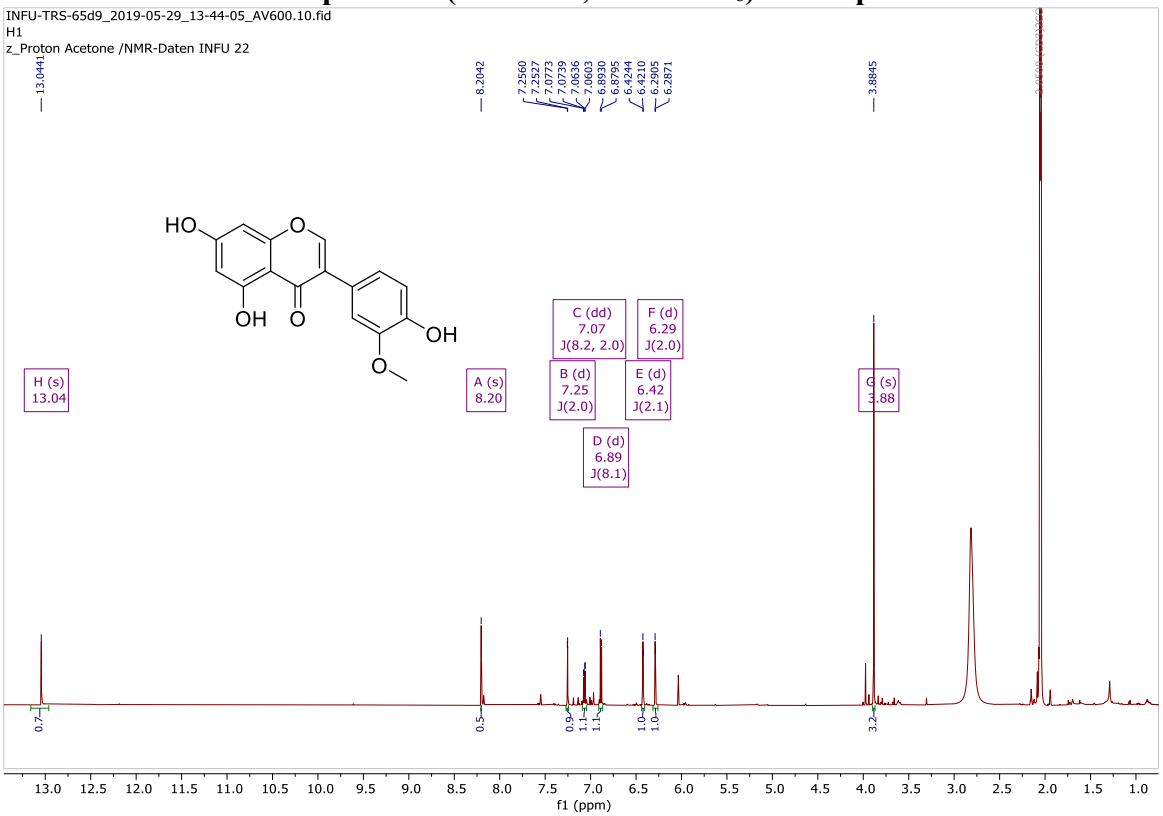


Appendix A54: Spectra for compound 54

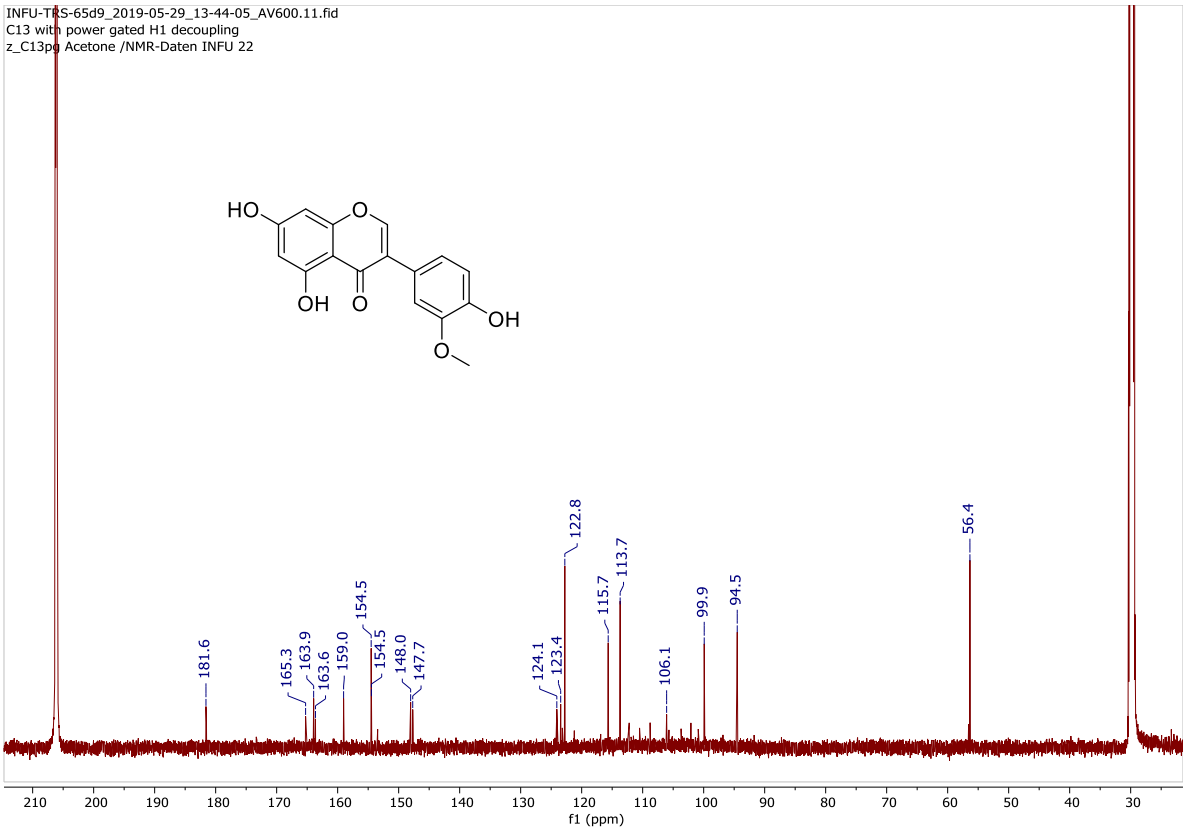
HRESIMS spectrum of compound 54



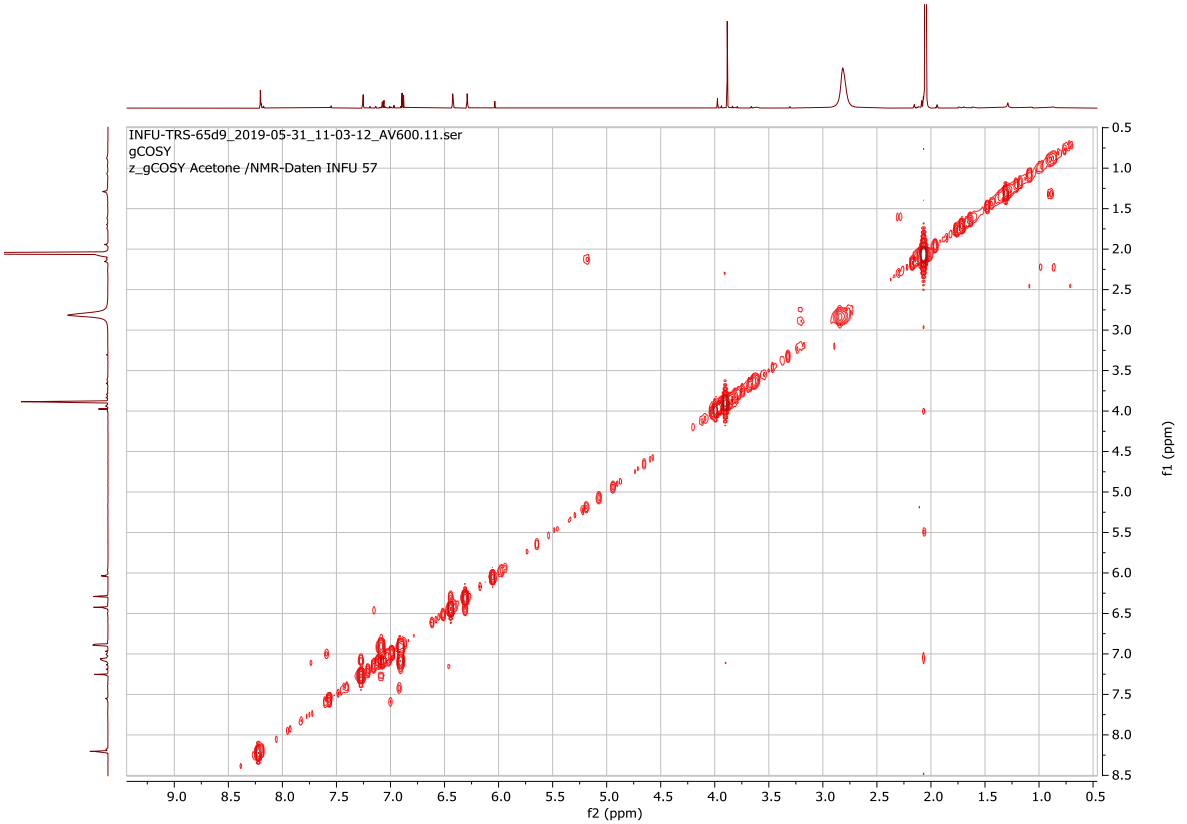
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 54



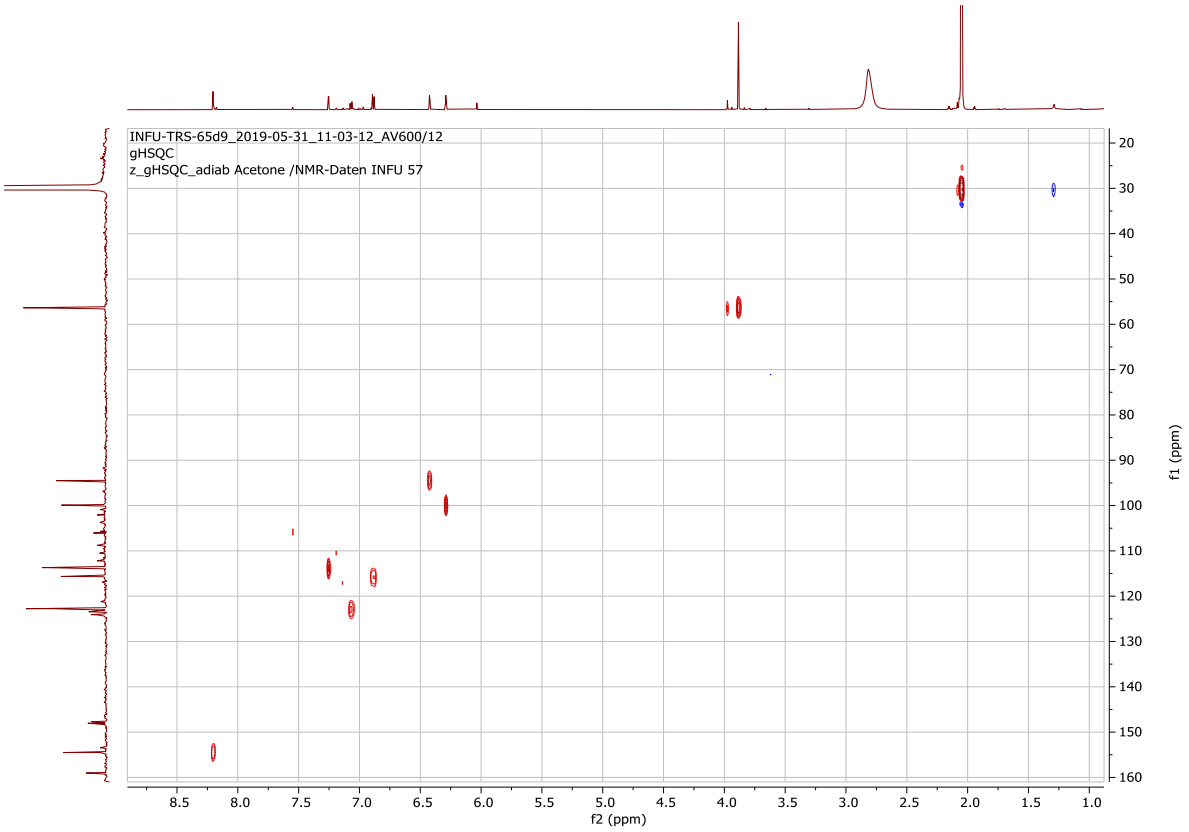
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 54



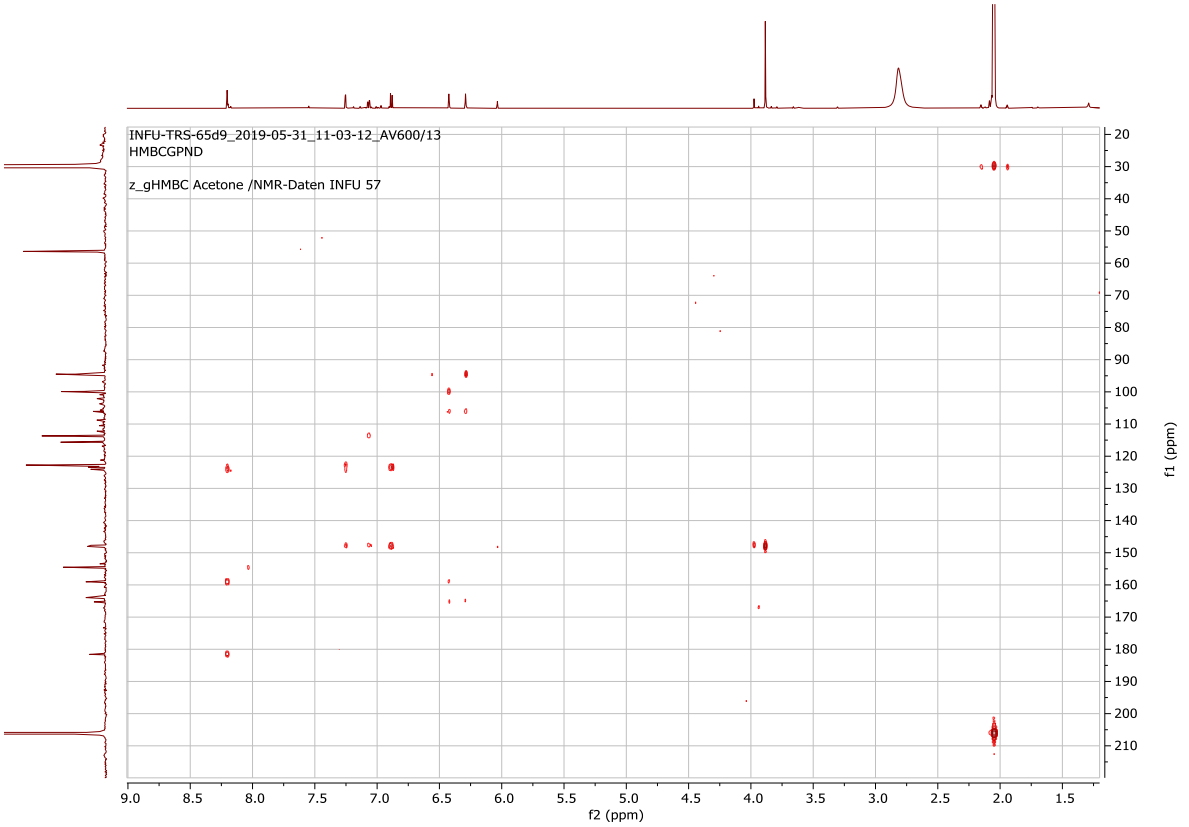
^{1}H-^{1}H-COSY spectrum of compound 54



HSQC spectrum of compound 54

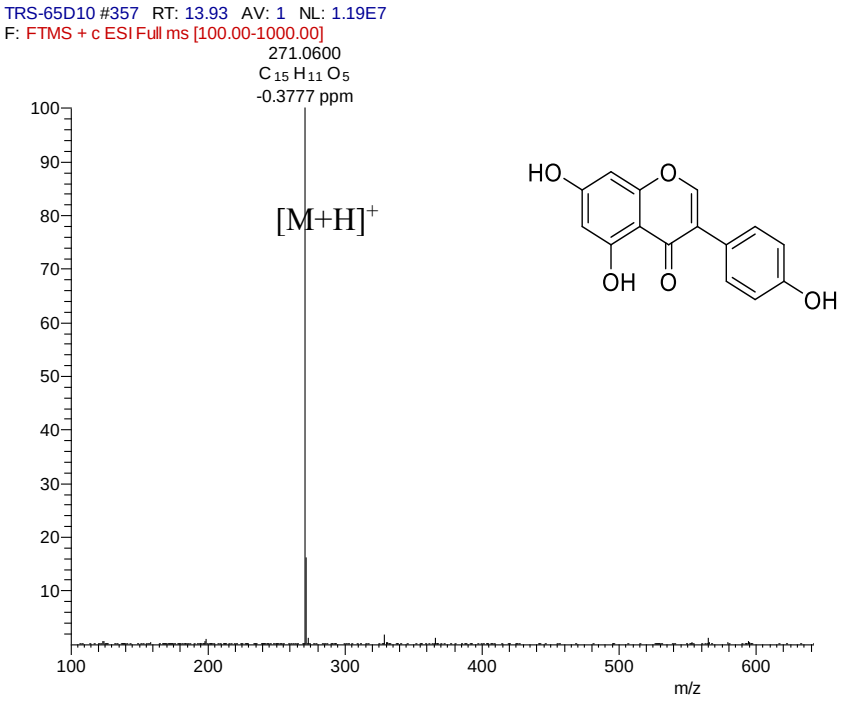


HMBC spectrum of compound 54

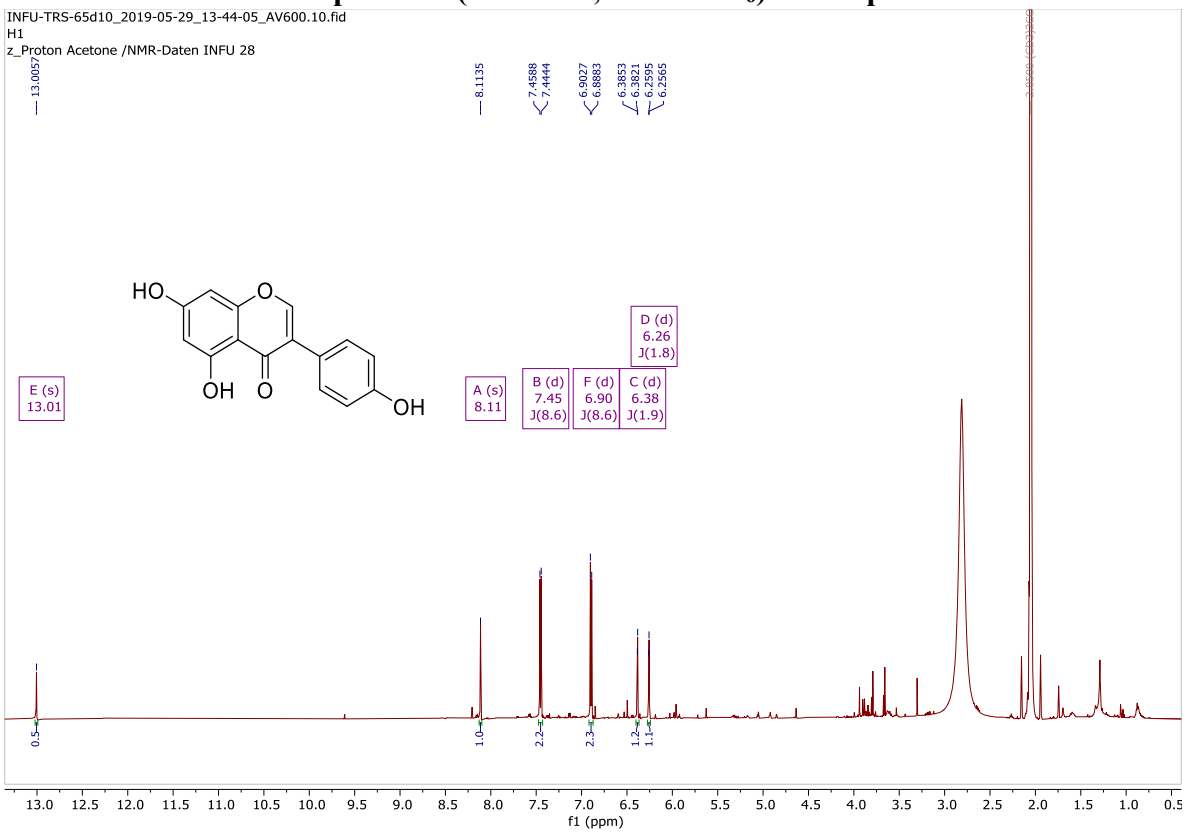


Appendix A55: Spectra for compound 55

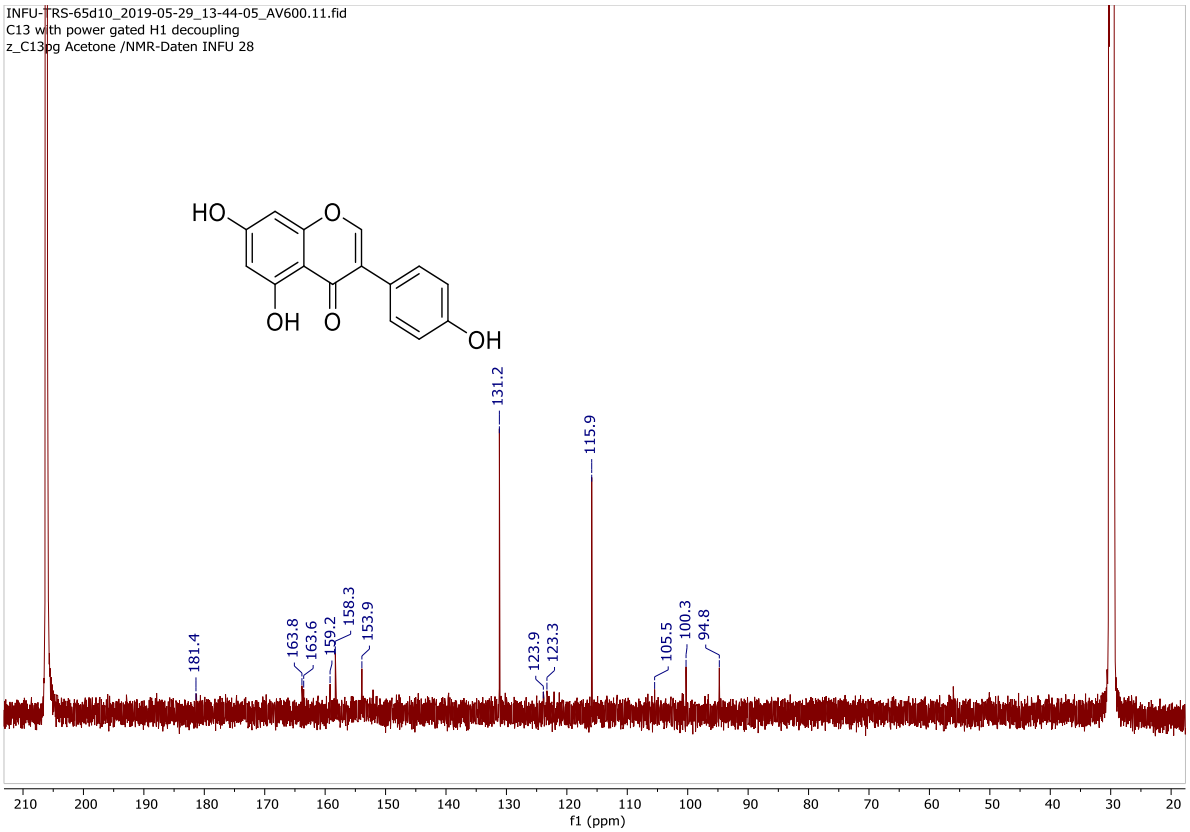
HRESIMS spectrum of compound 55



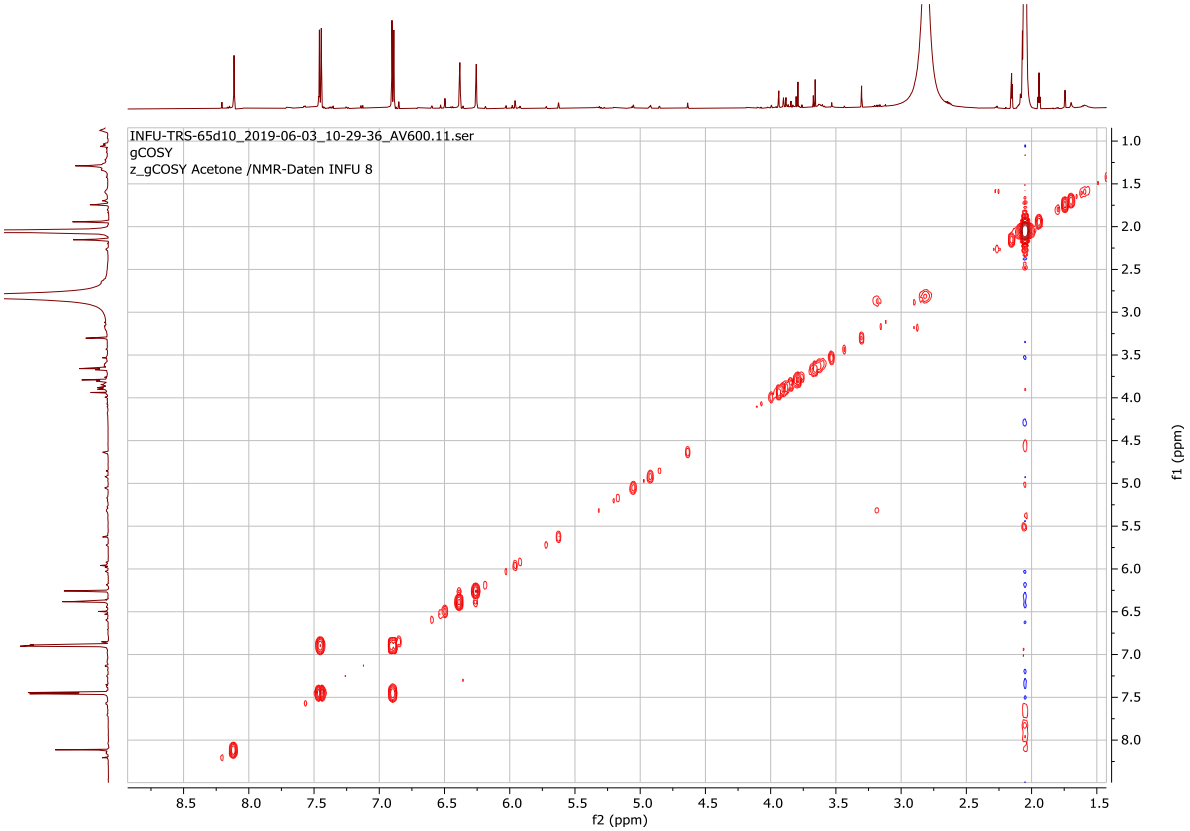
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 55



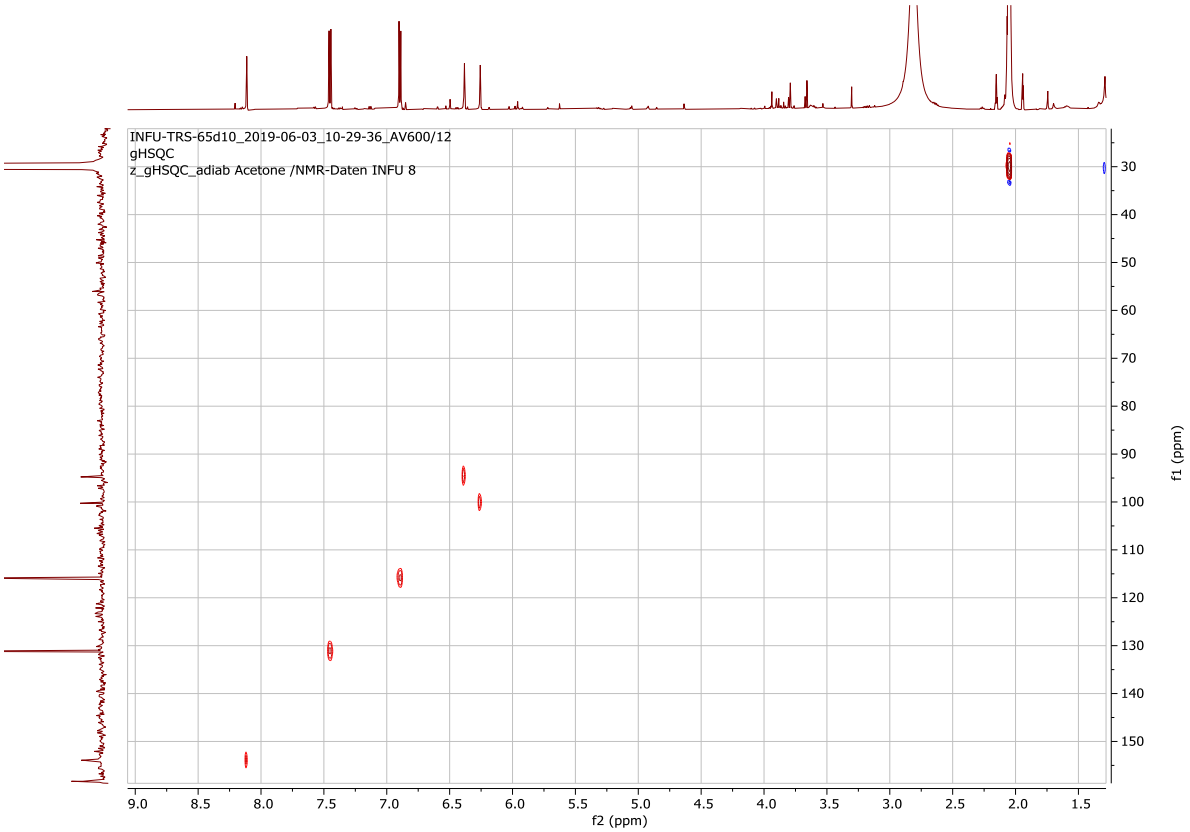
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 55



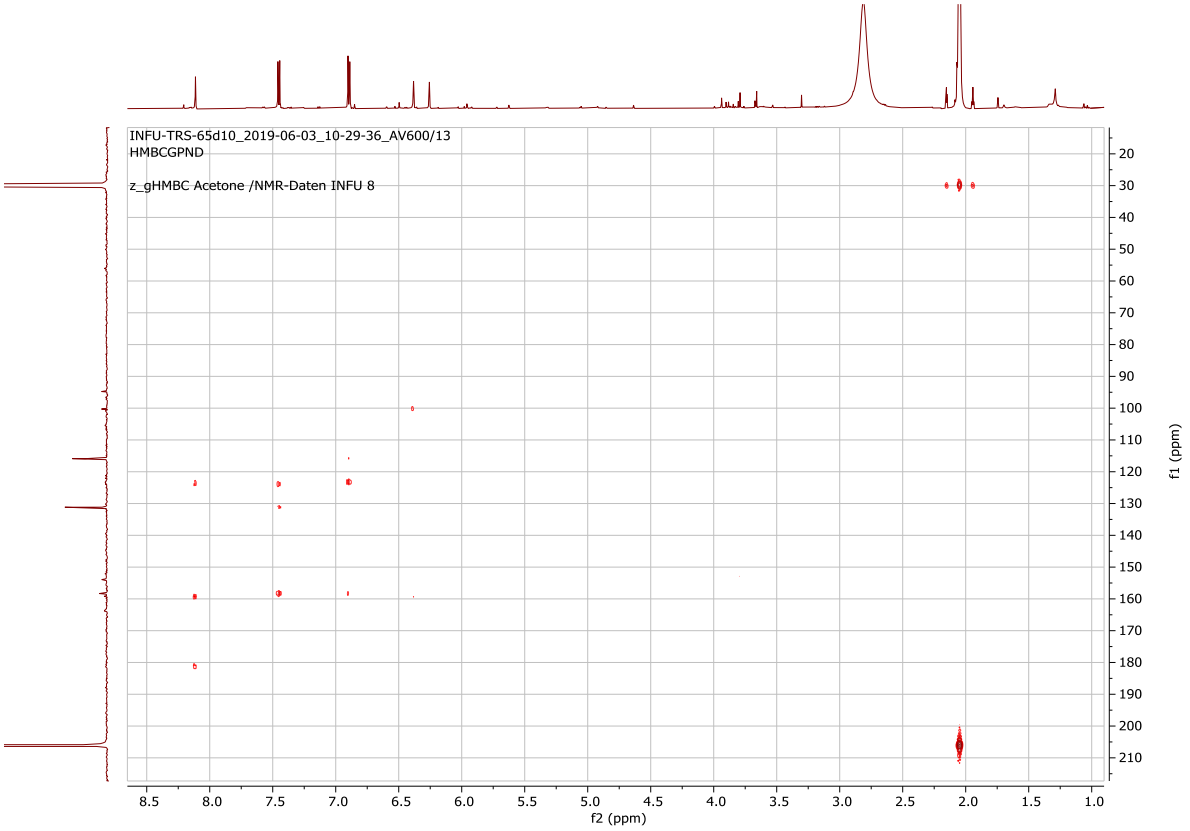
^{1}H-^{1}H-COSY spectrum of compound 55



HSQC spectrum of compound 55

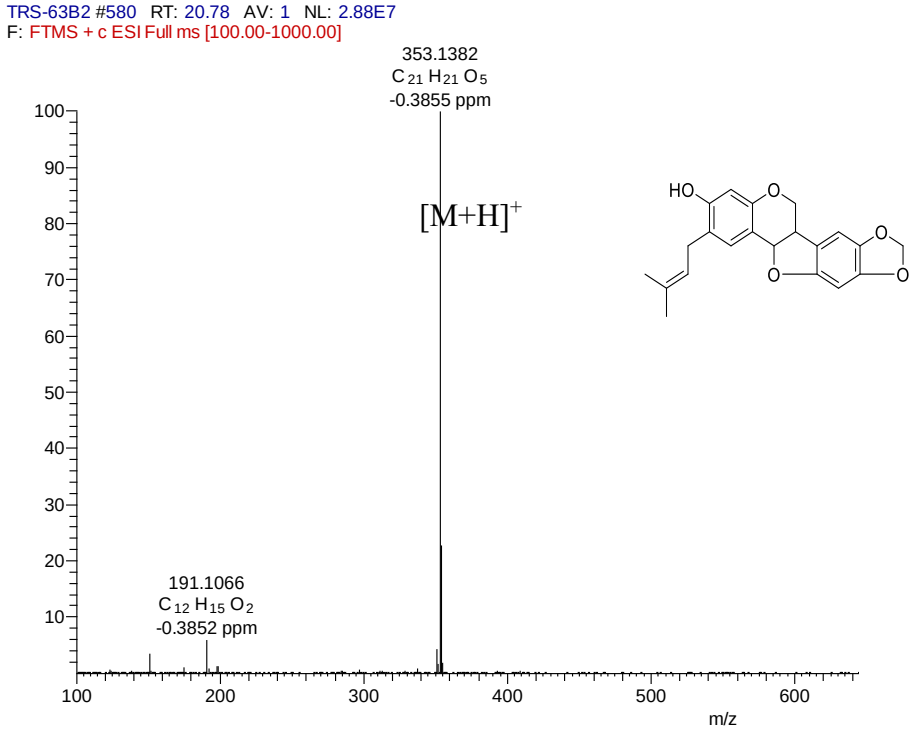


HMBC spectrum of compound 55

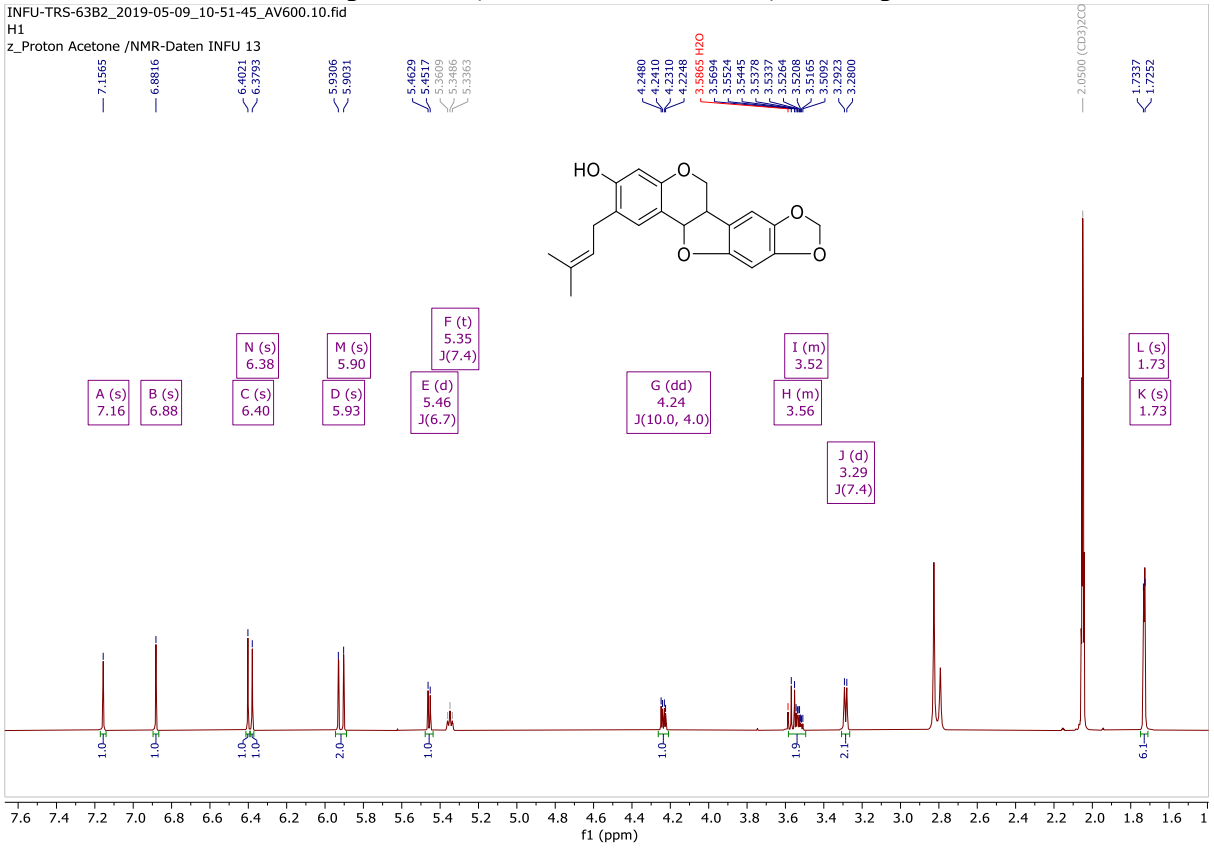


Appendix A56: Spectra for compound 56

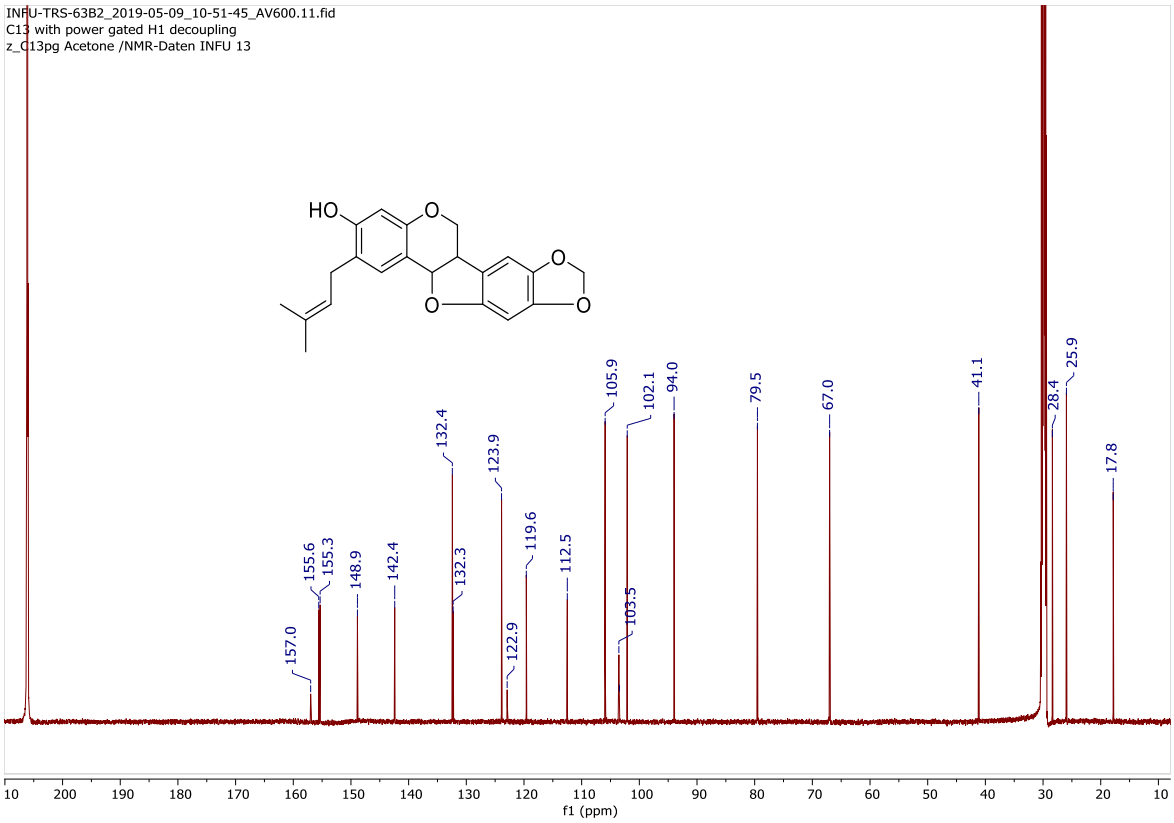
HRESIMS spectrum of compound 56



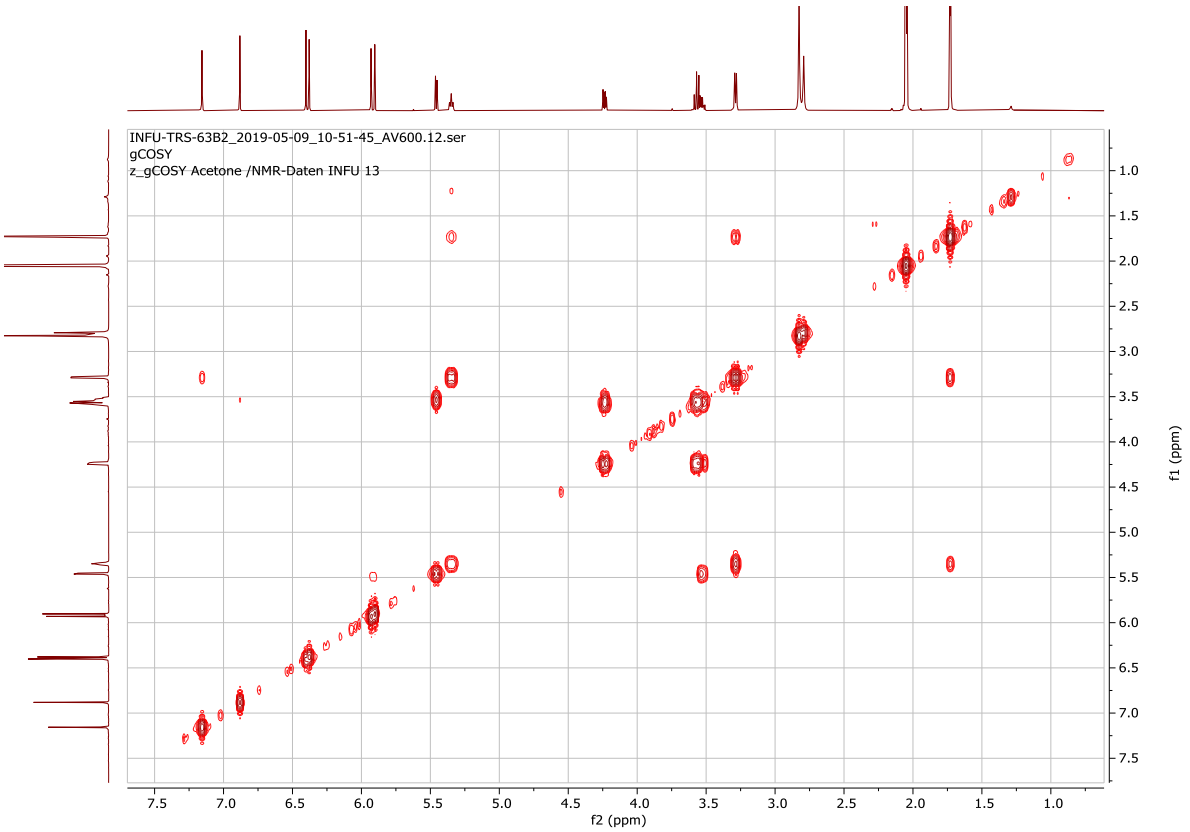
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 56



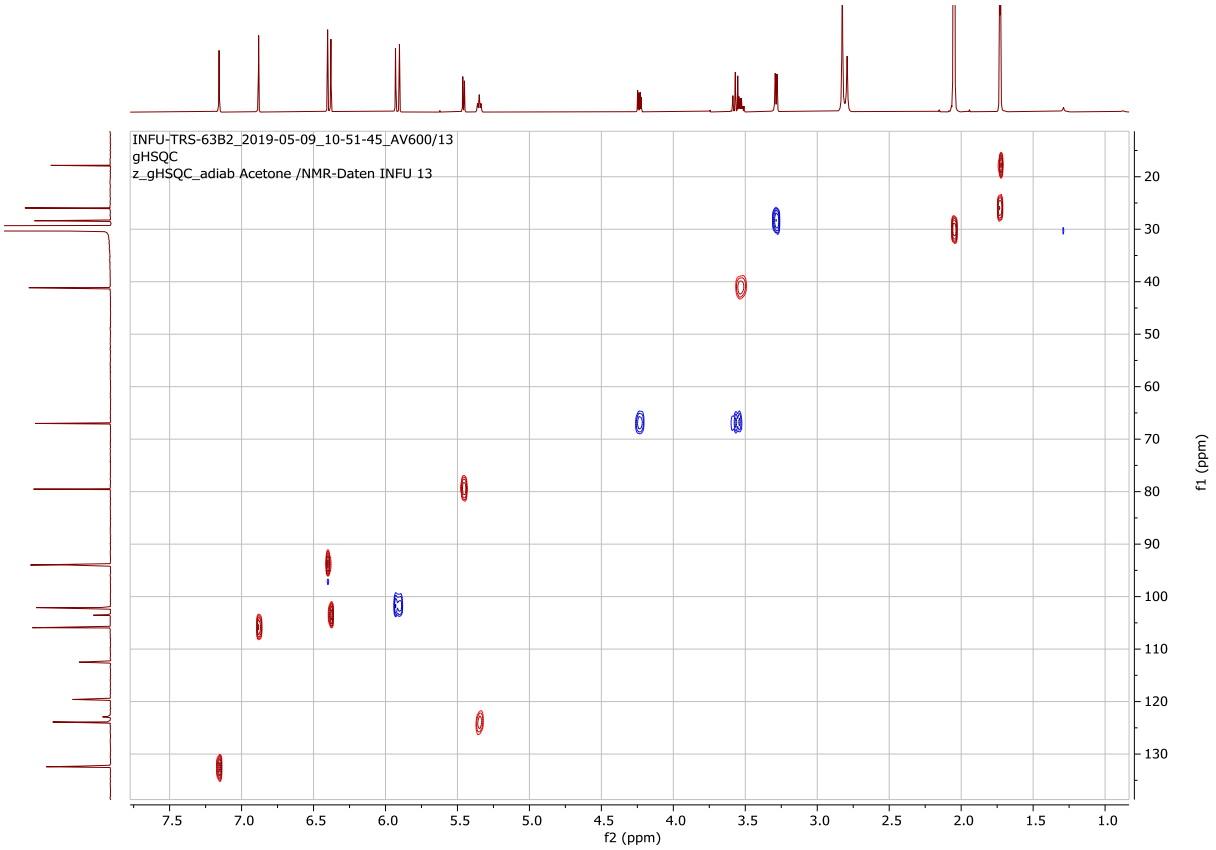
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 56



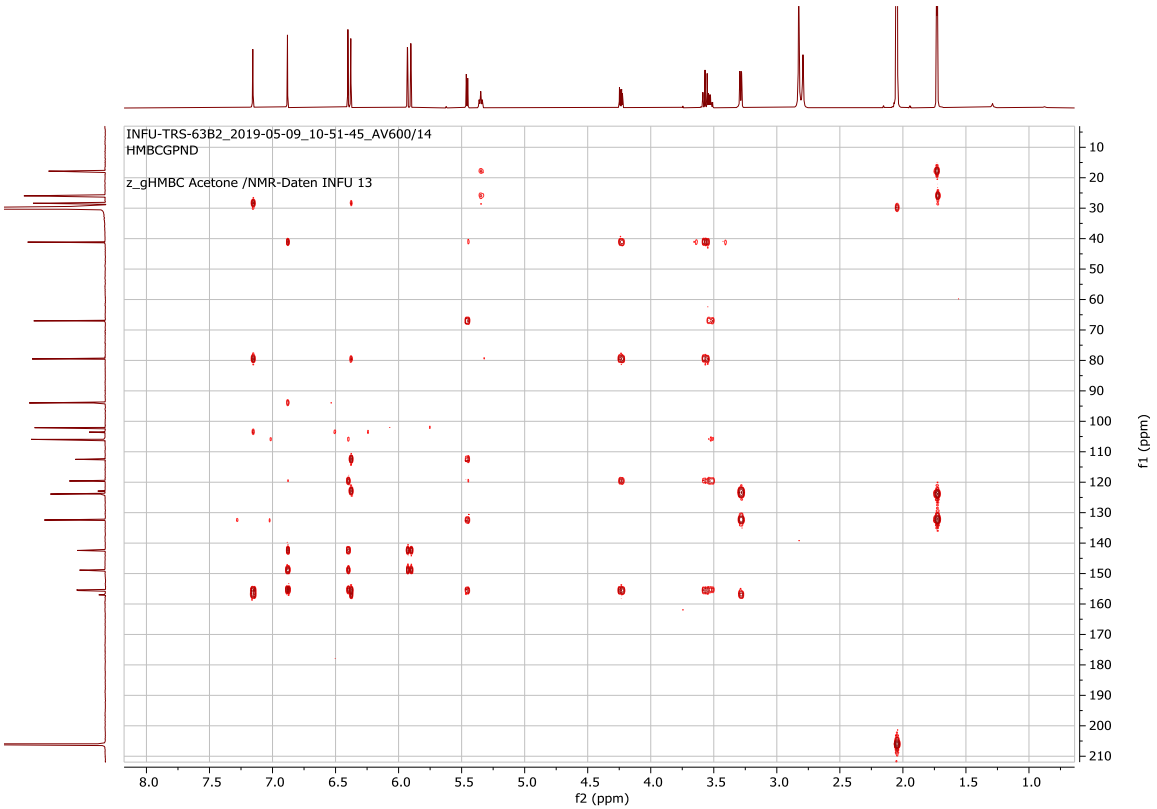
^{1}H-^{1}H-COSY spectrum of compound 56



HSQC spectrum of compound 56

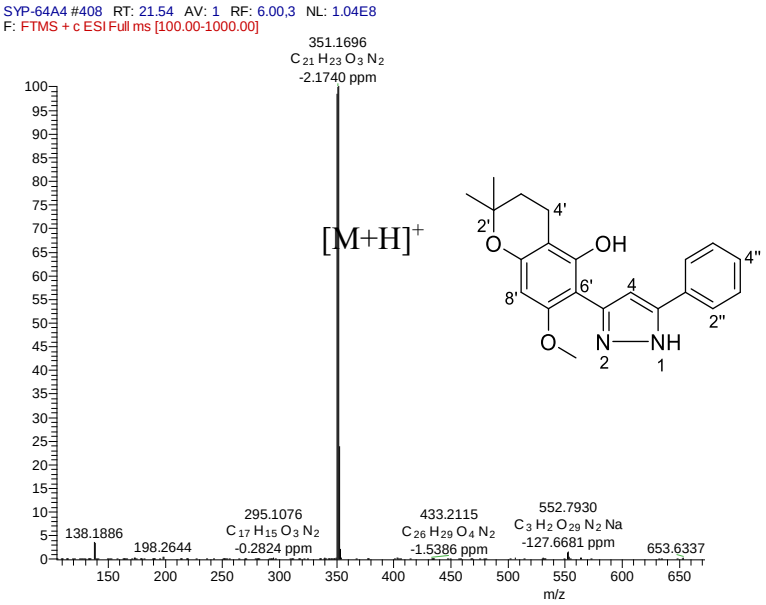


HMBC spectrum of compound 56

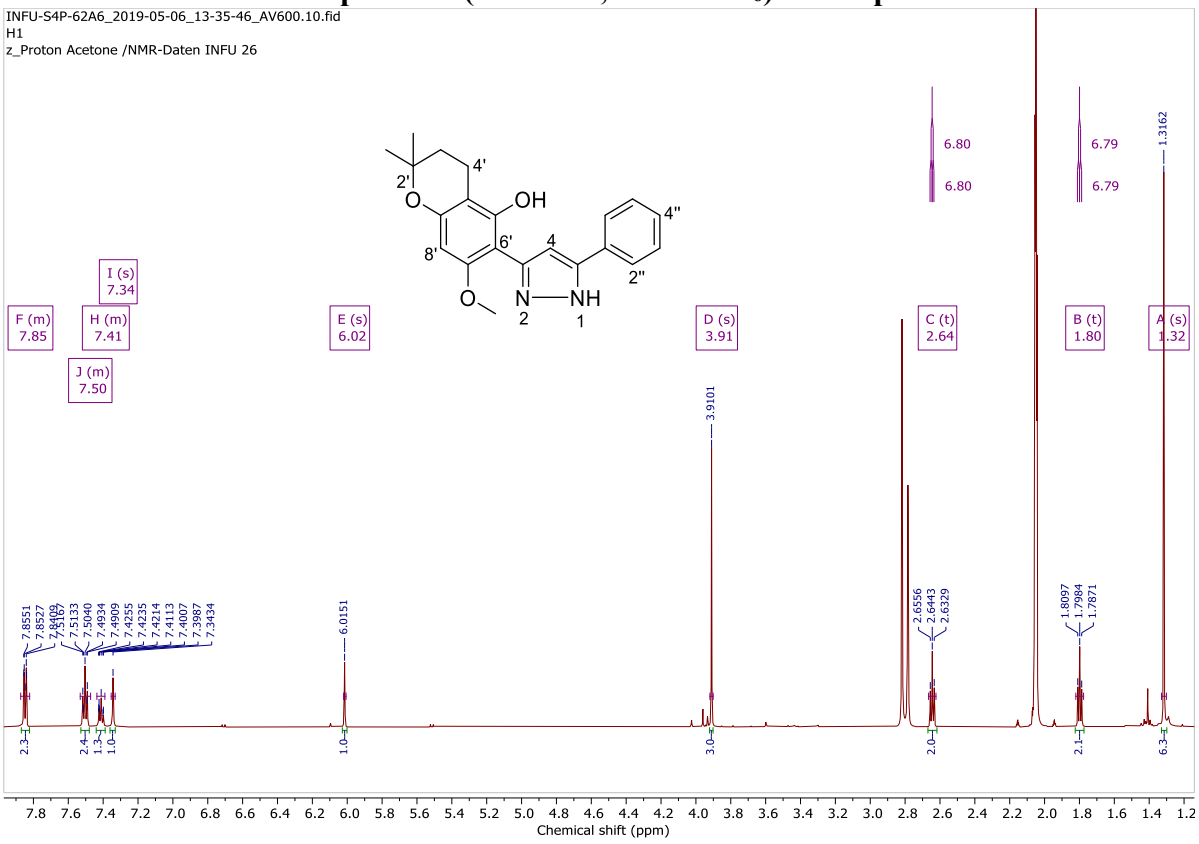


Appendix A57: Spectra for compound 57

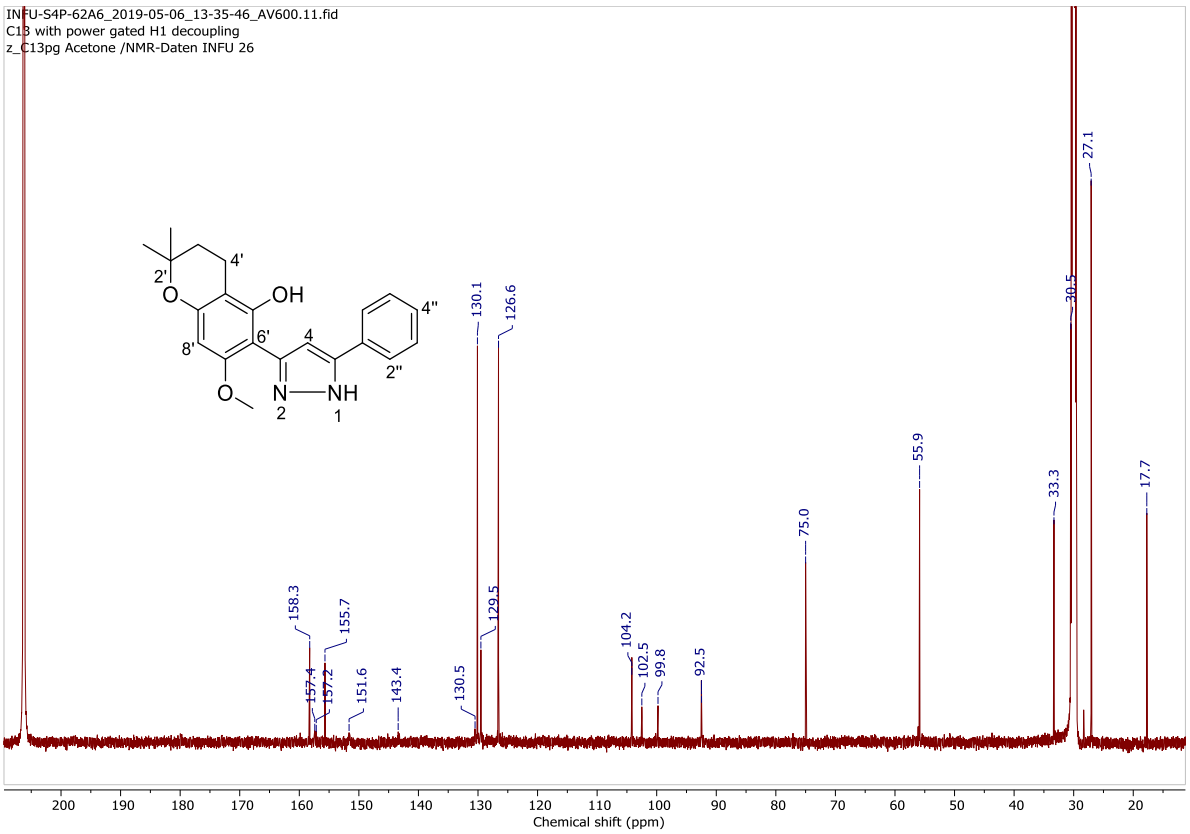
HRESIMS spectrum of compound 57



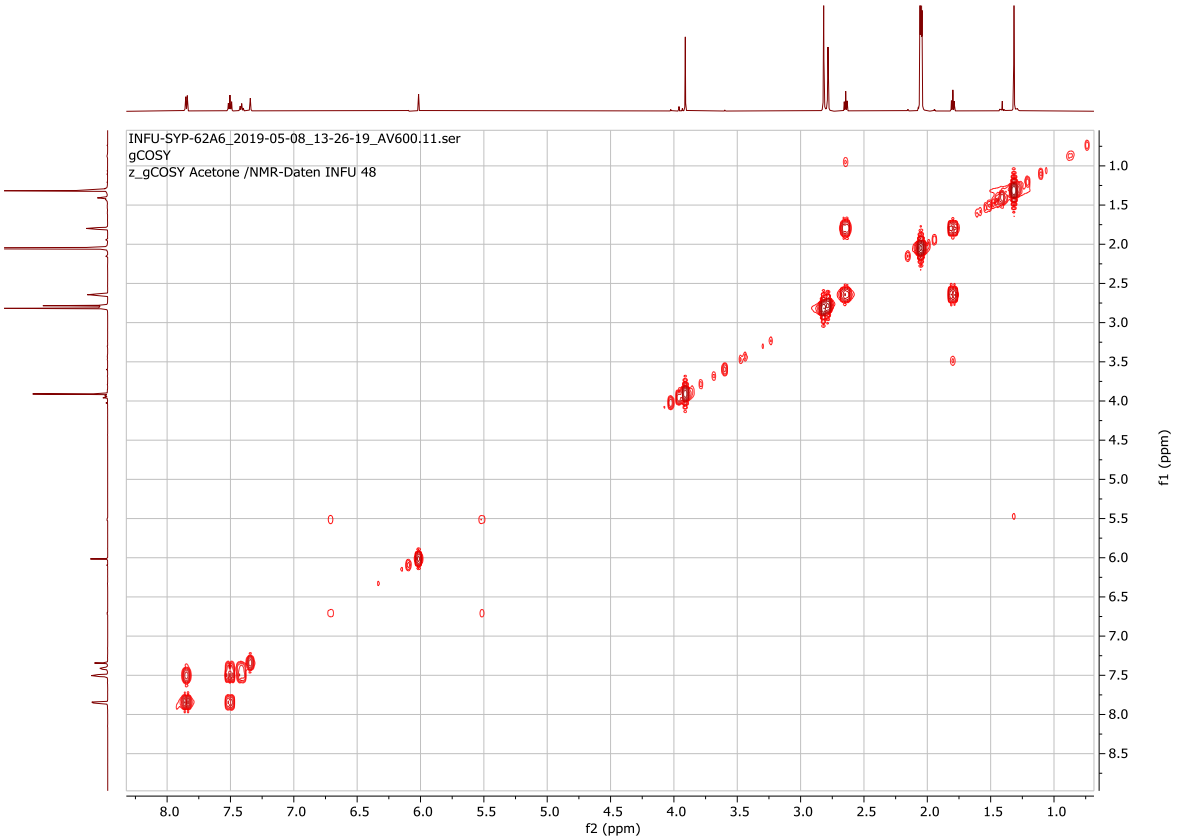
^{1}H NMR spectrum (600 MHz, Acetone-d_6) of compound 57



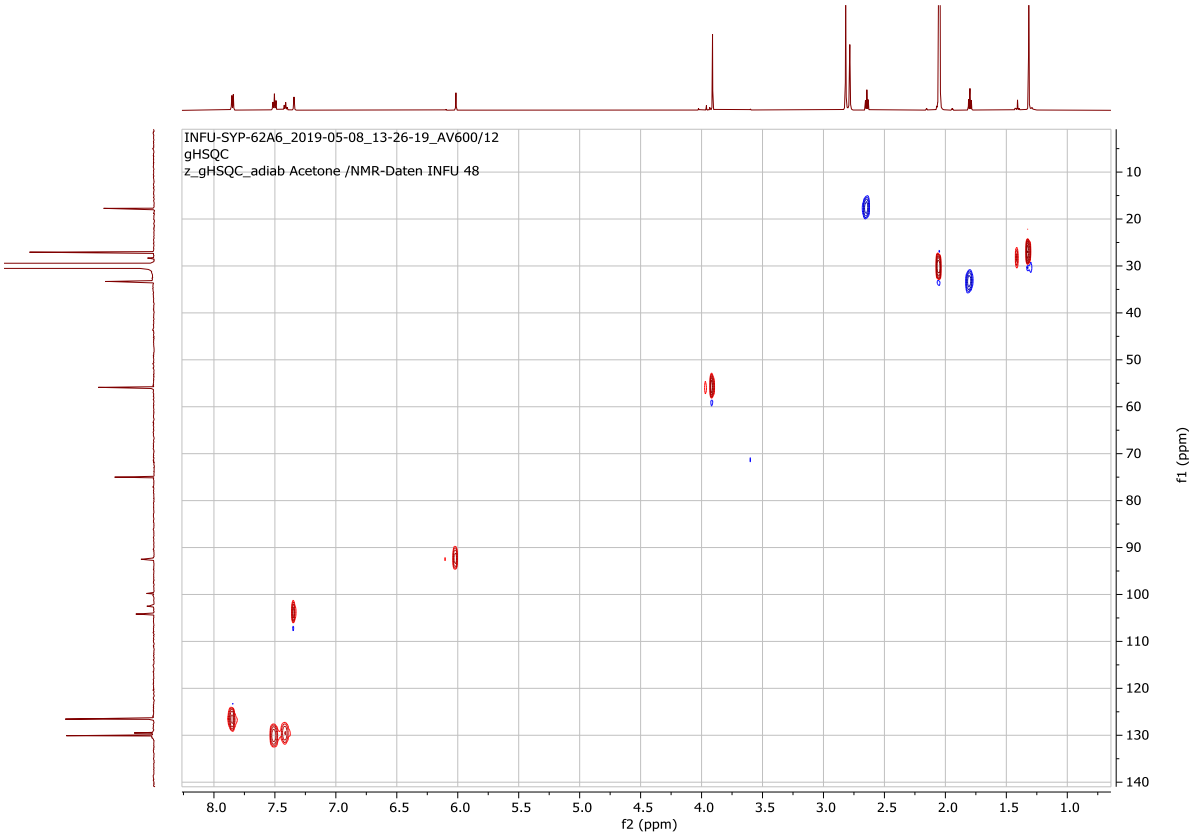
^{13}C NMR spectrum (150 MHz, Acetone-d_6) of compound 57



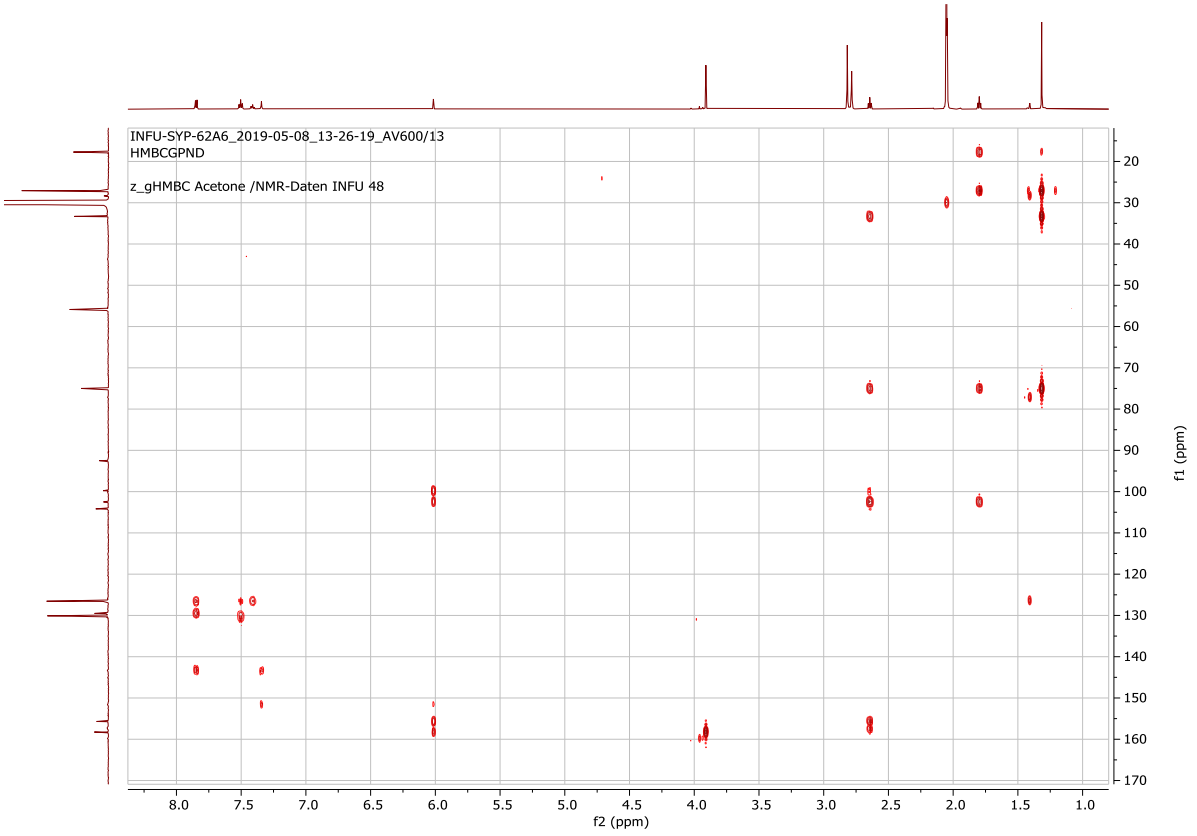
^{1}H-^{1}H-COSY spectrum of compound 57



HSQC spectrum of compound 57



HMBC spectrum of compound 57



APPENDIX B: Data for Anti-Inflammatory Assay

APPENDIX B1: Table Results for Anti-inflammatory Assay

Table B1: Results of controls (mean ± SD, n=3 for medium and n=4 for LPS and ibuprofen)

Controls		Cytokine release [pg/ml]					
		IL-1β	IL-2	IL-6	IFN-γ	GM-CSF	TNFα
Medium	mean	**616.95**	**6.59**	**29213.73**	**27.04**	**163.32**	**417.09**
	SD	178.00	1.51	22861.52	8.10	2.39	356.67
LPS	mean	**651.20**	**9.91**	**42900.00**	**42.60**	**168.60**	**656.66**
	SD	343.02	1.99	0.00	18.84	47.34	48.84
Ibuprofen	mean	**1297.77**	**7.96**	**19931.71**	**21.82**	**80.53**	**80.29**
	SD	54.04	1.59	5357.24	12.44	8.18	6.78

Table B2: Results for Cytokine release (pg/ml) after LPS stimulated PMBCs incubated with isolated compounds and crude extracts from *Tephrosia linear* and *T. hidebrandtii*[14]

Samples	**Cytokine release [pg/ml]**					
	IL-1β	**IL-2**	**IL-6**	**IFN-γ**	**GM-CSF**	**TNF-α**
Medium	820.55	5.71	2821.58	35.53	165.71	14.93
Medium	539.48	5.71	41919.60	26.17	160.93	695.07
Medium	490.81	8.34	42900.00	19.41	163.33	541.28
LPS	148.06	8.34	42900.00	15.11	12.08	723.15
LPS	867.29	8.34	42900.00	57.09	210.89	657.19
LPS	717.47	12.46	42900.00	51.79	177.44	607.52
LPS	872.00	10.52	42900.00	46.43	117.47	638.77
Ibuprofen	1248.97	8.34	22460.36	10.28	82.25	84.40
Ibuprofen	1356.05	8.34	18570.75	39.20	88.51	74.54
Ibuprofen	1331.35	5.71	25559.62	16.44	82.25	87.70
Ibuprofen	1254.72	9.47	13136.10	21.37	69.08	74.54
1	444.56	5.71	4670.13	15.11	29.33	6.07
2	7.65	5.71	0.00	15.11	<14.55	1.09
3	1106.02	1.76	266973.99	1.15	132.16	12.39
4	493.96	5.71	41795.72	15.11	141.17	20.67
5	1110.37	1.76	18492.90	1.15	29.33	26.44
6	1022.97	1.76	18614.32	1.15	69.08	17.48
7	350.15	1.76	11062.37	15.11	54.75	26.44
8	666.47	5.71	8393.81	15.11	79.05	41.92
9	1435.44	5.71	42900.00	15.11	60.29	82.42

[14] Isolated compounds and ibuprofen were evaluated at 100 µm and the plant crude extract was evaluated at 100 µg/mL.

Samples	Cytokine release [pg/ml]					
	IL-1β	**IL-2**	**IL-6**	**IFN-γ**	**GM-CSF**	**TNF-α**
10	265.90	1.76	10603.96	15.11	82.25	32.88
11	672.59	5.71	102332.57	14.42	65.63	34.17
12	684.87	<5.71	20732.99	<15.11	54.75	27.73
13	4.37	<5.71	18.17	<15.11	<14.55	1.09
14	314.85	5.71	3102.34	<15.11	8.21	7.33
15	711.26	5.71	>42900.00	2.44	156.09	23.87
16	31.34	<5.71	273.39	<15.11	<14.55	3.56
17	966.63	1.76	10699.86	13.40	82.25	25.16
TLC	701.13	1.76	5965.31	<15.11	62.09	30.30
25	211.81	1.76	3178.91	<15.11	72.47	18.75
26	1002.29	1.76	5583.01	<15.11	82.25	41.92
27	176.44	<5.71	252.14	<15.11	24.21	2.32
28	501.32	1.76	7503.57	<15.11	72.47	14.93
29	913.40	1.76	29074.63	1.15	82.25	20.03
30	131.49	1.76	2485.24	<15.11	18.58	7.33
31	951.61	5.71	12621.06	3.65	67.36	43.22
32	375.02	<5.71	4290.28	<15.11	3.22	9.85
THC	905.08	10.52	>42900.00	3.65	97.57	21.31

TLC – crude extract from *T. linearis*

THC – crude extract from *T. hildebrandtii*

Table B3: Results of controls (mean ± SD, n=3)

Controls		Cytokine release [pg/ml]			
		IL-1β	**IFN-γ**	**GM-CSF**	**TNFα**
Medium	mean	892.01	178.61	40.90	139.39
	SD	72.62	38.29	7.02	65.45
LPS	mean	14532.78	8876.79	427.98	8295.07
	SD	1354.00	1764.07	77.51	988.00
Ibuprofen	mean	3703.12	1493.90	103.75	2672.63
	SD	441.66	146.93	17.54	333.15
Ibuprofen	% of LPS control	25.48	16.83	24.24	32.22

Table B4: Results for Cytokine release (pg/ml) after LPS stimulated PMBCs incubated with isolated compounds and crude extracts from *Tephrosia vogelii, T. elata* and *T. rhodesica*[15]

Samples	Concentration	Cytokine release [pg/ml]			
		IL-1β	IFN-γ	GM-CSF	TNF-α
35	100 µM	9462.05	897.55	370.99	1878.78
36	100 µM	2323.22	43.06	75.31	183.83
38	100 µM	8418.14	121.10	30.16	61.57
44	100 µM	9537.92	1058.28	253.42	2364.94
45	100 µM	4361.17	27.51	125.49	278.81
46	100 µM	13251.15	4371.17	187.76	4701.73
47	100 µM	6810.24	314.62	212.69	1380.12
48	100 µM	6337.58	85.40	341.78	1064.32
52	100 µM	8367.57	512.77	300.30	1477.27
53	100 µM	11061.86	72.73	148.61	228.65
54	100 µM	10534.45	82.18	259.46	422.54
55	100 µM	9219.65	317.65	1061.74	2770.18
56	100 µM	8526.89	217.13	609.91	823.50
57	100 µM	10846.16	341.83	636.70	701.32
TVC	100 µg/ml	10926.34	93.51	283.08	267.69
TRC	100 µg/ml	6534.53	44.32	805.18	1705.53

TVC – the crude extract of *T. vogelii*

TRC – the crude extract of *T. rhodesica*

[15] Isolated compounds and ibuprofen were evaluated at 100 µm and the plant crude extract was evaluated at 100 µg/mL.

Table B5: Results for Cytokine release (pg/ml) after LPS stimulated PMBCs incubated with combination of isolated compounds and crude extracts from

			Cytokine release [pg/ml]			
Group	**Compound**	**Concentration**	**IL-1ß**	**IFN-γ**	**GM-CSF**	**TNF-α**
1	**1**	100 µM	35.93 ± 7.90	15.11 ± 0.00	14.55 ± 0.00	2.39 ± 0.00
	2					
	3					
	4					
	8					
	9					
2	**5**	100 µM	11.35 ± 2.84	15.11 ± 0.00	14.55 ± 0.00	8.59 ± 0.00
	12					
	13					
	14					
	15					
	16					
	17					
	6					
3	**7**	100 µM	489.92 ± 77.43	15.11 ± 0.00	8.87 ± 5.93	10.59 ± 1.75
	10					
	11					
4	**25**	100 µM	3068.36 ± 550.46	7.44 ± 2.61	15.75 ± 13.77	78.84 ± 14.95
	31					
	32					
5	**26**	100 µM	11544.17 ± 1529.60	107.70 ± 9.38	788.55 ± 111.83	657.12 ± 138.41
	27					
6	**28**	100 µM	5816.37 ± 1925.60	21.88 ± 9.33	51.78 ± 34.14	136.77 ± 130.10
	29					
	30					
Crude extract of *T. hidebrandtii*		10 µg/ml	2594.11 ± 147.62	950.60 ± 240.84	72.71 ± 10.48	1638.64 ± 86.94
Crude extract of *T. linearis*		10 µg/ml	2745.63 ± 305.94	582.78 ± 12.53	84.40 ± 16.59	1417.03 ± 307.13

APPENDIX B2: Certificate of Analysis

C.T.L.

EINGANG: 13.06.2019
LAGERUNG: RAA, Pos. 79/80/81

PRODUCT DATA SHEET – ePBMC® Uncharacterized Cryopreserved Human PBMC

Catalog No.: CTL-UP1

Product name: ePBMC® - Uncharacterized Cryopreserved Human PBMC

Size: >10x10^6 cells / vial

Description: Human PBMC (Peripheral Blood Mononuclear Cells) isolated from leukopacks and frozen in CTL-CryoABC™ serum-free freezing medium. These leukopacks were ethically collected from healthy donors with no risk of breaching privacy. Tested negative for HBsAg, HBcAb, HCV, HTLV I/II and STS by serology; as well as HIV I, HCV and WNV by NAT (nucleic acid testing)

Performance: T cell functionality by ELISPOT equivalent to fresh cells

Applications: PBMCs are suited for T cell monitoring in ELISPOT, ELISA, cytokine bead arrays, tetramer/ pentamer, and cytokine capture assays or any assay that requires live functional PBMC

Recommended test concentration: Investigators are advised to determine optimal concentrations for individual applications.
CTL recommends of 100,000 to 800,000 cells / well concentration for ELISPOT

Stability and Storage: Cryopreserved cells are shipped in a dry cryoshipper, and should be unpacked immediately upon receipt. Short-term storage of cells (24h) at -80°C is acceptable, but should be minimized to ensure maximum stability. For long-term storage, cryopreserved cells should be stored in liquid nitrogen. Thawed samples must be used immediately and have a finite life span in culture. Avoid repeated freeze-thaw cycles!

Long-term Storage: -169°C to -196°C (must be on liquid nitrogen (LN2) vapor)

Thawing: Thaw protocol included

Usage: **FOR RESEARCH USE ONLY! Not intended for direct therapeutic or diagnostic use in humans or animals, or for use in in vitro diagnostic procedures!**

Characterization results: PBMCs from 3 donors, 1 vial each to Pharmacelsus GmbH, Dr. Tanja Wolf

Sample ID#	HHU20181025	HHU20181023	HHU20181002	x	x
Ethnicity	African/American	Pacific Islander	Caucasian	---	---
Age	38	38	34	---	---
Gender	Male	Male	Female	---	---
ABO/Rh	0/Pos	B/Pos	A/Pos	---	---

SFC- Spot Forming Cells

RI — June 10, 2019
CTL Representative — Date

C.T.L., 20521 Chagrin Blvd., Shaker Heights, Ohio 44122 USA
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SCIENTIFIC
Bender MedSystems GmbH
Campus Vienna Biocenter 2
A-1030 Vienna, Austria
www.thermofisher.com

Certificate of analysis

ProcartaPlex

Cat. number: **PPX-07-MXXGRJX**
96 tests/7 analytes

Name: **Human Custom ProcartaPlex 7-plex**

Lot number: **214139000**

Expiry date: **2020-06**

Components

		Quantity	Lot	Store at
S10011EX	Standard Mix A	2 each	187440101	2-8°C
B-07-MXXGRJX-EX	7-plex Beads	1 x 5ml (1x)	213797000	2-8°C
BK-07-MXXGRJX-EX	7-plex det.AB	1 x 3,5ml (1x)	213798000	2-8°C
RBEX/46	Reading Buffer	1 x 40ml	18103753	2-8°C
WBEX/28	10x Wash Buffer	1 x 25ml	18093505	2-8°C
SA-PE	Streptavidin-PE	1 x 5ml	202532000	2-8°C
UABEX/11	Universal Assay Buffer 1x	1 x 10ml	18124434	2-8°C
SVM104	Black Microplate Lid	1 each		2-8°C
SVM16	Plate Covers	8 each		2-8°C
SVM183	PCR 8-Tube Strip	2 each		2-8°C
SVM182	Flat bottom Plate (black)	1 each		2-8°C

Standard Mix A Lot#187440101

Provided below is a table of Standard 1 (Std1) value for each analyte in each tube when prepared according to the "Preparing Standard" procedure of the Manual.

Analyte	Std1 Concentration (pg/ml)	ULOQ / LLOQ (pg/ml) Determined in cell culture medium
GM-CSF	59600	59600 / 15
IFN-gamma	61900	61900 / 15
TNF-alpha	35200	35200 / 8,59
IL-10	6950	6950 / 1,70
IL-12p70	25200	25200 / 6,15
IL-13	13800	13800 / 3,37
IL-17A	9300	9300 / 2,27
IL-18	67800	67800 / 17
IL-1beta	8150	8150 / 1,99
IL-2	23400	23400 / 5,71
IL-21	41500	41500 / 10
IL-22	77900	77900 / 19
IL-23	70700	70700 / 17
IL-27	71500	71500 / 17
IL-4	40900	40900 / 9,99
IL-5	42000	10500 / 10
IL-6	42900	42900 / 10
IL-9	24600	24600 / 6,01

7-plex Beads Lot#213797000

Target Name	Bead Number	Std1 Concentration pg/ml	Standard
GM-CSF	44	59600	Standard Mix A
IFN-gamma	43	61900	Standard Mix A
IL-1beta	18	8150	Standard Mix A
IL-12p70	34	25200	Standard Mix A
IL-2	19	23400	Standard Mix A
IL-6	25	42900	Standard Mix A
TNF-alpha	45	35200	Standard Mix A

Analytical information:

This product has been tested by Quality Control and passed internal specifications.

Quality control:

For Research Use Only. Not for use in diagnostic procedures. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1-800-955-6288 (US and Canada) or 1-760-603-7200, x2 (all other countries).
For inquiries, contact us at "thermofisher.com/askaquestion"

PRODUCT DATA SHEET – ePBMC® Uncharacterized Cryopreserved Human PBMC

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Applications: PBMCs are suited for T cell monitoring in ELISPOT, ELISA, cytokine bead arrays, tetramer/ pentamer, and cytokine capture assays or any assay that requires live functional PBMC

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Thawing: Thaw protocol included

Usage: **FOR RESEARCH USE ONLY! Not intended for direct therapeutic or diagnostic use in humans or animals, or for use in in vitro diagnostic procedures!**

Characterization results: PBMCs from 3 donors, 1 vial each to Pharmacelsus GmbH, Dr. Tanja Wolf

Sample ID#	HHU20190620	HHU20190709	HHU20190624	x	x
Ethnicity	Hispanic	Caucasian	Caucasian	---	---
Age	22	54	41	---	---
Gender	Female	Male	Male	---	---
ABO/Rh	O/Pos	AB/Pos	AB/Pos	---	---

SFC- Spot Forming Cells

ZSB
CTL Representative

Aug. 16, 2019
Date

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Bender MedSystems GmbH
Campus Vienna Biocenter 2
A-1030 Vienna, Austria
www.thermofisher.com

Certificate of analysis

ProcartaPlex

Cat. number: **PPX-06-MXKA3ZU**
96 tests/6 analytes

Name: **Human Custom ProcartaPlex 6-plex**

Lot number: **218832-000**

Expiry date: **2020-08**

Components		Quantity	Lot	Store at
S10011EX	Standard Mix A	2 each	187440101	2-8°C
B-06-MXKA3ZU-EX	6-plex Beads	1 x 5ml (1x)	218571-000	2-8°C
BK-06-MXKA3ZU-EX	6-plex det.AB	1 x 3,5ml (1x)	218572-000	2-8°C
RBEX/46	Reading Buffer	1 x 40ml	19045685	2-8°C
WBEX/28	10x Wash Buffer	1 x 25ml	19045686	2-8°C
SA-PE	Streptavidin-PE	1 x 5ml	202532000	2-8°C
UABEX/11	Universal Assay Buffer 1x	1 x 10ml	18124434	2-8°C
SVM104	Black Microplate Lid	1 each		2-8°C
SVM16	Plate Covers	8 each		2-8°C
SVM183	PCR 8-Tube Strip	2 each		2-8°C
SVM182	Flat bottom Plate (black)	1 each		2-8°C

6-plex Beads Lot#218571-000

Target Name	Bead Number	Std1 Concentration pg/ml	Standard
GM-CSF	44	59600	Standard Mix A
IFN-gamma	43	61900	Standard Mix A
IL-1beta	18	8150	Standard Mix A
IL-2	19	23400	Standard Mix A
IL-6	25	42900	Standard Mix A
TNF-alpha	45	35200	Standard Mix A

Analytical information:

This product has been tested by Quality Control and passed internal specifications.

Quality control:

For Research Use Only. Not for use in diagnostic procedures. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1-800-955-6288 (US and Canada) or 1-760-603-7200, x2 (all other countries).
For inquiries, contact us at "thermofisher.com/askaquestion"

APPENDIX C: Extract of First Page of Publications from this Thesis

JOURNAL OF NATURAL PRODUCTS

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Article

Anti-inflammatory Flavanones and Flavones from *Tephrosia linearis*

Richard Oriko Owor, Kibrom Gebreheiwot Bedane, Sebastian Zühlke, Solomon Derese, George Otieno Ong'amo, Albert Ndakala,* and Michael Spiteller*

Cite This: *J. Nat. Prod.* 2020, 83, 996−1004

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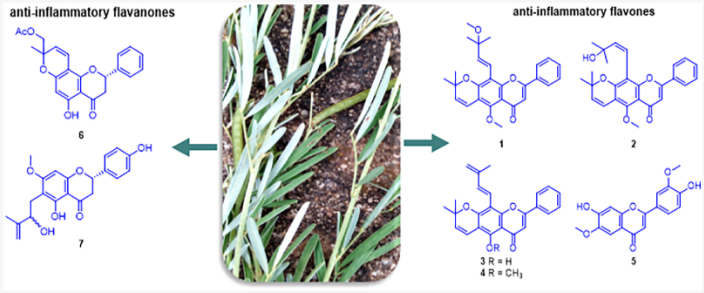

ABSTRACT: Phytochemical analysis of a methanol−dichloromethane (1:1) extract of the aerial parts of *Tephrosialinearis* led to the isolation of 18 compounds. Seven of these, namely, lineaflavones A−D (**1**−**4**), 6-methoxygeraldone (**5**), 8″-acetylobovatin (**6**), and 5-hydroxy-7-methoxysaniculamin A (**7**) are new compounds. The compounds were characterized based on their NMR and HRMS^n data. The anti-inflammatory effects of the crude extract and isolated compounds were evaluated by measuring the levels of interleukins (IL-1β, IL-2, and IL-6), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) in lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs). The crude extract inhibited the release of all cytokines except IL-1β, which slightly increased in comparison to the LPS control. All the tested compounds suppressed the production of IL-2, GM-CSF, and TNF-α. Whereas compounds **1**, **2**, **4**−**8**, **10**−**15**, **17**, and **18** decreased production of IL-6, compounds **1**, **2**, **4**, **7**, **10**, **13**−**15**, and **17** inhibited the release of IL-1β. It is worth noting that most of the compounds tested showed a superior reduction in cytokines release compared to the reference drug ibuprofen.

Plants of the genus *Tephrosia* Pers. (Fabaceae) mainly inhabit tropical and subtropical regions with over 30 species occurring in Kenya.[1] Many of the species have been used ethnomedicinally to alleviate diverse illnesses.[2,3] Phytochemical investigations of some of these plants by our research group have led to the isolation of a chalcone,[4] rotenoids,[5] flavanonols,[6] and flavones[7] that are biologically active. Also, the genus *Tephrosia* is reported to elaborate other bioactive flavonoids such as flavanones, isoflavones, and pterocarpans.[8,9] Some of these flavonoids also exhibit anti-inflammatory properties. For instance, genistein found in *Tephrosia* toxicaria[10] reduces peripheral and central nuclear factor-κB (NF-κB) and the nitric oxide system as well as pro-inflammatory cytokine over-activation,[11] while naringenin, common in the family Fabaceae, decreases the production of TNF-α,[12] and apigenin inhibits TNF-α-induced NF-κB.[13]

In Kenya, the juice of boiled leaves of *Tephrosialinearis* (Willd.) Pers. is used traditionally to treat a broad spectrum of ailments in infants.[14] An earlier phytochemical investigation on the roots of this plant led to the isolation of rotenone, deguelin, tephrosin, and 12a-hydroxyrotenone.[15] In our continued effort to investigate *Tephrosia* species, the aerial parts of *T. linearis* were investigated phytochemically leading to the isolation and identification of 18 compounds, of which seven are new. Herein, are discussed the isolation, structure elucidation, and anti-inflammatory effects of these compounds. To evaluate the anti-inflammatory effects of the crude plant extract and the isolated compounds, lipopolysaccharide (LPS)-stimulated cytokine release of peripheral blood mononuclear cells (PBMCs) was quantified through measurement of the levels of IL-1β, IL-2, IL-6, GM-CSF, and TNF-α.

RESULTS AND DISCUSSION

The crude extract of the aerial parts of *T. linearis* was subjected to silica gel and Sephadex LH-20 column chromatography,

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NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1736065

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Synergistic anti-inflammatory activities of a new flavone and other flavonoids from *Tephrosia hildebrandtii* vatke

Richard Oriko Owor[a,b,c], Kibrom Gebreheiwot Bedane[b,d], Yolande Ikala Openda[a], Sebastian Zühlke[b], Solomon Derese[a], George Ong'amo[e], Albert Ndakala[a] and Michael Spiteller[b]

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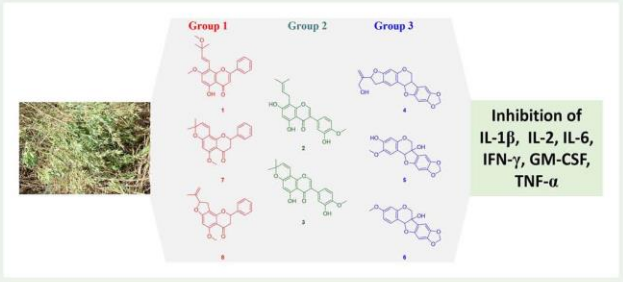
ABSTRACT

A new flavone, named hildeflavone (**1**) along with 7 other known flavonoids were isolated from the aerial parts of *Tephrosia hildebrandtii* Vatke. Their characterisation was based on NMR and MS data analysis. The anti-inflammatory properties of the crude extract, isolated compounds and combination of the compounds were investigated in lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs). Treatment of the LPS-stimulated PBMCs with the isolated flavonoids at a concentration of 100 μM significantly reduced the production of interleukins (IL-1β, IL-2 and IL-6), interferon-gamma (IFN-γ), granulocyte macrophage-colony stimulating factor (GM-CSF) and tumour necrosis factor-alpha (TNF-α). It was also found that the combination of a flavone and flavanones exhibited remarkable synergistic anti-inflammatory effects on the production of the cytokines.



ARTICLE HISTORY
Received 26 December 2019
Accepted 10 February 2020

KEYWORDS
Fabaceae; *Tephrosia hildebrandtii*; flavonoid; anti-inflammatory; hildeflavone; cytokine; synergy

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1736065.

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Isoflavones from the seedpods of *Tephrosia vogelii* and pyrazoisopongaflavone with anti-inflammatory effects

Richard Oriko Owor[a,b,c,*], Solomon Derese[a], Kibrom Gebreheiwot Bedane[d], Sebastian Zühlke[b], Albert Ndakala[a,**], Michael Spiteller[b,**]

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[d] Department of Chemistry, Addis Ababa University, P.O. Box 33658, Addis Ababa, Ethiopia

ARTICLE INFO

Keywords:
Tephrosia vogelii
Vogelisoflavone
Pyrazoisopongaflavone
Isopongaflavone
Anti-inflammatory
Cytokine

ABSTRACT

Phytochemical investigation of *Tephrosia vogelii* seedpods led to the isolation of twelve compounds: vogelisoflavone A (1), vogelisoflavone B (2), isopongaflavone (3), onogenin, luteolin, 4′,7-dihydroxy-3′-methoxyflavanone, *trans-p*-hydroxycinnamic acid, tephrosin, 2-methoxygliricidol, dehydrorotenone, 6a,12a-dehydro-α-toxicarol and pinoresinol. Compounds 1 and 2 are reported as new natural products. Isopongaflavone (3) was structurally modified using hydrazine to pyrazoisopongaflavone (4). These compounds were characterized based on their NMR and HRESIMS data. Further, four compounds (1–4) were evaluated for their anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs). Treatment of the LPS-stimulated PBMCs with the compounds at a concentration of 100 μM suppressed the secretion of interleukin IL-1β interferon-gamma (IFN-γ), granulocyte macrophage-colony stimulating factor (GM-CSF) and tumour necrosis factor-alpha (TNF-α).

1. Introduction

Tephrosia vogelii Hook.f. Pers. is widely distributed in tropical Africa and is commonly known as fish bean or Vogel's tephrosia [1,2]. This plant is widely cultivated in Africa for fishing, protecting crops against mole rats, pests and for soil enrichment [3–7]. It is also an important ethnomedicinal plant used to manage a variety of ailments including scabies, yaws and constipation [8,9]. The diverse traditional uses of *T. vogelii* have stimulated various studies on its phytochemical and pharmacological properties. Extracts and isolates from *T. vogelii* have exhibited varied activities including insecticidal, mollusicidal and piscidal activities [1,4,6,10–12]. Most of the entomotoxic and ichthotoxic properties of *T. vogelii* are attributed to rotenoids especially deguelin, rotenone, tephrosin, α-toxicarol and sarcolobine that have been reported to be abundant in the plant [11,13,14]. The plant also elaborates other flavonoids that are non-entomotoxic and non-ichthotoxic such as rutin, quercetin-3-arabinopyranoside, obovatin-5-*O*-methylether, quercetin-3-*O*-galactoside and *Z*-tephrostachin [13–16]. Interestingly, some of these flavonoids such as rutin, quercetin-3-*O*-galactoside have been shown to possess anti-inflammatory properties through modulation of inflammatory mediators especially cytokines [17,18].

In our continued efforts to study the anti-inflammatory flavonoids from *Tephrosia* plants [19,20], the phytochemical constituents of *T. vogelii* seedpods were investigated leading to isolation of two new isoflavones along with ten known compounds. Considering that conversion of some natural products such as curcumin into their pyrazole derivative enhanced their anti-inflammatory activity [21,22], in this study, one of the flavonoids (isopongaflavone) was structurally modified to its pyrazole derivative and evaluated for its anti-inflammatory activity. It is also worth noting that pyrazole derivatives are potent anti-inflammatory agents and some have been developed into anti-inflammatory drugs such as celecoxib, lonazolac and mepirizole [23–25]. Herein, the isolation, structural elucidation, structure modification, and anti-inflammatory activities of the compounds isolated from the seedpods of *T. vogelii* are described.

2. Experimental

2.1. General experimental procedures

The instrumentation and reagents used were as described in our

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