**UNIVERSITY OF NAIROBI**

**COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES**

**DEPARTMENT OF CHEMISTRY**

# SYNTHESIS OF ANTIMICROBIAL BENZENE-1,4-DIOL ANALOGUES OF A BENZOQUINONE METABOLITE FROM THE ENDOPHYTIC FUNGI *XYLARIA Sp.* PBR 30

**BY**

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**2017**

# DECLARATION

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

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# DEDICATION

To Maggie, a friend and mentor

# ACKNOWLEDGMENT

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Finally but most importantly, I thank the Almighty God for the gift of life, care, protection and more so for the time, resources and energy to pursue my academic career. His name be honored and glorified forever. Amen.

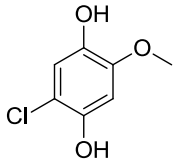
# ABSTRACT

The ever increasing problem of microbial resistance to currently available antimicrobial agents has led to a pressing need for development of new therapeutic and disinfecting agents. Although bioactive natural products continue to be a new source of antimicrobial agents, their use as antimicrobial drugs has often been restricted due to their low potency and high toxicity. This has necessitated a rationalized mechanistic approach of structural modification of such bioactive natural products to improve their potency, pharmacodynamics and safety. In this study, benzene-1,4-diol analogues of a bioactive benzoquinone metabolite, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**), were synthesized and assessed for antimicrobial activity.

Synthesis and antimicrobial evaluation of 2-chloro-5-methoxy-1,4-benzenediol (**59**), 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**), 2-methoxy-1,4-benzenediol (**79**), 2,5-dichloro-1,4-benzenediol (**81**) and 2,5-dinitro-1,4-benzenediol (**82**) and their intermediates was achieved. Compounds **59** and **60** were the most active against *Streptococcus infantarius* with minimum inhibition at 25µg/disc each, 5-chloro-2-hydroxyaniline (**62**) was the most active against *Candida albicans* (minimum inhibition at 12.5µg/disc) while **79** was the most active against *Staphyloccocus aureus* and *Escherichia coli* with minimum inhibition at 12.5µg/disc and 25µg/disc, respectively.

The *in vitro* antiplasmodial activity of compound **62**, 5-chloro-2-methoxyacetanilide (**61**) and 5-chloro-2-hydroxyacetanilide (**63**) were found to fall within the WHO scale of active compounds with $IC_{50}$ values of 2.70 ± 0.14, 2.85 ± 0.25 and 9.06 ± 1.09 µg/mL against the chloroquine sensitive 3D7 and 1.24 ± 0.47, 1.29 ± 0.48 and 7.09 ± 1.94 µg/mL against chloroquine sensitive

D6 isolate, respectively. On the basis of the observed activity, it is recommended that these compounds be further optimized through structure modification to identify more potent antimicrobial and antiplasmodial compounds for drug discovery.

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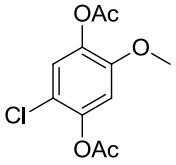


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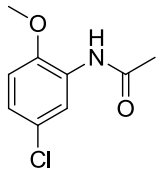


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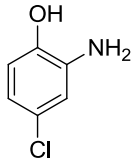


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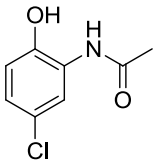


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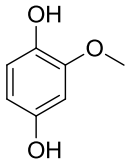


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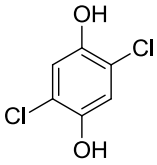


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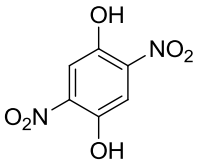


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# LIST OF ABBREVIATIONS AND ACRONYMS

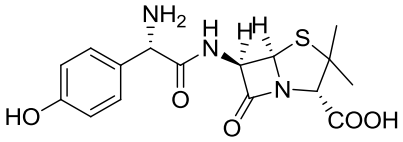
| | |
|---|---|
| **$Ac_2O$:** | Acetic anhydride |
| **ACT's:** | Artemisinin-based Combination Therapies |
| **ATCC:** | American Type Culture Collection |
| **$^{13}$C-NMR:** | $^{13}$Carbon Nuclear Magnetic Resonance |
| **° C:** | Degrees Celsius |
| **CC:** | Column Chromatography |
| **CDC:** | Center for Disease Control and Prevention |
| **CDDEP:** | Center for Disease Dynamics, Economics and Policy |
| **CLSI:** | Clinical Laboratory Standards Institute |
| **COSY:** | Homonuclear Correlation Spectroscopy |
| **CQ:** | Chloroquine Sensitive |
| ***d*:** | Doublet |
| **$\delta_C$:** | Chemical shift for $^{13}$C in ppm |
| **DCM:** | Dichloromethane |
| ***dd*:** | Doublet of Doublet |
| **$\delta_H$:** | Chemical shift for $^{1}$H in ppm |
| **D6:** | Chloroquine sensitive strain of *Plasmodium falciparum* |
| **3D7:** | Chloroquine sensitive strain of *Plasmodium falciparum* |
| **DCMX:** | 4, 6-Dichloro-3,5-xylenol |
| **DMSO:** | Dimethylsulfoxide |
| **EtOAc:** | Ethyl Acetate |
| **EtOH:** | Ethanol |
| **g:** | Grams |
| **GARP:** | Global Antibiotic Resistance Partnership |
| **GC-MS:** | Gas Chromatography-Mass Spectrometry |
| **H, H-COSY:** | Proton, Proton Correlation Spectroscopy |
| **HIV:** | Human Immunodeficiency Virus |
| **HMBC:** | Heteronuclear Multiple Bond Correlation |
| **HMQC:** | Heteronuclear Multiple Quantum Correlation |
| **$^{1}$H-NMR:** | Proton Nuclear Magnetic Resonance |
| **HSQC:** | Heteronuclear Single Quantum Coherence |
| **Hz:** | Hertz |
| **$IC_{50}$:** | Concentration of 50% Inhibition |
| ***J*:** | Coupling Constant |
| **KEMRI:** | Kenya Medical Research Institute |
| **K1:** | Multidrug resistant strain of *Plasmodium falciparum* |
| **MeOH:** | Methanol |
| **mg:** | Milligrams |
| **MHz:** | Megahertz |
| **MHA:** | Mueller Hinton Agar |

| | |
|---|---|
| **MIC:** | Minimum Inhibitory Concentration |
| **$MIC_{50}$:** | Minimum Inhibitory Concentration for 50% Inhibition |
| **mL:** | Milliliters |
| **mm:** | Millimeters |
| **MRSA:** | Methicillin-resistant *Staphylococcus aureus* |
| **ng/ mL :** | Nanogram per milliliter |
| **NIAID:** | National Institute of Allergy and Infectious Diseases |
| **NMR:** | Nuclear Magnetic Resonance |
| **NOESY:** | Nuclear Overhauser Effect Spectroscopy |
| **NQCL:** | National Quality Control Laboratory |
| **PCMX:** | *p*-Chloro-*meta*-xylenol |
| **Pd/ C:** | Palladium on Carbon |
| **PHPT:** | Department of Public Health Pharmacology & Toxicology |
| **ppm:** | Parts per million |
| ***q*:** | Quartet |
| **QAC's:** | Quaternary Ammonium Compounds |
| **$R_f$:** | Retention Factor |
| **RFU:** | Relative Fluorescence Units |
| **RT:** | Room Temperature |
| ***s*:** | Singlet |
| **SD:** | Standard Deviation |
| ***t*:** | Triplet |
| **TB:** | Tuberculosis |
| **THF:** | Tetrahydrofuran |
| **TLC:** | Thin Layer Chromatography |
| **TMS:** | Tetramethylsilane |
| **µg:** | Micrograms |
| **µg/ mL:** | Microgram per milliliter |
| **µL:** | Microlitres |
| **µM:** | Micromolar |
| **UV:** | Ultra Violet |
| **v/v:** | Volume by Volume |
| **WHO:** | World Health Organization |
| **wt/wt:** | Weight by Weight |

# CHAPTER 1
# INTRODUCTION

## 1.1. Background Information

Microbial infections caused by bacteria, fungi and protozoa remain a significant cause of mortality and morbidity globally. Globally, they remain the leading cause of death, accounting for an annual average of over 17 million of the estimated 52 million deaths (WHO, 2014; CDC, 2013). Antimicrobial agents such as antibiotics, antiseptics and disinfectants are regarded as the panacea to cure and prevent the spread of these microbial infections. Notable antimicrobial agents used as human drugs include amoxicillin (**1**) used in the treatment of pneumonia and bronchitis, metronidazole (**2**), an antibacterial and antiprotozoal drug used in the management of amoebic infections and trichomoniasis and quinine (**3**), a renowned antimalarial drug (CDDEP, 2015; Saga and Yamaguchi, 2009; WHO, 2016).

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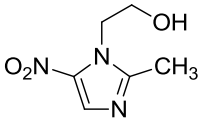


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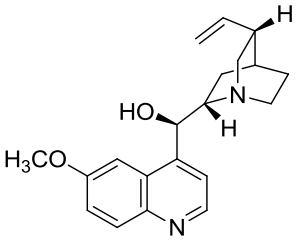
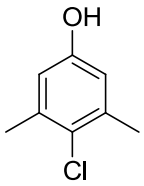



**3**

Disinfectants and antiseptics are widely used to control the spread of pathogenic microbes by sterilization of the skin, wounds, surfaces and equipment in medical and household settings (Saha *et al*., 2009; Payne *et al*., 1999). One of the most commonly used disinfectant, Dettol®, has *p*-chloro-*meta*-xylenol (PCMX) (**4**) as the active chemical constituent (El-Mahmood and Doughari, 2008). The other notable phenolic-type disinfectant is triclosan (**5**), an antiseptic compound useful in medicated soap formulation and toiletries (McDonnell and Russell, 1999).

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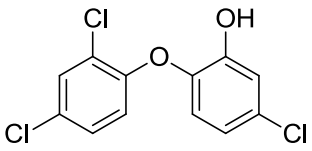


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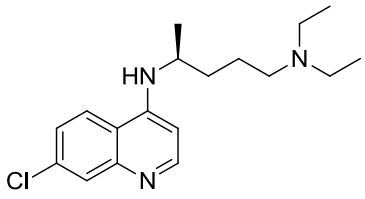
The continuous use of specific antimicrobial agents commonly leads to emergence of resistant microbes arising from a process of selection to the extent that some of the once-treatable microbial infections have become difficult to manage using the currently available drugs (Ventola, 2015). Notable examples of drug resistant microbes include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and gram-negative bacilli (Schlegelova *et al*., 2002; McDonnell and Russell, 1999). Microbes tolerant to PCMX (**4**) and triclosan (**5**) include *Staphylococcus aureus, Pseudomonas stutzeri*, *Citrobacter freundii* and *Acinetobacter johnsonii* (Lear *et al.*, 2002; Farzana *et al*., 2011). Moreover, resistance to first line antimalarial drugs like chloroquine (**6**) and artemisinin (**7**) has also been observed in *Plasmodium falciparum*, the deadliest malaria parasite (WHO, 2014; Winter *et al*., 2008).

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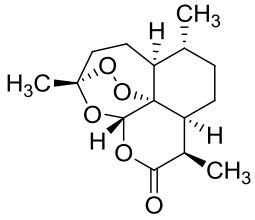


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This problem of drug resistance has necessitated a continuing search for new antimicrobial agents. Secondary metabolites from natural sources continue to act as a fundamental and integral component of today's pharmaceutical compendium (Saleem *et al.*, 2010). From time immemorial, secondary metabolites from plants and other sources have been used in curing microbial infections and related ailments (Cowan, 1999; Selim *et al.*, 2012). With scientific

development, they have also acted as a good source for new and more effective drugs and as potential lead compounds towards development of novel compounds active against pathogenic microbes through structural modification (Visalakchi and Muthumary, 2010).

An intensive search for alternative sources of newer, safer and more effective agents to deal with diseases eliciting drug resistance needs to be accelerated. Endophytes, a type of fungi that live symbiotically with plants but cause no apparent disease, are a novel source of potentially useful lead compounds and remain one such viable alternative (Verma and Gange, 2014; Suryanarayanan, *et al*., 2009). This study explored a bioactive benzoquinone metabolite derived from an endophytic fungus as a template for development of potential antimicrobial and antiplasmodial agents.

### 1.2. Statement of the Problem

Drug resistance is increasing amongst pathogenic microbes posing a serious health problem worldwide (WHO, 2014; CDC, 2013). For instance, microbes such as *Staphylococcus aureus Pseudomonas stutzeri*, *Citrobacter freundii* and *Acinetobacter johnsonii* have shown tolerance to phenolic-type antiseptics such as *p*-chloro-*meta*-xylenol (PCMX) (**4**) and triclosan (**5**), posing a major global health concern (Lear *et al.*, 2002; Farzana *et al*., 2011). Moreover, *Plasmodium falciparum* which causes cerebral malaria, the most dangerous form of the disease, has developed resistance to the common antimalarial drugs, chloroquine (**6**) and artemisinin (**7**) (WHO, 2015; Winter *et al*., 2008; Olliaro and Yuthavong, 1999).

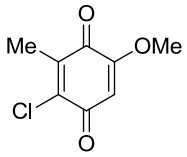
Due to the emerging problem of microbial drug resistance, there is need for the identification of new potent antimicrobial agents and viable lead compounds for drug development.

### 1.3. Objectives

This study was guided by the following general and associated specific objectives.

#### 1.3.1. General Objective

The general objective of this study was to identify antimicrobial phenolic analogues of 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**), a benzoquinone secondary metabolite from *Xylaria sp*.




**8**

#### 1.3.2. Specific Objectives

The specific objectives of this study were:

i. To synthesize and characterize various analogues of the benzoquinone metabolite, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione.

ii. To evaluate the antibacterial, antifungal and antiplasmodial activities of the synthesized analogues.

### 1.4. Justification and Significance of the Study

There are various reasons for the quest for new and more effective antimicrobial agents that can be used as antiseptics and in chemotherapy (Snow *et al*., 2001). Although natural and synthetic antimicrobial agents have been the panacea to cure microbial infections, their efficacy has been severely compromised by the emergence of antimicrobial resistance. Microbial resistance

continues to be a major global health concern since it reduces the effectiveness of antimicrobial treatment, leading to increased mortality and healthcare expenditure and if not urgently addressed will leave medical personnel virtually helpless in the prevention and treatment of many microbial infections (CDDEP, 2015; Ventola, 2015).

Use of bioactive natural products as human drugs in treatment of bacterial infections often has restricted application due to their low activity and high toxicity (Cowan, 1999; Carey *et al*., 2006), while natural products with low toxicity rarely progress through pre-clinical trials due to low bio-availability and poor solubility (Meshnick, 2002; Wiesner *et al*., 2003). To overcome these challenges, a rational approach to structural modification of natural products is required to enhance the activity and reduce toxicity (Carey *et al*., 2006; Andayi, 2005).

One such bioactive natural product that attracted our attention was the benzoquinone metabolite 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**) elucidated from *Xylaria sp.*, which exhibited some antimicrobial activity (Tansuwan *et al*., 2007; Rosa *et al.*, 2011; Bero *et al*., 2009) and therefore worth further investigation. It is noteworthy that the reduced form of the benzoquinone metabolite is structurally related to the antiseptic agent PCMX (Figure 1).

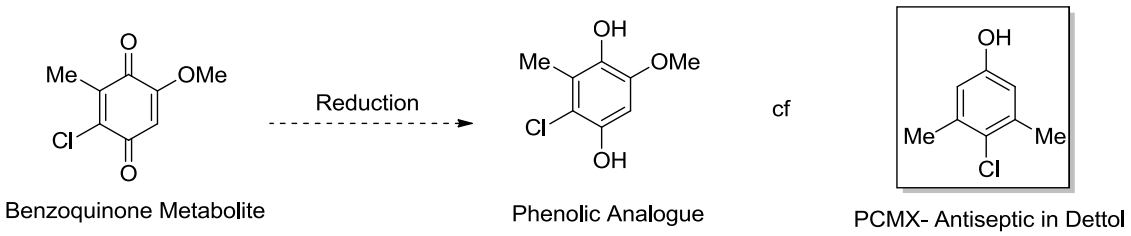



Figure 1: Similarities between PCMX and reduced benzoquinone metabolite

Considering that phenolic compounds have a good reputation for their antimicrobial activity, with notable examples of *p*-chloro-*meta*-xylenol (PCMX) (**4**), triclosan (**5**) and chlorophene, there exists great potential for identification of related compounds with similar or better activity

(McBain *et al.*, 2004; Shipton *et al.*, 1984). Consequently, diverse phenolic analogues of 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**) were synthesized in this study and evaluated for their efficacy against various microbes.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Microbial Diseases Affecting Humans

Microbial infections are the leading cause of death in humans, accounting for an annual average of over 17 million of the estimated 52 million deaths worldwide. Microbial infections in humans are caused by bacteria, protozoa, viruses and fungi. Common human microbial infections include intestinal disorders caused by bacteria such as *Vibrio cholerae* (cholera) and *E. coli*, teeth decay (caused by *Streptococcus infantarius* and other Streptococcal strains), respiratory, skin and urinary tract infections (Kampf and Kramer, 2004; NIAID, 2014).

Of all the deaths associated with bacterial infections, pneumonia which is the largest cause of infant mortality globally, accounts for 4.4 million deaths, about 4 million of whom are children. Cholera, typhoid and dysentery which spread chiefly through contaminated food, water and direct contact from person to person account for over 3.1 million cases, most of them being children, while tuberculosis accounts for nearly 3.1 million deaths (CDC, 2013; WHO, 2014).

The most common protozoan infection and the leading cause of infant mortality is cerebral malaria, caused by the parasite *Plasmodium falciparum* transmitted by the anopheles mosquito (Winter *et al*., 2008; Olliaro and Yuthavong, 1999). Globally, malaria continues to affect over 200 million people leading to over 400,000 fatalities annually with 90% of the deaths occurring in Africa (WHO, 2016)**.** It continues to impart devastating social and economic burdens to the population in regions prone to it especially to expectant mothers and children below the age of five (WHO, 2014; Winter *et al.*, 2008; Snow *et al*., 2001).

Infections caused by viruses include respiratory tract infections such as common cold, flu, hepatitis, HIV, pneumonia and vaginal herpes (Fridkin and Jarvis, 1996; Payne *et al.*, 1999) Globally, the most frequent viral infections include genital herpes accounting for over 500 million cases annually, HIV with over 36 million people living with the virus and viral hepatitis (WHO, 2014).

Fungal infections are diverse and affect both the body surfaces and internal organs (Mensah *et al*., 2000). Such infections include *Candida*, a common opportunistic infection in immune-compromised patients, which invades the skin and mucous membrane and *Aspergillus sp*. which infect the respiratory tract and parts of the skin. Candida accounts for 30% of all urogenital infections with about 75% of all women experiencing *candidiasis* at least once in their lifetime with nearly half, having recurring infections (CDC, 2013; NIAID, 2014).

The spread of pathogens associated with these microbial infections can be prevented by good hygiene practices or treated using either natural or synthetic antimicrobial agents (Weber *et al*., 2007; Kampf and Kramer, 2004; Grabley and Thiericke, 1999).

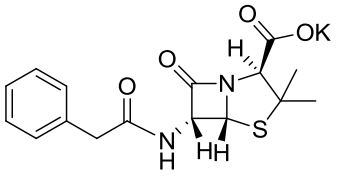
### 2.2. Antimicrobial Agents

Antimicrobial agents may be classified based on either their target class of microbes or their application, and include classes such as antibiotics, antimalarials, antiseptics and disinfectants (Bryskier, 2005; Weber *et al*., 2007; Brewer, 2011).

#### 2.2.1. Antibiotics

These are antimicrobial agents used in the treatment and prevention of bacterial infections. They include β-lactam antibiotics such as penicillins (e.g. penicillin G (**9**)), sulfonamides (such as

sulfanilamide (**10**) and sulfacetamide (**11**)), cephalosporins, tetracyclines, erythromycins and miscellaneous drugs having one or two representatives such as chloramphenicol (**12**) (NIAID, 2014; Bryskier, 2005).

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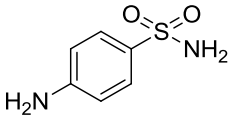


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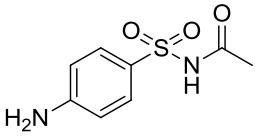


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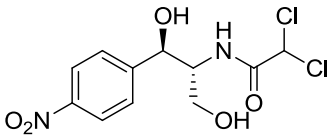



**12**

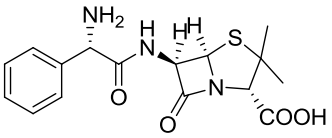
Since the discovery of penicillins in the late 1920s, antibiotics have been regarded as the panacea to cure both community and hospital-acquired bacterial infections. However, once-treatable bacterial infections are now becoming difficult to cure due to emergence of microbial resistance to available drugs, raising healthcare costs and patient's mortality (Ventola, 2015). For instance, CDC estimates that antibiotic resistance is responsible for over 2 million infections and 23,000 deaths annually in the USA alone (CDC, 2013).

Microbial resistance to available antibiotics continues to be a major global health concern. It has been attributed to non-compliance to prescription dosage (under-dosage by patients) and extensive antibiotics use in farm animals leading to progressive selection for tolerant microbes (CDDEP, 2015). Specific microbes with developed antibiotic resistance include cephalosporin-resistant *Escherichia coli*, methicillin-resistant *S. aureus*, amoxicillin-resistant *Streptococcus pneumonia* and multi-drug resistant TB (Saga and Yamaguchi, 2009; Ventola, 2015).

Drug resistant TB and *Shigella* infections continue to pose serious life threatening situations in developing countries especially to children under the age of 5 years. In 2014, WHO estimated that of the 9.6 million cases of TB experienced globally, 3.6% of new TB cases and 20.2% of

previously treated cases had developed resistance to multiple drugs. In 2012 alone, over 170,000 people succumbed to drug-resistant tuberculosis infections (WHO, 2014).

*Shigella* species, a major cause of diarrhea and dysentery, have developed resistance against traditional first-line drugs such as ampicillin (**13**) and cotrimoxazole (trimethoprim (**14**) and sulfamethoxazole (**15**) combination) necessitating use of alternative drugs like ciprofloxacin (**16**) and azithromycin, which have since experienced cases of resistance (CDDEP, 2015; WHO, 2014; WHO, 2016).

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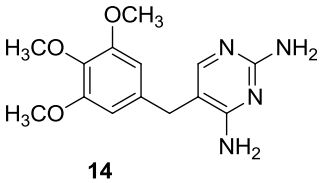


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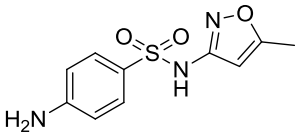


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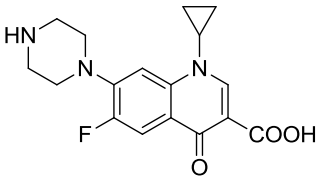


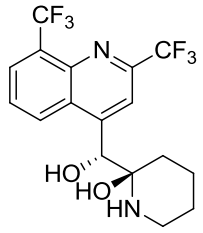

**16**

Limited data is available on the extent of drug resistance in sub-Saharan Africa with most data available being on resistance of clinical isolates (Leopold *et al*., 2014). In Kenya, microbial infections that contribute most to human infections include multi-drug resistant *Salmonella typhi*, *Mycobacterium tuberculosis*, methicillin-resistant *Staphylococcus aureus* and *plasmodium* parasites (GARP, 2011). By 2003, resistance of penicillin to *S. pneumonia* isolates was at 43% while half of the children with severe pneumonia were resistant to penicillin by 2005. By 2001, over 75% diarrheal cases were multi-drug resistant to three or more drugs while prevalence of

multidrug resistance to non-typhi *Salmonella* exceeded 40% in 2003 and 78% to *Salmonella typhi* by 2004 (CDDEP, 2015; GARP, 2011).

### 2.2.2. Antimalarial Agents

These are antimicrobial agents used in the treatment and prevention of malaria. Currently, the artemisinin-based combination therapies (ACT's) that comprise of a mixture of artemisinin (**7**) or its derivatives and antibiotics or other antimalarial drugs remain the most effective combination in malaria chemotherapy. Drugs incorporated in ACT's include mefloquine (**17**), amodiaquine (**18**) and a combination of sulfadoxine (**19**) and pyrimethamine (**20**) (WHO, 2015; Clarke and Moffat, 1986).

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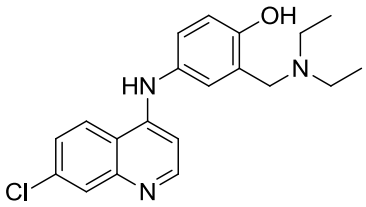


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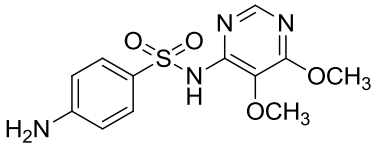


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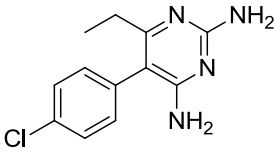



**20**

Introduction of ACT's has been necessitated by the decline in efficacy of mono-therapeutic antimalarial drugs such as chloroquine (**6**) due to the evolution of drug-resistant strains of both *P. falciparum* and *P. vivax* (WHO, 2014; Bero *et al.,* 2009). The risk of total collapse in malaria chemotherapy remains high, as experienced at the Cambodia-Thailand border where the parasite

has become resistant to almost all available antimalarial drugs, necessitating an urgent need for development of new anti-malarial drugs (WHO, 2014; Grimberg and Mehlotra, 2011; Winter *et al.*, 2008).

### 2.2.3. Antiseptics and Disinfectants

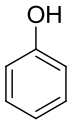
Unlike antibiotics which are used to treat both internal and surface infections, antiseptics and disinfectants are typically used for controlling and preventing infections on body surfaces. They are useful in healthcare and household settings for sterilization of external wounds and inanimate objects. In particular, they form an integral part in controlling hand-to-hand and surface spread of pathogens thus reducing the spread of both endemic and epidemic infections and/or diseases (Weber *et al*., 2007; Famurewa *et al*., 2014).

Pathogens whose transmission can be prevented by use of appropriate disinfectants include *Streptococci*, *Staphylococci* and *Mycobacterium tuberculosis* (Payne *et al.*, 1999). Some of the most important antiseptics and disinfectants are phenols, alcohols, peroxides, aldehydes, quaternary ammonium compounds (QACs), halogen-releasing agents and chlorhexidine (Russell, 2002; Farzana *et al.*, 2011).

#### 2.2.3.1. Synthetic Phenols as Antiseptics and Disinfectants

Phenols and their derivatives have from time immemorial been recognized for their antimicrobial properties particularly as antiseptics and disinfectants (McDonnell and Russell, 1999; Bryskier, 2005). Just like antibiotics, they are effective against a wide range of disease-causing pathogens but are typically used in decontamination of surfaces and household equipment (Farzana *et al*., 2011; Famurewa *et al*., 2014). Phenol (**21**) and phenolic derivatives such as 2-chlorophenol (**22**)

and 4-chlorophenol (**23**) are some of the oldest antiseptics commercially used exhibiting activity against bacteria, fungi and viruses (Bryskier, 2005; Russell *et al.*, 1999).

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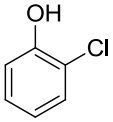


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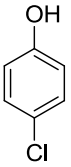
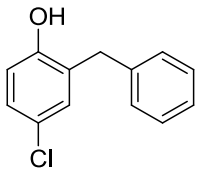



**23**

One of the most notable phenolic antiseptic and disinfectant of household surfaces and equipment is *p*-chloro-*m*-xylenol (PCMX) (**4**). PCMX is present in the commercial disinfectant Dettol® as an aqueous solution in 4.8% w/v concentration. It is highly preferred because besides its efficacy against a wide range of pathogenic micro-organisms, it is relatively cheap, easily available, exhibits low short-term mammalian toxicity and, in the recommended dilutions, is generally non-irritant (Shipton *et al.*, 1984; Opara *et al.*, 2012).

Other notable phenols with good reputation for their antiseptic ability are triclosan (**5**) and chlorophene (2-benzyl-4-chlorophenol) (**24**). They are useful in medicated soap formulation, toiletries and as medical, household and industrial disinfectants (Weber *et al*., 2007; Rekha and Kotchevar, 2010).



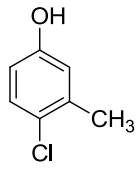

**24**

PCMX (**4**) and triclosan (**5**) remain the most potent antibacterial and antifungal phenols and have found relevance in formulations of many pharmaceuticals and disinfectants (Latosinska *et al.*, 2012; El-Mahmood and Doughari, 2008). PCMX (**4**) and chlorophene (**24**) are primarily used as

germicides in disinfectant solutions and soap formulations in hospitals (Opara *et al.*, 2012; Werner *et al.*, 1983).

Triclosan (**5**) is commonly used in the manufacture of personal care products. It is highly effective against a wide range of pathogenic micro-organisms such as *staphylococcus*, *streptococcus*, *mycobacterium*, fungi and protozoa such as *Entamoeba hystolytica* and has found use in a wide range of personal hygiene products such as soaps and detergents, toothpastes and mouthwash (Rekha and Kotchevar, 2010; Bryskier, 2005).

Other commercially useful phenolic antimicrobial agents are 4-chloro-*m*-cresol (PCMC) (**25**) used in medicated soaps, 4,6-dichloro-3,5-xylenol (DCMX) (**26**) used in domestic households and hospitals, 6-pentyl-*m*-cresol (**27**), an active ingredient in Strepsils® lozenges used to kill bacteria that cause sore throat and mouth infections, resorcinol (**28**), a dermatological agent in skin products such as Acnil®, Acnomel® and Eskamel® and 4-hexylresorcinol (**29**), an antihelmintic, anti-aging and antibacterial agent found in Benylin® and Strepsils Extra® sore throat lozenges (Clarke and Moffat, 1986; Rekha and Kotchevar, 2010; Russell *et al.*, 1999) .

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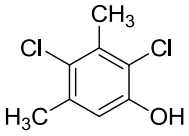


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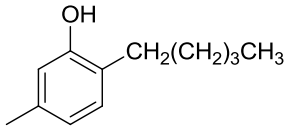


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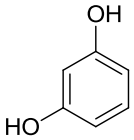


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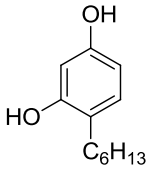



**29**

### 2.2.3.1.1. Mode of Action of Synthetic Phenols

The mode of action of phenolic antiseptics such as PCMX and triclosan has been extensively investigated (McDonnell and Russell, 1999). At high concentration, triclosan and PCMX are

known to cause damage to the outer cell membrane of bacteria and yeasts leading to leakage of intracellular constituents and eventually leading to death of the cell (Kojima *et al*., 2008; Novais *et al*., 2008). High concentrations of phenolics such as triclosan and chlorhexidine cause coagulation of intracellular constituents and lowers membrane permeability by partitioning or displacement of phospholipid layers (McDonnell and Russell, 1999; Denyer and Stewart, 1998).

At lower concentration, the agent permeates through the cells outer membrane and subsequently attacks the bacterial cytoplasmic or inner membrane. Moreover, phenols have also been demonstrated to lyse rapidly growing cultures of *E. coli*, *Staphylococci* and *Streptococci* by acting against young daughter cells at cell replication (Denyer and Stewart, 1998; Kojima *et al*., 2008).

#### 2.2.3.1.2. Side Effects and Microbial Resistance against Synthetic Phenols

PCMX and triclosan have for a long time been considered relatively safe due to their low mammalian toxicity (McDonnell and Russell, 1999). However, various side effects associated to these agents have been identified due to constant usage and exposure. They are now known to have a long-term allergic and neuro-toxic effect. Moreover, they have recently been discovered to cause skin irritation and have a depressive effect on the central nervous system (Mwambete and Lyombe, 2011; Latosinska *et al.*, 2012).

These side effects have triggered an advocacy for the ban of triclosan and PCMX from non-medical applications. For instance, the Food and Water Watch Organization and various agencies dealing with food, drugs and the environment across Europe and the US have petitioned for their ban or restriction in cosmetics. This has necessitated countries like Japan, Sweden and Germany to enact laws on such restrictions (McBain *et al.*, 2004; Latosinska *et al.*, 2012).

The other challenge that has arisen with the use of synthetic phenols as antiseptics and disinfectants is that of microbial resistance (Rekha and Kotchevar, 2010; El-Mahmood and Doughari, 2008). It has been established that constant exposure of microorganisms to sub-lethal concentrations of biocides such as PCMX, chlorhexidine gluconate and triclosan found in medicated soaps and other consumer products decreases microbial susceptibility over hundred folds leading to progressive microbial resistance (McBain *et al.,* 2004; Hay *et al*., 2001). For instance, tolerance to PCMX and triclosan has been reported in *Pseudomonas stutzeri*, *Citrobacter freundii* and *Acinetobacter johnsonii* with elevated tolerance observed in *P. stutzeri* and *A. johnsonii* (Lear *et al.,* 2002; Ahmad *et al.,* 2013). Table 2.1 shows other examples of bacteria that have developed resistance against commonly used antiseptics and disinfectants.

Table 2. 1: Resistant bacteria to commonly used antiseptics and disinfectants

| **Bacteria** | **Antiseptics against which resistance has developed** |
|---|---|
| Gram-negative bacteria | Triclosan |
| Mycobacteria | Chlorhexidine, glutaraldehyde |
| Gram-positive | Chlorhexidine |
| Bacteria spores | Phenolics, chlorhexidine |

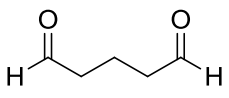
(McDonnell and Russell, 1999; Lear *et al.,* 2002)

Various mechanisms are involved in the development of microbial resistance with the main common being mutation**,** reduced diffusion of the antiseptic across the cell wall and enzymatic inactivation or degradation of the antiseptic (Latosinska *et al.,* 2012; Mwambete and Lyombe, 2011; McDonnell and Russell, 1999). Thus, with time, the disease-causing pathogens develop intrinsic resistance leading to the wider problem of resistance to available antimicrobial agents (Hay *et al.,* 2001).

### 2.2.3.2. Alcohols and Aldehydes as Antiseptics and Disinfectants

The most important and commonly used alcohols are ethanol, isopropanol and *n*-propanol. They exhibit broad-spectrum activity and are effective against Gram positive and Gram negative bacteria, fungi and enveloped viruses. They are most effective at concentrations above 50% with the optimum bactericidal activity at 60-90% concentration (Latosinska *et al.*, 2012). Their antimicrobial activity is due to their ability to denature proteins leading to cell lyses. For hand sanitization in hospitals and workplaces, alcohol-based hand sanitizers in gel or rub delivery systems have recently become popular. Alcohols are however not effective against bacterial spores and have limited effectiveness against non-enveloped viruses. Their volatility also reduces concentration and hence antibacterial efficacy (Larson and Morton, 1991; McDonnell and Russell, 1999).

Formaldehyde, gluteraldehyde (**30**) and *o*-phthalaldehyde (**31**) are aldehydes of wide germicidal spectrum commonly used in hospitals. Formaldehyde is traded and used principally as a water-based solution containing 37% formaldehyde by weight and is commonly referred to as formalin. The aqueous solution of these aldehydes is highly potent against bacteria, fungi and viruses (Weber *et al*., 2007; Ascenzi, 1996). Although these compounds are highly effective, formaldehyde and gluteraldehyde are highly toxic, potential carcinogens and produce pungent, irritating odors while *o*-phthalaldehyde stains unprotected parts of skin (Walsh *et al*., 1999).

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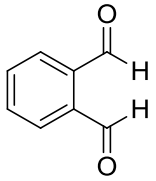
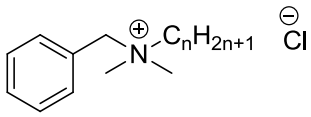



**31**

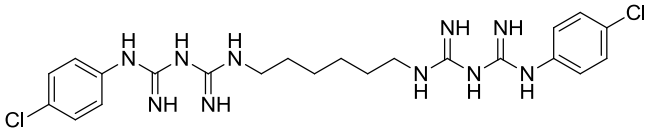
### 2.2.3.3. Halogenated Antiseptics and Disinfectants

Although quaternary ammonium compounds (QAC's) are commonly used as cationic detergents, some serve as primary broad-spectrum disinfectants with the most common being benzalkonium chlorides (**32**) (Larson and Morton, 1991; Russell, 2002). Due to their antimicrobial potency, they are often used in disinfection of unbroken skin and surfaces such as floors. However, quaternary ammonium compounds are almost exclusively effective against gram positive bacteria only and form lather in hard water reducing their effectiveness (Farzana *et al.,* 2011; Russell *et al*., 1999).



**32** n = 8, 10, 12, 14, 16, 18

Chlorhexidine (**33**), chlorine- and iodine-based compounds are the most significant microbicidal halogen-related compounds used as antiseptics and disinfectants. Chlorhexidine (**33**) is widely used in antiseptic products, particularly in hand-wash and oral products due to its broad-spectrum efficacy and low irritation (Weber *et al*., 2007; Larson and Morton, 1991).



**33**

For chlorine-releasing agents, the most important agents are chlorine dioxide, potassium-, calcium- and sodium hypochlorite while iodine-releasing agents include tincture of iodine and iodophors (Ascenzi, 1996). The main disadvantage of hypochlorites is that they have limited use and are mainly used for hard-surface disinfection while iodine releasing agents and iodophors are

associated with irritation and stained tissues. Chlorhexidine (**33**) is associated with low oral activity, neurotoxicity and low water solubility (McDonnell and Russell, 1999; Denyer and Stewart, 1998).

#### 2.2.3.4. Peroxides as Antiseptics and Disinfectants

Aqueous solutions of peroxyacetic acid and hydrogen peroxide are the most common antiseptic peroxides. They are characterized by rapid action against a wide range of microorganisms (Latosinska *et al.*, 2012). The most unique advantage of peroxides is that they form no harmful decomposition products since they decompose to produce harmless compounds such as acetic acid, water and oxygen. They are commonly used in sterilization of medical, surgical and dental equipment. However, peroxides corrode metals such as copper, zinc and aluminium and are unstable due to decomposition thus lose their efficacy over time (McDonnell and Russell, 1999). The shortcomings associated with currently used antibiotics and antiseptics have necessitated an urgent need to identify new antibacterial agents.

### 2.3. Strategies in Antimicrobial Drug Discovery

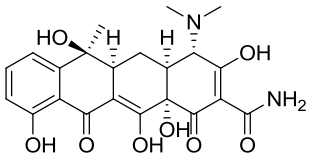
Currently, all existing antimicrobial agents are either natural or synthetic products. Natural products have played a key role in pharmaceutical research as about 40% of all existing drugs are either natural products or their synthetic derivatives (Selim *et al.*, 2012; Grabley and Thiericke, 1999).

#### 2.3.1. Natural Antimicrobial Agents

Natural products (secondary metabolites) are a fundamental and integral component of today's pharmaceutical compendium (Saleem *et al.*, 2010; Grabley and Thiericke, 1999). Since secondary

metabolites are produced by plants to counter stressed conditions such as wound healing and infection control (Moiz *et al*., 2013), they have been used since time immemorial in curing human bacterial infections and related ailments. Indeed many current and traditionally used antimicrobial drugs are derived from natural sources (Cowan, 1999; Selim *et al.*, 2012).

These natural antimicrobial drugs are of diverse chemical structures as evidenced in streptomycin, an aminoglycoside antibiotic initially isolated from the soil bacteria *Streptomyces griseus*, glycopeptides such as vancomycin isolated from soil bacteria (Saga and Yamaguchi, 2009), tetracyclines, a broad spectrum antibiotic family comprising of tetracycline (**34**) and 7-chlortetracycline (**35**), both isolated from *Streptomyces aureofaciens* and cephalosporins, β-lactam antibiotics initially isolated from *Cephalosporium acremonium* (Chopra and Marilyn, 2001).

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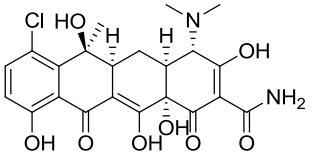
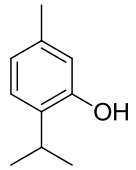



**35**

Natural phenols play a significant role in human health as antibacterial, antifungal, antioxidant and anti-cancer agents (Moiz *et. al*., 2013; Gutiérrez-Larraínzar *et al*., 2012). Phenols such as thymol (**36**), carvacrol (**37**), vanillin (**38**), gallic acid (**39**), eugenol (**40**) and their derivatives thymoquinone (**41**) and menthol (**42**), extracted from medicinal herbs and dietary plants, have antimicrobial activity (Table 2.2) (Brewer, 2011; Hayriye and Melissa, 2015).

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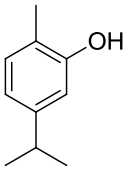


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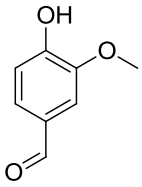


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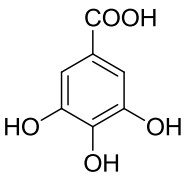


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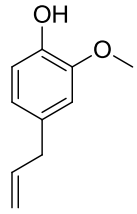


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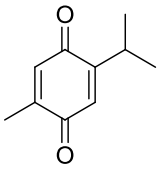


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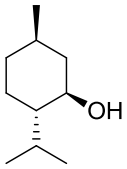



42

Table 2. 2: $MIC_{50}$ values (µg/mL) of some natural phenols against various disease-causing microbes

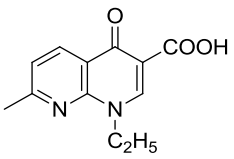
| **Microorganism** | **Natural Phenols** | | | | | |
|---|---|---|---|---|---|---|
| | **36** | **37** | **38** | **39** | **40** | **Reference** |
| *Fusarium oxysporum* | 31 | 42 | 528 | >1000 | 121 | (Zabka and Pavela, 2013) |
| *Aspergillus flavus* | 52 | 57 | >1000 | >1000 | 98 | (Zabka *et al*., 2009) |
| *S. aureus* (CECT 5192) | 400 | 300 | - | 1600 | 1600 | (Gutiérrez-Larraínzar *et al*., 2012) |
| *Aspergillus fumigatus* | 30 | 37 | 357 | >1000 | 49 | (Zabka and Pavela, 2013) |
| *Penicillium expansum* | 44 | 76 | 405 | >1000 | 134 | (Zabka *et al*., 2009) |

Although their potency may not justify their direct application as antimicrobial agents, with scientific development through structure modification, they can act as lead compounds for development of novel compounds active against pathogenic microbes (Meshnick, 2002; Grabley and Thiericke, 1999). Many other bioactive natural products have restricted use as human drugs due to high toxicity, low bio-availability and poor solubility (Wiesner *et al*., 2003; Cowan, 1999).

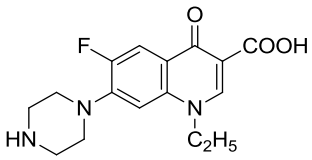
### 2.3.2. Structural Modification of Natural Antimicrobial Agents

Structural modification of natural products remains an integral part of drug development. It aims to realize novel scaffolds through structure-activity relationships (Adams *et al.*, 1993; Rao, 1993),

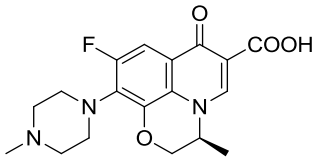
enhance potency and selectivity, improve physico-chemical, biochemical and pharmacokinetic properties and eliminate or reduce side-effects (Visalakchi and Muthumary, 2010; Carey *et al*., 2006). For instance, nalidixic acid (**43**), the first quinolone antimicrobial drug was only active against Gram-negative bacteria, had low blood solubility and was limited to treatment of urinary tract infections. However, modification led to development of related drugs such as norfloxacin (**44**) and levofloxacin (**45**) with broad range activity, improved pharmacodynamics and safety (Saga and Yamaguchi, 2009).




**43**

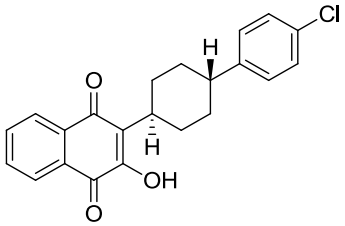



**44**

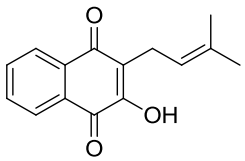



**45**

Structural modification not only serves to supply lead compounds for development of new drugs, but also for the development of scale-up protocols from cheap and readily available reagents for commercial or industrial production. Structural modification of bioactive natural products and existing drugs remains the most common strategy in drug discovery as it provides shorter development time to new drugs at reduced costs (Nordberg *et al.*, 2013; Carey *et al*., 2006). For instance, atovaquone (**46**), an effective antimalarial drug, is a synthetic napthoquinone analogue of lapachol (**47**), a natural antimalarial compound (Sebisubi and Ghee, 2011).

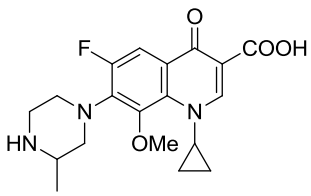



**46**

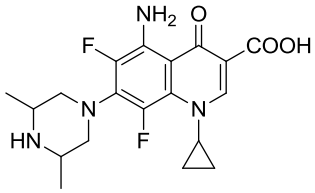



**47**

Structural modification of existing drugs has also been employed to curb the problem of microbial resistance (Coates and Hu, 2007). For instance, third generation quinolones gatifloxacin (**48**), sparfloxacin (**49**), gemifloxacin (**50**) and moxifloxacin (**51**) have been developed through modification of nalidixic acid (**43**) and are currently principally used in the treatment of penicillin- and multi drug resistant pneumonia (CDDEP, 2015; Asif, 2014; Oliphant and Green, 2002).




**48**

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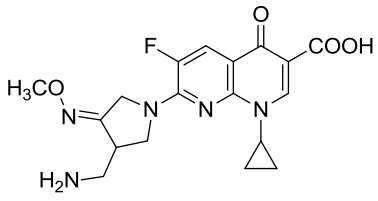
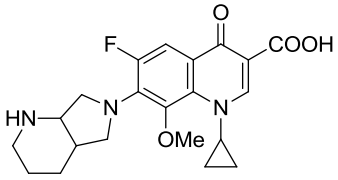



**50**




**51**

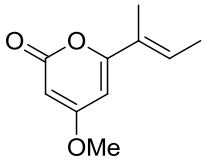
Due to the problem of microbial resistance to currently existing drugs that has complicated the treatment of microbial diseases (Strobel, 2003), structure modification of natural products from diverse sources (plants and marine sources) continues to be a dependable strategy for development of new drugs.

### 2.4. Fungal Metabolites as Potential Antimicrobial Agents

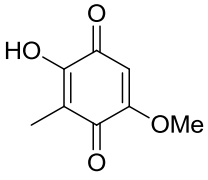
Natural products from endophytic fungi (fungi that live symbiotically with plants) are used as antibiotics and antimalarial agents (Guo *et al.,* 2008; Kharwar *et al*., 2011). They also continue to

be lead compounds for exploitation in the pharmaceutical sector for development of new drugs. The study of the metabolites of fungal endophytes and their analogues thus represents a highly viable area for discovery of new and more potent drugs (Suryanarayanan *et al*., 2009). For instance, pestalopyrone (**52**) has an $IC_{50}$ value of 37µM against *P. falciparum* and continues to attract great attention as a promising prototype for antimalarial drugs (Rosa *et al.,* 2011; Verma and Gange, 2014).

In another study, Tansuwan *et al*., (2007) isolated an antiplasmodial benzoquinone metabolite, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**8**) from *Xylaria sp.*, an endophytic fungi, with an $IC_{50}$ value of 1.84 µM against K1, the multidrug-resistant strain of *P. falciparum.* A related endophytic benzoquinone analogue, 2-hydroxy-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**53**), has also been isolated (Rosa *et al.*, 2011; Song *et al.*, 2014) but does not exhibit any antiplasmodial activity (Weber and Anke, 2009; Tansuwan *et al*., 2007).




**52**




**53**

Although the benzoquinone metabolite (**8**) displayed weak antibacterial activity against MRSA with an MIC value of 128 µg/mL (Song *et al.*, 2014; Klaiklay *et al.* 2011; Tansuwan *et al*., 2007), the fact that diverse phenols show impressive antimicrobial activity and are related to quinones prompted us to establish the antimicrobial potency of the phenolic analogues of the metabolite (Figure 2).

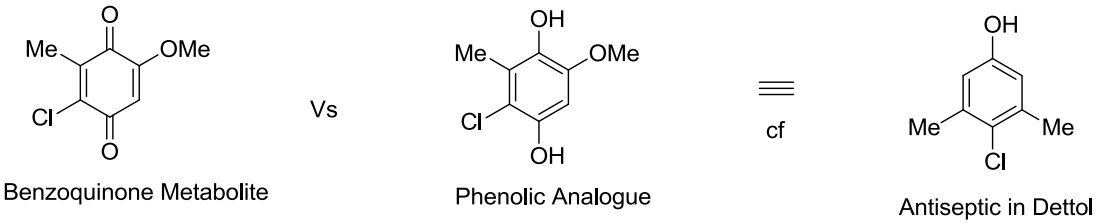



Figure 2 : Relationships between quinones and phenols and comparison with PCMX

In this study, benzene-1,4-diol analogues of the endophytic benzoquinone metabolite (**8**) and several key intermediates were synthesized to assess their antimicrobial potency.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1. General Experimentation and Instrumentation

All reactions were done in oven dried glass apparatus. The starting reagents, catalysts and solvents were purchased from Sigma-Aldrich, BDH, Loba Chemie, Finar and Alfa Aesar Limited and used without further purification. Hexane, DCM, EtOAc, acetone and MeOH used for reactions and/ or column chromatography were distilled in glass apparatus before use. All other solvents and reagents were used as purchased. Analogues were synthesized in glass flasks and subjected to appropriate purification techniques such as column chromatography and re-crystallization. Glass distilled solvents were used for extraction, crystallization and chromatographic separation.

Column chromatography (CC) was done on Merck silica gel 60 (70-230 mesh) as stationary phase. Analytical TLC on Merck silica gel 60 $F_{254}$ pre-coated aluminium plates was used to monitor the profile of reaction mixtures, eluent fractions from the columns and the purity of the compounds. The chromatographic spots were visualized under UV light at 254 or 366 nm.

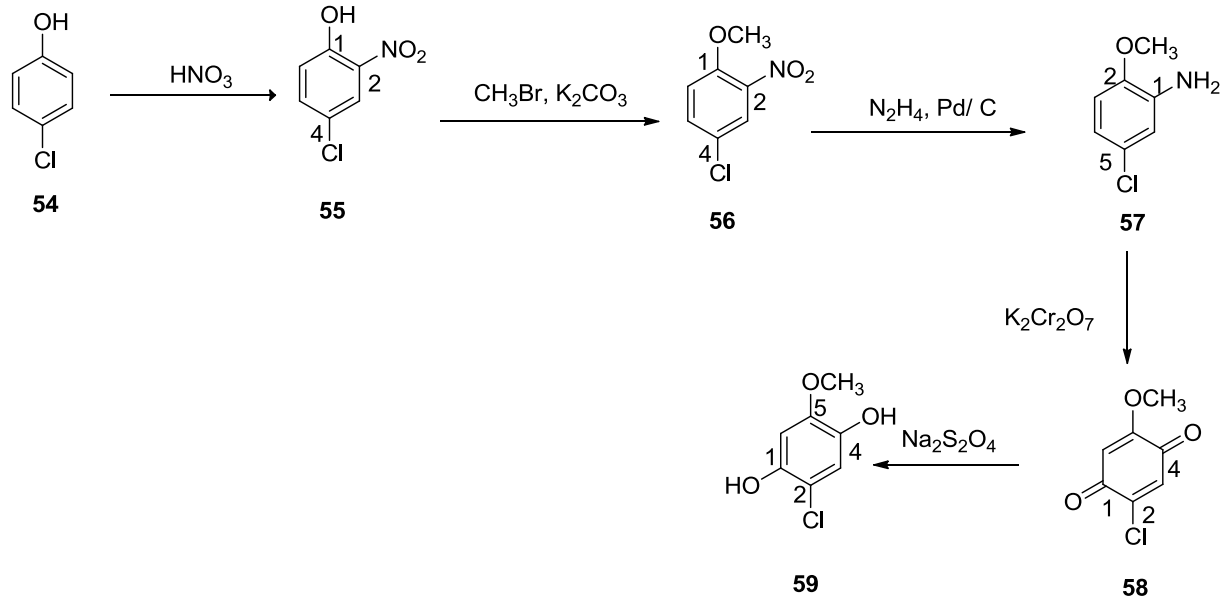
The $^{1}H$ and $^{13}C$ NMR spectra were recorded on 400, 500, 600 or 800 MHz Bruker Avance spectrometers using tetramethylsilane (TMS) as the internal standard. For chemical bond connectivity, the Homonuclear Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Connectivity (HMBC) spectra were acquired and processed using standard Bruker® software. Melting points of the compounds were determined in an oil bath using a mercury thermometer.

### 3.2. Synthetic Approach to 2-Chloro-5-methoxy-1,4-benzenediol (59) and Related Phenolic Analogues

The methodological approach in the synthesis of the substituted benzenediol, 2-chloro-5-methoxy-1,4-benzenediol (**59**), the benzoquinone, 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**), and analogues 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**), 5-chloro-2-hydroxyaniline (**62**), 5-chloro-2-hydroxyacetanilide (**64**) and 5-chloro-2-methoxyacetanilide (**61**) is highlighted in Schemes 3.1, 3.2, 3.3, 3.4 and 3.5, respectively.

#### 3.2.1. Synthesis of 2-Chloro-5-methoxy-1,4-benzenediol (59)

The synthesis of 2-chloro-5-methoxy-1, 4-benzenediol (**59**) was a multi-step process beginning from *p*-chlorophenol and involved nitration, *O*-methylation, reduction of the nitro group, oxidation to a benzoquinone derivative and then reduction to obtain the target compound as highlighted in Scheme 3.1. The individual synthetic procedures for the intermediate compounds are as highlighted below.




Scheme 3. 1: Synthetic scheme of 2-chloro-5-methoxy-1,4-benzenediol (**59**) and intermediate compounds

### 3.2.1.1. 4-Chloro-2-nitrophenol (55)

A solution of *p*-chlorophenol (**54**) (5.03 g, 39.13 mmol) in glacial acetic acid (40 mL) was added drop-wise to a solution containing conc. nitric acid (3.8 mL, 59 mmol) in 20 mL glacial acetic acid and the reaction mixture stirred at room temperature for 1 hour. The reaction mixture was then cooled and added to 100 mL of ice-cold water. The yellowish solid obtained was filtered off, washed thrice in distilled water to remove any acetic and nitric acid residues and dried to give a crude product which was further re-crystallized in EtOH to afford 4-chloro-2-nitrophenol (**55**) (6.63 g, 98% yield) as a yellow, crystalline solid.

$R_f$ = 0.45 (10% MeOH: DCM), tailing, mp 87 - 89° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 10.44 (1H, *s*, 1-OH), 8.13 (1H, *d*, *J* = 2.4 Hz, H-3), 7.27 (1H, *d*, *J* = 9.0 Hz, H-6), 7.73 (1H, *dd*, *J* = 9.0, 2.4 Hz, H-5). **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 153.2 (C-1), 137.0 (C-5), 134.6 (C-2), 124.2 (C-3, C-4) and 121.7 (C-6).

### 3.2.1.2. 4-Chloro-2-nitroanisole (56)

4-Chloro-2-nitrophenol (**55**) (5.0 g, 28.82 mmol) was added to a suspension of anhydrous $K_2CO_3$ (3.5 g) in acetone (65 mL). Methyl-iodide (2.5 mL, 40 mmol) was then added and the reaction mixture heated under reflux at 85° C for 3 hours. The mixture was then cooled, filtered under gravity and concentrated *in vacuo* to remove the solvent.

The crude product obtained was dissolved in ethyl acetate, washed thrice with 30 mL of 10% NaOH and the organic extract dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the crude product which was later re-crystallized in EtOH to afford 4-chloro-2-nitroanisole (**56**) (3.79 g, 70% yield) as a pale yellow crystalline solid.

$R_f$ = 0.49 (20% EtOAc: Hexane), mp 96 - 97° C; **$^1$H-NMR** (Acetone-$d_6$, 500 MHz): $\delta_H$ 7.91 (1H, *d*, *J* = 3.0 Hz, H-3), 7.69 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-5), 7.42 (1H, *d*, *J* = 9.0 Hz, H-6), 4.02 (3H, *s*, 1-OMe); **$^{13}$C-NMR** (Acetone-$d_6$, 125 MHz): $\delta_C$ 151.3 (C-1), 140.2 (C-2), 133.7 (C-5), 124.5 (C-4), 124.4 (C-3), 115.7 (C-6) and 56.5 (1-OMe).

### 3.2.1.3. 5-Chloro-2-methoxyaniline (57)

Hydrazine monohydrate (1.3 mL, 23.7 mmol) and 5% Pd/C (50 mg) were added to a solution of 4-chloro-2-nitroanisole (**56**) (0.8 g, 4.22 mmol) in a mixture of EtOH and toluene (15 mL, 3:1 v/v), and the reaction mixture refluxed at 100° C for 30 minutes. The solution was then allowed to cool to room temperature, filtered under gravity and the filtrate concentrated *in vacuo* to obtain the crude product which was further purified by column chromatography (EtOAc: Hexane 5-20% v/v) to afford 2-amino-4-chloroanisole (**57**) (0.57 g, 86% yield) as a yellowish-pink crystalline solid.

$R_f$ = 0.56 (20% EtOAc: Hexane), mp 82 - 85° C; **$^1$H-NMR** ($CD_2Cl_2$, 500 MHz): $\delta_H$ 6.73 (1H, *d*, *J* = 8.0 Hz, H-3), 6.69 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-4), 6.66 (1H, *d*, *J* = 2.0 Hz, H-6), 3.85 (3H, *s*, 2-OMe); **$^{13}$C-NMR** ($CD_2Cl_2$, 125 MHz): $\delta_C$ 145.9 (C-2), 137.7 (C-1), 125.6 (C-5), 117.1 (C-6), 114.0 (C-4), 111.1 (C-3) and 55.7 (2-OMe).

### 3.2.1.4. 2-Chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (58)

A solution of 2-amino-4-chloroanisole (**57**) (116 mg, 0.74 mmol) in 30% $H_2SO_4$ (25 mL) was cooled in ice after which $K_2Cr_2O_4$ (0.2 g, 0.68 mmol) was added and the reaction mixture left to react overnight at 0° C. A second portion of $K_2Cr_2O_7$ (0.2 g, 0.68 mmol) (excess) was then added, the mixture stirred for 45 minutes at the same temperature and extracted in ether (3 *x* 50

mL). The combined organic extract was dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to afford the expected product (**58**) (126.7 mg, 99% yield) as a dark brown crystalline solid.

$R_f$ = 0.39 (20% EtOAc: Hexane), mp 172 - 173° C; **$^1$H-NMR** ($CD_2Cl_2$, 500 MHz): $\delta_H$ 6.97 (1H, *s*, H-3), 6.10 (1H, *s*, H-6), 3.88 (3H, *s*, 5-OMe); **$^{13}$C-NMR** ($CD_2Cl_2$, 125 MHz): δc 179.5 (C-4), 179.2 (C-1), 159.2 (C-5), 145.0 (C-2), 131.5 (C-3), 107.1 (C-6) and 56.8 (5-OMe).

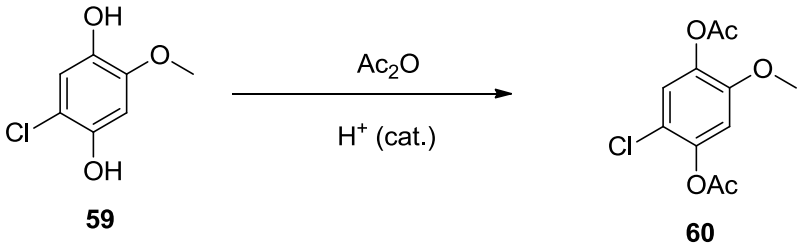
### 3.2.1.5. 2-Chloro-5-methoxy-1,4-benzenediol (59)

In portions, sodium dithionite (2.0 g, large excess) was added to a solution containing 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**) (0.16 g, 0.93 mmol) in EtOAc/ $H_2O$ mixture (25 mL, 3:2 v/v) and the reaction mixture stirred for 24 hours at room temperature. The organic phase was separated and the aqueous layer extracted in EtOAc (3 *x* 50 mL). The combined organic extract was washed thrice with aqueous $NaHCO_3$ (50 mL), water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the crude product which was further purified by column chromatography (EtOAc: Hexane 5-20% v/v) to afford the expected product (**59**) (98 mg, 61% yield) as a black crystalline solid.

$R_f$ = 0.42 (50% EtOAc: Hexane), mp 127 - 129° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 8.02 (1H, *s*, 4-OH), 7.45 (1H, *s*, 1-OH), 6.79 (1H, *s*, H-3), 6.66 (1H, *s*, H-6), 3.83 (3H, *s*, 5-OMe); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 147.2 (C-5), 145.8 (C-4), 140.2 (C-1), 115.4 (C-3), 110.4 (C-2), 101.4 (C-6) and 55.5 (5-OMe).

### 3.2.2. Synthesis of 2-Chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (60)

Synthesis of the compound was achieved in a one step reaction involving acylation of 2-chloro-5-methoxy-1,4-benzenediol (**59**) in excess acetic anhydride as highlighted in Scheme 3.2 and the succeeding procedure.




**59** **60**

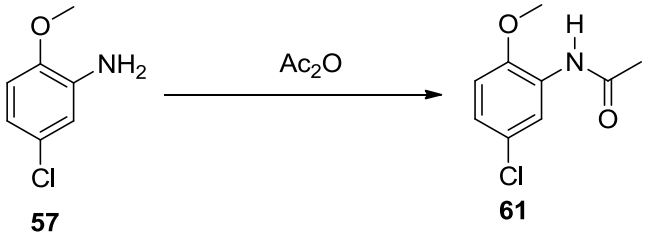
Scheme 3. 2: Acylation of 2-chloro-5-methoxy-1,4-benzenediol (**59**) to 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)

2-Chloro-5-methoxy-1,4-benzenediol (**59**) (10 mg, 0.057 mmol) was dissolved in acetic anhydride (5 mL, large excess), 98% $H_2SO_4$ (3 drops) added and the reaction mixture heated to reflux for 4 hours at 60° C. The reaction mixture was then allowed to cool, slowly poured in ice cold water (100 mL) and extracted in EtOAc (3 *x* 50 mL). The combined organic extract was then washed thoroughly in saturated $NaHCO_3$, then water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the expected product (**60**) (12.7 mg, 86% yield) as a brownish crystalline solid.

$R_f$ = 0.48 (20% EtOAc: Hexane) mp 127 - 129° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 7.28 (1H, *s*, H-3), 7.09 (1H, *s*, H-6), 3.72 (3H, *s*, 5-OMe), 2.33 (3H, *s*, 2'-$CH_3$), 2.27 (3H, *s*, 2''-$CH_3$); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 167.9 (1''-C=O), 167.7 (1'-C=O), 151.1 (C-5), 145.3 (C-1), 137.9 (C-4), 123.6 (C-3), 116.8 (C-2), 108.6 (C-6), 55.9 (-OMe), 19.6 (2'-$CH_3$) and 19.5 (2''-$CH_3$).

### 3.2.3. Synthesis of 5-Chloro-2-methoxyacetanilide (61)

The synthesis of 5-chloro-2-methoxyacetanilide (**61**) involved *N*-acylation of **57** as highlighted below.




**57** **61**

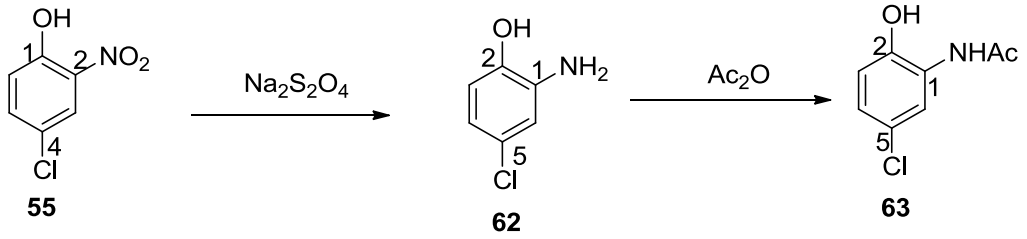
Scheme 3. 3: One-step acylation reaction of 2-amino-4-chloroanisole (**57**) to 5-chloro-2-methoxyacetanilide (**61**)

2-Amino-4-chloroanisole (**57**) (57 mg, 0.36 mmol) was dissolved in 10 mL EtOH/ water mixture (3:2) by refluxing at 80° C. This was followed by the drop-wise addition of acetic anhydride (0.04 mL, 0.4 mmol) in 5 mL ethanol over a period of 10 minutes. The mixture was then refluxed at the same temperature until all the starting material was deemed to be consumed by constant monitoring with TLC (about 1 hour). The reaction mixture was then cooled to room temperature and concentrated *in vacuo* to obtain the crude product which was later re-crystallized in EtOH to afford the expected product (**61**) (65 mg, 90% yield) as a yellowish-pink crystalline solid.

$R_f$ = 0.39 (50% EtOAc: Hexane), mp 104 - 106° C; **$^1$H-NMR** ($CD_2Cl_2$, 500 MHz): $\delta_H$ 8.42 (1H, *d*, *J* = 2.5 Hz, H-6), 7.03 (1H, *dd*, *J* = 8.5, 2.5 Hz, H-4), 6.85 (1H, *d*, *J* = 8.5 Hz, H-3), 5.36 (1H, *s*, -NH-), 3.90 (3H, *s*, 2-OMe), 2.20 (3H, *s*, C-1'',-$COCH_3$). **$^{13}$C-NMR** ($CD_2Cl_2$, 125 MHz): $\delta_C$ 168.3 (C-1', -C=O), 146.4 (C-2), 128.9 (C-1), 125.6 (C-5), 122.7 (C-4), 119.1 (C-6), 110.8 (C-3), 55.9 (2-OMe) and 24.6 (C-1'', -$COCH_3$).

### 3.2.4. Synthesis of 5-Chloro-2-hydroxyacetanilide (63)

Synthesis of 5-chloro-2-hydroxyacetanilide (**63**) was a multistep reaction involving reduction and *N*-acylation using acetic anhydride as highlighted in Scheme 3.4 following the individual synthetic procedures below.




Scheme 3.4: Synthetic scheme of 5-chloro-2-hydroxyacetanilide (**63**) and intermediate compounds

#### 3.2.4.1. 5-Chloro-2-hydroxyaniline (62)

Sodium dithionite ($Na_2S_2O_4$) (3.5 g, large excess) was added in portions to a suspension of 4-chloro-2-nitrophenol (**55**) (0.7 g, 4 mmol) in 30 mL EtOH/ $H_2O$ mixture (1:1 v/v) and the reaction mixture heated to reflux at 65° C for 4 hours. The solution was then cooled, extracted in EtOAc (3 *x* 50 mL) and the combined organic extract washed with brine, then with water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo*. The crude product obtained was purified by column chromatography (EtOAc: Hexane 5-20%) to afford 5-chloro-2-hydroxyaniline (**62**) (0.4 g, 70% yield) as a brown crystalline solid.

$R_f$ = 0.68 (50% EtOAc: Hexane), mp 138 - 139° C; **$^1$H-NMR** ($CDCl_3$, 800 MHz): $\delta_H$ 6.75 (1H, *d*, *J* = 2.4 Hz, H-3), 6.67 (1H, *d*, *J* = 8.0 Hz, H-6), 6.64 (1H, *dd*, *J* = 8.0, 2.4 Hz, H-4). **$^{13}$C-NMR** ($CDCl_3$, 200 MHz): $\delta_C$ 142.2 (C-2), 135.9 (C-1), 126.2 (C-5), 118.6 (C-4), 116.3 (C-6), and 115.9 (C-3).

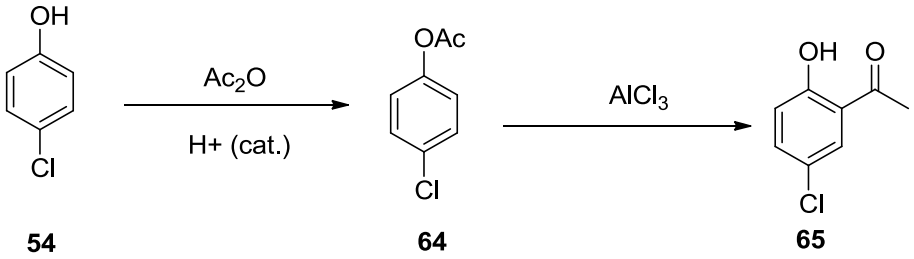
### 3.2.4.2. 5-Chloro-2-hydroxyacetanilide (63)

A 100 mL solution containing 99% acetic anhydride (1.5 mL) in MeOH was prepared and 1.5 mL of the solution (equivalent to 0.15 mmol acetic anhydride) added to a suspension of 2-amino-4-chlorophenol (**62**) (20 mg, 0.14 mmol) in 15 mL of MeOH. The reaction mixture was then stirred at room temperature until all the starting material was consumed (about 45 minutes, constantly monitored by TLC). The reaction mixture was then concentrated *in vacuo* to obtain the crude product which was later re-crystallized in EtOH to afford 4-chloro-2-hydroxyacetanilide (**63**) (24 mg, 93% yield) as a dark brown solid.

$R_f$ = 0.50 (50% EtOAc: Hexane), mp 181 - 183° C; **$^1$H-NMR** ($CDCl_3$, 800 MHz): $\delta_H$ 7.02 (1H, *d*, *J* = 2.4 Hz, H-6), 7.01 (1H, *dd*, *J* = 8.0, 2.4 Hz, H-4), 6.86 (1H, *d*, *J* = 8.0 Hz, H-3), 5.23 (1H, *s*, -*N*-H), 2.20 (3H, *s*, -$CH_3$). **$^{13}$C-NMR** ($CDCl_3$, 200 MHz): $\delta_C$ 169.5 (-C=O), 146.3 (C-2), 129.9 (C-4), 125.9 (C-1), 123.9 (C-3), 120.7 (C-6), 119.8 (C-5) and 22.8 (-$CH_3$).

### 3.2.5. Synthesis of 2-Acetyl-4-chlorophenol (65)

Synthesis of 2-acetyl-4-chlorophenol (**65**) was achieved in a one pot reaction as highlighted in Scheme 3.5 following the synthetic procedures below.




**54** **64** **65**

Scheme 3.5: Synthetic scheme of 2-acetyl-4-chlorophenol (**65**)

To a mixture of *p*-chlorophenol (**54**) (2 g, 15.56 mmol) in acetic anhydride (4.5 mL, excess), was added 2 drops of 98% $H_2SO_4$ and the reaction mixture stirred for 3 hours at room temperature.

The mixture was added slowly to saturated $NaHCO_3$ (100 mL) and extracted in EtOAc (3 *x* 50 mL). The combined organic extract was then washed thoroughly in saturated $NaHCO_3$, then in water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the intermediate as a pale brownish liquid.

$AlCl_3$ (4.14 g, 31 mmol) was added slowly over a period of 20 minutes to the oily liquid with the flask immersed in ice. After the evolution of HCl gas had subsided, the reaction mixture was heated to reflux at 130° C for 24 hours. The mixture was then cooled, 10% HCl (50 mL) slowly added, allowed to stir at room temperature for a further 1 hour and extracted with EtOAc (3 *x* 50 mL). The combined organic extract was washed with saturated $NaHCO_3$ followed by water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to provide the crude product which was purified by column chromatography (EtOAc: Hexane 0-20% v/v) and re-crystallized in EtOH to afford **65** (1.6 g, 61% yield) as a brown, low melting point solid.

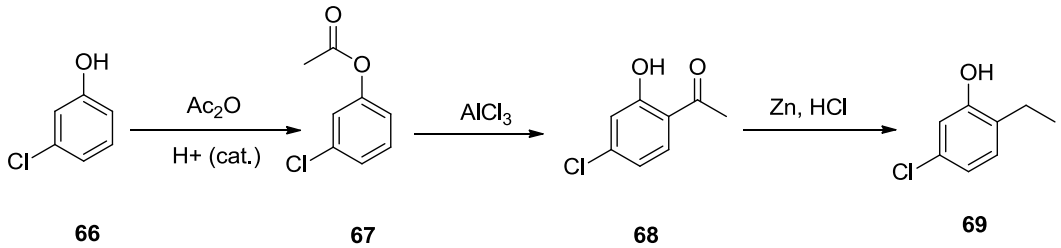
$R_f$ = 0.59 (25% EtOAc: Hexane), mp 51 - 52° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 8.00 (1H, *d*, *J* = 3.0 Hz, H-3), 7.76 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-5), 7.62 (1H, *d*, *J* = 9.0 Hz, H-6) and 2.44 (3H, *s*, -$CH_3$): **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 175.5 (-C=O), 154.9 (C-1), 133.6 (C-5), 124.4 (C-4), 124.2 (C-3), 120.3 (C-6), 109.9 (C-2) and 19.5 (-$CH_3$).

### 3.3. Synthesis of 5-Chlorophenols

The methodological approach employed in the synthesis of substituted 5-chlorophenol derivatives from *m*-chlorophenol (**66**) is highlighted in Schemes 3.6 and 3.7.

### 3.3.1. Synthesis of 5-Chloro-2-ethylphenol (69) and Key Intermediates

Synthesis of 5-chloro-2-ethylphenol (**69**) was a multistep reaction involving Fries rearrangement, a form of Friedel-Craft acylation reaction, followed by Clemmensen reduction as highlighted in Scheme 3.6 and the individual synthetic procedures that follow below.




**66** **67** **68** **69**

Scheme 3.6: Synthetic scheme of 5-chloro-2-ethylphenol (**69**) and key intermediates of interest

#### 3.3.1.1. 2-Acetyl-5-chlorophenol (68)

2 Drops of conc. $H_2SO_4$ were added to a solution of *m*-chlorophenol (**66**) (3 g, 23.35 mmol) in acetic anhydride (2.3 mL, 24 mmol) and the reaction mixture stirred at room temperature for 2 hours. 100 mL of saturated $NaHCO_3$ solution was then added and the mixture extracted in ether (3 *x* 50 mL). The combined organic layer was washed with a saturated aqueous $NaHCO_3$, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to afford the intermediate (**67**) as a pale brownish oily liquid which was relatively pure to be used in the subsequent step.

Anhydrous $AlCl_3$ (6.24 g, 46.8 mmol) was cautiously and slowly added in portions to the ice-cold oily liquid obtained resulting in evolution of gaseous HCl. After the gaseous evolution had subsided, the reaction mixture was heated under reflux at 160° C for 10 hours. The mixture was then cooled, 50% HCl (100 mL) slowly added and the solution extracted in EtOAc (3 *x* 50 mL). The combined organic extract was washed with saturated $NaHCO_3$, water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the crude product which was then purified by

column chromatography (EtOAc: Hexane 0-10%) to afford the expected product (**68**) as an off-white solid (3.79 g, 70% yield).

$R_f$ = 0.45 (20% EtOAc: Hexane), mp 50 - 51° C; **$^1$H-NMR** ($CD_2Cl_2$, 600 MHz): $\delta_H$ 12.44 (1H, *s*, -OH), 7.73 (1H, *d*, *J* = 9.0 Hz, H-3), 7.01 (1H, *d*, *J* = 2.4 Hz, H-6), 6.94 (1H, *dd*, *J* = 9.0, 2.4 Hz, H-4) and 2.64 (3H, *s*, -$CH_3$): **$^{13}$C-NMR** ($CD_2Cl_2$, 150 MHz): $\delta_C$ 204.1 (-C=O), 162.9 (C-1), 141.8 (C-5), 131.9 (C-3), 119.5 (C-4), 118.4 (C-2), 118.1 (C-6) and 26.6 (-$CH_3$).

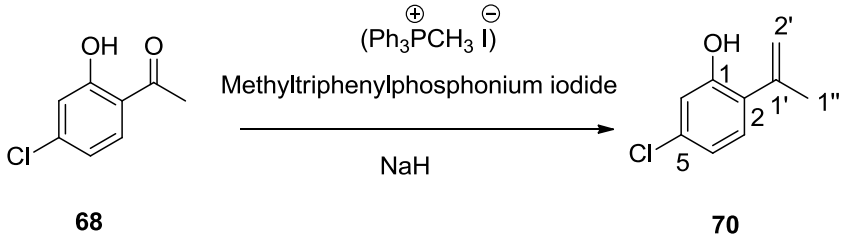
### 3.3.1.2. 5-Chloro-2-ethylphenol (69)

Zn dust (5.5 g, 83 mmol) was slowly added to a solution containing 2-acetyl-5-chlorophenol (**68**) (2.0 g, 11.73 mmol) in 50% HCl (30 mL) and the reaction mixture heated under reflux at 85° C for 8 hours. During the reflux, 1 mL of concentrated HCl was added after every 1 hour. The mixture was then cooled and extracted in ether (3 *x* 50 mL). The combined organic extract was washed with water, dried over anhydrous $Na_2SO_4$, filtered and then concentrated *in vacuo* to obtain the crude product, which was further purified by column chromatography on silica gel (ethyl acetate: hexane; 0-20%) to afford the desired product (**69**) (127 mg, 7% yield) as a dark red oily liquid.

$R_f$ = 0.39 (20% EtOAc: Hexane); **$^1$H-NMR** ($CDCl_3$, 800 MHz): $\delta_H$ 6.97 (1H, *d*, *J* = 8.0 Hz, H-3), 6.78 (1H, *dd*, *J* = 8.0, 1.6 Hz, H-4), 6.69 (1H, *d*, *J* = 1.6 Hz, H-6), 2.51 (2H, *q*, *J* = 15.2, 8.0 Hz, C-1',-$CH_2CH_3$), 1.13 (3H, *t*, *J* = 15.2, 8.0 Hz, -$CH_3$) ; **$^{13}$C-NMR** ($CDCl_3$, 200 MHz): $\delta_C$ 153.8 (C-1), 131.7 (C-5), 130.0 (C-3), 128.5 (C-2), 120.8 (C-4), 115.3 (C-6), 22.4 (-$CH_2$-) and 13.7 (-$CH_3$).

### 3.3.2. Synthesis of 5-Chloro-2-isopropenylphenol (70) via the Wittig Reaction

Synthesis of 5-chloro-2-isopropenylphenol (**70**) was achieved based on the Wittig reaction as highlighted in Scheme 3.7. This reaction required methyltriphenylphosphonium iodide (Wittig salt) and since the salt was unavailable, it was prepared from triphenylphosphine as detailed below.




**68** **70**

Scheme 3.7: Wittig transformation of 2-acetyl-5-chlorophenol (**68**)

#### 3.3.2.1. Methyltriphenylphosphonium Iodide (Wittig salt)

Triphenylphosphine (6.0 g, 23 mmol) was completely dissolved in anhydrous toluene (25 mL) and methyl iodide (4.0 g, 28 mmol) in 8 mL toluene was added drop-wise over a period of 15 minutes and the mixture stirred at ambient temperature. Immediately the methyl iodide was added, the mixture warmed up and a white precipitate started to form. The solution was stirred for 1 hour, 10 mL toluene added and the solution stirred for a further 1 hour. 20 mL hexane was added and the reaction mixture stirred for a further 1 hour. The resulting solid was filtered, washed several times with hexane and dried. The Wittig salt obtained (9.32 g, 88% yield) was used in the next step without further purification.

#### 3.3.2.2. 5-Chloro-2-isopropenylphenol (70)

Methyltriphenylphosphonium iodide (2.62 g, 6.45 mmol) was slowly added to a suspension of NaH (0.59 g, 14.7 mmol) in anhydrous THF (20 mL) at $0^{\circ}$ C. The mixture was stirred for 1 hour at that temperature and 2-acetyl-5-chlorophenol (**68**) (0.50 g, 2.93 mmol) added. The reaction

mixture was then allowed to warm to room temperature and stirred for 3 days. Aqueous $NH_4Cl$ (100 mL) was added and the mixture extracted in ether (3 *x* 50 mL). The combined organic extract was washed with brine, water and dried over anhydrous sodium sulfate. Concentration of the extract followed by purification by column chromatography on silica gel (5-20% ethyl acetate: hexane) afforded **70** (0.12 g, 24% yield) as a reddish oily liquid.

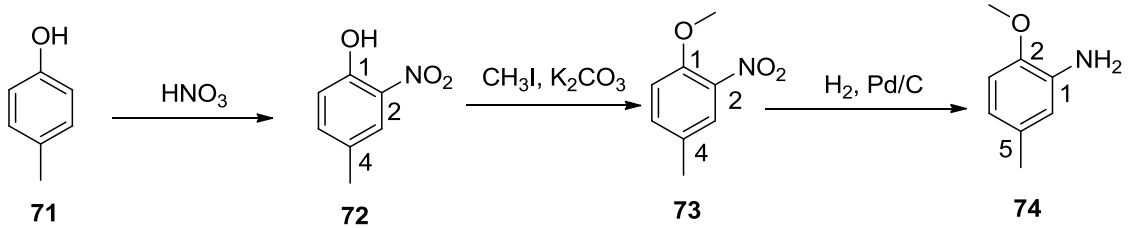
$R_f$ = 0.39 (20% EtOAc: Hexane); **$^1$H-NMR** (600 MHz, $CD_2Cl_2$): $\delta_H$ 7.39 (1H, *s*, -OH), 7.13 (1H, *d*, *J* = 8.4 Hz, H-3), 6.97 (1H, *d*, *J* = 2.4 Hz, H-6), 6.92 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-4), 5.45 (1H, *d*, *J* = 0.6 Hz, H-2') and 5.17 (1H, *d*, *J* = 0.6 Hz, H-2'), 2.13 (3H, *s*, $-CH_3$); **$^{13}$C-NMR** (150 MHz, $CD_2Cl_2$) $\delta_C$ 152.9 (C-1), 141.4 ($-C=CH_2$), 133.3 (C-5), 131.9 (C-3), 128.9 (C-2), 120.2 (C-4), 116.1 ($=CH_2$), 115.7 (C-6) and 23.8 ($-CH_3$).

### 3.4. Synthesis of Mono- and Di-substituted 4-Methylanisoles

The methodological approach employed in the synthesis of substituted 4-methylanisoles and their key intermediates is highlighted in Schemes 3.8 and 3.9.

#### 3.4.1. Synthesis of 2-Amino-4-methylanisole (74) and Related Intermediates

Synthesis of 2-amino-4-methylanisole (**74**) was achieved through a linear multi-step reaction as highlighted in Scheme 3.8 following the individual synthetic procedures given below.




**71** **72** **73** **74**

Scheme 3.8: Synthetic scheme of 2-methoxy-5-methylaniline (**74**) and other analogues of interest

### 3.4.1.1. 4-Methyl-2-nitrophenol (72)

To a solution of *p*-cresol (**71**) (3.6 g, 30.56 mmol) in acetic acid (20 mL) was added conc. $HNO_3$ (2 mL, 31 mmol) and the reaction stirred for 2 hours at room temperature. The reaction mixture was poured in ice cold water (100 mL), stirred for a further 30 minutes and filtered to obtain the crude product which was further re-crystallized in EtOH to obtain the expected product (**72**) (4.64 g, 99%) as a yellow crystalline solid.

$R_f$ = 0.73 (20% EtOAc: Hexane), mp 36 - 39° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 10.32 (1H, -OH), 7.94 (1H, *d*, *J* = 2.4 Hz, H-3), 7.56 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-5), 7.12 (1H, *d*, *J* = 8.4 Hz, H-6) and 2.38 (3H, *s*, -$CH_3$). **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 146.0 (C-1), 138.5 (C-5), 130.9 (C-2), 129.7 (C-4), 124.3 (C-3), 119.5 (C-6) and 19.0 (-$CH_3$).

### 3.4.1.2. 4-Methyl-2-nitroanisole (73)

4-Methyl-2-nitrophenol (**72**) (0.6 g, 3.92 mmol) and methyl iodide (1 mL, 16 mmol) was added to a suspension of anhydrous $K_2CO_3$ (4.1 g, 30 mmol) in acetone (20 mL) and the reaction mixture heated under reflux for 3 hours at 85° C. The mixture was then cooled, filtered and the filtrate extracted in EtOAc (3 *x* 50 mL). The combined organic extract was washed thrice with 10% NaOH (30 mL), water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the expected product (**73**) (0.51 g, 78% yield) as a brownish oily liquid.

$R_f$ = 0.45 (20% EtOAc: Hexane); **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 7.64 (1H, *d*, *J* = 3.0 Hz, H-3), 7.46 (1H, *dd*, *J* = 8.4, 3.0 Hz, H-5), 7.23 (1H, *d*, *J* = 8.4 Hz, H-6), 3.95 (3H, *s*, -OMe) and 2.36 (3H, *s*, -$CH_3$). **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 150.4 (C-1), 139.8 (C-2), 134.5 (C-5), 130.2 (C-4), 124.9 (C-3), 113.8 (C-6), 56.1 (-OMe) and 19.1 (-$CH_3$).

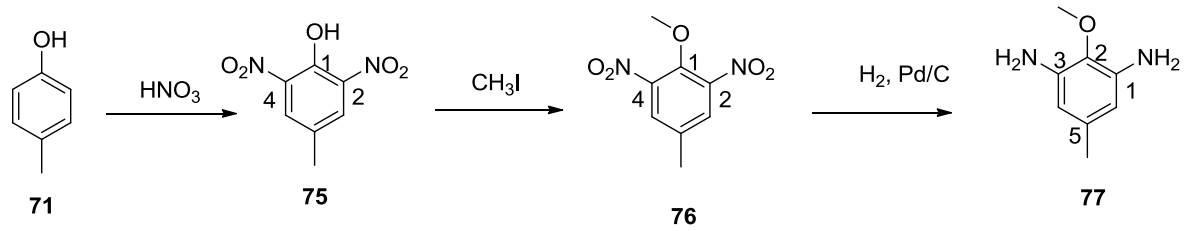
### 3.4.1.3. 2-Methoxy-5-methylaniline (74)

A suspension of 2-nitro-4-methylanisole (**73**) (0.2 g, 1.2 mmol) and 5% wt/wt Pd/ C (40 mg) in ethanol (10 mL) was purged with $N_2$ to deoxygenate and stirred for 24 hours over a $H_2$ balloon at room temperature. The mixture was then filtered, the filtrate washed thoroughly in ethanol and concentrated *in vacuo* to afford the expected product (**74**) (0.16 g, 95% yield) as a pale yellow oily liquid.

$R_f$ = 0.45 (20% EtOAc: Hexane); **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 7.65 (1H, *d*, *J* = 3.0 Hz, H-6), 7.47 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-4), 7.24 (1H, *d*, *J* = 9.0 Hz, H-3), 3.96 (3H, *s*, -OMe), 2.37 (3H, *s*, -$CH_3$); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 150.4 (C-2), 134.5 (C-4), 130.2 (C-1), 129.8 (C-5), 124.9 (C-6), 113.8 (C-3), 56.1 (-OMe) and 19.1 (-$CH_3$).

### 3.4.2. Synthesis of 2-Methoxy-5-methylbenzene-1,3-diamine (77) and Key Intermediates

Synthesis of 2-methoxy-5-methylbenzene-1,3-diamine (**77**) was achieved as highlighted in Scheme 3.9 and following the individual procedures below.




Scheme 3.9: Synthetic scheme of 2-methoxy-5-methylbenzene-1,3-diamine (**77**) from *p*-cresol (**71**)

### 3.4.2.1. 4-Methyl-2, 6-dinitrophenol (75)

A solution containing conc. nitric acid (2 mL, 30.95 mmol) in glacial acetic acid (7 mL) was added drop-wise to a solution of *p*-cresol (**71**) (1.1 g, 10.19 mmol) in glacial acetic acid (25 mL) and the reaction mixture stirred at room temperature for 1.5 hours. The reaction mixture was then cooled to room temperature and added to 100 mL of ice-cold water. The yellowish fibrous solid

obtained was filtered off, washed thoroughly in distilled water to remove any acetic and nitric acid and dried to give a crude product which was further re-crystallized in EtOH to afford 4-methyl-2,6-dinitrophenol (**75**) (1.85 g, 92% yield) as a yellow crystalline solid.

$R_f$ = 0.55 (30% EtOAc: Hexane), mp 82 - 85° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 10.79 (1H, *s*, -OH), 8.04 (1H, *d*, *J* = 2.4 Hz, H-3), 7.49 (1H, *d*, *J* = 2.4 Hz, H-5) and 2.43 (3H, *s*, -$CH_3$); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 150.9 (C-1), 140.3 (C-5) 133.6 (C-2), 129.7 (C-4), 127.2 ( C-6), 124.6 (C-3) and 20.1 (-$CH_3$).

### 3.4.2.2. 4-Methyl-2,6-dinitroanisole (76)

A solution of methyl iodide (1.7 g, 12 mmol) in acetone (5 mL) was added drop wise to a suspension of 4-methyl-2,6-dinitrophenol (**75**) (0.6 g, 3.03 mmol) and anhydrous $K_2CO_3$ (4.1 g, 30 mmol) in acetone (25 mL). The reaction mixture heated to reflux at 85° C for 4 hours. The solution was cooled to room temperature, filtered and the filtrate washed severally with acetone and then ethyl acetate (50 mL). The organic layer was washed thrice in 10% NaOH solution (3 *x* 50 mL), distilled water (3 *x* 50 mL), dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to afford the expected product (**76**) (0.43 g, 67%) as a whitish crystalline solid.

$R_f$ = 0.52 (25% EtOAc: Hexane), mp 122 - 124° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 8.09 (2H, *d*, *J* = 1.2 Hz, H-3 and H-5), 4.03 (3H, *s*, -$OCH_3$) and 2.54 (3H, *s*, -$CH_3$); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 144.9 (C-1), 144.5 (C-2 and C-6) 135.9 (C-4), 129.4 (C-3 and C-5), 64.1 (-OMe) and 19.5 (-$CH_3$).

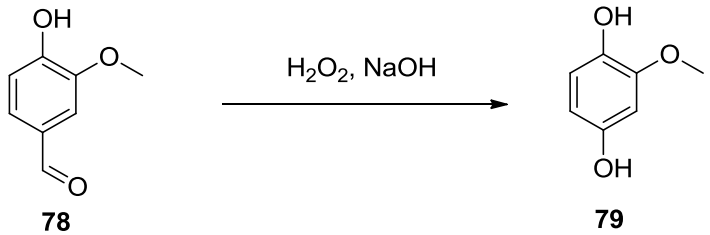
### 3.4.2.3. 2-Methoxy-5-methylbenzene-1,3-diamine (77)

A suspension of 2,6-dinitro-4-methylanisole (**76**) (0.15 g, 0.707 mmole) and 5% wt/wt Pd/ C (40 mg) in MeOH (10 mL) was purged with $N_2$ to deoxygenate and stirred for 24 hours under a $H_2$ balloon at room temperature. The mixture was then filtered, the residue washed thoroughly in ethanol and the combined extract concentrated *in vacuo* to afford the expected product (**77**) (0.1 g, 94%) as a pale yellow crystalline solid.

$R_f$ = 0.57 (30% EtOAc: Hexane), decomposes ; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 8.09 (2H, *s*, H-4 and H-6), 3.99 (3H, *s*, -OMe), 2.50 (3H, *s*, -$CH_3$); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 144.8 (C-2), 144.4 (C-1 and C-3), 135.9 (C-5), 129.3 (C-4 and C-6), 64.0 (-OMe) and 19.5 (-$CH_3$).

## 3.5. Synthesis of 2-Methoxy-1,4-benzenediol (79)

The methodological approach employed in the synthesis of 2-methoxy-1,4-benzenediol (**79**) from vanillin (**78**) was the single step Dakins reaction (Floyd *et al.*, 2005; Grant, 1993) highlighted in Scheme 3.10 and the succeeding procedure.




**78** **79**

Scheme 3. 10: Dakins oxidation of vanillin (**78**)

Vanillin (**78**) (3.0 g, 19.74 mmol) was added to 2 N NaOH (20 mL, 19.75 mmol) (prepared by dissolving 1.6 g NaOH in water to make a solution of 20 mL) and stirred vigorously to ensure complete dissolution. With vigorous stirring, 6% $H_2O_2$ (13 mL, 22 mmol) (prepared by diluting 5 g of 27.5% wt/wt $H_2O_2$ with water to 45 mL solution) was added drop-wise over a period of 10

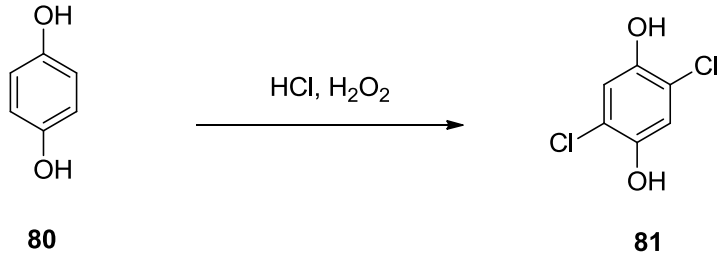
minutes and the reaction mixture heated to reflux for 3 hours at 45° C. The mixture was then cooled to room temperature and quenched by adding 50 mL of 50% HCl dropwise and stirring for 1 hour.

The reaction mixture was then extracted in EtOAc (3 *x* 50 mL). The combined organic extract was washed thoroughly in brine, then water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the crude product which was purified by column chromatography (EtOAc: Hexane 2-35% v/v) to afford the expected product (**79**) (0.83 g, 30% yield) as a black solid.

$R_f$ = 0.53 (50% EtOAc: Hexane), mp 87 - 88° C; **$^1$H-NMR** (Acetone-$d_6$, 600 MHz): $\delta_H$ 7.79 (1H, *s*, 1-OH), 6.92 (1H, *s*, 4-OH), 6.65 (1H, *d*, *J* = 9.0 Hz, H-6), 6.48 (1H, *d*, *J* = 3.0 Hz, H-3), 6.30 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-5), 3.80 (3H, *s*, 2-OMe); **$^{13}$C-NMR** (Acetone-$d_6$, 150 MHz): $\delta_C$ 150.7 (C-4), 147.9 (C-1), 139.5 (C-2), 114.9 (C-6), 106.4 (C-5), 100.2 (C-3) and 55.2 (2-OMe).

### 3.6. Synthesis of 2, 5-Dichloro-1,4-benzenediol (81)

Synthesis of 2,5-dichloro-1,4-benzenediol (**81**), from hydroquinone (**80**) was achieved in a single-step reaction (Song *et al*., 2008) as highlighted in Scheme 3.11 and the corresponding procedure:




**80** **81**

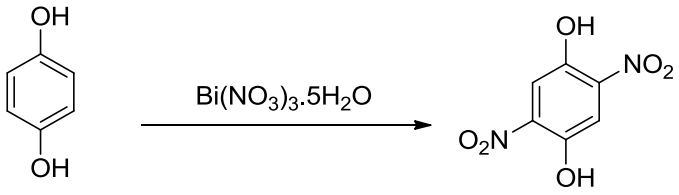
Scheme 3. 11: Oxidative chlorination of hydroquinone (**80**)

30% Hydrogen peroxide (15 mL, 145 mmol) was added over a period of 1 hour to a rapidly stirred solution of hydroquinone (**80**) (3.85 g, 35 mmol) in EtOAc (30 mL) and 36.5% HCl (24 mL, 280 mmol). The reaction mixture was stirred for 16 hours at room temperature and then extracted in EtOAc (3 *x* 50 mL). The combined organic layer was washed thoroughly with 5% $NaHCO_3$ to remove any traces of HCl, then water, dried over anhydrous $Na_2SO_4$ and concentrated *in vacuo* to obtain the crude product which was purified by column chromatography (EtOAc: Hexane 2-35% v/v) to afford the expected product (**81**) (0.98 g, 16% yield) as a pale yellowish solid.

$R_f$ = 0.58 (25% EtOAc: Hexane), mp 169 - 171° C; **$^1$H-NMR** (Acetone-$d_6$, 500 MHz): $\delta_H$ 7.45 (2H, *s*, H-3, H-6), 7.37 (2H, *s*, 1-OH, 4-OH). **$^{13}$C-NMR** (Acetone-$d_6$, 125 MHz): $\delta_C$ 169.9 (C-1, C-4), 140.2 (C-2, C-5), 133.3 (C-3, C-6).

### 3.7. Synthesis of 2, 5-Dinitro-1,4-benzenediol (82)

Synthesis of 2,5-dinitro-1,4-benzenediol (**82**) from hydroquinone (**80**) was achieved in a single step reaction of Scheme 3.12 using the succeeding procedure:




**80** **82**

Scheme 3. 12: Nitration of hydroquinone (**80**) in bismuth (III) nitrate pentahydrate

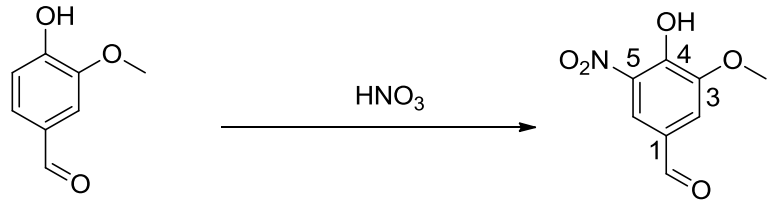
To a stirred solution of hydroquinone (**80**) (4.0 g, 36.4 mmol) in acetone (20 mL), $Bi(NO_3)_3.5H_2O$ (8.82 g, 18.2 mmol) in 10 mL acetone was added dropwise over a period of 10 minutes and the reaction mixture stirred at room temperature for 2 hours. Ice cold water (100 mL) was added to the reaction mixture, stirred for a further 30 minutes and filtered to obtain the

crude product which was further re-crystallized in EtOH to obtain the expected product (**82**) (1.13 g, 24%) as a black crystalline solid.

$R_f$ = 0.18 (20% EtOAc: Hexane), mp 207 - 209° C; **$^1$H-NMR** (Acetone-$d_6$, 500 MHz): $\delta_H$ 6.85 (2H, *s*, 1-OH, 4-OH), 6.67 (2H, *s*, H-3, H-6), **$^{13}$C-NMR** (Acetone-$d_6$, 125 MHz): $\delta_C$ 150.3 (C-1, C-4), 136.5 (C-2, C-5), 115.7 (C-3, C-6).

### 3.8. Synthesis of 4-Hydroxy-3-methoxy-5-nitrobenzaldehyde (83)

Synthesis of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**) was achieved in the single step reaction of Scheme 3.13 using the following procedure:




**78** **83**

Scheme 3. 13: Nitration of vanillin (**78**) to 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)

To a solution of vanillin (**78**) (4.0 g, 26.32 mmol) in acetic acid (40 mL) was added $HNO_3$ (69%, 2 mL, 30 mmol) slowly at 0° C. Afterwards the solution was warmed up to room temperature and allowed to stir for another 30 min. The mixture was then poured in 150 mL ice cold water and the precipitate obtained filtered off, washed with cold water to give a crude product which was later re-crystallized in EtOH to afford the expected product (**83**) (3.32 g, 64% yield) as yellow crystalline solid.

$R_f$ = 0.60 (10% MeOH: DCM), tailing, mp 170 - 172° C; **$^1$H-NMR** (Acetone-$d_6$, 500 MHz): $\delta_H$ 9.98 (1H, *s*, H-1'), 8.26 (1H, *s*, H-6), 7.73 (1H, *s*, H-2), 4.06 (3H, *s*, 3-OMe). **$^{13}$C-NMR**

(Acetone-$d_6$, 125 MHz): $\delta_C$ 189.4 (C-1'), 150.7 (C-4), 149.5 (C-3), 135.2 (C-1), 127.9 (C-5), 120.6 (C-6), 113.2 (C-2), and 56.4 (3-OMe).

### 3.9. Antimicrobial Assays

The antimicrobial assays were done at the Department of Microbiology, National Quality Control Laboratory (NQCL), Kenya Medical Research Institute (KEMRI), Kisumu and at the Department of Public Health Pharmacology and Toxicology (PHPT), University of Nairobi following the procedures elaborated below.

#### 3.9.1. Disc Diffusion Antimicrobial Susceptibility Test

The antimicrobial assay of the pure compounds was evaluated by disc diffusion method. *Staphyloccocus aureus* (ATCC 25923), *Streptococcus infantarius* (438209), *Escherichia coli* (ATCC 25922), *Pseudomonas aeroginosa* (ATCC 27853), *Salmonella typhimurium* (7222569-1) and *Candida albicans* (ATCC 10231) were used as the test microorganisms.

All the procedures were done according to the Clinical Laboratory Standards Institute (2008).

##### 3.9.1.1. Preparation of Test Discs

Test samples (10 mg for each pure compound) were dissolved in 20% DMSO/ $H_2O$ (250 µL) to make a 40,000 µg/mL concentration stock solution. Filter paper discs (5 mm diameter) were prepared and sterilized by autoclaving. The sterile filter paper discs were impregnated with the test sample material (10 µL) (400 µg compound) and allowed to dry at room temperature overnight in closed petri dishes to prevent contamination.

20% DMSO/$H_2O$ solution was used as a negative control while pre-coated 5mm impregnated disc (Oxoid, Oxoid Ltd, Basingstoke, Hampshire, England) containing ceftriaxone (active

against *S. typhimurium*), clotrimazole (*Candida albicans*), gentamicin (*Pseudomonas aeroginosa*), chloramphenicol (*Staphylococcus aureus*), erythromycin (*Streptococcus infantarius*) and ampicillin (*Escherichia coli*) were used as positive controls for the tests.

### 3.9.1.2. Media Preparation for Disc Diffusion Method

Mueller Hinton Agar (MHA) (OXOID, UK) was used for both the bacterial and fungal bioassay. MHA was prepared by dissolving 19 g in distilled water (500 mL) and boiling to dissolve completely. Sterilization was achieved by autoclaving at 121° C for 15 minutes. The media (20 mL) was then dispensed onto the pre-sterilized petri-dishes yielding uniform depths. The petri dishes were covered and the media allowed to cool and solidify at room temperature.

### 3.9.1.3. Microbial Growth Conditions and Preparation of Test Petri-dishes

Both the bacterial and fungal strains were maintained on Mueller Hinton Agar (MHA) petri-dishes at 4° C and 25° C respectively. Suspensions of test microorganisms were prepared from fresh cultures using normal saline and turbidity adjusted to 0.5 McFarland.

### 3.9.1.4. Methodology for the Disc Diffusion Method

Freshly prepared agar petri-dishes were aseptically streaked with the test microorganism suspensions using a sterile swab and allowed to dry for a few minutes. The impregnated discs were then placed aseptically on the inoculated agar plates using sterile forceps and incubated for 24 hours at 37° C for bacteria and 25° C for fungi. The experiments were carried out in duplicates with a single plate containing between 4 and 6 discs. Presence of a clear circular zone around the sample impregnated disc (i.e. the zone of inhibition) was used as indicator of activity. The results (mean values) were recorded by measuring zone diameter in millimeters. The test

samples which showed significant activity were subsequently diluted with 20% DMSO/ $H_2O$ and the experiments repeated.

### 3.9.2. *In vitro* Antiplasmodial Assay

The *in vitro* antiplasmodial activity of selected compounds was evaluated against chloroquine-sensitive 3D7 and D6 strains of *P. falciparum* using a non-radioactive assay technique (Yenesew *et al.,* 2009; Smilkstein *et al*., 2004). The method uses the flourochrome called "SYBR Green 1", a non-radioactive DNA dye that accurately depicts the *in vitro* parasite replication. Concurrently, two-fold serial dilutions of the drugs chloroquine (1.953-1000 ng/mL), mefloquine (0.488-250 ng/mL) and test samples (97.7-50,000 ng/mL) were prepared on a 96 well plate. The culture-adapted *P. falciparum* were reconstituted to 1% parasitemia and added onto the plate containing dose range of the reference drugs and test samples. They were incubated in a gas mixture (5% $CO_2$, 5% $O_2$ and 90% $N_2$) at 37° C. The assay was terminated after 72 hours by freezing at -80° C for 24 hours.

After thawing, lysis buffer containing SYBR Green 1 (1 x final concentration) was added directly to the plates and gently mixed by using the Biomek 2000 Automated Laboratory Workstation (Beckman Coulter, Inc., Fullerton, CA). The plates were incubated for 5-15 minutes at room temperature in the dark. Parasite growth inhibition was quantified by measuring per-well relative fluorescence units (RFU) of SYBR Green 1 dye using a Tecan Genios Plus Microplate Reader (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 nm and 535 nm respectively and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating 50% inhibition concentration ($IC_{50}$'s) for each drug and sample using GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA). A minimum of three

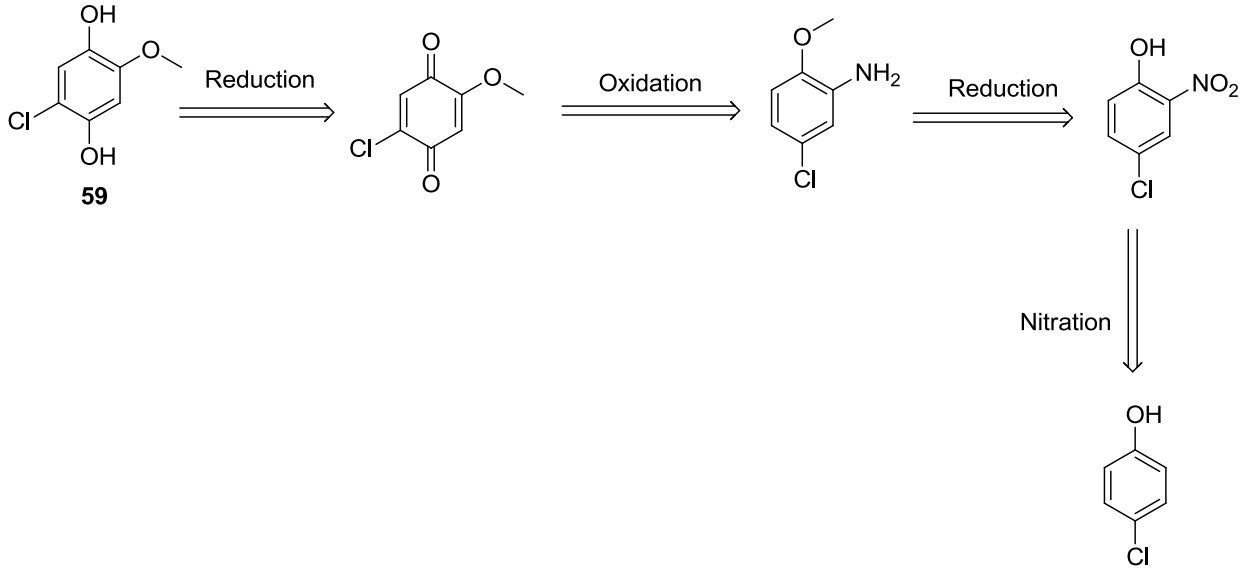
separate determinations was carried out for each sample and replicates of narrow data ranges presented as mean ± S.D. This antiplasmodial assay was done at Kenya Medical Research Institute (KEMRI)**,** Kisumu.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1. Synthesis of Benzene-1,4-diol Derivatives

There are several strategies for the synthesis of benzene-1,4-diol derivatives. Most of the synthetic strategies for benzene-1,4-diols involve at least a two-step process of oxidation of a phenol or aniline derivative with an appropriate oxidizing agent to a benzoquinone intermediate and subsequent reduction to a benzene-1,4-diol (Chu *et al.*, 2006; Sato *et al*., 1968). This was the strategy employed in the synthesis of one of the benzene-1,4-diol derivative, 2-chloro-5-methoxy-1,4-benzenediol (**59**), as highlighted in the retrosynthetic Scheme 4.1.

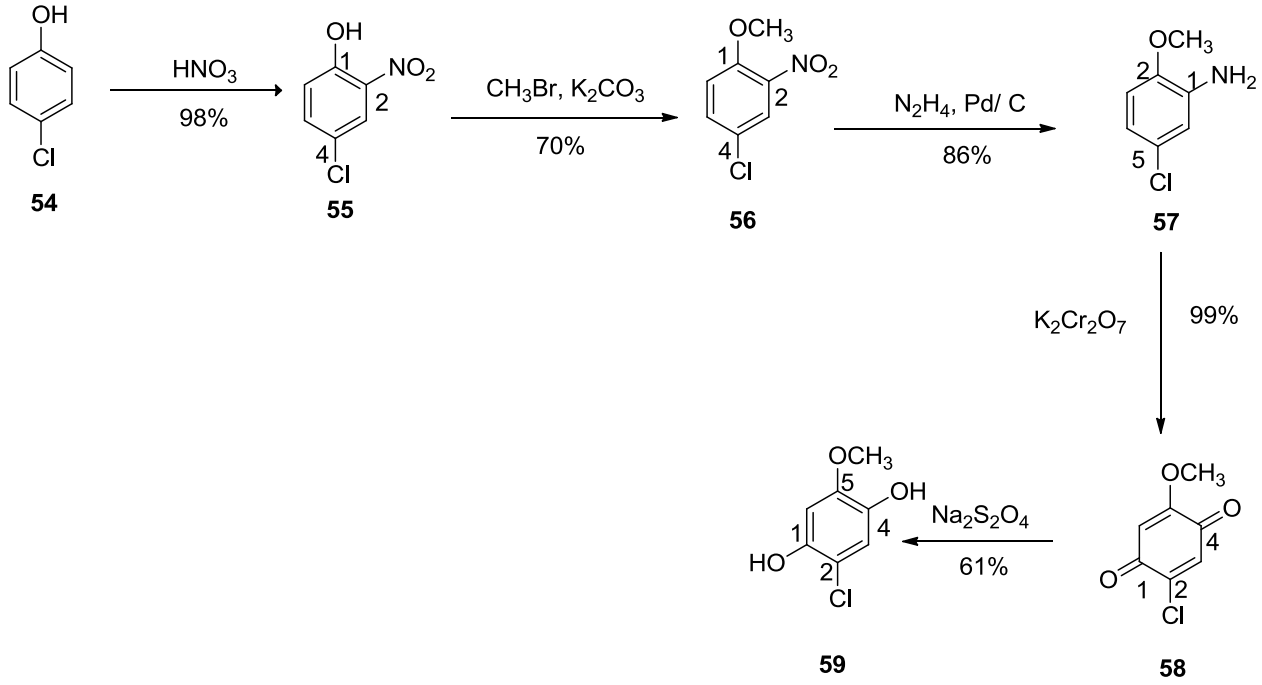



Scheme 4. 1: Retrosynthetic pathway for 2-chloro-5-methoxy-1,4-benzenediol (**59**)

Synthesis of other benzene-1,4-diol derivatives was based on Dakins oxidation of aldehydes in basic $H_2O_2$ (Floyd *et al.*, 2005; Grant, 1993) and through nitration and oxidative chlorination of hydroquinone (Jacoway *et al*., 2012; Song *et al*., 2008) with some modifications.

### 4.1.1. Synthesis of 2-Chloro-5-methoxy-1,4-benzenediol (59)

2-Chloro-5-methoxy-1,4-benzenediol (**59**) was synthesized from *p*-chlorophenol (**54**) as illustrated in the multi-step linear synthesis in Scheme 4.2.




Scheme 4. 2: Synthetic transformation of *p*-chlorophenol (**54**) to 2-chloro-5-methoxy-1,4-benzenediol (**59**)

4-Chloro-2-nitrophenol (**55**) was obtained from the nitration of *p*-chlorophenol (**54**) as a pale yellow crystalline solid in 98% yield. The $^{1}$H NMR data (Table 4.1) showed three mutually coupled aromatic protons and a chelated hydroxyl resonating at $\delta_H$ 10.44 (1H, *s*). The 2D NMR (Appendix 1) spectra showed $^{3}J$ and $^{2}J$ correlations of H-3 with C-2 ($\delta_C$ 134.6), C-4 ($\delta_C$ 124.2) and C-5 ($\delta_C$ 137.0) and correlation between H-5 with C-3, C-4 and C-6 consistent with mono-nitration.

Compound **55** was *O*-methylated to 4-chloro-2-nitroanisole (**56**) based on the Williamson ether synthesis reaction. It was obtained as a pale yellow crystalline solid in 70% yield. As in the starting material (**55**), the $^{1}$H NMR data of compound **56** showed three mutually coupled aromatic protons (Table 4.1). However, the chelated hydroxyl observed in the starting material was not detected but

instead three methoxy protons resonating at $\delta_H$ 4.02 (3H, *s*) with the corresponding carbon observed at $\delta_C$ 56.5 as would be consistent with the expected Williamson ether transformation.

Table 4. 1: $^{1}$H NMR and $^{13}$C NMR data for compounds **55** and **56**

| | **Compound 55** | | **Compound 56** | |
|---|---|---|---|---|
| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$(ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$(ppm)** |
| 1 | 10.44 (1H, *s*, 1-OH) | 153.2 | | 151.3 |
| 1 (-OMe) | | | 4.02 (3H, *s*) | 56.5 |
| 2 | | 134.6 | | 140.2 |
| 3 | 8.13 (1H, *d*, *J* = 2.4 Hz) | 124.2 | 7.91 (1H, *d*, *J* = 3.0 Hz) | 124.4 |
| 4 | | 124.2 | | 124.5 |
| 5 | 7.73 (1H, *dd*, *J* = 9.0, 2.4 Hz) | 137.0 | 7.69 (1H, *dd*, *J* = 9.0, 3.0 Hz) | 133.7 |
| 6 | 7.27 (1H, *d*, *J* = 9.0 Hz) | 121.7 | 7.42 (1H, *d*, *J* = 9.0 Hz) | 115.7 |

Compound **56** was reduced to 5-chloro-2-methoxyaniline (**57**) by transfer hydrogenation using hydrazine monohydrate in the presence of Pd/C catalyst (Orlowska *et al*., 2010). Compound **57** was obtained as yellowish-pink crystalline solid in 86% yield. The $^{1}$H and $^{13}$C NMR data of compounds **56** (Table 4.1) and **57** (Table 4.2) were similar, showing presence of three aromatic protons and a methoxy group. However, the $^{1}$H and $^{13}$C spectra of compound **57** appeared more shielded as would be expected with the formation of an amino group, a stronger electron donor than a nitro group. The transformation was also supported by HSQC and HMBC experiments (Appendices 2 and 3).

Compound **57** was subsequently oxidized to 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**) using acidified potassium dichromate in a one-pot reaction (Sato *et al*., 1968). Over-oxidation was controlled by using a stoichiometric amount of the strong oxidizing agent at low temperature. The product was obtained as a dark brown crystalline solid in 99% yield. The $^{1}$H and $^{13}$C data (Table 4.2) of the compound showed two *para* protons resonating at $\delta_H$ 6.97 (1H, *s*, H-3) and 6.10 (1H, *s*, H-6) with the corresponding carbons observed at $\delta_C$ 131.5 and 107.1, respectively. There was also a methoxy resonating at $\delta_H$ 3.88 (3H, *s*, 5-OMe) and two carbonyls at $\delta_C$ 179.5 and 179.2.

The downfield H-6 was attributed to the methoxy attached at the *ortho* position {$\delta_H$ 3.88 (3H, *s*, 5-OMe); $\delta_C$ 56.8}. The structure was also supported by the HMBC spectra (Appendix 4) which showed $^3J$ and $^2J$ correlations of H-3 and H-6 with C-1 ($\delta_C$ 179.2), C-2 ($\delta_C$ 144.9), C-3 ($\delta_C$ 131.5) and C-4 ($\delta_C$ 179.5).

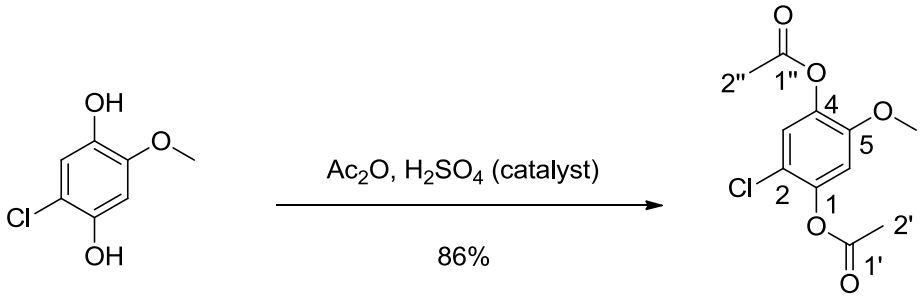
Compound **58** was subsequently reduced to 2-chloro-5-methoxy-1,4-benzenediol (**59**) using sodium dithionite, a mild reducing agent (Chu *et al*., 2006). The product was obtained as a black crystalline solid in 61% yield. The $^1$H and $^{13}$C NMR data of compound **59** (Table 4.2) was similar to that of compound **58** except for the incorporation of two hydroxyls resonating at $\delta_H$ 7.44 (1H, *s*, 1-OH) and 8.01 (1H, *s*, 4-OH). In the $^{13}$C NMR, the signals for carbonyls in the starting material (**58)** were not observed and the carbons appear to be more shielded at $\delta_C$ 140.2 and 145.7 consistent with oxygenated aromatic carbons. The structure was also supported by the HMBC spectra which showed $^3J$ and $^2J$ correlations of 1-OH with C-1 ($\delta_C$ 140.2), C-2 ($\delta_C$ 110.3), C-6 ($\delta_C$ 101.4) and 4-OH with C-3, C-4 and C-5 (Appendix 5).

Table 4. 2: $^1$H NMR and $^{13}$C NMR data for compounds **57**, **58** and **59**

| **Position** | **Compound 57** | | **Compound 58** | | **Compound 59** | |
|---|---|---|---|---|---|---|
| | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** |
| 1 | | 137.7 | | 179.2 | 7.44 (1H, *s*, 1-OH) | 140.2 |
| 2 | | 145.9 | | 144.9 | | 110.3 |
| 2-OMe | 3.85 (3H, *s*) | 55.7 | | | | |
| 3 | 6.73 (1H, *d*, *J* = 8.0 Hz) | 111.1 | 6.97 (1H, *s*) | 131.5 | 6.79 (1H, *s*, H-3) | 115.4 |
| 4 | 6.69 (1H, *dd*, *J* = 8.0, 2.0 Hz) | 114.0 | | 179.5 | 8.01 (1H, *s*, 4-OH) | 145.7 |
| 5 | | 125.6 | | 159.2 | | 147.1 |
| 5-OMe | | | 3.88 (3H, *s*) | 56.8 | 3.81 (3H, *s*, 5-OMe) | 55.5 |
| 6 | 6.66 (1H, *d*, *J* = 2.0 Hz) | 117.1 | 6.10(1H, *s*) | 107.1 | 6.66 (1H, *s*, H-6) | 101.4 |

### 4.1.2. Synthesis of 2-Chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (60)

2-Chloro-5-methoxy-1,4-benzenediol (**59**) was exhaustively acetylated in excess acetic anhydride to 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**) in 86% yield as highlighted in Scheme 4.3 (Liu *et al*., 2012).




**59** **60**

Scheme 4. 3: Acid catalyzed acylation of 2-chloro-5-methoxy-1,4-benzenediol (**59**)

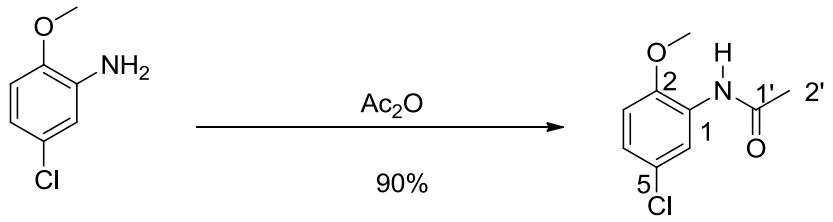
The product was obtained as a brown crystalline substance in 86% yield. The $^{1}H$ and $^{13}C$ NMR spectra of compound **60** (Table 4.3) was similar to that of compound **59** (Table 4.2) except for the incorporation of two acetyl groups with the two carbonyls resonating at $\delta_C$ 167.9 and 167.7 and two methyl groups observed at $\delta_C$ 19.6 and 19.5 while the corresponding protons appeared at $\delta_H$ 2.33 (3H, *s*, 2'-$CH_3$) and 2.27 (3H, *s*, 2''-$CH_3$), respectively. Moreover, the hydroxyl signals in the starting material (**59**) were not observed. The structure was confirmed by HMBC spectra (Appendix 6) which showed $^{2}J$ correlations of the 2'-$CH_3$ with the carbonyl ($\delta_C$ 167.7) and 2''-$CH_3$ with carbonyl ($\delta_C$ 167.9), consistent with the structure of a di-acetylated benzene-1,4-diol.

Table 4. 3: $^{1}$H NMR (600MHz) and $^{13}$C NMR data for compound **60** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 145.3 | C-1, C-2, C-6 |
| 1' (-C=O) | | 167.7 | |
| 2' (-$CH_3$) | 2.33 (3H, *s*, 2'-$CH_3$) | 19.6 | -C-1' (-C=O) |
| 2 | | 116.8 | |
| 3 | 7.28 (1H, *s*, H-3) | 123.6 | C-1, C-2, C-4, C-5 |
| 4 | | 137.9 | |
| 1'' (-C=O) | | 167.9 | |
| 2'' (-$CH_3$) | 2.27 (3H, *s*, 2''-$CH_3$) | 19.5 | -C-1'' (-C=O) |
| 5 | | 151.1 | |
| 5-OMe | 3.72 (3H, *s*, 5-OMe) | 55.9 | C-5 |
| 6 | 7.09 (1H, s, H-6) | 108.6 | C-1, C-2, C-4, C-5 |

### 4.1.3. Synthesis of 5-Chloro-2-methoxyacetanilide (61)

5-Chloro-2-methoxyaniline (**57**) was *N*-acylated with acetic anhydride to 5-chloro-2-methoxyacetanilide (**61**) (Pisaneschi *et al*., 2011) as highlighted in Scheme 4.4. Compound **61** was obtained as yellowish-pink crystalline solid in 90% yield.




**57** **61**

Scheme 4. 4: Acylation of 5-chloro-2-methoxyaniline (**57**)

The $^{1}$H and $^{13}$C NMR data of compound **61** (Table 4.4) and compound **57** (Table 4.2) were similar, showing presence of three aromatic protons and a methoxy group. However, the $^{13}$C spectra of compound **61** showed an increase in the number of carbons from seven to nine suggesting that a group containing two carbons had been incorporated. A carbonyl and methyl peaks observed at $\delta_C$ 168.3 and 24.6 [$\delta_H$ 3.85 (3H, *s*)], respectively and an amide proton resonating at $\delta_H$ 5.36 (*s*, -NH-)

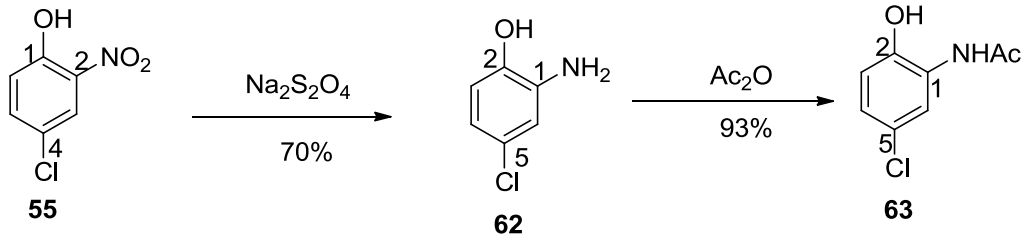
actually infer that acetylation took place. The transformation was also supported by HSQC and HMBC experiments (Appendix 7).

Table 4. 4: $^{1}$H NMR (600MHz) and $^{13}$C NMR data for compound **61** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 128.9 | |
| -NH- | 5.36 (1H, *s*, -NH-) | | |
| 1' (-C=O) | | 168.3 | |
| 2'(-$CH_3$) | 2.20 (3H, *s*) | 24.6 | |
| 2 | | 146.4 | |
| 2 - OMe | 3.90 (3H, *s*, 2-OMe) | 55.9 | C-2 |
| 3 | 6.85 (1H, d, *J* = 8.5 Hz) | 110.8 | C-1, C-5 |
| 4 | 7.03 (1H, *dd*, *J* = 8.5, 2.5 Hz) | 122.7 | C-2, C-6 |
| 5 | | 125.6 | |
| 6 | 8.42 (1H, *d*, *J* = 2.5 Hz) | 119.1 | C-2, C-4 |

### 4.1.4. Synthesis of 5-Chloro-2-hydroxyaniline (62) and 5-Chloro-2-hydroxyacetanilide (63)

The synthesis of compounds **62** and **63** was achieved by reduction of the nitro group in sodium dithionite and subsequent acetylation in acetic anhydride as highlighted in Scheme 4.5.




Scheme 4. 5: Synthetic transformation of 2-nitro-4-chlorophenol (**55**) to 5-chloro-2-hydroxyacetanilide (**63**)

Compounds **62** and **63** were obtained as brown and dark brown crystalline solids in 70% and 93% yield, respectively. The $^{1}$H and $^{13}$C spectra (Table 4.5) of compound **62** showed three mutually coupled protons at $\delta_H$ 6.75 (1H, *d*, *J* = 2.4 Hz, H-3), 6.67 (1H, *d*, *J* = 8.0 Hz, H-6) and 6.64 (1H, *dd*, *J* = 8.0, 2.4 Hz, H-4) with the $^{13}$C carbons resonating at $\delta_C$ 142.2 (C-2), 135.9 (C-1), 126.2 (C-5), 118.6 (C-4), 116.3 (C-6) and 115.9 (C-3) consistent with literature (Pisaneschi *et al*., 2011).

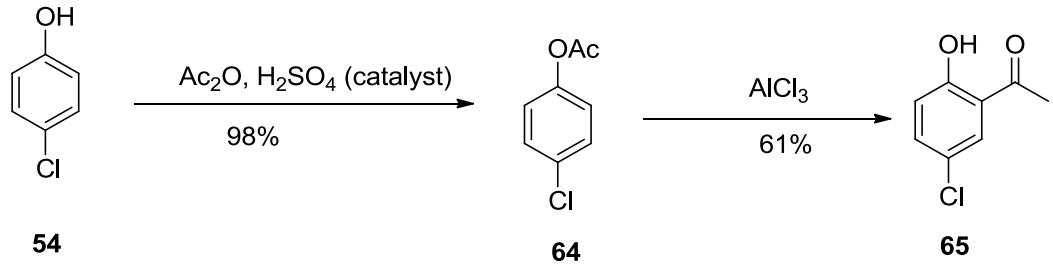
The NMR signal (Table 4.5) for compound **63** was similar to that of compound **62** except for the incorporation of an amino proton $\delta_H$ 5.23 (1H, *s*, -NH-), an acetyl methyl at $\delta_H$ 2.20 (3H, *s*, -$CH_3$), $\delta_C$ 22.8 (-$CH_3$) and a carbonyl resonating at $\delta_C$ 169.5. Compound **63** also showed an upfield shifted C-1 resonating at $\delta_C$ 125.9 while that of compound **62** appears at $\delta_C$ 135.9 attributed to resonance. This was also supported by HSQC and HMBC experiments (Appendix 8 and 9).

Table 4. 5: $^1$H NMR and $^{13}$C NMR data for compounds **62** and **63**

| | **Compound 62** | | **Compound 63** | |
|---|---|---|---|---|
| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** |
| 1 | | 135.9 | | 125.9 |
| -NH- | | | 5.23 (1H, *s*, -N-H) | |
| 1' (-C=O) | | | | 169.5 |
| 2'(-$CH_3$) | | | 2.20 (3H, *s*, -$CH_3$) | 22.8 |
| 2 | | 142.2 | | 146.3 |
| 3 | 6.75 (1H, *d*, *J* = 2.4 Hz) | 115.9 | 6.86 (1H, *d*, *J* = 8.0 Hz) | 123.9 |
| 4 | 6.64 (1H, *dd*, *J* = 8.0, 2.4 Hz) | 118.6 | 7.01 (1H, *dd*, *J* = 8.0, 2.4 Hz) | 129.9 |
| 5 | | 126.2 | | 119.8 |
| 6 | 6.67 (1H, *d*, *J* = 8.0 Hz) | 116.3 | 7.02 (1H, *d*, *J* = 2.4 Hz) | 120.7 |

### 4.1.5. Synthesis of 2-Acetyl-4-chlorophenol (65)

Synthesis of 2-acetyl-4-chlorophenol (**65**) was achieved in a one-pot reaction through esterification (*O*-acylation) followed by Fries rearrangement as highlighted in Scheme 4.6.




**54** **64** **65**

Scheme 4. 6: Synthetic scheme to 2-acetyl-4-chlorophenol (**65**)

The product was obtained as a brown, low melting point solid in 61% yield. As opposed to the starting material (**54**), the $^1$H NMR data (Table 4.6) showed presence of three mutually coupled aromatic protons resonating at $\delta_H$ 8.00 (1H, *d*, *J* = 3.0 Hz, H-3), 7.76 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-5)

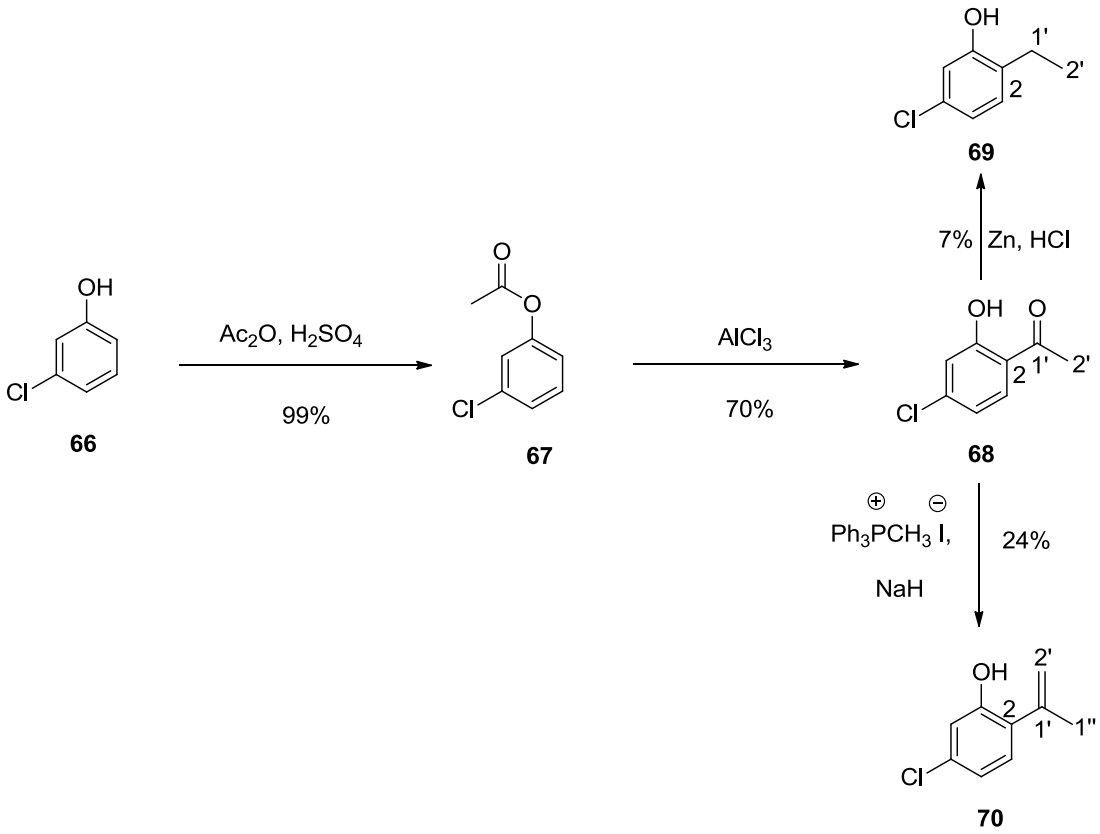
and 7.62 (1H, *d*, *J* = 9.0 Hz, H-6). Three methyl protons were also observed resonating at $\delta_H$ 2.44 (3H, *s*, -$CH_3$) with the associated carbon at $\delta_C$ 19.5 (-$CH_3$). The $^{13}C$ spectra also showed presence of a carbonyl at $\delta_C$ 175.5 (-C=O) with the transformation also supported by HMBC, HSQC and H, H-COSY experiments (Appendix 10).

Table 4. 6: $^{1}H$ NMR (600MHz) and $^{13}C$ NMR data for compound **65** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 154.9 | |
| 1' (-C=O) | | 175.5 | |
| 2'(-$CH_3$) | 2.44 (3H, *s*, -$CH_3$) | 19.5 | C-2 |
| 2 | | 109.9 | |
| 3 | 8.00 (1H, *d*, *J* = 3.0 Hz) | 124.2 | C-1, C-5 |
| 4 | | 124.4 | |
| 5 | 7.76 (1H, *dd*, *J* = 9.0, 3.0 Hz) | 133.6 | C-1, C-3 |
| 6 | 7.62 (1H, *d*, *J* = 9.0 Hz) | 120.3 | C-2, C-4 |

### 4.1.6. Synthesis of 5-Chloro-2-ethylphenol (69) and 2-Isopropenyl-5-chlorophenol (70)

5-Chloro-2-ethylphenol (**69**) and 2-isopropenyl-5-chlorophenol (**70**) were synthesized as highlighted in Scheme 4.7. *m*-Chlorophenol (**66**) was acetylated under acid catalyzed condition and subsequently converted to 2-acetyl-5-chlorophenol (**68**) through Fries rearrangement with $AlCl_3$ as the Lewis acid (Sebile *et al*., 2005). Compound **68** was isolated as an off-white solid in 70% yield.




Scheme 4. 7: Synthesis of 5-chloro-2-ethylphenol (**69**) and 2-isopropenyl-5-chlorophenol (**70**) based on Clemmensen and Wittig reactions

Compounds **69** and **70** were obtained from compound **68** through Clemmensen and Wittig reactions, respectively (Chittimalla *et al*., 2014; Seoane *et al*., 2014). Compounds **69** and **70** were isolated as dark red oily and reddish oily liquids in 7% and 24% yields, respectively. The low yield of compound **69** could be due to the fact that the reaction was not catalyzed in mercuric (II) salts or amalgams while that of compound **70** is due to the production of large amounts of highly non-polar triphenylphosphonium oxide which necessitated repetitive purification through column chromatography.

The $^{1}$H NMR spectra of compounds **68**, **69** and **70** showed the presence of three mutually coupled protons, H-3, H-4 and H-6 (Table 4.7). $^{1}$H NMR and $^{13}$C spectra of compound **68** showed presence

of a chelated hydroxyl at $\delta_H$ 12.44 (1H, *s*), a carbonyl signal resonating at $\delta_C$ 204.1 (-C=O) and acetoxy methyl resonating at $\delta_H$ 2.64 (3H, *s*) and 26.6 ($-CH_3$).

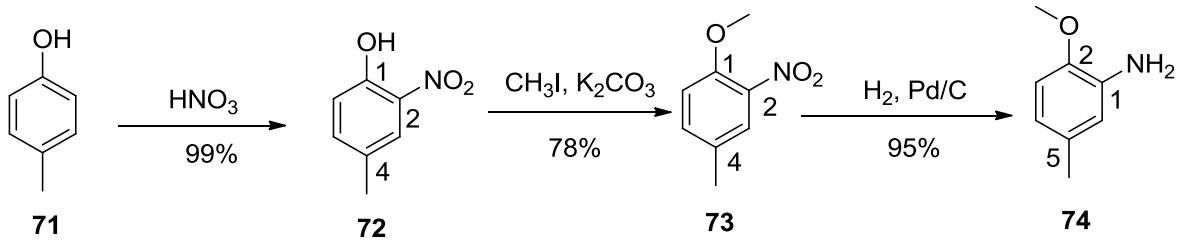
Table 4. 7: $^{1}H$ and $^{13}C$ spectral data of compounds **68**, **69** and **70**

| **Pos.** | **68** | | **69** | | **70** | |
|---|---|---|---|---|---|---|
| | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** |
| 1 | 12.44 (1H, *s*, -OH) | 162.9 | | 153.8 | 7.39 (*s*, -OH) | 152.9 |
| 2 | | 118.4 | | 128.5 | | 128.9 |
| 1’ | | 204.1 | 2.51 (2H, *q*, *J* = 15.2, 8.0 Hz, $-CH_2-$) | 22.4 | | 141.4 |
| 1’’ | | | | | 2.13 (3H, *s*) | 23.8 |
| 2’ | 2.64 (3H, *s*) | 26.6 | 1.13 (3H, *t*, *J* = 15.2, 8.0 Hz) | 13.7 | 5.45 (*d*, *J* = 0.6 Hz, 1H), 5.17 (*d*, *J* = 0.6 Hz,1H) | 116.1 |
| 3 | 7.73 (1H, *d*, *J* = 9.0 Hz | 131.9 | 6.97 (1H, *d*, *J* = 8.0 Hz) | 130.0 | 7.13 (*d*, *J* = 8.4 Hz) | 131.9 |
| 4 | 6.94 (1H, *dd*, *J* = 9.0, 2.4 Hz, | 119.5 | 6.78 (1H, *dd*, *J* = 8.0, 1.6 Hz) | 120.8 | 6.92 (*dd*, *J* = 8.4, 2.4 Hz) | 120.2 |
| 5 | | 141.8 | | 131.7 | | 133.3 |
| 6 | 7.01 (1H, *d*, *J* = 2.4 Hz | 118.1 | 6.69 (1H, *d*, *J* = 1.6 Hz) | 115.3 | 6.97 (*d*, *J* = 2.4 Hz) | 115.7 |

Following the reduction of the carbonyl of the acetyl of **68**, the NMR signals of the resulting ethyl group in **69** appear at $\delta_H$ 2.51 (2H, *q*) and 1.13 (3H, *t*) with the corresponding carbons resonating at $\delta_C$ 22.4 ($-CH_2$) and 13.8 ($-CH_3$). The conversion of the carbonyl in **68** to a methylene based on the Wittig reaction provides a propenyl group in **70** whose two olefinic protons resonate at $\delta_H$ 5.45 (*d*, *J* = 0.6 Hz, 1H) and 5.17 (*d*, *J* = 0.6 Hz, 1H), with the corresponding carbons resonating at $\delta_C$ 141.4 and 116.1. The structures of these compounds were confirmed by HMBC and HSQC experiments (Appendix 11, 12 and 13).

### 4.1.7. Synthesis of 2-Methoxy-5-methylaniline (74) and 2-Methoxy-5-methylbenzene-1,3-diamine (77)

The synthesis of 2-methoxy-5-methylaniline (**74**) from *p*-cresol (**71**) was accomplished as highlighted in Scheme 4.8. This was achieved through nitration, *O*-methylation and catalytic reduction of nitro group using hydrogen gas in the presence of Pd/C catalyst. Compounds **72** (yellowish crystals), **73** (brownish oil) and **74** (pale yellow oil) were obtained in 99, 78 and 95% yield, respectively.



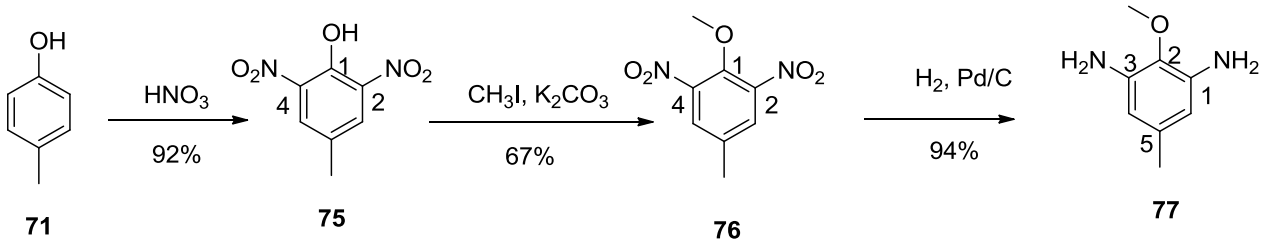

Scheme 4. 8: Synthetic transformation of *p*-cresol (**71**) to 2-methoxy-5-methylaniline (**74**)

The $^{1}H$ spectra of compounds **72**, **73** and **74** (Table 4.8) all showed presence of a methyl group and three mutually coupled aromatic protons. However, compounds **73** and **74** showed a methoxy resonating at $\delta_H$ 3.95 and 3.96 (3H, *s*), respectively. The difference observed in $^{13}C$ spectra for C-2 ($\delta_C$ 139.8) and C-1 ($\delta_C$ 130.2) for compounds **73** and **74**, respectively was attributed to electron withdrawal of the nitro group compared to the amino group which donates electrons through resonance. The structures of the compounds were also supported by HSQC and HMBC experiments (Appendix 14, 15 and 16).

Table 4. 8: $^{1}H$ and $^{13}C$ spectral data of compounds **72**, **73** and **74**

| **Pos.** | **72** | | **73** | | **74** | |
|---|---|---|---|---|---|---|
| | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** |
| 1 | 10.32 (1H, -OH) | 146.0 | | 150.4 | | 130.2 |
| 1-OMe | | | 3.95 (3H, *s*, -OMe) | 56.1 | | |
| 2 | | 130.9 | | 139.8 | | 150.4 |
| 2-OMe | | | | | 3.96 (3H, *s*, -OMe) | 56.1 |
| 3 | 7.94 (1H, *d*, *J* = 2.4 Hz) | 124.3 | 7.64 (1H, *d*, *J* = 3.0 Hz) | 124.9 | 7.24 (1H, *d*, *J* = 9.0 Hz) | 113.8 |
| 4 | | 129.7 | | 130.2 | 7.47 (1H, *dd*, *J* = 9.0, 3.0 Hz) | 134.5 |
| -$CH_3$ | 2.38 (3H, *s*, -$CH_3$) | 19.0 | 2.36 (3H, *s*, -$CH_3$) | 19.1 | 2.37 (3H, *s*, -$CH_3$) | 19.1 |
| 5 | 7.56 (1H, *dd*, *J* = 8.4, 2.4 Hz) | 138.5 | 7.46 (1H, *dd*, *J* = 8.4, 3.0 Hz) | 134.5 | | 129.8 |
| 6 | 7.12 (1H, *d*, *J* = 8.4 Hz) | 119.5 | 7.23 (1H, *d*, *J* = 8.4 Hz) | 113.8 | 7.65 (1H, *d*, *J* = 3.0 Hz) | 124.9 |

The synthetic route of 2-methoxy-5-methylbenzene-1,3-diamine (**77**) (Scheme 4.9) was similar to that of compound **74** except for the di-nitration to compound **75**. Compounds **75** (yellow crystals), **76** (white crystals) and **77** (pale yellow crystals) were obtained in 92, 67 and 94% yield, respectively.




Scheme 4. 9: Synthetic transformation of *p*-cresol (**71**) to 2, 6-diamino-4-methylanisole (**77**)

All the three compounds showed two *meta* coupled protons and a methyl group (Table 4.9) with those of compound **75** resonating at $\delta_H$ 8.05 (1H, *d*, *J* = 2.4 Hz, H-3), 7.49 (1H, *d*, *J* = 2.4 Hz, H-5) and 2.43 (3H, *s*, -$CH_3$). The $^{1}H$ and $^{13}C$ data of compound **75** also showed presence of a chelated

hydroxyl at $\delta_H$ 10.79 (1H, *s*) with the carbons resonating at $\delta_C$ 150.9 (C-1), 140.3 (C-5) 133.6 (C-2), 129.7 (C-4), 127.2 (C-6), 124.6 (C-3) and 20.1 (-$CH_3$).

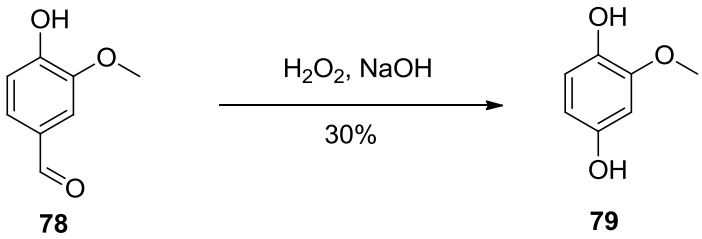
The spectra of compounds **76** and **77** were similar with the protons of methoxy observed at around $\delta_H$ 4.0 (3H, *s*). As opposed to compound **75** which lacks symmetry due to chelation, the $^{13}C$ spectra of compound **76** showed symmetry with the carbons resonating at $\delta_C$ 144.9 (C-1), 144.5 (C-2 and C-6), 135.9 (C-4), 129.4 (C-3 and C-5), 64.1 (-OMe) and 19.5 (-$CH_3$). This was also observed in the $^{13}C$ spectra of compound **72** with the data supported by HSQC and HMBC experiments (Appendix 17, 18 and 19).

Table 4. 9: $^{1}H$ and $^{13}C$ spectral data of compounds **75**, **76** and **77**

| **Pos.** | **75** | | **76** | | **77** | |
|---|---|---|---|---|---|---|
| | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **$\delta_H$ (ppm)** | **$\delta_C$(ppm)** |
| 1 | 10.79 (1H, *s*, OH) | 150.9 | | 144.9 | | 144.4 |
| 1-OMe | | | 4.03 (3H, *s*, -OMe) | 64.1 | | |
| 2 | | 133.6 | | 144.5 | | 144.8 |
| 2-OMe | | | | | 3.99 (3H, *s*, -OMe) | 64.0 |
| 3 | 8.0 (1H, *d*, *J* = 2.4 Hz) | 124.6 | 8.09 (1H, *d*, *J* = 1.2 Hz) | 129.4 | | 144.4 |
| 4 | | 129.7 | | 135.9 | 8.06 (1H, *s*) | 129.3 |
| -$CH_3$ | 2.43 (3H, *s*, -$CH_3$) | 20.1 | 2.54 (3H, *s*, -$CH_3$) | 19.5 | 2.50 (3H, *s*, -$CH_3$) | 19.5 |
| 5 | 7.49 (1H, *d*, *J* = 2.4 Hz) | 140.3 | 8.09 (1H, *d*, *J* = 1.2 Hz) | 129.4 | | 135.9 |
| 6 | | 127.2 | | 144.5 | 8.06 (1H, *s*) | 129.3 |

### 4.1.8. Synthesis of 2-Methoxy-1,4-benzenediol (79)

Synthesis of 2-methoxy-1,4-benzenediol (**79**) was achieved in a single step Dakins oxidation reaction in basic $H_2O_2$, a form of Baeyer-Villiger oxidation reaction, (Floyd *et al.*, 2005; Grant, 1993) with some modifications (Scheme 4.10).




Scheme 4. 10: One pot Dakins oxidation reaction of vanillin (**78**) in basic hydrogen peroxide

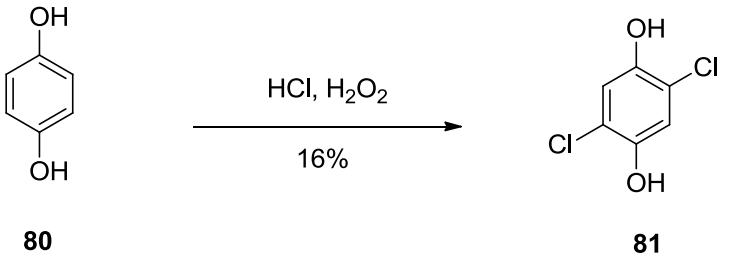
The product was obtained as a dark-purplish low melting point solid in 30% yield. The relatively low yield was attributed to formation of side products which necessitated purification by column chromatography. The $^{1}H$ and $^{13}C$ NMR data (Table 4.10) showed presence of three mutually coupled aromatic protons resonating at $\delta_H$ 6.65 (1H, *d*, *J* = 9.0 Hz, H-6), 6.48 (1H, *d*, *J* = 3.0 Hz, H-3), 6.30 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-5) and 2 aromatic hydroxyl protons resonating at 7.79 (1H, *s*, 1-OH) and 6.92 (1H, *s*, 4-OH). The signals for the carbonyl and the aldehydic proton in the starting material are not observed but instead the $^{13}C$ spectra shows 3 shielded carbons resonating at $\delta_C$ 150.7 (C-4), 147.9 (C-1) and 139.5 (C-2) consistent with oxygenated aromatic carbons inferring the expected transformation. The structure was also supported by the HMBC spectra which showed $^{3}J$ and $^{2}J$ correlations of 1-OH with C-1 ($\delta_C$ 147.9), C-2 ($\delta_C$ 139.5), C-6 ($\delta_C$ 114.9) and 4-OH with C-3, C-4 and C-5 (Appendix 20).

Table 4. 10: $^{1}H$ NMR (600MHz) and $^{13}C$ NMR data for compound **79** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 147.9 | |
| 1 - OH | 7.79 (1H, *s*, 1-OH) | | C-1, C-2, C-6 |
| 2 | | 139.5 | |
| 2 - $OCH_3$ | 3.80 (3H, *s*, 2-OMe) | 55.2 | C-2 |
| 3 | 6.48 (1H, *d*, *J* = 3.0 Hz) | 100.2 | C-1, C-5 |
| 4 | | 150.7 | |
| 4 - OH | 6.92 (1H, *s*, 4-OH) | | C-3, C-4, C-5 |
| 5 | 6.30 (1H, *dd*, *J* = 9.0, 3.0 Hz) | 106.4 | C-1, C-3 |
| 6 | 6.65 (1H, *d*, *J* = 9.0 Hz) | 114.9 | C-2, C-4 |

### 4.1.9. Synthesis of 2, 5-Dichloro-1,4-benzenediol (81)

2,5-Dichloro-1,4-benzenediol (**81**) was synthesized from hydroquinone (**80**) in a single step as highlighted in Scheme 4.11 (Song *et al*., 2008). The product was obtained as a pale-yellowish solid in 16% yield. The relatively low yield was attributed to formation of side products arising from oxidation by $H_2O_2$ which necessitated purification by column chromatography.




**80** **81**

Scheme 4. 11: Oxidative chlorination of hydroquinone (**80**)

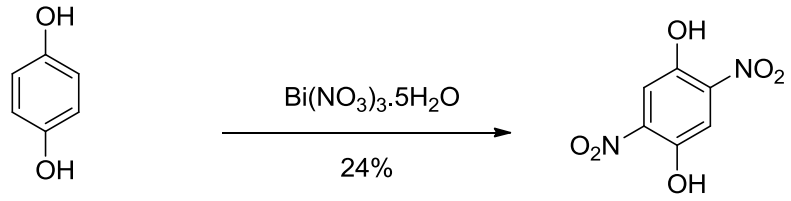
The $^{1}H$ NMR data (Table 4.11) of the compound showed two aromatic protons and two hydroxyl protons resonating at $\delta_H$ 7.45 (2H, *s*, H-3, H-6) and 7.37 (2H, *s*, 1-OH, 4-OH). The *para* coupled aromatic protons were expected to resonate singly but this is not observed due to symmetry of the molecule. Moreover, the $^{13}C$ data (Table 4.11) shows 2 de-shielded carbons resonating at $\delta_C$ 140.2 (C-2, C-5) consistent with the halogenated aromatic carbons and 2 aromatic carbons resonating at $\delta_C$ 133.3 (C-3, C-6) as opposed to 4 aromatic carbons expected in the starting material inferring success in the transformation. The structure was also supported by the HMBC and HSQC experiments (Appendix 21).

Table 4. 11: $^{1}H$ NMR (500MHz) and $^{13}C$ NMR data for compound **81** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 169.9 | |
| 1 - OH | 7.37 (1H, *s*) | | C-1, C-2, C-6 |
| 2 | | 140.2 | |
| 3 | 7.45 (1H, *s*) | 133.3 | C-1, C-5 |
| 4 | | 169.9 | |
| 4 - OH | 7.37 (1H, *s*) | | C-3, C-4, C-5 |
| 5 | | 140.2 | |
| 6 | 7.45 (1H, *s*) | 133.3 | C-2, C-4 |

### 4.1.10. Synthesis of 2, 5-Dinitro-1,4-benzenediol (82)

2,5-Dinitro-1,4-benzenediol (**82**) was synthesized from nitration of hydroquinone (**80**) in single step using bismuth (III) nitrate pentahydrate as highlighted in Scheme 4.12 (Jacoway *et al*., 2012). The nitration at positions 2 and 5 was regio-specifically directed by the two hydroxyl which are strong *ortho*, *para* directors.




**80** **82**

Scheme 4. 12: Nitration of hydroquinone (**80**) in bismuth (III) nitrate pentahydrate

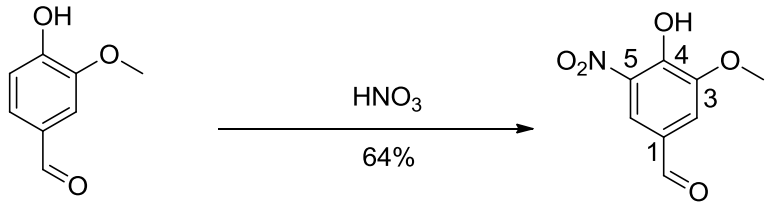
The product was obtained as a dark-brown crystalline substance in 24% yield. Similar to compound **81**, the $^{1}$H NMR data (Table 4.12) of the compound showed two aromatic protons and two hydroxyl protons resonating at $\delta_H$ 6.67 (2H, *s*, H-3, H-6), and 6.85 (2H, *s*, 1-OH, 4-OH), respectively. The hydroxyl protons were expected to be highly chelated and to resonate above $\delta_H$ 10 but this was not observed and was attributed to acetone-$d_6$, the solvent used to run the sample which suppresses the chelation. The $^{13}$C spectra showed 2 de-shielded carbons (low-field) resonating at $\delta_C$ 136.5 (C-2, C-5), consistent with nitrated carbons and 2 aromatic carbons resonating at $\delta_C$ 115.7 (C-3, C-6) as opposed to 4 aromatic carbons expected in the starting material. The structure was also supported by the HMBC and HSQC experiments (Appendix 22).

Table 4. 12: $^{1}$H NMR (500MHz) and $^{13}$C NMR data for compound **82** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 150.3 | |
| 1 - OH | 6.85 (1H, *s*) | | C-1, C-2, C-6 |
| 2 | | 136.5 | |
| 3 | 6.67 (1H, *s*) | 115.7 | C-1, C-5 |
| 4 | | 150.3 | |
| 4 - OH | 6.85 (1H, *s*) | | C-3, C-4, C-5 |
| 5 | | 136.5 | |
| 6 | 6.67 (1H, *s*) | 115.7 | C-2, C-4 |

### 4.1.11. Synthesis of 4-Hydroxy-3-methoxy-5-nitrobenzaldehyde (83)

4-Hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**) was synthesized from vanillin (**78**) in a single step as highlighted in Scheme 4.13. Compound **83** was obtained as a pale yellow crystalline solid in 64% yield.




**78** **83**

Scheme 4. 13: Transformation of vanillin (**78**) to 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)

The $^{1}$H NMR data (Table 4.13) showed two aromatic protons $\delta_H$ 8.26 (1H, *s*, H-6) and 7.73 (1H, *s*, H-2) as opposed to the three mutually coupled protons in the starting material. This is also supported by $^{13}$C spectra which shows four low-field carbons resonating at $\delta_C$ 150.7 (C-4), 149.5 (C-3), 135.2 (C-1) and 127.9 (C-5) as opposed to the expected three in the starting material. The aldehydic proton was found to resonate at $\delta_H$ 9.98 (1H, *s*, H-1'), and just like compound **77**, the chelated proton expected to resonate above $\delta_H$ 10 was not observed due to the solvent used to run the sample. The success of the transformation was also supported by the HMBC and HSQC experiments (Appendix 23).

Table 4. 13: $^{1}H$ NMR (500MHz) and $^{13}C$ NMR data for compound **83** in acetone-$d_6$

| **Position** | **$\delta_H$ (ppm)** | **$\delta_C$ (ppm)** | **HMBC** |
|---|---|---|---|
| 1 | | 135.2 | |
| 1 - COH | 9.98 (1H, *s*) | 189.4 | C-2, C-6 |
| 2 | 7.73 (1H, *s*) | 113.2 | C-4, C-6 |
| 3 | | 149.5 | |
| 3 - $OCH_3$ | 4.06 (3H, *s*, 3-OMe). | 56.4 | C-5 |
| 4 | | 150.7 | |
| 5 | | 127.9 | |
| 6 | 8.26 (1H, *s*) | 120.6 | C-2, C-4 |

### 4.2. Antimicrobial Activity

The antibacterial, antifungal and antiplasmodial activities of the compounds synthesized were evaluated and the results are discussed below.

#### 4.2.1. Antibacterial Activity

Based on the disc diffusion method, the compounds were assayed against two gram-positive bacteria (*Staphyloccocus aureus* ATCC 25923 and *Streptococcus infantarius* 438209) and three gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853 and *Salmonella typhimurium* 7222569-1). The inhibition zones of the compounds against the various microbes at varying concentrations are presented in Table 4.14.

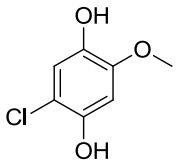
Table 4. 14: Antibacterial activity of the test samples against various microbes by the disc diffusion method

| Compound | Conc. (µg/disc) | Inhibition zone (mm) | | | | | Compd. | Conc. (µg/disc) | Inhibition zone (mm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SA | SI | PA | EC | ST | | | SA | SI | PA | EC | ST |
| **55** | 400 | - | - | - | - | - | **70** | 400 | 10 | 11 | - | 8 | 8 |
| **56** | 400 | - | - | - | - | - | | 200 | 7 | 8 | | 7 | - |
| **57** | 400 | - | - | - | - | - | | 100 | - | 7 | | 7 | |
| **58** | 400 | 13 | 11 | - | - | - | **72** | 400 | - | - | - | - | - |
| | 200 | 12 | 9 | | | | **73** | 400 | - | - | - | - | - |
| | 100 | 10 | 8 | | | | **74** | 400 | 19 | - | 8 | 9 | 9 |
| | 50 | 8 | - | | | | | 200 | 7 | | - | - | - |
| **59** | 400 | 21 | 18 | 10 | 14 | 9 | **75** | 400 | - | - | - | 8 | - |
| | 200 | 16 | 14 | 7 | 11 | 7 | **76** | 400 | - | - | - | - | - |
| | 100 | 14 | 13 | - | 10 | - | **77** | 400 | NT | NT | NT | NT | NT |
| | 50 | 11 | 10 | | 7 | | **79** | 400 | 22 | 19 | 11 | 18 | 14 |
| | 25 | 9 | 8 | | - | | | 200 | 13 | 16 | 9 | 13 | 8 |
| **60** | 400 | 15 | 14 | 8 | 10 | 10 | | 100 | 10 | 13 | 7 | 11 | - |
| | 200 | 9 | 13 | - | 8 | 9 | | 50 | 9 | 8 | - | 9 | |
| | 100 | 8 | 10 | | 7 | - | | 25 | 8 | - | | 7 | |
| | 50 | 7 | 8 | | - | | | 12.5 | 7 | | | - | |
| | 25 | - | 7 | | | | **81** | 400 | 18 | 15 | 8 | 11 | 8 |
| **61** | 400 | - | - | - | - | - | | 200 | 11 | 10 | - | 9 | - |
| **62** | 400 | 17 | - | 9 | 18 | 11 | | 100 | 8 | 8 | | 8 | |
| | 200 | 13 | | 7 | 8 | 8 | **82** | 400 | 16 | 14 | 8 | 8 | - |
| | 100 | 12 | | - | - | - | | 200 | 8 | 13 | - | - | |
| | 50 | 10 | | | | | | 100 | - | 10 | | | |
| | 25 | 7 | | | | | | 50 | | 8 | | | |
| | 12.5 | - | | | | | | 25 | | - | | | |
| **63** | 400 | - | - | - | 9 | 7 | **83** | 400 | - | - | - | - | - |
| **65** | 400 | 15 | - | - | 9 | 7 | **CRO*** | 30 | | | | | 30 |
| | 200 | 7 | | | - | - | **C*** | 30 | 23 | | | | |
| **68** | 400 | - | - | - | 8 | - | **E*** | 15 | | 27 | | | |
| **69** | 400 | 9 | - | - | 8 | 7 | **GN*** | 10 | | | 18 | | |
| | 200 | - | | | | - | **AMP*** | 10 | | | | 19 | |

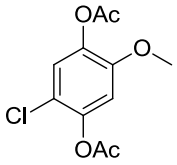
Standard Reference Drugs: CRO* = Ceftriaxone, C* = Chloramphenicol, GN* = Gentamicin, E* = Erythromycin, AMP* =Ampicillin
Microorganisms: SA = *Staphyloccocus aureus* (ATCC 25923), SI = *Streptococcus infantarius* (438209), PA = *Pseudomonas aeroginosa* (ATCC 27853), EC = *Escherichia coli* (ATCC 25922), ST = *Salmonella typhimurium* (7222569-1)
Key: "-" = No inhibition and "NT" = Not tested.

To aid in evaluation of the antimicrobial activity of the compounds in comparison to the standards, a susceptibility criteria based on the zone of inhibition was adopted. Any microorganism found to exhibit an inhibition zone < 7 mm at 400 µg/disc was inferred as not susceptible to the compound, 7 to < 10 mm at 400 µg/disc as low susceptibility, 10 to <14 mm as being of intermediate susceptibility and those exhibiting a zone of inhibition ≥ 14 mm at 400 µg/disc categorized as having high susceptibility.

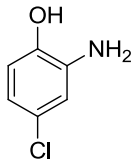
Amongst all the compounds assayed, compounds **59**, **60**, **62** and **79** were found to be the most active against the five bacterial strains they were tested against.



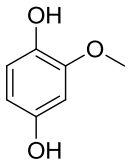

**59**




**60**




**62**




**79**

They exhibited high activity against either gram positive microorganisms, gram negative or both at a concentration of 200µg/disc. Compound **59** displayed moderately high activity against gram positive bacteria, *Staphyloccocus aureus* and *Streptococcus infantarius* with an inhibition zone of 9 and 8 mm respectively at 25µg/disc, each. Moreover, the compound exhibited a relatively high activity against *Escherichia coli*, a gram negative bacterium, with an inhibition zone of 7mm at 50µg/disc. A similar trend in activity was observed for compound **60** in comparison to the phenolic compound **59**.

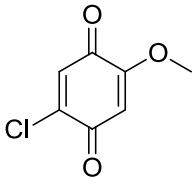
Amongst all the compounds assayed, compounds **59** and **60** were the most active against *Streptococcus infantarius* with an inhibition zone of 8 and 7mm, respectively at 25µg/disc. This might be attributed to the two compounds possibly sharing a similar mechanism of action, as de-acylation of the acetate group of compound **60** would yield compound **59**. Erythromycin, the

standard reference drug used against *S. infantarius* exhibited an inhibition zone of 27mm at a concentration of 15µg/disc.

With exception to *Streptococcus infantarius,* compound **62** exhibited activity against the other gram +ve and gram -ve bacteria at a concentration of 200µg/ disc. Interestingly, it exhibited high activity against *Staphyloccocus aureus* with an inhibition zone of 7 mm at a concentration of 25µg/disc. Similar activity against *Staphyloccocus aureus* was observed for compound **59**, a 1,4-benzenediol derivative, which exhibited an inhibition zone of 7 mm at a concentration of 25µg/disc.

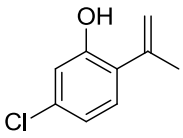
Compound **79**, a benzene-1,4-diol derivative, displayed broad spectrum activity against all the microorganisms at 200µg/ disc. Interestingly, it was the most active against *Staphyloccocus aureus* with an inhibition zone of 7mm at a concentration of 12.5µg/disc. Chloramphenicol, the standard reference drug used against *S. aureus,* exhibited an inhibition zone of 23mm at a concentration of 30µg/disc. Moreover, the compound was the most active against *Escherichia coli* with an inhibition zone of 7 mm at a concentration of 25µg/disc. Ampicillin, the standard reference drug used against *E. coli,* exhibited an inhibition zone of 19mm at a concentration of 10µg/disc.

Compound **58**, a benzoquinone derivative, showed moderate activity against gram positive bacteria only (*Staphyloccocus aureus* and *Streptococcus infantarius*) exhibiting an inhibition zone of 8 mm at 100 µg/disc and 50µg/disc against the two bacteria, respectively but showed no activity against gram negative bacteria at the same concentration.

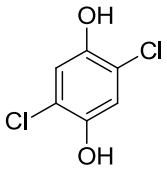



**58**

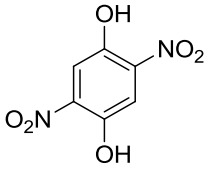
Compound **81**, a benzene-1,4-diol derivative, and compound **70** generally exhibited low to intermediate susceptibility against four microorganisms at 200µg/disc. At a concentration of 100µg/disc, they displayed intermediate susceptibility with an inhibition zone of around 7 mm against *Escherichia coli* and *Streptococcus infantarius*. Compound **82**, a benzene-1,4-diol derivative, exhibited an inhibition zone of 8 mm against *Streptococcus infantarius* at a concentration of 50µg/disc but was generally of low susceptibility against other microbes.




**70**




**81**




**82**

Most of the other compounds displayed insignificant activity or were inactive at 400µg/disc.

### 4.2.2. Antifungal Activity

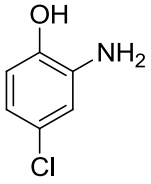
Similar to antibacterial activity, antifungal activity was evaluated based on the disc diffusion method. The compounds were assayed against the fungal strain *Candida albicans* ATCC 10231 that causes candidiasis in humans. The results based on the inhibition zones of the compounds at various concentrations are presented in Table 4.15.

Table 4. 15: Antifungal activity of the test samples against *Candida albicans* based on the disc diffusion method

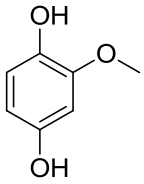
| Compound | Concentration (µg/disc) | Inhibition (mm) | Compound | Concentration (µg/disc) | Inhibition (mm) |
|---|---|---|---|---|---|
| **55** | 400 | - | **70** | 400 | 13 |
| **56** | 400 | - | | 200 | 10 |
| **57** | 400 | - | | 100 | 7 |
| **58** | 400 | - | | 50 | - |
| **59** | 400 | 9 | **72** | 400 | - |
| | 200 | 7 | **73** | 400 | - |
| | 100 | - | **74** | 400 | 7 |
| **60** | 400 | 16 | | 200 | - |
| | 200 | 10 | **75** | 400 | 7 |
| | 100 | 8 | **76** | 400 | - |
| | 50 | - | **77** | 400 | NT |
| **61** | 400 | - | **79** | 400 | 22 |
| **62** | 400 | 29 | | 200 | 14 |
| | 200 | 22 | | 100 | 9 |
| | 100 | 17 | | 50 | 7 |
| | 50 | 15 | | 25 | - |
| | 25 | 10 | **81** | 400 | 21 |
| | 12.5 | 7 | | 200 | 10 |
| **63** | 400 | 7 | | 100 | 8 |
| **65** | 400 | 7 | **82** | 400 | 12 |
| **68** | 400 | - | | 200 | - |
| **69** | 400 | 8 | **83** | 400 | - |
| | 200 | - | **CT*** | 25 | 21 |

*Standard reference drug: CT***** = clotrimazole, "-" = No inhibition, "NT" = Not tested

Amongst the compounds tested, compounds **62** and **79** showed the most promising antifungal activity.




**62**




**79**

Compound **62** was found to be the most active against *Candida albicans* with an inhibition zone of 7 mm at a concentration of 12.5µg/ disc. Clotrimazole, the standard reference drug used against *Candida albicans* exhibited an inhibition zone of 21mm at a concentration of 25µg/disc.

Compound **79**, a benzene-1,4-diol derivative, displayed moderate activity against *Candida albicans* with an inhibition zone of 7 mm at 50µg/ disc, while compounds **60**, **70** and **81** were found to have an inhibition zone of 8, 7 and 7 mm, respectively each at 100 µg/disc. Most of the other compounds displayed insignificant activity or were inactive at 200µg/disc.

### 4.2.3. *In vitro* Antiplasmodial Activity

*In vitro* antiplasmodial assay of selected synthesized compounds was carried out using a non-radioactive assay technique against chloroquine sensitive 3D7 and D6 strains of *Plasmodium falciparum* with chloroquine and mefloquine used as positive control drugs. The activity ($IC_{50}$ values) of the compounds is summarized in Table 4.16.

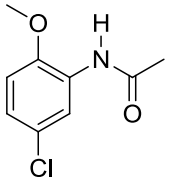
Table 4. 16: *In vitro* antiplasmodial activity of selected compounds against chloroquine sensitive 3D7 and D6 strains

| Sample | $IC_{50}$ value in µg/mL CQ 3D7 | $IC_{50}$ value in µg/mL CQ D6 |
|---|---|---|
| **57** | 23.19 ± 1.77 | 26.90 ± 1.97 |
| **59** | 26.24 ± 1.70 | 21.61 ± 4.27 |
| **61** | 2.85 ± 0.25 | 1.29 ± 0.48 |
| **62** | 2.70 ± 0.14 | 1.24 ± 0.47 |
| **63** | 9.06 ± 1.09 | 7.09 ± 1.94 |
| Chloroquine* | 0.00872 ± 0.00047 | 0.0108 ± 0.0022 |
| Mefloquine* | 0.0303 ± 0.0050 | 0.0275 ± 0.0101 |

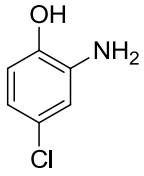
* Standard reference drugs

Amongst the five compounds assayed, compounds **61**, **62** and **63** were found (Table 4.16) to fall within the WHO recommended scale of compounds which are highly active against the two plasmodium strains (i.e. compounds with $IC_{50}$ values less than 10 µg/mL) (WHO, 2014; Lekana-

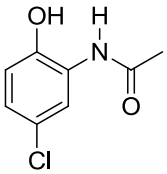
Douki *et al*., 2011). Both compound **57** and **59** were found to be moderately active (compounds with $IC_{50}$ values between 10 and 50 µg/mL) (Jonville *et al*., 2008; Lekana-Douki *et al*., 2011).




**61**




**62**




**63**

It is noteworthy that, amongst the compounds that were evaluated, the aminophenol derivatives exhibited higher antiplasmodial activity.

# CHAPTER 5

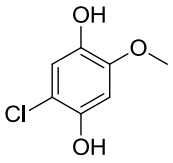
# CONCLUSIONS AND RECOMMENDATIONS

The main objective of this study was to synthesize phenolic analogues of the benzoquinone metabolite and evaluate their antimicrobial and antiplasmodial activity. Also evaluated were selected intermediates generated in the course of the synthesis of the phenolic analogues.

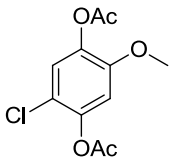
## 5.1. CONCLUSIONS

In this study:

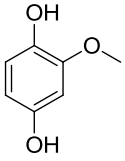
i. Five benzene-1,4-diol derivatives of the benzoquinone metabolite namely 2-chloro-5-methoxy-1,4-benzenediol (**59**), 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**), 2-methoxy-1,4-benzenediol (**79**), 2,5-dichloro-1,4-benzenediol (**81**) and 2,5-dinitro-1,4-benzenediol (**82**) were successfully synthesized and characterized.



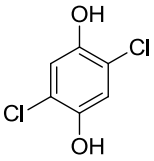

**59**



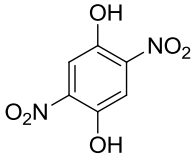

**60**




**79**



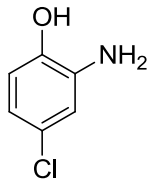

**81**




**82**

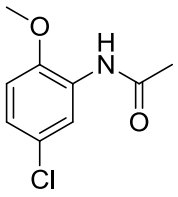
ii. Most of the synthesized benzene-1,4-diol derivatives exhibited promising antibacterial activity. Compounds **59** and **60** were found to be the most active against *Streptococcus infantarius* with minimum inhibition at 25µg/disc each, while **79** was the most active against *Staphyloccocus aureus* and *Escherichia coli* with minimum inhibition at 12.5µg/disc and 25µg/disc, respectively.

iii. 5-Chloro-2-hydroxyaniline (**62**) and 2-methoxy-1,4-benzenediol (**79**) were found to possess promising antifungal activity against *Candida albicans* with **62** being the most active (minimum inhibition at 12.5µg/disc) followed by **79** (minimum inhibition at 50µg/disc).

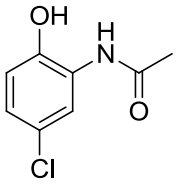



**62**

iv. Three aniline intermediates (5-chloro-2-methoxyacetanilide (**61**), 5-chloro-2-hydroxyaniline (**62**) and 5-chloro-2-hydroxyacetanilide (**63**)) in the preparation of benzene-1,4-diols were found to be highly active against 3D7 and D6 strains of the plasmodium parasite.




**61**




**63**

## 5.2. RECOMMENDATIONS

Based on the outcome of this study, I recommend the following:

i. Considering that benzene-1,4-diols displayed the highest antibacterial activity, I recommend the synthesis of diverse benzene-1,4-diol derivatives of the benzoquinone metabolite to assess the scope of their antibacterial potency.

ii. Bearing in mind that phenols have been shown to possess varied biological activity including anti-oxidant (e.g. quercetin) and anti-inflammatory effects (e.g.

acetaminophen and mesalamine), I recommend the assessment of the cytotoxicity, anti-inflammatory and anti-oxidant potency of the benzene-1,4-diol derivatives.

iii. To guide the rational choice of more potent benzene-1,4-diol derivatives for synthesis, I recommend target identification and subsequent computational modeling and docking studies be employed for identification of more potent benzene-1,4-diol derivatives.

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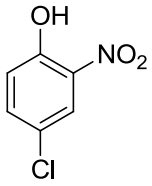
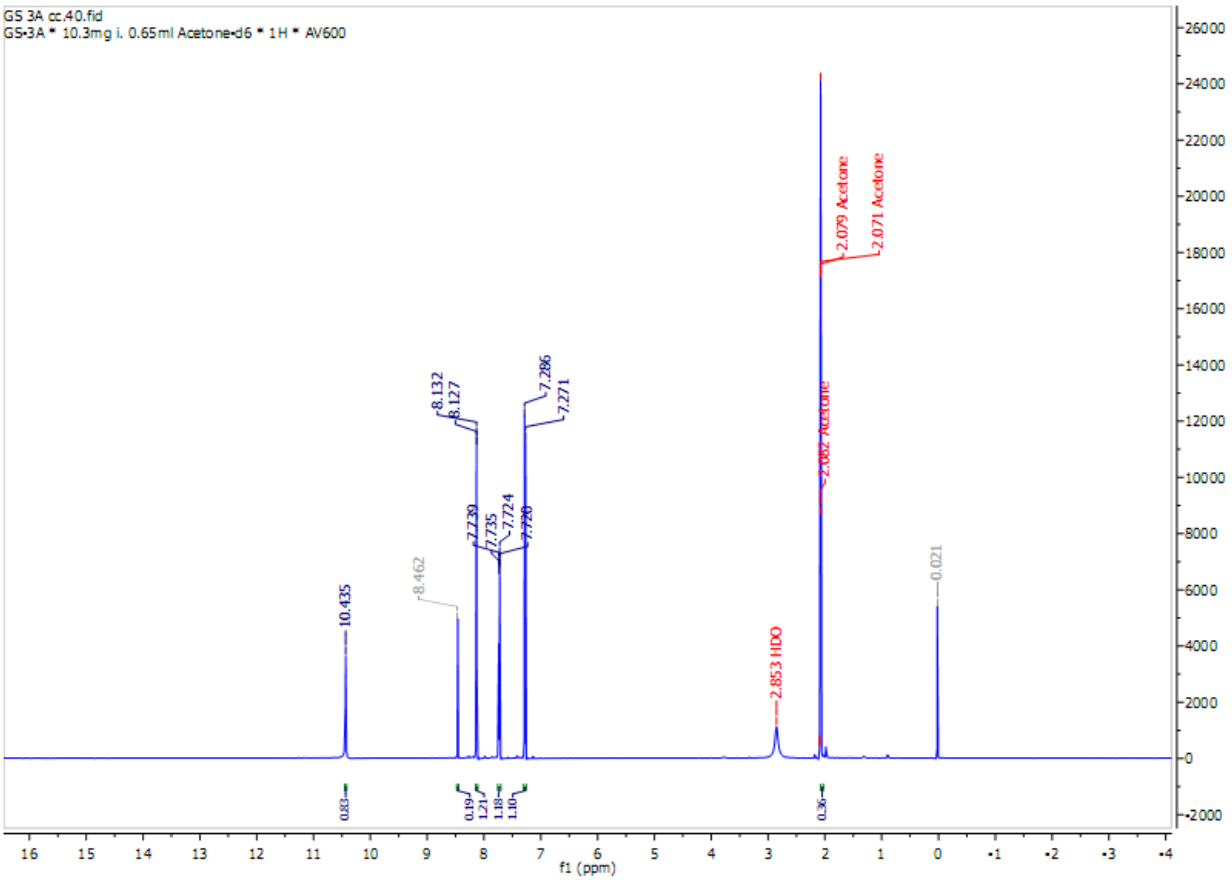
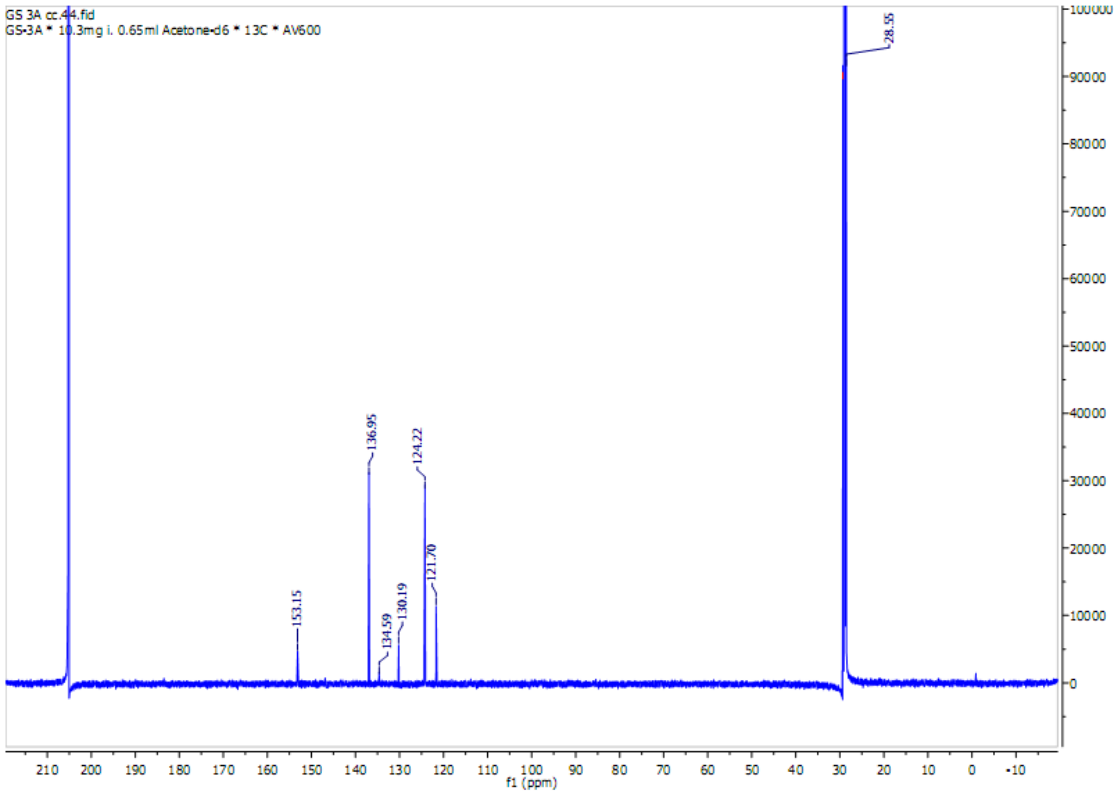
# APPENDICES

Appendix 1: The spectra of 4-chloro-2-nitrophenol (**55**)

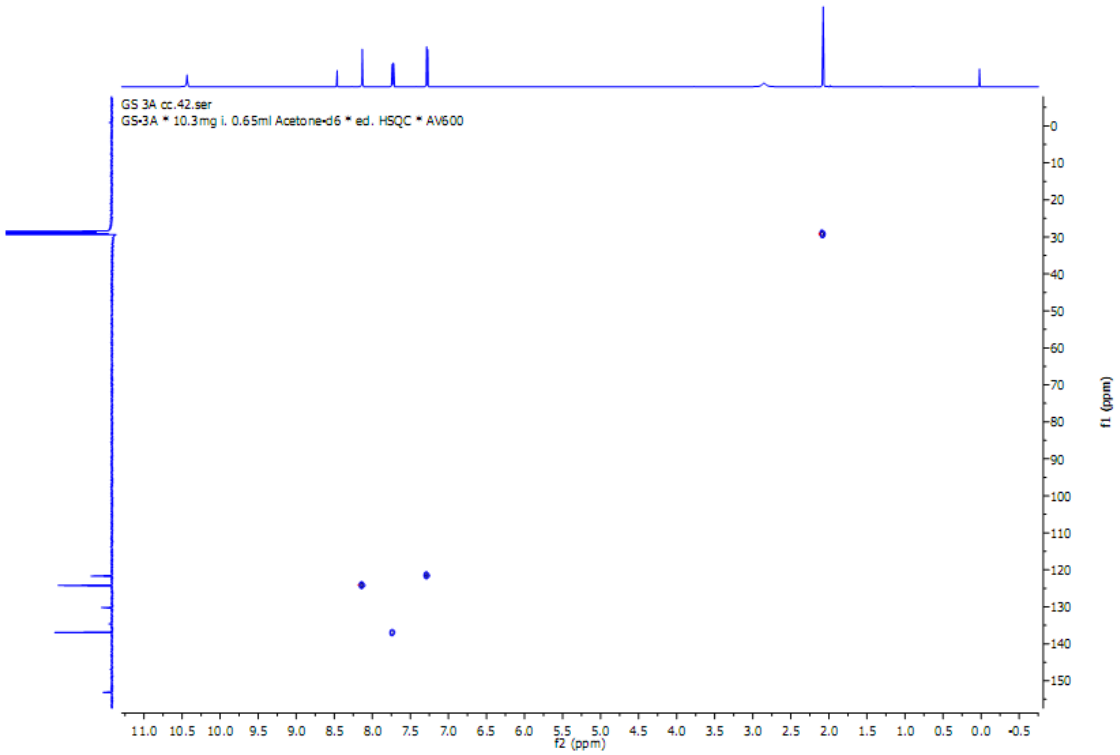


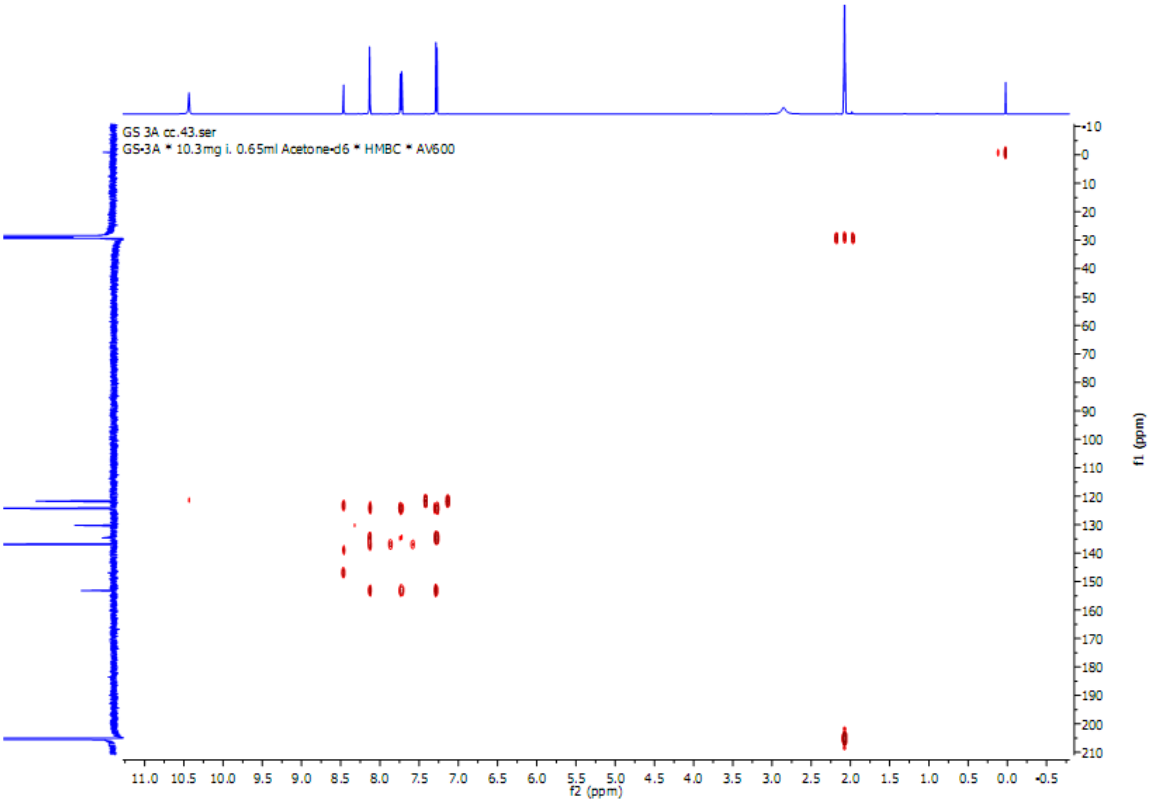

$^{1}$H NMR spectra of 4-chloro-2-nitrophenol (**55**)



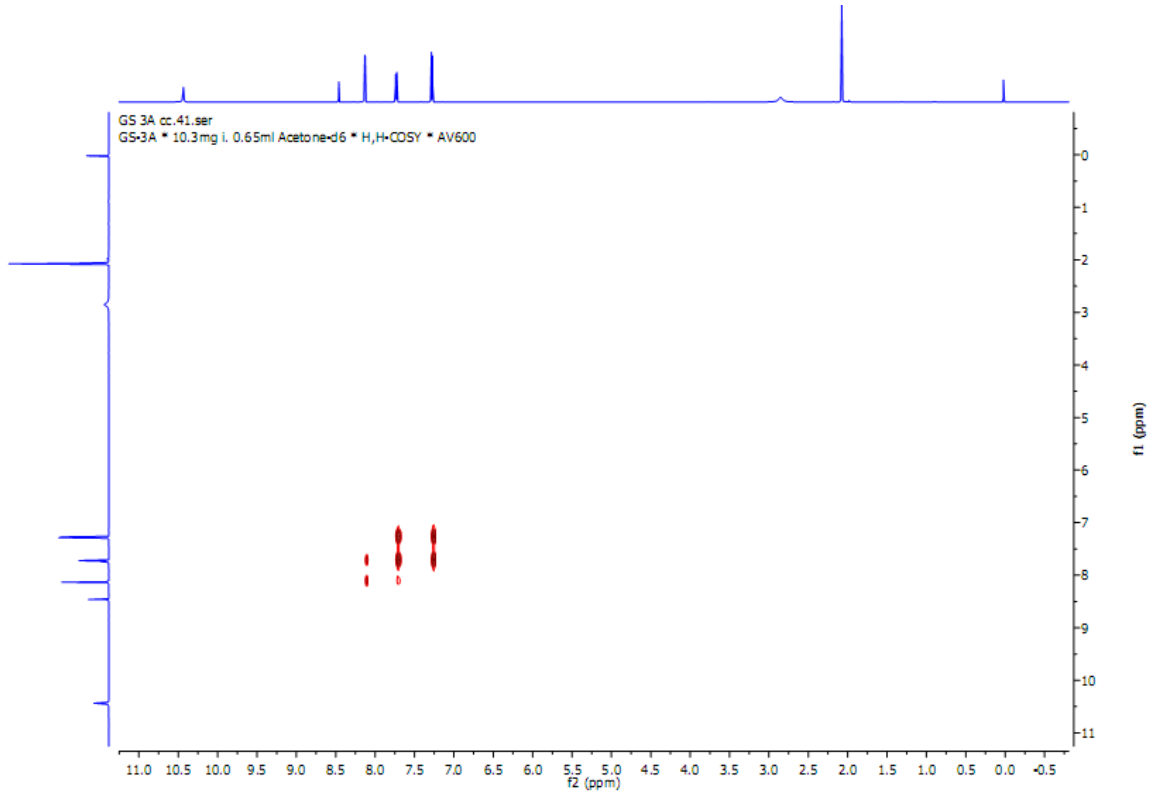

$^{13}$C NMR spectra of 4-chloro-2-nitrophenol (**55**)




HSQC spectra of 4-chloro-2-nitrophenol (**55**)

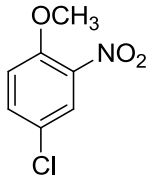


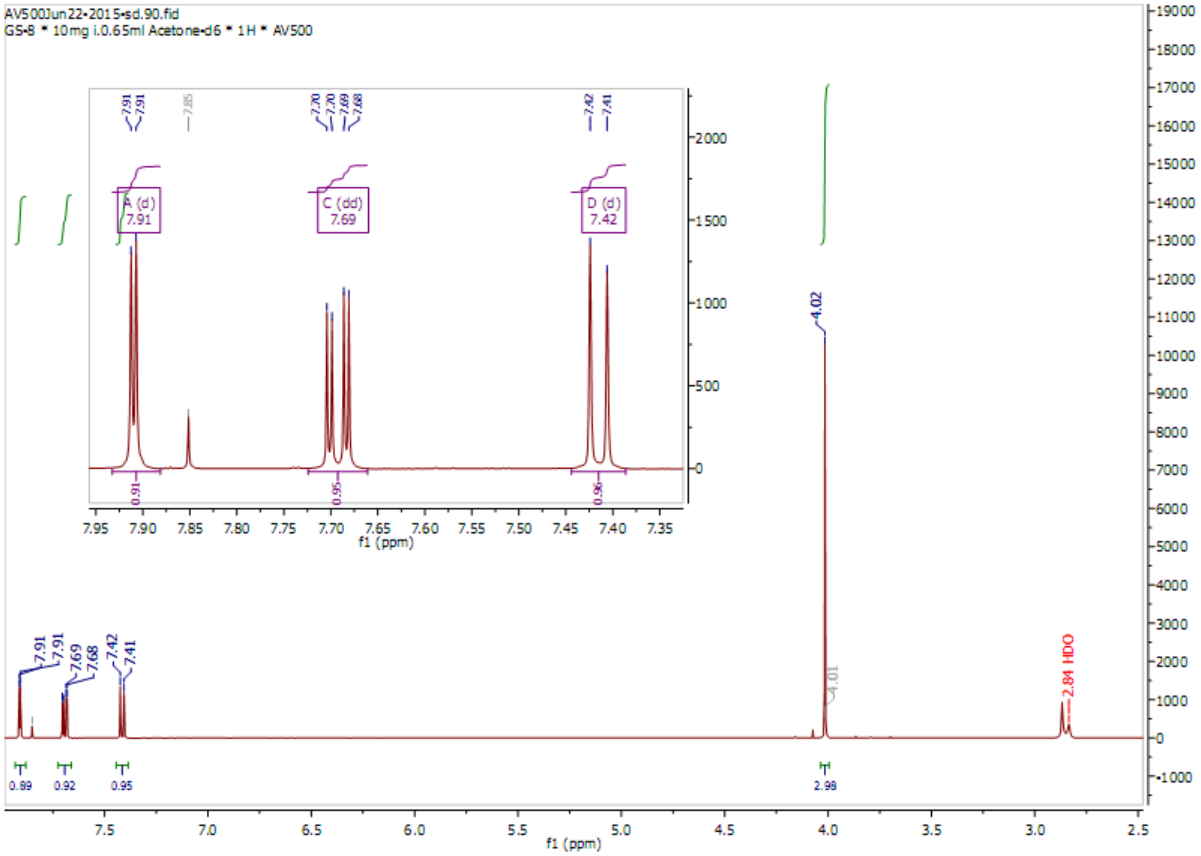

HMBC spectra of 4-chloro-2-nitrophenol (**55**)



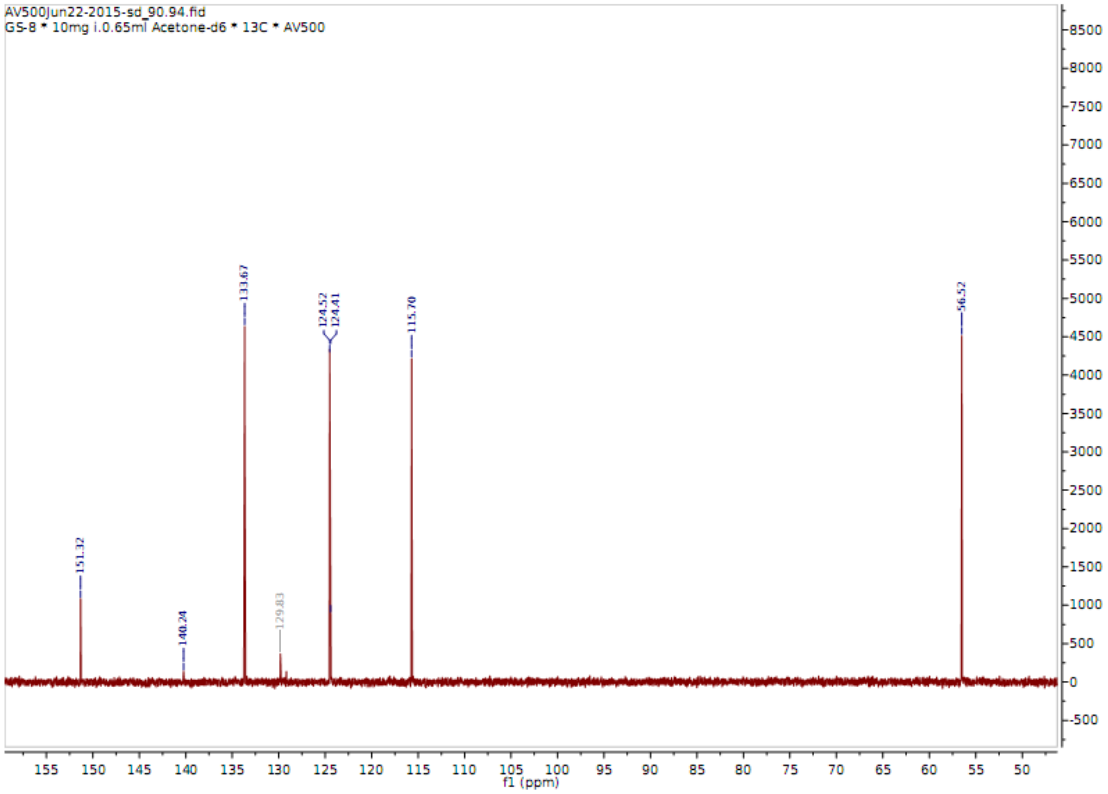

H, H-COSY spectra of 4-chloro-2-nitrophenol (**55**)

Appendix 2: The spectra of 4-chloro-2-nitroanisole (**56**)

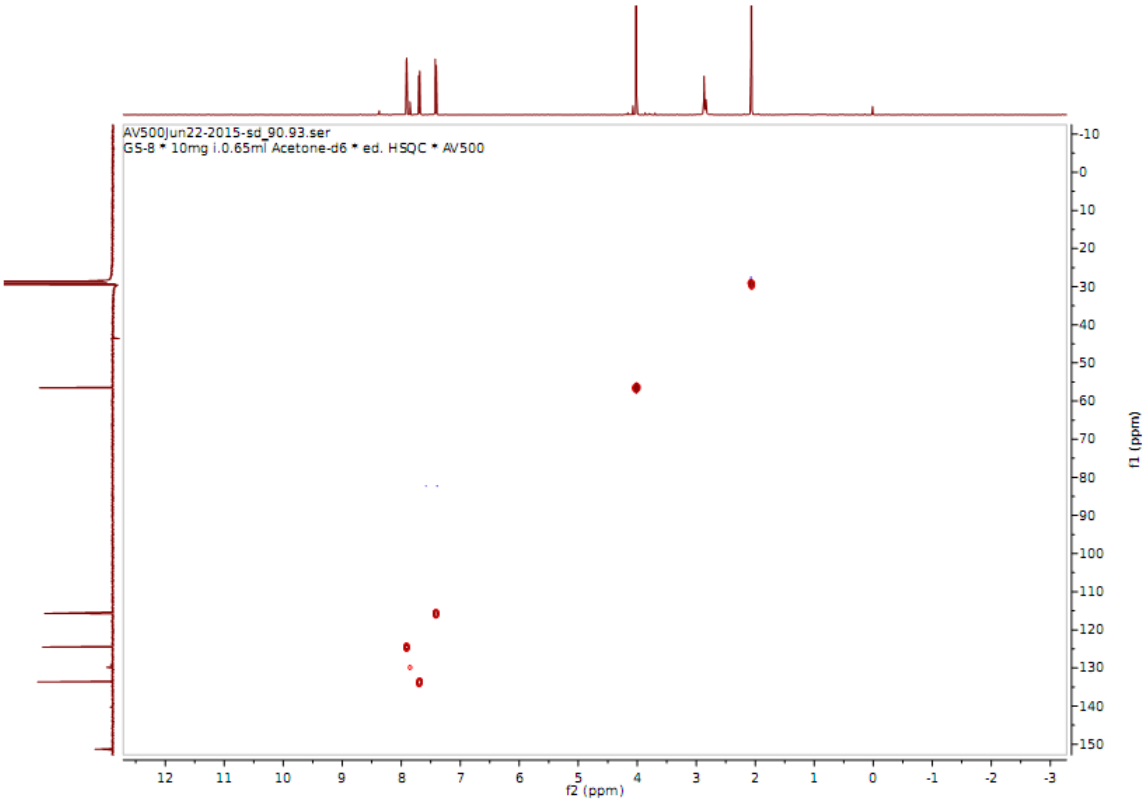



$^{1}$H NMR spectra of 4-chloro-2-nitroanisole (**56**)

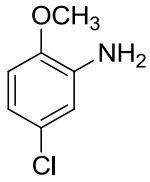


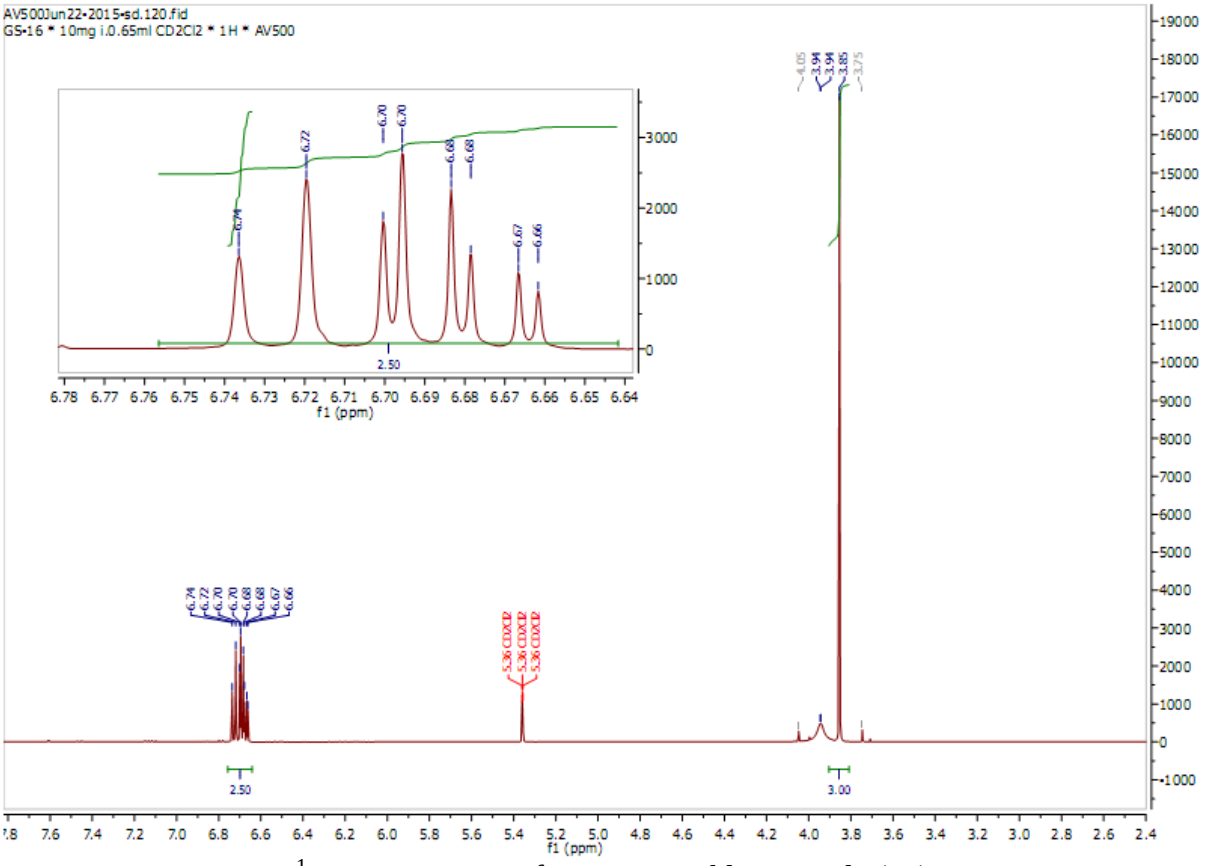

$^{13}$C NMR spectra of 4-chloro-2-nitroanisole (**56**)



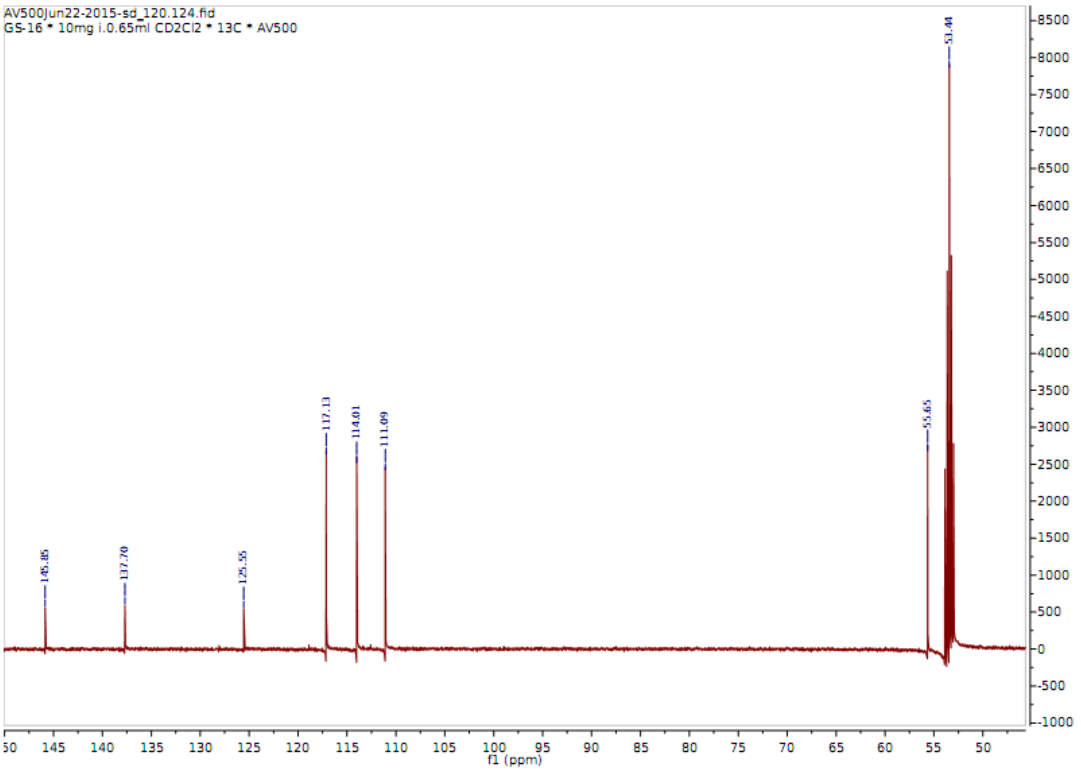

HSQC spectra of 4-chloro-2-nitroanisole (**56**)

Appendix 3: The spectra of 2-amino-4-chloroanisole (**57**)

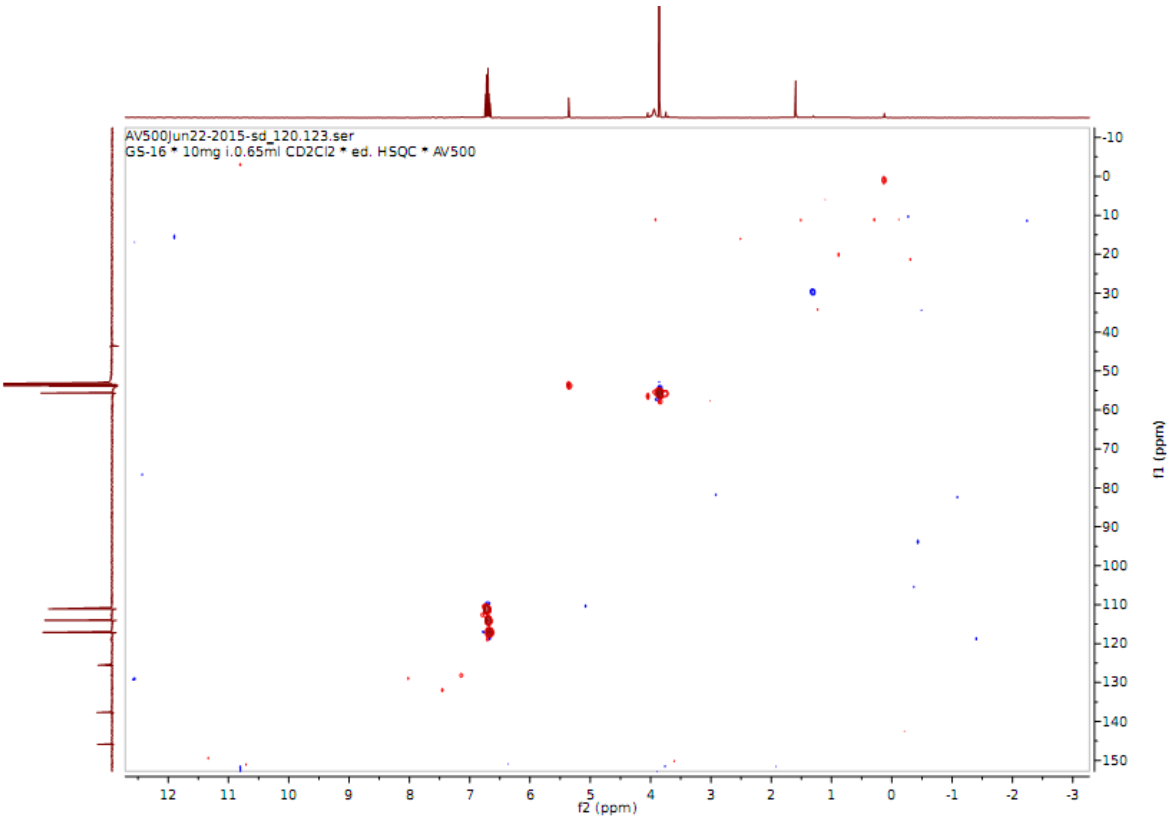



$^{1}$H NMR spectra of 2-amino-4-chloroanisole (**57**)

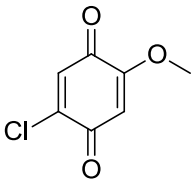


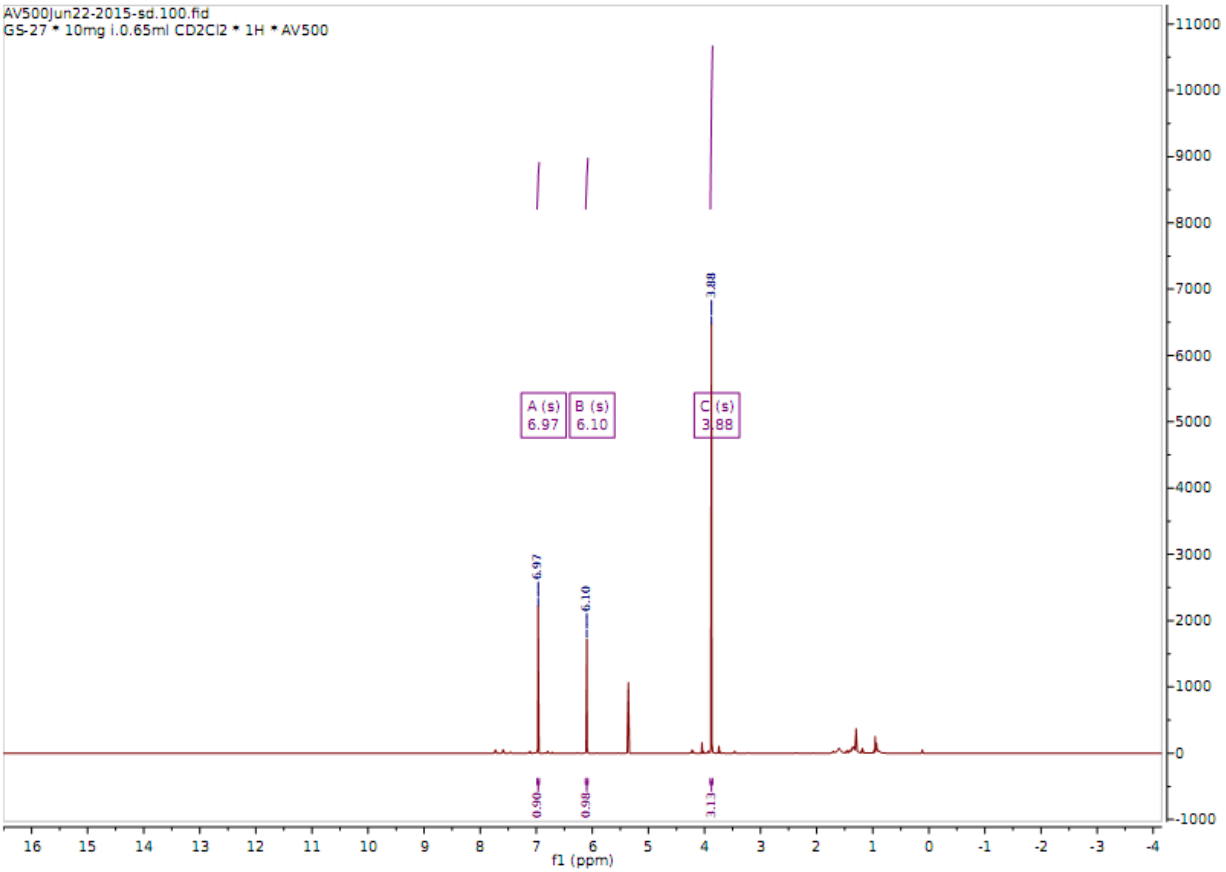

$^{13}$C NMR spectra of 2-amino-4-chloroanisole (**57**)



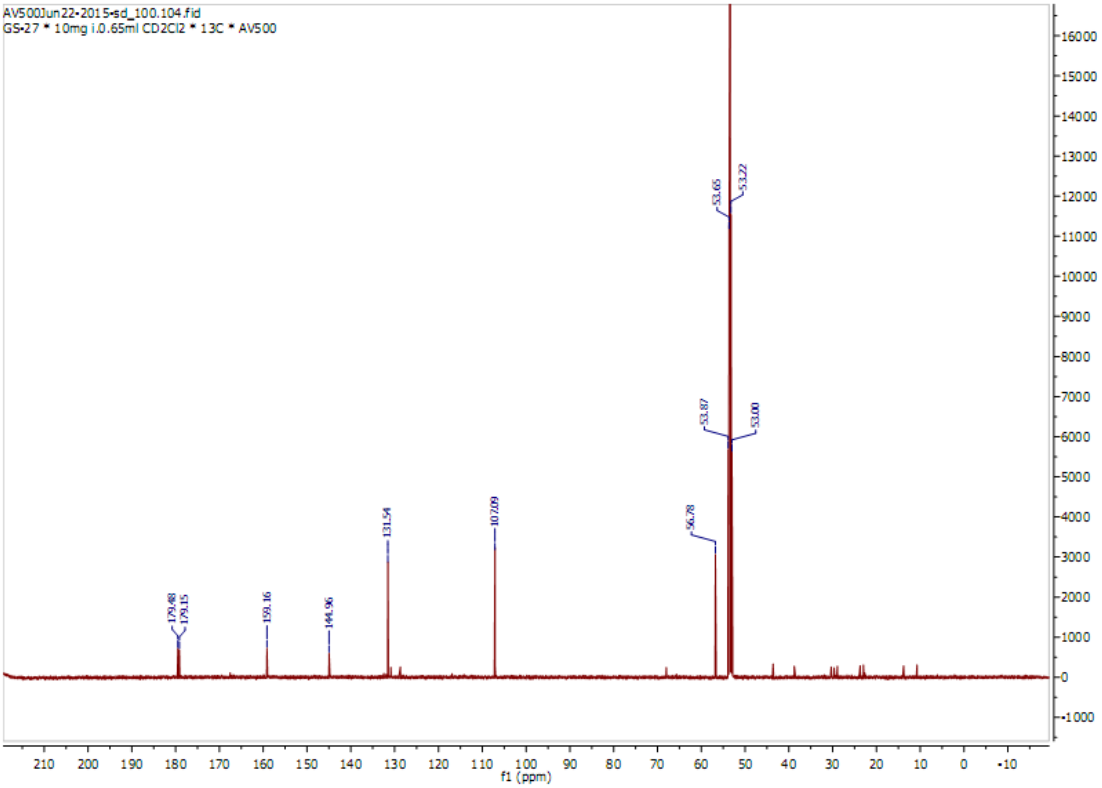

HSQC spectra of 2-amino-4-chloroanisole (**57**)

Appendix 4: The spectra of 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**)

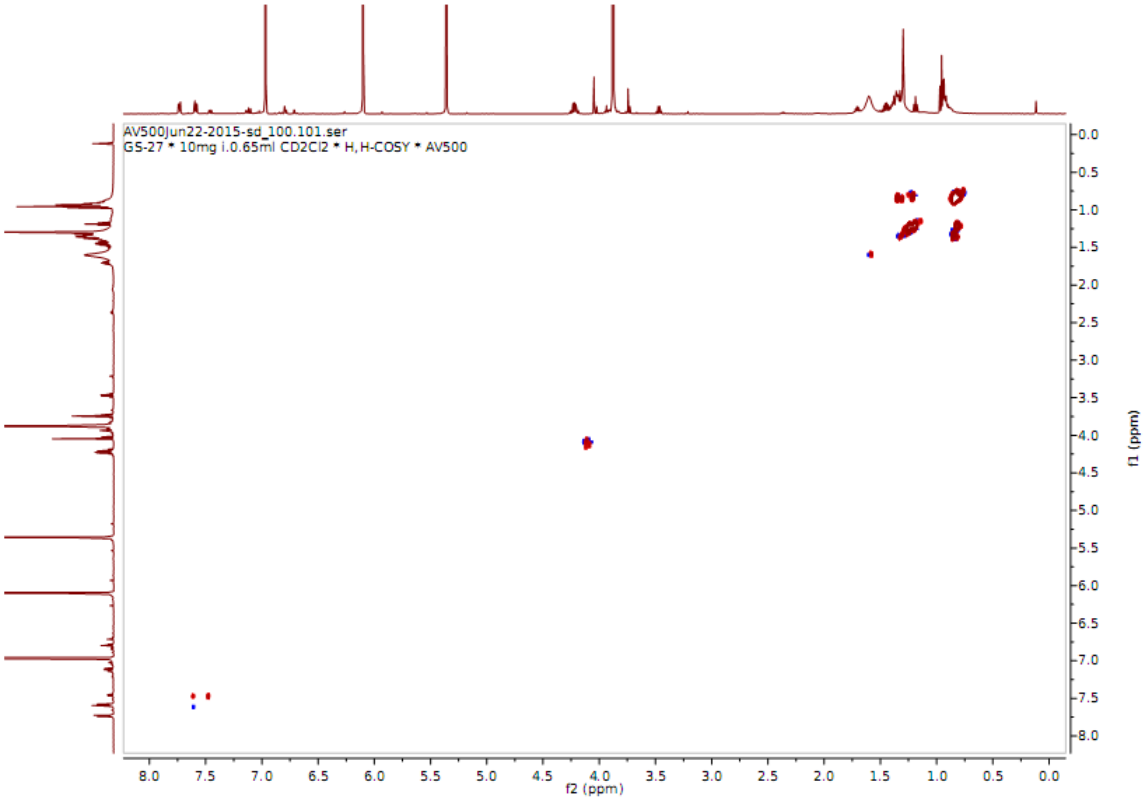


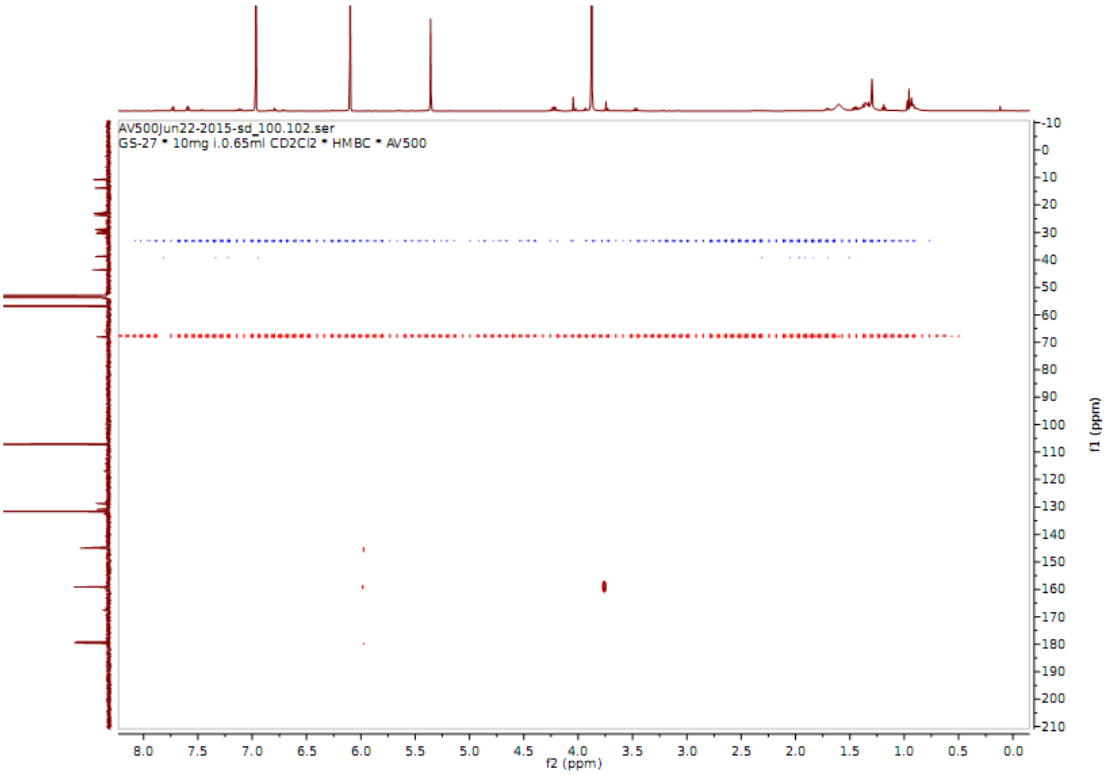

$^{1}$H NMR spectra of 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**)



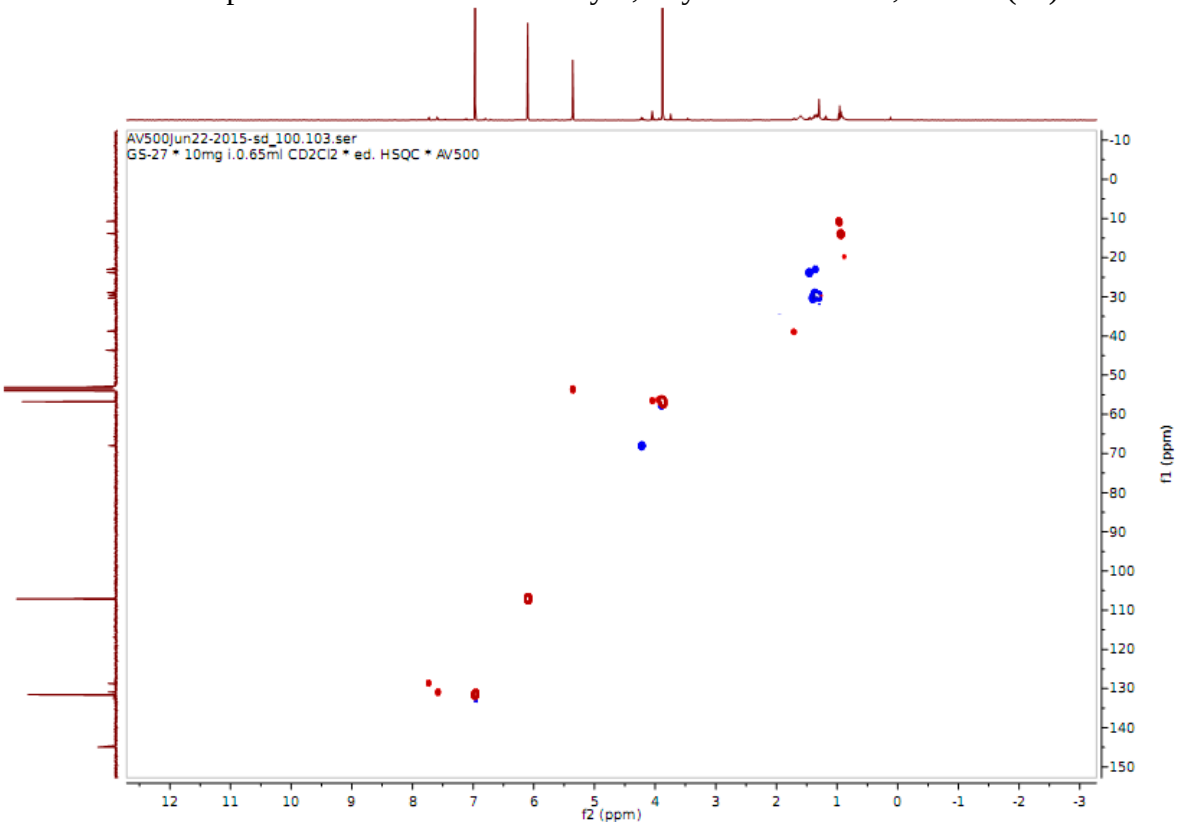

$^{13}$C NMR spectra of 2-chloro-5-methoxy-2, 5-cyclohexadiene-1,4-dione (**58**)




H,H-COSY spectra of 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**)

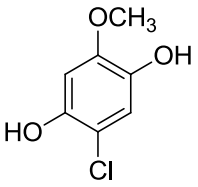


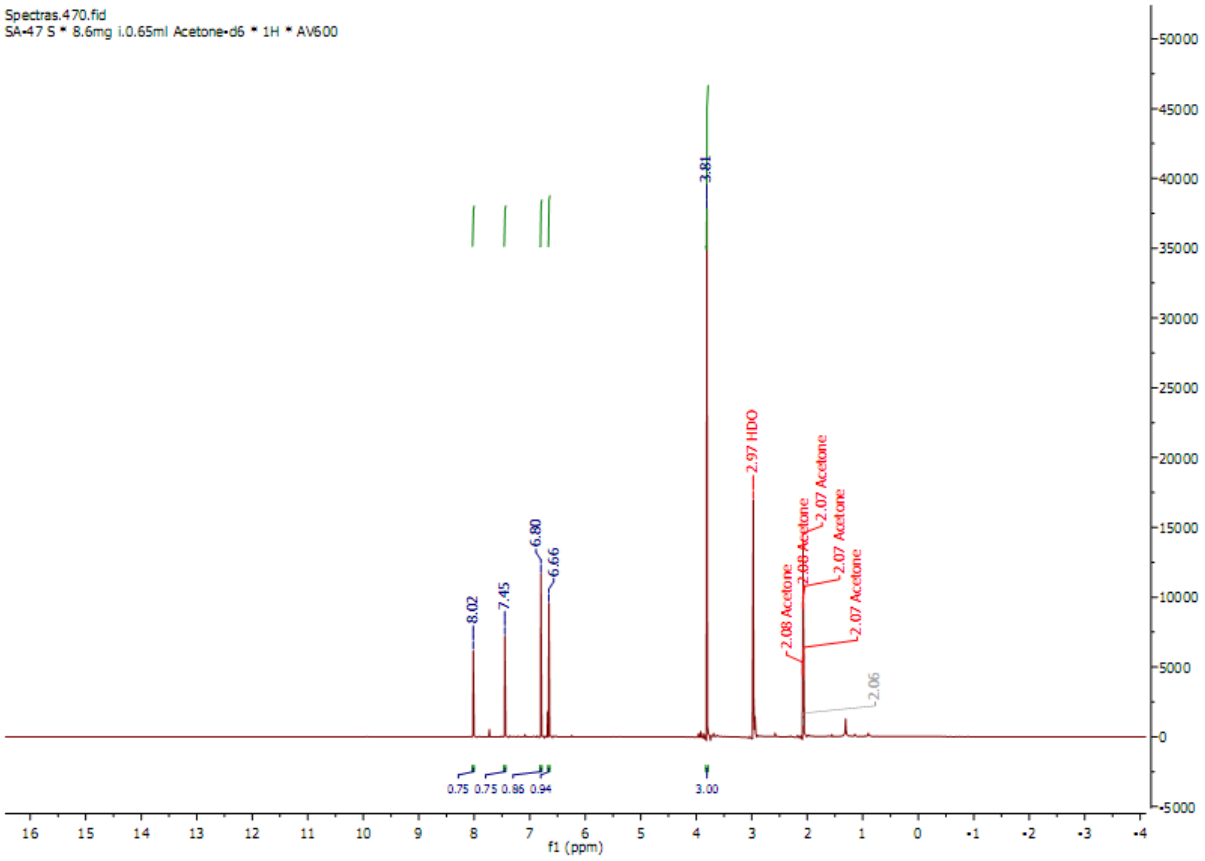

HMBC spectra of 2-chloro-5-methoxy-2,5-cyclohexadiene-1,4-dione (**58**)



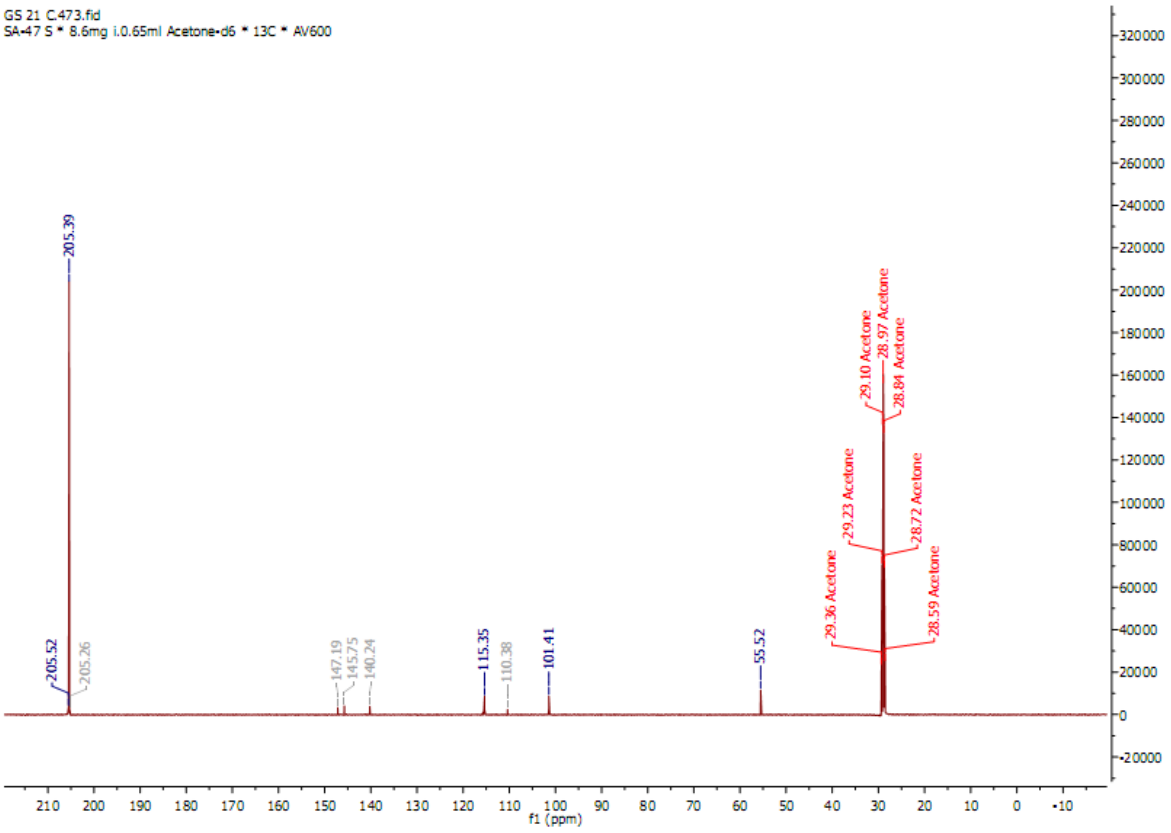

HSQC spectra of 2-chloro-5-methoxy-2, 5-cyclohexadiene-1, 4-dione (**58**)

Appendix 5: The spectra of 2-chloro-5-methoxy-1,4-benzenediol (**59**)

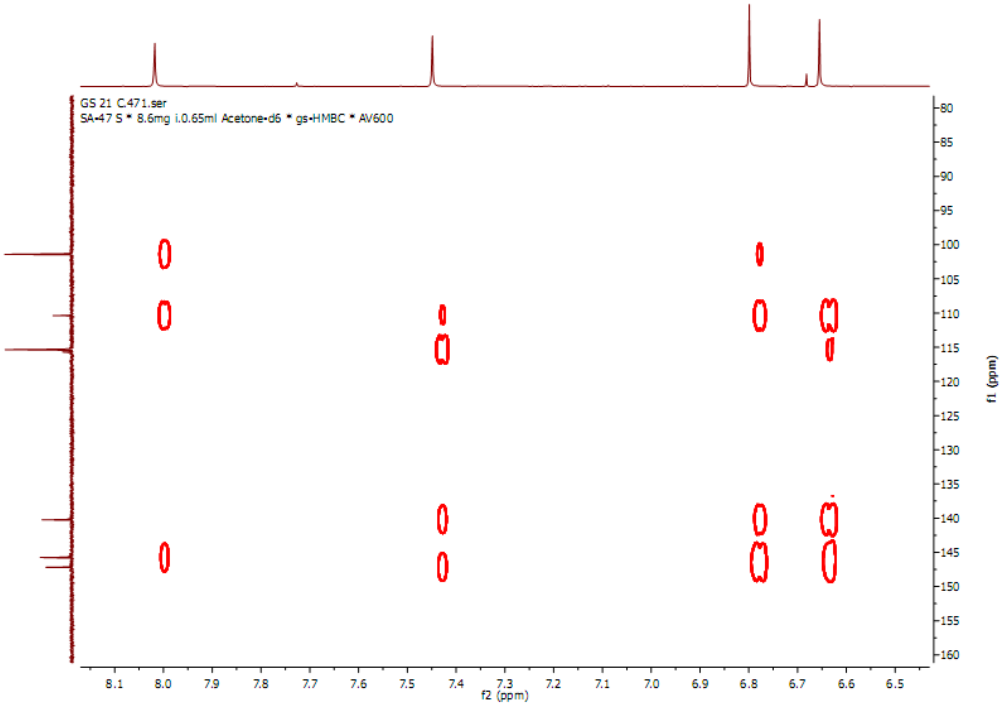


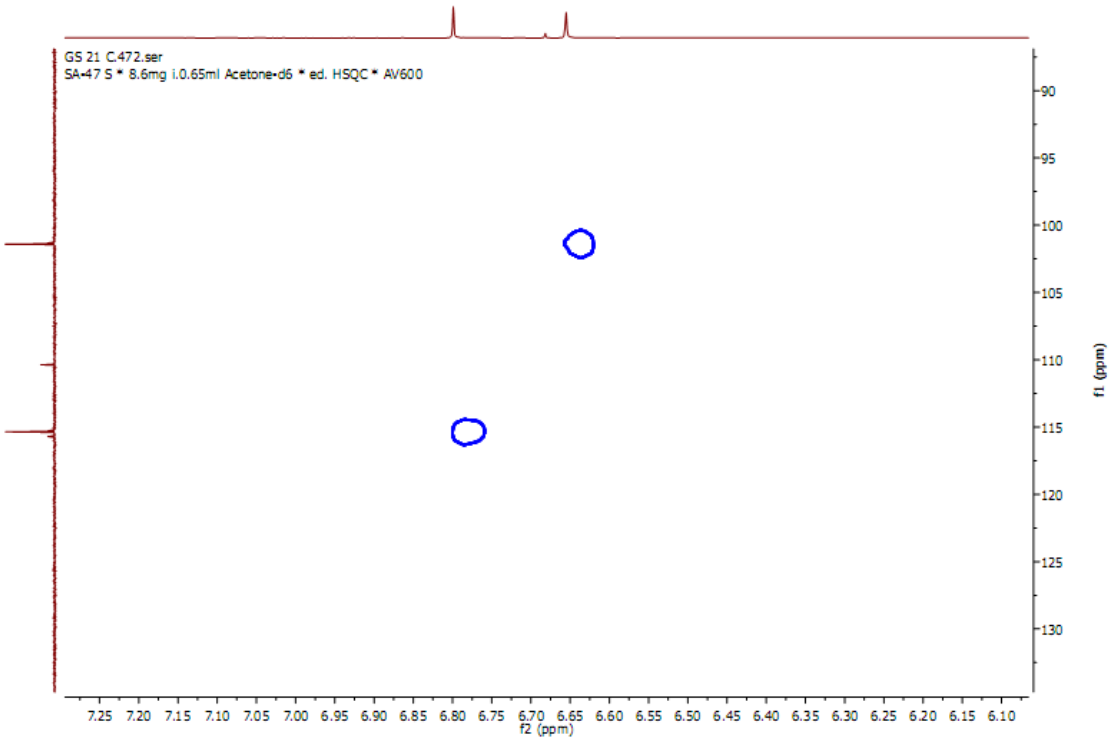

$^{1}$H NMR spectra of 2-chloro-5-methoxy-1,4-benzenediol (**59**)




$^{13}$C NMR spectra of 2-chloro-5-methoxy-1,4-benzenediol (**59**)

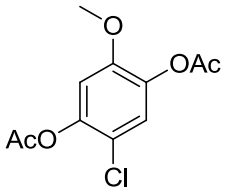


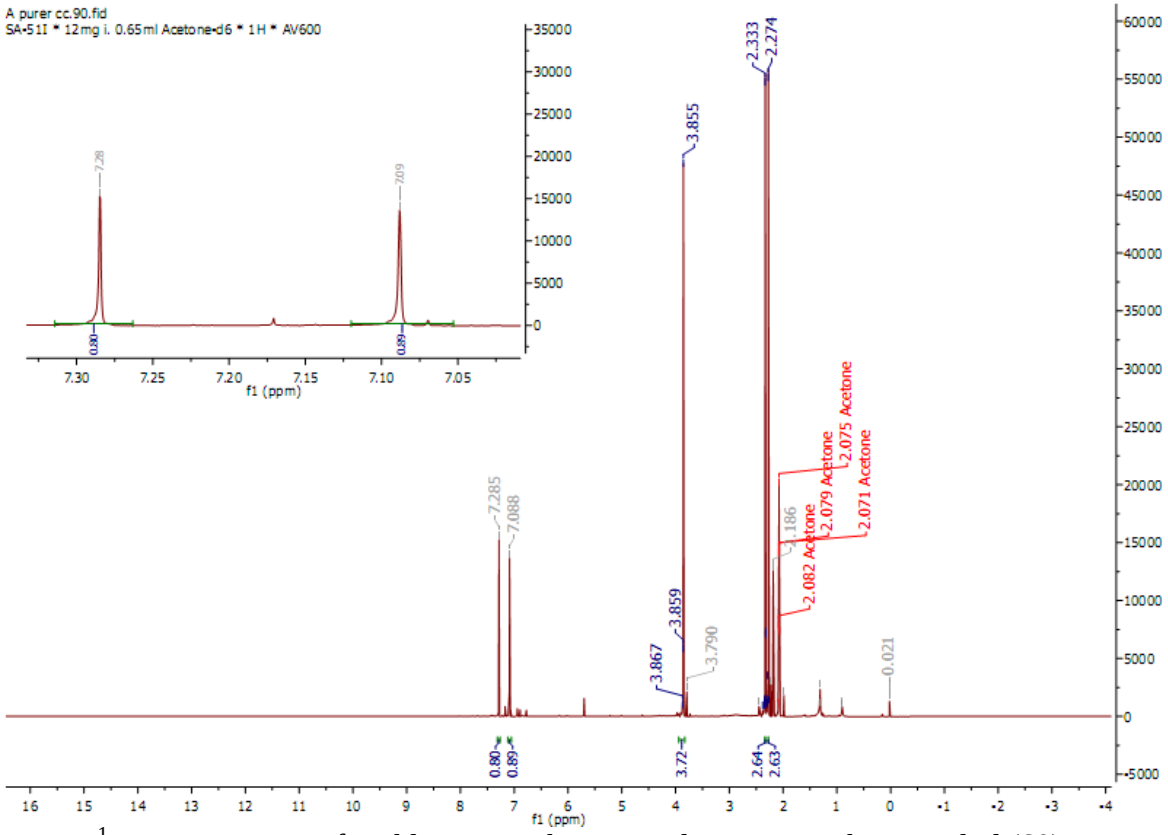

HMBC spectra of 2-chloro-5-methoxy-1,4-benzenediol (**59**)



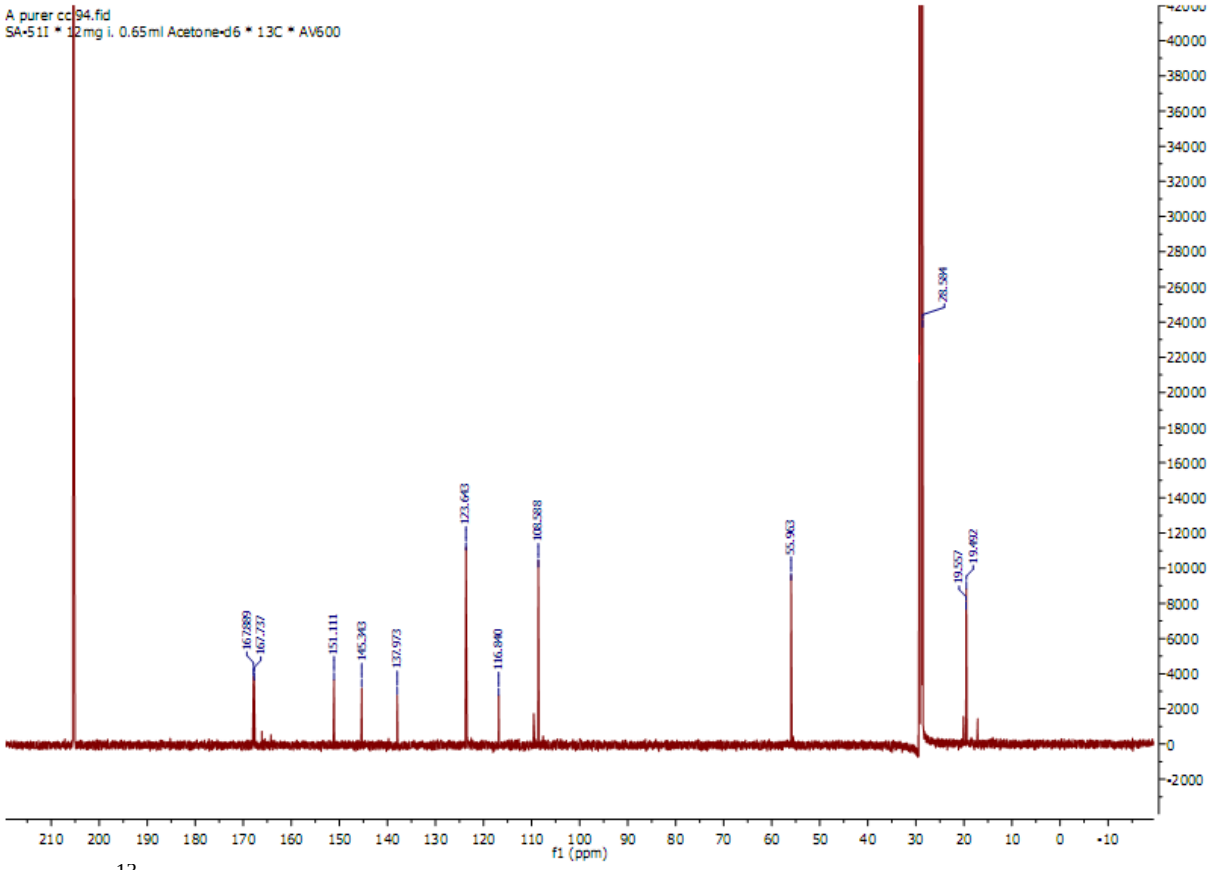

HSQC spectra of 2-chloro-5-methoxy-1,4-benzenediol (**59**)

Appendix 6: The spectra of 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)

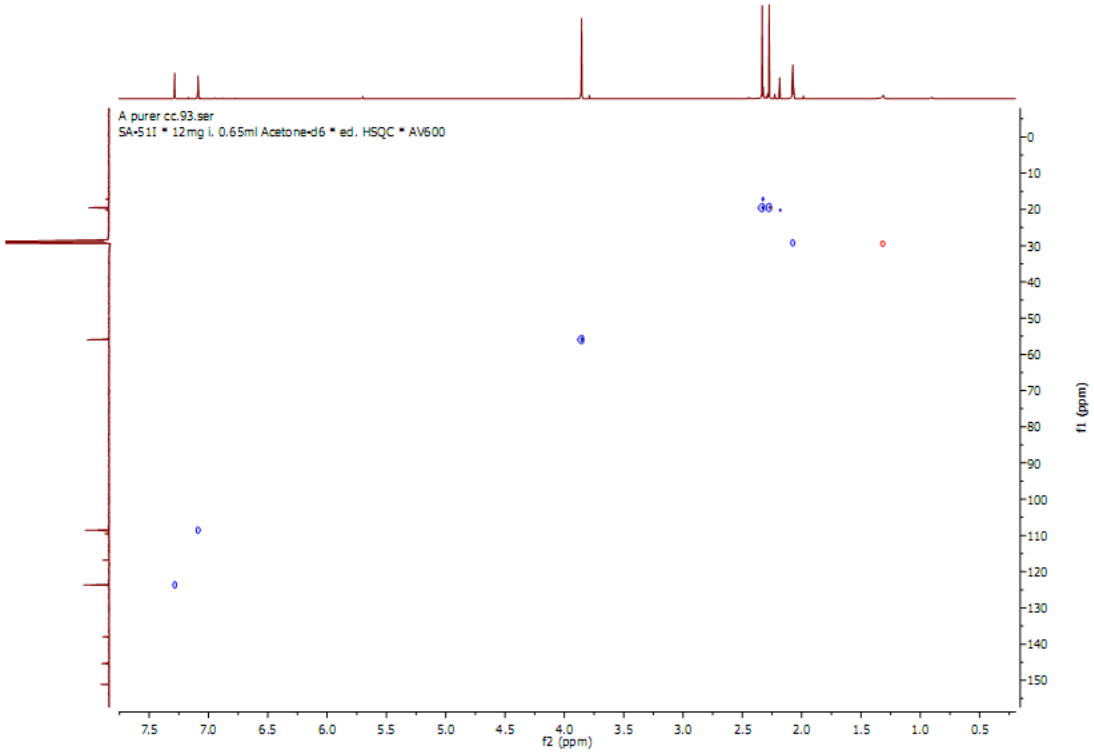


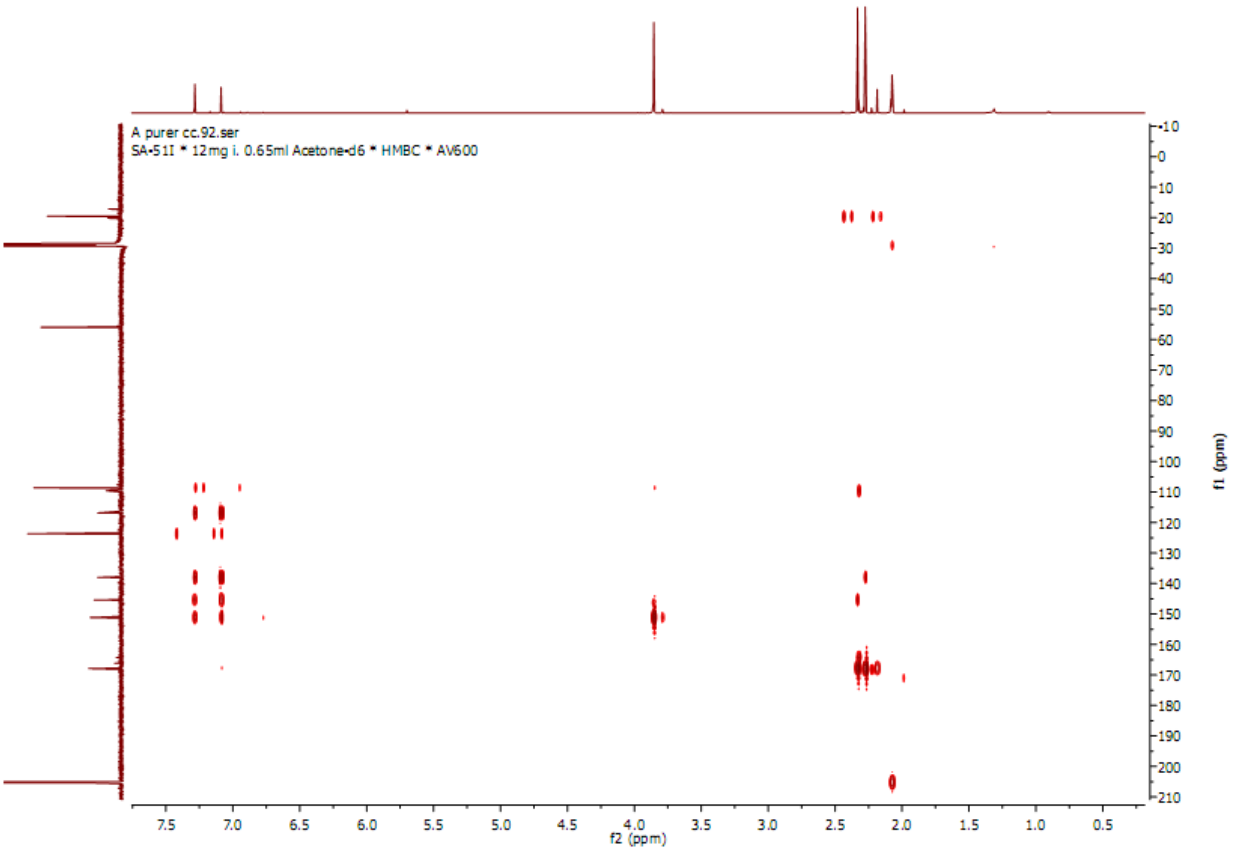

$^{1}$H NMR spectra of 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)




$^{13}$C NMR spectra of 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)

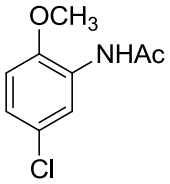


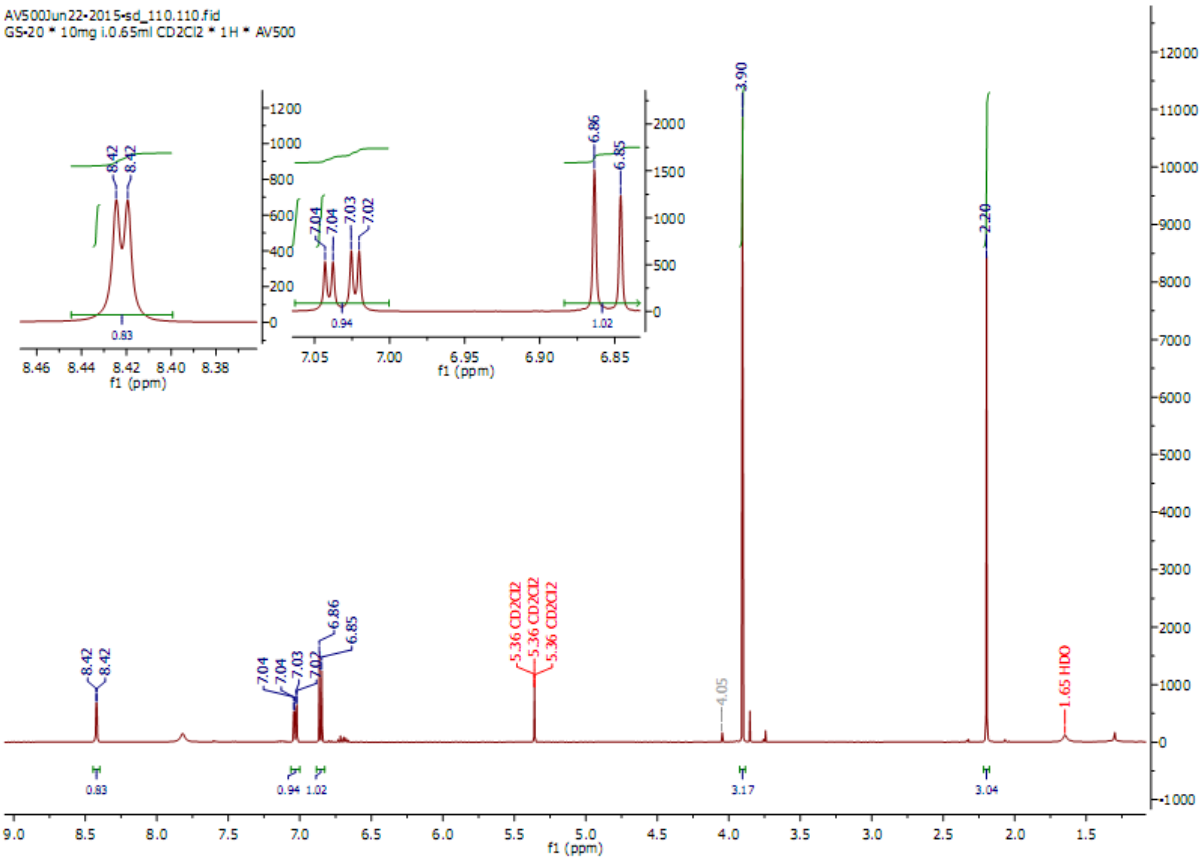

HSQC spectra of 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)



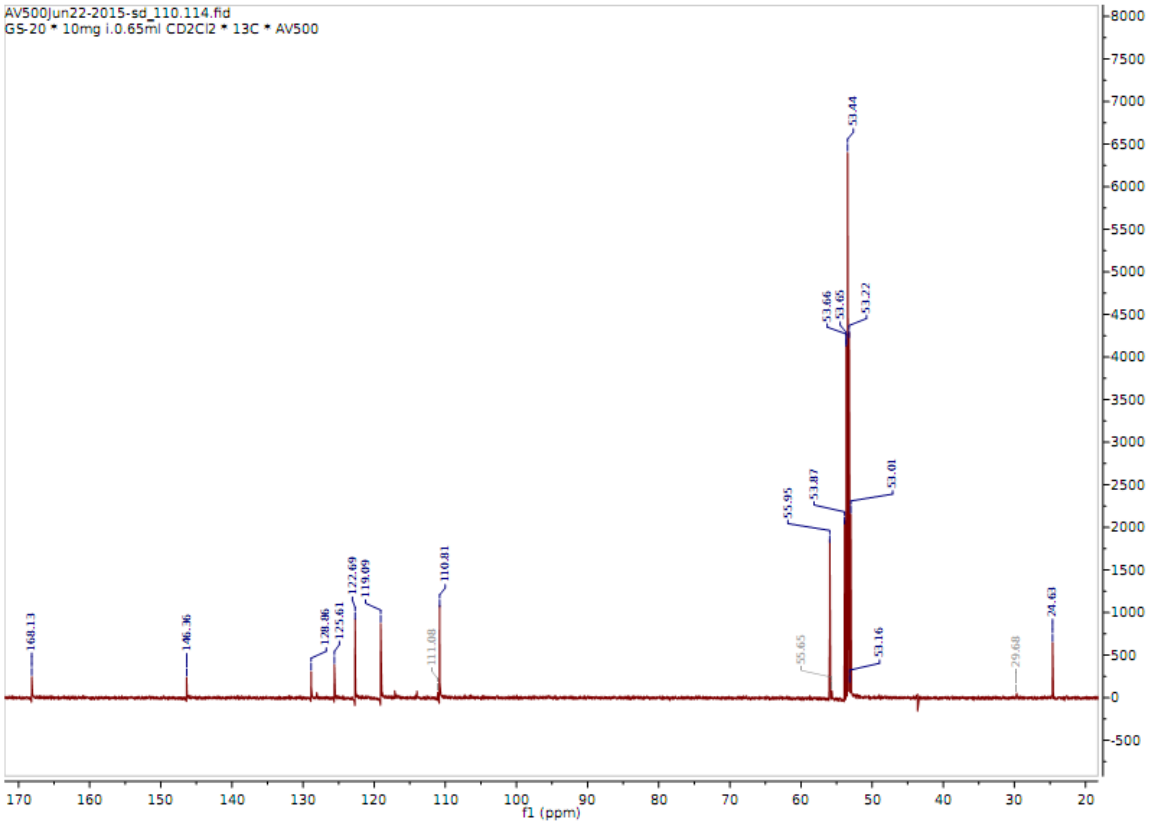

HMBC spectra of 2-chloro-5-methoxy-1,4-diacetate-1,4-benzenediol (**60**)

Appendix 7: The spectra of 5-chloro-2-methoxyacetanilide (**61**)

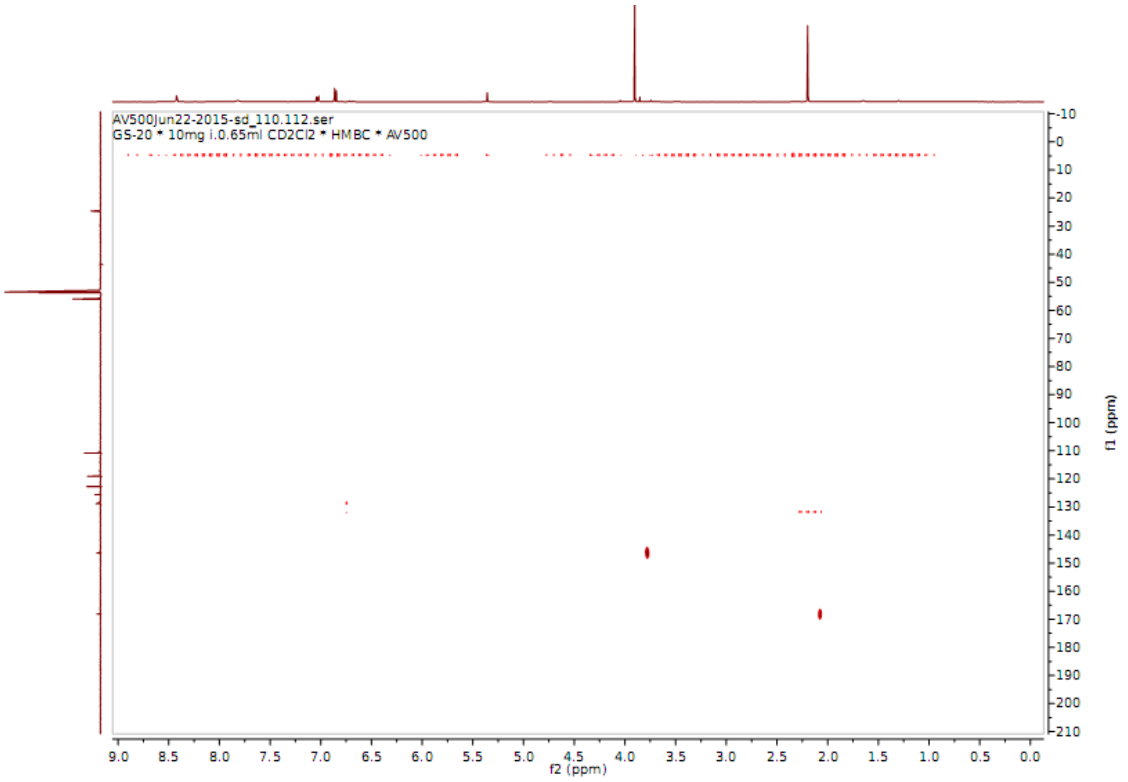


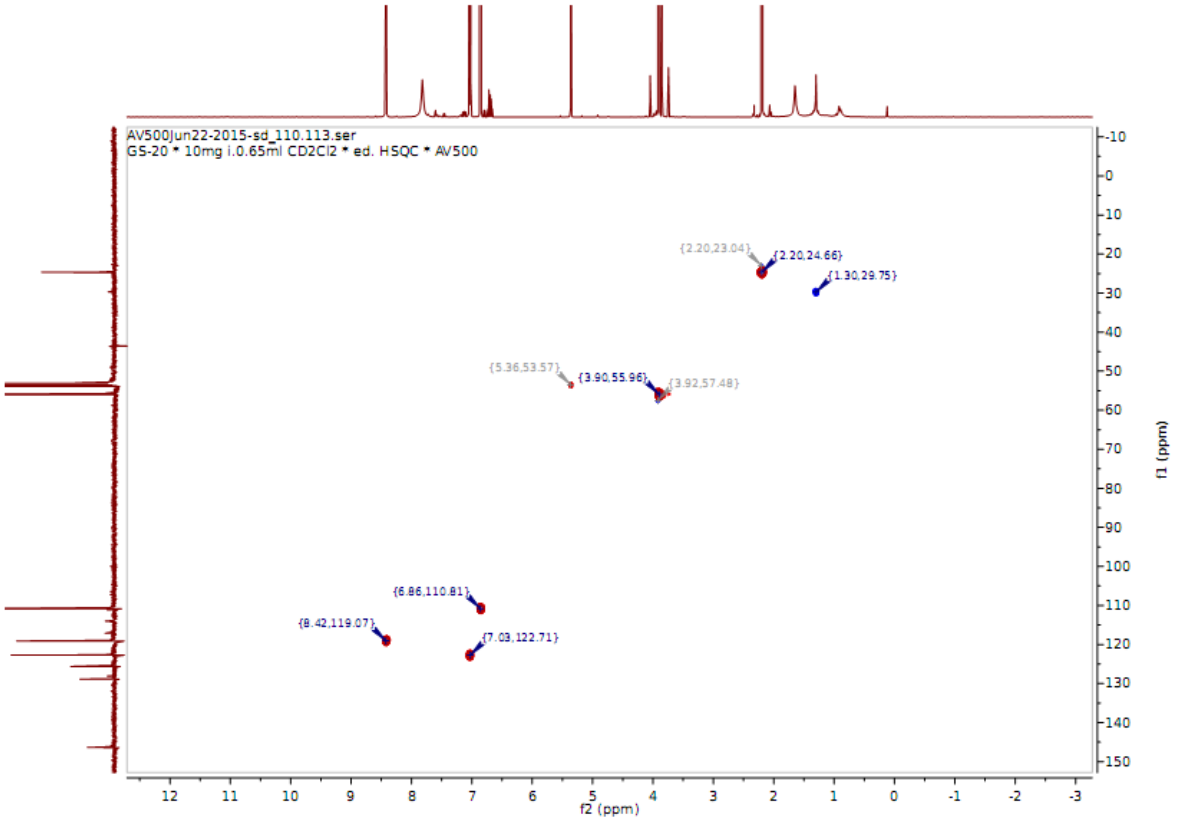

$^{1}$H NMR spectra of 5-chloro-2-methoxyacetanilide (**61**)




$^{13}$C NMR spectra of 5-chloro-2-methoxyacetanilide (**61**)

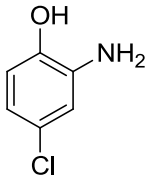


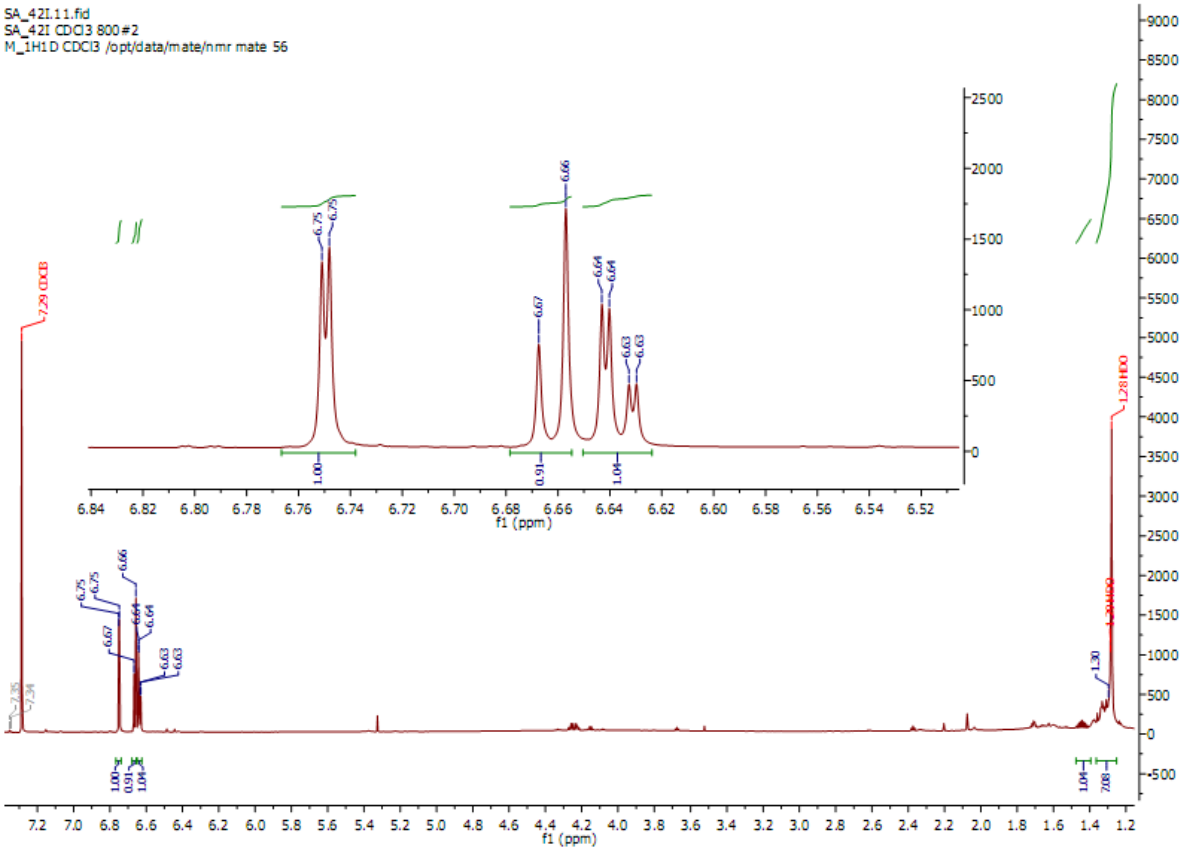

HMBC spectra of 5-chloro-2-methoxyacetanilide (**61**)



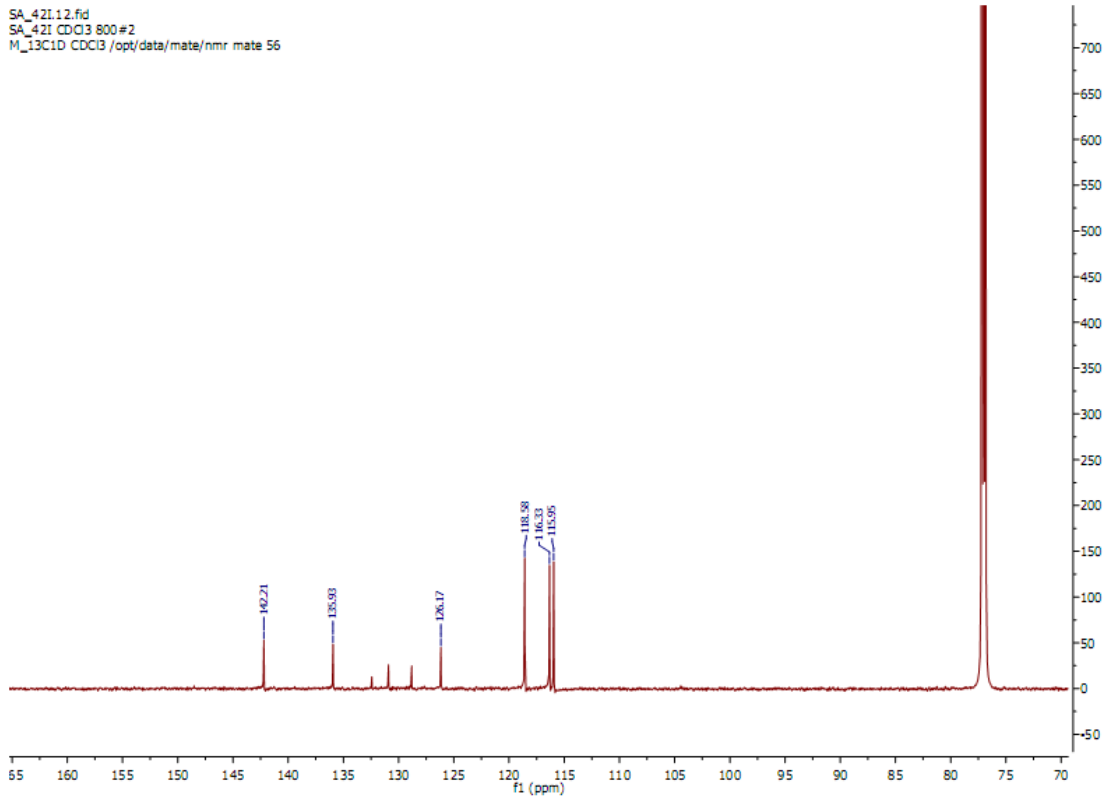

HSQC spectra of 5-chloro-2-methoxyacetanilide (**61**)

Appendix 8: The spectra of 5-chloro-2-hydroxyaniline (**62**)

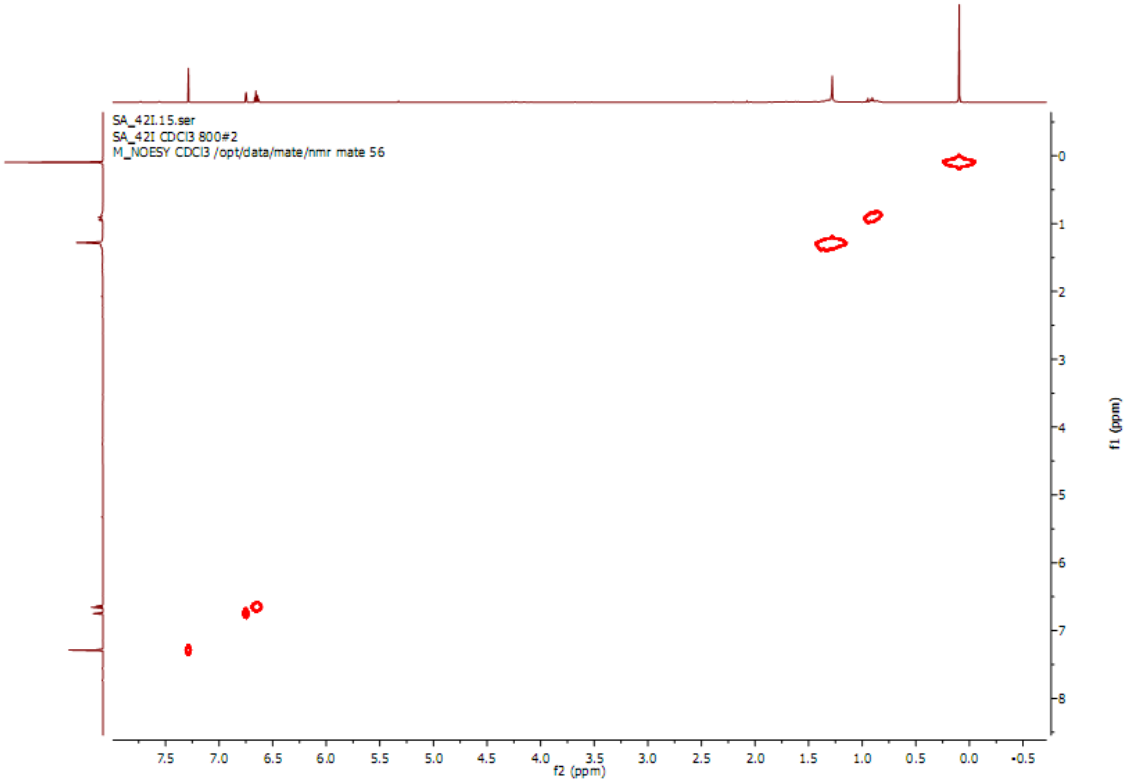


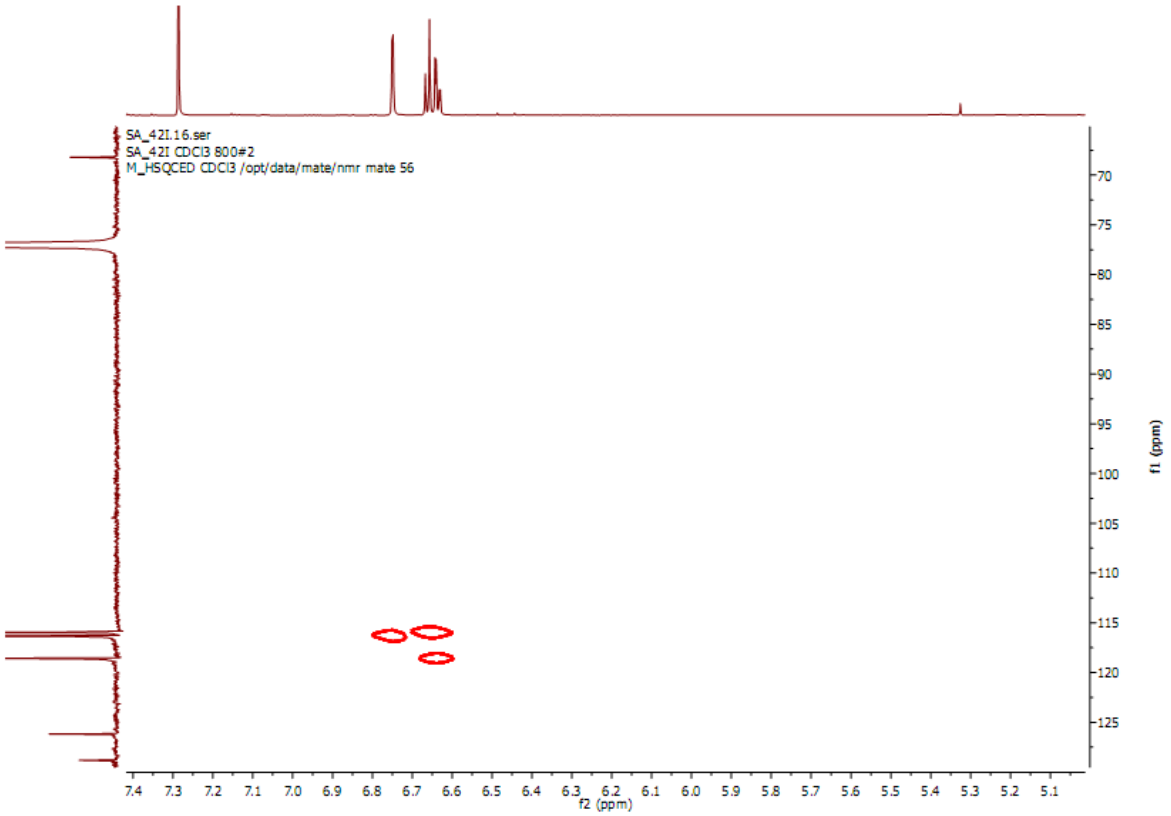

$^{1}$H NMR spectra of 5-chloro-2-hydroxyaniline (**62**)



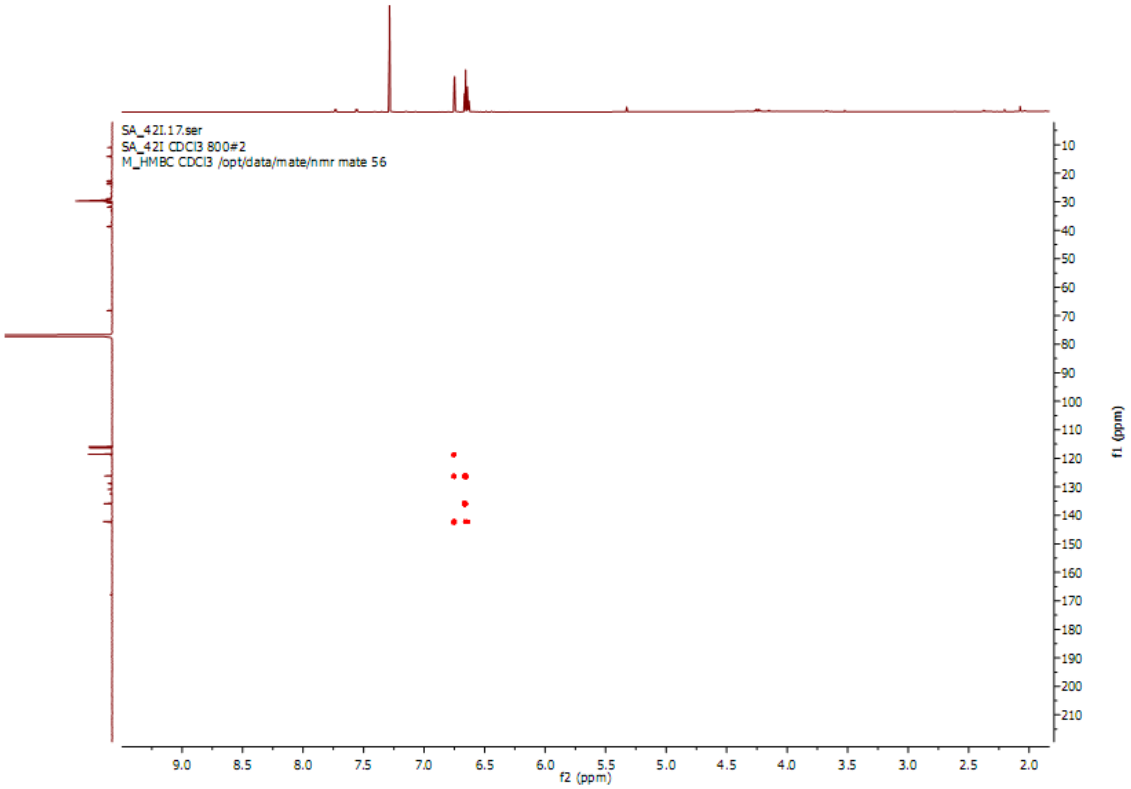

$^{13}$C NMR spectra of 5-chloro-2-hydroxyaniline (**62**)




NOESY spectra of 5-chloro-2-hydroxyaniline (**62**)

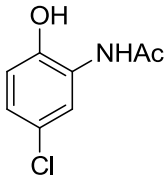


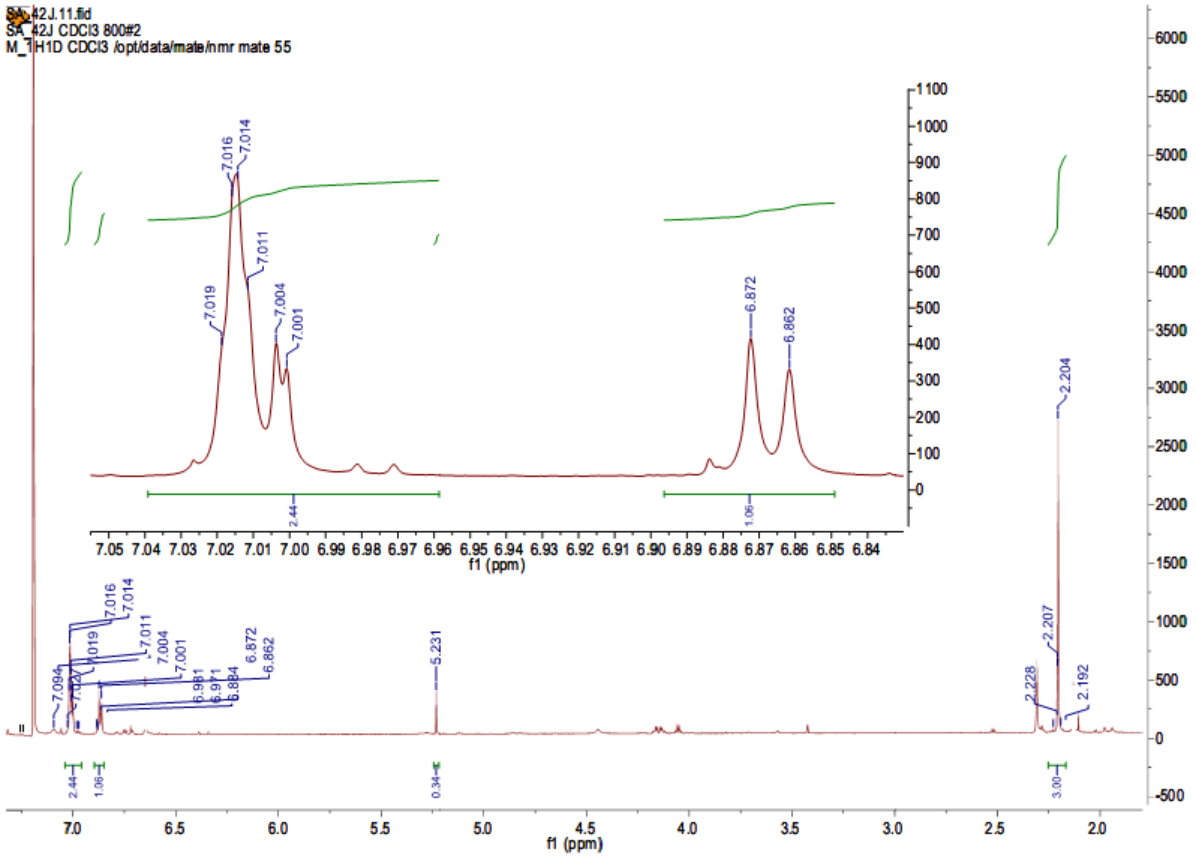

HSQC spectra of 5-chloro-2-hydroxyaniline (**62**)



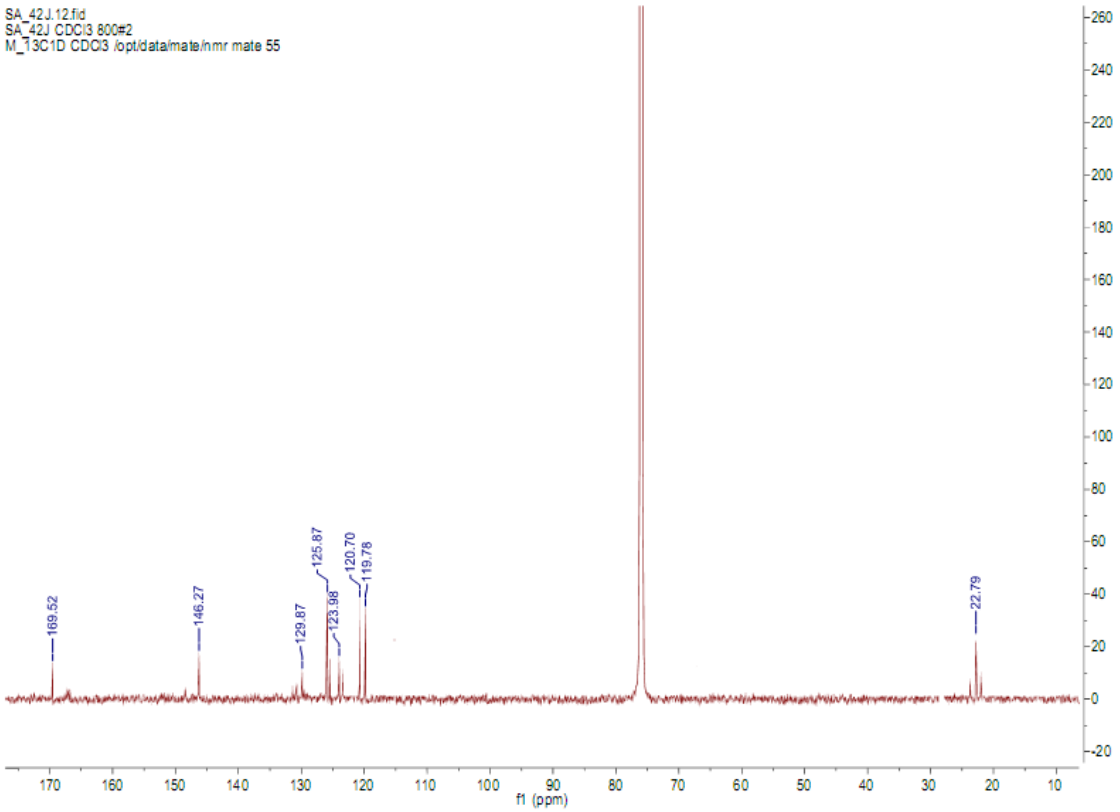

HMBC spectra of 5-chloro-2-hydroxyaniline (**62**)

Appendix 9: The spectra of 5-chloro-2-hydroxyacetanilide (**63**)

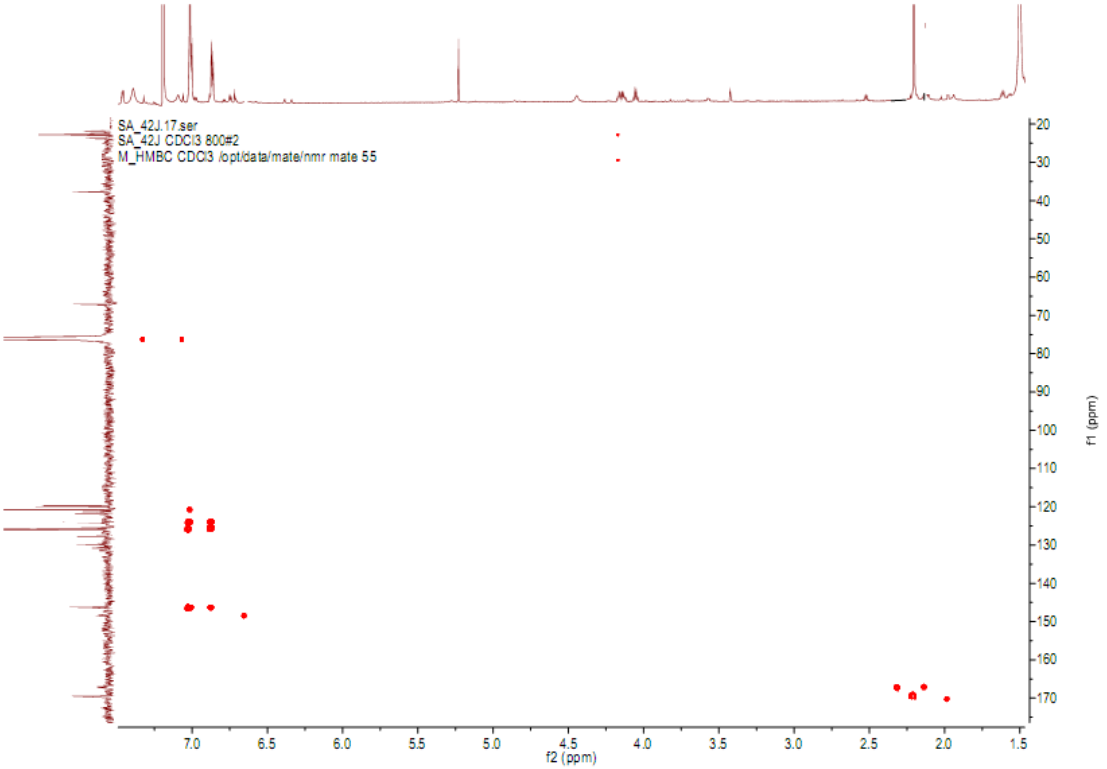


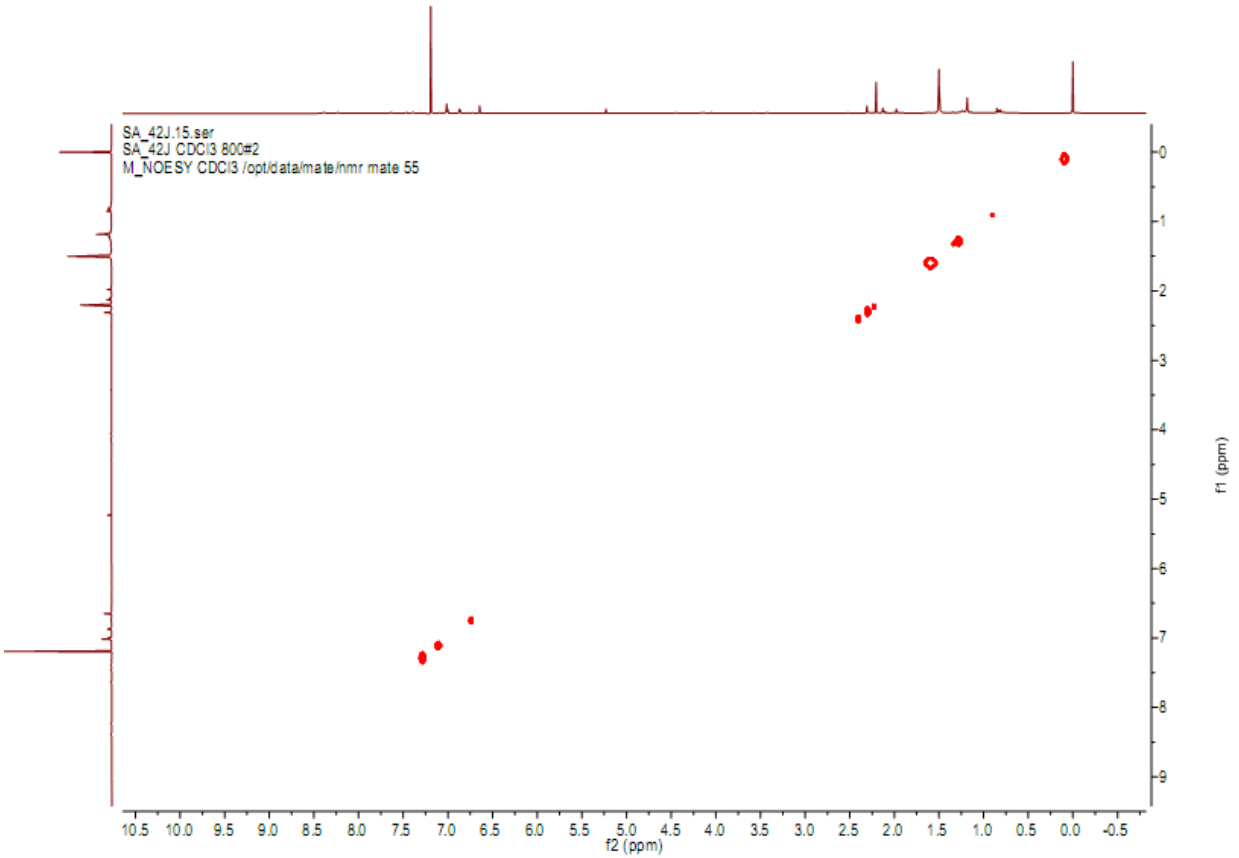

$^{1}$H NMR spectra of 5-chloro-2-hydroxyacetanilide (**63**)




$^{13}$C NMR spectra of 5-chloro-2-hydroxyacetanilide (**63**)

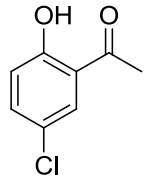


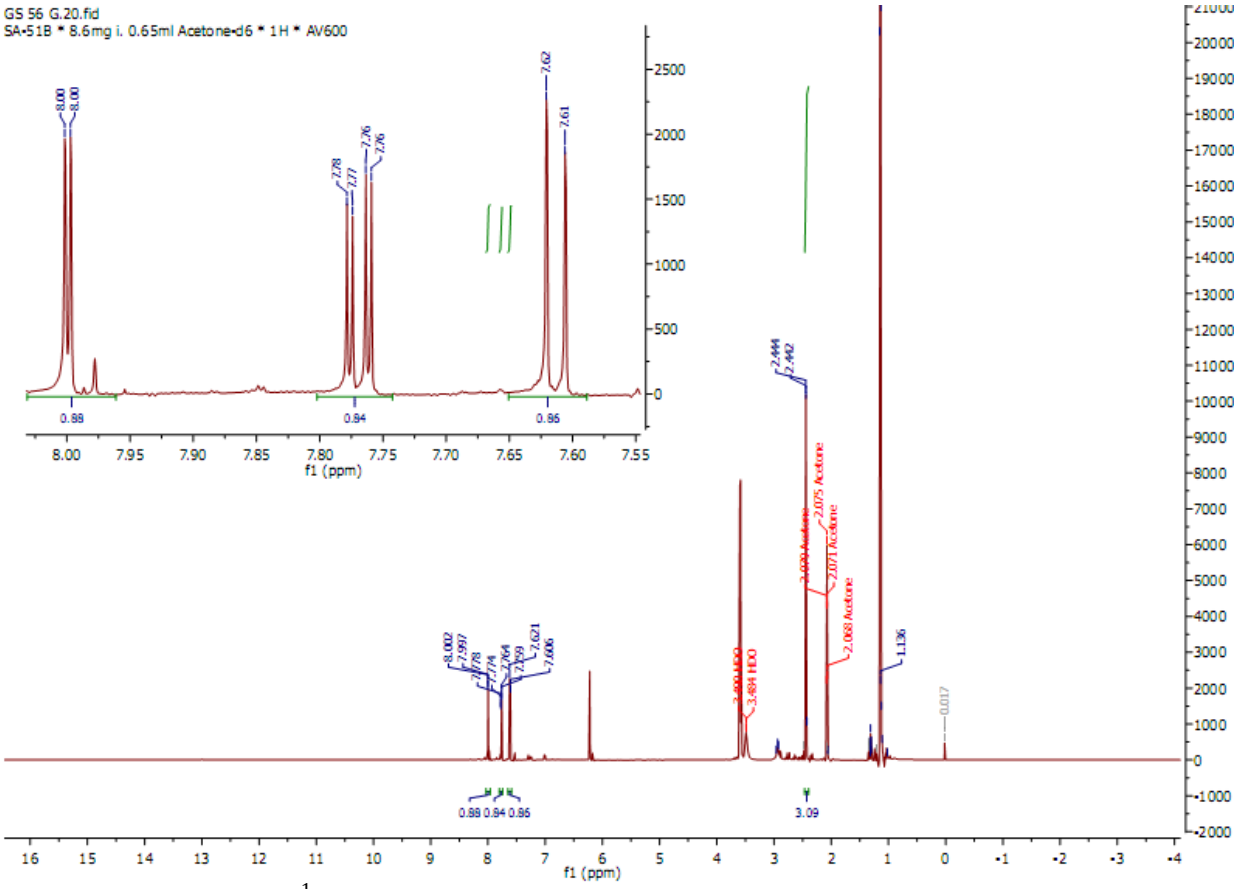

HMBC spectra of 5-chloro-2-hydroxyacetanilide (**63**)



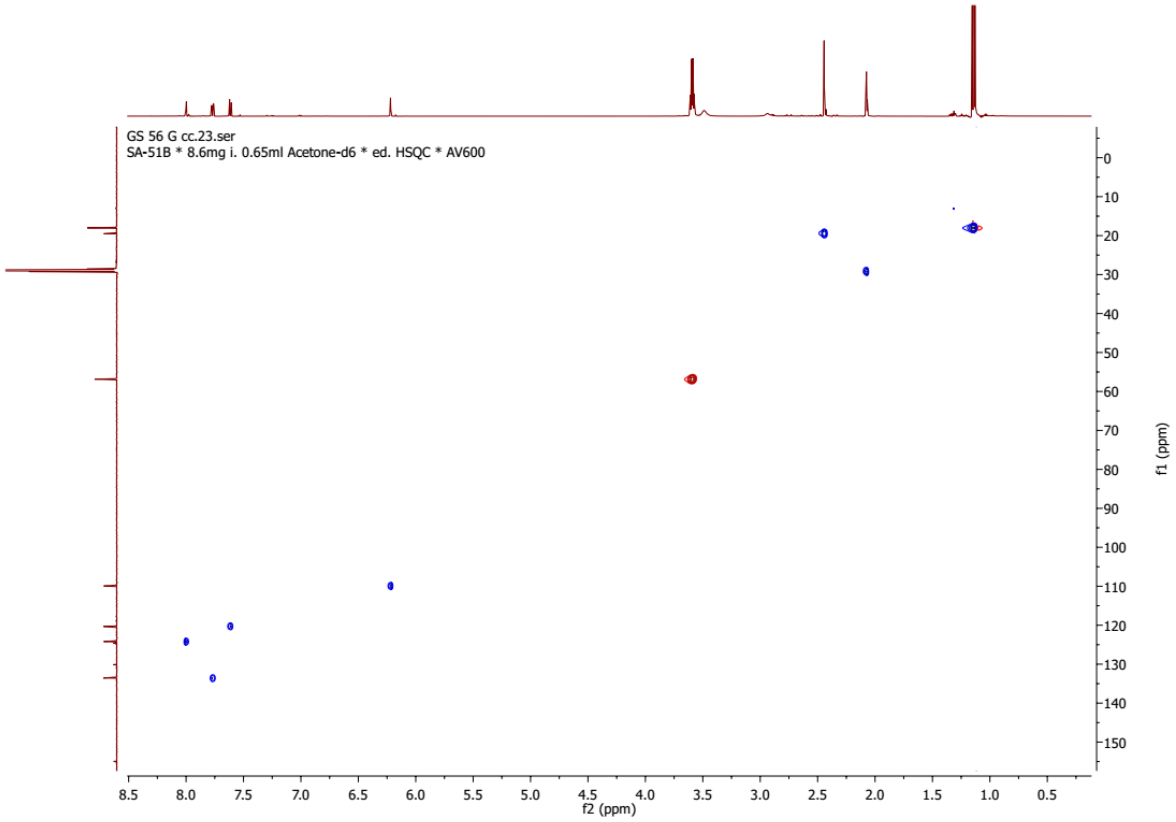

NOESY spectra of 5-chloro-2-hydroxyacetanilide (**63**)

Appendix 10: The spectra of 2-acetyl-4-chlorophenol (**65**)

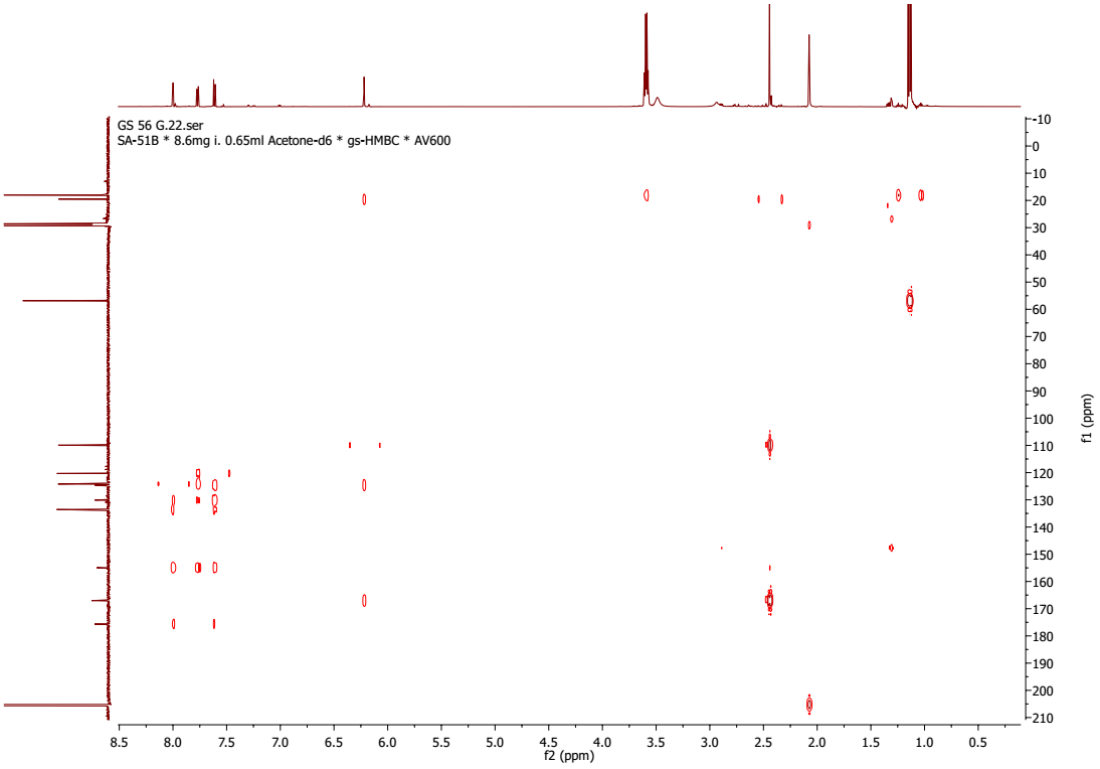


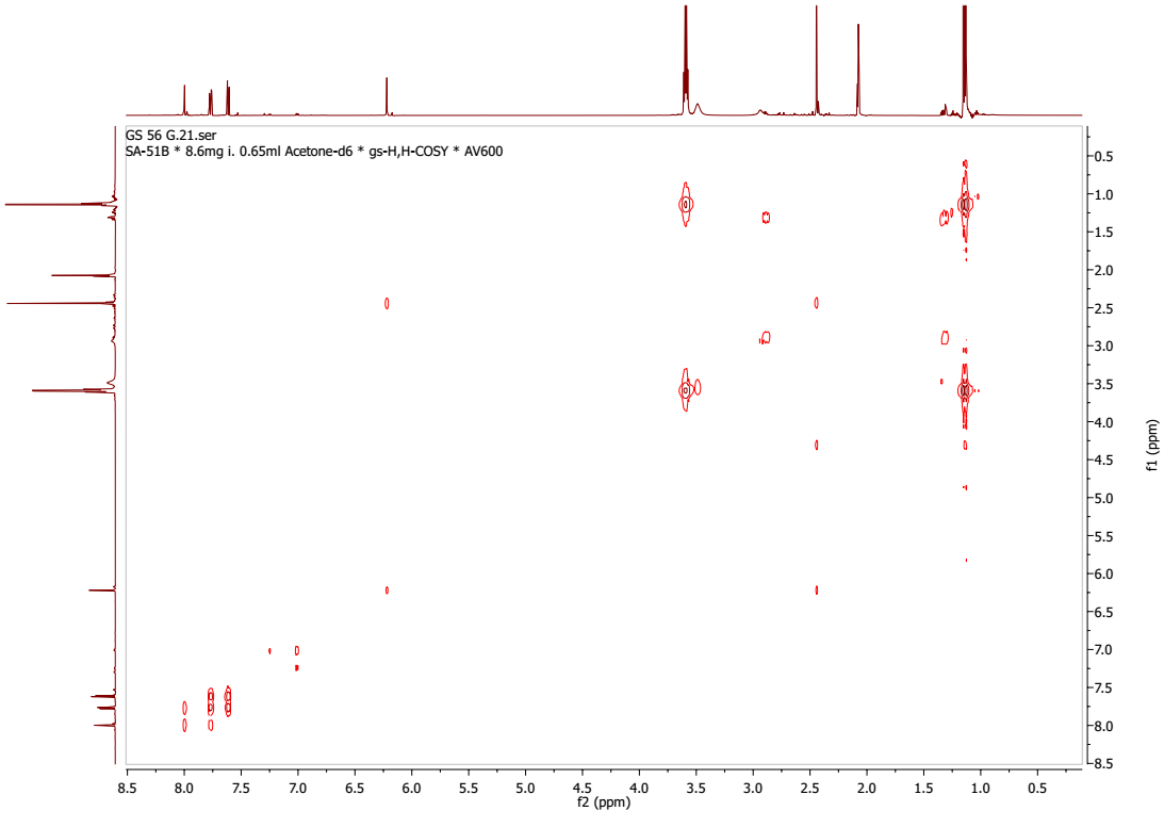

$^{1}$H NMR spectra of 2-acetyl-4-chlorophenol (**65**)



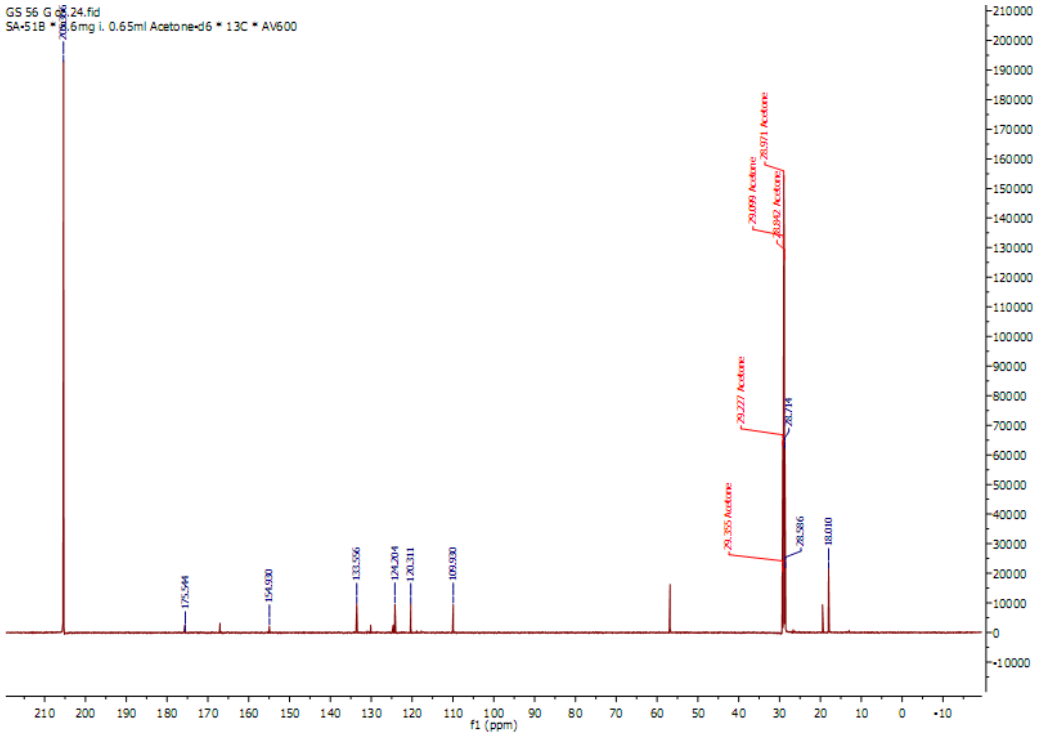

HSQC spectra of 2-acetyl-4-chlorophenol (**65**)




HMBC spectra of 2-acetyl-4-chlorophenol (**65**)

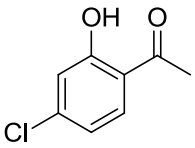


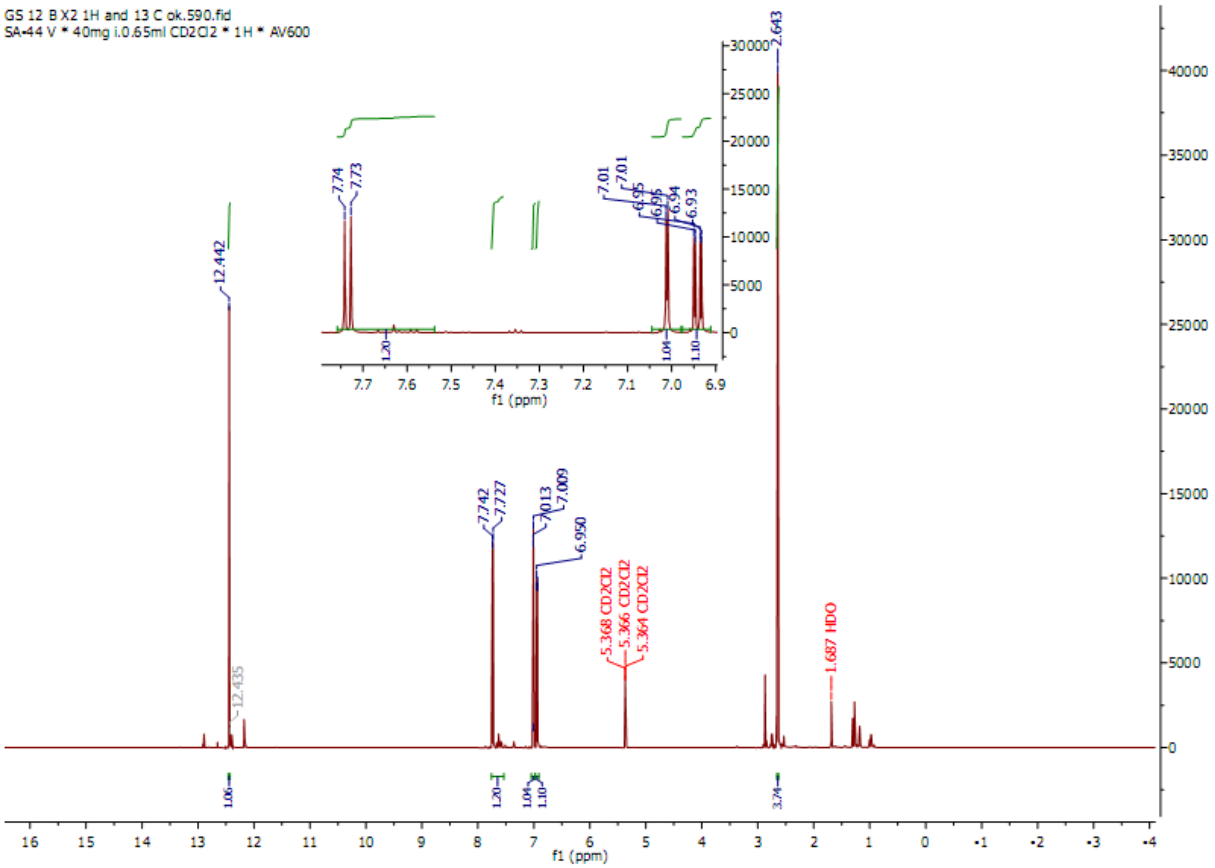

H, H - COSY spectra of 2-acetyl-4-chlorophenol (**65**)



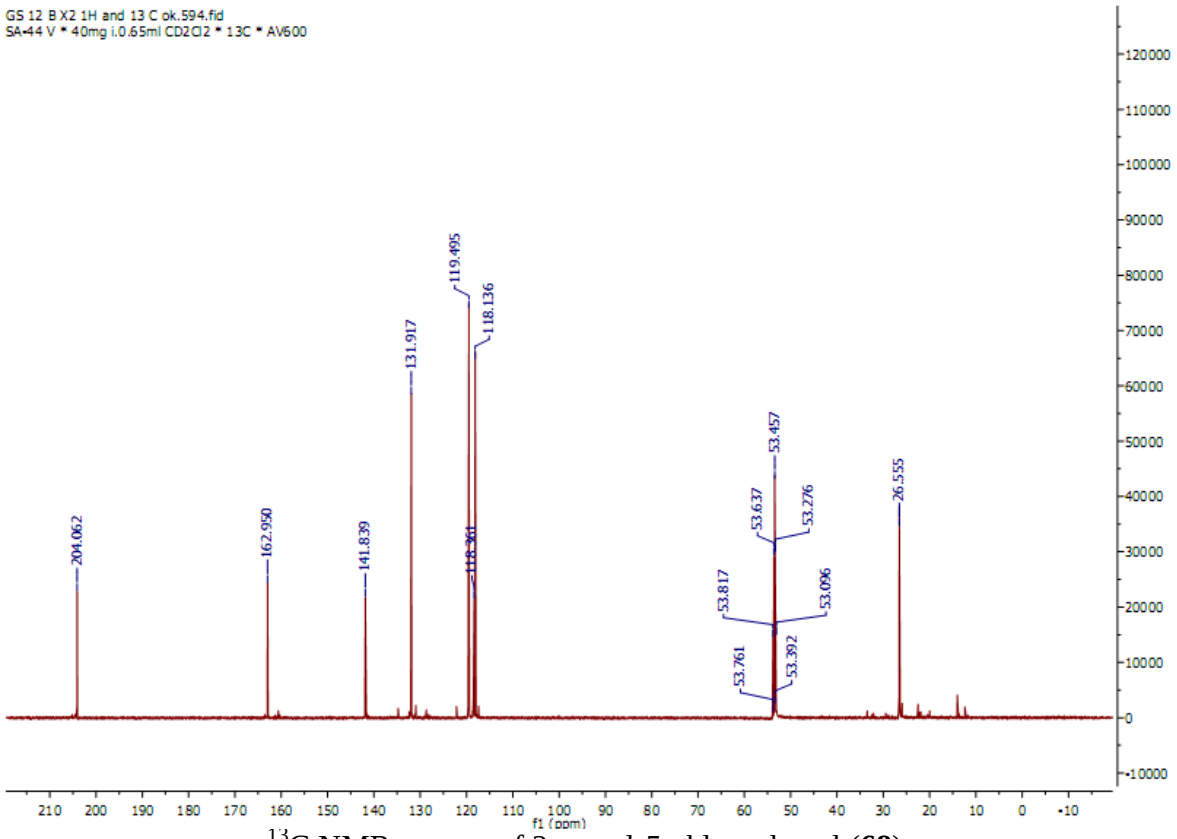

$^{13}$C NMR spectra of 2-acetyl-4-chlorophenol (**65**)

Appendix 11: The spectra of 2-acetyl-5-chlorophenol (**68**)

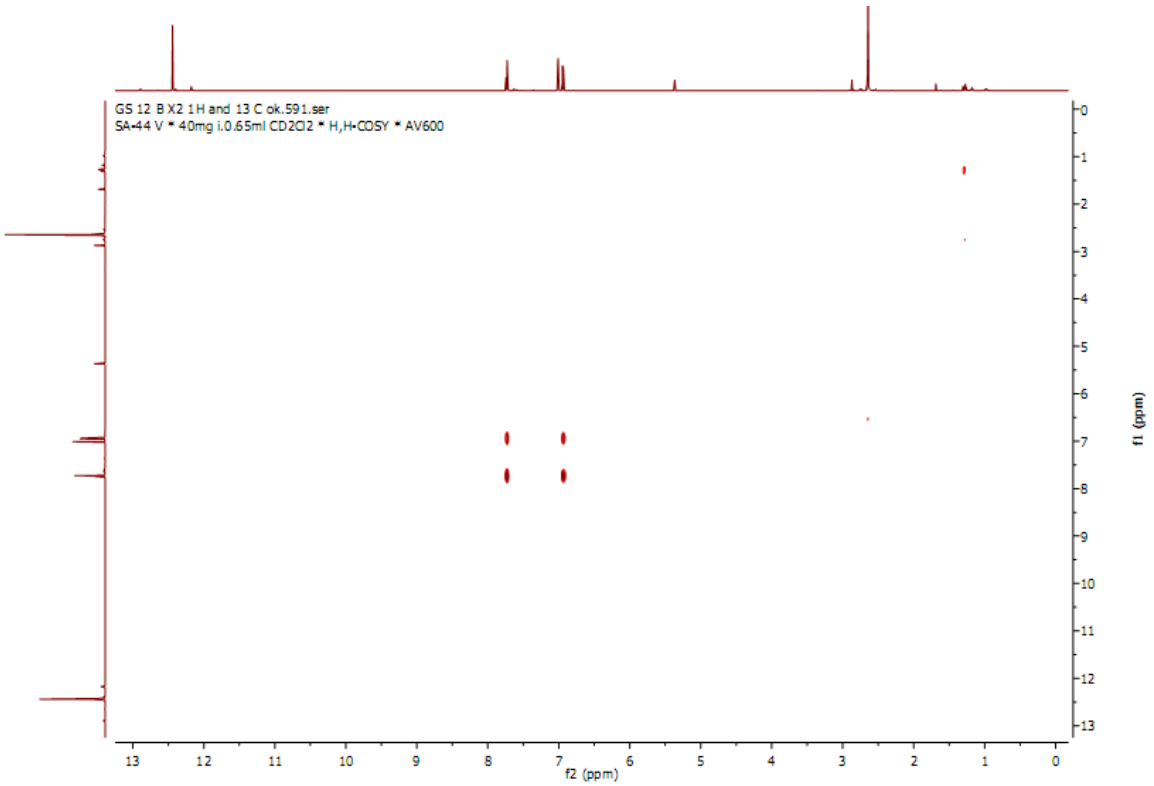


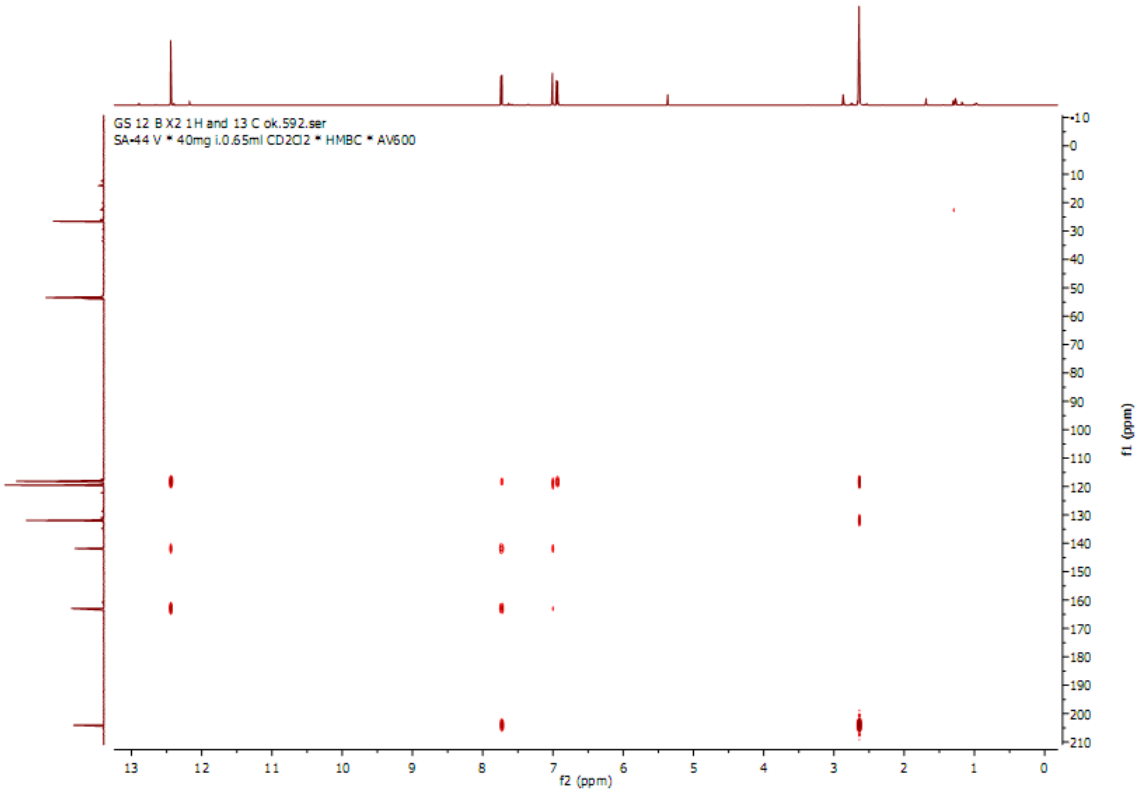

$^{1}$H NMR spectra of 2-acetyl-5-chlorophenol (**68**)



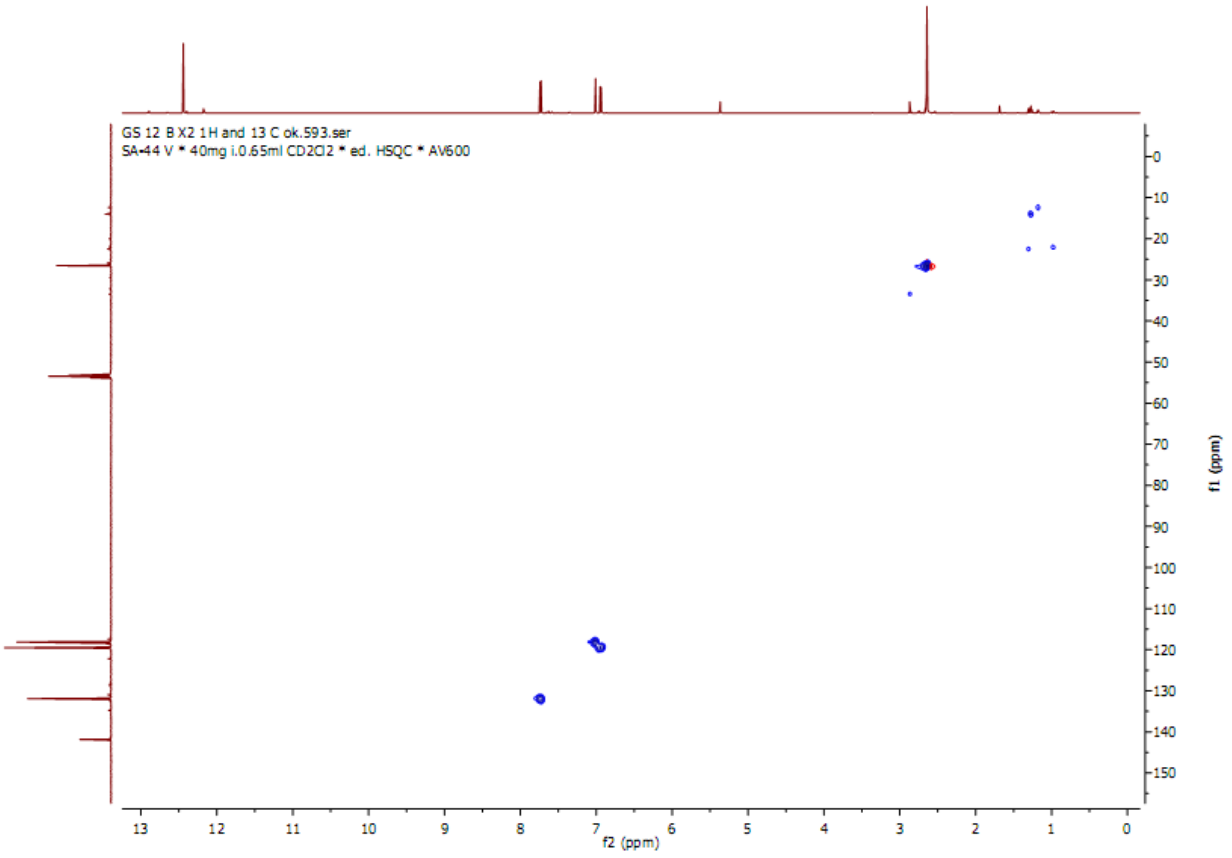

$^{13}$C NMR spectra of 2-acetyl-5-chlorophenol (**68**)




H,H-COSY spectra of 2-acetyl-5-chlorophenol (**68**)

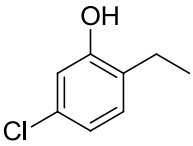


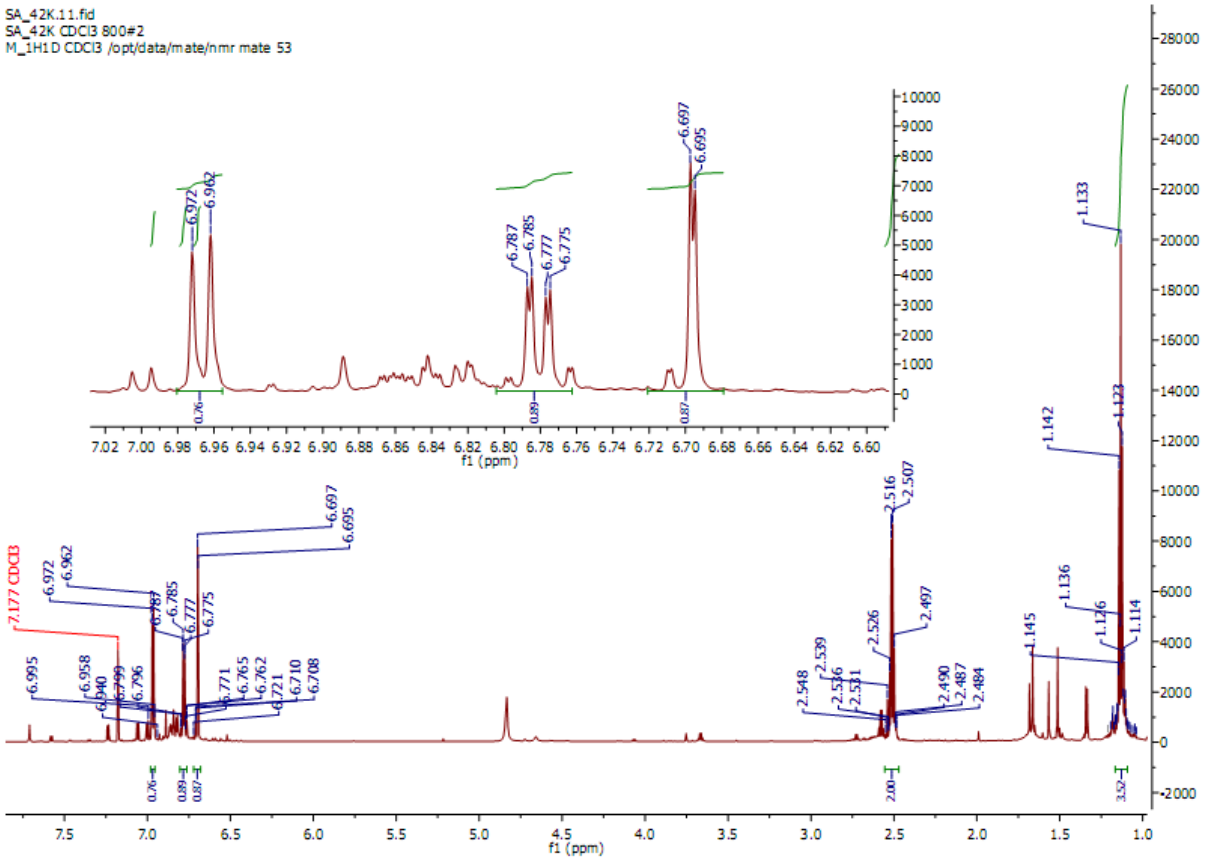

HMBC spectra of 2-acetyl-5-chlorophenol (**68**)



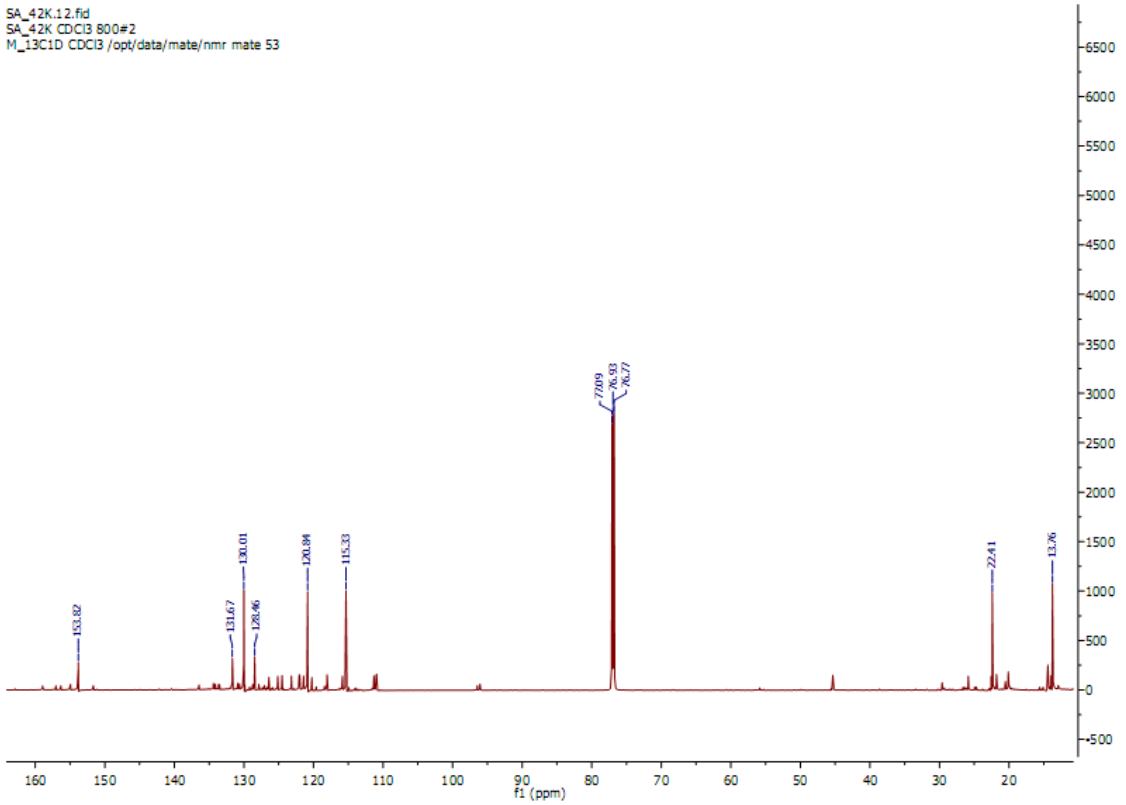

HSQC spectra of 2-acetyl-5-chlorophenol (**68**)

Appendix 12: The spectra of 5-chloro-2-ethylphenol (**69**)

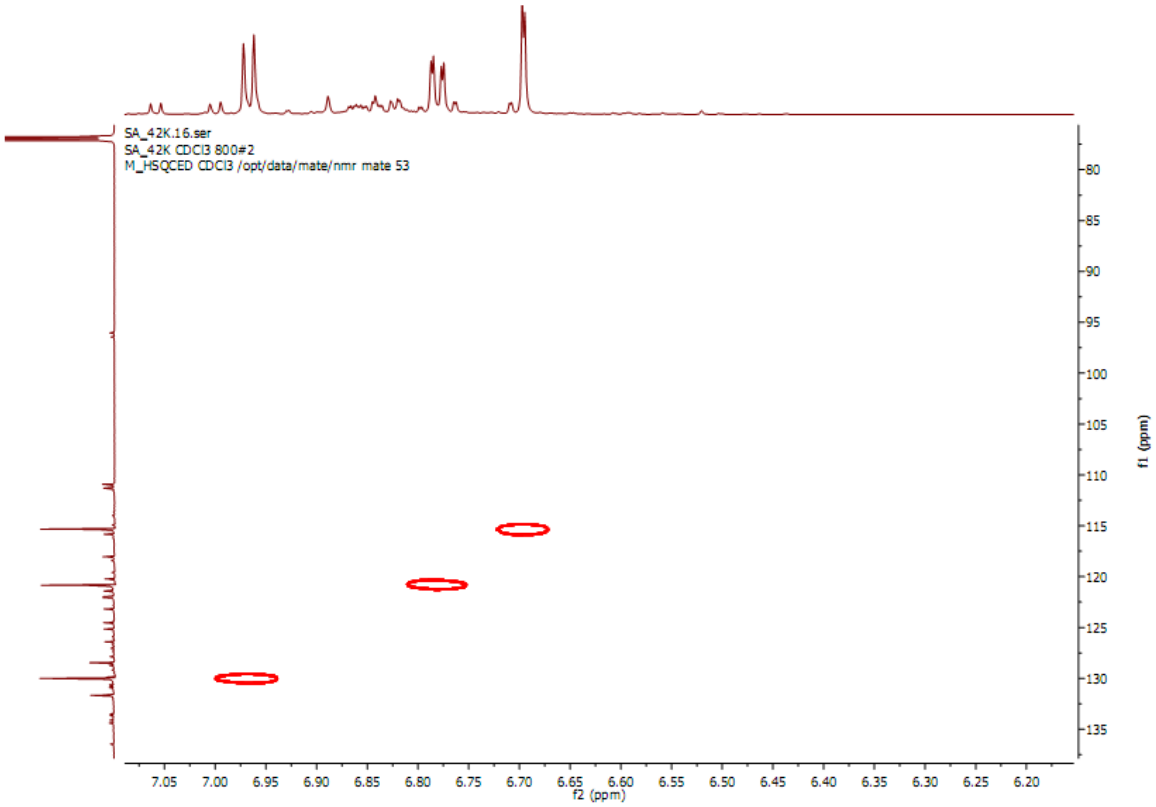


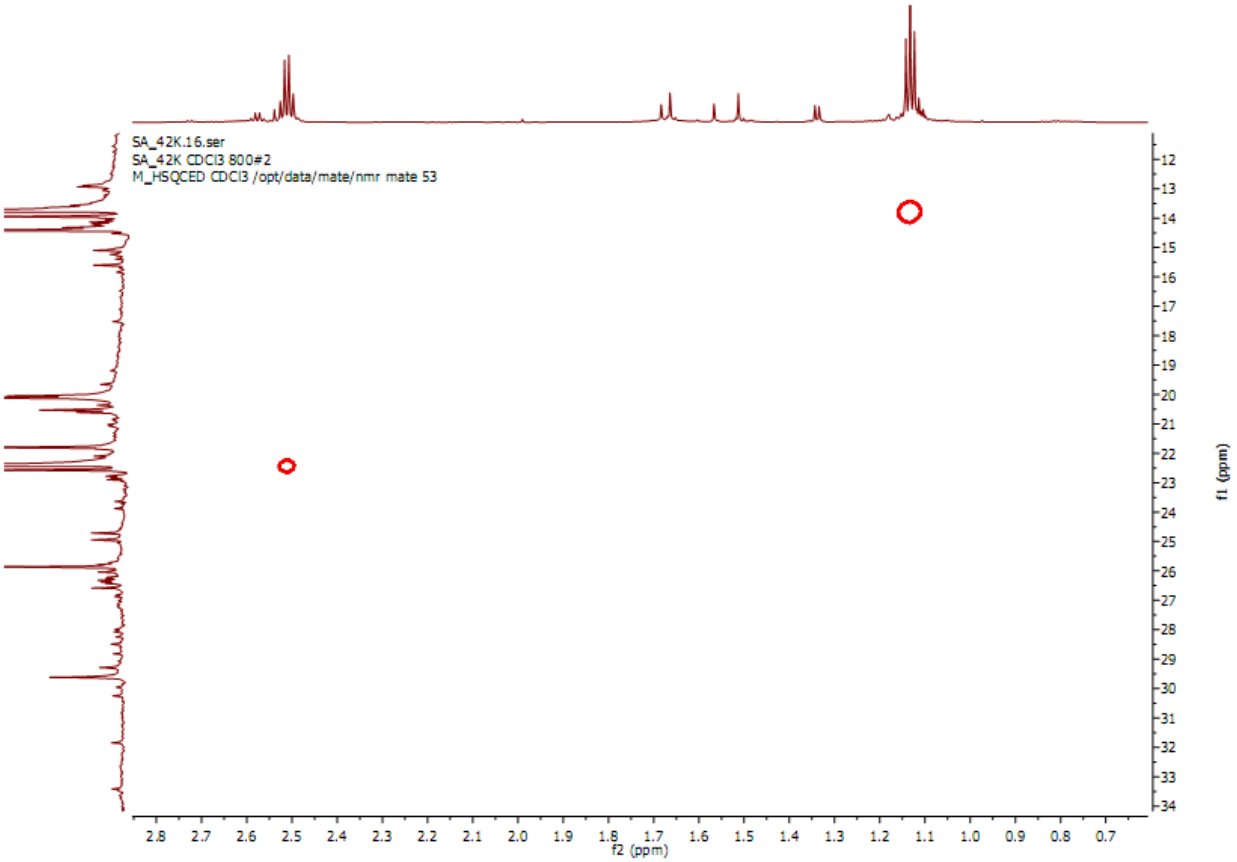

$^{1}$H NMR spectra of 5-chloro-2-ethylphenol (**69**)



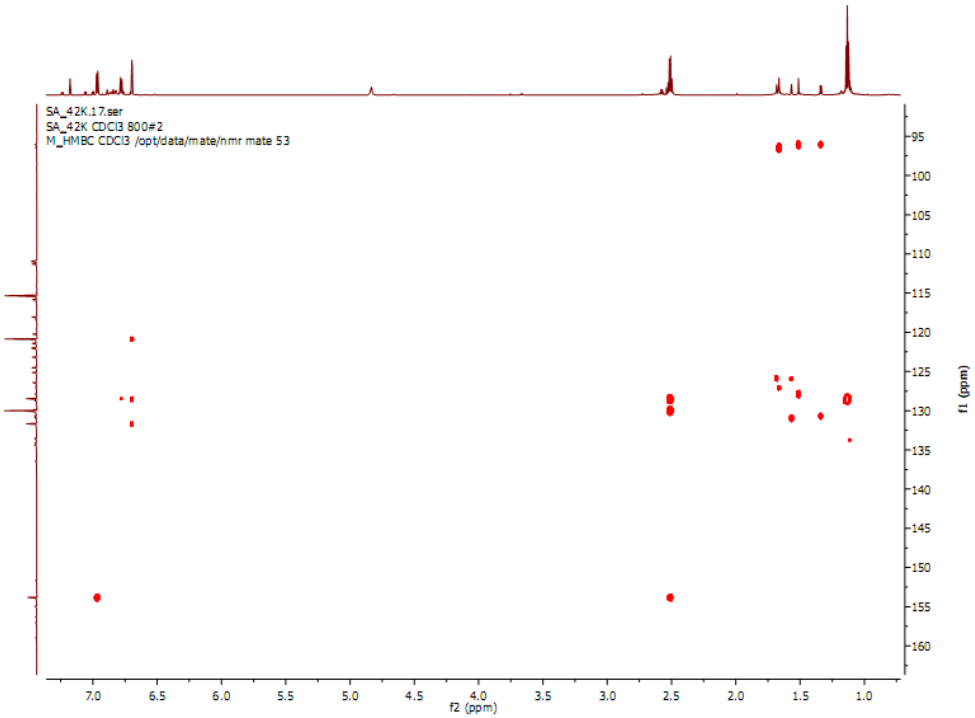

$^{13}$C NMR spectra of 5-chloro-2-ethylphenol (**69**)



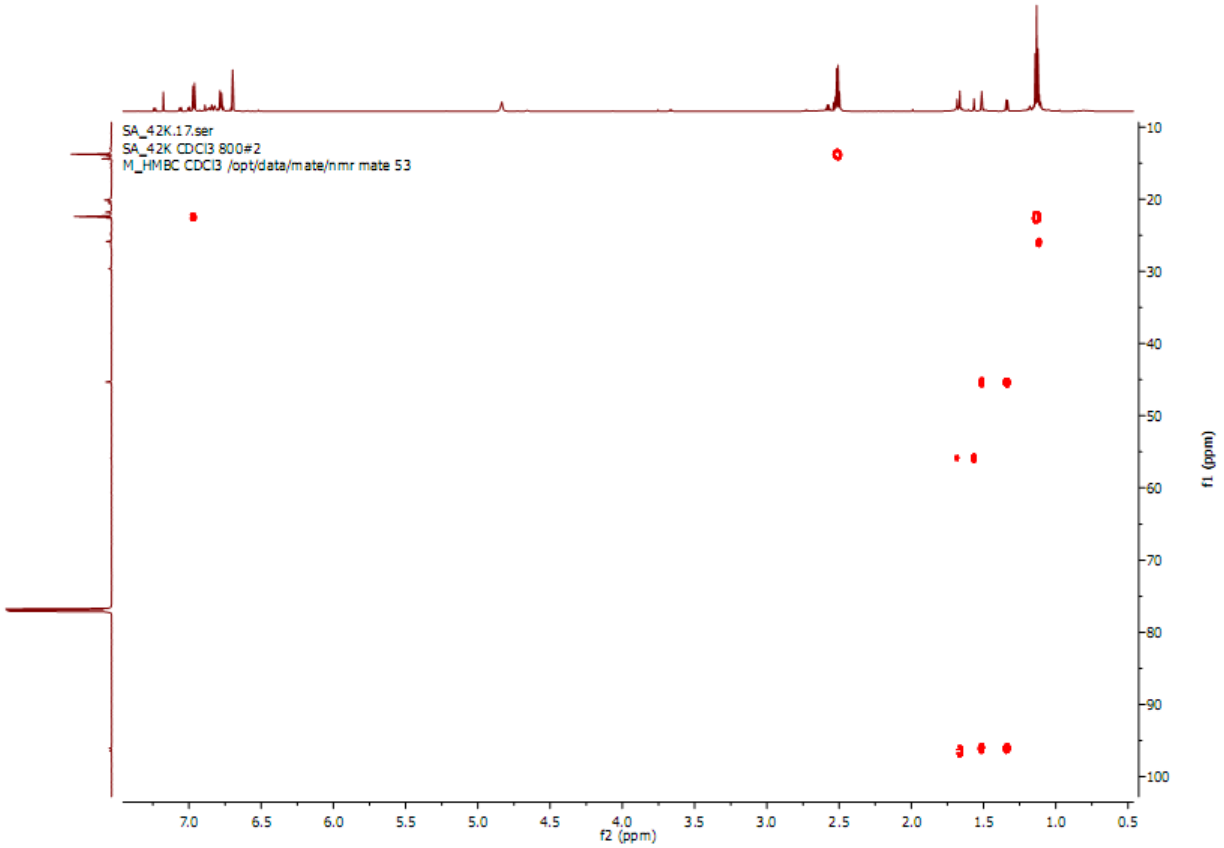

HSQC spectra of 5-chloro-2-ethylphenol (aromatic region) (**69**)




HSQC spectra of 5-chloro-2-ethylphenol (aliphatic region) (**69**)

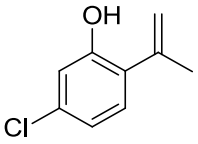


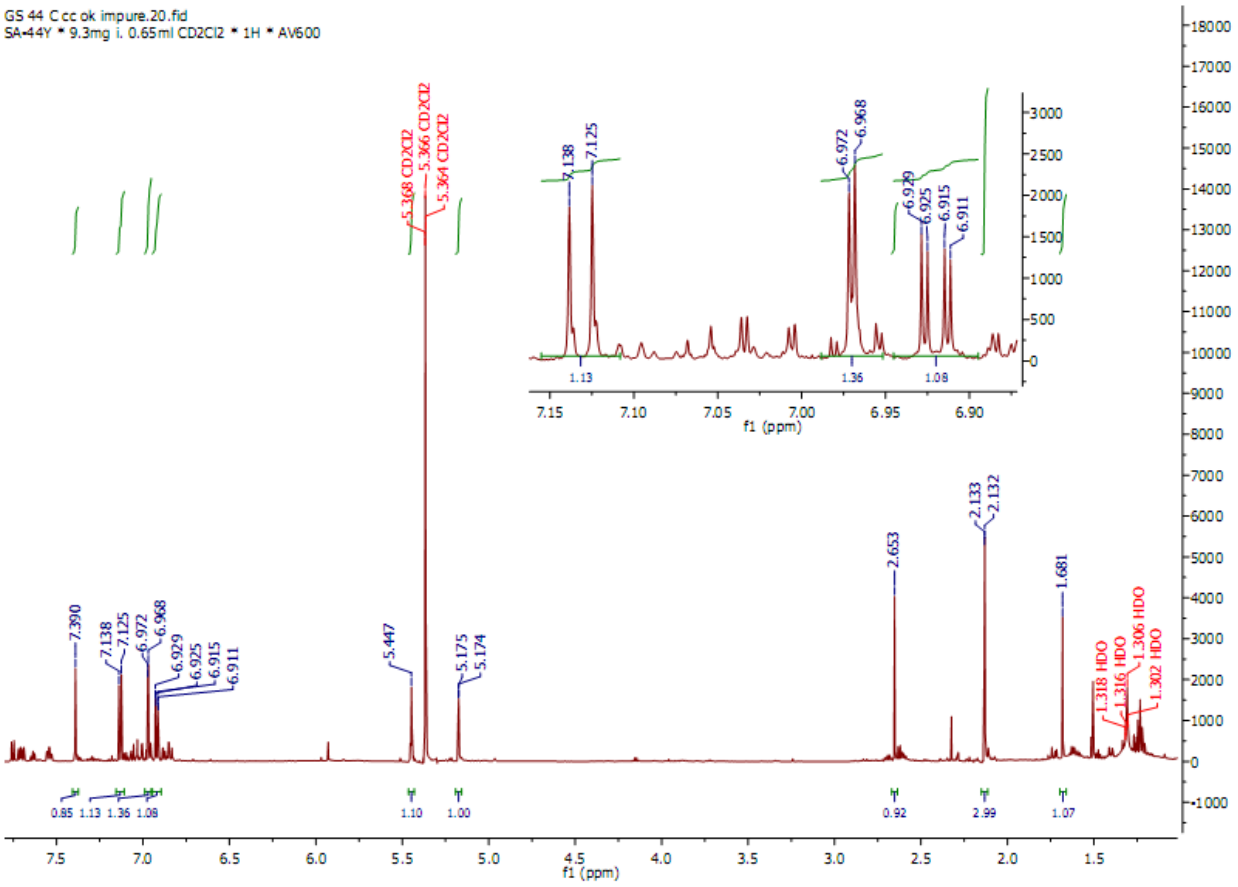

HMBC spectra 1 of 5-chloro-2-ethylphenol (**69**)



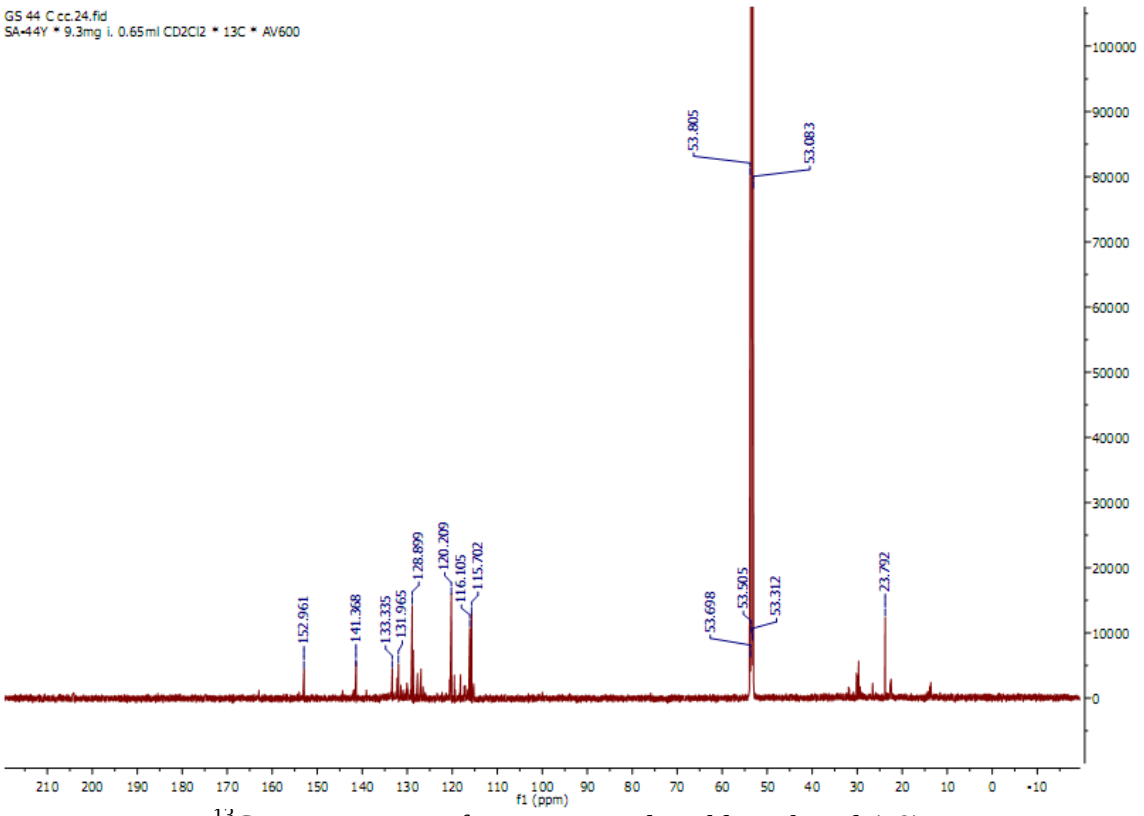

HMBC spectra 2 of 5-chloro-2-ethylphenol (**69**)

Appendix 13: The spectra of 2-isopropenyl-5-chlorophenol (**70**)

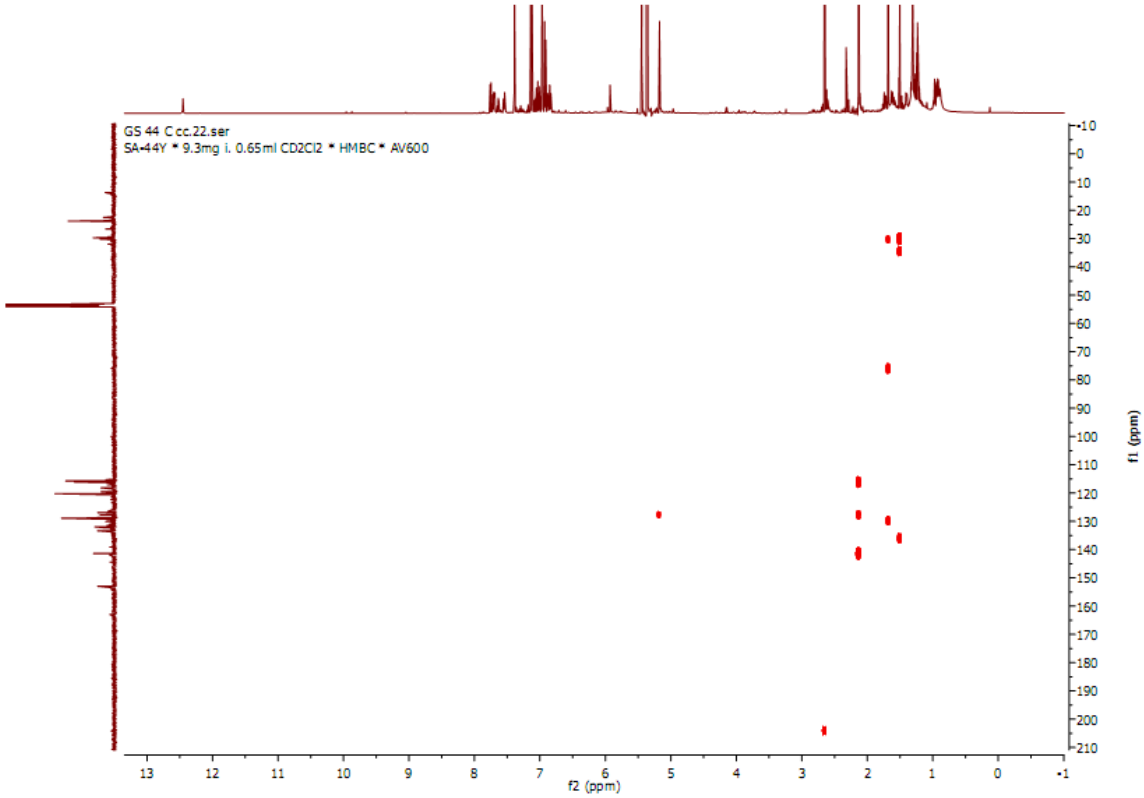


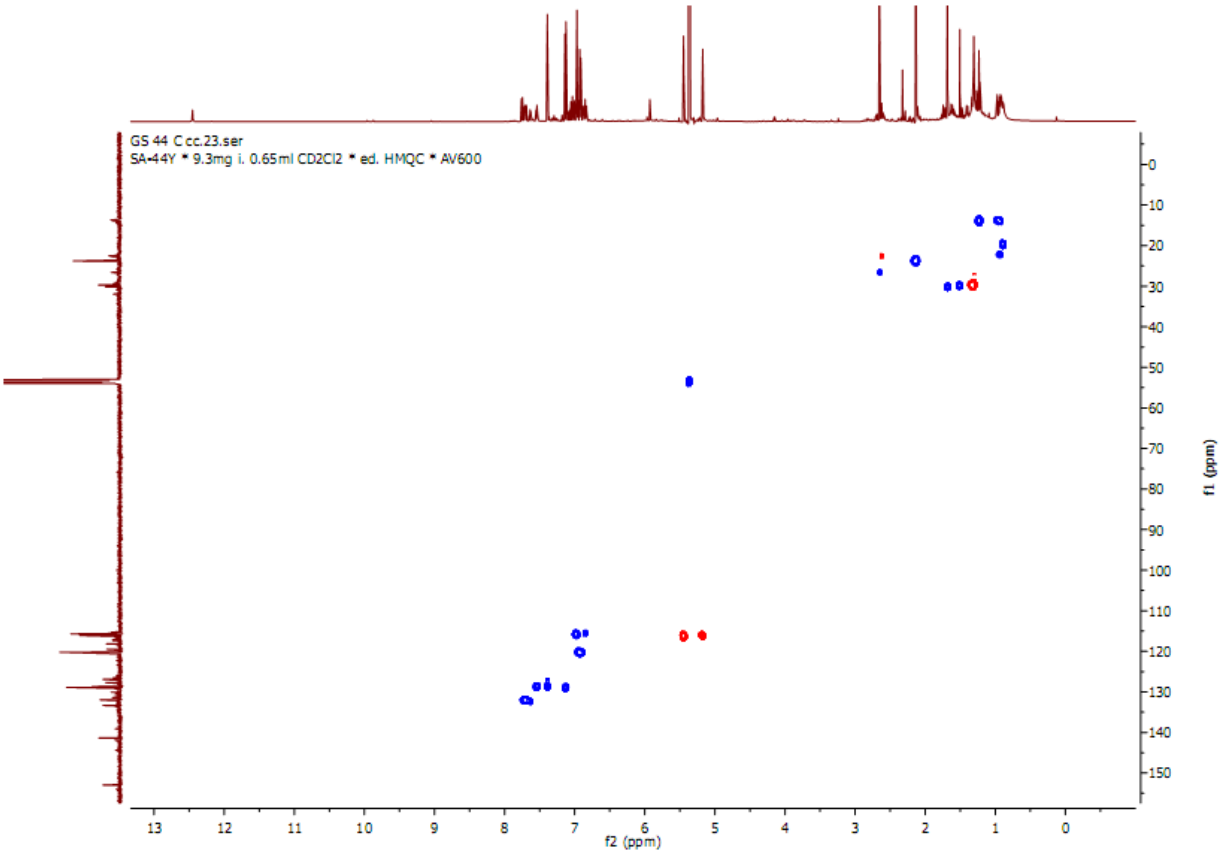

$^{1}$H NMR spectra of 2-isopropenyl-5-chlorophenol (**70**)




$^{13}$C NMR spectra of 2-isopropenyl-5-chlorophenol (**70**)

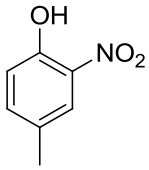


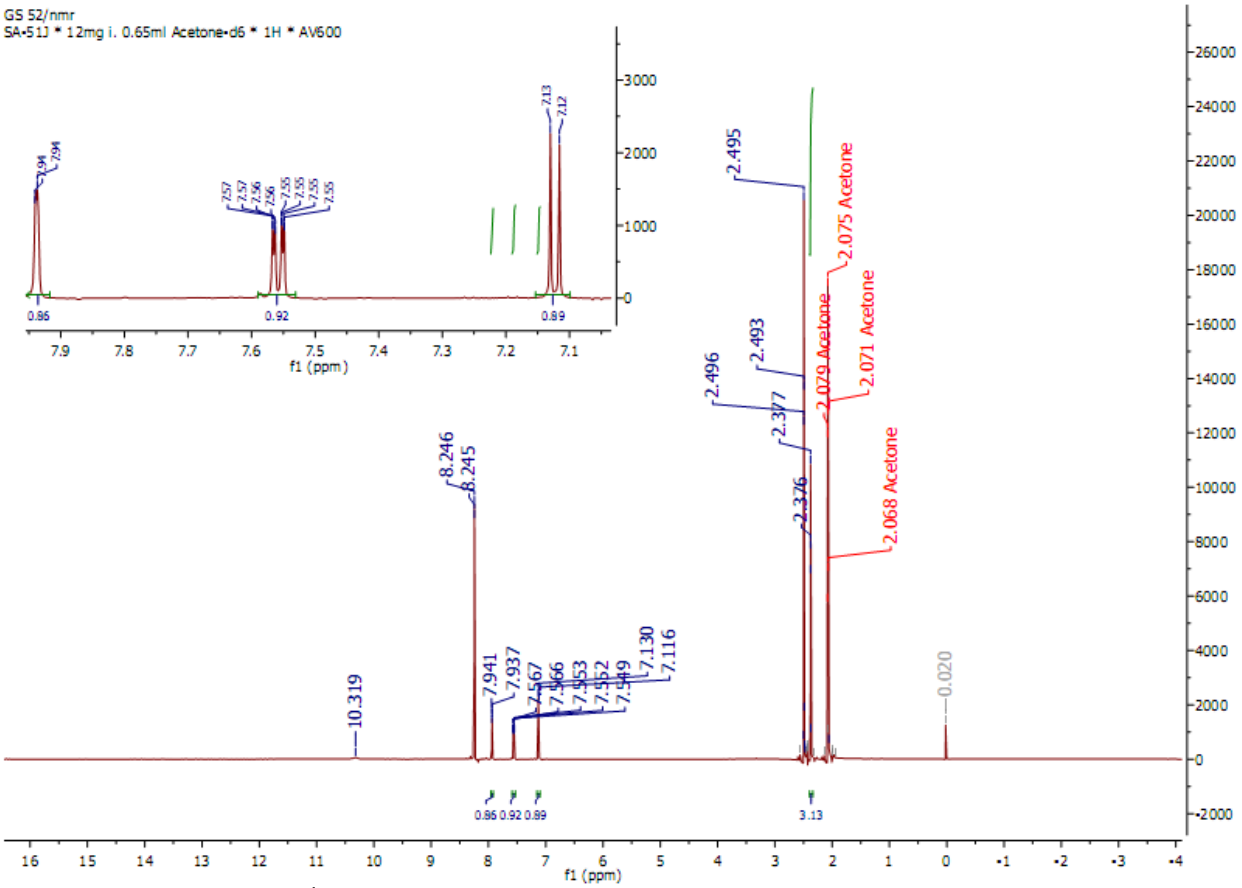

HMBC spectra of 2-isopropenyl-5-chlorophenol (**70**)



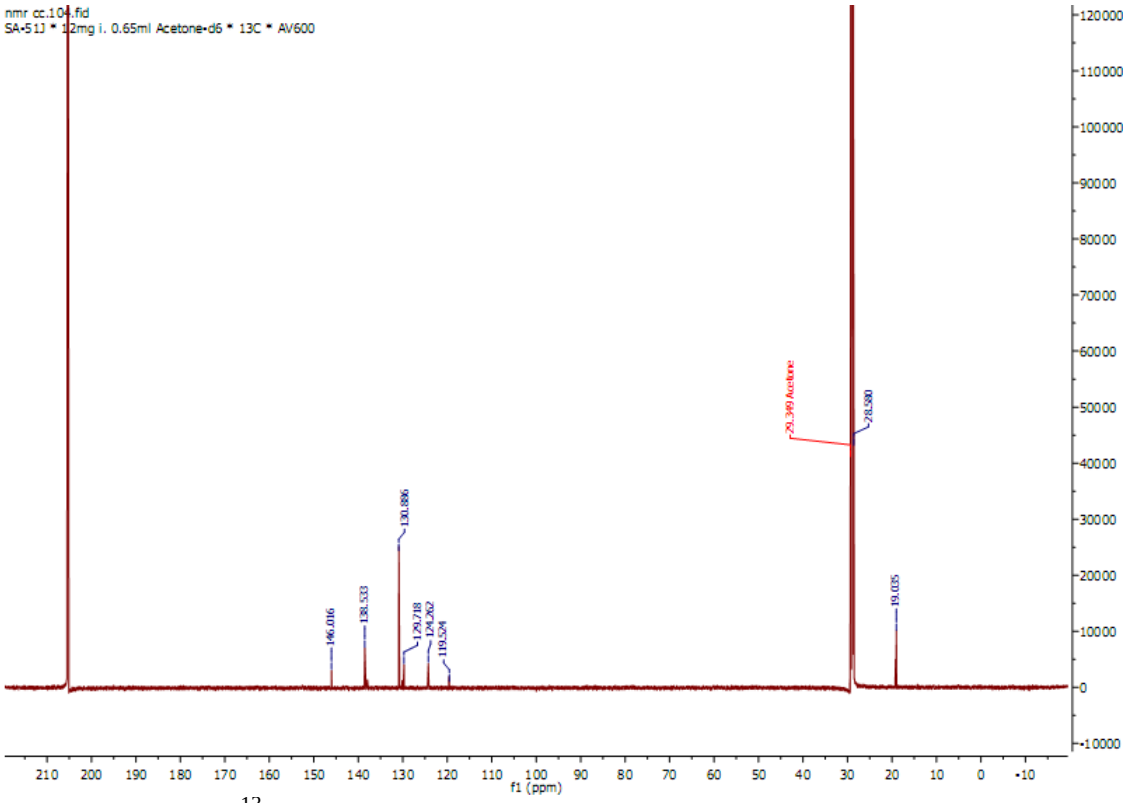

HMQC spectra of 2-isopropenyl-5-chlorophenol (**70**)

Appendix 14: The spectra of 4-methyl-2-nitrophenol (**72**)

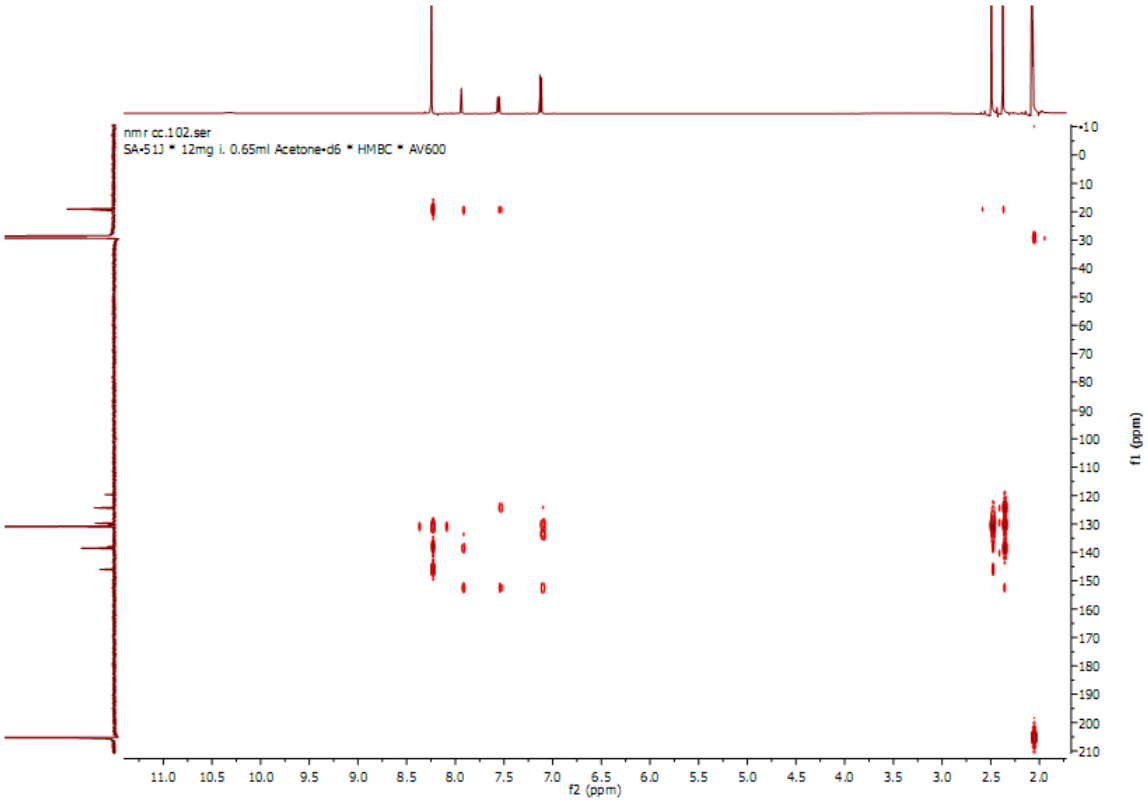


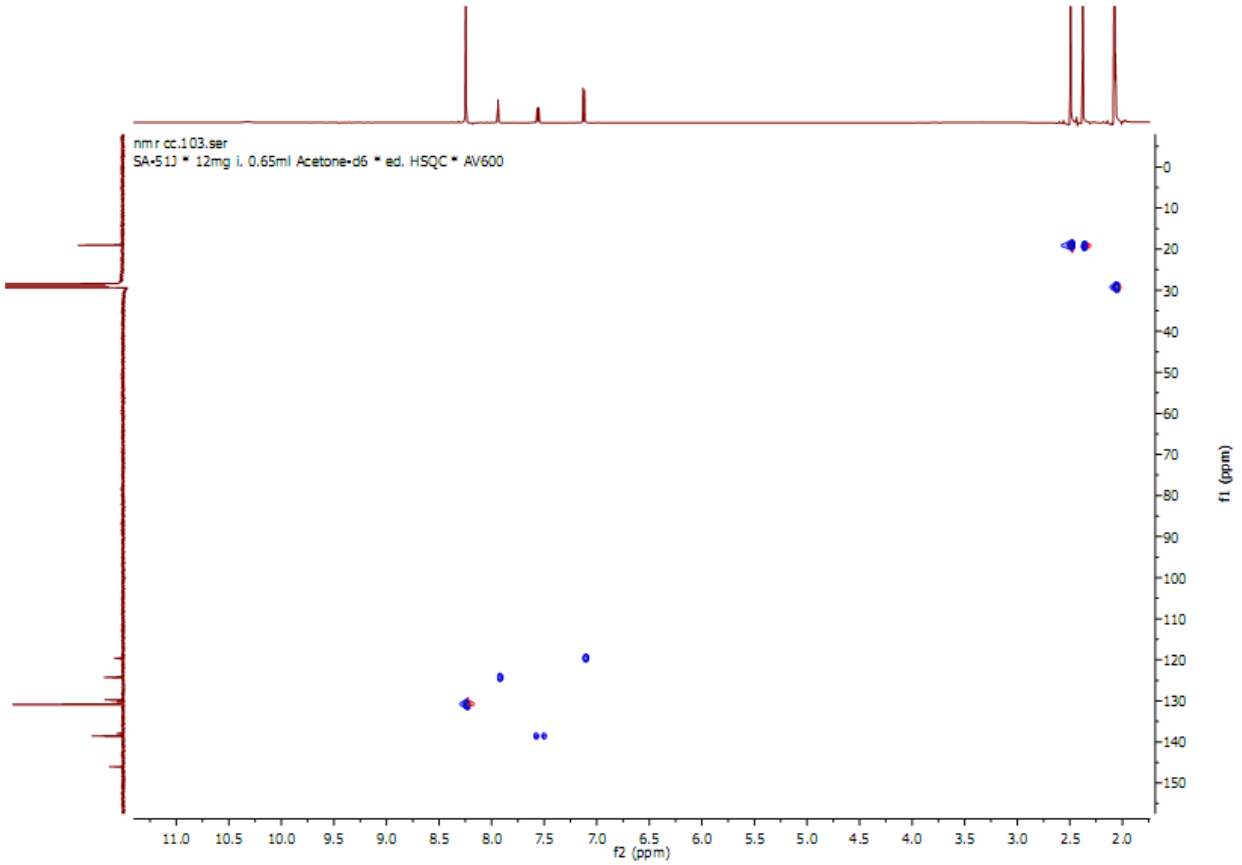

$^{1}$H NMR spectra of 4-methyl-2-nitrophenol (**72**)




$^{13}$C NMR spectra of 4--methyl-2-nitrophenol (**72**)

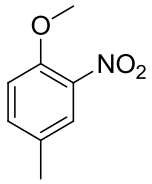


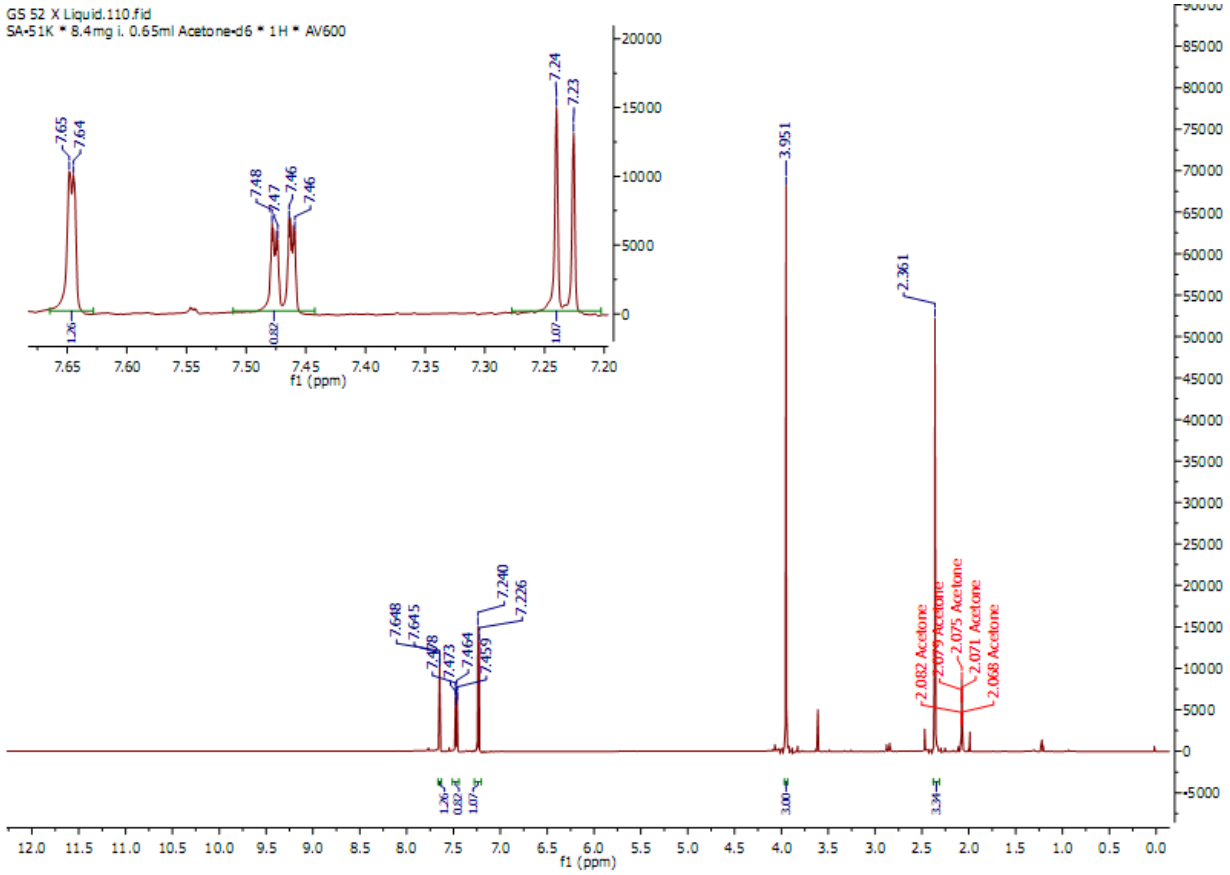

HMBC spectra of 4--methyl-2-nitrophenol (**72**)



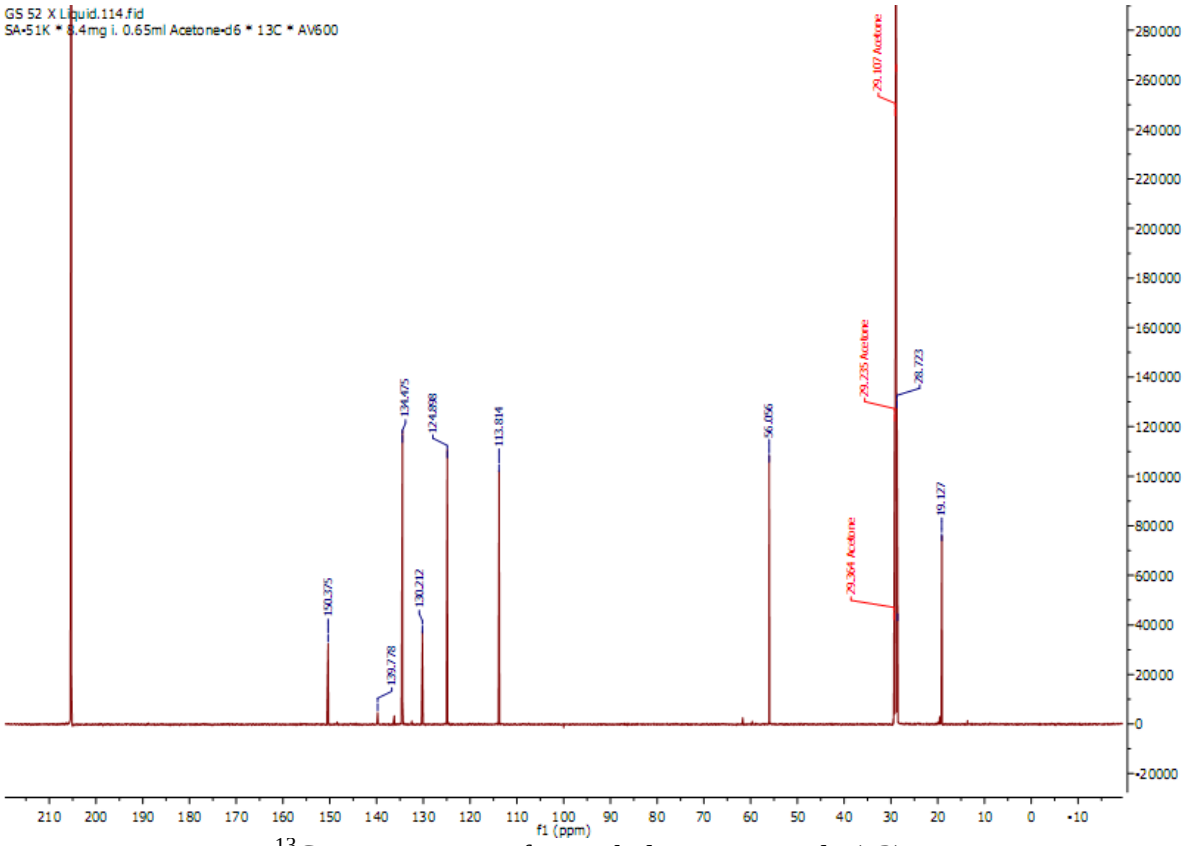

HSQC spectra of 4--methyl-2-nitrophenol (**72**)

Appendix 15: The spectra of 4-methyl-2-nitroanisole (**73**)

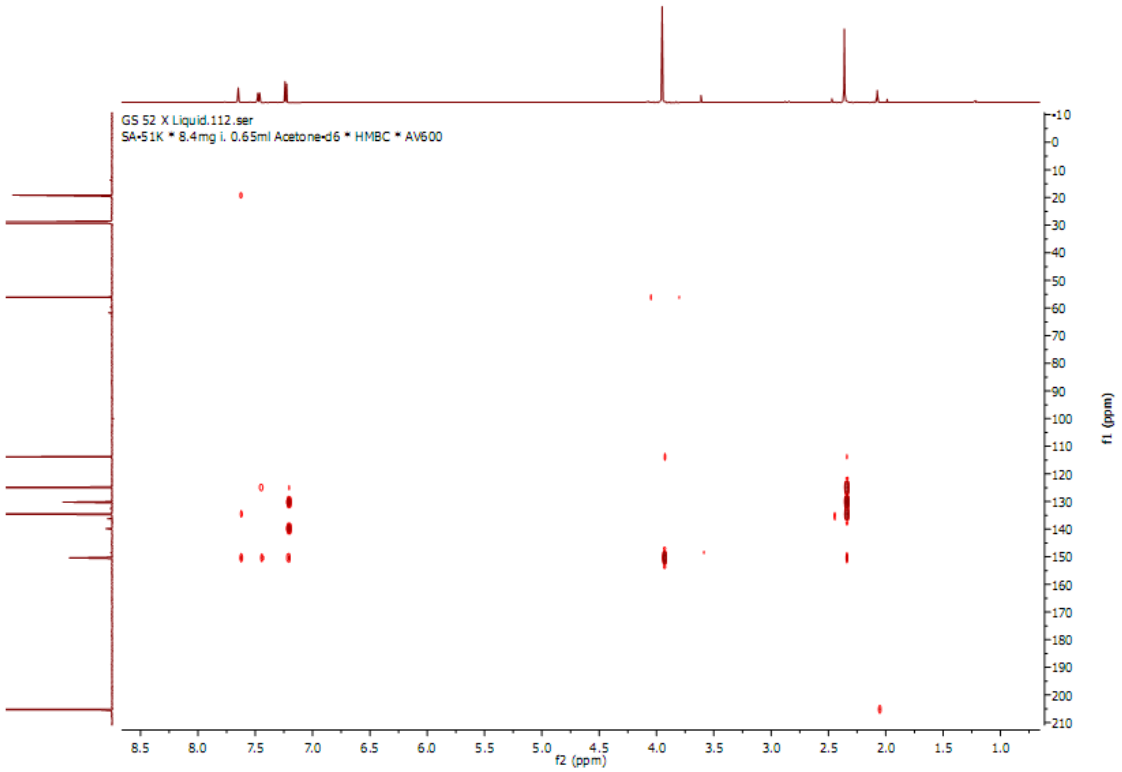


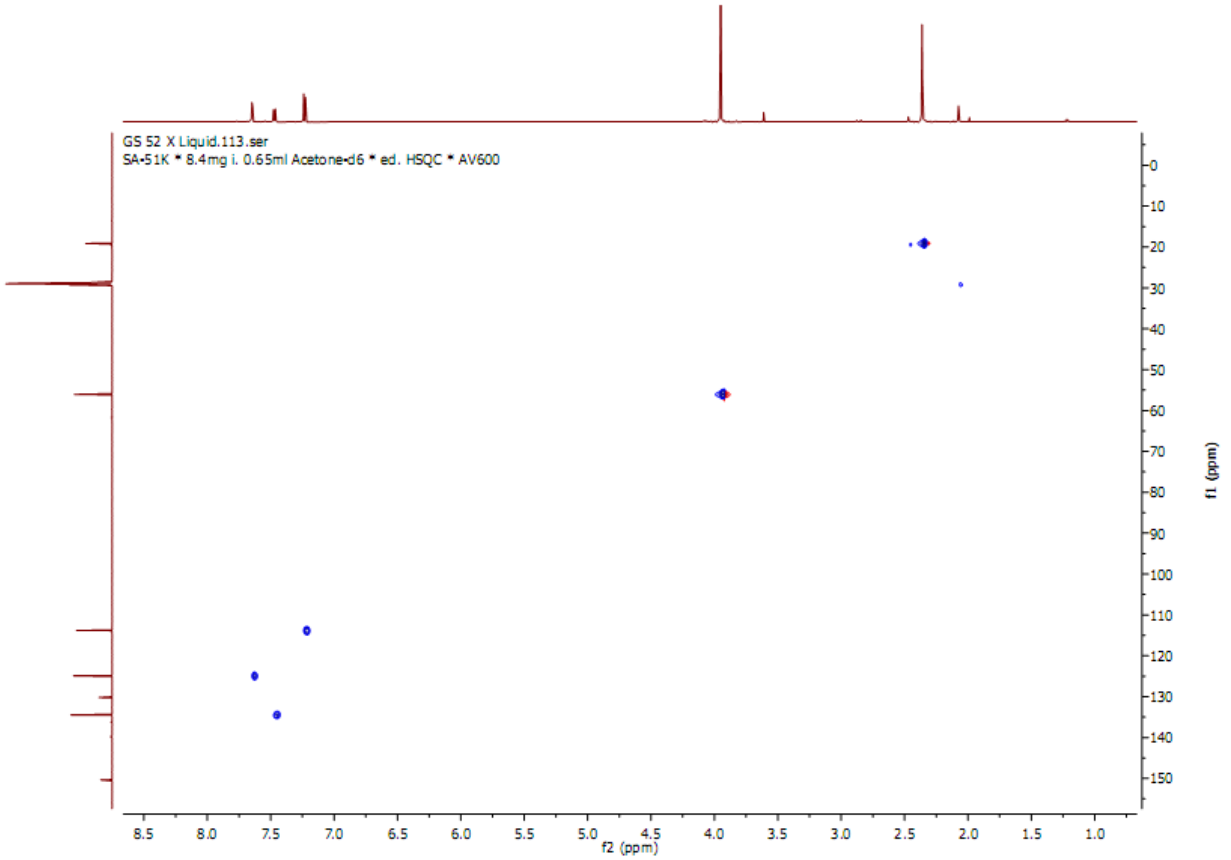

$^{1}$H NMR spectra of 4-methyl-2-nitroanisole (**73**)




$^{13}$C NMR spectra of 4-methyl-2-nitroanisole (**73**)

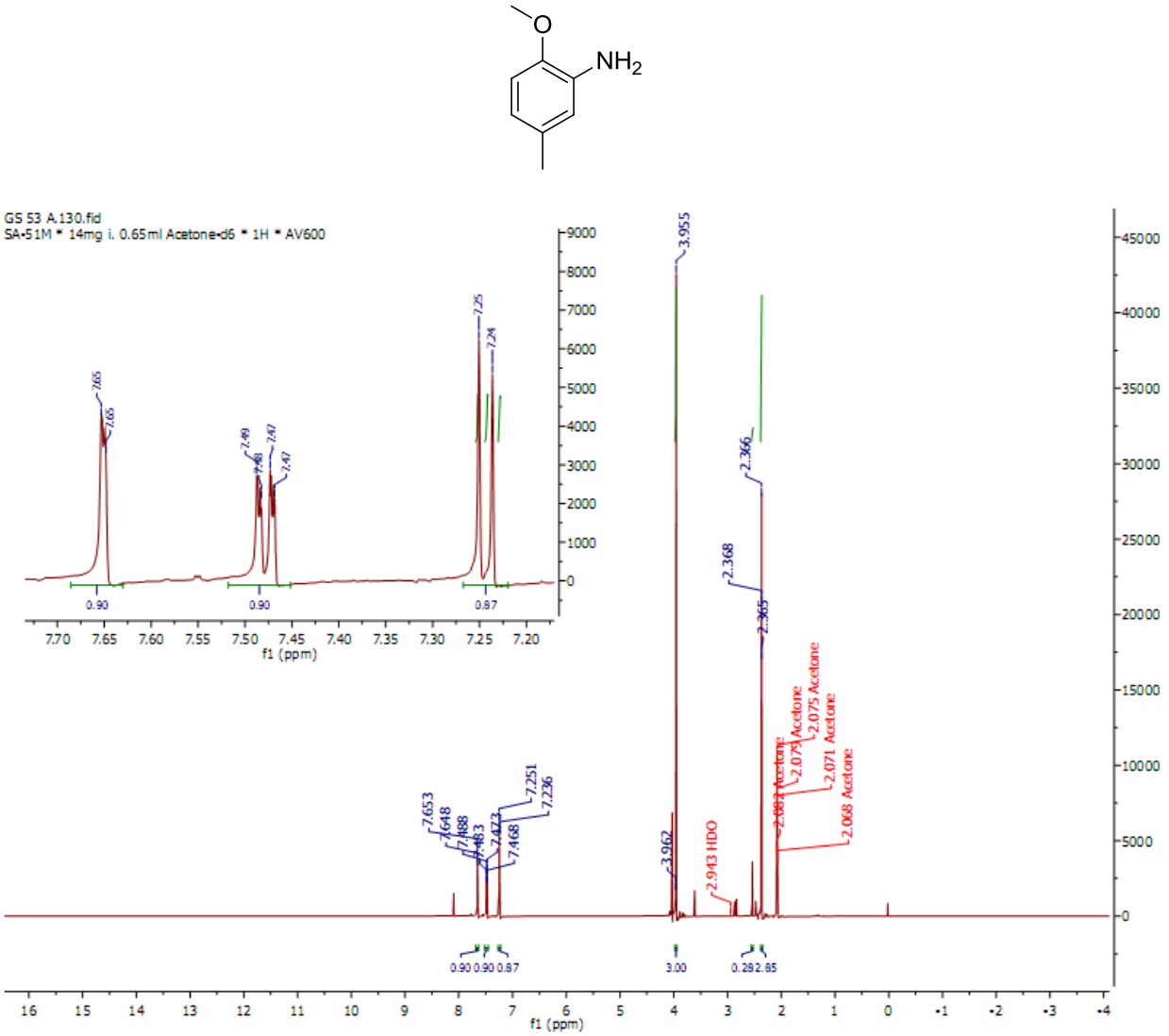



HMBC spectra of 4-methyl-2-nitroanisole (**73**)

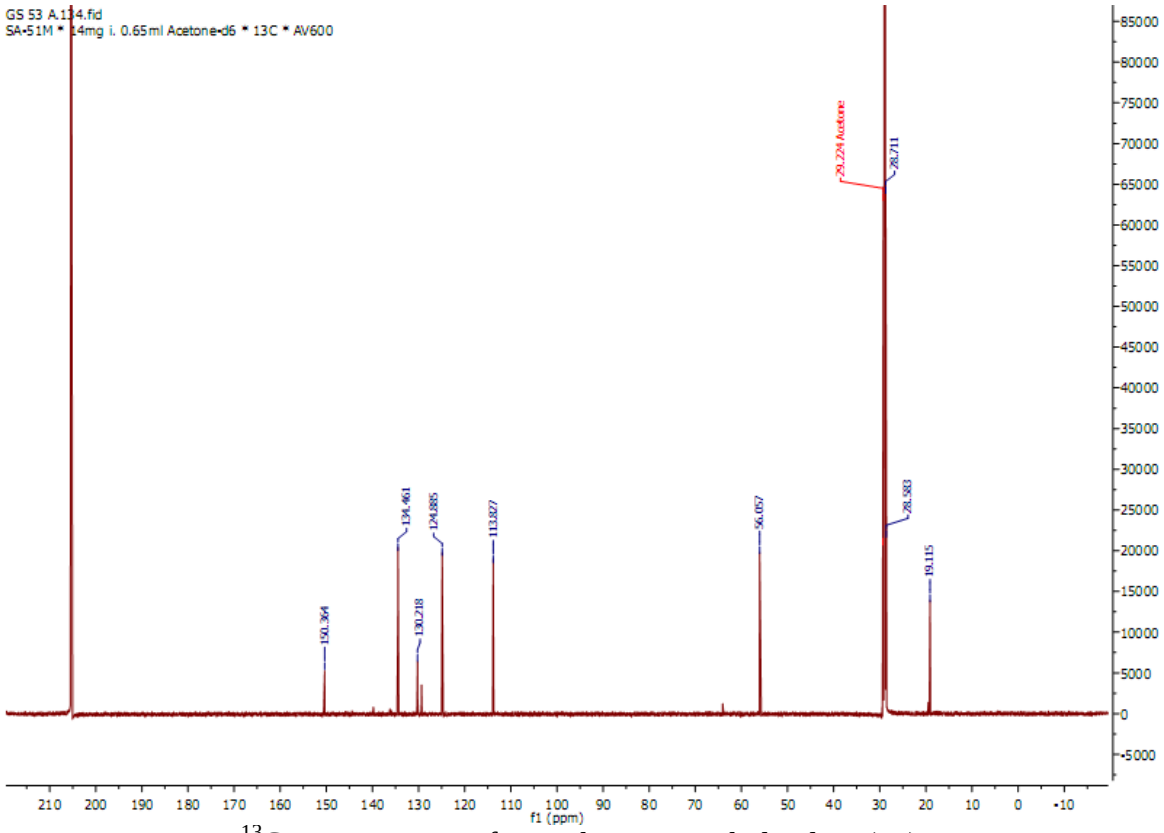



HSQC spectra of 4-methyl-2-nitroanisole (**73**)

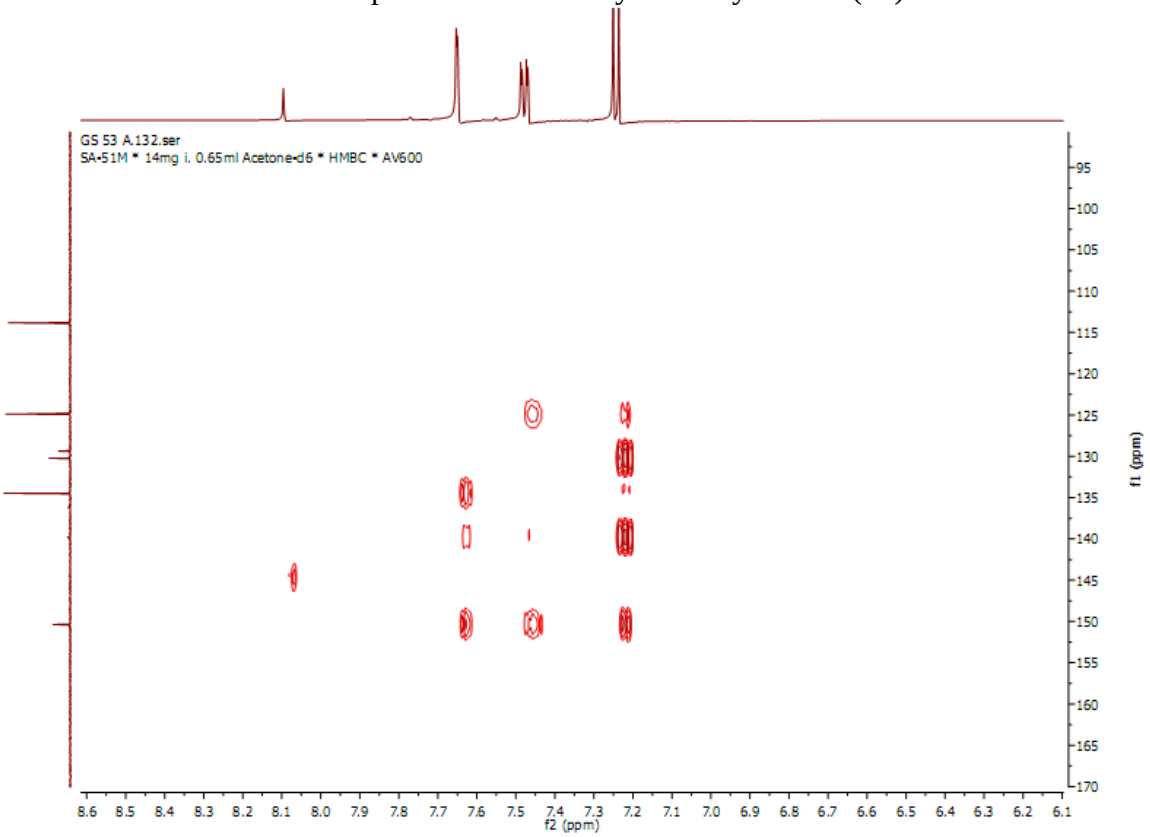
Appendix 16: The spectra of 2-methoxy-5-methylaniline (**74**)



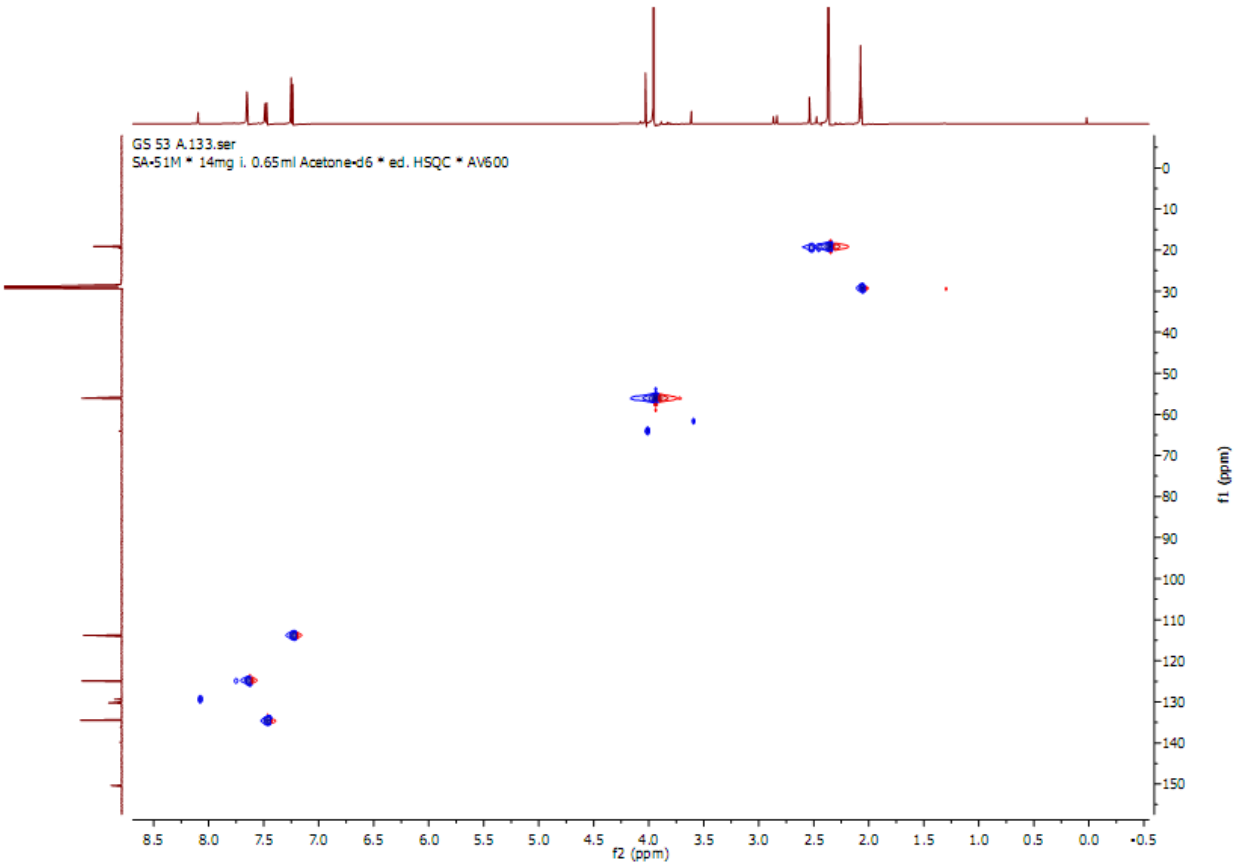

$^{1}$H NMR spectra of 2-methoxy-5-methylaniline (**74**)




$^{13}$C NMR spectra of 2-methoxy-5-methylaniline (**74**)

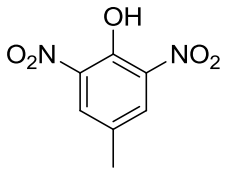


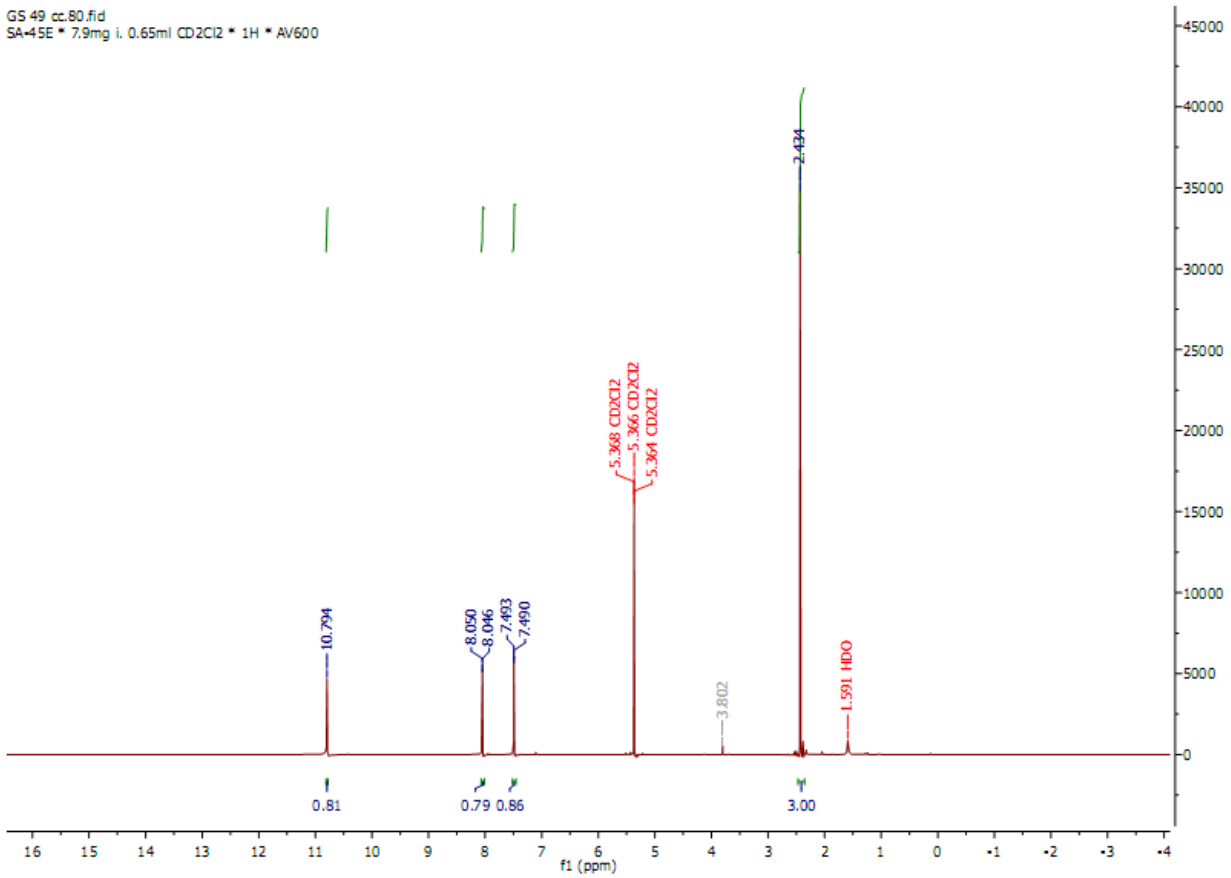

HMBC spectra of 2-methoxy-5-methylaniline (**74**)



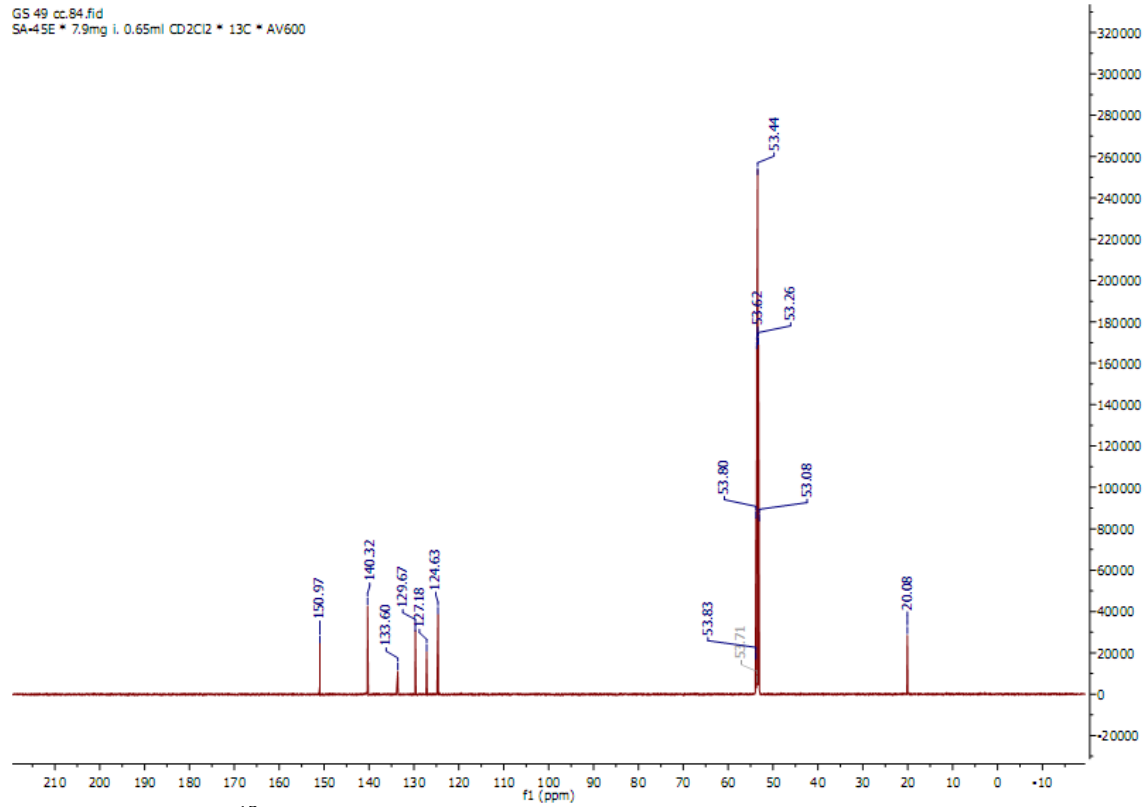

HSQC spectra of 2-methoxy-5-methylaniline (**74**)

Appendix 17: The spectra of 4-methyl-2,6-dinitrophenol (**75**)

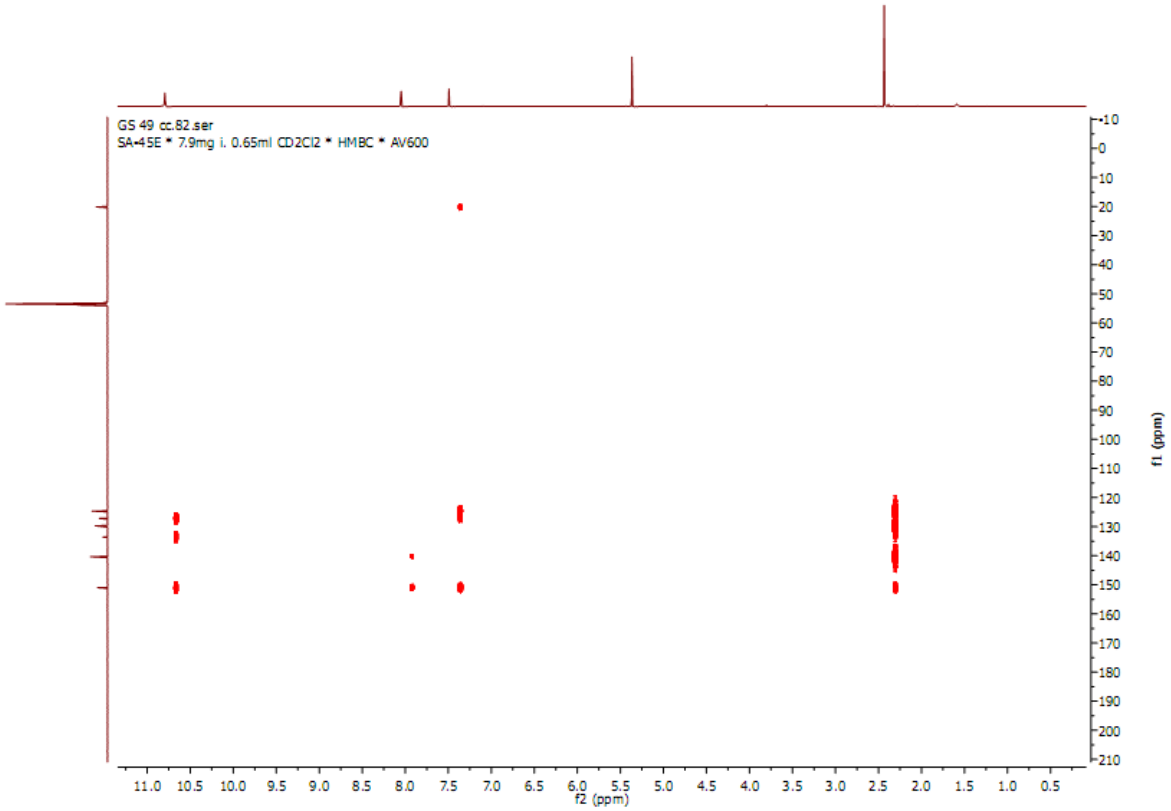


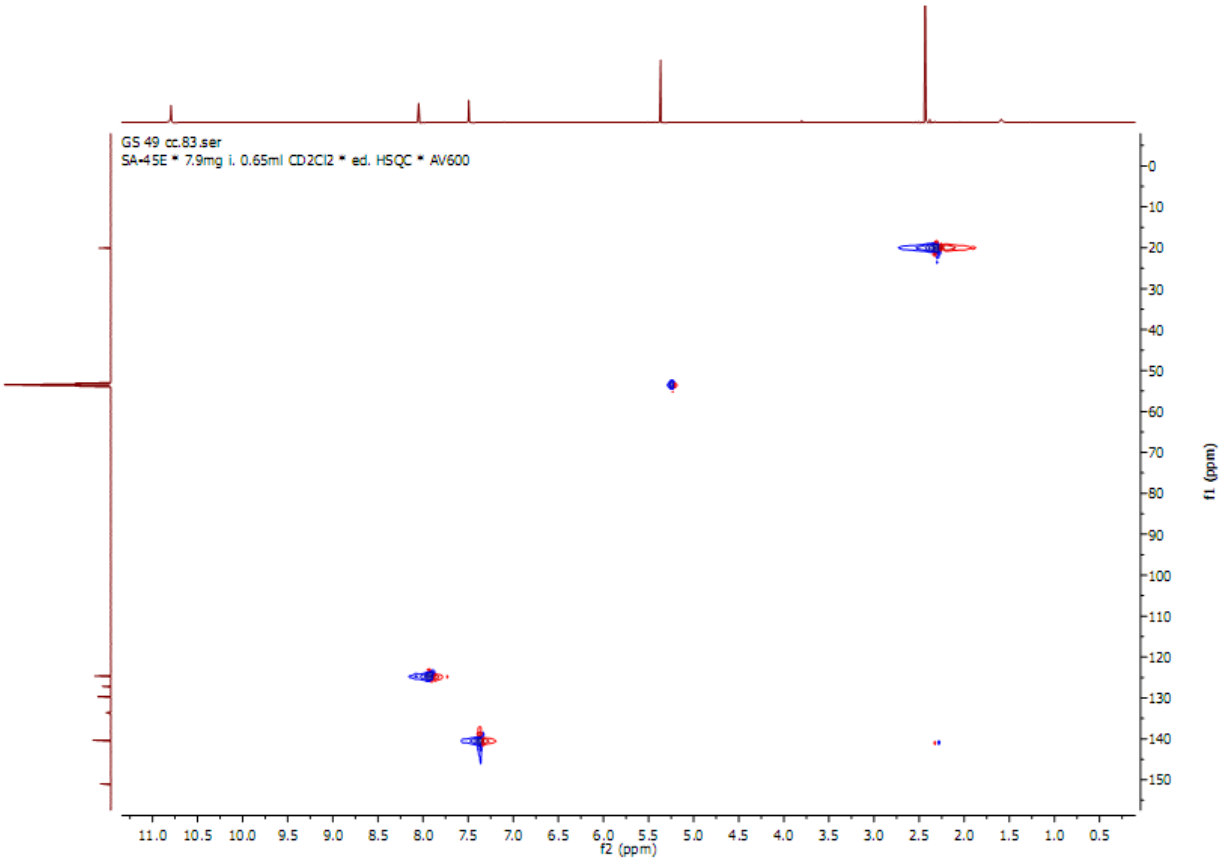

$^{1}$H NMR spectra of 4-methyl-2,6-dinitrophenol (**75**)




$^{13}$C NMR spectra of 4-methyl-2,6-dinitrophenol (**75**)

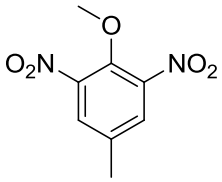


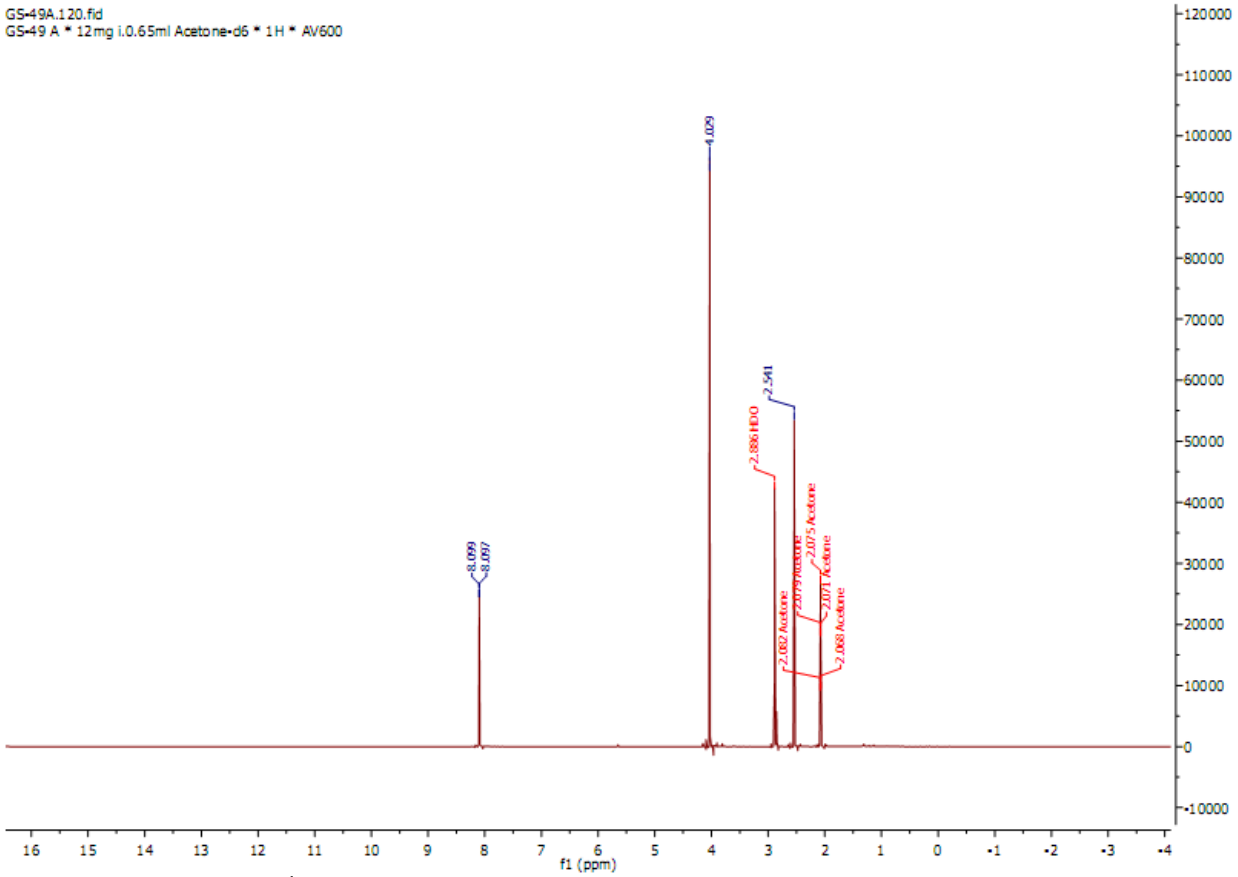

HMBC spectra of 4-methyl-2,6-dinitrophenol (**75**)



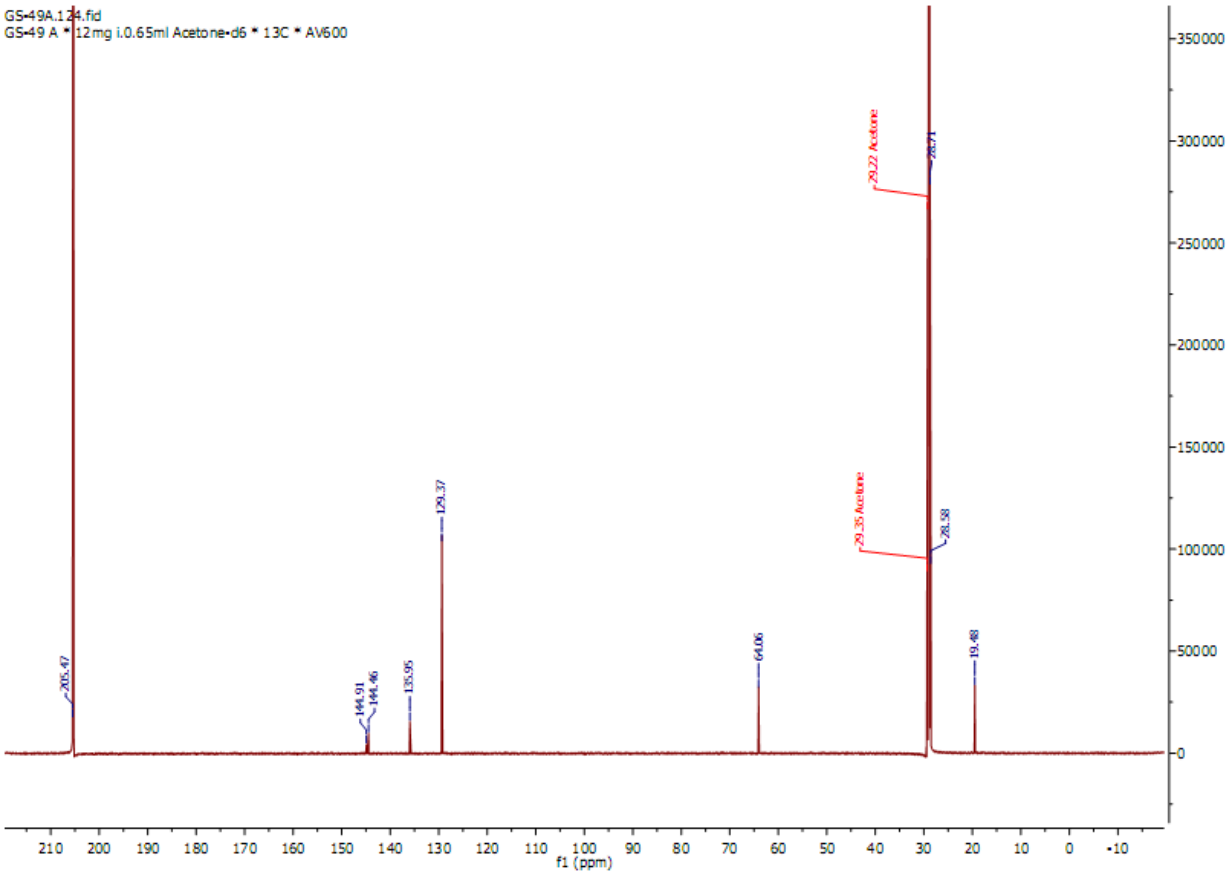

HSQC spectra of 4-methyl-2,6-dinitrophenol (**75**)

Appendix 18: The spectra of 4-methyl-2,6-dinitroanisole (**76**)

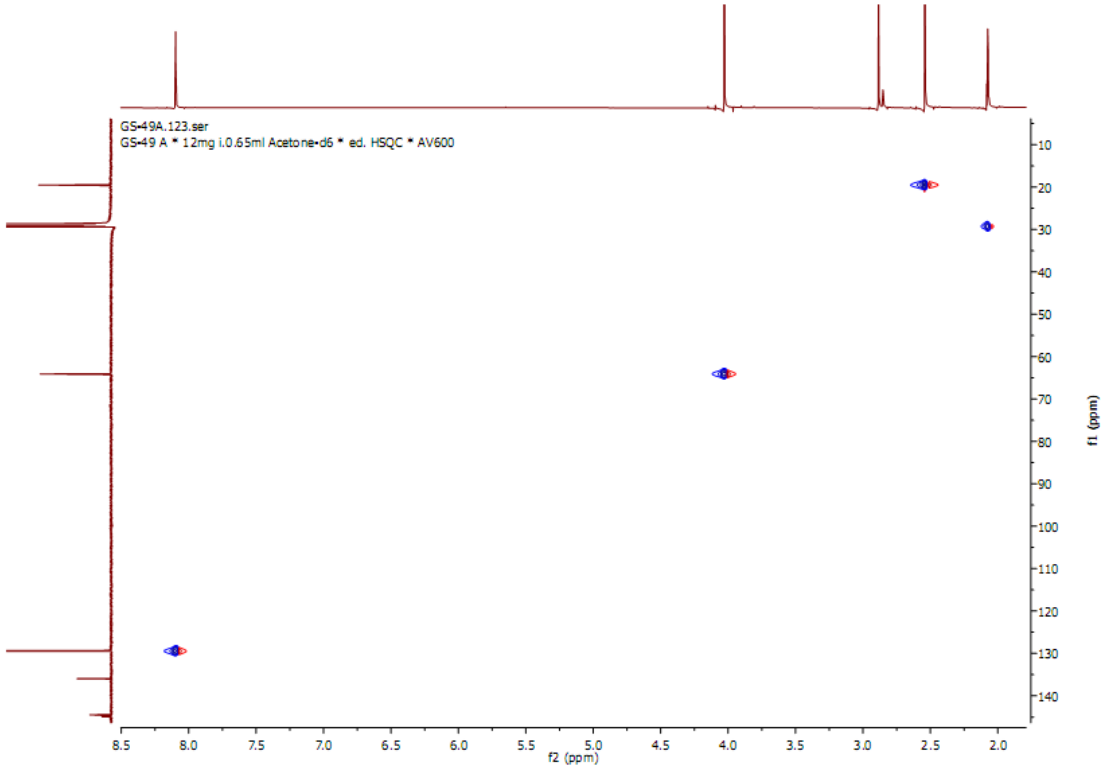


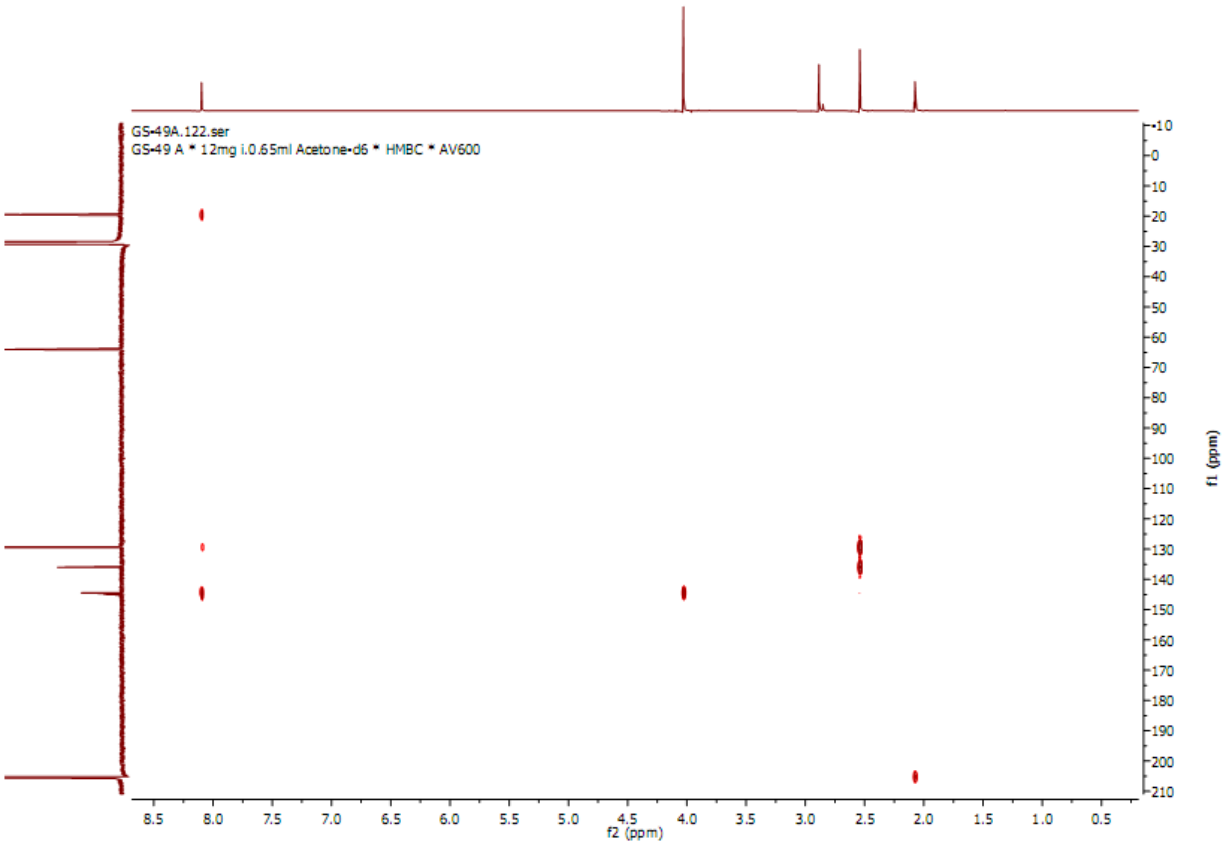

$^1$H NMR spectra of 4-methyl-2,6-dinitroanisole (**76**)




$^{13}$C NMR spectra of 4-methyl-2,6-dinitroanisole (**76**)

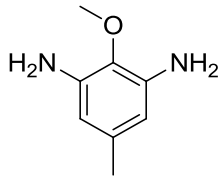


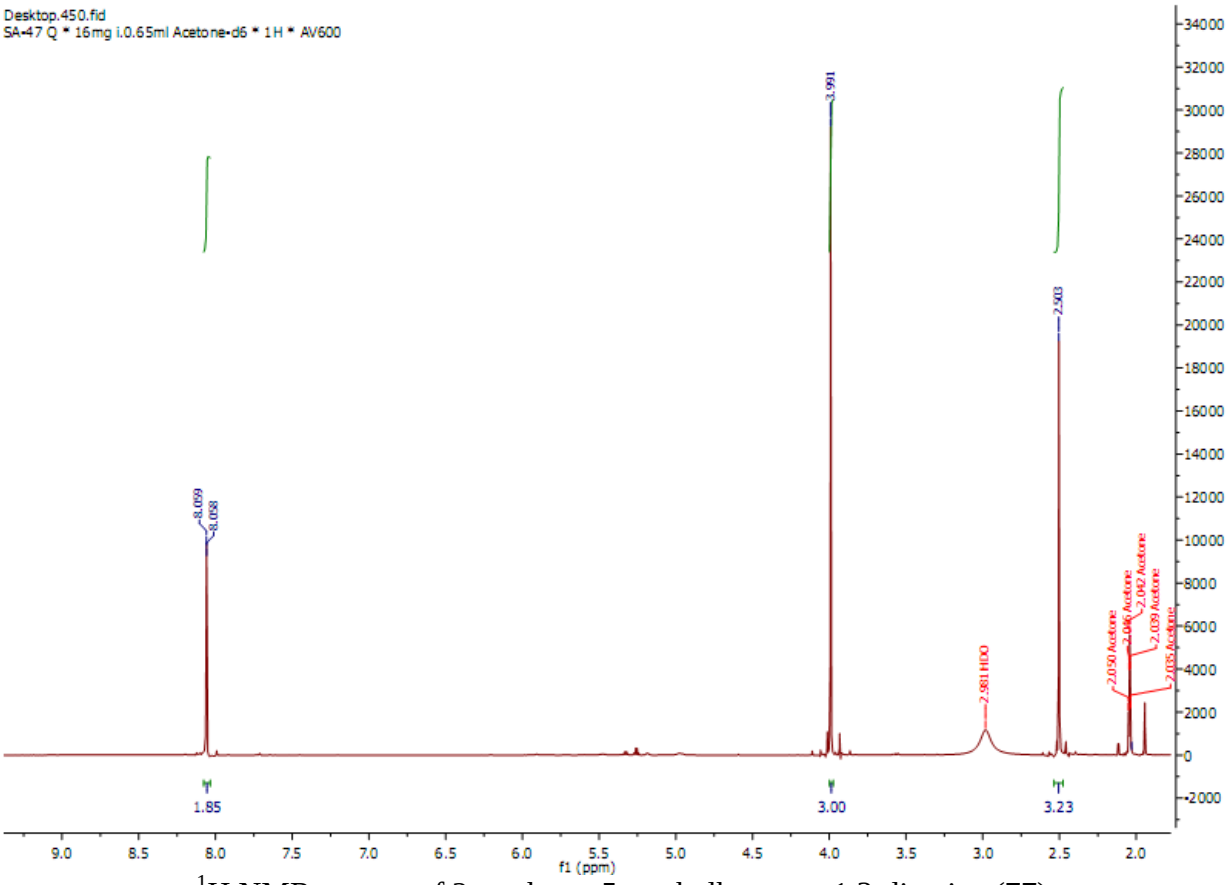

HSQC spectra of 4-methyl-2,6-dinitroanisole (**76**)



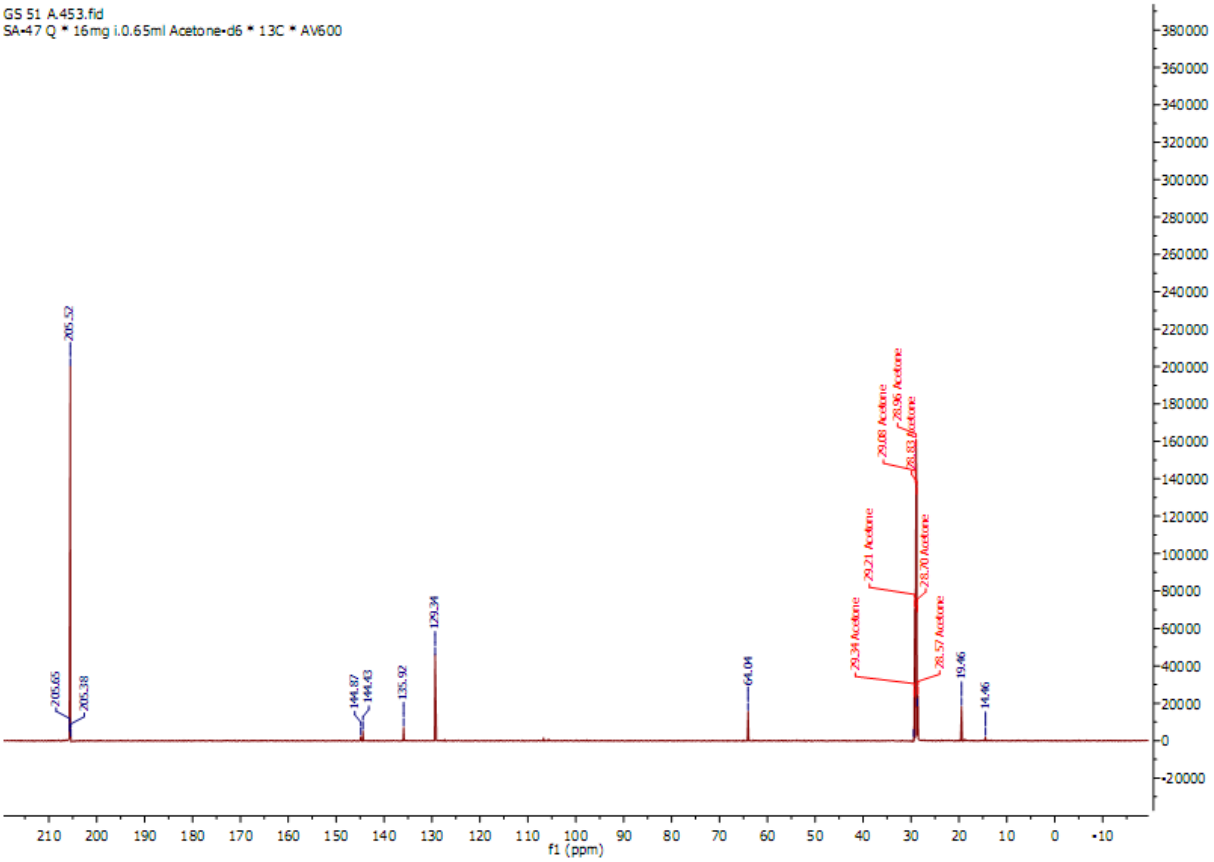

HMBC spectra of 4-methyl-2,6-dinitroanisole (**76**)

Appendix 19: The spectra of 2-methoxy-5-methylbenzene-1,3-diamine (**77**)

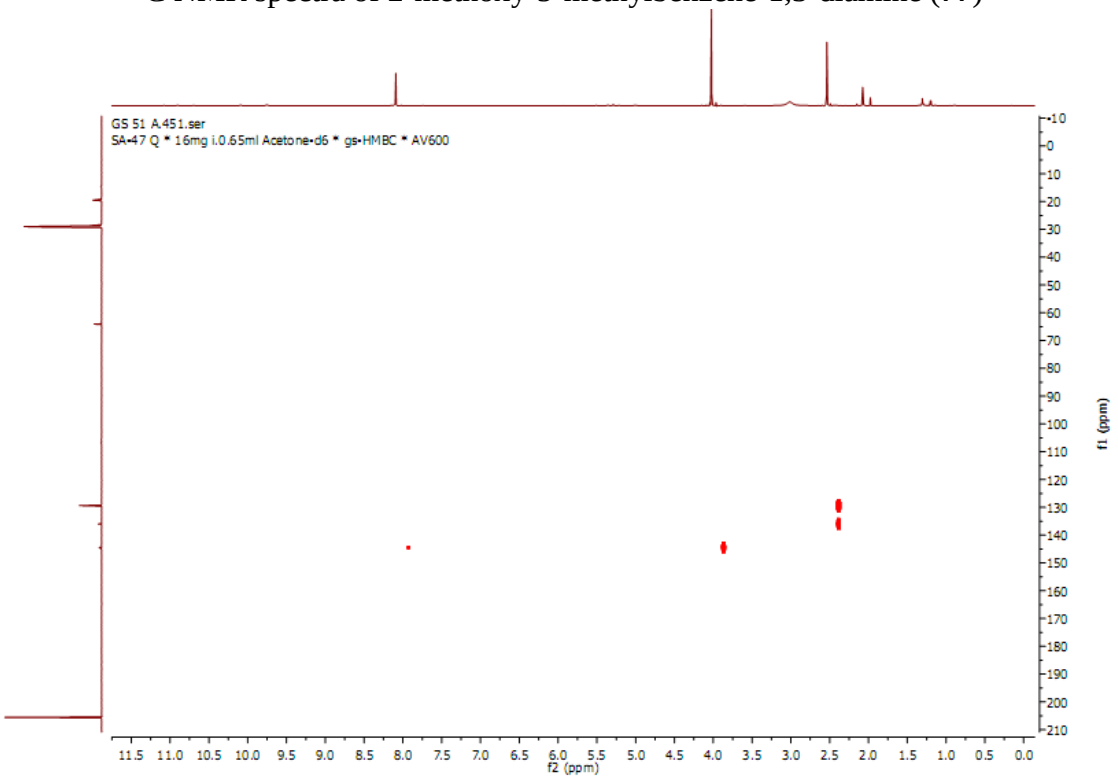


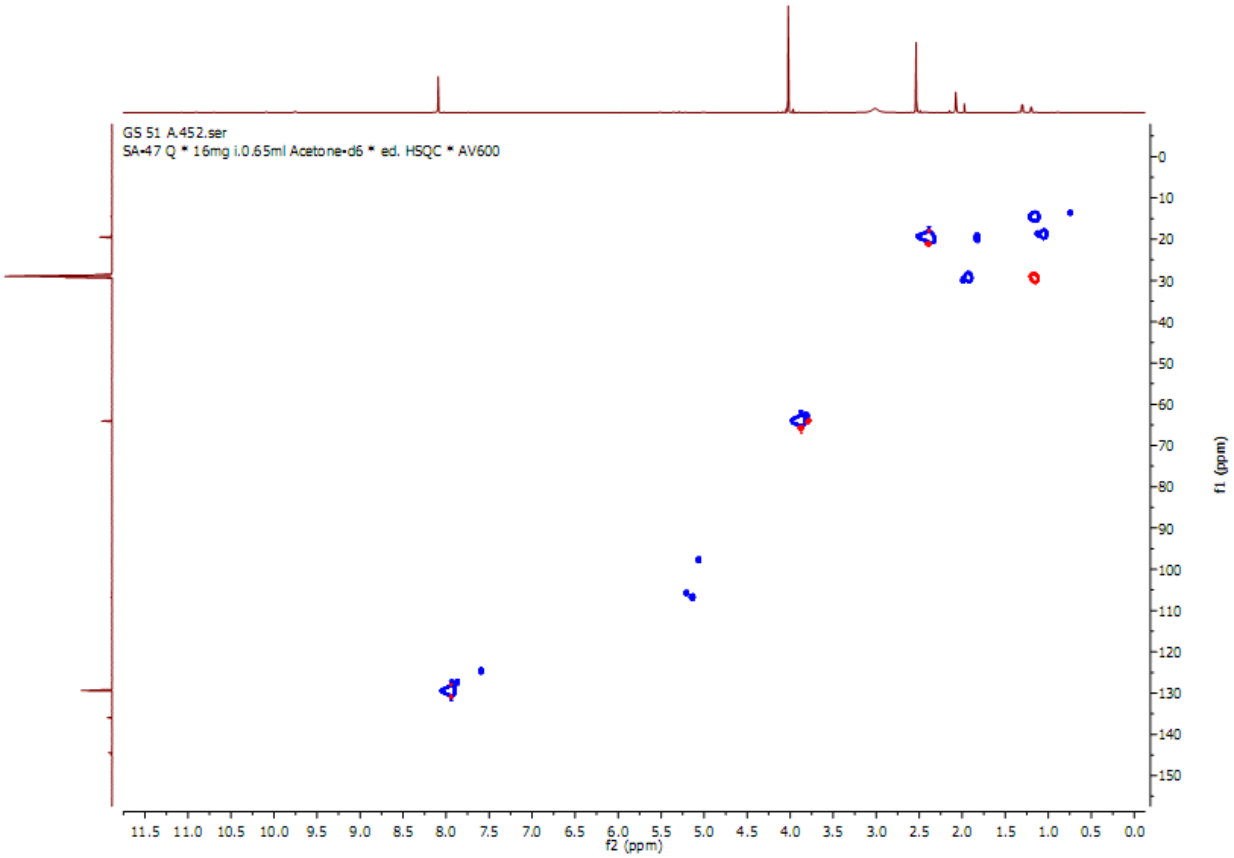

$^{1}$H NMR spectra of 2-methoxy-5-methylbenzene-1,3-diamine (**77**)




$^{13}$C NMR spectra of 2-methoxy-5-methylbenzene-1,3-diamine (**77**)

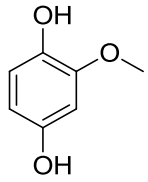


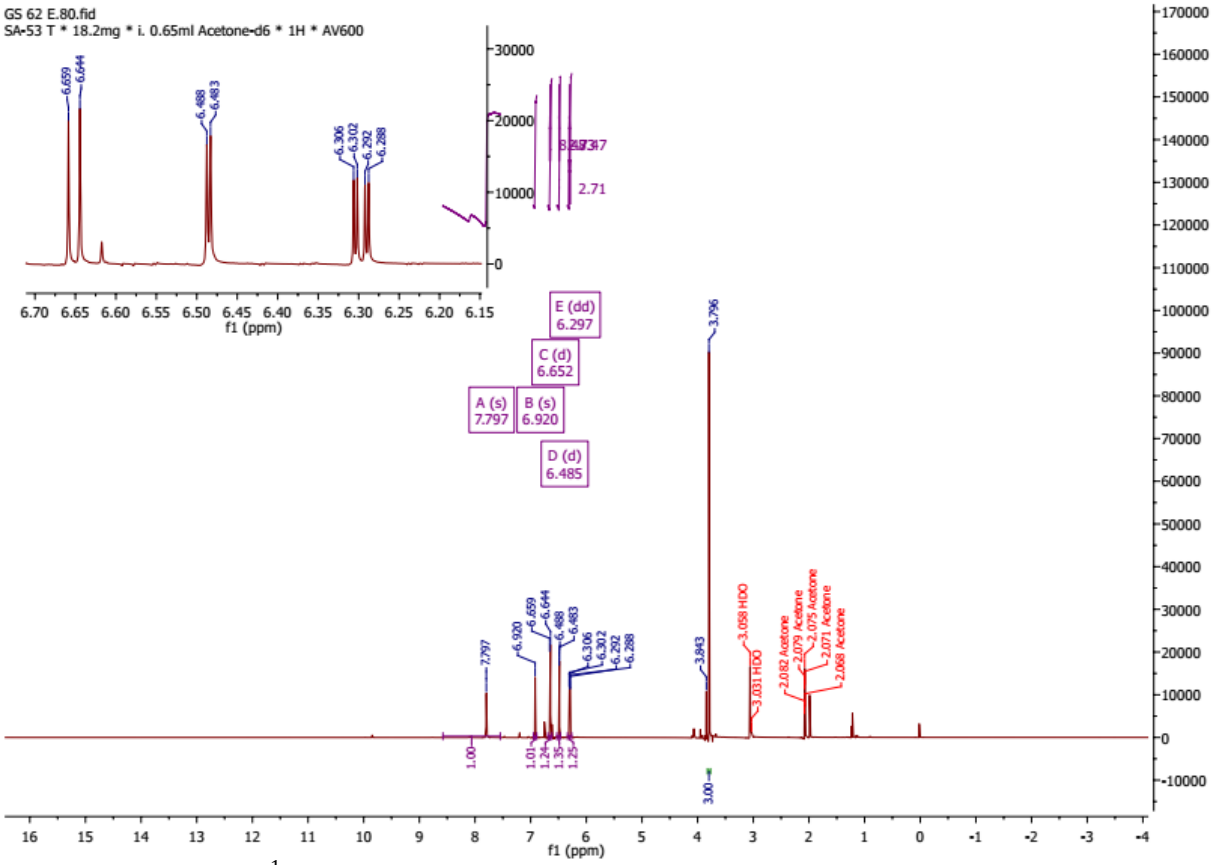

HMBC spectra of 2-methoxy-5-methylbenzene-1,3-diamine (**77**)



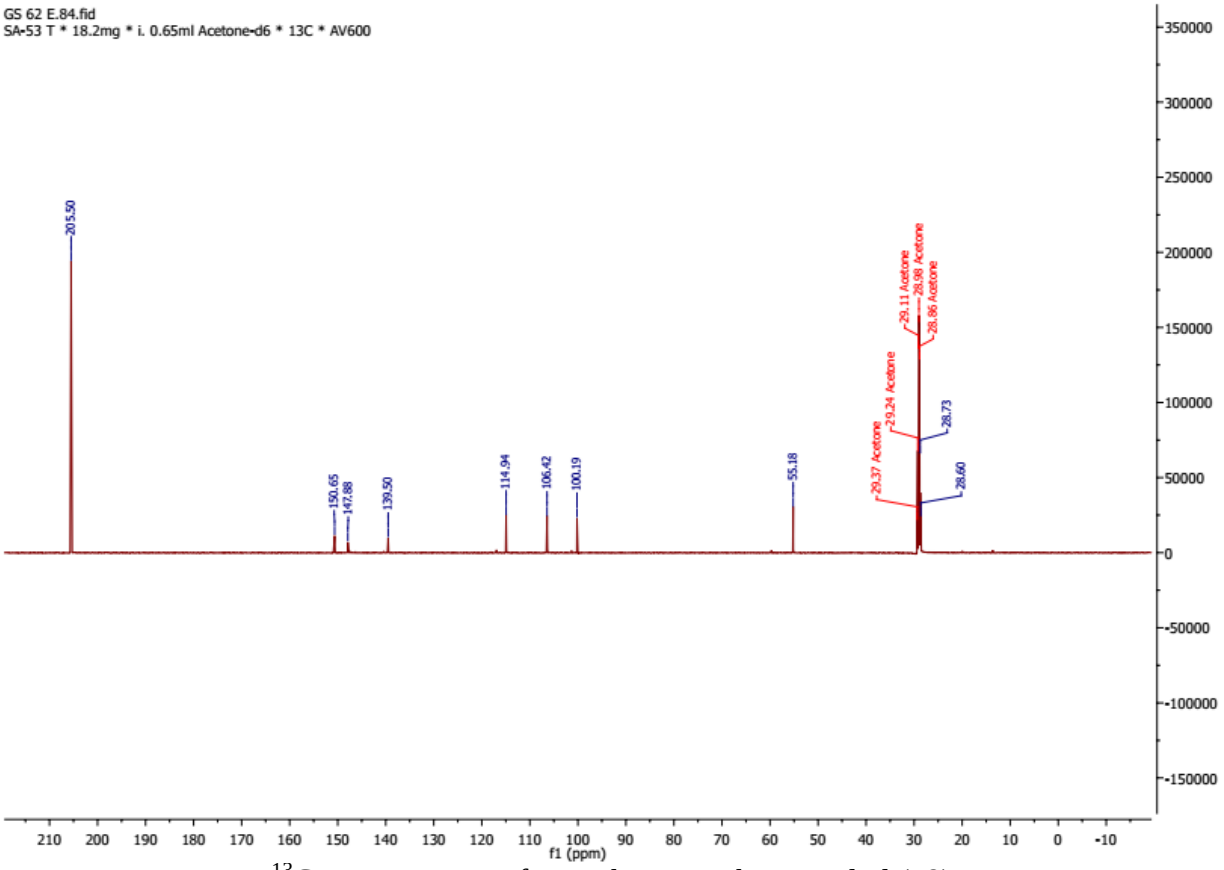

HSQC spectra of 2-methoxy-5-methylbenzene-1,3-diamine (**77**)

Appendix 20: The spectra of 2-methoxy-1,4-benzenediol (**79**)

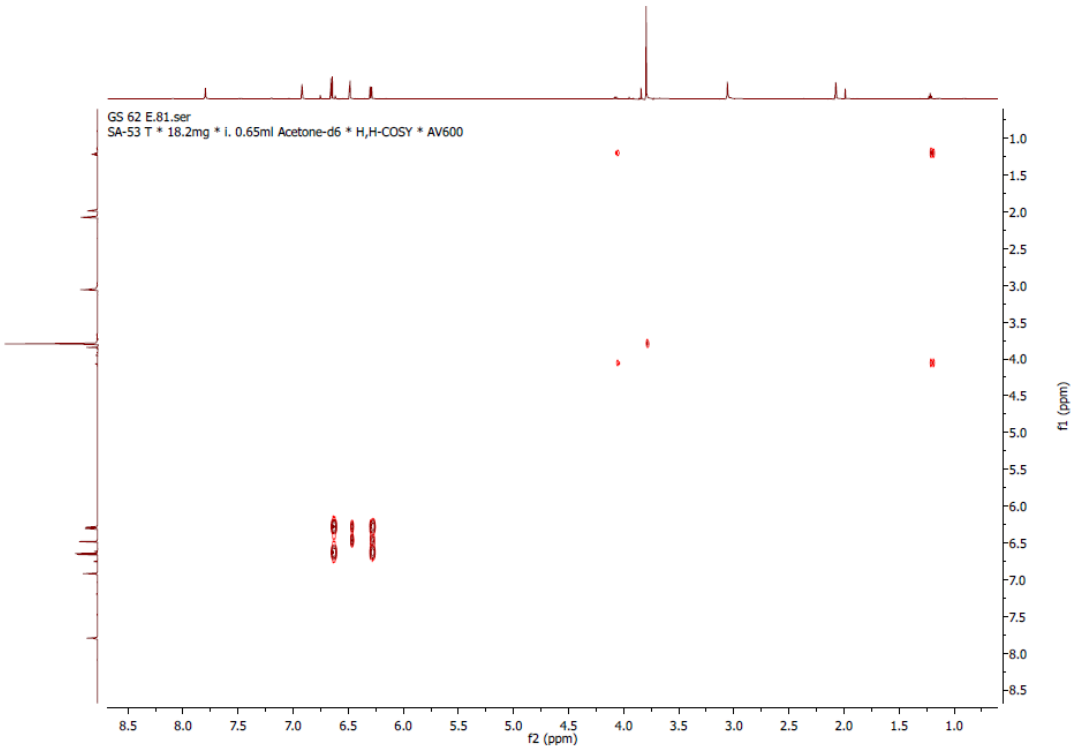


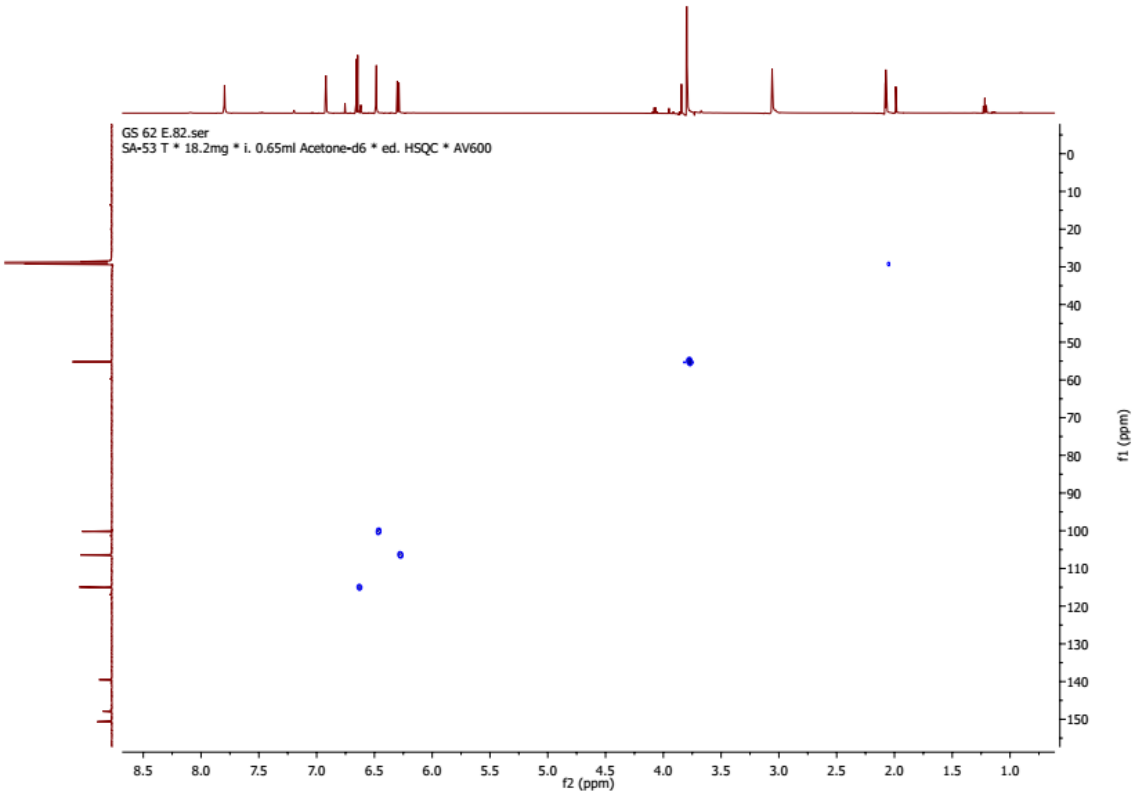

$^{1}$H NMR spectra of 2-methoxy-1,4-benzenediol (**79**)



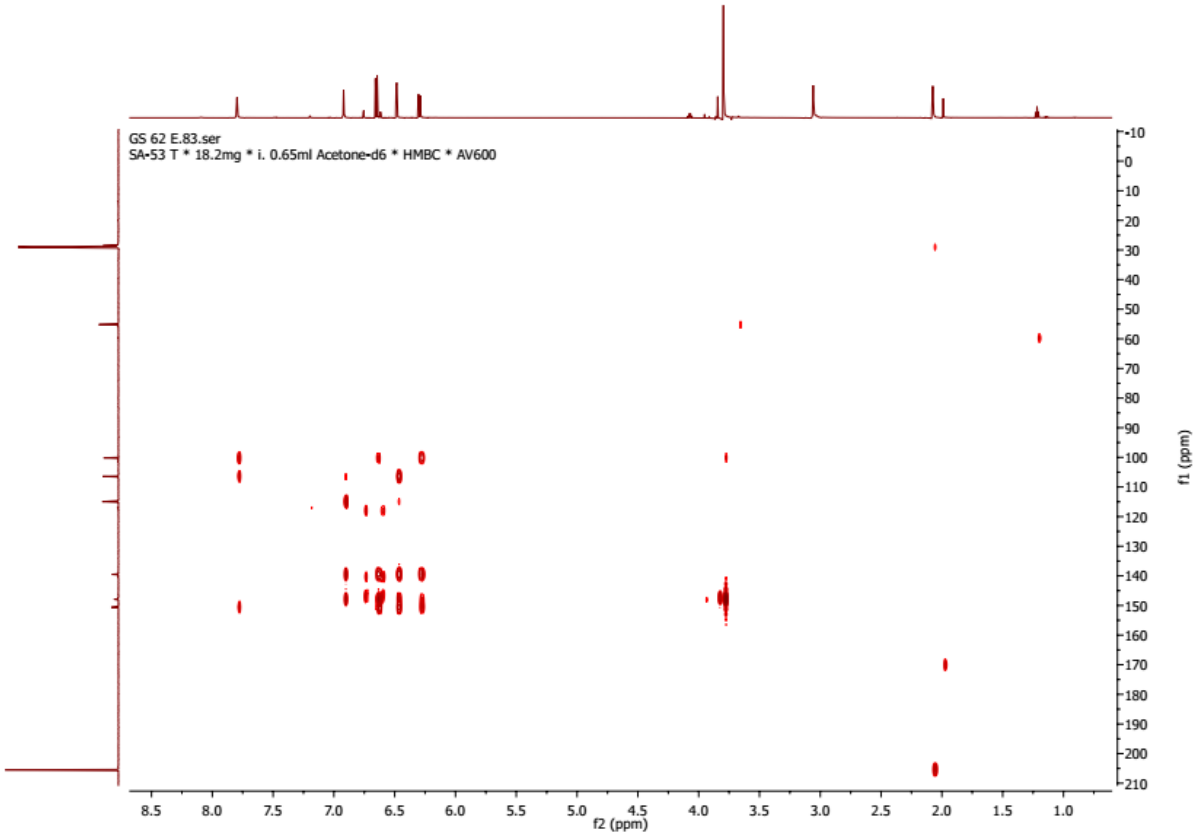

$^{13}$C NMR spectra of 2-methoxy-1,4-benzenediol (**79**)




H,H-COSY spectra of 2-methoxy-1,4-benzenediol (**79**)

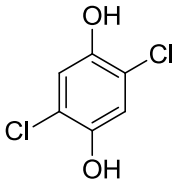


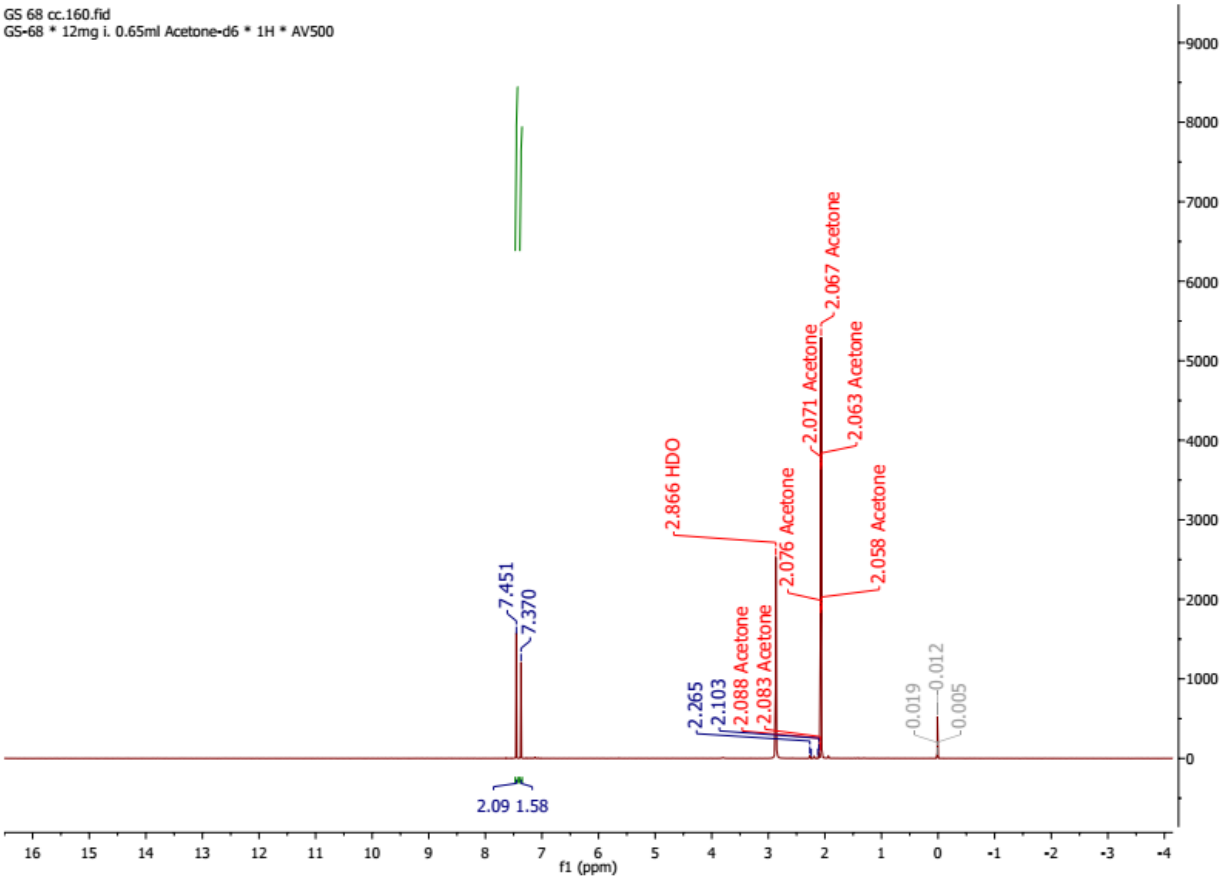

HSQC spectra of 2-methoxy-1,4-benzenediol (**79**)



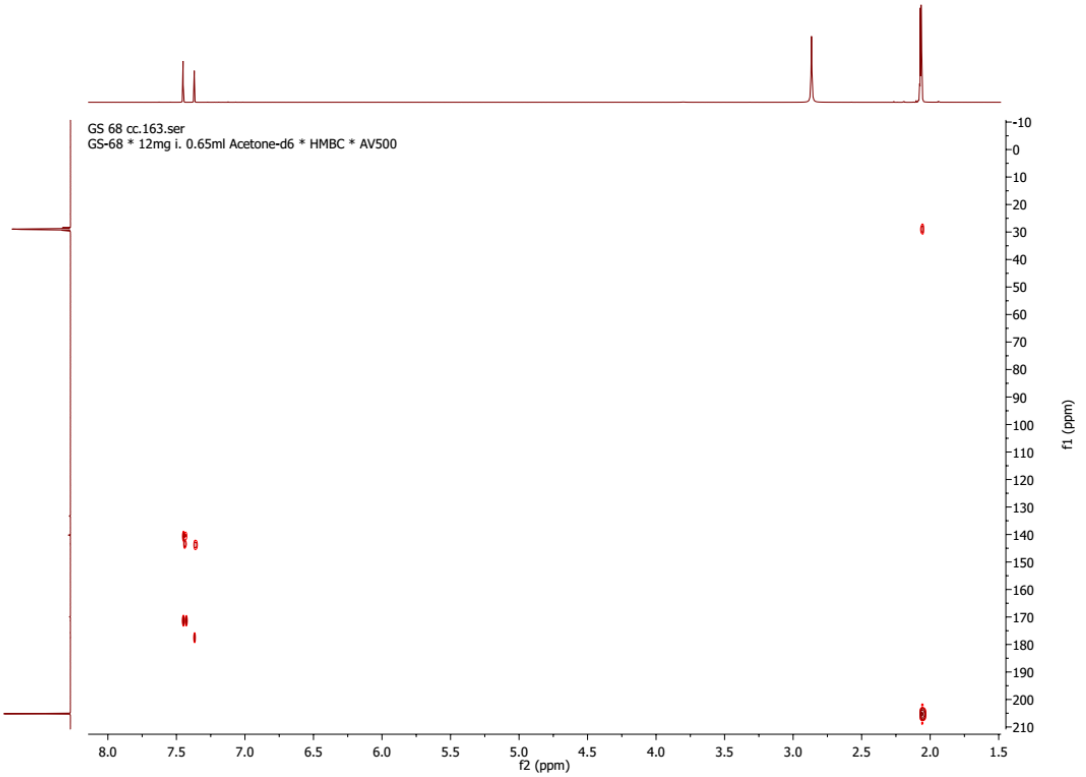

HMBC spectra of 2-methoxy-1,4-benzenediol (**79**)

Appendix 21: The spectra of 2,5-dichloro-1,4-benzenediol (**81**)

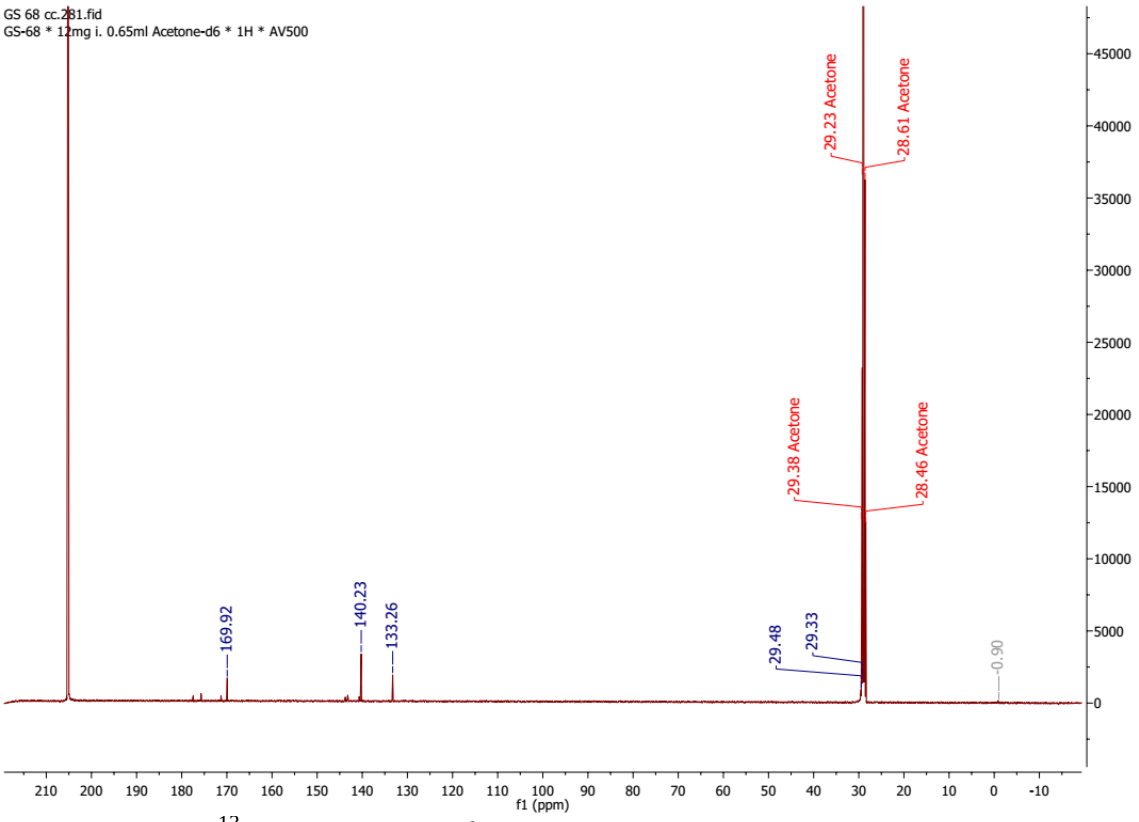



$^{1}$H NMR spectra of 2,5-dichloro-1,4-benzenediol (**81**)

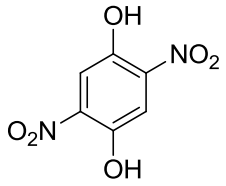


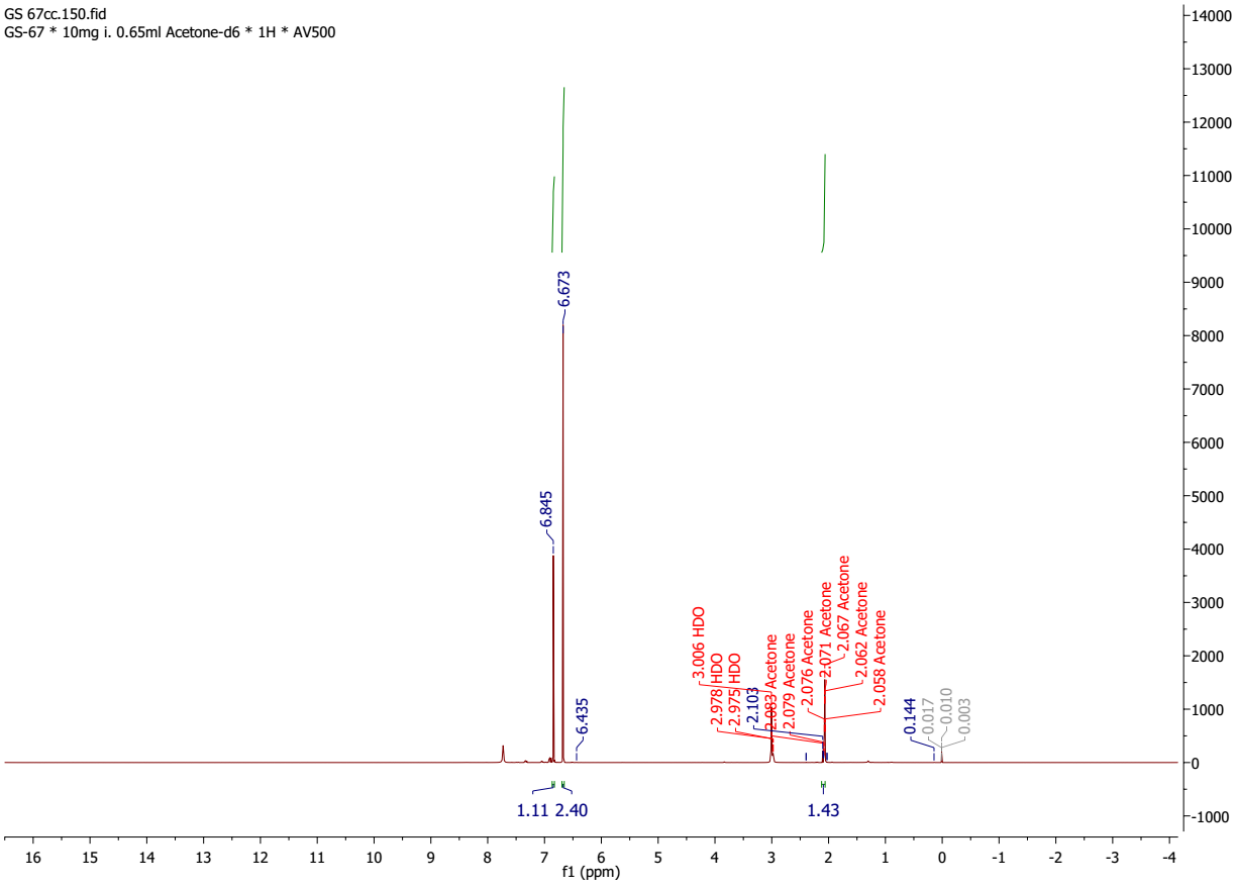

HMBC spectra of 2, 5-dichloro-1,4-benzenediol (**81**)



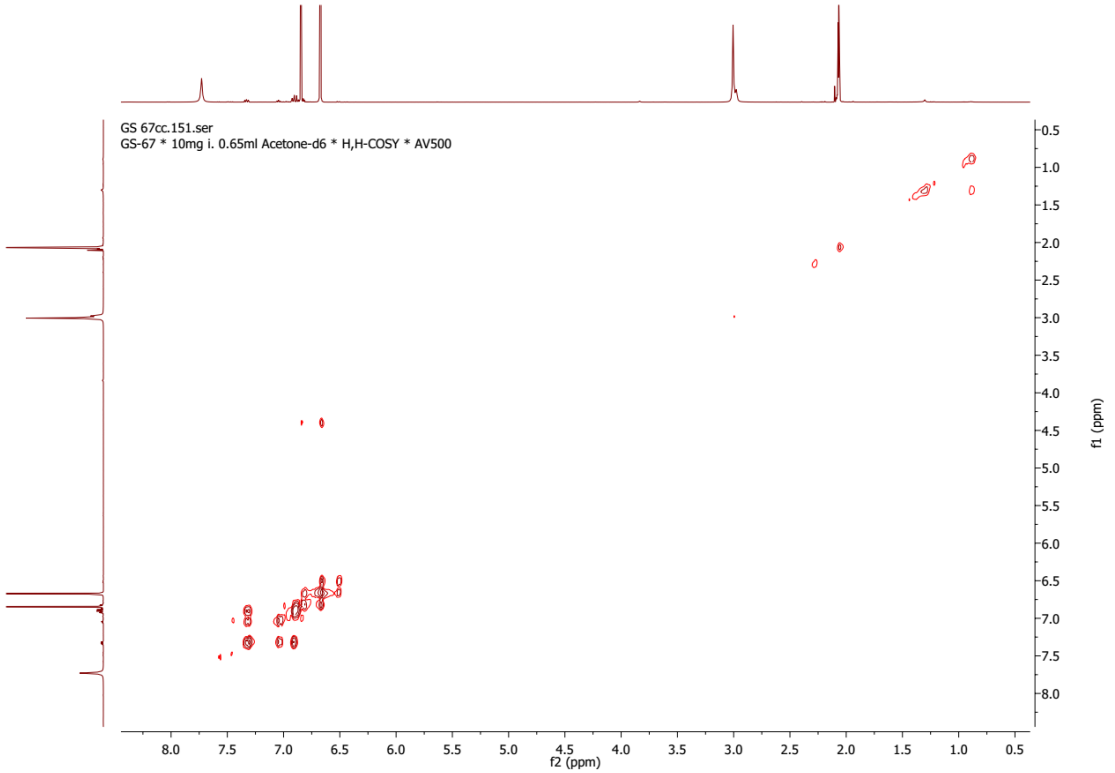

$^{13}$C NMR spectra of 2,5-dichloro-1,4-benzenediol (**81**)

Appendix 22: The spectra of 2,5-dinitro-1,4-benzenediol (**82**)

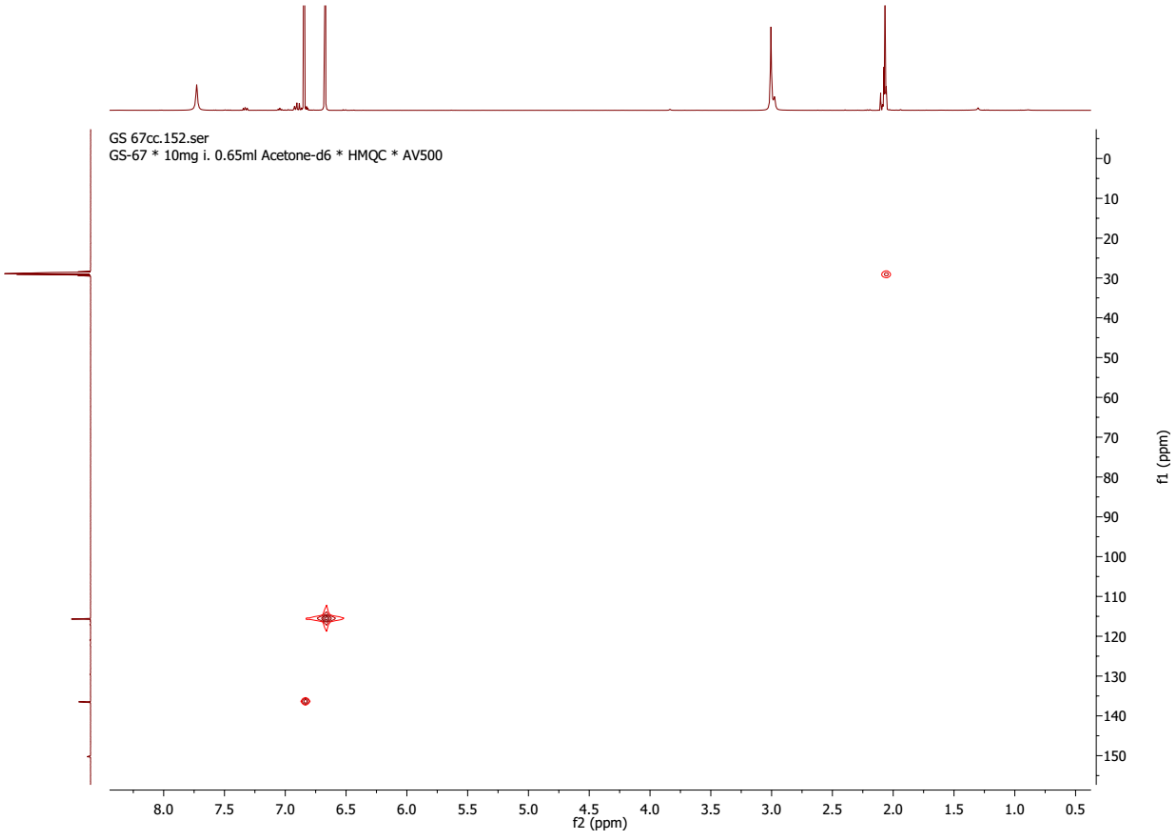


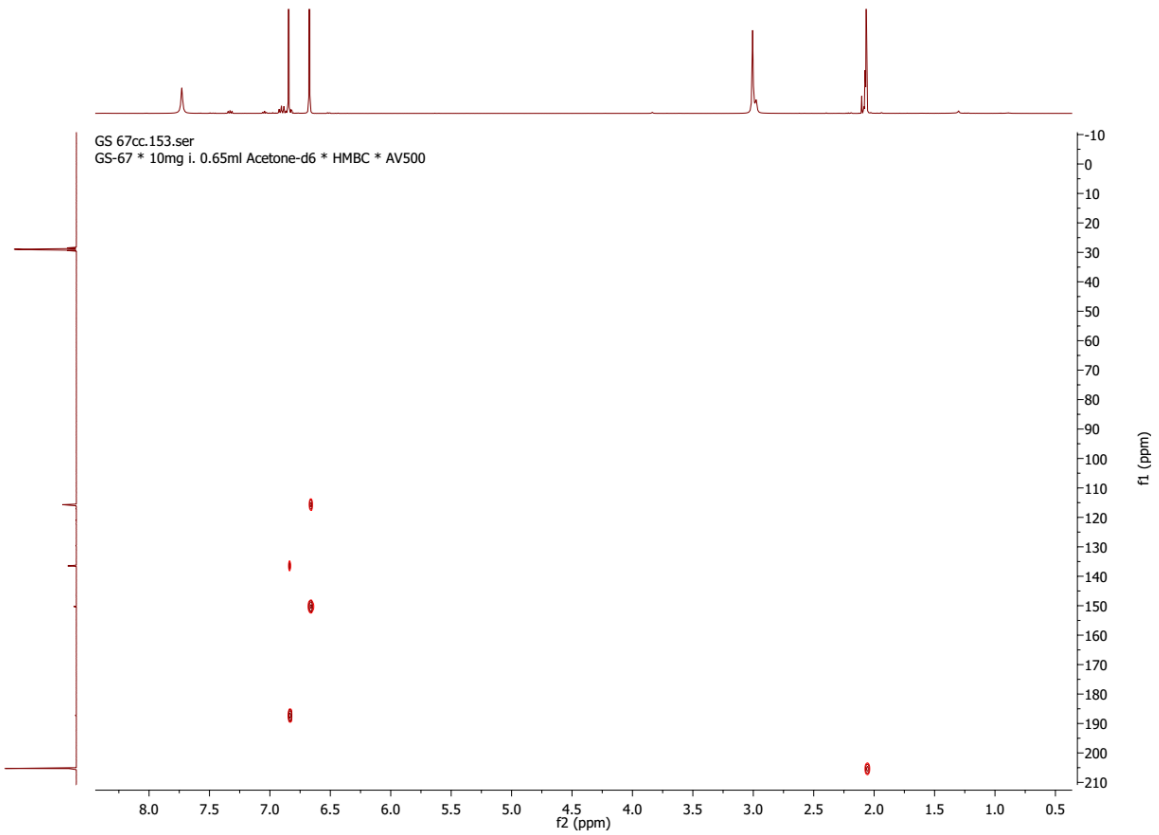

$^{1}$H NMR spectra of 2,5-dinitro-1, 4-benzenediol (**82**)



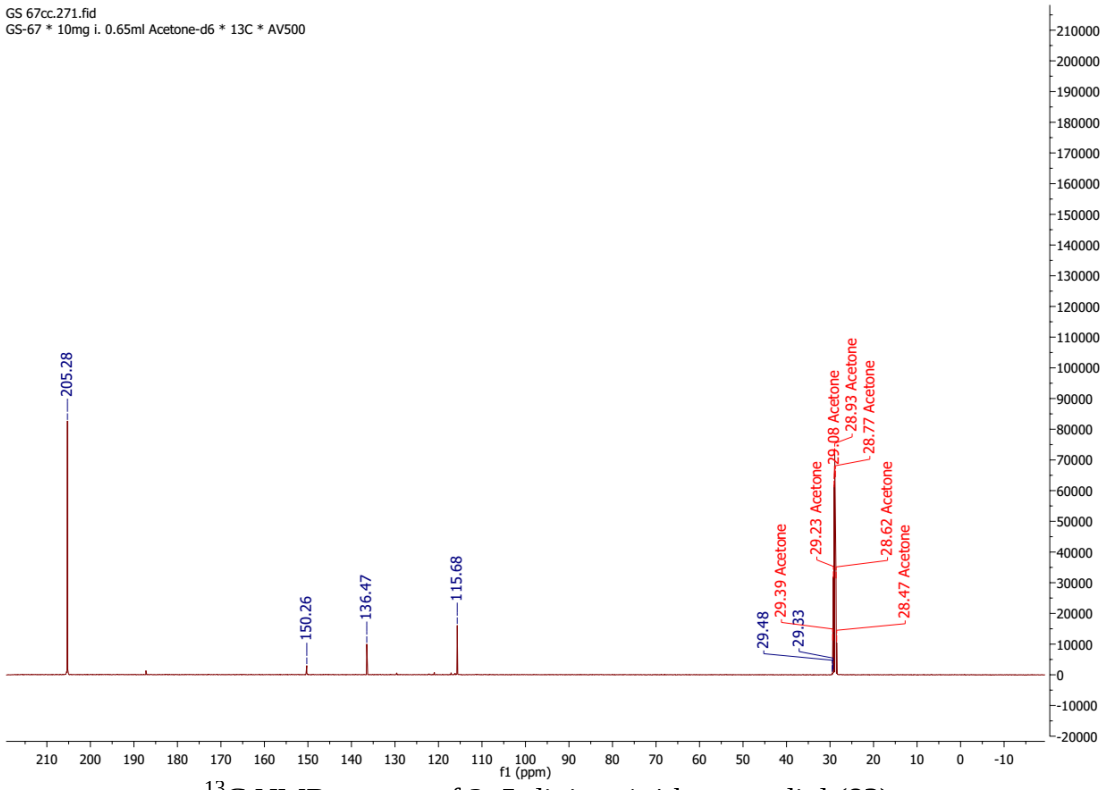

H,H-COSY spectra of 2,5-dinitro-1,4-benzenediol (**82**)




HMQC spectra of 2,5-dinitro-1,4-benzenediol (**82**)

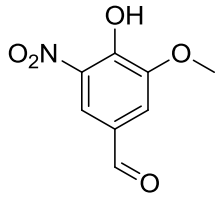


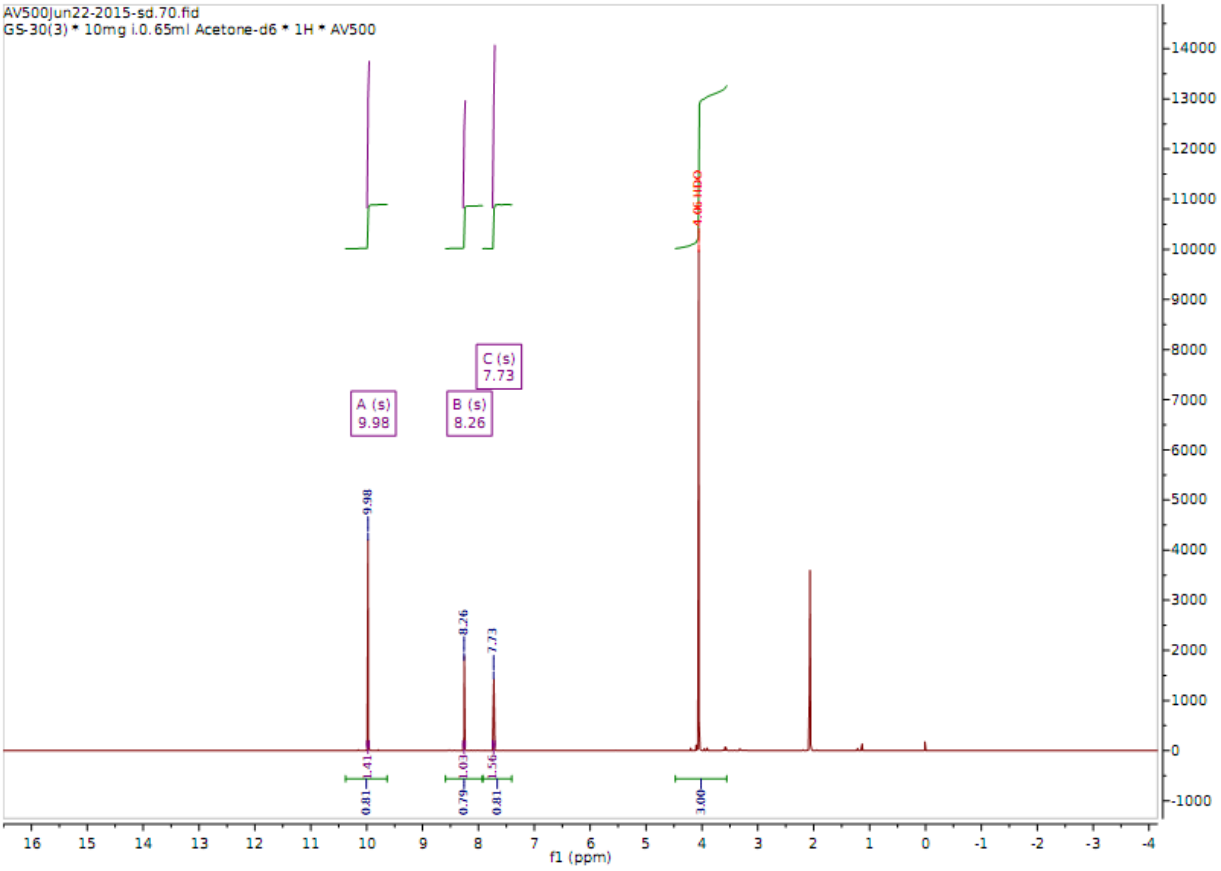

HMBC spectra of 2, 5-dinitro-1,4-benzenediol (**82**)



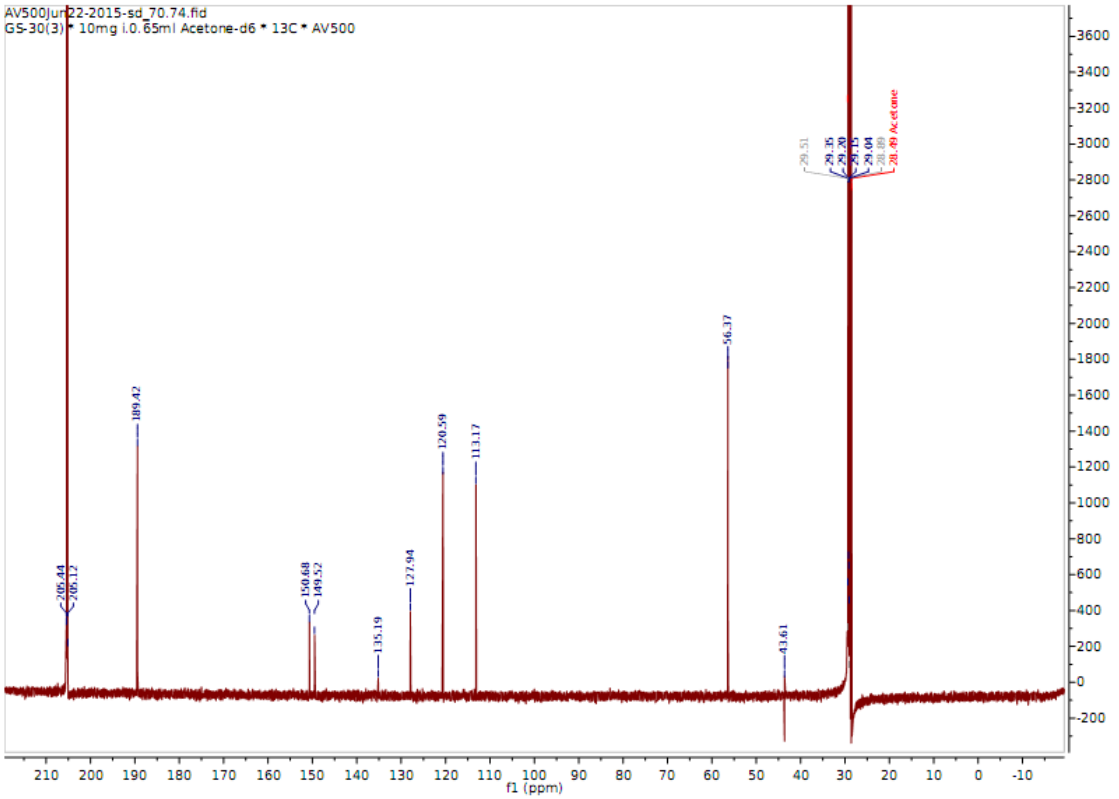

$^{13}$C NMR spectra of 2, 5-dinitro-1,4-benzenediol (**82**)

Appendix 23: The spectra of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)

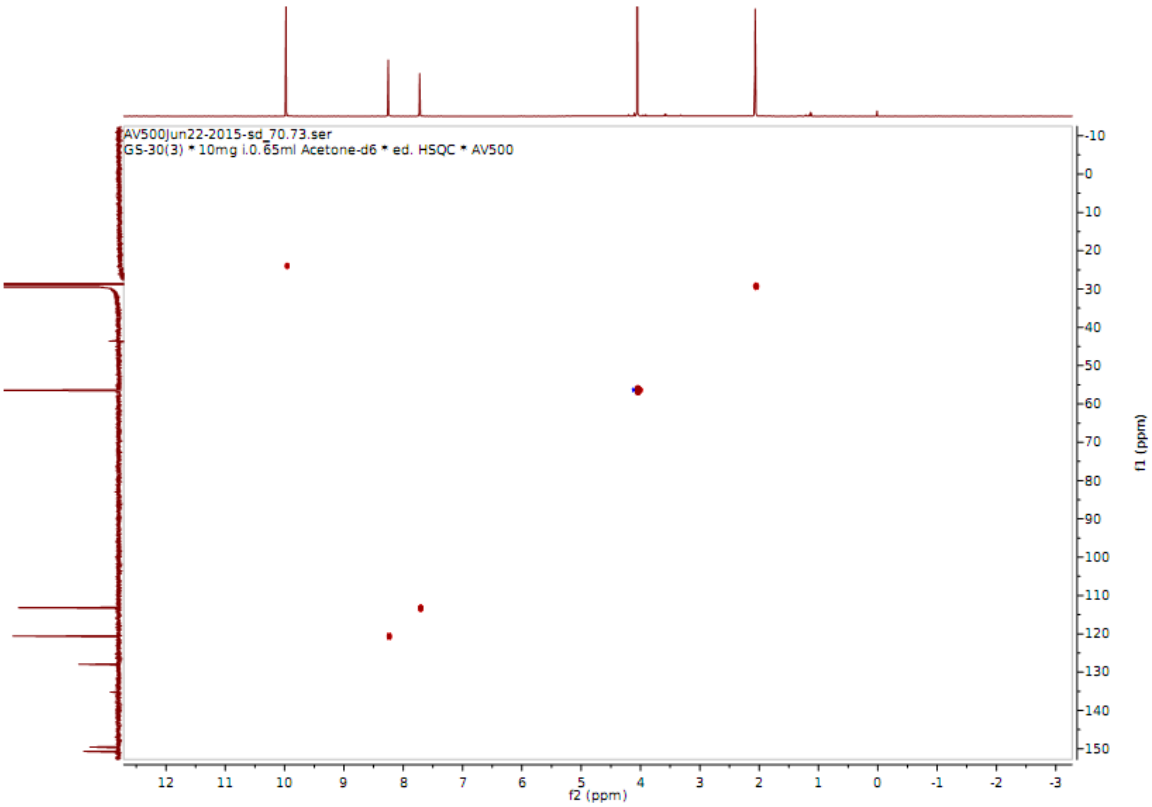



$^{1}$H NMR spectra of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)




$^{13}$C NMR spectra of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)




HSQC spectra of 4-hydroxy-3-methoxy-5-nitrobenzaldehyde (**83**)

Appendix 24: Disc diffusion antimicrobial test of some of the synthesized compounds against various microorganisms