SYNTHESIS AND ANTICONVULSANT STUDIES OF 3 ISOMERS OF 4-[(DIMETHYLPHENYL)AMINO]-4-OXOBUTENOIC ACID

BY

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BY

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FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

AUGUST 2016

DECLARATION

I declare that the work in this Thesis entitled **SYNTHESIS AND ANTICONVULSANT STUDIES OF 3 ISOMERS OF 4-[(DIMETHYLPHENYL) AMINO]-4-OXOBUTENOIC ACID** has been carried out by me in the Department of Pharmaceutical and Medicinal Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Ahmadu Jibril Oludare		
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled **SYNTHESIS AND ANTICONVULSANT STUDIES OF 3 ISOMERS OF 4-[(DIMETHYLPHENYL)AMINO]-4-OXOBUTENOIC ACID** by AHMADU JIBRIL OLUDARE meets the regulations governing the award of the degree of Master of Science in Pharmaceutical and Medicinal Chemistry of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to ALMIGHTY ALLAH (Subhaanahoo Wata'ala)

ABSTRACT

Epilepsy is a disorder of public concern worldwide and has been widely treated via pharmacotherapy, which are faced with challenges such as cost of therapy, side effects, toxicity and lack of selectivity. As such, researchers agree on the need to identify more selective, readily available and lesser toxic antiepileptic drugs. Three isomeric forms was synthesized using equimolar amount of furan-2,5-dione and 2,4-; 2,5- and 2,6- dimethyl isomers of aniline via a nucleophile-electrophile addition reaction at a temperature of 70^0C and refluxed. The compounds A, B and C were synthesized with good yields of 87, 85 and 80 % respectively and melting points of 105, 75 and 90^0C respectively. The structures were elucidated using spectroscopic techniques, particularly 1D and 2D NMR. Acute toxicity studies were carried out using Lorke's method while neurotoxicity was assessed using the beam walk assay. The compounds were screened for anticonvulsant activity using Maximum Electro-Shock Test (MEST) and subcutaneous Pentelenetetrazole (scPTZ) induced seizure test. Compound A, B and C has a median lethal dose of 774.6, 1131.4, and 1131.4 mg/kg respectively. In the neurotoxicity test, the compounds B and C showed no falls when tested against diazepam 1.5mg/kg (40 falls) showing it was not neurotoxic at test doses. In the MES test distilled water 2 ml/kg exhibited a mean time of recovery of 8.3 minutes with 0% protection while the Phenytion 20mg/kg recovered in 2 mins with a 80% protection. Compound A shows a 0% protection and mean time of recovery of 14.7, 10.2 and 10.4 minutes at test dose of 50, 100 and 200mg/kg. Compound B at test doses of 75, 150 and 300 mg/kg showed 0% protection and mean time of recovery of 5.9, 5.2 and 6.5 minutes respectively at all test doses. Compound C at test doses of 75, 150 and 300 mg/kg showed a mean time of recovery of 7.7, 5.8 and 5.8 minutes respectively with a 20%

protection at all test doses. Therefore, compound B and C has activity gainst MEST by decreasing the mean time of recovery from seizure. In the scPTZ test, the distilled water 2ml/kg ip and compound A, B and C showed no protection likewise, no increase in onset against scPTZ induced seizure. Therefore compound A, B and C shows no activity against scPTZ induced seizure. The 3 isomers were synthesized with good yields, characterised, and found to be 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid (A), 4-[(2, 5-dimethylphenyl) amino]-4-oxobutenoic acid (B) and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid (C). They were not neurotoxic and showed no significant quantal protection at test doses but reduced the mean time of recovery from seizure in the MES test. No significant activity was observed in the scPTZ test

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LIST OF ABBREVIATIONS

AEDs = Anti Epileptic Drugs

EEG = Electro Encephalograph

CT = Computerized Tomography

MERRF = Myoclonic Epilepsy with Ragged-Red Fibers

AMRF = Action Myoclonus-Renal Failure Syndrome

NREM = Non Rapid Eye Movement

FTIR = Fourier Transform Infrared Spectroscopy

^{1}HNMR = Proton Nuclear Magnetic Resonance

13CNMR = Carbon-13 Nuclear Magnetic Resonance

COSY = Correlated Spectroscopy

GABA = Gamma Aminobutyric Acid

HSQC = Heteronuclear Single Quantum Coherence Spectroscopy

DEPT 135 = Distortionless Enhancement by Polarization Transfer

MEST = Maximum Electroshock Test

ScPTZ = Subcutaneous Pentylene Tetrazole

IP = Intraperitoneal

mg = Milligramme

kg = Kilogramme

g = Gramme

ml = Milliliter

TLC = Thin Layer Chromatograghy

UV = Ultra Violet Spectroscopy

LD_{50} = Median Lethal Dose

Ca = Calcium ions

Na^+ = Sodium ions

K^+ = Potassium ions

Cl^- = Chloride ions

PWE = people with epilepsy

W H O = World Health Organization

nH 1: 2 EA = N Hexane 1:2 Ethylacetate

HLTE = hind limb tonic extension

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Epilepsy is a chronic disorder of the brain that affects people in every country of the world. It is characterized by recurrent seizures from episodic discharge. Seizures are brief episodes of involuntary shaking which may involve a part of the body (partial) or the entire body (generalized) and sometimes accompanied by loss of consciousness and control of bowel or bladder function. (WHO, 2016)

Brain cells communicate with each other and other cells through electrical signals. Seizure occurs when there is excessive electrical discharge from a group of brain cells (WHO 2016)

There is no recognized cause of epilepsy, although it may develop following a brain damage caused by infection, tumor, trauma or other kinds of neurological defects and diseases (Rang *et al.*, 2007).

Over 50 million people have been estimated to have epilepsy in the world of which about 75% or more are in developing countries and underdeveloped countries with little or no access to proper medical services (Meinardi *et al.,* 2001; Ngugi *et al.,* 2010; WHO 2016).

Communication and coordination of the entire body is partly by the nervous system which consists of the brain the spinal cord. This system is consisting of millions of microcellular unit called the neuron (a nerve cell). This neuron as seen in figure 1.1 consists of a cell

body, an axon (branch) and dendrites which connect one neuron to another at a junction called the synapses by communication of electrical signals across channels in the nerve cell membrane (Shield *et al.,* 2000)

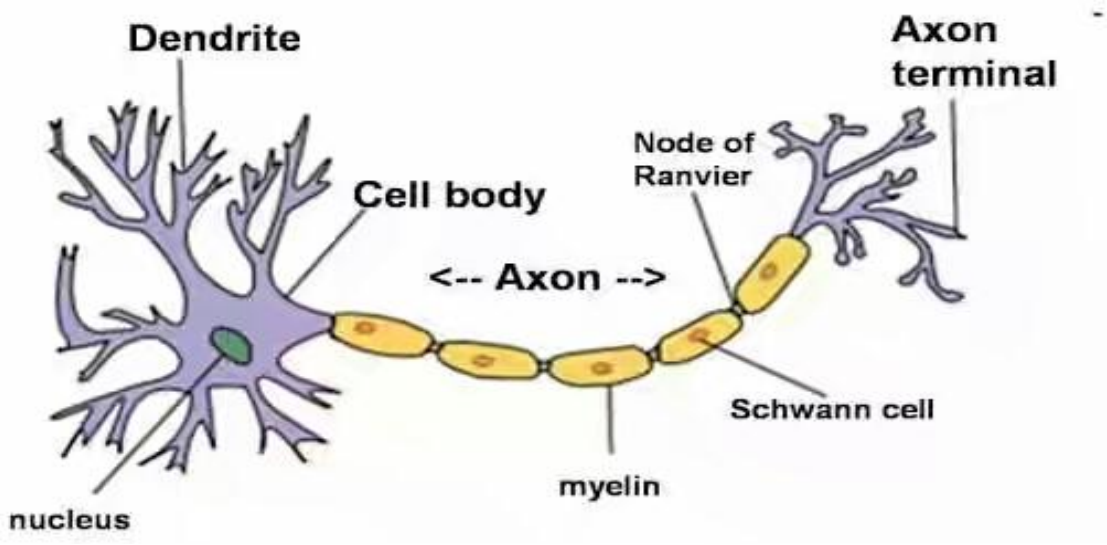


Figure 1.1 The Neuron with the Nerve Cell Body, Dendrites and Axon (Wikipedia)

Transmission from one neuron to another via the synapse is facilitated by a neurotransmitter. Some could be excitatory such as glutamate while others such as GABA are inhibitory to the joining neuron there by electrically inhibiting signals from passing down the neuron it is by this chemical and electrical pathway that millions of neurons communicate and function normally in the brain (Shield *et al.,* 2000).

Along the axon of a neuron from outside are calcium ions while from within are Na and Cl ions which are controlled by gated channels. This complex of ions and its relative balance makes the axon depolarized as such no message or impulse is transmitted. On receipt of a signal, there is a potentiation of the axon called action potential which opens the gated

channels and thereby allowing influx of calcium ion and outflux of sodium and chloride ion. Thereby, giving way for the transmission of impulses.

The relative inability of the brain and the spinal cord to maintain the balance between the chemicals in the synapse and the electrical impulses result in an uncoordinated electrical and chemical trigger within the brain thus resulting in seizures. Antiepileptic drugs work by ways of either decreasing excitation or enhancing inhibition. Specifically, antiepileptic drugs (AEDs) acts via the following ways; alteration of chemical transmission between neurons (GABA, glutamate), alteration of electrical activity in the neuron (Na, Ca, K, Cl) and via unknown mechanisms

1.2 Statement of Research Problem

The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or the need for treatment) at a given time is between 4 to 10 per 1000 people (WHO, 2016). Epilepsy is a public health issue especially in developing countries and is limited to certain factors such as; methods of therapy such as surgery, vagal nerve stimulation, deep brain stimulation, avoidance therapy , alternative medicine and dietary changes have been employed to treat epilepsy, but anti epileptic drugs (AEDs) remain the most widely utilized treatment strategy (Gerlach and Krajewski, 2010). However, pharmacotherapy of epilepsy is confronted with four major problems -

1. About 20-40 percent of epilepsy patients do not respond at all to currently available AEDS (Pavia *et al.*, 1987; Avanzini and Fanceschetti, 2003; Sirven , 2016).

2. Currently available AEDS are associated with toxicity and serious side effects, notably cognitive dysfunction, ataxia, behavioral disorder, hepatotoxicity, gingival hyperplasia megaloblastic anaemia and teratogenesis (Theunissen *et al.,* 1994; Tomson *et al.,* 1997; Zahn *et al.,* 1998; Battino *et al.,* 2000).
3. Continous medication is necessary even after the seizures have long been suppressed (Sasa, 2006).
4. Available AEDS merely treat the symptoms and not the disorder (Sasa, 2006).

1.3 Justification of the Study

The impact of epilepsy to the society goes beyond just the patient but the family and indirectly the society. Stigma has also long been recognized as a major burden to people with epilepsy and their families (Temkin, 1971; Whitman, 1986). In Asia and India the discovery of epilepsy in a couple is a reason for nullification of marriage (WHO 2016). The nature and degree of this stigma vary depending on an individual's premorbid/ baseline status within society and the societal conceptualization of the condition. (Morrell, 2002, Baskind, 2005; Jacoby *et al.*, 2005).

Epilepsy also has serious physical, psychosocial and economic consequences on the concerned persons and their families.Reports from West Africa generally indicate that people believe seizures to be contagious, could be spread by saliva, urine, feaces or flatus expelled during convulsions (Rwiza *et al.,* 1993; Andermann, 2000).

Furthermore, children and adolescent with epilepsy are at increased risk of emotional, behavioral and cognitive disorders (Alwash *et al,* 2000; Oostrom *et al.*, 2000; Adewuya and Ola, 2005).

Indeed the burden produced by epilepsy is enormous and of global concern. Yet, a large proportion of the patients particularly in the developing world go without treatment. This informed the establishment of the Global Campaign against Epilepsy by the World Health Organization (WHO), the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) in the year 2002. This study is aimed at addressing the above problems via research and exploration of other anticonvulsant agent through the synthesis and evaluate the 3 isomeric forms of the synthesized compounds (which are derivatives of carboxamide). To the best of our knowledge the existence, synthesis and anticonvulsants evaluation of these compounds have not been reported in any literature as at the time of commencement of this work. Therefore, this research work is the first of its kind.

1.4 Aim of the study

To synthesize and screen the anticonvulsant activities of 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6-dimethylphenyl) amino]-4-oxobutenoic acid.

1.5 Objectives of the Study

1. To Synthesize and characterize the isomeric forms of the compound
2. To establish acute toxicity as well as the neurotoxicity of the synthesized compounds

3. To evaluate the anticonvulsant activity of the synthesized compounds using maximal electro shock (MES) and subcutaneous pentylenetetrazol (scPTZ) test models.

1.6 Research Hypothesis

The synthesized compound, viz: 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5-dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid poses anticonvulsant activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Epilepsy

Epilepsy is derived from the greek word “epilambanein” which means to “seize or attack” it is a neurological disorder that knows no geographical, social, or racial boundaries occurring in men and women and affecting people across all age ranges especially in the first two decades of life and people over the age of 60 (Sander, 2003).

The disease of Epilepsy has existed for thousands of years but only in the past hundred years or so has it begun to be understood. The only symptom of epilepsy is the epileptic seizure and which can be frightening and strange especially without the knowledge of modern science (Statler, *et al.,* 2005). Recordings of seizures have been recovered as far back as the first days of history and can even be found in the Bible (Statler, *et al.*, 2005). These records tend to be highly superstitious and religious though a few of the more scientifically minded have provided more empirical observations. All of them have attempted to explain and cure this strange phenomenon through a wide variety of methods (Statler, *et al.*, 2005).

In 400 B.C. Hippocrates, the father of medicine, offered another view of epilepsy, that it was just another natural disease and could be treated through natural methods. He supported the use of medicine and control of the diet in order to cure this disease based on his theories of medical methodology. While his methods were hardly scientific he was the first to consider epilepsy to be a natural disorder and would be the only one to do so for

centuries. After the Greeks the Romans took a view similar to that of the Babylonians as to the nature of epilepsy (Statler, *et al.,* 2005).

They believed that the disease was also caused by the presence of demons in a person and believed that if the person breathed on or touched another the demon would spread to the other person unless that person spit immediately. Romans also developed a novel and highly pervasive treatment for epilepsy, using the blood of a murdered individual, particularly the blood of a gladiator. Different philosophers also commented on other things that would be helpful in addition, this included things from the human body such as the liver which made the process of curing the disease a form of cannibalism. This form of treatment was used for quite awhile, the last known incident of it happened in 1908. The introduction of anti-epileptic drugs was what put an end to the blood-cure (Moog and Karenberg, 2003).

Throughout the middle ages and the renaissance epilepsy took on religious connotations again as it did in Greece though there was a difference in opinion between the common people and the nobility as to the nature of the disease. The nobility and the church believed epilepsy not to be a disease at all but rather a sign of prophetic powers and great intelligence. The common people found it to be a terrible illness and sought a cure for it through contact with holy relics. The Malleus Mallificarum, a guide to witch hunting, provided a third take on seizures by designating them as the sign of witch hood and many died because of it. The highly varied social perception of the disease occurred because of both the highly variable nature of the seizures and because of the high degree of difference between the classes (Ahern, 1989).

The first electrical theory of epilepsy was described by Robert Bentely Todd in 1849 in Lumleian lectures he delivered to the Royal College of Physicians (Reynolds, 2001). The first person actually credited with the electrical theory was John Hughlings Jackson in 1873. Between 1859 and 1906 under the leadership of three English neurologists-John Hughlings Jackson, Russell Reynolds, and Sir William Richard Gowers, the modern medical era of epilepsy begins. In a study, Jackson defines a seizure as *"an occasional, excessive and a disorderly discharge of nerve tissue on muscles.*" He also recognizes that seizures can alter consciousness, sensation, and behaviour. In the 1870s Fritsch, Hitzig, Ferrier, and Caton evoked and recorded epileptic seizures in the cerebral cortex of animals. In 1929, Berger discovered that electrical brain signals could be recorded from the human head by using scalp electrodes. This discovery led to the use of electroencephalography (EEG) to study and classify epileptic seizures. Gibbs, Lennox, Penfield, and Jasper further advanced the understanding of epilepsy and developed the system of the 2 major classes of epileptic seizures currently used (Goldersohn *et al.*, 1997).

In 1939 the first animal model for epilepsy was developed allowing the disease to be studied and treatment methods experimented. The same year the first drug (phenytoin III) for the treatment of epilepsy was developed (Janz *et al.*, 1985). After this, a variety of drugs were synthesized and produced and other treatments were developed and refined to create the current range of treatment available for this disease.

2.2 Classification of Epileptic Seizures

Understanding the classification of epileptic seizures is the first step towards the correct diagnosis, treatment and prognosis of the condition (Rudzinski *et al.,* 2011). The initial management of a patient with seizures begins with an understanding of the patient's seizure type.

The classification of epileptic seizures is still largely based on clinical observation and expert opinions. The modern classification of the epilepsy is based upon the nature of the seizures rather than the presence or absence of an underlying cause.

Traditionally, epileptic seizures are divided into "grand mal" and "petit mal'' seizures. ILAE classification of epileptic seizures in 1981 based on clinical observation and EEG findings Seizures were divided into partial and generalized seizures based on loss of consciousness. Partial seizures were divided into simple partial and complex partial based on alteration of consciousness. While in 1989 ILAE classified epilepsy and epileptic seizures on 2 distinctions; Known or presumed etiology Idiopathic, symptomatic, provoked, cryptogenic and Location of pathology (localized or generalized)

2.2.1 Location of pathology (Localized or generalized)

These accounts for 80% of adult epilepsies which are subdivided into; Simple partial seizures, Complex partial seizures and Partial seizures secondarily generalized.

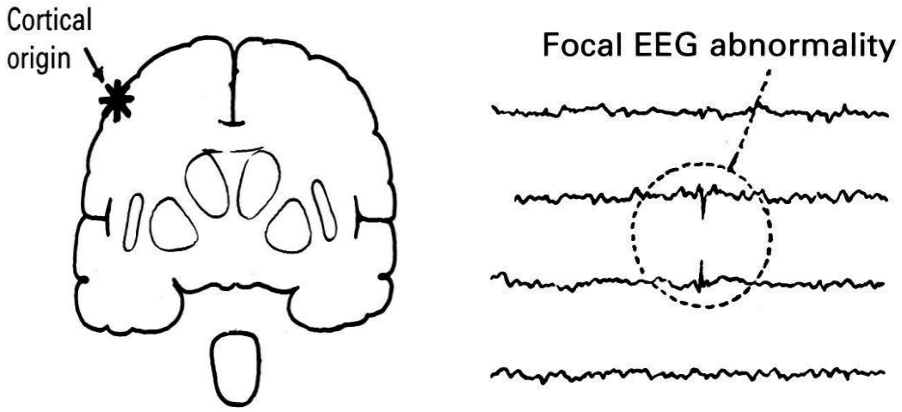


Figure 2.1 showing the point of origin of partial seizure from the Cerebrum and its EEG

Partial or focal seizures comprise one of the two main classes of epileptic seizures, with generalized seizures being the other. Partial seizures are subdivided between simple and complex partial seizures, which are distinguished by the presence or absence of impairment of consciousness.

2.2.2 Complex partial seizures

Complex partial seizures are partial seizures with impairment of consciousness. They may start as simple partial seizures (auras) and progress to complex partial seizures or may begin as complex partial seizures with impairment of consciousness at the onset of the seizure. They may or may not involve automatisms.

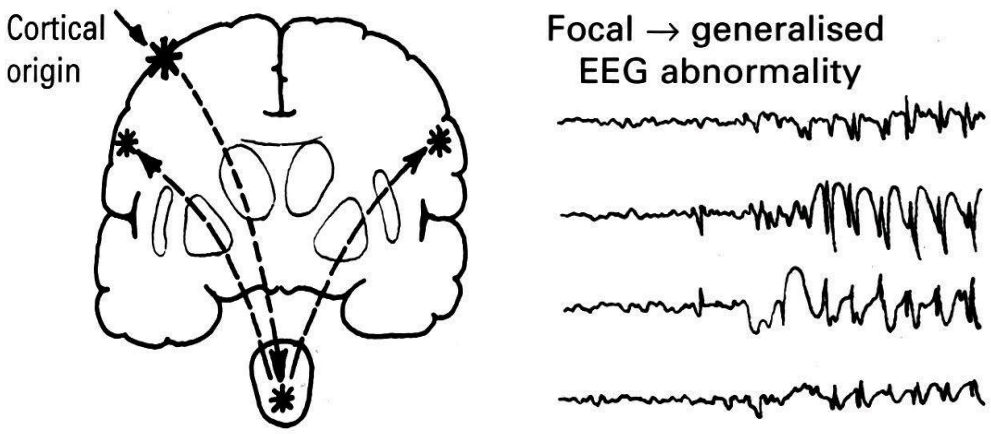


Figure 2.2 showing the point of origin of complex seizure from the Cerebrum and its EEG

The clinical features of the complex partial seizure depend on the region affected by abnormal electrical activity. Complex partial seizures usually originate in the frontal or temporal lobes but can occur in the parietal or occipital lobes.

2.2.3 Generalized seizures

Absence Seizures are characterized by a sudden onset behavioral arrest, a blank stare, unresponsiveness, and sometimes a brief upward rotation of the eyes. The duration is typically a few seconds to half a minute. There is little to no post-ictal confusion, and the patient typically resumes the activity he/she was doing prior to the seizures. This seizure type is also referred to as simple absence.

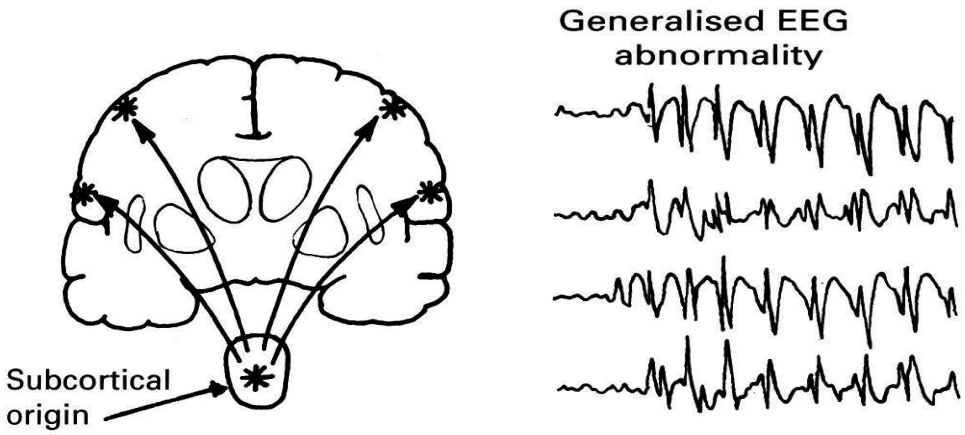


Figure 2.3 showing the point of origin of generalized seizure from the Cerebrum and its EEG

2.3 Early Therapeutic Attempt to Treat Epilepsy

Seizure was, at one time, thought to be due to excessive sexual activity, so bromide was introduced as an aphrodisiac without realizing the depressant action of bromide on the cortical neuron (Locock, 1857). Patients on bromide experienced reduction in a number of fits. So bromide was the first drug to be of proven value despite its obvious drawback of providing benefits at almost incapacitating dose. The bromide soon disappeared from general use because of their unpleasant side effects especially bromism which is characterized by skin rash, irritability, confusion, stupidity, hallucination etc.

Phenobarbitone (I) was first synthesized as superior hypnotic (Fischer and Meringh, 1911).

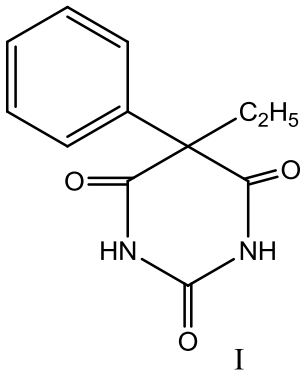
The inability to explain or interpret accurately the basic mechanism of epilepsy can be attributed to the failure of the early physician to control epilepsy. Sir Charles Locock provided the first intimation that seizure was an abnormality of the neuronal function or activity, which could be controlled without seriously interfering with other aspects of the

central nervous function. The discovery of phenobarbitone (I) led to three important advancements:

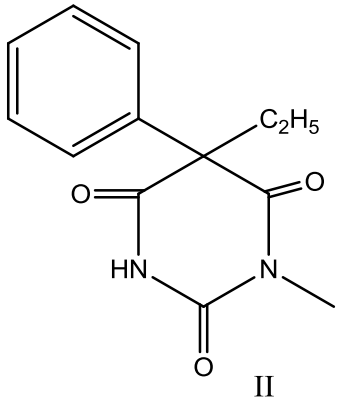
1. It served as substitute for bromides whose toxic effects were just being tolerated because there was no substitute.

2. It was the first drug that proved effective against grandmal epilepsy.

3. It was the first synthetic compound shown to be effective in the treatment of the disease (Swinyard and Goodman, 1972).



I



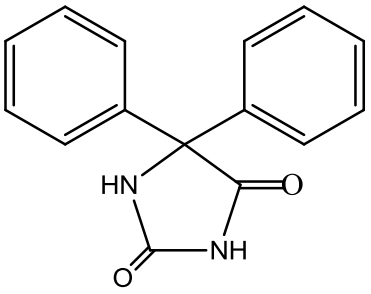
II

Phenobarbital, however, has sedative side effect, a property that often prevents the clinician from using sufficient doses to ensure full protection against epileptic seizures. The second barbiturate drug, mephobarbital (II) was introduced 20 years later for the treatment of grand mal epilepsy (Vida, 1977).

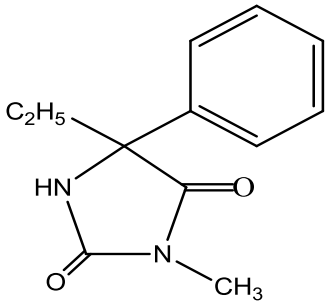
The need for a drug with little or no hypnotic properties arises. In 1934 Putnam and Gibbs established the first encephalographic laboratory for routine clinical study of brain wave, where they observed that epileptic seizures were accompanied by electric storm in the brain and concluded that it was possible to induce convulsion in laboratory animals by

applying electric current to the brain. This led to the discovery of phenytoin due to its similar benzene ring with the phenobarbs. Phenytoin proved to protect cats from electrically induced convulsion. It is also effective in psychomotor epilepsy, which was refractory to previous drugs (Merrit and Putnam, 1938).

Phenytoin (III) was followed by other hydantoins such as mephenitoin (IV)

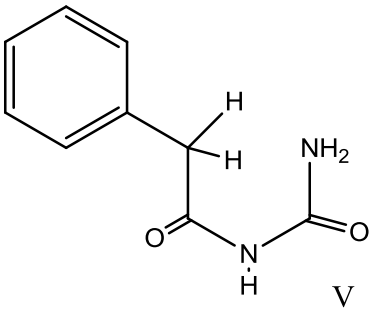


III

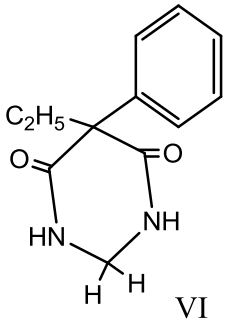


IV

A highly promising anticonvulsant drug, phenacemide (V), was the first noncyclic ureid to gain acceptance in 1954 (Vida, 1977). Initially, this drug was considered to be effective in treatment of grand mal and petit mal psychomotor seizures. Later, it was used primarily for the treatment of psychomotor seizures (Vida, 1977). Ultimately, however, it became obvious that the toxic effects of phenacemide (V) outweighed its advantage as an anticonvulsant.



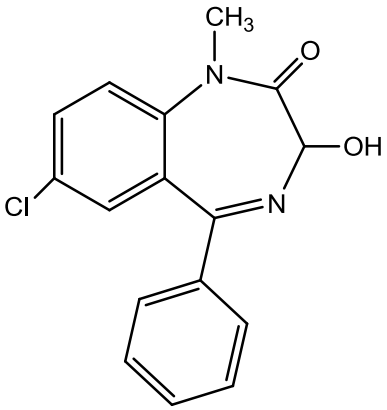
V



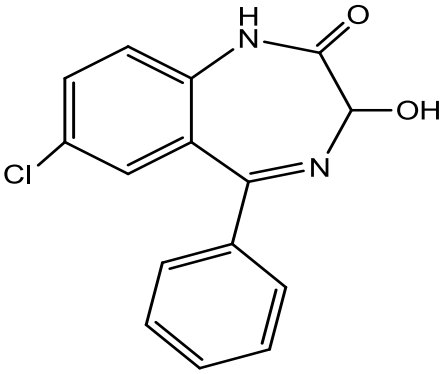
VI

The numerous side effects of hydantoins warranted for search and synthesis of barbiturate analoques. Primidon (VI), a deoxybarbiturate, was introduced in the 1950s as an effective antivonvulsant particularly against grand mal epilepsy.

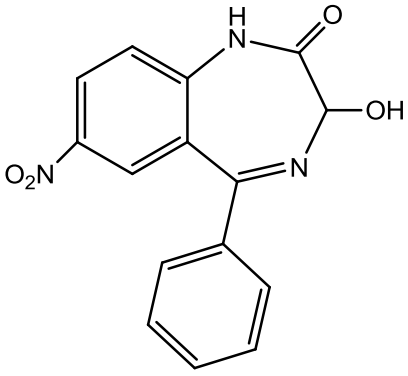
Later, diazepam (VII) was found and particularly very useful in status epilepticum. Other benzodiazepines that were used clinically include oxazepan (VIII), nitrazepan (IX) and clonazepan(X).



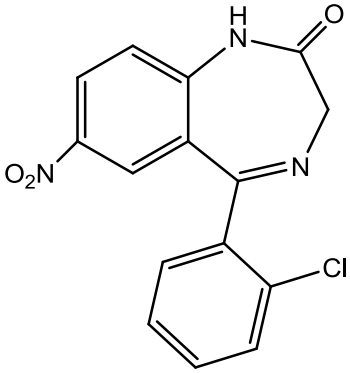
VII



VIII

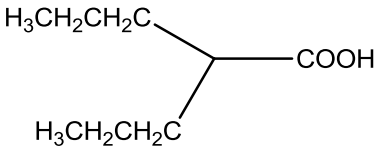


IX

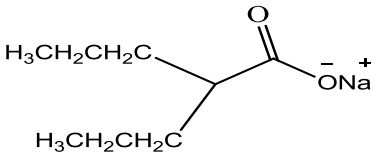


X

Valproic acid (XII) had its anticonvulsant activity discovered serendipitously in an attempt to get suitable solvent to dissolve compounds for biological test (Carraz *et al.,* 1963). The sodium salt of valproic acid (valproate) (XI) is used for the control of various epileptic seizures.

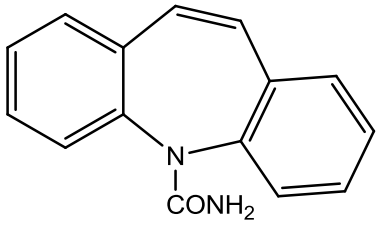


XII



XI

Carbamazepine (XIII) is a tricyclic compound structurally related to the imipramine type of antidepressants. Carbamazepine is the drug of choice for the treatment of grand mal epilepsy and is effective against temporal lobe and generalized seizures (Liu *et al.,* 2006).



XIII

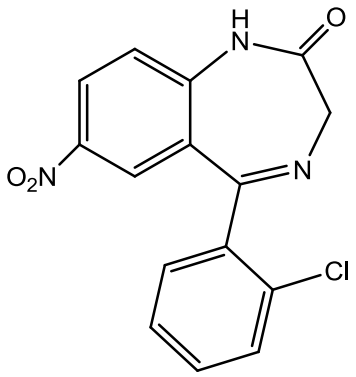
It acts by blockade of voltage sensitive sodium channels and have proven to posses anticonvulsant activities in animals with chemical and electrical induced seizures by reducing or abolishing the pain induced (Marvin, 2010).

Modification of the drug has resulted in oxacarbazepine and eslicarbamazepine, the two most clinically effective drugs for the treatment of epilepsy (Ghalmi, *et al.*, 2003; Dulsat, *et al.,* 2009).

2.4 Newer Antiepileptic Drugs

2.4.1 Clonazepam

Clonazepam (X) belongs to a class of drugs known as the benzodiazepens with diazepam (VII) being its most widely known member. Clonazepam (X) is a derivative of diazepam in an attempt to reduce its addictive effect. It is used either as monotherapy or in combination in the treatment of Lennox-Gastaut syndrome (a variant of petit mal), myoclonic and akinetic syndrome that are not responsive to succinimides.



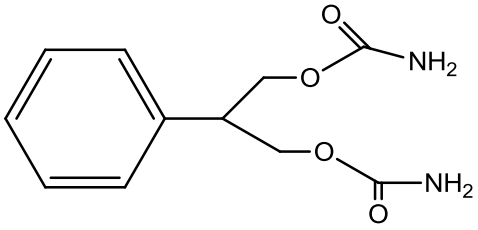
X

Its mechanism is believed to be related to its ability to enhance the activity of GABA, a major inhibitory neurotransmitter in the brain. In some studies about 30% of patients have shown a loss of ant seizure activity due to tolerance (Marvin, 2010).

2.4.2 Felbamate

Felbamate (2-phenyl-1,3-propanediol dicarbamate) (XIV) is a broad spectrum anti seizure agent ,it has a variety of effects on ion channels that could contribute to its therapeutic activity. The drug has been reported to inhibit sustained repetitive spike firing in cultured

neurons, suggesting that it modulates voltage-activated sodium channels (White *et al.*, 1992).

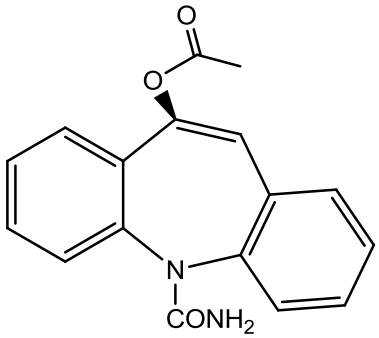


XIV

Felbamate is not a first line drug use in the treatment of epileptic seizures as adverse effects such as aplastic anaemia, hepatic failure are attributed to the drug (Porter and Meldrum, 2007). Its mechanism is unknown. The drug may increase the seizure threshold, and it has weak inhibitory effects on GABA- receptor binding site (Marvin, 2010).

2.4.3 Eslicarbamazepine

This drug is a derivative of carbamazepine. It's a novel CNS- active drug with anticonvulsant activity used in the treatment of adults with refractory, partial-onset seizures. In clinical trials ESL was associated with a significant reduction in seizure frequency compared with placebo in patients with partial-onset epilepsy. The drug acts by blockage of sodium gated channels inhibiting sodium dependent release of neurotransmitter (Marvin, 2010).

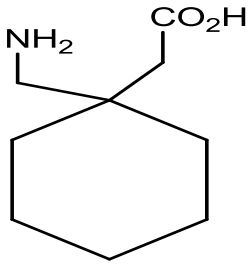


XVI

The drug is an active metabolite of oxacarbamepine and has the same mechanism of action of carbamazepine. In March 2009, (XVI) was recommended as an adjunctive therapy for adults with partial-onset seizures. Ultimately, results from ongoing studies will reveal the clinical utility of this agent as adjunctive therapy for partial onset seizures (Marvin, 2010).

2.4.4 Gabapentin

Gabapentin (XVII) was developed to have a structure similar to that of GABA; however, experimental evidence showed that it has, in fact, little or no action on the GABA receptor. It is highly soluble in water. It enhances the enzyme GAD, but does so weakly. It binds with the α2δ subunit of calcium channels in the cerebral cortex, hippocampus, and spinal cord; this mechanism of action may be important for its efficacy in pain.

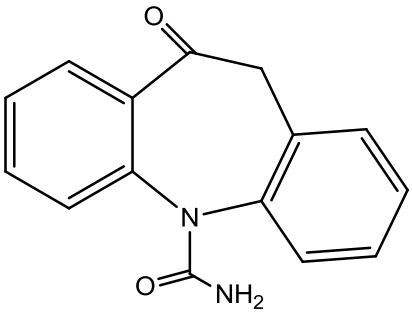


XVII

Gabapentin does not exhibit affinity for benzodiazepine, glutamate, NMDA, strychnine-insensitive or strychnine-sensitive glycine; alpha1, alpha2, or beta-adrenergic, adenosine A1 or A2, cholinergic muscarinic or nicotinic, dopamine D1 or D2; histamine H1; serotonin S1, or S2; or voltage-sensitive, calcium-channel receptor sites (Marvin, 2010).

2.4.5 Oxcarbazepine

Oxcarbazepine (XVIII) is a recently developed analog of carbamazepine (XIII). Oxcarbazepine (XVIII) was developed in an attempt to hold on to the benefits of primidon (VI) while avoiding its auto-induction and drug interaction properties. Licensed in over 50 countries, including the United States, (XVIII) is now considered as one of the first-line therapies in some countries

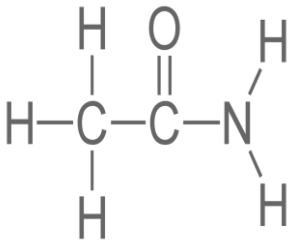


XVIII

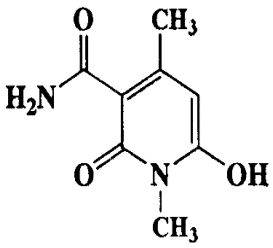
It is a competitive inhibitor of the enzyme-branched chain amino acid transferase, which metabolizes the branch chain amino acids (l-leucine, lisoleucine, and l-valine) to glutamate. Through this mechanism, may reduce brain glutamate levels (Kondo *et al.*, 1991; Backonja *et al.*, 1998; Petroff *et al.*, 2001).

2.5 Carboxamides as anticonvulsants

Carboxamides also known as amino carbonyls are functional groups with the general structure R-CO-NR'R″ with R, R', and R″ as organic substituents, or hydrogen; linear or cyclic, aliphatic or aromatic. The properties and reactivity of the group of compounds arise from the hydrogen bonding capabilities of the -NH_2 group as well as the carbonyl oxygen. The carbon atom in a carboxamide has a characteristic low-lying LUMO that is capable of accepting electron density from the nonbonding lone pair on the nitrogen, weakening the carbon-oxygen bond. Acetamide (XIX) is an example of a simple carboxamide .



XIX

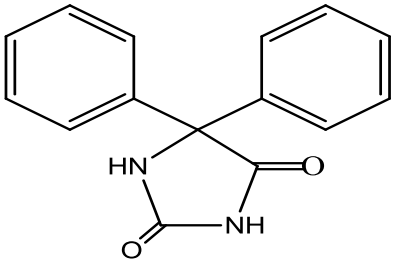


XX

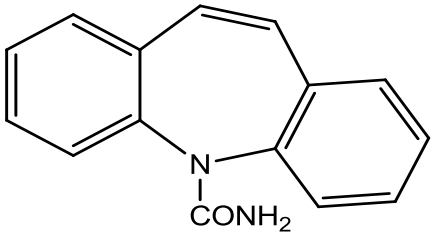
Figure 2.4 showing various forms of carboxamides

Several carboxamides have been launched on the market. The early drug carbamazepine is the drug of choice for the treatment of grand mal epilepsy and is effective against temporal lobe and generalized seizures (Liu *et al.,* 2006). Modification of the drug has resulted in oxacarbazepine and eslicarbamazepine being the two most clinically effective drugs for the treatment of epilepsy (Nain *et al.,* 2015; Dulsat *et al.,* 2009).

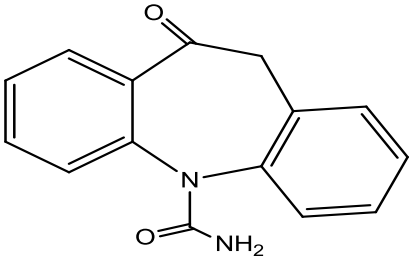
Progabide is the first GABA agonist that is effective in all the three types of epilepsy, generalized tonic-clonic, myoclonic, and partial seizures in both children and adults (Schmidt, D. *et al.*, 1986). The success recorded prompt the need for other studies on possible carboxamides



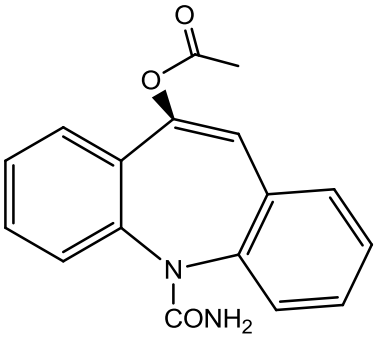
III



XIII



XVIII



XVI

2.5.1 Mechanism of action of carboxamides

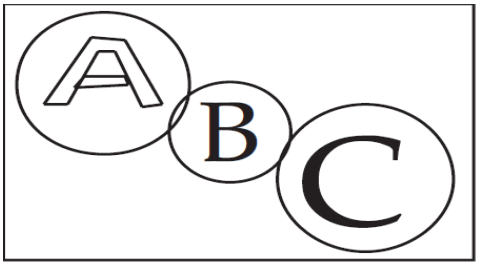
Carboxamides act by blockade of voltage-dependent sodium channels leading to inactivation thereby reducing the ability of the neurons to fire at high frequencies. Carbamazepine, oxacarbazepine, and eslicarbamazepine belong to this group (Benes *et al.*, 1999). Progabide is a GABA agonist as well as blocks the voltage-dependent sodium channels and effective in the treatment of three types of epilepsy viz generalized tonic-clonic, myoclonic and partial seizures occurring in both children and adults. (Nain *et al.*, 2015; Schmidt *et al.*, 1986).

As advancement in pharmaceutical research continued and the need for a structure guided pharmacologic activity of a compound is required, pharmacophores were established by Lemont Kier, who first mentioned the concept in 1967 and used the term in his publication (Kier 1971).

Dimmock proposed the following structural features of an anticonvulsant: aryl binding site responsible for hydrophobic interaction (HPB site);belectron donor system (C=N); and most importantly a hydrogen bonding area as represented by carboxamide $CONH_2$ function (HBD) (Dimmock *et al.*, 1995)

However, the model was not able to explain the Anti Epileptic Drugs having an aryl/ alkyl substituent in close proximity to the electron donor system, like progabide, phenytoin and arylsemicarbazones having substitution in the carboxamide amino function. Therefore, Pandeya (2002) proposed a versatile model to explain the anticonvulsant activity of a large number of AEDs. In addition to the above mentioned three sites, an additional aryl/alkyl site was proposed as pharmacokinetic site. This site was responsible for the

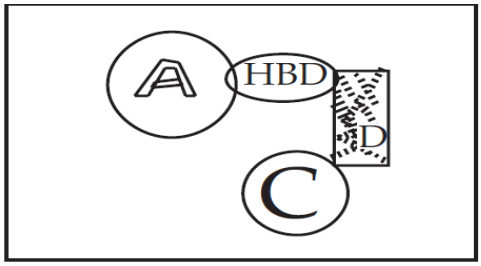
pharmacokinetic properties of the molecule and included a phenyl ring with or without *ortho* substitution like benzodiazepines (Pandeya *et al.*, 2002).



Dimmock model
(graphical)

A & C: Hydrophobic bonding areas

B: Hydrogen bonding site



Pandeya's model

A: Hydrophobic bonding site(HPB)
HBD: Hydrogen bonding domain
D: Two electron donor group
C: Hydrophobic-Hydrophilic site (PKS)

Figure 2.4 Dimmock and Pandeya's Pharmacophore model for Anticonvulsants

However, in 2012 Mohammed and Mohammed *(2012)* proposed a general pharmacophore model for all anticonvulsants as depicted below (Mohammed and Mohammed, 2012).

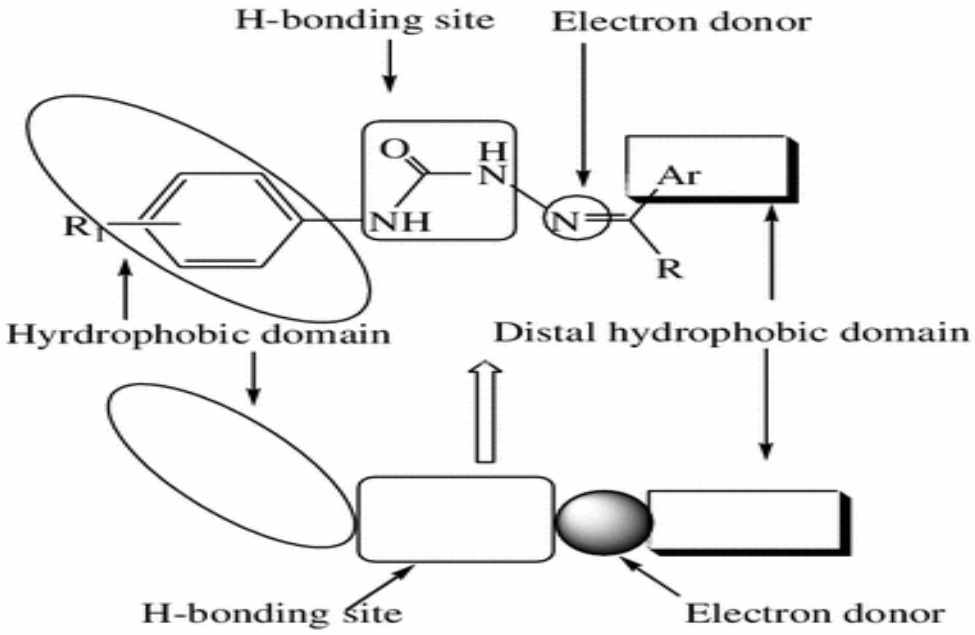


Figure 2.5 Mohammed and Mohammed Pharmacophore model for anticonvulsants

In the conformational analysis of the older generation clinically active anticonvulsant drugs such as Phenytoin, Carbamazepine, Lamotrigene, Rufinamide, Remacemide and Phenobarbitone on the basis of molecular dynamics distance estimations, a molecular model was suggested. According to this, an electron donor (D) in a distance range of 3.2-5.1 Å to an aryl ring or any other hydrophobic unit (R) and of 3.9-5.5 Å to hydrogen bonding domain (HBD) (Yogeeswari *et al.,* 2005). This led to several attempts made to define the structural requirement for anticonvulsants acting at the voltage sensitive sodium channels. Brouillette and co-workers (Brouillette *et al.,* 1990; 1994; and 1988) investigated the sodium channel blocking activity of several mono and bicyclic phenytoin analogues and concluded that high log P values, a free amide group, and a specific aromatic ring orientation are optimal for high binding affinity to the sodium channel. Although these

criteria were well fulfilled in monocyclic hydantoins, the conclusions of these studies were not applicable to other classes acting at the same receptor site.

Unverferth *et al.* (1998) employed five well known but structurally different anticonvulsants with sodium channel blockade activity [carbamazepine, phenytoin, lamotrigine, Zonisamide, and Rufinamide to propose a 3-point pharmacophore for sodium blockade activity. The model comprises an electron donor D in relatively limited distance ranges of 3.2-5.1 A^{o} to and aryl ring or other hydrophobic units R and of 3.9-5.5 A^{o} to a hydrogen bond acceptor/donor unit. The distance between R and HAD spans a wider range of 4.2-8.5 A^{o}. The hydrophobic unit R is not necessarily oriented in the same plane like the other essential elements.

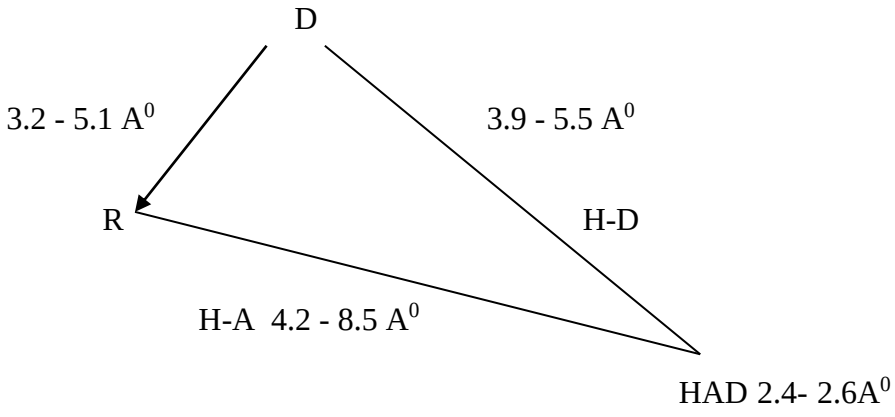


Figure 2.6 Unverferth *et al.* (1998) proposed Pharmacophore

This pharmacophore model is well fulfilled by many other structurally different anticonvulsants compounds that act at the voltage sensitive sodium channels including vinpocetine, dezinamide, ramacemide and the 3- aminopyrroles (Unverferth *et al.*, 1998)

Shindikar *et al.*, (2006) proposed a two-point pharmacophore (figure 2.8), consisting of an aromatic center separated from an H-Bonding acceptor/donor group within a distance range of 4.18-6.88 A^o, for anticonvulsants active at the sodium chanels. This pharmacophore agrees with Unverferth's proposal.

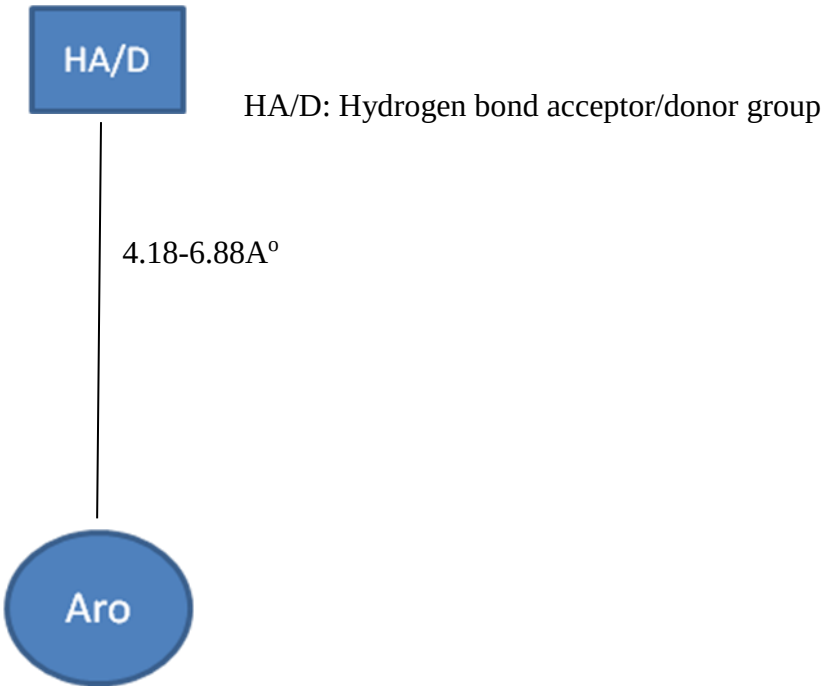


Figure 2.7 Shindikar *et al.*, (2006) proposed Pharmacophore for anticonvulsant activity.

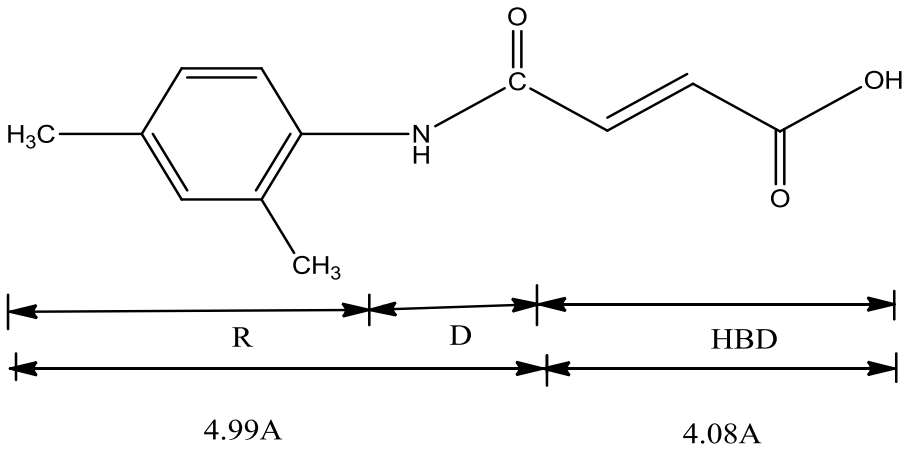


Figure 2.8 Distances between the various groups essential for anticonvulsant activity

The 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid are carboxamides that consist essentially of an hydrophobic aromatic ring, an electron donor (D) and a hydrogen bond acceptor/donor group HA/HD (carboxyl group) and the distances between R to HAD for 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid is 6.965 A^o; the distance between R to D , D to HAD is 3.28A^o and 5.23A^o respectively.

The dicloro isomeric forms of these compounds, that is: 4-[(2, 4- dichlorophenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dichlorophenyl) amino]-4-oxobutenoic acid have been synthesized and found to have proconvulsive activities (Abdullahi *et al.,* 2013). This could be due to a decrease in lipohilicity of the dichloro substitute on the aromatic ring thus, reducing the ability of the drug to cross the blood-brain barrier that is necessary for activity. On further studies, the need to structurally modify these compounds to a more lipophylic state led to the replacement of the dichloro- substituents to a dimethyl substituent on the aryl moety as seen in this work.

Theoretically, the 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5-dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid appear to satisfy Unverferth *et al., (*1998) and Shindikar *et al.,* (2006) proposed pharmacophores for compounds acting at voltage sensitive sodium channels. In this work 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid are synthesized and screened for anticonvulsant activity using the Maximal Electroshock (MES) and Subcutaneous Pentylenetetrazol (ScPTZ) seizure test models. Moreover, since alkyl or aryl substitutions on the aromatic ring may increase lipophilicity thus enhancing the penetration into the blood brain barrier for effective anticonvulsant activity. It was thought that a dimethyl substituents on the aryl ring could lead to a very promising AEDs and its rotation from the various positions on the ring might help determine which of the isomers best interact with the receptor sites.

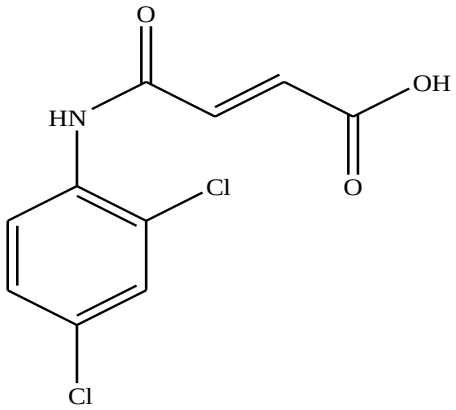


Figure 2.9 Structure of the Dichloro Isomeric form of the compound to be synthesized

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Gallenkamp melting point apparatus, UV absorption spectrophotometer, 400MHz Bruker ULTRASHIELD PLUSTM 400 NMR spectrometer, Shimadzu FITR spectrometer, Ugo Basile electroconvulsive unit (ECT800) fitted with ear clip electrodes, Analytical balance (Mettler AE-240), Steam/water bath (Gallenkamp), Oven (BS model OV33 Gallenkamp), Thin layer chromatographic (aluminium) plates pre-coated with 0.2mm silica gel (Merck) and Glass wares

3.1.2 Chemicals

2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, furan 2,5-dione, Methanol, Ethylacetate, N-Hexane, Phenytoin, (Sigma-Aldrich, St Louis MO), Valproic acid (Sigma-Aldrich, St Louis MO), pentylenetetrazol, distilled water, toluene and acetone. All the solvents purchased were of analytical grades and were used without further purification

3.1.3 Animals

200 locally bred Swiss albino mice obtained from the Department of Pharmacology and Therapeutics, ABU, Zaria and 200 day-old white cockerels obtained from the National Animal Production Research Institute, NAPRI, Zaria were used. Ethical approval was obtained and the animals were kept under standard temperature and pressure with unrestricted access to food and water.

3.2 Methods

3.2.1 Percentage Yield

The percentage yield of compound A, B and C using the formula below;

(Actual yield / theoretical yield) X 100

3.2.2 Chemistry of the reaction

The starting materials for the synthesis of 4-[(2, 4- dimethylphenyl) amino]-4-oxobutenoic acid, 4-[(2, 5- dimethylphenyl) amino]-4-oxobutenoic acid and 4-[(2, 6- dimethylphenyl) amino]-4-oxobutenoic acid are 2,4 dimethylaniline, 2,5 dimethylaniline, 2,6 dimethylaniline and furan 2,5 dione (maleic anhydride). The reaction mechanism conforms Nucleophile-electrophile addition reaction (the dimethylaniline is the nucleophile and the furan 2,5 dione (maleic anhydride) is the α,β-unsaturated carbonyl compound, the electrophile as illustrated in figure 3.1and 3.2

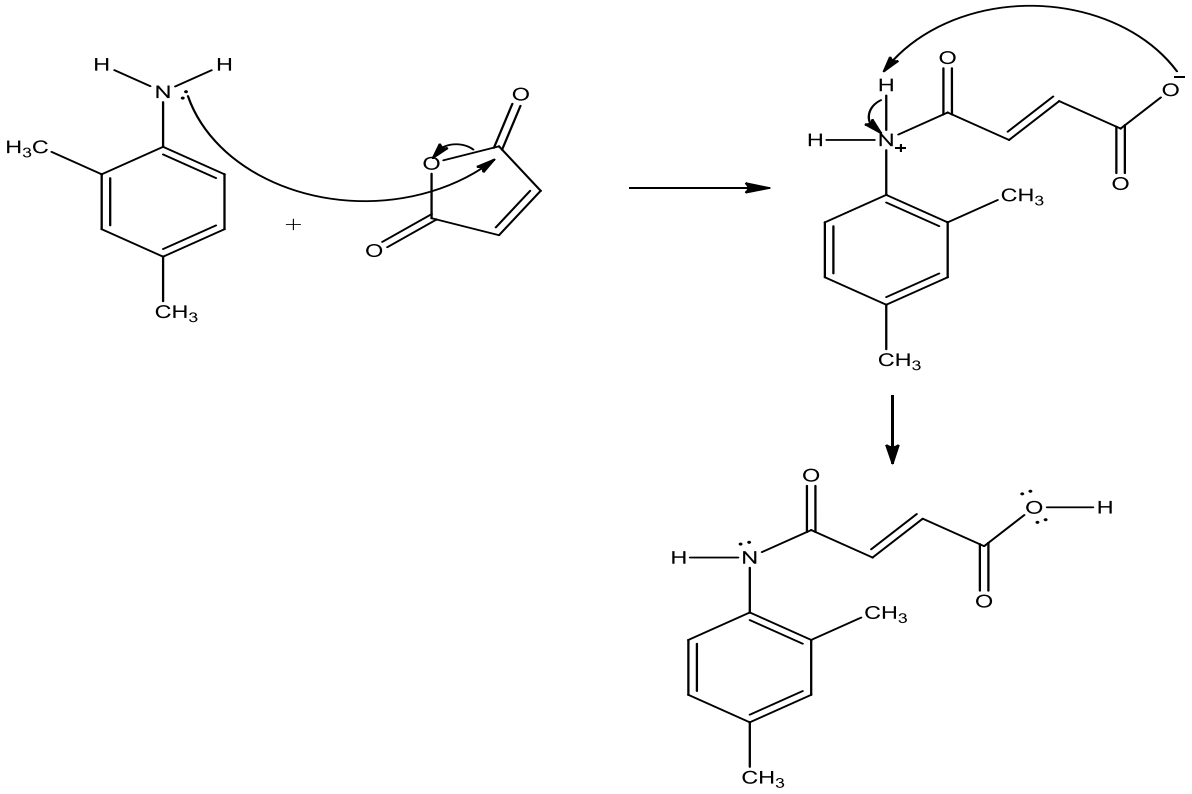
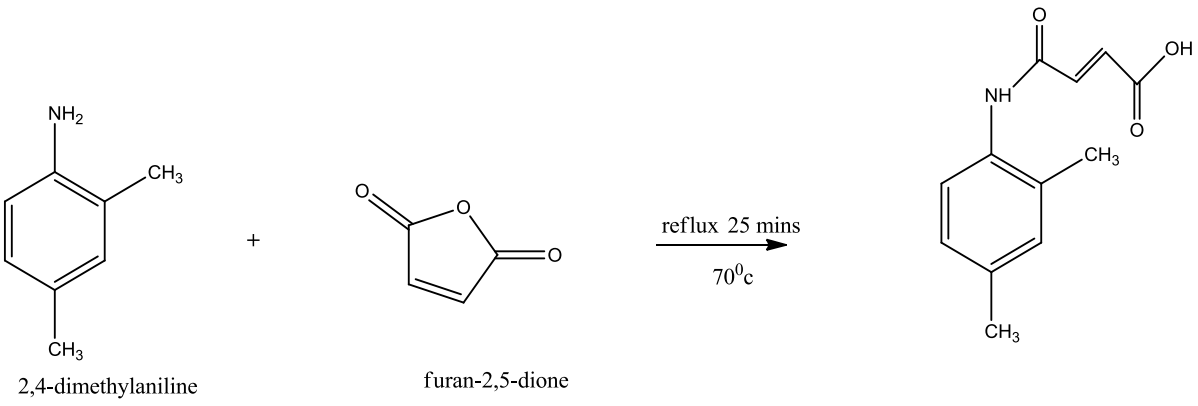
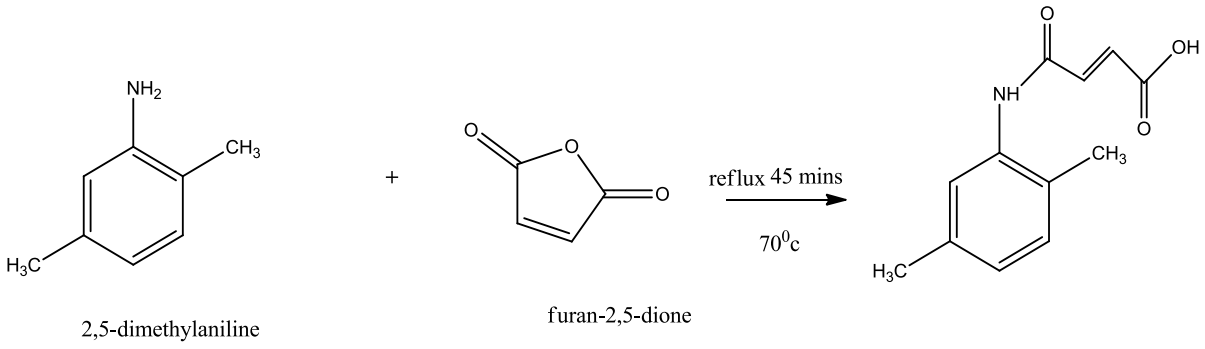


Figure 3.1 Scheme showing the mechanism for the synthesis of isomeric form of 4-(dimehylphenyl)amino]-4-oxobut-2-enoic acid.

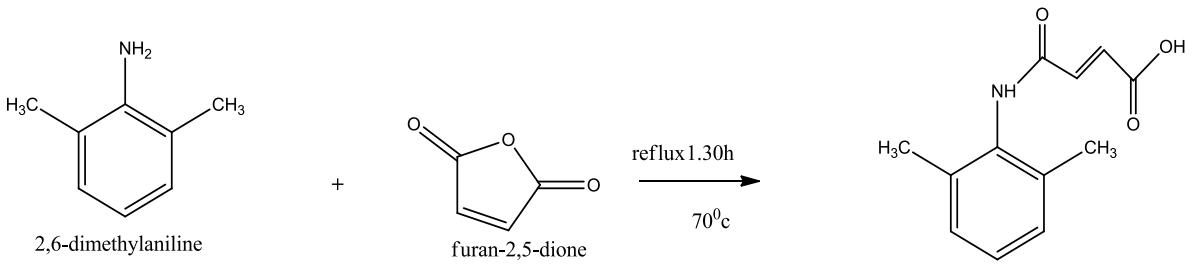




2,5-dimethylaniline

furan-2,5-dione

4-[(2,5-dimehylphenyl)amino]-4-oxobut-2-enoic acid (B)



2,6-dimethylaniline

furan-2,5-dione

4-[(2,6-dimehylphenyl)amino]-4-oxobut-2-enoic acid (C)

Figure 3.2 Scheme showing the synthesis of isomeric forms of 4-(dimehylphenyl)amino]-4-oxobut-2-enoic acid.

3.2.3 Procedure for the synthesis of isomeric forms of 4-[(dimehylphenyl) amino]-4-oxobut-2-enoic acid**.**

Dimethyl substituted anilines (2,4 ; 2,5 and 2,6) 0.4865g equivalent to 4.015mmol were dissolved in 5mls toluene and each separately reacted with Furan 2,5 dione (maleic anhydride) 0.3937g equivalent to 4.015mmol dissolved in 10 mls toluene in a round

bottom flask. The mixtures were refluxed with a magnetic stirrer for 25, 45 and 90 minutes respectively at 70^0C.

The mixtures were allowed to cool to room temperature for about 1 hour and filtered. The residues were washed with toluene, dried and labeled accordingly.

3.3 Characterization of isomeric forms of 4-[(dimethylphenyl) amino]-4-oxobut-2-enoic acid.

3.3.1 Melting Point Determination of the synthesized compounds

All melting points were determined using a Gallenkamp melting point apparatus (Electrothermal^R) by inserting a small quantity into the capillary tube and inserted into the apparatus. The temperature when the powder starts melting and that which the powders completely melt was recorded as a range for the 3 isomeric forms.

3.3.2 UV- Visible Spectroscopy

UV-Visible spectra were obtained and recorded using a Biomate Thermo Scientific UV-Visible Spectrophotometer (Part No. 9423BIO1102E, Serial No. Bio-164312) of the Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University Zaria, Nigeria.

3.3.3 Infrared (IR) Spectroscopy

FTIR Spectra were recorded on a BRUKER TENSOR 27 platinum ATR scan (wave length) : 500-4000 per cm. Base line correction was performed before each scan at the Polymer Science Laboratory of the Nelson Mandela Metropolitan University, South Africa. The samples were each at a time placed crushed into a thin film of the powder with

Potassium Bromide (KBr) salt to enable the film formation. KBr was used for the base line correction before the film containing the sample was subjected to Infrared radiation and observed for the various vibration frequencies of its corresponding functional groups.

3.3.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Proton and carbon-13 NMR (1H NMR and 13C NMR) spectra were recorded on a Brůker ultrashield plus^{tm} (400 MHz), Nelson Mandela metropolitan University South Africa; $CDCl_3$ (Deuterated chloroform) was used as solvent, Data for 1HNMR are reported as follows; chemical shift (δ ppm), Integration was done on the ^1H NMR and the coupling constant (measured in Hz); Data for 13CNMR are reported in terms of chemical shifts.

3.4 Biological Studies

3.4.1 Acute Toxicity Studies in Mice (LD_{50})

LD_{50} determination was conducted using the method of Lorke, (1983). In the initial investigation mice were divided into three groups of three mice each and treated with the drugs at 10, 100 and 1000mg/kg body weight i.p. each of compound 2,4; 2,5 and 2,6 coded as A, B, and C and observed for 24 hours. The results of these tests were used as the bases for selecting the subsequent dosages for the second phase of the study. The LD50 was calculated as the square root of the product of the lowest dosage that kill all the animals and the highest dosage survived by the entire animal in the group.

3.4.2 Neurotoxicity Studies in Mice

Beam walking neurotoxicity test was carried out as described by Stanley *et al.* (2005) by training 48 Swiss albino mice severally (about 6 times) to walk from a start platform along

a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports to a metal box at the other end until a memory of the path has been established in the mice. They mice were grouped into 8 sub-groups each containing 6 mice, group I was administered ip diazepam 1.5 mg/kg as positive control and group II was administered distilled water10 ml/kg ip as negative control. Group III-VI were administered different doses of 300, 150 and 75 mg/kg ip of compound B and 300, 150 and 75 mg/kg ip of compound C respectively and placed to walk a narrow beam 30 minutes after the drug was administered. The mice were carefully observed for three basic parameters namely; time taken to complete the task, number of foot slips and number of falls for a period of 30 minutes.

The time taken to complete the task and foot slips predict motor coordination deficit with the number of foot slips having the potential to predict clinical sedation and is being used to evaluate for motor coordination deficit. The fall from the beam is an index of neurotoxicity (Stanley, *et al.,* 2005).

3.5 Anticonvulsant Screening

3.5.1 Maximal Electroshock Induced Seizure test in Chicks

The method of Swinyard *et al.,* (1989) was employed. UGO Basile electroconvulsive unit ECT 800 fitted with ear clip electrodes and shock duration of 0.6 seconds, frequency 100Hz, pulse width 0.6ms, and current 70mA was used. The standard drug (positive control) was phenytoin and distilled water was used as the negative control.

110 day-old white ranger cockerels were randomly divided into eleven groups of 10 chicks each. Group 1 (negative control) were administered distilled water interperitoneally i.p. Group II (positive control) were administered 20 mg/kg phenytoin. Groups III-V were administered compound A 50,100 and 200 mg/kg (dissolved in distilled water) i.p. respectively. Groups VI-VIII were given compound B 75,150 and 300 mg/kg i.p. (dissolved in distilled water) respectively and Groups IX-XI were given compound C 75, 150 and 300 mg/kg i.p. (dissolved in distilled water) respectively.

Thirty minutes later, maximum electroshock was delivered to induce seizure in all the groups hind limb tonic extension (HLTE) and observed for the occurrence of seizures; the abolition of the HLTE was considered as protection from electroshock induced convulsion.

3.5.2 Pentylenetetrazol (PTZ) Induced Seizure in Mice

The method of Swinyard *et al.,* (1989) was employed. Sixy six (66) mice were divided into 11 groups of 6 mice each. Group 1 (negative control) received distilled water i.p. Group II (positive control) received valproic acid 200 mg/kg i.p. Groups III-V received compound A 50, 100, and 200 mg/kg i.p. respectively. Groups VI-VIII were given compound B 75,150 and 300 mg/kg i.p. respectively and Groups IX-XI were given compound C 75, 150 and 300 mg/kg i.p. respectively.

After 30 minutes, all the groups were administered freshly prepared PTZ subcutaneously. All the mice were observed for a period of 30 minutes after scPTZ for episodes of clonic spasms of at least 5 seconds duration with loss of righting reflex.

The time of onset of seizure were observed and recorded. Absence of clonic spasms in the 30 minutes observation period indicates compound ability to abolish the effect of pentylenetetrazol on seizure threshold (Swinyard *et al.*, 1989).

3.6 Statistical Analysis

The mean onset of seizure and mean recovery time were presented as mean ±standard error of mean (SEM). The mean values of the control were compared with the mean values of the groups treated with the test compounds using one-way ANOVA, Bonferonni and Kruskarl-Wallis statistical kit. The result was considered significant at $p \leq 0.05$

CHAPTER FOUR

4.0 RESULT

4.1 Yield Obtained

The percentage yield of compound A, B and C were calculated to be 87%, 85% and 80% respectively

4.2 Physical Properties of Compound A, B and C

The melting points of compound A, B and C all had narrow ranges and their physical appearance are as tabulated below;

Table 4.1 showing physical properties of compound A, B and C

	Compound A	**Compound B**	**Compound C**
Melting point ^{0}C	105-107	75-77	90-92
Colour	Yellow	Light Green	Chocolate Brown
Form	Powder	Powder	Spiky Crystals
R_f value : (N-Hexane 1:2 ethylacetate)	0.42	0.64	0.46

A = 4-[(2,4-dimehylphenyl)amino]-4-oxobut-2-enoic acid; **B** = 4-[(2,5-dimehylphenyl)amino]-4-oxobut-2-enoic acid and **C** = 4-[(2,6-dimehylphenyl)amino]-4-oxobut-2-enoic acid

4.2 TLC Profile of reactants, intermediates and products as reaction progresses

Progress and purity of all the reactions was monitored using thin layer chromatography (TLC) as shown in the Figure 4.1;4.2 and 4.3 below.

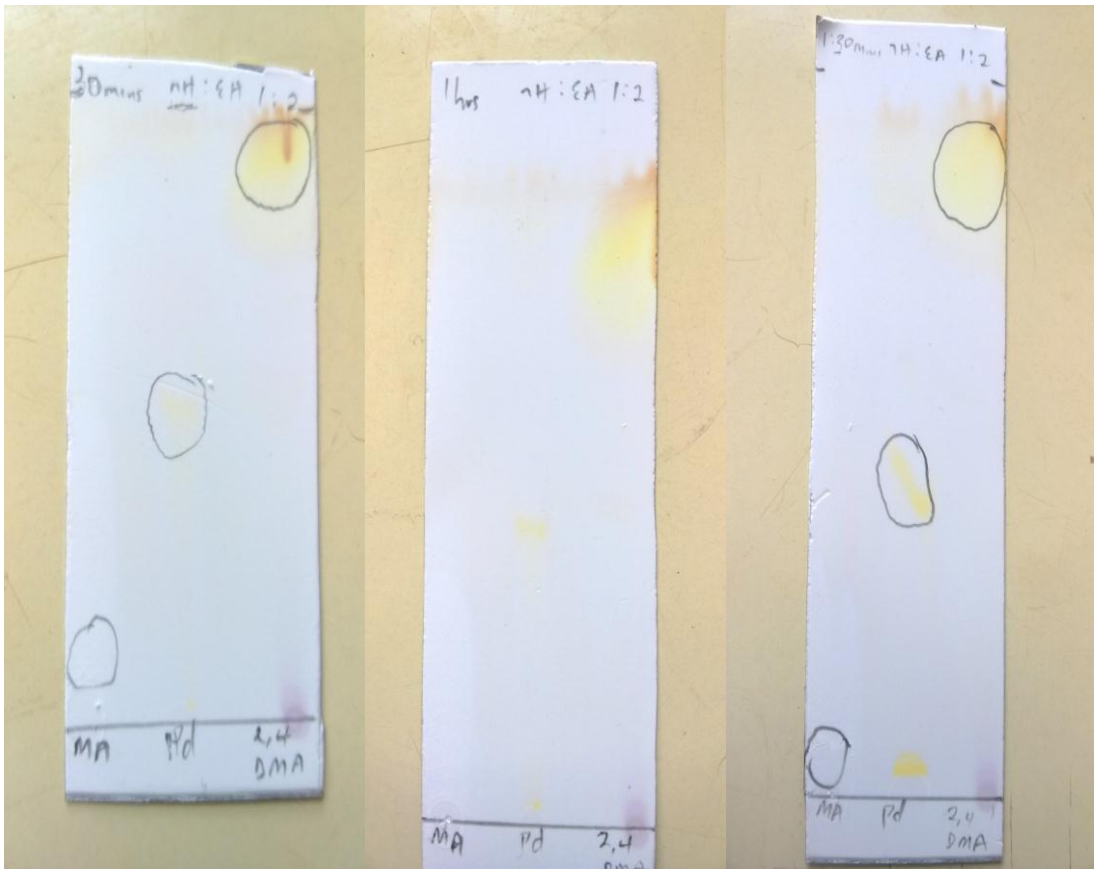


Figure 4.1 TLC Profile of Reactants and Product for compound A using N-Hexane 1:2 Ethylacetate Solvent systems

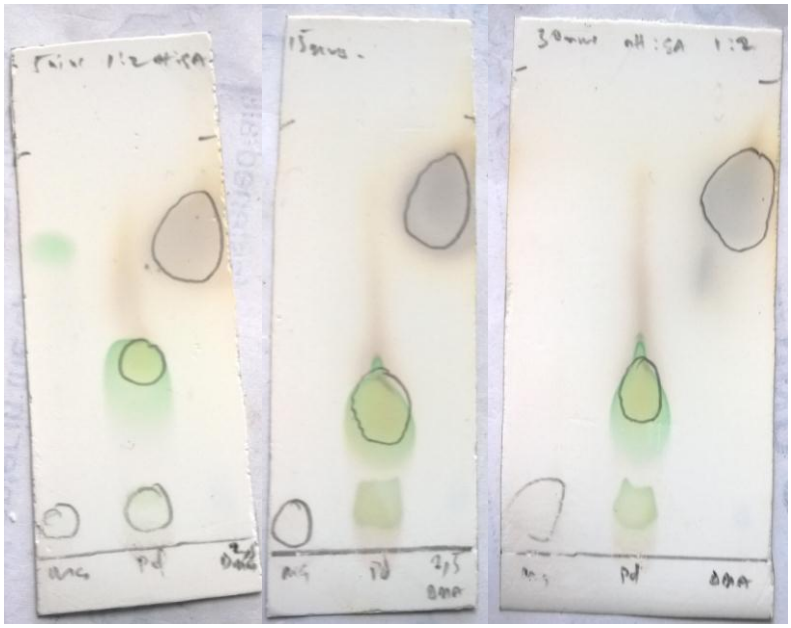
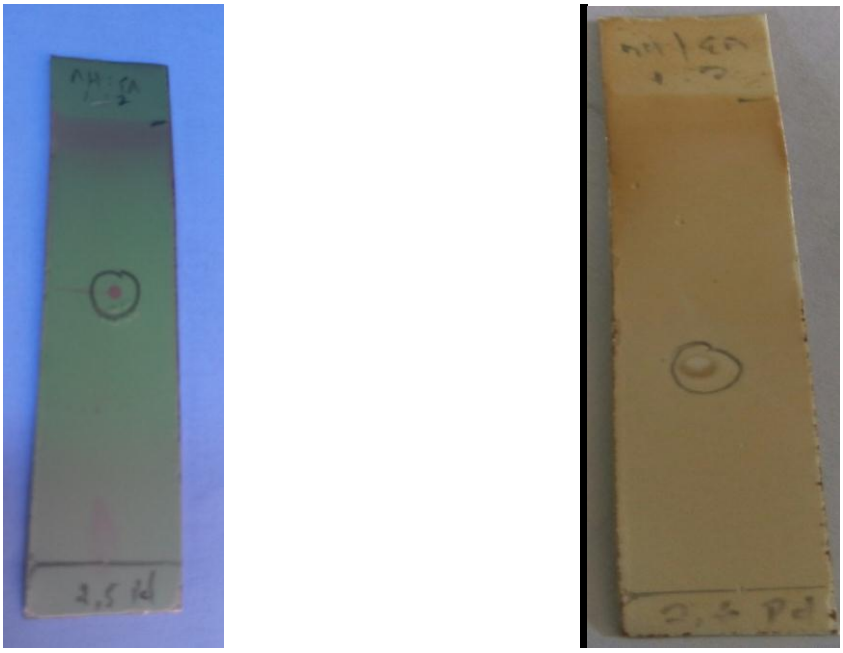


Figure 4.2 TLC of Reactants and Product as reaction progresses for compound B using nHexane 1: 2 Ethylacetate



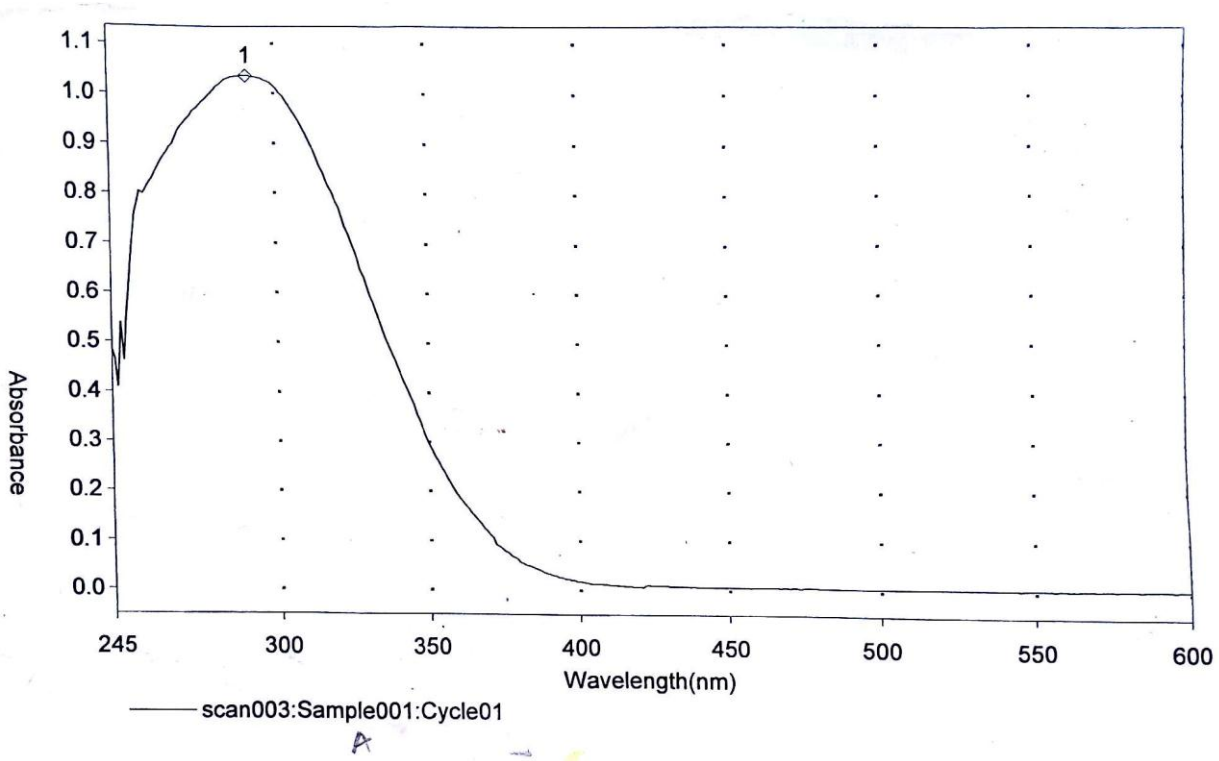
A B

Figure 4.3 TLC of final products B (R_f=0.64) and C (R_f=0.46) after recrystallization nH 1: 2 EA

4.3 UV-Visible Spectroscopy

The UV-Visible spectra of the compounds A, B and C exhibited maximum absorbance at 291, 285 and 257 nm respectively as shown below in Figure 4.4; 4.5 ad 4.6

The wavelength for maximum absorption λmax for compound A was found to be 291nm



Results Table - scan003,Sample001,Cycle01

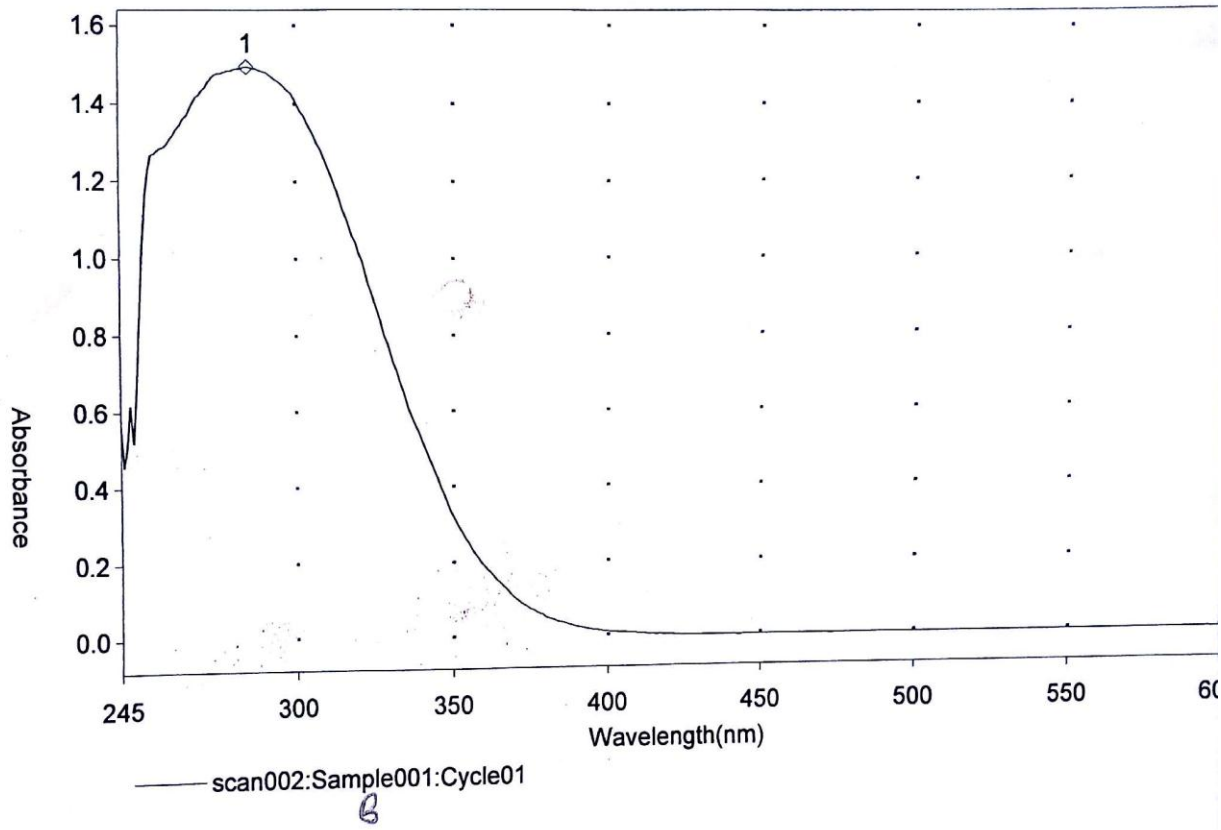
nm	A
291.00	1.036

Peak Pick Method
Find 8 Peaks Above -3.0000 A
Start Wavelength 245.00 nm
Stop Wavelength 600.00 nm
Sort By Wavelength

Sensitivity Auto

Figure 4.4 UV-Visible Spectrum of Compound A

The wavelength for maximum absorption λmax for compound B was found to be 285nm



Results Table - scan002,Sample001,Cycle01

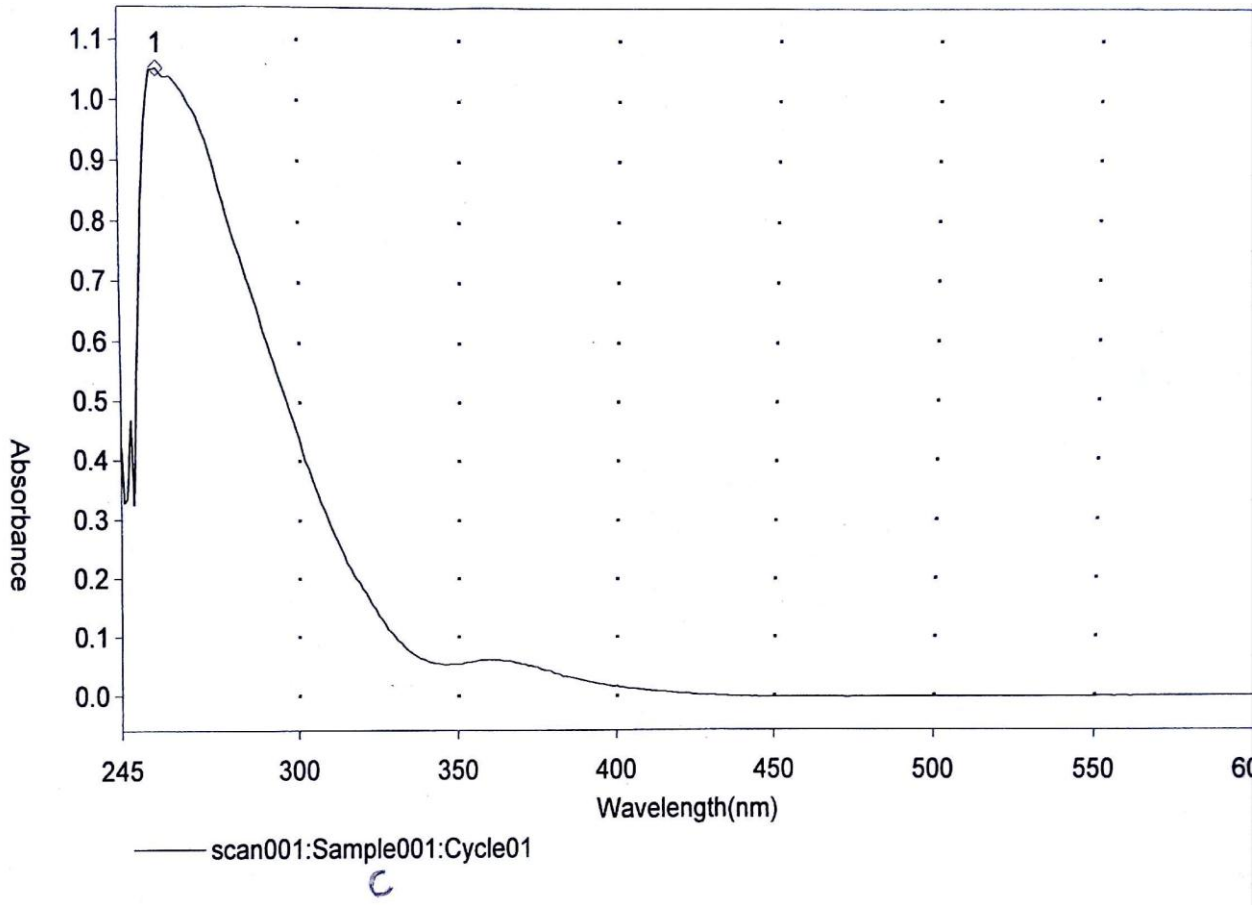
nm	A
285.00	1.496

Peak Pick Method
Find 8 Peaks Above -3.0000 A
Start Wavelength 245.00 nm
Stop Wavelength 600.00 nm
Sort By Wavelength

Sensitivity Auto

Figure 4.5 UV-Visible Spectrum for compound B

The wavelength for maximum absorption λmax for compound C was found to be 257nm



Results Table - scan001,Sample001,Cycle01

nm	A	**Peak Pick Method**
257.00	1.052	Find 8 Peaks Above -3.0000 A
		Start Wavelength 245.00 nm
		Stop Wavelength 600.00 nm
		Sort By Wavelength
Sensitivity	Auto	

Figure 4.6 UV-Visible Spectrum for compound C

4.4 FTIR Spectra of compound A, B and C

The FTIR Spectra shows vibration frequencies of various functional groups for the 3 isomeric compounds A, B and C are shown in Figure 4.7; 4.8; and 4.9 while the three isomeric forms FTIR individual spectrum were superimposed to easily compare similarity in functional groups as shown in Figure 4.10. Figure 4.11 shows the spectrum of the 3 isomeric forms mixed stacked together.

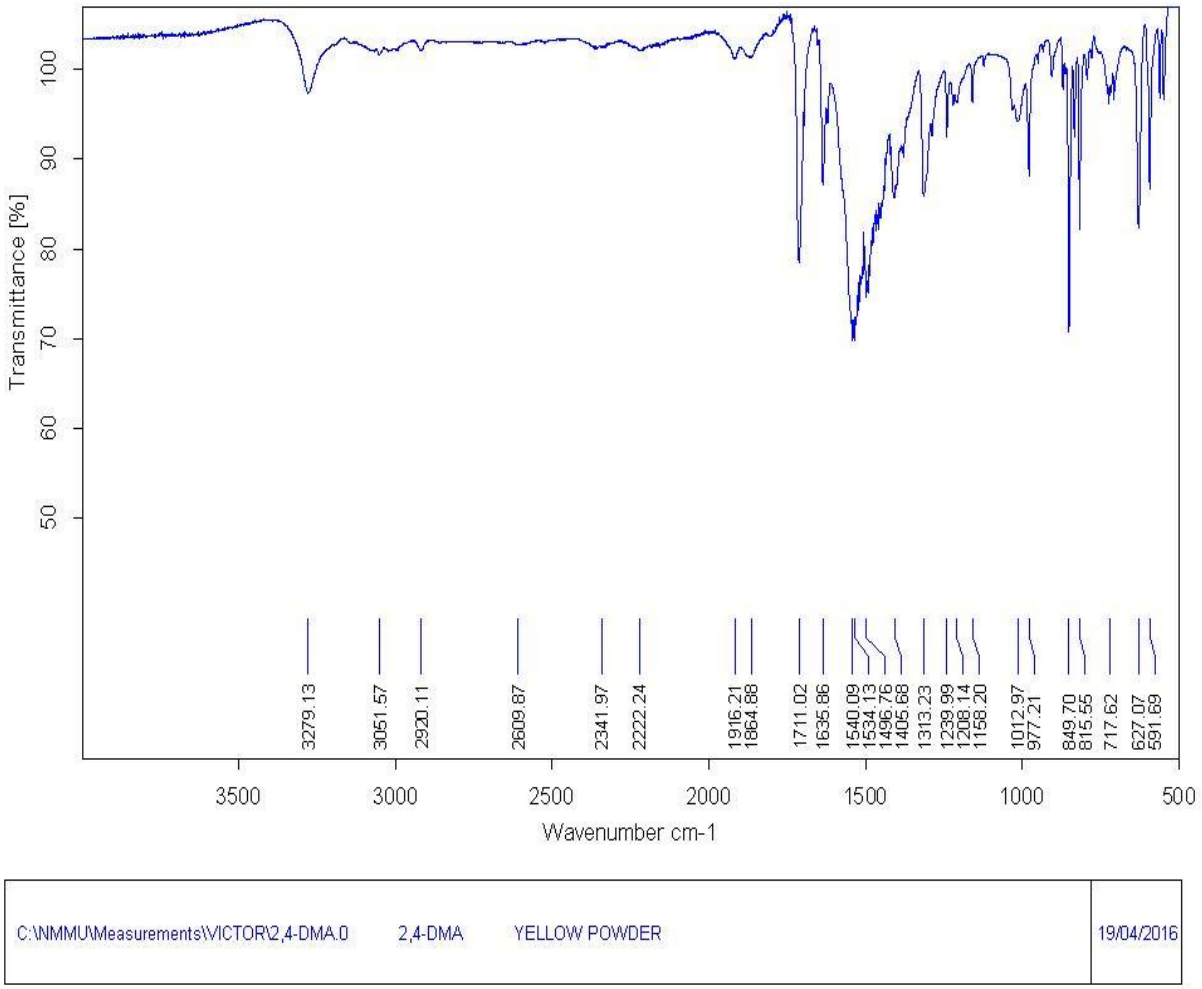


Figure 4.7 FTIR Spectrum of compound A

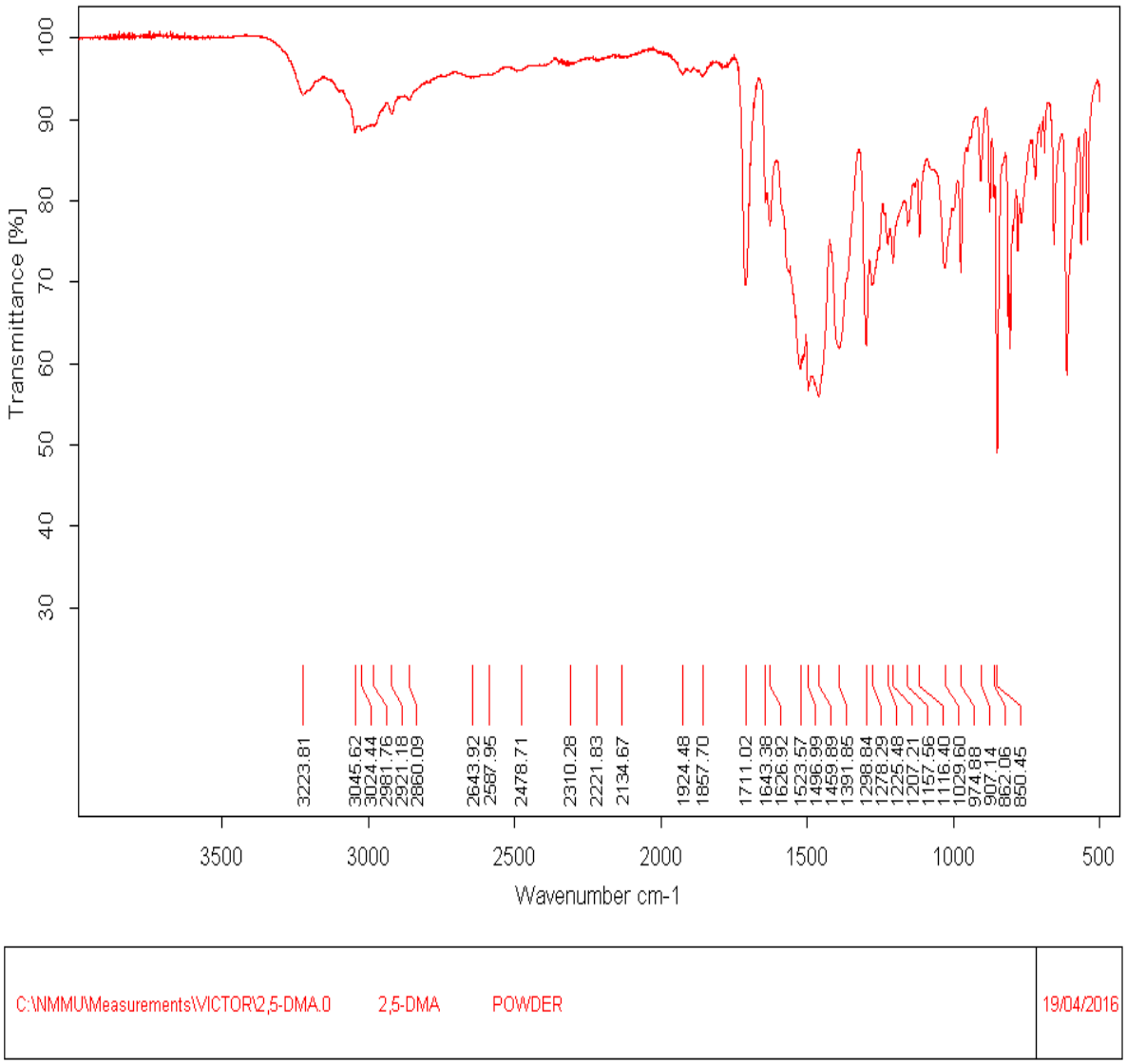


Figure 4.8 FTIR Spectrum of compound B

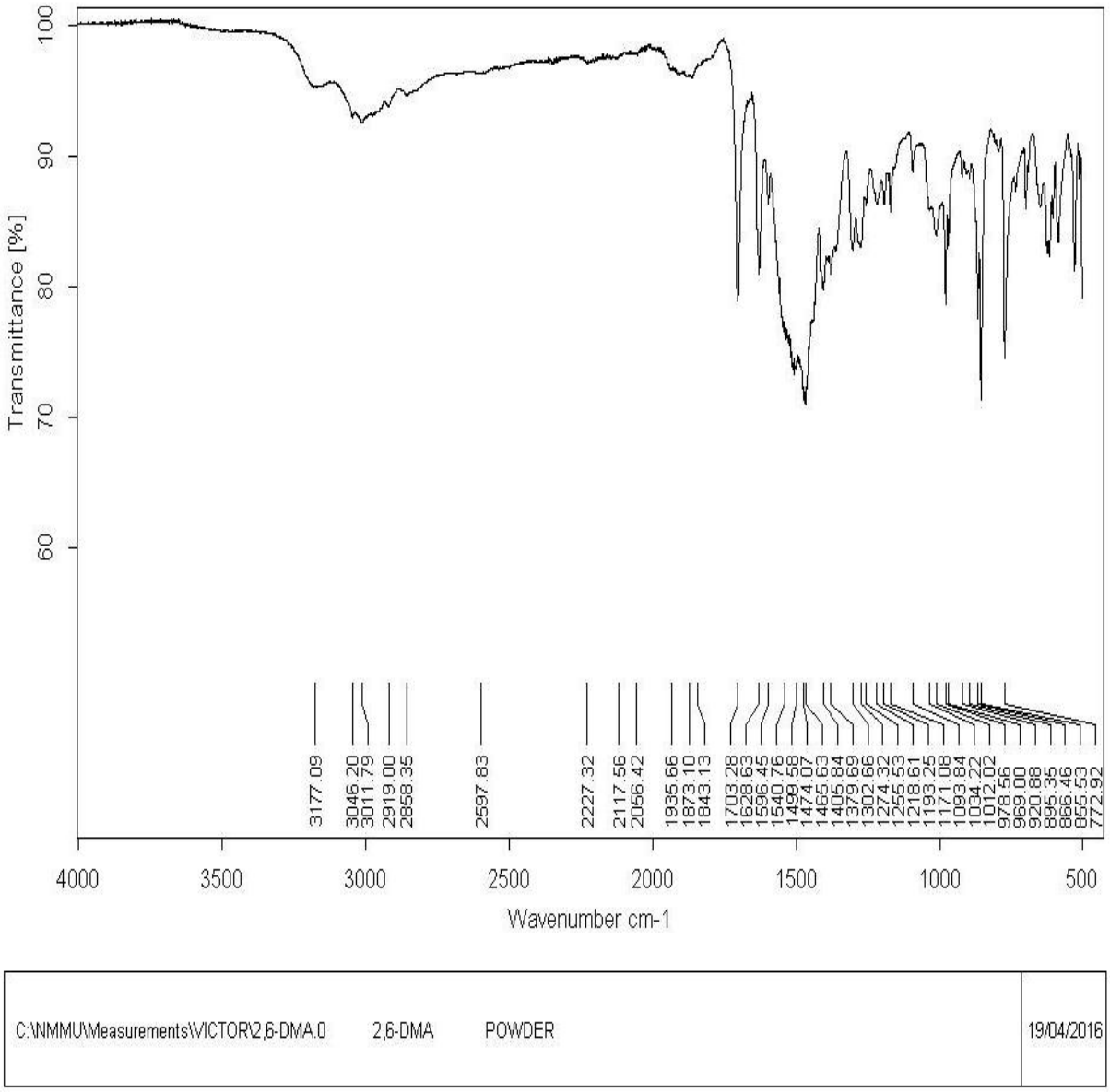
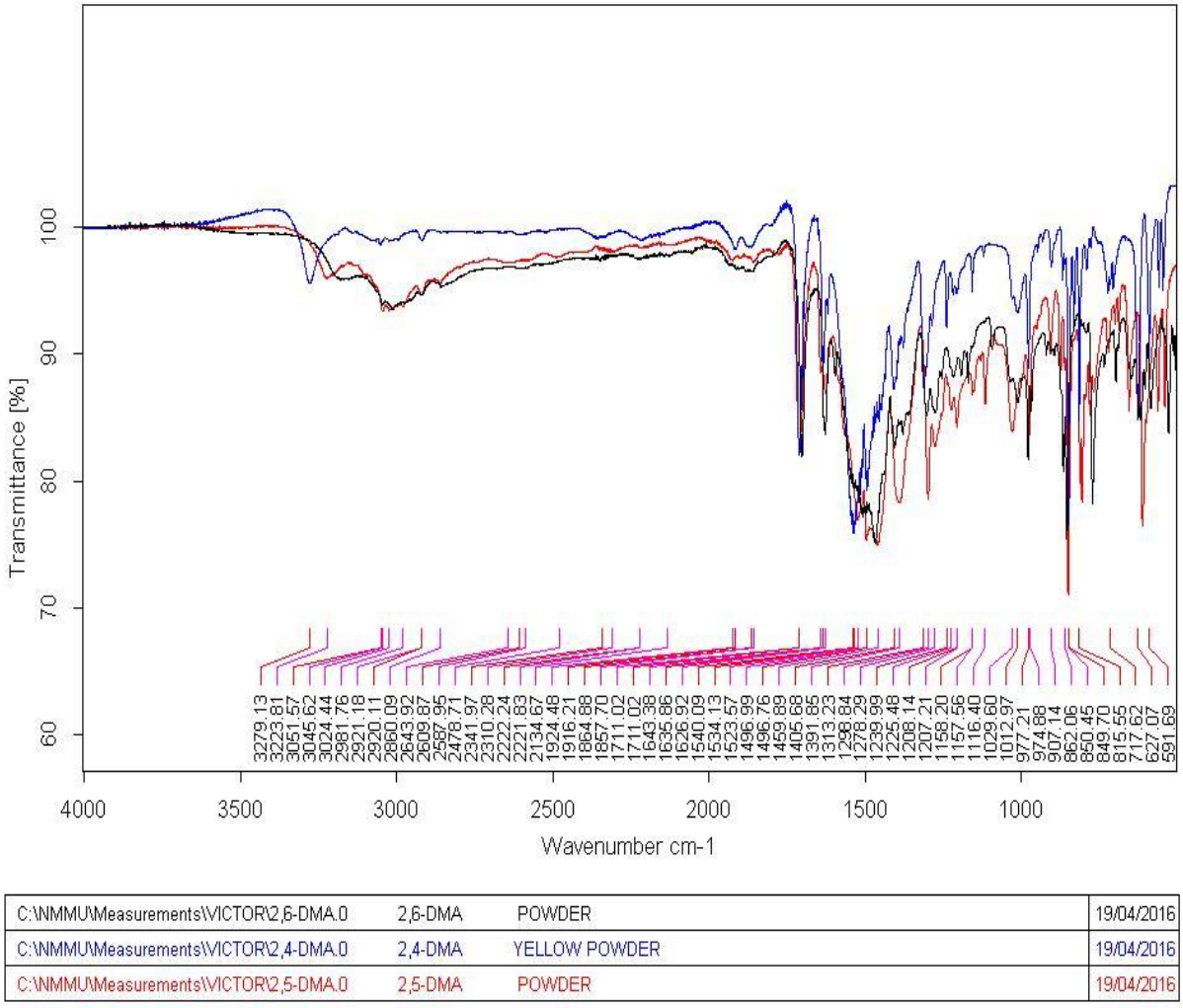
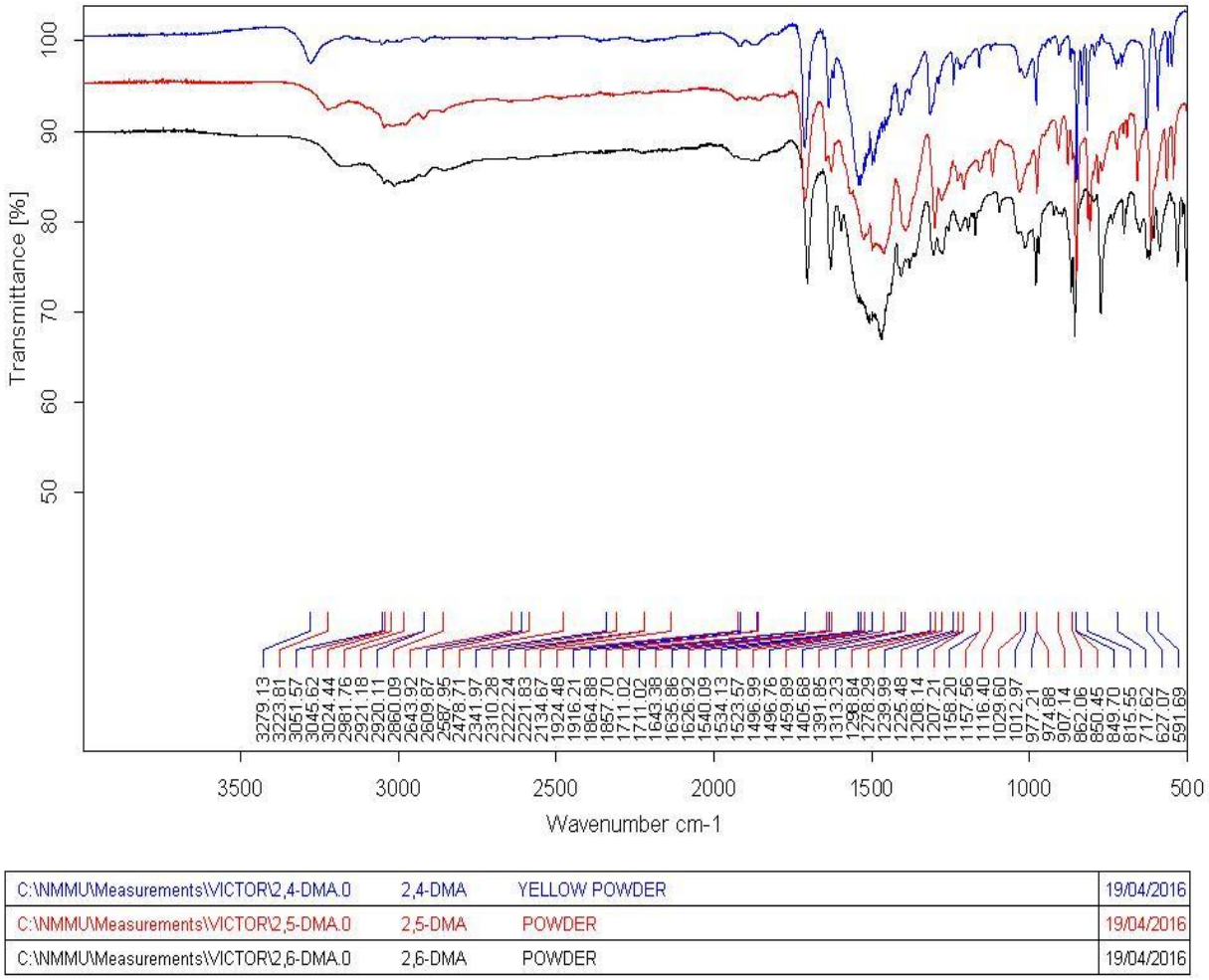


Figure 4.9 FTIR Spectrum of compound C



C:\NMMU\Measurements\VICTOR\2,6-DMA.0	2,6-DMA	POWDER	19/04/2016
C:\NMMU\Measurements\VICTOR\2,4-DMA.0	2,4-DMA	YELLOW POWDER	19/04/2016
C:\NMMU\Measurements\VICTOR\2,5-DMA.0	2,5-DMA	POWDER	19/04/2016

Figure 4.10 FTIR Spectrum of compound A, B and C all stacked



C:\NMMU\Measurements\VICTOR\2,4-DMA.0	2,4-DMA	YELLOW POWDER	19/04/2016
C:\NMMU\Measurements\VICTOR\2,5-DMA.0	2,5-DMA	POWDER	19/04/2016
C:\NMMU\Measurements\VICTOR\2,6-DMA.0	2,6-DMA	POWDER	19/04/2016

Figure 4.11 FTIR Spectrum of compound A, B and C Overlaid

The summery of the Infrared vibration frequencies of various functional groups for the 3 isomeric forms as indicated from Fig 4.7 to 4.9 are as shown in Table 4.2 below;

Table 4.2 Analysis of the functional group region on the FTIR Spectrum of Compound A, B and C

Vibrations Compound A (cm^{-1})	**Vibrations Compound B (cm^{-1})**	**Vibrations Compound C (cm^{-1})**	**Functional Group**
3279	3224	3177	N-H stretch (amides)
3051	3045	3046	C=C-H asymmetric stretch (olefinic)
2920	2921	2919	O-H Stretching (-COOH)
1711	1711	1703	C=O stretch (-COOH)
1643	1643	1628	C=O stretch (amide)
1540	1524	1540	N-H bend (amides)
1496	1496	1499	C-C=C stretch (aromatic)
1405	1392	1405	C-H ($-CH_3$)
1313	1299	1302	C-N stretch
1208	1207	1218	C-O stretch
849	850	855	C-H out of plane bend (aromatic)

4.5 NMR Spectra of Compound A

4.5.1 ^{1}H NMR Spectra of Compound A

The ^{1}H NMR Spectrum revealed the presence of proton signals at δ 2.27, 2.34, 6.32, 6.45, 6.57, 6.99, 7.06, 7.46 and 8.43 as seen in Figure 4.12 below;

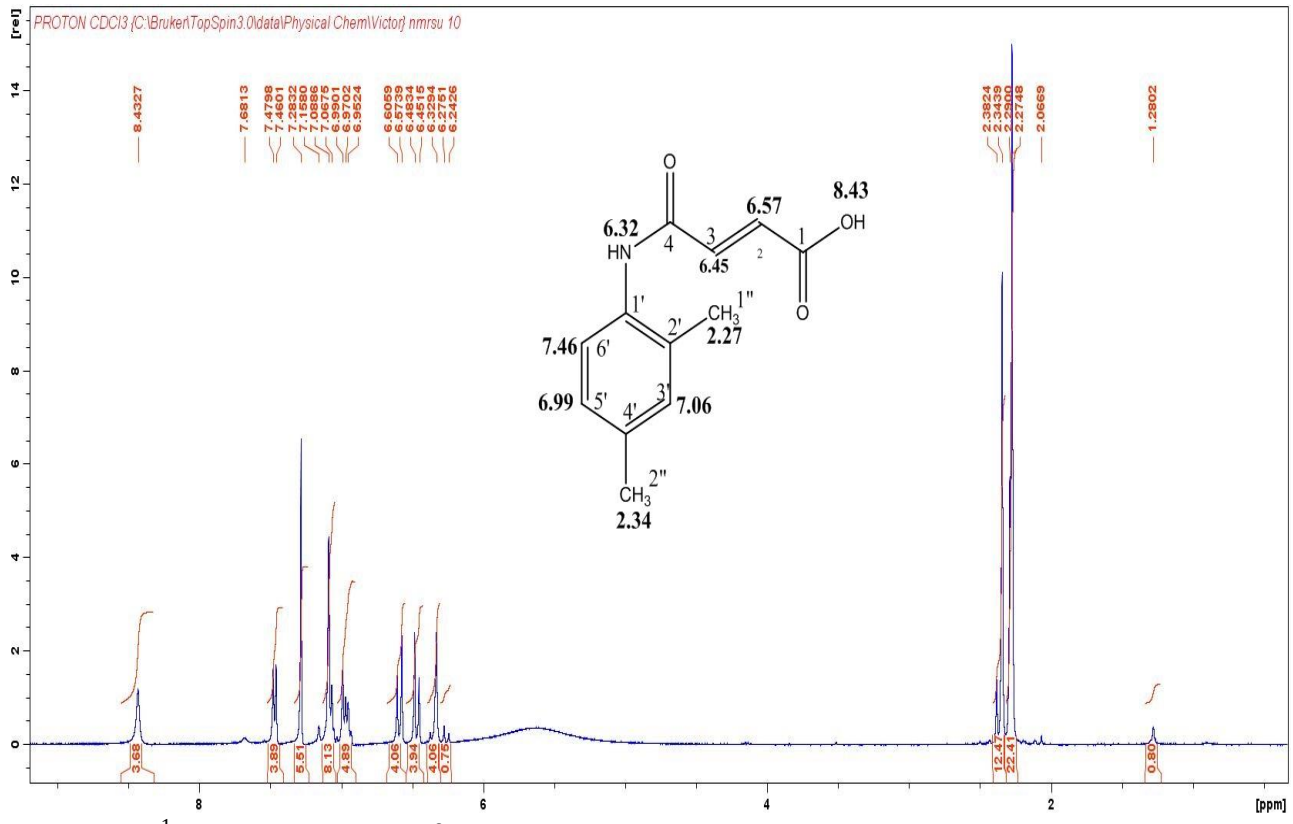


Figure 4.12 ^{1}H NMR Spectrum of Compound A

4.5.2 Carbon 13 NMR Spectra of Compound A

The result of the ^{13}C NMR revealed the presence of 12 carbon atoms at δ 17.75, 20.99, 124.58, 127.49, 130.68, 131.64, 131.70, 131.78, 136.82, 137.70, 164.81 and 165.35 as indicated in Figure 4.13 below;

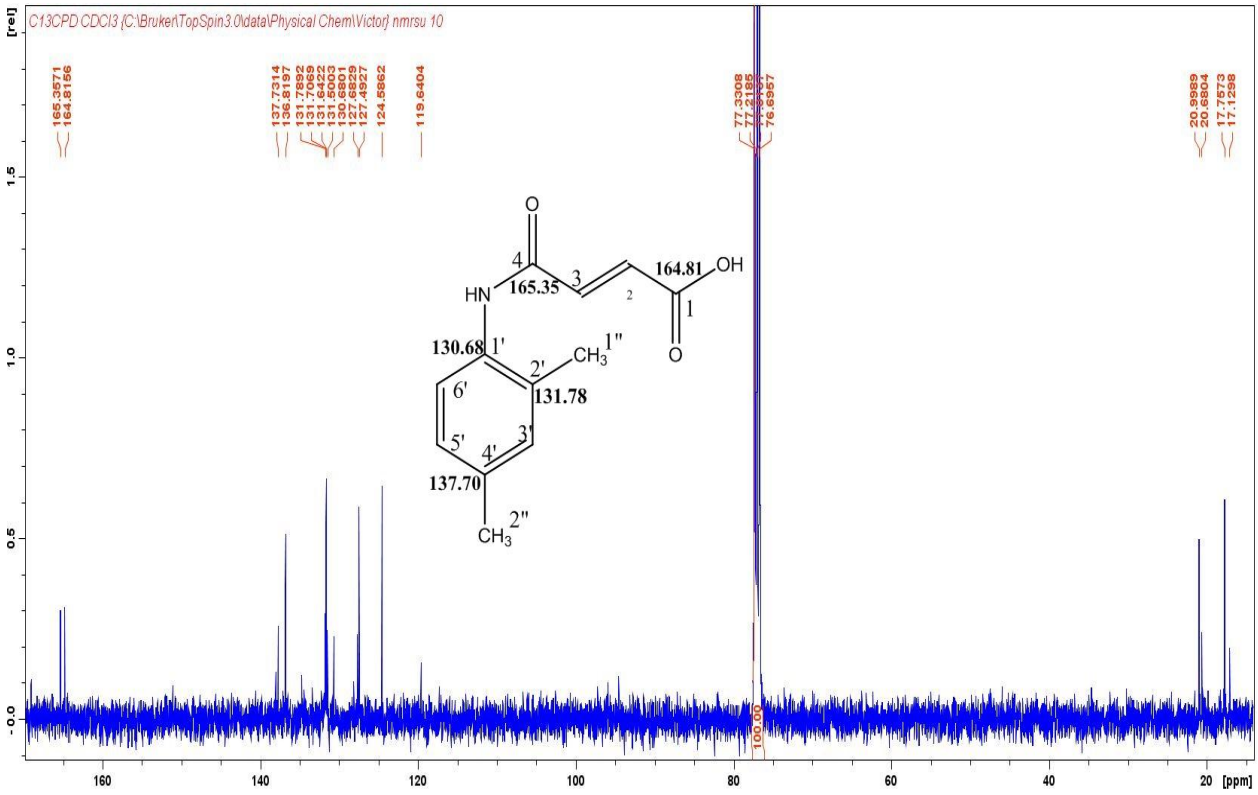


Figure 4.13 ^{13} C NMR Spectrum of Compound A

4.5.3 DEPT 135 Spectra of Compound A

The result of the DEPT 135 Spectra indicates the presence of 5 –CH at δ 124.58, 127.49, 131.64, 131.70 and 136.82 and 2 $–CH_3$ at δ 20.7 and 21.00 and 5 quaternary Carbons at 127.68, 130.68, 137.73, 164.82 and 165.36 as shown in Figure 4.14 below;

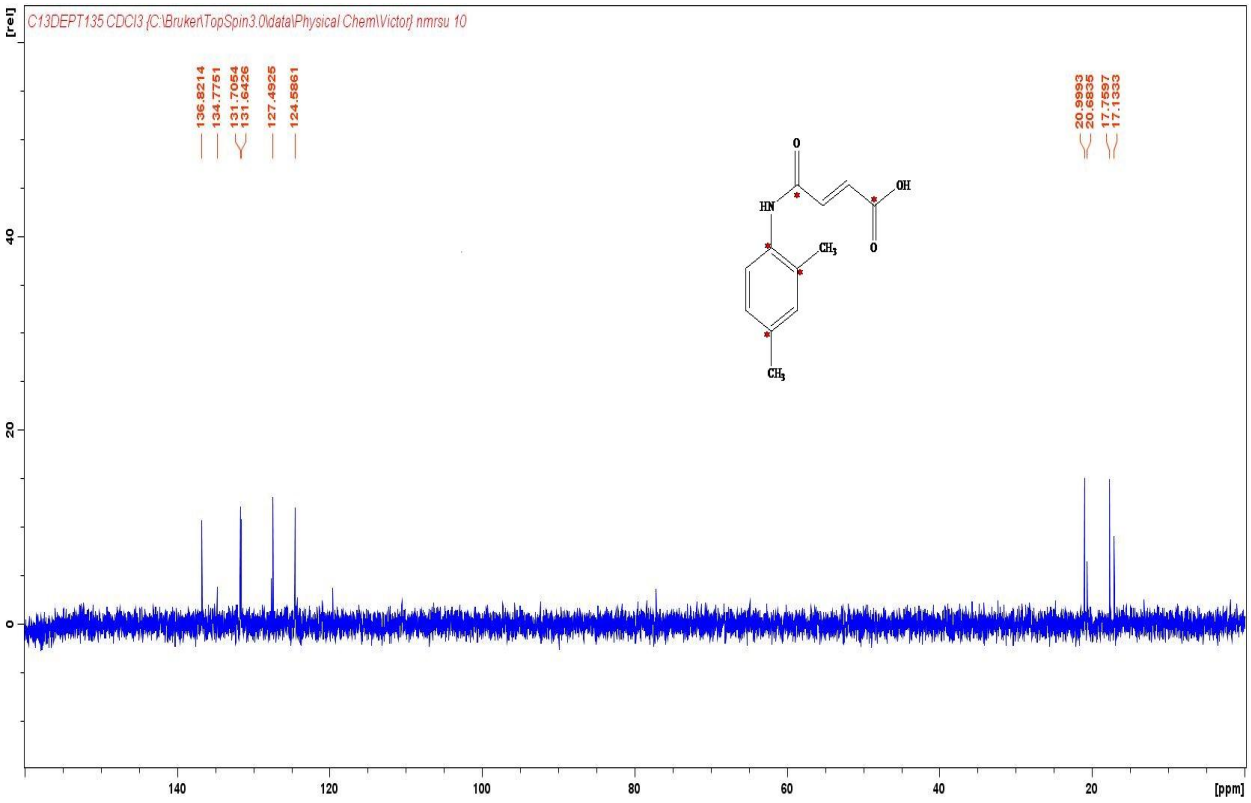


Figure 4.14 DEPT 135 135 Spectrum of Compound A

4.5.3 HSQC Spectra of Compound A

The HSQC Spectrum of Compound A was used to assign protons to their respective carbons. As shown if Figure 4.15 below;

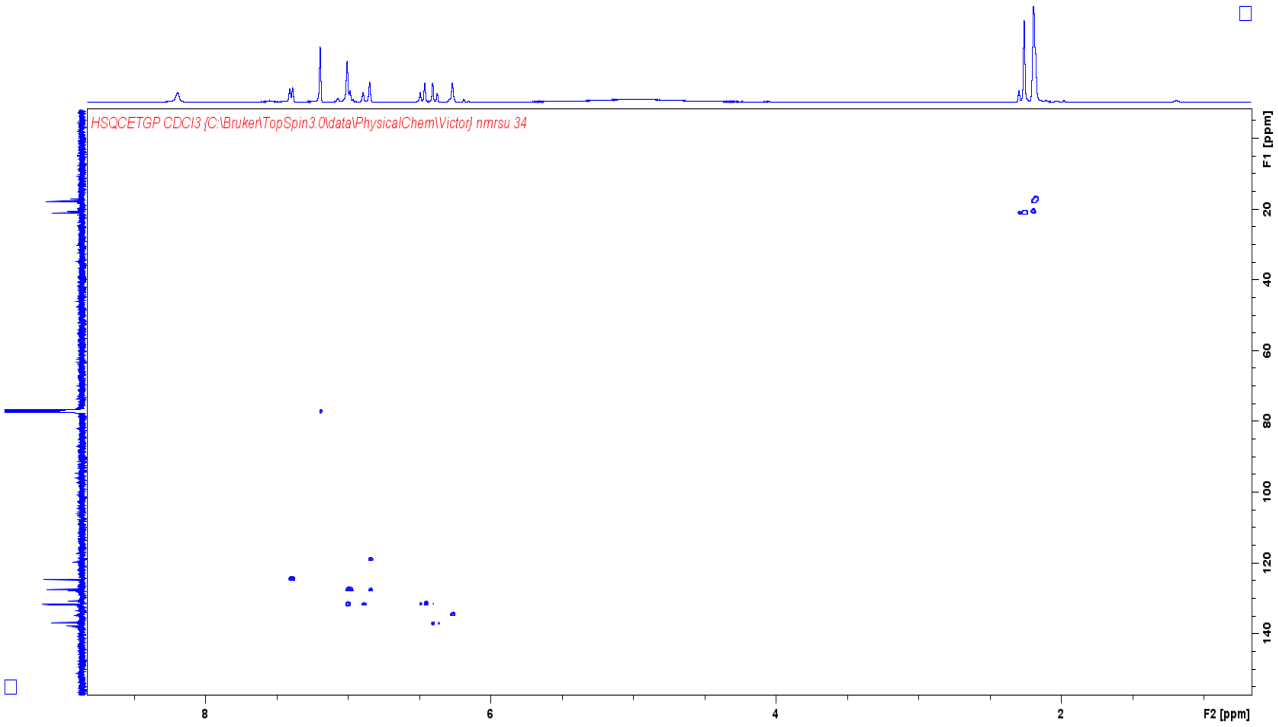


Figure 4.15 HSQC Spectrum of Compound A

4.5.4 COSY Spectra of Compound A

The COSY spectrum showed some cross peaks such as δ_H 6.99 and 7.4; δ_H 6.45 and 6.57 as shown if Figure 4.16 below;

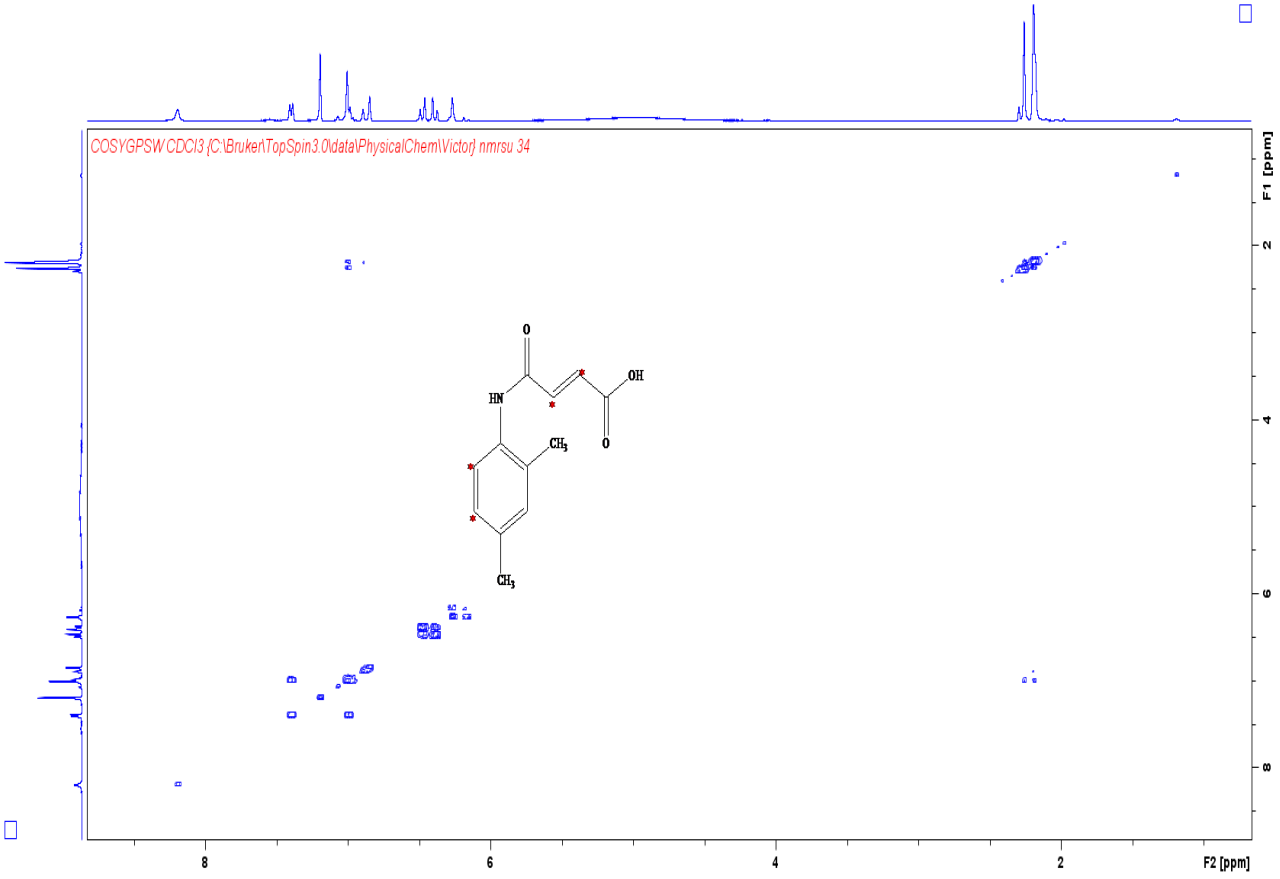


Figure 4.16 COSY Spectrum of Compound A

4.3.8 Result of ^{1}H, ^{13}C, DEPT 135 Spectra of Compound A

The ^{1}H NMR indicates the presence of 9 proton signals. The ^{13}C spectra revealed 12 carbons and DEPT 135 spectrum indicates 5 –CH and 5 quaternary carbons as shown in Table 4.3 below;

Table 4.3 Result of ^{1}H, ^{13}C, DEPT 135 Spectra of Compound A

Position	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT 135**
1’	-	130.68	C
2’	-	131.78	C
3’	7.06	131.70	CH
4’	-	137.7	C
5’	6.99 $_{J=7.9}$	127.49	CH
6’	7.46 $_{J=7.9}$	124.58	CH
4	-	165.35	C
3	6.45 $_{J=12.8}$	136.82	CH
2	6.57 $_{J=12.8}$	131.64	CH
1	-	164.81	C
1’’	2.27	17.75	CH_3
2’’	2.34	20.99	CH_3
OH	8.43		
NH	6.32		

Compound A : 4-[(2,4-dimehylphenyl)amino]-4-oxobut-2-enoic acid

4.6 Spectra of Compound B

4.6.1 Proton NMR Spectra of Compound B

The ^{1}H NMR Spectrum revealed the presence of protons signals at δ 2.27, 2.36, 6.46, 6.51, 6.52, 7.07, 7.22, 7.48 and 8.10 As shown if Figure 4.17 below;

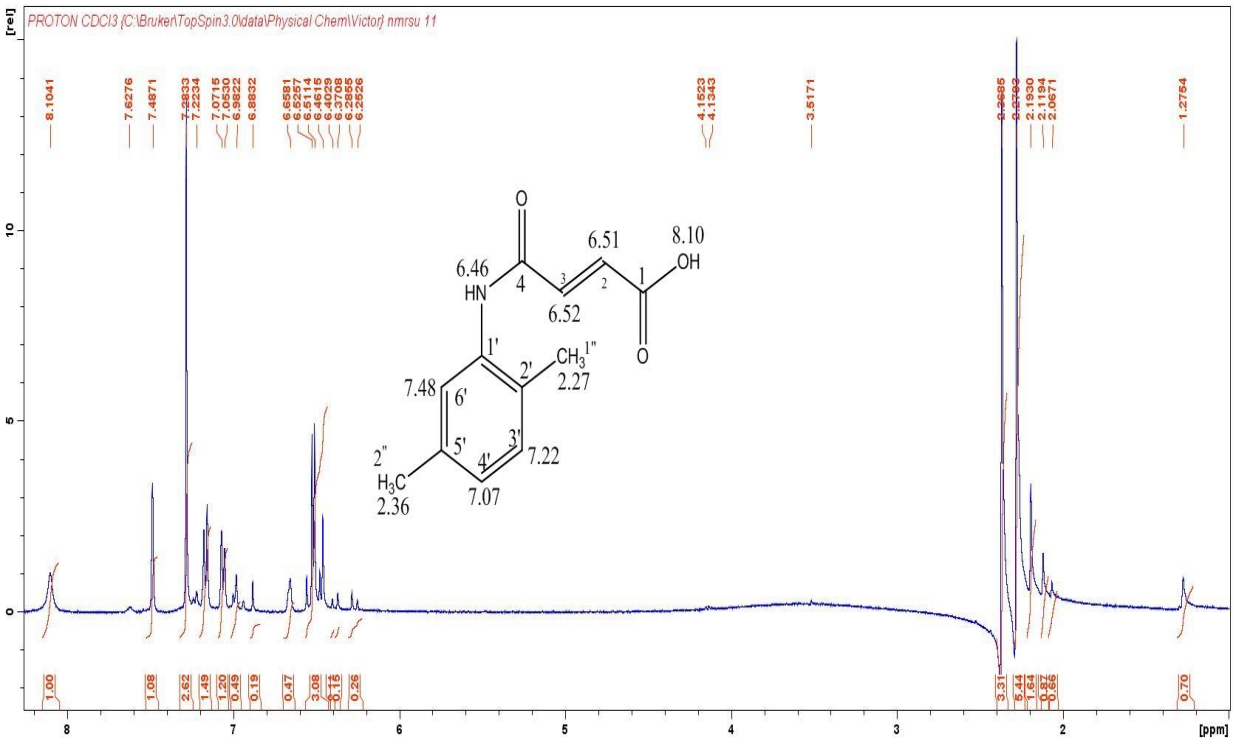


Figure 4.17 Proton NMR Spectrum of Compound B

4.6.2 Carbon 13 NMR Spectra of Compound B

The result of the ^{13}C NMR revealed the presence of 12 carbon atoms at δ 17.37, 21.02, 124.88, 128.01, 128.50, 130.78, 131.42, 133.05, 136.92, 137.22, 164.59 and 164.91 As shown if Figure 4.18 below;

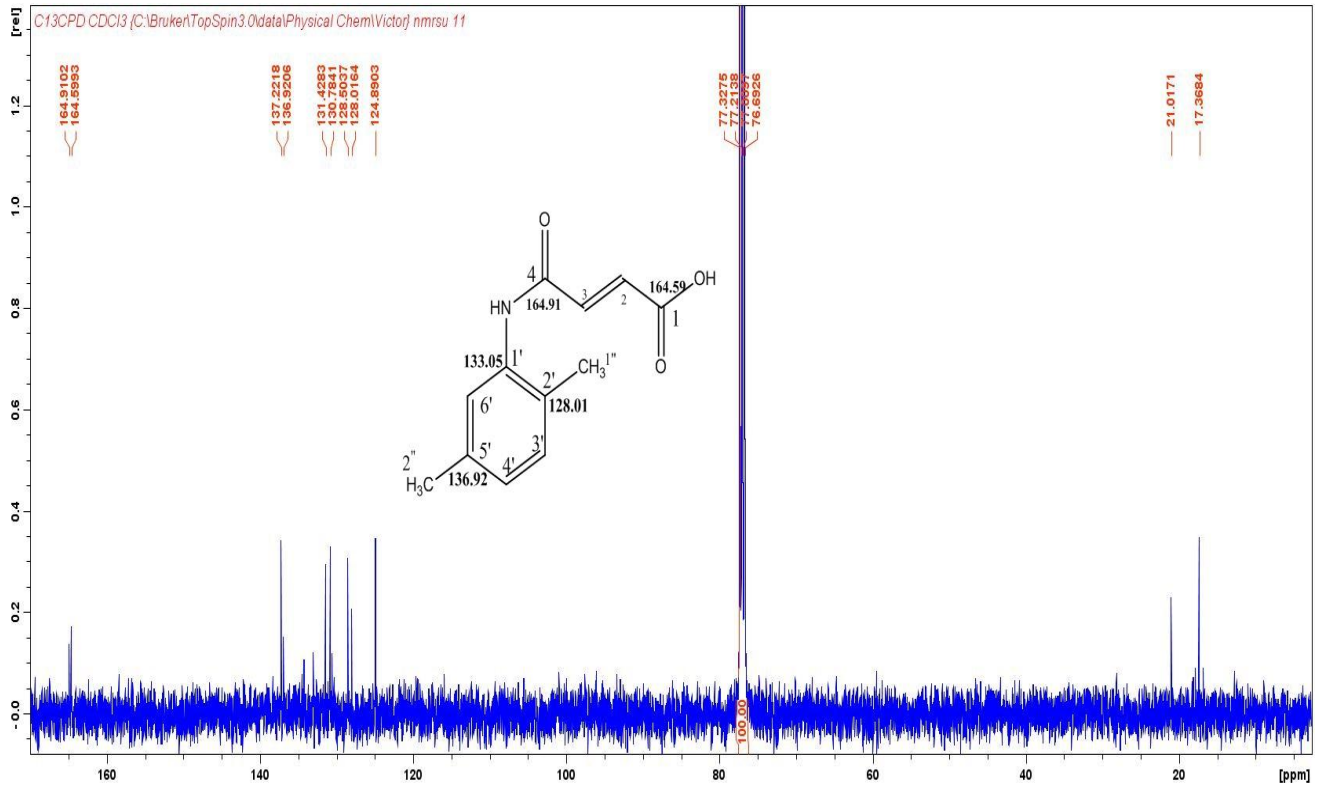


Figure 4.18 Carbon 13 spectrum of Compound B

4.6.3 DEPT 135 Spectra of Compound B

The result of the DEPT 135 Spectra indicates the presence of 5 –CH at δ 124.88, 128.50, 130.78, 131.42 and 137.22 and 2 $–CH_3$ at δ 17.37 and 21.02 and 5 quaternary Carbons at 128.01, 133.05, 136.92, 164.59 and 164.91 As shown if Figure 4.19 below;

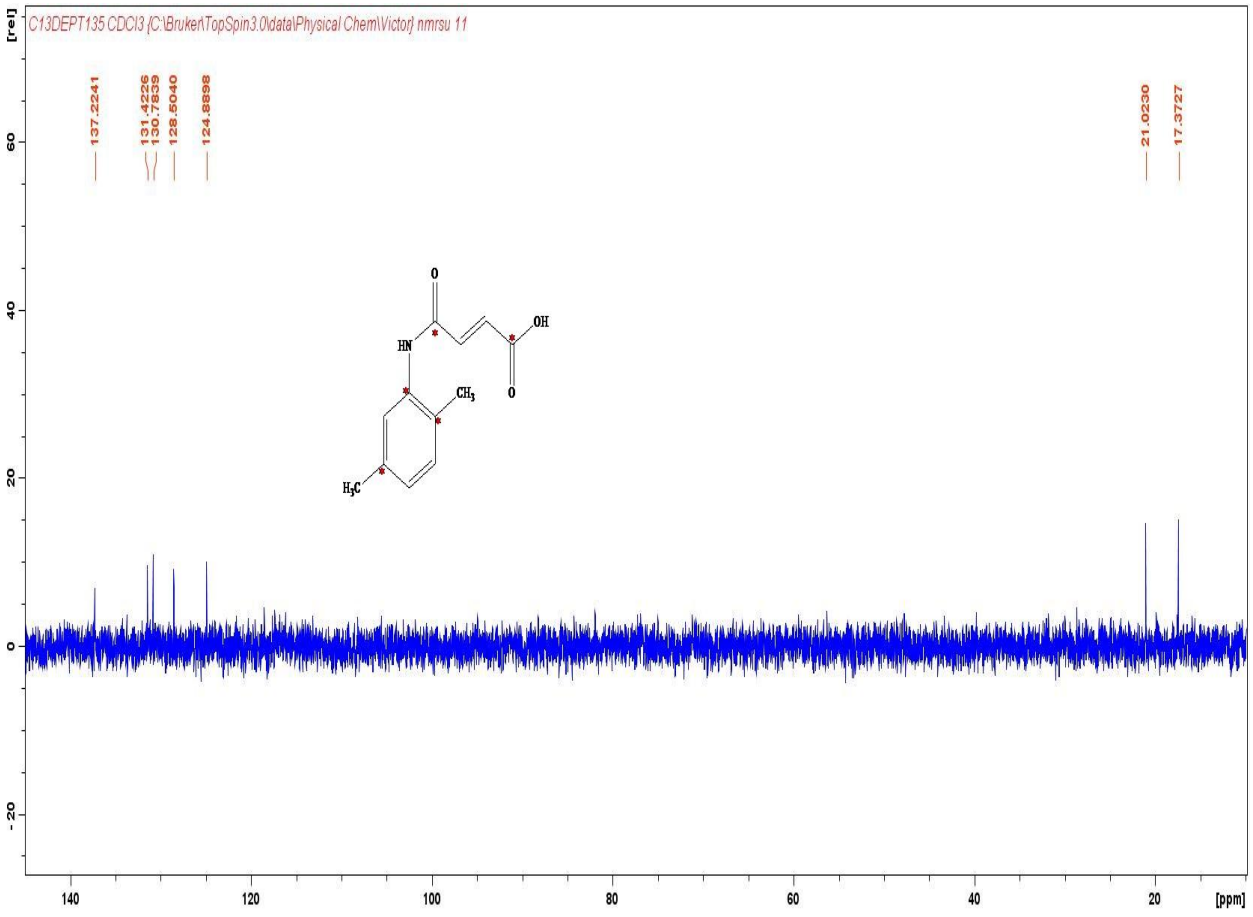


Figure 4.19 DEPT 135 Spectrum of Compound **B**

4.6.4 HSQC Spectra of Compound B

The HSQC Spectrum of Compound B was used to assign protons to their respective carbons. As shown if Figure 4.20 below;

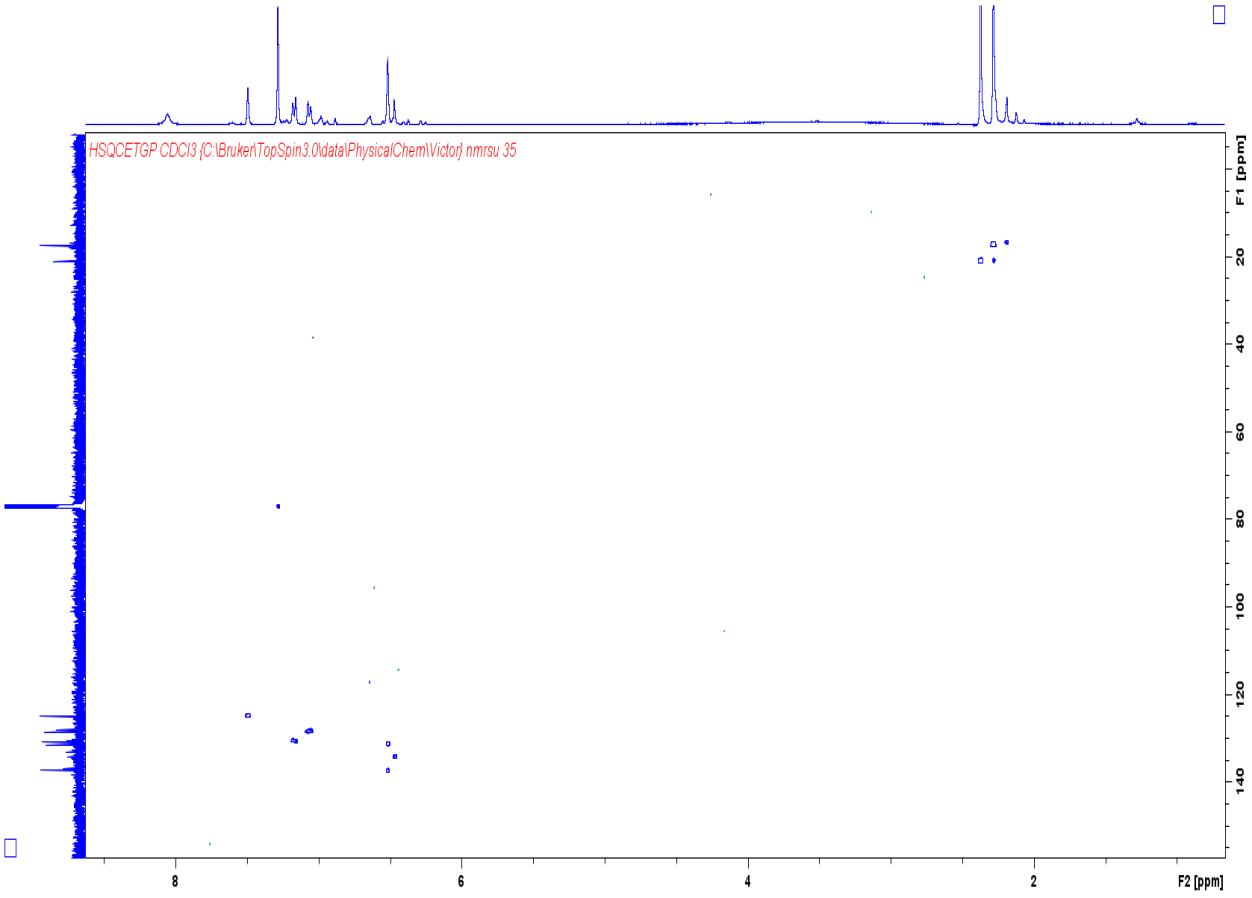


Figure 4.20 HSQC Spectrum of Compound B

4.6.5 COSY Spectra of Compound B

The COSY spectrum showed some cross peaks such as δ_H 6.51 and 6.52; 7.1 and 7.2 ; δ_H 7.1 and 7.48 As shown if Figure 4.21 below;

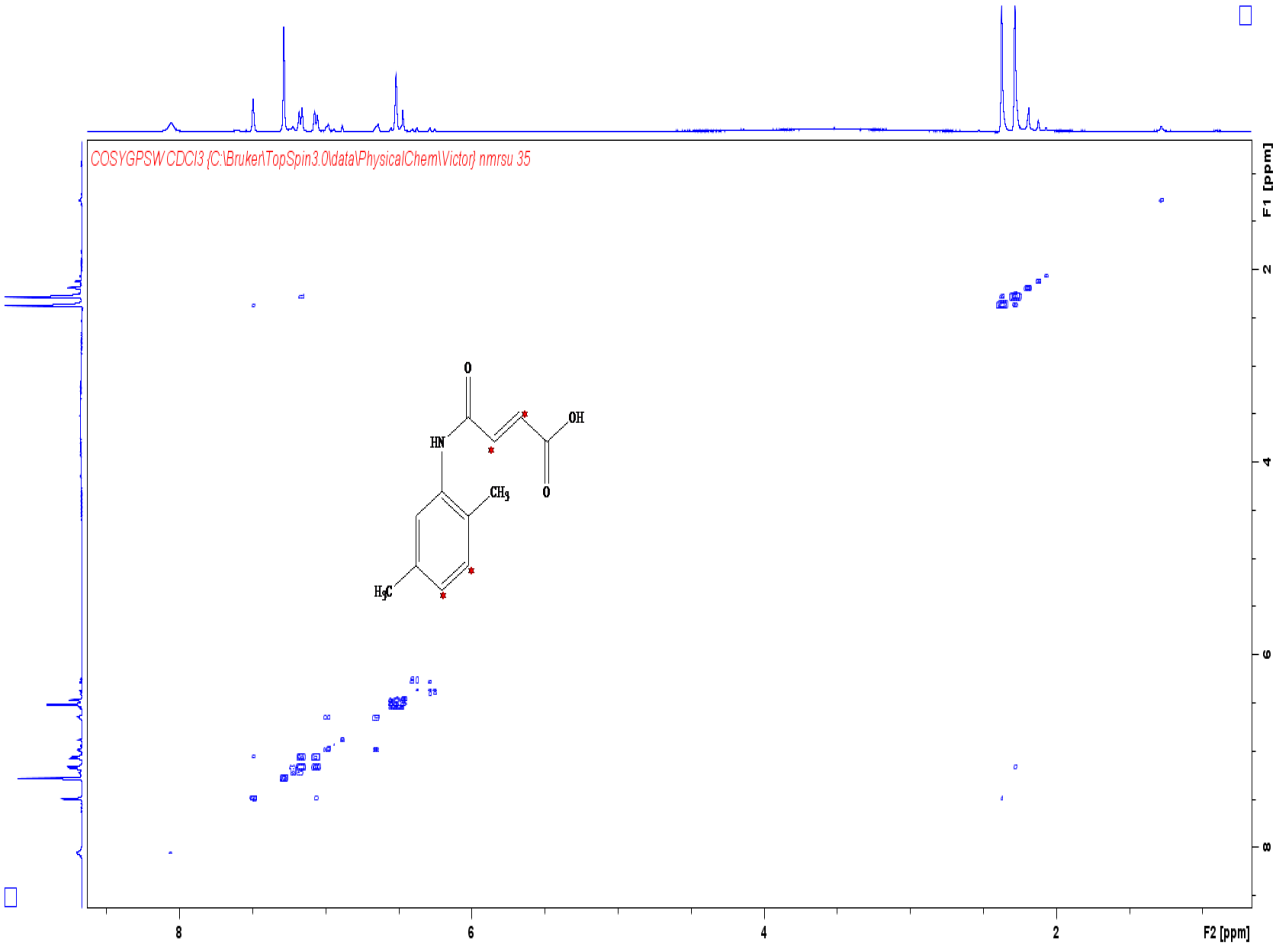


Figure 4.21 COSY Spectrum of Compound B

4.6. ^{1}H, ^{13}C, DEPT 135 Spectra of Compound B

The ^{1}H NMR indicates the presence of 9 proton environments. The ^{13}C spectra revealed 12 carbons and DEPT 135 spectrum indicates 5 –CH and 5 quaternary carbons as shown in table 4.4 below;

Table 4.4 Result of ^{1}H, ^{13}C, DEPT 135 Spectra of Compound B

Position	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT 135**
1’	-	133.05	C
2’	-	128.01	C
3’	7.22 $_{J=7.4}$	130.78	CH
4’	7.07 $_{J=7.4}$	128.50	CH
5’	-	136.92	C
6’	7.48	124.88	CH
4	-	164.91	C
3	6.52 $_{J=12.84}$	137.22	CH
2	6.51 $_{J=12.84}$	131.42	CH
1	-	164.59	C
1’’	2.27	17.37	CH_3
2’’	2.36	21.02	CH_3
OH	8.10		
NH	6.46		

Compound B : 4-[(2,5-dimehylphenyl)amino]-4-oxobut-2-enoic acid

4.7 Spectra of Compound C

4.7.1 Proton NMR Spectra of Compound C

The ^{1}H NMR Spectrum revealed the presence of proton signals at δ 2.27 (2 protons in the same environment), 6.35, 6.63, 6.90, 6.6, 7.14 (2 protons in the same environment) and 8.30 as shown if Figure 4.22 below;

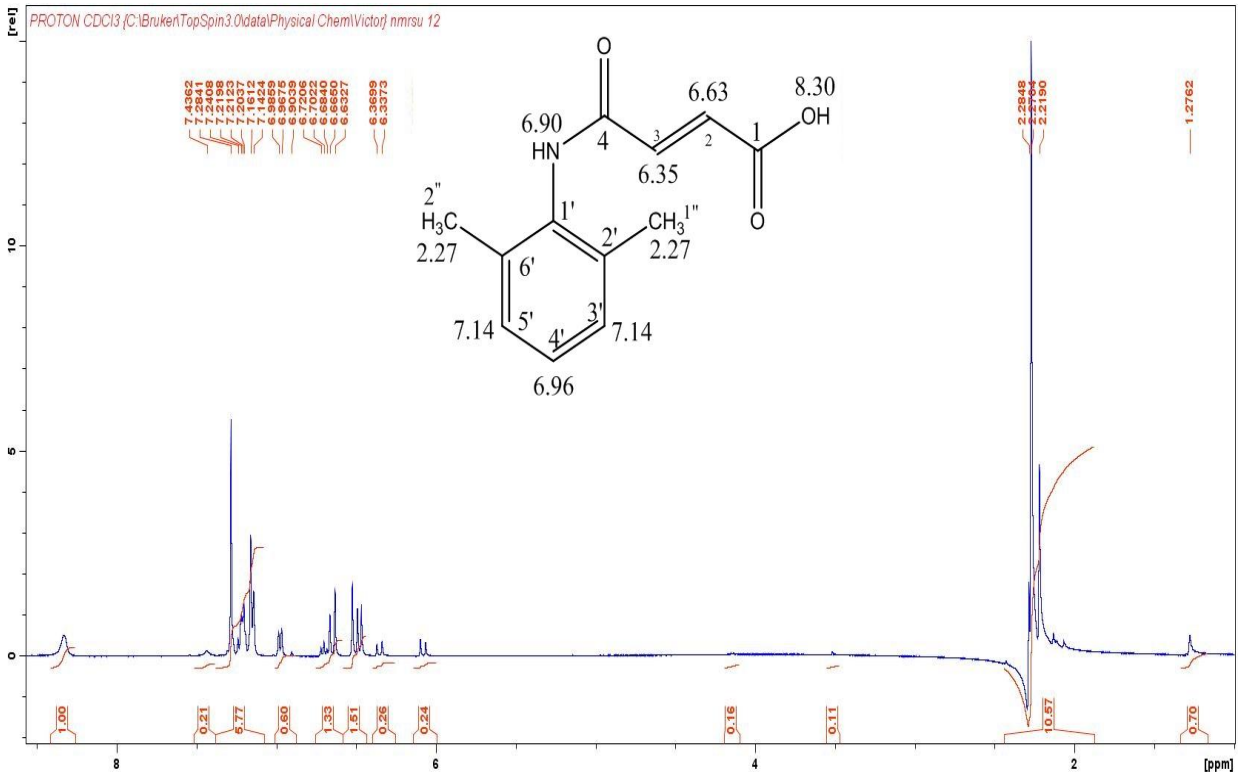


Figure 4.22 Proton NMR Spectrum of Compound C

4.7.2 Carbon 13 NMR Spectra Analysis of Compound C

The result of the ^{13}C NMR revealed the presence of 12 carbon atoms at δ 18.30 (2 carbons in the same environment), 128.52, 128.70 (2 carbons in same environment), 130.84, 135.10 (2 carbons in the same environment), 137.35, 138.95, 165.11 and 165.26 as shown if Figure 4.23 below;

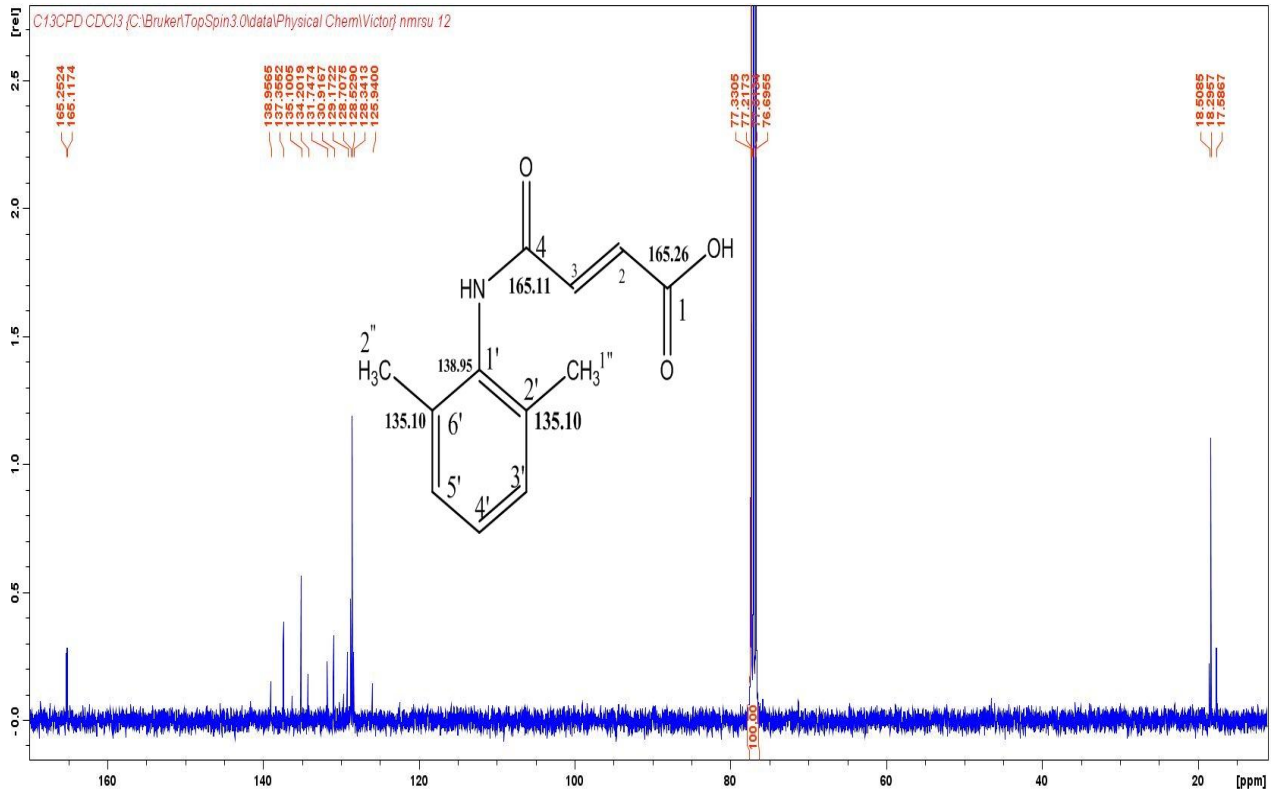


Figure 4.23 ^{13}C NMR Spectrum of Compound C

4.7.3 DEPT 135 Spectra of Compound C

The result of the DEPT 135 Spectra indicates the presence of 4 –CH at δ 128.53, 128.72 (2 CH same environment), 130.84, and 137.41 and 2 $–CH_3$ at δ 18.30 and 18.51 and 4 quaternary Carbons at 135.10 (2 C same environment),138.95 165.11 and 165.26 As shown if Figure 4.24 below;

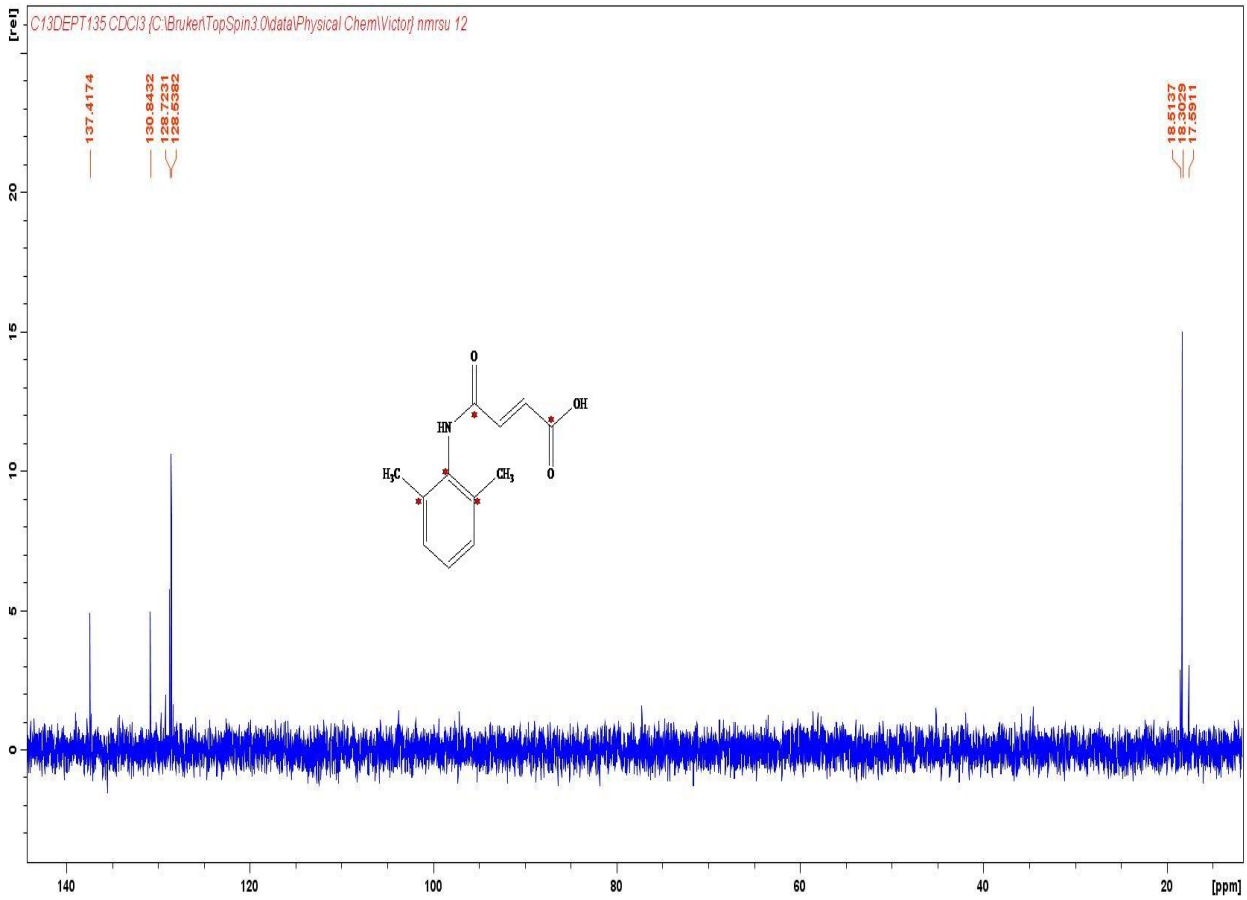


Figure 4.24 DEPT 135 Spectrum of Compound C

4.7.4 HSQC Spectra of Compound C

The HSQC Spectrum of Compound C was used to assign protons to their respective carbons. As shown if Figure 4.25 below;

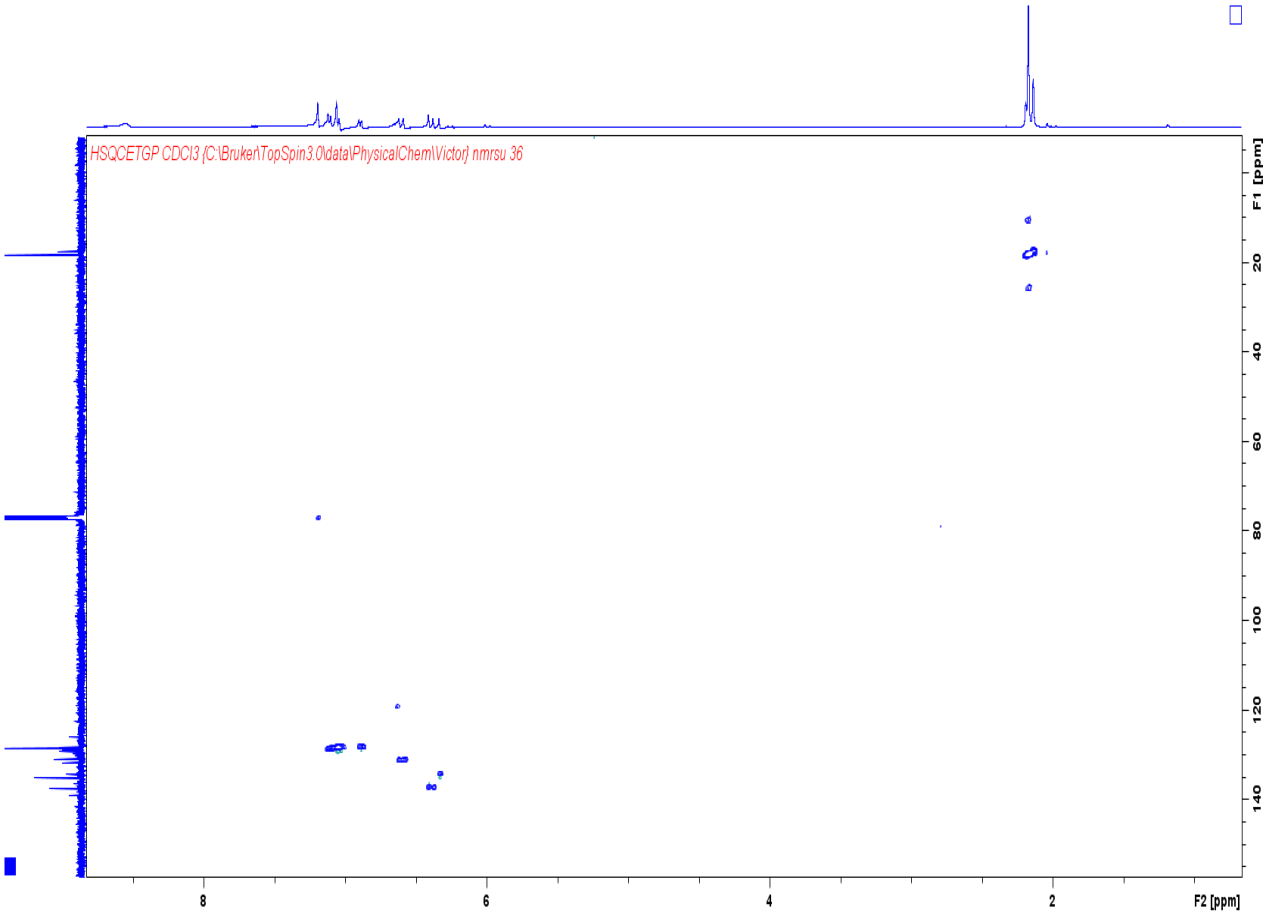


Figure 4.25 HSQC Spectrum of Compound C

4.7.5 COSY Spectra of Compound C

The COSY spectrum showed some cross peaks such as δ_H 6.36 and 6.63 As shown in Figure 4.26 below;

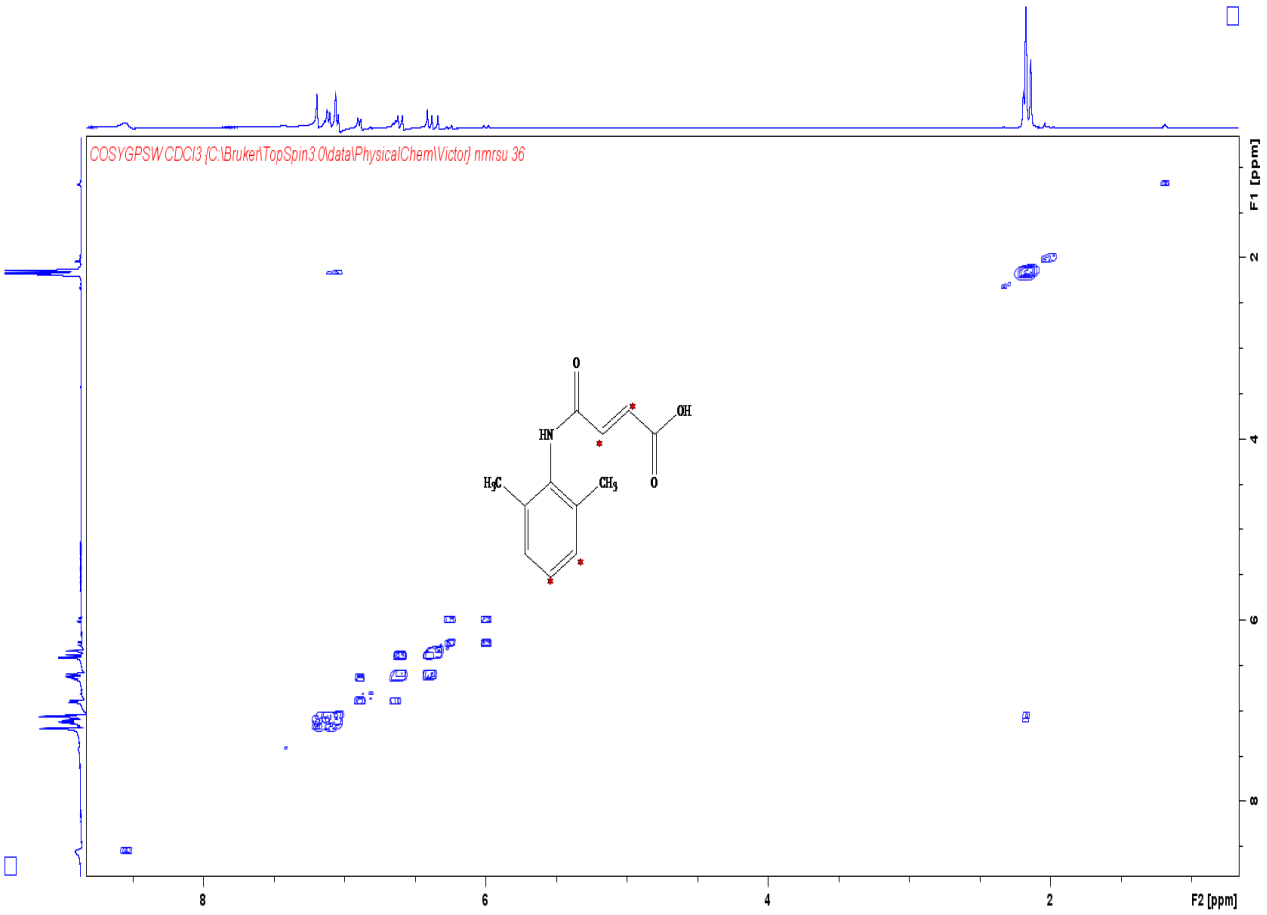


Figure 4.26 COSY Spectrum of Compound C

4.7.6 Result of ^{1}H NMR, ^{13}C, DEPT 135 Spectra of Compound C

The ^{1}H NMR indicates the presence of 9 proton signals. The ^{13}C spectra revealed 12 carbons and DEPT 135 spectrum indicates 4 types of –CH and 4 types quaternary carbons as shown in Table 4.5 below;

Table 4.5 showing the result of Result of ^{1}H, ^{13}C, DEPT 135 Spectra of Compound C

Position	**^{1}H (ppm)**	**^{13}C (ppm)**	**DEPT 135**
1’	-	138.95	C
2’ and 6’	-	135.10	C
3’ and 5’	7.14 J=7.3	128.72	CH
4’	6.96 J=7.3	128.52	CH
4	-	165.11	C
3	6.35 J=13	137.35	CH
2	6.63 J=13	130.84	CH
1	-	165.26	C
1’’ and 2’’	2.27	18.30	CH_3
OH	8.30		
NH	6.90		

Compound C : 4-[(2,6-dimehylphenyl)amino]-4-oxobut-2-enoic acid

The ^{1}H NMR spectra of compound A, B and C were compared with each other and observed for similarity in the signals of possible protons sharing similar environments across the three isomeric forms as seen in the Table 4.6 below;

Table 4.6 Comparison in ^{1}H NMR spectra of compound A, B and C

Position	**^{1}H Compound A**	**^{1}H Compound B**	**^{1}H Compound C**
1’	-	-	-
2’	-	-	-
3’	7.06	7.22 $_{J=7.4}$	7.14 $_{J=7.3}$
4’	-	7.07 $_{J=7.4}$	6.96 $_{J=7.3}$
5’	6.99 $_{J=7.9}$	-	7.14
6’	7.46 $_{J=7.9}$	7.48	-
4	-	-	-
3	6.45 $_{J=12.8}$	6.52 $_{J=12.84}$	6.35 $_{J=13}$
2	6.57 $_{J=12.8}$	6.51 $_{J=12.84}$	6.63 $_{J=13}$
1	-	-	-
1’’	2.27	2.27	2.27
2’’	2.34	2.36	2.27
OH	8.43	8.10	8.30
NH	6.32	6.46	6.90

The ^{13}C spectra of compound A, B and C were compared with each other and observed for similarity in the signals sharing similar environments across the three isomeric forms as seen in the Table 4.7 below;

Table 4.7 Comparison in ^{13}C spectra of compound A, B and C

Positions	**^{13}C Compound A**	**^{13}C Compound B**	**^{13}C Compound C**
1’	130.68	133.05	138.95
2’	131.78	128.01	135.10
3’	131.70	130.78	128.72
4’	137.7	124.5	128.52
5’	127.49	136.92	128.72
6’	124.58	124.88	135.10
4	165.35	164.91	165.11
3	136.82	137.22	137.35
2	131.64	131.42	130.84
1	164.81	164.59	165.26
1’’	17.75	17.37	18.30
2’’	20.99	21.02	18.30

4.8 Result of Acute toxicity study

The Median lethal dose was determined to be 774.6, 1131.4, and 1131.4 mg/kg for compound A, B, and C respectively.

4.9 Neurotoxicity Study of Mice

Beam walking neurotoxicity test was conducted using diazepam as positive control and distilled water as negative control. Since the mean number of foot slip is used as an indices for measuring clinical sedation, it observed that the number of foot slips seen in compound C were similar to that of the negative control. Significant increase in foot slips are observed for compound B at test doses of 300 and 150 mg/kg but much lesser at test dose of 75 mg/kg like that of the negative control as seen in Table 4.8 below;

Table 4.8 Neurotoxicity Study of Mice Using the Beam Walking Assay

Treatments % Mg/kg	**Mean time taken to complete task (secs)**	**Mean number of foot slips**	**Mean number of falls**
Distilled water:	13.33 ± 2.24	1.00 ± 0.68	0.00
Compound B:			
300	24.50 ± 5.53	11.16 ± 1.5*	0.00
150	18.16 ± 4.60	3.33 ± 1.02	0.00
75	8.83 ± 0.83	1.83 ± 0.47	0.00
Compound C:			
300	14.50 ± 2.15	3.66 ± 0.76	0.00
150	12.00 ± 1.75	3.16 ± 1.04	0.00
75	5.50 ± 1.83	0.17 ± 0.17	0.00
Diazepam 1.5	36.66 ± 16.1	7.50 ± 2.06*	3.00

- Shows significant difference at $p<0.05$, n=6, Data presented as Mean±SEM (Standard Error of Mean).

4.10 Effect of the Compounds on Maximal Electro Shock Induced Seizures in Chicks

The Maximal Electro Shock Induced Seizures in Chicks test was done using Positive Control (Phenytion) 20 mg/kg and distilled water as negative control. A 20% Quantal protection was observed for compound C while compound A and B shows no quantal protection. But difference was observed in their mean time of recovery showing a shorter time of recovery in compound B and C relatively similar to that of positive control, while compound A shows a relatively higher time of recovery similar to the negative control as seen in Table 4.9 below;

Table 4.9 Effect of the Compounds A, B and C on Maximal Electro Shock Induced Seizures in Chicks

Treatments, %(mg/kg)	**Mean Time of Recovery (min)**	**Quantal protection**	**Percentage protection (%)**
Distilled water	8.30 ± 0.99*	0/10	0.00
Compound A:			
200	14.70 ± 2.53*	0/10	0.00
100	10.20 ± 1.39*	0/10	0.00
50	10.40 ± 1.50*	0/10	0.00
Compound B:			
300	5.90 ± 0.43	0/10	0.00
150	5.20 ± 0.36	0/10	0.00
75	6.50 ± 0.92	0/10	0.00
Compound C:			
300	7.7 ± 1.51	2/10	20.00
150	5.80 ± 1.01	2/10	20.00
75	5.80 ± 1.08	2/10	20.00
Phenytion 20	2.00 ± 1.37	8.0/10	80.00

Values are presented as mean +SEM, n=10, $p<0.05$ using ANOVA

*shows significant statistical difference, with positive control (phenytion) thus indicating that the compounds A has no anticonvulsant activity at test doses.

4.11 Subcutaneous Pentylenetetrazol Induced Seizures in Mice

The Subcutaneous Pentylenetetrazol Induced Seizures in Mice test was done using Positive Control (Sodium Valproate) 200 mg/kg and distilled water as negative control. The was no significant quantal protection across all test doses of compound A, B and C. the mean onset of seizure was also observed to be significantly lower than that of the positive control as seen in Table 4.10 below;

Table 4.10 Effect of Compound A, B and C on Subcutaneous Pentylenetetrazol Induced Seizures in Mice

Treatments, %(mg/kg)	**Mean Onset of seizure (min)**	**Quantal Protection**	**Percentage protection(%)**
Distilled water	5.40 ± 0.51	0/5	0.00
Compound A:			
200	8.80 ± 1.07	0/5	0.00
100	8.80 ± 1.71	0/5	0.00
50	6.40 ± 2.30	0/5	0.00
Compound B:			
300	5.6 ± 1.63	1/5	20.00
150	16.0 ± 0.89	0/5	0.00
75	5.20 ± 0.80	0/5	0.00
Compound C:			
300	10.80 ± 2.10	0/5	0.00
150	4.80 ± 0.66	0/5	0.00
75	3.20 ± 0.49	0/5	0.00
Sodium valproate 200	27.00 ± 2.80*	4/5	80.00

Values are presented as mean +SEM, n=5, $p<0.05$ using Bonferonni test

No significant statistical difference when compared with negative control, thus showing a no anticonvulsant activity at test doses.

CHAPTER FIVE

5.0 DISCUSSION

The synthesis of the three isomeric forms of 4[(dimethylphenyl) amino] 4-oxo but-2-enoic acids were achieved via a nucleophile-electrophile addition reaction with good yield obtained in high percentages of 87, 85.3 and 79.7 for compound A, B and C respectively. This agrees with the Siddiqui, *et al.*, (2010) on the yields of carboxamides.

The melting point ranges of the 3 isomeric forms were narrow thus indicating the compounds are pure. The UV spectra showed absorption maxima at 291, 285 and 257nm for compound A, B and C respectively. The spectra also indicate that compounds have similar chromophores (Olaniyi, 2000).

FTIR Spectroscopy results obtained, revealed the following vibration frequencies at 3279, 3224 and 3177 indicating the presence of N-H stretch in amide group (Habib., 2013). Vibrations at 3051, 3045 and 3046 indicates C=C-H olefinic asymmetric stretch. While vibration frequencies at 2920, 2921, and 2919 indicates -OH groups. Vibrations at 1711, 1711 and 1703 C=O stretch (in -COOH). Vibrations at 1643, 1643 and 1628 indicates groups C=O stretch in amides (Habib, 2013; Abdullahi M, *et al.*, 2013). 1540, 1524, and 1540 indicates groups of N-H bend in amides (Habib, 2013). 1313, 1299, and 1302 are frequencies of vibrations indicating C-N stretch. The absence of two bands (3400-3500) for both symmetric and asymmetric stretch as should be seen in amines and the presence of the amide and carboxylic bands indicates new bond formation in products. The broad –OH in carboxylic acid pick indicates product formation. The all stacked and overlaid spectra show

that the 3 compounds have similar functional groups as they share a very similar vibration pattern.

The ^{1}H NMR spectrum of compound A exhibited signals for ortho coupled hydrogen at δH 6.99 (dd, 1H, $J_{5',6'}$=7.9 H-5') and 7.47 (d, 1H, $J_{6',5'}$=7.9 H-6') and a hydrogen coupled at δH 7.06 (d, 1H, H-3') due to the 1',2' and 4' trisubstituted aromatic ring; this result agrees with that obtained by Habib, (2013); Abdullahi M, *et al.,* (2013). The presence of two protons at δH 6.46 (1H, $J_{3,2}$=13 H-3) and 6.57 (1H, $J_{2,3}$=13 H-2) is typical of olefinic hydrogen in trans conformation. (Metin, 2005). The signal at δH 8.43 (s, 1H H-in -COOH) this result agrees with that obtained by Mariam, (2013); Laurie, (2012) but contrary to Habib, (2013), δH 2.27 and 2.34 are signals for (3H each for H-1'' and 2'' respectively) while signals at δH 6.32 (s, 1H, H-in –NH for amides) this result agrees with that obtained by Laurie, (2012); Habib, (2013).

The results of the ^{13}C spectra of compound A showed signals for 12 carbon atoms at δ 17.75 (C-1"), 20.99 (C-2"), 124.58(C-6'), 127.49(C-5'), 130.68(C-1'), 131.64(C-2), 131.70(C-3'), 131.78(C-2'), 136.82(C-3), 137.73(C-4'), 164.82(C-1) and 165.36(C-4) which is similar with that obtained by Habib, (2013) and Laurie, (2012).

The DEPT 135 spectrum for compound A indicates the presence of five methine carbon –CH at δ 124.58, 127.49, 131.64, 131.70 and 136.82 at the upper region and two methyl carbons $–CH_3$ at δ 20.7 and 21.00 at the lower region. Since the expected compound contains no $–CH_2$ carbons as indicated by absence of signals below the DEPT 135 baseline. The resultant remaining carbon from the ^{13}C NMR spectrum reveals the presence of five quaternary carbons -C at 127.68, 130.68, 137.73, 164.82 and 165.36 The nature of the

carbon atoms (–CH,-CH_2 or-CH_3) were established by means of Distortionless Enhancement by Polarization Transfer (DEPT 135) spectroscopy where upon peaks due to –CH and-CH_3 are up the base line while peaks due to-CH_2 fall below the base line in the spectrum.

The COSY spectrum of compound A showed two important cross peaks at δ_H 6.99 (C-5'H) and 7.4(C-6'H) ; δ_H 6.45(C-3 H) and 6.57(C-2 H). The HSQC spectrum was used to assign protons to their respective carbons.

The ^{1}H NMR spectrum of compound B exhibited signals for ortho coupled hydrogen at δH 7.07 (dd, 1H, $J_{4',3'}$=7.4 H-4') and 7.22 (d, 1H, $J_{3',4'}$=7.4 H-3') and a hydrogen coupled at δH 7.48 (d, 1H, H-6') due to the 1',2' and 5' trisubstituted aromatic ring; this result agrees with that obtained by Habib, (2013); Abdullahi , *et al.,* (2013). The presence of two protons at δH 6.52 (1H, $J_{3,2}$=13 H-3) and 6.51 (1H, $J_{2,3}$=13 H-2) is typical of olefinic hydrogen in trans conformation. (Metin 2005). The signal at δH 8.10 (s, 1H H-in -COOH) agrees with that obtained by Abdullahi , *et al.,* (2013); Laurie, (2012) but contrary to Habib, (2013), δH 2.27 and 2.36 are signals for (3H each for H-1'' and 2'' respectively) while signals at δH 6.46 (s, 1H, H-in –NH for amides) which is similar to that obtained by Laurie, (2012); Habib, (2013).

The results of the ^{13}C spectra of compound B showed signals for 12 carbon atoms at δ 17.37 (C-1"), 21.02 (C-2"), 124.88 (C-6'), 136.92 (C-5'), 133.05 (C-1'), 131.42 (C-2), 130.78 (C-3'), 128.01 (C-2'), 137.22 (C-3), 124.5 (C-4'), 164.59 (C-1) and 164.91 (C-4) this result agrees with that obtained by Habib, (2013) and Laurie, (2012).

The DEPT 135 for compound B indicates the presence of 5 methine carbons (–CH) at δ 124.88, 128.50, 130.78, 131.42 and 137.22 at higher region and 2 methyl carbons ($–CH_3$) at δ 17.37 and 21.02 at the lower region. Since the expected compound contains no $–CH_2$ Carbons as indicated by absence of signals below the DEPT 135 baseline. The resultant remaining carbon from the ^{13}C NMR spectrum reveals the presence 5 quaternary Carbons (C) at 128.01, 133.05, 136.92, 164.59 and 164.91.

The COSY spectrum of compound B showed two important cross peaks at δ_H 7.07 (C-4'H) and 7.22(C-3'H) ; δ_H 6.52(C-3 H) and 6.51(C-2 H). The HSQC spectrum was used to assign protons to their respective carbons.

The ^1H NMR spectrum of compound C exhibited signals for ortho coupled hydrogen at δH 6.98 (dd, 1H, $J_{4',3'}$=7.3 H-4') and 7.14 (dd, 2H, $J_{3',4'}$=7.3 H-3' and H-5') due to the 1',2' and 6' trisubstituted aromatic ring; this result agrees with that obtained by Habib, (2013); Abdullahi, *et al.,* (2013). The presence of two protons at δH 6.6 (1H, $J_{3,2}$=13 H-3) and 6.4 (1H, $J_{2,3}$=13 H-2) is typical of olefinic hydrogen in trans conformation (Metin, 2005). The signal at δH 8.4 (s, 1H H-in -COOH) this result agrees with that obtained by Abdullahi, *et al.,* (2013); Laurie, (2012) but contrary to Habib, (2013), δH 2.27 are signals for (6H 3H for CH_3 at C-1'' and 2'' respectively) while signals at δH 6.90 (s, 1H, H-in –NH for amides). This result is similar with that obtained by (Laurie, 2012; Habib, 2013).

The ^{13}C spectra of compound C showed signals for 10 carbon environments at δ 18.30 (C-1" and C-2"), 138.95 (C-1'), 135.1 (C-2' and C-6'), 128.72 (C-3' and C-5'), 128.52 (C-4'), 130.84 (C-2), 137.35 (C-3), 165.26 (C-1) and 165.11 (C-4) this result is similar with that obtained by Habib, (2013) and Laurie, (2012).

The DEPT 135 Spectra of compound C indicates the presence of 4 –CH at δ 128.53, 128.72 (2 CH same environment), 130.84, and 137.41 at the higher region and 1 –CH_3 environment at δ 18.30 (2 CH_3 same environment) at the lower region. Since the expected compound contains no –CH_2 carbons as indicated by absence of signals below the DEPT 135 baseline. The resultant remaining carbon from the ^{13}C NMR spectrum reveals the presence of 4 quaternary Carbons environment at 135.10 (2 C same environment), 138.95 165.11 and 165.26

The COSY spectrum of compound C showed two important cross peaks at δ_H 7.14 (C-3'H) and 6.96(C-4'H) ; δ_H 6.35(C-3 H) and 6.63(C-2 H). The HSQC spectrum was used to assign protons to their respective carbons. From all the NMR spectra it is observed that traces of a cis-trans interconversion of the 3 isomeric forms of the compound at position 2 and 3 occurs with the trans form more predominant. From Table 4.9 comparison in ^{13}C NMR values of the three isomeric carbons it is observed that there is a similarity in the values of the compound A, B and C from position 4, 3, 2, 1, 1'' and 2''. This is because the positions are identical between the 3 isomeric forms of the compounds synthesized as such show similar signals.

The Lethal Dose; LD_{50} using Lorkes method for compound A was 774.6 mg/kg, compound B 1131.4mg/kg and compound C 1131.4mg/kg after which their respective 30, 15, and 7.5 % graded doses were obtained to be 200, 100 and 50mg/kg for compound A ; 300, 150, and 75 mg/kg for compound B; and 300, 150, and 75 mg/kg for compound C (Maiha *et al.*, 2009). This all falls within the range of drugs classified as slightly toxic as all the LD_{50} values fall between 500-5000 mg/kg (Hollinger, 2003)

The Beam walk Neurotoxicity test was conducted on compound B and C following a pilot study revealing possibilities of anticonvulsant activity. All test doses of compound C; 75 and 150mg/kg of compound B proved to be neurologically non toxic by having a shorter time to complete task and fewer foot slips as similar with distilled water thus making it safe for use which agrees with the result obtained in the work of Bin H, *et al.,* (2001) on the neurological safety carboxamides. While test dose of 300 mg/kg of compound B showed significant motor coordination impairment as seen with diazepam proved to be Neurotoxic. This was ascertained using statistical tools to analyse test results. Since number of animals per group are less than 10 and the values failed the homogeneity of variance test, then Anova cannot be used and thus a non-parametric test ; Kruskarl-Wallis was used to analyze the result at $p \leq 0.005$.

Anticonvulsant Screening results for MES and scPTZ test models are given in tables 4.11 and 4.12 respectively. In the MES test model; the result showed no quantal protection against occurrence of seizures between the groups that were given distilled water, compound A and B. Likewise there was a 20% protection against seizures in the group that received compound C, which is not significant or dose dependent. Therefore, compound A, B and C did not have protective capacity as seen in phenytion against maximal electro-induced seizures.

There was significant decrease in both the onset and mean time of recovery for compound B and C when compared to distilled water and the result obtained by dichloro- substitutes in the work of Abdullahi, *et al.,* (2013). It could be said that compound B and C has the ability to increase the mean time and reduce frequency of GABA gated chloride ion

channel (Vanquelin *et al.,* 2002). Likewise, It could also be said that an increase in lipophilic moieties (dihydroxyl- to dimethyl-) on the compound increases its ability to reduce mean time of recovery in MES test models due to enhance permeabimility through the blood-brain barrier. This further explains that carboxamides are preferentially active against the type of seizure induced by MES test model (Bin *et al.,* 2001).

In the scPTZ test model; the result showed no quantal protection against occurrence of seizures between the groups that were given distilled water, compound A, B and C. Likewise there was a 20% protection against seizures in the group that received compound B at test dose of 300 mg/kg, which is not significant. Therefore, compound A, B and C did not have protective capacity as seen in valproate against scPTZ induced seizures and no significant increase in the mean time for onset of seizure for compound A, B and C when compared to distilled water.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The compound A, B and C were synthesized in good yields of 87, 85 and 80 % and were screened in mice for anticonvulsant activity and toxicity studies were carried out. In the preliminary investigation the compound B and C were screened at three dosage levels; 75, 150 and 300 mg/kg and compound A was screened at three dose levels of 50, 100 and 200 mg/kg using the MES and scPTZ seizure test models. Neurotoxicity was assessed using the beam walking assay for neurological deficit.

Compounds that were active at a graded dosage of 7.5, 15 and 30% of LD_{50} or less were considered for neurotoxicity evaluation.

Structural modification to increase lipophilicity can enhance drug activity via improved permeabilibity through the blood-brain barrier.

6.2 Conclusion

The 3 isomeric compounds; A: 4-[(2,4-dimehylphenyl)amino]-4-oxobut-2-enoic acid, B: 4-[(2,5-dimehylphenyl)amino]-4-oxobut-2-enoic acid, and C: 4-[(2,6-dimehylphenyl)amino]-4-oxobut-2-enoic acid were synthesized with high yields.

Compound A is a yellow powder with a melting point of 105-107 ^{0}C and a wavelength of maximum absorbance of 291 nm. Compound B is a light green powder with a melting point of 75-77 ^{0}C and a wavelength of maximum absorbance of 285 nm. Compound C is a

yellow cbocolate brown spiky powder with a melting point of 90-92 ^{o}C and a wavelength of maximum absorbance of 257 nm.

The median lethal dose for compound A, B and C is 774.6, 1131.4 and 1131.4 mg/kg respectively.

Compound A, B and C did not have protective capacity as seen in phenytion against maximal electro-induced seizures. There was significant decrease in both the onset and mean time of recovery for compound B and C when compared to distilled water and the result obtained by dichloro- substitutes in the work of Abdullahi, *et al.,* (2013).

Therefore, accepting the hypothesis that the compound B and C has anticonvulsant activity against MES test model.

All the compounds exhibit no activity against scPTZ seizure test by providing no protection to the animals and no increase in time of onset of seizure.

Compound B and C proved anticonvulsant activity thus affirming Unverferth *et al.,* (1998) and Shindikar *et al.,* (2006) proposed pharmacophores models for anticonvulsant activity while Compound A did not show anticonvulsant activity across both test models.

6.3 Recommendation

A more lipophilic enhancing structural modification should be carried out on the 2,5 and 2,6 isomeric forms of the synthesized compounds in order to make it usable for treating epilepsy.

Chronic toxicity studies should be carried out on compounds that have shown anticonvulsant activity.

Catalyzed reaction should be carried out to help increase yield and shorten reaction time in the synthesis of these compounds

Further pharmacological (both in vivo and in vitro) investigation on compound B and C; and the compounds can be tested for other CNS disorders

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