SYNTHESIS AND ANTICONVULSANT SCREENING OF THREE ISOMERIC 3-(DIMETHYLPHENYL)AMINOPROPANAMIDES

BY

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACEUTICAL CHEMISTRY

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SEPTEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled: Synthesis and anticonvulsant screening of three isomeric 3-(dimethylphenyl)aminopropanamides has been performed by me in the Department of Pharmaceutical and Medicinal Chemistry Ahmadu Bello University, Zaria under the supervision of Dr Abdullahi Idris and Dr A. M. Musa.

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 at this or any other university.

_____ _____

Shuaibu Ahmad Date

CERTIFICATION

This thesis entitled: Synthesis and anticonvulsant screening of three isomeric 3-(dimethylphenyl)aminopropanamides by Shuaibu Ahmad meets the regulations governing the award of a Master of Science degree in Pharmaceutical and Medicinal Chemistry of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my beloved father, Ahmad Abdullahi and my late mother, Barakatu Ado.

LIST OF ABBREVIATIONS

cm	centimeter
cm^{-1}	wave number unit
g	gram
g/kg	gram per kilogram
ml/kg	milliliter per kilogram
ml	milliliter
nm	nanometer
ppm	parts per million
UV	ultraviolet spectroscopy
IR	infrared spectroscopy
^{1}H-NMR	proton nuclear magnetic resonance spectroscopy
mg/kg	milligram per kilogram
%	percentage
EEG	electroencephalography
R_f	retention factor
^{13}C-NMR	carbon-13 nuclear magnetic resonance

LD_{50}	median lethal dose
AED	antiepileptic drug
GABA	gamma amino butyric acid
TLC	thin layer chromatography
MES	maximum elecro shock
PTZ	pentylenetetrazole
i.p	intraperitoneal
3-(2,4-DMP)AP	3-(2,4-dimethylphenyl)aminopropanamide
3-(2,5-DMP)AP	3-(2,5-dimethylphenyl)aminopropanamide
3-(2,6-DMP)AP	3-(2,6-dimethylphenyl)aminopropanamide
NMDA	N-methyl-D-aspartate
DMPA	dimethyl phenyl amine

ABSTRACT

Tremendous effort was made in this work to synthesize the three isomeric 3-(dimethylphenyl)aminopropanamides through the Michael nucleophilic substitution reaction. However, some of the results obtained after spectroscopic analysis did not satisfactorily conform to the structure of the compounds, this was attributed perhaps to the lack of control of the experimental conditions, the methodology adopted or some instrumental constrains. The anticonvulsant screening of the hypothetical compounds was also carried out via the maximal electroshock (MES) and pentylenetetrazole seizure models.

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CHAPTER ONE

1.1 Introduction

Epilepsy is one of the most common neurological disorders that affect approximately 70 million people worldwide (Yemadje *et al.,* 2011), of which 10.5 million are children (Guerrini, 2006). Nearly 80% of people with epilepsy are found in developing countries, where epilepsy remains a major public health problem, not only because of its health implications but also for its social, cultural, psychological and economic effects (Kabir *et al.,* 2005; Yemadje *et al.,* 2011). However, worldwide prevalence of epilepsy varies from 2.8 to 19.5 per, 1000 of the general population (Dantas *et al.,* 2001). In Nigeria the prevalence of active epilepsy is 3 – 14 per 1000 population (W.H.O. 2004).

Epilepsy is basically a chronic brain disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge of neurons that result in almost instantaneous disturbance of sensation and loss of consciousness (Kabir *et al.,* 2005; Yoo *et al.,* 2009).

Epilepsy causes seizure to occur and these seizures can cause a variety of symptoms depending on the areas of brain affected. Symptoms can vary from mild to severe and can include complete or partial loss of consciousness, loss of speech, uncontrollable motor behavior, and unusual sensory experiences (Atshunler, 1990).

Religious and socio-cultural beliefs influence the nature of treatment and care received by people with epilepsy (Kabir *et al.,* 2005, Njamnshi *et al.,* 2009). Wrong perception and beliefs about epilepsy create serious negative social and psychological consequences for people with epilepsy such as fear, humiliation and limitations in social interaction (Lua and Neni 2011). One of the greatest challenges facing the optimal management of

epilepsy is stigma which makes the lives of people with epilepsy difficult (Aydemir, 2011). Many communities in Africa believe that epilepsy result from insanity, witchcraft or possession by evil spirits (Kabir *et al.,* 2005; Hassan *et al.,* 2010). Persons with epilepsy are shunned and discriminated against in employment and marriage because epilepsy is seen as a highly contagious and shameful disease in the eyes of the public (Kabir *et al.,* 2005). This discrimination against epileptic patients could also be due to lack of knowledge and understanding about epilepsy (Hassan *et al.,* 2010).

Epilepsy tends to be more common in the lower socio-economic groups than in higher economic class (Danesi *et al.,* 1981). Shomansky and Glaser (1979) reported high incidence of epilepsy among the poor blacks in United States of America. Several studies reported in Africa also confirmed a higher prevalence of epilepsy among rural dwellers (Osuntokun 1978; Watts 1992; Schoenberg, 1978).

Several carboxamides have been found to possess good anticonvulsant activity. These include toluidine propamides, anisidinopropamides (Idris *et al.,* 2008), phthalimide (Vamecq *et al.,* 2000), and chalcone of N-phenylacetamide (Singh and Rana, 2010). They are capable of preventing seizure spread through blockade of voltage-dependent sodium channels. The anticonvulsant properties of the 3-(dimethylphenyl)aminopropanamides have not been reported in the literature to the best our knowledge.

1.2 Historical Background of Epilepsy

Epileptic seizures have been recognized for millennia. One of earliest descriptions of a secondary generalized tonic-clonic seizure was recorded over 3000 years in Mesopotamia. The seizure was attributed to the god of the moon (Owsei, 1994). Epileptic seizures were described in other ancient cultures, including those of China, Egypt, India

and Africa (Osuntokun, 1978). An ancient Egyptian papyrus described a seizure in man who had previous head trauma. Hippocrates wrote the first book about epilepsy almost 2500 years ago (Owsei, 1994). He rejected ideas regarding the Devine etiology of epilepsy and concluded that the cause was excessive phlegm that causes abnormal brain consistency. Hippocratic teachings were forgotten, and Devine etiologies again dominated beliefs about epileptic seizures during medieval times. Even at the turn of the 19th century, excessive masturbation was considered a cause of epilepsy. This hypothesis is credited as leading to use of the first effective anticonvulsant (i.e bromides) in 1850. Modern investigation of the etiology of epilepsy began with the work of Fritsch, Hitzig, Ferrier and Caton in the 1870s (Goodkin, 2007). They reported 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; 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 two major classes (Generalized and Partial) of epileptic seizures currently used (Goodkin, 2007).

1.3 The Michael addition reaction

The Michael addition reaction, which is also commonly termed conjugate addition, has recently attracted attention as a synthetic strategy for the formation of many carboxamides. The Michael addition, named after Arthur Michael the American organic chemist, is a facile reaction between nucleophiles and activated olefins and alkynes in which the nucleophiles add across a carbon-carbon multiple bonds (Rubio *et al*., 1998). The Michael addition benefits from mild reaction conditions, high functional group

tolerance, a large host of functional precursors as well as high conversions and favorable reaction rates (Vernon *et al.* 2003). The Michael addition involves the addition of a nucleophile, also called a `Michael donor' to an activated electrophile usually olefin, `the Michael acceptor', resulting in Michael adduct. Although, the Michael addition is generally considered the addition of enolate nucleophiles to activated olefins, a wide range of functional groups possess sufficient nucleophilcity to act as Michael donors. Reactions involving non enolate nucleophiles such as amines, thiols and phosphines are typically referred to as Michael-type additions (Mather et al., 2006). The Michael acceptor possesses an electron withdrawing and resonance stabilizing activating group, which stabilizes the anionic intermediate. Michael addition acceptors are far more numerous and varied than donors, due to the plethora of electron withdrawing activating groups that enable the Michael addition to olefins and alkynes. Acrylate ester, acrontriles, acrylamides, maleimides, alkyl methacrylates, cyanoacrylates and vinyl sulphonates serves as Michael acceptors. Less common, but equally important, vinyl ketones, nitro ethylenes, α, ß-unsaturated aldehydes, vinyl sulphonates, acrylonitrile, vinyl pyridines, azo compounds and even acetylene esters also serve as Michael acceptors (Vaccaro and Scola, 1990).

1.4 Theoretical Framework

The brain, spinal cord, and musculature interact via nerve cells called neurons, the functional unit of the CNS. The neuron is made up of a soma or cell body; dendrites that receives information from other axons or various receptors; and axons that transmit information from the axons. Neurons interface with other nerve cells via small gaps called synapses. At the synapse, an axonal terminal bout on is in close proximity to

dendrite of another axon. At the synapse, a chemical neurotransmitter is released from the axonal terminal bout on as a result of an action potential, the electro physiologic voltage change manifested in the axon due to a transient variation in the sodium and potassium permeability of the axon. This neurotransmitter diffuses across the synapse and binds to receptors on the dendrites of the next axon (Bohdan, 2007).

When the permeabilities of the membrane's ionic channels for sodium and potassium are varied sequentially, a fluctuation in the membrane voltage occurs, which is termed the action potential. As the sodium attempts to enter the nerve cell, the potassium permeability increases as the potassium channels open, and the membrane begins to repolarise to the resting membrane potential. The nerve cell repolarises and is ready for the next action potential to come along. These action potentials are also modified by the flux of chloride ions and the presence or absence of the GABA activity in the membrane of the axon (Bohdan, 2007).

A neuron or group of neurons in the brain can become hyper excitable or irritable due to hypoxia, ischemia, hypoglycememia, or electrolyte abnormalities that affect the action potential and cause these nerve cells to discharge action potential irregularly without adequate suppression and attenuation of the abnormal activity. If this occurs, the corresponding muscle fascicle may begin to contract inappropriately, thus producing seizure-like activity (Bohdan, 2007). Depending on where the focus of this aberrant discharge is in a particular region of the brain, the corresponding motor or sensory area will be affected, leading to either motor symptoms such as tonic-clonic contractions or sensory manifestations of seizure-like activity. These foci of aberrant electrical activity (the seizure) may be isolated, or the focus may spread and involve various areas of the

brain, leading to chaotic, uninhibited discharge of electrical activity of various neurons in the brain. The resultant and /or sensory activity manifested by and experienced by the patient is clinically described as a seizure (Bohdan, 2007).Control of the seizure can be accomplished by suppressing the action potential via manipulation of sodium and potassium ion permeabilities, rendering the axon refractory to the action potential, or blocking transmission of impulses at the synapse by blocking the neurotransmitter from binding to its receptor site, or preventing its release and/or synthesis (Bohdan, 2007).

AEDs are characterized by their ability to block seizures in patients with epilepsy, mediated by their interaction and inhibition of brain Na^+ channels (Rogawski and Loscher, 2004; McNamara, 2006). AEDs have little effect on normal brain activity, and they blocked Na^+ poorly at slower firing rates from normal resting potentials. Seizures, however, are characterized by depolarized resting potentials and high frequency trains of action potentials. The drugs are thought to suppress seizures by inhibiting this high frequency repetitive firing, allowing the drug to have selective action on hyperactive rather than normal neurons (MacDonald and Kelly, 1993; MacNamara, 2006). AEDs have little effect on skeletal muscle or heart, even though excitation in those tissues depends on Na^+ channels with similar structures.

1.5 Pharmacophore model

The conformational analysis of currently used AEDs such as phenytoin, carbamazepine, lamotrigine, rufinamide and Phenobarbital has led to the proposal of many pharmacophore models for anticonvulsant activity (Pendeya *et al.*, 1999), these include model of Jones & Woodbury (1982), Codding *et al.*, (1989), Brouillette *et al.*,(1990) and Dimmock and Baker 1994 and that of Shindikar for carboxamides.

Shindikar *et al.,* (2006) proposed a two point pharmacophore (figure 1.1) that consists of aromatic ring separated by 4.18-6.88 Å from a hydrogen bonding domain for an anticonvulsants acting on sodium channels. The model also works well with so many compounds that were synthesized and tested for anticonvulsant activity. Such compounds include: anisidopropanamides, aniline propanamides (Idris *et al.,* 2008), N-Phenyl acetamide (Shindikar *et al.,* 2006).

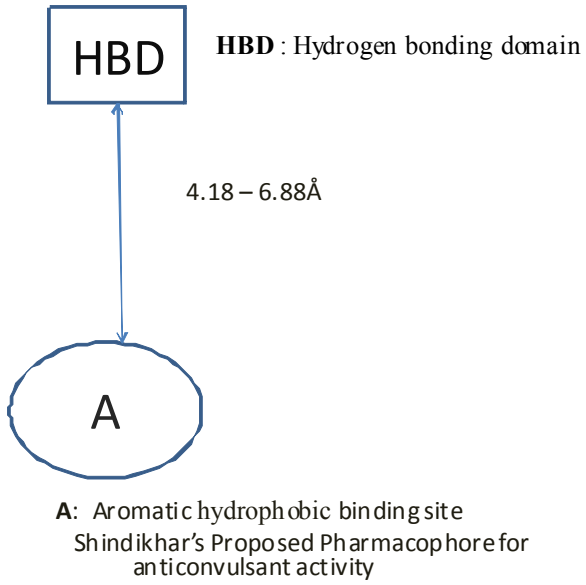


Figure 1.1: Shindikar´s Proposed pharmacophore for anticonvulsant activity

The three isomeric 3-(methylphenyl)aminopropanamides contain a hydrophobic aromatic (phenyl group) and a HBD unit (amide group). The distance between the aromatic hydrophobic (A) and the hydrogen bonding domain (HBD) is 5.38-6.63 Å which is in line with Shindikar´s proposed pharmacophore model for anticonvulsant activity.

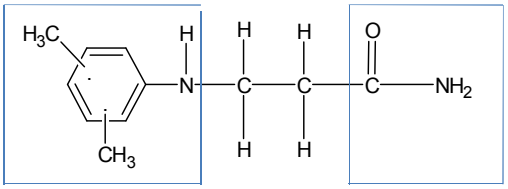


Figure 1.2: 3-(dimethyl phenyl)aminopropanamides showing aromatic Hydrophobic binding site **(A)** and the amide as the hydrogen bonding domain (**HBD**) adopted from Shindikar´s model.

Distance between A and HA/D in conformity with Shindikhar´s model

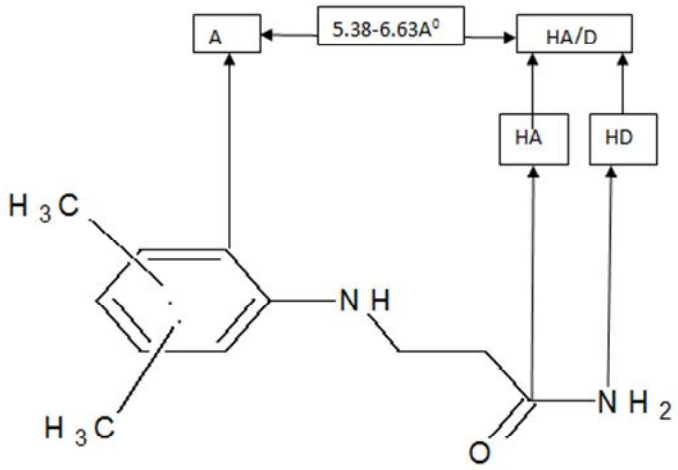


Figure 1.3: showing distances between structural units

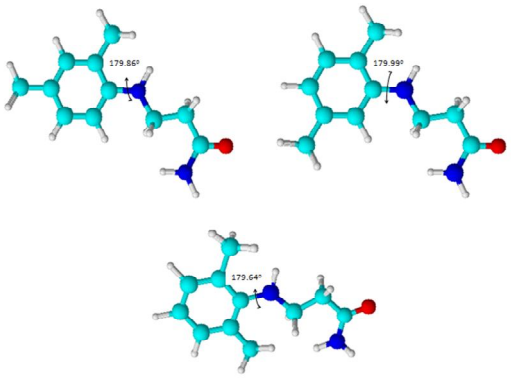


Figure 1.4: 3-dimensional structures showing co- planarity of methyl groups with aromatic ring

In this work three isomeric 3-(dimethylphenyl)aminopropanamides were synthesized through the Michael addition reaction. The synthesized compounds were screen for anticonvulsant activity using the maximum electroshock (MES) and pentelynetetrazole (PTZ) seizure models in order to ascertain their anticonvulsant activity.

1.6 Research hypothesis

The three isomeric 3-(dimethylphenyl)aminopropanamides have anticonvulsant activity

1.7 Statement of the problem.

AEDs are associated with numerous side effects including ataxia, hepatotoxicity, gingival hyperplasia and megaloblastic anemia. (Brunton *et al*., 2009). There is a significant group of patients (up to 30%) who are resistant to the available antiepileptic drugs. Available anticonvulsant drugs merely treat the symptoms and not the disorder (Bell and Sander, 2002).

1.8 Aim of the study.

To synthesize and evaluate the anticonvulsant potentials of three isomeric 3-(dimethyl phenyl)aminopropanamides.

1.9 Objectives of the study

1) To synthesize and characterize three isomeric 3-(dimethylphenyl)aminopropanamides
2) To estimate the LD_{50} of the synthesized compounds.
3) To screen the three compounds for anticonvulsant effect in maximal electroshock (MES) and pentylenetetrazole (PTZ) seizure test models.

1.9.1 Significance of the study

1) Epilepsy is a neurological disorder with far-reaching effects on the lives of those afflicted.
2) Uncontrolled epileptic seizures can be detrimental to a person's general well being and above all his quality of life (Epilepsy Foundation, 2010).
3) The anticonvulsant properties of the three isomeric compounds have not been reported in the literature.

CHAPTER TWO

LITERATURE REVIEW

2.1 Types of epileptic seizures

The numerous epileptic seizures types are most commonly defined and grouped according to scheme proposed by the Epilepsy Foundation (EF), (2009). Distinguishing between seizure types is important since different types of seizures may have different causes, prognosis and treatments (Holland *et al.,* 1992). The Epilepsy Foundation states that seizures are classified under four different categories: Generalized, partial, non epileptic and status epilepticus. This classification is based on observation (Clinical and EEG) rather than the underlying pathophysiology or anatomy.

2.1.1 Generalized seizures

Generalized seizures differ from others in that both sides of the brain are affected throughout the life of the seizure (Svoboda, 2004). During this type of seizure consciousness is lost for a period of time. Generalized seizures are broken into tonic-clonic, myclonic, absence and atonic by the Epilepsy Foundation (2009). Searson, (2008) also added tonic and clonic seizures separately. Tonic-clonic seizures are those which are generally the most recognizable and are the most common type of seizure, also called grand mal seizures (Epilepsy Foundation, 2009). These generally last for a period of 1 to 2 minutes. There are two phases to grand mal seizures which gives its name: tonic-clonic. During the tonic phase, the limbs stiffen and it is often accompanied by the person falling to the ground while very rigid. During the clonic phase the face and limbs jerk. If the tonic phase or the clonic phase of the grand mal seizure is all that is experienced, this is

like a “mini” grand mal seizure. Searson (2008) found that it is often the arm or the leg that is affected by this type of seizure. It usually affects both sides of the body but not always. The body muscle jerk rapidly but usually look like a twitch to others, rather than a seizure. According to the Epilepsy Foundation, (2009), for those who are not epileptic the closest experience may be when during sleep, a body part jerks. Absence seizures, or petit mal seizures, are usually not noticeable to outsiders. They start and stop suddenly, causing the affected person to “zone out” for several seconds. Searson (2008) described them as “a short cessation of physical movement and loss of attention”. They usually last somewhere from 2 to 15 seconds and the person will resume whatever activity he was doing beforehand usually without realizing that the event just occur (EF 2009). The person may stare, flutter their eyes, or do certain motions such as smacking the lips or fumbling repeatedly. Atonic seizures are somewhat similar. Searson (2008) stated that they are often characterized by the affected person dropping to the floor, or dropping their head suddenly. The cause of this is a sudden lack of muscle tone in the body. These occur very suddenly and can cause head or face injuries because they happen so quickly and the person has no warning to protect themselves from the fall (Searson, 2008).

2.1.2 Partial Seizures

According to Searson (2008), partial seizures are those in which the activity in the brain affects only a portion of the brain, unlike generalized seizures, and may not spread to the other areas. There are two subcategories of this type of seizure; simple partial seizures with no alteration of consciousness or memory and complex partial seizures with alteration of consciousness or memory. Simple partial seizures can be motor seizures with twitching, abnormal vision, sounds or smells, and distortion of perception. Seizure

activity can spread to the autonomic nervous system, resulting in flushing, tingling, or nausea. All such simple partial seizures will be in clear consciousness and with full recall on the part of the patient. If the patient becomes confused or cannot remember what is happening during the seizure, then the seizure is classified as a complex partial seizure.

Complex partial seizures previously were called psychomotor seizure, temporal lobe seizure or limpic seizures. Complex partial seizure may have an aura, which is a warning for the seizure, typically a familiar feeling, nausea, heat or tingling, or distortion of sensory perceptions. During the complex partial seizures patients may fumble or perform automatic fragments of activity such as lip smacking, picking at their clothes, walking around aimlessly, or saying nonsense phrases over and over again. These purposeless activities are called automatisms (Robert and Maslah, 2010).

2.1.3 Non epileptic Seizures

Non epileptic seizures can look like epileptic seizures outwards but there are no electrical disruptions to the brain occurring in this instance (EF, 2009).

2.1.4 Status Epilepticus

Status epilepticus is what need to be watched for during a seizure. This is when a seizure lasts more than thirty minutes in length (Klepping and Zaagman 2009). When the seizure continuous indefinitely, it can be very dangerous to the brain, as the entire body is deprived of oxygen, it may cause permanent damage (Klepping and Zaagman 2009).

2.2 Historical background of Antiepileptic drugs

Modern treatment of seizures started in 1850 with the introduction of bromides (Dreifuss, 1995) which was based on the theory that epilepsy was caused by excessive sex drive. In 1910, Phenobarbital, which then was use to induce sleep, was found to have anti seizure activity and became the drug of choice for many years (Dreifuss, 1995). A number of medications similar to Phenobarbital were developed, including primidone. In 1938, Merrit and Putnam described animal models for screening multiple compounds for antiepileptic activity in Journal of American Medical Association. In 1940, phenytoin was found to be an effective drug for the treatment of epilepsy, and since then it has become a major first-line antiepileptic drug in the treatment of partial and secondary generalized seizures (Dreifuss, 1995).

In 1968, carbamazepine was approved, initially for the treatment of neuralgia; later, in 1974, it was approved for partial seizures. Ethosuximide has been used as a first-choice drug for the treatment of absence seizures without generalized tonic-clonic seizures. Valproate was licensed in Europe in 1960 and in the United States in 1978, and now is widely available throughout the world. It became the drug of choice in primary generalized epilepsies and in the mid-1990s was approved for treatment of partial seizures. These anticonvulsants were the mainstays of seizure treatment until the 1990s, when newer antiepileptic drugs with good efficacy, fewer toxic effects, better tolerability, and no need for blood level monitoring were developed. These newer antiepileptic drugs

include lamotrigine, oxcarbamazepine, topiramate, gabapentin and levetiracetam (Molgaard-Nelsen and Hviid, 2011).

2.3 Field of AEDs research

The field of AEDs research is very dynamic. Numerous compounds of varying chemical classes are reported each year as possessing anticonvulsant activity. One of such class of compounds is the carboxamides, which include several Benzamides (Bruce and Lynes, 1992). Ethanamides (Johnson *et al.,* 1962; Carraz *et al*., 1964) and Piperidine carboxamides (Haj-Yahia and Bialer, 1996; Hinko and Crider, 2001). In particular, the anilide function yields potent anticonvulsant compounds such as Ameltolide (Clark *et al.,* 1986), Ralitoline (Leppik, 1994), certain N-phenylthalimides (Bailleux *et al*., 1995; Vamecq *et al.,* 2000), N-(2,6-dimethylphenyl)-2-piperidine carboxamides (Ho *et al.,* 1998). In addition, all currently used antiepileptic drugs, such as hydantoins, deoxybarbiturates, succinimides, benzodiazepines, iminostilbenes and carboxylic acids, have been obtained through chemical synthesis (Sander and Shorvon, 1996). The maximum electroshock (MES) test is a predictor of compounds that are active against grand mal seizures (Krall *et al.,* 1978). The pentylenetetrazole (PTZ) test is used to detect compounds useful in treating petit mal seizures.

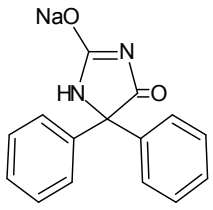
2.4 Mechanism of action of Antiepileptic drugs

Many structures and processes are involved in the development of a seizure, including neurons, ions channels, receptors, glia, and excitatory synapses. The antiepileptic drugs are designed to modify these processes so as to favor inhibitory over excitatory and thereby stop or prevent seizure activity. At cellular levels, three basic mechanisms are recognized: modulation of voltage-dependent ion channels (Na^+, Ca^{2+}, K^+), enhancement of GABA mediated inhibitory neurotransmission and attenuation of excitatory transmission (Porter and Rogawski, 1992).

2.4.1 Modulation of ion channels by antiepileptic drugs

Phenytoin

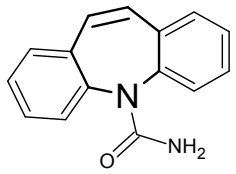
Phenytoin was synthesized as a barbiturate analogue. It has become the first line treatment for partial and generalized tonic-clonic seizures. Phenytoin exerts its anticonvulsant effect primarily by action on voltage- dependent Na^+ channels. Phenytoin inhibits high frequency repetitive firing in a voltage-dependent manner, with limitation of firing increased after depolarization and removed by hyperpolarisation. This is achieved by prolonging the inactivated state of voltage sensitive neuronal Na^+ channel that governs the refractory period of the neuron. As a result high frequency discharges are inhibited with little effect on normal low frequency discharges which allow Na^+ channel to recover even when inactivation is prolonged. Phenytoin has also been reported to block high Voltage-activated Ca^{2+} channels to attenuate glutamate release and, paradoxically, to reduce K^+ current (Davies, 1995).



Phenytoin

Carbamazepine

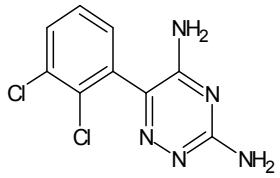
Carbamazepine is widely used in the treatment of partial and generalized tonic-clonic seizures and chemically related to the tricyclic antidepressants. Its value in the treatment of epilepsy was discovered by chance. Carbamazepine's by blocking sodium channels during rapid, repetitive, sustained neuronal firing thereby preventing post tetanic potentiation (Lang *et al.,* 1993).



Carbamazepin

Lamotrigine

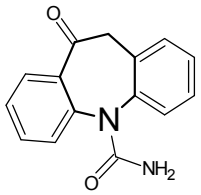
Lamotrigine is a new antiepileptic drug; it is a derivative of phenyl triazine from the group of folic acid antagonists. The main action of lamotrigine is blockade of sodium channels. Lamotrigine acts pre- and postsynptically. Presynaptically, it inhibits the release of neurotransmitters, like excitatory amino acids Glutamate and aspartate. Postsynaptically, it diminishes the excitability of neurons like other anticonvulsive sodium channel blockers. Apart from inhibiting the sodium conductance lamotrigine may reduce high voltage activated calcium currents. Lamotrigine is a broad spectrum antiepileptic agent (Davies, 1995).



Lamotrigine

Oxcarbazine

Oxcarbazine is a relatively novel antiepileptic drug, which closely resembles carbamazepine in structure. It inhibits voltage-dependent fast sodium channels. In particular its frequency dependent effect gives the substance special significance in seizures. Moreover, in contrast to carbamazepine; oxcarbazine also have favorable influences on potassium channels, calcium channels. It is an effective drug for partial seizures but may aggravate myoclonic or absence seizures (Davies, 1995).

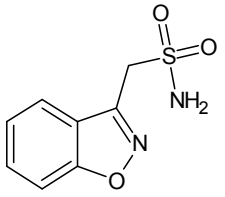


Oxcarbazine

Zonasimide

Zonasimide was synthesized as a benzisoxazole in 1974.It is chemically unrelated to any of the other antiepileptic drugs. It has sulfonamide side chain. The major mechanism of action of zonasimide is reduction of neuronal repetitive firing by blocking sodium channels and preventing neurotransmitter release. It also exerts influence on T-type

calcium channels and prevents influx of calcium. In addition, zonasimide exhibits neuroprotective effects through free radical scavenging (Davies, 1995).

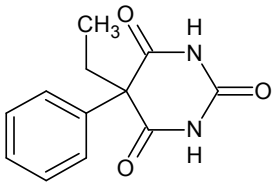


Zonasimide

2.4.2 Potentiation of gamma amino butyric acid by antiepileptic drugs

Phenobarbital

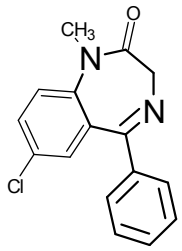
Phenobarbital was the first efficacious antiepileptic introduced in 1912. It is effective for both generalized tonic-clonic and partial seizures in patients of all ages (Mattson *et al.,* 1985). Phenobarbital is used to treat status epilepticus. The drug potentiates inhibitory neurotransmission by increasing the duration of time that GABA-mediated chloride channels remain open and reduces neurotransmitter release from nerve terminals, probably through its effect on calcium channels (Twyman *et al*., 1989). Phenobarbital also diminishes excitatory neurotransmission by reducing the effects of glutamate (White, 1999).



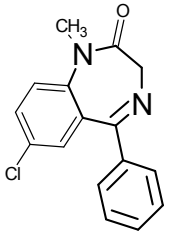
Phenobarbital

Benzodiazepines

A number of benzodiazepines are presently marketed in the world. The most commonly used anticonvulsant drugs are Diazepam, Larazepam, Clobazam and Clonazepam. These antiepileptic benzodiazepines have the efficacy in the potential idiopathic generalized epilepsies and for the acute epilepsies. The benzodiazepines bind with α-subunit of $GABA_A$ receptor, which leads to the increase in the frequency of Cl^- channel opening without affecting duration or channel conductance (Dichter and Brodie 1996).



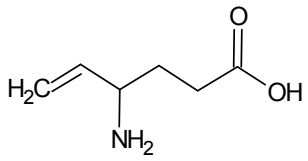
Diazepam



Diazepam

Vigabatrin

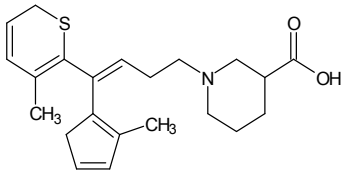
Vigabatrin is an inhibitor of GABA-transaminase, the enzyme which degrades GABA. Anticonvulsant action may be due to increase in synaptic GABA concentration. It suppresses maximal electroshock and kindles seizures, and is effective in many patients with refractory epilepsy, especially partial seizures with or without generalization (Dichter and Brodie 1996).



Vigabatrin

Tiagabine

Tiagabine is a novel antiepileptic drug, recently licensed widely for the adjunctive treatment of partial seizures with or without secondary generalization. Tiagabine enhances the activity of GABA, the major inhibitory neurotransmitter in the CNS. Tiagabine binds to recognition sites associated with the GABA the GABA uptake carrier, suggesting that this activity blocks GABA reuptake into presynaptic neurons and thus permitting more GABA to be available for receptor binding on the surface of post synaptic cells (Dichter and Brodie, 1996).



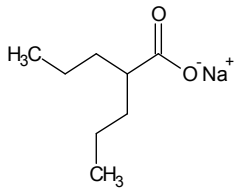
Tiagabin

2.4.3 Antiepileptic drugs with multiple mechanism of action

Sodium valproate

It is a branched chain aliphatic carboxylic acid with a broad spectrum anticonvulsant action. Valproate appears to act by multiple mechanisms: (a) phenytoin like frequency

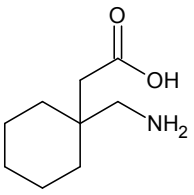
dependent prolongation of Na^+ channel activation, (b) weak attenuation of Ca^{2+} mediated `T'current, (c) augmentation of release of inhibitors transmitter GABA by inhibiting degradation as well as probably by increasing its synthesis from glutamic acid (Davies, 1995).



Sodium valproate

Gabapentin

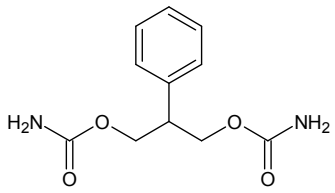
Gabapentin is a novel compound, structurally related to GABA, which is effective in the adjunctive treatment of partial seizures, with or without secondary generalization. This lipophilic GABA derivative crosses the brain and enhances GABA release, but is not an agonist at $GABA_A$ receptor. Early efforts to identify the mechanism of action of Gabapentin proposed an interaction with L-amino acid transport system, resulting in alterations in the cytosolic and extracellular concentrations of several amino acids, including L-leucine and L-valine (Dichter and Brodie, 1996).



Gabapentin

Felbamate

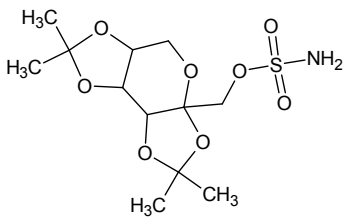
Felbamate is a potent anticonvulsant, very effective against multiple seizure types. It blocks the N-methyl-D-aspartate receptors and voltage-gated calcium channels and also modulates sodium channel conductance, but also no effect on GABA receptors (Pellock and Brodie, 1997).



Felbamate

Topiramate

Topiramate is active against partial-onset and generalize seizures in humans. Topiramate is a newly developed anticonvulsant approved for use since 1998. It is characterized by a complex mechanism of action. It exerts an inhibitory effect on sodium conductance, decreasing the duration of spontaneous bursts and frequency of generated action potentials, enhances GABA, inhibits the 5-methyl-4-isoxazole proprionate subtype glutamate receptor, and is a weak inhibitor of carbonic anhydrase (Dichter and Brodie, 1996).

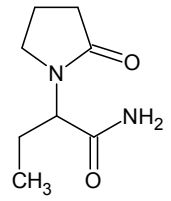


Topiramate

2.4.4 Antiepileptic Drugs with unknown mechanism of Action

Levetiracetam

Levetiracetam is a piracetam derivative. It is effective in several models of seizures, including tonic and clonic audiogenic in mice, tonic seizures in maximum electroshock-seizure test in mice and tonic seizures induced in rodents by chemo-convulsants. The mechanism of action is possibly related to a brain specific stereo-selective binding site, synaptic vesicle protein 2A. Thus, Levetiracetam possibly enhances the release of inhibitory neurotransmitter (Xu, and Bajjalieh, 2002).



Levitiracetam

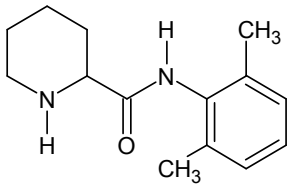
2.5 Amides as anticonvulsant Agents

2.5.1 N-Benzyl amides of gamma amino butyric acid

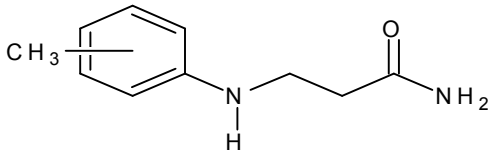
Derivatives of α- substituted gamma- amino, gamma-phthalimido, gamma-acetoxy, and gamma-hydroxy butyric acid, esters and amides have been investigated as new potent anticonvulsants (Malawska and Gobaille, 1995). It has been shown that α- substitutes N-benzylamides of gamma-hydroxybutyric acid are the most potent compounds in this group, possessing anticonvulsant activities in MES screen (Malawska and Gobaille, 1995).

2.5.2 2,6-dimethyl anilides, carboxamides

The activity of several 2-piperidinecarboxamides in the MES test in mice has been reported (Hinko *et al.,* 1996). Using N-(2,6 dimethyl) phenyl-2-piperidinecarboximide and N-(α-methyl benzyl)-2-piperidinecarboxamide as structural leads, a variety of analogues have been synthesized and evaluated for anticonvulsant activity (Hinko and Crider, 2001).The following modifications led to an increase in MES activity: replacement piperidine ring with pyridine and movement of the carboxamide group to the 4-position, then opening the piperidine ring. The 2,6-dimethyl anilides were the most potent compounds in the MES test in each group of compounds.



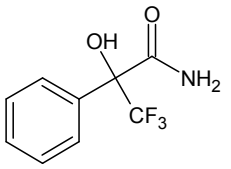
N-(2,6-dimethylphenyl)piperidine-2-carboxamide



3-Toluidinopropanamides

2.5.3 Hydroxyamides

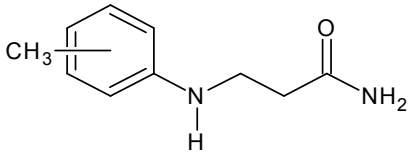
Brown and coworkers have evaluated a series of novel hydroxyamides (Brown *et al.* 1999). Anticonvulsant screening of these compounds revealed the, 3,3,3-triflouro-2-hydroxy-2-phenyl propionamide, to have potent anticonvulsant activity.



3,3,3-trifluoro-2-hydroxy-2-phenylpropanamide

2.5.4 3-Anilinopropamides, 3-toluidinopropamide, 3-anisidopropamide and their N-benzyl derivatives

Idris *et al.,* (2008) designed and synthesized a series of novel 3-anilinopropamides, 3-toluidinopropamides, 3-anisidinopropanamide, and their N-benzyl derivatives. The compounds were tested for anticonvulsant activity in various animal models; most of the compounds were potent with minimal neurotoxicity.



3-Toluidinopropanamides

CHAPTER THREE

3.0 EXPERIMENTAL

3.1 MATERIALS

3.1.1 Solvents/Reagents

All reagents and solvents (n-hexane, methanol, benzene, ethyl acetate, acrylamide, dimethylanilines and iodine) for the experiments were of analytical grade, and were purchased from Sigma Chemicals (Germany).

3.1.2 Experimental Animals

One hundred and two locally bred (in the department pharmacology and therapeutics, ABU Zaria) adult albino mice of either sex weighing between 18-28 g were used in this study. The animals were housed under standard laboratory conditions and allowed free access to food and water. One hundred and twenty (day-old) Ranger Cockerels weighing 28-34 g were also used. The Cockerels were obtained from the National animal production research institute, Shika Zaria.

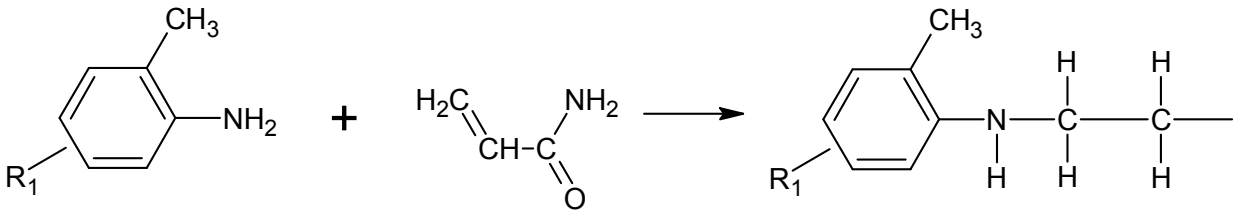
3.2 Determination of 3D structure of the supposed synthesized compounds

The 3-dimensional structure of the compounds were determined using Chemsketch software in order to calculate the distances between the aromatic hydrophobic binding site and the hydrogen bonding domain and identify centers of co- planarity.

3.3 Synthesis of test Compounds

The three isomeric 3-(dimethylphenyl)aminopropanamides were synthesized via Michael nucleophilic addition reaction between dimethyl anilines (Michael donors) and acrylamide (Michael acceptor), solvent free and uncatalyzed. The chemical equations for the synthesis are shown in scheme 1

Scheme 1. Synthesis of 3-(dimethylphenyl)aminopropanamide



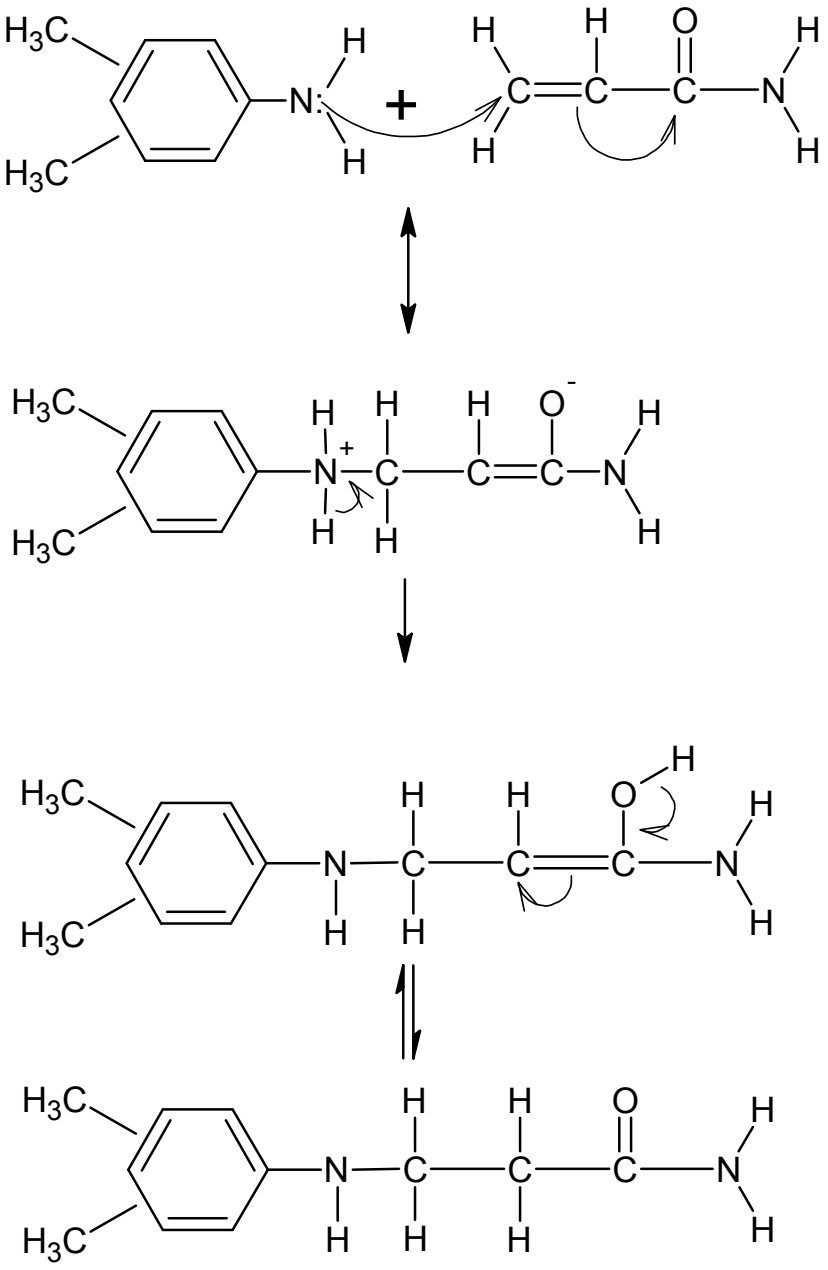
R_1
4-methyl
5-methyl
6-methyl

R_1	Product
4-methyl	3-(2,4-DMP)AP
5-methyl	3-(2,5-DMP)AP
6-methyl	3-(2,6-DMP)AP

3.3.1 General Procedure for synthesis of 3-(dimethylphenyl)aminopropanamide

Equimolar quantities of dimethyl aniline (12.12g, 0.1mole), and acrylamide (7.11g, 0.1mole), were refluxed on a water bath (maintained at about 80^{o}C) for 30 minutes until the mixture was homogenous. The resultant liquid was left to stand at room temperature for 2 weeks. The crystals formed were digested in benzene, filtered and the residue washed with several portions of 5 ml ice-cold benzene. The crystals formed were recrystallized in benzene several times until a single spot on TLC was obtained. The crystals were dried on filter paper. The melting point was determined and the percentage yield calculated.

Scheme 2: Mechanism of the reaction



3.4 TLC Analysis

TLC analysis was used to monitor the progress of the reaction and to confirm the purity of the product. The solvent system for the development of the TLC used was ethyl acetate and n-hexane (1:8) and (1:5). The single spot was visualized using iodine vapor.

3.5 Determination of melting point

Melting point was determined using Gallenklamp melting point apparatus. The results were uncorrected.

3.6 Spectral analysis

Proton and carbon-13 Nuclear Magnetic Resonance (NMR) experiment were performed using AV 400 MHz NMR spectrometer. ^{1}H and ^{13}C NMR data were reported in term of chemical shifts. Fourier Transform Infrared (FTIR) spectra were recorded using Shimadzu spectrometer at the National Research Institute for Chemical Technology, Zaria. Ultraviolet-visible spectroscopy was recorded using Thermo electron Vision prosoftware V 3.00.

3.7 Pharmacological Studies

3.7.1 Acute toxicity studies

LD_{50} was determined using the method of Lorke (1983). All mice were injected intraperitoneally (*i.p*). The study was divided into two phases. In the first phase, twenty

seven mice of either sex were divided into nine groups of three mice each (1-3, 4-6, 7-9), the first group received 10, 100 1000 mg/kg of the first compound; supposed 3-(2,4-DMP)AP, The second group were given 10, 100 and 1000 mg/kg dose levels of the second compound; supposed 3-(2,5-DMP)AP and the last group the same dose of 10, 100 and 1000 mg/kg supposed 3-(2,6-DMP)AP. The animals were observed for signs and symptoms of toxicity including mortality for twenty four hours after treatment.

In the second phase, nine mice of either sex were divided into three groups of three mice each. The first group of three mice received the first compound; supposed 3-(2,4-DMP)AP at dose of 1,600, 2,900 and 5000 mg/kg respectively. The second group of three mice received supposed 3-(2,5-DMP)AP at again a dose of 1,600, 2,900 and 5000 mg/kg. The last group of three mice were given similar dose of supposed 3-(2,6-DMP)AP.

The animals were also observed for twenty four hours. The medium lethal dose was calculated as the square root of the product of the highest dose survived by all the animals and the lowest dose that killed all the animals in the group. The results obtained were used as a guide to select the dose for the MES and PTZ tests.

3.7.2 Anticonvulsant Evaluation of the supposed test compounds

3.7.2.1 Pentylenetetrazole (PTZ) induced seizure test

The method previously described by Swinyard et al., (1989) was employed. Sixty six mice were randomly divided into eleven groups of six mice each. Group I (negative control) were given 10 ml/kg *i.p* of normal saline. Group II (positive control) received sodium valproate 200 mg/kg body weight i.p. Groups III, IV and V received 75, 150 and 300 mg/kg of supposed 3-(2,4-dimethylphenyl)aminopropanamide respectively. While groups VI, VII and VIII received 75, 150, and 300 mg/kg i.p of supposed 3-(2,5-dimethylphenyl)amino propanamide respectively. Groups IX, X and XI received 75, 150 and 300 mg/kg i.p of supposed 3-(2,6-dimethykphenyl)aminopropanamide respectively. After 30 minutes groups (I-X1) were administered with 90 mg/kg freshly prepared PTZ (*i.p)*. All the mice were observed for a period of 30 minutes after PTZ for onset and duration of different phases of epileptic seizure. The quantal protection, time of onset of convulsion and time of death were observed and recorded.

3.7.2.2 Maximum Electroshock Induced seizure test in Chicks

The modified methods of Swinyard *et al.,* (1989) were employed. One hundred and ten (day-old) chicks were randomly divided into 11 groups of 10 chicks each. Group I (negative control) was given normal saline *i.p*. Group II (positive control) was given 20 mg/kg body weight of phenytoin. Groups III-XI were divided into 9 sets and each set was injected with the test compounds at three dose levels; 75, 150, and 300 mg/kg body weight. Thirty minutes later, maximum electroshock was delivered to induced seizure in all the groups using UGO-basile electroconvulsive machine. Corneal electrodes were placed in the upper eye lids of the chicks. The current, shock duration, frequency and

pulse width were set and maintained at 80 mA, 0.8 s, 100 pulses per second (100 Hz) and 0.6 ms respectively. Hind limb tonic extension (HLTE) was selected as the end point of the experiment and the abolition of the HLTE was considered as protection from electroshock induced convulsion (Swinyard *et al.*, 1989).

3.8 Statistical Analysis

The mean onsets of seizure, mean time of recovery, mean time of death were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). $P < 0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 Yield and some physical properties of supposed 3-(2,4-DMP)AP,3-(2,5-DMP)AP and 3-(2,6-DMP)AP

Compound	Physical appearance	Melting point °C	Yield %	R_f
3-(2,4-(DMP) AP	White crystals	91-92	85	0.40
3-(2,5-(DMP)AP	White crystals	89-90	96	0.30
3-(2,6-DMP) AP	White crystals	83-84	95	0.38

4.1 Spectroscopy

Table 4.2 Analysis of the functional group region in IR spectra of supposed 3-(2,4-DMP)AP, 3-(2,5-DMP)AP and 3-(2,6-DMP)AP

Compound	$N\text{-}H_{(S)\ amide}$ cm^{-1}	$C{=}O_{(S)\ amide}$ cm^{-1}	$N\text{-}H_{(S)\ amine.}$ cm^{-1}	$C\text{-}N_{(S)}$ cm^{-1}
3-(2,4-(DMP)AP	3355	1672	3189	1134.18
3-(2,5-(DMP)AP	3353	1668	3191	1134.18
3-(2,6-DMP)AP	3354	1672	3189	1134.15

The N-H stretching and C=O stretching of acrylamide 3410cm^{-1} 1685cm^{-1} respectively.

Table 4.3 Results of ^{1}H-NMR (Chemical shift (ppm) of the supposed synthesized Compounds

Compounds	H-3'	H-4'	H-5'	H-6'	H-7'	H-8'	-NH-	$-NH_2$
3-(2,4-DMP)AP	6.8	—	6.8	6.9	2.2	2.3	7.2	5.6
3-(2,5-DMP)AP	6.6	6.9	—	6.7	2.2	2.3	7.2	5.6
3-(2,6-DMP)AP	6.9	6.7	6.9	—	2.2	2.3	7.2	5.6

Table 4.4 Results of ^{13}C-NMR (δ ppm, MeOH, 400 MH_Z) of the supposed synthesized Compounds

Compounds	C-1	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C-7'	C-8'
3-(2,4-DMP)AP	170	124	129	131	144	127	116	17	20
3-(2,5-DMP)AP	170	146	127	120	132	121	127	17	21
3-(2,6-DMP)AP	170	144	127	131	129	131	127	17	17

Table 4.5 Result of acute toxicity test (LD_{50}) for the supposed 3-(2,4-DMP)AP, 3-(2,5-DMP)AP and 3-(2,6-DMP)AP

Compound	LD_{50} (mg/kg)
3-(2,4-DMP)AP	1264.91
3-(2,5-DMP)AP	1264.91
3-(2,6-DMP)AP	1264.91

Table 4.6 Effect of the supposed 3-(2,4-DMP)AP, 3-(2,5-DMP)AP, 3-(2,6-DMP)AP and phenytoin on Maximal electroshock induced seizure in chicks

Treatment Mg/kg	Quantal protection	Mean time of against seizure	% mortality recovery (min)
Normal saline	0/10	10 ± 2.83	0.00
10 mg/kg			
3-(2,4-DMP)AP			
75	0/10	8.30 ± 0.72	0.00
150	0/10	7.00 ± 1.20	0.00
300	0/10	6.00 ± 0.60	0.00
3-(2,5-DMP)AP			
75	0/10	7.50±0.86	0.00
150	0/10	7.30±0.70	0.00
300	0/10	12.44±2.40	0.00
3-(2,6-DMP)AP			
75	0/10	7.70±1.50	0.00
150	0/10	7.10±1.30	0.00
300	0/10	9.40±1.33	0.00
Phenytoin	8/10	6.00 ± 3.00	0.00

Table 4.7 Effect of the supposed 3-(2,4-DMP)AP, 3-(2,5-DMP)AP , 3-(2,6-DMP)AP and Sodium valproate on pentylenetetrazole induced seizure in mice

Treatment mg/kg	Quantal protection against s seizure	Mean onset of seizure (min)	Quantal protection against mortality	Mean time of death
Normal saline 10mg/kg	0/6	10, 00± 2.04	0/6	15 ± 0.71
3-(2,4-DMP)AP				
75	2/6	6.67 ± 1.15	2/6	11.83 ± 1.12
150	2/6	12.00 ± 3.03	2/6	13.75 ± 2.50
300	1/6	10.80 ± 1.77	1/6	11.40 ± 1.96
3-(2,5-DMP)AP				
75	1/6	6.2 ± 1.24**	1/6	8.80 ± 0.73
150	0/6	6.5 ± 1.50*	0/6	5.33 ± 1.20
300	1/6	6.00 ± 0.55	1/6	11.40 ± 1.03
3-(2,6-DMP)AP				
75	0/6	6.67 ± 0.92*	0/6	8.67 ± 0.42
150	2/6	5.50 ± 1.50**	2/6	6.00 ± 1.60
300	1/6	11.20 ± 1.20	1/6	11.40 ± 1.90
Sodium valproate	6/6	_	6/6	_

n= 6, **p< 0.05 when compared to control

*p< 0.01

CHAPTER FIVE

DISCUSSION

5.1 Synthesis and characterization

The newly supposed synthesized compounds were characterized on the basis of their melting points, IR, ^{1}H NMR, and ^{13}C NMR. The supposed compounds were synthesized through the Michael Nucleophilic addition reaction in satisfactory yields (85–96 %). The supposed compounds were purified by recrystallization from benzene. The purity of the compounds and progress of the reaction were checked by thin layer chromatography (TLC) using ethyl acetate and n-hexane in 1:8 and 1:5 ratios as the solvent system. Yields and physical properties of the compounds are presented in table 4.1. It is worth noticing however, that the R_f values for acrylamide, supposed 2,4-DMA, 2,5-DMA and 2,6-DMA are 0.60, 0.40, 0.30, 0.38 respectively, in comparism to the R_f values of the synthesized compounds (Table 4.1). A clear indication for the formation of the target compounds.

The UV spectrum of the supposed compounds showed λ max at 216 nm and 237 nm for 3-(2,4-DMP)AP, 234 nm for 3-(2,5-DMP)AP and 218nm and 238nm for 3-(2,6-DMP)AP; this indicates the presence of conjugation of the aromatic ring. Although NH_2 group does not absorb UV, but its presence increases wavelength of absorption, hence the reason for two peaks of absorption in two of the UV spectra. However, the two reactants (acrylamide and dimethyl anilines) are also susceptible to UV absorption owing to possession of chromophoric groups; but acrylamide and dimethyl phenyl aniline absorbed at 200 nm and 280nm respectively. The IR spectrum however, showed characteristic stretching band at 1672 cm^{-1} for supposed 3-(2,4-DMP)AP, 1688 cm^{-1} for supposed 3-

(2,5-DMP)AP and 1672 cm^{-1} for supposed 3-(2,6-DMP)AP an indication for the presence of C=O (amide) (Ravi et al., 2010). The stretching band at 3355 cm^{-1}, 3353 cm^{-1} and 3354 cm^{-1} showed the presence of NH (s) vibration of amide in all the supposed synthesized compounds. NH stretching of secondary amine occurred at 3189cm^{-1} for supposed 3-(2,4-DMP)AP, 3191 cm^{-1} for supposed 3-(2,5-DMP)AP and 3189 cm^{-1} for supposed 3-(2,6-DMP)AP. C-N stretching bands at 1135-1134 cm^{-1} occur in all the supposed compounds.

The ^{1}H NMR spectrum of supposed 3-(2,4-DMP)AP showed chemical shift at 2.2 and 2.3 are due to two separate singlet peaks of two methyl protons ($C_{7'}$ and C8'). 6.6 is chemical shift value for singlet of two equivalent aromatic protons ($C_{3'}$ and $C_{5'}$). The singlet aromatic proton ($C_{6'}$) has a chemical shift value at 6.7. 5.6 and 7.2 are chemical shift values due to a singlet secondary amine proton and singlet of two primary amide protons respectively.

The ^{1}HNMR spectrum of supposed 3-(2,5-DMP)AP also showed chemical shift values for two methyl protons ($C_{7'}$ and $C_{8'}$), secondary amine proton and two primary amide protons with corresponding integration similar to 3-(2,4-DMP)AP. However, chemical shift values at 6.6, 6.9, and 6.7 are due to one doublet proton ($C_{3',}$) one doublet proton ($C_{4'}$) and one singlet proton ($C_{6'}$) respectively. Methyl protons, secondary amine proton and the primary amide protons in terms of their chemical shift values and their integration just like in the preceding compounds. However, the aromatic region has the following chemical shift assignment: 6.7 is for a single (triplet) proton ($C_{4'}$) and that at 6.9 for two doublet equivalent protons ($C_{3'}$ and $C_{5'}$).

The ^{13}CNMR of the supposed synthesized compounds elaborated the number and position of each carbon atoms. The chemical shift value at 170 ppm is due to the carbonyl carbon. $C_{7'}$ and $C_{8'}$ are the two methyl carbons attached to the aromatic ring and have chemical shift of 17 ppm and 20 ppm respectively. The remaining chemical shift values are the aromatic carbons $C_{1'}$-$C_{6'}$. The non appearance of chemical values for C_2 and C_3 in the appropriate positions in ^{13}C-NMR spectra has to some extent indicated the non formation of test compounds satisfactorily, nonetheless, the results obtained in this is presented as above.

5.2 Anticonvulsant screening

All the supposed compounds were screened for their anticonvulsant potential through Maximal electroshock (MES) and pentylenetetrazole (PTZ) models in doses of 75, 150 and 300 mg/kg intraperitoneal injection. The doses were selected from their LD_{50} data. The MES induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. MES induced seizure can however, be prevented either by drugs that inhibit voltage depended Na^+ channel, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor, such as felbamate (Mohammed et al., 2002). By contrast, the PTZ test represents valid model for human generalized myoclonic and absence seizures (Loscher and Schmdt, 1988). According to Desarro et al, PTZ may be exerting its convulsive effect by inhibiting the activity of GABA at GABA receptors, the major inhibitory neurotransmitter which is implicated in epilepsy.

The data indicates the failure of the supposed compounds to protect the animals against MES induced seizure at all the doses tested, these suggest that the compounds do not act by inhibiting voltage dependent Na^+ channels and cannot therefore, be effective against generalized tonic-clonic seizure (Mohammed et al., 2002).

Supposed 3-(2,4-dimethylphenyl)aminopropanamide exhibits anticonvulsant in the PTZ screening at the three doses tested: 300,150 and 75 mg/kg body weight. 33.33% of the animals were protected at 150 and 75 mg/kg body weight; also 16.67% of the animals were protected at 300 mg/kg body weight.

Supposed 3-(2,5-dimehylphenyl)aminopropanamide exhibited slight anticonvulsant activity at dose of 75 and 300 mg/kg body weight in which 16.67% of the animals were protected. On the other hand, supposed 3-(2,6-dimethylphenyl)aminopropanamide exhibited anticonvulsant activity at 150 and 300mg/kg where 33.33% and 16.67% of the animals were again protected respectively.

Although, the three compounds have similar pharmacophoric unit, their pharmacological profiles are slightly different. According to the propose pharmacophore of Shindikar (2006), the compounds should display better activity profile because the distance between the centre of aryl group and the amide hydrogen bonding domain falls in to the proposed range 4.18-6.88 Å (Figure 1.3).

The slight activity of the compounds in the PTZ and their lack of activity in the MES can be attributed to their structures shown in figure 1.4. In all the three compounds, the phenyl ring, methylene groups, amide group and the two aromatic methyl groups all lie in the same plane. It can therefore, be suggested that, the relatively low anticonvulsant activity of the compounds may be caused by their co-planarity.

The results of anticonvulsant screening of the supposed compounds presented in this thesis may suggest that the substituted methyl groups on the aryl moiety did not significantly increase anticonvulsant effect as indicated by their lack of activity in the MES test (Idris et al., 2008). The intraperitoneal median lethal dose of the supposed synthesized compounds has been found to be 1264.91 mg/kg in mice.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The hypothetical three isomers were synthesized through the Michael Nucleophilic addition reaction. Their structures were established by means of UV, IR, ^{1}HNMR and ^{13}CNMR spectroscopy. In the carboxamide series Shindikar et al., (2006) had proposed a pharmacophore model with two binding sites essential for anticonvulsant activity and the compounds under investigation also possess two similar binding sites. However, the anticonvulsant screening indicated that supposed 3-(2,4-DMP)AP and supposed 3-(2,6-DMP)AP exhibited slight activity (33.33 %) in the PTZ test at the dose levels of 75, 150 and 300 mg/kg respectively and no activity in all the doses administered in the MES induced seizure test.

6.2 Conclusion

The three supposed isomeric 3-(dimethylphenyl)aminopropanamides were synthesized in excellent yields and tested for anticonvulsant activity. supposed 3-(2,4-dimethylphenyl)aminopropanamide has shown slight activity against PTZ seizure test model in two of the doses administered than the remaining two compounds, perhaps due to the Para position of the second methyl group on the aromatic ring. The supposed three compounds however, fit to the Shindikar`s postulated pharmacophore model. From these data, ideas pertaining to substituent modification or rearrangement may lead to compounds with greater favorable pharmacological properties and lesser side effects. From what has come to light in the ^{13}C-NMR spectra, the lack of conspicuous appearance of signals for C_2

and C_3 has made us to believe and conclude that the compounds under investigation have not been satisfactorily formed (they thymodynamically unstable) under the prevailed experimental conditions carried out.

6.3 Recommendation

Despite limitation to the operation of the NMR spectrometer by myself, the in ability to control some the experimental conditions and other instrumental constrains, it is suggested that synthesis of these compounds be carried out perhaps under modified experimental conditions in the near future.

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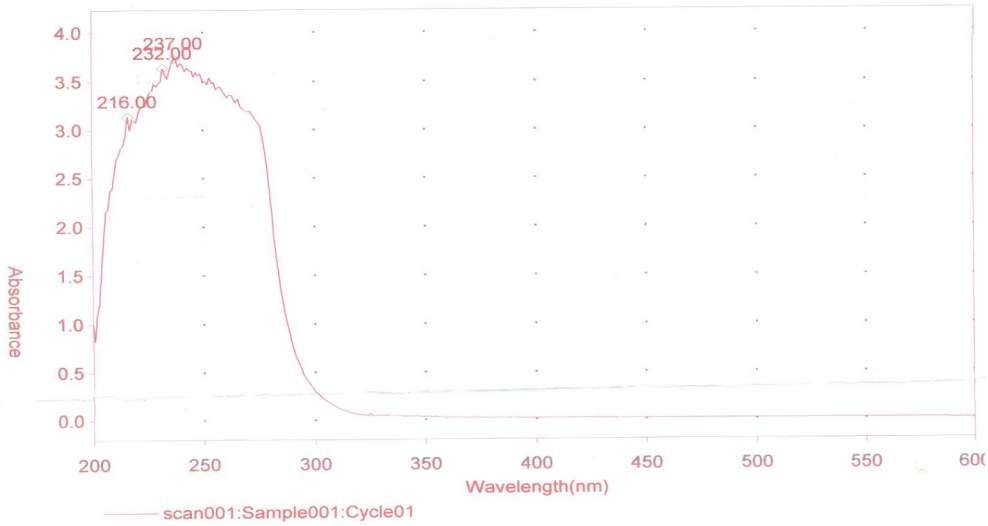
Appendix 1: UV spectrum of 3-(2,4-DMP)AP

SA1

THERMO ELECTRON ~ VISIONpro SOFTWARE V3.00

Operator Name	Umar U.Usman	Date of Report	6/18/2013
Department	Pharm Chemistry	Time of Report	1:57:26AM
Organisation	Ahmadu Bello University Zaria		
Information	(None Entered)		

Scan Graph



Results Table - scan001,Sample001,Cycle01

nm	A
216.00	3.139
232.00	3.636
237.00	3.735

Peak Pick Method
Find 8 Peaks Above -3.0000 A
Start Wavelength 200.00 nm
Stop Wavelength 600.00 nm
Sort By Wavelength

Sensitivity Auto

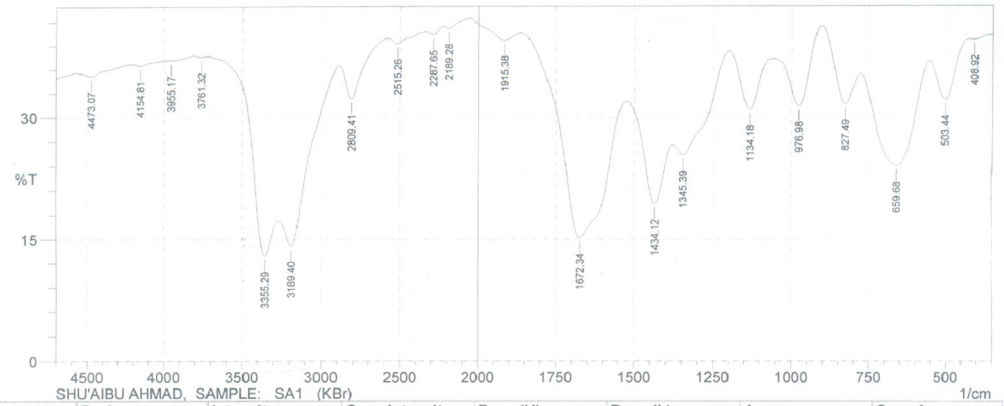
Page 1, Scan Graph

Appendix: 2 IR spectrum of 3-(2,4-(DMP)AP

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



SHU'AIBU AHMAD, SAMPLE: SA1 (KBr)

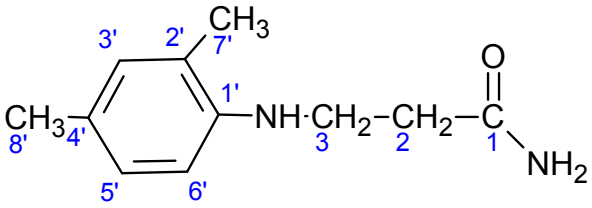
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	408.92	39.741	0.167	424.35	357.81	26.489	0.055
2	503.44	32.206	5.934	554.55	425.32	57.792	4.104
3	659.68	24.047	12.267	778.3	555.52	120.298	22.133
4	827.49	31.651	6.138	903.68	779.27	56.259	4.454
5	976.98	31.405	8.167	1061.85	904.64	70.257	6.611
6	1134.18	30.95	6.868	1198.8	1062.81	62.656	5.185
7	1345.39	25.323	3.421	1378.18	1199.76	93.9	5.539
8	1434.12	19.465	9.163	1524.78	1379.15	87.772	9.828
9	1672.34	15.191	20.495	1864.26	1525.74	199.278	49.096
10	1915.38	39.667	1.384	2057.15	1865.23	74.435	1.132
11	2189.28	41.179	0.456	2224.96	2058.11	63.247	0.292
12	2287.65	40.352	0.679	2336.84	2225.93	43.263	0.401
13	2515.26	39.272	0.858	2579.88	2337.8	96.428	0.939
14	2809.41	32.33	5.013	2885.6	2580.84	133.71	6.268
15	3189.4	14.18	7.069	3268.49	2886.57	239.879	14.398
16	3355.29	12.923	8.231	3720.81	3269.45	261.51	15.49
17	3761.32	37.562	0.166	3809.54	3721.77	37.236	0.086
18	3955.17	37.174	0.108	3982.17	3810.5	73.355	0.192
19	4154.81	36.508	0.329	4211.71	3983.14	99.147	0.334
20	4473.07	35.17	0.744	4568.55	4212.67	158.367	1.186

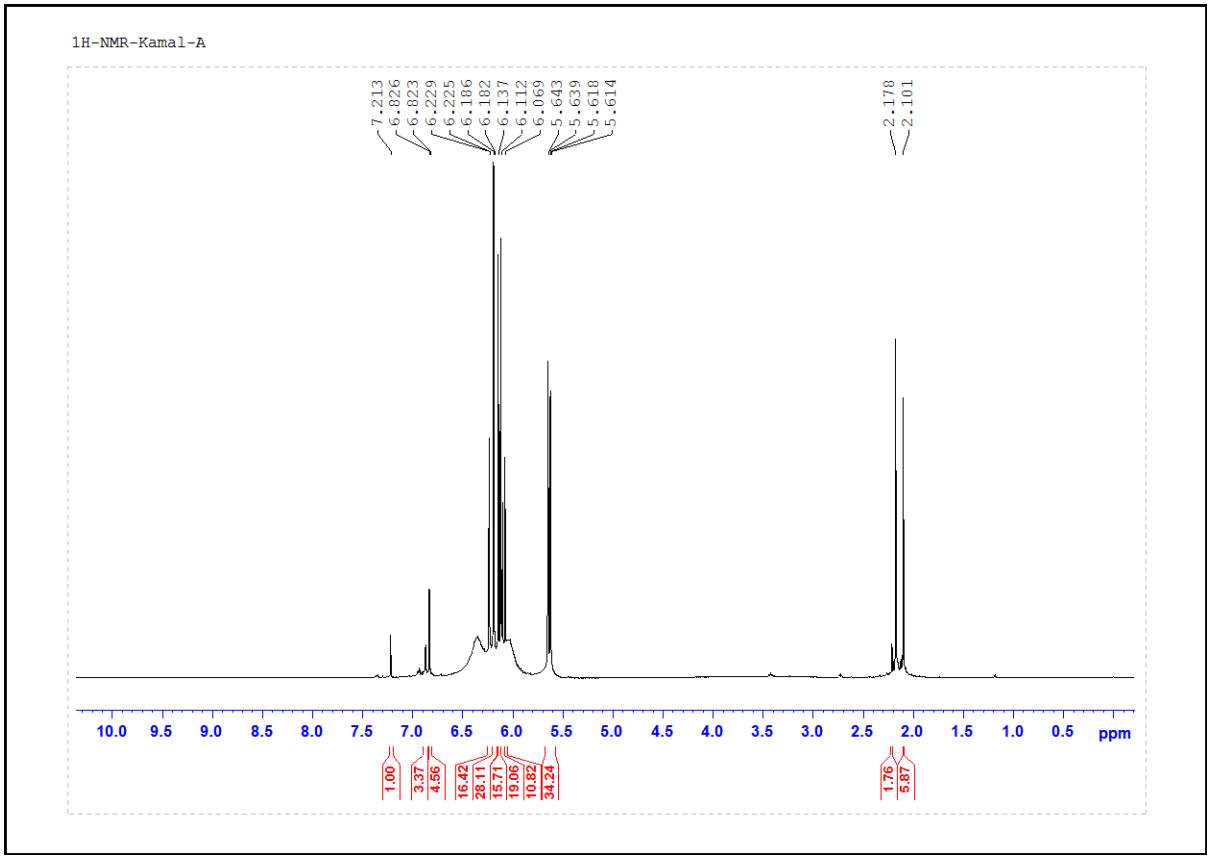
Comment;
SHU'AIBU AHMAD, SAMPLE: SA1 (KBr)

User; Administrator

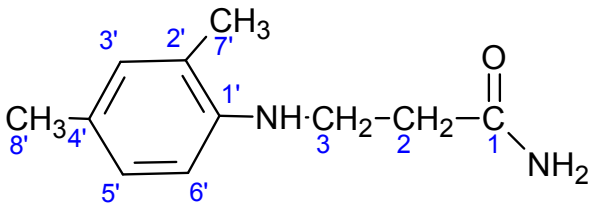
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Date/Time; 6/11/2013 1:08:42 PM
Resolution;

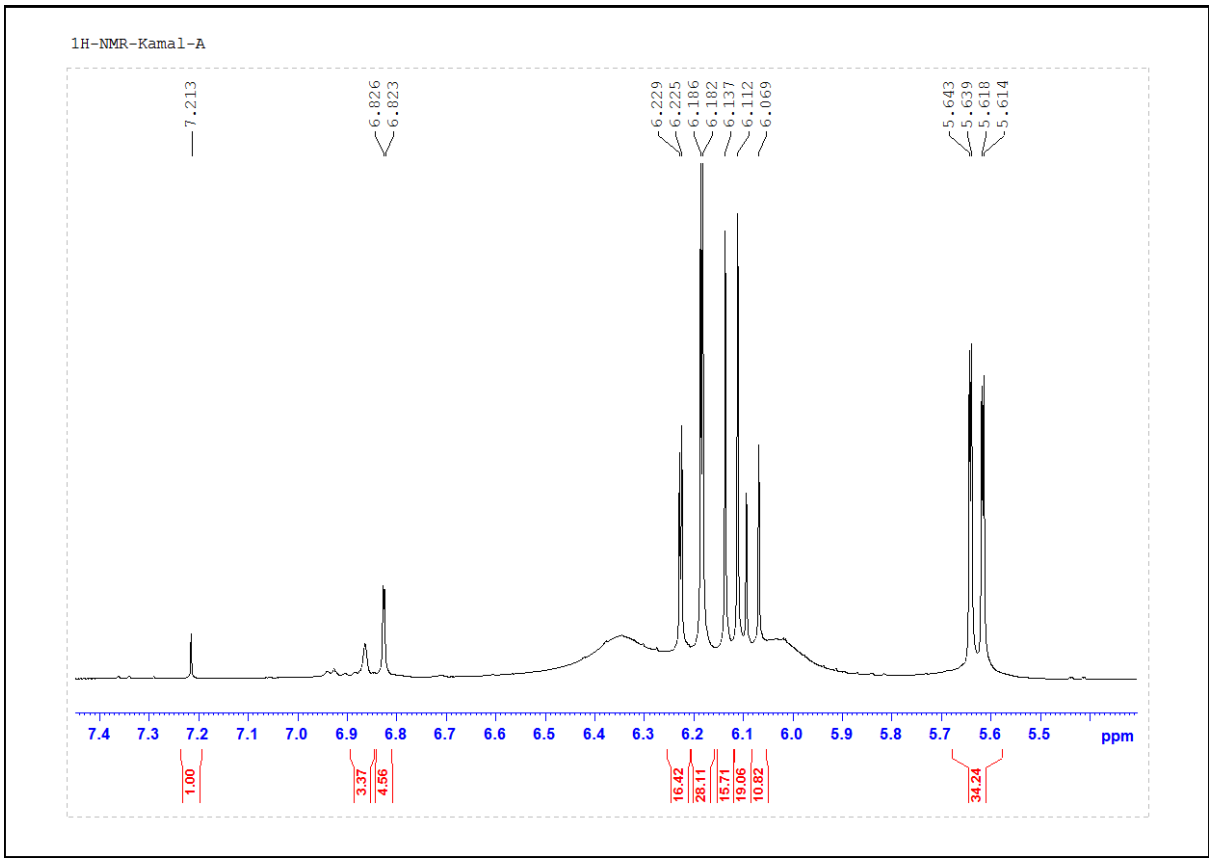
Appendix: 3 Proton NMR of 3-(2,4-DMP)AP



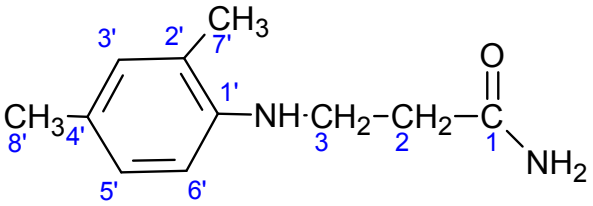


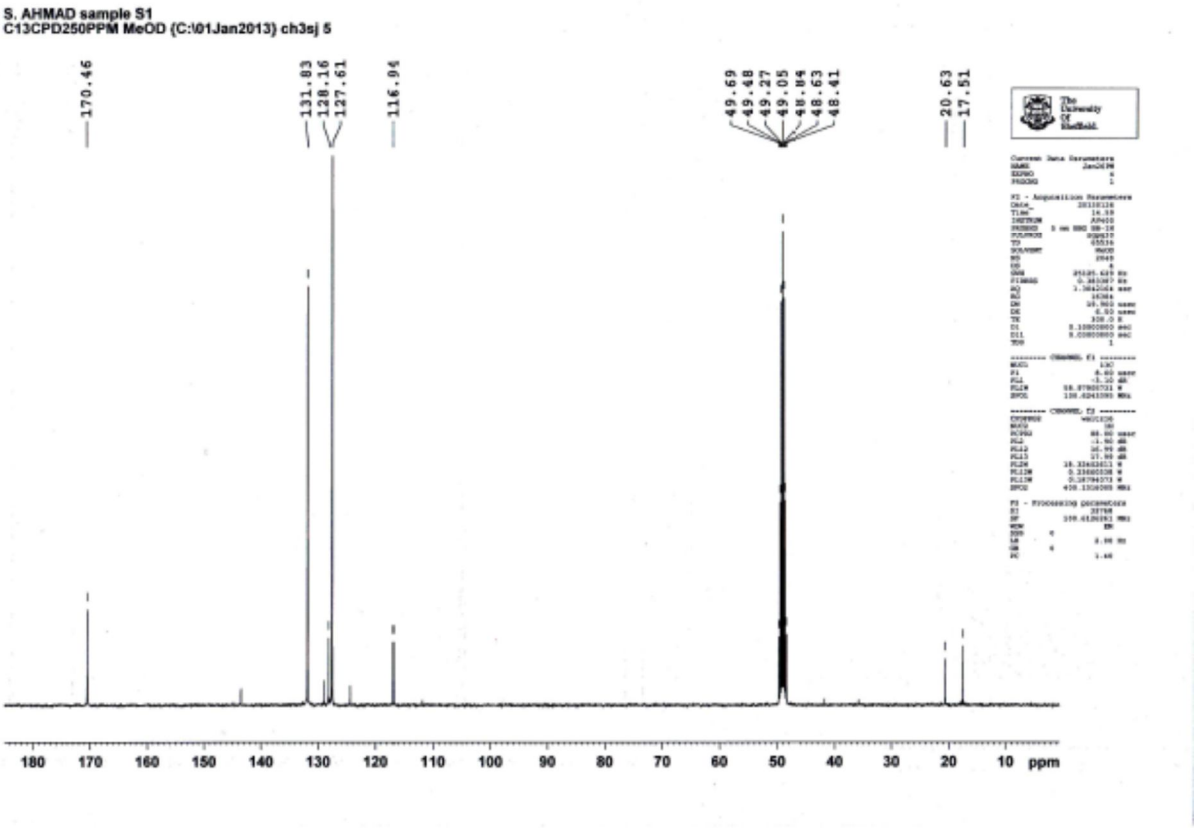
Appendix: 4 Expanded proton NMR spectrum of 3-(2,4-DMP)AP





Appendix: 5 ^{13}C NMR spectrum of 3-(2,4-DMP)AP





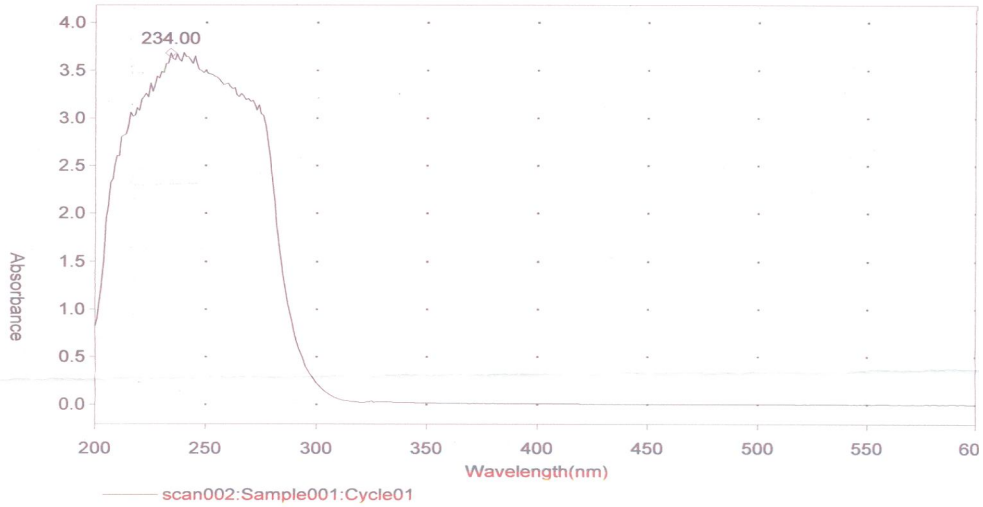
Appendix: 6 UV spectrum of 3-(2,5-DMP)AP

SA2

THERMO ELECTRON ~ VISIONpro SOFTWARE V3.00

Operator Name	Umar U.Usman	Date of Report	6/18/2013
Department	Pharm Chemistry	Time of Report	2:01:03AM
Organisation	Ahmadu Bello University Zaria		
Information	(None Entered)		

Scan Graph



Results Table - scan002,Sample001,Cycle01

nm	A
234.00	3.686

Peak Pick Method
Find 8 Peaks Above -3.0000 A
Start Wavelength 200.00 nm
Stop Wavelength 600.00 nm
Sort By Wavelength

Sensitivity Auto

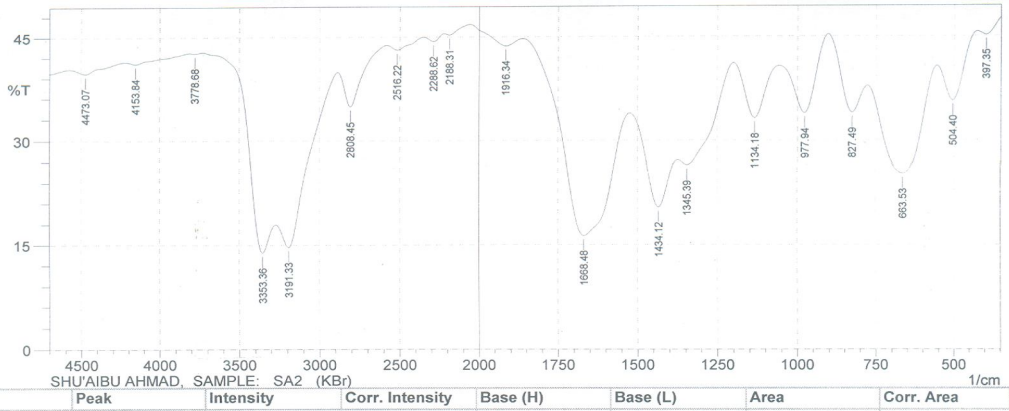
Page 1, Scan Graph

Appendix: 7 IR spectrum of 3-(2,5-(DMP)AP

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



SHU'AIBU AHMAD, SAMPLE: SA2 (KBr)

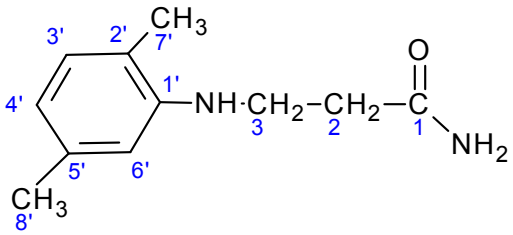
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	397.35	45.153	1.222	425.32	349.13	25.751	0.527
2	504.4	35.605	7.002	555.52	426.28	51.796	4.536
3	663.53	25.253	14.013	778.3	556.48	113.714	23.489
4	827.49	33.919	6.769	903.68	779.27	52.338	4.62
5	977.94	33.851	9.206	1057.03	904.64	63.125	7.154
6	1134.18	33.15	7.746	1199.76	1057.99	60.865	5.784
7	1345.39	26.451	3.07	1374.33	1200.73	87.735	5.331
8	1434.12	20.458	9.376	1523.82	1375.29	87.103	10.161
9	1668.48	16.342	22.139	1862.33	1524.78	189.541	51.166
10	1916.34	43.659	1.649	2058.11	1863.3	67.354	1.242
11	2188.31	45.295	0.445	2221.11	2059.08	54.66	0.262
12	2288.62	44.311	0.9	2352.27	2222.07	45.424	0.546
13	2516.22	43.064	1.065	2581.81	2353.23	81.863	1.22
14	2808.45	34.843	6.008	2886.57	2582.77	122.193	7.059
15	3191.33	14.685	7.749	3269.45	2887.53	231.278	15.744
16	3353.36	13.862	8.586	3728.53	3270.42	242.363	14.47
17	3778.68	42.601	0.093	3813.4	3729.49	31.049	0.041
18	4153.84	41.079	0.504	4217.5	3814.36	152.765	0.951
19	4473.07	39.671	0.958	4575.3	4218.46	140.243	1.51

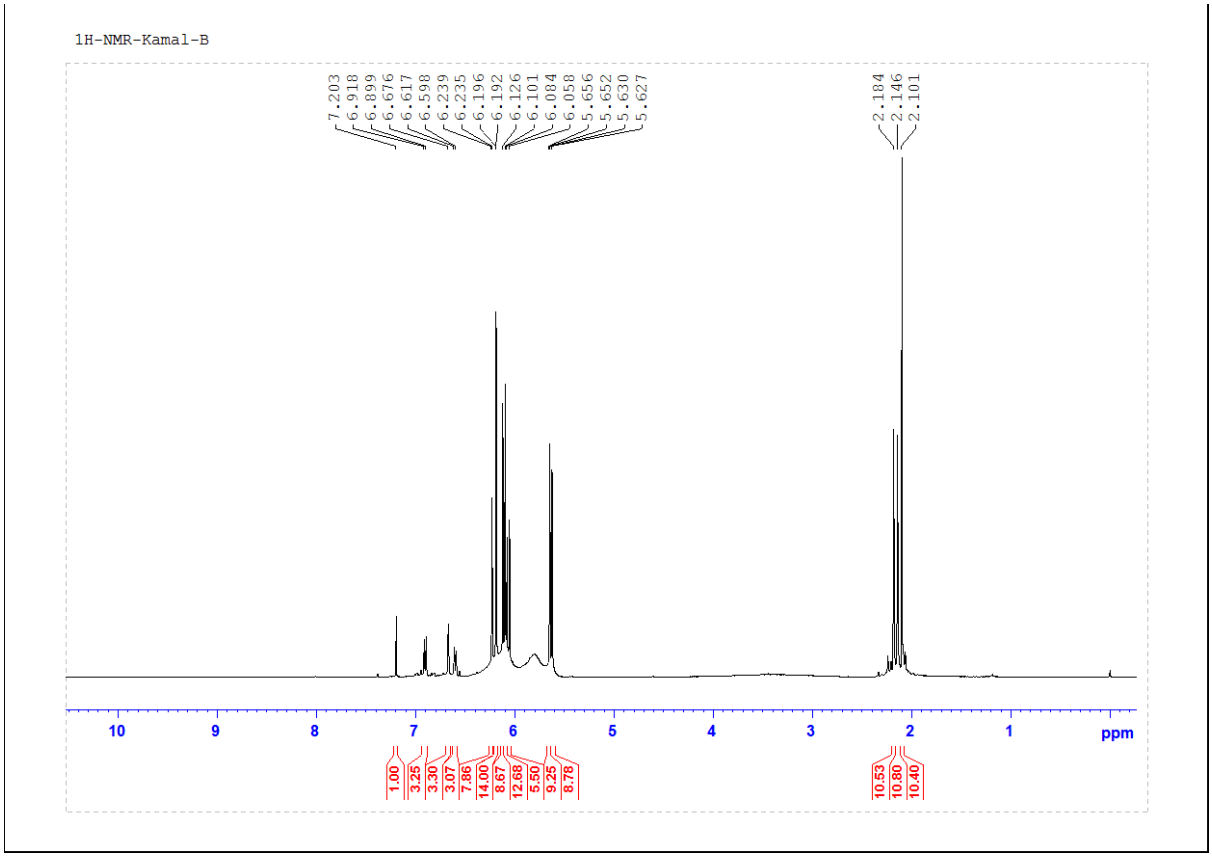
Comment;
SHU'AIBU AHMAD, SAMPLE: SA2 (KBr)

User; Administrator

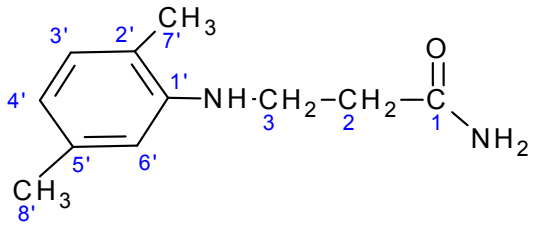
No. of Scans;
Date/Time; 6/11/2013 1:04:58 PM
Resolution;

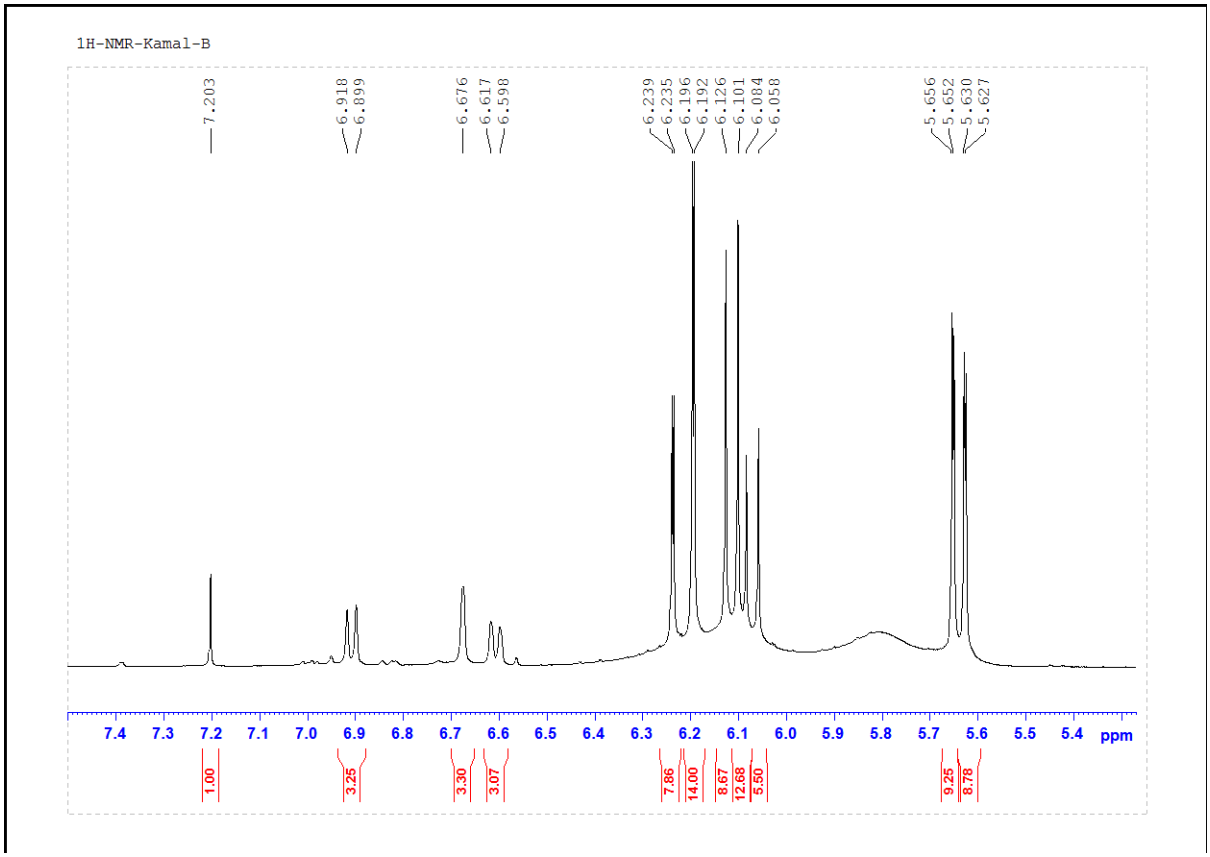
Appendix: 8 Proton NMR of 3-(2,5-DMP)AP



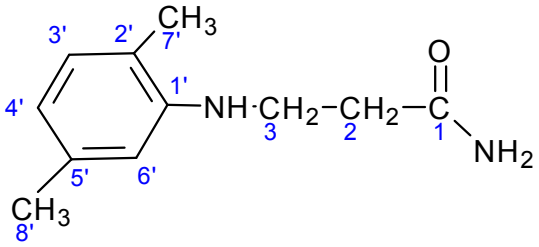


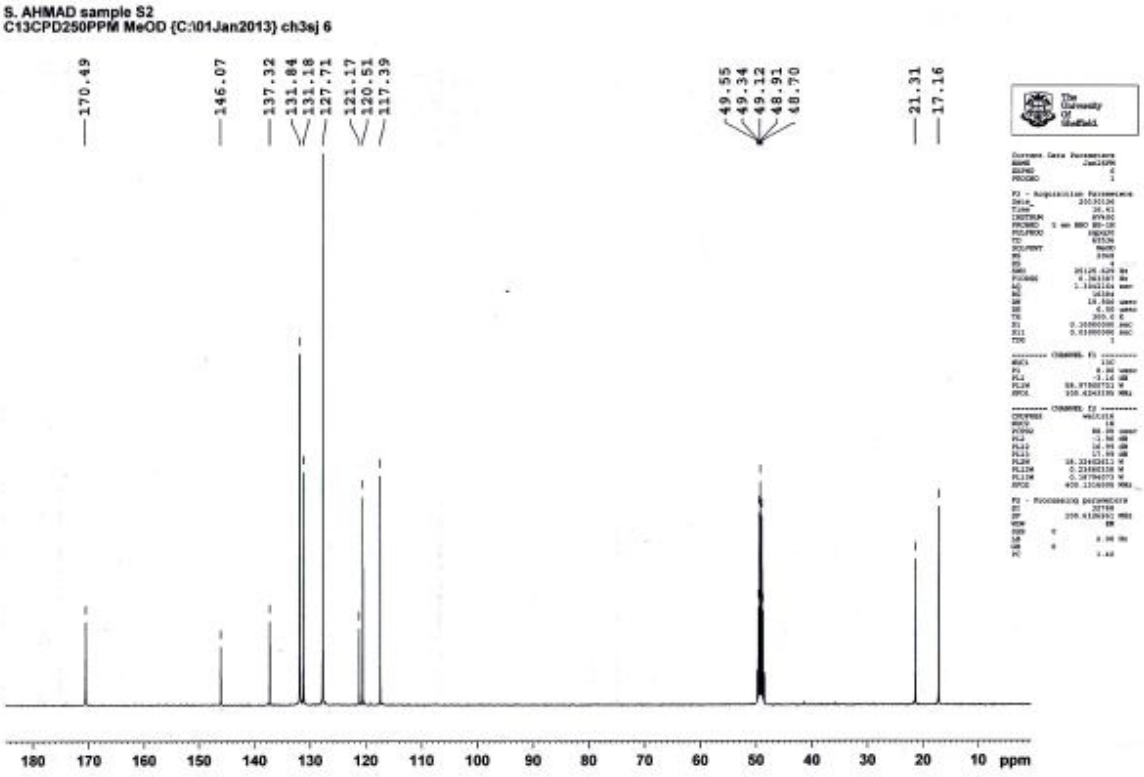
Appendix: 9 Expanded Proton NMR of 3-(2,5-DMP)AP





Appendix: 10 ^{13}C- NMR spectrum of 3-(2,5-DMP)AP



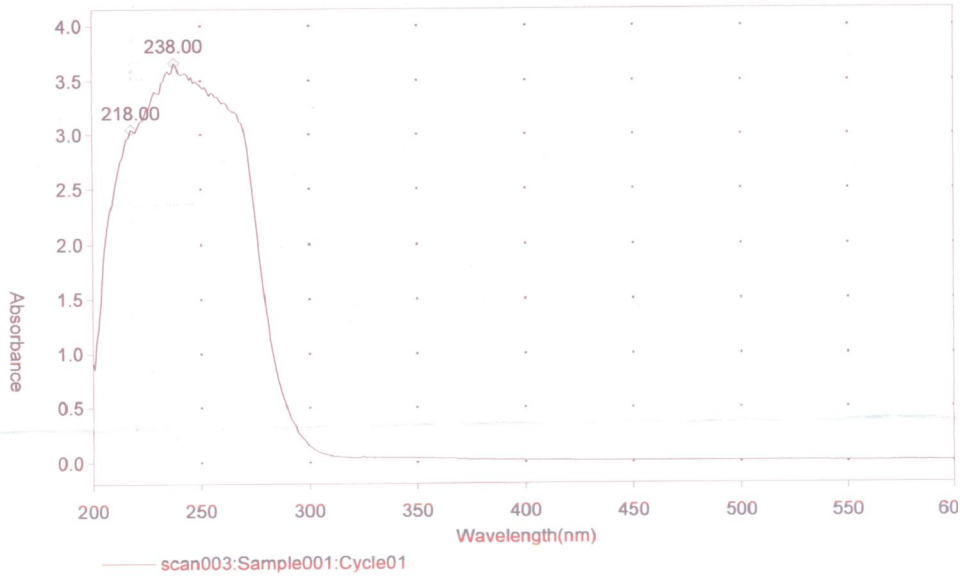


Appendix: 11 UV spectrum of 3-(2,6-DMP)AP

THERMO ELECTRON ~ VISIONpro SOFTWARE V3.00

Operator Name	Umar U.Usman	Date of Report	6/18/2013
Department	Pharm Chemistry	Time of Report	2:04:43AM
Organisation	Ahmadu Bello University Zaria		
Information	(None Entered)		

Scan Graph



Results Table - scan003,Sample001,Cycle01

nm	A
218.00	3.049
238.00	3.664

Peak Pick Method
Find 8 Peaks Above -3.0000 A
Start Wavelength 200.00 nm
Stop Wavelength 600.00 nm
Sort By Wavelength

Sensitivity Auto

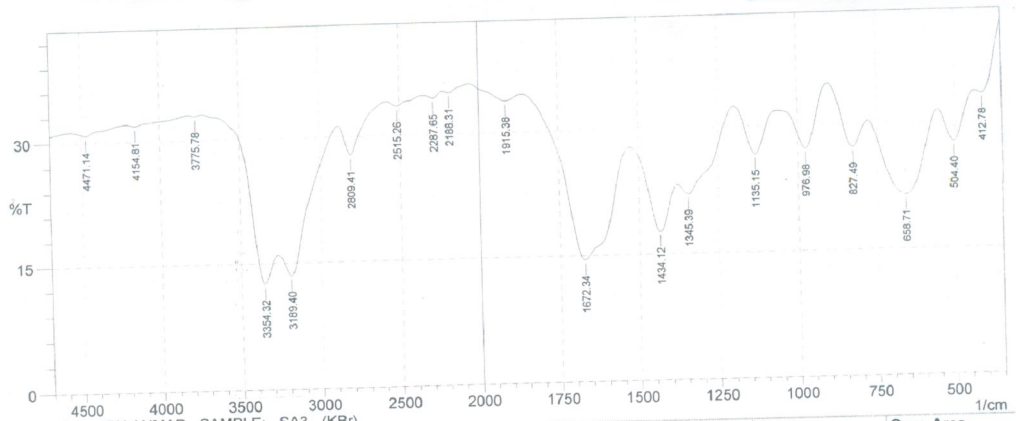
Page 1, Scan Graph

Appendix: 12 IR spectrum of 3-(2,6-DMP)PA

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



SHU'AIBU AHMAD, SAMPLE: SA3 (KBr)

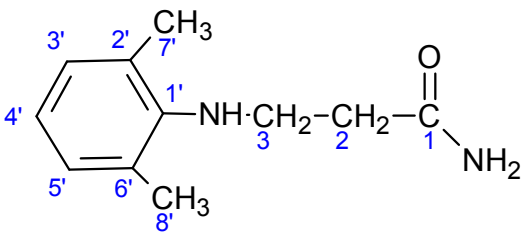
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	412.78	33.314	2.493	434.96	349.13	38.184	1.789
2	504.4	27.693	4.636	554.55	435.93	61.663	3.747
3	658.71	21.517	9.372	778.3	555.52	133.264	19.464
4	827.49	27.249	4.824	903.68	779.27	64.828	4.094
5	976.98	27.198	6.246	1063.78	904.64	81.928	5.879
6	1135.15	26.665	5.412	1198.8	1064.74	70.948	4.713
7	1345.39	22.17	2.784	1378.18	1199.76	105.267	5.27
8	1434.12	17.688	7.298	1526.71	1379.15	96.497	8.842
9	1672.34	14.511	16.214	1863.3	1527.67	214.346	43.654
10	1915.38	33.77	1.133	2057.15	1864.26	88.463	1.088
11	2188.31	34.978	0.382	2224.96	2058.11	75.158	0.286
12	2287.65	34.342	0.582	2341.66	2225.93	53.262	0.413
13	2515.26	33.536	0.7	2579.88	2342.62	110.903	0.892
14	2809.41	27.855	4.144	2885.6	2580.84	154.019	5.965
15	3189.4	13.461	5.681	3268.49	2886.57	258.808	13.176
16	3354.32	12.516	6.612	3729.49	3269.45	285.896	13.433
17	3775.78	32.935	0.151	3820.15	3730.45	43.174	0.09
18	4154.81	31.912	0.33	4212.67	3821.11	191.665	0.96
19	4471.14	30.853	0.671	4570.48	4213.64	179.271	1.228

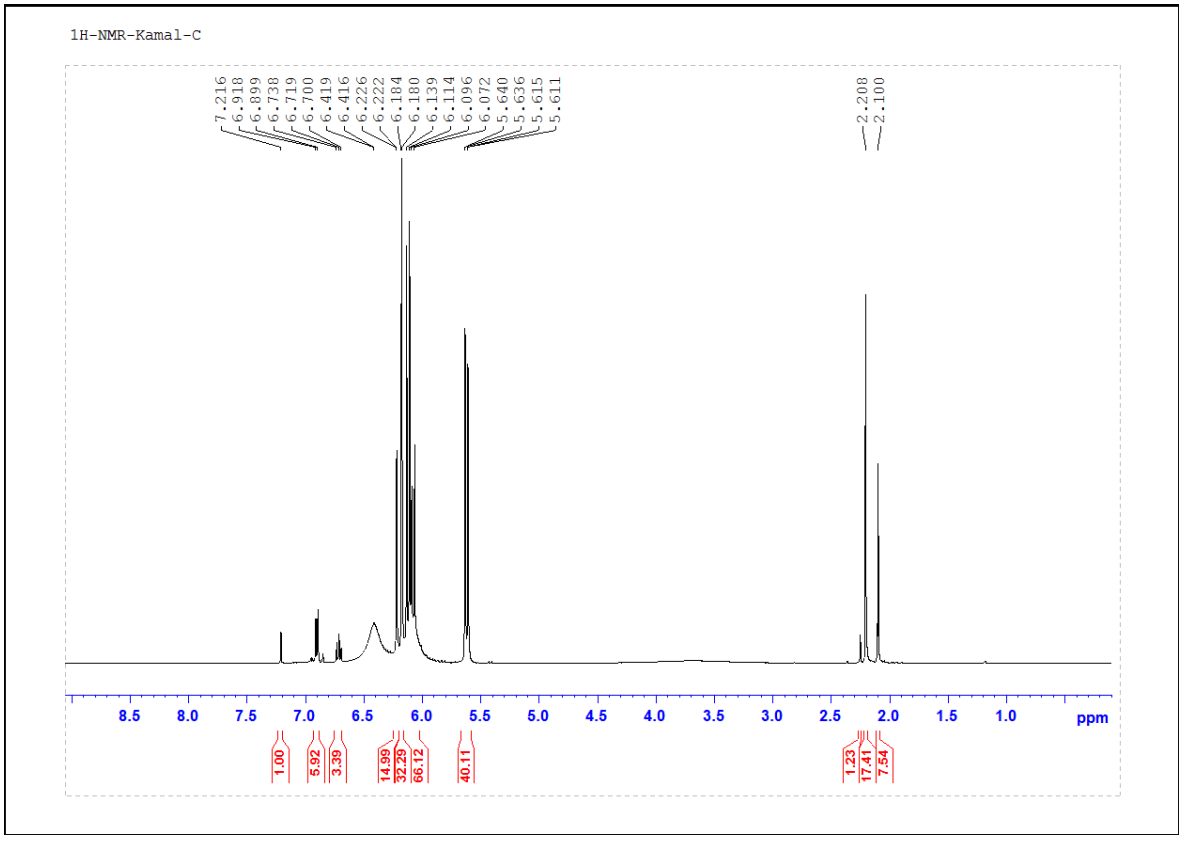
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User; Administrator

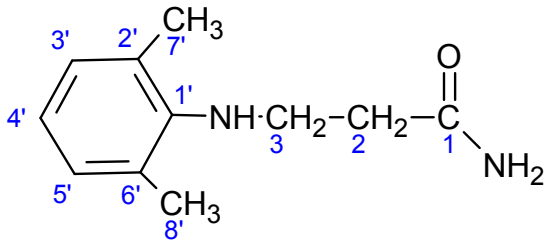
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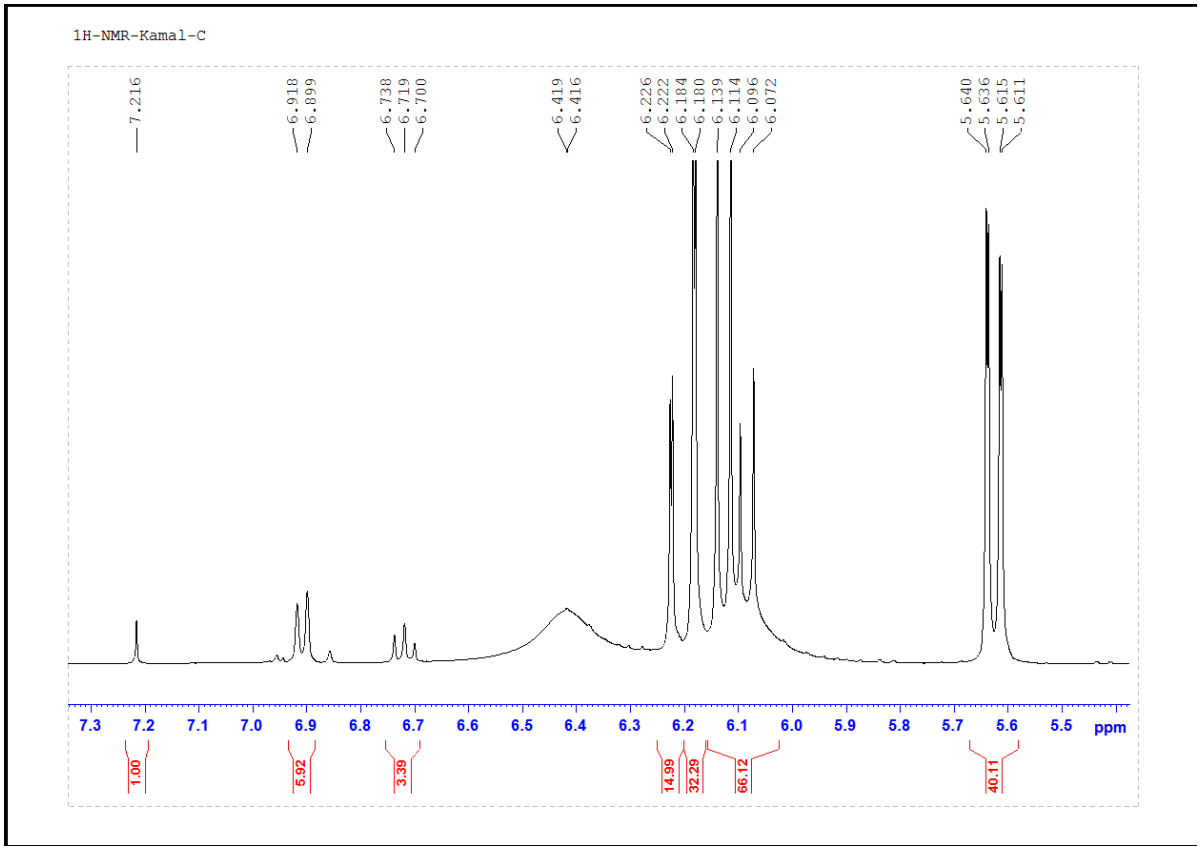
Appendix: 13 Proton NMR of 3-(2,6-DMP)AP





Appendix: 14 Expanded Proton NMR of 3-(2,6-DMP)AP





Appendix: 15 ^{13}C NMR spectrum of 3-(2,6-DMP)AP

