SYNTHESIS AND ANTICONVULSANT STUDIES OF ALKYL AND ARYL SUBSTITUTED N-PHENYLFORMAMIDE

BY

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DECLARATION

I declare that the work in the dissertation entitled "Synthesis and Anticonvulsant Studies of Alkyl and Aryl Substituted N-Phenylformamide" has been performed by me in the Department of Pharmaceutical and Medicinal Chemistry, under the supervision of Dr. Idris Abdullahi Yunusa and Dr. M. I. Sule. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation report was previously presented for another higher degree at any university.

Asmau Hamza Nasiru

	Signature	**Date**

CERTIFICATION

This project entitled "Synthesis and Anticonvulsant Studies of Alkyl and Aryl Substituted N-Phenylformamide" by Asmau Hamza Nasiru meets the regulation governing the award of master's degree of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Alhamdulillah

DEDICATION

This Work Is Dedicated to my Brother, Umar Hamza

ABSTRACT

N-phenylacetamide and N-phenybenzamide were synthesized by modified Schotten Bauman reaction. The structures of the two compounds were established using infrared (IR) and various proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The structural analysis is further supported by two dimensional NMR spectroscopy (COSY and HETCOR). The two compounds were screened in chicks and mice against maximum electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure test models, respectively. N-phenylacetamide displayed a good and broad spectrum of activity, comparable to that of phenytoin (20 mg/kg) at a dose of 75mg/kg in MES test and to that of valproate (200 mg/kg) at a dose of 300mg/kg. However, N-phenybenzamide shows insignificant activity ($p<0.05$) against MES and little activity against scPTZ induced seizure (33% protection). The pharmacological activity of the two synthesized compound is discussed in terms of their optimized three dimensional structures. The two compounds are recommended for further screening and optimization.

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ABBREVIATIONS

AED	Anti Epileptic Drug
GABA	Gamma Amino Butyric Acid
NMR	Nuclear Magnetic Resonance
IR	Infrared
TLC	Thin Layer Chromatography
MES	Maximum Electro Shock
sc PTZ	Subcutaneous Pentylenetetrazole
IP	Intraperitoneal
sc	Subcutaneous

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Epilepsy is one of the worlds oldest recognized conditions. It is a chronic disorder of the brain that affects people all over the world (WHO, 2009). Approximately, 1% of world's population have epilepsy. It is also the second most common neurologic disorder after stroke (Porter and Meldrum, 2007). About 50 million people worldwide have epilepsy; almost 90 % of these people live in developing countries including Nigeria. Generally, epilepsy can affect people of all ages. However, it is more likely to affect young children or people over the age of 65 years. (http://www.epilepsynse.org.uk). Most people suffering from epilepsy in Africa prefer anonymity and are reluctant to disclose their condition because of the stigma attached to the disease (Osuntokun, 1978). This factor affects the prevalence rates. Hence, there is like hood that most of the reported prevalence rates represent the tip of the iceberg as the chances of under reporting are very high. Thus the prevalence of epilepsy reported in Africa are based on surveys of certain defined communities and hospital admissions. In Nigeria, epilepsy prevalence (based on defined communities) is in the range of 15 – 37 incidents per 1000 people. The age distribution in the Nigerian case appears to be similar to that of the Caucasians. Between 70 to 85 % of people with epilepsy have onset of seizures below 30 years of age. Most African studies reported a slight male excess (Osuntokun, 1978). Epilepsy tend to be more common among the lower socio-economic groups (Danesi *et al*, 1981). Showmanisky and Glaser reported a very high incidence of epilepsy among the poor blacks in the United States of America. Several studies conducted in Africa also confirmed a higher prevalence of epilepsy among rural dwellers (Osuntokun, 1978; Watts, 1992).

Epilepsy is a chronic disorder characterized by recurrent seizure which takes various forms and result from episodic neuronal discharge. The form of seizure depend on the part of the brain that is affected. There is no recognizable cause of epilepsy, although it may develop after brain damage caused by trauma, infection or tumour growth, or other kinds of neurological diseases (Rang *et al*, 2007; Brunton *et al*, 2009). The seizure is caused by synchronous high frequency discharge of a group of neurons, starting locally and spreading to a varying extent to affect other parts of the brain. Partial seizures affect localized brain regions and the attack may involve mainly motor, sensory, or behavioural phenomena.

Unconsciousness occurs when the reticular formation is involved. Generalized seizures affect the whole brain. Two common types of generalized seizures are the tonic-clonic (grand mal) and the absence seizure (petit mal). Mutations in several genes have been linked to some types of epilepsy. Several genes that code for protein sub-units of voltage gated and ligand gated ion channels have been associated with forms of generalized epilepsy (Meisler, 2005). Epileptic seizures causes transient impairment of consciousness, leaving the patient at the risk of bodily harm and often interfering with patient's education and job. It also increases the patients risk of premature death (WHO, 2009).

Epilepsy is usually controlled, but not cured, with medication. In some cases the implantation of a stimulator of the vagus nerve and a special diet (ketogenic diet) can be helpful. Neurosurgical operation for epilepsy can be palliative, reducing the frequency or severity of seizures. In some patients an operation can be curative (Porter and Meldrum, 2007). However, over 30% of people with epilepsy do not have seizure control even with the best available medication . Therefore, there is a need to improve the efficacy of therapy (Porter and Meldrum, 2007; Rang *et al,* 2007; Engel *et al,* 1996).

Many of the current antiepileptic drugs (AEDs) were developed empirically on the basis of activity in animal models. Long established anti epileptic drugs include phenytoin, carbamazepine, valproate, ethosuximide and phenobabital, together with various benzodiazepams such as diazepam, clonozepam. New drugs include vigabatrin, gabapentin, lamotrigin, felbamate, tiagbine, topiramate, levatiracetam and zonisamide. The length of this list reflects the efforts being made to improve the properties of the earlier drugs (Rang *et al,* 2007).

Despite the introduction of many second generation AEDs in the past 15 years, still some patients remain refractory to available treatments. In view of these facts, most epileptologists agree on the definite need for more effective and less toxic anticonvulsant drugs. Present study is aimed at synthesizing and determining the anticonvulsant potential of two carboxamides: N-phenylacetamide and N-phenylbenzamide.

Several carboxamides have been found to possess good anticonvulsant activity. These include toluidinopropanamides, anisidinopropanamides (Idris *et al,* 2008), phthalimide (Vamecq *et al,* 2000], chalcone of N-phenylacetamide (Singh and Rana, 2010). Most of these compounds are active against maximal electroshock seizure but have little or no activity against subcutaneous pentylenetetrazole seizure. They are capable of preventing seizure

spread through blockade of voltage-dependent sodium channels. Although many derivatives of substituted N-phenylbenzamide were synthesized and evaluated for anticonvulsant effect (Clark *et al*, 1985, 1987; Bruce and Lyne, 1962), there is no report on anticonvulsant screening of the unsubstituted N-phenylbenzamide.

N-phenylacetamide is a synthetic organic compound introduced into therapy in 1886 as a fever reducing drug. It's effectiveness in relieving pain was discovered thereafter, and was used as an alternative to aspirin for many years. Later, it was removed from the market because of it's toxicity (Encyclopedia Britannica, 2010).

1.2 Theoretical framework

The characteristic event in epilepsy is the seizure which is associated with episodic high frequency discharge of impulses by a group of neurons in the brain. The underlying neurochemical abnormality in epilepsy is not yet fully understood. The pivotal role of synapses in mediating communication among neurons in the mammalian brain suggests that defective synaptic function might lead to a seizure, that is a reduction of inhibitory synaptic activity or enhancement of excitatory synaptic activity mediated by GABA or glutamate receptors, respectively. Other factors include volume of the extracellular space, and most importantly, intrinsic properties of neurons such as voltage-regulated ion channels including those gating potassium, sodium, and calcium ions (Voskoyl and Clincker, 2009).

Progress in the techniques of electrophysiology has fostered the progressive refinement of the level of analysis of the seizure mechanism from the EEG to population of neurons, to individual neurons, to individual synapses, and individual ion channels on individual neurons. Electrophysiological analysis of individual neurons demonstrate that the neurons undergo depolarization and fire action potential at high frequencies; this pattern is uncommon during normal physiological neuronal activity. Thus, selective inhibition of this pattern of firing is expected to reduce seizure with minimal side effects (Rang *et al*, 2007). The inhibition is thought to be mediated by reducing the ability of sodium ion channels to recover from inactivation. That is, depolarization triggered opening of the sodium ion channels in the axonal membrane of a neuron is required for an action potential. However, reducing the rate of recovery of sodium ion channels from inactivation would limit the ability of a neuron to fire action potential at high frequencies, an effect that likely underlies the

action of carbamazepine, lamotrigine, phenytoin, topiramate, valproic acid and zonisamide (Brunton *et al*, 2009). These drugs were found to inhibit preferentially the excitation of cells that are firing repetitively without unduly interfering with the low frequency firing of neurons in the normal state. The higher the frequency of firing, the greater the block produced. This property arises from the ability of the drugs to discriminate between sodium ion channels in their resting, open, and inactivated state. High depolarization of neurons that occurs during seizure increases the proportion of the sodium ion channels in the inactivated state. The AEDs that target this channel bind preferentially to channels in this state, preventing them from returning to the resting state, and thus reducing the number of of functional channels available to generate action potential (Rang *et al*, 2007).

Since only the primary structures of several types of voltage dependent sodium channels are known, a study of the various anticonvulsants with sodium blockage activity may define structural elements which are essential for pharmacological activity (Noda *et al*, 1986; Catteral *et al*, 1988)). Several attempts were made to postulate a general pharmacophore for different anticonvulsants acting on sodium channels. The various postulated pharmacophore models show no uniform picture. Jones and Woodburry (1982) on the basis of some ideas of Camerman and Camerman (1980) developed a model with two electron donors in some proximity to a bulky hydrophobic moiety. By selecting other compounds as those of Jones and Woodburry, Codding *et al* (1989) postulated a pharmacophore model consisting a linear arrangement of rotated phenyl rings, an electron donor atom, and a hydrogen donor site, which partially agrees with the model of Jones and Woodburry.

Brouillett *et al* (1990) investigated the sodium ion channel blocking activity of several mono and bicyclic phenytoin analogues and concluded that high (LogP values), a free amide group, and a specific aromatic ring orientation are optimal for high binding affinity to the sodium ion channel. These criteria were well fulfilled in monocyclic hydantoins. However, the conclusions of these studies were not related to other substance classes acting at the same receptor site.

Recently, Unverferth *et al* (1998) suggested a pharmacophore model for structurally different anticonvulsants containing an aryl ring, electron donor, and hydrogen bonding function (Singh and Rana, 2010). The Unverferth model for sodium blockade activity comprises an electron donor, D, in limited distance ranges of 3.2 -5.1Å to an aryl ring or

other hydrophobic unit, R, and of 3.9-5-5 Å to hydrogen bond acceptor/donor unit (HAD). The distance between R and HAD spans a wide range of 4.2 – 8.5Å. The hydrophobic unit is not necessarily oriented in the same plane like the other essential elements. Unverferth further concluded that possibly the presence of only one component of the HAD unit at the postulated position; for example, only the hydrogen donor part as it is also postulated by other authors. In addition to the two other essential structural elements R and D may be sufficient for activity as a blocker of the voltage dependent sodium channel.

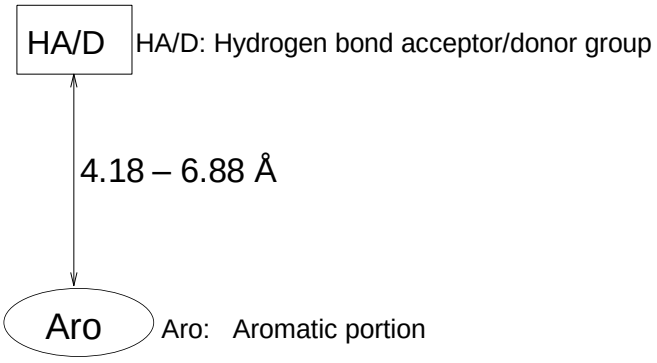


Figure 1.1 Proposed pharmacophore for anticonvulsant activity (Shindikar *et al*, 2006)

More recently, Shindikar *et al*. proposed a two-point pharmacophore (Figure 1.1) that consists of an aromatic ring separated by 4.18 – 6.88 Å from a HAD for anticonvulsants acting on the sodium channels (Shindikar *et al*, 2006). Shindikar's pharmacophore model agrees well with Unverferth's model. The model also works well with so many compounds that were synthesized and tested for anticonvulsant activity. Such compounds include: anisidinopropanamides, anilinopropanamides (Idris *et al*, 2008), N-phenylacetamides (Shindikar *et al*, 2006), and many other synthesized compounds tested by other researchers.

N-phenylacetamide and N-phenylbenzamide contain a hydrophobic aromatic ring (phenyl group) and a HAD unit (amide group). There are two isomers for the two compounds: cis and trans. The trans isomer is the most stable because of the intramolecular hydrogen bonding between the phenyl ortho hydrogen atom and the amide oxygen atom. The distance between the phenyl ortho hydrogen atom and the amide oxygen atom is short enough (about 1.75 Å) to allow for the weak intramolecular hydrogen bonding to occur. Indeed, many structural studies have shown that N-phenylacetamide and N-phenylbenzamide exist exclusively in the trans form in the gaseous, liquid, and solid phases. The structures of the trans isomers of N-phenylacetamide and N-phenylbenzamide optimized using the molecular mechanics tools contained in ACD/3D viewer software are shown in Figure 1.3. As apparent

from Figure 1.3, the distance between aromatic ring and amide units of the N-phenylbenzamide fall into the range of 4.18 – 6.88 Å as proposed by Shindikar *et al*. Substituted derivatives of N-phenylacetamide and N-phenylbenzamidehave been synthesized and found to be active against seizure (Clark *et al*, 1985; Clark and Davenport 1987; Singh and Rana, 2010). Although N-phenylacetamide was used for its antipyretic activity, there is no published report on the anticonvulsant activity of N-phenylacetamide. However, several alkanamides have been shown to possess potent and broad spectrum anticonvulsant activity.

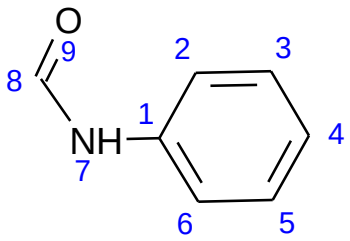
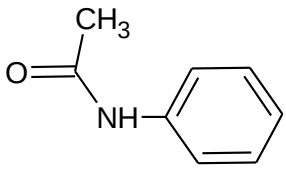
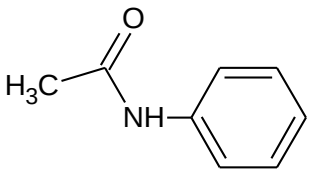


Figure 1.2 N-phenylformamide

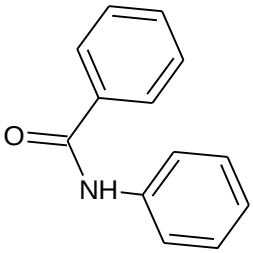
In this work unsubstituted N-phenylacetamide and N-phenylbenzamide were synthesized using Schotten-Baumann reaction. The synthesized compounds were screened for anticonvulsant activity using maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure model in order to ascertain their anticonvulsant activity.



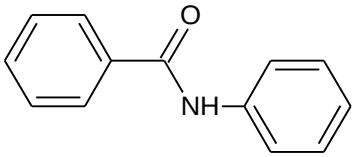
(a) Cis- N-phenylacetamide



(b) Trans- N-phenylacetamide

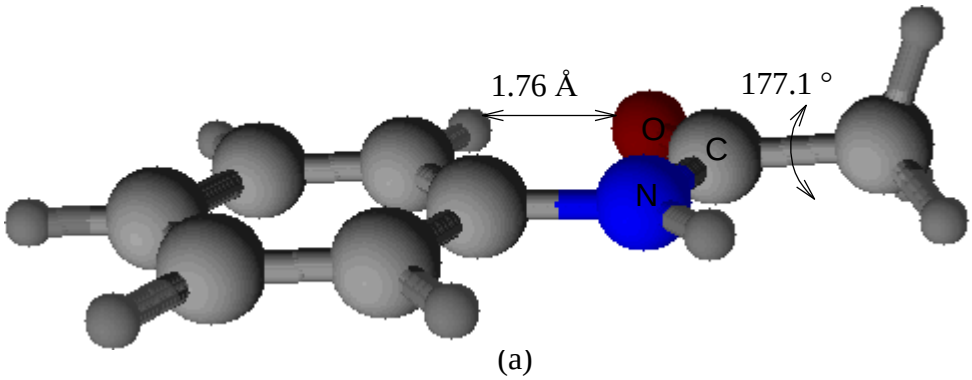


(c) Cis- N-phenylbenzamide

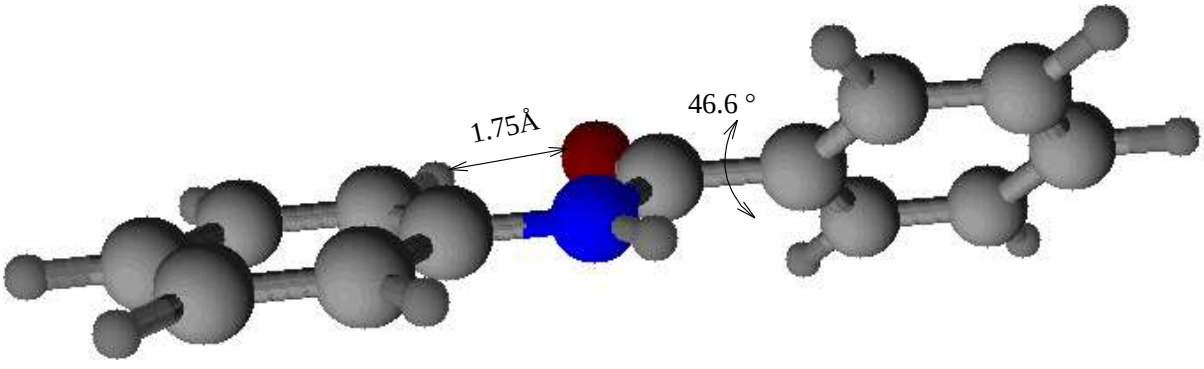


(d) Trans- N-phenylbenzamide

Figure 1.3 Structural isomers of N-phenylacetamide and N-phenylbenzamide.



(a)



(b)

Figure 1.4 Optimized three dimensional structures of the trans isomers of :

(a) N-phenylacetamide and (b) N-phenylbenzamide

1.3 Research Hypothesis

1. N-phenylacetamide –although an antipyretic drug has anticonvulsant activity.
2. N-phenylbenzamide has anticonvulsant activity.

1.4 Statement of the problem

Although methods such as surgery, vagal nerve stimulation, deep brain stimulation, and dietary changes have been employed to treat epilepsy, antiepileptic drugs (AEDs) remain the most widely utilized treatment strategy (Gerlach and Krajewski, 2010]. Often, antiepileptic medication treatment is life long. For some patients, the treatment is frequently associated with serious side effects and toxicity, ranging from minimal impairment of the central nervous system to death (Brunton *et al*, 2009). Other side effects include: aplastic anaemia, hepatic failure, cognitive dysfunction, ataxia, hepatotoxicity, and gingival hyperplasia, etc. In Europe, 88% of epilepsy patients were reported to be affected by at least one anticonvulsant drug related side effect (Baker *et al*, 1997). Furthermore, compliance with medication is a major problem because of the need for life long therapy together with the side effects.

Additionally, over 30 % of people with epilepsy do not have seizure control even with the best available medications (Brunton *et al*, 2009). Up to today, antiepileptic therapy is symptomatic in the sense that available drugs inhibit seizures, but neither effective prophylaxis nor cure is available. In view of these facts, most epileptologists agree on the need for more selective and less toxic AEDs (Voskoyl and Clincker, 2009).

1.5 Aims and objectives of the study

The aims and objectives of this study are as follows:

1. To synthesize and characterize N-phenylacetamide and N-phenylbenzamide.
2. To assess the acute toxicity of the synthesized compounds.
3. To screen the two compounds for anticonvulsant effect in maximal electroshock (MES) and subcutaneous pentylene tetrazole (Sc PTz) seizure test models.

1.6 Significance of the study

Epilepsy is a major public health concern that is associated with significant socio-economic effects (WHO, 2009; Susanta and Durga, 2010). Although the social effects vary from one country to another, the discrimination and social stigma that surround epilepsy worldwide are often more difficult to overcome than the seizures themselves. In Nigeria, epilepsy is a considerable source of stigma and misery for those affected by the disease. A study carried out in the university teaching hospital Enugu shows that more than 25 % of patients complained about socio-economic effects including stigmatization and job loss (Onwuekwe *et al*, 2009). Suicide or attempted suicide is not uncommon among Nigerian epilepsy patients (Osuntokun, 1978; Osuntokun *et al*, 1970). In the developed countries, people with epilepsy experience reduce access to health and life insurance, denial of driving licence, and barriers to engage in many occupations. For example, in the United States of America, until 1970s, it was legal to deny people with seizures access to restaurants, theatres, recreational centres, and other public buildings (WHO, 2009). People with epilepsy are often a target of prejudice.

Furthermore, emotional consequences occur in about 25 – 75 % of people with epilepsy. The most common emotional responses are:

1. Fear of unexpected seizure.
2. Acute humiliation after seizure, especially if incontinence occurs.
3. Feeling of alienation at work and in social situations.

The mortality and morbidity of epilepsy is very significant worldwide. Beyond symptoms of the underlying diseases that can be part of certain epilepsies, people with epilepsy are at the risk of premature death by about two to three times compared to the general population (WHO, 2009; Walczak et al, 2001; Lathers and Schraeder, 1990).

Certain diseases also seem to occur at higher than expected rates in people with epilepsy. These diseases include: depression, migraine and other headaches, infertility, and low sexual libido. There have been studies suggesting that up to two third of people with epilepsy experience sexual disturbances (www.about.com).

Attention deficit/hyperactivity disorder affects three to five times more children with epilepsy than healthy children. Several studies have documented problems in academic

performance of this group of patients when compare with their healthy counterparts (Adewuya *et al*, 2006; Austin *et al*, 1998).

WHO and partners recognize that epilepsy is a major public health concern. The international league against epilepsy (ILAE) and the international bureau for epilepsy (IBE) are carrying out a global campaign to provide better information and raise awareness about epilepsy, and strengthen public and private effort to improve care and reduce the disorder's impact (WHO, 2009). This study might contribute to the above effort to improve care for epileptic patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical Background Of Epilepsy

Epilepsy is one of the oldest recognized disorder that was described in the early scriptures. In the Gospel, according to saint mark (4) “it was a fool spirit that was cast out of the young man with fits”. Epilepsy was believed to be a sacred disease that is the result of the invasion of a body by god. It was thought that only god can deprive a healthy person of his senses, throw him to the ground, and convulse him and then rapidly restore him to his former self again (Reynolds, 1988). The word ‘lunatic’ was first applied to sufferers of epilepsy as gods that were thought to occupy heavenly spheres, one of which was the moon (Olubunmi, 2006). The “Dictator Perpetus” of the Roman empire, the great Julius Caesar likely had epilepsy on the basis of documented four attacks that were probably complex partial seizure. The historical legacy has continued to influence public attitude to epilepsy making it a dreaded disease. These beliefs have resulted in people with epilepsy being ostracized, stigmatized and misunderstood (Olubunmi, 2006).

In Africa, there is a belief that the disease results from visitation of the devil, effect of witch craft, the revenge of an aggrieved ancestral spirit or consumption of something harmful in utero (Dada and Odeku, 1966; Awaritefe *et al*, 1985). The remarkable issue raised by the Hippocrates famous treatise on the scared disease about 2500 years was his belief that epilepsy was not sacred, that the brain was the seat of the disease. He marked that epilepsy would cease to be considered divine, the day it will be understood.

In the later half of the nineteenth century, Hughling Jackson (Olubunmi, 2006) define epilepsy on the basis of neuronal theory. He defines seizure as an occasional, excessive and disorderly discharge of nerve tissue on muscles. Jackson also recognized that seizures can alter consciousness. This insightful proposal provided a valuable framework for thinking about the mechanisms of partial epilepsy.

The advent of the electroencephalogram (EEG) in the 1930s by Berger permitted the recording of electrical activity from the scalp of humans with epilepsy and demonstrated that the epilepsies are disorders of the neuronal excitability (Brunton *et al*, 2009). Merritt and Putnam developed electroshock seizure test in 1934. The electroshock seizure is extremely valuable because drugs that are effective against tonic- hind limb extension induced by

electroshock generally have proven to be effective against partial and tonic-clonic seizures in humans. Another screening test is the seizure induced by the chemoconvulsant, pentylenetetrazole, and is most useful in identifying drugs that are effective against myoclonic seizures in humans. These screening tests are still used.

During the 1980s, a diversity of in vitro models of seizures were developed in isolated brain slice preparation in which many synaptic connections are preserved and this permitted mechanistic investigations into the induction of seizures (Brunton *et al,* 2009).

2.2 Types of Epilepsy

The clinical classification of epilepsy defines two major categories, namely, partial and generalized seizures, although there is some overlap and many varieties of each. Either form is classified as simple (if consciousness is not lost) or complex (if consciousness is lost).

2.2.1 Partial seizures

Partial seizures are those in which the discharge begins locally and often remain localized. The symptoms depend on the brain region involved, and include: involuntary muscle contractions, abnormal sensory experiences or autonomic discharge, or effects on mood and behaviour.

The EEG discharge in this type of epilepsy is normally confined to one hemisphere. Partial seizures are often attributed to local cerebral lesions, and their incidence increases with age. In complex partial seizures, loss of consciousness may occur at the outset of the attack, or somewhat later, when the discharge has spread from it's site of origin to regions of the brain stem reticular formation.

2.2.2 Generalized Seizures

Generalized seizures involve the whole brain, including the reticular system, thus producing abnormal electrical activity throughout the hemispheres. Immediate loss of consciousness is characteristic of generalized seizures. Two important categories are: tonic-clonic seizures (grand mal) and absence seizures (petit mal).

A tonic-clonic seizure consists of an initial strong contraction of the whole musculature, causing a rigid extensor spasm and an involuntary cry. Respiration stops, and defecation, micturation, and salivation often occur. This tonic phase lasts for about 1 minute, during which the face is suffused. This is followed by a series of violent, synchronous jerks that gradually die out in 2 – 4 minutes. The patient stays unconscious for a few more minutes and then gradually recovers. Injuries may occur during the convulsive episode. The EEG shows generalized continuous high-frequency activity in the tonic phase and an intermittent discharge in the clonic phase.

Children hardly experience seizures, which are much less dramatic but may occur more frequently than tonic-clonic seizures. The patient abruptly ceases whatever he/she is doing; sometimes stops speaking in the mid-sentence, and snores vacantly for a few seconds with little or no motor disturbance. The EEG pattern shows a characteristic rhythmic discharge during the period of the seizure.

2.3 Antiepilectic Drugs (AEDs)

The first AED was a bromide, suggested in 1857 by Charles Locock, who used it to treat women with 'hysterical epilepsy'. It was noted to cause impotence. It also suffered from the way it affects behaviour, introducing the idea of the 'epileptic personality'. Phenobarbitone was the first synthetic organic agent recognized in 1912 as having anti seizure activity (Brunton *et al*, 2009). Phenobarbitone was the main anticonvulsant from 1912 till 1938, when Merrit and Putman developed the electroshock seizure test in experimental animals to screen chemical agents for anti seizure effectiveness in the course of screening a variety of chemical substances. They discovered that diphenylhydantoin (later renamed phenytoin) suppressed seizure in the absence of sedative effects (Brunton *et al*, 2009). Phenytoin was synthesized in 1908 by Bilts.

The chemical structures of most of the drugs introduced before 1965 were closely related to that of phenobarbital. These included the hydantoins and succinimides. Between 1965 and 1990, the chemically distinct structures of the benzodiazepines, an iminostilbene (carbamazepine), and a branched-chain carboxylic acid (valproic acid) were introduced. The ant seizure properties of valproic acid was discovered serendipitously when it was used as a vehicle for other compounds that were being screened for anti seizure activity (Brunton *et al*,

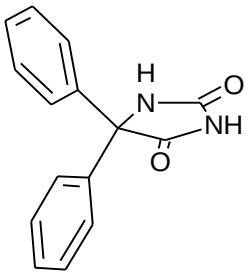
2009). In the 1990s, a phenyltriazine (lamotrigine), a cyclic analogue of GABA (gabapentin), a sulfamate substituted monosaccharide (topiramate), a nipecotic acid derivative (tiagbine), and a pyrrolidine derivative (levetiracetam) were introduced. Despite the lunch of over ten drugs since 1990, no new drug has been shown to be more effective than the older set, which include carbamazepine valproate and phenytoin.

2.4 Drugs Used In Partial Seizures and Generalized Tonic-Clonic Seizures

2.4.1 Classic Drugs

2.4.1.1 Phenytoin

This is a diphenyl substituted hydantoin that was introduced in 1938 as an antiseizure drug. It has much lower sedative properties than compounds with alkyl substituents at the 5 position. Phenytoin has major effects on several physiologic systems. The mechanism of phenytoin's action probably involves a combination of actions at several levels. At therapeutic concentrations, the major action of phenytoin is to block sodium ion channels and inhibit the generation of rapidly repetitive action potentials. Action on glutamate and GABA release probably arise from actions other than those on voltage-gated sodium ion channels. Phenytoin is associated with several dose-related adverse effects like gingival hyperplasia, hirtutism atoxia, and unpredictable pharmacokinetics (Porter and Meldrum, 2007).

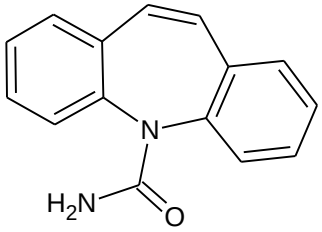


Phenytoin

2.4.1.2 Carbamazepine

This is a tricyclic compound effective in the treatment of bipolar depression and neuralgia but has proved useful for epilepsy as well. The ureide moiety (-N-CO-NH_2) present in the heterocyclic ring of most AEDs is also present in carbamazepine. Three-dimensional studies indicate that its spatial conformation is similar to that of phenytoin. The

mechanism of action appears to be similar to that of phenytoin; it blocks sodium ion channels at therapeutic concentrations and also acts presynaptically to decrease synaptic transmission. Carbamazepine also exhibits dose related side effects like drowsiness, hyponatremia and water intoxication among many others (Porter and Meldrum, 2007).

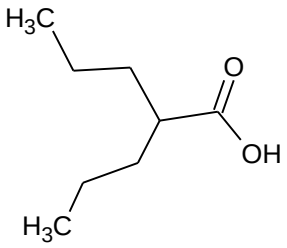


Carbamazepine

2.4.1.3 Valproic Acid and Sodium valproate

Valproic acid is fully ionized at body pH, and for that reason the active form of the drug may be assumed to be the valproate ion regardless of whether valproic acid or a salt of the acid is administered. Valproic acid is one of a series of fatty carboxylic acids that have antiseizure activity; this activity appears to be greatest for carbon chain lengths of five to eight atoms. The amides and esters of valproic acid are also active antiseizure agents. The time course of valproate's anticonvulsant activity appears to be poorly correlated with blood or tissue levels of the parent drug, an observation giving rise to considerable speculation regarding both the active species and the mechanism of action of valproic acid. Valproate is active against both pentylenetetrazol and maximal electroshock seizures. Like phenytoin and carbamazepine, valproate blocks sustained high-frequency repetitive firing of neurons in culture at therapeutically relevant concentrations. Its action against partial seizures may be a consequence of this effect on Na^+ currents. Blockade of NMDA receptor-mediated excitation may also be important. Much attention has been paid to the effects of valproate on GABA. Several studies have shown increased levels of GABA in the brain after administration of valproate, although the mechanism for this increase remains unclear. An effect of valproate to facilitate glutamic acid decarboxylase (GAD), the enzyme responsible for GABA synthesis, has been described (Porter and Meldrum, 2007). An inhibitory effect on the GABA transporter GAT-1 may contribute. At very high concentrations, valproate inhibits GABA transaminase in the brain, thus blocking degradation of GABA. However, at the relatively low doses of valproate needed to abolish pentylenetetrazol seizures, brain GABA levels may remain unchanged. Valproate produces a reduction in the aspartate content of rodent brain,

but the relevance of this effect to its anticonvulsant action is not known (Porter and Meldrum, 2007).



Valproic acid

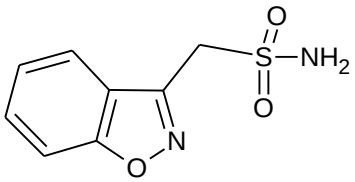
Valproic acid is a potent inhibitor of histone deacetylase and through this mechanism changes the transcription of many genes (Porter and Meldrum, 2007). A similar effect, but to a lesser degree, is shown by some other antiseizure drugs (topiramate, carbamazepine, and a metabolite of levetiracetam). The most common dose-related adverse effects of valproate are nausea, vomiting, and other gastrointestinal complaints such as abdominal pain and heartburn (Porter and Meldrum, 2007). The drug should be started gradually to avoid these symptoms. Sedation is uncommon with valproate alone but may be striking when valproate is added to phenobarbital. A fine tremor is frequently seen at higher levels. Other reversible adverse effects, seen in a small number of patients, include weight gain, increased appetite, and hair loss. The idiosyncratic toxicity of valproate is largely limited to hepatotoxicity, but this may be severe; there seems little doubt that the hepatotoxicity of valproate has been responsible for more than 50 fatalities in the USA alone. The risk is greatest for patients under the age of 2 years and for those taking multiple medications. Initial aspartate aminotransferase values may not be elevated in susceptible patients, although these levels do eventually become abnormal. Most fatalities have occurred within 4 months after initiation of therapy. Some clinicians recommend treatment with oral or intravenous L-carnitine as soon as severe hepatotoxicity is suspected. Careful monitoring of liver function is recommended when starting the drug; the hepatotoxicity is reversible in some cases if the drug is withdrawn. The other observed idiosyncratic response with valproate is thrombocytopenia, although documented cases of abnormal bleeding are lacking. It should be noted that valproate is an effective and popular antiseizure drug and that only a very small number of patients have had severe toxic effects from its use.

Several epidemiologic studies of valproate have confirmed an increased incidence of spina bifida in the offspring of women who took valproate during pregnancy. In addition, an

increased incidence of cardiovascular, orofacial, and digital abnormalities has been reported. These observations must be strongly considered in the choice of drugs during pregnancy (Porter and Meldrum, 2007).

2.4.1.4 Zonisamide

The drug is effective against partial and generalized tonic-clonic seizures and may also be useful against infantile spasms and certain myoclonias. It has good bioavailability, linear kinetics, low protein-binding, renal excretion, and a half-life of 1-3 days. Doses range from 100 mg/day to 600 mg/day in adults and from 4 mg/day to 12 mg/day in children. Zonisamide does not interact with other antiseizure drugs. Adverse effects include drowsiness, cognitive impairment, and potentially serious skin rashes (Porter and Meldrum, 2007).



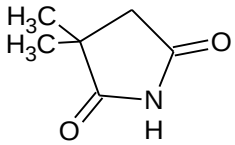
Zonisamide

2.4.2 Drugs Used In Generalized Seizures

2.4.2.1 Ethosuximide

Ethosuximide was introduced in 1960 as the third of three marketed succinimides in the USA. Ethosuximide has very little activity against maximal electroshock but considerable efficacy against pentylenetetrazol seizures, and it was introduced as a "pure petit mal" drug. Its continued popularity is based on its safety and efficacy, and its role as the first choice anti-absence drug remains undiminished in part because of the idiosyncratic hepatotoxicity of the alternative drug, valproic acid. Ethosuximide is the last antiseizure drug to be marketed whose origin is in the cyclic ureide structure. The three antiseizure succinimides marketed in the USA are ethosuximide, phensuximide, and methsuximide. Methsuximide and phensuximide have phenyl substituents, whereas ethosuximide is 2-ethyl-2-methylsuccinimide. Ethosuximide has an important effect on Ca^{2+} currents, reducing the low-threshold (T-type) current. This effect is seen at therapeutically relevant concentrations

in thalamic neurons. The T-type calcium currents are thought to provide a pacemaker current in thalamic neurons responsible for generating the rhythmic cortical discharge of an absence attack. Inhibition of this current could therefore account for the specific therapeutic action of ethosuximide. As predicted from its activity in laboratory models, ethosuximide is particularly effective against absence seizures, but has a very narrow spectrum of clinical activity. Documentation of its effectiveness in human absence seizures was achieved with long-term electroencephalographic recording techniques. Absorption is complete following administration of the oral dosage forms. Peak levels are observed 3-7 hours after oral administration of the capsules (Porter and Meldrum, 2007).



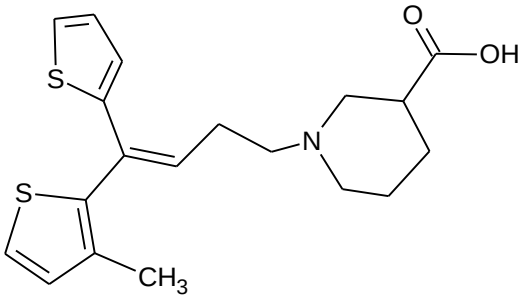
Ethosuximide

Ethosuximide is not protein-bound. It is completely metabolized, principally by hydroxylation, to inactive metabolites. The drug has a very low total body clearance (0.25 L/kg/d). This corresponds to a half-life of approximately 40 hours, although values from 18 to 72 hours have been reported. Therapeutic levels of 60-100 mcg/mL can be achieved in adults with dosages of 750-1500 mg/d, although lower or higher dosages and blood levels may be necessary and tolerated (up to 125 mcg/mL) in some patients. Ethosuximide has a linear relationship between dose and steady-state plasma levels. The drug might be administered as a single daily dose were it not for its adverse gastrointestinal effects; twice-a-day dosage is common. Administration of ethosuximide with valproic acid results in a decrease in ethosuximide clearance and higher steady-state concentrations owing to inhibition of metabolism. No other important drug interactions have been reported for the succinimides. The most common dose-related adverse effect of ethosuximide is gastric distress, including pain, nausea, and vomiting. When an adverse effect does occur, temporary dosage reductions may allow adaptation. Ethosuximide is a highly efficacious and safe drug for absence seizures; the appearance of relatively mild, dose-related adverse effects should not immediately call for its abandonment. Other dose-related adverse effects include transient lethargy or fatigue and, much less commonly, headache, dizziness, hiccup, and euphoria. Behavioral changes are usually in the direction of improvement. Non-dose-related or

idiosyncratic adverse effects of ethosuximide are extremely uncommon. Skin rashes have been reported, including at least one case of Stevens-Johnson syndrome. The development of systemic lupus erythematosus has also been reported, but other drugs may have been involved.

2.4.2.2 Tiagabine

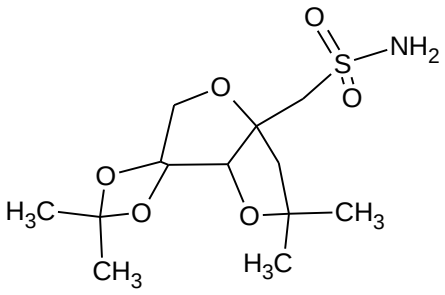
Tiagabine is a derivative of nipecotic acid and was "rationally designed" as an inhibitor of GABA uptake (as opposed to discovery through random screening). Tiagabine is an inhibitor of GABA uptake in both neurons and glia. It preferentially inhibits the transporter isoform 1 (GAT-1) rather than GAT-2 or GAT-3 and increases extracellular GABA levels in the forebrain and hippocampus. It prolongs the inhibitory action of synaptically released GABA. In rodents, it is potent against kindled seizures but weak against the maximum electroshock model. Tiagabine is indicated for the adjunctive treatment of partial seizures and is effective in doses ranging from 16 mg/d to 56 mg/d. Divided doses as often as four times per day are sometimes required. Some patients appear to do well with tiagabine monotherapy, which is generally well tolerated. Minor adverse events are dose-related and include nervousness, dizziness, tremor, difficulty in concentrating, and depression. Excessive confusion, somnolence, or ataxia may require discontinuation. Psychosis occurs rarely. Rash is an uncommon idiosyncratic adverse effect. Hepatic impairment causes a slight decrease in clearance (and may necessitate a lower dose), but the drug does not cause inhibition or induction of hepatic enzymes. The drug is oxidized in the liver by CYP3A. Elimination is primarily in the urine. Tiagabine is 90-100% bioavailable, has linear kinetics, and is highly protein-bound. The half-life is 5-8 hours and decreases in the presence of enzyme-inducing drugs. Food decreases the peak plasma concentration but not the area under the concentration curve (Rang *et al*, 2007).



Tiagabine

2.4.2.3 Topiramate

Topiramate is a substituted monosaccharide that is structurally different from all other antiseizure drugs. Topiramate blocks repetitive firing of cultured spinal cord neurons, as do phenytoin and carbamazepine. Its mechanism of action, therefore, is likely to involve blocking of voltage-gated sodium channels. Topiramate also appears to potentiate the inhibitory effect of GABA, acting at a site different from the benzodiazepine or barbiturate sites. Topiramate also depresses the excitatory action of kainate on glutamate receptors. It is possible that all three of these actions contribute to topiramate's anticonvulsant effect. Clinical trials of topiramate demonstrated a dose-response relationship, and monotherapy trials showed the drug to be effective against partial and generalized tonic-clonic seizures. Good evidence suggests that the drug has a broader spectrum, with effectiveness against Lennox-Gestaut syndrome, West's syndrome, and even absence seizures. Topiramate is also approved for the treatment of migraine headaches. Dosages typically range from 200 mg/d to 600 mg/d, with a few patients tolerating dosages higher than 1000 mg/d. Most clinicians begin at a low dose (50 mg/d) and increase slowly to avoid adverse effects. Although no idiosyncratic reactions have been noted, dose-related adverse effects occur most frequently in the first 4 weeks and include somnolence, fatigue, dizziness, cognitive slowing, paresthesias, nervousness, and confusion. Acute myopia and glaucoma may require prompt drug withdrawal. Urolithiasis has also been reported. However, the discontinuation rate is apparently only about 15%. The drug is teratogenic in animal models, and hypospadias has been reported in male infants exposed in utero to topiramate; however, no causal relationship could be established. Topiramate is rapidly absorbed (about 2 hours) and is 80% bioavailable. There is no food effect on absorption, minimal (15%) plasma protein binding, and only moderate (20-50%) metabolism; no active metabolites are formed. The drug is primarily excreted unchanged in the urine. The half-life is 20-30 hours. Although increased levels are seen with renal failure and hepatic impairment, there is no age or gender effect, no autoinduction, no inhibition of metabolism, and kinetics are linear. Drug interactions do occur and can be complex, but the major effect is on topiramate levels rather than on the levels of other antiseizure drugs. Birth control pills may be less effective in the presence of topiramate, and higher estrogen doses may be required (Porter and Meldrum, 2007).

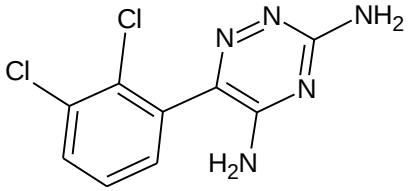


Topiramate

2.4.2.4 Lamotrigine

Lamotrigine was developed when some investigators thought that the antifolate effects of certain antiseizure drugs (eg, phenytoin) may contribute to their effectiveness. Several phenyltriazines were developed, and although their antifolate properties were weak, some were active in seizure screening tests. Lamotrigine, like phenytoin, suppresses sustained rapid firing of neurons and produces a voltage- and use-dependent inactivation of sodium channels. This action probably explains lamotrigine's efficacy in focal epilepsy. It appears likely that lamotrigine has another mechanism of action to account for its efficacy in primary generalized seizures in childhood, including absence attacks; this mechanism may involve actions on voltage-activated Ca^{2+} channels. Lamotrigine also decreases the synaptic release of glutamate. Although most controlled studies have evaluated lamotrigine as add-on therapy, some suggest that the drug is effective as monotherapy for partial seizures, and the drug is now widely prescribed for this indication. Some authorities feel that the drug is also active against absence and myoclonic seizures in children. Adverse effects include dizziness, headache, diplopia, nausea, somnolence, and skin rash. The rash is considered a typical hypersensitivity reaction. Although the risk of rash may be diminished by introducing the drug slowly, pediatric patients are at high risk; some studies suggest that a potentially life-threatening dermatitis will develop in 1-2% of pediatric patients. Lamotrigine is almost completely absorbed and has a volume of distribution in the range of 1-1.4 L/kg. Protein binding is only about 55%. The drug has linear kinetics and is metabolized primarily by glucuronidation to the 2-*N*-glucuronide, which is excreted in the urine. Lamotrigine has a half-life of approximately 24 hours in normal volunteers; this decreases to 13-15 hours in patients taking enzyme-inducing drugs. Lamotrigine is effective against partial seizures in adults, with dosages typically between 100 and 300 mg/d and with a therapeutic blood level near 3 mcg/mL. Valproate causes a twofold increase in the drug's

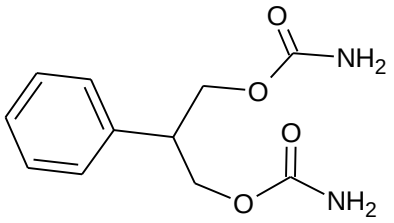
half-life; in patients receiving valproate, the initial dosage of lamotrigine must be reduced to 25mg` every other day (Porter and Meldrum, 2007).



Lamotrigine

2.4.2.5 Felbamate

Felbamate has been approved and marketed in the USA and in some European countries. Although it is effective in some patients with partial seizures, the drug causes aplastic anemia and severe hepatitis at unexpectedly high rates and has been relegated to the status of a third-line drug for refractory cases. Felbamate appears to have multiple mechanisms of action. It produces a use-dependent block of the NMDA receptor, with selectivity for the NR1-2B subtype. It also potentiates $GABA_A$ receptor responses. Felbamate has a half-life of 20 hours (somewhat shorter when administered with either phenytoin or carbamazepine) and is metabolized by hydroxylation and conjugation; a significant percentage of the drug is excreted unchanged in the urine. When added to treatment with other antiseizure drugs, felbamate increases plasma phenytoin and valproic acid levels but decreases levels of carbamazepine. In spite of the seriousness of the adverse effects, thousands of patients worldwide remain on the medication. Usual dosages are 2000-4000 mg/day in adults, and effective plasma levels range from 30 mcg/mL to 100 mcg/mL. In addition to its usefulness in partial seizures, felbamate has proved effective against the seizures that occur in Lennox-Gastaut syndrome (Porter and Meldrum, 2007).



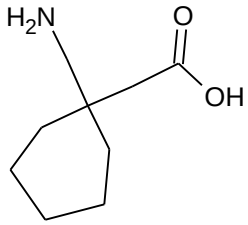
Felbamate

2.4.2.6 Gabapentin and Pregabalin

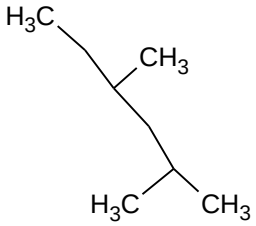
Gabapentin is an amino acid (an analog of GABA), that is effective against partial seizures. Originally planned as a spasmolytic, it was found to be more effective as an antiseizure drug. Pregabalin is another GABA analog, closely related to gabapentin. This drug was recently approved in the USA for both antiseizure activity and for its analgesic properties. In spite of their close structural resemblance to GABA, gabapentin and pregabalin do not act directly on GABA receptors. They may, however, modify the synaptic or nonsynaptic release of GABA. An increase in brain GABA concentration is observed in patients receiving gabapentin. Gabapentin is transported into the brain by the L-amino acid transporter. Gabapentin and pregabalin bind avidly to the subunit of voltage-gated Ca^{2+} channels. Gabapentin and pregabalin also act presynaptically to decrease the release of glutamate; this effect is probably dependent on reduced presynaptic entry of Ca^{2+} via voltage-activated channels. Gabapentin is effective as an adjunct against partial seizures and generalized tonic-clonic seizures at dosages that range up to 2400 mg/day in controlled clinical trials. Open follow-on studies permitted dosages up to 4800 mg/day, but data are inconclusive on the effectiveness or tolerability of such doses. Monotherapy studies also document some efficacy. Some clinicians have found that very high dosages are needed to achieve improvement in seizure control. Effectiveness in other seizure types has not been well demonstrated. Gabapentin has also been found effective in the treatment of neuropathic pain and is now indicated for postherpetic neuralgia in adults at doses of 1800 mg and above. The most common adverse effects are somnolence, dizziness, ataxia, headache, and tremor. Pregabalin is approved (as an adjunct) for the treatment of partial seizures, with or without secondary generalization; controlled clinical trials have documented its effectiveness. It is available only in oral form, and the daily dose ranges from 150 mg/day to 600 mg/day, usually in two or three divided administrations. Pregabalin is also approved for use in neuropathic pain, including painful diabetic peripheral neuropathy and postherpetic neuralgia. Absorption is nonlinear and dose-dependent at very high doses, but otherwise the elimination kinetics are linear. The drug is not bound to plasma proteins. Drug-drug interactions are negligible. Elimination is via renal mechanisms; the drug is excreted unchanged. The half-life is short, ranging from 5 hours to 8 hours; the drug is typically administered two or three times per day.

Pregabalin, like gabapentin, is not metabolized and is almost entirely excreted unchanged in the urine. It is not bound to plasma proteins and has virtually no drug-drug interactions, again

resembling the characteristics of gabapentin. Likewise, other drugs do not affect the pharmacokinetics of pregabalin. The half-life of pregabalin ranges from about 4.5 hours to 7.0 hours, thus requiring more than once-per-day dosing in most patients (Porter and Meldrum, 2007).



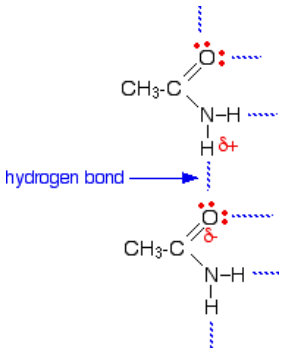
Gabapentin



Pregabalin

2.5 Chemistry of Amides

Amides are derivatives of carboxylic acids containing the functional group consisting of an carboxyl group (-C=O) linked to a nitrogen atom. Simple amides from benzoic acids are called carboxamides or benzamides. The amide moiety is an important constituent of many important pharmacologically active compounds. This stimulates continuous interest in the chemistry of amides. With the exception of methanamide which melts at 3°C, all amides are solids at room temperature. The melting points of the amides are high because they can form hydrogen bonds among themselves. The hydrogen atoms in the -NH_2 group form a hydrogen bond with a lone pair on the oxygen atom of another amide molecule as shown below.



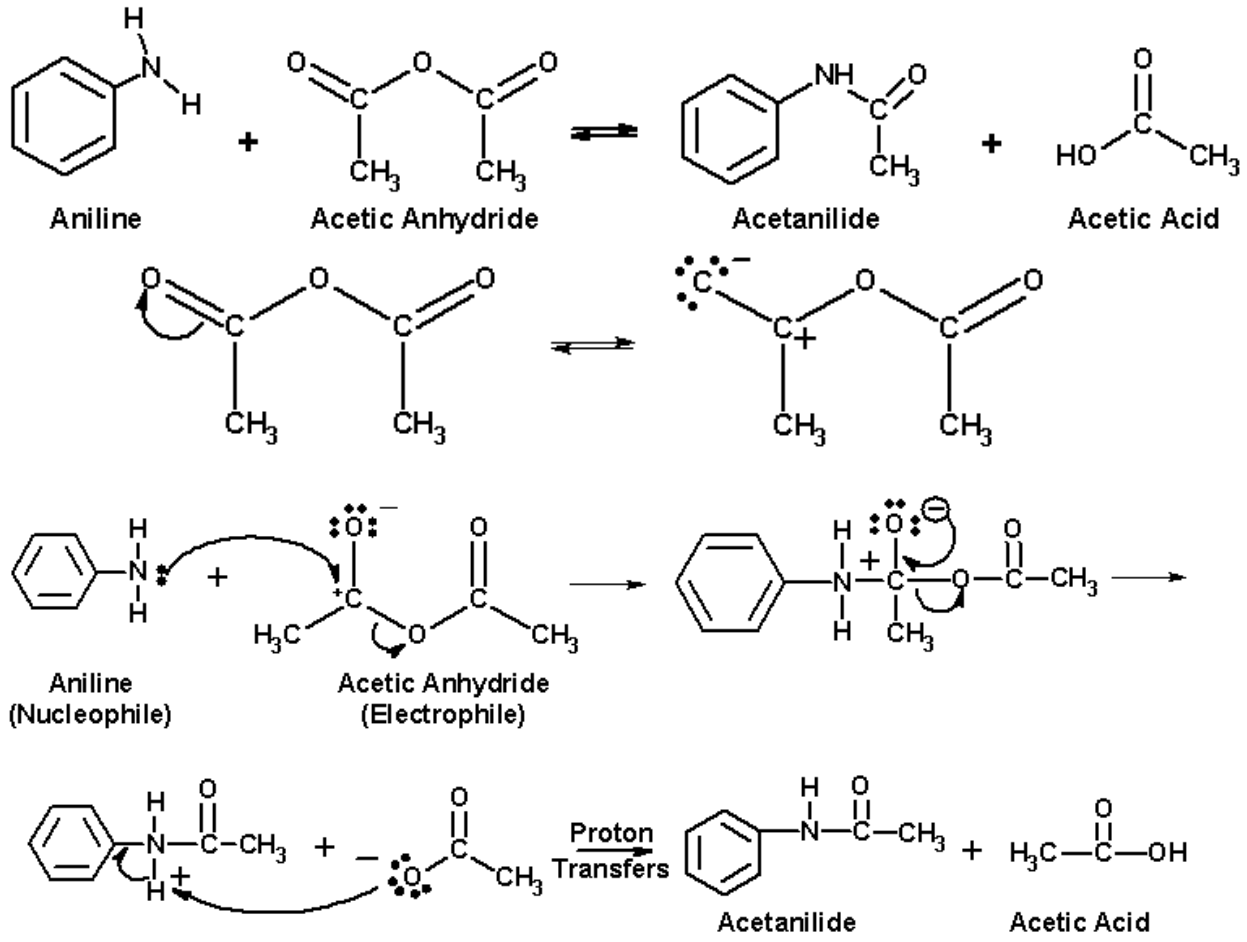
Hydrogen bonding in amides

Low molecular weight amides are soluble in water because they can form hydrogen bond with the water molecules. The electron pair on the nitrogen in an aromatic amide is less likely to move to aromatic ring. An amide is stabilized by the resonance involving the nonbonding pair of electrons on the nitrogen atom and the strong electron-withdrawing effect of the carbonyl group. The strong electron withdrawing carbonyl oxygen takes on a partial negative charge. Hence, protonation of an amide occurs on the oxygen rather than the nitrogen. The lone pair of electrons on the nitrogen is delocalized onto the carbonyl, thus forming a partial double bond between nitrogen and the carbonyl carbon. Consequently the nitrogen in amides is not pyramidal. Amides can be described by two resonance structures A and B; B with (+) and (-) charges is less stable than the neutral A.

The planar nature of amide group lead to the existence of two configurational isomers: cis and trans. The existence of these isomers has been established by the use of NMR, IR and Raman spectroscopic techniques, as well as by dipole moment measurement. In the condensed phase, amides tend to associate with themselves and solvent molecules because of their dipolar nature. Both concentration of amides and the nature of solvents affects the observed physical parameters such as chemical shifts and the IR absorption frequency. The three types of association products that are well documented in the literature are: hydrogen bonded dimers, hydrogen bonded polymers, and dipole-dipole dimers. The latter may be restricted to tertiary amides where hydrogen bonding is impossible. Complex formation between amides and aromatic solvents readily occurs.

2.5.1 Preparation of Aromatic amides

The most common method for preparing amides involves treatment of ammonia, primary, secondary, amines with acylating agents to yield primary, secondary, and tertiary amides, respectively. Aromatic amides are usually made through a nucleophilic acyl substitution (addition/elimination) reaction between a nucleophile (usually aniline), and a carboxylic acid derivative as the electrophile. The following reaction mechanism for N-phenylacetamide exemplifies mechanism of nucleophilic acyl substitution of aromatic amides (Bell *et al*, 1997)..



Other methods for the synthesis of aromatic amides include:

i. Direct hydration of nitriles to form secondary amides: this reaction involves strong heating in an autoclave in order to obtain a good yield of amides. The reaction is catalyzed by both mineral acids and alkalis. The acid catalyzed reaction proceed by the attack of water molecule on protonated nitrile; whereas, the base catalyzed reaction involves attack of hydroxide ion on the free nitrile.

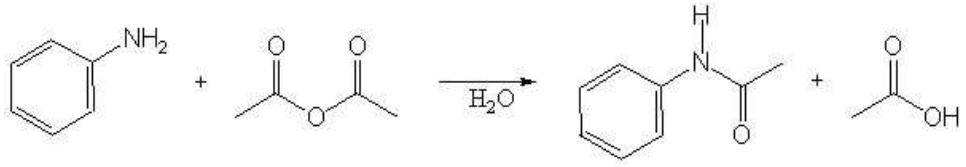
ii. The Ritter reaction is an extension of hydration reaction leading to a secondary amide. The reaction involves the alkylation of the nitrile nitrogen followed by the formation of carbonyl function. Carbonium ion is generated from an olefin or alcohol, which with concentrated sulphuric acid as catalyst, react with nitriles to form an intermediate nitrilinium salt which is hydrolysed directly to secondary amide.

iii. Rearrangement reactions: Most of the rearrangement reactions that lead to the formation of secondary and tertiary amides involve migration of alkyl or aryl substituent to nitrogen. The migration group may be electron rich migrating to electron deficient nitrogen atom, or may be electron deficient migrating or interacting with lone pair of electron of nitrogen as in the Beckman and Chapman reactions, respectively.

iv. Alkylation of amides: Amides can be modified by alkylation to give more highly substituted analogues. Direct alkylation using reagents such as alkylhalides often leads to a mixture of O and N substituted products. To obtain pure N-alkylated products, pure nucleophilic amide ion has to be formed by using sodium hydride prior to treatment with alkylating agent. This permits easy conversion of secondary amides into tertiary amides.

2.5.1.1 Synthesis of N-phenylacetamide and N- phenylbenzamide

For over hundred years, the commonly used procedures for the synthesis of N-phenylacetamide and several aromatic amides are the modified versions of the Schotten (1884) and Baumann (1886) reactions. N-phenylacetamide is made by reacting aniline with acetic anhydride in chlorinated solvents (usually dichloromethane) in the presence of pyridine. Usage of acetic anhydride banned in some places, due to its utility in narcotic business. The side product of the reaction is acetic anhydride.

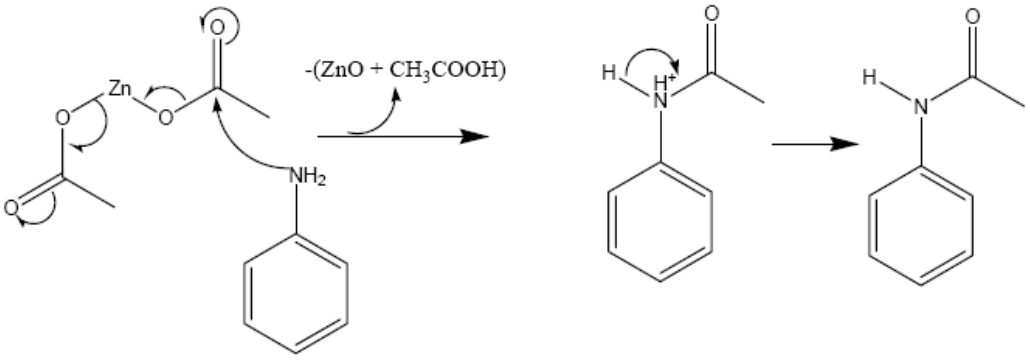


Schotten-Baumann reaction is found in most organic chemistry laboratory manuals for university students. For instance, the typical procedure for Schotten-Baumann reaction is as follows: 2.15 mmol of aniline is placed in a test tube. 5 ml of distilled water is then added to the test tube with swirling, followed by 20 drops of acetic anhydride. The mixture is stirred until solid forms (about 5 minutes). The product is crystallized in the same test tube. 5 ml of water is then added and the test tube is heated in a hot water bath (250 mL beaker) with occasional stirring until the entire solid dissolved. The test tube is allowed to cool for 3-5

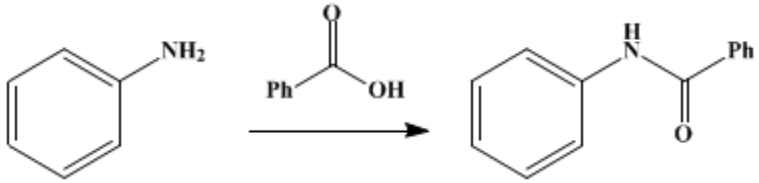
minutes and then chilled in an ice bath. The product is collected by vacuum filtration using a Büchner funnel. The yield of the dried product is 75% (Bell *et al*, 1997).

Schotten-Baumann reaction has three major drawbacks: chlorinated solvents and pyridine are very toxic chemicals, the acetic acid produced is wasted because it is very difficult to purify it.

In recent years, there have been several attempts to eliminate and/or minimize the pitfalls of the Schotten-Baumann reaction (Anastas and Warner, 1998). For example, Bhagat (2006) developed a procedure for the synthesis of N-phenylacetamide which doesn't require acetic anhydride and hazardous chlorinated solvents, and also minimizes waste by-products. The procedure involves mixing of aniline (10 ml) and zinc dust (0.5 g) in acetic acid (30 ml) in a 100 ml round bottom flask. The mixture is then heated for about 2 hrs. The reaction mixture is then carefully poured in cold water (100 ml) in a 250 ml beaker with cooling and vigorous stirring. The crystals of N-phenylacetamide are separated slowly, and collected by filtration. The yield of the dried product is 91% (Bhagat *et al*, 2006). The proposed mechanism of the zinc dust catalyzed reaction is shown below.



The synthesis of N-phenylbenzamide from aniline and benzoic acid is also well documented in the literature (www.orgsynth.org).



One such synthetic procedure is as follows: 8.1 moles of aniline and 8.2 moles of benzoic acid are placed in a round-bottomed flask. The flask is placed in an oil bath and

connected to a condenser for distillation. The temperature of the oil is raised quickly to 180–190°C, at which point distillation starts. The bath is held at this temperature until practically no more aniline and water distil (about two hours), and then the temperature is slowly raised to 225°C and maintained at this temperature until no further distillation takes place (one to two hours). The oil bath is then removed and the contents of the flask are allowed to cool below 180°C and 5.9 moles of aniline is added. The distillations at 190°C and 225°C are repeated (about six hours). The hot mixture is poured into an evaporating dishes and allowed to cool. The purplish-gray solid is grounded in a mortar and is poured with vigorous stirring into a container containing 6 litres of 1M hydrochloric acid). The stirring is continued for an hour after all the N-phenylbenzamide. has been added, and then the solid is filtered on a Büchner funnel. The process of stirring with acid and filtering is repeated twice to remove the excess of aniline. The solid is then stirred for two hours with 6 litres of water and is filtered. It is stirred for one hour with 6 litres of 1M sodium hydroxide solution to remove the excess of benzoic acid and is then filtered. The stirring with sodium hydroxide and filtering is repeated. The solid is stirred for two hours with 7 litres of water and filtered, sucked dry, and air-dried overnight on paper. After drying on paper the purplish solid is dried to constant weight in evaporating dishes at 90–100°C (about two days) and is then repowdered. The product is light purplish-gray and the yield is 80–84 per cent of the theoretical amount. It melts at 157–160°C. When 100 g of N-phenylbenzamide. is dissolved in 750 ml of hot alcohol and the solution is boiled with about 10 g. of decolorizing carbon (Norite), filtered, and cooled at 10°C overnight, 80–86 g. of an almost colourless product melting at 160–161°C separates. A second crystallization from alcohol using decolorizing carbon gives a white product with approximately the same loss in the mother liquors as in the first crystallization (www.orgsynth.org). The major disadvantage of this procedure is the large number of operations involved. In this work, a new procedure is developed for one step synthesis of N-phenylbenzamide from aniline and benzoyl chloride (more reactive than benzoic acid) under milder conditions.

2.6 Amides as Anticonvulsants

Structurally many of the simplest compounds possessing anticonvulsant properties are carboxylic acids and their amides. Valproic acid is perhaps the best known example of this class of compounds. Some of the various carboxamides reported to have anticonvulsant properties are as follows:

i. Valproic acid amide

Half a dose of valproic acid amide has been shown to be as effective as the dose of valproic acid.

ii. N-alkyl-α,β-dimethyl cinnamides

Balsamo *et al* (1977) reported the anticonvulsant property of this compound, with activity against MES induced seizure. The anticonvulsant activity was attributed to the cis isomer.

iii. 4-Amino-N-cyclopropyl N-phenylbenzamides

Bruce and Lyne (1962) reported the anticonvulsant profile of the several aromatic amides. 4-amino-N-cyclopropyl-3,5-dichloroN-phenylbenzamide and 4-amino-N-cyclopropyl-3,5-dibromoN-phenylbenzamide were the most potent among the compounds tested. Bruce and Lyne emphasized the importance of the benzamide moiety for anticonvulsant activity.

iv. 4-AminoN-phenylbenzamides and their derivatives

Using the 4-aminobenzoylamino template, Clark and co-workers (1985, 1987, 1988), through a series of successive works, demonstrated the significant anticonvulsant potential in animal epilepsy models for the amino-substituted N-phenylbenzamides with one of them, (4-amino-N-(2,6-dimethylphenyl)N-phenylbenzamide) being comparable to phenytoin. Structure activity relationship studies suggest that amides having a primary amine in the 4-position of the N-phenylbenzamide moiety and an aromatic N-substitution of the 4-aminoN-phenylbenzamide pharmacophore possess optimal antiepileptic activity. Studies on a series of 4-aminophenylN-phenylacetamides have shown significant loss of anticonvulsant activity resulting from insertion of a methylene between the aromatic and the amide carbonyl of the aminoN-phenylbenzamides (Clark and Davenport, 1987). From this work, ameltolide which exhibit a phenytoin like profile emerged. Using the batrachotoxin affinity assay, it was noted that the 4-amino moiety played an important role in the molecular recognition process at the level of the receptor modulating the voltage-dependent sodium channels. The moiety was therefore preserve in most pharmacophore modulation. It was then thought that the 4-amino moiety is necessary for the anticonvulsant activity.

v. N-phenylphthalimide and their derivatives

Vamecq et. al. (1998) found that the 4-aminoN-phenylbenzamide moiety is actually not essential for anticonvulsant activity. They designed N-phenylphthalimide

derivatives that were highly potent and active in the MES test. SAR studies of the compounds suggest that the best results require substitution of the N-phenyl moiety. The work of Vamecq *et al* (1998, 2000) led to the design of the phthalimide counterpart of ameltolide: the 4-amino-N-(2,6-dimethylphenyl) phthalimide.

vi. C(α)-acetoamido-N-benzyl N-phenylacetamide and their derivatives.

Bardel *et al* (1994) demonstrated that α-substituted C(α)-acetoamido-N-benzylacetanilide displayed excellent anticonvulsant activities in mice. The various derivatives exhibit potencies comparable to or greater than phenytoin in the MES test.

vii. Milacemide and their derivatives

Due to marginal effects seen in clinical trials of milacemide (1,2-(n-pentyl) amino N-phenylacetamide), development of the compound was no longer pursued. Peverello *et al* (1998) used the compound to design novel compounds that are more potent and with a wider range of anticonvulsant spectrum. Some of the compounds were orally active and safe anticonvulsants. Preliminary results point toward a potent sodium channel blocking activity.

viii. 3-Anilinopropanamides, 3-toluidinopropanamide, 3-anisidopropanamide, and their N-benzyl derivatives

Idris *et al* (2008) designed and synthesized a series of novel 3-anilinopropanamides, 3-toluidinopropanamide, 3-anisidopropanamide, 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.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Equipments

All starting reagents and solvents for the experiments were of analytical grade, and were used without further purification.

Table 3.1 Reagents and solvents used for the experiments

	Reagents/Solvents	Source
1	Aniline	Department of Pharmaceutical Chemistry, ABU, Zaria.
2	Benzoyl chloride	Department of Pharmaceutical Chemistry ABU, Zaria.
3	Acetic anhydride	Purchased from Lagos
4	Activated charcoal	Department of Pharmaceutical Chemistry, ABU, Zaria.
5	Sodium hydroxide	Department of Pharmaceutical Chemistry, ABU, Zaria.
6	Iodine crystals	Department of Pharmaceutical Chemistry, ABU, Zaria.
7	Methanol	Department of Pharmaceutical Chemistry ABU, Zaria.
8	Absolute ethanol	Department of Pharmaceutical Chemistry, ABU, Zaria.
9	Ethyl acetate	Department of Pharmaceutical Chemistry, ABU, Zaria.
10	Chloroform	Department of Pharmaceutical Chemistry, ABU, Zaria.
11	Distilled water	Department of Pharmaceutical Chemistry ABU, Zaria.
12	Acacia powder	Department of Pharmaceutical Chemistry, ABU, Zaria.
13	Pentylenetetrazole	Department of Pharmacology, ABU, Zaria.
14	Phenytoin	Department of Pharmacology, ABU, Zaria.
15	Valproic acid	Department of Pharmacology, ABU, Zaria.

Pyrex made glassware were used. The glassware was properly cleaned with distilled water and dried in an oven before use. Most of the equipments were sourced from the Department of Pharmaceutical and Medicinal Chemistry, ABU, Zaria. Nuclear Magnetic Resonance (NMR) analysis were performed in the Department of Chemistry, Kwazulu Natal University, South Africa. Fourier Transform Infrared (FTIR) spectra were performed in the National Research Institute for Chemical Technology (NARICT), Zaria.

Table 3.2 Equipments used for the experiments

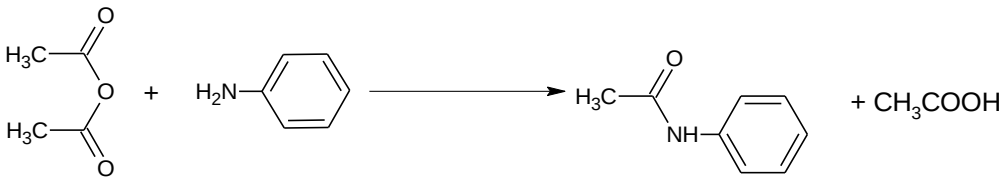
	Equipment	Source
1	Analytical balance (METLER AE-240)	Department of Pharmaceutical Chemistry, ABU, Zaria.
2	Steam water bath (Gallenklamp)	Department of Pharmaceutical Chemistry, ABU, Zaria.
3	Oven (13s model OV 33; Gallenkamp)	Department of Pharmaceutical Chemistry, ABU, Zaria.
4	Micro capillary tubes	Department of Pharmaceutical Chemistry, ABU, Zaria.
5	Thin layer chromatographic plates	Department of Pharmaceutical Chemistry, ABU, Zaria.
6	Reflux condenser	Department of Pharmaceutical Chemistry, ABU, Zaria.
7	Top loading balance (W and T Avery England)	Department of Pharmaceutical Chemistry, ABU, Zaria.
8	Syringes	Department of Pharmaceutical Chemistry, ABU, Zaria.
9	Mortar and pestles	Department of Pharmaceutical Chemistry, ABU, Zaria.
10	Beakers of various sizes	Department of Pharmaceutical Chemistry, ABU, Zaria.
11	Plastic cages of various sizes	Department of Pharmacology, ABU, Zaria.
12	Hotplate (Gallenkamp)	Department of Pharmaceutical Chemistry, ABU, Zaria.
13	Melting point apparatus	Department of Pharmaceutical Chemistry, ABU, Zaria.
14	UV absorption spectrophotometer	Department of Chemistry, ABU, Zaria.
15	400MHz Bruker 400 (Avance lll, Germany)NMR spectrometer	Department of Chemistry, Kwazulu Natal University, South Africa.
16	Shimadzu FTIR spectrometer	NARICT, Basawa, Zaria
17	UGO basile electroconvulsive unit ECT 800 fitted with ear clip electrodes	Department of Pharmacology, ABU, Zaria.

3.2 Experimental Animals

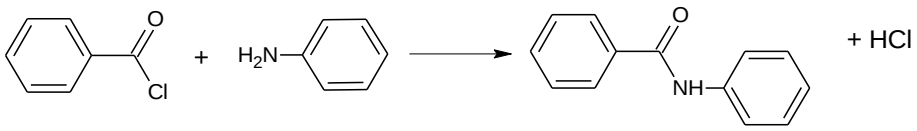
157 locally bred (in the Department of Pharmacology and Therapeutics , ABU Zaria) adult albino mice of either sex weighing between 16- 36g were used in this study. The animals were housed under standard laboratory conditions (temperature of 25±3 °C, humidity of 45-60 %, and 12 hours of light/dark circle) and allowed free access to food and water. 150 day-old white cockerels weighing 22 – 39g were used. The cockerels were obtained from the national animal production research institute, ABU Zaria.

3.3 Synthesis of N-phenylacetamide and N-phenylbenzamide.

N-phenylacetamide and N-phenylbenzamide were synthesized via nucleophilic addition (A_N) reaction using NaOH as the catalyst. The chemical equations for the synthesis are shown in schemes 1 and 2.



Scheme 1. Synthesis of N-phenylacetamide



Scheme 2. Synthesis of N-phenylbenzamide.

3.3.1 Procedure for the synthesis of N-phenylacetamide

A mixture of 9.1 ml of aniline (0.1 mol) and 0.5 g zinc dust in 10ml of acetic acid (0.106 mol) in a 100 ml round bottom flask was refluxed using water condenser. for about 30 minutes. The reaction mixture was then carefully poured in cold water (100 ml) in a beaker with vigorous stirring. The crystals of N-phenylacetamide were separated slowly. After 15 min. the N-phenylacetamide crystals were collected by filtration. The crystals were washed

over the Buchner funnel with water and the product was dried. The percentage yield and properties of the product are reported in Table 4.1.

3.3.2 Procedure for the synthesis of N-phenylbenzamide.

To 0.020 moles (2.0 ml) of aniline, 20 ml of 10% sodium hydroxide solution was added in a well corked conical flask. 0.040 moles (4.5 ml) of benzoyl chloride was then added onto the mixture slowly while shaking gently. The mixture was then shaken vigorously until the disappearance of the odour of benzoyl chloride (about 10 minutes). The mixture was filtered using a Buchner funnel. The solid product was washed with cold distilled water, and then recrystallized several times from absolute ethanol until a single spot on TLC was obtained. The product was dried on a paper. The percentage yield was then calculated as reported in Table 4.1.

3.3.3 Characterization of N-phenylacetamide and N-phenylbenzamide.

Melting point were determined using Gallenklamp melting point apparatus as described in the manufacturer's manual. The reported melting points are uncorrected.

Proton and Carbon-13 one- and two-dimensional Nuclear Magnetic Resonance (NMR) experiments were performed using 400MHz Bruker 400 (Avance lll, Germany)NMR spectrometer. ^{1}H and ^{13}C NMR data is reported in terms of chemical shifts.

Fourier Transform Infrared (FTIR) spectra were recorded using Shimadzu spectrometer at the National Research Institute for Chemical Technology, Zaria.

ACD/Chemsketch software downloaded from www.acdlabs.com was used for drawing two-dimensional chemical structures. The structures of the trans isomers of N-phenylacetamide and N-phenylbenzamide optimized using the molecular mechanics tools contained in ACD/3D viewer software that was also downloaded from www.acdlabs.com.

3.4 Anticonvulsant Screening

For the MES screening UGO basile electroconvulsive unit ECT 800 fitted with ear clip electrodes was used. The standard drugs were phenytoin injection and valproic acid. Acacia powder was used as the negative control. For the scPTz screening, pentylenetetrazole was the chemical agent used to induce seizure, and the standard drug was valproic acid.

3.4.1 Determination of Acute Toxicity in Mice LD_{50}

LD_{50} was determined using the method of Lorke (1983). In the first phase of the experiment, 36 mice were divided into 12 groups and treated with 10, 100, and 100mg/kg body weight of the each of the synthesized compounds. The mice were observed for 24 hours. The second phase of the experiment was conducted in order to obtain the highest dose survived by all the animals.

The LD_{50} was calculated as the square root of the product of the lowest dosage that killed all the animals and the highest dosage survived by all the animals in the group. The results obtained were used as a guide to select the dose for the MES and PTz tests.

3.4.2 Primary (Qualitative) Evaluation of the Test Compounds

3.4.2.1 Pentylenetetrazole (Ptz) Induced Seizure Test

The method of Swinyard *et al* (1989) was employed. 84 mice were divided into 14 groups of 6 mice each. Group I (negative control) received 1.0 mg/kg body weight of 2 % acacia gum intraperitoneally (i.p). Group II (positive control) received valproic acid 200mg/kg body weight i.p. Group III, IV, and V received 600, 300, and 150mg/kg of compound N-PHENYLACETAMIDE, respectively. While group VI, VII, and VIII received 300, 150, and 75mg/kg body weight (i.p) of compound N-PHENYLBENZAMIDE., respectively. After 30 minutes post treatment, all the groups (I-VIII) were administered 85mg/kg body freshly prepared PTz subcutaneously. All the mice were observed for a period of 30 minutes after sc PTz (that is an episode of clonic spasm of at least 5 second duration with loss of righting reflex). The percentages of protection, time of onset of convulsion and time of death if any, were observed and recorded.

3.4.2.2 Maximal Electroshock Induced Seizure Test in Chicks

The modified methods of Swinyard *et al* (1989) were employed. 90 day-old white ranger cockerels were randomly divided into 9 groups of 10 chicks per group. Group I (negative control) was given normal saline i.p. Group II and III (positive control) were given 20mg and 200mg/kg body weight of phenytoin and valproic acid, respectively. Group IV-IX were divided into 4 sets and each set received different doses of the test compounds (compound N-phenylacetamide 600, 300, and 150mg/kg bodyweight, and compound N-phenylbenzamide, 300, 150, and 75mg/kg bodyweight).

Thirty minutes later, maximum electroshock was delivered to induce seizure in all the groups using ugo- basile electroconvulsive machine. Corneal electrodes were placed in the upper eyelids of the chicks. The current, shock duration, frequency, and pulse width were set and maintained at 90 mA, 0.6 sec, 100 pulses per second (100 Hz), and 0.6 ms, respectively. Hind limb tonic extension (HLTE) was selected as the endpoint of the experiment and the abolition of the HLTE was considered as protection from electroshock induced convulsion (Swinyard *et al*, 1989).

3.5 Statistical Analyses

The mean onset of seizure, mean time of death, and mean recovery time were presented as mean±standard error of mean (SEM). The mean values of the control group were compared with the mean values of the groups treated with the test compounds (N-phenylacetamide, N-phenylbenzamide.) using student t test. The results were considered significant at $p < 0.05$.

CHAPTER 4.

RESULTS

Table 4.1 Yield and some physical properties of

N-phenylacetamide and N-phenylbenzamide

	N-phenylacetamide	N-phenylbenzamide
Yield, %	97.3	96.5
Melting point, °C	113 – 114	127
R_f[a]	3.7	3.9
Appearance	White crystals	White crystals
Molecular weight	135	197

[a] Silica TLC plate with ethylacetate/chloroform in 1:2 ratio as the development solvent

Table 4.2 Analysis of the functional group region in the FTIR spectra of

N-phenylacetamide and N-phenylbenzamide

Wavenumber in cm^{-1} and Relative Intensity[a]		Assignment
N-phenylacetamide	N-phenylbenzamide.	
3340 (s)	3286 (s)	N-H stretching vibration
3045 (s)	3070 (m)	aromatic C_{sp2}-H stretching vibration
1655 (s)	1660 (s)	C=O stretching vibration
1516	1539 (s)	N-H deformation vibration

[a]Relative Intensity in parenthesis (m=medium intensity, s=strong intensity)

Table 4.3 Analysis of the ^{1}H spectra of

N-phenylacetamide and N-phenylbenzamide

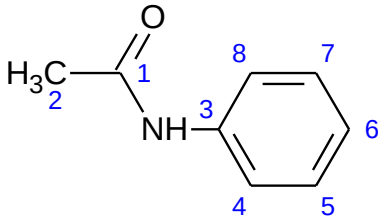
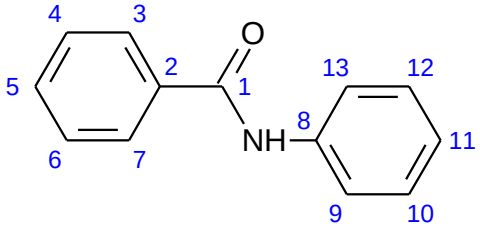
N-phenylacetamide		N-phenylbenzamide	
			
Chemical shift, Ppm	Assignment	Chemical shift, ppm	Assignment
	C_2-**H**	4.8	N-**H**
4.8	N-**H**	7.2	C_{11}-**H**
7.0	C_6-**H**	7.6	C_4-**H**, C_6-**H**
7.2	C_5-**H**, C_7-**H**	7.9	C_3-**H**, C_7-**H**
7.4	C_4-**H**, C_8-**H**	7.5	C_5-**H**
		7.4	C_{10}-**H**, C_{12}-**H**
		7.7	C_9-**H**, C_{13}-**H**

Table 4.4 Analysis of the ^{13}C spectra of

N-phenylacetamide and N-phenylbenzamide

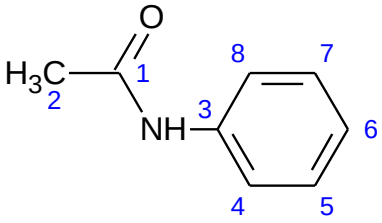
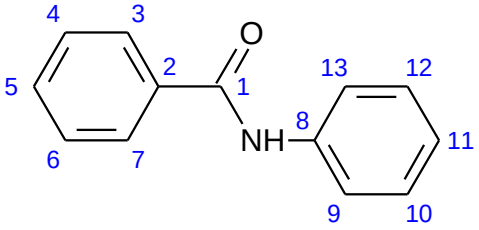
N-phenylacetamide		N-phenylbenzamide	
			
Chemical shift, Ppm	Assignment	Chemical shift, ppm	Assignment
23	C_2	121	C_6 and C_{11}
120	C_6	124	C_{10} and C_{12}
124	C_5 and C_7	127	C_4 and C_6
128	C_3, C_4 and C_8	128	C_9 and C_{13}
171	C_1	131	C_3 and C_7
		135	C_8
		139	C_2
		167	C_1

Table 4.5 Effect of N-phenylacetamide, N-phenylbenzamide and phenytoin on maximum electroshock induced seizure in chicks

Treatments, mg/kg	Mean time of recovery, mins	Quantal protection	Percentage protection, %	Mortality, %
Negative control (acacia solution)	6.1±1.33	0/10	0.00	0.00
N-phenylacetamide:				
300	-	10/10	100	0.00
150	-	10/10	100	0.00
75	-	10/10	100	0.00
N-phenylbenzamide.				
600	8.22±1.43	1/10	10	0.00
300	6.33±0.31	1/10	10	0.00
150	6.23±1.03	2/10	20	0.00
Positive control (phenytoin) 20	-	10/10	100	0.00

Table 4.6 Effect of N-phenylacetamide, N-phenylbenzamide and valproate on subcutaneous pentylenetetrazole induced seizure in mice

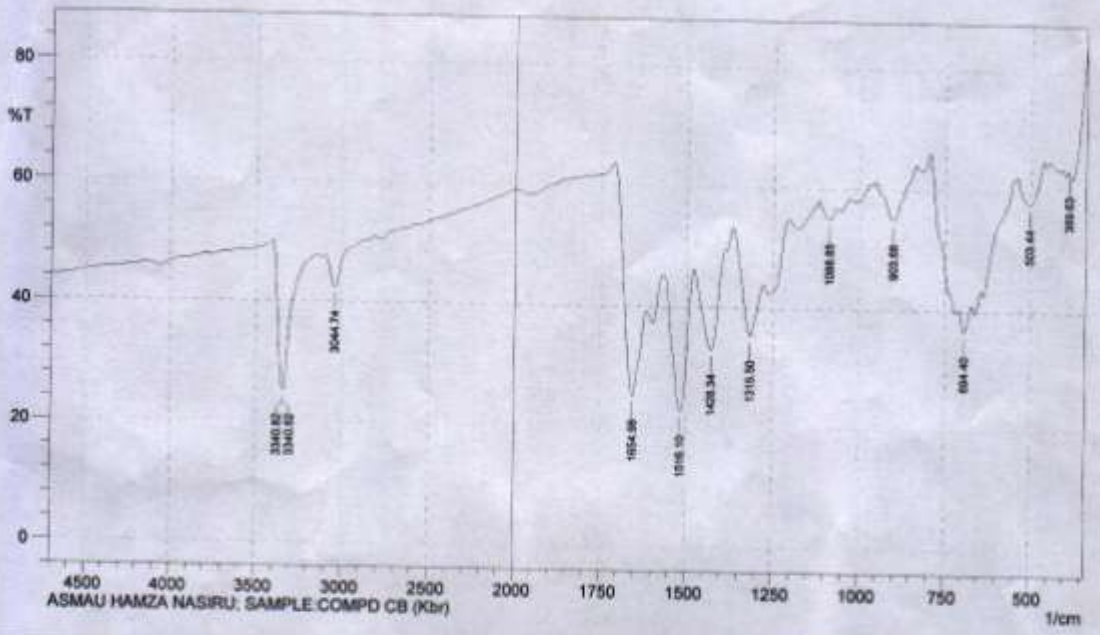
Treatments, mg/kg	Mean onset of seizure, mins	Mean time of death, mins	Quantal protection	Percentage protection, %	Mortality, %
Negative control (acacia solution)	6.33±1.50	5.00±1.26	0/6	0.00	83.33
N-phenylacetamide:					
300	23.00 ±0.00	0.00	5/6	83.33	16.66
150	17.33 ±3.31	16.00±0.00	3/6	50.00	
75	9.00±0.00	-	4/6	66.66	
N-phenylbenzamide:					
600	9.75 ±2.16	16.00±0.00	2/6	33.33	66.66
300	8.75 ±0.96	6.50±1.75	2/6	33.33	66.66
150	11.00±1.84	10.00±0.00	2/6	33.33	33.66
Positive control (valproate) 200	14.00±3.53	10/10	6/6	0.00	

Table 4.7 Results of acute toxicity test (LD_{50}) for N-phenylacetamide and N-phenylbenzamide

Compound	LD_{50} ($mgkg^{-1}$)
N-phenylacetamide	2154.1
N-phenylbenzamide.	1264.9

SHIMADZU

FTIR ANALYSIS RESULT
NARICT,ZARIA



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	389.63	63.51	0.09	391.56	387.7	0.759	0.001
2	503.44	57.877	0.352	505.37	463.9	9.082	0.307
3	694.4	36.513	4.099	710.79	672.21	15.972	0.894
4	903.68	55.081	5.625	932.61	838.1	21.928	2.073
5	1088.85	55.057	2.315	1119.71	1069.56	12.598	0.547
6	1315.5	35.369	12.595	1366.61	1278.85	33.022	5.45
7	1428.34	33.002	15.232	1478.49	1388.79	36.06	7.487
8	1516.1	22.604	23.417	1568.18	1478.49	42.901	12.593
9	1654.98	25.17	23.9	1712.85	1616.4	40.6	11.606
10	3044.74	42.4	5.841	3098.75	2954.08	48.928	3.355
11	3340.82	25.133	24.387	3405.44	3151.79	101.083	22.328
12	3340.82	25.133	24.387	3405.44	3151.79	101.073	22.328

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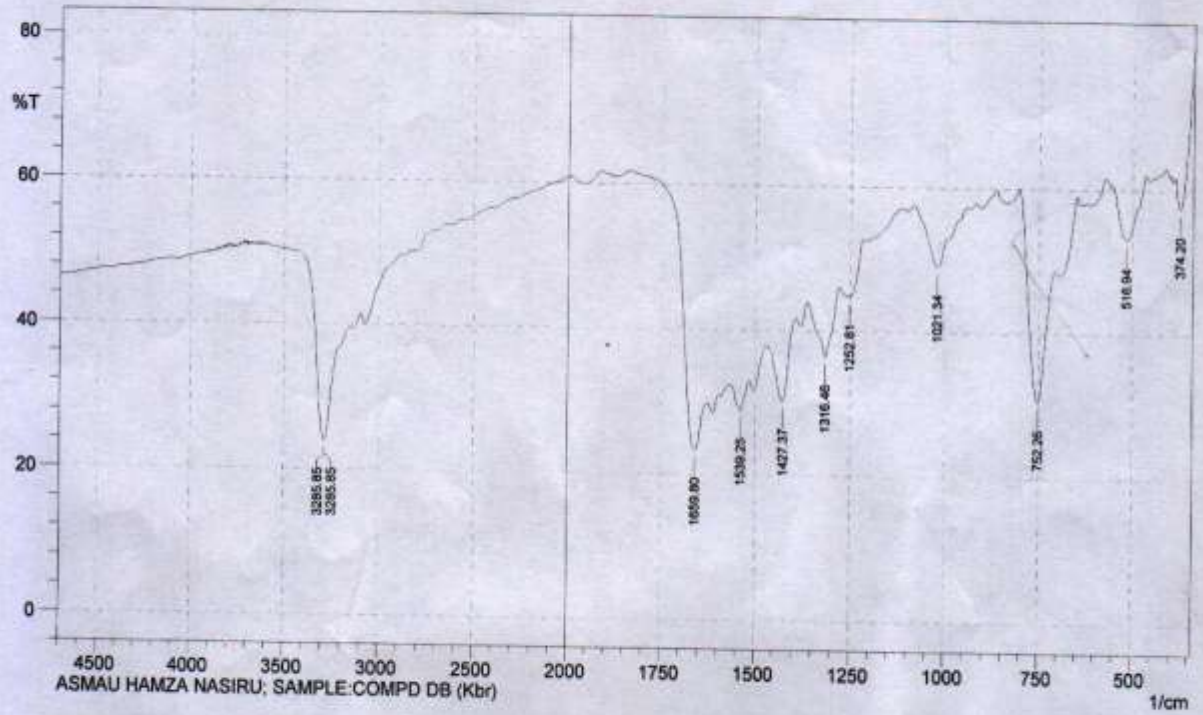
Figure 3.1 FTIR spectrum of N-phenylacetamide

SHIMADZU

FTIR ANALYSIS RESULT
NARICT,ZARIA



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	374.2	57.44	9.62	387.7	339.48	9.436	2.122
2	516.94	53.13	5.68	555.52	488.97	16.901	1.673
3	752.26	30.615	23.246	805.31	707.9	36.972	10.959
4	1021.34	49.053	8.393	1084.03	947.08	37.198	4.216
5	1252.81	44.827	4.275	1279.81	1219.05	20.134	1.503
6	1316.46	36.383	8.863	1363.72	1279.81	32.942	3.926
7	1427.37	30.338	9.629	1469.81	1393.62	34.56	3.99
8	1539.25	28.944	3.752	1563.36	1517.06	23.746	1.261
9	1659.8	23.331	14.388	1752.39	1627.01	51.534	4.699
10	3285.85	23.894	20.583	3432.44	3154.68	120.582	23.051
11	3285.85	23.894	20.586	3433.41	3153.72	121.211	22.757

Comment;
ASMAU HAMZA NASIRU; SAMPLE:COMPD DB (Kbr)

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No. of Scans;
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Figure 3.2 FTIR spectrum of N-phenylbenzamide

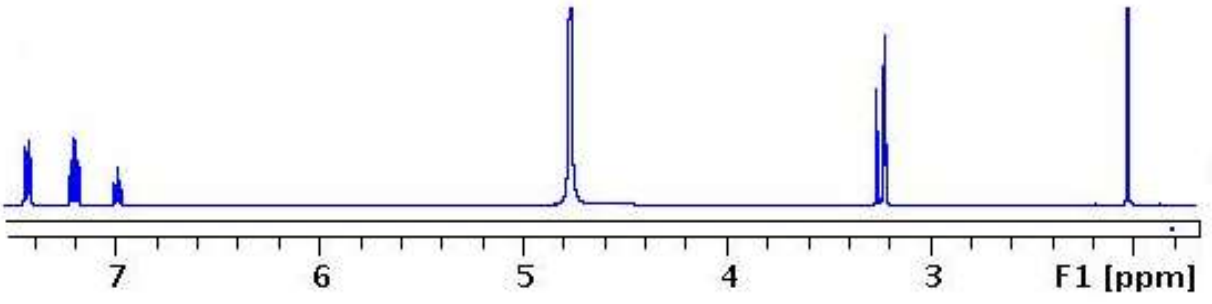

Figure 3.3 ^{1}H spectrum of N-phenylacetamide

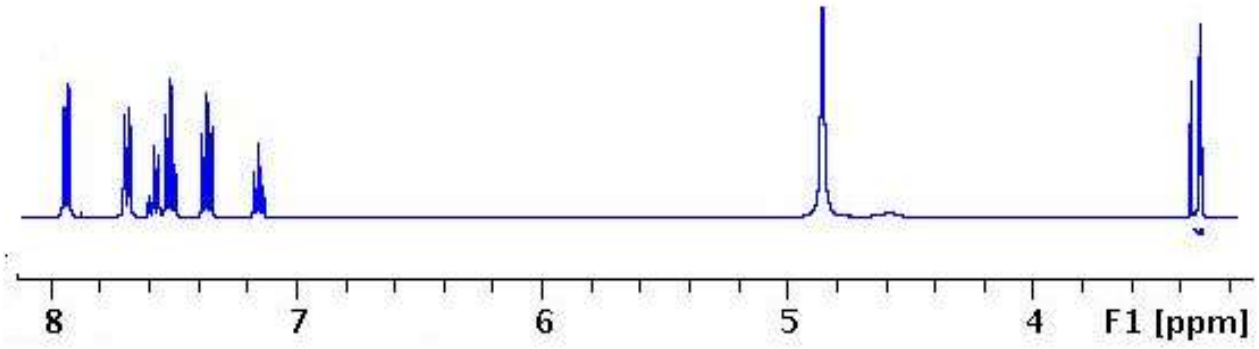

Figure 3.4 ^{1}H spectrum of N-phenylbenzamide

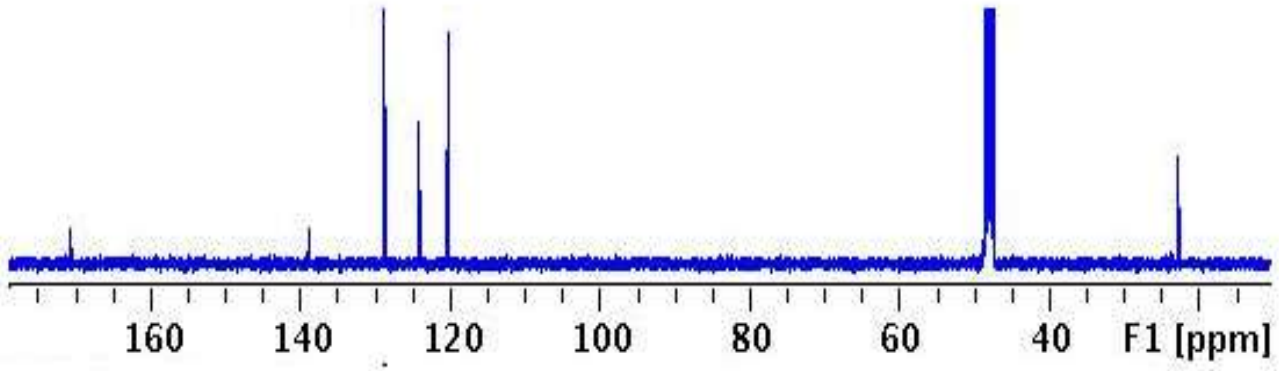

Figure 3.5 ^{13}C spectrum of N-phenylacetamide

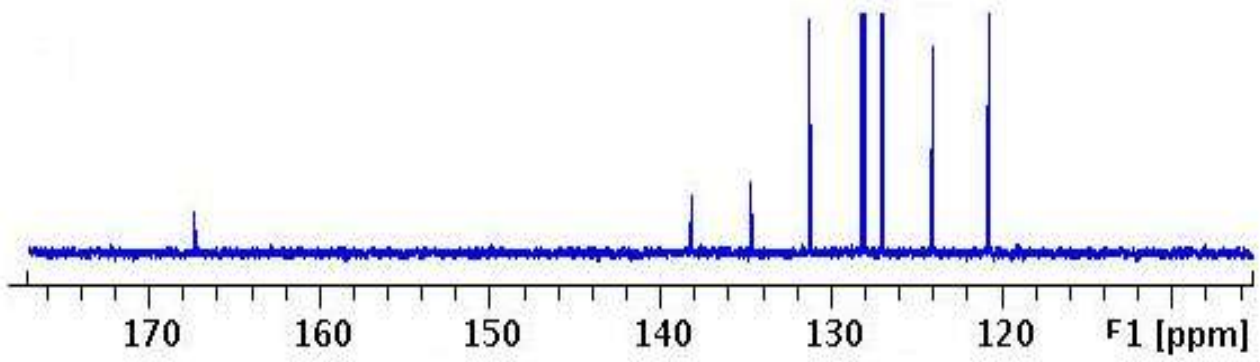

Figure 3.6 ^{13}C spectrum of N-phenylbenzamide

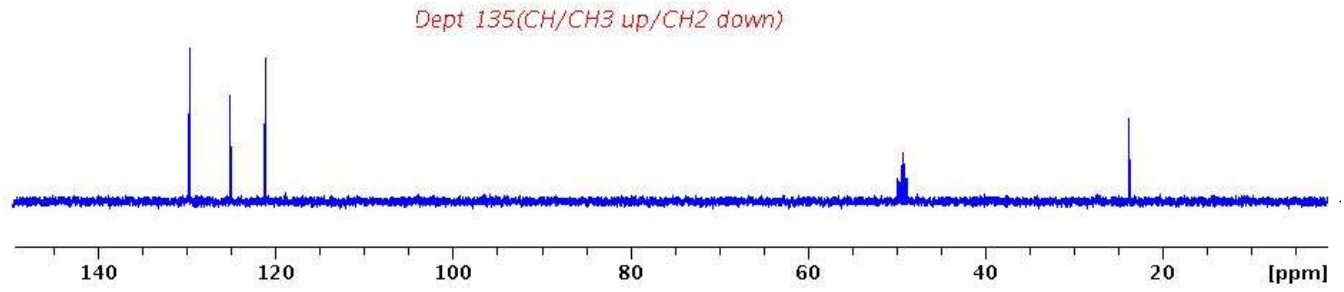

Figure 3.7 DEPT spectrum of N-phenylacetamide (CH_3 and CH up; CH_2 down)

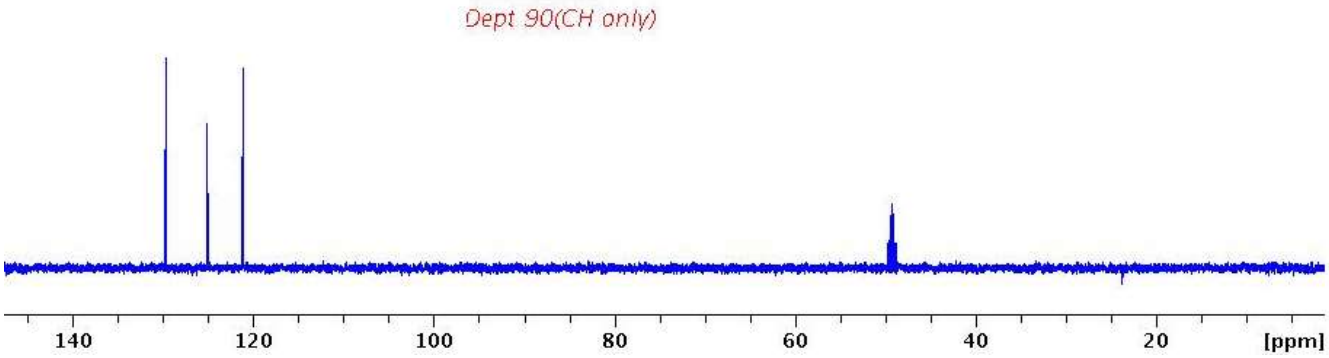

Figure 3.8 DEPT spectrum of N-phenylacetamide (CH only)

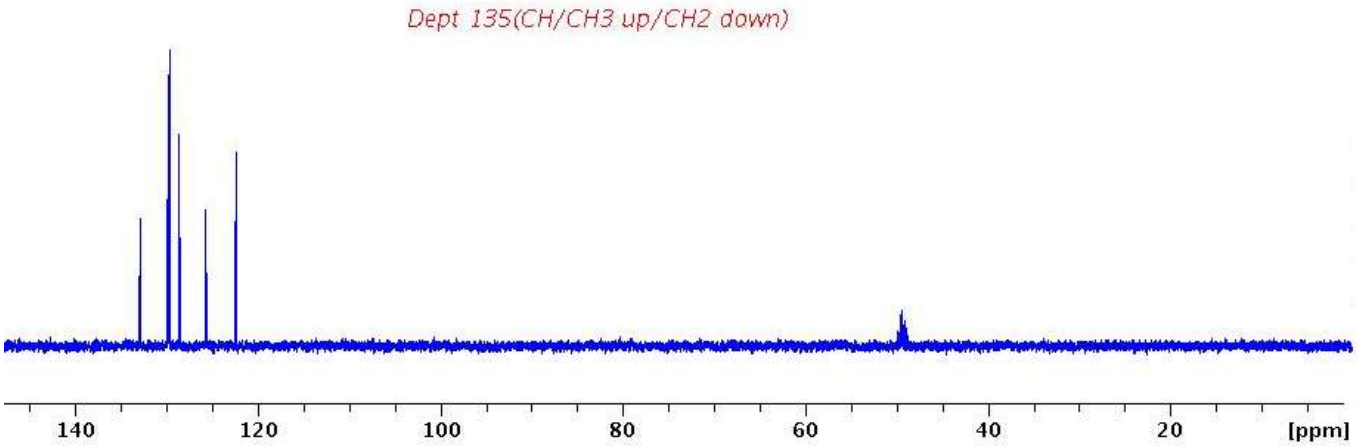

Figure 3.9 DEPT spectrum of N-phenylbenzamide (CH_3 and CH up; CH_2 down)

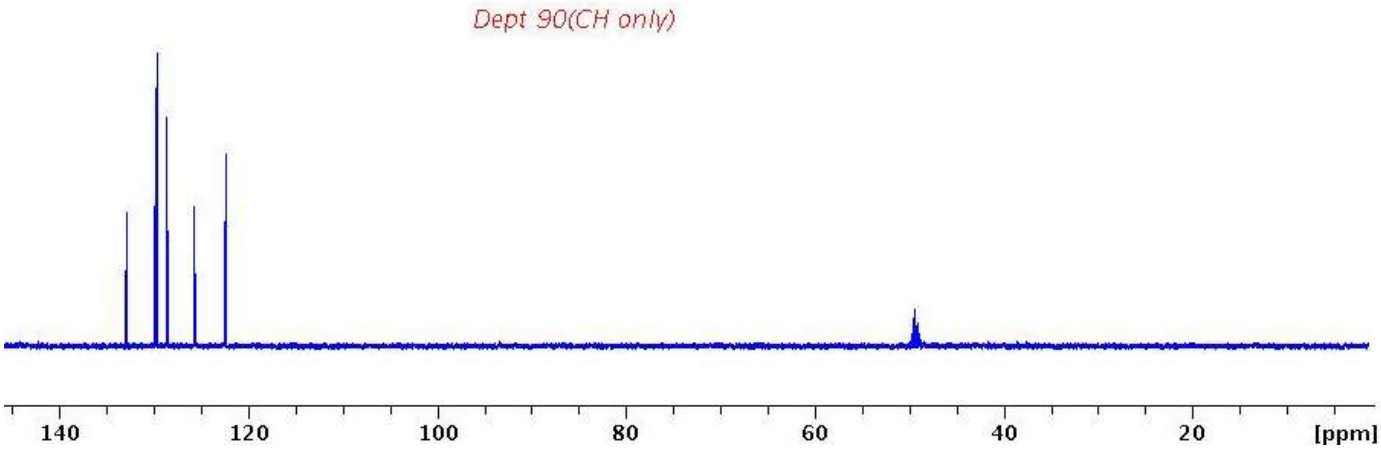

Figure 3.10 DEPT spectrum of N-phenylbenzamide (CH only)

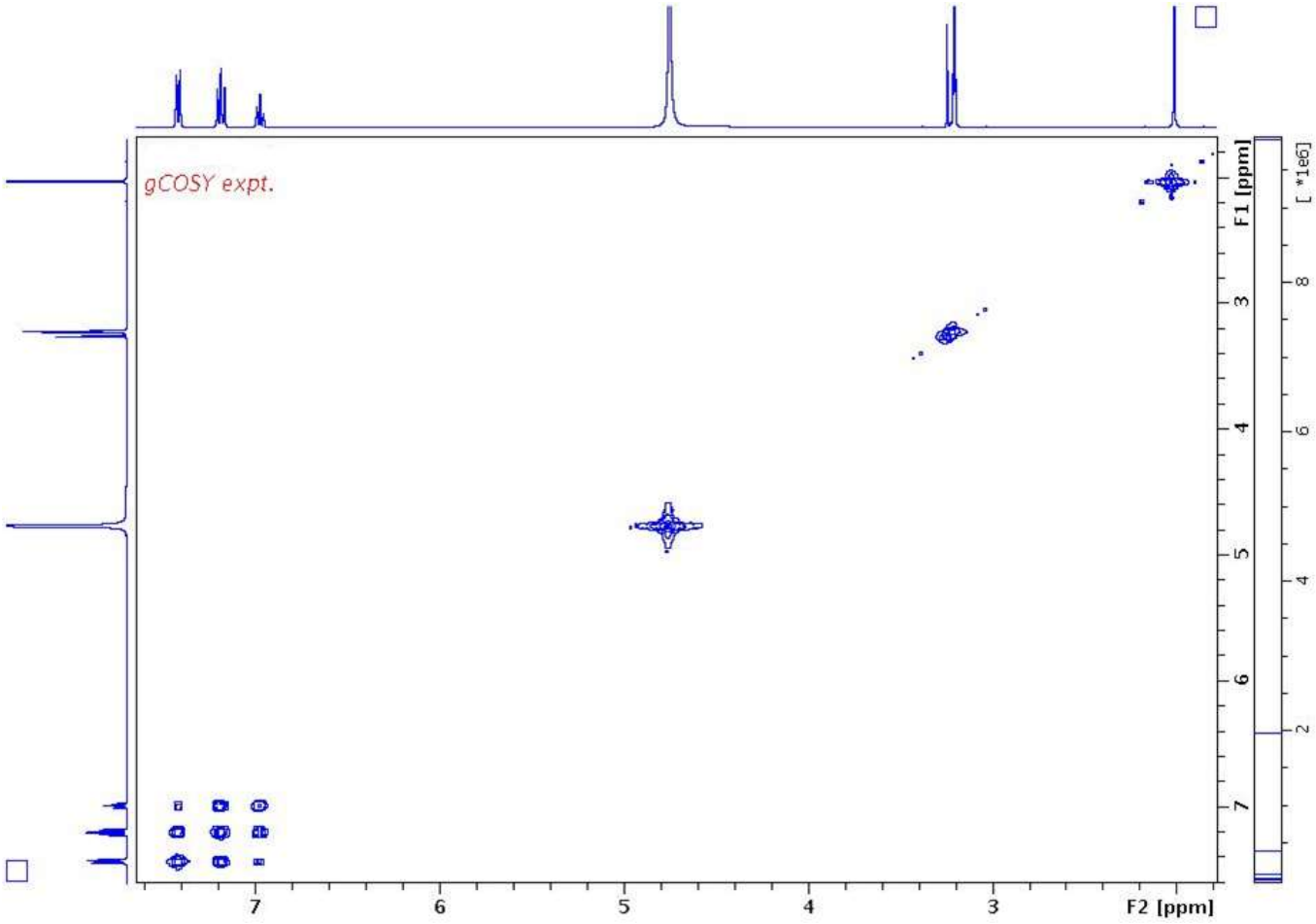

Figure 3.11 COSY spectrum of N-phenylacetamide

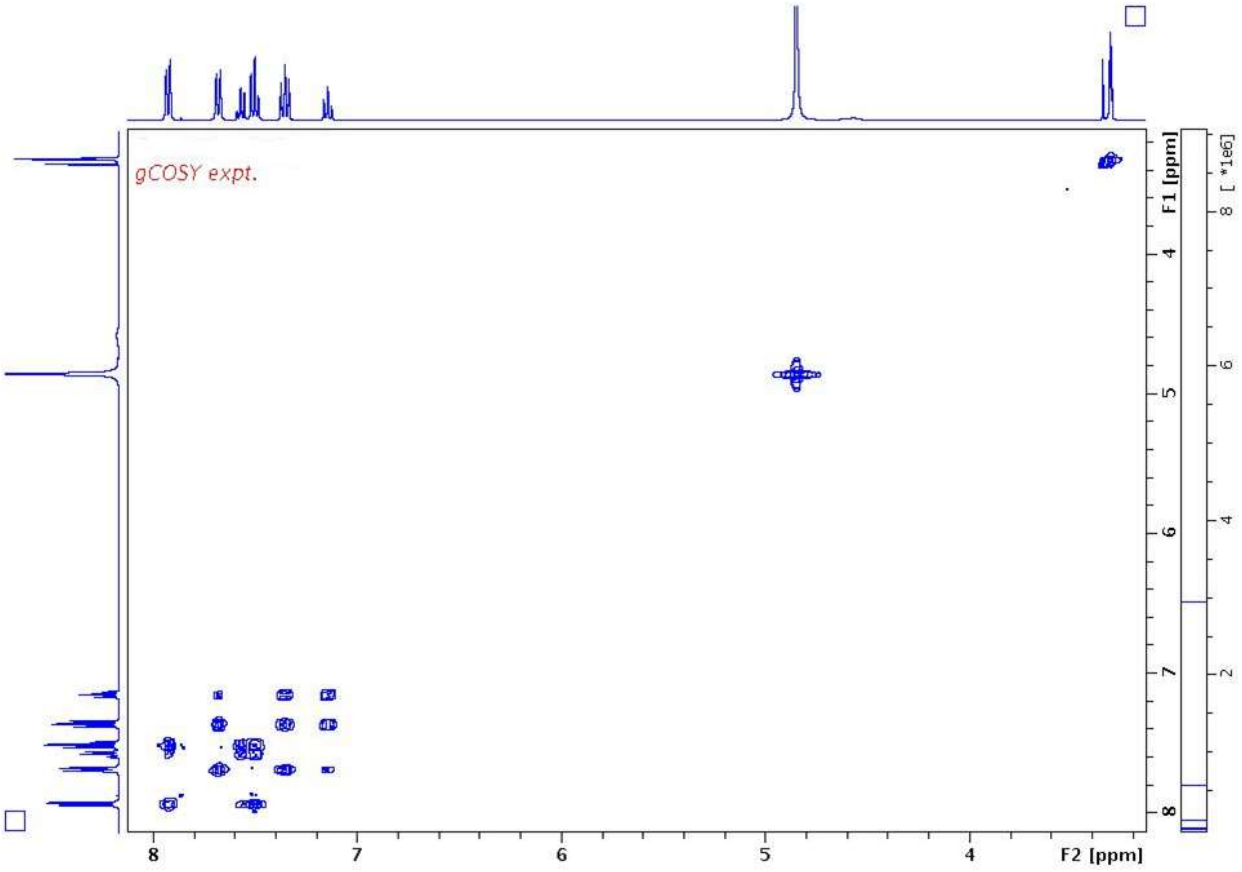

Figure 3.12 COSY spectrum of N-phenylbenzamide

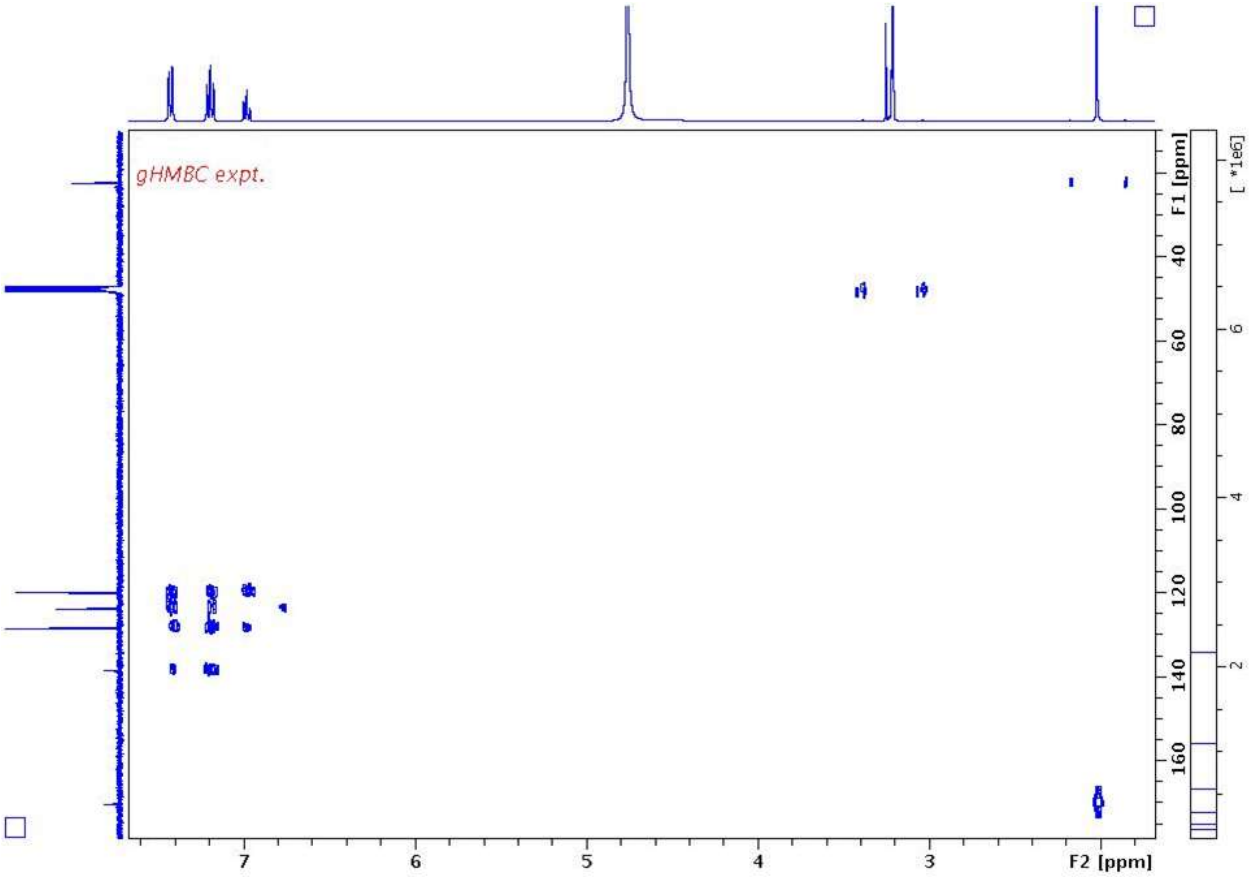

Figure 3.13 HETCOR spectrum of N-phenylacetamide

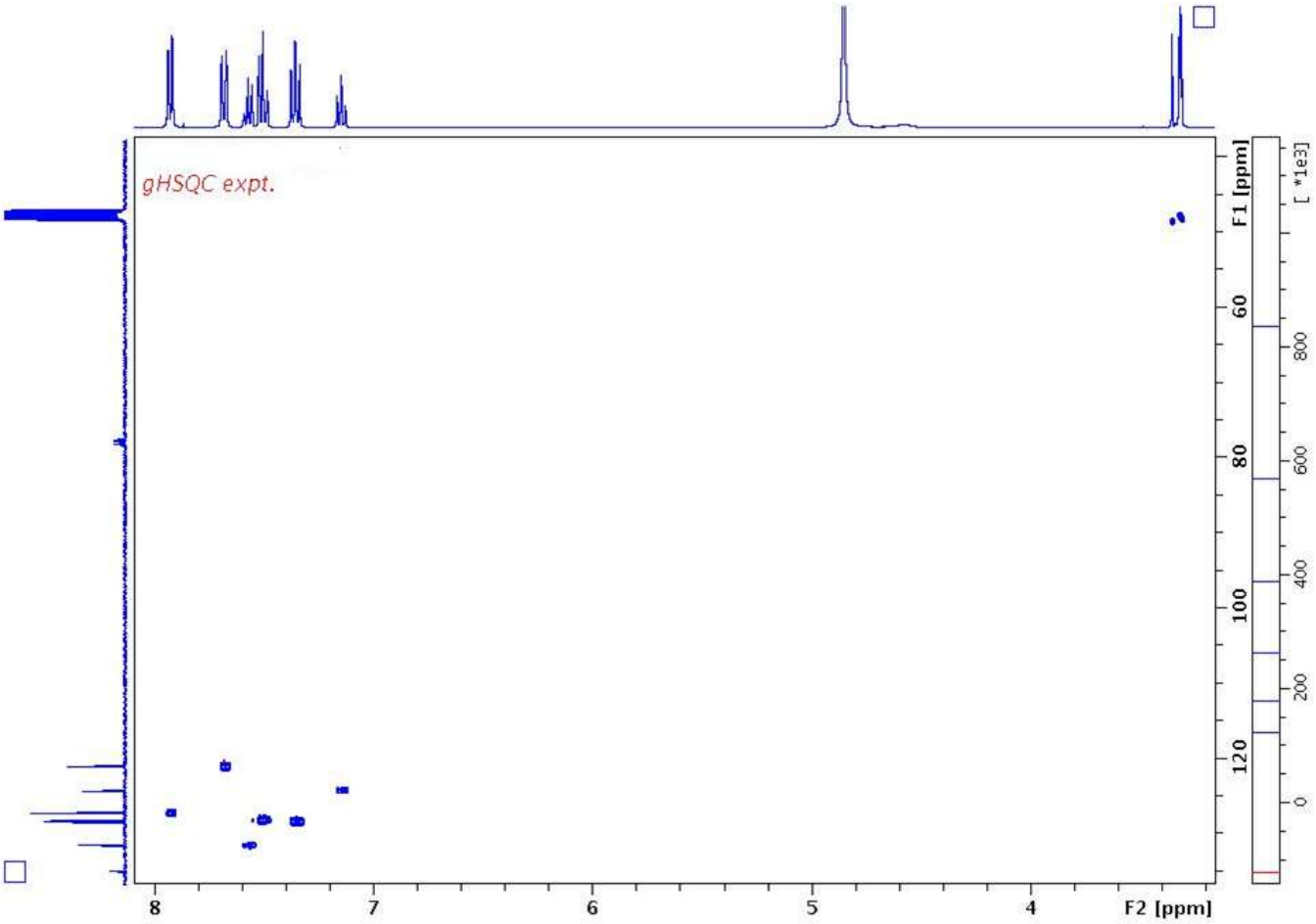

Figure 3.14 HETCOR spectrum of N-phenylbenzamide

CHAPTER FIVE

DISCUSSION

5.1 Synthesis and Characterization

As seen in Table 4.1, the modified procedure for the synthesis of N-phenylacetamide and N-phenylbenzamide furnish very high yields (up to 97 %). Thin layer chromatography (TLC) with silica gel was used to monitor the progress of the reaction. The best solvent system for TLC was found by trial and error. A mixture of ethylacetate and chloroform in 1:2 ratio was found to be the best TLC solvent for monitoring the reaction. For both N-phenylacetamide and N-phenylbenzamide, completion of the reaction was determined by the disappearance of reactant spots in the thin layer chromatogram and appearance of a single product spot. The very narrow range of the uncorrected melting points of the two synthesized recrystallized compounds indicates their high purity.

Detailed structural analysis of N-phenylacetamide and N-phenylbenzamide was performed using Fourier Transformed Infrared Spectroscopy (FTIR)and various types of Nuclear Magnetic Resonance (NMR). The latter includes: proton (^{1}H) NMR, carbon-13 (^{13}C) NMR, Correlated SpectroscopY (COSY) and HETeronuclear Chemical shift CORrelation (HETCOR). Table 4.2 summarizes the analysis of the FTIR data. The ^{1}H and ^{13}C NMR data are analysed in Table 4.3 and 4.4.

Only the functional group region (with wavenumbers > 1500 cm^{-1}) of the FTIR spectra were analysed because the finger print region (with wavenumbers < 1500 cm^{-1}) is very complicated with many bands to the extent that unambiguous analysis of the finger print region requires special skills that are beyond the scope of this work. The FTIR data confirms the presence of the aromatic and secondary amide functional groups in both N-phenylacetamide and N-phenylbenzamide , as evidenced by the appearance of prominent bands at 3285-3341 cm^{-1} (N-H stretching vibrations), 3040-3080 cm^{-1} (aromatic C_{sp2}-H stretching vibrations), 1654-1660 cm^{-1} (C=O stretching vibrations, often referred to as amide band l), 1516-1540 cm^{-1} (N-H deformation vibration, often referred to as amide band ll). The N-H stretching bands of N-phenylacetamide and N-phenylbenzamide at 3285-3341 cm^{-1} appear as singlets, indicating that N- phenylacetamide and N-phenylbenzamide are secondary amides. Analysis of FTIR spectra alone can only provide information about the

functional groups present in a compound, but cannot reliably be used to establish the chemical structure of a compound. Nuclear Magnetic Resonance technique allows one to map out the arrangement of hydrogen, carbon, and other hetero atoms in a given molecule. This permits one to establish the chemical structure of the compound under investigation.

The atom numbering scheme of N- phenylacetamide and N-phenylbenzamide included in Tables 4.3 and 4.4 facilitates the analysis of the ^{1}H and ^{13}C NMR. The analysis was done by comparison with the published ranges of chemical shifts of H and C atoms in various electronic environments. As seen in Tables 4.3 and 4.4, the positions of all H and C atoms in N- phenylacetamide and N-phenylbenzamide have been assigned. The nature of carbon atoms (CH_3-, -CH_2, or –CH) was established by means of the Distortionless Enhancement by Polarization Transfer (DEPT) spectroscopy. The DEPT spectra of N-phenylacetamide and N-phenylbenzamide are presented in Figures 3.7-3.10 In one set of the DEPT spectra (DEPT 135), the signals due to CH_3 and CH are above the baseline; while, the signals due to CH_2 are below the baseline. In the second set of the DEPT spectra (DEPT 90), only the CH signals appeared. The low resolution of the NMR spectrometer does not permit determination of coupling constants which may provide further support for the structural analysis. However, the two dimensional (COSY and HETCOR) NMR spectroscopic data shown in Figures 3.11-3.14 confirm the tentative structural assignment that was made using ^{1}H and ^{13}C NMR. COSY correlates two identical ^{1}H NMR spectra; whereas, HETCOR correlates ^{1}H NMR spectrum and ^{13}C NMR spectrum of the same molecule. The connection between each carbon atom and the attached hydrogen atom (atoms) is unambiguously established using HETCOR as seen in Tables 4.3 and 4.4.

5.2 Preliminary Anticonvulsant Screening

The synthesized compounds were tested in vivo to ascertain their anticonvulsant activity. Both MES and scPTZ test models for anticonvulsant activity were employed. The results obtained are summarized in Tables 4.5-4.7 . The preliminary screening was done at the dosage levels of: 600, 300, and 150 $mgkg^{-1}$ body weight and 300, 150, and 75 $mgkg^{-1}$ body weight administered i.p for N- phenylacetamide and N-phenylbenzamide, respectively. The respective doses were selected from their LD_{50} data as shown in Table 4.7.

N-phenylacetamide exhibits anti MES activity at a dose levels of 300, 150, and 75 $mgkg^{-1}$ body weight, in which there is 100 % protection of the animals. This observation indicates the ability of N-phenylacetamide to prevent seizure spread. The preliminary anti MES activity of N-phenylacetamide is comparable to that of phenytoin – a commonly prescribed AED that is utilized at a dose of 20 $mgkg^{-1}$ body weight. The inhibitory effect of N-phenylacetamide (100 % protection at a dose of 75 $mgkg^{-1}$ i.p) is an indicator of its anticonvulsant activity and its potential for the treatment of generalized tonic-clonic and complex partial seizures.

scPTZ test model identifies compounds that elevate seizure threshold. N-phenylacetamide also exhibits anticonvulsant activity in the preliminary scPTZ screening at the three doses tested: 300, 150, and 75 $mgkg^{-1}$ body weight. 83.33 % of the animals were protected at a dose of 300 $mgkg^{-1}$, which is comparable to valproate at a dose of 200 $mgkg^{-1}$. 50 % and 66 % of the animals were protected at a dose of 150 $mgkg^{-1}$ and 75 $mgkg^{-1}$, respectively. There is also a significant ($p>0.05$) increase in latency in mean onset of seizure for the animals that were not protected at the doses of 300 $mgkg^{-1}$ and 150 $mgkg^{-1}$ bodyweight. Thus, the preliminary profile of N-phenylacetamide is characterized by its high ability to modify the maximal electroshock seizure pattern and elevate the PTZ seizure threshold.

When compared with N- phenylacetamide and N-phenylbenzamide exhibits a weak anticonvulsant activity against scPTZ induced seizure in mice. Only 33 % of the animals were protected at the three doses tested: 600, 300, and 150 $mgkg^{-1}$ bodyweight. But there is no significant ($p<0.05$) increase in mean onset of convulsion in all the three doses. In the MES test, anticonvulsant activity was found to be almost absent in N-phenylbenzamide. The maximum protection is only 20 % at a dose of 20 $mgkg^{-1}$. Moreover, there is no statistically significant ($P>0.05$) difference between the control group and the three doses tested for the mean time of recovery. Therefore, the usefulness of this compound for anticonvulsant activity is not established for both generalized tonic-clonic, partial and absence seizure.

Although, N- phenylacetamide and N-phenylbenzamide have similar pharmacophoric unit (N-phenylformamide), their pharmacological profiles are pretty much different. Both N-phenylacetamide and n-phenylbenzamide fall into Codding's postulated pharmacophore because they both contain amide and aryl groups (Codding *et al,* 1989). N-phenylbenzamide has a bulky hydrophobic substituent (phenyl group); whereas, N-phenylacetamide has a

small hydrophobic substituent (methyl group). According to the proposed pharmacophore models of Unverferth (1998) and Shindikar (2006), N-phenylbenzamide would display better activity profile because the distance between the centre of aryl substituent and the amide hydrogen bond acceptor/donor unit (Figure 1.4) falls into the proposed range of 3.2- 5.1 Å for anticonvulsants containing aryl ring, electron donor and hydrogen bonding unit. All pharmacophore anticonvulsant models emphasize the important effect of hydrogen bonding on anticonvulsant activity. The higher activity of N-phenylacetamide relative to that of N-phenylbenzamide can be attributed to their optimized structures shown in Figure 1.4. In both molecules, the phenyl ring that is bonded to nitrogen atom is coplanar with the amide group. The methyl group of N-phenylacetamide is nearly coplanar (lies on the same plane) with the phenyl and amide groups. The torsion angle of the methyl group is 177.1° ; full coplanarity requires a torsion angle of 180°. On the other hand, the phenyl substituent group of N-phenylbenzamide is significantly out of the plane of the phenyl and amide groups with a torsion angle of 46.6°. Thus, N-phenylacetamide is essentially a planar molecule; N-phenylbenzamide is non planar. The non planarity of N-phenylbenzamide is caused by the bulkiness of the phenyl substituent group. It can be suggested that the relatively low anticonvulsant activity of N-phenylbenzamide is caused by its non planarity.

The preliminary results of anticonvulsant screening of N- phenylacetamide and N-phenylbenzamide presented in this thesis, do not allow for a clear cut conclusions concerning the structure-activity relationship. However, the good profile of anticonvulsant activity (anti MES and anti sc PTz) observed in N-phenylacetamide would trigger more anticonvulsant research on this simple amide.

SUMMARY

This work presents the first synthesis and anticonvulsant screening of alkyl and aryl substituted N-phenylformamide. The investigated compounds (N-phenylacetamide and N-phenylbenzamide) fall into the Codding's postulated pharmacophore models, having an electron donor (-C=O) and/or hydrogen bonding donor (-NH-) sites and a phenyl ring in a linear arrangement. Theoretical calculations show that the minimum energy structure of N-phenylacetamide is planar; i.e. the methyl (CH_3), amide (-NHC=O), and phenyl (C_6H_5-) groups are all lying on the same plane. Whereas, the minimum energy structure of N-

phenylbenzamide is nonplanar; one of the phenyl rings is rotated by 46° from the plane of the amide and the phenyl ring bonded to amide nitrogen.

Examination of the in vivo activity profile of the compounds against electrical and chemical model of convulsion shows that N-phenylacetamide has ability to inhibit the maximum electroshock seizure and elevate the scPTZ seizure threshold. Thus, N-phenylacetamide possess a broad spectrum of activity and can be used for generalized tonic-clonic, partial, and absence seizures. Whereas, N-phenylbenzamide has a weak activity against sc PTz seizure; Hence, its potential for use in the treatment of absence seizure cannot be ascertain.

Although the two compounds were designed for sodium ion blockage activity; the results turned out to be different, this is because the animal models for AEDs evaluation are unbiased with respect to mechanism of action.

N-phenylbenzamide which was expected to give better activity against electrically induced seizure, shows insignificant activity ($p<0.05$). However, N-phenylbenzamide has little activity against sc PTz induced seizure. N-phenylacetamide exhibits a broad spectrum of activity against both MES and scPTZ seizure tests. N-phenylacetamide is as active as phenytoin and valproate at a minimum dose of 75 $mgkg^{-1}$ in scPTz and MES seizure test, respectively. This may not be a surprise because the mechanism of action of most available AEDs is either unknown or involve multiple interaction with the receptors (Aaron and Jeffery, 2010). Despite the fact that N-phenylacetamide had a major drawback when it was used as an analgesic, this work shows that N-phenylacetamide is a potential anticonvulsant. Further optimization might yield a potent and safer N-phenylacetamide based anticonvulsant.

The search for new anticonvulsants continues perhaps until the pathophysiology of epilepsy, the composition of ion channels, and a standard pharmacophore are fully established.

CONCLUSION

1. N- phenylacetamide and N-phenylbenzamide were synthesized in excellent yields of about 97 % via nucleophilic addition reaction, and tested for anticonvulsant activity.
2. N-phenylacetamide has shown a good activity against both sc PTz and MES seizure tests.
3. N-phenylbenzamide has shown little activity against sc PTz seizure test.
4. N- phenylacetamide and N-phenylbenzamide fit to the Coddling's postutaled pharmacophore model. Alkyl substituted N-phenylformamide (N-phenylacetamide) possess a broader spectrum of anticonvulsant activity than aryl substituted N-phenylformamide (N-phenylbenzamide).

RECOMMENDATION

Further pharmacological (both in vivo and in vitro) investigations and optimisation should be carry out on N-phenylacetamide in order to make it usable for treating epilepsy . Neurotoxicity studies should also be carried out to determine its protective index.

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