SYNTHESIS AND ANTICONVULSANT STUDIES OF 3-ANILINOPROPANAMIDES AND THEIR N-BENZYL DERIVATIVES

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AHMADU BELLO UNIVERSITY, ZARIA

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TITLE PAGE

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BY

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DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

MARCH, 2008

DECLARATION

I declare that the work in the dissertation entitled Synthesis and Anticonvulsant Studies of 3-Anilinopropanamides and their N-Benzyl Derivatives has been performed by me in the Department of Pharmaceutical and Medicinal Chemistry, under the supervision of Prof. A. K. Haruna and Prof. Magaji Garba. 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.

Idris, Abdullahi March, 2008

Idris Abdullahi Yunusa	**Signature**	**Date**

CERTIFICATION

This dissertation Entitled Synthesis and Anticonvulsant Studies of 3-anilinopropanamides and their N-Benzyl derivatives by Idris, Abdullahi Yunusa meets the regulation governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Abstract

The 3-ANPs (A01-A10) were prepared through acid catalysed Michael addition while the N-benzyl derivatives (A11-A15) were obtained through an uncatalysed amine exchange reaction with benzylamine. The structures of these compounds were established using various spectroscopic techniques. Compounds (A01-A15) were screened in mice against maximal electroshock (MES) and subcutaneous pentylene tetrazole (scPTZ) seizure models as well as the righting reflex test for neurological deficit. All the 15 compounds were active in the MES test with A04, A05, A07 and A13 being more potent than phenytoin. Compounds A05, A06 and A11-A15 were active in both MES and scPTZ seizures models and therefore have valproate-like activity. Compound A04 is the most potent (ED_{50} = 0.63mg/Kg) anti-MES compound ever reported in the literature while compound A13 is the most potent (MES ED_{50} = 3.25mg/Kg and scPTZ ED_{50} = 20.40mg/Kg) in the valproate class. The results of this work agrees with Unverferth et al (1998) and Shindikar et al (2006) proposed pharmacophores for anticonvulsants active at the voltage-dependent sodium channels. The compounds mentioned above are recommended for further studies.

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ABBREVIATIONS

3-ANP	=	3-anilinopropanamide
NBDANP	=	N-benzyl Derivatives of the 3-anilinopropanamide
IR	=	Infrared
^{1}HNMR	=	Proton Nuclear Magnetic Resonance
^{13}CNMR	=	Carbon-13 Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
GABA	=	Gamma Aminobutyric Acid
HETCOR	=	Heteronuclear Chemical Shift Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
ED_{50}	=	Median Effective dose
TD_{50}	=	Median toxic dose
PI	=	Protective index
MES	=	Maximum Electroshock
ScMET	=	Subcutaneous Metrazole
ScPTZ	=	Subcutaneous Pentylene Tetrazole
IP	=	Intraperitoneal
mg	=	Milligramme
kg	=	Kilogramme
g	=	Gramme
ml	=	Milliliter
TLC	=	Thin Layer Chromatograghy

CHAPTER ONE

1.0 INTRODUCTION

Epilepsy is the most common serious neurologic disorder (Scott *et al*., 2001; Shindikar, 2006) and one of the world's most prevalent non-communicable diseases (Scott *et al.,* 2001). At least 50 million people suffer from epilepsy worldwide (Coleman *et al*., 2002) of which 10.5 million are children (Guerrini, 2006). Over 80 percent of the cases are from the developing world (Scott, *et al*., 2001); about 10 million from Africa (Coleman *et al*., 2002). The age-adjusted incidence of epilepsy is 40-70 and 100-190 per 100,000 populations in developed and developing countries respectively (Pond *et al*, 1960; Placentia *et al*, 1992a and Hauser *et al.,* 1993). However, the prevalence of active epilepsy (4-10 per 1,000 populations) is remarkably uniform in most countries of the world, although higher prevalence rates (14-57 per 1,000 populations) have been reported from African and some South American countries (Hauser and Annegers, 1993; Sander *et al*., 1999). In Nigeria the prevalence of active epilepsy is 3-14 per 1,000 populations (fig 1.1and appendix 1)

Epilepsy is a disorder in which the balance between cerebral excitation and inhibition is tipped towards uncontrolled excitability. Despite their different nature and causes, the common feature of recurrent seizure attacks characterizes them all. Seizures are due to abrupt paroxysmal discharge of a large population of neurons firing synchronously and are divided into two major categories depending on whether the initial discharge arises within a system of neurons localized in a discrete area of the cerebral grey matter (partial or focal seizures)

or diffusely in both hemispheres (generalized seizures). Generalised seizures may be convulsive (tonic, myoclonic, tonic-clonic depending on the characteristics of the muscle contration) or non-convulsive, as the case of the so-called petit mal, where the paroxysmal discharge can be accompanied by only a suspension of consciousness without motor phenomena. Untreated epilepsy can lead to impaired intellectual function or death and is typically accompanied with psychosocial prejudices and other psycho pathological consequences such as loss of self-esteem and poor quality of life (Shindikar *et al*., 2006).

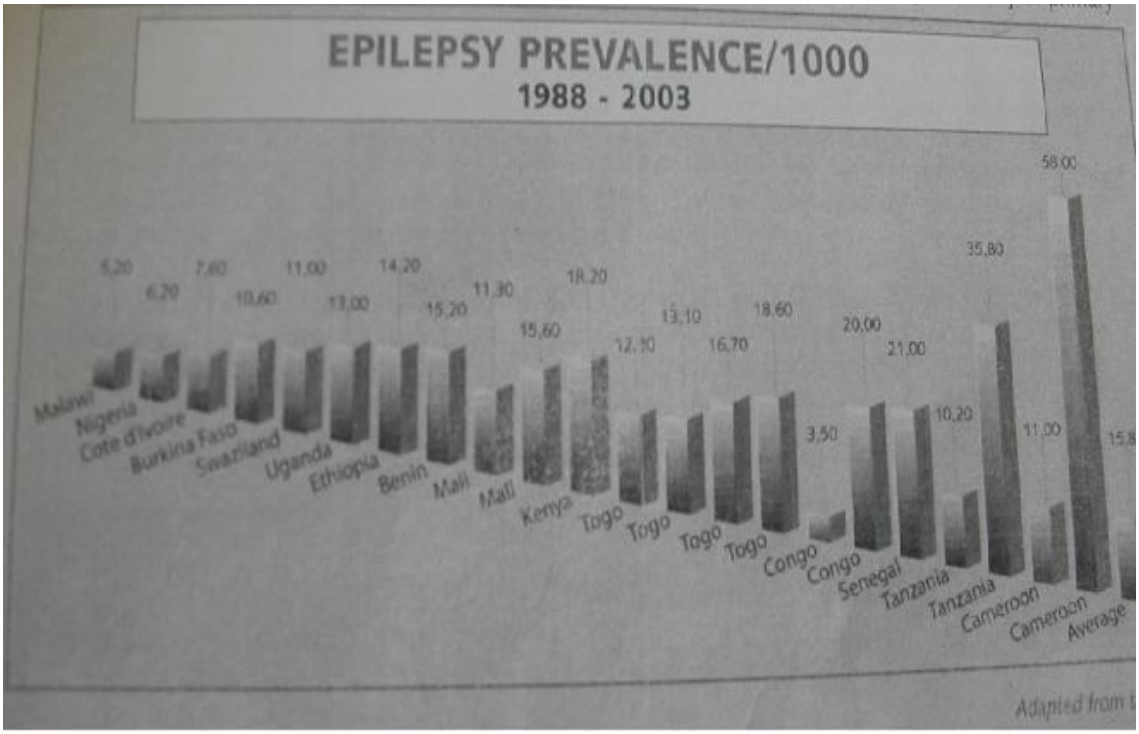


FIGURE 1.1: SHOWING THE EPILEPSY PREVALENCE/1000

Since the introduction of Phenobarbital (luminal) by Hauptman in 1912 as an effective anticonvulsant agent a number of antiepileptic drugs (AEDs) have been introduced into the market. The first generation AEDs (Phenytoin,

carbamazepine, valproate, Phenobarbital, primidone, ethosuximide and benzo-diazepines) are capable of preventing seizure generation irrespective of the underlying etiology. Moreover, advances in the understanding of the biological causes of epilepsy over the last three decades have allowed the development of the second generation AEDs (oxcarbazepin, Gabapentin, lamotrigine, felbamate, Tiagabin, Vigabatrin, Topiramate, Levetiracetam, and Zonisamide) that target the cellular mechanism that is responsible for epileptic discharges. With optimal use of both generations of AEDS complete seizure control can be achieved in 60-70 per cent of the patients (Kwan and Brodie, 2000; Sander and Perucca, 2003).

The field of AED research is very dynamic. Numerous compounds of varying chemical classes are reported each year as possessing anticonvulsant activity. One of such class of compounds is the carbonxamides, which include several Benzamides (Bruce and Lynes, 1962), Ethanamides (Johnson *et al*., 1962, Carraz *et al*, 1964) and Piperidine carboxamides (Haj-Yahia and Bialer, 1989; Bialer *et al*, 1996; Ho et al, 2001). In particular, the anilide function yields potent anticonvulsant compounds such as Ameltolide (Clark *et al*, 1986), Ralitoline (Leppik, 1994), D2916 (Lepage *et al*, 1992), certain N-phenylpthalim-ides (Bailleux *et al*, 1995; Vamecq *et al*, 2000), N-(2, 6-dimethylphyenyl)-2-piperidin carboxamides (Ho ***et al,*** 1998) and w-(IH-I-imidazolyl)-N-phenylalkanoic acid amide derivatives (Akturk ***et al,*** 2002). Most of these compounds exhibit phenytoin-like anticonvulsant profile (i.e, 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. The anticonvulsant properties of the anilinopropanamides (which are a group of carboxamides) have not been reported in the literature. In this study, a series of analogues of anilinopropanamides and their N-benzyl derivatives are prepared and screened for anticonvulsant activity.

1.1 THEORETICAL FRAME WORK

The typical behavioural manifestation common to all forms of epilepsy is the aberrant synchronized discharge of a neuronal population termed the 'seizures'. Seizures result from phasic changes in the firing properties of groups of neurons, usually within a descrete focal point, to an intermittent high-frequency burst-firing mode resulting in synchronized disruptive 'electrical' discharges owing to changes in neuronal excitability. The first determinant of neuronal excitability in any given neuron is the balance of synaptic input from neighbouring cells within the network in the form of chemical transmission (through ligand gated ion channels and G-protein coupled metabotropic receptors) and aplastic but extremely rapid electrical transmission (through gap junctions). Secondly and perhaps most importantly are the electronic properties intrinsic to each neuron. These are determined to a great extent by the complement and localization of functional voltage gated ion channels expressed in the cell membrane (Traub *et al,* 1996). It is intuitive, therefore, that these determinants of physiological excitability would be crucially involved in the pathological hyperexcitability occurring during the seizure discharges characteristic of the epilepsies. Indeed both excitatory and inhibitory neurotransmission as well as voltage gated

channels (that can be modulated selectively by natural or synthetic toxins), have been shown to be crucial for seizure-like activity in acute chemoconvulsant models in rodent brain slices and in vivo (Jefferys, 2003). It is not surprising, therefore that many of the drugs used in the treatment of seizures and epilepsy target the channels and receptors that are vital to the initiation and spread of seizure activity.

The blockade of sustained repetitive firing (SRF) of Na-dependent action potential is one of the basic mechanisms of action of currently available AEDs including phenytoin, carbamazepine, lamotrigine, topiramate, zonisamide, vaproate, oxcarbazepine, zonisamide and felbamate (Meldrum, 1996). The firing of an action potential by an axon is accomplished through sodium channels each of which dynamically exists in 3 states viz: a resting state during which the channel allows passage of sodium into the cell, an active state in which the channel allows increased influx of sodium into the cell and an inactive state in which the channel does not allow passage of sodium into the cell.

During an action potential, these channels exist in the active state and allow influx of sodium ions. Once the activation or stimulus is terminated, a percentage of these sodium channels become inactive for a period of time known as the refractory period. With constant stimulus or rapid firing, many of these channels exist in the inactive state, rendering the axon incapable of propagating the action potential. AED^{s} that target these sodium channels prevent the return of these

channels to the active state by stabilizing the inactive form of these channels. In doing so repetitive firing of the axons is selectively prevented.

Several attempts have been made to define the structural requirement for anticonvulsants acting at the voltage sensitive sodium channels. Brouillette and co-workers (Brouillette *et al,* 1990, Broullette *et al*., 1994, Broullette *et al*., 1998) investigated the sodium channel blocking activity of several mono and bicyclic phenytoin analogues and concluded that high log P values, a free amide group, and a specific aromatic ring orientation are optimal for high binding affinity to the sodium channel. Although these criteria were well fulfilled in monocyclic hydantoins, the conclusions of these studies were not applicable to other substance classes acting at the same receptor site.

More recently, Unverferth *et al* (1998) employed five well-known but structurally different anticonvulsants with sodium channel blockade activity [carbamazepine (XXIII), phenytoin (IV), lamotrigine (XLVI), Zonisamide (XXXIII), and Rufinamide (LXXV) to propose a 3-point pharmacophore for sodium blockade activity. The model comprises an electron donor D in relatively limited distance ranges of 3.2-5.1 A^o to and aryl ring or other hydrophobic units R and of 3.9-5.5 A^o to a hydrogen bond acceptor/donor unit. The distance between R and HAD spans a wider range of 4.2-8.5 A^o (fig 1.2). The hydrophobic unit R is not necessarily oriented in the same plane like the other essential elements.

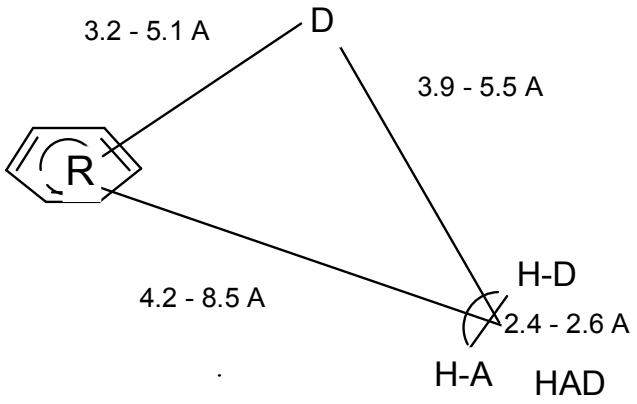


Figure 1.2: Unverferth *et al* (1998) proposed Pharmacophore

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

Most recently Shindikar *et al* (2006) proposed a two-point pharmacophore (fig. 1.3), consisting of an aromatic center separated from an H-Bonding acceptor/donor group within a distance range of 4.18-6.88 A^o, for anticonvulsants active at the sodium chanels. This pharmacophore agrees with Unverferth's proposal.

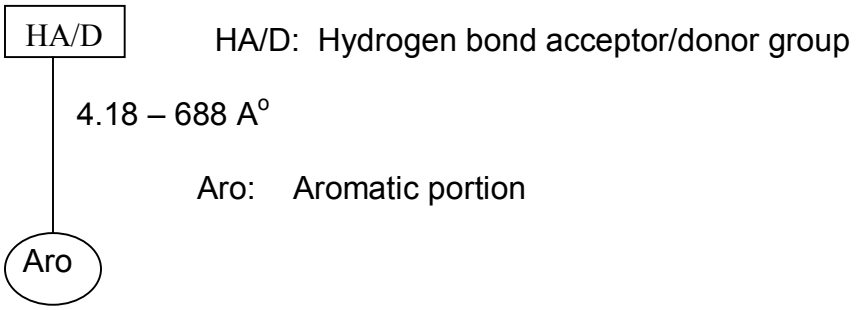
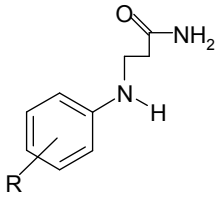
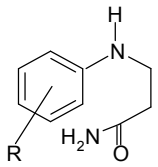


Figure 1.3: Shindikar *et al* (2006) proposed Pharmacophore for anticonvulsant activity.

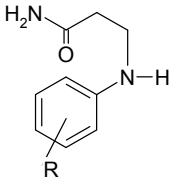
The 3-anilino propanamides (I) are carboxamides that consist essentially of an aryl ring, an electron donor atom (N) that is separated from a hydrogen bond acceptor/donor group (*–$CONH_2$)* by (just) two carbon atoms. The propanamide wing of the molecule is flexible and can assume different orientations (1a, 1b and 1c etc.) relative to the anilino moiety. R group may be an alkyl, aryl, alkoxy or a halogen group at positions ortho, meta and para to the moiety.



(Ia)

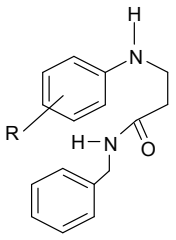


(Ib)

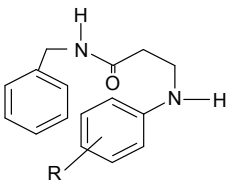


(Ic)

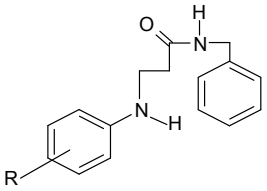
Theoretically, the 3-anilinopropanamides appear to satisfy Unverferth *et al* (1998) and Shindikar *et al* (2006) proposed pharmacophores for compounds acting at voltage sensitive sodium channels. In this work analogues of 3-anilinopropan-amides are prepared and screened for anticonvulsant activity using the Maximal Electroshock (MES) and Subcutaneous Pentylene Tetrazole (ScPTZ) seizure test models. Moreover, since alkyl or aryl substitutions on the amido nitrogen of carboxamides increase anticonvulsant potency (Clark *et al,* 1984; Choi *et al*, 1996; Ho *et al*, 2001), N-benzyl derivatives (Id, Ie and If) of the very promising 3-anilinopropanamides are also prepared and assessed.



(Id)



(Ie)



(If)

1.2 RESEARCH HYPOTHESIS

1. The 3-anilinopropanamides yield potent anticonvulsant compounds.
2. N-benzyl substitution on 3-anilinopropanamides increase anticonrulsant potency.

1.3 STATEMENT OF THE PROBLEM

Although non-pharmacological treatment options such as surgery Stefan, 2001), vagus nerve stimulation (Stefan *et al*, 2001), Ketogenic diet (Freeman *et al*, 1998), avoidance therapy (Loiseau, 1997) and, most recently, trigeminal nerve stimulation (DeGiorgio, 2006) are available for epilepsy, pharmacotherapy is still considered the main stay of treatment for most epileptic patients. The primary goal of the treatment is to achieve complete seizure freedom, ideally without adverse events (Kwan and Brodie, 2000; Sander, 2003), reduce morbidity and mortality and improve quality of life (Perucca, 2000). However, pharmacotherpy of epilepsy is confronted with four major problems –

1. About 30-40 percent of epilepsy patients do not respond at all to currently available AEDS (Pavia et al., 1987; Avanzini and Fanceschetti, 2003).
2. Currently available AEDS are associated with toxicity and serious side effects, notably cognitive dysfunction, ataxia, behavioural disorder, hepatotoxicity, gingival hyperplasia megaloblastic anaemia and tetratogenesis. (Theunissen *et al*, 1994; Tomson *et al*, 1997; Zahn *et al*, 1998; Battino *et al*, 2000)
3. Continous medication is necessary even after the seizures have long been suppressed (Sasa, 2006).

4. Available AEDS merely treat the symptoms and not the disorder (Sasa, 2006).

1.4 OBJECTIVES OF THE STUDY

The anticonvulsant potential of the carboxamides are well documented (Bruce and Lynes 1962; Borsey *et al*, 1968; Balsmo *et al*, 1975, 1977; Clark *et al*, 1984-1989; Haj-Yahia and Bialer, 1989; Ho *et al*, 2001; Akturk *et al*, 2006). There are no published reports on the anticonvulsant properties of the 3-anilinopropana-mides (APMs) and their N-alky or N-aryl derivatives. Hypothetically, these compounds appear to fulfil the basic requirements for Unverferth *et al* (1998) and Shidikar *et al* (2006)-proposed pharmacophores for anticonvulsant compounds acting at the voltage-sensitive sodium channels. In this study we are concerned with the synthesis and anticonvulsant screening of some 3-APMs and their N-benzylderivatives (NBDAPMs). Specifically the aim of the study is –:

1. To synthesis of 3-APMs with alkyl-, halo-, and methoxy-, derivatives at *ortho* (o), *Meta* (m) and *para* (p) positions of the anilino moiety.
2. To screen the APMs (above) for anticonvulsant activity in MES and scPTZ seizure test models.
3. To synthesis NBDAPMs from candidate 3-APMs
4. To screen the NBDAPMs (above) for anticonvulsant effect in MES and scPTZ seizure test models.
5. To assess the effect (if any) of substitution pattern on the anilino moiety on the anticonvulsant potency of the 3-APMs.
6. To assess the acute toxicity of the 3-AMPs and NBDAPMs.

7. To assess the neurotoxicity of the 3-APMs and NBDAPMs using the righting reflex test for neurological deficit.
8. To establish the ED_{50}, TD_{50} and protective Indices (PI) of the promising candidate 3-APMs and the NBDAPMs

1.5 SIGNIFICANCE OF THE STUDY

World-wide epilepsy is a major cause of morbidity and mortality; the standard mortality rate being 2 to 4 times higher than normal (Sridharan, 2002) and the incidence of sudden unexpected death ranging between 1 and 5 per 1000 patients (Perucca, 2002). In sub-Saharan Africa, the combination of poverty, limited medical care, ignorance, traditional beliefs and social role expectations coalesce to further limit the life of people with epilepsy (Baskind, 2005).

Stigma has also long been recognized as a major burden to people with epilepsy and their families (Temkin, 1971; Whitman, 1986). The nature and degree of this stigma vary depending on an individual's premorbid/baseline status within society and the societal conceptualization of the condition. Excellent reviews of epilepsy-associated stigma from the United States, the United Kingdom and sub-Saharan Africa are available (Morrell*,* 2002, Baskind, 2005; Jacoby *et al,* 2005). Epilepsy also has serious physical, psychosocial and economic consequences on the concerned persons and their families. A Canadian study found that people with epilepsy (PWE) had more days off work, a lower annual income and a lower quality of life (Wiebe, 1999). In China epilepsy has been reported to threaten the aspirations of the entire family as well as the opportunities of the

individual with the disorder (Kleiman *et al*., 1995). Reports from West Africa generally indicate that people belief seizures to be contagious, spread by saliva, urine, feces or flatus expelled during convulsions (Rwiza *et al,* 1993; Andermann, 2000). Therefore PWE are frequently required to use separate eating utensils or use separate sleeping mats away from the rest of the household (Baskind, 2005). In Ethiopia PWE were found living in cemeteries and church courtyard (Giel, 1968). Osuntokun (1970) described patients abandoned by their wives because of nocturnal anuresis.

Further, children and adolescent with epilepsy are at increased risk of emotional, behavioural and cognitive disorders (Alwash *et al,* 2000; Oostrom *et al,* 2000; Adewuya and Ola, 2005). Several studies have documented problems in academic performance of this group of patients when compared with their healthy counterparts or children with other chronic diseases (Austin *et al*, 1998; William *et al,* 2001; Fastenau *et al,* 2004 and Adewuya *et al,* 2006). Other reports indicate that teachers frequently expel children from school solely because they have seizures (Osuntokun and Odeku, 1970; Matuja and Rwiza, 1994; Birbeck and Calichi, 2003).

Indeed the burden produced by epilepsy is enormous and of global concern. Yet, a large proportion of the patients particularly in the developing world go without treatment. This informed the establishment of the Global Capaign Against Epilepsy by the World Health Organisation (WHO), the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE) in the year 2002. The Aim is to bring epilepsy 'out of the Shadows' so that the physical and socioeconomic burden on the individual and society is reduced.

CHAPTER TWO

LITERATURE REVIEW

2.0 HISTORICAL BACKGROUND OF EPILEPSY

The word epilepsy derives from the Greek verb *epilambanein, meaning to be seized, to be overwhelmed by surprise.* Epilepsy has afflicted human beings since the dawn of our species and has been recognised since the earliest medical writings. In fact, few medical conditions have attracted so much attention and generated so much controversy as epilepsy.

One of the earliest descriptions of a secondarily generalized tonic-clonic seizure was recorded over 3000 years ago in Mesopotamia. The seizure was attributed to the god of the moon. Epileptic seizures were described in ancient cultures, including those of China, Egypt, and India. Hippocrates wrote the first book about epilepsy almost 2500 years ago. He rejected ideas regarding the divine etiology of epilepsy and concluded that the cause was excessive phlegm that caused abnormal brain consistency. Hippocratic teachings were forgotten, and divine etiologies again dominated beliefs about epileptic seizures during medieval times. “In the Gospel according to Mark (9:14-29), Jesus Christ casts out a devil from a young man with epilepsy: Teacher, I brought you my son, who is possessed by a spirit that has robbed him of speech. Whenever it seizes him, it throws him to the ground. He foams at the mouth, gnashes his teeth, and becomes rigid. I asked your disciples to drive the spirit out, but they could not.” In 1494 a handbook on witch-hunting, *Malleus Maleficarum*, brings a wave of persecution and torture, leading to the death of more than 200,000 women.

Written by two Dominican friars under papal authority, the book identifies the presence of seizures as a characteristic of witches.

Between 1859 and 1906 under the leadership of three English neurologists-John Hughlings Jackson, Russell Reynolds, and Sir William Richard Gowers, the modern medical era of epilepsy begins. In a study, Jackson defines a seizure as *"an occasional, excessive and a disorderly discharge of nerve tissue on muscles.*" He also recognizes that seizures can alter consciousness, sensation, and behaviour. In the 1870s Fritsch, Hitzig, Ferrier, and Caton evoked and recorded epileptic seizures in the cerebral cortex of animals. In 1929, Berger discovered that electrical brain signals could be recorded from the human head by using scalp electrodes. This discovery led to the use of electroencephalogralphy (EEG) to study and classify epileptic seizures. Gibbs, Lennox, Penfield, and Jasper further advanced the understanding of epilepsy and developed the system of the 2 major classes of epileptic seizures currently used. An excellent historical review of seizures and epilepsy has been published (Goldersohn *et al,* 1997).

2.1 EARLY THERAPEUTIC ATTEMPT TO TREAT EPILEPSY

Duretus (1527-1586) reported success with a boy who had suffered depression of the skull as a result of a fall, and whose injured bone of the skull hindered the growth of the patient's brain, and as a result of pressure created by the non-growing skull, the boy suffered from epilepsy. He was however cured by the perforation of the depressed bone (Temkin, 1971).

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

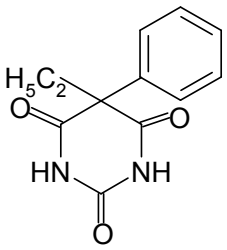
Seizure was, at one time, thought to be due to excessive sexual activity, so bromide was introduced as an anaphrodisiac without realizing the depressant action of bromide on the cortical neuron (Locock, 1857). Patients on bromide experienced reduction in a number of fits. So bromide was the first drug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

Phenobarbitone (II) was first synthesized as superior hypnotic (Fischer and Meringh, 1911). The anticonvulsant activity was discovered accidentally. When used, it reduces the incidence of number of fits among patients in both day and night at sub hypnotic dose.The discovery of phenobarbitone (II) represented an important advancement for three reasons:

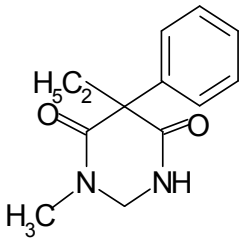
i. It served as substitute for bromides whose toxic effects were just being tolerated because there was no substitute.

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

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



(II)



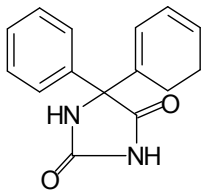
(III)

Phenobarbital, however, has the disadvantage of being hypnotic, a property that often prevents the clinician from using sufficint doses to ensure full protection agaist epileptic seizures. The second barbiturate drug, mephobarbital (III) was introduced 20 years later for the treatment of grand mal epilepsy (Vida, 1977).

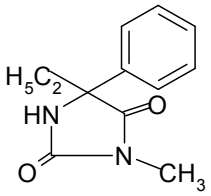
Putnam and Gibbs (1934) initiated an experiment to find a less sedating anticonvulsant agents than phenobarbitone (II), by establishing the first encephalographic laboratory for routine clinical study of brain wave, where they observed that epileptic seizures were accompanied by electric storm in the brain and concluded that it was possible to induce convulsion in laboratory animals by applying electric current to the brain. These breakthroughs afforded a method of recognising whether a drug could afford some degree of protection to the animals or not and was responsible for the major advancement in the treatment of epilepsy.

Screening of phenyl compounds hoping that phenyl group in phenobarbitone was responsible for its activity led to the discovery of phenytoin (IV), which was active and with no hypnotic properties. It protects cats from electrically induced

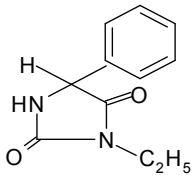
convulsion. It is also effective in psychomotor epilepsy, which was refractory to previous drugs (Merrit and Putnam, 1938). Phenytoin (IV) was followed by other hydantoins such as mephenitoin (V) and ethotoin (VI).



(IV)



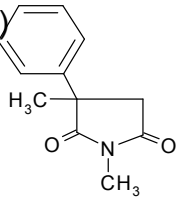
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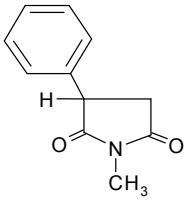
(VI)

The next therapeutic class of compounds reported for epilepsy therapy were the oxalidinediones like trimethadione (VII) and paramethadione (VIII) followed by the succinimides such as phensuximide (IX), methsuximide (X) and ethosuximide (XI). These drugs were quickly accepted and incorporated into clinical pratice because they were the first effective drugs for the management of absences (Vida, 1977).

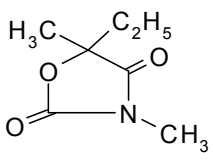
A highly promising anticonvulsant drug, phenacemide (XII), was the first noncyclic ureid to gain acceptance in 1954 (Vida, 1977). Initially, this drug was considered to be effective in treatment of grand mal and petit mal psychomotor seizures. Later, it was used primarily for the treatment of psychomotor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



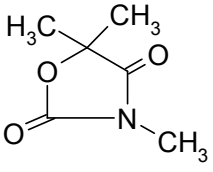
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IX



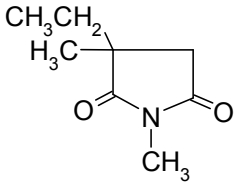
VIII



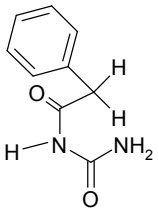
VII

The discovery that metabolic acidosis causes amelioration of epilepsy and that acetazolamide (XVI) can induce this metaboli change led to the use of carbonic anhydrase inhibitors as anticonvulsants. However, not all carbonic anhydrase inhibitors are anticonvulsants because most of them fail to cross the blood brain barier. Moreover, tolerance to the anticonvulsant effect of acetazolamide was rapid (Vida, 1977).

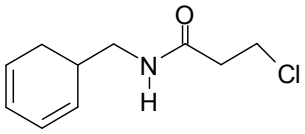
The numerous side effects of hydantoins warranted for search and synthesis of barbiturate analoques. Primidon (XVII), a deoxybarbiturate, was introduced in the 1950s as an effective antivonvulsant particularly against grand mal epilepsy.



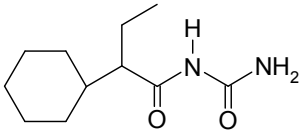
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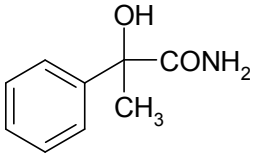
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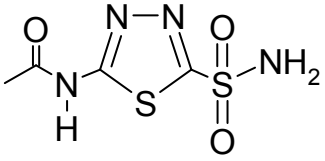
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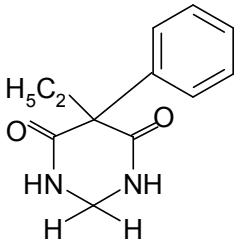
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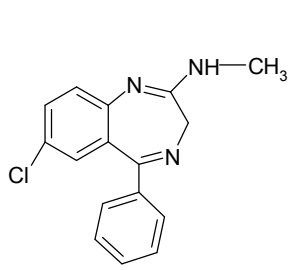


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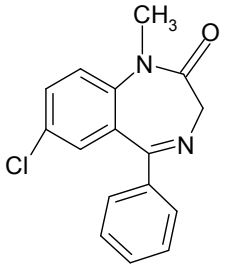


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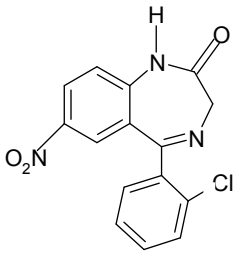
The benzodiazepines became popular after the approval of chlordiazepoxide (XVIII) for use as an anticonvulsant in the U. S. A. in 1960. Later, diazepam (XIX) was found to be more selective than chlordiazepoxide (XVIII) and particularly very useful in status epilepticum. Other benzodiazepines that were used clinically include oxazepan (XX), nitrazepan (XXI) and clonazepan (XXII).



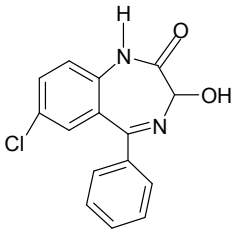
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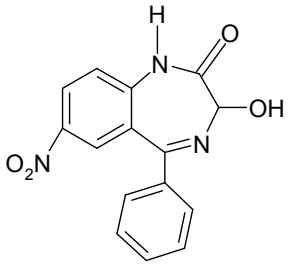
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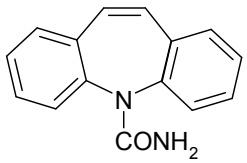
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(XX)



(XXI)

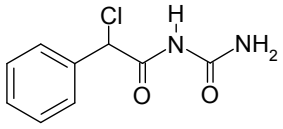


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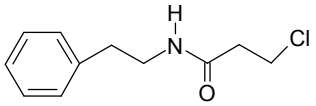
Carbamazeprie (XXIII) a tricyclic compound structurally related to the imipramine type of antidepressants was marketed in the United States in the early 1960s for the treatment of trigeminal neuralgia. Later, the drug was investigated and found to be effective in the treatment of epileptic patients with grand mal and psychomotor seizures (Cereghino *et al*., 2000).

Other drugs marketed outside the United States between the late 1960s and early 1970s include chlorphenacemide (XXIV) which was marketed in Austria, fanaclone (XXV) in Soviet Union, chlormethiazole (XXVI) in Scandinavia and the

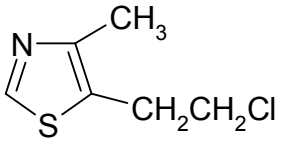
United Kingdom and ethylphenacemide (XIV) in Poland, Spain and the United Kingdom (Vida, 1977).



(XXIV)

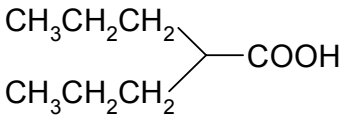


(XXV)

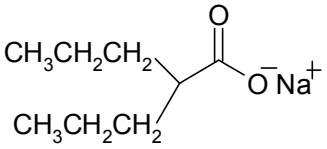


(XXVI)

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

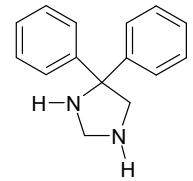


(XXVII)

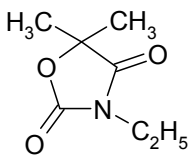


(XXVIIa)

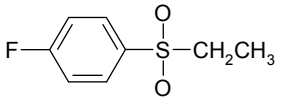
Doxenitoine (XXVIII), a deoxyhydantoin, was marketed in France as a derivative of phenytoin (II) with higher margine of safety than the parent compound (Vida, 1977). Ethadione (XXIX), an effective drug against absences, was marketed in Belgium, France and Sacndinavia. Fluoresone (XXX) was marketed in France as an adjuct in treatment of grand mal epilepsy while methindione (XXXI) was used in the Soviet Union for the treatment of grand mal and psychomotor epilepsy.



(XXVIII)

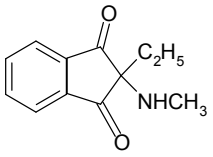


(XXX)

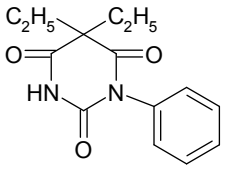


(XXIX)

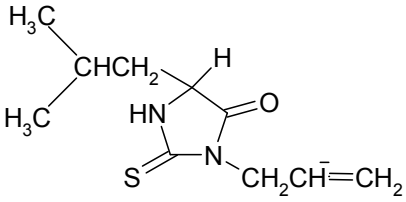
Phetarbita (XXXII) was use in controlling absences both in Hungary and the United Kingdom in the early 1960s. Albutoin (XXXIII) was also reported (Vida, 1977), to be useful in controlling grand mal epilepsy but was later abandoned because a great percentage of adult patients displayed nausea, vomiting and abdominal pain. Sulthiame (XXXIV) was used in Europe both as an adjunct, drug in the treatment of psychomotor epilepsy and singly for the treatment of grand mal epilepsy.



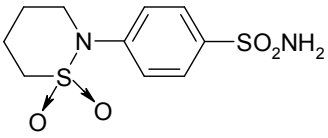
(XXXI)



XXII)

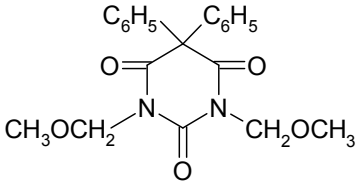


(XXXIII)

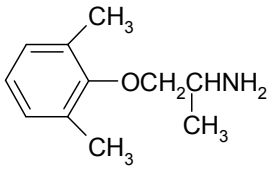


(XXXIV)

In the early seventies both Eterobarb (XXXV) and mexiletine (XXXVI) were tried both in the Europe and the United States but later abandoned (Vida, 1977)



(XXXV)

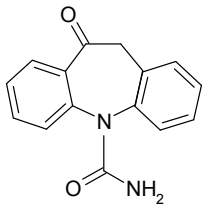


(XXXVI)

2.2 NEWER ANTIEPILEPSY DRUGS

2.2.1 Oxcarbazepine (XXXVII)

Oxcarbazepine (XXXVII) is a recently developed analog of carbamazepine (XXIII). Oxcarbazepine (XXXVII) was developed in an attempt to hold on to the benefits of (XXIII) while avoiding its auto-induction and drug interaction properties. Licensed in over 50 countries, including the United States, (XXXVII) is now considered as one of the first-line therapies in some countries. Oxcarbazepine (XXXVII) does not produce the epoxide metabolite, which is largely responsible for the adverse effects reported with (XXIII). Like (XXIII), (XXXVII) blocks the neuronal sodium channel during sustained rapid repetitive firing (Schachter *et al*, 1999; Beydoun *et al*, 2000; Gelisse *et al*, 2004).

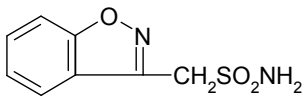


XXXVII

2.2.2 Zonisamide (XXXVIII)

Zonisamide (XXXVIII) was synthesized as a benzisoxazole in 1974. It is chemically unrelated to any of the other AEDs. It is a small ringed structure related to sulfonamide antibiotics with pH-dependent solubility in water. Although the US Food and Drug Administration (FDA) approved (XXXVIII) in March 2000 for the indication of partial seizures in patients older than 12 years as adjunctive therapy to other AEDs, it has been approved and studied in Japan for more than

10 years. The major mechanism of action of (XXXVIII) is reduction of neuronal repetitive firing by blocking sodium channels and preventing neurotransmitter release. It also exerts influence on T-type calcium channels and prevents influx

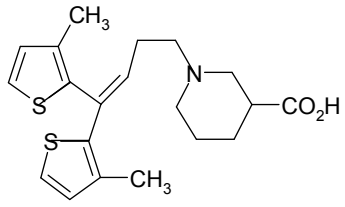


XXXVIII

of calcium. Zonisamide (XXXVIII) also exhibits neuroprotective effects through free radical scavenging (Kyllerman *et al*, 1998; Leppik, 2001; Kothare *et al*, 2004).

2.2.3 Tiagabine (XXXIX)

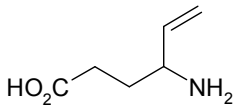
Tiagabine (XXXIX) is a derivative of the GABA uptake inhibitor nipecotic acid. It acts by inhibition of the GABA transporter-1 (GAT-1). This inhibitory effect is reversible. XXXIX is lipid soluble and thus is able to cross the blood-brain barrier. It was introduced into clinical practice in 1998. Measurements in human and experimental models have confirmed that extracellular GABA concentrations increase after administration of (XXXIX). Studies have shown little or no effect at other receptor systems.



XXXIX

2.2.4 Vigabatrin (XL)

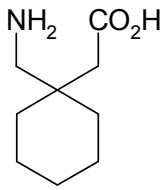
In the 1970s, GABA was recognized as an important inhibitory neurotransmitter in the CNS. Favoring the balance toward the GABA system was a major target of drug research, and soon XL was developed. The drug was licensed worldwide, except in the United States because of its toxicity. It is a close structural analog of GABA, binding irreversibly to the active site of GABA-T. Newly synthesized enzymes take 4-6 days to normalize the enzymatic activity. In vivo studies in human and animal subjects have shown that XL significantly increases extracellular GABA concentrations in the brain. XL has no other known action (Aicardi *et al*, 1996; Thomas *et al*, 1996; Malmgren *et al*, 2001; Spence *et al*, 2001)



XL

2.2.5 Gabapentin (XLI)

Gabapentin (XLI) was developed to have a structure similar to that of GABA; however, experimental evidence showed that (XLI) has, in fact, little or no action on the GABA receptor. It is highly soluble in water. It enhances the enzyme GAD, but does so weakly. It binds with the $\alpha_2\delta$ subunit of calcium channels in the cerebral neocortex, hippocampus, and spinal cord; this mechanism of action may be important for its efficacy in pain.



XLI

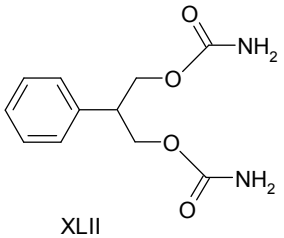
At this time, the exact mechanism by which (XLI) increases the intracellular concentration of GABA is unknown. In vivo MR spectroscopy studies have shown that (XLI) increases brain levels of GABA and its metabolites homocarnosine and pyrrolidinone. It also may reduce monoamines and affect serotonin release.

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

2.2.6 Felbamate (XLII)

The dicarbamate felbamate, a broad spectrum AED, has a variety of effects on ion channels that could contribute to its therapeutic activity. The drug has been reported to inhibit sustained repetitive spike firing in cultured neurons, suggesting that it modulates voltage-activated sodium channels (White *et al.,* 1992). However, an effect on recombinant sodium channels was observed only at relatively high (1mM) concentrations (Taglialatela *et al*., 1996). Felbamate also , inhibits high voltage-activated calcium currents (Stefani *et al*., 1996), but the significance of this action for seizure protection is uncertain. At therapeutic serum concentrations (100–300 μM), felbamate acts as a positive allosteric modulator of

$GABA_A$ receptors (Rho *et al*., 1994; Kume *et al*., 1996). However, this effect appears to be specific for $GABA_A$ receptors containing α1 or α2 subunits along with β2 or β3 subunits (Simeone and McClellan, 2000).



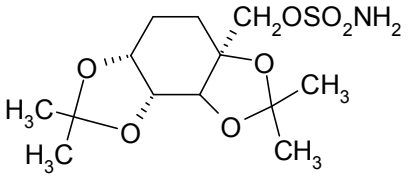
XLII

In addition, felbamate at a concentration of 100 mM, has been found to block NMDA receptor mediated synaptic responses (Pugliese *et al*., 1996) and to inhibit NMDA receptor currents in isolated neurons (*K*d, ~1 mM; Rho *et al*., 1994; Subramaniam *et al.,* 1995; Kuo *et al*., 2004). Studies with recombinant NMDA receptor subunit combinations have indicated that felbamate selectively bocks NMDA receptors composed of NR2B subunits at lower concentrations (IC_{50}, 0.5 mM), than other subunit combinations (Kleckner *et al*., 1999a; Harty and Rogawski, 2000). This selectivity could contribute to the relatively low neurobehavioral toxicity of felbamate in relation to other NMDA receptor antagonists (Loscher and Rogawski, 2002).

2.2.7 Topiramate (XLIII)

Topiramate (XLIII) is a very potent anticonvulsant. It is structurally different from other AEDs. It is derived from D-fructose and initially was developed as an antidiabetic drug. In animal models, it was found to have potent antiepileptic effects. Topiramate (XLIII) has multiple mechanisms of action. It exerts an

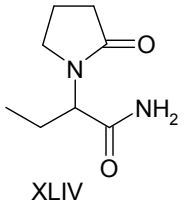
inhibitory effect on sodium conductance, decreasing the duration of spontaneous bursts and the frequency of generated action potentials, enhances GABA by unknown mechanisms, inhibits the AMPA subtype glutamate receptor, and is a weak inhibitor of carbonic anhydrase (Shank *et al*, 1994; Privitera *et al*, 1996; Zona *et al*, 1997; Glauser *et al*, 2000; Ochoa *et al*, 2000).



XLIII

2.2.8 Levetiracetam (XLIV)

Levetiracetam (XLIV) is a piracetam (S-enantiomer pyrrolidone) derivative. It was developed in the 1980s to enhance cognitive functions and as anxiolytic. Levetiracetam is not active in the traditional MES or PTZ models when conducted according to standard protocols (Klitgaard *et al*., 1998; Klitgaard, 2001). Its anticonvulsant activity was discovered in audiogenic seizure-susceptible mice, and it was subsequently found to have activity in a range of chemoconvulsant models, including seizures induced by submaximal PTZ doses (Gower *et al*., 1992), the 6-Hz model, a rat genetic model of absence epilepsy (Gower *et al*., 1995) and also various kindling models (Loscher and Honack, 1993). Levetiracetam also seems to have “antiepileptogenic” activity, in that it retards the development of pentylenetetrazol-kindled seizures (Gower *et al*., 1992) as well as convntional amygdala-kindled seizures (Loscher et al., 1998)



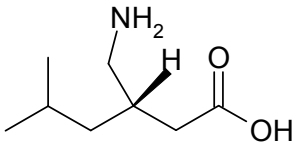
XLIV

SV2A, a synaptic vesicle protein, has recently been identified as the likely target for levetiracetam (Lynch *et al*., 2004). The mechanism whereby binding to SV2A results in anticonvulsant activity is unknown. SV2A is an abundant protein component of synaptic vesicles that is structurally similar to 12-transmembrane domain transporters, although a transporter activity for SV2A has not yet been identi-fied. SV2A is not essential for synaptic transmission, but mice in which the protein has been deleted by gene targeting exhibit seizures (Janz *et al.,* 1999). In addition, it reduces bicuculline-induced hyperexcitability in rat hippocampal CA3 neurons, suggesting a non-GABAergic mechanism. XLIV inhibits Ca^{2+} release from the IP3-sensitive stores without reducing Ca^{2+} storage, which could explain some of XLIV antiepileptic properties (Gower *et al*, 1995; Noyer *et al*, 1995; Loscher *et a*l, 1998; Cereghino *et a*l, 2000; Privitera *et al*, 2001).

2.2.9 Pregabalin (XLV)

Pregabalin (XLV) was designed as a lipophilic analogue of GABA to facilitate diffusion across the blood-brain barrier. Although structurally related to GABA, pregabalin is not functionally related and does not directly affect the GABAergic system. (XLV) is inactive at GABA receptors, does not affect GABA uptake or degradation, and does not elevate GABA levels acutely in rat forebrain. It shows

affinity for receptor sites or alters responses associated with the action of several common drugs for treating seizures. XLV exhibits potent anticonvulsant, analgesic, and anxiolytic activity in various animal models. Similar to gabapentin, pregabalin's potent binding to the alpha-2-delta site and its structural analogs has been shown to reduce depolarization-induced calcium influx at nerve terminals, with a consequential reduction in the release of several excitatory neurotransmitters (eg, glutamate, noradrenaline, substance P, calcitonin gene-related peptide, but it does not completely block calcium channel function or

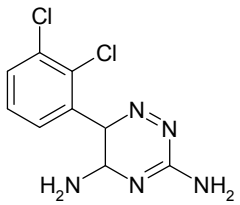


XLV

transmitter release even at high concentrations. XLV shows higher binding affinity than gabapentin (XLI) and is also more potent in both animal models and clinical studies. XLV prevents tonic extensor seizures in maximal electroshock animal models, generalized tonic-clonic seizures induced by sound in genetically susceptible DBA/2 mice, seizures induced by pentylenetrazol in hippocampal-kindled rats, but it does not reduce the incidence of spontaneous absence seizures in genetically susceptible rats (GAERS) (Ben-Menachem *et al*, 2004; Brodie, 2004; Matsuo *et al*, 2004; Cunnington, 2004).

2.2.10 Lamotrigine (XLVI)

This was synthesized in drug development to exploit antiepileptic effect of novel antifolate agents. Lamotrigine (XLVI) has weak antifolate properties, but its



XLVI

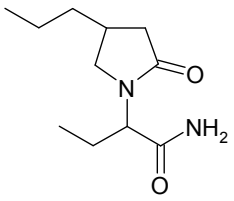
antiepileptic properties are unrelated to the antifolate activity. Lamotrigine (XLVI) inhibit voltage dependent sodium ion channel resulting in decrease in the release of excitatory neurotransmitters such as aspartate and glutamate (Harden, 1994)

2.3 ANTIEPILEPTIC DRUGS IN THE DEVELOPMENT PIPELINE AND MECHANISMS

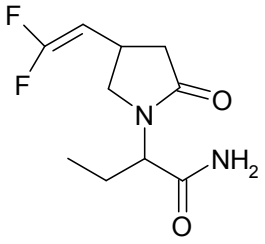
2.3. I ENHANCEMENT OF PRESENTLY MARKETED DRUGS

2.3.1.1. Levetiracetam analogs

Seleteracetam (XLVII) and brivaracetam (XLVIII) are derivatives of levetiracetam (XLIV) that are substituted at the 4-position on the 2-pyrrolidinone ring (Kenda *et al*., 2004). Both have 10-fold greater affinity for SV2A than does levetiracetam but brivaracetam (XLVIII) may also have modest sodium channel blocking activity. Like levetiracetam (XLIV), seleteracetam (XLVII) is inactive in the MES and PTZ tests. However, it is 10-fold more potent than levetiracetam (XLIV) in the corneal kindling model and 25-fold more potent in the genetic absence epile-



(XLVIII)



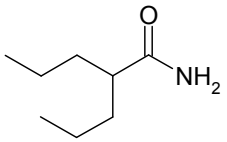
(XLVII)

psy rat of Strasbourg (GAERS). In contrast, brivaracetam (XLVIII) is slightly less potent than seletracetam (XLVII) in corneal kindling, but is weakly active in the MES and PTZ tests. Overall, seletracetam (XLVII) seems to have a similar pharmacological profile to levetiracetam (XLIV), but is more potent. In animal models, it exhibits an even higher protective index than levetiracetam (XLIV). (Protective index, the ratio of TD_{50} for rotorod toxicity to ED_{50} for seizure protection, is a measure of the separation between anticonvulsant potency and potency to induce motor impairment; Loscher and Nolting, 1991.) In contrast, brivaracetam has a broader spectrum of activity and may be more effective than levetiracetam in the rat amygdala kinding model for suppressing both behavioural kindled seizures and the after discharge. However, it has a lower protective index than levetiracetam. In a placebo-controlled phase IIa clinical trial in patients with photosensitive epilepsy brivaracetam was effective in reducing the photoparoxysmal electroencephalogram response (Kasteleijn-Nolst Trenit`e *et al.,* 2004). Seleteracetam and brivaracetam provide examples of how it is possible for analogs of a prototype anticonvulsant structure to either optimize the inherent activity of the molecule or maintain its high target site selectivity (seleteracetam) or, alternatively, broaden its target specificity so that it exerts therapeutically desirable actions on additional targets (brivaracetam).

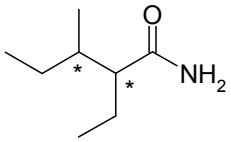
2.3.1.2. *Valproate-like agents*

Valproate (di-*n*-propylacetic acid, isooctanoic acid) (XXVIIa) has a unique place in epilepsy therapy because of its efficacy in generalized seizures including, absence, primary generalized tonic-clonic, and myoclonic seizures, as well as in

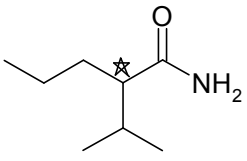
partial seizures (Perucca, 2002a). It is also an important therapeutic option for migraine prophylaxis and in the treatment of acute mania. However, valproate (XXVIIa) has substantial disadvantages in that it is a teratogen, causes weight gain and reproductive dysfunction, and occasionally is associated with hepatic and pancreatic toxicity. Therefore, there has been considerable interest in the development of an agent that has the same broad spectrum of clinical efficacy as valproate without these undesirable characteristics. Because the precise mechanism of action of valproate is unknown, it is difficult to predict base on preclinical studies alone whether an analog will have the same broad spectrum of clinical efficacy as the parent. Valproate (XXVIIa) exhibits protective activity in virtually all animal AED screening models, but its potency in any specific model varies depending upon the species and route of administration (Loscher, 2002). These factors complicate attempts to prove that a compound is “valproate-like.” Valproate (XXVIIa) is especially active in genetic models of absence seizures, such as the GAERS rat (Marescaux *et al*., 1992). It is also protective against seizures induced by the inverse benzodiazepine receptor agonist DMCM (Petersen, 1983). At higher doses, valproate XXVIIa) is protective in the MES and PTZ models. Valproate (XXVIIa) has diverse pharmacological actions, but none of these actions has been unequivocally linked to its broad protective activity against diverse seizure types in animals and in man. Among these various actions, Loscher (2002) has concluded that increases in GABA turnover



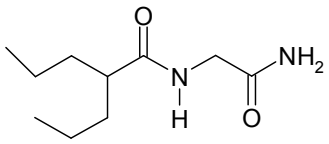
(XLIX)



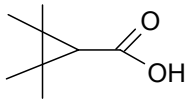
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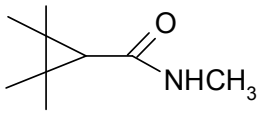
(LI)



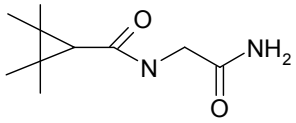
(LII)



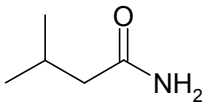
(LIII)



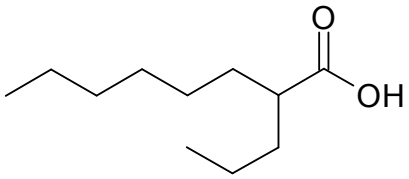
(LIV)



(LV)



(LVI)



(LVII)

which are produced in specific brain regions is of particular importance in the ability of valproate (XXVIIa) to control seizure generation and propagation in diverse epilepsy models.

A variety of compounds that are more or less structurally similar to valproate (XXVIIa) have been shown to have a valproate-like profile in animal seizure models. In some cases, the compounds have greater potency than valproate (XXVIIa). For example, valpromide (XLIX)), which is marketed as an AED and antipsychotic agent in several European countries (Bialer, 1991), is approximately two-fold more potent than valproate (XXVIIa) in the MES and PTZ models (Loscher and Nau, 1985). The enhanced potency is believed to be due to greater blood–brain barrier permeability, with corresponding greater brain concentrations (Blotnick *et al.,* 1996). The higher brain penetration also contributes to greater potency for inducing motor impairment, so that, if anything, the protective index of valpromide is reduced compared with valproate. Valpromide (XLIX) is not converted to valproate (XXVIIa) in mice, and there is no

evidence that it is teratogenic in this species (Nau and Loscher, 1986). In humans, however, valpromide (XLIX) is rapidly biotransformed to valproate (XXVIIa) (Bialer, 1991).

Because of its conversion to valproate (XXVIIa), valpromide (XLIX) does not offer a substantial therapeutic advantage. However, Bialer and colleagues have identified analogs of valpromide (XLIX) that resist metabolic hydrolysis to their corresponding acids (Isoherranen *et al.,* 2003b). Two such analogs, valnoctamide (L) and diisopropyl acetamide (LI), exhibit greater anticonvulsant potency than valproate (XXVIIa) and their corresponding acids (Winkler *et al.,* 2005). Studies in human subjects have confirmed that valnoctamide undergoes only minimal biotransformation to valnoctic acid (Bialer *et al.,* 1990; Barel *et al.,* 1997). Valnoctamide (L) is a chiral compound with two stereogenic carbons, so that the molecule exists in two enantiomeric pairs. Only small differences were found between the anticonvulsant potencies and pharmacokinetic properties of the (2*S*, 3S) and (2*R*, 3S) forms (Isoherranen *et al.,* 2003a).Valnoctamide (a mixture of both enantiomeric pairs) (L) has been marketed as anxiolytic and sedative (as Axiquel and Nirvanil) in the U.S., Italy, the Netherlands and Switzerland. Whether it is useful in epilepsy remains to be determined. Despite having been marketed as a sedative, the drug was well tolerated and produced only mild sedation in volunteers (Pisani *et al*., 1992). Animal studies reveal a striking difference from valproate (XXVIIa) in terms of teratogenicity. Thus, in pregnant NMRI mice where valproate (XXVIIa) produces more than 50% embryotoxicity, valnoctic acid and valnoctamide (L) induce embryotoxicity at

frequencies no greater than control (Radatz *et al.,* 1998). One disadvantage of valnoctamide (L) is that it is a potent inhibitor of microsomal epoxide hydrolase so that concomitant administration with carbamazepine (XXIII) causes five-fold increases in the levels of carbamazepine epoxide and clinical signs of toxicity (Pisani *et al.,* 1992, 1993).

Diisopropylacetamide (LI), which is expected to enter clinical development, is also a chiral compound with a single stereocenter. As in the case of valnoctamide (L) only small differences were found in the relative anticonvulsant potencies and pharmacokinetic properties of the enantiomers. Thus, while the (*R*)-enantiomer was found to be modestly more potent than the (*S*)-enantiomer in the rat MES test (ED_{50} values, 25 and 16 mg/kg, p.o., respectively; Spiegelstein *et al.,* 1999), this enantioselectivity was not observed in all models (Isoherranen *et al*., 2003c). In mice, the (*R*)-enantiomer had a modestly longer half-life, but pharmacokinetic differences between the isomers were less evident in the rat. Whether the enantiomeric selectivity is due to pharmacokinetic differences is yet to be determined.

Another potential valproate-like AED is valrocemide (LII). This compound is modestly more active in the mouse MES test than valproate (XXVIIa) (ED_{50}, 151 mg/kg versus 287 mg/kg, i.p, for valproate) and is similarly more active in the mouse PTZ test (ED_{50}, 132 mg/kg versus 209 mg/kg, i.p. for valproate) (Isoherranen *et al.,* 2001). Valrocemide (LII) is also more potent in other chemoconvulsant models, including seizures induced by bicuculline and picrotoxin, but not by strychnine, where valproate (XXVIIa) is weakly active

(ED_{50}, 293 mg/kg, i.p.) but valrocemide (LII) is inactive. It has been proposed that the greater potency of valrocemide (LII) is due to its increased accumulation in the brain. Assuming valrocemide has valproate-like activity in the clinic, the question remains whether the drug will have reduced teratogenicity or side effects. Valrocemide (LII) does not cause embryotoxicity in rats and rabbits. The main metabolic pathway is enzymatic amide hydrolysis yielding the acid valproylglycine (Hovinga, 2004; Bialer *et al.,* 2004). In rats and dogs, minimal levels of valproate (XXVIIa) are produced. However, in humans treated with valrocemide (LII) (3g/day) mean valproate (XXVIIa) levels are substantial (15 µg/ml), but below the therapeutic range (50–100 µg/ml). Therefore, valrocemide (LII) could be safer than valproate (XXVIIa), but the risk of teratogenicity is not eliminated. The half-life of valrocemide (LII) is relatively short so that three-times daily dosing will be required.

Bialer and colleagues have extensively investigated the anticonvulsant activity of cyclic analogs of valproate in an attempt to avoid the hepatotoxicity and teratogenicity of valproate (XXVIIa). Starting with 2,2,3,3-tetramethylcyclopropan-carboxylic acid (LIII), which itself is not a potent anticonvulsant, several amides were discovered with excellent anticonvulsant activity, including *N*-methyl-tetramethylcyclopropanecarboxamide (LIV) and 2,2,3,3-tetramethylcyclopropan-ecarbonylurea (LV) (Isoherranen *et al.,* 2003b; Sobol *et al*., 2004). These cyclic analogs possess two quaternary carbons in the β-position to the carbonyl and cannot be biotransformed into metabolites with terminal double bond, which is presumed to be the source of hepatotoxicity (Sobol *et al.,* 2005). Like valproate (XXVIIa), these compounds are effective in both the MES and PTZ tests, but

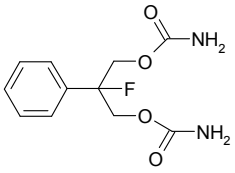
some show a greater relative potency in the PTZ test raising the possibility that they are mechanistically different from valproate (XXVIIa). For example, (LIV) has ED_{50} values of 98 and 39 mg/kg in the mouse MES and PTZ tests compared with values for valproate (XXVIIa) of 263 and 220 mg/kg. In contrast, (LV) is preferentially active in the MES test (ED_{50} values, 90 and 125 mg/kg, respectively).

Eadie (2004) has recently proposed that valerian, the dried root of various *Valeriana* species with purported antiepileptic properties, is active as a result of `constituent isovaleric acid, a simple branched-chain monocarboxylic acid. Isovaleric acid has undesirable odour and taste, but its amide isovaleramide (LVI) does not. Indeed, isovaleramide (LVI) has been found to have activity in the MES and PTZ models as well as in kindling models and other chemoconvulsant and absence epilepsy models (Bialer *et al*., 2001). Overall, it appears similar to valproate (XXVIIa) but is weaker in potency in animal testing (e.g., MES and PTZ ED_{50} values, 913 and 748 mg/kg, p.o., respectively). Phase I clinical trials have indicated that isovaleramide (LVI) is safe and well tolerated; whether it is truly valproate-like will require further clinical evaluation.

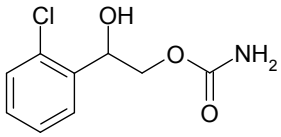
An additional valproate analog that is currently in early stage clinical trial is arundic acid [(*R*)-(-)-2-propyloctanoic acid (LVII) (de Paulis, 2003; Asano *et al.,* 2005). This compound is being evaluated for stroke and neurodegenerative diseases, but not epilepsy.

2.3.1.3 Carbamates: fluorofelbamate and RWJ-333369

Felbamate (XLII) use is limited by hematological and hepatic toxicities, which are attributed to the formation of an aldehyde (atropaldehyde or 2-phenylpropenol) intermediate that reacts with proteins (Thompson *et al.,* 2000). Substitution of a fluorine atom for hydrogen at the 2-position of the propanediol moiety of felbamate (XLII) to form fluorofelbamate (LVIII) prevents the formation of this reactive aldehyde. In some animal models fluorofelbamate (LVIII) is equivalent in potency to felbamate (XLII) and in others it is substantially more potent (Bialer *et al.,* 2004). Notably, in the rat MES test, the ED_{50} of flurofelbamate (LVIII) is 3-mg/kg p.o. whereas felbamate (XLII) is eight-fold less potent (ED_{50}, 25 mg/kg). There is only limited information to address the question of whether the action of fluorofelbamate (LVIII) on ion channels is similar to that of felbamate (XLII). Preliminary evidence indicates that fluorofelbamate (LVIIII) does not enhance GABA receptor responses (Bialer *et al.,* 2004). However, like felbamate, fluorofelbamate may inhibit NMDA receptors and might also affect sodium channels.



(LVIII)



(LIX)

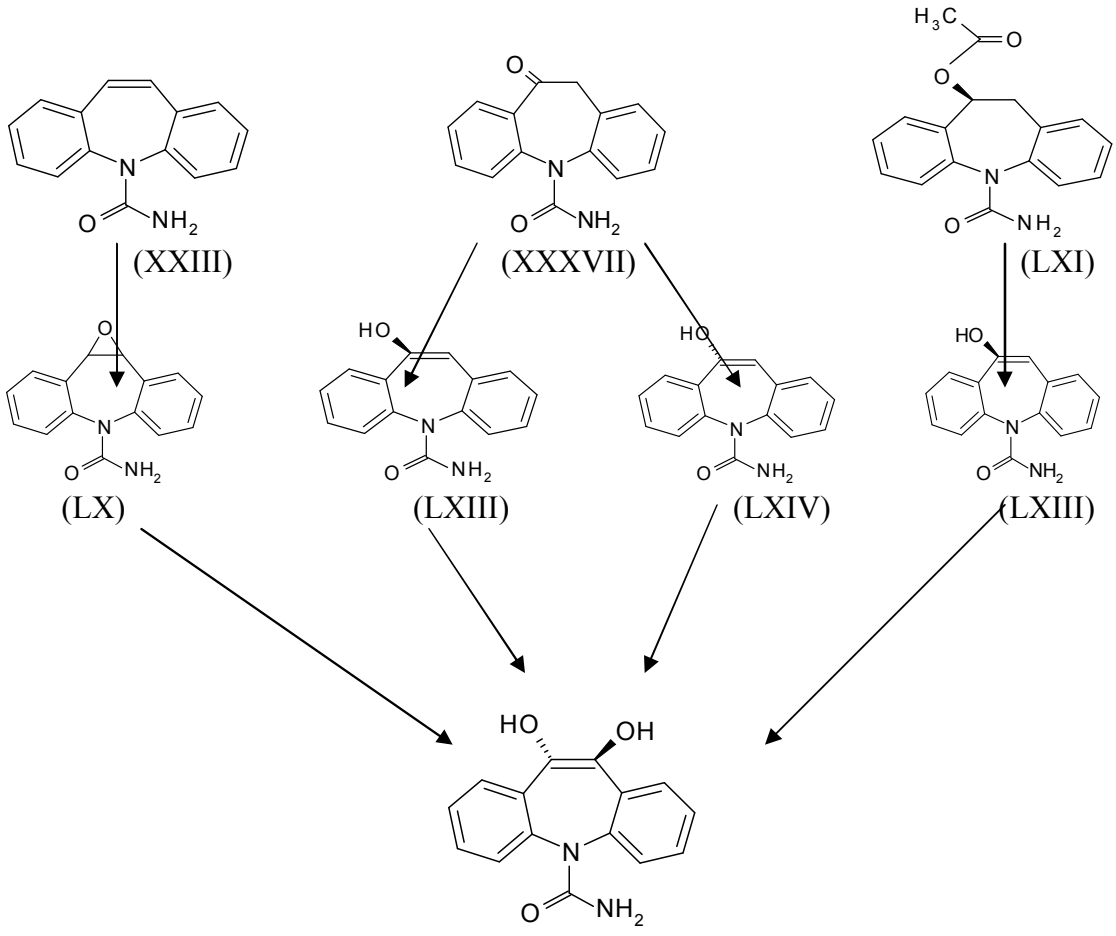
A series of monocarbamates of the general structure 2-carbamoyl-1-chlorophenylethanol have been described with activity in the mouse MES test (ED_{50} values, ≥7.4 mg/kg, p.o.; (Choi *et al.,* 1997, 2000). One of these compounds 2-carbamoyloxy-1-(2-chlorophenyl)ethanol (LIX) was selected for

clinical development. (LIX) has activity in the mouse MES (ED_{50}, 7.9 mg/kg, i.p.) and PTZ (ED_{50}, 20.4 mg/kg, i.p.) tests and is also protective against seizures induced by bicuculline and picrotoxin, and in some kindling models.(LIX) is also active in the GAERS model of absence epilepsy, albeit at doses that are modestly greater than those active in the MES test (doses of 30 and 60 mg/kg attenuated spike-wave discharges but 10 mg/kg was inactive; Nehlig *et al.,* 2005). It therefore differs from other marketed AEDs in having a broader spectrum of activity even than felbamate (XLII), which is not active in the bicuculline model. There is preliminary evidence that the drug is effective in protecting against spontaneous recurrent seizures in kainate-treated animals (Grabenstatter and Dudek, 2004) and that it confers antiepileptogenic and neuroprotective activity when administered repeatedly after lithium-pilocarpine status epilepticus in rats (Francois *et al.,* 2005). LIX is currently in Phase II clinical development for the treatment of partial epilepsy and migraine headache. Whether the drug will ultimately prove to have clinically useful antiepileptogenic properties is of considerable interest (Rogawsky, 2006).

2.3.1.4 Carbamazepine analogs: licarbazepine and (S)-licarbazepine acetate

Carbamazepine (XXIII) is extensively metabolized through several distinct biotransformation pathways. Quantitatively, the most important biotransformation is the expoxide-diol pathway in which carbamazepine (XXIII) is first oxidized at the 10,11-double bond of the azepine ring to form the chemically stable epoxide [carbamazepine-10,11-epoxide (LX)] , which has potent anticonvulsant activity in its own right. Although several studies have implicated the epoxide in side effects

(Rogawsky, 2006), other studies have disputed this conclusion and at least one study suggested an improvement in side effects when patients are switched to the epoxide (Holmes, 2002). In any case, there has been an interest in analogs of carbamazepine (XXIII) that are not converted to the epoxide, as is the case for oxcarbazepine (XXXVII), the 10-keto analog of carbamazepine (XXIII). Oxcarbazepine (XXXVII) serves as a prodrug for its 10-hydroxy metabolites (*R*)- and (*S*)-licarbazepine, which appears in plasma and urine in a 4:1 ratio (Faigle and Menge, 1990; Volosov *et al.,* 1999). (*S*)-Licarbazepine acetate (LXI) is also in Phase III clinical trials. LXI only forms (*S*)-licarbazepine (LXIII), which is then converted to the trans-diol (LXII) (Benes *et al.,* 1999; Almeida *et al*., 2005). In rats, orally administered LXI (2 hr; ED_{50}, 4.7 mg/kg) is as potent as carbamazepine (XXIII) (ED_{50}, 5.4 mg/kg) in the MES test and, at the same time, more potent than oxcarbazepine (XXXVII) (ED_{50}, 10.0 mg/kg).

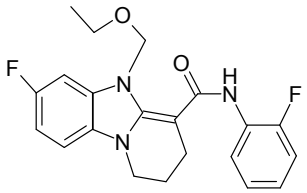


Interestingly, there is some stereoselectivity as (*R*)-licarbazepine acetate (LXI) is about one-half potent (ED_{50}, 10.9 mg/kg). In the mouse MES test LXI is less potent than XXII. LXI, like XXIII and XXXVII, is weak or inactive in the PTZ and kindling models. LXI causes a voltage-dependent block of voltage-dependent sodium currents with similar potency to XXIII (Bonif´acio et al., 2001). In addition, LXI, as is the case for other sodium channel blocking AEDs (Rogawski and L¨oscher, 2004a), inhibits sodium channel-dependent release of neurotransmitters with similar potency to carbamazepine (XXIII) and oxcarbazepine (XXXVII) (Ambr´osio *et al.,* 2001; Parada and Soares-da-Silva, 2002). Although LXI is chemically distinct from licarbazepine and could potentially have distinct pharmacodynamic properties related to its novel structure, there is little evidence that it is substantially different mechanistically.

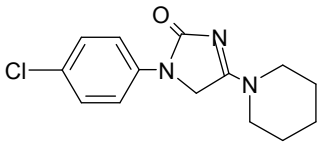
2.3.1.5 Benzodiazepine-like agents

Benzodiazepines, which act as positive allosteric modulators of $GABA_A$ receptors, including diazepam, lorazepam and clonazepam, have potent anticonvulsant activity in animal seizure models and are used clinically in the treatment of status epilepticus (Rogawski, 1996). These classical benzodiazepines act on $GABA_A$ receptors containing γ2 subunits and α1, α2, α3 or α5 subunits. Such benzodiazepine-sensitive $GABA_A$ receptors are believed to comprise more than three-quarters of all brain $GABA_A$ receptors (Whiting, 2003). The development of tolerance as well as mechanism-related side effects, most notably sedation, limits the use of classical benzodiazepines in chronic epilepsy therapy. Benzodiazepine-like agents that act as partial agonists at the

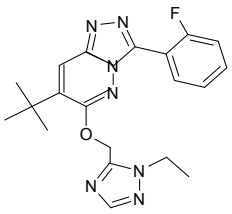
benzodiazepine recognition site, such as LXV may show less propensity towards tolerance (Costa and Guidotti, 1996). LXV is of particular interest as it has highly potent anticonvulsat activity and favourable protective index (Dubinsky *et al,* 2002). None of the partial benzodiazepine agonists, however, has been proven to lack tolerance in clinical trials (Rogawsky, 2006).



(LXV)



(LXVII)



(LXVI)

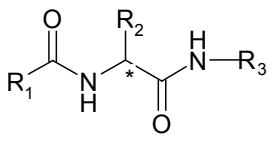
Another approach toward obtaining improved clinical performance is through the development of subtype selective benzodiazepine-like agents.LXVI bind to α1-containing $GABA_A$ receptors but has no intrinsic activity and indeed acts as an antagonist at these isoforms; it is a partial agonist for other $GABA_A$ receptor subtypes and has potent anticonvulsant activity against PTZ seizures and in other seizure models (Rogawsky, 2006).

Another subtype selective benzodiazepine receptor agonist is LXVII [1-(4-chlorophenyl)-4-piperidin-1-yl-1,5-dihydro-imidazol-2-on], which has potency equivalent to diazepam on α3-subunit containing $GABA_A$ receptors, but is 50-fold weaker at α1 and α5 and inactive at α2 and α4 (Langen *et al*., 2004, 2005). It has reduced efficacy for potentiation of α3, α1 and α5 receptors, and is therefore a partial agonist. LXVII is currently in Phase II clinical trials as an anxiolytic. However, it also has anticonvulsant activity albeit with lower potency than classical benzodiazepines (Dost *et al*., 2005).

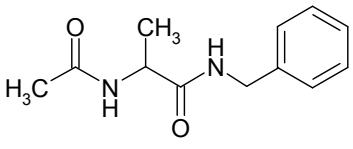
2.3.2.0 NEW SCAFFOLDS AND MECHANISMS

2.3.2.1 Functionalized amino acids

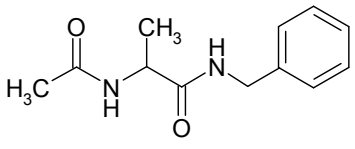
In 1985, Cortes *et al.* reported that the amino acid derivative *N*-acetyl-dl-alanine-*N*-benzylamide was protective in the mouse MES test with modest potency (ED_{50}, 77 mg/kg; Conley and Kohn, 1987). Examination of a large number of derivatives that conform to the general *N*-benzyl-2-acetamido-propionamide structure (R_1 =CH_3; R_3 = phenyl in LXVIII) revealed that certain substitutions at the 2-carbon markedly increased potency. For example, the 2-CH_2OCH_3 derivative (*N*-benzyl-2-acetamido- 3-methoxypropionamide) (LXIX) had ED_{50} of 8.2 mg/kg (mouse) and rotorod toxicity (TD_{50}) of 43 mg/kg, so that its potency and protective index (TD_{50}/ED_{50}, 5.2) was comparable to phenytoin (Choi *et al*., 1996). These compounds—which are referred to as *functionalized amino acids*—have an asymmetric carbon at position 2. In general, the (*R*)-enantiomers exhibit greater potency than the (*S*)-enantiomers. In the case of the 2-CH_2OCH_3 derivative, the (*R*)-enantiomer [lacosamide (formerly harkoseride)] has about twice the potency of the racemic mixture (ED_{50}, 4.5 mg/kg in mouse MES test) whereas the (*S*)-enantiomer is inactive (Hovinga, 2003). Functionalised amino acids generally lack activity in the PTZ test.



(LXV III)



(LXX)(



(LXIX)

Indeed, lacosamide (LXIX) is also inactive against clonic seizures induced by bicuculline and picrotoxin at doses below those that cause rotorod impairment. Lacosamide (LXIX) has activity against hippocampal-kindled seizures, as do marketed AEDs including phenytoin, carbmazepine and valproate, although there is a suggestion that it may be modestly more efficacious. Overall, lacosamide (LXIX) does not appear to have a profile of activity in animal seizure models that distinguishes it from sodium channel blocking agents. However, cellular electrophysiological studies have failed to reveal an effect of the drug at concentrations as high as 100 μM on voltage-activated sodium channels or on other channel types, including voltage-activated calcium-channels (L-, N-, P- or T-type) or voltage-activated potassium channels (delayed rectifier, KCNQ2/3). The drug also does not block synaptic transmission in the hippocampal slice preparation, although it does produce a stereoselective block of spontaneous and evoked epileptiform discharges in the presence of 4-AP that resembles the action of carbamazepine (Lees *et al.,* 2006). It has been suggested that lacosamide may act as an antagonist of NMDA receptors through an interaction with the strychnine-insensitive glycine site. Radioligand binding experiments have provided inconsistent results and generally do not support this conclusion, as do recordings from recombinant NMDA receptors expressed in *Xenopus* oocytes (Troughton and Kleckner, 2005). However, these recording indicate that the drug may allosterically block NMDA receptors with a specific action on receptors containing the NR2B subunit. If this observation can be confirmed, lacosamide (LXIX) would have some similarity with felbamate (XLII), which also is low affinity antagonist of NMDA receptors with preference for the NR2B

subunit (Kleckner *et al.,* 1999b; Harty and Rogawski, 2000). Lacosamide (LXIX) has good oral bioavailability (~100%), linear pharmacokinetics, a half-life (~12 h) that will allow twice-daily dosing, insignificant protein binding (<1%), and its metabolites are largely eliminated by the kidney so that there are no significant interactions with other AEDs (Hovinga, 2003). Thus, lacosamide (LXIX) has nearly ideal pharmacokinetic properties. Phase II clinical studies have been promising and Phase III trials are in progress.

Harold Kohn's laboratory has prepared 250 distinct functionalized amino acids. Among these structures, 12 provide protection in the MES test at equal or greater potency to phenytoin (Shen *et al.,* 2004). Compounds within several related structural series including amino acid amides (Beguin *et al.,* 2004) and functionalized amino ketones (Beguin *et al*., 2003) also exhibit anticonvulsant activity in the MES test. Like lacosamide (LXIX), these compounds are inactive in the PTZ model, suggesting that they act through a similar mechanism. Despite the comprehensive effort, it has not been possible to identify structures with superior properties to lacosamide (LXIX).

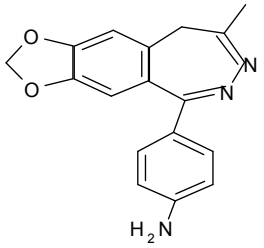
2.3.2.1 AMPA receptor antagonists

AMPA receptors are key mediators of seizure spread and may also play a role in seizure-induced brain damage. Moreover, AMPA receptor antagonists have a broad spectrum of activity in animal seizure models. Thus, AMPA receptor antagonists could have broad utility in epilepsy therapy, both as conventional AEDs and also for the acute treatment of status epilepticus (Rogawski and Donevan, 1999). The first selective AMPA receptor antagonists to be identified

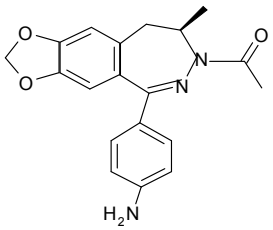
were quinoxalinedione derivatives which block AMPA receptors through a competitive interaction at the glutamate recognition site of the receptor. Such agents are effective in the MES and PTZ tests and also in kindling models (Yamaguchi *et al*., 1993; Loscher *et al.,* 1993; Loscher and Honack, 1994; Rogawski *et al*., 2001; Sarro *et al.,* 2003; Barton *et a*l., 2003). However, they tend to produce motor impairment at doses similar to those that are protective against seizures. The early quinoxalinediones had poor solubility so that they precipitated in the kidney, leading to crystaluria and nephrotoxicity. However, newer quinoxalinedione analogs and non-quinoxalinedione congeners have been synthesized that largely overcome this problem (Loscher and Rogawski, 2002). Other problems with the quinoxalinediones include sedation and negative impact on the level of conciousness (Walters *et al*.,2005) At present, no competitive AMPA receptor antagonists are under development for the chronic treatment of epilepsy.

Some years after the development of quinoxalinedione competitive AMPA receptor antagonists, a novel class of AMPA antagonists was identified that exert their blocking action in a mechanistically distinct fashion (Rogawski, 1993). These 2,3-benzodiazepines, such as GYKI 52466 (LXXI), do not interact with the AMPA recognition site, but appear to block AMPA receptors in a non-competitive fashion via an allosteric site on the receptor–channel complex (Donevan and Rogawski, 1993). Like competitive AMPA antagonists, allosteric antagonists such as LXXI have anticonvulsant activity in a broad spectrum of animal seizure models, including the kindling model (Yamaguchi *et al.,* 1993; Loscher and

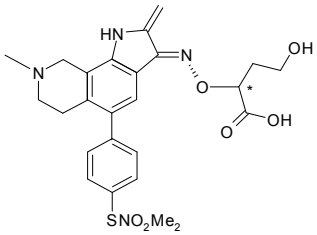
Rogwsky, 2002). However, as is the case for the quinoxalinediones, the protective index of 2,3-benzodiazepines is low. At present, the LXXI analog talampanel (LXXII) is undergoing Phase III clinical trials. LXXII is protective in the MES test in mice and also in various chemoconvulsant models, but only at doses near those that cause motor side effects (Andrasi, 2001). In clinical trials talampanel has been reported to be well tolerated, but sedation may occur, especially with initial dosing (Chappell *et al.,* 2002; Langan *et al*., 2003; Danielsson *et al*., 2004).NS1209 [8-methyl-5-(4-(*N*,*N*-dimethylsulfamoyl)phenyl)-6,7,8,9,-tetrahydro-1*H*-pyrrolo[3,2-*h*]-iso-quinoline2,3-dione-3-*O*-(4-hydroxybuty-ricacid-2-yl)oxime] (LXXIII) is a structually novel, water soluble competitive AMPA receptor antagonist with good central nervous system bioavailability (Nielsen *et*



(LXXI)



(LXXII)



(LXXIII)

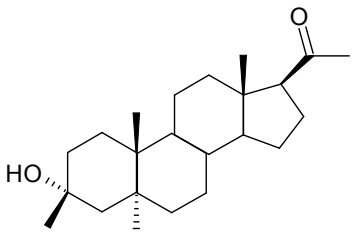
al., 1999). LXXIII elevates the threshold for tonic electroshock-induced seizures in mice with prolonged duration of action. The drug is also able to effectively terminate kainate-induced status epilepticus in rats even when administered 2 h after the onset of seizures. LXXIII has been reported to be well tolerated in Phase I/II clinical trials and is being evaluated for the treatment of refractory status epilepticus under the theory that it may not only terminate seizures, but also protect against status epilepticus-induced brain damage (Roawsky, 2006).

Since sedation may be a limiting factor in the use of AMPA receptor antagonists, the focus on the acute treatment of status epilepticus is reasonable.

2.3.2.2 Neuroactive steroids

Endogenous steroid hormones including progesterone, deoxycorticosterone and testosterone, when reduced at the 5- and 3-positions of the steroid A-ring, form metabolites that lack classical hormonal activity but instead are high affinity, positive allosteric modulators of $GABA_A$ receptors. These neuroactive steroids have all of the expected properties of $GABA_A$ receptor modulators in animal models, including anticonvulsant activity (Rogawski and Reddy, 2004). The precursor hormones progesterone and deoxycorticosterone are also anticonvulsants, by virtue of their conversion to the neuroactive steroid products (Kokate *et al.,* 1999; Reddy and Rogawski, 2002). In fact, a precursor loading strategy with progesterone has shown an indication of efficacy, at least for the treatment of catamenial epilepsy (Herzog, 1995). A more direct approach is to use neuroactive steroids themselves. A large number of analogs of the natural steroids have been demonstrated to be $GABA_A$ receptor modulators (Kokate *et al.,* 1994; Hamilton, 2002). Among these analogs, ganaxolone (LXXIV), the 3β-methyl analog of the endogenous neurosteroid allopregnanolone has been most extensively evaluated, and early clinical trials have been completed (Monaghan *et al.,* 1999). Ganaxolone (LXXIV) is a potent positive modulator of GABA receptors containing α1, α2 or α3 subunits and does not show dramatic α-subunit selectivity (Carter *et al.,* 1997). Indeed, unlike benzodiazepines, neuroactive steroids have activity on all $GABA_A$ receptor

isoforms, including those composed of α4 and α6 subunits that lack γ^2 (Lambert *et al*., 2001); δ-subunit containing receptors are especially sensitive to neurosteroids (Wohlfarth *et al.,* 2002). Such δ-containing receptors are mainly localized persynaptically or extrasynaptically (Nusser *et al.,* 1998; Farrant and Nusser, 2005) where they mediate tonic current (Stell *et al*., 2003), and likely represent an important target for the anticonvulsant activity of neuroactive steroids.



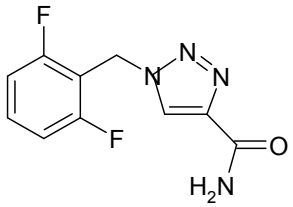
(LXXIV)

Ganaxolone (LXXIV) has a profile in seizure models similar to that of other $GABA_A$ receptor modulators. It is highly active in the PTZ model and only at higher doses in the MES test (Carter *et al.,* 1997). Ganaxolone (LXXIV) and related neurosteroids also have activity in the 6-Hz model (Kaminski *et al.,* 2004), against amygdale-kindled seizures (Reddy *et al*., 2004), and in various models of status epilepticus (Kokate et al., 1996). In contrast to benzodiazepines whose clinical utility in the chronic treatment of epilepsy is limited by tolerance, the anticonvulsant efficacy of neuroactive steroids, at least in animal models, does not diminish with chronic treatment (Kokate *et al*., 1998; Reddy and Rogawski, 2000a). Open label clinical studies have provided indications of efficacy in infantile spasms and complex partial seizures (Monaghan *et al*., 1999). There is evidence from studies in an animal model of perimenstrual catamenial epilepsy

that neurosteroids, including ganaxolone, have greater activity at the time of enhanced seizure susceptibility (Reddy and Rogawski, 2000b, 2001). Although there is anecdotal evidence that ganaxolone may be useful in catamenial epilepsy (McAuley *et al.,* 2001), controlled clinical trials are required.

2.3.2.3 Rufinamide (LXXV)

Rufinamide [1-(2,6-difluorophenyl)methyl-1*H*-1,2, 3-triazole-4-carboxamide;] (LXXV) is a triazole compound that bears some structural similarity with lamotrigine (XLVI). Rufinamide (LXXV) is effective in the mouse MES test (ED_{50}, 15.5 mg/kg, i.p.) and at higher doses in the PTZ test (54 mg/kg) (Schmutz *et al.,* 1993; White *et al.,* 2005). It is ineffective against absence-like seizures in WAG/Rij rats. The drug has very low propensity for toxicity in the rotorod test TD_{50}, >500 mg/kg, i.p.). Overall, rufinamide (LXXV) has a distinctive profile in animal testing—activity in both MES and PTZ models and a very high protective index—that differentiates it from marketed AEDs. The efficacy of rufinamide (LXXV) in the MES test is compatible with sodium channel blocking activity. In fact, the drug has been reported to prolong the inactivation of sodium channels and to limit the frequency of action potential firing in cultured and acutely isolated neurons (Karolchyk and Schmidt, 2002; McLean *et al*., 2005). In other studies, there were no effects on sodium channels at the concentrations tested (Vickery *et al.,* 2004). In view of its unique profile, it seems unlikely that effects on sodium channels entirely explain the anticonvulsant activity of rufinamide. To date, no alternative mechanisms have emerged: there is no evidence for interactions with GABA or glutamate systems.

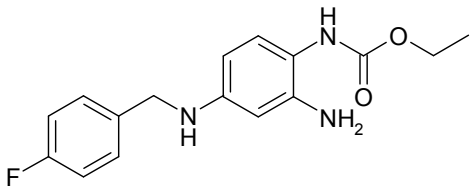


(LXXV)

Several late stage clinical trials of rufinamide have been completed in patients with partial seizures, primary generalized tonic–clonic seizures and the Lennox–Gastaut syndrome. Some of these studies reached statistically significant endpoints and applications with regulatory agencies to market the drug have been filed.

2.3.2.4 Retigabine (LXXVI)

Retigabine [*N*-(2-amino-4-[fluorobenzylamino]- phenyl)carbamic acid; D-23129] (LXXVI), the desaza-analog of flupirtine (a non-opiate analgesic approved in Europe for general nocioceptive pain), was originally identified as an anticonvulsant, because of its activity in the MES test (ED_{50}, 9.3 mg/kg, i.p., mice) (Blackburn-Munro *et a*l., 2005). Retigabine (LXXVI) also has activity in the PTZ test and various other epilepsy models, including seizures induced by kainate, NMDA and picrotoxin (Rostock *et al.,* 1996); it is exceptionally potent in the amygdala kindling model (Tober *et al.,* 1996). Retigabine (LXXVI) has undergone clinical testing in several Phase II clinical trials, largely in patients with partial seizures with or without secondary generalization who were refractory to available therapies. Indications of efficacy were obtained in open label trials, with responder rates in the range of 35–44% (=50% reduction in seizure frequency) (Sachdeo *et al*., 2005).



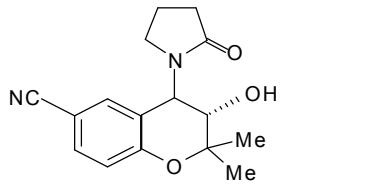
LXXVI

The principal action of retigabine is to shift the activation of KCNQ M-current to more hyperpolarized membrane potentials, and also to slow their deactivation and accelerate their activation (Tatulian *et al*., 2001; Tatulian and Brown, 2003). The critical action of the drug is to increase potassium current near resting potential, which reduces excitability and presumably is responsible, at least in part, for the anticonvulsant effect of retigabine.

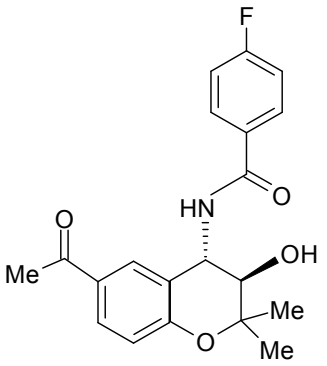
2.4 NEW ANTICONVULSANT AGENTS: STRUCTURE ACTIVITY RELATIONHIPS

2.4.1 Heterocyclic Analogs of Carabersat

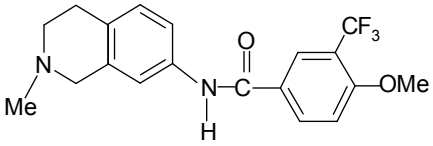
Carabersat (SB-204269) (LXXVII) was originally designed by modifying the structure of cromakalim LXXVIII). This later drug represents a class of antihypertensive agents which act via the relaxation of vascular smooth muscle caused by the opening of ATP-sensitive potassium channels. The potassium channel activators which more readily penetrate the central nervous system (CNS) may have therapeutic potential in the treatment of epilepsy (Coford *et al.,* 2002). Replacement of the 2-pyrrolidinone group of cromakalim by the fluorobenzoylamino group has introduced anticonvulsant activity (Blackburn, *et al*., 1995). Carabersat, the benzopyran derivative, is a chemically novel AED with a novel mechanism of action and a stereospecific CNS binding site. It has potent oral anticonvulsant properties in a range of rat seizure models, with potency and efficacy equivalent to or better than carbamazepine and lamotrigine. Carabersat



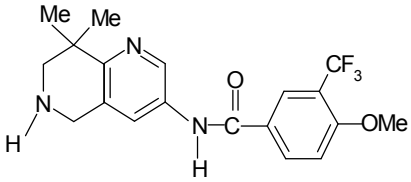
LXXVII



LXXVIII



LXXIX



LXXX

is currently being proposed for the treatment of epilepsy and migraine prophylaxis (Chan*, et al.,* 1996; Herdon, *et al.,* 1997). Subsequent exploration of structure activity-relationships, using high-throughput screening of the novel SB-204269 (LXXVII) binding site led to the identification of anticonvulsant active series of the isomeric tetrahydro- isoquinolinyl (THIQ) benzamides 5-, 7- and 8-substituted (Chan, *et al*., 1998; Chan *et al.,* 2000). Compound SB-270664 (LXXIX), the 7-substituted THIQ was reported as a promising key lead compound with high affinity at the [^{3}H]-SB-204269 (LXXVII) binding site (p*K*i 8.9), but was later found to have poor pharmacokinetic properties (Chen *et al.,* 2000). The major routes of metabolism of this compound were hydroxylation of the THIQ benzo ring, *N*-demethylation and aromatization to the isoquinolinium species. Starting from a series of 7-linked THIQ derivatives, a new series of 8,8-dimethylnaphthyridine compounds has been identified.In this series a *gem* dimethyl group is incorporated to prevent aromatization, and replacement of the

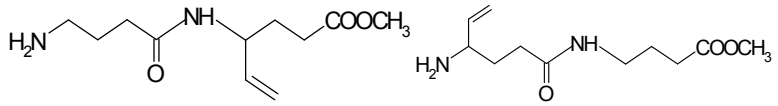
THIQ benzo ring with pirydyl represents an attempt to reduce hydroxylation. In this new series, compound LXXX was identified as new potential agent displaying excellent anticonvulsant activity and an encouraging pharmacokinetic profile *in vivo*. LXXX has high affinity at the [^{3}H]-SB-204269 (LXXVII) binding site (p*K*i 8.7), suggesting a novel mechanism of action, comparable with carabersat. *In vivo*, the rat maximal electroshock threshold (MEST) test at 2 mg kg-1 p.o., compound LXXX showed a good level of anticonvulsant activity. In the rat supraMES model, LXXX produced an ED_{50} value of 3.9 mg kg-1 when analysed by 'ALLFITrd-quo'. This figure is comparable with that obtained with carabersat in the same model under identical conditions (ED_{50} of 6.3 mg kg-1). LXXX has excellent aqueous solubility (>1 mg ml^{-1}) and has been shown to have an encouraging pharmacokinetic profile and good *in vivo* activity in preclinical anticonvulsant models in rats [Austin, *et al.,* 2003].

2.4.2.0 AMIDES

2.4.2.1 Analogues of γ-vinyl GABA

Vigabatrin (γ-vinyl GABA) is an anticonvulsant agent with a mechanism reportedly based on an inhibitor of GABA aminotransferase (Czuczwar *et al.,* 2001). Lee and coworkers have developed several analogs of vigabatrin as potential dual acting prodrugs which were covalently coupled with an amide bond of vigabatrin and GABA mimetic substances such as GABA, γ-vinyl GABA, valproic acid, isonipecotic acid, nipecotic acid and 2-pyrrolidinone (Kim *et al.,* 2000; Zhao *et al.,* 2004). Most of these compounds have shown moderate anticonvulsive activities. Among them, compounds LXXXI and LXXXII displayed the most potent anticonvulsive activity and a broader spectrum when compared

to vigabatrin (XL).Compounds LXXXI and LXXXII were active against MES (ED_{50} = 0.64, 0.76), scPTZ (ED_{50} = .58, 0.40), BIC (ED_{50} = 0.47, 0.34) and PCR (ED_{50} = 0.47, 0.49 mmol kg-1) seizure tests respectively.

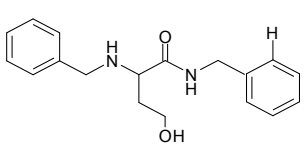


LXXXII LXXXI

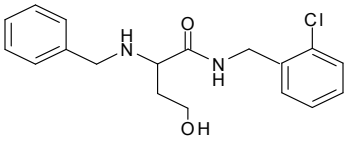
2.4.2.2 N-Benzylamides of γ-hydroxybutyric Acid

Derivatives of α-substituted γ-amino-, γ-phthalimido-, γ-acetoxy- and γ-hydroxy butyric acid, such as acids, esters and amides, have been investigated as new potent anticonvulsants (Malawska *et al.,* 1995, 1997, 1999 a,b, 2003, 2001, 2004; Mendyk *et al.,* 2001). It has been shown that α-substituted *N*-benzylamides of γ-hydroxybutyric acid are the most potent compounds in this group, possessing anticonvulsant activities in MES (i.p. in mice) screens. The most potent anticonvulsants were α-(benzylamino)-γ-hydroxybutyric acid *N*-benzylamide LXXXIII and *N*-(2-chlorobenzylamide) LXXXIV; their ED_{50} being respectively 63.0 and 54.0 mg kg-1. In the MES screen, these compounds were less active than the commonly used anticonvulsants carbamazepine and phenytoin, but possessed higher activity than sodium valproate (Malawska *et al*. 1999). Biochemical tests have indicated that the active amides act as allosteric modulators of the γ-aminobutyric acid, $GABA_A$ complex, and have an affinity to voltage-sensitive calcium channel receptors. *N*-(4-methylbenzyl)amide of α-(4-phenylpiperazin-1-yl)-γ- hydroxybutyric acid (LXXXV) displayed the binding of [^{35}S] TBPS ([^{35}S] *tert*-buthylbicyclophosphorothionate) to the chloride channel of the $GABA_A$ receptor complex (IC_{50} = 95 μM) (Malawska *et al*. 1999). This may be the possible mechanism mediating the anticonvulsant effect of these compounds.

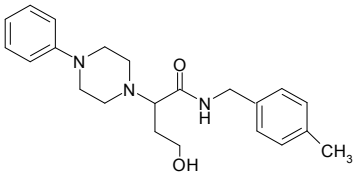
The results of pharmacological *in vivo* experiments with the γ- hydroxybutyric acid amide analogues LXXXIII and LXXXIV have shown that the compounds possess variable influence on the CNS in mice (Salat *et al*, 2002).



LXXXIII



LXXXIV



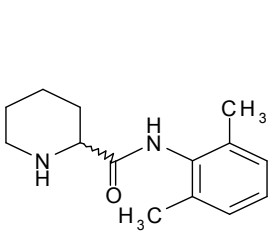
LXXXV

SAR studies have enabled the definition of structural elements responsible for the anticonvulsant activity of these several series of compounds (Malawska *et al.* 2001, 2004). These features are as follows: the presence of the *N*-benzylamide fragment, a hydrophobic unit (aryl ring) as a distal binding site and a group which could act as an H-bond donor. It was also concluded that a hydroxyl group was necessary for MES activity, and more lipophylic compounds showed better anticonvulsant properties (Malawska *et al*. 2003).

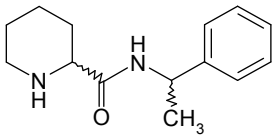
2.4.2.3 2,6-dimethylanilides, Carboxamides

The activity of several 2-piperidinecarboxyamides in the MES test in mice has been reported (Hinko *et al*., 1996; Ho, *et al.,* 1998). Receptor binding studies indicate that these amides demonstrated weak binding affinity at the phencyclidine (PCP) site on the *N*-methyl-*D*-aspartate (NMDA) receptor complex; however, a correlation between affinity and seizure protection in the MES test was not observed. Using *N*-(2,6-dimethyl)phenyl-2-piperidinecarboxyamide

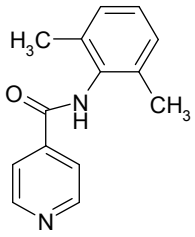
(LXXXVI) and *N*(α-methylbenzyl)-2-piperidinecarboxamide (LXXXVII) as structural leads, a variety of analogues have been synthesized and evaluated for



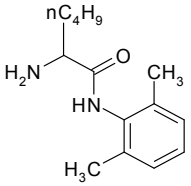
LXXXVI



LXXXVII



LXXXVIII

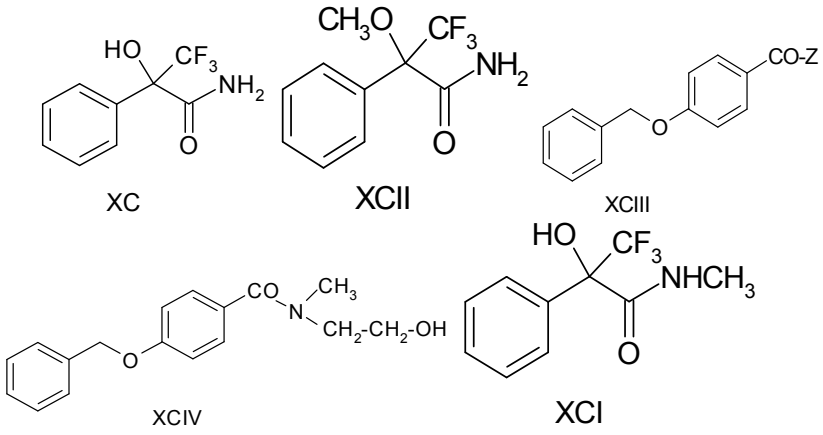


LXXXIX

anticonvulsant activity in the MES test in mice (Ho*, et al*. 2001). The following modifications led to an increase in MES activity: replacement of the piperidine ring with pyridine and movement of the carboxamide group to the 4-position, then opening the piperidine ring. The 2,6-dimethylanilides were the most potent compounds in the MES test in each group of compounds. The 4-pyridine-carboxamide (LXXXVIII) (ED_{50} = 9.7 mg kg-1, TD_{50} = 53.3 mg kg-1, PI = 5.5) was highly active in the MES test in mice. The norleucine derivative (LXXXIX), obtained by opening the piperidine ring between the 1- and 6-positions was among the most potent compounds in the MES test in these series of compounds (ED_{50} = 5.8 mg kg-1, TD_{50} = 36.4 mg kg-1, PI = 6.3). In these derivatives, the lipophilicity of the compound and the substitution at the α-position of the amino acid derivative played key roles in quantitative anticonvulsant activity. Pyridinecarboxamide (LXXXVIII) and norleucinecarboxamide (LXXXIX) were selected as useful leads in the development of compounds with therapeutic potential in the treatment of tonic-clonic and partial seizures in humans.

2.4.2.4 Hydroxyamides

Brown and coworkers have evaluated a series of novel hydroxyamides (Brown *et al*., 1999; Schenck *et al,*. 2004]. Anticonvulsant testing of these compounds revealed the lead, 3,3,3-trifluoro-2-hydroxy-2- phenyl-propionamide (XC), to have potent nticonvulsant activity. Anticonvulsant evaluation of XC administered i.p. in mice demonstrated complete (3/3 mice) protection at a dosage of 100 mg kg-1 up to 4 hr when challenged with MES. XC also demonstrated effectiveness against scPTZ (5/5 mice protected) for 0.5 hr at the same dosage. In rats p.o. test (XCprotected): the MES ED_{50} value was found to be 9.9 mg kg-1, the scPTZ ED_{50} was 34 mg kg-1 and the TD_{50} noted at 100 mg kg-1, yielding a therapeutic index of 10 for the MES model and 2.6 for the scPTZ model. In this new series of compounds, two, XCI and XCII, were the most active. Analogue XCI's MES ED_{50} was also determined to be 62.4 mg kg-1 but has notably longer-lasting effects



(up to 6 hr) and a TD_{50} over 100 mg kg-1. Patch clamp electrophysiology studies demonstrated significant tonic blockade of T-type calcium current by compounds XC-XCII at 1mM. Furthermore, compounds XC and XCI induced a significant use-dependent blockade of T-type calcium current. These results suggest that the mechanism of anticonvulsant activity may include blockade of T-type calcium

currents. Compounds XCI and XCII are methylated versions of compound XC at the amide and alcohol, respectively. Summing up, compound XC is an active orally available anticonvulsant with similar activity to phenytoin, and its methylated alcohol and amide have shown similar activity.

2.4.2.5 Alkanolamides

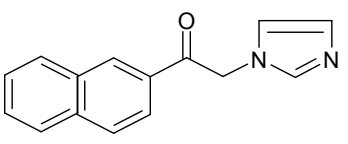
In the group of alkanolamides XCIII, the most interesting results were yielded by 2-*N*-methylaminoethanol derivative (compound XCIV), which displayed anti-MES activity (i.p. in mice) with a PI of 2.54 (ED_{50} of 51.8 mg kg-1), higher than that for valproate (ED_{50} = 287, PI 1.7) in the same test (Marona, *et a*l. 2003).

2.4.3.0 HETEROCYCLIC AGENTS

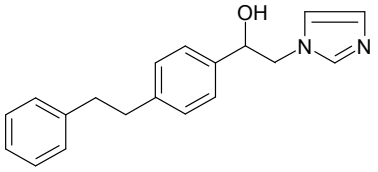
2.4.3.1 (Arylalkyl) imidazoles.

One of the structurally distinct classes of antiepileptic drugs is the (arylalkyl)imidazoles.Denzimol (+/-)-N-[β-[4(β̃-phenylethyl)phenyl]-βhydroxyethyl]-imidazole (XCVI) and nafimidone (1-[2-naphthoylmethyl)imidazole (XCV) are examples of a class of anticonvulsants; the (arylalkyl)imidazoles (Edafiogho and Scott, i996).SAR studies show that anticonvulsant properties of this group are associated with the presence of a small oxygen functional group (such as carbonyl, ethylene dioxy, methoxy, acyloxy and hydroxyl substituents) in the alkylene bridge in addition to an imidazole ring and a lipophilic aryl portion facilitating penetration of the blood barrier. The introduction of oxime and oxime ether groups to the alkylene bridge of (arylalkyl)imidazole as a small oxygen functional group led to new compounds, which displayed various levels of activity

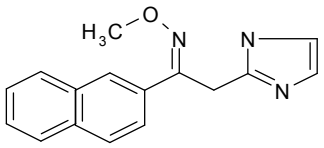
(Karakurt *et al.* 2001). Nafimidone oxime did not exhibit any anticonvulsant activity. *O*-Alkylation of nafimidone oxime, i.e. addition of an oxime ether functional group to the Alkylene Bridge of nafimidone, resulted in new compounds possessing anticonvulsant properties. The size of the alkyl moiety on the oxime group appears to be important for these properties. The *O*-alkyl substituted ompounds (XCVII-XCVIII) were found to be more active than the *O*-arylalkyl substituted compounds. Anticonvulsant activity against MES-induced convulsion showed that compound XCVII *E* (trans) isomer, was the most active with ED_{50} 17.95 mg kg-1 and TD_{50} < 150 when the drug was administered i.p. in rats. Compound XCVII and XCVIII showed ED_{50} values of 46.77 and 36.99 mg kg-1 i.p. and PI = 1.417 and 1.411 respectively in MES test in mice.



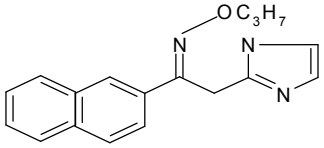
XCV



XCVI



XCVII

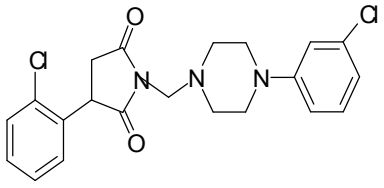


XCVIII

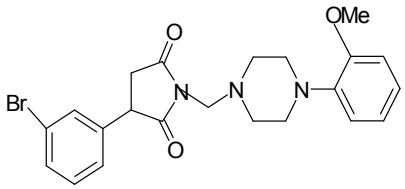
2.4.3.2 Pyrrolidin-2,5-diones

Ethosuximide (XI), a derivative of pyrrolidin-2,5-dione, belong to a group of old antiepileptic drugs and is still used in the treatment of epilepsy. A great number of 3-phenylpyrrolidine-2,5-dione derivatives with pyridyl-, aryl- and aminophenyl-moiety at the nitrogen atom, as well as 3-arylpyrrolidine-2,5-dione containing a 4-

arylpiperazinyl-1-yl-alkyl moiety at the nitrogen atom and 2-aza-spiro[4.4]nonane-1,3-dione have been investigated by Obniska, Zejc and coworkers (Zejc, *et al*., 1990; Obniska *et al.* 1998,1999, 2002, 2003). Pharmacological studies have shown anticonvulsant activity in some compounds of that group. Recently, the most potent in the series of N-[(4-arylpiperazin-1-yl)methyl derivatives of 3-arylpyrrolidine-2,5-dione were compounds XCIX and C. In the MES test in rats (after p.o. administration) the best ED_{50} = 14.18 mg kg-1 and PI = 8.8 were recorded for compound XCIX. Compound C was less active, with ED_{50} =133.64 mg kg-1 and PI = 2.47. SAR studies in this group of pyrrolidin-2,5-dione derivatives have led to a conclusion that the following structural elements are required for anticonvulsant activity: an aromatic ring at the 3-position of pyrrolidine-2,5-dione moiety and a 4-arylpiperazine fragment with selected



XCIX



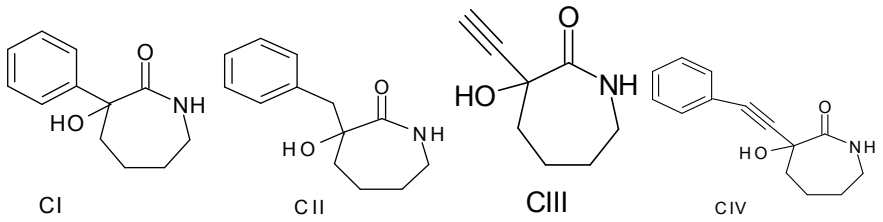
C

substituents at the phenyl ring. The introduction of a spirocyclopentyl ring at the 3-position of pyrrolidine-2,5- dione did not enhance the anticonvulsant activity.

2.4.4 Lactams

Derivatives of cyclic lactams were synthesized and evaluated for antiepileptic activity (Brouillette *et al.,* 1984; Reddy *et al.,* 1996, 1997; Grimm *et al*., 2003]. One of the compounds, α-hydroxy-α-phenylcaprolactam (CI) was particularly interesting. I.p. administration of CI in mice, resulted in potent anticonvulsant

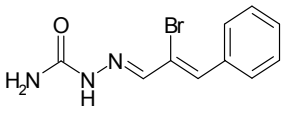
protection in both anticonvulsant models tested (MES- and scMet-induced convulsions), exhibiting an anti-MES ED_{50} = 63 mg kg-1 and anti-scMet ED_{50} 74 mg kg-1. New synthesized analogues of this compound, α-benzyl-α-hydroxy-caprolactam (CII), α- ethynyl-α-hydroxycaprolactam (CIII) and α-hydroxy-α-(phenylethynyl)caprolactam (CIV) displayed significant anticonvul-sant activity (Grimm, *et al*., 2003). For these compounds (i.p. in mice) anti-MES activity was seen at 100 mg kg-1 for CII, anti-scMet at 300 mg kg-1 for CIII and anti-MES and anti-scMet at 100 mg kg-1for CIV. Compound CII exhibited (oral activity in rats) an anti-MES ED_{50} = 86 mg kg-1 and anti-scMet ED_{50} < 83 mg kg-1. Given that the Rotorod TD_{50} was greater than 330 mg kg-1, this particular lactam exhibited therapeutic indices exceeding 3.9 (MES) and 4.0 (scMet). These results showed that α-benzyl-α-hydroxycaprolactam (CII) was effective in halting seizures, while



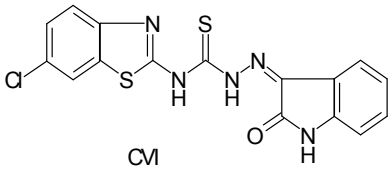
α-ethynyl-α- hydroxycaprolactam (CIII) was a selective inhibitor of petite mal seizures. Compound CIV protected rats against MES-seizures at 30 mg kg-1; no toxicity was noted. The difference in activity between CIII and CIV was postulated to be a result, at least partly, of the significant change in log *P* from the unsubstituted terminal alkyne (-0.27) to the phenyl-substituted alkyne (1.52). The potent activity of CIV in all models indicated that the substituted alkynylcapro-lactams represent a new anticonvulsant structural class.

2.4.5 Semi- and Thiosemicarbazones

Semicarbazones and related compounds have a documented and consistent role in the design of novel anticonvulsant agents, through the work of Dimmock's and Pandeya's research group (Dimmock *et al*., 1995 a,b; 1996 and 2000 a,b; Puthucode *et al.*, 1998; Pandeya, 1999, 2000 a, b, and 2003; Yogeeswari *et al.,* 2002). A number of semicarbazones, thiosemicarbazones, *bis*-carbohydrazones, aryl, arylidene, aryloxyarylsemicarbazones, acetylhydrazones and oxamoylhydrazones have been synthesized and evaluated for anticonvulsant activity. Extensive SAR studies have led to postulating a specific binding site of semicarbazones. The proposed pharmacophoric requirements in the semicarbazone molecules are: 1) aryl binding site with a hydrophobic group; 2) hydrogen bonding domain exemplified by the presence of the –NHCO- grouping; 3) two electron donor systems; 4) hydrophobic binding site whose size determines the type of activity.



CV



CVI

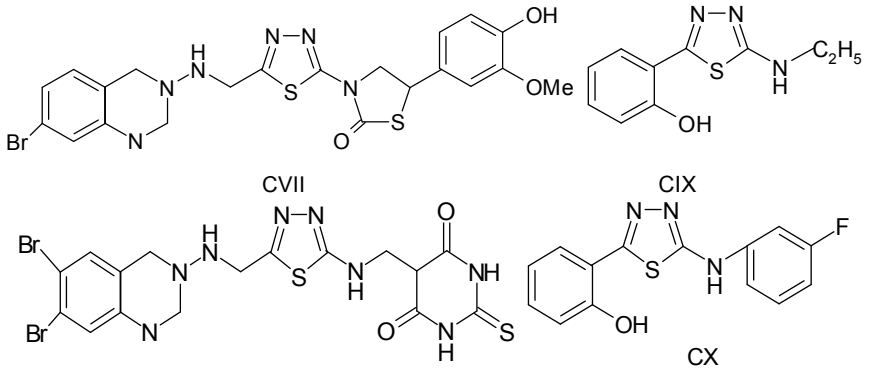
Derivatives of (aryloxy)aryl semicarbazones displayed greater protection in the MES test than the scPTZ screen (Dimmock *et al* 1996).Quantification of approximately one-third of over 100 compounds tested revealed an average protection index of approximately 9. Following oral administration to rats, a number of compounds displayed significant potencies in the MES screen (ED_{50} value of 1-5 mg kg-1) accompanied by a very high protection index. Later studies enabled the selection, in the series of aryloxyaryl semicarbazones, of a lead

molecule (CV). ED_{50} values of compound CV in the MES and scPTZ screens (i.p. in mice) were 48.6 and 94.1 mg kg-1, with PI figures of 4.20 and 2.17 respectively (Puthucode *et al.,* 1998). Recently, compound CVI [4-(6-chlorobenzo-thiazol-2-yl)-1-(3-isatinimino)thiosemicarbazone] has also shown strong activity in MES seizures and scPTZ screens (Yogeeswari, *et al*., 2002). Knowing that isatine derivatives possess anticonvulsant properties (Popp, 1984; Pendaya *et al.,* 2001), compound CVI was designed as hybrid molecule incorporating a thiosemicarbaz-one fragment and an isatine molecule. Compound CVI was more potent than valproate against MES and scPTZ tests and was more effective than ethosuximide against MES-induced seizures, with an ED_{50} of 17.86 and 6.07 mg kg-1 i.p. in mice and rat p.o., respectively. Compound CVI exhibited a PI value of 8.77 in the rat oral identification in the MES screen. In the preliminary hippocampal-kindling screen in rats (i.p.), compound CVI showed weak ability to block the expression of fully kindled seizures. The potency and spectrum of activity of these 6- chlorobenzothiazolyl thiosemicarbazones were comparable to those of standard drugs, and represent a structurally novel class for subsequent molecular modifications.

2.4.6 Thiadiazoles and Quinazolin-4(3H)-ones

New thiadiazolyl and thiazolidinonyl quinazolin-4(3*H*)-ones have been synthesized and screened for their anticonvulsant activity comparing with the standard AEDs (Srivastava *et al*., 2002). These hybrid molecules comprise two fragments, quinazolinone and thiazolidinone, whose derivatives have been found to show anticonvulsant properties (Chapleo, *et al.,* 1986). Out of the 30 new

hybrid compounds, the most active was CVII**.** Compound CVII at three graded doses of 7.5, 15 and 30 mg kg-1 (i.p. in mice) in MES models was found to possess more potency than phenytoin and to be equipotent to lamotrigine at all the three dosage levels. In the scPTZ model, CVII exhibited better anticonvulsant activity than sodium valproate at all the three tested dosages (18.5, 37 and 74 mg kg-1). SAR studies have indicated that compounds having a 3-amino-2-methyl-6-bromoquinazolin- 4(3*H*)-aryl moiety showed more protection than compounds with a 3-amino-2-methyl-quinazolin-4(3*H*)-aryl moiety. Another group of new hybrid molecules represent derivatives of 1,3,4-thiadiazoles has been obtained by adding two heterocyclic nuclei possessing anticonvulsant activity, namely barbituric acid and quinazolinone (Srivastava, *et al.,* 2004). Of the compounds studied, the most active one (CVIII) displayed activity against MES and scPTZ seizure test in mice (i.p.) that was more potent than the standard drug. Compound CVIII at three graded dosages of 17.5, 25 and 50 mg kg^{-1} has been found to incur 20, 40 and 90% protection in the MES model and 20, 50 and 90% protection against seizure in the scPTZ model (respectively).



In the series of 2,5-disubstituted 1,3,4-thiadiazoles, two active anticonvulsant agents (CIX**,** CX) have been found (Dogan *et al.,* 2002). Compounds CIX and

CX have shown maximum protection (90 and 70%, respectively) comparative to sodium valproate 80%) in the scPTZ screen. The ED_{50} values of the most effective compounds CIX and CX were 33 and 66 mg kg-1 respectively.

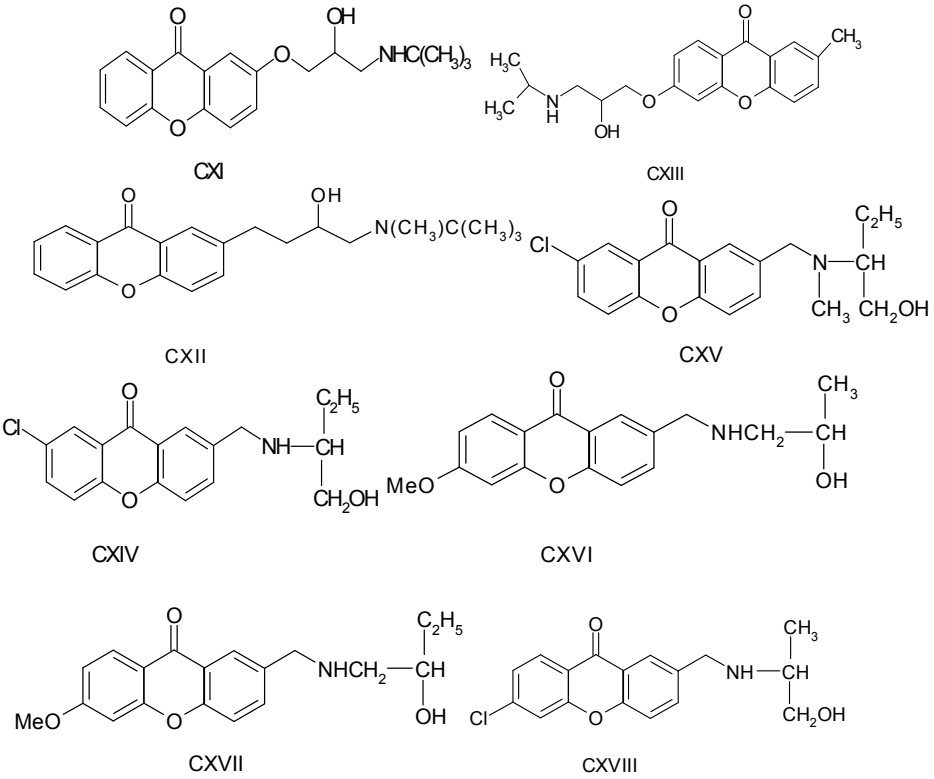
2.4.7 Xanthones

A different group of substituted derivatives of xanthone as new anticonvulsant agents has been discovered by Marona and coworkers (Marona, *et al.,* 1998 a,b,c; 200; Jastrzebska *et al*., 2003). A series of alkanolamides, alkanoamines and aminoalkanolic derivatives of 2- 3-, 6-, 7- xanthone have been obtained and evaluated for anticonvulsant activity in the MES and scPTZ assays. Several compounds have been found to be active in both anticonvulsant tests. In a series of aminoisopropanoloxy derivatives of 2-, 3-, or 6-xanthone, the most promising compounds were the 3-(*tert*-buthyl-amino) (CXI) and 3-[*N*methyl-(*tert*-buthyl)-amino] (CXII) substituted 2-hydroxy-1-(2- xanthonoxy)-propane and compound CXIII (Marona*, et al.,* 2001), of which CXII was active in both anticonvulsant tests (Marona, *et al.,* 1998). Results of quantitative anticonvulsant activity in the MES screen (i.p. in mice) for the tested compounds were as follows: for CXI ED_{50} = 42.2 mg kg-1 PI = 1.9, for CXII ED_{50} = 110.0 mg kg-1 PI > 4.5, for CXIII ED_{50} = 173.45 mg kg-1 PI = 0.80.

In a series of alkanolamides and alkanoamines (Marona, *et al.,* 1998 a,b])2-amino- or 2-*N*-methylamino-1-butanol and derivatives of 7-chloroxanthone (CXIV and CXV) have been found active in both anticonvulsant tests, displaying anti-MES activity (i.p. in mice) with a PI in the range of 2.84-3.64 corresponding with

that for phenytoin, carbamazepine and valproate. In the MES test, both racemate CXV (*R,S*) and CXV (*R*) isomer displayed activity in the similar range [ED_{50} = 27 mg kg-1 PI = 3.28 for CXV (*R,S*) and ED_{50} = 33.4 mg kg-1 PI = 2.848 for CXV (*R*)].

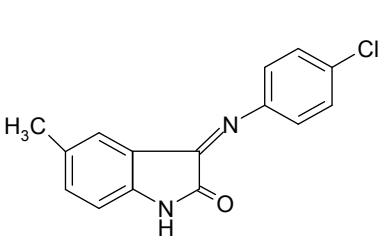
Among derivatives of 6-methoxy- or 6-chloroxanthone, the most active were 2-amino-1-propanol-, 1-amino-2-propanol- and 1-amino-2-butanol derivatives, which displayed anti-MES activity with a PI in the range of 2,21- 5.88. In the MES test (i.p. in mice), ED_{50} values were 33.89, 37.4, 41.2 and 56.2 mg kg-1 for



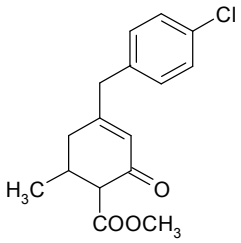
compounds CXVI (*R,S*), CXVI (*S*), CXVII (*R,S*) and CXVIII (*R,S*) respectively (Marona, et al 1998). Recent studies of some central effects of chiral xanthone derivatives demonstrated differing potencies of enantiomers and a racemic mixture of compound CXIII (Jastrzebska, *et al*., 2003).

2.4.8 Derivatives of Isatin

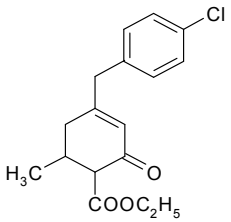
Derivatives of 1,3-dihydro-indol-2-one (isatin) have been reported to possess anticonvulsant activity (Popp, *et al.* 1984; Pandeya, *et al*., 2001). Recent studies of hydrazones, Schiff and Mannich, bases of isatin, have led to the discovery of a new anticonvulsant agent (CXIX) effective in both MES and scPTZ tests (Sridhar *et al*., 2002; Sridhar and Ramesh, 2001b; Verma *et al.,* 2004). Compound CXIX [3-(4-hloro-phenylimino)-5-methyl-1,3-dihydro-indol-2-one] yielded 87% protect-tion at 100 mg kg-1 with an ED_{50} value of 53.61 mg kg-1 in the scPTZ screen after i.p. administration in mice. This new agent was less neurotoxic than phenytoin, and showed greater protection than sodium valproate (Sridhar, *et al.,* 2002).



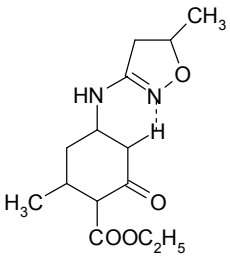
CXIX



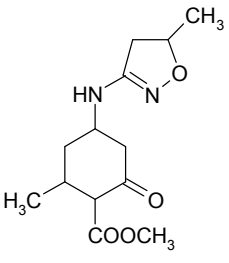
CXX



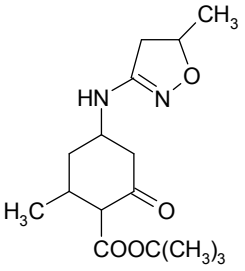
CXXI



CXXII



CXXIII



CXXIV

2.4.9 Enaminones

Scott and coworkers have synthesized a series of enaminones bearing the aniline, benzylamine moieties and various aromatic heterocycles such as the pyridine, phenothiazines, and currently the isoxazole nucleus (Alexander, *et al.,*

1996; Foster, *et al*., 1999; Eddington *et al*., 2000, 2002, 2003; Cox *et al.* ,2001, 2002; Edafiogho, *et al.,* 2002). The prototype compound in the aniline series of enaminone was methyl 4-[(4-chlorophenyl)amino]-6-methyl-2-oxo-3-cyclohex--ene-1-carboxylate (CXX). Their ethyl analogue (CXXI) was found to be the most promising enaminone in the aniline series in both rat and mouse models during preliminary pharmacological evaluation. In MES model seizu-res, in mice, i.p. administration of CXXI resulted in an ED_{50} value of 16.7 mg kg-1 and TD_{50} of 110.7 mg kg-1, PI = 6.6 and an ED_{50} value of 3.0 mg kg^{-1} and TD_{50} > 250 mg kg-1, PI = 83.3 p.o.administration in rats

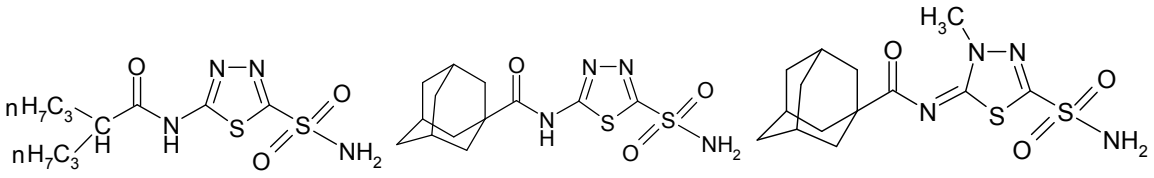
The anticonvulsant activity of a series of enaminone derived isoxazoles provided a potent orally active class of compounds (Eddignton, *et al.,* 2002). Two series of enaminones possessing the isomeric 5-methyl-substituted isoxazoles and 3-methylsubstituted isoxazoles revealed both similarities and differences with regard to anticonvulsant activity. The most potent anti-MES were three compounds in the 3- amino series: ethyl ester CXXII (ethyl 4-(5-methyl-3-isoxazolyl)amino]-6-methyl-2-oxo-3-cyclohexene-1-carboxylate), ED_{50} 68.9 mg kg-1, p.o. in rats TD_{50} > 500 mg kg-1, PI > 49.6; methyl ester (CXXIII), ED_{50} 68.9 mg kg-1 i.p. in mice, TD_{50} > 500 mg kg-1, PI > 7.3, and *tert*-buthyl ester (CXXIV), ED_{50} 28.1 mg kg-1 p.o. in rats, TD_{50} > 500 mg kg-1, PI > 17.8.

2.4.10 Sulfonamides

Acetazolamide, topiramate and zonisamide possess a sulfamate moiety, which is essential for their anhydrase inhibition. Several new carbonic anhydrase (CA)

inhibitors derivatives of sulfonamide have been developed by Scozzafava, Supuran and coworkers (Spuran *et al.,* 1998; Ilies *et al*., 2000, 2004; Scozzafava, *et a*l., 2000; Casini *et a*l., 2000; Mascreel *et a*l., 2001; Ilies, 2004). A series of aromatic/heteroaromatic sulfonamides incorporating valproyl and other lipophylic moieties has been found to possess potent CA inhibitory properties as well as anticonvulsant *in vivo* effects. The hybride ompound, valproyl derivative of acetazolamide (5-valproylamido-1,3,4- thadiazole-2-sulfonamide) (CXXV) was one of the best hCA I and hCA II (human cloned isoenzymes) inhibitors in the series and it exhibited very strong anticonvulsant properties in the MES test in mice (Mascreel *et al.,* 2002). Several other 1,3,4-thiadiazole-sulfonamide derivatives possessing potent CA inhibitory properties and substituted with various alkyl/arylcarboxamido/sulfonamide/ureido moieties in the 5-position have been investigated for their anticonvulsant effects in the same animal model. It was observed that some lipophilic derivatives, such as 5-benzoylamido-, 5-toluenesulfonylamido-, 5-adamantylcarboxamido-, and 5-pivaloylamido1,3,4-thiadiazole-2-sulfonamide, show promising *in vivo* anticonvulsant properties and that these compounds may be considered as interesting leads for developing anticonvulsant or selective cerebrovasodilator drugs (Ilies *et al.*, 2004).

A new series of sulfonamides incorporating adamantyl moieties attached to the scaffolds of aromatic/heteroaromatic sulfonamides have shown good inhibitory potency against two human CA isozymes, hCA I and hCA II (compound CXXVI



CXXV CXXVI CXXVII

and CXXVII). The anticonvulsant activity of these two CA inhibitors against MES tests in mice revealed that after i.p. injection (30 mg kg-1), compounds CXXVI and CXXVII exhibit good protection against electrically-induced convulsions (>90%). Their ED_{50} values were 3.5 and 2.6 mg kg-1, respectively.

2.5 SOURCES OF NEW DRUGS

Broadly speaking, one can discern four main sources of new drugs which include natural products, existing drugs, screens and physiological mechanism.

2.5.1 NATURAL PRODUCTS AS SOURCE OF NEW DRUGS

Historically, natural products provide the oldest source of new drugs. Natural selection during evolution and competition between species has produced powerful biologically active natural products, which can serve as chemical leads, to be refined by the chemist to give more specifically active drugs. Indeed several plants including Viscum capsense (Amabeoku *et al.,* 1998), Spondias mombin Linn (Ayoka *et al.,* 2006), Carimiora edalis (Ruiz *et al*., 1995), Delphinium denadatum (Mohsin, 2003) and Harpagophytum procumbens (Ismail *et al.,* 2006) have been reported to have anticonvulsant activity. Ketogenic diet, a predominantly fatty food, has been employed in the management of epilepsy in

children since 1920. However, to date, no plant product has received official recommendation for use against any form of epilepsy.

2.2.2 EXISTING DRUGS AS A SOURCE OF NEW DRUGS

One of the most fruitful bases for the discovery of a new drug is to start with an old drug. The existing drug may need to be improved, e.g., to obtain a better dosage form, to improve drug absorption, to increase potency, to reduce the daily dose or to avoid certain side effects. Valpromide (XLIX) is twice as potent as valproate (XXVIIa) and has no evidence of tetratogenesis, but is rapidly transformed to valproate (XXVIIa) in humans (Bialer, 1991). However, valnoctamide (L) and disopropylacetamide (LI) exhibit greater anticonvulsant potency than vaproate XXVIIa) and their corresponding acids (Winkler et al., 2005). Studies in human subjects have confirmed that valnoctamide (L) undergo only minimal biotransformation to valnoctic acid (XXVII) (Bialer *et a*l., 1990). Also from levetiracetam (XLIV), brivaracetam (XLVIII) and selectracetam (XLVII) have been prepared.

2.5.3 PHYSIOLOGICAL MECHANISM

With the advent of greater understanding of physiological mechanism it has become possible to take a more mechanistic approach to research and start from a rationally argued hypothesis to design drugs. Progress depends largely upon the current state of understanding of physiology in relation to disease. This is the modern rational approach to drug design which is becoming increasingly

important with the development of information in cell biochemistry and cell biology especially where this is understood at the molecule level.

The rational approach to new drug discovery requires several essential ingredients for success including

1. Evidence of a physiological basis for understanding a diseases, so that one my hypothesize that a drug with a particular action should be therapeutically beneficial;
2. An explicit chemical starting point;
3. Bioassay systems which measure the desired drug activity in the laboratory;
4. A test which measures the activity of the drug in humans that can be related to a potential therapeutic treatment.

The targeted sites for drug action include enzymes, receptors, transport systems, cell replication and protein synthesis and storage sites (e.g., mast cell, blood, platelets, neurons, etc.).

The body is controlled by chemical messengers (physiological mediators). There is a very complex communication system and each messenger has specific functions and is recognized at specialized sites where it acts. In disease, something has got out of balance, and in drug therapy the aim is to redress that balance.

2.5.4 DISEASE MODELS AS SCREENS

The engine that drives AED discovery is screening in animal models. For practical reasons, most testing, at least at the compound identification stage, is carried out in normal rodents induced to have a seizure by an electrical or chemical stimulus. Chronic models, in which the animal has a genetic or acquired propensity for seizures, are arguably closer to human epilepsy and commonly are included in the battery of tests for early stage characterization of AED (White *et al.,* 2002).

Although there are a large number of models that could potentially be used to screen for anticonvulsant activity, the maximal electroshock model (MES), and the subcutaneous pentylenetetrazole model (scPTZ) remain the “gold standard” in early stages of testing (Schmidt and Rogwski, 2002). Compounds active in either MES or scPTZ tests have generally been efficatious in clinical trials, although inactivity in these tests does not necessarily indicate lack of efficacy. An alternative electroshock test, the 6 Hz model, may detect compounds like levetiracetam that fail to be identified by the MES or PTZ models (Barton, *et al.,* 2001). Kindling models, where animals have acquired succeptibility for limbic seizures, are now routinely a part of the battery for preclinical characterization. Other methods commonly used in early stage development include audiogenic seizure susceptible mice (Rogowski, 2000); the gamma hydroxy butyrate spike and wave model of generalized absence seizure (Snead, 1992); the phenytoin-resistant kindled rat (Loscher *et al*., 2000), the carbamazepine kindled rat (Nissinen *et al.,* 2000); the methylazoxy methanoate (MAM)-treated rat (Smyth *et*

al., 2002) and the in vitro low magnesium entorhinal-hippocampal slice preparatice (Armand, 2000).

2.6 COMPUTER AIDED DRUG DESIGN

Computers augument the scientific process in drug discovery by assisting the chemist, with collecting, storing, manipulating, analyzing and viewing data. Further, computers provide a link to theoretical chemistry and graphic modeling, providing calculated estimates of molecular properties, models of molecules, models of biological sites and sometimes even models of drug-receptor interactions. The development of a quantative structural activity relationship (QSAR) acts as a catalyst in the hypothesis-testing-analysis cycle of the normal scientific process as it applied to drug discovery and drug design (Fig 2.1).

The process of drug design begins with the identification of a compound with interesting biological activity. A hypothes is conceived suggesting which chemical features are related to the bioactivity. The hypothesis is evaluated by synthesizing and testing compounds in an appropriate biological assay.On the basis of the analysis of this biological result, the chemist can modify the original hypothesis and then repeat the process if necessary.

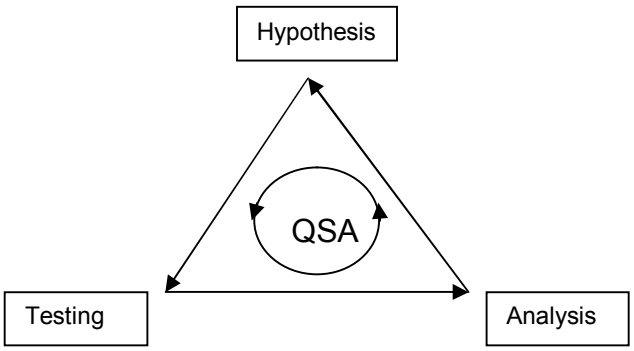


Fig.2.1 Role of QSR in the scientific

Part of this analysis is intended to ascertain a structure activity relationship (QSAR), that is, to identify whether there is a pattern within the changes at molecular features which may explain the changes in biological activity. QSAR can be used to 'design-in' biological properties such as potency, efficacy, selectivity and bioavailability. It can also assist the scientist by increasing the efficiency of the discovery process (Maliski and Bradshaw, 1999) using combinatorial.

2.7 MOLECULAR TARGETS FOR ANTIEPILEPTIC DRUG DEVELOPMENT

Molecular targets for antiepileptic drug development have recently been reviewed (Medrum and Rogawsky, 2007). They include voltage-gated ion channels, ligand-gated ion channels, acid-sensing ion channels, G-protein-coupled receptors, neurotransmitter transporters, presynaptic proteins influencing synaptic function, enzymes and gap junctions (connexins).

VOLTAGE-GATED ION CHANNELS

Neuronal excitability is determined by the properties of the ion channels in the neuronal membrane such that the aberrant excitability associated with an epileptic discharge will necessarily be mediated by voltage-gated and ligand-gated ion channels, and may even be the result of defects in the function of these channels. Because of the pivotal role played by these ion channels in the physiology of all forms of epilepsy, they are obvious AED targets and it is

perhaps no surprise that several of these channels represent the critical sites of action for AEDs that were originally identified by empirical screening in animal models.

Voltage-gated ion channels are now seen as forming a superfamily (referred to as the "voltage-gated-like ion channels") comprising 143 genes (Yu and Cartterall, 2004 and Yu *et al.,* 2005). This superfamily encompasses the S4 family in which the pore-forming subunits are built on a motif of six transmembrane segments (S1–S6) the fourth of which (S4) contains a voltage-sensing element. In voltage-gated Na^+ and Ca^{2+} channels, four such domains (referred to as I–IV or D1–D4) are expressed as a single polypeptide arranged around a central pore that conducts the ionic current. In voltage-gated K^+ channels, the channel is a tetramer of four individual subunits, each of which contains a single S1–S6 domain. In all of the voltage-gated channels, four re-entrant pore loops (P-loops) between the S5 and S6 segments form the external mouth of the ion channel. A highly conserved sequence within this loop confers the specificity for the conducted ion (Na^+, Ca^{2+} or K^+). The tetrameric organization of voltage-gated K^+ channels is also present in calcium-activated K^+ channels and cyclic nucleotide-gated and the hyperpolarization-activated cyclic nucleotide-modulated cation channels.

Voltage-gated-like ion channels not only control excitability in the central nervous system, but also in the peripheral autonomic nervous system, the cardiovascular system, and the digestive system. They also control all secretory functions including the release of hormones. Thus, in selecting an ion channel as a

molecular target, it is necessary to be cognizant of which tissues express the channel. Importantly, the voltage-gated Na^+, Ca^{2+} and K^+ channels expressed in the heart are often distinct from, but closely homologous to, those expressed in the brain. Drugs need to be selected that preferentially select for the isoforms in the brain over those in the heart.

2.7.1.1 Voltage-gated sodium channels

The mammalian voltage-gated Na^+ channels that have been functionally expressed so far form a single family of nine genes encoding the pore-forming α-subunits (Catterall *et al*., 2005). Four of these are expressed predominantly in the central nervous system-$Na_v1.1$ (*SCN1A*), $Na_v1.2$ (*SCN2A*), $Na_v1.3$ (*SCN3A*), and $Na_v1.6$ (*SCN8A)*-and two in the peripheral nervous system and dorsal root ganglia-$Na_v1.7$ (*SCN9A*) and $Na_v1.8$ (*SCN10A*). $Na_v1.4$ (*SCN4A*) is expressed in skeletal muscle. $Na_v1.5$ (*SCN5A*) is expressed principally in cardiac muscle, but is also found in some limbic neurons in the rat brain, including the piri-form cortex. $Na_v1.3$ is significantly expressed in the brain only early in development. $Na_v1.9$ (*SCN11A*) is expressed widely in the brain and spinal cord and contributes to the persistent, tetrodotoxin-resistant sodium current in small-diameter dorsal root ganglion neurons. It is also expressed in peripheral tissues including the spleen, small intestine, and placenta (Meldrum and Rogawski, 2004).

In the mammalian brain, the fast, transient Na^+ currents that generate action potentials are mediated by $Na_v1.1$ and $Na_v1.2$ isoforms (predominant in the rostral brain, globus pallidus, hippocampus, thalamus, and cerebellum) and by the $Na_v1.6$ isoform (prominent in the somatodendritic regions of output neurons

of the cerebellum, cortex and hippocampus). These channels also mediate the persistent, resurgent, or late Na^+ current, which (although much smaller than the fast inactivating Na^+ current) may play a significant role in epilepsy and in the action of AEDs (Rogawski, 2004).

The persistent current is greater for $Na_v1.6$ than for $Na_v1.1$ or $Na_v1.2$. Persistent Na^+ currents are thought to contribute to burst discharges in the hippocampus after pilocarpine-induced status epilepticus by enhancing after-depolarizing potentials. There are four auxiliary (β) subunits ($Na_vβ1$–$Na_vβ4$; genes *SCN1B*–*SCN4B*) that have an intramembrane segment and an immunoglobulin-like extracellular element. All four can be found in association with the α-units expressed in the brain.

Mutations in the α subunits have been found in various clinical syndromes, including the long QT syndrome, periodic paralysis, paramyotonia congenita, and various forms of idiopathic generalized epilepsy, including generalized epilepsy with febrile seizures plus (GEFS+). The first mutation identified in a family with GEFS+ was in *SCN1B* (C121W) (Table 2.1). This mutation, when expressed in oocytes, depresses fast inactivation and increases the Na^+ current through loss of the modulatory function of the β subunit (Wallace *et al*, 2002). Subsequently, more than 100 different mutations in *SCN1A* and *SCN2A* were identified in GEFS+ families, including some in the S4 voltage sensor. The functional consequences of these mutations in expression systems have proven to be diverse, and not simply related to enhanced excitability, although some enhance persistent current (Lossin *et al.,* 2002). Computer simulations, however, suggest that the changes can explain increased neuronal firing (Sampanato *et al,* 2004). Two other severe syndromes of early life are associated with mutations in *SCN1A*. Severe myoclonic epilepsy of infancy (SMEI) manifests many seizure

Table 2.1 Voltage Gated ion Channel Genes Bearing Mutations which have been Linked to Inherited Epilepsy.

Voltage-gated ion channel family	Gene name	Syndrome	
Potassium channels	KCNQ2 KCNQ3 KCNQ3	BFNC BFNC JME	
Sodium channels	SCN1A SCN1A SCN2A SCN2A SCN1B	GEFS+ SMEI GEFS+ BFNIC GEFS+	
Calcium channels	CACNA1A	IGE	
	CACNA1H	CAE	

BFNC: Benign familial neonatal convulsions
BFNIC: Benign familial neonatal/infantile convulsions
CAE: Childhood absence epilepsy
GEFS+: Generalized epilepsy with febrile seizures plus
IGE: Idiopathic generalised epilepsy
JME: Juvenile myoclonic epilepsy
SMEI: Severe myoclonic epilepsy of infancy

types often evolving into a malignant syndrome. A large number of mutations, some occurring de novo and some involving the ion pore, have been identified in *SCN1A* (Claes *et al*, 2003 and Kanai *et al,* 2004*)*. Intractable childhood epilepsy with generalized tonic–clonic seizures (ICEGTCS), also due to mutations in *SCN1A*, is similar clinically to SMEI except that there is reduced severity of psychomotor impairment and no myoclonus (Fujiwara *et al.,* 2003). Mutations in *SCN2A* have recently been associated with benign familial neonatal–infantile, (BFNIS) (Berkovic *et al*, 2004). The functional effects of these mutations are not known.

Studies of expressed α subunits with single site mutations have demonstrated that local anesthetics, antiarrythmics, and Na^+ channel-blocking AEDs act on overlapping sites on the α subunit of voltage-gated Na^+ channels (Ragsdale *et*

al., 1994,1996; Yarov-Yarovoy *et al.,* 2001 and Lui *et al.*, 2003) effects on channel function. AEDs that modulate voltage-dependent Na^+ channels produce a voltage- and use-dependent block of the channels by binding predominantly to the inactivated state of the channels, thus suppressing high-frequency repetive action potential firing. The critical downstream effect may be to reduce action potential–dependent synaptic neurotransmitter release during the high-frequency firing that occurs with epileptic discharges (Lingamaneni and Hemmings, 1999 and He, 2002).

Localization of the precise site of AED action on the $Na_v1.2$ channel was provided by studies of the effects of single site-directed mutations in expressed recombinant α subunits. The binding of phenytoin and the frequency-dependent block of Na^+ current was diminished by mutations F1764A and Y1771A in transmembrane segment IVS6 (Ragsdale *et al.,* 1994 and 1996). Further studies with lamotrigine and some related compounds revealed that the nearby I1760 and additional residues in IIIS6 are also critical to drug binding (Yarov-Yarovoy *et al.,* 2001; Lui, 2003 and Cronin, 2003). During gating, the structure of the Na^+ channel changes such that the critical amino acid residues in IIIS6 and IVS6 become exposed to the pore in the activated and inactivated states. This enables docking of lamotrigine within the pore in a use-dependent fashion.

Why do different Na^+ channel-blocking AEDs have distinct clinical profiles? There are at least three possibilities. First, the biophysical parameters for Na^+ channel block may differ in terms of binding kinetics and selectivity for the different gating states of the channel. In fact, there are marked differences in these parameters

among the classical Na^+ channel-blocking AEDs phenytoin, carbamazepine and lamotrigine, which could, in part, account for their distinctive clinical properties (Ragsdale *et al.,* 1991 and Kuo, 1998). Recently, there has been some success in attempts to optimize the biophysical parameters of Na^+ channel blocking drugs. For example, the semicarbazone anticonvulsant V102862 (Co 102862), discovered by empirical screening in animal models, was found to block Na^+ channels. Analogs with enhanced Na^+ channel blocking potency were discovered using an electrophysiological screen (Sun *et al.*, 2003). Recent biophysical studies of one of these analogs, PPPA {2-[4-(4-chloro-2-fluorophenoxy) phenyl]-pyrimidine-4-carboxamide}, demonstrated enhanced state-dependence (selectivity for inactivated *versus* resting channels) compared to carbamazepine and lamotrigine, and, correspondingly, a greater protective index (comparing potency for rotarod toxicity with activity in the MES test) (Ilyin *et al.,* 2006). Thus, it may be possible to identify compounds with potentially improved clinical activity based on a detailed characterization of their biophysical parameters for block of Na^+ channels.

A second way in which Na^+ channel blockers can differ is in their selectivity for Na^+ channel isoforms. $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ have very different patterns of expression in neurons and among brain regions, so that selective targeting might protect against seizures with reduced side effects compared with nonselective antagonists. Selective targeting of isoforms, such as $Na_v1.6$, that contribute more to persistent current than other isoforms might also optimize anticonvulsant

activity. Designing drugs to achieve such selectivity among paralogs may be challenging, but the examples provided by tetrodotoxin and pyrethroid insecticides indicate that it is achievable. Thus, tetrodotoxin shows exquisite selectivity, blocking Na^+ channel isoforms with an aromatic residue in the first intracellular linker (phenylalanine 383 in $Na_v1.1$, tyrosine 401 in $Na_v1.4$, cysteine 374 in $Na_v1.5$), but not channels in which a polar residue is present at this site (as in $Na_v1.8$ and $Na_v1.9$). Similarly, pyrethroid sensitivity can be altered by single amino acid substitutions.

Na^+ channels are present at distinct sites in neurons, where they subserve different functions and play distinct roles in epileptic discharges. Thus, selective targeting of Na^+ channels isoforms expressed in different parts of the neuron may produce markedly different functional effects. Some Na^+ channels, especially those at the initial axon segment, initiate action potentials and control firing thresholds and the thresholds of burst discharges. Postsynaptic somatodendritic Na^+ channels act in concert with a range of ligand-gated and voltage-gated channels to generate interictal and ictal neuronal discharges. In contrast, presynaptic Na^+ channels contribute to the regulation of neurotransmitter release. All Na^+ channel blocking AEDs will act to some extent on these three Na^+ channel populations, but there may be differences in the relative effects on one population. For example, some Na^+ channel blocking AEDs may preferentially inhibit glutamate release as a result of selective interactions with Na^+ channels that are located on presynaptic glutamatergic terminals (Prakriya and Menneric, 2000).

The third way in which Na^+ channel blocking AEDs may differ is in their secondary effects on other voltage-gated or ligand-gated ion channels. Several marketed AEDs that are known to block Na^+ channels (including felbamate, topiramate, and zonisamide) also have prominent interactions with other ion channel targets. The combination of actions may contribute to the unique clinical efficacies of each of these drugs.

In addition to explaining differences in the clinical spectrum of action among currently available Na^+ channel blocking AEDs, these principles suggest that it may be possible to optimize the activity of drugs that target Na^+ channels, with the prospect that new compounds may have improved activity or reduced side effects.

2.7.1.2 Voltage-gated calcium channels

Like other voltage-gated ion channels, voltage-gated Ca^{2+} channels contribute to the membrane potential behavior of neurons; they are particularly important in rhythm generation and burst firing. Because Ca^{2+} serves as a messenger, Ca^{2+} channels have the unique ability among ion channels to couple membrane electrical events to diverse cellular functions, including exocytosis of neurotransmitters. The pore-forming α_1 subunits of the voltage-gated Ca^{2+} channels are homologous in structure to the α subunits of voltage-gated Na^+ channels.They fall into three subfamilies, corresponding to channel types earlier classified according to their biophysical and pharmacological properties: L-type (high voltage-activated, generating a long-lasting current); N, P/Q, and R-types (expressed in nerve terminals and responsible for the Ca^{2+} entry that triggers

neurotransmitter release); and T-type (low voltage-threshold, generating a transient current, somatodendritic localization, and critical to pacemaker activity and some patterns of burst firing)(Tsien *at al.*, 1988; Cattell *et a.l*, 2005). The α_1 subunits are variably associated with auxiliary subunits, including the intracellular β subunits (β1–β4), the largely intramembranal γ subunits (γ1–γ8), and the intramembranal/extracellular α2–δ subunits (types 1–4) (Wolf *at al.*, 2003).

A surprising variety of mutations involving voltage-gated Ca^{2+} channels have been identified in mice that exhibit absence-like seizures with 5- to 7-Hz spike-and-wave cortical discharges (Felix, 2002; Jones, 2002 and Pietrobon, 2005). These are all recessive syndromes and involve several different specific Ca^{2+} channel subunit types, but not L-type or T-type channels. L-type channels have not been associated with epilepsy syndromes in mice or humans and are not considered to be targets for AEDs. T-type Ca^{2+} channels are believed to be the targets of antiabsence agents such as ethosuximide, which weakly block native and recombinant T-type Ca^{2+} channel currents (Coulter *et al*, 1989; Crunelli, 2002 and Gomora *et al,* 2001). As expected from this pharmacological observation, mice that lack α_{1G} T-type Ca^{2+} channels are resistant to absence seizures (Kim *et al,* 2001 and Song *et al,* 2004).

The three recessive mutations in *Cacna1a* ($Ca_v2.1$) that produce absence-like syndromes in *tottering*, *leaner* and *rocker* mice all impair channel function, reducing P/Q-type Ca^{2+} currents. These and *rolling Nagoya* mice (with a further mutation on *Cacna1a* but not showing absence seizures) show ataxia and a wide variety of other central nervous system changes. The *stargazer* mouse has a

mutation in *Cacng2* that alters the function of $Ca_v2.1$ and $Ca_v2.2$ (Kang *et al*, 2001) and modifies the cell surface expression of AMPA receptors (Chen *et al*, 2000). The mutation in the *lethargic* mouse interferes with the β_4 subunit modulatory action on the α_1 subunit $Ca_v2.1$ and $Ca_v2.2$ although some compensatory subunit insertions ("reshuffling") may occur (Burges *et al*, 1999). In *ducky* mice, the gene for the α2–δ-2 subunit is mutated, and this also alters the function of the $Ca_v2.1/2.2$ channels (Gao *et al*, 2000). Targeted disruption of the *Cacna2d2* gene leads to ataxia and enhanced seizure susceptibility (Ivanov *et al*, 2004). In sum, a common factor in these six very different mouse syndromes expressing absence-like seizures is impaired function in presynaptic Ca^{2+} channels (P/Q-type) controlling neurotransmitter release. Proteins involved in the SNARE complex that link P/Q-type Ca^{2+} channels to synaptic vesicle release (SNAP-25, syntaxin, synaptotagmin) (Zhong *et al*, 1999) play a neurotransmitter release. Note that *coloboma* mouse, which bears an autosomal dominant mutation affecting the SNAP25 protein, exhibits spike-and-wave discharges (Zhang *et al,* 2004).

Only recently have mutations in voltage-activated Ca^{2+} channel subunits been associated with human epilepsy syndromes. A syndrome of absence epilepsy with episodic ataxia similar to that observed in mice has been described in a family with a mutation in the α_{1A} subunit ($Ca_v2.1$)(Imbrici *et al*,. 2004). In childhood absence epilepsy (CAE), 12 mutations involving *CACNA1H* (encoding the α_1 subunit $Ca_v3.2$) have been reported (Chen and Pan, 2003 and Heron et al, 2004). Functional expression studies with several of these mutations have revealed a gain of function (Peloquin *et al,* 2006 and Vitco *et al,* 2005). At least

30 mutations in *CACNA1H*, some involving splicing defects, are associated with CAE and other idiopathic generalized epilepsies (Zhong *et al*, 2006). Five mutations in *EFHC1*, a gene encoding a protein with an EF-hand motif, have been found in families with juvenile myoclonic epilepsy (JME) (Suzuki *et al*, 2004). This protein associates with the R-type Ca^{2+} channel $Ca_v2.3$. EFHC1 increases R-type Ca^{2+} currents, but this activity is lost when the protein bears the mutations associated with JME. A tentative association between polymorphisms in a further EF-hand containing gene (*EFHC2*) has also been found in families with JME (Gu *et al*, 2005).

Many AEDs have been reported to inhibit Ca^{2+} currents. Only in the case of agents such as ethosuximide, which act on T-type Ca^{2+} channels, is there firm evidence that Ca^{2+} channels are the primary target for seizure protection. The anticonvulsant action of the barbiturate phenobarbital may, however, be due, at least in part, to inhibition of Ca^{2+} current, as well as an action on $GABA_A$ receptors (Ffrench-Mullen *et al*, 1993). Similarly, although the anticonvulsant activity of lamotrigine is believed to be mediated primarily by effects on voltage-gated Na^+ channels, lamotrigine also inhibits high voltage-activated (N- and P/Q-type) Ca^{2+} channels (but inhibits R-type minimally, and T- or L-type Ca^{2+} channels not at all) (Leews and Leach 1993; Stefani *et al* ,1996; Hainsworth *et al,* 2003 and Pisani *et al,* 2004). Whereas effects on Na^+ channels are believed to be responsible for the inhibitory action of Na^+ channel-blocking AEDs on synaptic glutamate release, inhibitory actions on voltage-gated Ca^{2+} channels may also contribute, especially for lamotrigine (through its blocking action on N-type channels) (Wang *et al,* 1996 a,b) .

Recently, it has become apparent that the molecular targets for gabapentin and pregabalin are α2–δ proteins, specifically α2–δ-1 and α2–δ-2(Gee *et al,* 1996 and Belliotti, 2005). The evidence supporting this concept has been reviewed (Cunningham *et al,* 1994). At present, the exact way in which binding to these proteins protects against seizures is not fully understood. Some investigators (but not all) have observed inhibitory effects on voltage-gated Ca^{2+} currents and have reported that these effects can be partially occluded by toxins that selectively block either P/Q- or N-type Ca^{2+} channels (Fink *et al*, 2002; Sutton et al, 2002 and Brown and Randall 2005). Other studies have shown inhibition of the release of glutamate and other neurotransmitters (Van Hoft et al, 2002; Bayer *et al,* 2004 qnd Cunningham *et al,* 2004).

The variability in the effects on Ca^{2+} current may relate to differences in expression of the α2–δ subunit in different cell types or in response to different conditions. For example, in chronic neuropathic pain states, α2–δ subunits may be upregulated, conferring gabapentin and pregabalin sensitivity (Rogawski and Loscher 2004 and Li *et al,* 2006). Whether there are similar plastic changes in α2–δ subunit expression in epilepsy remains to be determined. Although gabapentin and pregabalin inhibit neurotransmitter release in many systems, there is evidence that this may not require inhibition of calcium influx and may instead be mediated by an interaction of α2–δ (or the calcium-channel complex containing α2–δ) with synaptic proteins that are involved in the release or trafficking of synaptic vesicles.

2.7.1.3 Voltage-gated potassium channels

The most diverse group of ion channels, K^+ channels serve to limit excitability in neural cells. They are formed from α subunits, which comprise the ion-conduction pore, selectivity filter, and gating apparatus, and various auxiliary subunits that serve modulatory roles. The typical K^+ channel is a tetramer of individual α subunits, each one of which is homologous to a domain of the pore-forming α or α_1 subunits of voltage-gated Na^+ or Ca^{2+} channels.

More than 70 genes for K^+ channel α subunits have been identified in mammals. Their common feature is a sequence motif TVGYG in the P-loop that confers selectivity of the pore for K^+ over Na^+. K^+ channels fall into several families: the six-transmembrane-helix voltage-gated (K_v) channels, the two-transmembrane-helix inward-rectifier (K_{ir}) channels, the Ca^{2+}-activated K^+ channels (K_{Ca}), and the tandem-pore domain (K_{2P}) channels. Tandem-pore domain K^+ channels are comprised of two subunits each containing two P-loops, thus forming a channel homogenous to the tetrameric structure of other K^+ channels.

The K_v and K_{Ca} families are of particular relevance in epilepsy. The K_v family has more than 40 members (Gutman, 2005), and the channels that they form include the delayed-rectifier K^+ current (I_K) responsible for the repolarization phase of action potentials, as well as other voltage-gated K^+ currents that have diverse functions in neurons, including the A-current (K_v1, K_v3, and K_v4) and the M-current (KCNQ). A-currents and M-currents play important roles in regulating the excitability of neurons in brain regions relevant to epilepsy, such as the neocortex

and the hippocampus (Rogawski, 1985). Further details can be found in the work of Meldrum and Rogawski, (2007).

2.7.1.4 The hyperpolarization cyclic nucleotide-gated cation (HCN) channels

The hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels are Na^+-permeable and K^+ -permeable channels that participate in pacemaker currents in cardiac cells and neurons. The channels are opened by hyperpolarization to negative membrane potentials (more negative than −50 mV). In addition, they are also modulated by cAMP binding to a consensus cyclic nucleotide binding domain in the carboxy terminus. Binding of cAMP shifts the voltage dependence of activation to more positive potentials; it can also directly open the channels.

There are four known subunits, HCN1-4, each of which has six transmembrane segments. The subunits combine to form homomeric or heteromeric tetramers, as do K_v channels. HCN1 channels are prominently expressed in the cortex and the hippocampus, particularly in dendrites. In contrast, HCN2 channels, which are highly responsive to cAMP, are expressed mainly in the thalamus, where they are believed to limit burst firing. In recordings from hippocampal or cortical neurons, the current produced by opening of the HCN channels is referred to as I_h. Many neurotransmitters (including monoamines, serotonin, and acetylcholine) can modulate I_h through cAMP.

A role for HCN channels in epilepsy has been widely proposed, but the evidence is complex (Pools, 2005).

2.7.1.5 Voltage-gated chloride channels

The mammalian chloride channel (ClC) gene family encodes nine Cl^- channels with diverse functions in plasma membranes and intracellular organelles. One of these channels, ClC-2, a homodimeric channel found in neurons and glia (encoded by the *CLCN2* gene), has been implicated in epilepsy (Jentsch *et al.,* 2005). ClC-2 is a plasma membrane channel activated by hyperpolarization, cell swelling and extracellular acidification. ClC-2 knockout mice do not have epilepsy (Bosl *et al.,* 2001). In humans, however, ClC-2 mutations cosegregated in three families with various idiopathic generalized epilepsy syndromes, including JME, juvenile absence epilepsy, CAE, and epilepsy with grand mal seizures on awakening (EGMA) (Heil, 2005). Functional studies in transfected cells suggest that the mutations cause a loss of function (Niemeyer, 2004). At present, there is no definitive evidence that these mutations are responsible for the epilepsy syndromes; searches for ClC-2 mutations in other cohorts with epilepsy revealed sequence changes that were probably only polymorphisms. In any case, it has been proposed that the epilepsy-associated ClC-2 mutations may lead to alterations in the Cl^- gradient in neurons such that $GABA_A$-mediated inhibition is impaired or may even become excitatory.

2.7.2 LIGAND-GATED ION CHANNELS

Ligand-gated ion channels in the mammalian brain fall into two major superfamilies, the cys-loop receptors (comprising the $GABA_A$, glycine, nicotinic, cholinergic, and 5-HT3 receptors) and the glutamate ionotropic receptors

(comprising the AMPA, kainate, and NMDA receptors). There are two additional superfamilies: the ATP-gated P2X channels and the TRP channels. Binding of agonist to these receptors induces a conformational change that opens the channel.

The cys-loop receptors vary in their ion selectivity. $GABA_A$ and glycine receptors are permeable to Cl^- and HCO_3^-, whereas nicotinic cholinergic receptors are permeable mainly to Na^+ and K^+, but also to Ca^{2+}. The ionotropic glutamate receptors are also cation permeable, with significant variation in the extent of Ca^{2+} permeability. The majority of known convulsant compounds (natural or synthetic) act via the ligand-gated ion channels, with the greatest number and variety acting to diminish GABA-mediated transmission either by direct action on $GABA_A$ receptors or by other effects on GABAergic function.

2.7.2.1 Cys-loop ligand-gated channels

$GABA_A$ receptors, GABA serves as the main fast inhibitory neurotransmitter in the brain. Inhibitory inter-neurons that make use of GABA as their neurotransmitter are found throughout the brain, but in any region they may comprise a wide range of morphological and functional types that participate in different circuits with principal neurons. Thus, in the CA1 area of the rat hippo-campus it is possible to distinguish 16 different types of GABAergic interneurons on the basis of their morphology, specific protein content (e.g., calbindin, calretinin, parvalbumin), and pattern of firing in relation to ongoing rhythms and oscillatory firing of pyramidal neurons(Somogyi and Klausberg, 2005). Through the mechanism of recurrent inhibitory feedback, GABAergic interneurons in the

cortex terminate local sustained burst firing and, through inhibitory surround, limit the lateral spread of seizure activity. Chemical agents that impair GABAergic inhibition are powerful convulsants.

$GABA_A$ receptors are pentameric in structure, with the five subunits arranged like spokes of a wheel around a central Cl^--selective pore (Barnard, 2001). The 19 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–2) are encoded by 19 distinct genes. Each subunit has four transmembrane segments, with both the amino and carboxy termini located extracellularly. These extracellular segments form the recognition sites (two per channel) for GABA and also, in some channel types, the recognition site (one per channel) for benzodiazepine-like allosteric modulators. The subunit composition determines both the biophysical properties of the receptor–channel complex and its pharmacology, most notably the sensitivity to benzodiazepines (Johnston, 2005 and Rudolph, 2004). A typical benzodiazepine-sensitive $GABA_A$ receptor consists of two α1, α2, α3, or α5 subunits, two β2 or β3 subunits (or one each), and a γ2 subunit. Classically, $GABA_A$ receptors have been recognized as mediating phasic (synaptic) inhibition through the generation of fast, transient, rapidly desensitizing currents (IPSCs) in postsynaptic neurons in response to synaptically released GABA. More recently, it has been recognized that $GABA_A$ receptors also contribute to tonic (extrasynaptic) inhibition, representing the Cl^- conductance activated at non-synaptic sites in response to background concentrations of GABA (Farrant and Nusser,2005).

2.7.2.2 Ionotropic Glutamate Receptors

The ionotropic glutamate receptors are a superfamily of ligand-gated cation channels that encompass three receptor families identified by the agonists that selectively activate them: AMPA, kainate, and NMDA (Dingledine *et al.*, 19995; Mayer and Armstrong, 2004; Kew and Kemp, 2005). These channels mediate most of the fast excitatory transmission in the central nervous system and are thus involved in all brain functions. Their excessive activation plays a large role in epileptic phenomena, including the paroxysmal depolarization shift (the single-cell correlate of interictal spikes) and seizure discharges.

Neurotransmitter glutamate serves to activate all ionotropic glutamate receptors at synapses. However, NMDA receptors require the presence of a coagonist, either glycine or D-serine (Mothet *et al.,* 2000). The coagonists are not released during synaptic transmission. Rather, their concentrations are regulated by various processes. For example, the levels of glycine are maintained at levels below those that saturate NMDA receptors by glycine transporter-1 (GlyT1), a Na^+/Cl^--dependent carrier molecule that exists in several isoforms (a–e) in glial and neuronal cell membranes. GlyT1 can operate bidirectionally: decreasing synaptic glycine concentrations when operating in the forward direction and releasing glycine from glial cells when operating in reverse mode. GlyT1b is found in astrocytes and colocalizes with NMDA receptors in the forebrain; it can powerfully modulate NMDA receptor-mediated responses (Supplission *et al.,* 1997). D-Serine production is regulated by its synthesizing enzyme serine racemase (Martineau *et al.*, 2006). The various regulatory mechanisms for

NMDA receptors related to coagonists suggest a variety of strategies for modulating NMDA receptor function.

2.7.3 ACID-SENSING ION CHANNELS ($ASIC^S$)

Acid-sensing ion channels are a family of proton-gated cation channels related to the degenerin/epithelial Na^+ channels, which have diverse functions in metazoan cells (Krishtal, 2003). Six isoforms (ASIC1–6) have been cloned; these are widely expressed as homomeric or heteromeric channels in the central and peripheral nervous system (Babinski *et al.,* 2000). ASICs in sensory neurons are believed to be involved in nociception when injury or inflammation causes acidification. The functional roles of ASICs in the brain are less well understood.

It has been suggested that protons released during high-frequency stimulation of excitatory synapses activate ASICs to cause postsynaptic depolarization (Wemmie *et al*., 2002). Among the consequences of this depolarization may be reduction in the Mg^{2+} block of NMDA receptors, which would promote epileptic activity. Thus, pharmacological inhibition of ASICs might reduce excitatatory synaptic transmission under these circumstances and have anticonvulsant actions. Moreover, acidification occurs during intense seizure activity, which could activate ASICs and contribute to seizure-induced brain damage, particularly because many ASICs are Ca^{2+} permeable (Xiong *et al*., 2004). ASIC antagonists might minimize these adverse consequences of seizures.

ASIC disruption in mice had no adverse developmental or behavioral consequences, and did not affect ordinary synaptic transmission (Wemmie *et al.,*

2002). Thus, ASIC antagonists may have fewer adverse effects than other antagonists of excitatory neurotransmission. At present, no selective ASIC antagonists are available to test the role of ASICs as anticonvulsant targets; however, the potassium-sparing diuretic amiloride does act as an ASIC antagonist and it appears to have anticonvulsant properties (Ali *et al.,* 2007). The peripheral cardiotoxicity of amiloride caused by its hyperkalemic action can be reduced by a liposome encapsulation. Status epilepticus has been shown to cause downregulation of ASIC mRNA (types 2b and 1a) in the hippocampus, raising the possibility that resistance to ASIC antagonists could occur under some circumstancesbecause many ASICs are Ca^{2+} permeable (Xiong *et al.,* 2004) ASIC antagonists might minimize these adverse consequences of seizures.ASIC disruption in mice had no adverse developmental or behavioral consequences, and did not affect ordinary synaptic transmission (Wemmie *et al.,* 2002). Thus, ASIC antagonists may have fewer adverse effects than other antagonists of excitatory neurotransmission. At present, no selective ASIC antagonists are available to test the role of ASICs as anticonvulsant targets; however, the potassium-sparing diuretic amiloride does act as an ASIC antagonist and it appears to have anticonvulsant properties (Ali *et al*., 2007). The peripheral cardiotoxicity of amiloride caused by its hyperkalemic action can be reduced by a liposome encapsulation. Status epilepticus has been shown to cause downregulation of ASIC mRNA (types 2b and 1a) in the hippocampus, raising the possibility that resistance to ASIC antagonists could occur under some circumstances

2.7.4 G-Protein-Coupled Receptors

G-protein-coupled receptors (GPCRs) are the largest family of receptors with as many as 1000 members, including many that have been identified from the sequencing of the human genome but are of unknown function. It is claimed that they comprise the largest set of therapeutic drug targets for known medicinal agents (Fang *et al.*, 2003; Piece *et al.*, 2002).

2.7.4.1 Metabotropic glutamate receptors

The metabotropic glutamate metabotropic receptors are a family of eight G-protein-linked receptors that fall into three groups defined by their sequence homology, second messenger effects, and common pharmacology (Pin and Acher, 2002 and Conn, 2003). They have seven transmembrane segments, with an intracellular loop between segments 3 and 4 that binds the G protein and a large “venus flytrap” extracellular element that is homologous with bacterial periplasmic proteins. In neurons, group I receptors are primarily located postsynaptically and have excitatory effects mediated by several ion channels. Group II and III receptors are located presynaptically, where their activation reduces transmitter release through inhibition of voltage-gated Ca^{2+} channels or potentiation of inwardly rectifying K^{+} channels (Schoepp, 2001). Astrocytes also express mGluR3 and mGluR5. mGluR2, a member of group II, is located some distance from the synaptic cleft on the axon and has high affinity for glutamate, whereas mGluR7, a member of group III, is located within the cleft and has a low glutamate affinity.

There is little evidence to indicate that genetic alterations of mGluRs play a role in the pathogenesis of epilepsy. Spontaneous mutations causing epilepsy are not known in mice or in humans; however, mice in which GluR7 has been deleted by gene targeting show an increased susceptibility to seizures and increased cortical excitability(Sansig*, et al.*, 2001).

Acquired forms of epilepsy show marked changes in the expression and function of mGluRs. In the kindling model of epilepsy, there is upregulation of group I receptors that can be demonstrated by biochemical or electrophysiological functional assays (Akiyama, 1992 and Keele *et al.,* 2000). There is also upregulation of mGluR5 in the hippocampus of patients with complex partial seizures (Notenboom *et al.,* 2006). In contrast, downregulation of hippocampal group II mGluRs was seen in chronically epileptic rats that experienced pilocarpine-induced status epilepticus (Pacheco *et al.,* 2006). A downregulation of group III mGluRs has also been reported in hippocampal slices from kindled rats (Klaptein and Meldrum, 1999)' and from patients with complex partial seizures (Dietrich *et al.,* 1999). The possible role of these changes in mediating the epileptic state has not been well defined, but is potentially important, in that such changes all tend to enhance excitability.

2.7.4.2 $GABA_B$ receptors

$GABA_B$ receptors were originally identified as the receptors mediating the bicuculline-insensitive, Cl^--independent inhibitory effects of GABA and baclofen on sympathetic ganglion neurons and presynaptic terminals (Lynch, 2003 and Bowery *et al.,* 1980). They were subsequently shown to play an important role in

mediating the actions of GABA in the central nervous system. $GABA_B$ receptors are expressed presynaptically at GABAergic and glutamatergic synapses, where they act to decrease neurotransmitter release by reducing Ca^{2+} entry. $GABA_B$ receptors are also expressed postsynaptically at GABAergic synapses, where they produce a late hyperpolarization in response to synaptically released GABA by enhancing K^+ permeability through GIRKs ($K_{ir}3.x$).

Like metabotropic glutamate receptors, $GABA_B$ receptors are G-protein-coupled receptors and they share the "venus flytrap" extracellular ligand-binding domain. The functional receptor is a heteromeric dimer made up of a $GABA_{B1}$ and $GABA_{B2}$ subunit (Jones *et al.*, 1998; Pin J-P *et al.,* 2004). $GABA_{B1}$ binds GABA, and $GABA_{B2}$ is required for the heteromer to reach the cell surface and plays a key role in G protein coupling. In the thalamus, $GABA_B$ receptors, activated by strong firing of thalamic reticular nucleus (TRN) neurons, contribute to the generation of rhythmic activity and are required for oscillations at 3–4 Hz, which is similar to the spike-and-wave seizure frequency range in humans. (Blumenfeld *et al.,* 2003). In the GAERS model of absence epilepsy, there is an upregulation of both $GABA_{B1}$ and $GABA_{B2}$ protein in corticothalamic circuits, which may contribute to the seizure phenotype (Princivalle *et al,* 2003).

2.7.5 Neurotransmitter Transporters

There are three neurotransmitter transporter families (Gether *et al.,* 2006). Vesicular transporters serve to transport specific neurotransmitters into synaptic vesicles, including glutamate (VGLUT1–3), GABA and glycine (VGAT/VIAAT), acetylcholine (VAC), and biogenic amines (catecholamines and serotonin)

(VMAT). Changes in the function or expression of these transporters can modify the quantal size of synaptic potentials (Wojcik *et al.,* 2006). Plasma membrane transporters regulate the activity of neurotransmitters including GABA and biogenic amines by sequestering them into cells after they are released from nerve terminals. They couple transmembrane movement of Na^+ and Cl^- (and in some systems, K^+) to the reuptake of the neurotransmitter. The third family transports glutamate and related substances, including D- and L-aspartate, across the plasma membrane and is coupled to Na^+.

Both vesicular and plasma membrane transporters move transmitters uphill, against a concentration gradient, and require energy from the hydrolysis of ATP. At the plasma membrane, the energy is utilized by (Na^+,K^+)-ATPase in generating ion gradients that provide the driving force for neurotransmitter uptake. In the case of the vesicular transporters, ATP is hydrolyzed by a H^+ pump (V-type H^+-ATPase) that acidifies the vesicle interior, generating a proton gradient that drives transport. Neurotransmitter transporters engage in complex functional interactions with themselves and with many other proteins (e.g., syntaxin 1A), modifying their localization and function. Neurotransmitter transporters are electrogenic because of the ions that are coupled to transport of the solute. They generate currents that exceed predictions, and are believed to have channel-like properties in addition to the transporter function (Sonders *et al.,* 1996).

2.7.6 PRESYNAPTIC PROTEINS INFLUENCING SYNAPTIC FUNCTION

2.7.6.1 *Synaptic vesicle proteins*

Several proteins are known to influence synaptic vesicle formation and function. These include the synapsin proteins (synapsin I–III) that coat the vesicles and regulate transmitter release (Hilfiker *et al.,* 1999). Mice deficient in synapsin I exhibit enhanced seizure-like electrographic and behavioral responses with amygdala stimulation (Li *et al.,* 1995). Synaptic function in these animals is altered in a variety of ways. In particular, there is a loss of immediately releasable GABA, which could account for the hyperexcitability (Gitler *et al.,* 2004; Bogen *et al.,* 2006). The synapsins are regulated by phosphorylation, which might provide a mechanism for pharmacological manipulation.

2.7.6.2 *Synaptic anchoring proteins*

Many proteins are involved in the anchoring and trafficking of ion channels, receptors, and transporter molecules. A large group of these proteins are found in postsynaptic densities, including spectrin, actin, calcineurin, contactin, PSD-95, SAP-90, gephyrin, and Homer 1A. The expression of many of these molecules changes during the course of kindling or status epilepticus-induced epileptogenesis. Such changes may contribute to or oppose epileptogenesis. For example, overexpression of Homer 1A during kindling may modulate glutamatergic transmission and oppose seizure activity and epileptogenesis (Potschka *et al*., 2002). In addition to Homer 1A, the many other proteins that participate in the trafficking of receptor subunits are potential targets for epilepsy therapy, because trafficking undoubtedly plays a part in epileptogenesis.

2.8.7 ENZYMES

Two enzymes, GABA-transaminase and carbonic anhydrase, are recognized AED targets. Enzymes involved in the regulation of receptor phosphorylation are other potential AED targets.

2.7.7.1 GABA-transaminase

Synaptically released GABA is transported into astrocytes and nerve terminals, where it is either taken up into synaptic vesicles or further metabolized by two mitochondrial enzymes, GABA-transaminase (4-aminobutyrate-2-oxoglutarate aminotransferase) and succinic semialdehyde dehydrogenase, to yield succinate, a tricarboxylic acid cycle intermediate. After it was shown that inhibitors of GABA-transaminase raise brain GABA content and inhibit seizures in rodents, (Anlezark *et a.l.*, 1976) two GABA analogs, *γ*-acetylenic GABA and *γ*-vinyl GABA (vigabatrin), were identified as irreversible inhibitors of GABA-transaminase with prolonged anticonvulsant activity in rodent and primate models of epilepsy (Jung *et al.,* 1977; Schechter *et al.,* 1977; Meldrum *et al.,* 1978). They initially bind reversibly to the cofactor pyridoxal-5-phosphate and then bind covalently to the active site of the enzyme (De Biase *et al.,* 1991). Vigabatrin is a racemic mixture; the *S*(+)-enantiomer possesses the anticonvulsant activity (Meldrum *et al.,* 1983). In mice, an anticonvulsant dose of vigabatrin increases brain GABA content five- to sixfold (Jung et al., 1977). In humans, vigabatrin increases cerebrospinal fluid GABA concentration by 180% (Kalviainen *et al.,* 1993) and occipital cortical GABA content threefold (Petroff *et al.,* 1998; Verhoeff *et al.,* 1999).

The hypothesis that the anticonvulsant action of vigabatrin is a consequence of enhanced GABAergic inhibition resulting from an augmented releasable pool of neurotransmitter has been abandoned, following the observation that IPSCs are diminished in frequency and amplitude in cultured hippocampal neurons exposed to vigabatrin (Overstreet *et al.,* 2001; Wu *et al.*, 2003). What is clear from *in vitro* and *in vivo* studies is that the extracellular concentration of GABA is increased, leading to some desensitization of synaptic $GABA_A$ receptors, but also to an increase in tonic Cl^- conductance due to activation of nonsynaptic $GABA_A$ receptors (Wu *et al.*, 2003). The increase in extracellular GABA is not dependent on vesicular release, but rather on reverse transport of GABA by GAT-1 or GAT-3.

The clinical use of vigabatrin has been severely restricted by the discovery that it causes ganglion cell damage, resulting in a high incidence of visual field defects (Lawden *et al.,* 1999). It is not clear whether the retinal pathology is a direct consequence of GABA-transaminase inhibition or is related to some other effect of vigabatrin, such as glutamic acid decarboxylase inhibition. Until it is shown that chronic inhibition of GABA-transaminase sufficient to suppress seizures can be achieved without risk of retinal damage, the utility of GABA-transaminase as an AED target will remain uncertain.

2.7.7.2 Carbonic anhydrase

Carbonic anhydrase (carbonate dehydratase) is an enzyme found in diverse isoforms in most tissues, including blood and brain. By controlling the rate of hydration of CO_2 and dehydration of bicarbonate (HCO_3^-), it modulates intracellular and extracellular pH. Specifically, carbonic anhydrase inhibition

causes extracellular acidification because of interference with H^+ buffering; there may also be parallel intracellular acidification.

Neuronal excitability is influenced by pH in many different ways. Probably most important in epilepsy is modulation of the functional consequences of $GABA_A$ receptor activation, which occurs through intraneuronal carbonic anhydrase VII (Rivera *et al.*, 2005). Although $GABA_A$ receptor currents are carried mainly by Cl^-, HCO_3^- normally carries an opposing outward (depolarizing) current that is one fifth the size of the Cl^- current. During intense $GABA_A$ receptor activation, intracellular accumulation of Cl^- ions and the CO_2-mediated redistribution of HCO_3^- ions can lead to a situation in which outward HCO_3^- current exceeds inward Cl^- current, resulting in depolarizing GABA response (Staley et al., 1995). This effect, which is dependent on the intraneuronal generation of HCO_3^- by carbonic anhydrase VII, probably plays a significant role in hippocampal epileptiform activity (Fujiwara-Tsukamoto *et al.,* 2003). Carbonic anhydrase in astrocytes also can influence neuronal excitability by regulating extracellular pH. Decreasing pH tends to depress epileptiform burst firing, through a variety of actions (Church *et al,* 1987), including effects on NMDA receptors, which are inhibited by extracellular H^+ (Tang *et al.*, 1990).

Acetazolamide and various related sulfamates were shown in the 1950s to inhibit carbonic anhydrase and to be anticonvulsant in rodent seizure models (more effective against MES than against PTZ seizures) and in humans against generalized and focal motor seizures and absence epilepsy (Millichap *et al.,* 1955; Golla *et al.,* 1957). Tolerance develops relatively rapidly, however, largely

due to enhanced carbonic anhydrase activity. Two more recently introduced AEDs, zonisamide and topiramate; also inhibit carbonic anhydrase, which may contribute to their side effects, including kidney stones and perhaps also oligohydrosis and hyperthermia (Dodgson *et al.,* 2000; Leppik *et al.*, 2004).

Because of the problem of tolerance, carbonic anhydrase has not been considered a promising target for new AED development. This situation may change if the propensity for tolerance can be overcome, perhaps by selective targeting of isoforms with intracellular–extracellular or neuronal–astrocytic specificity.

2.7.7.3 Protein kinases and phosphatases

Activation of either ionotropic or metabotropic receptors have immediate effects on ionic conductances and delayed effects involving second messenger systems. These longer-term effects may be triggered by changes in intracellular Ca^{2+} that influence the activity of a large number of enzymes (such as Ca^{2+}/calmodulin-dependent protein kinase) or through changes in second messengers, such as cAMP and cGMP, that affect protein kinases.

Phosphorylation sites are present on most voltage-gated ion channels, receptors, and membrane transporter molecules and the phosphorylation state of these sites may modulate the activity of the effectors. There is no firm evidence that altered phosphorylation of ion channels or other excitability molecules are responsible for epileptogenesis. There are, however, numerous reports of functional changes in voltage-gated ion channels, GABA and glutamate

receptors, and transporters in brain tissue from rodents with kindled seizures and humans with intractable temporal lobe epilepsy (Mody *et al,* 1993; Rogawski *et al*, 1995; Avoli *et al,* 2005). Some of these probably depend on altered gene expression, but many could result from altered phosphorylation. Mutations involving kinase or phosphatase systems would have such widespread effects on development and cellular function that they are unlikely to be found associated with idiopathic epilepsy. Similarly, pharmacological agents that influence these enzymes might not be expected to have sufficiently specific actions to be useful anticonvulsants. As noted previously, however, topiramate may act indirectly on ion channels by effects on phosphorylation (Shank *et al.,* 2005).

2.7.8 GAP JUNCTIONS (CONNEXINS)

Evidence for a role of gap junctions in epilepsy has come to the fore in the last decade (Traub *et al.,* 2004; Nakase *et al.,* 2004; Nemani *et al.,* 2005). Electrotonic coupling between vertebrate neurons was described in the 1960s and 1970s, prior to the description from electron micrographs of gap junctions between neurons. Structurally these comprise two hemichannels (connexons), each made up of six connexin proteins surrounding a central pore that permits the passage of ions, second messengers, and other small molecules between cells. Gap junctions can be between neurons or between astrocytes (homocellular) or between astrocytes and neurons (heterocellular). Hemichannels may also permit the release of compounds such as glutamate and ATP from glia into the extracellular fluid.

About 20 connexins have been identified in mammals. Connexins (Cx) 26, 29, 30, 32, 36, 37, 40, 43, 45, 46, and 47 are expressed in the brain, with marked variation according to developmental stage and cell type (Nakase *et al.*, 2004). In astrocytes, where a key function for gap junctions is calcium wave signaling, Cx43 is the major connexin (Nedergaard et al, 2003). In neurons, Cx36 and Cx45 play an important role and provide electrical coupling between neurons in cortex and hippocampus (Venance *et al.,* 2000). In oligodendrocytes, Cx32 and Cx47 provide nutrients to the myelin sheaths (Nagy *et al.,* 2005).

Connexin knockout studies in mice present a very mixed picture of developmental defects and some changes in oscillatory potentials in slice preparations, (Maier *et al,* 2002; Pais *et al,* 2003), but are difficult to interpret because of possible compensatory changes in other connexins. Recent studies indicate that knockout of the major neuronal gap junction protein Cx36 promotes epileptic hyperexcitability, suggesting that specific inhibitors would not be useful as AEDs. Other connexins may be relevant, however. Changes in the expression of connexins may play a role in epileptogenesis (Fonseca *et al.*, 2002; Samoilova et al., 2003). Moreover, there is strong evidence that gap junctions play a role in the fast oscillations that precede the onset of seizure discharges in the hippocampus (Traub *et al.*, 2004; Timofeev *et al.,* 2004).

In brain slice preparations, drugs that block gap junction function (such as halothane, octanol, or carbenoxolone) suppress epileptiform discharges, including those induced by zero Ca^{2+} (Perez-Velazquez *et al.*, 1994; Jahromi *et al.,* 2002; Gigout *et al.*, 2006). Opening channels with trimethylamine or

alkalinization augments discharges (Köhling *et al.,* 2001). *In vivo* studies with gap junction blockers and openers have recently been performed in genetic and acquired rodent models of absence seizures (Tian *et al.,* 2005; Gigout *et al.,* 2006; Proulx *et al.,* 2006). These studies have established that systemically administered carbenoxolone can diminish spike-and-wave discharges and indicate that gap junctions play a role in the spread of seizure activity in the thalamus and the cortex. Moreover, the data identify connexins as plausible molecular targets for AED development.

The selective expression of the isoforms in specific neurons and astrocytes provides the opportunity for precise molecular targeting. Recent evidence implicating astrocytes in the generation of epileptic activity suggest that selective targeting of astrocytic gap junctions, such as Cx43, may be a fruitful strategy (Tian *et al.,* 2004). The novel benzoylamino benzopyran tonabersat [SB-220453; an analog of the anticonvulsant carabersat (SB-204269) (Upton *et al.,* 1997)], which is currently in clinical development for migraine, is believed to block gap junctions (Parsons *et al.,* 2001).

2.8 THE MICHAEL REACTION

Nuclophlic addition of a variety of reagents, HZ to activated alekene of type CH_2=CH-X to form Z-CH_2-CH_2-X is known as the Micheal reaction. The pre-requisite for this mode of addition to the alkene is that X be an electron-attracting group such as an aldehyde, ketone, carboxylic acid, ester, amide, nitrile, nitro or sulphenyl group to mention a few. Therefore it is expected that the carboxamide

group in acrylamide (XXXVII) would stimulate and direct the addition of nucleophilic reagents to the alkene.

$CH_2CHCONH_2$

CXXVIII

The orginal scope of Micheal reacton has been widened. Apart from active methylene compound, HZ now includes others like acetophenone, nitriles, nitrocompounds, sulfones, amines and certain hydrocarbon such as cyclopentadiene, and indenes that contain sufficiently reactive hydrogen atoms. Thus the reacton of HZ with acrylamide (CXXVIII) would yield the β-propanamide derivative (CXXIX)

$Z–CH_2CH_2CONH_2$

(CXXIX)

2.8.1 MICHAEL REACTION WITH ACRYLAMIDE

Generally speaking, only very few cases have been reported of Micheal condensation in which acrylamide acts as an acceptor. The very first was reported by Bruson in 1945.In this case, the active hydrogen atom of 2-nitropropane was replaced by the carbomoylethyl group on treatment with acrylamide (CXXVIII) in the presence of 40 percent trimethylbenzyl-ammonium hydroxide. No yields were given.

$O_2N–CH(CH_3)_2 + H_2C=CH–CONH_2 \longrightarrow O_2NCH(CH_3)_2–CH_2CH_2CONH_2$

(CXXVIII)

It has been observed that potassium fluoride catalyses the replacemnt of all the active hydrogen atoms of a number of nitroalkanes by the 2-carbamoylethyl group (Patterson and Barnes, 1967). Thus a mixture of nitroethane and

acrylamide in ethanol stirred for five days at 65-70^0 in the presecen of potassium fluoride gives 45 percent of the disubstituted product. Similarly, nitromethane reacts to give the trisubstituted product.

$$CH_3NO_2+CH_2=CHCONH_2 \longrightarrow O_2N–C(CH_2CH_2CONH_2)_3$$

$$CH_3CH_2NO_2+CH_2=CHCONH_2 \longrightarrow CH_3C(NO_2)(CH_2CH_2CONH_2)_2$$

A number of ketones have also been condensed with acrylamide (CXXVIII) in the Micheal type reaction with yields ranging from 10 to 50 percent (Elad and Ginsburg, 1983). In each instance, the nucleophilic moiety attaches to the β-carbon of acrylamide to form a new carbon bond. The ketones used include cyclohexanone, acetophenone, 2-phenycyclohexanone and dibenzylketone. A number of catalysts such as sodium hydride, potassium t-butoxide and sodamide were used.

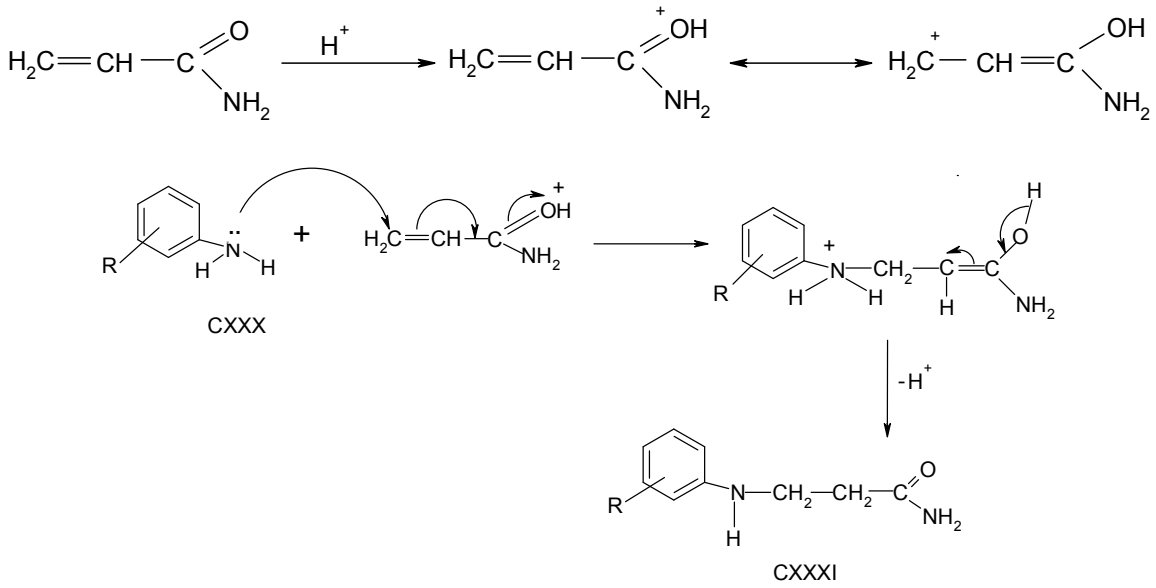
Micheal-like additions have also been reported between acrylamide and primary aliphatic nitramines to give secondary nitramine products (Kissinger and Schwartz, 1958). Amines and indoles have been added to acrylamide as well (Bukus *et al.,* 1968). In one of such reactons, addition of aromatic amines such as aniline, p-toluidine or p-anisidine was said to proceed without catalysis to give either the mono- or di-carbamoylethylated product depending on the reaction conditions. The reacton of acrylamide with indole gave β-(N-indolyl) propannamide, but only in the presence of potassium hydroxide at 100^{0}C in dioxane.

The influence of some solvents and catalytic mixtures on the rate of adition of a number of amines to acrylamide has been studied (Suminov, 1967). It was

reported that addition of the amino group to acrylamide took place most easily in water, worse in alcohols and, in aprotic solvents, the reaction rate decreased. Proton donors such as ammonium chloride increased the rate of addition of 3,5,-trimethylpyrazolone to acrylamide where as sodium or potassium fluoride or acetate did not affect the addition.

2.8.2 MECHANISM FOR THE ACID-CATALYSED MICHAEL ADDITION OF ACRYLAMIDE TO ANILINE/SUBSTITUTED ANILINES

As usual, the first step is the protonation of the carboxamide oxygen, followed by the attack of the amide on the β-carbon of the acrylamide (CXXVIII)



Scheme 1:

2.8.3 Mechanism for the synthesis of 3-anilinopropanamides (CXXXI)

2.8.4 CONVERSION OF 3-ANILINOPROPANAMIDES TO N-BENZYL-3-ANILINO-PROPANAMIDES BY AMINE EXCHANGE REACTION

It is well known that under suitable conditions, amides are hydrolysed by water in the presence of acid or base to generate the parent carboxylic acid and an amine (Zabicky, 1970).

$$R–CONR^1{}_2+H_20 \xrightarrow{H^+/^-OH} RCOOH + R^1{}_2NH$$

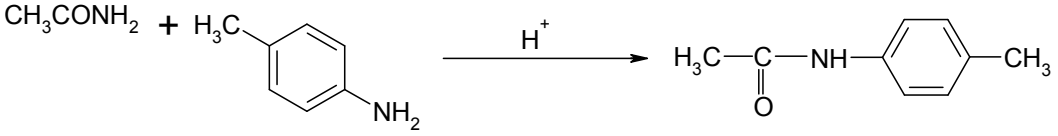
Similarly, amides react with nucleophilic solvents (other than water) such as amines, alcohols, hydroxylamides and carboxylic acids under suitable conditions. Amino group exchange occurs on heating amides with primary amines often without additional catalysts, but usually, the reactions only proceed to completion where the expelled amine is relatively volatile (Hurd *et al.,* 1932). It was found that amides such as acetamide, propanamide and isobutyramide do not react with aniline at room temperature or at 100^0C, but continued refluxing gives rise to small yields of anilides with evolution of ammonia. Fairly higher yields (45-49 percent), were obtained when the amide vapours were conducted directly into cold aniline.

$$R—\underset{\underset{O}{\|}}{C}—NH_2 + H_2NC_6H_5 \longrightarrow RCONHC_6H_5 + NH_3$$

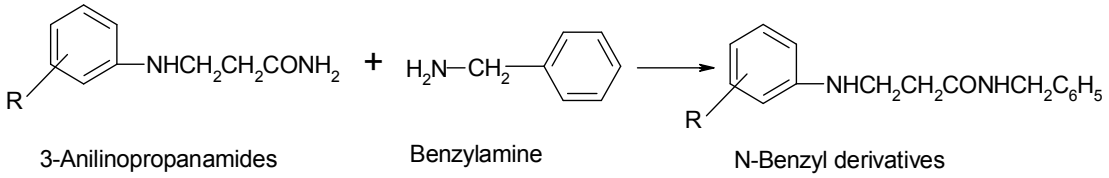
A number of mono- and di-alkylated amides have also been obtained by this process in the presence of borontrifluoride which removes the ammonia as monoamminoboron trifluordie (NH3-PF_3) (Sowa and Nieuland, 1937). The raction is represent-ted as follows:

$$CH_3CONH_2 \xrightarrow[(b)\ RNH_2]{(a)\ BF_3} CH_3CONHR + NH_3\,BF_3$$

Davidson and karten (1956) have also reported that ammonolysis of amides is accelarated considerably in the presence of acid catalysts. In the experiment, they reported that addition of catalytic amounts of glacial acetic acid gives 65 – 70 percent of aceto-p-toluidide on refuluxing.The role of the acid catalyst is that of protonation of the oxygen atom of the amide group with the formation of an iminohydrin structure.This results in an increase in the electrophilicity of the carboxamide carbon atom.

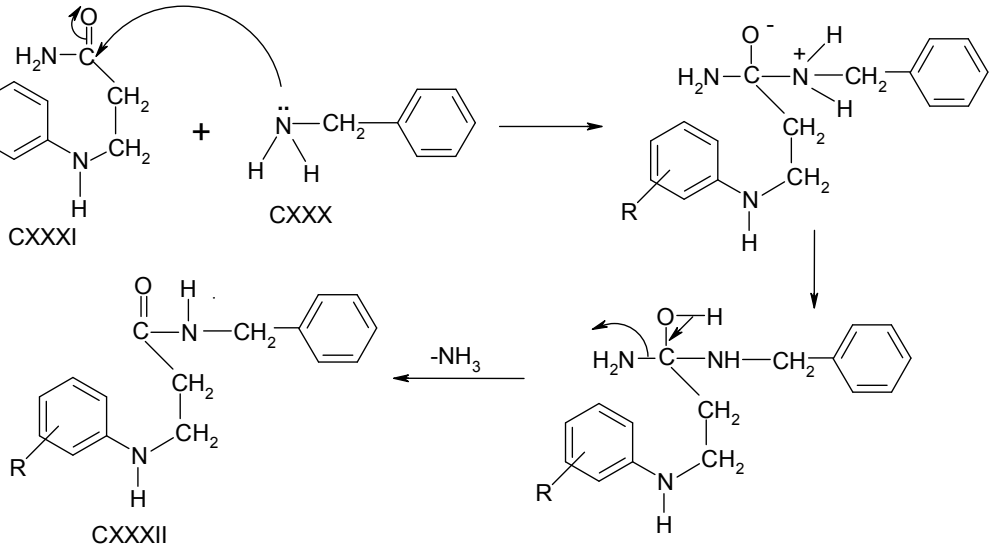


In this studies conversion 3-anilinopropanamides to the N-benzyl-3-anilinopro-pamanides was acehived by an uncatalysed amine exchange reaction with benxylamine according to scheme 2 below.



SCHEME 2: Conversion of 3-anilinopropanamides to N-Benzyl-3-anilinopropanamide (CXXXII)

The mechanism of the above reaction is as given below;



CHAPTER THREE

EXPERIMENTAL

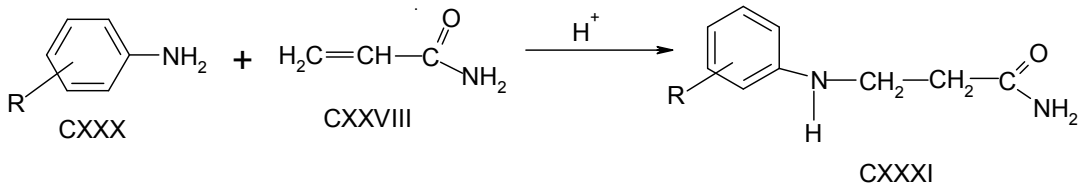
3.0 SYNTHESIS

3.1 MATERIALS (Reagents, equipments and glass wares)

Starting materials were Analar grade and used without further purification. Aniline; 2-,3- and 4-chloroanilines; 2-,3- and 4-anisidines; 2-,3- and 4-Toluidines, acrylamide, benzylamine, p-toluenesulphonic acid, iodine crystals and potassium permanganate were all obtained from BDH chemicals. The solvents (benzene, toluene, methanol, chloroform, ethylacetate and n-hexane) were also obtained from BDH chemicals. Glass wares (including measuring cylinders, conical flaks, round bottom flasks, beakers and test tubes) were of Pyrex made and were properly cleaned, rinsed (with distilled water) and dried before use. Other materials used include analytical balance (Mettler AE-240), steam/water bath (Gallenkamp), magnetic stirrer (Gallenkamp), ovun, (BS model OV33 Gallenkamp), microcapillary tubes (Drumen scientific company, U.S.A), thin layer chromatographic plates (5X20CM), reflux condenser (Pyrex) and melting point apparatus (Gallenkamp).

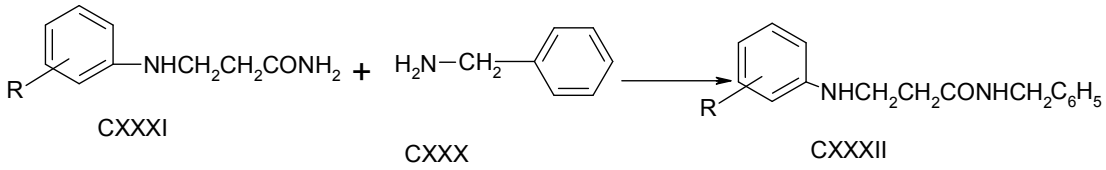
3.2 METHOD

The anilinopropanamides (1-10) were prepared through an acid-catalysed Michael reaction between substituted anilines (CXXX) and acrylamide (CXXVIII) in the presence of catalytic amounts of p-toluensulphonic acid (P-TOSA), according to scheme 1



Scheme 1 Synthesis of 3-anilinopropanamides (CXXXI)

Conversion of the 3-anilinopropanamides to the corresponding N-benzyl derivatives (11-15) was achieved by an uncatalysed amine exchange reaction with benzylamine (scheme 2).



Scheme 2 Synthesis of N-Benzyl derivatives of 3-anilinopropanamides (CXXXII)

3.3 PROCEDURE

Progresses of all the reactions were monitored by Tin Layer Chromatography (TLC) using ethylacetate as development solvent. Purity of starting materials and of end products were also assessed using TLC. The TLC was performed on Merck aluminum plates pre-coated with 0.2mm silica gel (60-F). Chromatograms were visualized under U.V fluorescence quenching at 254m or by staining with potassium permanganate solution or iodine vapour.

3.3.1 GENERAL PROCEDURE (A) FOR THE PREPARATION OF THE 3- ANILINOPROPANAMIDES

A mixture of 0.5mole of acrylamide (CXXVIII) and 0.5mole substituted aniline (CXXX) was heated together (in the presence of catalytic amount of P-Toluene-sulphonic acid) on a water bath maintained at about 80^0C until the mixture became homogenous. The mixture was then allowed to stand at room temperature until the reaction was complete (as indicated on the TLC). The product formed was digested in benzene, filtered, and the residue washed with several portions of chilled benzene (5ml). Recrystallisation of the product in benzene, Toluene or ethanol gave the appropriate 3-anilino-propanamide. The yields and physical properties of the compounds are given in table 4.1.

3.3.1.1 PREPARATION OF 3-CHLOROANILINOPROPANAMIDES (1, 2, 5)

A mixture of acrylamide (35.54g, 0.5mol), the appropriate chloroaniline (63.79, 0.5mol) and P-Toluenesulphonic acid (2.0g, 0.012mol) contained in a 250ml conical flask was clamped unto a water bath maintained at about 80^0C for about 30 minutes with constant stirring. The resultant syrupy liquid was allowed to stand at room temperature and a thick mass of substance was formed within 5-10 minutes. After cooling the flask was covered with aluminium foil and the product was allowed to stand for 24-48 hours depending on the TLC findings. During this period the mass crystallised progressively. The crystals were filtered (hot), washed with benzene and dried for 15-20 minutes. The filtrate (mother liquor) was allowed to cool and crystallized. The products were recrystallized several times in benzene until a single spot was obtained on TLC.

3.3.1.2 PREPARATION OF 3-TOLUIDINOPROPANAMIDES (3, 4, 7)

A mixture of the appropriate toluidine (53.50, 0.5mol), acrylamide (35.55, 0.5mol) and P-toluenesulphonic acid (2.0g, 0.012mol) was refluxed on a water bath (maintained at about 80^0C) until the mixture was homogenous. The resultant liquid was left to stand at room temperature for about 2 weeks. The cake formed was digested in benzene, filtered and the residue was washed with several portions of 5ml ice-cold benzene. The products were recrystallized in benzene several times until a single spot on TLC was obtained.

3.3.1.3 PREPARATION OF 3-ANISIDINOPROPANAMIDES (6, 8, 9)

A mixture of the anisidine (61.58, 0.5mol), acrylamide (35.54g, 0.5mol) and P-Toluenesulphonic acid (2.0g, 0.012mol) contained in a 250ml conical flask was refluxed on water bath (80^oC) for about 45 minutes. The homogenous mixture was allowed to cool to room temperature whence a syrupy liquid separated out. The flask was placed on an ice-bath for 2 hours. The solid formed was dispersed in benzene, filtered, and washed with fresh cold portions of benzene. The products were recrystallized in benzene several times until a single spot on TLC was obtained.

3.3.1.4 PREPARATION OF ANILINOPROPANIMIDE (10)

A mixture of aniline (46.55g, 0.5mol), acylamidess (35.54, 0.5mol) and p-toluenesulphonc acid (2.0g, 0.012mol) contained in a 250ml conical flask was reflused on water bath (80^0C) for 45 minutes. The homogeneous mixture was

allowed to cool to room temperature whence a syrupy honey-like liquid separated out. The flask was placed on an ice-bath for 2 hours. The solid formed was dispersed in benzene, filtered and washed with fresh cold portions of benzene. Recrystallisation from benzene gave grey granutes.

3.3.2 GENERAL METHOD (B) FOR THE PREPARATION OF THE N-BENYLDERIVATIVES OF THE ANILINOPROPANAMIDES (11-15)

A mixture of about 0.22mole each of the 3-anilinopropanamide (CXXXI) and benzylamine (CXXX) was refluxed on oil bath (170-180^{0}C) for about 24 houres. The set up was allowed to cool to room temperature and the solid formed was digested in chloroform and collected by filtration. The products were recrystallized in benzene/chloroform until a single spot was obtained on TLC. The yields and physical properties of compounds are given in Table 4.1.

3.3.2.1 PREPARATION OF N–BENZYL–3–ANILINOPROPANAMIDE (11)

A mixture of **10** (36.08g, 0.22nol) and benzylanine (23.54g, 0.22mol) was measured into a 250ml round bottom flask. The mixture was refluxed on an oil bath (170-180^{0}C) for about 25 houres.The content of the flask was allowed to cool to room temperature. The solid formed was dispersed in chloroform, filtered and washed with several 5ml portions of ice-cold chloroform. The product was recrystalized several times until a single spot on TLC was obtained.

3.3.2.2 PREPARATION OF N–BENZYL–3–δ–TOLUIDINOPROPANAMIDE (12)

A mixture of **4** (39.16g, 0.22mol) and benzylamine (23.54g, 0.22mol) was refluxed for 25 hours. The product was recrystallized several times in benzene until a single spot on TLC was obtained.

3.3.2.3 PREPARATION OF N–BENZYL-3-(δ-CHLORO)ANILINOPROPANAMIDE (13)

A mixture of **5** (43.69g, 0.22mol) and benzylamine (23.54g, 0.22mol) was refluxed for 12 hours as described in the general method (B). The product was recrystalized several times in benzene until a single spot on TLC was obtained.

3.3.2.4 PREPARATION OF N–BENZYL –3–δ– ANISIDINOPROPANAMIDE (14)

A mixture of **9** (42.68, 0.22mol) and benzylamine (23.54g, 0.22mol) was refluxed according to the general method (B) for 45 hours. The product obtained was recrystalised in benzene resulting in a white fluffy compound.

3.3.2.5 PREPARATION OF N–BENZYL–3–M– ANISIDINOPROPANAMIDE (15)

A mixture of 6 (42.68, 0.22mol) and benzylamine (23.54g, 0.22mol) was refluxed for an hour (Gen. Method B). The compound obtained was recrystalised in benzene to give a dirty white but fluffy compound. The yield and other physical characteristics are given on table 4.1

3.4 CHARACTERIZATION OF THE 3-ANILINOPROPANAMIDES

1. *Melting Point Determination*

   All melting points were determined using a Gallenkamp melting point apparatus and were uncorrected.

2. *Microanalysis (Elemental Analysis)*

   Elemental carbon, hydrogen and nitrogen were determined with a Parkin Elmer model 240 elemental analyzer; chlorine were determined by the Schröniger combustion (University of leads, England).

3. *Infrared (IR) Spectroscopy*

   IR were recorded on a Parkin Elmer paragon 1000 or Parkin Elmer 881 spectrometer as a thin film between sodium chloride plates or as KBr disc. All absorptions are reported in terms of frequency of absorption CM^{-1} (University of Readings, U. S. A)

4. Mass spectra were measured on an AP2000 (IS, 70ev) instrument

   (University of Leads, England)

5. *Nuclear Magnetic Resonance (NMR) Spectroscopy*

   Proton and carbon-13 NMR (^{1}H NMR and ^{13}CNMR) spectra were recorded on a Brůker CWM 250 (250 MHz), a Brůker DPX 250 (250 MHz) or a Brůker AMX 400 (400 MHz) (University of Readings England) or Joel AL-300 spectrometer (JAPAN) with TMS as an internal standard. Data for ^{1}HNMR are reported as follows; chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet q = quartet, m = multiplet); Integration, coupling constant (in Hz). Data for ^{13}CNMR are reported in terms of chemical shift.

3.5 SCREENING FOR ANTICONVULSANT ACTIVITY

Anticonvulsant screening was established by both electrical and chemical procedures which have been previously described (Swinyard *et al.,* 1989)

3.5.1 Materials

The equipment for the MES screening is an Ugo Basile electroconvulsive unit ECT 800 fitted with ear clip electrodes. Phenytoin and pentylenetetrazole were obtained from Sigma-Aldrich (St Louis MO), Valprioc acid from Sanofi-synthelabo and propylene glycol from BDH chemical (Poole England). Other materials used include Avery top loading balance (W. and T, Avery England) and plastic cages of various sizes (E. K. brown, London) Avery, England) and plastic cages of various sizes (E. K. Brown, London).

3.5.2 Animals

Swiss albino (male) mice in the weight range 18-30g were used. The animals were inbred in the animal house of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were kept under standard laboratory conditions (temperature 25 ± 3^0C; humidity 45 – 60% and 12 hours light/dark cycle) and were allowed free access to both food (Vital feeds mixed with fish and groundnut cake) and water except when they were removed from their cages for the experiments.

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

LD_{50} determination was conducted using the method of Lorke (1983). In the initial investigation mice were divided into three groups of three mice each and treated with the drugs at 10, 100 and 1000mg/kg body weight i.p. and observed for 24 hours. The results of these tests were used as the bases for selecting the

subsequent dosages. The LD_{50} was calculated as the square root of the product of the lowest dosage that kill all the animals and the highest dosage survived by the entire animal in the group.

3.5.2 Determination of Anticonvulsant Activity

Determination of time of peak anticonvulsant effect:

Maximum electroshock (MES) method was employed for the determination. 100mg/kg of the compound was injected intraperitoneally to four groups of mice, each group consisting of six mice. The first group was subjected to MES at 15 minutes, while the second, third and fourth, group were given at 30, 45 and 60, minutes respectively. In each case the number of mice protected was recorded. From the data the time of peak anticonvulsant activity was determined (stables and Kupferberg, 1977)

3.5.4 Primary (Qualitative) Evaluation of the 3-anilinopropanamides and their N-benzyl Derivatives

3.5.4.1 The Maximal Electroshock Seizure (MES) Test:

The method of Swinyard *et al* (1989) was employed. 48 male albino mice (18-30g) were ramdomly divided into six groups of 8 mice per group. Three groups received intraperitoneal injection of 30mg, 100mg and 300mg respectively of the test compound (dissolved in propylene glycol) while the control group received 25mg/Kg propylene glycol solution i.p. The other two groups received 20mg/Kg (i.p) of phenytoin and valproic acid respectively. An electrical stimulus enough to produce a hind limb tonic extensor in at least 97% of control animals (50mA at 60Hz for 0.2sec) was delivered through clip electrodies using an Ugo Basile

electroconvulsive generator. Abolition of the hind limb tonic extensor component indicates the compounds ability to inhibit MES induced seizures spread. The tests were conducted at 0.5hr and 4hrs after injection of the test compound.

3.5.4.2 The subcutaneous Pentylenetetrazol (scPTZ) Seizure Test

The method of Swinyard *et al* (1989) was employed. 48 male albino mice (18-30g) were randomly divided into six groups of 8 mice per group. Three groups received intraperitoneal injection of 30mg, 100mg and 300mg respectively of the test compound (dissolved in propylene glycol) while the control group received 25mg/kg propylene glycol solution IP. The other 2 groups received 20mg/kg (i.p) of phenytion and valproic acid respectively. 85mg/kg metrazole (which produces clonic seizures lasting for a period of at least five seconds in 97 percent of control animals) was injected subcutaneously. The mice were observed for 30 minutes for the occurrence of seizures. Absence of clonic spasms in the observed time period indicates a compounds ability to abolish the effect of pentylenetetrazole on seizure threshold. The tests were conducted at 0.5hr and 4hr after the injection of the test compound.

3.5.4.3 Determination of minimal neurotoxicity

The method of Swinyard *et al* (1952) was employed. 32 male albino mice were randomly divided into 4 groups of eight animals per group. Each animal was examined for its neurological status before the administration of the test drug. The first second and third groups were given 30mg, 100mg and 300mg of the compound respectively, while the last group was given 25mgkg^{-1} propylene glycol (ip). The mice were placed or their back. Nerological deficit was indicated by inability of the animal to quickly resume their normal posture. The tests were

conducted at 0.5hr and 4hrs after injection of the compounds. The number of mice that showed signs of nerotoxicity at various dose levels was recorded.

3.5.5 Secondary (Quantitative) Evaluation of the 3-anilinopropanamides and their N-benzyl derivatives

All quantitative determinatons were carried out at the estimated time of peak anticonvulsant activity of the candidate compounds.

3.5.5.1 Determination of time of peak anticonvulsant effect:

Maximum electroshock (MES) method was employed for the determination. 100mg/kg of the compound was injected intraperitoneally to four groups of mice, each group consisting of six mice. The first group was subjected to MES at 15 minutes, while the second, third and fourth, group were given at 30, 45 and 60, minutes respectively. In each case the number of mice protected was recorded. From the data the time of peak anticonvulsant activity was determined (Stables and Kupferberg, 1977)

3.5.5.2 Determination of Minimal Neurotoxicity (TD_{50}):

The method of Swinyard (1952) was employed. Each animal was examined for its neurological status before the administration of the test drug.Groups of eight mice were given graded doses of the candidate compound such that two points were established between limits of 100 percent toxicity and zero percent toxicity. The animals were subjected to righting test as described above. The number of mice that showed sign of neurotoxicity at various dose levels was recorded and the TD_{50} was determined using graphical method of Miller and Tainter (1944).

3.5.5.3 Determination of the median effective dose ED_{50}

The method of Swinyard (1989) was used. Several groups of at least eight mice per group received various doses of the candidate compound until at least two points were established between the limits of 100 percent protection and zero percent protection using the MES, and ScPTZ tests as described above. The control group received 25mg/kg of the vehicle (propylene glycol). The ED_{50} was calculated using the graphical method of Miller and Tainter (1944).

3.6 STATISTICAL ANALYSIS

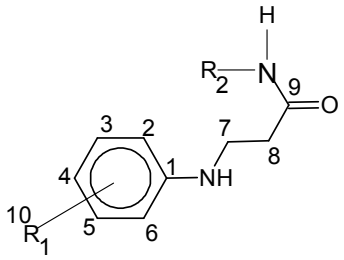
Student's T-test was used to determine level of significance of all results obtained. Results were regarded as significant at $P<0.05$.

CHAPTER FOUR

4.0 RESULTS

TABLE 4.1A PHYSICOCHEMICAL PROPERTIES OF THE

3- ANILIONOPROPANAMIDES AND THEIR N-BENZYL DERIVATIVES



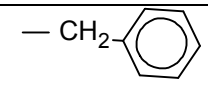
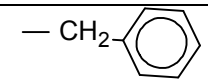
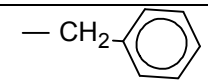
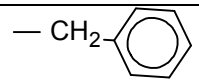
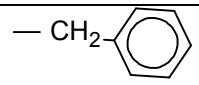
Comp.	R_1	R_2	M.pt^{O}C	%Yield	RT. in mins.
1.	4-Cl	H	105.107	95.00	35.393
2.	3-Cl	H	82-83	96.32	35.603
3.	4-CH_3	H	99-100	90.70	32.752
4.	2-CH_3	H	97-98	85.20	34.440
5.	2-Cl	H	110-113	78.4	35.191
6.	3-OCH_3	H	62-63	94.50	33.124
7.	3-CH3	H	91.50	96.45	32.184
8.	4-OCH_3	H	113-114	92.00	32.916
9.	2-OCH_3	H	105-106	65.00	34.498
10.	H	H	54-55	25.00	39.99
11.	2-H		211-212	65.00	31.728
12.	2-CH_3		148-149	25.00	30.184
13.	2-Cl		127-128	65.0	29.516
14.	2-OCH_3		120-121	56.20	28.532
15.	3-OCH_3		151-152	48.30	29.431

TABLE 4.1B PHYSICOCHEMICAL PROPERTIES OF THE 3-ANILINOPROPA-NAMIDES AND THEIR N-BENZYL DERIVATIVES.

Compound	Elemental Composition	Physical Nature/colour	Formula	MWt
A01	C,H,N,Cl	Ash coloured granule	$C_9H_{11}N_2ClO$	198
A02	C,H,N,Cl	Light purple crystals	$C_9H_{11}N_2ClO$	198
A03	C,H,N	Dirty white flakes	$C_{10}H_{14}N_20$	178
A04	C,H,N,N,Cl	Purple granules	$C_{10}H_{14}N_20$	178
A05	C,H,N	Dark purple prismatic needles	$C_9H_{11}N_2ClO$	198
A06	C,H,N	Cream coloured crystals	$C_{10}H_{14}N_20_2$	194
A07	C,H,N	Deep purple crystals	$C_{10}H_{14}N_20$	178
A08	C,H,N	Brown crystals	$C_{10}H_{14}N_20$	194
A09	C,H,N	White flakes	$C_{10}H_{14}N_20$	194
A10	C,H,N	Grey granules	$C_9H_{12}N_20$	164
A11	C,H,N	White fluffy material	$C_{16}H_{18}N_20$	254
A12	C,H,N	Deep brown granules	$C_{17}H_20N_20$	268
A13	C,H,N,Cl	Dirty white crystals	$C_{16}H_{17}N_2ClO$	288
A14	C,H,N	White fluffy granules	$C_{17}H_20N_20_2$	284
A15	C,H,N	Light yellow crystals	$C_{17}H_20N_20_2$	284

MWt = Molecular weidgt

TABLE 4.2 RESULTS OF MICROANALYSIS OF 3-ANILINOPRONAMIDES (A01-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15).

COMP.	CARBON	HYDROGEN	NITROGEN	CHLORINE
	Found Calcd	Found Calcd	Found Calcd	Found Calcd
A01	54.58 (54.55)	5.61 (5.56)	14.26 (14.14)	17.87 (11.68)
A02	54.64 (54.55)	5.59 (5.56)	14.34 (14.34)	17.76 (17.68)
A03	67.51 (67.42)	7.89 (7.87)	15.85 (15.85)	‘
A04	67.45 (67.42)	7.92 (78.7)	15.79 (15.79)	‘
A05	54.64 (54.55)	5.61 (5.56)	14.27 (14.27)	17.83 (17.68)
A06	61.92 (61.86)	7.25 (7.22)	14.48 (14.48)	‘
A07	67.52 (67.42)	7.96 (7.87)	15.81 (15.81)	‘
A08	61.89 (61.86)	7.28 (7.22)	14.60 (14.43)	‘
A09	61.87 (61.86)	7.34 (7.22)	14.58 (14.43)	‘
A10	65.93 (65.65)	7.41 (7.32)	17.27 (17.07)	‘
A11	75.65 (75.59)	7.16 (7.09)	11.16 (11.02)	‘
A12	76.34 (76.12)	7.53 (7.46)	10.56 (10.45)	‘
A13	66.69 (66.67)	5.99 (5.90)	9.89 (9.72)	12.24 (12.15)
A14	71.91 (71.83)	7.14 (7.04)	9.97 (9.86)	‘
A15	71.87 (71.83)	7.12 (7.04)	9.91 (9.86)	‘

Calctd: calculated values are given in parenthesis

TABLE 4.3 INFRARED SPECTRA DATA FOR 3-ANILINOPROPANAMIDES AND THEIR N-BENZYL DERIVATIVES

SAMPLE	N-H-str	=C-H_{ar} str	C=C	C=O Amide Band I	N-Hdef Amide Band II	C-Nstr Ar 1280	C—H_{ipb}	Para	Meta	ortho
A0 1	3445.59(m) 3319.56(s)	3175.56(m)	1602.45(m)	1654.39(s)	1513.37(s) 1490.90(s)	1296.45(m) 1266.13(m)	1084.56(m) 1050.93(m)	813.86(m) 795.81(m)		
A02	3391.88(m)	3176.39 (m)	1596.23(m)	1654.42(s)	1596.23(s)	1283.99(m)	1088.20(m) 1076.85(m)		767.46(s)	
A03	3396.94(s)	3159.06(m)	1612.11(m)	1650.34(s)	1517.58(s)	1306.12(m)	1125.06(s) 1100.09(s)	807.90(s)		
A04	3410.62(m)	3202.59(m)	1603.69(m)	1645.10(s)	1514.56(s)	1219.91(s)	1126.69(s) 1022.95(s)		730.10(s)	
A05	3398.00(m)	3178.25(m)	1606.80(m)	1653.08(s)	1517.47(s)	1286.12(s)	1177.47(s)		774.76(s)	
A06	3358.98(m)	3180.41(m)	1613.90(m)	1644.11(s)	1517.02(s)	1264.67(s)	1035.84	803.89(s)		
A07	3411.23(m)	3202.61(m)	1603.44(m)	1645.34(s)	1513.46(s)	1297.25(s)	1022.33(m) 1175.69(m)		729.95(s)	
A08	3332.10(s)	3160.57(m)	1609.95(m)	1659.08(s)		1135.88(s)	1279.01(m)	815.89(s)		
A09	3409.00(s) 3335.50(s)	3187.9(s)	1609.30(m)	1656.30(vs)	1519.50(s)	1287.20(s)	1177.80(s)			697.10(s)
A10	3356.10(s/d)	3184.10(s/b)	1600.21(m)	1651.80(vs)	1521.00(s)	1252.90(s)	1265.00(m)		733.40(s)	
A11	345.90(m)	3209.40(s/b)	1600.82(m)	1651.60(vs)	1621.10(s)	1285.33(s)	1252.80(m)			670.00(s)
A12	3415.80(m)	3005.30(s/b)	1600.50(m)	1651.60(vs)	1531.00(s)	1299.20(s)	1129.60(m)		732.90(s)	
A13	3330.26(m)	3050.29(s/b)	1606.80(m)	1653.08(vs)	1589.44(s)	1246.29(s)	117.47(m)			696.05(s)
A14	3385.00(m)	3063.71(s/b)	1592.16(m)	1620.09(vs)	1514.56(s)	1280.65(s)	1101.40(m)			7001.62(s)
A15	3386.43(m)	3062.00(s/b)	1619.98(m)	1645.34(vs)	1513.46(s)	1297.25(s)	1055.53(m)		729.95(s)	

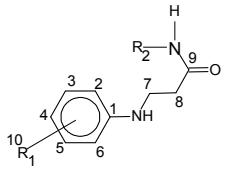


TABLE 4.4 ^{1}HNMR SPECRA DATA FOR THE 3-ANILINOPROPANAMIDES (A01-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15).

AO1 δ(ppm): 7.53(brs, 1H,-N**H**), 7.16-7.18(dd, 2H, $^{3}J_{3,2}$ = 8.51, 2Ar-H, **H**-2 and**H**-6), 6.55-6.60(dd, 2H, $^{3}J_{3,2}$ = 8.51, $^{3}J_{5,6}$ =8.51, **H**-3 and **H**-5), 4.90(s, 2H,-N**H**$_2$), 3.30-3.39 (m, 2H, $^{3}J_{7,8}$ = 7.30, **H**-7) 2.50 (t, 2H, $^{3}J_{8,7}$ = 7.34, **H**-8).

AO2 δ(ppm): 8.86(brs, 1H-N**H**), 7.05-6.96(t, Ar-H, $^{3}J_{5,4}$ = 8.04, $^{3}J_{5,6}$ = 8.20,**H**-**5**), 6.93(s, 1H, Ar-H, **H-2**) 6.58-6.54 (d, 1H, $^{3}J_{6,5}$ = 8.20, Ar-H , **H-6**), 6.53-6.40(d, 1H, $^{3}J_{4,5}$ = 8.04, Ar-H, **H-4**), 4.80(s, 2H,-N**H**$_2$), 3.35 - 3.25(t, 2H, $^{3}J_{7,8}$ = 7.34,-NH-C**H**$_2$, **H-7**), 2.42 – 2.40 (t, 2H, $^{3}J_{8,7}$ = 7.34-NH-CH$_2$-C**H**$_2$-,**H-8**). Meta substitution confirmed since $^{4}J_{6,2}$ = 2.09(for meta subst., J= 2 – 3)

AO3 δ(ppm): 7.53 (brs, 1H,-N**H**), 6.85-6.82 (dd, 2H, $^{3}J_{3,2}$ = 8.33, $^{3}J_{5,6}$ = 8.33 2Ar-H, **H-3** and **H-5**), 6.60-6.55 (dd, 2H, $^{3}J_{2,3}$ = 8.33, $^{3}J_{6,5}$ = 8.33 2Ar-H, **H-2** and **H-6**), 4.80 (5, 2H,-N***H$_2$***), 3.35-3.40 (t, 2H, $^{3}J_{8,7}$ = 6.64,-NHCH$_2$-C**H$_2$,H-8**), 2.35 (5, 3H, -C**H$_3$**, **H-10**). Since $^{5}J_{2,5}$ = 0.45 and $^{5}J_{3,6}$ = 0.45 para substitution is confirmed (J = 0-1 for para substitution).

AO4 δ(ppm): 6.82-6.75 (m, 1H, $^{3}J_{4,3}$ = 759, Ar-H, **H-4**), 6.60 (dt, 2H, $^{3}J_{3,4}$ = 7.59, $^{3}J_{5,4}$ = 7.27, $^{3}J_{5,6}$ = 7.92, 2Ar-H, **H-3** and **H-5**), 4.85 (5, 2H, N**H$_2$**), 3.80 (t, 2H, $^{3}J_{7,8}$ = 6.64 –NH-C**H$_2$, H-7**), 3.40 (t, 2H, $^{3}J_{8,7}$ = 6.64 -NH-CH_2-**CH_2-H-8**), 2.45 (5, 3H, -C**H$_3$**). Since $^{3}J_{5,4}$ = 7.27, $^{3}J_{5,6}$ = 7.92 and $^{3}J_{4,3}$ = 7.59, ortho substitution is confirmed. (J = 7-10 for σ-subst.)

AO5 δ(ppm): 7.1- 7.15 (d, 1H, $^{3}J_{3,4}$ = 7.84, Ar-H, **H-3**), 7.12-7.10 (t, 1H, $^{3}J_{4,5}$ = 7.24, $^{3}J_{5,6}$ = 8.11, Ar-H, **H-5**), 7.09-6.75 (d, 1H, $^{3}J_{6,5}$ = 8.11, Ar-H,**H-6**), 6.82 (5, 2H,-N**H$_2$**), 6.77-6.75 (t, 1H, $^{3}J_{4,3}$ = 8.11, $^{3}J_{4,5}$ = 7.24, Ar-H, **H-4**), 3.26-3.24 (t, 2H, $^{3}J_{7,8}$ = 7.34 $^{3}J_{8,7}$ = 7.34 –NH-C**H$_2$**-, **H-7**), 2.58 (t, 2H, $^{3}J_{8,7}$ = 7.34 – NH-CH_2-C**H$_2$**-, **H-8**).

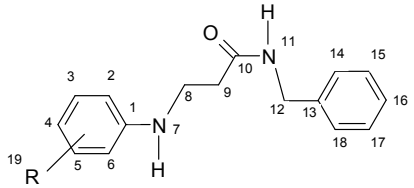
AO6 δ(ppm): 7.25 (5, 2H, -N**H$_2$**-), 7.10-7.05 (t, 1H, $^{3}J_{5,6}$ = 8.20, $^{3}J_{5,4}$ = 8.03, Ar-H, **H-5**), 6.60-6.55 (d, 1H, $^{3}J_{6,5}$ = 8.2, Ar-H, **H-6**), 6.45-6.40 (dd, 2H,$J_{4,5}$ = $^{3}J_{5,6}$ = 8.2, Ar-H, **H-2** and **H-4**), 3.45-3.40 (t, 2H $_{3}J_{7,8}$ =7.34 -NH-C**H$_2$ H-7**), 2.50 (t, 2H, $^{3}J_{8,7}$ = 7.34 -NH,-CH_2-C**H$_2$**-,**H-8**), 2.28 (s, 3H,OC**H$_3$**, **H-10**). Since $^{4}J_{6,2}$ = 2.38 and $^{4}J_{4,2}$ = 2.38, meta substitution is confirmed.

AO7 δ(ppm): 7.41 (brs, 1H, -N**H**-), 6.98-6.94 (d, 1H, $^{3}J_{6,5}$ = 8.2, Ar-H, **H-6**) 6.77 (s, 2H, –N**H$_2$**), 6.77-6.75 (t, 1H, $^{3}J_{5,4}$ = 7.72, $^{3}J_{5,6}$ = 8.20, Ar-H, **H-5**), 6.65-6.63 (d, 1H, $^{3}J_{4,5}$ = 7.72, Ar-H, **H-4**), 6.46 (s, 1H, Ar-H, **H-2**), 3.26-3.24 (t, 2H, $^{3}J_{7,8}$ = 7.34, -NH-CH_2-CH_2- **H-8**) $^{4}J_{2,6}$ = 2.54, $^{4}J_{2,4}$ = 1.85, $^{4}J_{4,6}$ = 1.95 meta substation

AO8 δ(ppm): 7.40 (s, 1H, – N**H**,-), 7.30 (s, 2H, – N**H$_2$**), 7.05-7.00 (dd, 2H $^3J_{2,3}$ = 8.93, $^3J_{6,5}$ = 8.93, 2Ar-H, **H-2** and **H-6**), 6.60-6.55 (dd, 2H, $^3J_{3,2}$ = 8.93, 2Ar-H, **H-3** and **H-5**), 3.45-3.40 (t, 2H, $^3J_{7,8}$ = 7.34, –NH-C**H$_2$** -**H-7**), 2.60-2.55 (t, 2H, $^3J_{8,7}$ = 7.34, -NH-CH$_2$-C**H$_2$**-, **H-8**), 2.30-2.25 (s, 3H, – OC**H$_3$**).

AO9 δ(ppm): 7.25 (s, 1H, -N**H**), 6.90 (d, 1H, $^3J_{3,4}$ = 8.54, Ar-H, **H-3**), 6.85 (t, 1H,$^3J_{4,5}$ = 7.53, Ar-H, **H-4**), 6.70 (t, 1H, $^3J_{5,6}$ = 8.27, Ar-H, **H-5**), 6.6(d, 1H, $^3J_{6,5}$ = 8.27, Ar-H, **H-6**), 5.92 -5.75 (brd, 2H, – N**H$_2$**), 3.88 (5, 3H, – OC**H$_3$**), 3.49-3.51 (t, 2H, $^3J_{7,8}$ = 7.34, -NH-C**H$_2$**-,**H-7),** 3.49 –3.51 (t, 2H, $^3J_{7,8}$ = 7.34, -NH-C**H$_2$**-, **H-7**), 2.55-2.52 (t, 2H, $^3J_{8,7}$ = 7.34, -NH-CH$_2$-C**H$_2$**-, **H-8**)

AO10 δ(ppm):7.30 (s, 1H, -N**H**), 6.25-5.75 (m, 5H, $^3J_{5,6}$ = 8.2 $^3J_{5,4}$ = 7.38, $^3J_{4,3}$ = 7.38, $^3J_{3,2}$ = 8.2, 5Ar-H, **H-1**, **H-2**, **H -3, H-4**, **H -5** & **H -6**) 4.8 (s, 2H, –NH$_2$), 3.45 (t, 2H, $^3J_{7,8}$ = 7.34,-NH-CH-C**H$_2$**-, **H-7**), 2.50 (t, 2H, $^3J_{8,7}$ = 7.34, -NH-CH$_2$-C**H$_2$**-, **H-8**)



AO11 δ(ppm):7.67 (s, 1H, Ar-NH, **H-7**), 6.95 (s, 1H,-CO-NH-, **H-11**), 6.73-6.70 (dd, 2H, $^3J_{14,5}$ = 7.83, $^3J_{18,17}$ = 7.83, Ar-H**, H-14** and **H-18**), 6.68-6.64 (m, 4H, $^3J_{3,2}$ = 8.20, $^3J_{3,4}$ = 7.38, $^3J_{4,5}$ = 7.38, $^3J_{5,6}$ = 8.20, **H-2**, **H-4**, **H-5**, **H-6**), 6.62-6.60 (t, 1H, $^3J_{16,15}$ = 7.05, $^3J_{16,17}$ = 7.05, Ar-H, **H-16**), 6.61-6.58 (dd, 2H, $^3J_{15,14}$ = 7.83, $^3J_{15,16}$ = 7.05, $^3J_{17,18}$ = 7.83, $^3J_{17,16}$ = 7.05 **H-15** and **H-17**), 6.57-6.55 (t, $^3J_{14,13}$ = 7.83,

$^{3}J_{14,15}$ = 7.05, Ar-H, **H-14**), 6.53-6.50 (dd, 2H, $^{3}J_{12,13}$ =7.83, $^{3}J_{16,15}$ =7.83, 2Ar-H, **H-12** and **H-16**), 4.54-4.52 (s, 2H, $^{3}J_{10,11}$ = 5.0, -CO-NH-CH_2-, **H-12**), 3.23-3.21 (t, 2H, $^{3}J_{7,8}$ = 7.34, Ar-NH-C**H_2**-,**H-8**), 2.55-2.52 (t, 2H, $^{3}J_{8,7}$ = 7.34, -NH-CH_2-C**H_2-, H-9**)

AO12 δ(ppm):7.60-7.57 (t, 1H, $^{3}J_{11,12}$ = 5.00 –CO-N**H**-, **H-11**), 7.28-7.24 (dt,1H each, $^{3}J_{15,16}$ = 7.05, $^{3}J_{17,18}$ = 7.83 $^{3}J_{17,16}$ = 7.05, 2Ar-H, **H-15, H-17**) 7.26-7.12 (t, 1H, $^{3}J_{16,15}$ = 7.05, $^{3}J_{17,18}$ = 7.05, Ar-H**, H-16**), 6.87- 6.85 (dd, 2H, $^{3}J_{14,15}$ = 7.83, $^{3}J_{18,17}$ = 7.83, 2Ar-H, **H-14**, **H-18**), 6.80- 6.79 (m, 2H, $^{3}J_{4,3}$ = 7.59, $^{3}J_{4,5}$ = 7.27, 2Ar-H, **H-3**, **H-4**), 6.77-6.73 (t, 1H, $^{3}J_{5,6}$ = 7.92, Ar -H, **H-5**), 6.53-6.50 (d, 1H, $^{3}J_{6,5}$ = 7.92, Ar-H, **H-6**), 5.68 (s, 2H, Ar-N**H**-CH_2-CH_2 -, **H-7**) 4.54 (s, 2H, -CO-CH_2 -,**H-11**) 3.16-3.12 (t, 2H, $^{3}J_{8,9}$ = 7.34, Ar-NH-C**H_2**-, **H-8**) 2.55-2.51 (t, 2H $^{3}J_{9,8}$ = 7.34, Ar-NH-CH_2-**CH_2**-, **H-9**), 2.43 (s, 3H, –C**H_3**, **H-19**).

AO13 δ(ppm):7.60-7.57 (t, 1H, $^{3}J_{11,12}$ = 5.00, -C-N**H**–, **H-11**), 7.28 – 7.24 (m, 2H, $^{3}J_{15,14}$ = 7.83, $^{3}J_{15,16}$ = 7.05, $^{3}J_{17,18}$ = 7.83, $^{3}J_{17,16}$ = 7.05, 2Ar-H, **H-15**, **H-17**), 7.17-7.12 (t, $^{3}J_{16,17}$ = 7.05, $^{3}J_{5,4}$ = 7.24, $^{3}J_{5,6}$ = 8.11, 2Ar- H, **H-3** and **H-5**), 6.96-6.93 (t,1H, $^{3}J_{6,5}$ = 8.11, Ar-H, **H-6**), 6.87-6.85 (m, 2H, $^{3}J_{14,15}$ = 7.83, $^{3}J_{18,17}$ = 7.83, 2Ar-H, **H-14**, **H-18**), 6.77 – 6.72 (t, 1H, $^{3}J_{4,3}$ = 7.84, $^{3}J_{4,5}$ = 7.24, Ar-H, **H-4**), 4.54-4.52 (d, 2H, $^{3}J_{11,12}$ = 5.00 – CO-NH-C**H_2**- **H-12**), 3.17-3.14 (t, 2H, $^{3}J_{8,9}$ = 7.34, Ar-NH – C**H_2**-, **H-8**), 2.55-2.51 (t, 2H, $^{3}J_{9,8}$ = 7.34, Ar-NH-CH_2-C**H_2**-,**H-9**).

AO14 δ(ppm):7.60-7.57 (t, 1H, $^{3}J_{12,13}$ = 5.0, -NH-,**H-11**), 7.28–7.24 (m, 2H, $^{3}J_{15,14}$ = 7.83, $^{3}J_{15,16}$ = 7.05, $^{3}J_{17,18}$ = 7.83, $^{3}J_{17,16}$ = 7.05 2Ar–H**, H–15** and **H–17**), 7.17–7.12 (t, 1H, $^{3}J_{16,15}$ = 7.05, $^{3}J_{16,17}$ = 7.05, Ar–H, **H–16**), 6.87–6.85 (m, 2H, $^{3}J_{14,13}$ = 7.83, $^{3}J_{18,17}$ = 7.83, 2Ar–H, **H–14**, **H–18**), 6.69–6.67 (d 1H, $^{3}J_{3,4}$ = 8.54, Ar–H, **H–3**), 6.60–6.57 (t, 1H, $^{3}J_{4,3}$ = 8.54, $^{3}J_{4,5}$ = 7.53, Ar–H, **H–4**), 6.55–6.53 (t, 1H, $^{3}J_{5,4}$ = 7.53, $^{3}J_{5,6}$ = 8.27, Ar–H, **H–5**), 6.23 (5, 1H, Ar–NH, **H–7**), 6.04–.04 (d, 1H, $^{3}J_{6,5}$ = 8.27, Ar–H, **H–6**), 4.54–4.52 (d, 2H, $^{3}J_{12,11}$ = 5.0, – CO–NH–C**H$_2$**– **H–12**), 3.81 (s, 3H, – OC**H$_3$**, **H–19**) 3.28–3.24 (t, 2H, $^{3}J_{8,9}$ = 7.34, Ar –NH–C**H$_2$**–, **H–8**), 2.55–2.5 (t, 2H, $^{3}J_{9,8}$ = 7.34, NH-CH_2-C**H$_2$**-, **H–9**).

AO15 δ(pmm):8.85 (s, 1H, Ar–NH, **H–7**), 7.60–7.57 (t, 1H, $^{3}J_{12,13}$ = 5.00, – CO–NH, **H–11**), 7.28–7.24 (m, 2H, $^{3}J_{15,14}$ = 7.83, $^{3}J_{15,16}$ = 7.05, $^{3}J_{17,18}$ = 7.83, $^{3}J_{17,16}$ = 7.05, 2Ar–H, **H–15, H–17**), 7.17–7.13 (t, 1H, $^{3}J_{16,15}$ = 7.83, $^{3}J_{16,17}$ = 7.05, Ar–H, **H–16**), 6.97–6.93 (dd, 2H, $^{3}J_{14,15}$ = 7.83, $^{3}J_{18,17}$ = 7.83, 2Ar–H, **H–14**, **H–18**), 6.87 – 6.85 (d, 1H, $^{3}J_{5,6}$ = 8.20, Ar–H, **H–6**), 6.80–6.76 (t, 1H, $^{3}J_{5,4}$ = 8.20, $^{3}J_{5,4}$ = 8.03, Ar–H, **H–5**), 6.74 – 6.71 (d, 1H, $^{3}J_{4,5}$ = 8.03, Ar – H, **H–4**) 6.64 (s, 2H, $^{3}J_{12,11}$ = 5.00, –C–NH–C**H$_2$**,– **H–12**), 3.85 (s, 3H, – OCH_3, **H–19**), 3.368–3.35 (t, 2H, $^{3}J_{10}$ = 7.34, Ar–NH–C**H$_2$**,**H–8**), 2.28–2.25 (t, 2H $^{3}J_{11,10}$ = 7.34, Ar- NH–CH_2–C**H$_2$**–, **H–9**).

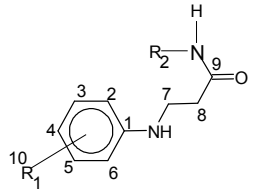


TABLE 4.5 ^{13}CNMR DATA FOR THE 3-ANILINOPROPANAMIDES (A01-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15)

A01

δ(ppm): 190.00(1C, —C(=O)—, **C-9**), 161.60(1C,Ar-**C**-NH, **C-1**), 143.00(2C, Ar-C, **C-2** & **C**-**6**), 135.70(1C, Ar-C, Cl-C, **C-4**), 128.30(2C, Ar-C, **C-3**, **C-5**), 54.40(1C, -NH-C, **C-7**), 49.10(1C, -NH-C-C, **C-8**).

AO2

δ (ppm): 177.56(1C, —C(=O)—, **C-9**), 151.60(1C, Ar-C-NH, **C-1**), 136.36(1C, Ar-C-Cl, **C-3**), 131.68(1C, Ar-C, **C-5**), 117.98(1C, Ar-C, Cl-C, **C-4**), 113.74(1C, Ar-C- **C-2**), 112.65(1C, Ar-C, C-6), 41.31(1C, -NH-C, **C-7**), 36.39 1C, -NH-C-C**, C-8**).

A03

δ (ppm): 177.50(1C, —C(=O)—, **C-9**), 147.25(1C, Ar-C-NH, **C-1**), 130.58(2C, Ar-C, **C-3** and **C-5**), 127.93(1C, Ar-C, **C-4**), 114.81(2C, Ar-C, C-2**, C-5**), 41.85 (1C, -NH-C, **C-7**), 35.97(1C, -NH-C-C**-, C-8**), 20.51(1C, **C**H_3).

A04

δ (ppm): 177.42(1C, , **C-9**), 148.36(1C, Ar-**C**-NH, **C-1**), 107.57(1C, Ar-C, **C-3**), 121.78(1C, Ar-C, **C-5**), 117.95(1C, Ar-C, **C-2**), 111.33(1C, Ar-C, **C-4**), 110.42 55.50(1C, -NH-C-, **C-7**), 40.69(1C, -NH-C-C-, **C-8**), 35.32, (1C, **C**H_3).

A05

δ (ppm): 77.86(1C, , **C-9**), 186.63(1C, Ar-**C**-NH, **C-1**), 146.38(1C, Ar-C- **C-3**), 143.17(1C, Ar-C **C-5**), 139.72(1C, Ar-C C-4), 125.57(1C, Ar-CL **C-2**), 110.1(1C, Ar-C **C-6**), 41.73(1C, -NH-**C**H_2-CH-, **C-7**), 36.27(1C, -NH-CH_2-**C**H_2-, **C-8**).

A06

δ (ppm): 174.51 (1C, ,**C-9**), 148.00 (1C, Ar-**C**-OCH_3, **C-3**), 139.57 (1C, Ar-**C**- NH-, **C-1**), 129.62 (1C, Ar-C, **C-5**), 119.36 (1C, Ar-C, **C-6**), 114.54 (1C, Ar-C, **C-4**), 110.84 (1C, Ar-C **C-2**), 40.31 (1C, 0**C**H_3), 35.38 (1C, -NH-**C**H_2-, **C-7**),22.02 (1C, -NH-CH_2 -**C**H_2 **C-8**).

A07

δ (ppm): 77.97(1C, , **C**-9), 147.52(1C, Ar-**C**-NH, **C-1**), 138.31(1C, Ar-C- CH_3, **C-3**), 128.63 (1C, Ar-C **C-5**), 110.43 (1C, Ar-C **C-4**), 115.11(1C, Ar-C **C-6**), 113.24(1C, Ar-C **C-2**), 40.37(1C, -NH-**C**H_2-**C-7**), 23.02(1C, -NH-CH_2-**C**H_2-**C-8**), 20.95 (1C, **C**H_3).

A08

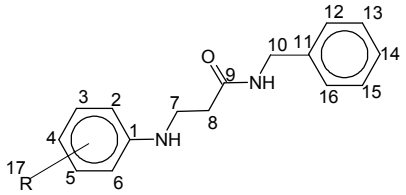
δ (ppm): 174.43(1C, , **C-9**), 147.60(1C, Ar-C-OCH_3, **C-4**), 137.87(1C, Ar-C- NH-, **C-1**), 121.63(1C, Ar-C **C-3**), 117.64(1C, Ar-C **C-5**), 110.62(1C, Ar-C **C-2**), 110.08(1C, Ar-C **C-6**), 55.82(1C, 0**C**H_3), 40.13(1C, -NH-**C**H_2-, **C-7**), 35.721C, -NH-CH_2**C**H_2-**C-8**).

AO9

δ (ppm): 74.47(1C, , **C-9**), 147.65(1C, Ar-**C**-OCH_3**, C-2**), 137.74(1C, Ar-C- NH-, **C-1**), 121.64(1C, Ar-C **C-5**), 117.77(1C, Ar-C, **C-4**), 110.75(1C, Ar-C **C-6**) 110.03(1C, Ar-C **C-3**), 55.83(1C, 0**C**H_3), 40.27(1C, -NH-**C**H_2-,**C-7**), 35.68(1C, -NH-CH_2 -**C**H_2, **C-8**).

A10

δ (ppm): 175.66 (1C, , **C-9**), 147.87 (1C, Ar-C -NH, **C-1**) 127.71 (2C, Ar-C- **C-3** &, **C-5**), 112.74 (2C, 2Ar-C, **C-2** and **C-6**), 39.90 (1C, -NH-**C**H_2-**C-7**) 32.33 (1C, -NH-CH_2 -**C**H_2, **C-8**)



A11

δ (ppm): 172.02 (1C, —C(=O)—, **C-9**), 148.35 (1C, Ar-**C** -NH, C-1), 138.11 (1C, Ar-C- **C-11**), 128.95 (2C, Ar-C **C-12** & **C-16**), 128.74 (2C, 2Ar-C, **C-13** and **C-5**), 127.78 (2C, 2Ar-C, **C-2** and **C-6**), 123.00 (1C –Ar-C, **C-4**), 113.11(2C, 2Ar-C, **C-2** and **C-6**) 45.61 (1C, -NH-**C**H_2, -**C-10**), 40.18 (1C, -NH-**C**H_2,**C-7**), 33.43 (1C, -NH-CH_2 -**C**H_2, **C-8**).

A12

δ (ppm): 172.72 (1C, —C(=O)—, **C-9**), 147.62 (1C, Ar-**C**-NH, **C-1**), 138.53 (1C, Ar-C-, **C-11**), 131.17 (2C, Ar-C, **C-3**), 128.58 (2C, 2Ar-C, **C-12** and **C-16**), 128.42 (2C, 2Ar-C, **C-13** and **C-15**), 127.93 (1C –Ar-C, **C-14**), 125.61(1C, 2Ar-C, **C-2**), 122.01(1C, 2Ar-C, **C-2**), 118.13(1C, Ar-C, **C-4**), 110.15 (1C, Ar-C, **C-6**), 42.88 (1C, -NH-**C**H_2-,**C-10**) 39.97 (1C, -NH-**C**H_2,**C-7**), 32.43 (1C, -NH-CH_2 –**C**H_2-, **C-8**), 18.14 (1C-**C**H_3, C-**17**)

A13

δ (ppm): 172.69 (1C, —C(=O)—, **C-9**), 147.51 (1C, Ar-C -NH, **C-1**) 138.17 (1C, Ar-C-,**C-11**), 134.02 (1C, Ar-C, **C-3**), 299.61 (1C, 2Ar-C, **C-5**), 128.97 (2C, 2Ar-C, **C-12** and **C-16**),128.89 (2C –2Ar-C, **C-13** and **C-15**), 127.72(1C,

Ar-C, C-14) 122.56 (1C, Ar-C, **C-4**), 117.51(1C, Ar-C, **C-2**), 115.22 (1C, Ar-C, **C-6**), 42.73 (1C, -NH-CH_2-,**C-10**) 39.93 (1C, -NH-**C**H_2-**C-7**), 32.62 (1C, -NH-CH_2 –**C**H_2, **C-8**)

A14

δ (ppm): 172.72 (1C, , **C-9**), 148.01 (1C, Ar-**C** –OCH_3, **C-2**), 138.41 (1C, Ar-C, **C-11**), 136.23 (2C, Ar-C**, C-1**), 129.11 (2C, 2Ar-C, **C-12** and **C-16**), (127.64 (1C, Ar-C, **C-14**) 122.03 (1C –Ar-C, **C-5**), 121.91(1C, Ar-C, **C-4**), 117.01(1C, Ar-C, **C-6**), 114.37(1C, Ar-C, **C-3**), 59.04 (1C, -O-**C**H_3,**C-17**), 43.13 (1C, -NH-**C**H_2,**C-10**), 39.96 (1C, -NH-**C**H_2 -, **C-7**), 33.33 (1C-NH- CH_2-**C**H_2, **C-8**).

A15

δ (ppm): 172.11 (1C, , **C-9**), 164.61 (1C, Ar-**C** –OCH_3, **C-3**), 148.49 (1C, Ar-C-, **C-1**), 138.60 (1C, Ar-C, **C-11**), 129.21 (2C, 2Ar-C, **C-12** and **C-16**), 129.15 (2C, 2Ar-C, **C-13** and **C-15**), 128.22 (1C –Ar-C, **C-14**), 124.07 (1C, Ar-C, **C-5**), 110.11 (1C, Ar-C, **C-6**), 108.17(1C, Ar-C, **C-4**), 106.19 (1C, Ar-C, **C-2**), 55.94 (1C, -OCH_3-, **C-17**), 42.83 (1C, -NH-**CH_2**, **C-10**), 40.19 (1C, -NH-**C**H_2, **C-7**), 32.71 (1C, -NH-CH_2 –**C**H_2-, **C-8**).

TABLE 4.6 E1MS DATA FOR THE ANILINOPROPANAMIDES

Compound	EIMS m/z (% intensity)
AO1	198 (31, M^+), 140 (100)
AO2	198 (31, M^+), 140 (100)
AO3	178 (28, M^+), 120 (100)
AO4	178 (28, M^+), 120 (100)
AO5	198 (31, M^+), 140 (100)
AO6	194 (40, M^+), 136 (100)
AO7	178 (28, M^+), 120 (100)
AO8	194 (40, M^+), 136 (100)
AO9	194 (40, M^+), 136 (100)
AO10	- - - - -
AO11	- - - - -
AO12	- - - - -
AO13	- - - - -
AO14	- - - - -
AO15	- - - - -

M^+ = Molecular ion

TABLE 4.7 TIME OF PEAK ANTICONVULSANT ACTIVITY OF THE 3–ANILINOPROPANAMIDES AND THEIR N–BENZYL DERIVATIVES

QUANTAL PROTECTION (QP)/PERCENT PROTECTION (PP)								
Compd	15 MIN		30 MIN		45 MIN		60 MIN	
	QP	PP	QP	PP	QP	PP	QP	PP
AO1	2/6	33.3	3/6	50	2/6	33.3	3/6	50
AO2	2/6	33.3	4/6	66.7	3/6	50	4/6	66.7
AO3	2/6	33.3	4/6	66.7	3/6	50	3/6	50
AO4	6/6	100.0	6/6	100.0	6/6	100.0	6/6	100.0
AO5	6/6	100.0	6/6	100.0	6/6	100.0	6/6	100.0
AO6	5/6	88.3	6/6	100.0	4/6	66.7	5/6	88.3
AO7	1/6	16.7	4/6	66.7	3/6	50	2/6	33.3
AO8	2/6	33.3	3/6	50	3/6	50	2/6	33.3
AO9	6/6	100.0	6/6	100.0	6/6	100.0	6/6	100.0
AO10	1/6	16.7	2/6	50	3/6	50	2/6	33.3
AO11	2/6	33.3	4/6	66.7	4/6	66.7	4/6	66.7
AO12	6/6	100.0	6/6	10.0	6/6	100.0	6/6	100.0
AO13	6/6	100.0	6/6	100.0	5/6	88.3	6/6	100.0
AO14	6/6	100.0	6/6	100.0	6/6	100.0	6/6	100.0
AO15	2/6	33.3	3/6	50	3/6	50	3/6	50

QP = Quantal Protection
PP = Percentage Protection

TABLE 4.8 EFFECT OF 3-ANILINOPROPANAMIDES (A1-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15) ON ELECTROSHOCK INDUCED SEIZURES IN MICE

	0.5hr							4hr					
Comp	30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}			30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}	
	QP	PP	QP	PP	QP	PP		QP	PP	QP	PP	QP	PP
AO1	5/8	62.5	8/8	100.0	8/8	100.0		6/8	75.0	8/8	100.0	8/8	100.0
AO2	7/8	87.5	8/8	100.0	8/8	100.0		6/8	75.0	8/8	100.0	8/8	100.0
AO3	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO4	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO5	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO6	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO7	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO8	6/8	75.0	8/8	100.0	8/8	100.0		7/8	87.5	8/8	100.0	8/8	100.0
AO9	7/8	87.5	8/8	100.0	8/8	100.0		7/8	87.5	8/8	100.0	8/8	100.0
AO10	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO11	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO12	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO13	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO14	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO15	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
PH	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
VAP	2/8	25.0	5/8	62.5	8/8	100.0		2/8	25.0	5/8	62.5	8/8	100.0
CONT	0/8	00	0/8	00	0/8	00		0/8	00	0/8	00	0/8	0.0

QP = Quantal Protection. PP = percentage protection

TABLE 4.9 EFFECT OF 3-ANILINOPROPANAMIDES (A01-A10) AND THEIR N-BENZYLDERIVATIVES (A11-A15) ON METRAZOLE INDUCED SEIZURES IN MICE

	0.5hr							4hrs					
Compd	30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}			30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}	
	QP	PP	QP	PP	QP	PP		QP	PP	QP	PP	QP	PP
AO1	2/8	25.0	3/8	37.5	7/8	87.5		2/8	25.0	3/8	37.5	6/8	75.0
AO2	3/8	37.5	4/8	50.0	8/8	100.0		3/8	37.5	4/8	50.0	8/8	100.0
AO3	1/8	12.5	3/8	37.5	6/8	75.0		1/8	12.5	3/8	37.5	6/8	75.0
AO4	2/8	25.0	3/8	37.5	7/8	87.5		2/8	25.0	3/8	37.5	8/8	100.0
AO5	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO6	4/8	50.0	8/8	100.0	8/8	100.0		4/8	50.0	8/8	100.0	8/8	100.0
AO7	1/8	12.5	3/8	37.5	4/8	50.0		2/8	25.0	3/8	37.5	4/8	50.0
AO8	1/8	12.5	3/8	37.5	5/8	62.5		1/8	12.5	3/8	37.5	5/8	62.5
AO9	1/8	12.5	3/8	37.5	6/8	75.0		1/8	25.0	3/8	37.5	6/8	75.0
AO10	2/8	25.0	4/8	50.0	8/8	100.0		1/8	12.5	3/8	37.5	8/8	100.0
AO11	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO12	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO13	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO14	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
AO15	8/8	100.0	8/8	100.0	8/8	100.0		8/8	100.0	8/8	100.0	8/8	100.0
PHT	0/8	0.0	1/8	12.5	2/8	25.0		0/8	0.0	1/8	12.5	1/8	12.5
VAP	3/8	37.0	6/8	75.0	8/8	100.0		2/8	25.0	5/8	62.5	8/8	100.0
CONTR	0/8	0.0	0/8	0.0	0/8	0.0		0/8	0.0	0/8	0.0	0/8	0.0

QP = Quantal Protection

PP = Percentage Prptection

TABLE 4.10 RIGHTING REFLEX TEST FOR NEUROLOGICAL DEFICIT

Compd	0.5hr							4hr					
	30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}			30mg kg^{-1}		100mg kg^{-1}		300mg kg^{-1}	
	QP	PP	QP	PP	QP	PP		QP	PP	QP	PP	QP	PP
AO1	0/8	0.00	0/8	0.00	1/8	12.5		0/8	0.00	1/8	12.5	1/8	12.5
AO2	0/8	0.00	3/8	37.5	7/8	87.5		0/8	0.00	3/8	37.5	7/8	87.5
AO3	0/8	0.00	3/8	37.5	7/8	87.5		0/8	0.00	4/8	50.0	7/8	87.5
AO4	0/8	0.00	4/8	50.0	8/8	100.0		0/8	0.00	4/8	50.0	8/8	100.0
AO5	0/8	0.00	4/8	50.0	6/8	75.0		0/8	0.00	3/8	37.5	6/8	75.0
AO6	0/8	0.00	3/8	37.5	8/8	100.0		0/8	0.00	4/8	50.0	8/8	100.0
AO7	0/8	0.00	4/8	50.0	8/8	100.0		0/8	0.00	4/8	50.0	8/8	100.0
AO8	0/8	0.00	2/8	25.0	8/8	100.0		0/8	0.00	2/8	25.0	8/8	100.0
AO9	0/8	0.00	4/8	50.0	3/8	37.5		0/8	0.00	4/8	50.0	3/8	37.5
A10	0/8	0.00	3/8	37.5	8/8	100.0		0/8	0.00	4/8	50.0	8/8	100.0
A11	0/8	0.00	4/8	50.0	3/8	37.5		0/8	0.00	3/8	37.5	3/8	37.5
A12	0/8	0.00	3/8	37.5	3/8	37.5		0/8	0.00	5/8	62.5	3/8	37.5
A13	0/8	0.00	5/8	62.5	8/8	100.0		0/8	0.00	5/8	62.5	8/8	100.0
A14	0/8	0.00	3/8	37.5	7/8	87.5		0/8	0.00	3/8	37.5	7/8	87.5
A15	0/8	0.00	2/8	25.0	8/8	100.0		0/8	0.00	2/8	25.0	8/8	100.0
CONTR	0/8	0.00	0/8	0.00	0/8	0.00		0/8	0.00	0/8	0.00	0/8	0.00

QP = Quantal protection
PP = Percentage protection

TABLE 4.11 RESULTS FOR ANTICONVULSANT SCREENING OF 3-ANILINOPROPA-NAMIDES (A01-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15) IN MES AND scPTZ TESTS AND THE RIGHTING REFLEX TEST IN MICE.

COMPD	Protection against MES Induced Seizures		Protection against scPTZ Induced Seizures		Righting Reflex Text	
	0.5hr	4hrs	0.5hr	4hrs	0.5h	4hrs
AO1	++	+++	-	-	-	-
AO2	+++	+++	+	+	+	+
AO3	+++	+++	-	-	++	++
AO4	+++	+++	-	-	++	+
AO5	+++	+++	++	++	--	-
AO6	+++	+++	++	++	++	++
AO7	+++	+++	-	-	++	++
AO8	+++	+++	-	-	++	++
AO9	+++	+++	+	+	--	+
A10	++	++	-	-	++	++
A11	+++	+++	-	-	++	++
A12	+++	+++	+	+	++	++
A13	+++	+++	+++	+++	+++	+++
A14	+++	+++	-	-	++	+
A15	++	++	-	-	+++	+++
CONTROL	-	-	-	-	-	-

Statistical analysis was conducted by the student T-test $p < 0.05$ compare to vehicle treated control groups; Key. +++: activity at 30mg kg^{-1}; ++; activity at 100 mg kg^{-1}; +; activitiy at 300 mgkg^{-1}; - no activity even at 300 mg kg^{-1} or not determined

TABLE 4.12 QUANTITATIVE ANTICONVULSANT ACTIVITY OF SELECTED 3-ANILINOPROPANAMIDES AND THEIR N-BENZYL DERIVATIVES.

Compd	ED_{50}[a,b] MES	ED_{50}[a,b] ScPTZ	TD_{50}[a,b,c]	PI[b] MES	PI[b] scPTZ
AO1	42.31 (38.02 – 50.30)	>100	194.14 (156.61 – 251.07)	4.6	–
AO2	30.70 (26.23 – 36.18)	>100	85.25 (78.13 – 93.01)	2.8	–
AO3	14.40 (13.00 – 16.80)	>100	72.40 (59.50 – 88.20)	5.0	–
AO4	0.63 (0.26 – 1.64)	>100	6.48 (5.70 – 7.20)	10.3	–
AO5	5.07 (1.95 – 17.38)	12.70 (6.13 – 26.2)	25.30 (16.00 – 40.00)	5.0	2.0
AO6	10.30 (8.60 – 12.40)	37.40 (35.40 – 40.10)	59.40 (45.20 – 78.2)	5.8	1.6
AO7	6.76 (2.14 – 23.55)	>300	40.60 (30.10 – 21.90)	6.0	–
AO8	33.00 (28.00 – 38.00)	>300	63.80 (45.41 – 89.72)	1.9	–
AO9	30.40 (27.00 – 34.10)	>100	103.90 (88.86 – 119.92)	3.4	–
A10	15.20 (11.20 – 20.70)	>100	43.30 (34.4 – 54.6)	2.8	–
A11	17.30 (14.81 – 20.41)	24.08 (21.62 – 26.02)	85.20 (78.10 – 92.90)	4.9	3.5
A12	18.02 (13.41 – 21.43)	41.78 (38.85 – 46.02)	69.03 (62.84 – 72.90)	3.8	1.7
A13	3.25 (1.61 – 6.56)	16.67 (14.70 – 19.13)	20.40 (13.30 – 31.00)	6.3	1.2
A14	13.20 (6.42 – 27.30)	15.7 (8.98 – 27.30)	56.60 (39.30 – 81.60)	4.8	3.6
A15	23.40 (15.50 – 35.40)	25.30 (16.00 – 40.00)	72.40 (59.50 – 88.20)	3.1	2.9
PHT	9.51 (8.14 – 10.45)	>300	65.45 (52.48 – 72.09)	6.9	–
VAP	271.54 (246.93 – 337.85)	148.49 (122.59 – 176.95)	424.57 (368.88 – 450.38)	1.6	2.9
CONT	–	–	–	–	–

[a]All data were calculated using the graphical method of Miller and Tainter (1944). [b]95% confidence limits are given parentheses. At least 32 animals administered ip were used to calculate each ED_{50} and TD_{50} value, [b]PI = TD_{50}/ED_{50}.c Righting Reflect Toxicity. All doses are measured in $mgkg^{-1}$. (–) Means no activity at $100mgkg^{-1}$

TABLE 4.13 RESULTS OF ACUTE TOXICITY TEST (LD_{50}) FOR THE ANILINOPROPANAMIDES (A01-A10) AND THEIR N-BENZYL DERIVATIVES (A11-A15)

Compounds	LD_{50} ($Mgkg^{-1}$)
AO1	113.1
AO2	729.6
AO3	1015.6
AO4	1131.4
AO5	523.6
AO6	471.0
AO7	526.2
AO8	1261.3
AO9	826.7
A10	1329.3
A11	624.9
A12	486.2
A13	587.5
A14	811.5
A15	714.8

CHAPTER FIVE

DISCUSSION

Melting points are uncorrected. The results are given in table 4.1A. The results for microanalytical studies are given in table 4.2 and are within ± 0.4 percent of theoretical values. The results for IR studies are given in table 4.3. The spectra show prominent peaks at 3450-3350cm^{-1} (-N-H stretching vibrations), 3300-3200cm^{-1} (aromatic =C-H stretching frequencies), 1600-1610cm^{-1} (aromatic carbon-carbon stretching vibrations), 1650-1660cm^{-1} (carbonyl freguency vibrations usually referred to as amide band I), 1500-1550cm^{-1} (N-H deformation usually referred to as amide band II), 1270-1300cm^{-1} (-C-N stretching frequencies of secondary amines), 1050-1210cm^{-1} (inplane bending vibrations of methylene groups) and 700 -850cm^{-1} (out of plane bending vibrations). The latter can be diagnostic of the pattern of substitution on an aromatic ring (para substitution around 800cm^{-1}, meta substitution at about 750cm^{-1} and ortho substitution at about 700cm^{-1}).

The data for mass spectra are presented in tale 5.4. The most important peaks are the molecular ion peaks (which range between 28-40 percent of the base peak) and the base peaks.The latter are formed by expulsion of a radical through α-cleavage characteristic of secondary amines. Both the base peaks and the expelled radicals are resonance stabilized.

H^1NMR Spectra Data

The proton positions are as indicated in table 4.4. Coupling protons were detected using correlated spectroscopy (COSY) and the J values were estimated using a computer ACD software.

^{13}CNMR Spectra Data

The carbon positions are as indicated in table 4.5. The nature of the carbon atoms (–CH,-CH_2 or-CH_3) were established by means of Distortionless Enhancement by Polarization Transfer (DEPT) spectroscopy where upon peaks due to –CH and-CH_3 are up the base line while peaks due to-CH_2 fall below. Further refinement of the structures was made by the application of Heteronuclear Chemical Shift Correlation (HETCOR) spectroscopy. This correlates the peaks of the ^{1}HNMR spectrum with that of its ^{13}CNMR spectrum there by showing which proton is attathed to which carbon atom. Long Range HETCOR was used in a few cases. Thus complete hydrogen and Carbon assignments were made as confirmed by structural simulation on a standard soft ware package (ACD software).

ANTICONVULSANT SCREENING

The compounds were initially screened for anticonvulsant activity in mice the mouse MES and scPTZ tests. The results are given in tables 4.8 and 4.9 respectively. The screenings were performed at three dosage levels; 30,100 and 300mgkg^{-1}, 0.5hr and 4.0hrs after intravenous injection of the drugs. Activity was defined by obtaining percentage response of at least 37.5 (or a quantal response of 3/8). Neurotoxicity was assessed by the righting reflex test (table 4.10). The

results of the preliminary screening are shown in table 4.11. Compounds that were active at 30mgkg^{-1} and non-toxic were then evaluated quantitatively. The results for the quantitative studies are shown in table 4.10. At least 32 mice (four points) were used in estimating each ED_{50} and TD_{50} from plots of log dose against (corrected) probits.

On a general note, the 3-anilinopropanamides and their N-benzyl derivatives were active in the MES test, indicative of their ability to prevent seizure spread. In addition to being active at the MES test, the N-benzyl derivatives were active also in the scPTZ test. The latter is used to identify compounds that elevate seizure threshold. The 3-aminopropanamides thus fall in a class with phenytoin and carbemazepine while their N-benzyl derivatives are similar to sodium valproate (XXVIIa).

In the quantitative tests, four compounds AO4 (MES ED_{50} = 0.63mgkg^{-1} i.p mice, PI = 10.3), AO5 (MES ED_{50} = 5.07mgkg^{-1}i.p. mice, P = 5.0), AO7 (MES ED_{50} = 6.76mgkg^{-1} i.p. mice, PI = 6.0) and A13 (MES ED_{50} = 3.25mgkg^{-1} i.p.mice, PI = 6.3) were more potent than phenytoin (MES ED_{50} = 9.51mgKg^{-1} i.p.mice, PI = 6.9) and have comparable therapeutic indeces. Compound AO4 is the most potent compound reported in the literature that has such a high therapeutic index. The N-benzyl derivatives (A11-A15) are are also far more potent than sodium vaproate in both MES and scPTZ with favourable therapeutic indices table4.12. In particular, A13 is the most potent compound reported in the literature in the sodium vaproate (XXVIIa) class of anticonvulsants. However, A13 appear to be a little toxic LD_{50} = 587.5mgKg^{-1}).

STRUCTURE/ACTIVITY RELATIONSHIP STUDIES

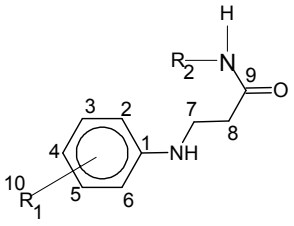


TABLE 5.1

STRUCTURE/ACTIVITY RELATIONSHIP OF THE 3-ANILINOPROPAMIDES

Position	**MES ED_{50} in mice**			**scPTZ ED_{50} in mice**		
R_1	–Cl	$-CH_3$	$-OCH_3$	–δ	$-CH_3$	$-OCH_3$
Ortho (2,6)	5.07	0.63	30.40	12.70	-	30.40
Meta (3)	30.70	6.76	10.30	-	-	37.40
Para (4)	42.31	14.40	33.30	-	-	-

From table 5.1 it could be seen that the 3-toluidino products are more potent than the 3-chloroanilino and the 3-anisidino products. It is also evident that among the toluidino product the order of potency is in accordance with the sequence δrtho> meta>para. The same observation holds for the chloroanilino products. This reveals that substitution pattern and the nature of substituent(s) on the anilino moiety is of vital importance for anticonvulsant activity and potency. Similar observations have been reported by Lapage *et al* (1992) and Bailleuxx *et al* (1994).

Another observation is that, except for the anisidino products (in the MES test) where the meta-substituted product is more potent than the ortho-substituted product, the latter is always the most potent. This reveals that ortho substitution

on the anilino moiety of the 3-anilinopropanamides is of particular importance for anticonvulsant potency. Since the benzamide structure has anticonvulsant activity in its own right (Bruce and Lynes, 1964; Borsey *et al*., 1968; Clark *et al*., 1984; Clark *et al*., 1986; Clark and Davenport, 1987; Clark, 1988) it was conceived that introduction of N-benzyl group into propanamide wing of the ortho derivatives of the 3-APMs would further increase their anticonvulsant potency. This led to the benzylation of the 3-APMs at the amido nitrogen to obtain the NBDAPMs.

In table 5.2 the effect of this N-benzylation is assessed by comparing the ED_{50} values of the unbenzylated and benzylated products. It could be seen that Introduction of an N-benzyl group does not necessarily increase the anticonvulsant potency of the 3-APM^{S}. Rather, it broadens their spectrum of activity by making them not only active in the MES test but also in the scPTZ test. The NBDAPMs therefore have the capability of raising seizure threshold in addition to preventing seizure spread; a property that is typical of valproate-like anticonvulsants. By implication the 3-APMs have the potential of being useful in the treatment of tonic-clonic seizures (i.e. grand mal epilepsy) while the NDBAPMs may be useful in treating generalized seizures.

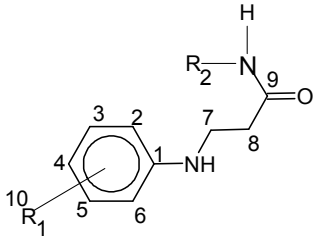
ACCEPTANCE/REJECTION OF THE HYPOTHESIS

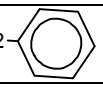
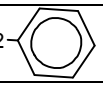
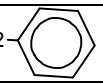
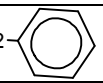
Hypothesis 1: Appliation of t-test reveals significant difference between the 3-APM treated groups and the vehicle treated control groups ($P< 0.05$). The

hypothesis that the 3-APM yield potent anticonvulsant compounds is therefor accepted.

Hypothesis 2: Application of t-test reveals no significant difference between the ED_{50} values of the 3-APMs and NBDAPMs in the MES test. Hence, hypothesis 2 is rejected. However, it should be noted that in the scPTZ test the ED_{50} values significally differ.

TABLE 5.2: EFFECT OF N-BENZYL SUBSTITUTION ON THE ANTICONVULSANT POTENCY OF THE 3-ANILINOPROPANAMIDES



COMP	R_1	R_2	MES ED_{50} ($mgkg^{-1}$)	SCPTZ ED_{50} ($mgkg^{-1}$)
A10	–H	–H	15.020	-
A11	–H	— CH_2 	17.30	24.08
AO5	2–Cl	–H	5.07	12.70
A13	2–Cl	— CH_2 	3.25	16.67
AO4	2–CH_3	–H	0.63	-
A12	–OCH_3	— CH_2 	18.02	41.78
AO6	–OCH_3	–H	10.30	37.40
A14	–OCH_3	— CH_2 	13.20	15.78

(–) Denotes lack of efficacy at 100$mgkg^{-1}$

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Although the understanding of the pathophysiolopy of epilepsy, particularly in terms of physiology and genetics has advanced tremendously in the last three decades, improvement in terms of clinical outcomes has fallen short of expectations with up to one-third of patients continuing to experience seizures or unacceptable medication related side effects in spite of efforts to identify uptimal treatment regimes with one or more drugs.

Neuronal excitability is determined by the properties of the ion channels in the neuronal membrane such that the aberrant excitability associated, with an epileptic discharge will necessarily be mediated by voltage-gated and ligand-gated ion channels and may even be the results of defects in the function of these channels. Because of the pivotal role played by these ion channels in the physiology of all forms of epilepsy they are obvious AED targets.

Unverferth (1998) and Shindikar (2000) have defined the basic structural requirements for a compound to be active at the voltage-dependent sodium channels. Hypothetically, the 3-anilinopropanamides (3-APMs) appear to satisfy these pharmacophore requirements and are therefore expected to yield potent anticonvulsant drugs. Derivatives of 3-APMs (A01-A10) were synthesized and screened in mice for anticonvulsant activity and toxicity studies.

In the preliminary investigation the compounds were screened at three dosage levels; 30, 100 and 300mgKg^{-1} using the MES and scPTZ seizure test models.

Neurotoxicity was assessed using the righting reflex test for neurological deficit. Compounds that were active at a dosage of 30mgKg-1 or less and not neurologically toxic were considered for the secondary evaluation.

Trend analyses of the results obtined from the preliminary investigation reveal that orth-substitution on the anilino moiety of the 3-AMPs favour anticonvulsant potency. Benzylation of these isomers at the amido nitrogen was expected to increase anticonvulsant activity since the benzyl group has anticonvulsant property on its own. This intuition led to the syntheses and testing of the N-benzyl derivatives of the σ-substituted 3-APM (A11-A15).

All the fifteen compounds prepared were found to be active in the MES test; four of them (A04, A05, A07 and A13) being more potent than phenytoin. On the other hand, while only two of the 3-APMs (A05 and A06) have appreciable activity in the scPTZ seizure test model, all the N-benzyl derivatives were active with favourable potencies compare to valproic acid. Thus, the 3-APMs fall in a class with phenytoin which is used primarily for the treatment of tonic clonic seizures. The NBDAPMs on the other hand may be compared with valproate which is good for the the treatment of generalized seizures.These findings affirm the pharmacopores proposed by Unverferth *et al* (1998) and Shindikar *et al* (2006) for anticonvulsants active at voltage-gated sodium channels.

6.2 CONCLUSIONS

The 3-anilinopropanamides and their N-benzyl derivatives yield potent anticonvulsant compounds thus affirming Unverferth *et al* (1998) and Shindikar *et al* (2006) proposed pharmacophores.

6.3 RECOMMENDATION

Compounds A03, A04, A06, A11, A13 and A15 are recommended for tertiary in-vivo evaluation.

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