Design and Synthesis of γ-Aminobutyric Acid Derivatives for the Treatment of Epilepsy

THESIS

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By

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CERTIFICATE

This is to certify that the thesis entitled, **"Design and Synthesis of γ-Aminobutyric Acid Analogues for the Treatment of Epilepsy"** was submitted by **A. Sunil Kumar Reddy, ID. No. 2006PHXF009** for award of Ph.D. degree of the Institute embodies the original work done by him under my supervision.

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I humbly dedicate my thesis to my wife and parents.

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List of Abbreviations

AEDs	Antiepileptic Drugs
BBB	Blood brain barrier
BIC	Bicuculine
BOC	Ditertiary butyl dicarbonate
CAMP	3'-5'-cyclic adenosine monophosphate
CBZ	Carbobenzoxy
CIP SYSTEM	Cahn-Ingold-Prelog System
CNS	Central nervous system
d	Doublet
DCC	Dicyclohexyl carbodimide
DCM	Dichloromethane
DBU	1, 8-Diazabicyclo[5.4.0]undec-7-ene
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EEG	Electroencephalogram
IAEC	Institutional Animal Ethical Committee
ILAE	International League against Epilepsy
IP	Intraperitoneal
IR	Infrared
IV	Intravenous
GAA	Glacial acetic acid
GABA	γ-Aminobutyric acid
GAD	Glutamicacid decarboxylase
^{1}HNMR	Proton Nuclear Magnetic Resonance
MES	Maximalelectroshock
MP	Melting point
NBS	N-Bromosuccinimide
NMDA	N-methyl-D-aspartic acid
PEG	Polyethyleneglycol

P-GABA	Phthaloyl GABA
PIC	Picrotoxin
PPM	Parts per million
PTZ	Pentylenetetrazole
s	Singlet
TACA	Trans-4-aminocrotonic acid
TEA	Triethyl amine
TLC	Thin layer chromatography
TMS	Tetramethylsilane

ABSTRACT

Several lipophilic analogues of N-Spiro GABA were synthesised and evaluated for their anticonvulsant activity. The structures of the synthesised compounds were confirmed by their spectral data besides elemental analyses.

The anticonvulsant evaluation was carried out using four animal models of seizure including MES, scPTZ, scSTY and scPIC tests. The acute neurological deficit was determined using the rotarod test.

Of a total of fifty eight compounds synthesized, fifty five compounds were found to be active in scPIC test indicative of the possible involvement of GABA-mediation in the anticonvulsant action. Fourty two compounds showed protection in scSTY test, ten compounds exhibited activity in the MES test and none were found to be active in scPTZ test.

Among all the series, with respect to effectiveness and neurotoxicity SH-series (4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl) butanoic acids) derivatives were more potent and less neurotoxic than other series of compounds. The order of anticonvulsant activity was SH series > MC series > CP series > BS series.

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CHAPTER 1.1
EPILEPSY

Epilepsy:

The term epilepsy, based on the greek word epilambanein (meaning to seize) was first mentioned by Hippocrates. In the world's first scientific monograph on epilepsy, entitled "On the sacred disease" (ca 400 B.C), Hippocrates disputed the myth that the cause of epilepsy is supernatural and the cure magic [1].

Seizures' and 'epilepsy' are often used synonymous and yet they are not; seizures are a symptom of epilepsy. While all epilepsies are characterized by seizures, not all seizures are epileptic [2].

Epilepsy is a chronic brain disorder characterized by recurrent seizures, A seizure (from the Latin *sacire*—to take possession of) is the clinical manifestation of an abnormal, excessive, hyper synchronous discharge of a population of cortical neurons, it affects 1-3% of the US and Canadian populations. The prevalence of epilepsy is 5—8.3 per 1000 population in India. Epilepsy has a lifetime prevalence of 3%—that is, 7.2 million persons become affected by this disorder. Almost 10% of the population experience at least one epileptic seizure in 80 years of life. Epilepsy is the second leading neurological disorder, exceeded only by stroke. Onset can occur at any age, although is most common in the young and the old, with an increasing incidence in those aged >60 years [3-6].

Epileptogenesis:

The term epileptogenesis refers to the transformation of the brain to a long-lasting state in which recurrent, spontaneous seizures occur. It may involve focal area of the brain (partial epilepsy) or the entire brain simultaneously (generalized epilepsy). Epileptogenesis must be distinguished from seizure expression, which is concerned with processes that trigger, generate seizures and can arise in non epileptic brain exposed to acute insults [7].

Aetiology :

About half of all seizures have no known cause, the other half are linked to a disease or injury of the brain.

a. During development and first few years of childhood, the brain undergoes lot of growth. During this growth the brain is at the risk of certain diseases due to infections, poor nutrition, and poor supply of oxygen. Some of the diseases are associated with epilepsy.
b. The neurons of the brain develop into complex webs of wires. Defects in wiring during brain development could lead to epilepsy. After a head injury or stroke the brain repairs itself by making new wiring. If the new wiring is abnormal it could cause seizures.
c. Diseases of the brain such as hydrocephalus and meningitis, could cause epilepsy.
d. Poisoning of the brain such as lead and carbon monoxide poisoning could lead seizures.
e. Exposure to street drugs and overdoses of antidepressants could also lead to seizures.
f. Older people sometimes develop diseases of the brain, such as brain tumours, infections and stroke and bleeding. These types of diseases could lead to epilepsy.
g. Sometimes epilepsy tends to run in families, suggesting hereditary causes [5].

Diagnosis:

EEG (Electroencephalograph) is a helpful diagnostic tool in the investigation of seizure disorder. It confirms the presence of abnormal activity gives information regarding the type of seizure disorder and discloses the location of the seizure focus. Electroencephalogram (EEG) is a recording of the electrical activity of the cerebral cortex, through electrodes placed on the scalp. The EEG measures the electrical potentials of cortical neuronal dendrites near the brain's surface.)

The EEG waveforms are divided into four major frequency bands: delta (0–3+ Hz), theta (4–7+ Hz), alpha (8–13+ Hz), and beta (>14 Hz). At first glance, the normal spontaneous electrical activity detected by the EEG appears somewhat chaotic. However, there is a certain organization and rhythmicity of the activity that depends on the level of alertness or sleep and the age of the subject. The physiological basis of at least some of

these rhythms seems to arise from intrinsic pacemaker cells in the cortex and thalamus. Several EEG rhythms can be characterized on the basis of the location, frequency and reactivity of the activity and the clinical state of the patient. For example, a symmetrical rhythm is observed over the posterior head regions during relaxed wakefulness with eyes closed, that undergoes amplitude attenuation with eye opening or mental alerting activities. It is called the posterior dominant rhythm or alpha rhythm, because in adults it has a frequency of 8–13 Hz however, its frequency in children may be in the theta range. Alterations of brain function often result in abnormally slow frequency activity in the EEG. Pathologic slowing, when localized, often correlates with focal brain lesions; when diffuse, slow activity often signifies an encephalopathy. Epileptiform activity characteristic of people with epilepsy includes abnormalities such as spikes, sharp waves and spike-wave complexes [8-10]. **(Fig. 1)**

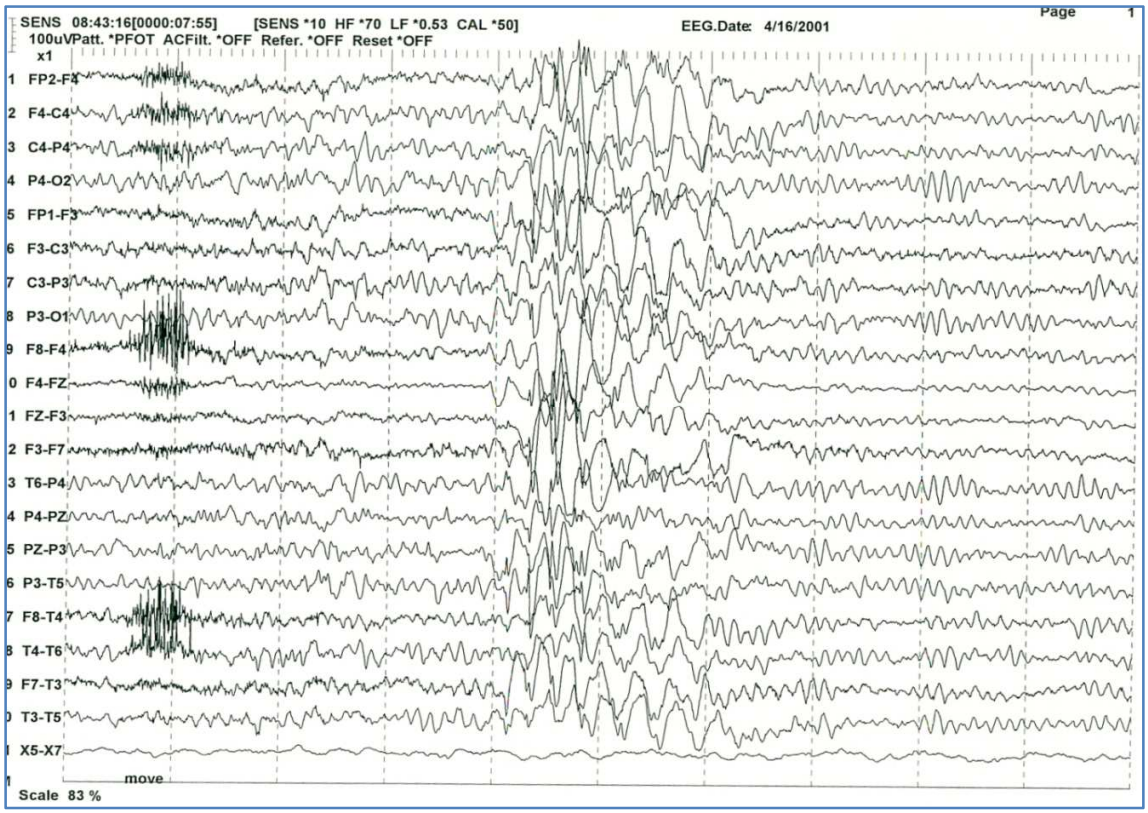


Fig. 1: EEG showing generalized spike wave discharge [10]

Mechanisms of epilepsy:

Epilepsy is a paroxysmal disorder characterized by abnormal neuronal discharges. Although the causes of epilepsy are many, the fundamental disorder is secondary to abnormal synchronous discharges of a network of neurons. Epilepsy can be secondary to either abnormal neuronal membranes or an imbalance between excitatory and inhibitory influences._Epileptogenesis is the sequence of events that turns a normal neuronal network into a hyperexcitable network. **(Fig-2)**

Two sets of changes can determine the epileptogenic properties of neuronal tissues. Abnormal neuronal excitability is believed to occur as a result of disruption of the depolarization and repolarization mechanisms of the cell (this is termed the excitability of neuronal tissue). Aberrant neuronal networks that develop abnormal synchronization of a group of neurons can result in the development and propagation of an epileptic seizure (this is termed the synchronization of neuronal tissue).

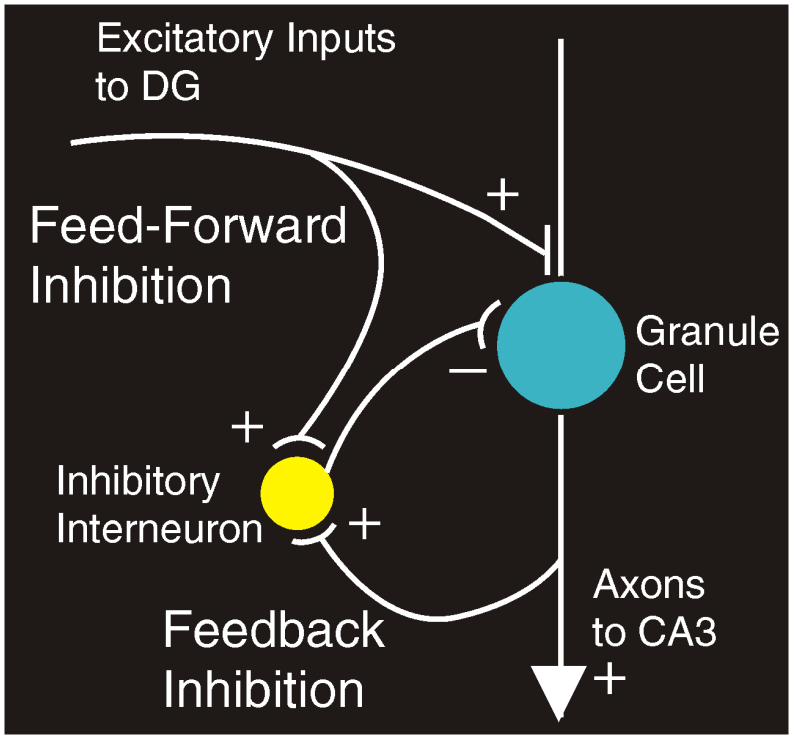


Fig. 2: Basic mechanism underlying seizures and epilepsy [10].

A hyperexcitability of neurons that results in random firing of cells, by itself, may not lead to propagation of an epileptic seizure. Indeed, both normal and abnormal patterns of behavior require a certain degree of synchronization of firing in a population of neurons.

Epileptic seizures originate in a setting of both altered excitability and altered synchronization of neurons. The excitability of individual neurons is affected by

1. Cell membrane properties and the microenvironment of the neuron
2. Intracellular processes
3. Structural features of neuronal elements
4. Interneuronal connections [9-13]

Seizures:

Epileptic seizures have been recognized for millennia. One of the earliest descriptions of a secondarily generalized tonic-clonic seizure was recorded over 3000 years ago in Mesopotamia. Epileptic seizures were described in ancient cultures, including those of China, Egypt, and India. An ancient Egyptian papyrus described a seizure in a man who had previous head trauma.

Modern investigation of the etiology of epilepsy began with the work of Fritsch, Hitzig, Ferrier, and Caton in the 1870s. They recorded and evoked epileptic seizures in the cerebral cortex of animals. In 1929, Berger discovered that electrical brain signals could be recorded from the human head by using scalp electrodes; this discovery led to the use of electroencephalography (EEG) to study and classify epileptic seizures [2].

Definition:

A seizure is a sudden change in behavior characterized by changes in sensory perception (sense of feeling) or motor activity (movement) due to an abnormal firing of nerve cells in the brain [1].

Parts of a seizure:

The period during which the seizure actually occurs is called the ictus or ictal period. The aura is the earliest portion of a seizure recognized, and the only part

remembered by the patient, it may act as warning. The time immediately after a seizure is referred as the postictal period. The interval between seizures is the interictal period [8].

Pathophysiology of Seizures:

The hypersynchronous discharges that occur during a seizure may begin in a very discrete region of cortex and then spread to neighboring regions. Seizure initiation is characterized by two concurrent events:

1) high-frequency bursts of action potentials.

2) hypersynchronization of a neuronal population.

The synchronized bursts from a sufficient number of neurons result in a so-called spike discharge on the EEG. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift. **(Fig-2)**

The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca^{++}, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+}, and generation of repetitive action potentials. The subsequent hyperpolarizing afterpotential is mediated by GABA receptors and Cl^{-} influx, or by K^{+} efflux, depending on the cell type.

Seizure propagation, the process by which a partial seizure spreads within the brain, **(Fig. 3)** occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surround inhibition and spread of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long association pathways such as the corpus callosum

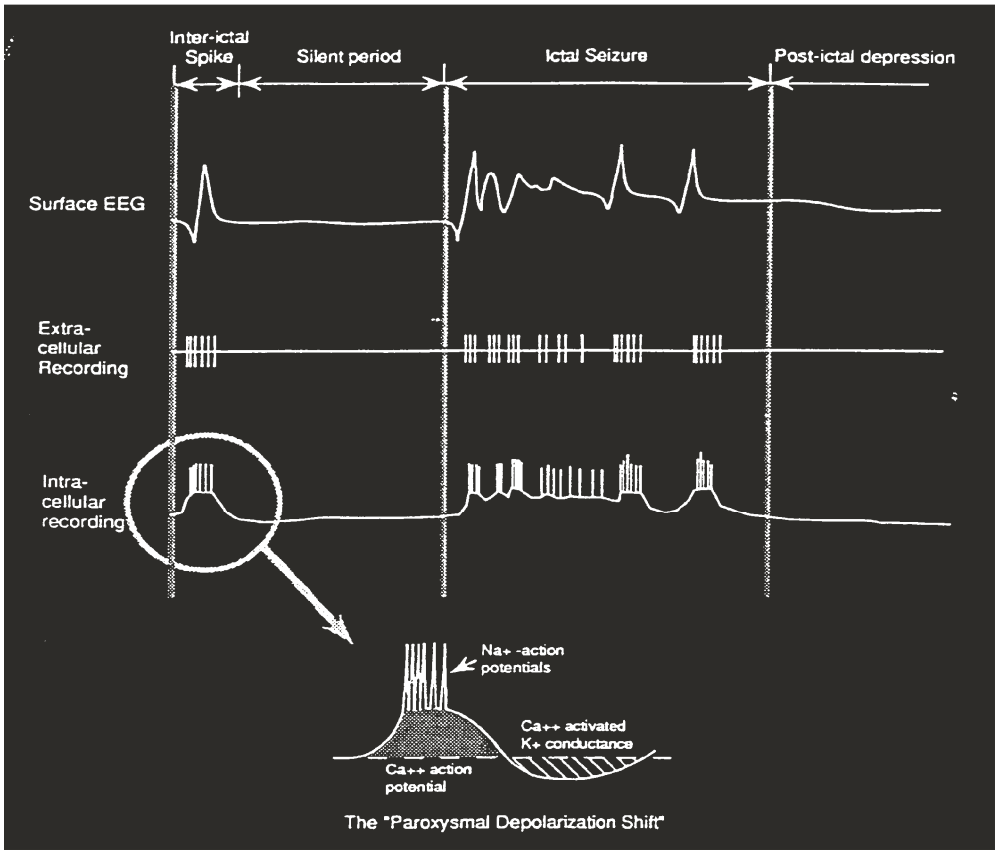


Fig. 3: EEG showing paroxysmal depolarization shift [10].

In 1981, the International League Against Epilepsy (ILAE) developed an international classification of epileptic seizures that divides seizures into two major classes: partial-onset seizures and generalized-onset seizures. Partial-onset seizures begin in a focal area of the cerebral cortex, whereas generalized-onset seizures have an onset recorded simultaneously in both cerebral hemispheres. Some seizures are difficult to fit into a single class, and they are considered unclassified seizures. This classification is still widely accepted.

Classification of seizures by International League against Epilepsy, 1981

1. Partial seizures
a. Simple partial seizures (with motor, sensory, autonomic, or psychic signs)
b. Complex Partial Seizures
c. Partial Seizures with secondarily generalization
2. **Primarily Generalized seizures**
a. Absence (petit mal)
b. Tonic-Clonic (grand mal)
c. Tonic
d. Atonic
e. Myoclonic
3. Unclassified Seizures
a. Neonatal seizures
b. Infantile spasms.

1. Partial seizures

a. Simple partial (focal) seizures: A focal (partial) seizure develops when a limited confined population of nerve cells fire their impulses abnormally on one hemisphere of the brain, without the impairment of consciousness. Simple partial seizures may consist of motor, sensory, autonomic, or psychic signs and symptoms.

b. Complex partial seizures: The central feature of complex partial seizures is impairment of Consciousness.

c. **Partial Seizures with secondarily generalization:** Secondarily generalized seizures often begin with an aura that evolves into a complex partial seizure and then into a generalized tonic-clonic seizure. However, a complex partial seizure may evolve into a generalized tonic-clonic seizure, or an aura may evolve into a generalized tonic-clonic seizure without an obvious complex partial seizure.

2. **Primarily Generalized seizures:**

a. Absence (petit mal): Absence seizures are classified as either typical or atypical. The typical absence seizure is characterized by unresponsiveness and behavioral arrest, abnormal muscular movements of the face and eyelids, and lasts less than 10 seconds.

b. Tonic-Clonic (grand mal): Tonic-clonic seizures are commonly referred to as grand mal seizures. They consist of several motor behaviors, including generalized tonic extension of the extremities lasting for few seconds followed by clonic rhythmic movements and prolonged postictal confusion.

c. Tonic: Tonic seizures consist of sudden-onset tonic extension or flexion of the head, trunk, and/or extremities for several seconds. These seizures typically occur in relation to drowsiness, shortly after the person falls asleep, or just after he or she awakens. They are often associated with other neurologic abnormalities.

d. Atonic: Atonic seizures, also called "drop attacks," are abrupt, with loss of muscle tone lasting one to two seconds, but with rapid recovery. Consciousness is usually impaired. The rapid loss of muscular tone could be limited to head and neck muscles, resulting in head drop, or it may be more extensive, involving muscles for balance and causing unexpected falls with physical injury.

e. Myoclonic: Myoclonic seizures consist of brief, arrhythmic, jerking, motor movements that last less than a second. Myoclonic seizures often cluster within a few minutes. If they evolve into rhythmic, jerking movements, they are classified as evolving into a clonic seizure.

3. Unclassified Seizures:

a. Neonatal seizures: Neonatal seizures refer to seizures that occur between birth and two months of age. The most prominent feature of neurological dysfunction in the neonatal period are seizures.

b. Infantile spasms (west syndrome): These are characterised by brief head nods or seizures consist of violent flexion of the trunk, arms and legs [2, 13 & 14].

Several shortcomings of ILAE classification have been described and recently the ILAE has officially acknowledged the need for a revision of the classification systems. Using the ILAE system, seizure classification tended to change significantly between pre- and post-monitoring (in more than a third of patients). This finding supports the contention that a semiological classification may be better suited for everyday clinic use, which is logical since it is based solely on clinical characteristics. Seizures can easily be classified based on the amount of information available, without having to assume the presence of certain symptoms (e.g. impairment of consciousness). More specifically, the ILAE has even acknowledged the necessity of a seizure classification based exclusively on seizure semiology.

Semiological seizure classification

1. Auras

a. Somato-sensory
b. Visual
c. Auditory
d. Gustatory
e. Olfactory
f. Autonomic
g. Abdominal
h. Psychic

2. Autonomic seizure

3. Dialeptic seizure (Isolated alteration of awareness)

4. Motor seizures

- **Simple motor seizures**

  Clonic
  Tonic
  Tonic–clonic
  Epileptic spasm
  Myoclonic

Versive

- **Complex motor seizuresa**

Automotor

Hypermotor

Gelastic

5. Special seizures (negative)

Aphasic

Astatic

Atonic

Akinetic

Hypomotor

Negative myoclonic.

Auras

Auras consist exclusively of subjective symptoms and usually occur at the beginning of a seizure ("warning symptoms"). In general, they are brief (seconds) and only rarely may persist longer (minutes).

In the SSC, auras are subdivided into the following subgroups:

a. Somatosensory auras.

Somatosensory auras consist of abnormal somatosensory sensations that are limited to a clearly defined somatosensory region of the body. Sensations that are poorly localized or consist of vague sensations should be classified as unclassifiable auras (just''auras").

b. Visual auras.

Visual hallucinations or illusions, when occurring in isolation, should be classified as visual auras.

c. Auditory auras.

Isolated auditory hallucinations or illusions should be classified as auditory auras.

d. Olfactory auras.

Perception of a smell as an epileptic phenomenon is classified as an olfactory auras.

e. Gustatory auras.

Perception of a taste as an epileptic phenomenon is classified as a gustatory auras.

f. Autonomic auras.

Autonomic alterations elicited by epileptic activation of autonomic cortical centers produce symptoms that the patient can detect but that observers have difficulty identifying, particularly from a videotape recording (palpitations, hot flashes, and so on).

g. Abdominal auras

Patients with temporal lobe epilepsy frequently have auras with abdominal sensations. **Psychic auras**.

Psychic auras consist of complex hallucinations and illusions that usually affect different senses.

Autonomic seizures

Autonomic seizures consist of episodic alterations of autbnomic function that are elicited by activation of autonomic cortical centers activated by an epileptiform discharge.

Cases in which the patient reports only sensations that most probably correspond to an autonomic alteration (hot flashes, palpitations, and so on) and for which there is no objective documentation should be classified as autonomic auras.

Dialeptic seizures

Dialeptic seizures is a new term coined to identify seizures in which the predominant symptomatology consists of an alteration of consciousness.

Motor seizures

Seizures in which the main symptomatology are motor signs are identified as motor seizures. Two major subgroups can be differentiated:

1. Simple motor seizures in which the motor movements are relatively "simple," unnatural, and consist of movements similar to movements elicited by electrical stimulation of the primary motor areas (Brodmann areas 4 and **6).**

2. Complex motor seizures, in which the movements are relatively complex and simulate natural movements, except that they are inappropriate for the situation.

Simple motor seizures

Simple motor seizures can be subdivided into the following groups.

a. Myoclonic seizures: Myoclonic seizures consist of short muscle contractions lasting <400 ms.

b. Tonic seizures: Tonic seizures consist of sustained muscle contractions, usually lasting >3 s**,** that lead to "positioning."

c. Epileptic spasms: The term epileptic spasm is used to identify muscle contractions of variable duration which affect predominantly axial muscles. Epileptic spasms frequently occur in clusters in which the duration of the muscle contractions may vary from a short myoclonic jerk to a sustained tonic posturing. Usually the epileptic spasm consists of abduction

of both arms in a "salaam" posture.

d. Clonic seizures: Clonic seizures are a series of myoclonic contractions that regularly recur at a rate 0.2 to 5 s.

e. Tonic-clonic seizures: Generalized tonic-clonic seizures are characterized by an initial tonic posturing of all limbs. The sustained muscle contractions that determined the tonic phase then tend to slow, evolving into a clonic phase with contractions of progressively decreasing frequency until the contractions disappear completely. The muscles included

in the tonic and clonic phase should be essentially the same. Focal motor seizures showing such a tonic-clonic evolution are infrequent.

f. Versive seizures: Versive seizures are seizures during which the patient either has a conjugate eye movement to one side or moves the head, and occasionally the whole body, to one side. Only conjugate eye movements or lateral head and body movements that are sustained and extreme should be classified as versive seizure.

Complex motor seizures

The following three types of complex motor seizures can be distinguished.

Again, "complex" herein refers to the complex characteristics of the movement and

does not mean that the patient loses awareness during the seizure.

a. Hypermotor seizures: Hypermotor seizures are seizures in which the main manifestations consist of complex movements involving the proximal segments of the limbs and trunk. This results in large movements that appear "violent" when they occur at high speeds. The "complex motor manifestations" imitate normal movements, but the movements are inappropriate for the situation and usually serve no purpose. Frequently, the movements are stereotypically repeated in more or less complex sequences (e.g., pedaling). Consciousness may be preserved during these seizures.

b. Automotor seizures: Automotor seizures are complex motor seizures in which the main manifestations consist of automatisms involving the distal segments of the hands and feet or the mouth and tongue. Consciousness is usually affected but may be preserved, particularly when the seizure originates from the nondominant hemisphere.

c. Gelastic seizures: Seizures in which the main motor manifestation is "laughing' ' are termed gelastic seizures. They may be preceded or followed by any other type of seizure. Only seizures in which the main ictal semiology is laughing should be classified as gelastic seizures. These seizures are classified separately because they are common in patients with hypothalamic hamartoma.

Special seizures

Seizures that cannot be classified in one of the four types described above are classified as

special seizures. All these seizures are "negative" or "inhibitory" motor seizures except the aphasic seizures that most probably represent ''negative cognitive" seizures.

a. Atonic seizures: Atonic seizures cause a loss of postural tone. The result is loss of posture (head drops, falls, and so on). Often these seizures are preceded by a short myoclonic seizure.

b. Astatic seizures: Astatic seizures consist of epileptic falls. Polygraphic studies show that only in a few patients are the falls the result of atonic seizures. In most patients, a myoclonic jerk causes the patient to lose balance, and the fall itself is produced by an atonia that occurs immediately after the initial myoclonic jerk. Pure generalized tonic seizures may also lead to an epileptic fa1l.

c. Hypomotor seizures. Hypomotor seizures have as their main manifestation a decrease or total absence of motor activity without the emergence of new motor manifestations. This classification is used exclusively in patients in whom it is not possible to test consciousness during or after the seizure (such as newborns, infants, and severely mentally retarded
patients).

d. Akinetic seizures. Akinetic seizures are characterized by the inability to perform voluntary movements. Therefore, they can actually be considered negative complex motor seizures. Muscle tone is also frequently lost, but the akinesia is the most prominent manifestation of the seizure.

e. Negative myoclonic seizures. Negative myoclonic seizures are seizures that consist of a brief interruption of tonic muscle activity due to an epileptiform discharge. The brief interruption of muscle activity may result in a short, sudden movement similar to a myoclonic jerk.

f. Aphasic seizures. During aphasic seizures, the patient cannot speak and often cannot understand spoken language. The seizures are probably a negative phenomenon produced by epileptic activation of a cortical language center, a phenomenon similar to that produced by cortical stimulation of language areas.

Paroxysmal events

Paroxysmal events are episodes in which the observer believes that there is not sufficient evidence to assume that a "seizure like" event was of epileptic nature. If an "ictal" EEG is available, it should not show an ictal EEG pattern. This classification of epileptic seizures is
based exclusively on semiology.

ADVANTAGES OF SEMIOLOGICAL SEIZURE CLASSIFICATION:

1. It provides a terminology that permits clear identification of ictal semiological features independent of any other rest results.
2. It clarifies the difference between seizure classification and epileptic syndrome classification.

3. A semiological seizure classification focuses the attention of the observer on clinical semiology.
4. It is comprehensive, can be applied to any age group. However, certain types of seizures will not occur or will seldom occur in newborn and infant system do not assume a one-to-one relationship between types of seizures (classified exclusively on the because of their incompletely developed nervous system [17, 18].

ANTIEPILEPTIC DRUGS:

Modern treatment of seizures started in 1850 with the introduction of bromides, on the basis of the theory that epilepsy was caused by an excessive sex drive. In 1910, phenobarbital, which then was used to induce sleep, was found to have antiseizure activity and became the drug of choice for many years. A number of medications similar to phenobarbital were developed, including primidone. Houston Merrit and Tracy Putnam introduced animal models for screening multiple compounds for antiepileptic activity, In 1940, phenytoin (PHT) was found to be an effective drug for the treatment of epilepsy, and since then it has become a major first-line antiepileptic drug (AED) in the treatment of partial and secondarily generalized seizures.

In 1968, carbamazepine (CBZ) was approved, initially for the treatment of trigeminal neuralgia; later, in 1974, it was approved for partial seizures. Ethosuximide has been used since 1958 as a first-choice drug for the treatment of absence seizures without generalized tonic-clonic seizures. Valproate was licensed in Europe in 1960 and in the United States in 1978, and now is widely available throughout the world. It became the drug of choice in primary generalized epilepsies and in the mid 1990s was approved for treatment of partial seizures. These anticonvulsants were the mainstays of seizure treatment until the 1990s.

The 1990s was an exciting era for physicians who treated patients suffering from intractable seizure disorders. Never before had so many new and novel AEDs been available for the management of epilepsy. For patients with epilepsy, these drugs represented renewed hope for complete seizure control and diminution of their AED-associated adverse events. Not only have many new compounds been identified for

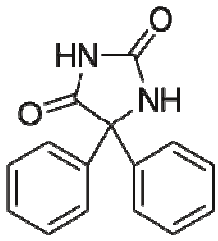
clinical use, but the potential mechanisms of action of older AEDs, including phenytoin, carbamazepine, valproate, ethosuximide, primidone, phenobarbital, and the benzodiazepines, are now more fully understood.when newer AEDs with good efficacy, fewer toxic effects, better tolerability, and no need for blood level monitoring were developed. The new AEDs have been approved in the United States as add-on therapy only, with the exception of lamotrigine, which is approved for conversion to monotherapy [2, 14].

The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry. A rational drug design process of a new anticonvulsant could be achieved in several ways.

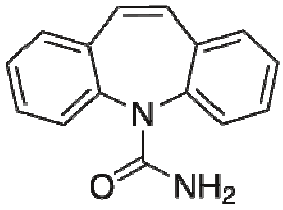
The first strategy is the identification of new targets through better understanding of molecular mechanisms of epilepsy. Another way is to modify already existing drugs and formulations. AEDs belong to many different chemical classes of compounds, including: hydantoins, iminostilbenes, barbiturates, benzodiazepines, valproate, imides, oxazolidine-2, 3-diones, sulfonamides and miscellaneous agents.

Currently available AEDs can be broadly classified into four categories:

1. Those whose main action relates to the inhibition of sustained repetitive firing, through blockage of voltage-dependent sodium channels and consequent inhibition of the release of excitatory neurotransmitters (phenytoin, carbamazepine, oxcarbazepine)

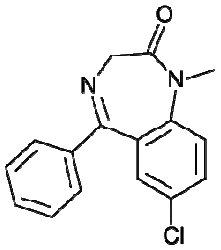


Phenytoin

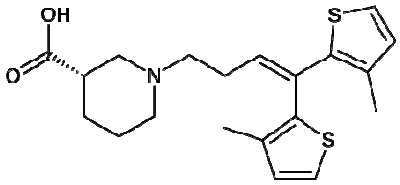


Carbamazepine

2. Those which enhance GABAergic transmission (benzodiazepines, barbiturates, vigabatrin, tiagabine)

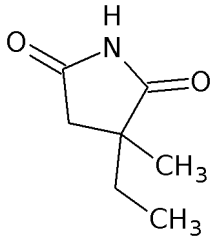


Diazepam



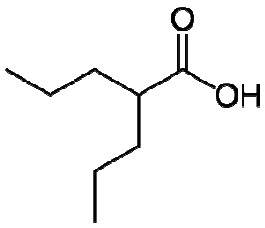
Tiagabine

3. Those stabilizing thalamic neurons through inhibition of T-type calcium channels (ethosuximide)

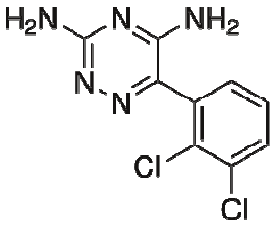


Ethosuximide

4. Those possessing a combination of the above actions, often coupled with additional mechanisms (valproic acid, gabapentin, lamotrigine, topiramate, zonisamide, felbamate).



Valproic acid



Lamotrigine

However, this classification has limited value because the majority of AEDs possess more than one mechanism of action, which may account for their efficacy, and it is also the fact that some of the clinically used drugs have not been linked with a specific site the brain, and the exact mechanisms of many AEDs remain unknown [19-27].

CLASSIFICATION OF AEDS

Classical (Introduced before 1979)	Newer (Introduced since 1993)
• Phenytoin • Phenobarbital • Primidone • Carbamazepine • Ethosuximide • Valproate (valproic acid) • Trimethadione (not currently in use)	• Lamotrigine • Felbamate • Topiramate • Gabapentin • Tiagabine • Vigabatrin • Oxycarbazepine • Levetiracetam • Fosphenytoin • Pregabalin.

Drugs in the developmental stage [26]**:**

- AWD 131 -138
- DP-VPA (DP16)
- Harkoseride (SPM 927)
- LY 300164
- NPS 1776
- NW-1015
- Remacemide
- Retigabine (D-23129)
- Rufinamide
- Valrocemide (TV 1901)

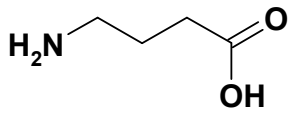
Table 1.1: Treatment with antiepileptic drugs, according to type of seizure and epileptic syndrome [23].

Type of seizure and epileptic syndrome	First line drug	Second line drug
Primary generalized Absence seizures	Ethosuximide, valproic acid	Lamotrigine
Myoclonic seizures	valproic acid	Acetazolamide, clonazepam, lamotrigine, primidone
Tonic–clonic seizures	Valproic acid, carbamazepine, phenytoin	Lamotrigine, phenobarbital, primidone
Absence epilepsy	Ethosuximide	Valproic acid, lamotrigine
Juvenile myoclonic epilepsy	Valproic acid	Acetazolamide, clonazepam, primidone, lamotrigine
Infantile spasms (West's syndrome)	Corticotropin	Clonazepam, valproic acid
Lennox–Gastaut syndrome	Valproic acid, lamotrigine	Carbamazepine
Partial, Simple partial seizures, complex partial seizures, secondarily generalized tonic clonic seizures and partial epileptic syndromes.	Carbamazepine, phenytoin	Gabapentin, lamotrigine, phenobarbital, primidone, tiagabine, topiramate, valproic acid

CHAPTER 1.2

GABA

While it had been known for some time that 4-aminobutyric acid (GABA) is present in biological tissue, it wasn't until 1950 that Roberts, Frankel and Udenfriend first positively identified large quantities of this amino acid in mammalian brain.



GABA

4-Aminobutyric acid (GABA), is the major inhibitory amino acid transmitter of the mammalian central nervous system and it is present in some 40% of all neurons, is widely distributed throughout the neuraxis. Given its ubiquity, and relatively high concentrations in brain and spinal cord, it is likely that GABA plays a major role in mediating or modulating most, if not all, central nervous system functions. Low levels of GABA in the brain are a major factor linked with epileptic phenomena and the regulation of GABA neurotransmission is the principal mode of action of antiepileptic drugs. Its role in maintaining normal neuronal activity is to regulate neuronal inhibition processes, counterbalancing neuronal excitation. GABA has therefore been described as the brain's natural calming agent. Consequently, GABA is implicated in a number of neurological disorders including epilepsy, depression, anxiety, Alzheimer's disease, Parkinson's disease, schizophrenia, and Huntington's chorea [27-31].

GABA and Neurologic disorders:

A decrease in GABAergic neurotransmission is involved in the pathogenesis of several neurologic disorders, including some forms of epilepsy, chronic pain, and anxiety and other mood disorders [31-33]. For example, a positron emission tomography (PET) study showed that patients with panic disorder have decreased GABA-A receptor binding [31]. Low plasma GABA may be characteristic of a subgroup of Mechanisms for enhancing GABAergic activity and their potential therapeutic implications patients with mood disorders [33, 34].

Similarly, research conducted at Yale showed that unipolar patients have GABA levels approximately 50% lower than those of normal controls. Drugs that enhance GABA activity, such as benzodiazepines, valproate, and phenobarbital, are often effective in the treatment of these disorders. GABAergic mechanisms appear to be important in both anxiolytic and sedative medications. Not surprisingly, GABAergic drugs that have anticonvulsant effects may also have clinically useful mood-stabilizing or antimanic effects (e.g., valproate) [35-37].

ROLE OF GABA IN EPILEPSY:

1. Abnormalities of GABA have been observed in genetic and acquired models of epilepsy.
2. Reduction of GABA mediated inhibition, activity of glutamate decarboxylase, binding to $GABA_A$ and benzodiazepine sites, GABA in cerebrospinal fluid and brain tissue, and GABA detected during microdialysis studies have all been reported in studies of human epileptic brain tissue.
3. GABA agonists suppress seizures and GABA antagonists produce seizures.
4. Drugs that inhibit GABA produce seizures.
5. Benzodiazepines and barbiturates which are effective anticonvulsants work by enhancing GABA nediated inhibition.
6. Drugs that inhibit synaptic GABA by inhibiting gaba catabolism (vigabatrin) or reuptake (tiagabine) are effective anticonvulsants [30].

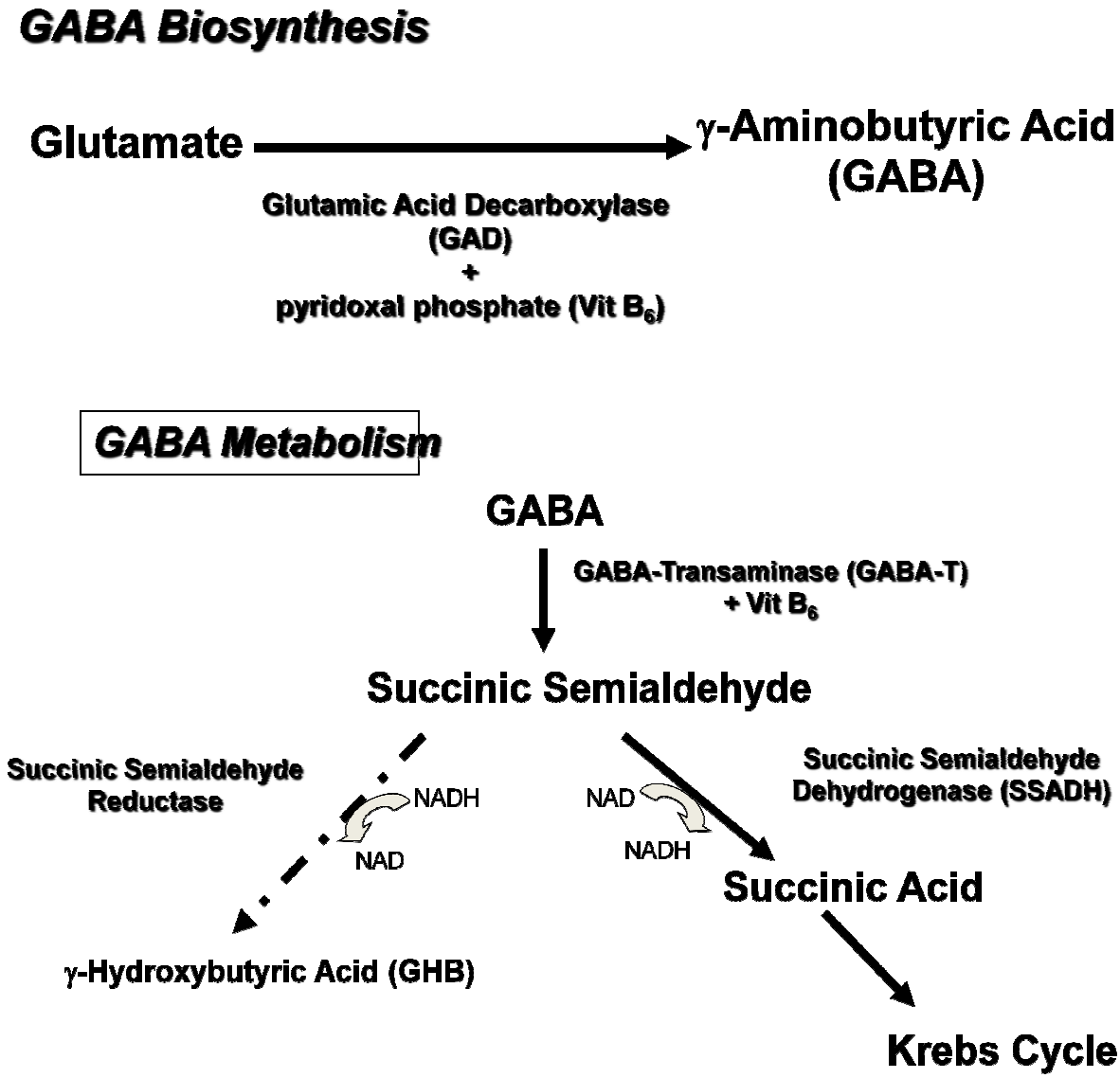


Fig: 4 Schematic representations of GABA biosynthesis and metabolism [30].

GABA RECEPTORS

GABA exerts its physiological actions through the interaction with three receptor subtypes, termed $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_C$ are ligand-gated ion channels permeable to anions. $GABA_A$ receptors mediate fast inhibitory synaptic transmissions **(Fig: 5**) they regulate neuronal excitability and rapid changes in mood. Thus, the seizure threshold, anxiety, panic, and response to stress (i.e., the "fight or flight" response) are regulated by GABA-A receptors**.**

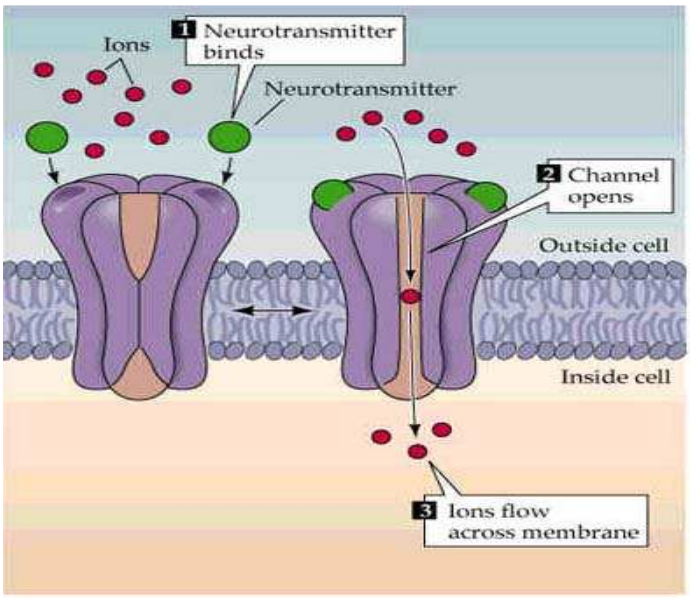


Fig: 5 Ionotropic Neurotransmitter Receptors ($GABA_A$ and $GABA_C$): Inhibitory Neuro- transmitter causes chloride influx and hyperpolaristion. **Excitatory Neurotransmitter causes sodium influx and depolarisation [38].**

GABA-B receptors mediate slow inhibitory transmissions**, (Fig: 6**) which appear to be important in memory, mood, and pain. GABA-C receptors have been identified, but their physiologic role has not yet been described [39, 40].

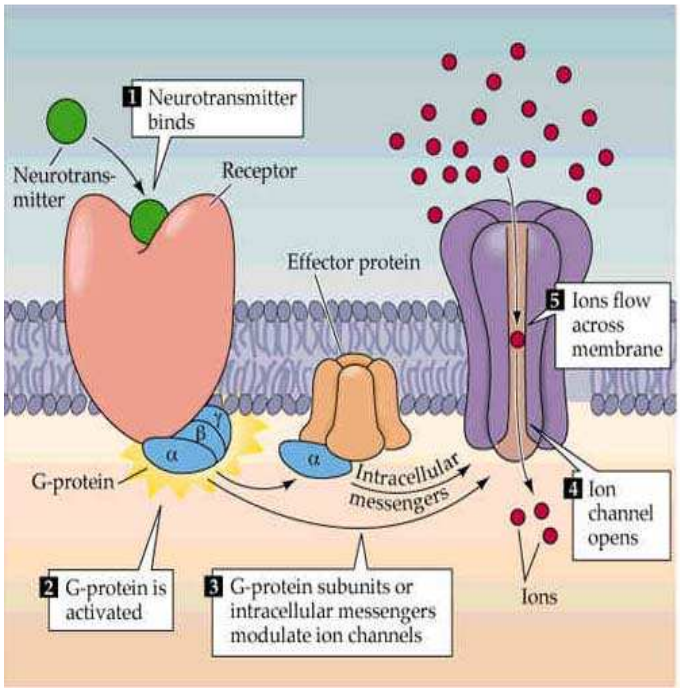


FIG: 6 Metabotropic Neurotransmitter Receptors ($GABA_B$). Slow neurotransmission neuromodulation, Receptor coupled to G-protein, Activates intracellular enzyme systems to produce an intracellular signal (the second messenger) [38].

GABA-A receptors:

The $GABA_A$ receptor's structure is typical of most ligand-gated (ionotropic) receptors. It is made up of five protein subunits arranged in a circle to form a pore, or channel, that remains closed until its specific ligand (in this case, GABA) binds to the recognition site**. (Fig: 7)**

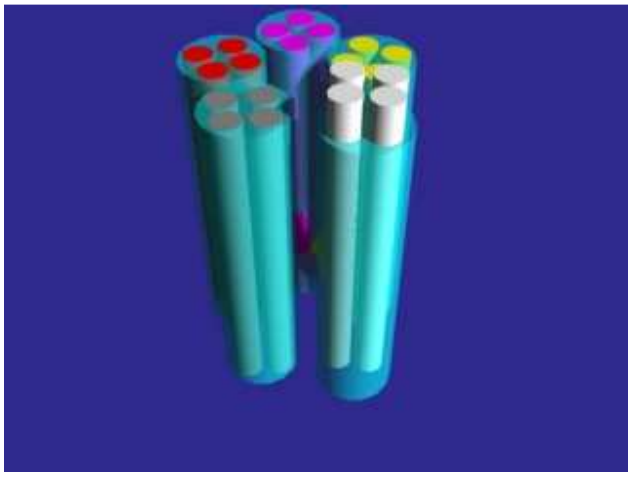


Fig: 7 GABA-A receptor (when GABA is present) [41]

Each protein subunit is actually a string of amino acids which passes in and out of the cell membrane four times. At the extracellular end of this string is a large N-terminal; this end-chain is thought to mediate GABA-channel interactions. In the middle of the string is a large intracellular loop of amino acids with four sites where phosphorylation occurs.

Each of the five subunits that make up the receptor is about 50,000 daltons in size. These subunits have been labeled with Greek letters, such as alpha, beta, gamma and delta. It appears as though GABA requires both alpha and beta components in order to bind. GABAa receptors are typically made up of two alpha and two beta subunits among the five subunits, though the particular subunit composition often varies widely among brain regions and species.

The five protein subunits of the GABAa receptor are arranged in circular formation to form a pore which transverses the membrane of the post-synaptic neuron. When GABA is not present, this pore is shaped somewhat like an hourglass. (**Fig: 8**)

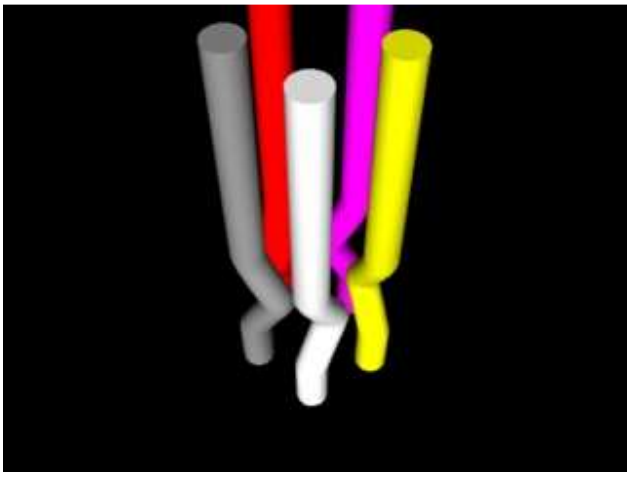
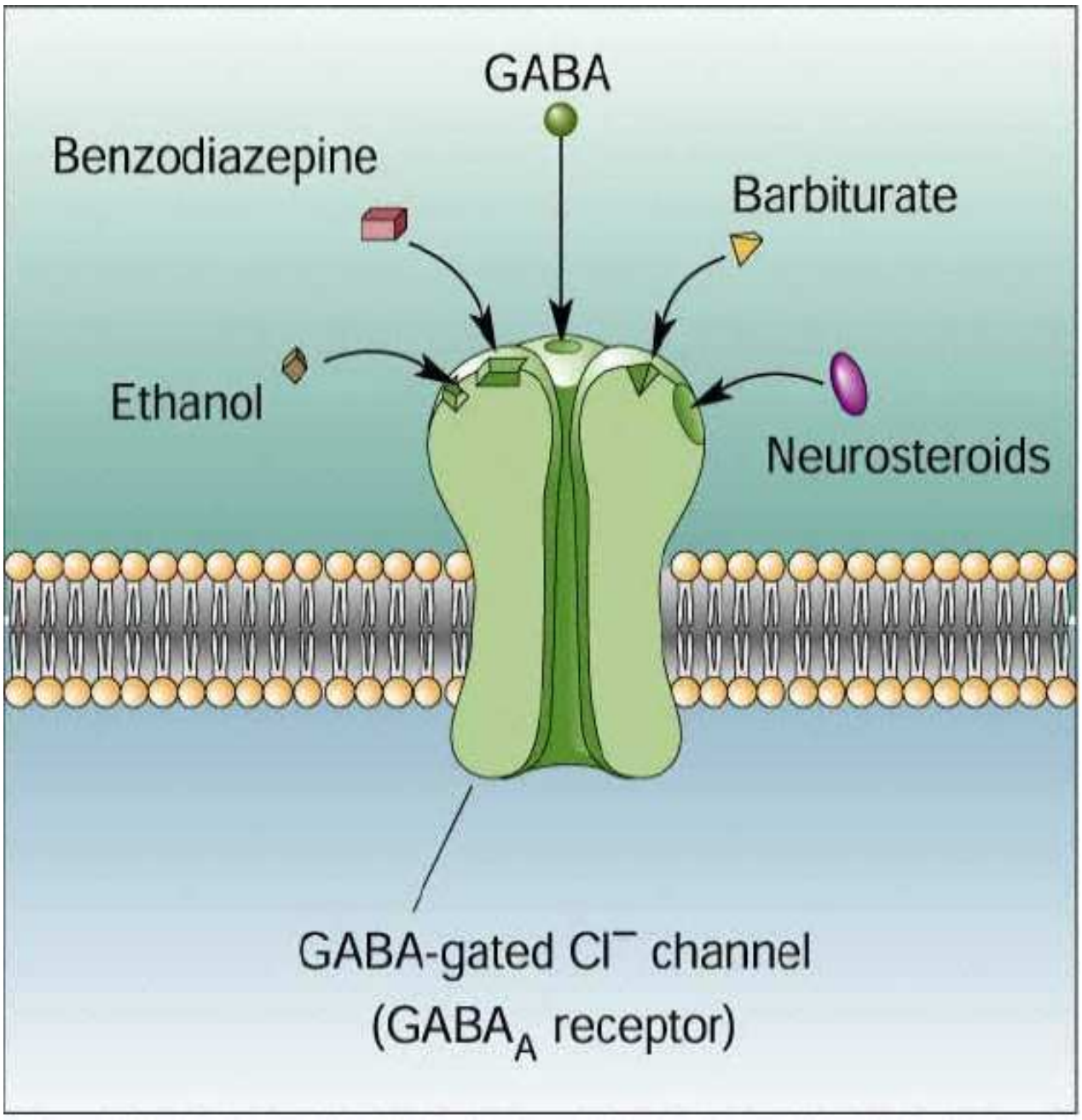


Fig: 8 GABA-A receptor (without GABA) [42]

The openings at either end are relatively large, reaching a diameter of about 3 nm, yet the inside of the channel narrows to about 5 nm. The narrowing of the channel is due to a kink in the three dimensional structure of the five protein units which form the receptor. It is this narrow region which prevents chloride ions from entering the neuron when GABA is not present. However, when GABA binds to the recognition site, it induces several conformational changes in the receptor molecule. One of these changes involves the rotation of the five protein subunits such that the diameter of the kink is widened. A wider channel makes it possible for choride ions to pass freely into the neuron. In addition to binding sites for GABA, $GABA_A$ receptors have binding sites for benzodiazepines, ethanol, barbiturates, and neurosteroids [43-47]**. (Fig: 9)**



(Fig. 6) Binding sites of GABA$_A$ receptor antagonists [14]

$GABA_A$ receptor agonists:

GABA receptors can be activated by a number of compounds (**Fig: 10**) such as muscimol **1**, isoguvacine, **2** Baclofen, **3**, Gabapentin, **4** 3-aminopropyl phosphinic acid, **5** of which were subsequently used as radioligands.

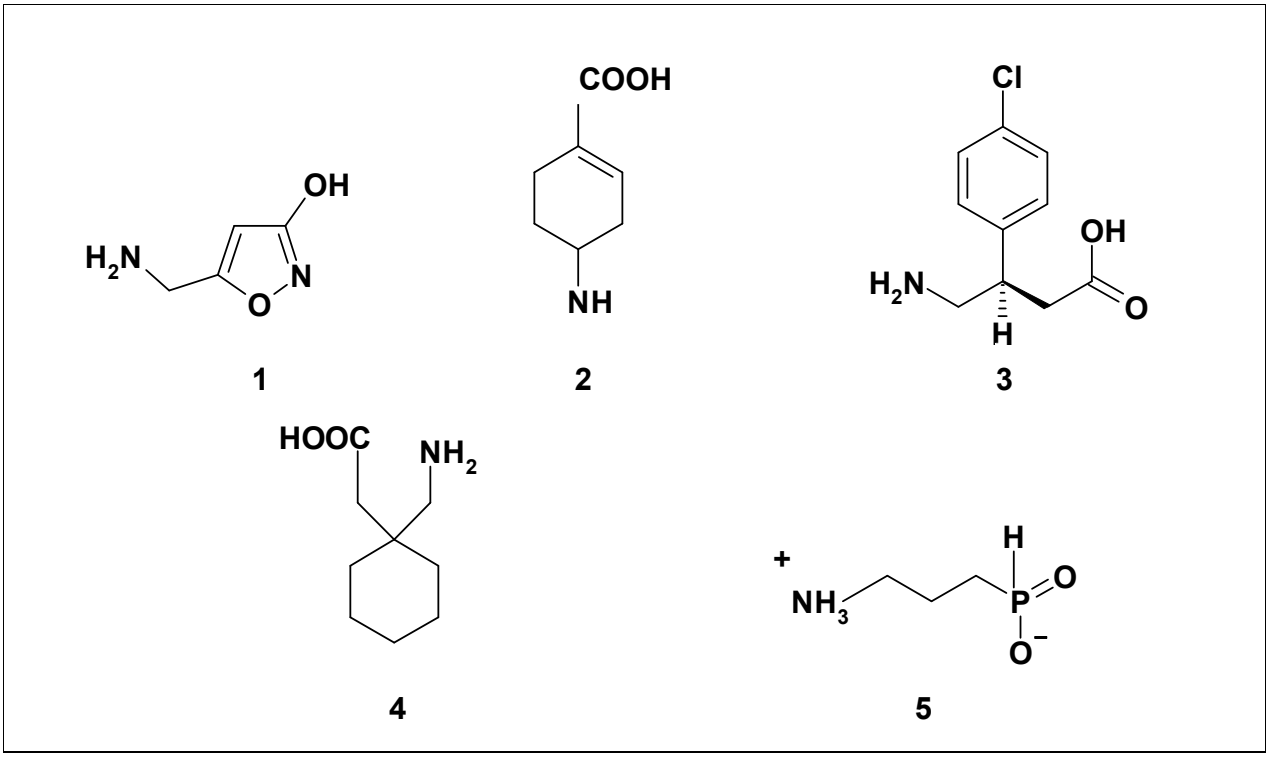


Fig: 10 $GABA_A$ receptor agonists

$GABA_A$ receptor antagonists:

GABA antagonists do not interact with the GABA binding site itself, but rather with separate sites on or near the Cl^- channel. Such noncompetitive antagonists block GABA-mediated Cl^- flux, and therefore act as potent convulsants just as the competitive blocker. One well-known drug of this type is the synthetic CNS stimulant pentylenetetrazole, **6** which is still used experimentally to induce seizures in animal subjects. Furthermore, picrotoxinin, **7** is a naturally

Occurring, found in the seeds of the East Indian shrub *Animirta cocculus*, noncompetitive $GABA_A$ antagonist. Specific receptor antagonists are essential tools for studies of the physiological role and pharmacological importance of the particular receptors. The

classical $GABA_A$ antagonist's bicuculline, **8** and its quaternized analogue bicuculline methochloride,

(BMC) **9** have played a key role in such studies on $GABA_A$ receptors. In recent years, new structural classes of $GABA_A$ antagonists have been developed. Whereas the bicyclic 5-isoxazole derivative, Iso-THAZ, **10** is a moderately potent $GABA_A$ antagonist, a series of arylaminopyridazine analogues of GABA, notably gabazine, **11** showed very potent and selective $GABA_A$ antagonist effects [48-55].

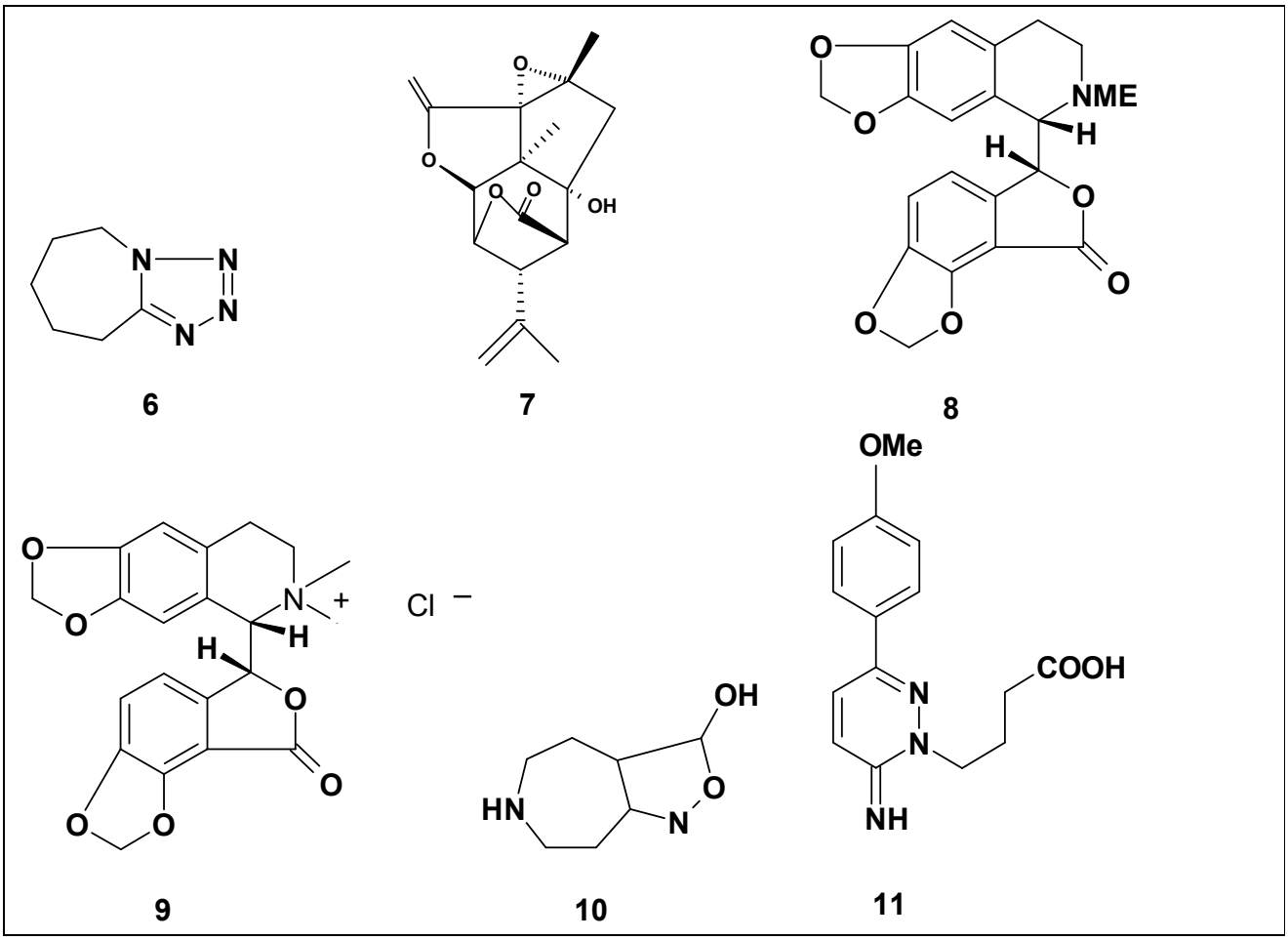


Fig: 11 $GABA_A$ receptor antagonists

$GABA_B$ RECEPTOR

Unlike $GABA_A$ receptors that form ion channels, $GABA_B$ receptors address second messenger systems through the binding and activation of guanine nucleotide-binding proteins (G proteins) $GABA_B$ is a G-protein coupled receptor which modulates the synaptic transmission through intracellular effector systems. More specifically, $GABA_B$ is involved in the presynaptic inhibition of transmitter release and mediates the slow synaptic inhibition by increasing the potassium conductance responsible for long-lasting inhibitory postsynaptic potentials.

$GABA_B$ receptor **(Fig: 12)** was not a single protein but instead consisted of two distinct subunits, neither of which was functional on its own.

The first indication of the structure of the $GABA_B$ receptor emerged in 1997 when Bettler and colleagues identified a large molecular weight (130 kDa), seven transmembrane spanning receptor protein, $GABA_{B1}$. This was obtained using an expression cloning technique which was dependent on the development of the high affinity radiolabelled iodinated receptor ligand [125I]-CGP64213. A year after this initial discovery, it was realized that $GABA_{B1}$ is not expressed on the surface of cells without the support of a second receptor protein, referred to as $GABA_{B2}$, which appears to couple to $GABA_{B1}$ at the level of the endoplasmic reticulum in order to facilitate surface expression. $GABA_{B2}$ also has a seven transmembrane spanning and links to $GABA_{B1}$ at their intracellular C-terminals. The combination of these two proteins forms a heterodimer that is crucial for full receptor function.

Numerous isoforms of $GABA_{B1}$ and $GABA_{B2}$ have been described with at least three forms of human $GABA_{B1}$ and $GABA_{B2}$ proteins. However, whether different combinations of these isoforms produce different pharmacological characteristics is not known [56-70].

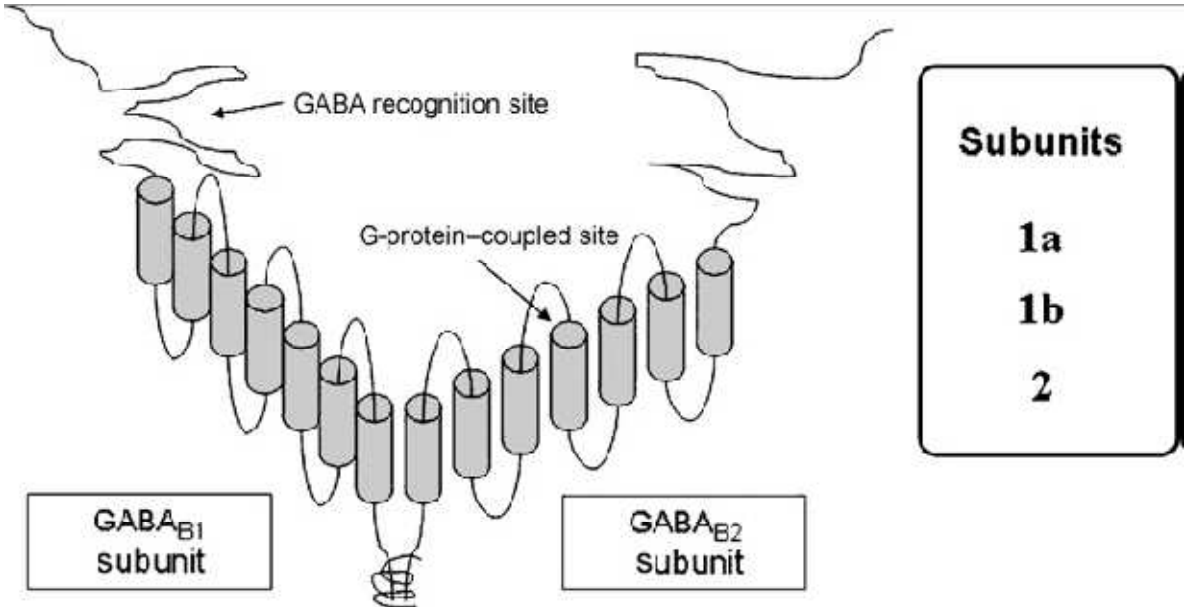
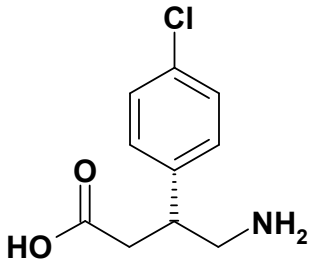


Fig: 12 Schematic representation of the $GABA_B$ receptor. Listed in the insert are the predominant GABAB receptor subunits [39].

$GABA_B$ receptor agonist:

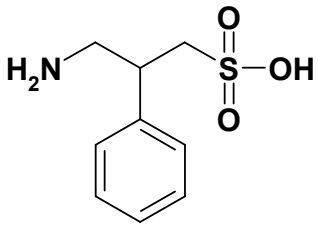
Baclofen, **12** is direct agonist at $GABA_B$ receptors, which are coupled to G proteins. $GABA_B$ receptors may regulate Ca2+ and K+ influx through the Gi/o family of G proteins and act presynaptically to inhibit the release of excitatory amino acids such as glutamate. Baclofen is orally active as a muscle relaxant and has been used in the treatment of rigidity and spasticity of cerebral palsy.



12

$GABA_B$ receptor antagonist:

Saclofen, 13 is a competitive antagonist for the $GABA_B$ receptor. This drug is an analogue of the $GABA_B$ agonist Baclofen. The action of saclofen on the central nervous system is understandably modest, because G-proteins rely on an enzyme cascade to alter cell behavior while ionotropic receptors immediately change the ionic permeability of the neuronal plasma membrane, thus changing its firing patterns. These particular receptors presynaptically inhibit N- and P/Q- VGCCs via a direct interaction of the dissociated beta gamma subunit of the g-protein with the intracellular loop between the 1st and 2nd domain of the VGCC's alpha-subunit; postsynaptically, these potentiate Kir currents. Both result in inhibitory effects [61].



13

$GABA_C$ RECEPTOR: $GABA_C$ receptors are also structurally distinct from $GABA_A$receptors. Although fully functional $GABA_A$ receptors require heterooligomeric formation of a-, b- and g-subunits $GABA_C$ receptors can assemble as homooligomers. To date, three different r-subunits (r1–r3) have been cloned from several mammalian and vertebrate species. The r-subunits share only 30–38% amino acid sequence identity with the $GABA_A$ receptor subunits and they mediate robust bicuculline-insensitive GAB_A responses in heterologous expression systems. There is no evidence so far from heterologous expression systems that the r-subunits coassemble with the $GABA_A$ receptor a-, b- and g-subunits, or with the glycine receptor b-subunit.

Agonists:

GABAC receptors share several agonists with $GABA_A$ receptors **(Fig: 12).** However, the sensitivity of GABAC receptors to GABA is much higher than that of GABAA receptors,

with EC50 values in the range 0.8–2.2 μM for recombinant ρ1 or ρ2 homomeric receptors and 7.5 μM for ρ3 homomeric. Muscimol, **1** also activates $GABA_C$ receptors with an EC50 of 1.3 μM (human6), 2.3 μM (bovine) and 4.81 μM (fish). However, the potency of muscimol is much less than that of GABA (42% and 73% of the potency of GABA in fish and cow, respectively). This is opposite to the findings with $GABA_A$ receptors (2–10-fold higher potency than GABA). Thus, muscimol acts as a partial agonist of $GABA_C$ receptors, similar to isoguvacine. Other $GABA_A$ receptor agonists, such as 4, 5, 6, 7-tetrahydroisoxazolo {5, 4-*c*} pyridin-3-ol (THIP), piperidine-4-sulfonic acid (P-4-S), isonipecotic acid and 3-aminopropanesulfonic acid (3-APS), are largely inactive as agonists. The pharmacological nuances of GABAC receptor agonists reflect subtle structural differences between the underlying ρ-subunits, and, together with selective antagonists, provide useful tools for characterizing native GABAC receptors.

Antagonists

An important characteristic of native and recombinant $GABA_C$ receptors is their insensitivity to bicuculline, which is a competitive antagonist of $GABA_A$receptors Other competitive $GABA_A$ receptor antagonists, such as strychnine and SR95531 (Gabazine), are much weaker inhibitors of $GABA_C$ receptors. Other selective agonists of $GABA_B$ receptors, such as baclofen and saclofen, have no effect on $GABA_C$ receptors [39, 71, and 72].

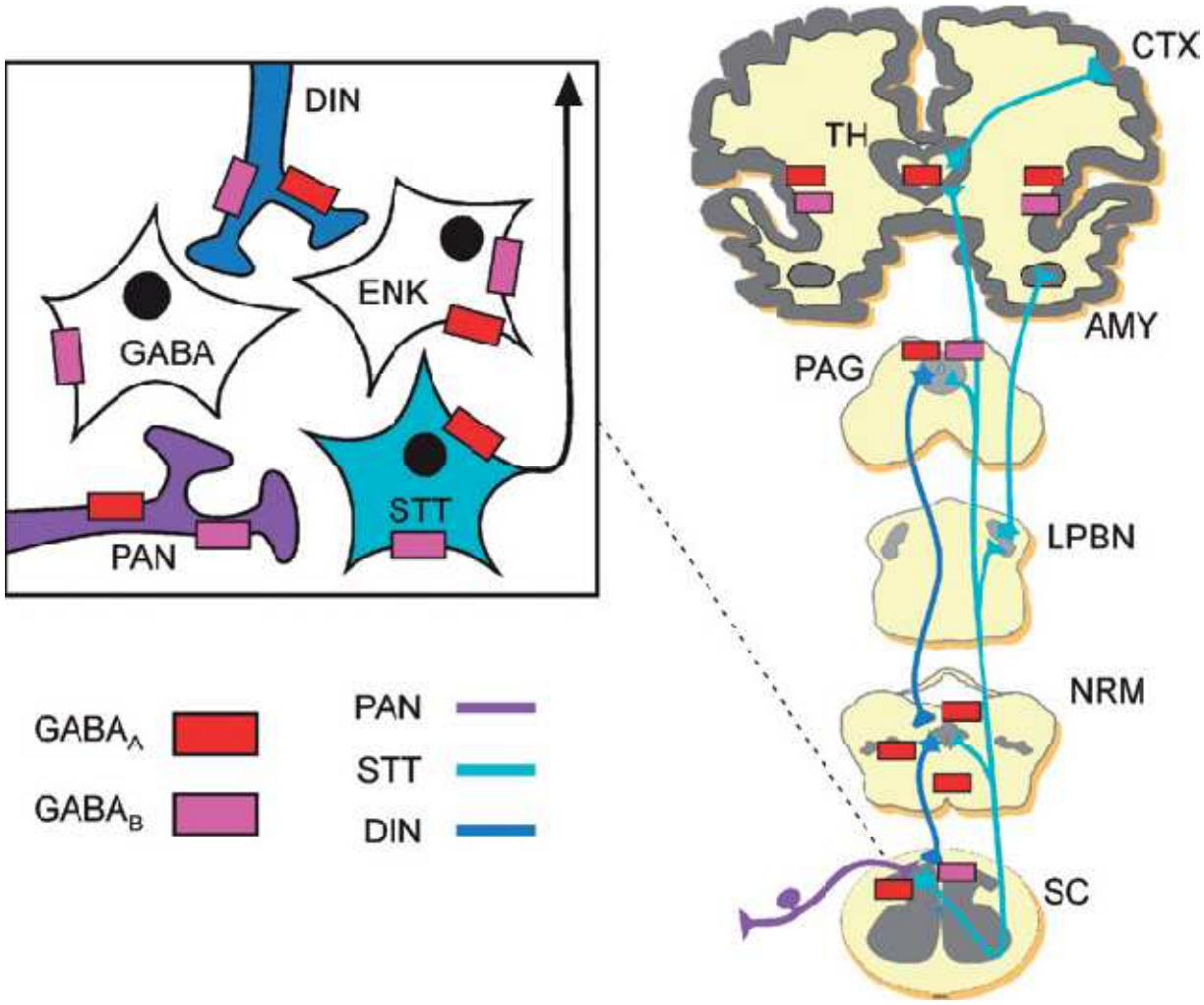


Fig: 13 Anatomical localization of GABA receptors in the central and peripheral nervous systems. Depicted in the inset is the circuitry within the dorsal horn of the spinal cord [68].

Abbreviations: CTX, primary sensory cortex; TH, thalamus; AMY, amygdala; PAG, periaqueductal gray; LPBN, lateral parabrachial nucleus; NRM, medullary raphe nucleus; SC, spinal cord; PAN, primary afferent nociceptor C and A-d fibers; DIN, descending inhibitory neuron; GABA, GABAergic interneuron; ENK, enkaphalinergic interneuron; STT, spinothalamic tract projection; GABA-A, GABA-A receptor; GABA-B, GABA-B receptor [39].

Mechanisms for enhancing GABA activity

There are at least five known mechanisms by which drugs can increase the availability and activity of GABA.

(1) **Stimulation of GABA-A receptors.**

GABA-A receptors are coupled to chloride ion channels; activation of the GABA-A receptor induces increased inward chloride ion flux, resulting in membrane hyperpolarization and neuronal inhibition. This can be effected by increasing either the frequency (benzodiazepines) or the duration (phenobarbital) of opening of the chloride ion channels.

(2) Increasing the release of GABA from glial cells.

This is believed to be the mode of action of gabapentin, which is structurally similar to GABA but does not interact with GABA-A receptors.

(3) **Inhibition of GABA transaminase,** (the enzyme that metabolizes GABA).

Vigabatrin (not approved for use in the U.S.) works primarily and valproate works in part through this mechanism.

(4) **Increases in GABA synthesis and release**.

This is also one of the multiple GABAergic mechanisms of valproate.

(5) Inhibition of reuptake of GABA by neurons and glial cells.

Tiagabine prevents GABA reuptake by inhibiting the action of GAT-1 GABA transporters.

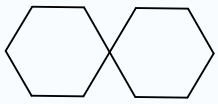
Table: 1 summarizes the mechanisms of potent GABAergic drugs that are currently used for neuropsychiatric disorders or have shown a potential for such use. The subsequent discussion of the potential clinical uses of these agents omits phenobarbital (because of its serious adverse cognitive effects) and vigabatrin (because it is not approved for U.S. use) [2, 73].

Table 1.2: Mechanisms of drugs with strong GABAergic activity [33]:

Drug	Gabaergic mechanism	Gabaergic potency	Comment
Benzodiazepines	Direct agonism at Increases frequency	strong	Increases frequency of GABA-A chloride channel opening
Phenobarbital	Direct agonism at GABA-A receptors.	strong	Prolongs duration of opening of GABA-A chloride channel
Gabapentin	Increases GABA release from glial cells	strong	Increases total cerebral gaba.
Vigabatrin	Inhibits gaba transaminase	strong	Increases total cerebral gaba.
Valproate	Multiple gabaergic mechanisms, including GABA transaminase inhibition and increases in GABA synthesis and release	Strong	Increases total cerebral gaba.
Tiagabine	Selective inhibition of gaba reuptake by neurons and glial cells.	Strong	GABA release remains under physiologic control; no increase in total CNS GABA

CHAPTER 1.3
SPIRO COMPOUNDS

A spiro compound is a bicyclic organic compound with rings connected through just one atom. The rings can be different in nature or identical. The connecting atom is also called the spiroatom, most often a quaternary carbon ("Spiro carbon").



Some spiro compounds exhibit axial chirality. Spiroatoms can be centers of chirality even when they lack the required four different substituents normally observed in chirality. When two rings are identical the priority is determined by a slight modification of the CIP system assigning a higher priority to one ring extension and a lower priority to an extension in the other ring. When rings are dissimilar the regular rules apply [74].

All spiro compounds have the infix *spiro* followed by square brackets [] containing the number of atoms in the smaller ring and the number of atoms in the larger ring excluding the spiroatom itself.

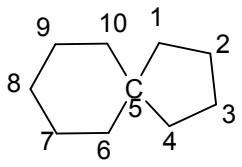
The Spiro compounds may be classified according to the number of Spiro atoms

1. Monospiro
2. Dispiro
3. Trispiro

Nomenclature:

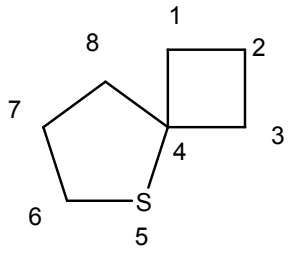
1. Spiro heterocycles with one Spiro atom consisting of one or both heterocyclic rings are named by prefixing Spiro to the name of normal alkane with same number of carbon atoms. The number of atoms in each ring are indicated by Arabic numbers separated by a full stop and enclosed in a square bracket in ascending order and are placed between Spiro prefix and the name of

hydrocarbon. The heteroatoms are indicated by the prefixes and are prefixed with their positions to the name of Spiro hydrocarbon.



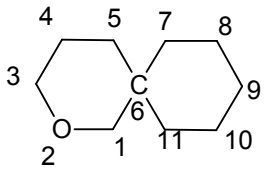
Spiro[4.5]decane

2. The numbering starts from the ring atom of the smaller ring (if rings are of unequal size) attached to the spiro atom and proceeds first around the smaller ring and then the larger ring through the spiro atom. The heteroatoms are assigned the lowest possible number locants.



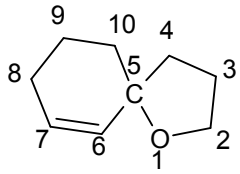
5-thiaspiro[3.4]octane

3. The heterocyclic ring is preferred over the carbocyclic ring of the same size. If both the rings are heterocyclic, the preference is given to the heterocyclic ring with heteroatom appearing first in table.



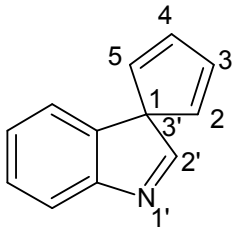
2-Oxaspiro[5.5] undecane.

4. If the unsaturation is present in aring, the pattern of numbering remains the same but the direction around the ring remains in such a way that the multiple bond is given number as low possible. However , the heteroatom is preferred over the multiple bond.



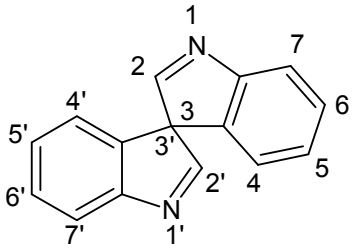
1-Oxaspiro[4.5]dec-6-ene.

5. When one or both the components of spiro heterocycle are fused polycyclic system, the names of both the components are cited after prefix ‘spiro’ in square bracket in alphabetical order and are separated by the numbers of spiroatom. The components in such spiro heterocyclic system retain their numbering, but the second component is numbered by primed numbers.



Spiro{cyclopenta-2,4-diene-1,3’-3H-indole]

6. If both the heterocyclic components are the same in spiroheterocyclic system, ‘spirobi-’ is prefixed to the name of heterocyclic component [75].



3,3’-spirobi(3H-indole)

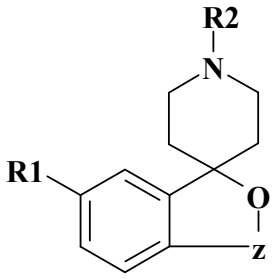
Biological activities of spiro compounds:

Spiro compounds having cyclic structures fused at a central carbon are of recent interest due to their interesting conformational features and their structural implications on biological systems. The asymmetric characteristic of the molecule due to the chiral spiro carbon is one of the important criteria of the biological activities.

The Spiro functionally has been known for a long time to be present in photochemicals either in alkaloids, lactones or terpenoids. The spirocyclic alkaloid (K)-histrionicotoxin

isolated from skin extract of the poison dart frog Dendrobats Histrionius, found in Columbia is a very potent nicotinic receptor antagonist. The spiro [pyrrolidin-3, 30-indole] ring system is a recurring structural motif in a number of natural products such as vinblastin and vincristine that function as cytostatics and are of prime importance in cancer chemotheraphy. Some Spiroheterocycles benzopyrans, which are aldose reductase inhibitors and found to be useful as antidiabetics. Several potent reductase inhibitors based on Spirosuccinimide, Spiropyridazine and Spiroazetidine have been reported for the prevention of secondary complications of diabetes [76, 77].

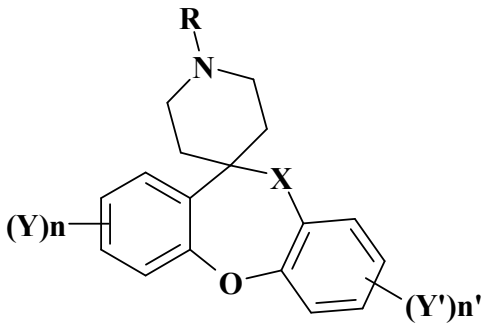
According to klioze et al 1, 3-dihydrospiro [isobenzofuran-1, 4-piperidine] s **(14)** are useful as analgesics, anticonvulsants and anti depressants. These compounds have substantial differences from the compounds of the prior art and exhibited unanticipated pharmacological activity and low toxicity levels [78].



(14)

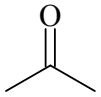
Where R1 is hydrogen, lower alkyl, lower alkoxy, halogen or triflouromethyl, R2 is hydrogen or benzyl and Z is –CH2-or-CO-.

Ong et al described methods for preparing spiro [dibenz (b, f) oxepin-piperidine]s **(15)** which are useful as analgesics, tranquilizers and anticonvulsants [79].



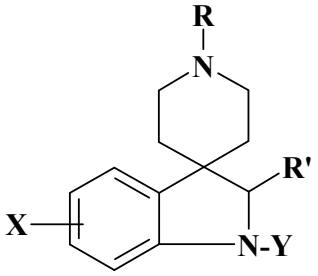
(15)

Where R is hydrogen, alkyl, alkenyl, alkynyl, hydroxyl alkyl, cyclo alkyl etc.

Where X is  or 

Y and Y' are the same or different and each can be chlorine, fluorine, bromine, methoxy, methylthio or triflouromethyl. n and n' are the same or different each can be an integer from 1 to 2.

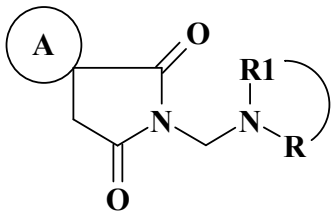
Novel spiro [indoline-3, 4'-piperidine] s **(16)** and related compounds were described by Ong et al. These compounds are useful as antidepressants, anticonvulsants and tranquilizers [80].



(16)

Where R is hydrogen, lower alkyl, cyano, lower alkanoyl, phenoxy carbonyl, phenoxy lower alkyl etc, R' is hydrogen lower alkyl, y is hydrogen, halogen, trifluoromethyl, lower alkyl, lower alkoxy, hydroxyl, nitro etc and X is hydrogen, halogen or lower alkyl.

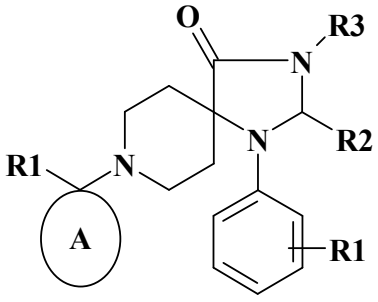
Mannich bases of spirosuccinimides (17) were reported to have anticonvulsant, sedative and antileukemic activities.



(17)

Where ring A is selected from the group consisting of saturated and unsaturated monocyclic and bicyclic carbon rings of atleast 5 carbon atoms, R and R_1 are each selected from the group consisting of lower alkyl, lower alkenyl, cycloalkyl and aryl groups, when taken together with the nitrogen atom to which they are attached, represent a hetrocyclic selected from the group consisting of morpholino, piperidino, pyrrolidino, piperazino and derivatives of these groups [81].

Adam et al. described the process for the synthesis of 1, 3, 8-Triazaspiro (4, 5) decan-4-one **(18)** derivatives. These compounds and their salts are having valuable therapeutic properties as they are agonists and/or antagonists of the Orphanin FQ receptor. They are useful for the treatment of memory and attention deficits, psychiatric, neurological and psychological disorders, amelioration of symptoms of anxiety and stress disorders, depression, trauma, memory loss due to alzheimer's disease or other dementias, epilepsy and anti convulsants, acute and/or chronic pain conditions and metabolic disorders such as obesity [82].

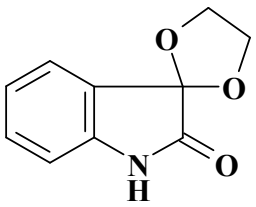


(18)

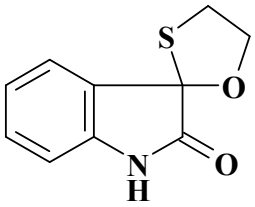
Where R_1 is hydrogen, lower alkyl, halogen, lower alkoxy, trifluromethyl, lower alkyl-phenyl or cycloalkyl, R_2 is hydrogen, lower alkyl, phenyl or lower alkyl phenyl, R_3 is hydrogen, lower alkyl, benzyl or lower alkyl phenyl etc. and A is a ring system consisting

of (c_{5-15})-cyclo alkyl which is unsubstituted or substituted by lower alkyl, trifluoromethyl, phenyl, (c_{5-7})-cycloalkyl, spiro-undecanyl or by 2-norbornyl.

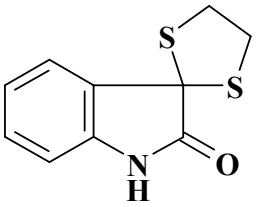
Structural analogues of spiro[1,3-dioxane-2,3'-indolin]-2'-one **(19)**, Spiro[1.3-dithiolane-2,3'-indolin]-2'-one **(20)** and spiro[indoline-3,2'-[1,3]-oxathiolan]-2-one **(21)**, were found to have good anticonvulsant activity [83].



(19)



(20)



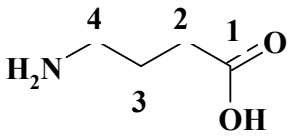
(21)

Chapter-2
Review of literature

CHAPTER 2.1
LITERATURE REVIEW

GABA DERIVATIVES:

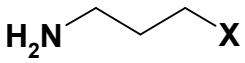
Since 1960 GABA and its derivatives have begun to be used as medicinal preparations in the treatment of nervous and psychiatric conditions. The successful use of GABA and its derivatives in the treatment of various neurotic and psychopathic states lead to the possibility of synthesizing new synthetic drugs with selective action and with a low toxicity by virtue of their similarity to the metabolic products in the brain [29].



GABA

MODIFICATIONS AT THE FIRST POSITION: (COOH TERMINUS):

Active analogs of GABA usually possess an acidic proton in a position comparable to that in GABA. Replacement of the carboxyl group with a sulfonic acid gives 3-aminopropane sulfonic acid **(2.1a)** which is a potent and selective GABA agonist in the CNS tissue (Skerritt et. al); it is more potent than GABA at depolarizing rat cervical ganglia (Bowery et. al)



2.1a X = SO_3H **2.1b** X = SO_2H

2.1c X = PO $(OH)_2$ **2.1d** X = BO_2OH

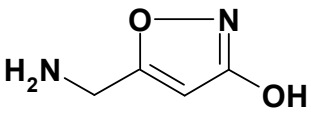
2.1e X = CONHOH **2.1f** X = CO_2CH_3

2.1g X = $CONH_2$ **2.1h** X = $CONHC_{12}H_{25}$

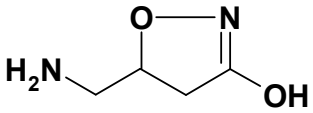
The sulfinic acid analogue **(2.1b)** is also a relatively potent receptor agonist in contrast to the phosphonic and boronic acids **(2.1c, d)** which are either weak or inactive. The hydroxamic acid **(2.1e)** has been reported as a weak inhibitor of GABA-T.

Compounds without an acidic proton, such as methylene ester **(2.1f)** and the amide **(2.1g)** generally have low activity on GABA receptors or uptake but considerable GABA uptake inhibition appeared in the detergent like amide **(2.1h)** esters of GABA with long chain alcohols such as cetyl gaba penetrate the BBB with resultant anticonvulsant action. Recently, ethylenediamine has been investigated as a GABA mimetic: it shows moderate activity as a Bicuculine sensitive inhibitor of neuronal firing in vivo and as an inhibitor of gaba binding invitro. Ethylene diamine is a weak inhibitor of neuronal GABA uptake but is more potent as an inhibitor of beta alanine uptake suggesting some selectivity for the dual GABA uptake system.

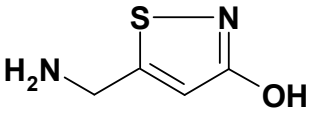
Muscimol **(2.2),** a centrally active constituent of the mushroom *Amanita muscaria,* is a conformationally restricted analog of GABA in which 3-hydroxy-isoxazole moiety replaces the carboxyl group. Muscimol is a very potent on bicuculline-sensitive post synaptic receptors and is a only a weak inhibitor of GABA uptake. Variations of heterocyclic ring of Muscimol as in dihydromuscimol **(2.3)** and thiomuscimol **(2.4)** lead to a potent receptor agonists [84].



2.2



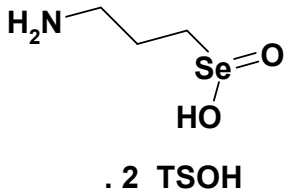
2.3



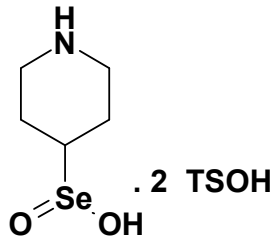
2.4

Nicolai stuhr-hansen et. al reported the synthesis and pharmacology of selenic acid analogues of 4-aminobutyric acid.

The concept of bioisosteric replacement continues to play an important role in bioorganic and medicinal chemistry in the design of novel pharmacological tools as well as therapeutic agents with optimal pharmacological profile and improved pharmacokinetic properties. A number of acidic groups have been shown to be useful carboxyl group bioisosteres. The bioisosteic potential of the selenic acid group was investigated. Of the four organic acids of selenium (selenol, selenenic, seleninic and selenonic acids), the seleninic acid group is the most stable.



2.5



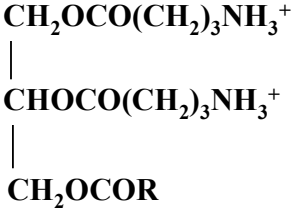
2.6

The affinity for the $GABA_A$ and $GABA_B$ receptors of the dihydrotosylate salts of **2.5** and **2.6** as well as their functional properties were characterized in binding studies, guinea pig ileum and cloned $GABA_A$ receptors. It has been experimentally established, that there is a high correlation between binding data obtained in cell lines expressing human GABA receptors and rat brain homogenates.

The 3-aminopropane seleninic acid **(2.5)** was shown to be a potent and relatively selective $GABA_B$ agonist. In contrast the piperidine-4-seleninic acid **(2.6)** was shown to be a potent and selective partial $GABA_A$ agonist with a maxium response of 3% of GABA [85].

James N. Jacob et. al reported synthesis and pharmacological properties of 4-aminobutyric acid esters **(2.7a – 2.7d)**. A series of c-14 labeled and unlabeled di-γ-aminobutyric acid esters of glycerly lipids having zero to three double bonds (stearoyl,oleoyl,linoleoyl and linolenoyl) were synthesized.

They have synthesized glyceryl lipids containing two GABA ester groups in a compound. The c-14 labeled GABA derivatives were also synthesized for measurements of the brain uptake properties. The results suggest that all the compounds can readily penetrate the blood brain barrier and that maximum pharmacology activity is associated with the compounds containing the highest degree of unsaturation [86].



2.7

a: R = $(CH_2)_{16}$ CH3[Stearoyl (SG_2)],

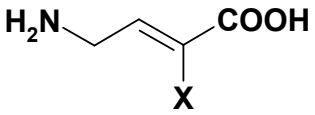
b: $(CH_2)_7(CH=CHCH_2)(CH_2)_6CH_3$[oleoyl ($OG_2$)]

c: R = $CH_2)_7(CH=CHCH_2)_2(CH_2)_3CH_3$[linolenyl ($L^2G_2$)],

d: R = $(CH_2)_7(CH=CHCH_2)_3(CH_2)_6CH_3$[linolenoyl ($LG_2$)].

MODIFICATIONS AT THE SECOND POSITION:

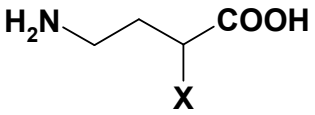
The steric bulk of a methyl group from C_2 to the N detracts from GABA mimetic activity,



2.8 a X = H, b X = F, c X = Cl, d X = Br, e = X = CH_3

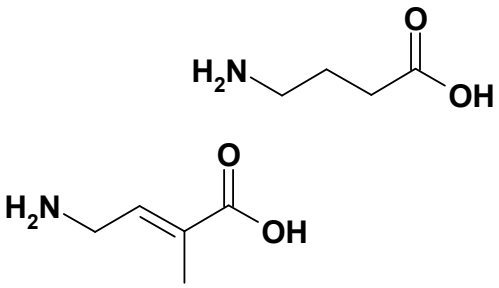
The 2 substituted derivatives **(2.8 a-e)** are potent inhibitors of GABA binding on GABA uptake, when the bulk of the substituent is increased to that of bromine the steric capacity of this position in the uptake receptor is apparently exceeded and **(2.8d)** is atleast 5 times less active than **(2.8a, b, c, e).** The fluoro derivative **(2.8b)** is also a potent time dependent inhibitor of GABA transaminase, while **(2.8e)** like **(2.5a)** is a more efficient substrate for the enzyme than GABA itself. The 2-methyl crotonic acid derivative **(2.8e**) is more potent on GABA uptake than the 3-methyl derivative.

Hydroxy and Amino substituents give rise to potent and selective GABA analogues. 2-hydroxy GABA **(2.9a)** and 2-amino GABA (2, 4-diaminobutyric acid, DABA) **(2.9b)** are potent inhibitors and the latter is more selective with considerably less post synaptic inhibitory activity. The S (+) isomer of DABA is over 28 times more potent as an inhibitor of GABA neuronal uptake than the R (-) isomer. DABA has found considerable use as a selective inhibitor of neuronal GABA uptake [84].



2.9 a X = OH, b = NH_2

Duke et. al reported the synthesis and resolution of 2-methyl analogues of GABA [85]. This enabled the preparation of 2-MeTACA **(2.12)**, -2-MeGABA **(2.13)** and its enantiomers **2.14** and **2.15**.



2.10

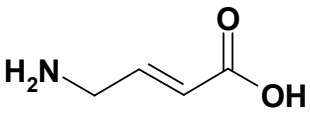
GABA

Agonist

2.11

TACA

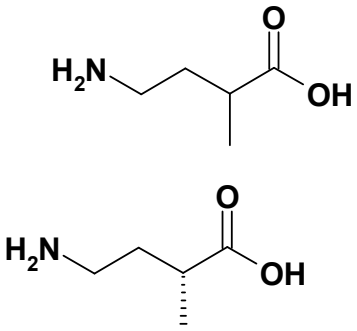
Agonist



2.12

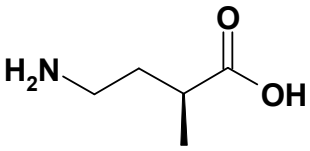
(-)-(2R)-Methyl TACA

Antagonist



2.13

2-Methyl GABA



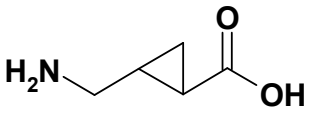
2.14

(+)-(2S)-Methyl GABA

2.15

(-)-(2R)-Methyl GABA

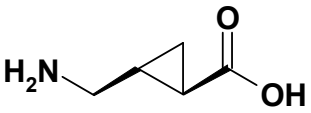
Partial agonists



2.16

CAMP

Agonist



2.17

(+)-CAMP

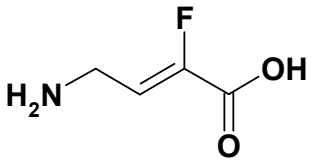
Agonist

2.18

(-)-CAMP

Antagonist

Mary Chebib et. al reported few 2-substituted gaba analogues which are active at the GABA C receptors. In this study, a known series of substituted analogues of GABA and TACA were studied to determine what position(s) of the carbon skeleton substitution is tolerated for the development of active and selective agonists and antagonists for the GABAC receptor. Halo- and methyl substitution on the C_2, C_3, C_4 and nitrogen of TACA, GABA and some cyclic analogues were tested by use of the two-electrode voltage- clamp method with human r_1 mRNA expressed in Xenopus oocytes. Molecular modeling was also used to map areas of steric interaction and to identify what features a molecule may require to interact with the binding site. Only the C_2 position of TACA and GABA was found to tolerate substitution of either a methyl or a halo group at GABAC receptors. Substitution may be tolerated in this position because the substituent is able to lie in the plane of the active site.



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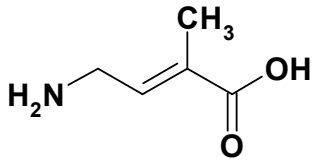

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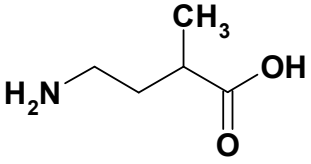

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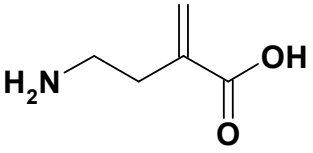

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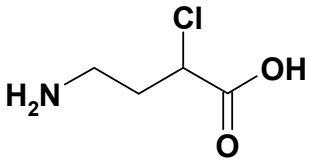

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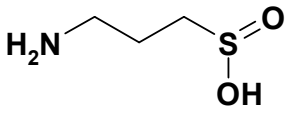


2.24

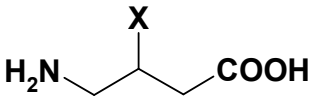
Most compounds used in this study have some effect as agonists at the GABA-A receptors but it was only compounds with substituents on the C_2 position that had some effect on GABA-C receptors. In general, antagonists are much larger than agonists. *trans*-4-amino-2-fluorobut-2-enoic acid **(2.19)** is a potent agonist while compound *trans*-4-amino-2-methylbut-2-enoic acid **(2.20)** is a moderately potent antagonist at GABA-C receptors. Replacement of the hydrogen from the C_2 position of TACA with a fluoro group maintains agonist activity.

Compounds 4-amino-2-methylbutanoic acid **(2.21)**, 4-amino-2-methylenebutanoic acid **(2.22)**, 4-amino-2-chlorobutanoic acid **(2.23)** and homohypotaurine **(2.24)** were weak partial agonists with low intrinsic activity. These compounds are flexible compounds and can adopt many more low energy conformations than either compounds **(2.19)** and **(2.20)**. Replacement of the carboxylic acid group with a sulphonic or sulphinic acid group results in potent agonists at the GABAA receptor. However, replacement of the carboxylic acid group with a phosphinic or methylphosphinic acid group results in a drop of affinity for the $GABA_A$ receptor [87-89].

Silverman et al., reported the synthesis and GABA Transaminase inhibitory activities of 4-amino-2-(substituted methyl)-2-butenoic acids. 4-amino-2-(substituted methyl)-2-butenoic acids, where **X** (the substituted group) = F, C1, OH, are synthesized from Cbz-protected tert-butyl 4-aminobutanoate. Successive substitutions at the α-carbon by phenylseleno and hydroxymethyl groups, followed by elimination of the selenoxide and halide substitution at the hydroxymethyl group, afford the compounds in good yields [90-92].

MODIFICATIONS AT THE THIRD POSITION:

Of the 3-substituted analogues, 3-hydroxy-GABA (GABOB), **2.25a** and 3, 4-diaminobutyric acid **(2.25b)**, the latter is least important being only a weak inhibitor of GABA uptake. GABOB is a potent inhibitor of GABA and muscimol binding of GABA uptake and has been extensively used for its moderate to potent GABA agonist activity on Bicuculine postsynaptic receptors.



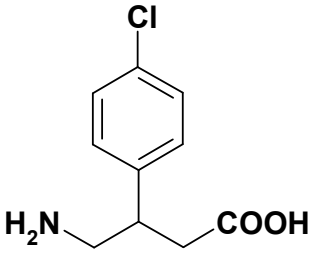
2.25, a: X = OH, d: X = SCH_3 g: X = F

b: X = NH2 e: X = SPh

c: X = Cl f: X = SO_3H

3-Chloro GABA **(2.25c)** is a moderately potent GABA agonist on cray fish stretch receptor neurons, as well as being a potent inhibitor of GABA binding, where as the sulfur substituted derivatives **(2.25d-f**) are considerably less potent and 3-fluoro GABA **(2.25g)** has not been tested [84].

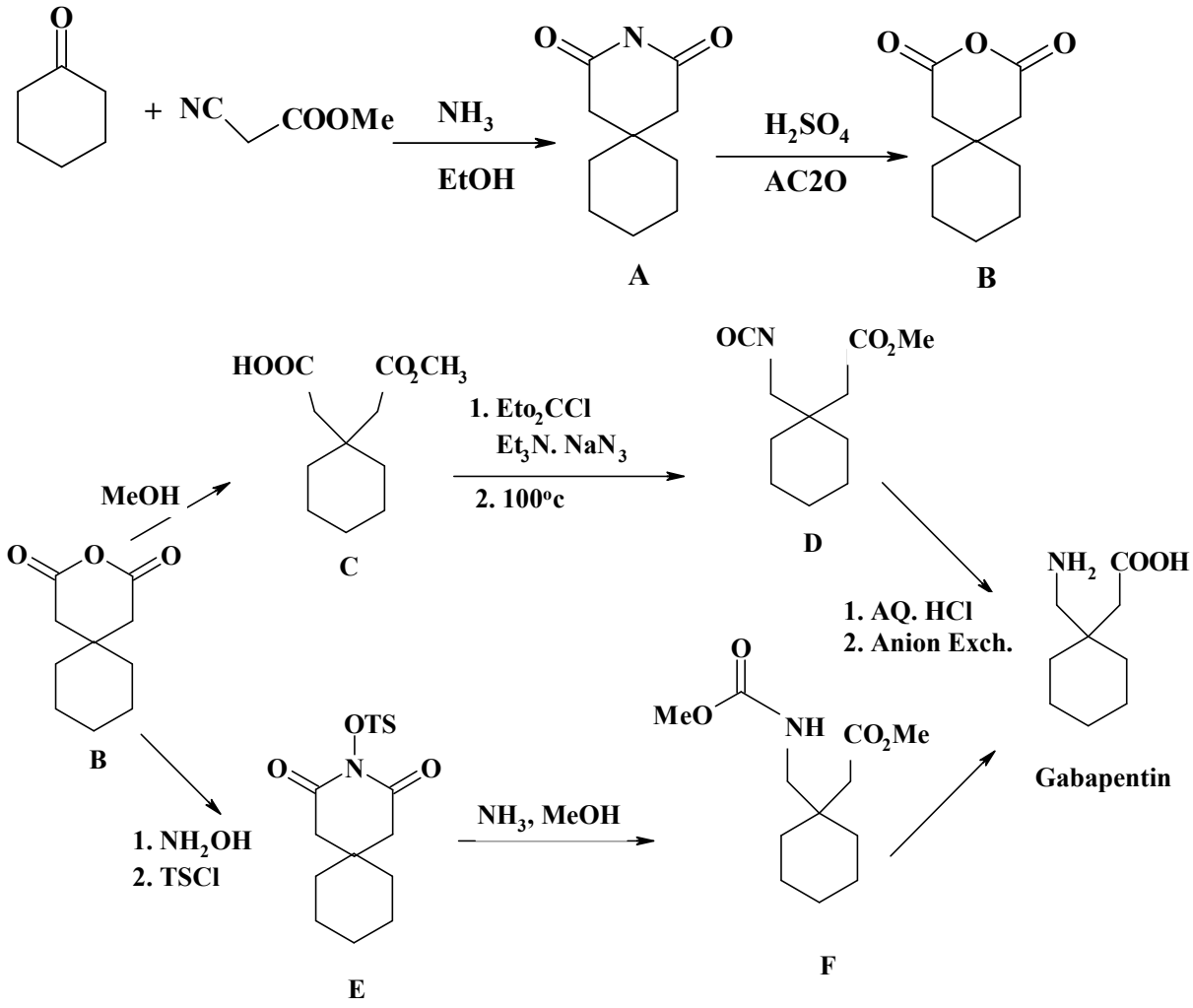
Aromatic substituents of GABA alter physicochemical properties so that derivatives are more likely to cross the BBB. Baclofen **(2.26**) is a centrally acting drug used to alleviate skeletal muscle spasticity of neuronal origin. Its actions are not those expected of a classical GABA agonist, electrophoretically its inhibitory action in CNS neurons being weak and Bicuculine insensitive.



2.26

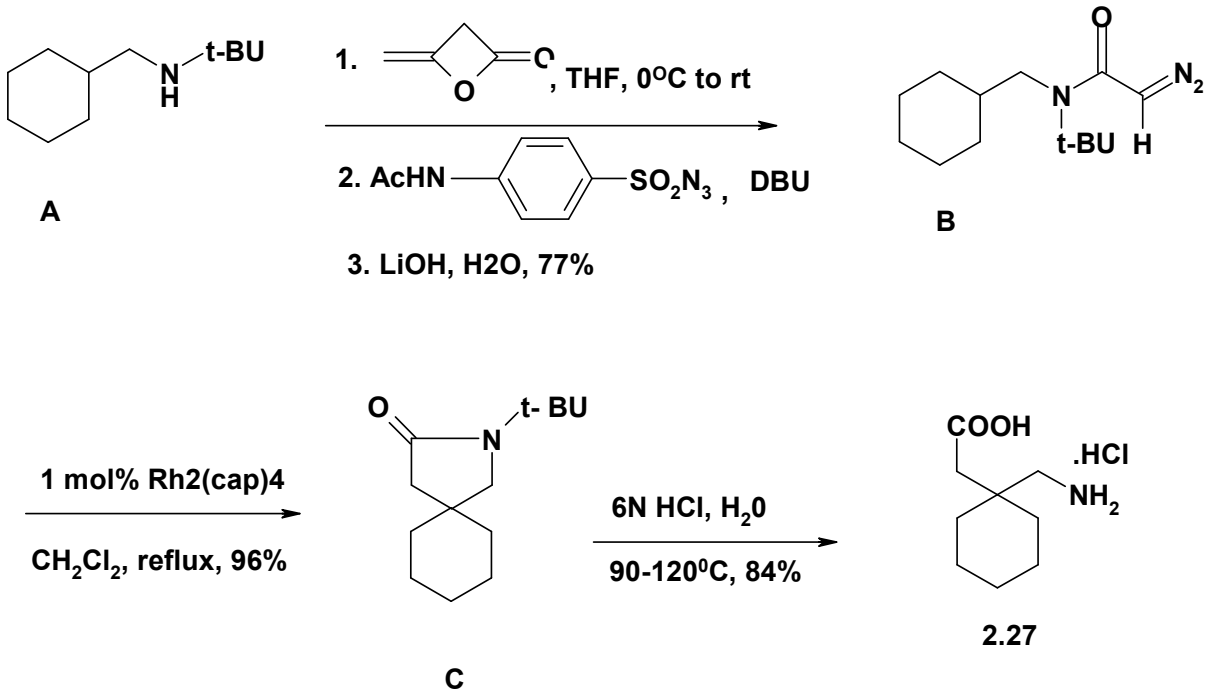
Other 3-aryl GABA analogues, example, 3-chlorophenyl and 4-fluoro phenyl, are less active at peripheral and central Bicuculine insensitive sites while others, example 4-isopropyl phenyl and β-napthyl are inactive.

Gabapentin was designed as a liphophilic GABA analog and was first synthesized as a potential anticonvulsant and was launched in 1994 as add-on therapy for the treatment of Epilepsy. There are several methods documented in the literature for the synthesis of gabapentin. Satzinger, Hartenstein, Herrmann, and Heldt disclosed the original synthesis in a 1976. This route started with the formation of Guareschi salt **A** and its conversion to the spiroanhydride **B**. The anhydride could be converted to the half ester **C** that was transformed to the isocyanate **D** via a Curtius rearrangement. Acidic hydrolysis followed by ionexchange chromatography gave Gabapentin. Alternatively the spiroanhydride **B** could be converted to the tosylamide **E** which is then induced to undergo a Lossen type rearrangement to give carbamate F which was converted to Gabapentin as before [84, 93-96]. **(Scheme - 2.1).**



Scheme - 2.1

In 2003, Hu and co-workers, of the Chengdu Institute of Organic Chemistry, investigated a transition metal catalyzed C–H insertion reaction to prepare gabapentin hydrochloride **(2.27)** (Chen et al., 2003, 2005). The α-diazoacetamide B was prepared by reacting the N-tert-butylamine **A** with diketene followed by 4-acetamidobenzenesulfonyl azide/DBU treatment. The a-diazoacetamide B was then subjected to an intramolecular C–H insertion reaction catalyzed by 1 mol% of Rh2(cap)4 to form N-tert-butyl gabapentin lactam C in good yield. This lactam was then hydrolyzed under refluxing aqueous hydro- chloric acid to give gabapentin hydrochloride [97] **(2.27)**. **(Scheme – 2.2).**

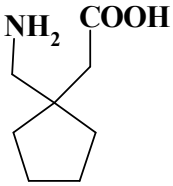


SCHEME – 2.2

S. A. R STUDIES OF GABAPENTIN:

Bryans et. al, Along with Gabapentin, several other 3-cycloalkyl substituted GABA analogues were prepared and evaluated in anticonvulsant paradigms. Compounds with ring sizes ranging from 5-8 carbon atoms were prepared and tested for their ability to control convulsions in rats caused either chemically by the GAD inhibitor thiosemicarbazide (TSCZ) or maximum electric shock results are shown in table Compounds from this series were studied for their ability to displace [^{3}H] gabapentin. Although the 5- and 7- member ring analogs **2.28** and **2.29** are active in anticonvulsant

models, the 6 member ring analog gabapentin prevents convulsions at much lower doses in these assays. Gabapentin is more potent in binding assay than **2.28** and **2.29** compound **2.30** having the three position of the GABA chain incorporated into an 8 membered ring, lacked anticonvulsant activity and was over 10 fold less potent in the binding assay. Placement of the cyclohexyl ring one carbon away from the GABA chain also resulted in a compound apparently devoid of anticonvulsant activity and greatly reduced binding affinity.



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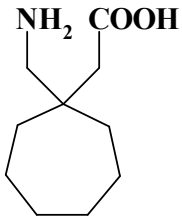

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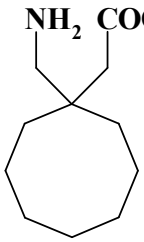

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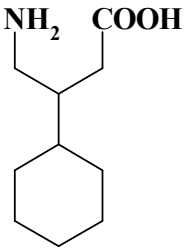
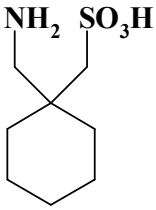


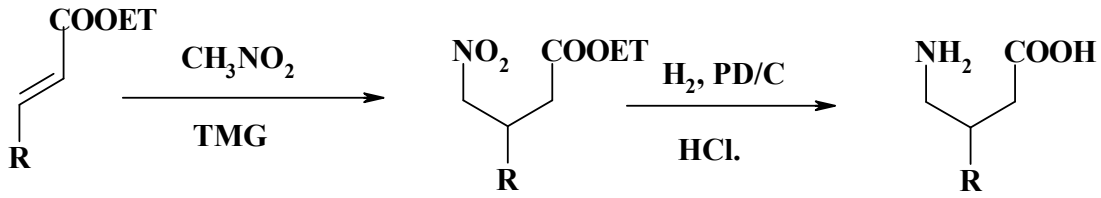
2.31



2.32

Although the 5 and 7 member analogues are active in the anticonvulsant models, the 6 member ring analogue Gabapentin prevents convulsions at much lower doses in these assays. Compound **2.30** having the 3 position of the GABA chain incorporated into an 8 membered ring, lacked anticonvulsant activity and was 10 fold less potent in the binding assay. Placement of the cyclohexyl ring 1 carbon away from the GABA **(2.31)** chain also resulted in a compound apparently devoid of anticonvulsant activity and greatly reduced binding affinity. This SAR suggested a narrow tolerance of size and substitution patterns in the 3 position of GABA chain for optimal anticonvulsant and binding activity. The lack of activity for **2.32**, the sulfonic isostere of Gabapentin indicated a specific requirement at the caboxy terminus of the molecule. Incorporation of methyl groups on to the cyclohexane ring if Gabapentin in various positions depicted for compound led to an improvement in vitro binding relative to Gabapentin [93].

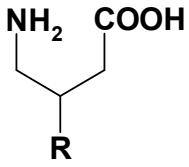
The story of the discovery of pregabalin began in 1991, when Silverman and Taylor published a paper on the anticonvulsant effect of 3-alkyl GABA analogs **2.33** (Silverman et al., 1991). They synthesized a series of analogues which were substituted in the three position **(Scheme - 2.4).**



SCHEME – 2.3

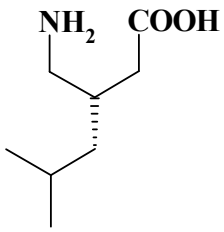
These compounds were studied for their ability to inhibit maximal electroshock seizures in mice. The racemic 3-isobutyl analog **(2.33g)** was shown to be significantly more potent in the assay than any of the other analogs. This analogue was shown to have peak activity 2h after dosing with the anticonvulsant effect greatly decreased by 8 hours. A number of these analogues also activated the enzyme L-glutamic acid decarboxylase (GAD). However **(2.33g)** was not very potent in this assay showing a significant activation of GAD activity only at a concentration of 2.5Mm [93].

Table: 2.1 3–Alkyl GABA Structure activity relationship:



NO	R	Dose mg/kg	Anticonvulsant effect % Protected
2.33a	Methyl	100	60
2.33b	3, 3 Dimethyl	100	50
2.33c	Ethyl	100	100
2.33d	n-propyl	100	30
2.33e	isopropyl	100	60
2.33f	n-butyl	100	20
2.33g	isobutyl	14.4	90
2.33h	Sec-butyl	30	20
2.33i	Ter-butyl	100	50
2.33j	isopentyl	100	0

The individual enantiomers of **(2.33g)** were synthesized utilsing the evans chiral auxiliary, which exemplifies the preparation of the S enantiomer. This strategy produced both enantiomers in extremely high optical purity. The anticonvulsant activity of racemic 3-isobutyl GABA was found to reside in the S enantiomer **(2.34**) called pregabalin. Pregabalin was active in preventing maximal electroshock-induced seizures in mice while its enantiomer **(2.35)** was inactive in this



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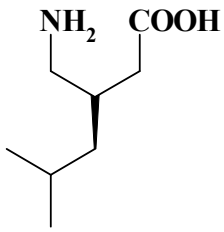


2.35

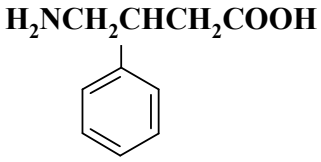
anticonvulsant model. Pregabalin also displaced [3H] gabapentin from partially purified rat neocortex with greater potency than R isomer [98].

Hu and co-workers, at Pfizer, filed a patent application on a process to prepare pregabalin (2) *via* enzymatic resolution (Hu et al., 2005). The Pfizer researchers investigated a large number of commercially available hydrolases to catalyze the hydrolysis of β-cyanodiester **A**, to form (3S)-3-cyano-2-ethoxcarbonyl-5-methylhexanoic acid potassium salt **B** enantioselectively. With this enzyme-screening study, they determined that LIPOLASE 100L, type EX, was the most effective hydrolase for the large-scale preparation of mono-ester **C**. Hydrogenation of nitrile **B** over Raney nickel followed by acid treatment gave the pyrrolidinone-3-carboxylic acid in 97% ee. Acid **D** was then decarboxylated and hydrolyzed with hydrochloric acid to give crystalline pregabalin **(F)** in greater than 99.5% ee**.** The (R)-3-cyano-2-ethoxycarbonyl-5-methylhexanoic acid ethyl ester **(C)** left over from the LIPOLASE-catalyzed hydrolysis reaction can be recycled back to the β-cyanodiester **A** by sodium ethoxide in ethanol treatment, providing a 50% savings in cost of goods over the malonate approach described above [97].

A French group has recently reported some SAR work carried out on 3-alkylated GABA compounds similar to pregabalin. In this study the compounds are evaluated for inhibition of GABA-T and for any activation of glutamic acid decarboxylase (GAB).

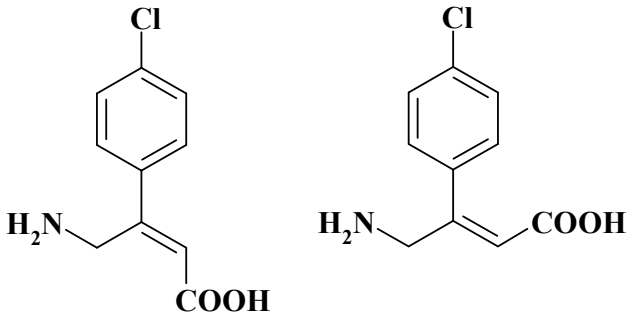
Range of compounds was synthesized based on the general structure, none of the compounds showed any significant effect on GABA-T or GAD [98, 99].

A search for the medicinal drugs among the phenyl derivatives of GABA was carried out in the pharmaceutical division of the Ceiba-Geigy company. γ-amino-β-phenybutyric acid **(2.36)** was synthesized. Intensive synthetic studies carried out by chemists led to the preparation of a several series of GABA derivatives. Among the derivatives obtained capable of permeating through the HEB, γ-amino-β-(*p*-chlorophenyl) butyric acid showed the greatest pharmacological activity [100].



2.36

Robin D. Allan reported the synthesis of Z and E isomers **(2.37)** and **(2.38)** of 4-amino-3-(4-chlorophenyl) but-2-enoic acid as conformationally restricted analogues of Baclofen [100, 101]

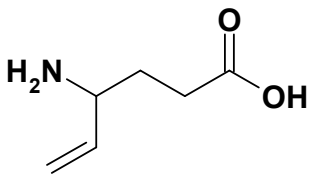


2.37 **2.38**

MODIFICATIONS AT THE FOURTH POSITION:

4-Aminohex-5-enoic acid (vinyl GABA) **2.39** and 4-aminohex-5-ynoic acid (acetylenic GABA) **2.40** have been developed as irreversible GABA-T inhibitors which have been extensively studied as possible therapeutic agents. They enter into the brain after systemic administration and are transformed into highly reactive intermediates such as **2.41** (PYCH=pyridoxal) after condensation with the pyridoxal phosphate co-enzyme of the GABA-T. These intermediates bind to the enzymes in irreversible fashion and thus **2.39 and 2.40** act as suicide inhibitors. A similar formation of an unsaturated reactive

intermediate after enzymatic abstraction of the C4 hydrogen is presumably the mode of action of compounds with fluorine on C4 methyl substituent such as **2.41** and **2.42**.



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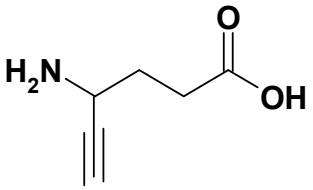
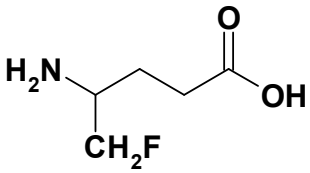
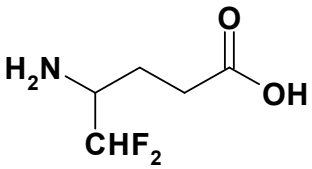


2.40

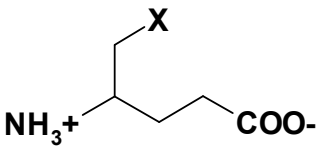


2.41



2.43

Richard B. Silverman reported the syntheses of a new class of γ-aminobutyric acid (γ-Abu) transaminase inactivators. They described the synthesis of a new class of transaminase inhibitors, the 5-substituted 4-aminopentanoic acids **2.44a-e**



2.44a, X = OH

b, X = F

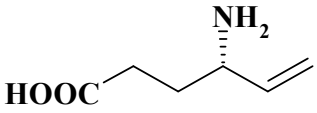
c, X = Cl

d, X = Br

e, X = CN

Vigabatrin which is highly selective enzyme activated inhibitor of GABA-T in mammalian brain crosses the BBB and is used clinically primarily to control seizures refractory to other anticonvulsant drugs. An efficient synthesis of (S) Vigabatrin **(2.45**) has been developed by Zhong-Yong Wei et. al., Advantages of this methodology include high optical and chemical yields, ease of operation, commercially available L-glutamate,

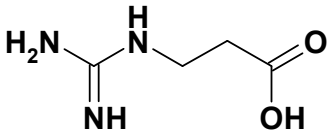
and a short synthetic sequence. In addition this procedure is applicable to the synthesis of other chiral (S)-GABA analogs which may also be useful as GABA-T inhibitors [84, 102-104].



2.45

MODIFICATIONS AT AMINO TERMINUS:

A basic functional group appears to be a prerequisite for substantial activity as a GABA agonist. The replacement of tetrahedral amino group with a planar guanidino group results in **2.46** which has low activity against GABA binding but moderate activity on neuronal uptake. The hydroxyaminogroup **2.47** synthesized appears not to have been tested on any GABA process. Low molecular weight N-acylated GABA analogues are only weakly active *in vitro*. But increasing the lipophilic nature of the molecule gives N-lauroyl-GABA **2.48a** which is only 15 times less active than GABA on receptor binding. Other hydrophobic derivatives, such as N-benzoyl-GABA **2.48b** and N-pivaloyl GABA **2.48c** which penetrate the BBB are hydrolysed to GABA, have anticonvulsant activity in rats. Analogues with a carbon-nitrogen double bond are hydrolysed in aqueous solution and the action of GABA mimetic compounds **2.48** may be due to decomposition to yield GABA [84].



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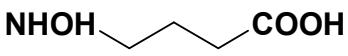
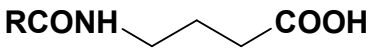


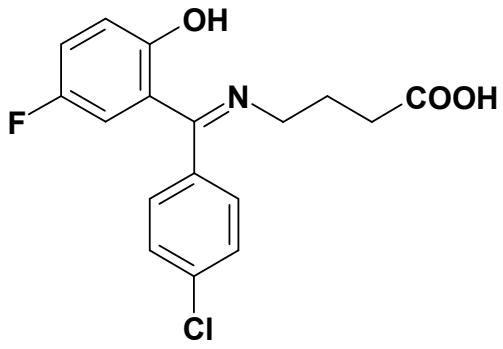
2.47



2.48a, R = C_{11} H_{23}

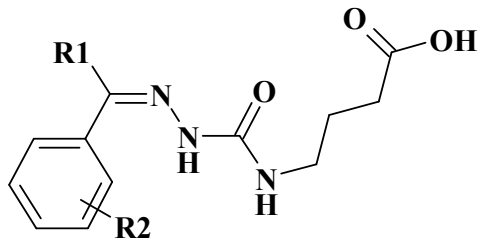
b, R = PH

c, R = $(CH_3)_3$ C

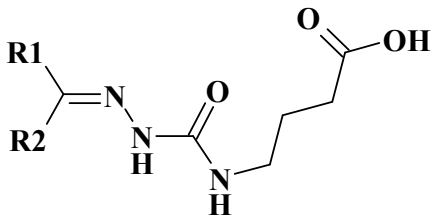


2.49

Perumal Yogeeswari et al., reported the design and synthesis of newer *γ*-aminobutyric acid (GABA) derivatives with the combination of aryl semicarbazone and the GABA pharmacophores in order to develop a multifunctional drug useful in the treatment of neurological disorders like epilepsy and neuropathic pain.



2.50 - 2.61



2.62 - 2.64

Table: 2.2 GABA derivatives with the combination of aryl semicarbazone and the GABA pharmacophores

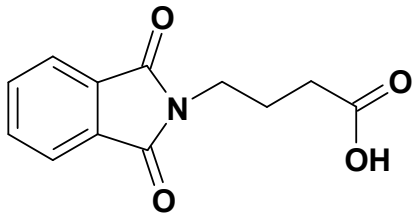
No	R1	R2
2.50	H	2-OH
2.51	H	4-NO_2
2.52	H	4-Cl
2.53	H	3-NO_2
2.54	H	4-$N(CH_3)_2$
2.55	CH_3	H

2.56	**CH_3**	**4-CH_3**
2.57	**CH_3**	**3-NH_2**
2.58	**CH_3**	**4-NO_2**
2.59	**C_6H_5**	**H**
2.60	**C_6H_5**	**4-Br**
2.61	**CH_2-C_6H_5**	**CH_2-C_6H_5**
2.62	**Cyclohexylene**	-
2.63	**Cyclopentylene**	-
2.64	**Isatinyl**	

The synthesized compounds **(1-15)** were evaluated at dose levels of 30, 100, 300mg/kg intraperitoneally for mice for anticonvulsant activity by the standard anticonvulsant drug development program protocols. Five compounds (8, 10, 11, 14, and 15) showed activity in the MES screen indicative of their ability to prevent seizure spread. Compounds **8** and **15** exhibited longer duration of action (activity until 4h interval)

Six compounds **(1, 3, 6 and 10-12)** showed protection in the scStrychnine induced seizure model. Compound 1 was the most effective in this model exhibiting protection at 30mg/kg for a longer duration [105].

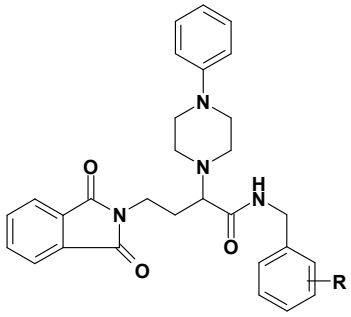
Bhowmick et al., reported a new γ-amino butyric acid derivative, N-Phthaloyl GABA **(2.65)** was synthesized and anticonvulsant activity was tested and compared with sodium valproate for efficacy against experimentally induced convulsions in mice. At a dose of 80mg/ kg, P-GABA rendered more protection than sodium valproate [106].



2.65

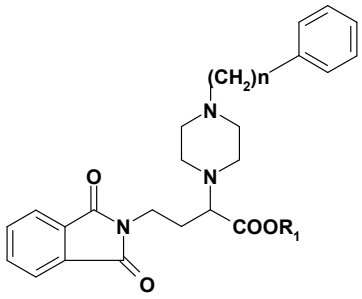
MODIFICATIONS AT COOH TERMINUS AND NH_2 TERMINUS:

In search for new anticonvulsant compounds Malwaska and coworkers synthesized several series of GABA derivatives and they were evaluated for anticonvulsant activity. In the year 1995 they reported the synthesis and anticonvulsant activities of new derivatives of α-substituted γ-phthalimidobutyric acid. The synthesis of N-substituted amides **(2.66)** and esters **(2.67**) of α-(4-phenylpiperazine)-γ-phthalimidobutyric acid was described.



2.66

NO	2.66a	2.66b	2.66c	2.66d	2.66e
R	-H	-Cl	$-CH_3$	$-OCH_3$	-F



2.67

NO	2.67a	2.67b	2.67c
R1	-C2H5	-C2H5	C
X	N n=0	-CH n=1	N n=0

These compounds were evaluated for anticonvulsant activity. Three N-substituted amides (2.66a, 2.66b and 2.66c) of the acid displayed protection against MES and scMet induced seizures. Instead esters of this acid were devoid of anticonvulsant activity.

In the year 1999 they reported synthesis and anticonvulsant activities of N-substituted amides of 2-(4-phenylpiperazino)-4-phthalimidobutyric acid and N-substituted amides of 2-(4 phenylpiperazino)-GABA.

N-substituted amides of 2-(4-phenylpiperazino)-4-phthalimidobutyric acid were prepared by the condensation of acid the corresponding derivatives of the benzylamine in the presence of different coupling agents (2-chloro-4, 6-dimethoxy-1, and 3, 5-triazine (CDMT) and Carbonyldi imidazole (CDI).

NO	**2.67d**	**2.67e**	**2.67f, 2.67i**	**2.67g, 2.67j**	**2.67h, 2.67k**
R	**2-Cl**	**3, 4 (OCH_3)**	**H**	**4-OCH_3**	**4-F**

Anticonvulsant activities were determined in mice and in rats using scMET and MES screens. The amides **(2.67i-2.67k)** showed protection against scMET and MES seizures in mice. N-(4-Methoxybenzyl)-2-(4-phenylpiperazin-1-yl)-4-aminobutyric amide was the most effective and displayed anticonvulsant activity in at doses of 100-300mg/kg in mice and at 30mg/kg in the MES screen in rats. The active compounds were tested for their ability to displace [^{3}H] nitrendipine binding sites (voltage-sensitive calcium channel receptors) from rat cortex. Amide **2.67j** was the most active in pharmacological and biochemical tests.

In the same year they reported the synthesis of two series of n-benzylamides of α-(benzylamino)-γ-hydroxybutyric acid (series A) and α-(2-phenylethylamino-γ-hydroxybutyric acid (series B).

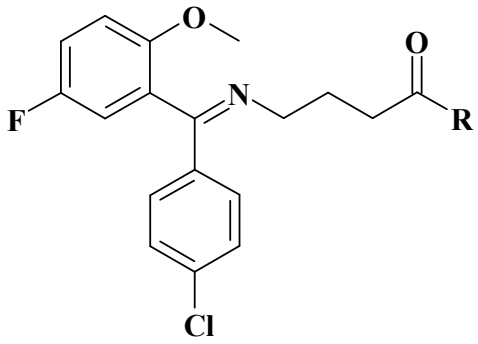
R	**H**	**2-Cl**	**4-Cl**	**4-F**	**4-CH_3**	**4-OCH_3**	**3, 4(OCH_3)2**
Series A n=1	**2.68a**	**2.68b**	**2.68c**	**2.68d**	**2.68e**	**2.68f**	**2.68g**
Series B n=2	**2.69a**	**2.69b**	**2.69c**	**2.69d**	**2.69e**	**2.69f**	**2.69g**

All the compounds were investigated in MES, scMET and rotarod toxicity assays. All the screened compounds except for compound 2.68g, had anticonvulsant properties, and showed protection against MES seizures in mice. Compounds 2.68a, 2.68e and 2.68f were active at 100mg/kg after 0.25hr while compounds 2.68a-2.68c and 2.69d were active after 0.5hr at the same dose. The other amides were less active. At the anticonvulsant dose of 100mg/kg no neurotoxicity was noted with any of the compound.

The most effective in the MES screen in rats at 30mg per kg were compounds 2.68e and 2.68f. Amides 2.68b, 2.68c, 2.68e, 2.69d and 12.69g displayed weak activity.

The N-benzyl amides 3-9 were also evaluated for their ability to displace [3H]-nitrendipine from voltage-sensitive calcium channel (VSCC) receptors isolated from rat cortex. Binding studies showed that all investigated amides possessed affinity for voltage-sensitive calcium channel receptors with Ki values of 3.5-37.8μM [107-108].

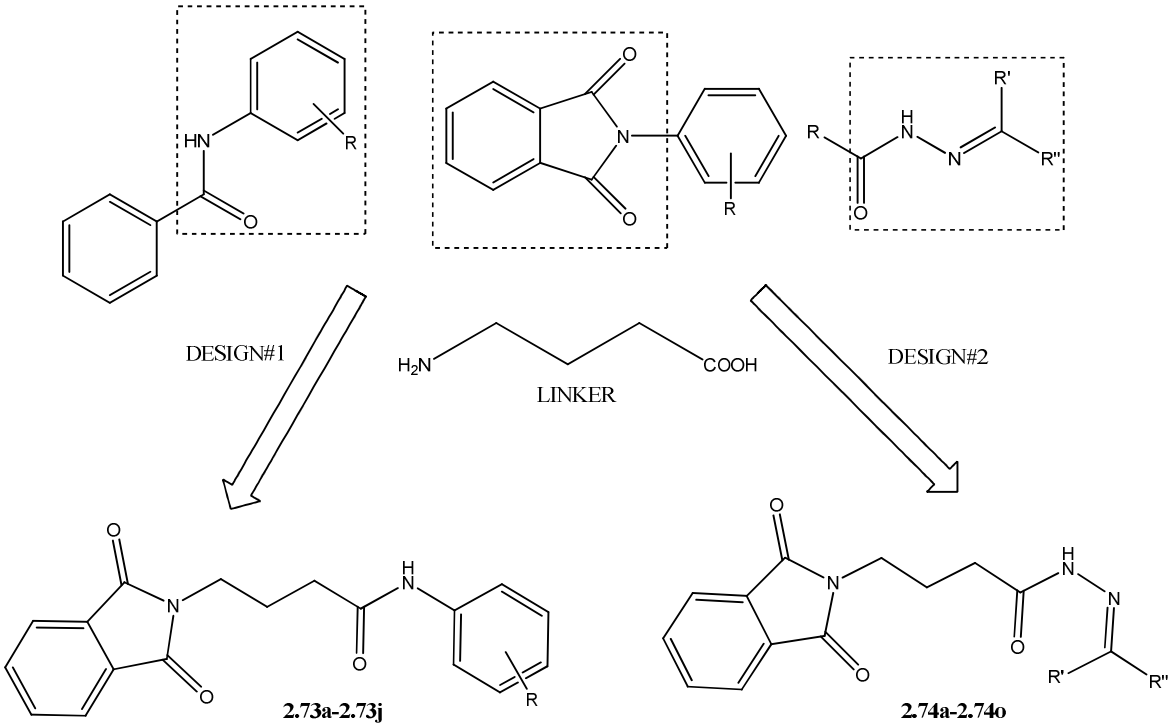
Jean-pierre Kaplan et al., reported the synthesis and anticonvulsant activity of Schiff bases (**2.70-2.72)** of a 4-aminobutyric acid and 4-aminobutyramide [109].



2.70, R = OH
2.71, R = ONa
2.72, R = NH_2

J. V. Ragavendran et al., designed and synthesised anticonvulsants from a combined phthalimide-GABA-anilide and hydrazone pharmacophore.

This study reports the synthesis of pharmacophoric combinations of phthalimide-GABA-anilide/hydrazones as candidate anticonvulsants.



Anticonvulsant Compounds designed by pharmacophore combination

N-aryl substituted 4-(1, 3-dioxo-1, 3-dihydro-2H-isoindol-2-yl) butanamides **(2.73a-2.73j)**

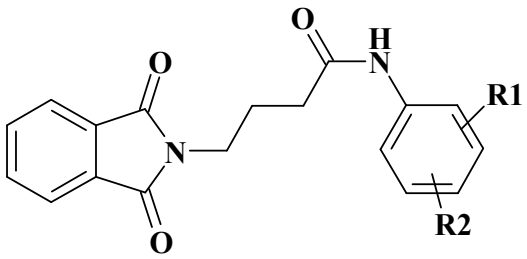
were obtained by reaction of N-protected GABA with different substituted anilines.The N-aryl/alkylidene-4-(1,3-dioxo-1,3-dihydro-2H-iso- indol-2-yl)butanoylhydrazones were obtained via reaction of N-protected GABA with hydrazine hydrate after activation of GABA with N,N0 -dicyclohexyl carbodiimide (DCC), and subsequently with variously substituted aldehydes and ketones to give **(2.74a-2.74o)**.

All the compounds were evaluated for anticonvulsant and neurotoxic properties. Anticonvulsant screening was performed using MES, scPTZ, scSTY and ipPIC seizure

threshold tests. Most of the compounds were found to be effective in the scSTY and ipPIC models and very few compounds showed protection in the scPTZ model.

Of the two series of pharmacophoric hybrids, the phthalimide-GABA-anilides were found to be more effective than the corresponding phthalimide-GABA-hydrazone derivatives [110].

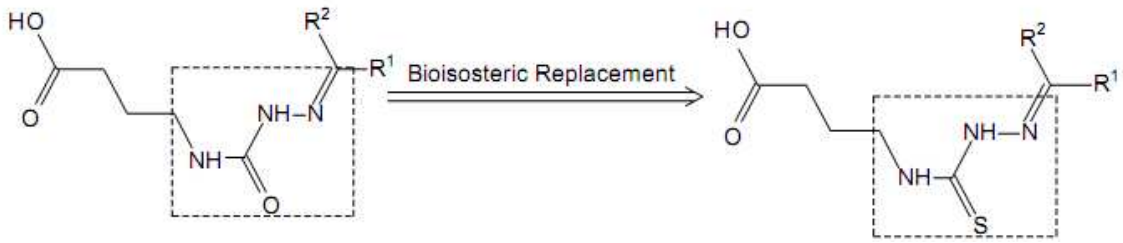
Perumal Yogeeswari et al., reported Newer N-Phthaloyl GABA Derivatives with antiallodynic and antihyperalgesic activities in both Sciatic Nerve and Spinal Nerve Ligation Models of Neuropathic Pain.



2.75 General Structures of compounds

This study was undertaken to assess the peripheral analgesic, antiallodynic and antihyperalgesic activities of the synthesized structural analogues of GABA. The screening study included acute tissue injury, chronic constriction injury (CCI), and spinal nerve ligation (SNL) models of neuropathic pain. All of the tested compounds suppressed the acetic acid-induced writhing response significantly in comparison to the control. In this study, they have demonstrated that combining phthalimide pharmacophore with GABA has evolved compounds effective for the treatment of neuropathic pain [111].

J. V. Ragavendran et al. disclosed Newer GABA derivatives for the treatment of epilepsy including febrile seizures. This was the first report on these new GABA derivatives in the treatment of febrile seizures.



Anticonvulsant compounds designed by bioisosteric replacement

Table: 2.3 Bioisosteric analogues of GABA semicarbazones.

Comp.	R1	R2
2.76	**H**	**2-OH**
2.77	**H**	**4-NO_2**
2.78	**H**	**4-Cl**
2.79	**H**	**3-NO_2**
2.80	**H**	**4-$N(CH_3)_2$**
2.81	**CH_3**	**H**
2.82	**CH_3**	**4-CH_3**
2.83	**CH_3**	**3-NH_2**
2.84	**CH_3**	**4-NO_2**
2.85	**C_6H_5**	**H**
2.86	**C_6H_5**	**4-Br**
2.87	**CH_2-C_6H_5**	**CH_2-C_6H_5**
2.88	**Cyclohexylene**	
2.89	**Cyclopentylene**	
2.90	**Isatinyl**	

The reported compounds were designed as bioisosteric analogues of GABA semicarbazones. Initial anticonvulsant screening was performed using intraperitoneal (i.p.) maximal electroshock-induced seizure (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine (scSTY), and subcutaneous picrotoxin (scPIC)-induced seizure threshold tests. A model involving 22-day old rat pups was also employed to further screen the effects of the test compounds against hyperthermia-induced febrile seizures. Only two compounds were found to be active in the MES test. Most of the compounds were found to be effective in the scPIC and febrile seizure models and very few compounds showed protection in scPTZ and scSTY models. These thiosemicarbazono derivatives of GABA were found to exhibit anticonvulsant activity in various animal models of seizure including the febrile seizure model. Overall, the synthesized compounds emerged as more active and less neurotoxic when compared to their semicarbazono counterparts [112].

Yogeeswari et al., reported series of pharmacophoric hybrids of ameltolide-γ-aminobutyric acid (GABA)-amides.

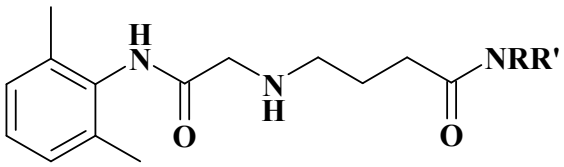
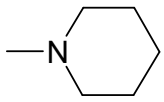
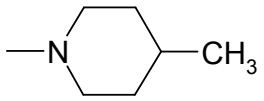
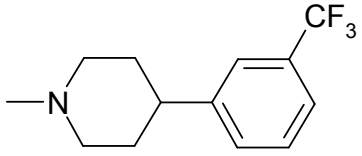


Table: 2.4 Pharmacophoric hybrids of ameltolide-γ-aminobutyric acid (GABA)-amides.

Comp	NRR’
2.91	**—**
2.92	**–NHPh**
2.93	**–NH(2-BrPh)**
2.94	**–NH(3-CH_3Ph)**
2.95	**–NH(3-Cl-2-CH_3Ph)**
2.96	**–NH(2,4-dimethylPh)**
2.97	**–NH(2,6-dimethylPh)**
2.98	**–N(CH_3)$_2$**
2.99	**–N(n-Bu)$_2$**
3.0	**–N(Ph)$_2$**
3.01	
3.02	
3.03	

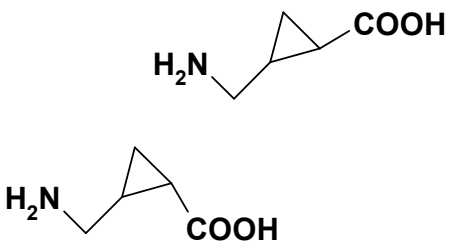
These compounds were designed, synthesized, and evaluated for their anticonvulsant and neurotoxic properties. Initial anticonvulsant screening was performed using

intraperitoneal(ip) maximal electroshock-induced seizure (MES), subcutaneous pentylenetetrazole (scPTZ), and subcutaneous picrotoxin (scPIC)-induced seizure threshold tests. All the compounds had improved lipophilicity and the pharmacological activity profile confirmed their blood–brain barrier penetration. These compounds showed promising activity in scPIC screen indicating the involvement of GABA-mediation. Compound **2.98** (4-(2-(2, 6-dimethylaminophenylamino)-2-oxoethylamino)-N-(2,6-dimethylphenyl) butanamide) emerged as the most potent derivative effective in all the three animal models of seizure with no neurotoxicity at the anticonvulsant dose. The pharmacological activity profile confirmed their blood–brain barrier penetration. Thus, these newer derivatives would be beneficial for a wide range of seizures and furthermore this logical thinking can be extended in combining other anticonvulsant pharmacophores in the future [113].

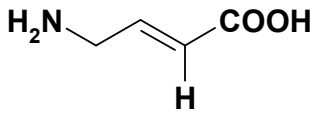
CONFORMATIONAL ANALOGUES OF GABA:

Restriction of conformation by linking C_2-C_3:

The use of a cyclopropane ring as a restriciting group has the advantage of low conformational flexibility as well as a minimal increase in steric bulk of the molecule. The incorporation of a methyl group as in the trans cyclopropane derivative **(3.04)** leads to a potent GABA agonist at bicuculine sensitive receptors, but which is considerably less active than the transcrotonic acid **(3.05**) against GABA uptake. The corresponding ciscyclopropane **(3.06)** derivative is considerably weaker, but antagonism by bicuculine could not be readily demonstrated when a cyclobutane carbocyclic ring was used as in **(3.07)**, activity was drastically reduced.

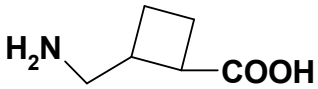


3.05



3.06

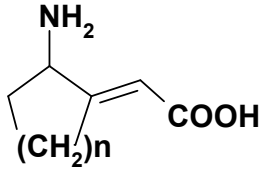
3.07



3.08

Restriction of conformation by linking C_3-C_4

The low activity of **3.09a** and **3.09b** on GABA systems has been interpreted in terms of steric hindrance of the confirmation restricting ring at the relevant active sites. The related aromatic derivative to- aminophenyl acetic acid is also inactive.

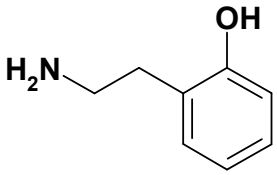


3.09a, n=1,

3.09b, n=2

Restriction of conformation by linking C_1-C_2

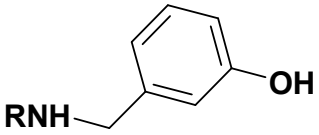
These analogues involve the replacement of the carboxyl group with a phenol as in the inactive **3.10.**



3.10

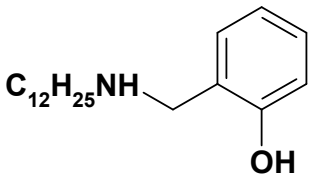
Restriction of conformation by linking C_1-C_3:

The unsubstituted phenolic analogue **(3.11)** proved to be almost inactive at inhibiting GABA binding to human cerebellar synaptic membranes.however, introduction of a lipophilic N-alkyl chain as in **(3.12**) conferred weak receptor binding activity on it and similar diphenols.the different separations of polar groups in receptor and uptake processes is exemplified by the potency of the related phenol **(3.13**).



3.11 R = H

3.12 R = $C_{12}H_{25}$

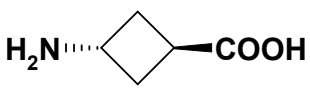


3.13

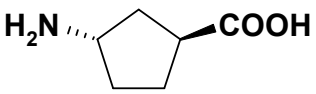
Restriction of conformation by linking C_2-C_4:

Of the two isomeric cyclobutane analogs **(3.14)** the *cis* isomer displayed moderate activity with respect to GABA uptake,binding, substrate activity for GABA-T, and neuronal depressant activity in vivo the lesser effect of the trans isomer has been interpreted in terms of the particular conformational restraints and steric factors in the one, three-substituted cyclo butane ring.

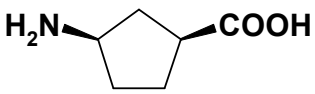
The cyclopentane analogs **3.15 and 3.16** interact potently with GABA systems. Investigations using racemic mixtures have shown that the Trans isomer **(3.15)** is more potent on GABA binding and bicuculline sensitive receptors, where as both isomers have a similar potency on GABA uptake. Interpretation of the result in terms of conformational requirement of binding sites and uptake sites has been clarified using resolved isomers. The (+) - *trans* – 1S,3S, isomer **(3.15)** is a potent and selective bicuculline sensitive receptor agonist between 5 and 25 times more potent than GABA on cat spinal neurons and also more potent than GABA on immature rat spinal cord preparations. Only muscimol and dihydromuscimol are more potent at inhibiting GABA binding. With respect to uptake the (+)-*cis*-1S, 3R **(3.16)** and (-)-*trans*-1R, 3R Stereoisomers were potent inhibitors being approximately twice as potent as GABA [29, 84].



3.14



3.15



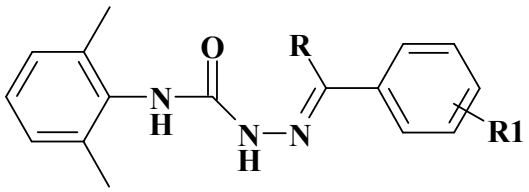
3.16

GABA TRANSAMINASE INACTIVATORS:

GABA transaminase is a mitochondrial enzyme, occurring in both neurons and glia, so that inactivation of the enzyme leads to an increased intracellular concentration of GABA, but the pharmacological actions must be presumed to be due to extracellular neurotransmitter interacting with receptors located on the outer surface of cell membranes. Hence, the increased content of intracellular GABA must be released to the exterior of the cell to produce the known antiepileptic activity [114].

Richard B. Silverman reported the syntheses of a new class of γ-aminobutyric acid (γ-Abu) transaminase inactivators [90].

Yogeeswari et al., discovered N-(2, 6-Dimethylphenyl)-Substituted Semicarbazones as Anticonvulsants by Hybrid Pharmacophore-Based Design, these compounds exhibited anticonvulsant activity and six compounds (3.07-3.12) showed 88% GABA-T enzyme inhibition *invitro* [115].



Comp	R	R1
3.17	**H**	**4-CH_3**
3.18	**H**	**4-$N(CH_3)_2$**
3.19	**H**	**4-OH, 3-OCH_3**
3.20	**H**	**2-OH**
3.21	**H**	**2-NO_2**
3.22	**CH_3**	**4-OH**

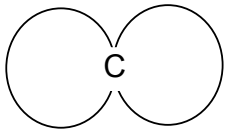
Several 2, 4-dimethylphenyl substituted semicarbazones were synthesized and these compounds were evaluated for anticonvulsant activity by using a series of test models, including maximal electroshock seizure, subcutaneous pentylenetetrazole and subcutaneous strychnine seizure threshold tests. Preliminary studies suggest that these compounds exhibit anticonvulsant activity via a GABA-mediated mechanism. Potent

compounds were evaluated for their effects on rat brain aminobutyric acid (GABA) levels and in vitro amino butyrate transaminase (Pseudomonas fluorescens) activity; two compounds **(3.23 & 3.24)** showed 90% inhibition [116].

Comp	R	R1
3.23	-	-
3.24	CH_3	C_2H_5

CHAPTER 2.2
BIOLOGICAL ACTIVITIES OF SPIRO COMPOUNDS

In 1900, Bayer created the first spiran described as a bicyclic hydrocarbon connected by a single carbon. The term spirocyclanes was used to describe the family of such hydrocarbon. Due to the tetrahedral nature of the spiro linked carbon, the ring planes are nearly perpendicular to each other.



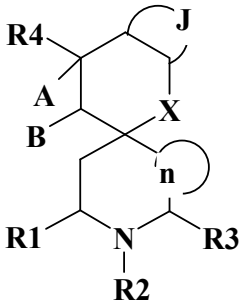
Spiro compounds having cyclic structures fused at a central carbon are of recent interest due to their interesting conformational features and their structural implications on biological systems. The presence of the sterically constrained spiro structure in various natural products also adds to the interest in the investigation of spiro compounds. Spiro compounds represent an important class of naturally occurring substances characterised by their highly pronounced biological properties [74, 77].

Spiro compounds can serve as privileged structures; Privileged structures are molecular scaffolds with versatile binding properties, such that a single scaffold is able to provide potent and selective ligands for a range of different biological targets through modification of functional groups. In addition, privileged structures typically exhibit good drug-like properties, which in turn lead to more drug-like compound libraries and leads. The net result is the production of high quality leads that provide a solid foundation for further development. Over the past 15 years the privileged structure concept has emerged as a fruitful approach to the discovery of novel biologically active molecules, since then, an increasing number of substructural frameworks have been described as privileged structures [77, 117-120].

Spiro derivatives find very wide application as antimicrobial, anticancer, antibiotic and as agents for C.N.S [121-124].

Dolle et al., in their patent US 7338962 B2 described the invention relating to Spirocyclic heterocyclic derivatives **(3.1)** (including derivatives of spiro (2H-1-benzopyran-2, 4'-

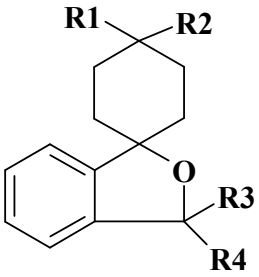
piperidines), pharmaceutical compositions containing these compounds and methods for their pharmaceutical use. The Spirocyclic heterocyclic derivatives are ligands of the δ opioid receptor and are useful for treating and/or preventing pain, anxiety, gastrointestinal disorders and other δ opioid receptor mediated conditions [125].



(3.1)

Where R^1 and R^3 are independently H, alkyl, alkenyl, alkynyl or aryl etc, R^2 is H, alkyl, alkenyl or aryl etc. R^1 and R^3 taken together form a 4-8 membered heterocyclic ring. N is integer 0, 1, 2 or 3. A and B are independently H, Fluoro or akyl or together form a double bond between two carbons. J forms 6 membered aryl or 5-6 membered heteroaryl ring.

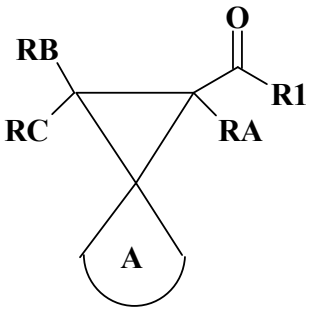
3'-Phenylspiro [cyclohexane-1, 1' (3'H)-isobenzofuran] s **(3.2)** are useful as antidepressants, tranquillizers and anticonvulsants.



(3.2)

Where R1 is hydrogen, R2 is NR5 & R6 (R5 is hydrogen or lower alkyl, R6 is hydrogen or lower alkyl), R3 is hydrogen, lower alkyl or cyclohexyl, R4 is hydrogen, lower alkyl or hydroxyl [126].

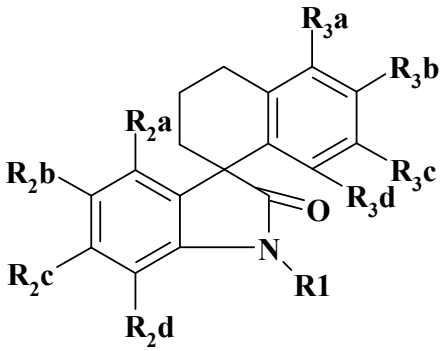
US2004/0077616 (Bennani et al.) described spirocyclopropylamides **(3.3)**, useful for treating epilepsy, bipolar disorder, psychiatric disorders, migraine, pain or movement disorders and also provide neuroprotection [127].



(3.3)

Where A is cycloalkyl or bicycloalkyl, R_A, R_B, R_C are independently Hydrogen or alkyl or aryl R_1 is OR_2 or NR_3R_4

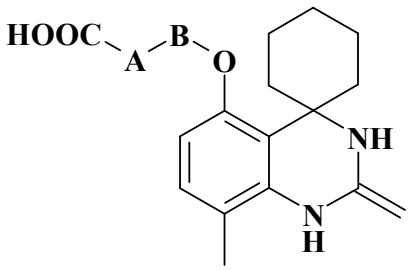
US 2006/0252812 by Chafeev et al. discloses Spiro-oxindole compounds**(3.4)**, useful in treating sodium channel mediated diseases or conditions, such as pain, as well as other diseases and conditions associated with the mediation of sodium channels [128].



(3.4)

Where in R_1, R_2a, R_2b, R_2c, R_2d, R_3a, R_3b, R_3c, R_3d are defined here in as a stereoisomer, enantiomer, tautomer or a mixture.

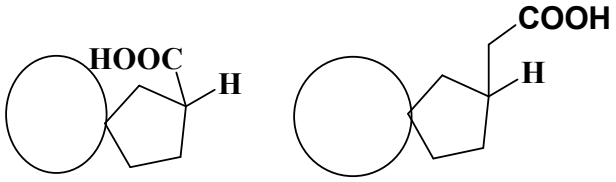
Rawson et al. in their patent US 2007/0129388 A1 described spirocyclic derivatives **(3.5)**, useful as PDE7 inhibitors and have a number of therapeutic applications, particularly in the treatment of pain, especially neuropathic pain [129].



(3.5)

Several analogues of novel anxiolytic buspirone were found to have tranquillising activity. The anxiolytic effects of buspirone are known to dependent on two distinct substructural pharmacophores with in the molecular frame work. The azaspirodecanedione imide and the aryl piperazine moiety found in its pyrimidinyl piperazine fragment [84].

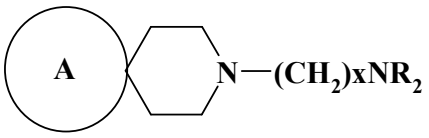
Carbocyclic spiranes **(3.6)** were found to have good anticonvulsant activity, they are found to resisit metabolic alteration. The efficacy of these compounds is comparable to valproic acid when tested for PTZ and picrotoxin induced seizures. Mode of action is due to intact COOH group [121].



Series a: cyclohexanone, **(3.6)**

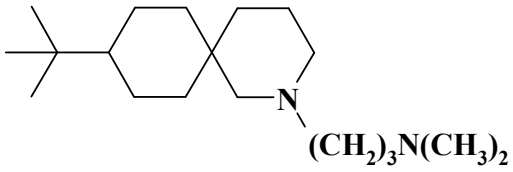
Series b: cycloheptanone.

Azaspiranes represented by general formula **(3.6a & 3.6b)** where ring A is attached to the 4^{th} position of piperidine ring or 3^{rd} position of pyrrolidine rinmg.(In all cases Nitrogen is removed by atleast one carbon atom from the spirocarbon) with in this group of compounds structural variations in the n-dialkyl aminoalkylsubstituent azaspirane nuclei and nuclear substituents were made. These compounds several have shown marked effects on growth of cancer cells invitro interested compound with good antineoplastic activity is n-dimethylaminopropyl-9-t-butyl-3-azaspiro [5, 5] undecane **(3.7)** [130].



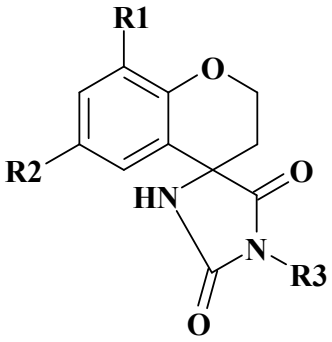
(3.31a)

(3.31b)

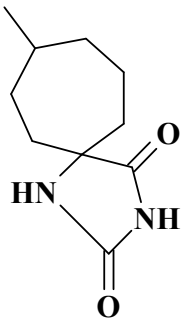


(3.7)

Several spirohydantoins **(3.8a & 3.8b)** were found to have good anticonvulsant activity [131].



(3.8a)



(3.8b)

Chapter-3
Objective and Plan of Work

CHAPTER: 3

OBJECTIVE AND PLAN OF WORK

The sucessful use of GABA and its derivatives in the treatment of various neurologic and psychopathic states led to the possibility of synthesising new synthetic drugs with selective action and with a low toxicity by virtue of their similarity to the metabolic products of the brain.

The widespread distribution of $GABA_B$ to the posssibility of synthesising receptors in both the CNS and the periphery is a clear clue of their physiological and physiopathological importance. In general, presynaptic $GABA_B$ receptors modulate synaptic transmission by depressing neurotransmitter release, including that of GABA itself, through autoreceptors, while postsynaptic $GABA_B$ receptors contribute to the inhibitory control of overall neuronal excitability. Thus, $GABA_B$ receptors play a critical role in fine-tuning the CNS synaptic transmission and are attractive targets for the treatment of epilepsy, anxiety, depression, cognitive deficits, sclerosis and nociceptive disorders.

Indeed, several potential therapeutic applications are associated with pharmacological control of GABA-B receptor. GABA-B agonists, in particular, may be employed as antispastic agents, in respiratory diseases such as asthma, in the pharmacological control of cocaine addiction, in migraine and in pain. On the other hand, GABA-B antagonists, when employed at doses that do not induce convulsion, increase neutrophin expression in the CNS and in the spinal cord and, thus may have therapeutic relevance in neurodegenerative diseases. As a result, the development of GABA-B receptor agonists and antagonists is of great therapeutic interest [29-32].

3.1: Rationale for choosing spiro compounds:

The special structural characters of Spiro compounds are of biological interest. Spiro compounds can serve as privileged structures; a privileged structure may be defined as "a single molecular framework able to provide ligands for diverse receptors." It was envisaged, that the privileged structures could be a valuable alternative in the search for

new receptor ligands by suitably decorating these substructures. Since then, an increasing number of sub structural frameworks have been described as privileged structures, including indoles, aryl piperazines, spiro phenylpiperidines, biphenyls, benzopyranes and 1, 4-dihydropyridines. These privileged structures have since then, deliberate or not, been used extensively in medicinal chemistry programs to identify new ligands.

3.2: Objectives: -

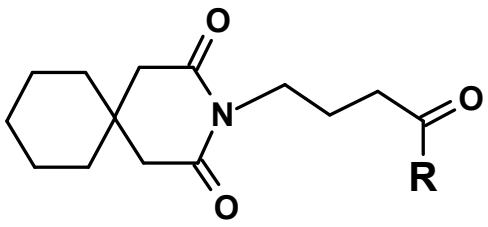
- To design and synthesize novel N-spiro analogues of 4-aminobutyric acid (GABA) with increased lipophilicity in order to treat epilepsy.
- To assess the anticonvulsant potential of the synthesized compound**s.**

3.2: Plan of work

The plan of work is briefly outlined as follows,

I. DESIGN AND SYNTHESIS

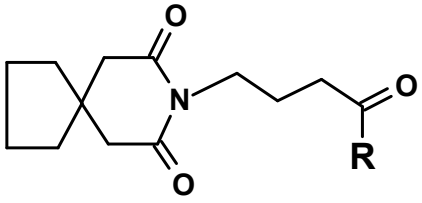
A) 4-(2, 4-DIOXO-3-AZASPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS:



R= Substituted phenyl.

SH (1-16)

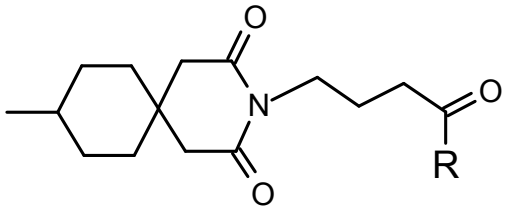
B) 4-(7, 9-DIOXO-8-AZA SPIRO[4.5]DECAN-8-YL)BUTANOIC ACIDS:



R= Substituted phenyl.

CP (1-16)

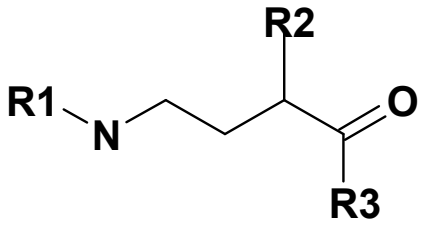
C) 4-(9-METHYL-2,4-DIOXO-3-AZASPIRO[5.5]UNDECAN-3-YL)BUTANOIC ACIDS.



R= Substituted phenyl.

MC (1-16)

D) Miscellaneous GABA derivatives:



R1= Phthaloyl or spiro, R2= Bromine or Hydrogen, R3 = OCH3 or OC2H5

BS (1-10)

II) CHARACTERISATION:

The synthesized compounds will be physically and chemically characterized. The homogeneity of the compounds will be assessed by thin layer chromatography and melting point determinations. Various instrumental techniques such as UV, IR, MASS and ^{1}H-NMR will be used to confirm the structure of the compounds. Elemental analyses will also be performed.

III) PHARMACOLOGY

The profile of anticonvulsant activity of the synthesized compounds was established using one electrically induced and three chemically induced seizure tests. Neurotoxicity was determined using rotarod test.

1. Anticonvulsant evaluation

1.1. Maximal electro-shock seizure test (MES)

1.2. Subcutaneous pentylenetetrazole seizure threshold test (scPTZ)

1.3. Subcutaneous strychnine-induced seizure threshold test (scSTY)

1.4. Subcutaneous picrotoxin-induced seizure threshold test (scPIC) and

2. Neurotoxicity (Rotarod assay)

Chapter-4
Materials and Methods

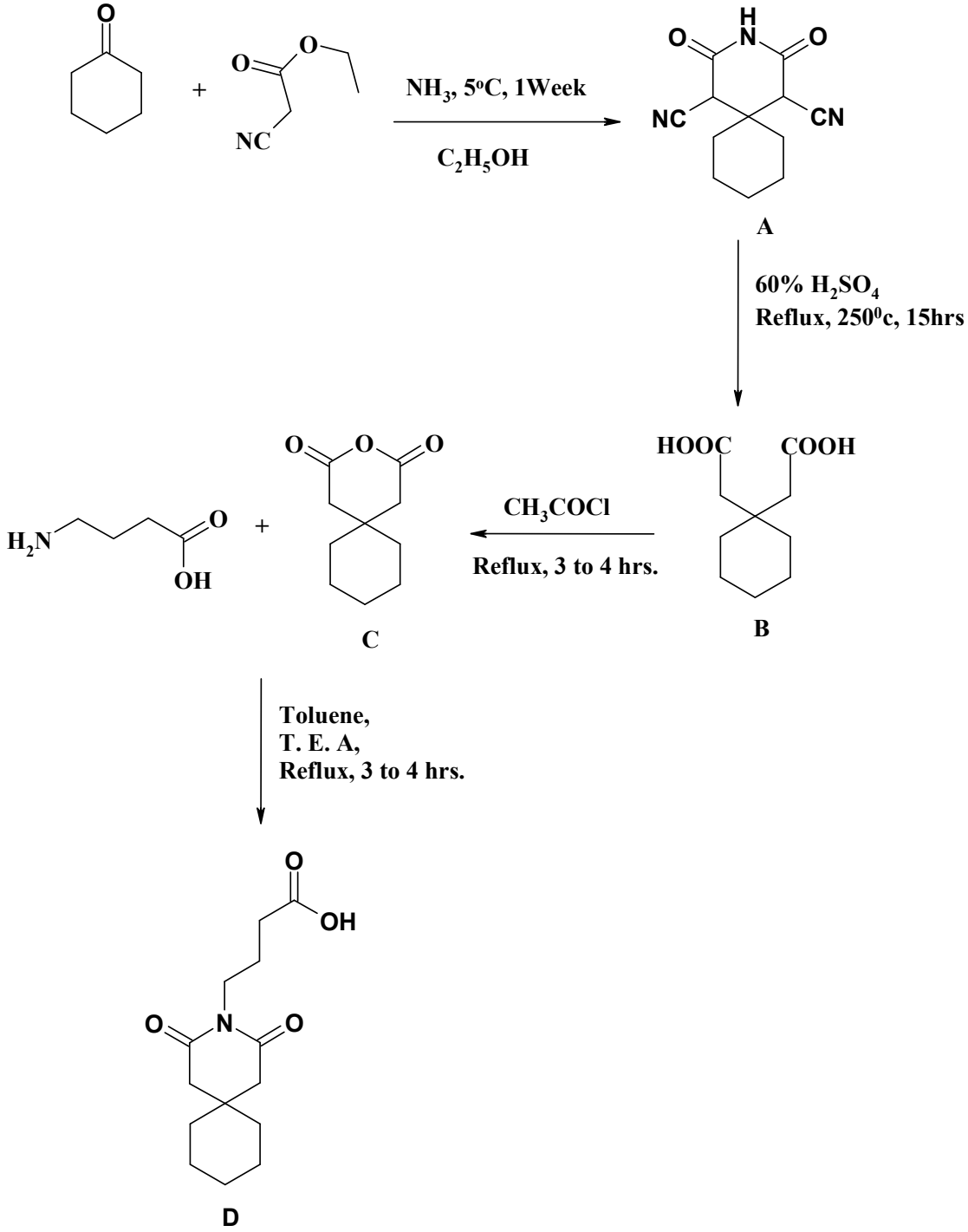
CHAPTER 4
MATERIALS & METHODS

4.1: Chemistry

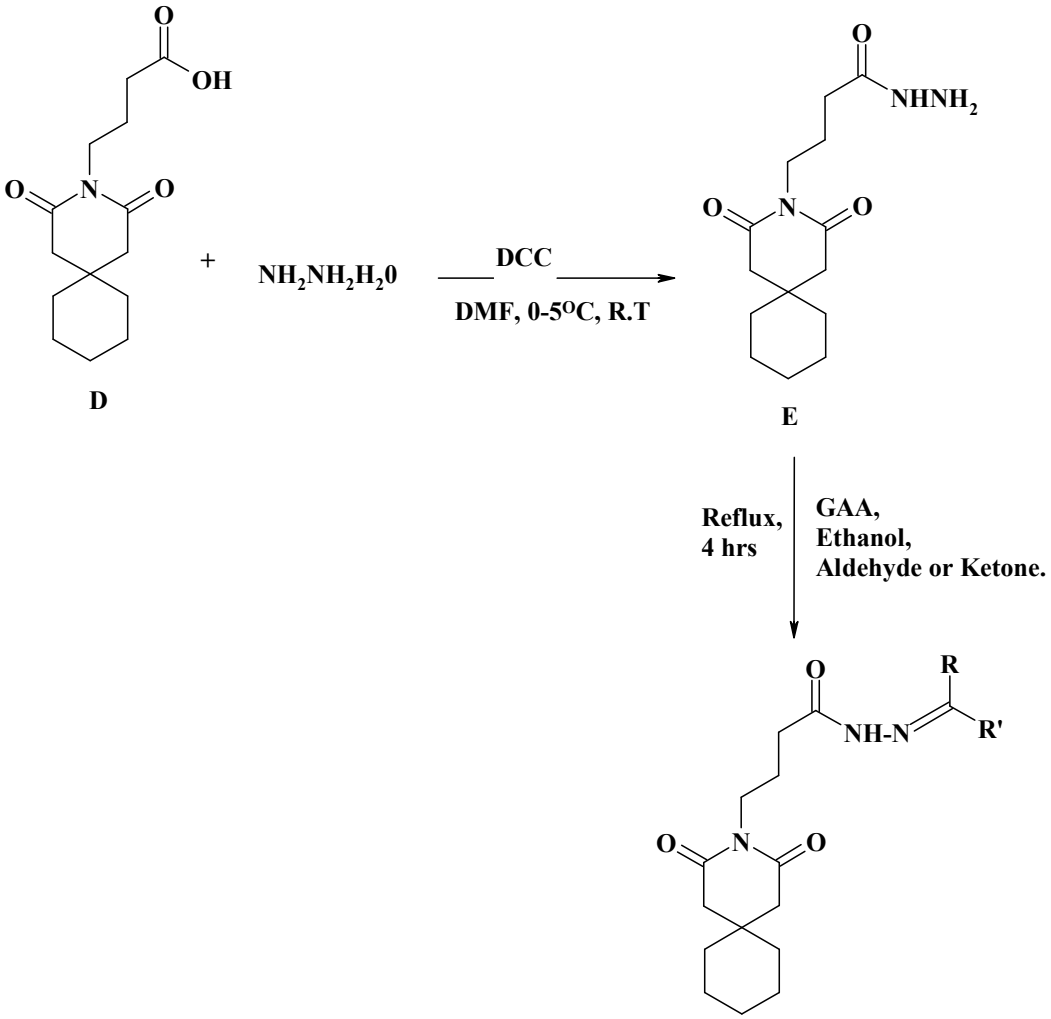
Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus and are uncorrected. Infra red spectra (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (400 MHz) spectrophotometers, respectively. Chemical shifts were reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. All exchangeable protons were confirmed by the addition of deuterated water (D_2O). Mass spectra were recorded with Shimadzu GC-MS-QP5000 spectrophotometer. Elemental analyses (C, H, and N) were undertaken with Perkin-Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates and visualized under UV irradiation or by using iodine vapor. The solvent system used was Ethyl acetate/Hexane (2:8). The log P values were determined using Chem Bio office software.

Synthesis:

SERIES I: 4-(2, 4-DIOXO-3-AZASPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS:



SCHEME-4.1



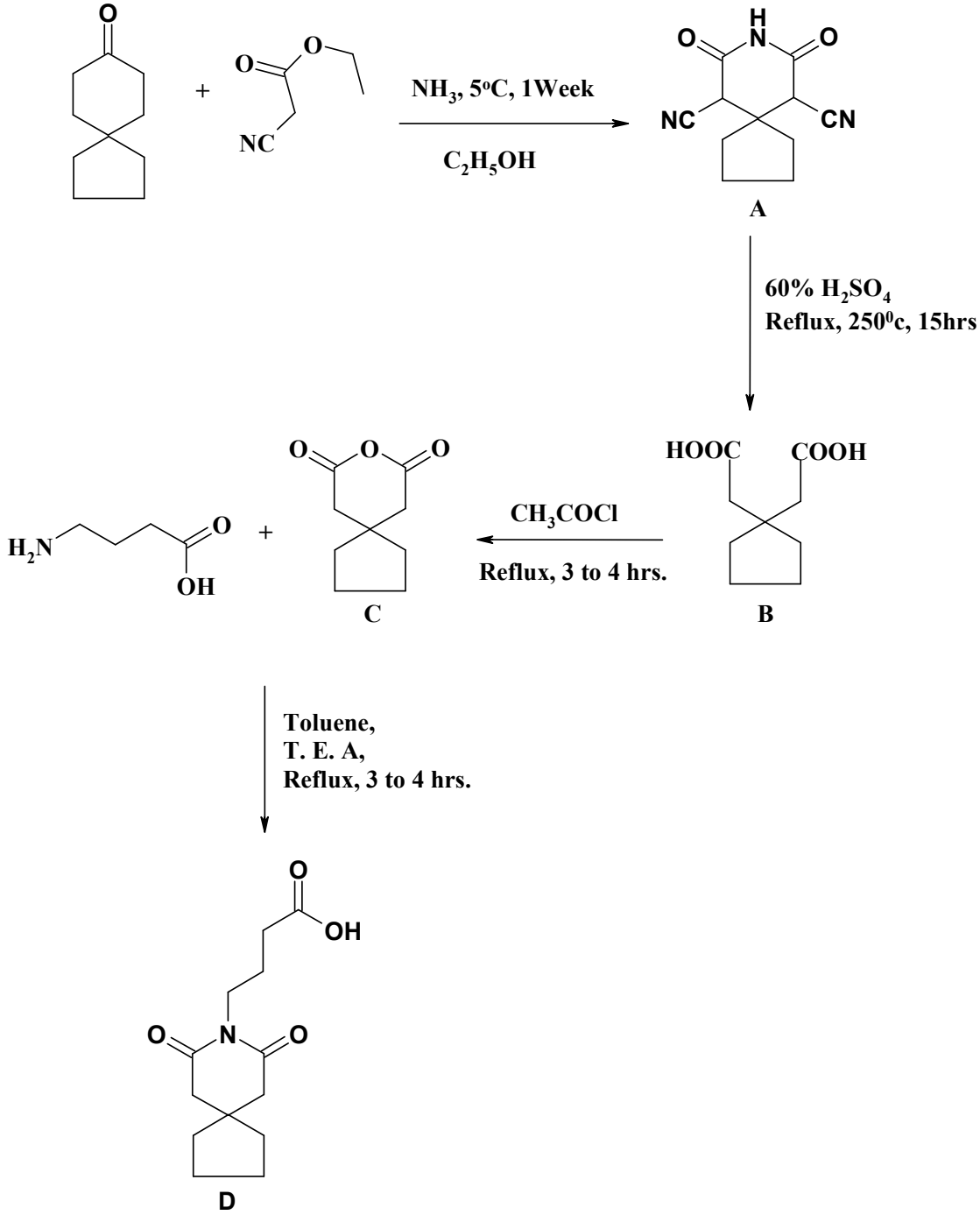
SH-3 to SH-15

SCHEME-4.2

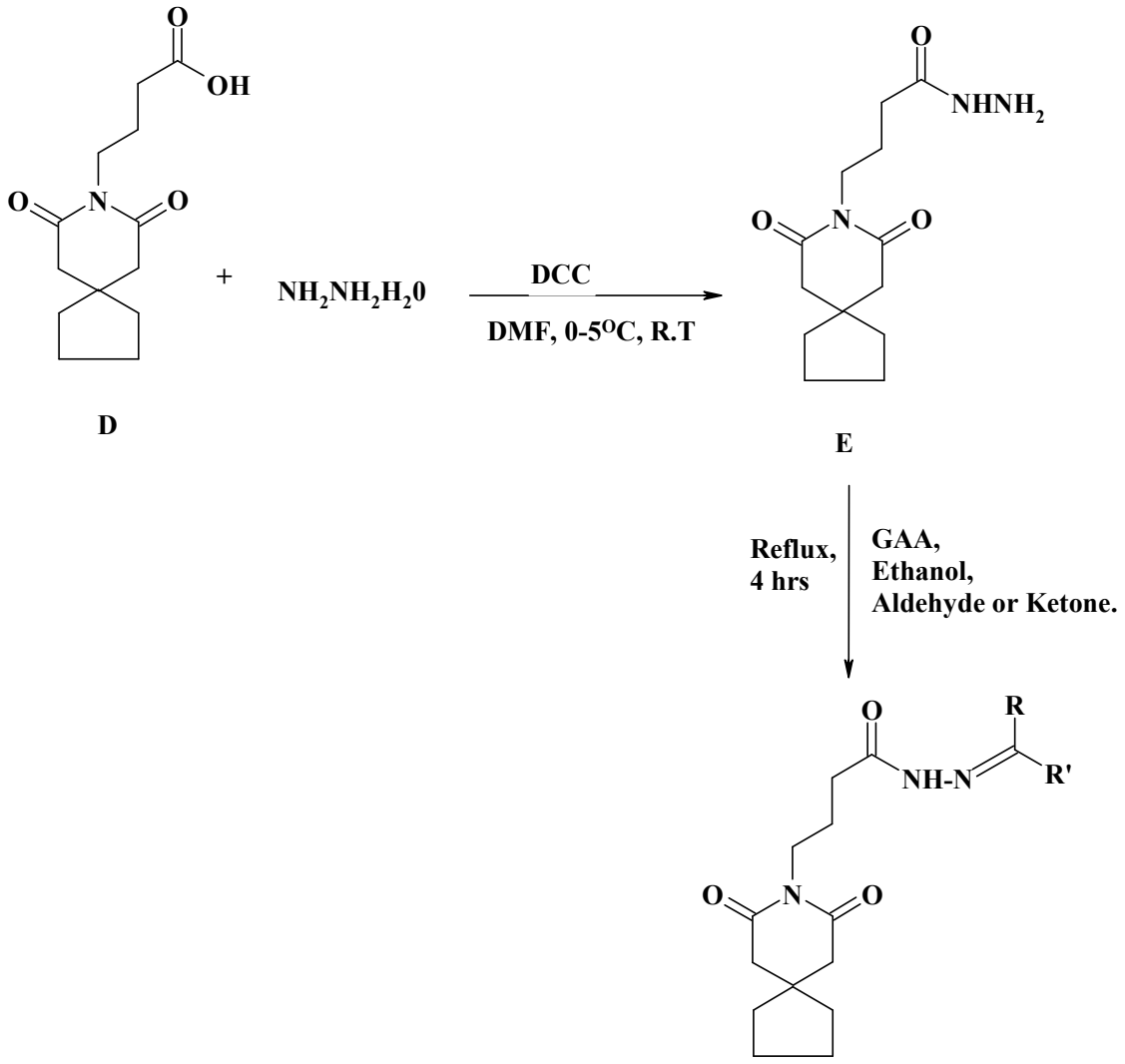
Guareschi imide **(A)** was synthesized by treating cyclohexanone with ethylcyanoacetate in the presence of alcohol and ammonia (0-5^{o}C) [134]. This imide was subjected to hydrolysis by soaking in concentrated sulphuric acid followed by refluxing with 60% sulphuric acid to give **(B)** [135], which was then cyclised by refluxing with acetyl chloride to give **(C)** [136]. The obtained spiroanhydride was coupled with GABA using TEA as base and toluene as solvent to yield spiro-GABA **(D)**, which was converted to hydrazide **(E)** using hydrazine hydrate, dicyclohexyl carbodimide and a mixture of DCM and DMF. This hydrazide was reacted with aldehyde or ketone to get the desired final product. **(SH-3 to SH-16)**.

SERIES II:

SYNTHESIS OF 4-(7, 9-DIOXO-8-AZA SPIRO [4.5] DECAN-8-YL) BUTANOIC ACIDS:



SCHEME-4.3



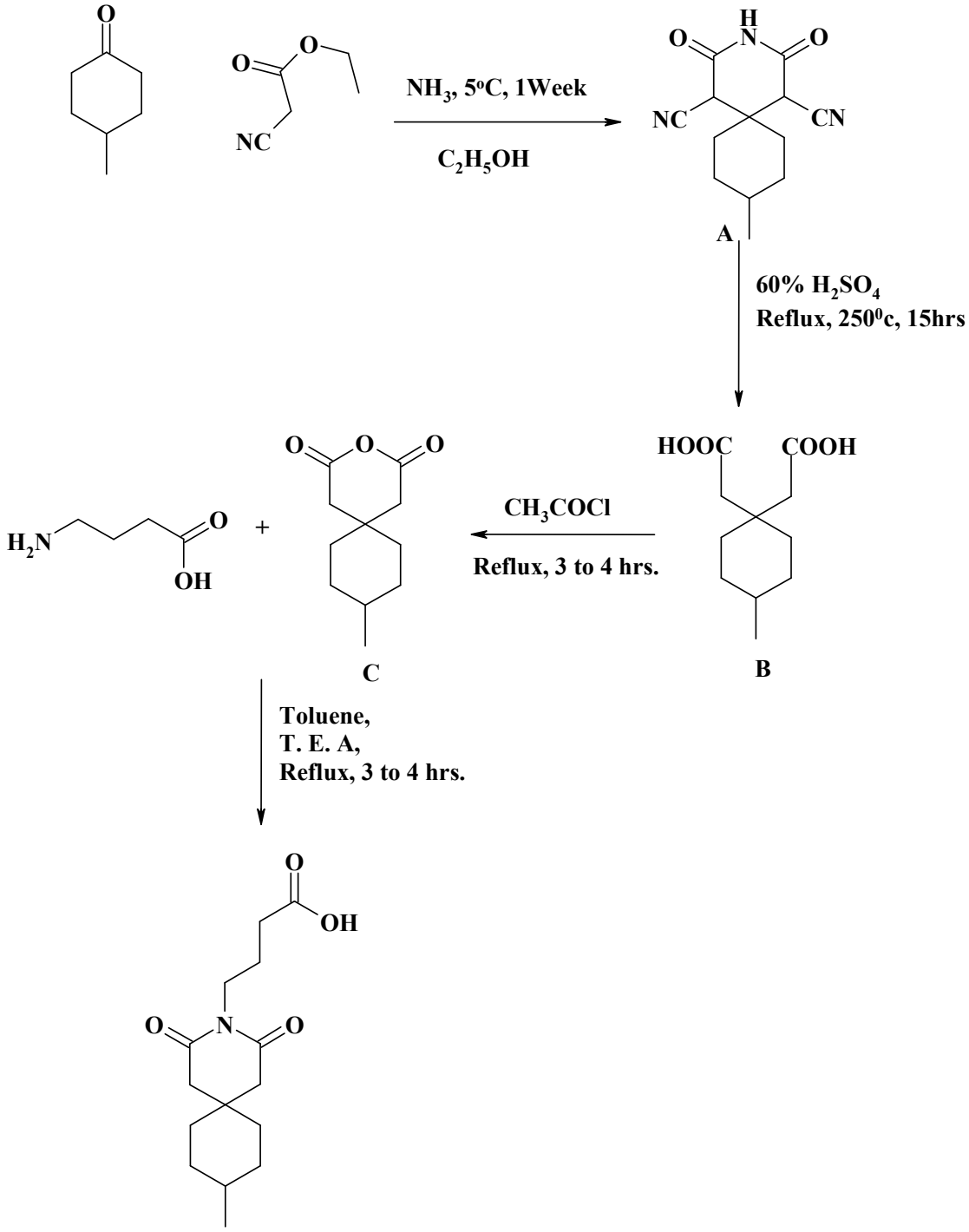
CP-4 to CP-16

SCHEME-4.4

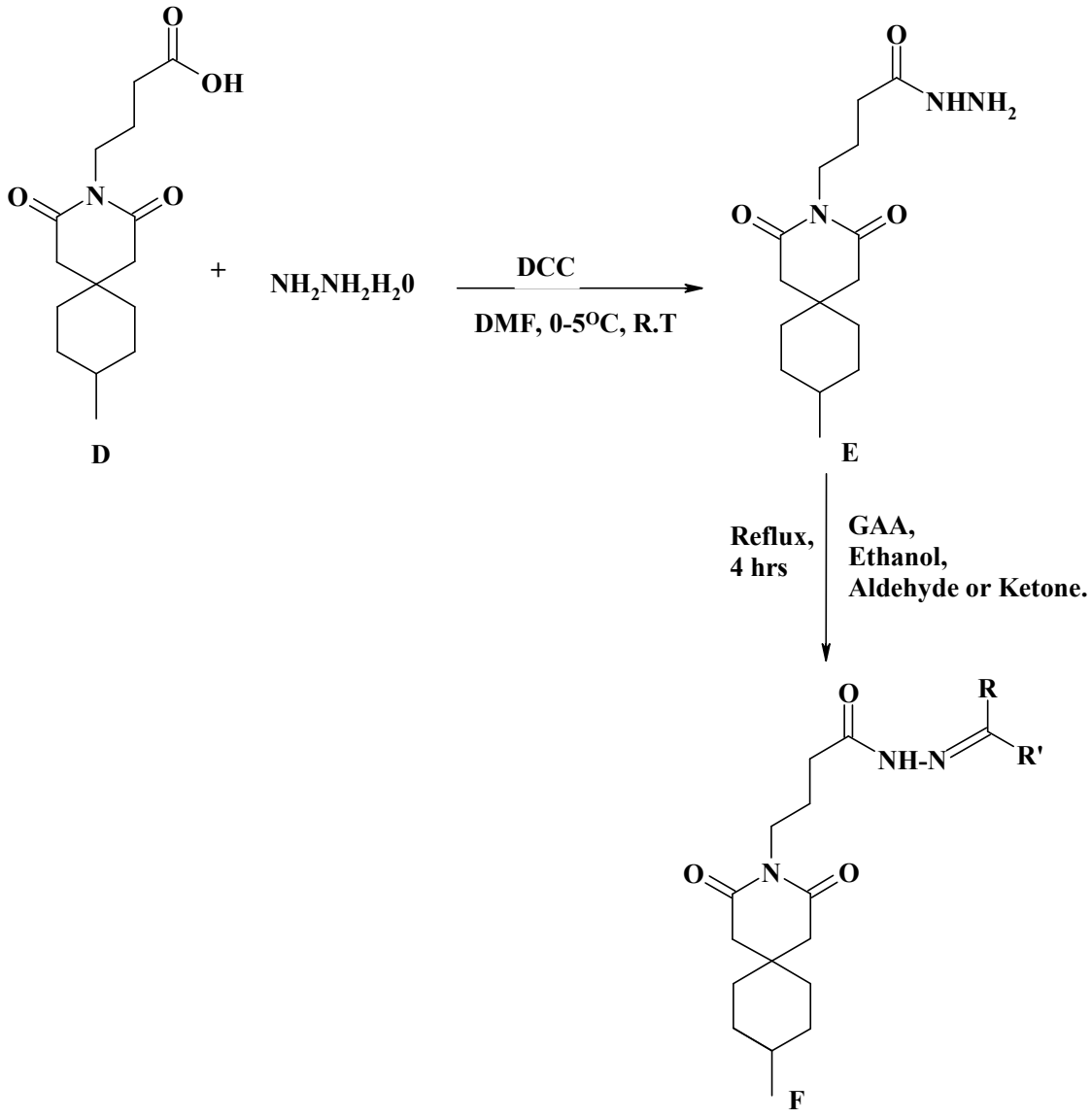
The Synthesis of 4-(7, 9-dioxo-8-aza spiro [4.5] decan-8-yl) butanoic acids **(CP-1 to CP-16)** was accomplished by taking cyclopentanone as Starting material and following the methods described for Series-1.

SERIES III:

SYNTHESIS OF 4-(9-METHYL-2, 4-DIOXO-3-AZA SPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS



SCHEME-4.5



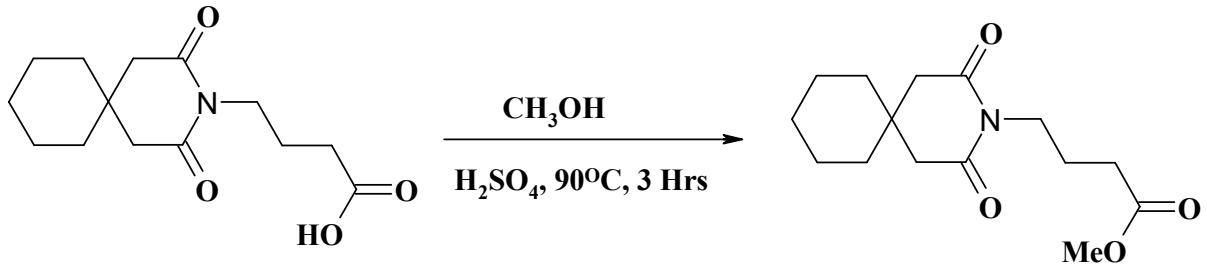
MC-4 to MC-16

SCHEME 4.6

The Synthesis of 4-(9-methyl-2, 4-dioxo-3-aza spiro [5.5] undecan-3-yl) butanoic acids (**MC-1 to MC-16)** was accomplished by taking 4-methylcyclohexanone as Starting material and following the methods described for Series-1.

SERIES IV:

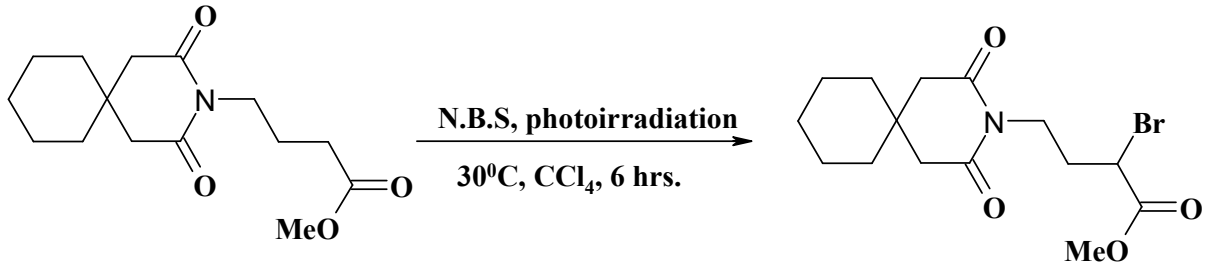
BS Series:



SH-1 **BS-1**

SCHEME 4.7

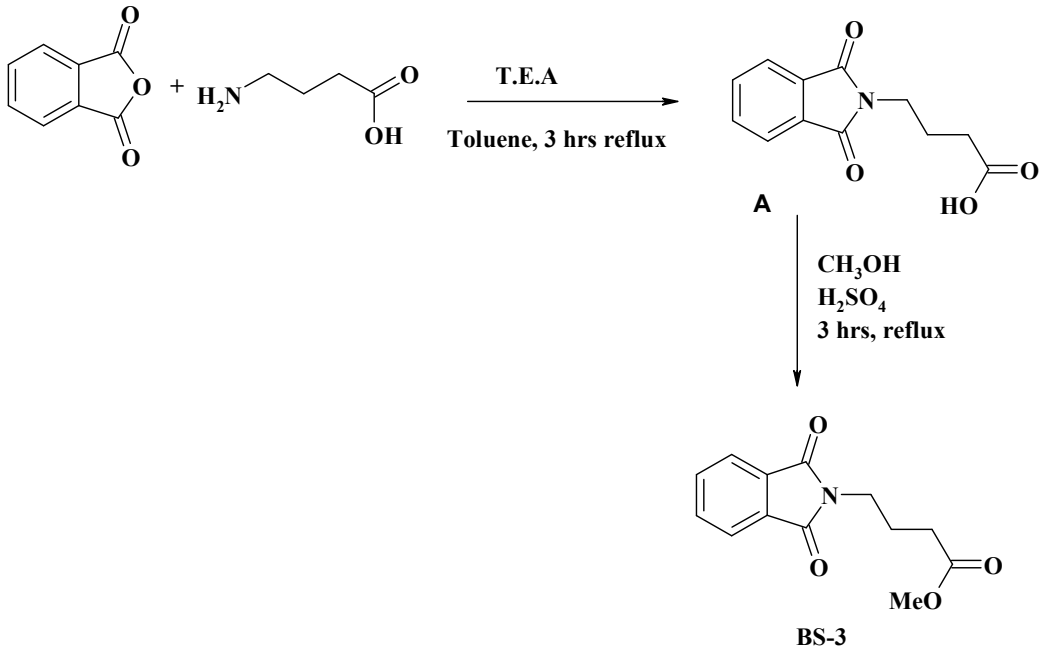
N-Spiro GABA was dissolved in methanol and 1 ml of concentrated sulphuric acid was added. The reaction mixture was refluxed for 3 hours and was evaporated and extracted with ethyl acetate to obtain the methoxy derivative.



BS-1 **BS-2**

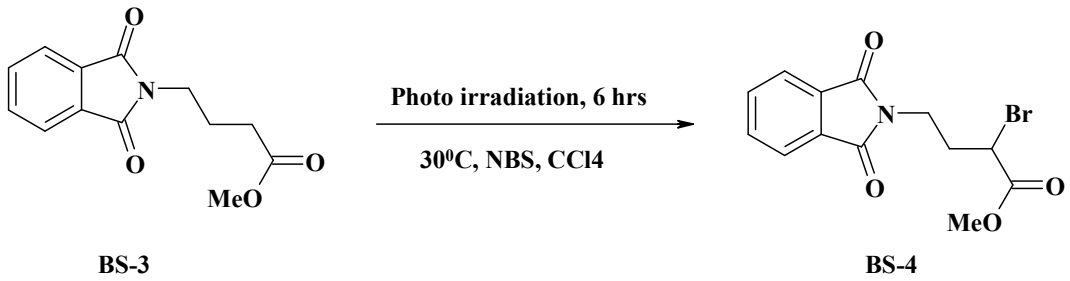
SCHEME 4.8

The compound obtained from scheme 4.7 was dissolved in carbon tetrachloride. N-Bromosuccinimide was added to the reaction mixture. The reaction mixture was illuminated with 500W bulb for 6 hours. After completion the reaction mixture was filtered to remove the succinimide and was extracted with ethyl acetate and evaporated.



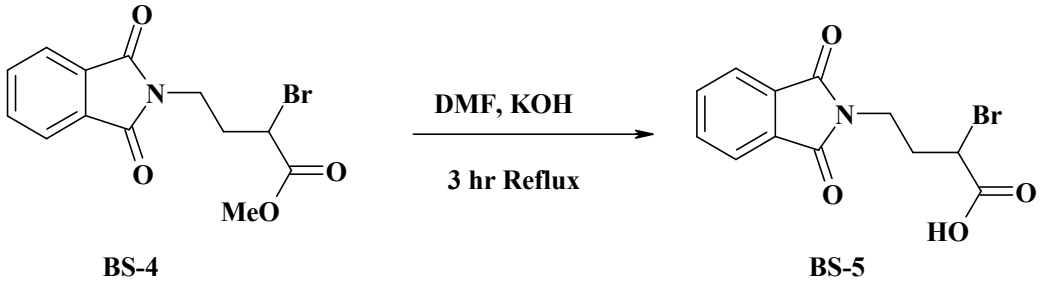
SCHEME 4.9

Phthalic anhydride and GABA were refluxed in toluene in the presence of triethylamine for 3 h to obtain N-phthaloyl GABA [137] **(A)** which is refluxed with methanol and sulphuric acid to yield its methoxy derivative **(BS-3)**.



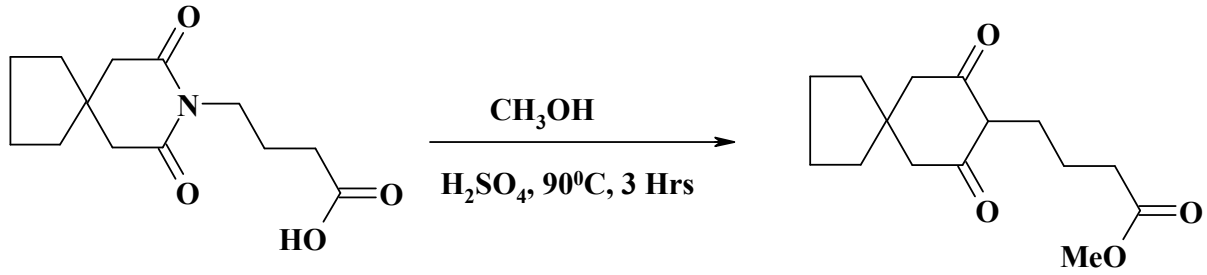
SCHEME 4.10

Compound **BS-3** scheme-4.9 was dissolved in CCl_4 followed by the addition of NBS, the reaction mixture was illuminated for 6 h using 500W bulb, to yield the bromo derivative.



SCHEME 4.11

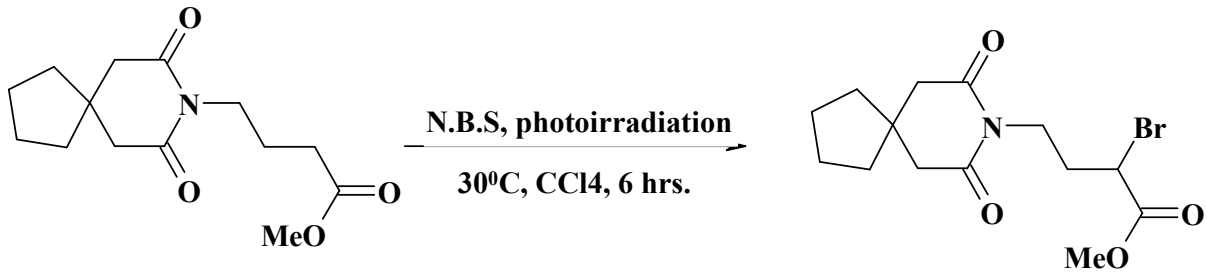
Bromo derivative **(BS-4)** was hydrolysed by refluxing it with KOH and DMF for 3 hrs, to yield Bromosubstituted N-phthaloyl-GABA **(BS-5)**.



CP-2 **BS-6**

SCHEME 4.12

BS-6 was obtained by taking **CP-2** as starting material and following the method described for scheme-4.7.



BS-6 **BS-7**

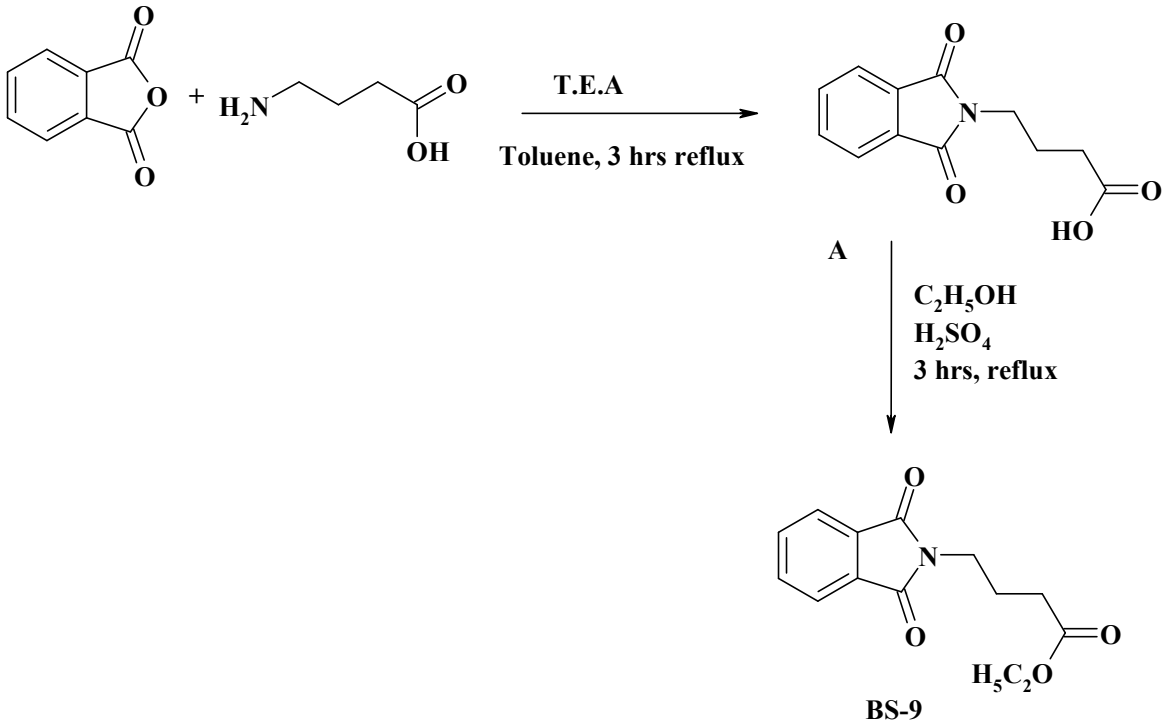
SCHEME 4.13

BS-7 was synthesized by using the method described for compound **BS-2** and **BS-4.**

BS-7 **BS-8**

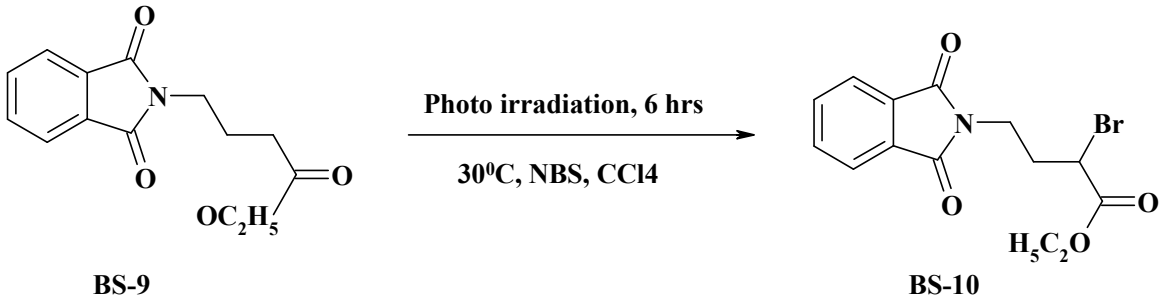
SCHEME 4.14

The synthesis of **BS-8** was accomplished by following the procedure described for **BS-5**.



SCHEME 4.15

Phthalic anhydride and GABA were refluxed in toluene in the presence of triethylamine for 3 h to obtain N-phthaloyl GABA **(A)** which is refluxed with ethanol and sulphuric acid to yield its ethoxy derivative **(BS-9)**.



SCHEME 4.16

The compound obtained from scheme 4.15 was dissolved in carbon tetrachloride. N-bromosuccinimide was added to the reaction mixture. The reaction mixture was illuminated with 500W bulb for 6 hours. After completion the reaction mixture was filtered to remove succinimide and was extracted with ethyl acetate and evaporated to obtain the bromo derivative **(BS-10).**

4.2: Pharmacology

Animals

The neuropharmacological studies were conducted on Swiss albino mice (20-25g) and Wistar albino rats (180-250g) of either sex. The animals were obtained from Hisar Agricultural University and were housed six (mice) and four (rats) per cage at constant temperature under a 12-h light/dark cycle (lights on at 7:00 AM), with food (standard laboratory pellet) and water *ad libitum*. All of the animals were acclimatized to the housing conditions for a period of one week before the start of experiments.

All experiments and procedures described in this thesis were reviewed and approved by the Institutional Animal Ethics Committee (Protocol Nos: IAEC/RES/6/3 and IAEC/RES/6/2).

The various neuropharmacological tests performed are as follows,

1. Anticonvulsant evaluation

Test solutions of all compounds were prepared in 0.5% w/v methyl cellulose (MES and scPTZ tests) and 30% v/v PEG 400, and the animals were dosed 30 min prior to testing. All of the compounds were administered intraperitoneally in a volume of 0.01 mL/g body weight for mice and 0.004 mL/g body weight for rats at doses of 30, 100 and 300 mg/kg to one to four animals. The profile of anticonvulsant activity was established by one electrically-induced and three chemically-induced seizure tests.

1.1. Maximal electroshock seizure test (MES)

Maximal seizures were elicited by a 60Hz alternating current of 50mA (five to seven times that is necessary to elicit minimal seizures) intensity delivered for 0.2 s via corneal electrodes. A drop of 0.9% w/v sodium chloride instilled in each eye prior to application of the electrodes assured adequate electrical contact. The animals were dosed intraperitoneally with the test compounds 30 min prior to testing. Abolition of the hind limb tonic extensor component of the seizure was defined as protection in the MES test [136].

1.2. Subcutaneous pentylenetetrazole seizure threshold test (scPTZ)

This test produces minimal clonic seizures. Compounds were tested for their ability to antagonize scPTZ-induced convulsions in mice after i.p. injection. After 30 min of administration of test compounds, pentylenetetrazole in 0.9% w/v sodium chloride, was administered subcutaneously at a dose of 85 mg/kg. The animals were placed in individual cages and observed for 30 min after pentylenetetrazole administration. A threshold convulsion was defined as one episode of clonic convulsion, which persisted for at least 5 seconds. Absence of a single 5 second episode of clonic spasm was taken as the end point in this test [137].

1.3. Subcutaneous strychnine-induced seizure threshold test (scSTY)

The test compounds were dosed intraperitoneally 30 min prior to testing. The convulsive dose of strychnine (1.2 mg/kg) was injected subcutaneously in a volume of 0.01 mL/g body weight into each of the mice. The mice were placed in isolated cages and observed for 30 min for the presence or absence of the hind limb tonic extensor component of the seizure. Abolition of the hind limb tonic extensor component was taken as the end point, which indicates that the test substance has the ability to prevent seizure spread [138].

1.4. Subcutaneous picrotoxin-induced seizure threshold test (scPIC)

The test compounds were evaluated for their ability to antagonize scPIC-induced convulsions in mice after i.p. administration. After 30 min of drug administration the in-house validated convulsive dose of picrotoxin (3.15 mg/kg) was injected subcutaneously in a volume of 0.01 mL/g body weight into each of the mice. The mice were placed in isolated cages and observed for the next 45 min for the presence or absence of threshold convulsion. Absence of a threshold convulsion was taken as the end point, which indicates that the test substance has the ability to elevate the picrotoxin seizure threshold [139].

2. Neurotoxicity (Rotarod assay)

The acute neurological deficit was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod that rotates at 6 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were given intraperitoneal injection of the test compounds. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials [140].

Chapter-5
Design and Synthesis

CHAPTER- 5
DESIGN AND SYNTHESIS

5.1: DESIGN:

Epilepsy, a chronic brain disorder characterized by recurrent seizures, affects 1-2% of world's population. It has been shown that seizures arise due to reduction in GABA concentration in the brain. GABA the major inhibitory neurotransmitter in the brain plays an important role in the etiology and control of epilepsy by mediating the inhibition processes of epilepsy. This endogenous ligand of GABA receptor (which itself is an anticonvulsant target) would be an ideal anticonvulsant but its poor CNS penetration property implicated GABA as a structural template for a number of substance that have various CNS effects. Advent of vigabatrin, progabide and gabapentin was strategically based on the above hypothesis. The criterion was to manipulate the GABA molecule to increase its lipophilicity there by aiding to cross BBB. and various other derivatives, enrichment of antiepileptic pharmacotherapy became available [2, 6, 93].

A Spiro compound is a bicyclic organic compound with rings connected through just one atom. A number of spiro compounds were found to have good CNS activity. Our present study was designed to synthesis and evaluates spiro derivatives of GABA [74].

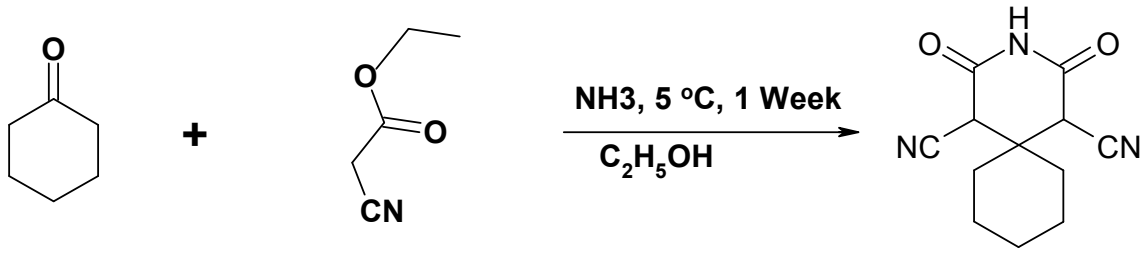
Three series of compounds were designed by combining the above pharmacophoric elements with various acid hydrazones and were synthesized as acid hydrazones of N-spiroGABA. This modification was carried out, as various acid hydrazones had been reported earlier as potent anticonvulsants [143, 144]. These series includes 48 compounds all of them were evaluated for their anticonvulsant activity.

One series was designed to make 2-substitution in GABA as this substitution improves the penetration to blood brain barrier. This series include 10 compounds and all of which were evaluated for anticonvulsant activity.

5.2: SYNTHESIS AND CHARACTERISATION

SERIES I: 4-(2, 4-DIOXO-3-AZASPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS:

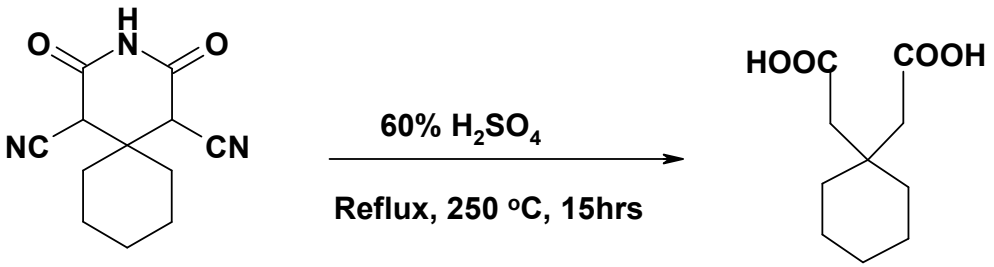
Step-1: Synthesis of 2, 4-dioxo-3-azaspiro [5.5] undecane-1, 5-dicarbonitrile (A):



A

Cyclohexanone (30 gm, 306.12 mmoles) and ethyl cyanoacetate (72.7 gm, 642 mmoles) were treated at 0 °C with an excess of ammonia in ethanol and the mixture was stored at 0-5 °C in a stoppered bottle for a period of 1 week. The precipitated ammonium salt was filtered off, pressed and washed several times with alcohol. After the filter cake had stopped dripping, the solid ammonium salt was dissolved in large amount of boiling water; the solution was filtered while hot and acidified with HCl. The free imide was filtered, washed with water and dried to obtain compound **(A)**. 75%, 210-212 ^{O}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 2225, 2175, 1780, 1725, 1450 and 1300. **MS**: $(M+1)^{+}$ 232.10. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.33(2H, m), 1.46(4H, t) 1.54(4H, p), 3.65(2H, s), 9.45(s, 1H, N*H*).

Step-2: Synthesis of 2, 2'-(cyclohexane-1, 1-diyl)diacetic acid (B):

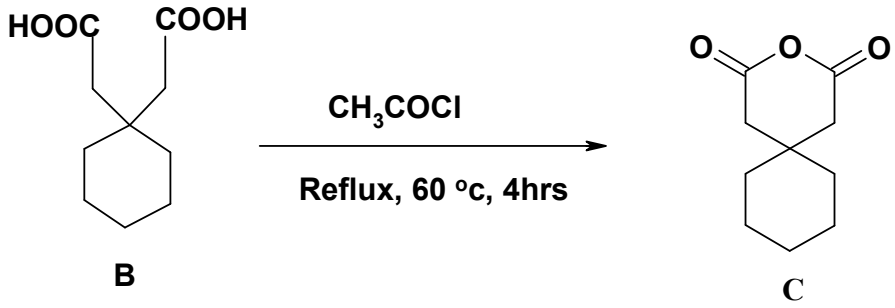


A **B**

Compound **(A)** (30 gm, 129.87 mmoles) was soaked in sulphuric acid (four times the weight of imide). After standing for 8 hours , water (three times the weight of imide) was

added with frequent shaking. The mixture was heated under reflux for 15 hours with intermittent shaking, until frothing had ceased. After the mixture was allowed to cool to room temperature, water was added to dilute the reaction mixture. The crude acid together with charred material was filtered off and washed well with water. The crude reaction product was then suspended in hot water and sufficient potassium bicarbonate was added to dissolve all the acid. After boiling with charcoal, the solution was filtered, acidified with concentrated hydrochloric acid. The precipitate was filtered off, washed with water and dried. 45%, 180-183 ^{O}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 1710 and 1300. **MS**: $(M+1)^{+}$ 201.10. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.33(4H, t), 1.46(2H, t), 1.48(4H, p), 2.25(2H, s), 2.27(2H, s), 11.15(s, 2H, COOH).

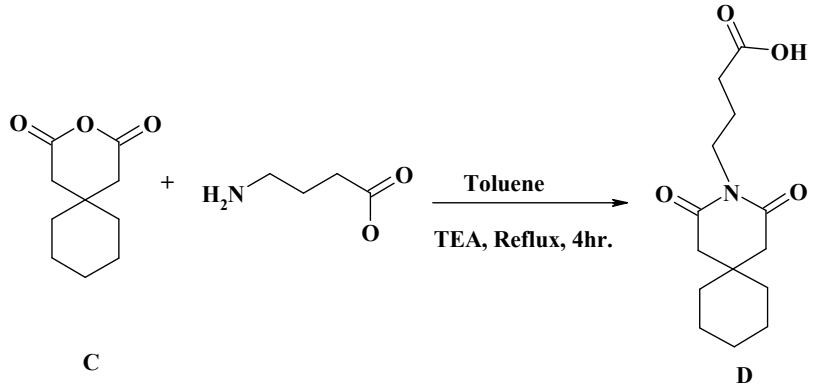
Step-3: Synthesis of 3-oxaspiro [5.5] undecane-2, 4-dione (C):



B C

Compound **(B)** (20 gm, 100 mmoles) was mixed with 3 times its weight of acetyl chloride and refluxed for 4 hours. The reaction mixture was cooled to room temperature and crushed ice was added to the reaction mixture. The precipitated solid was filtered off and washed several times with water and dried to obtain Compound **(C)**. 55%, 65-66 ^{O}C. **IR** (cm^{-1} KBr): 3100, 1780, 1720 and 1300. **MS**: $(M+1)^{+}$ 183.09. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.31(4H, t), 1.44(2H, t), 1.47(4H, p), 2.25(4H, s).

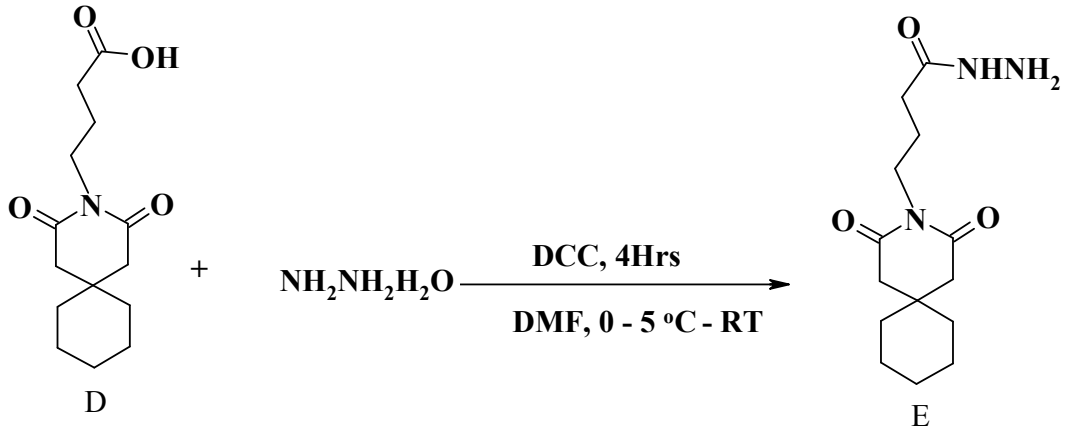
Step-4: Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanoic acid (D):



C D

Compound **(C)** (10 gm, 54.94 mmoles) and equimolar quantity of GABA (6.22 gm, 60.38 mmoles') was refluxed in toluene in the presence of triethylamine for 4 hours in Dean-stark apparatus. The organic solvents were removed *in vacuo*, water was added to the reaction mixture and acidified with concentrated hydrochloric acid and stirred for 30 minutes, filtered and dried to obtain compound (**D)**. 57%, 160-163 ^{O}C. **IR** (cm^{-1} KBr): 3300, 3175, 3100, 1712 and 1300. **MS**: $(M+1)^{+}$ 268.15 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.33(2H, m), 1.46(4H, t) 1.54(4H, p), 1.98(2H, p), 2.25(4H, s), 2.30(2H, t), 4.45(2H, t), 11.11(s, 1H, OH).

Step-5: Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanehydrazide (E):



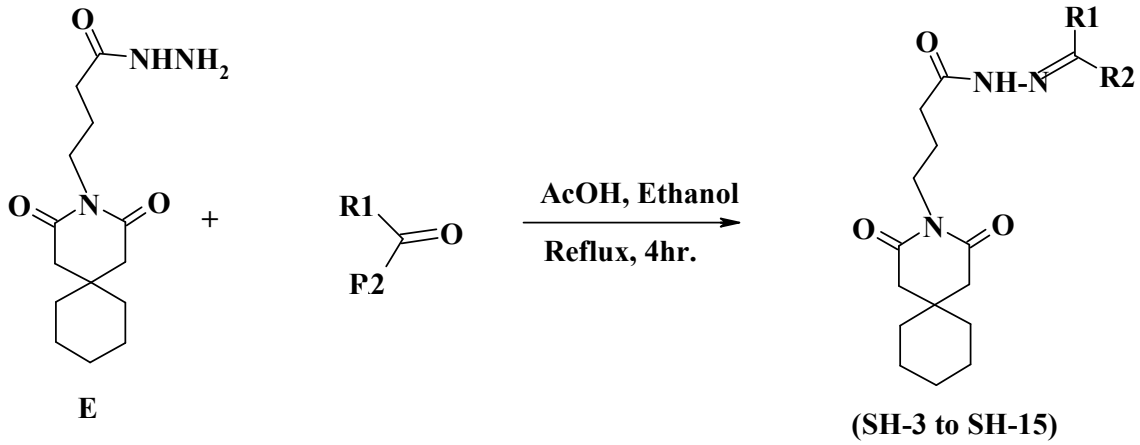
D E

Compound **(D)** (5 gm, 18.72 mmoles) was dissolved in dimethylformamide. The temperature of the reaction mixture was brought down to 0-5 °C by placing the round

bottom flask in ice bath. Dicyclohexylcarbodimide was added to the mixture, followed by 10 moles of hydrazine hydrate. The reaction mixture was brought to room temperature and stirred for 4 hours.

The mixture was filtered off to remove dicyclohexyl urea. The filtrate was extracted with ethyl acetate and concentrated in vacuo to obtain compound of formula (**E)**. 65%, 120-124 ^{O}C. **IR** (cm^{-1} KBr): 3230, 3100, 2930, 1650, 1450 and 1300. **MS**: (M+1) $^{+}$ 282.17. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.33(2H, m), 1.41(4H, t) 1.52(4H, p), 1.94(2H, m), 2.15(4H, s), 2.37(2H, t), 4.52(2H, t), 5.14(s, 2H, NH_2), 5.87(s, 1H, NH).

Step-6: Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl) butanoic acid hydrazones:



4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask, to this 10 ml of glacial acetic acid and 10 ml of absolute alcohol were added followed by the addition of aldehyde or ketone, the reaction mixture was refluxed on an oil bath for 4 hours, after the completion of reaction, the reaction mixture was distilled and then cold water was added to the reaction mass. The obtained precipitate was filtered, washed and dried to afford the acid hydrazone.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(4-nitrophenyl) ethylidenebutanoic acid hydrazide (SH-3):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask, to this 10 ml of glacial acetic acid and 10 ml of absolute alcohol were added followed by the addition of 4-nitroacetophenone (0.339 gm, 2.24 mmol), the reaction mixture was refluxed on an oil bath for 4 hours, after the completion of reaction, the reaction mixture was distilled and then cold water was added to the reaction mass. The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375 and 1300. **MS**: $(M+1)^+$ 415.19. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.01(2H, m), 1.18(2H, t) 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2Ht), 3.1(2H, t), 5.31(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{26}N_4O_5$ C: 60.86, H: 6.32, N: 13.52, O: 19.30 **Found**: C: 60.66, H: 6.47, N: 13.62, O: 19.26.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-1-(biphenyl) methylidene butanoic acid hydrazide (SH-4):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, (0.5 gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of benzophenone (0.280 gm, 2.05 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1590, 1550, 1500 and 1300. **MS**: $(M+1)^+$ 384.22. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(2H, s), 2.39(2Ht), 3.95(2Hs), 5.45(s, 1H, N*H*), 7.97-8.02 (m, 3H, aromatic), 8.09-8.13(m, 3H, aromatic), 8.21-8.26(m, 4H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{29}N_3O_3$ C: 68.90, H: 7.62, N: 10.96, O: 12.52 **Found**: C: 68.70, H: 7.51, N: 10.85, O: 12.43.

Synthesis of 4-(2, 4-dioxo-3-azaspiro[5.5] dec-3-yl)-N'-1-(4-methylphenyl ethylidene butanoic acid hydrazide (SH-5):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-methyl acetophenone (0.278 gm, 2.05 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400,

3050, 2900, 1675, 1590, 1550, 1500, 1375 and 1300. **MS**: $(M+1)^+$ 398. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99 (4H, m), 1.18 (2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(2H, s), 2.39(2H, t), 2.45(3H, s), 2.57(3H, s), 3.95(2H, m) 5.33(s, 1H, N*H*), 5.97(m, 2H, doublet), 8.05(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{31}N_3O_3$ C: 69.49, H: 7.86, N: 10.57, O: 12.07 **Found**: C: 69.55, H: 7.85, N: 10.41, O: 12.19.

Synthesis of 4-(2,4-dioxo-3-azaspiro[5.5] dec-3-yl)-N'-1-cyclopentylidene butanoic acid hydrazide (SH-6):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of cyclopentanone (0.166 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 348. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.37(1H, m), 1.52(4H, t), 1.93(2H, m), 2.52(4H, t), 2.29(4H, m), 2.39(2H, t), 3.1(2H, t), 5.28(s, 1H, N*H*). **Elemental Analyses Calcd for** $C_{19}H_{29}N_3O_3$ C: 65.68, H: 8.41, N: 12.09, O: 13.81 **Found**: C: 65.66, H: 8.47, N: 12.07, O: 13.80.

Synthesis of 4-(2,4-dioxo-3-azaspiro[5.5] dec-3-yl)-N'-cyclohexylidene butanoic acid hydrazide (SH-7):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of cyclohexanone (0.193 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 362.47. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.40(2H, p), 1.52(4H, t), 1.93(2H, m), 2.52(4H, t), 2.29(4H, m), 2.39(2H, t), 3.1(2H, t), 5.31(s, 1H, N*H*). **Elemental Analyses Calcd for** $C_{20}H_{31}N_3O_3$ C: 66.45, H: 8.64, N: 11.62, O: 13.18 **Found**: C: 66.63, H: 8.47, N: 11.69, O: 13.21.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(3-nitrophenyl) methylidene butanoic acid hydrazide (SH-8):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the

addition of 3-nitrobenzaldehyde (0.350 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1590, 1550, 1525, and 1300. **MS**: $(M+1)^+$ 415.45. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.39(2H, t), 3.1(2H, t), 4.36(2H, t), 4.5(1H, s), 5.31(s, 1H, N*H*), 8.03 (m, 2H, aromatic), 8.01(m, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{26}N_4O_5$ C: 60.86, H: 6.32, N: 13.52, O: 19.30 **Found**: C: 60.66, H: 6.47, N: 13.62, O: 19.26.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-phenylethylidene butanoic acid hydrazide (SH-9):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) followed by the addition of acetophenone (0.234 gm, 1.97 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 384.22. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01(2H, m), 1.04(4H, t), 1.18(2H, t), 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.35(3H, s), 2.39(2Ht), 3.13(2H, t), 5.34(s, 1H, N*H*), 8.04(d, 2H, aromatic), 8.09(m, 3H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{29}N_3O_3$ C: 68.90, H: 7.60, N: 10.96, O: 12.52 **Found**: C: 68.73, H: 7.77, N: 10.84, O: 12.66.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene] butanoic acid hydrazide (SH-10):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of isatin (0.287 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 411.21 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01(2H, m), 1.04(4H, t), 1.18(2H, t), 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.39(2Ht), 3.13(2H, t), 5.34(s, 1H, N*H*), 5.51(s, 1H, NH) 7.84(d, 2H, aromatic), 7.88(m, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{26}N_4O_4$ C: 64.37, H: 6.38, N: 13.65, O: 15.59 **Found**: C: 64.22, H: 6.48, N: 13.57, O: 15.73.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3Z)-5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene] butanoic acid hydrazide (SH-11):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-chloro-isatin (0.357 gm, 1.95 mmol) and further following the procedure

described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 445.16 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01(2H, m), 1.03(4H, t), 1.18(2H, t), 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.39(2H, t), 3.13(2H, t), 5.34(s, 1H, N*H*), 5.51(s, 1H, NH) 7.89(d, 2H, aromatic), 8.28(s, 1H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{25}ClN_40_4$ C: 59.39, H: 5.66, Cl: 7.97 N: 12.59, O: 14.38 **Found**: C: 59.46, H: 5.60, Cl: 7.93, N: 12.57, O: 14.44.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3Z)-5-methyl-2-oxo-1,2-dihydro-3H-indol-3-ylidene] butanoic acid hydrazide (SH-12):

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-methyl-isatin (0.315 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, 1375 and 1300. **MS**: $(M+1)^+$ 425.21 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01(2H, m), 1.04(4H, t), 1.18(2H, t), 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.39(2Ht), 3.13(2H, t), 3.49(3H, s), 5.36(s, 1H, N*H*), 5.53(s, 1H, NH) 7.80(d, 2H, aromatic), 7.83(m, 2H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{28}N_4O$ C: 65.08, H: 6.65, N: 13.20, O: 15.08 **Found**: C: 65.19, H: 6.67, N: 13.13, O: 15.01.

Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3Z)-5-fluoro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene] butanoic acid hydrazide (SH-13);

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-fluoro-isatin (0.322 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 429.19 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01 (2H, m), 1.04 (4H, t), 1.18 (2H, t), 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.39(2Ht), 3.13(2H, t), 5.34(s, 1H, N*H*), 5.51(s, 1H, NH) 7.84(d, 2H, aromatic), 7.88(m, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{25}FN_40_4$ C: 61.67, H: 5.88, F: 4.33, N: 13.08, O: 14.94 **Found**: C: 61.54, H: 5.80, F 4.39, N: 13.21, O: 15.06.

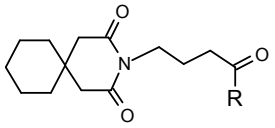
Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(4-nitrophenyl) methylidene butanoic acid hydrazide (SH-14):

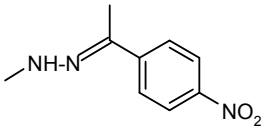
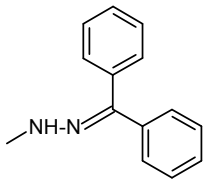
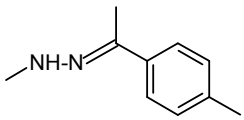
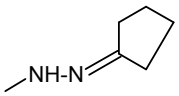
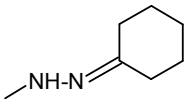
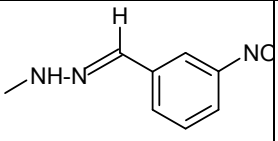
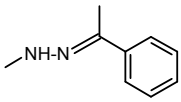
4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-nitrobenzaldehyde (0.350 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound.**FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 415.19. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.01(2H, m), 1.18(2H, t) 1.34(2H, m), 1.90(2H, m), 2.29(4H, m), 2.39(2Ht), 3.1(2H, t), 4.3(1H, s), 7.31(s, 1H, N*H*), 8.04(d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{26}N_4O_5$ C: 60.86, H: 6.32, N: 13.52, O: 19.30 **Found**: C: 60.64, H: 6.44, N: 13.64, O: 19.29.

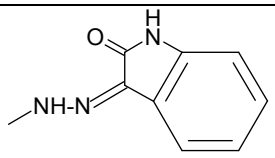
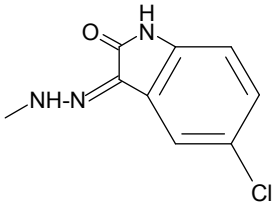
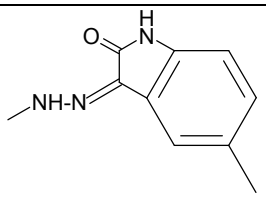
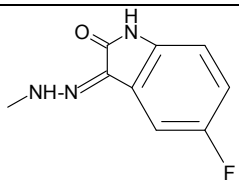
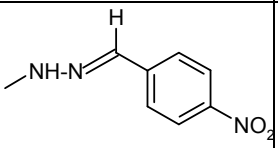
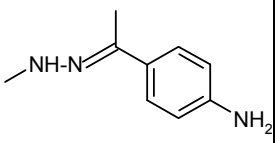
Synthesis of 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(4-aminophenyl) ethylidenebutanoic acid hydrazide (SH-15);

4-(2,4-Dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-aminoacetophenone (0.264 gm, 1.95 mmol) and further following the procedure described for **SH-3** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3300, 3050, 2900, 1675, 1590, 1550, 1525, and 1300. **MS**: $(M+1)^+$ 399.23. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.05(2H, m), 1.18(2H, t) 1.33(2H, m), 1.93(2H, m), 2.27(4H, m), 2.36(3H, s), 2.39(2Ht), 3.1(2H, t), 5.33(s, 1H, N*H*), 6.52(m, 2H, NH), 7.99(d, 2H, aromatic), 8.01(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{30}N_4O_3$ C: 66.31, H: 7.59, N: 14.06, O: 12.04 **Found**: C: 66.40, H: 7.47, N: 14.04, O: 12.09.

TABLE: 5.1 PHYSICAL DATA OF 4-(2, 4-DIOXO-3-AZASPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS



Comp	R	MF	MW	LOGP[b]	MP(°C)	Rf[a]	%YIELD
SH-1	– OH	$C_{14}H_{21}NO_4$	267.32	1.01	162-165	0.32	55.63
SH-2	– $NHNH_2$	$C_{14}H_{23}N_3O_3$	281.35	0.11	220-222	0.71	53.93
SH-3		$C_{22}H_{28}N_4O_5$	428.48	3.45	190-192	0.65	47.52
SH-4		$C_{27}H_{31}N_3O_3$	445.55	4.25	185-187	0.56	35.75
SH-5		$C_{23}H_{31}N_3O_3$	397	3.29	197-199	0.44	44.61
SH-6		$C_{19}H_{29}N_3O_3$	347.45	2.01	212-215	0.65	48.72
SH-7		$C_{20}H_{31}N_3O_3$	361.47	2.43	200-203	0.69	43.11
SH-8		$C_{21}H_{26}N_4O_5$	414.45	3.49	189-191	0.56	49.88
SH-9		$C_{22}H_{29}N_3O_3$	388.48	2.35	211-213	0.47	53.41

SH-10		$C_{22}H_{26}N_4O_4$	410.46	1.34	150-152	0.63	66.51
SH-11		$C_{22}H_{25}ClN_40_4$	444.91	1.9	168-170	0.66	63.23
SH-12		$C_{23}H_{28}N_4O_4$	424.49	1.83	177-180	0.56	65.12
SH-13		$C_{22}H_{25}FN_40_4$	428.45	1.5	202-204	0.61	65.67
SH-14		$C_{21}H_{26}N_4O_5$	414.45	3.49	188-191	0.54	57.90
SH-15		$C_{22}H_{30}N_4O_3$	398.49	1.55	169-171	0.62	43.27

[a]Mobile phase Ethyl acetate/Hexane (2:8).

[b]LogP was calculated using Chem-Biooffice software.

Results & Discussion: -

The design of 4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl) butanoic acids was based on the literature report in which the introduction of spiromoiety will result in good CNS activity.

4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl) butanoic acids were synthesized by a six-step process starting from cyclohexanone. The homogeneity of the compounds was monitored by performing thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were Ethylacetate: Hexane (2:8).

Most the compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 [log P > 2] except for compounds **SH-1, SH-2, SH-(10-13)**, **SH-15**. These compounds possessed log P < 2 since all substituted with polar groups like OH, NH_2 and substituted isatin **SH-(10-13)** derivatives.

With regard to the percentage yield of the synthesized compounds, the compounds with substituted aryl aldehydes have yields in the range of 49-57%, and those with substituted ketones and isatins gave 35-53% and 63-66% respectively.

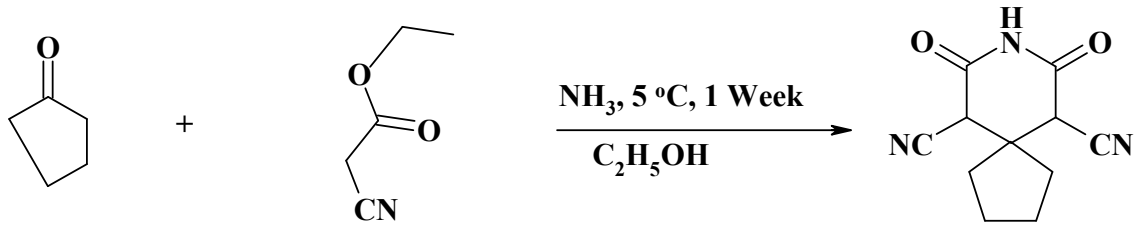
The IR spectrum 4-(2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-N'-1-(4-methylphenyl ethylidene butanoic acid hydrazide **(SH-5)** was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3400 cm^{-1} showed NH stretching, 3050 cm^{-1} showed aromatic CH stretching, 2900 cm^{-1} showed aliphatic CH stretching, 1675 cm^{-1} showed C=O stretching, 1590 cm^{-1} showed C=N stretching, 1550 $cm^{-1}$and 1500 cm^{-1} showed aromatic C=C stretching 1375 showed methyl group on the aromatic ring and 1300 cm^{-1} showed C–O stretching were observed.

^{1}H-NMR spectrum revealed a multiplet at δ 0.99(4H), triplet at δ 1.18(2H), multiplet at δ 1.34(2H), multiplet at δ 2.29(4H), a singlet at δ 2.32(2H) for 14H of CH_2 protons of the spiromoiety, singlet at 2.45 for protons of methyl group on the aromatic ring, singlet at 2.57 for protons of the methyl group, multiplet at δ 1.93(2H), triplet at δ 2.39(2H), triplet at δ 3.95(2H) for CH_2 protons of GABA, a singlet at δ 5.33(1H, NH) was D_2O exchangeable, two doublets at 7.97(2H) and 8.05(2H) for aromatic protons (Similarly, the structures of other compounds were confirmed according to their characteristic peaks)

The mass spectrum of compound **SH-5** showed a molecular ion peak at m/z 398.

SERIES II: 4-(7, 9-DIOXO-8-AZA SPIRO [4.5] DECAN-8-YL) BUTANOIC ACIDS:

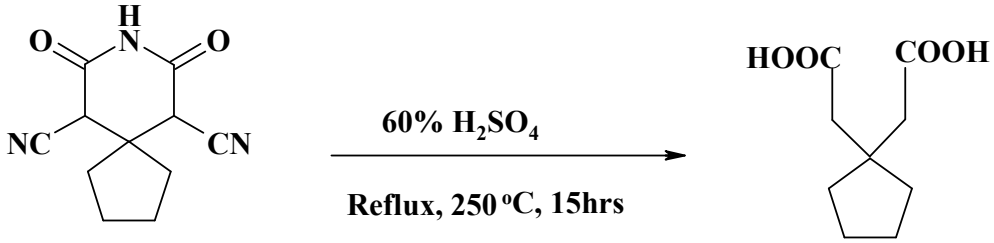
Step-1: Synthesis of 7, 9-dioxo-8-azaspiro [4.5] decane-6, 10-dicarbonitrile (A):



A

Cyclopentanone (30 gm, 356.63 mmoles) and ethyl cyanoacetate (85 gm, 75.221 mmoles) were treated at 0°C with an excess of ammonia in ethanol and the mixture was stored at 0-5 °C in a stoppered bottle for a period of 1 week. The precipitated ammonium salt was filtered off, pressed and washed several times with alcohol. After the filter cake had stopped dripping, the solid ammonium salt was dissolved in large amount of boiling water; the solution was filtered while hot and acidified with HCl. The free imide was filtered, washed with water and dried to obtain compound **(A)**. 60%, 179-181 ^{O}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 2225, 2175, 1780, 1725, 1450 and 1300. **MS**: $(M+1)^{+}$ 218.09. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.43(4H, t), 1.51(4H, t) 3.61(2H, s), 9.55(s, 1H, N*H*).

Step-2: Synthesis of 2, 2'-(cyclopentane-1, 1-diyl)diacetic acid (B):

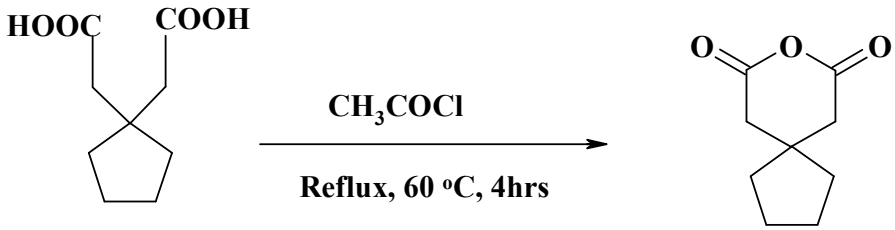


A **B**

Compound **(A)** (30 gm, 161.29 mmoles) was soaked in sulphuric acid (four times the weight of imide). After standing for 8 hours, water (three times the weight of imide) was added with frequent shaking. The mixture was heated under reflux for 15 hours with intermittent shaking, until frothing had ceased. After the mixture was allowed to cool to room temperature, water was added to dilute the reaction mixture. The crude acid

together with charred material was filtered off and washed well with water. The crude reaction product was then suspended in hot water and sufficient potassium bicarbonate was added to dissolve all the acid. After boiling with charcoal, the solution was filtered, acidified with concentrated hydrochloric acid. The precipitate was filtered off, washed with water and dried. 45%, 175-177 ^{O}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 1710 and 1300. **MS**: $(M+1)^{+}$ 187.09. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.43(4H, t), 1.51(4H, t), 2.15(2H, s), 2.17(2H, s), 11.11(s, 2H, COOH).

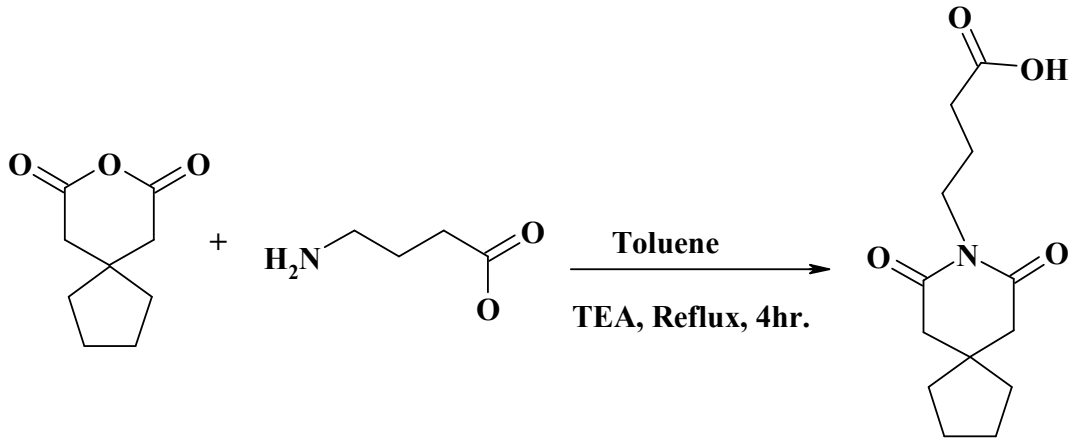
Step-3: Synthesis of 8-oxaspiro [4.5] decane-7, 9-dione (C):



B **C**

Compound **(B)** (20 gm, 0. 107 moles) was mixed with 3 times its weight of acetyl chloride and refluxed for 4 hours. The reaction mixture was cooled to room temperature and crushed ice was added to the reaction mixture. The precipitated solid was filtered off and washed several times with water and dried to obtain Compound of formula **(C)**. 45%, 62-64 °C. **IR** (cm^{-1} KBr): 3100, 1780, 1720 and 1300. **MS**: $(M+1)^{+}$ 169.08 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.41(4H, t), 1.53(4H, t), 2.16(4H, s).

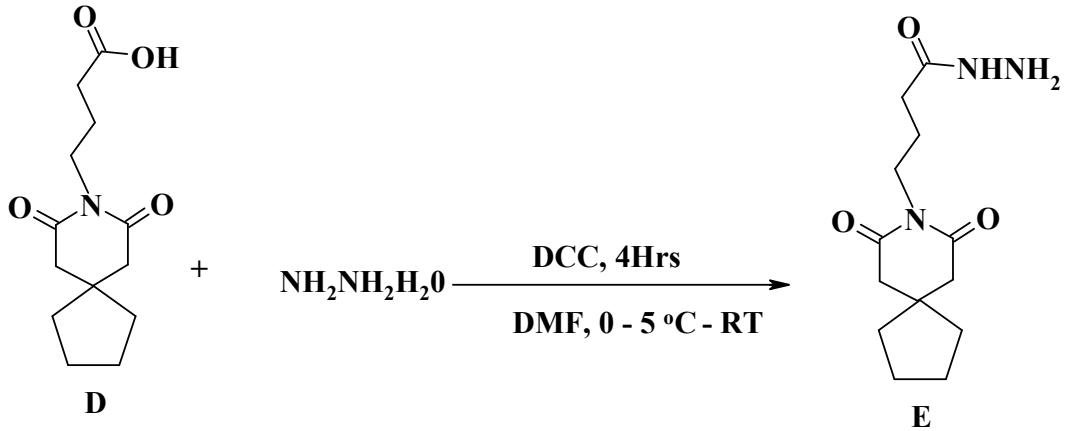
Step-4: Synthesis of 4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid (D):



C D

Compound **(C)** (10 gm, 59.52 mmoles) and equimolar quantity of GABA (6gm, 58.252) was refluxed in toluene in the presence of triethylamine for 4 hours in Dean-stark apparatus. The organic solvents were removed *in vacuo*, water was added to the reaction mixture and acidified with concentrated hydrochloric acid and stirred for 30 minutes, filtered and dried to obtain compound of formula **(D)**. 55.61, 172-174 ^{O}C. **IR** (cm^{-1} KBr): 3300, 3175, 3100, 1712 and 1300. **MS**: $(M+1)^{+}$ 254.13 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.43(4H, t), 1.52(4H, t) 1.95(2H, p), 2.10(4H, s), 2.25(2H, s), 4.45(2H, t), 11.15(s, 1H, OH).

Step-5: Synthesis of 4-(7, 9-dioxo-8-azaspiro[4.5]decan-8-yl)butanehydrazide (E):

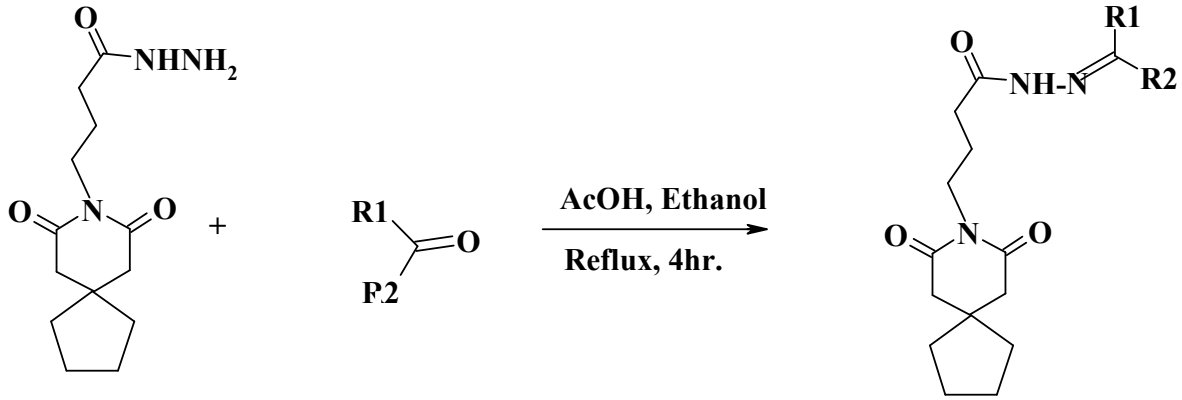


D E

Compound **(D) (**5 gm, 19.763 mmoles) was dissolved in DMF, the temperature of the reaction mixture was brought down to 0-5°C by placing the RB flask in ice bath. Dicyclohexylcarbodimide was added to the mixture, followed by 10 moles of hydrazine

hydrate (9.88 gm, 197.63 mmoles). The reaction mixture was brought to room temperature and stirred for 4 hours. The mixture was filtered off to remove dicyclohexyl urea. The filtrate was extracted with ethyl acetate and concentrated *in vacuo* to obtain compound of formula **(E)**. 53.92%, 158-160 °C. **IR** (cm^{-1} KBr): 3230, 3100, 2930, 1650, 1450 and 1300. **MS**: $(M+1)^+$ 268.16 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.43(4H, t), 1.52(4H, t), 1.94(2H, p), 2.15(4H, s), 2.37(2H, t), 4.52(2H, t), 5.12(s, 2H, NH_2), 5.83(s, 1H, NH).

Step-6: Synthesis of 4-(7, 9-dioxo-8-aza spiro [4.5] decan-8-yl) butanoic acids



E **(CP-4 to CP-16)**

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-1-(4-methylphenyl)ethylidine butanoic acid hydrazide (CP-4):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask, to this 10 ml of glacial acetic acid and 10 ml of absolute alcohol were added followed by the addition of 4-methyl acetophenone (0.280 gm, 2.05 mmol), the reaction mixture was refluxed on an oil bath for 4 hours, after completion of the reaction, the reaction mixture was distilled and then cold water was added to the reaction mass. The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575, 1550, 1375, and 1300. **MS**: $(M+1)^+$ 384.22. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2Ht),

3.01(3Hs), 3.1(2H, t), 5.31(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{29}N_3O_3$ C: 68.90, H: 7.62, N: 10.96, O: 12.52 **Found**: C: 68.70, H: 7.51, N: 10.85, O: 12.43.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-[(3*Z*)-5-methyl-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene] butanoic acid hydrazide (CP-5):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-methyl isatin (0.331 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575, 1550, 1375, and 1300. **MS**: $(M+1)^+$ 411.46. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99 (4H, m), 1.18 (2H, t) 1.20(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2Ht), 3.39(2H, t), 5.31(s, 1H, N*H*), 5.51(s, 1H, N*H*), 8.09 (d, 2H, aromatic), 8.13(d, 1H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{26}N_4O_4$ C: 64.37, H: 6.38, N: 13.65, O: 15.59 **Found**: C: 64.50, H: 6.20, N: 13.81, O: 15.49.

Synthesis of 4-(7,9-dioxo-8-azaspiro[4.5]dec-8-yl)-*N*'-[(1*Z*)-1-phenylethylidenebutanoic acid hydrazide (CP-6):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-methyl acetophenone (0.280 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575, 1550, 1375, and 1300. **MS**: $(M+1)^+$ 384.22. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2Ht), 3.01(3Hs), 3.1(2H, t), 5.31(s, 1H, N*H*), 8.04(d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{29}N_3O_3$ C: 68.90, H: 7.62, N: 10.96, O: 12.52 **Found**: C: 68.70, H: 7.51, N: 10.85, O: 12.43.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-[(1*Z*)-1-(4-nitrophenyl) ethylidine butanoic acid hydrazide (CP-7):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-nitroacetophenone (0.368 gm, 2.05 mmol) and further following the procedure

described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 401.42. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99 (4H, m), 1.18 (2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.39(2Ht), 4.1(2H, t), 4.62(1H, s), 5.31(s, 1H, N*H*), 7.94 (d, 2H, aromatic), 7.99(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{20}H_{24}N_4O_5$ C: 59.99, H: 6.04, N: 13.99, O: 19.98 **Found**: C: 60.09, H: 6.17, N: 13.88, O: 19.86.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-cyclohexylidene butanoic acid hydrazide (CP-8):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of cyclohexanone (0.203 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 348.45. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.55(4H, t), 1.63(2H, m), 1.93(2H, m), 2.29(4H, m), 2.39(2H, t), 2.52(4H, t), 3.1(2H, t), 5.31(s, 1H, N*H*),. **Elemental Analyses Calcd for** $C_{19}H_{29}N_3O_3$ C: 65.68, H: 8.41, N: 12.09, O: 13.81 **Found**: C: 65.66, H: 8.47, N: 12.02, O: 13.85.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-[(3*Z*)-2-oxo-1,2-dihydro-3*H*-indol-3-yliden]butanoic acid hydrazide (CP-9):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of isatin (0.302 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575, 1550, 1525 and 1300. **MS**: $(M+1)^+$ 397.43. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.19(2H, t) 1.23(2H, m), 1.91(2H, m), 2.26(4H, m), 2.37(2Ht), 3.41(2H, t), 5.34(s, 1H, N*H*), 5.55(s, 1H, N*H*), 8.01 (d, 2H, aromatic), 8.05(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{24}N_4O_4$ C: 63.62, H: 6.10, N: 14.13, O: 16.14 **Found**: C: 63.66, H: 6.13, N: 14.11, O: 16. 10.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-cyclopentylidene butanoic acid hydrazide (CP-10):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of cyclopentanone (0.173 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 334.42. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.09(4H, m), 1.21(2H, t) 1.31(2H, m), 1.57(4H, t), 1.90(2H, m), 2.26(4H, m), 2.43(2H, t), 2.57(4H, t), 3.13(2H, t), 5.33(s, 1H, N*H*), **Elemental Analyses Calcd for** $C_{18}H_{27}N_3O_3$ C: 64.84, H: 8.16, N: 12.60, O: 14.40 **Found**: C: 64.76, H: 8.19, N: 12.52, O: 14.53.

Synthesis of 4-(7, 9-dioxo-8-azaspiro[4.5]dec-8-yl)-*N*'-1-(biphenyl)methylidene butanoic acid hydrazide (CP-11):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of benzophenone (0.374 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575, 1550, 1375, and 1300. **MS**: $(M+1)^+$ 432.52. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 3.1(2H, t), 4.62(2H, t), 5.31(s, 1H, N*H*), 7.65(m, 3H, aromatic), 7.79(m, 3H, aromatic), 7.94 (d, 2H, aromatic), 7.99(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{26}H_{29}N_3O_3$ C: 72.37, H: 6.77, N: 9.74, O: 11.12 **Found**: C: 72.40, H: 6.51, N: 9.85, O: 11.24.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-1-(4-aminophenyl)ethylidine butanoic acid hydrazide (CP-12):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-aminoacetophenone (0.280 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3300, 3050, 2900, 1675, 1575 1550, 1525, 1375 and 1300. **MS**: $(M+1)^+$ 415.19. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(2H, s),

2.39(3H, t), 3.5(2H, t), 7.31(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{26}N_4O_5$ C: 60.86, H: 6.32, N: 13.52, O: 19.30 **Found**: C: 60.66, H: 6.47, N: 13.62, O: 19.26.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-[(1*Z*)-1-(4-nitrophenyl) ethylidine butanoic acid hydrazide (CP-13):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide compound (**E**), (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-nitroacetophenone (0.340 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, and 1300. **MS**: $(M+1)^+$ 415.45. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99 (4H, m), 1.19 (2H, t) 1.37(2H, m), 1.91(2H, m), 2.29(4H, m), 2.31(3H, s), 2.33(2Ht), 3.13(2H, t), 5.29(s, 1H, N*H*), 8.01 (d, 2H, aromatic), 8.04(d, 2H, aromatic).. **Elemental Analyses Calcd for** $C_{21}H_{26}N_4O_5$ C: 60.86, H: 6.32, N: 13.52, O: 19.30 **Found**: C: 60.66, H: 6.47, N: 13.62, O: 19.25.

Synthesis of 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-[(1*Z*)-1-(3-nitrophenyl) methylidine butanoic acid hydrazide (CP-14):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide compound **(E)**, (0.5 gm 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 3-nitrobenzaldehyde (0.368 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375 and 1300. **MS**: $(M+1)^+$ 401.42. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.39(2Ht), 3.1(2H, t), 4.5(1H, s) 5.31(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{20}H_{24}N_4O_5$ C: 59.99, H: 6.04, N: 13.99, O: 19.98 **Found**: C: 60.01, H: 6.14, N: 13.88, O: 19.97.

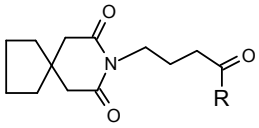
Synthesis of 4-(7, 9-dioxo-8-azaspiro[4.5]dec-8-yl)-*N*'-[(3*Z*)-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]butanoic acid hydrazide (CP-15):

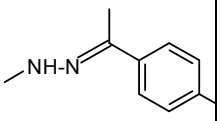
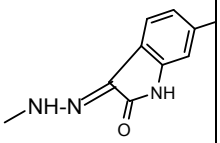
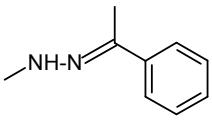
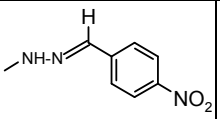
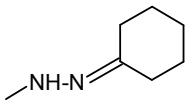
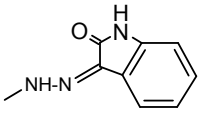
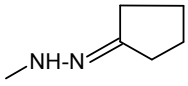
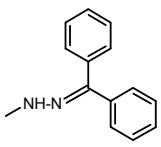
4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl) butanoic acid hydrazide, compound **(E)**, (0.5gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-chloroisatin (0.372 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575, 1550, 1525, and 1300. **MS**: $(M+1)^+$ 431.88. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2Ht), 3.01(3Hs), 3.1(2H, t), 5.28(s, 1H, N*H*) 5.36(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 1H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{23}ClN_4O_4$ C: 58.54, H: 5.38, Cl: 8.23, N: 13.0, O: 14.85.0 **Found**: C: 58.33, H: 5.51, Cl: 8.26, N: 13.03, O: 14.87.

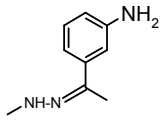
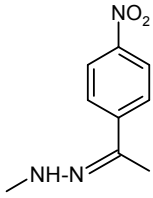
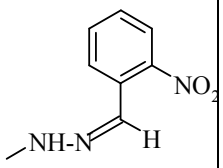
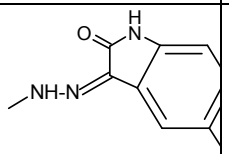
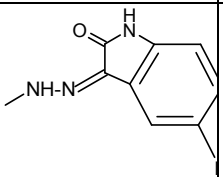
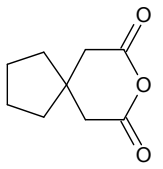
Synthesis of 4-(7, 9-dioxo-8-azaspiro[4.5]dec-8-yl)-*N*'-[(3*Z*)-5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]butanoic acid hydrazide (CP-16):

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid hydrazide, compound **(E)**, (0.5gm, 1.87 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-fluoroisatin (0.339 gm, 2.05 mmol) and further following the procedure described for **CP-4** afforded the title compound.. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575, 1550, 1525 and 1300. **MS**: $(M+1)^+$ 415.43. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(4H, m), 1.18(2H, t) 1.34(2H, m), 1.93(2H, m), 2.29(4H, m), 2.32(3H, s), 2.39(2H, t), 3.01(3Hs), 3.1(2H, t), 5.28(s, 1H, N*H*) 5.36(s, 1H, N*H*), 8.04 (d, 2H, aromatic), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{21}H_{23}CFN_4O_4$ C: 60.86, H: 5.59, F: 4.58, N: 13.52, O: 15.44 **Found**: C: 60.81, H: 5.61, F: 4.56, N: 13.55, O: 15.47.

TABLE: 5.2 PHYSICAL DATA OF 4-(7, 9-DIOXO-8-AZA SPIRO [4.5] DECAN-8-YL)BUTANOIC ACIDS



Comp	R	MF	MW	LOGP[b]	MP (°C)	Rf[a]	%YIELD
CP-2	– OH	$C_{13}H_{19}NO_4$	253.29	0.59	172- 174	0.35	55.61
CP-3	– $NHNH_2$	$C_{13}H_{21}N_3O_3$	267.32	-0.3	208-210	0.69	53.92
CP-4		$C_{22}H_{29}N_3O_3$	383.48	2.42	205-207	0.75	47.52
CP-5		$C_{22}H_{26}N_4O_4$	410.46	1.41	184-187	0.51	35.79
CP-6		$C_{21}H_{27}N_3O_3$	369.45	1.93	218-222	0.49	44.60
CP-7		$C_{20}H_{24}N_4O_5$	400.42	3.04	193-196	0.67	48.75
CP-8		$C_{19}H_{29}N_3O_3$	347.45	2.01	180-183	0.66	43.67
CP-9		$C_{21}H_{24}N_4O_4$	396.43	0.93	192-196	0.56	49.84
CP-10		$C_{18}H_{27}N_3O_3$	333.42	1.46	192-195	0.49	53.48
CP-11		$C_{26}H_{29}N_3O_3$	431.52	3.83	174-177	0.53	66.50

CP-12		$C_{21}H_{28}N_40_3$	384.47	1.13	211-214	0.51	63.29
CP-13		$C_{21}H_{26}N_4O_5$	414.45	3	189-191	0.63	65.45
CP-14		$C_{20}H_{24}N_40_5$	400.42	3.04	201-203	0.69	67.68
CP-15		$C_{21}H_{23}ClN_4O_4$	430.88	1.48	185-187	0.59	57.91
CP-16		$C_{21}H_{23}CFN_4O_4$	414.43	1.08	199-201	0.69	43.23
CP-1		$C_9H_{12}O_3$	168.18	1.2	62-64	0.81	45.12

[a]Mobile phase Ethyl acetate/Hexane (2:8).

[b]LogP was calculated using chem-biooffice software.

Results & Discussion: -

The design of 4-(7, 9-dioxo-8-aza spiro [4.5] decan-8-yl) butanoic acids was based on the literature report in which the introduction of spiromoiety will result in good CNS activity. 4-(7, 9-dioxo-8-aza spiro [4.5] decan-8-yl) butanoic acids were synthesized by a six-step process starting from cyclohexanone. The homogeneity of the compounds was monitored by performing thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were Ethylacetate: Hexane (2:8).

Few compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 [log P > 2] these are **CP-4, CP-7, CP-8, CP-11, CP-14**, **SH-15**. Rest of the compounds in this series possessed log P < 2 since all substituted with polar groups like OH, NH_2 and substituted isatin derivatives.

With regard to the percentage yield of the synthesized compounds, the compounds with substituted aryl aldehydes have yields in the range of 48.7-67.6%, and those with substituted ketones and isatins gave 43.6-66.5% and 35.7-57.9% respectively.

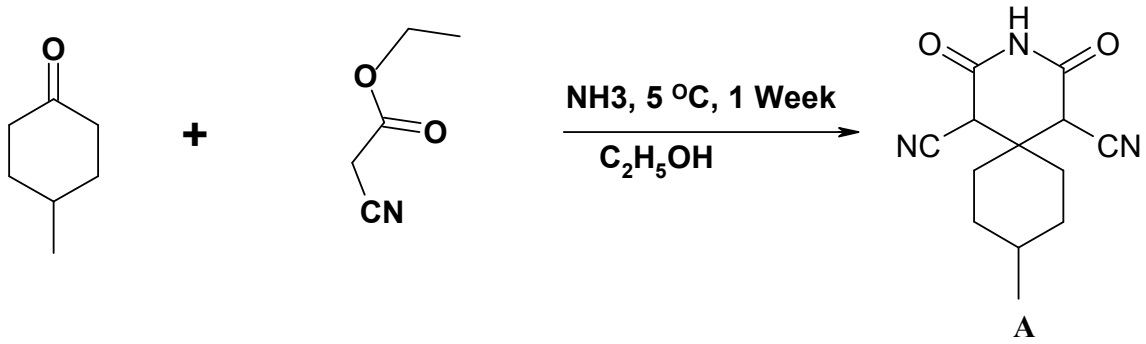
The IR spectrum 4-(7, 9-dioxo-8-azaspiro [4.5] dec-8-yl)-*N*'-1-(4-aminophenyl) ethylidine butanoic acid hydrazide **(CP-12)** was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3400 cm^{-1} showed NH stretching, 3050 cm^{-1} showed aromatic CH stretching, 2900 cm^{-1} showed aliphatic CH stretching, 1675 cm^{-1} showed C=O stretching, 1590 cm^{-1} showed C=N stretching, 1550 $cm^{-1}$and 1500 cm^{-1} showed aromatic C=C stretching and 1300 cm^{-1} showed C–O stretching were observed.

^{1}H-NMR spectrum revealed a multiplet at δ 0.99(4H), triplet at δ 1.18(2H), multiplet at δ 1.34(2H), multiplet at δ 2.29(4H), a singlet at δ 2.32(2H) for 14H of CH_2 protons of the spiromoiety, singlet at δ 2.39 for protons of the methyl group, multiplet at δ 1.93(2H), triplet at δ 2.39(2H), triplet at δ 3.95(2H) for CH_2 protons of GABA, a singlet at δ 5.33(1H, NH) and a singlet at δ 7.52(2H, NH_2) were D_2O exchangeable, two doublets at 8.04(2H) and 8.09(2H) for aromatic protons (Similarly, the structures of other compounds were confirmed according to their characteristic peaks)

The mass spectrum of compound **CP-12** showed a molecular ion peak at m/z 415.19.

SERIES III: 4-(9-METHYL-2, 4-DIOXO-3-AZA SPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS

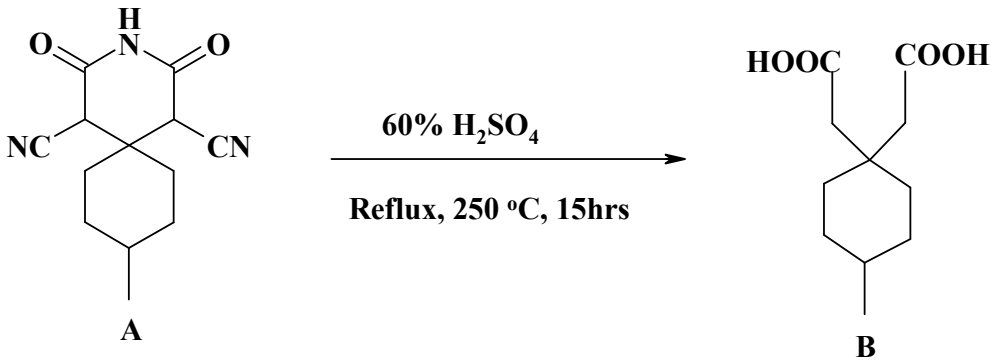
Step-1: Synthesis of 9-methyl-2, 4-dioxo-3-azaspiro [5.5] undecane-1, 5 dicarbonitrile (A):



A

4-Methyl cyclohexanone (30 gm, 267 mmoles) and ethyl cyanoacetate (63.56 gm, 562.47 mmoles) were treated at 0 °C with an excess of ammonia in ethanol and the mixture was stored at 0-5 °C in a stoppered bottle for a period of 1 week. The precipitated ammonium salt was filtered off, pressed and washed several times with alcohol. After the filter cake had stopped dripping, the solid ammonium salt was dissolved in large amount of boiling water; the solution was filtered while hot and acidified with HCl. The free imide was filtered, washed with water and dried to obtain compound **(A)**. 77%, 201-202 ^{o}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 2225, 2175, 1780, 1725, 1450 and 1300. **MS**: (M+1) $^{+}$ 246.12. **^{1}H-NMR** (DMSO-d$_6$): δ ppm 1.12(1H, d), 1.30(4H, d) 1.54(4H, d), 1.61(1H, m), 3.65(2H, s), 9.45(s, 1H, N*H*).

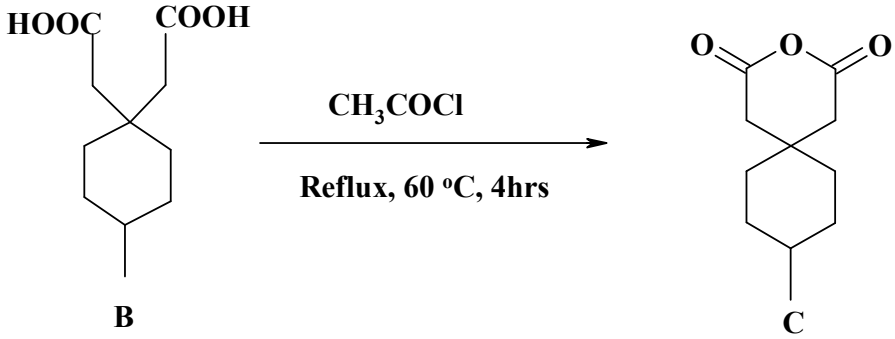
Step-2: Synthesis of 2, 2'-(4-methylcyclohexane-1, 1-diyl)diacetic acid (B):



A B

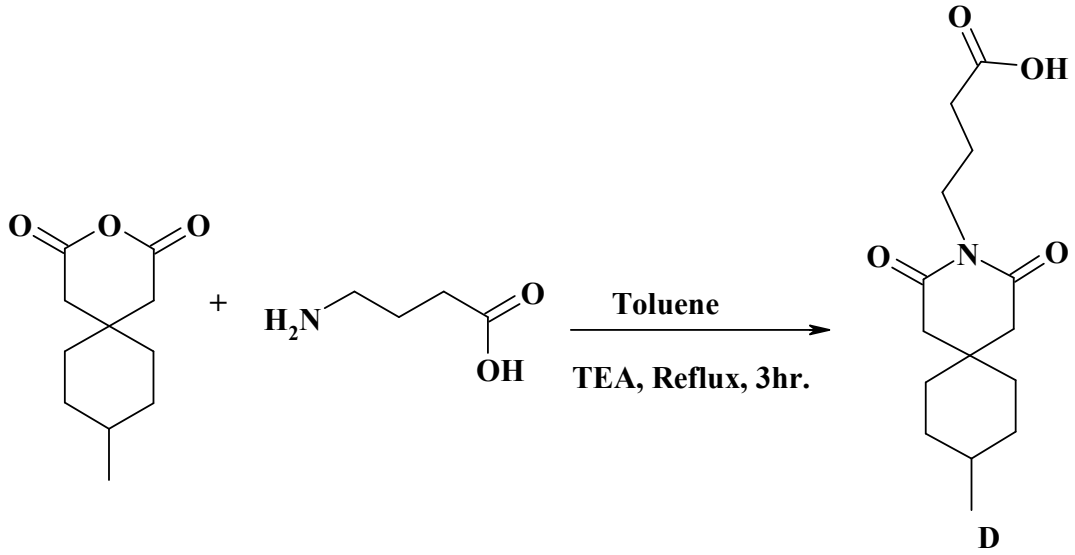
Compound **(A)** (30 gm, 122.44 mmoles) was soaked in sulphuric acid (four times the weight of imide). After standing for 8 hours, water (three times the weight of imide) was added with frequent shaking. The mixture was heated under reflux for 15 hours with intermittent shaking, until frothing had ceased. After the mixture was allowed to cool to room temperature, water was added to dilute the reaction mixture. The crude acid together with charred material was filtered off and washed well with water. The crude reaction product was then suspended in hot water and sufficient potassium bicarbonate was added to dissolve all the acid. After boiling with charcoal, the solution was filtered, acidified with concentrated hydrochloric acid. The precipitate was filtered off, washed with water and dried to obtain compound of formula **(B)**. 45%, 170-172 ^{o}C. **IR** (cm^{-1} KBr): 3200, 3175, 3100, 1710 and 1300. **MS**: $(M+1)^{+}$ 215.12. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.13(3H, d), 1.46(4H, t), 1.48(4H, t), 1.61(1H, m), 2.27(4H, s), 11.25(s, 2H, COOH).

Step-3: Synthesis of 9-methyl-3-oxaspiro [5.5] undecane-2, 4-dione (C), (MC-1):



Compound **(B)** (20 gm, 93.45 mmoles) was mixed with 3 times its weight of acetyl chloride and refluxed for 4 hours. The reaction mixture was cooled to room temperature and crushed ice was added to the reaction mixture. The precipitated solid was filtered off and washed several times with water and dried to obtain Compound **(C)**. 57.89%, 55-57^{o}C. **IR** (cm^{-1} KBr): 3100, 1780, 1720 and 1300. **MS**: $(M+1)^{+}$ 197.11. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.11(3H, d), 1.44(4H, t), 1.47(4H, t), 1.61(1H, m), 2.28(4H, s).

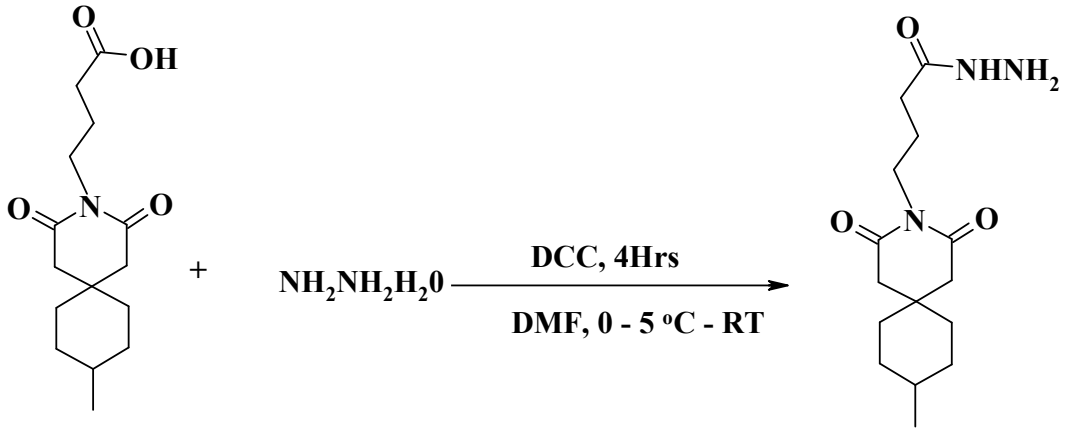
Step-4: Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanoic acid (D), (MC-2).



D

Compound **(C)** (10 gm, 51.02 mmoles) and equimolar quantity of GABA (5.78 gm, 56.12 moles) was refluxed in toluene in the presence of triethylamine for 4 hours in Dean-stark apparatus. The organic solvents were removed in vacuo, water was added to the reaction mixture and acidified with concentrated hydrochloric acid and stirred for 30 minutes, filtered and dried to obtain compound **(D)**. 55.61%, 133-134 ^{o}C. **IR** (cm^{-1} KBr): 3300, 3175, 3100, 1712 and 1300. **MS**: (M+1)$^{+}$ 282.16. **^{1}H-NMR** (DMSO-d$_6$): δ ppm 1.11(3H, d), 1.46(4H, t) 1.54(4H, t), 1.61(1H, s), 1.98(2H, p), 2.25(4H, s), 2.30(2H, t), 4.45(2H, t), 11.16(s, 1H, OH).

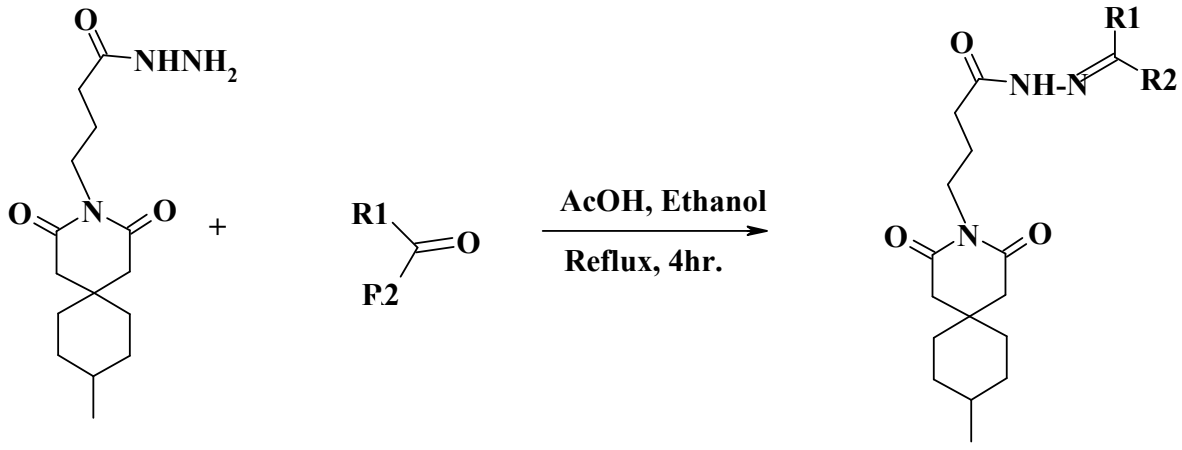
Step-5: Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanehydrazide (E) (MC-3).



D **E**

Compound **(D)** (5 gm, 17.79 mmoles) was dissolved in Dimethyl formamide. The temperature of the reaction mixture was brought down to 0-5°C by placing the RB flask in ice bath. Dicyclohexylcarbodimide was added to the mixture, followed by 10 moles of hydrazine hydrate (8.89 gm, 177.93 mmoles) The reaction mixture was brought to room temperature and stirred for 4 hours. The mixture was filtered off to remove dicyclohexyl urea. The filtrate was extracted with ethyl acetate and concentrated *in vacuo* to obtain compound of formula **(E)**. 53.91%, 165-168 ^{o}C. **IR** (cm^{-1} KBr): 3230, 3100, 2930, 1650, 1450 and 1300. **MS**: (M+1)$^{+}$ 296.19. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.13(3H, d), 1.41(4H, t) 1.52(4H, t), 1.63(1H, m), 1.94(2H, m), 2.15(4H, s), 2.37(2H, t), 4.52(2H, t), 5.19(s, 2H, NH_2), 5.97(s, 1H, NH).

Step-6: Synthesis of 4-(9-methyl-2, 4-dioxo-3-aza spiro [5.5] undecan-3-yl) butanoic acids.



E

(MC-4 to MC-16)

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(4-methyl phenyl)ethylidine butanoic acid hydrazide (MC-4):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask, to this 10 ml of glacial acetic acid and 10 ml of absolute alcohol were added followed by the addition of 4-methylacetophenone (0.251 gm, 1.85 mmol), the reaction mixture was refluxed on an oil bath for 4 hours, after completion of the reaction, the reaction mixture was distilled and then cold water was added to the reaction mass. The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 412.53 **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(1H, d), 1.37(4H, m), 1.57(4H, t), 1.60(4H, m), 1.96(2H, m), 2.32(2H, t), 2.87(3H, s), 3.36(2H, t), 3.57(3H, s), 5.33(s, 1H, N*H*), 8.17(d, 2H, aromatic), 8.19(d, 3H, aromatic). **Elemental Analyses Calcd for** $C_{24}H_{33}N_3O_3$ C: 70.04, H: 8.08, N: 10.21, O: 11.66. **Found**: C: 70.10, H: 8.27, N: 10.12, O: 11.60.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3*Z*)-5-methyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]butanoic acid hydrazide (MC-5):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by

the addition of 5-methylisatin (0.300 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 439.51. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.97(3H, s), 1.01(1H, \m), 1.37(4H, m), 1.57(4H, t), 1.60(4H, m), 1.96(2H, m), 2.32(2H, t), 2.87(3H, s), 3.36(2H, t), 5.33(s, 1H, N*H*), 5.49(s, 1H, NH), 8.11(d, 2H, aromatic), 8.14(d, 1H, aromatic). **Elemental Analyses Calcd for** $C_{24}H_{30}N_4O_4$ C: 65.73, H: 6.90, N: 12.78, O: 14.59 **Found**: C: 65.78, H: 6.85, N: 12.82, O: 14.55.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1*Z*)-1-phenyl ethylidine butanoic acid hydrazide (MC-6):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of acetophenone (0.223 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 398.24. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.90(3H, d), 1.34(1H, m), 1.44(4H, t), 1.53(4H, m), 1.93(2H, t), 2.23(4H, s), 2.39(2H, t), 3.95(2H, t), 5.33(s, 1H, N*H*), 7.93(d, 2H, aromatic), 8.09(d, 3H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{31}N_3O_3$ C: 69.49, H: 7.86, N: 10.57, O: 12.07 **Found**: C: 60.70, H: 6.27, N: 13.42, O: 19.21.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1Z)-1-(4-nitro phenyl)methylidine butanoic acid hydrazide (MC-7):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-nitrobenzaldehyde (0.281 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375, 1350, 1325 and 1300. **MS:** $(M+1)^+$ 429.21. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.90(1H, d), 1.34(4H, m), 1.53(4H, t), 1.57 (4H, m), 1.93(2H, m), 2.51(3H, s), 3.32(2H, t), 5.1(1H, s), 5.31(s, 1H, N*H*), 8.04 (d, 2H, aromatic

), 8.09(d, 2H, aromatic). **Elemental Analyses Calcd for** $C_{22}H_{28}N_4O_5$ C: 61.67, H: 6.59, N: 13.08, O: 18.67. **Found**: C: 61.70, H: 6.57, N: 13.11, O: 18.61.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5]dec-3-yl)-*N*' cyclohexylidene butanoic acid hydrazide (MC-8):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of cyclohexanone (0.186 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 376.20 **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(3H, d), 1.55(4H, q), 1.60(4H, t), 1.64(1H, m), 1.67(2H, p), 1.69(4H, m), 1.93(2H, p), 2.12(4H, s), 2.25(2H, t), 2.36(4H, t), 4.35(2H, t), 5.31(s, 1H, N*H*) **Elemental Analyses Calcd for** $C_{21}H_{33}N_3O_3$ C: 67.17, H: 8.86, N: 11.19, O: 12.78 **Found**: C: 67.23, H: 8.84, N: 11.22, O: 12.71.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3*Z*)-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene] butanoic acid hydrazide (MC-9):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of isatin (0.270 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 439.51. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.01(3H, d), 1.37(4H, q), 1.57(4H, t), 1.60(1H, m), 1.62(4H, s), 1.96(2H, m), 2.32(2H, t), 3.36(2H, t), 5.33(s, 1H, N*H*), 5.49(s, 1H, NH), 7.33(d, 1H, aromatic), 7.5(m, 1H, aromatic), 7.86(d, 2H aromatic). **Elemental Analyses Calcd for** $C_{23}H_{28}N_4O_4$ C: 65.08, H: 6.65, N: 13.20, O: 15.08 **Found**: C: 65.78, H: 6.85, N: 12.82, O: 14.55.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*' cyclopentylidene butanoic acid hydrazide (MC-10):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask

followed by the addition of cyclopentanone (0.160 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 362.47 **^{1}H-NMR** (DMSO-d_6): δ ppm 0.99(3H, d), 1.33(4H, t), 1.39(4H, t), 1.46(4H, q), 1.62(4H, s), 1.64 (1H, m), 1.96(2H, p), 2.38(4H, t), 2.45(2H, t), 4.35(2H, t), 5.36(s, 1H, N*H*) **Elemental Analyses Calcd for** $C_{20}H_{31}N_3O_3$ C: 66.45, H: 8.64, N: 11.62, O: 13.28 **Found**: C: 66.49, H: 8.60, N: 11.52, O: 13.39.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-1-(biphenyl) methylidene butanoic acid hydrazide (MC-11):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl) butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of benzophenone (0.339 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525, 1375, and 1300. **MS**: $(M+1)^+$ 460.57. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.98(3H, d), 1.51 (4H, t), 1.53(4H, q), 1.64(1H, m), 1.95(2H, p), 2.15(4H, s), 2.33(2H, t), 5.35(s, 1H, N*H*), 7.57 (t, 2H, aromatic), 7.58(m, 3H, aromatic), 7.62(3H, m), 7.97(2H, t). **Elemental Analyses Calcd for** $C_{28}H_{33}N_3O_3$ C: 73.18, H: 7.24, N: 9.14, O: 10.44 **Found**: C: 73.25, H: 7.27, N: 9.17, O: 10.31.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1*Z*)-1-(4-aminophenyl) ethylidine butanoic acid hydrazide (MC-12):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-aminoacetophenone (0.251 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525 and 1300. **MS**: $(M+1)^+$ 413.52. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.96(3H, d), 1.49(4H, t), 1.53(4H, q), 1.93(2H, p), 2.14(4H, s), 2.33(2H, t), 2.43(3H, s), 5.91(s, 1H, N*H*), 6.22(s, 1H, NH), 6.98 (q, 2H, aromatic), 7.58(t, 2H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{32}N_40_3$ C: 66.96, H: 7.82, N: 13.58, O: 11.64 **Found**: C: 66.98, H: 7.88, N: 13.48, O: 11.66.

Synthesis of 4-(9-methyl-2, 4-dioxo-3- azaspiro [5.5] dec-3-yl)-*N*'-1-(4-nitrophenyl) ethylidene butanoic acid hydrazide (MC-13):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 4-nitroacetophenone (0.309 gm, 1.86 mmol), and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525 and 1300. **MS**: $(M+1)^+$ 443.53. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.96(3H, d), 1.49(4H, t), 1.53(4H, q), 1.93(2H, p), 2.14(4H, s), 2.33(2H, t), 2.43(3H, s), 5.91(s, 1H, NH), 6.98 (q, 2H, aromatic), 7.58(t, 2H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{30}N_4O_5$ C: 62.43, H: 6.83, N: 12.66, O: 18.08 **Found**: C: 62.55, H: 6.82, N: 12.42, O: 18.21.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1z)-1-(2-nitrophenyl) methylidene butanoic acid hydrazide (MC-14):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 2-nitroacetophenone (0.309 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3050, 2900, 1675, 1575 1550, 1525 and 1300. **MS**: $(M+1)^+$ 443.53. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.96(3H, d), 1.49(4H, t), 1.53(4H, q), 1.93(2H, p), 2.14(4H, s), 2.33(2H, t), 2.43(3H, s), 5.91(s, 1H, N*H*), 7.58(m, 4H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{30}N_4O_5$ C: 62.43, H: 6.83, N: 12.66, O: 18.08 **Found**: C: 62.55, H: 6.82, N: 12.42, O: 18.21.

Synthesis of 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(3*Z*)-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] butanoic acid hydrazide (MC-15):

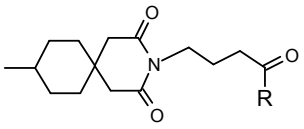
4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-chloroisatin (0.344 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525 and 1300. **MS**: $(M+1)^+$ 459.13. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.97(3H, s), 1.01(1H, m), 1.37(4H, m), 1.57(4H, t), 1.60(4H, m), 1.96(2H, m), 2.32(2H, t), 3.36(2H, t), 5.36(s, 1H, N*H*), 5.52(s, 1H, NH), 8.10(d, 2H, aromatic), 8.12(d, 3H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{27}ClN_4O_4$ C:

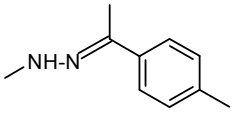
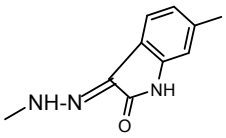
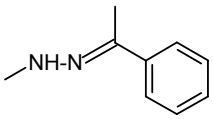
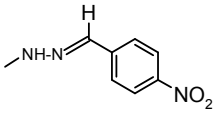
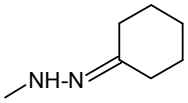
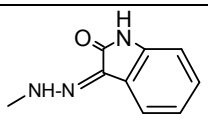
60.19, H: 5.93, Cl: 7.73, N: 12.21, O: 13.94. **Found**: C: 60.23, H: 5.85, Cl: 7.75, N: 12.31, O: 13.95.

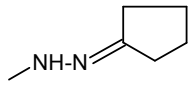
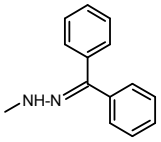
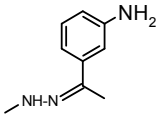
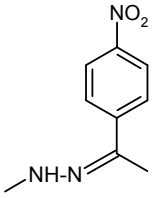
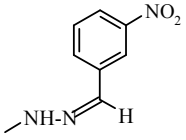
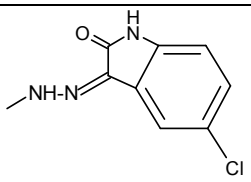
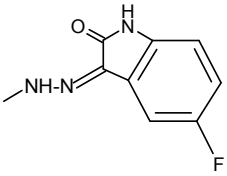
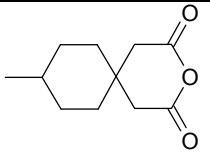
Synthesis of 4-(9-methyl-2,4-dioxo-3-azaspiro [5.5] dec -3-yl)-*N*'-[(3*Z*)-5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]butanoic acid hydrazide (MC-16):

4-(9-Methyl-2,4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoic acid hydrazide, compound **(E)**, Scheme-**5.6** (0.5 gm, 1.69 mmol) was taken in a 100 ml one neck round bottom flask followed by the addition of 5-fluoroisatin (0.307 gm, 1.86 mmol) and further following the procedure described for **MC-4** afforded the title compound. **FT-IR** (cm^{-1} KBr): 3400, 3350, 3050, 2900, 1675, 1575 1550, 1525 and 1300. **MS**: $(M+1)^+$ 443.48. **^{1}H-NMR** (DMSO-d_6): δ ppm 0.97(3H, s), 1.01(1H, m), 1.37(4H, m), 1.57(4H, t), 1.60(4H, m), 1.96(2H, m), 2.32(2H, t), 3.36(2H, t), 5.36(s, 1H, N*H*), 5.52(s, 1H, NH), 8.10(d, 2H, aromatic), 8.12(d, 3H, aromatic). **Elemental Analyses Calcd for** $C_{23}H_{27}FN_4O_4$ C: 62.43, H: 6.15, F: 4.29, N: 12.66, O: 14.46. **Found**: C: 62.33, H: 6.23, F: 4.25, N: 12.67, O: 14.52.

TABLE: 5.3 PHYSICAL DATA OF 4-(9-METHYL-2, 4-DIOXO-3-AZA SPIRO [5.5] UNDECAN-3-YL) BUTANOIC ACIDS



Comp	R	MF	MW	LOGP[b]	MP (°C)	Rf[a]	%YIELD
MC-2	$-OH$	$C_{15}H_{23}NO_4$	281.34	1.34	133-134	0.33	55.61
MC-3	$-NHNH_2$	$C_{15}H_{25}N_3O_3$	295.38	0.9	165-168	0.52	53.91
MC-4		$C_{24}H_{33}N_3O_3$	411.53	3.17	199-200	0.74	47.57
MC-5		$C_{24}H_{30}N_4O_4$	438.51	2.63	178-180	0.31	35.74
MC-6		$C_{23}H_{31}N_3O_3$	397.51	2.68	192-193	0.49	44.67
MC-7		$C_{22}H_{28}N_4O_5$	428.48	3.7	181-184	0.81	48.73
MC-8		$C_{21}H_{33}N_3O_3$	375.50	2.76	195-197	0.72	43.89
MC-9		$C_{23}H_{28}N_4O_4$	424.49	2.3	192-193	0.55	49.80

MC-10		$C_{20}H_{31}N_3O_3$	361.47	2.34	193-194	0.33	53.43
MC-11		$C_{28}H_{33}N_30_3$	459.57	4.58	190-192	0.41	66.52
MC-12		$C_{23}H_{32}N_40_3$	412.52	1.88	175-178	0.45	63.25
MC-13		$C_{23}H_{30}N_4O_5$	442.50	3.66	170-172	0.69	65.89
MC-14		$C_{22}H_{28}FN_40_5$	428.48	3.7	184-185	0.55	67.60
MC-15		$C_{23}H_{27}ClN_4O_4$	458.93	2.7	193-196	0.66	57.95
MC-16		$C_{23}H_{27}N_4O_4$	442.48	2.3	187-188	0.44	43.90
MC-1		$C_{11}H_{16}O_3$	196.24	1.94	55-57	0.8	57.89

[a]Mobile phase Ethyl acetate/Hexane (2:8).

[b]LogP was calculated using chem-biooffice software.

Results & Discussion: -

The design of 4-(9-methyl-2, 4-dioxo-3-aza spiro [5.5] undecan-3-yl) butanoic acids was based on the literature report in which the introduction of spiromoiety will result in good CNS activity.

4-(9-methyl-2, 4-dioxo-3-aza spiro [5.5] undecan-3-yl) butanoic acids were synthesized by a six-step process starting from 4-methyl cyclohexanone. The homogeneity of the compounds was monitored by performing thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were Ethylacetate: Hexane (2:8).

Most of the compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 [log P > 2] except some compounds **MC (1-3)** and **MC-(12)** possessed log P < 2 since all were substituted with polar groups like OH and NH_2.

With regard to the percentage yield of the synthesized compounds, the compounds with substituted aryl aldehydes have yields in the range of 48.7-67.6%, and those with substituted ketones and isatins gave 43.8-66.5% and 35.7-57.9% respectively.

The IR spectrum 4-(9-methyl-2, 4-dioxo-3-azaspiro [5.5] dec-3-yl)-*N*'-[(1*Z*)-1-phenyl ethylidine butanoic acid hydrazide **(MC-6)** was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3400 cm^{-1} showed NH stretching, 3050 cm^{-1} showed aromatic CH stretching, 2900 cm^{-1} showed aliphatic CH stretching, 1675 cm^{-1} showed C=O stretching, 1590 cm^{-1} showed C=N stretching, 1550 $cm^{-1}$and 1500 cm^{-1} showed aromatic C=C stretching and 1300 cm^{-1} showed C–O stretching were observed.

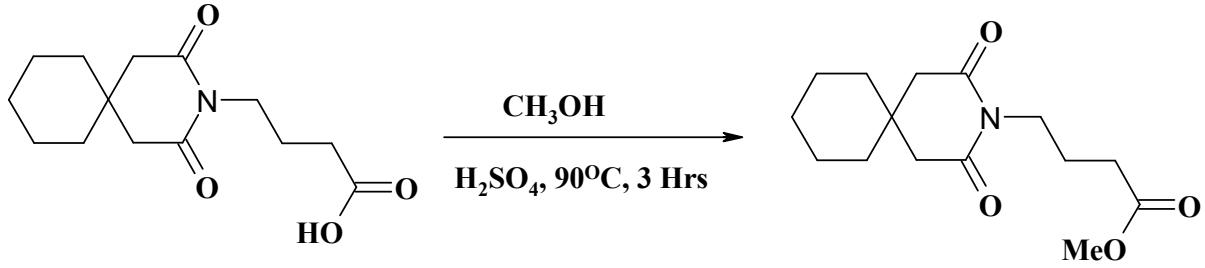
^{1}H-NMR spectrum revealed a doublet at δ 0.90(3H), multiplet at δ 1.34(1H), a triplet at δ 1.44(4H), triplet at δ 1.53(4H), a singlet at 2.23(4H) for 16H of CH_2 protons and methyl group of the spiromoiety, singlet at δ 2.59 for protons of the methyl group, multiplet at δ 1.93(2H), triplet at δ 2.39(2H), triplet at δ 3.95(2H) for CH_2 protons of GABA, a singlet at δ 5.33(1H, NH) was D_2O exchangeable, two doublets at 7.93(2H) and 8.09(3H) for aromatic protons (Similarly, the structures of other compounds were confirmed according to their characteristic peaks)

The mass spectrum of compound **MC-6** showed a molecular ion peak at m/z 398.24.

Series- IV.

Miscellaneous GABA Analogues:

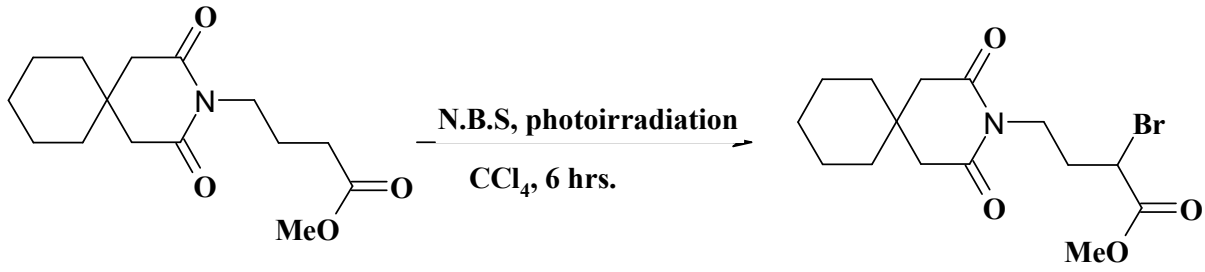
1. Synthesis of methyl 4-(2, 4-dioxo-3-azaspiro[5.5]undecan-3-yl)butanoate (BS-1):



SH-1 **BS-1**

4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanoic acid (**SH-1,** 2 gm, 7.49 mmoles) obtained from scheme-4.1 was dissolved in methanol, concentrated 1 ml of concentrated sulphuric acid was added, the reaction mixture was refluxed for 3 hours, the course of reaction mixture was followed by checking T.L.C, after completion, the reaction mixture was evaporated and then extracted with ethyl acetate The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1675, 1575 and 580. **MS**: $(M+1)^+$ 282.34. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.44(4H, t), 1.46(2H, t), 1.49(4H, q), 2.11(2H, p), 2.17(4H, s), 2.53(2H, t), 3.71(3H, s), 4.31(2H, t). **Elemental Analyses Calcd for** $C_{15}H_{23}NO_4$ C: 64.03, H: 8.24, N: 4.98, O: 22.75. **Found**: C: 64.13, H: 8.27, N: 4.93, O: 22.67.

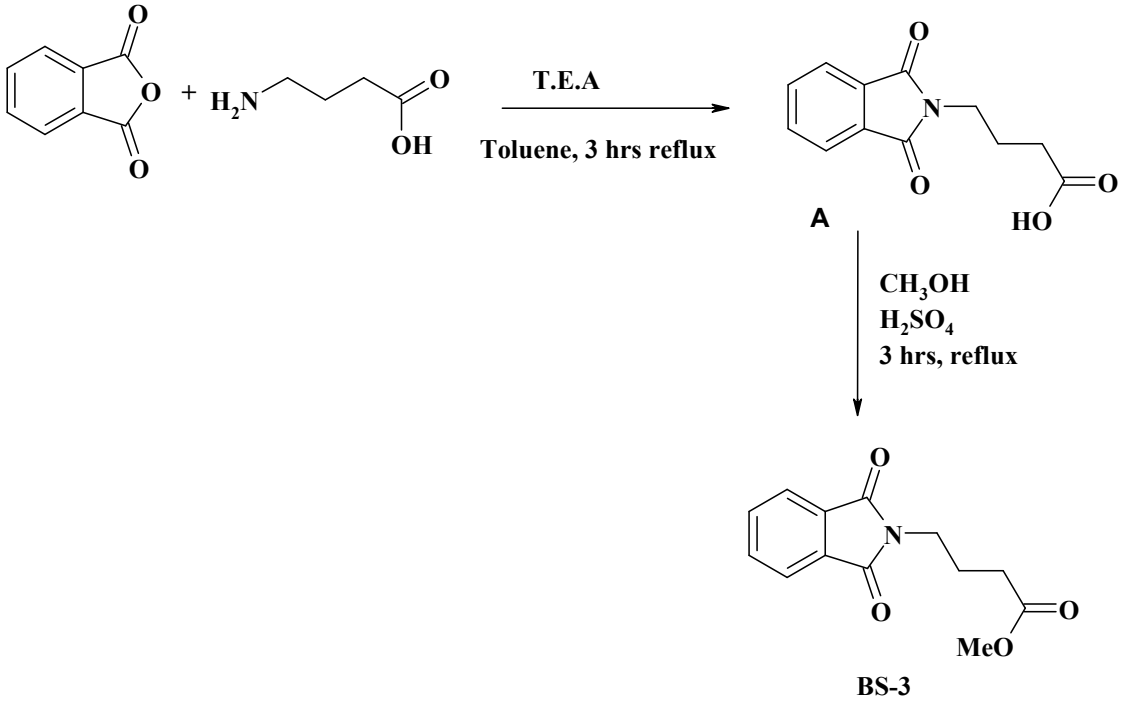
2. Synthesis of methyl 2-bromo-4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanoate (BS-2):



Methyl 4-(2, 4-dioxo-3-azaspiro [5.5] undecan-3-yl)butanoate **(BS-1**, 1 gm 3.55 mmol) was taken in an round bottom flask, carbon tetrachloride was added to dissolve the starting material. N-bromosuccinimide (6.5 gm, 36.63 mmol) was added to the reaction mixture. The reaction mixture was illuminated with 500 W bulb for 6 hours, keeping reaction mixture between 10-30 ^oC. The course of the reaction mixture was monitored by

T.L.C. After completion, the reaction mixture was filtered to remove succinimide, sodium metabisulfite was added and the reaction mixture was extracted with ethyl acetate, evaporated to yield orange solid. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1675, and 580. **MS**: $(M+1)^+$ 361.34, 363.34. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.41(4H, t), 1.49(2H, t), 1.51(4H, q), 2.13(2H, p), 2.15(4H, s), 2.53(2H, t), 3.73(3H, s), 4.35(1H, t). **Elemental Analyses Calcd for** $C_{15}H_{22}BrNO_4$ C: 50.01, H: 6.16, Br: 22.18, N: 3.89, O: 17.77. **Found**: C: 50.08, H: 6.14, Br: 22.22, N: 3.86, O: 17.70.

3. Synthesis of methyl-4-(1, 3-dioxoisoindolin-2-yl)butanoate (BS-3):



STEP: 1: Synthesis of 4-(1,3-dioxoisoindolin-2-yl)butanoic acid:

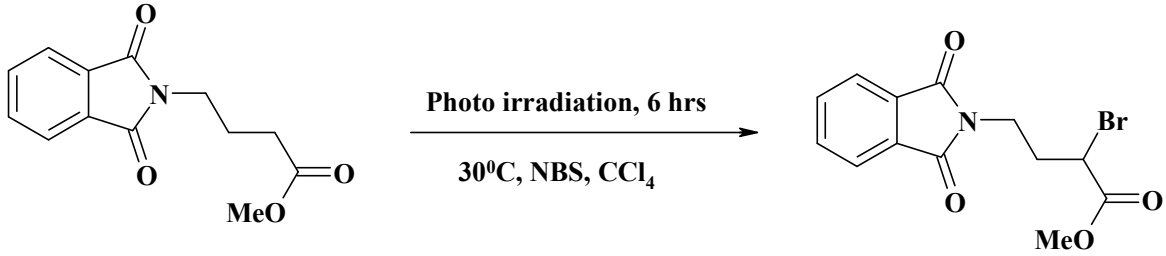
Phthalic anhydride (0.05 M) and GABA (0.05 M) were refluxed in 75 mL of toluene in the presence of the proton scavenger, triethylamine for 3 h. The solvent was then stripped off *in vacuo* and 75 mL of distilled water was added to the concentrate, followed by the addition of concentrated hydrochloric acid. The reaction mixture was then stirred under room temperature for 30 min. The product so formed as a precipitate was filtered under vacuum and dried to afford the title compound. M.P. 115 °C; Yield: 80%. **IR** (cm^{-1} KBr):

3230, 3100, 2950, 1650, 1450, 1380 and 1300. **MS**: $(M+1)^+$ 234.07. **^{1}H-NMR** (DMSO-d_6): δ ppm 2.16(2H, p), 2.30(2H, t) 4.55(2H, t), 7.82-7.88(m, 4H, Aromatic).

STEP: 2: Synthesis of methyl 4-(1, 3-dioxoisoindolin-2-yl)butanoate.

4-(1, 3-dioxoisoindolin-2-yl)butanoic acid (2 gm, 8.57 mmol) was dissolved in methanol (20 ml), concentrated 1 ml of concentrated sulphuric acid was added, the reaction mixture was refluxed for 3 hours, the course of reaction mixture was followed by checking T.L.C, after completion, the reaction mixture was evaporated and then extracted with ethyl acetate. The ethyl acetate layer was evaporated and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3200, 3100, 3050, 1690, 1675 and 1550. **MS**: $(M+1)^+$ 248.24. **^{1}H-NMR** (DMSO-d_6): δ ppm 2.13(2H, p), 2.53(2H, t), 2.11(2H, p), 2.57(2H, t), 3.65(3H, s), 7.87(4H, m). **Elemental Analyses Calcd for** $C_{15}H_{23}NO_4$ C: 64.03, H: 8.24, N: 4.98, O: 22.75. **Found**: C: 64.13, H: 8.27, N: 4.93, O: 22.67.

4. Synthesis of methyl 2-bromo-4-(1, 3-dioxoisoindolin-2-yl)butanoate (BS-4):

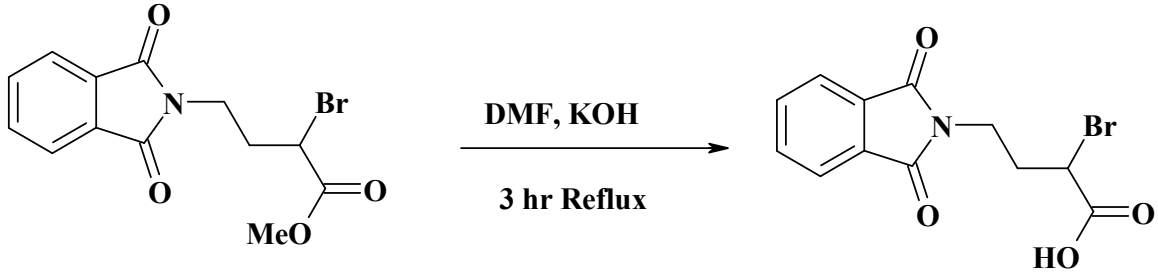


BS-3 **BS-4**

Methyl-4-(1, 3-dioxoisoindolin-2-yl)butanoate **(BS-3,** 1 gm 3.55 mmol) was taken in a round bottom flask, carbon tetrachloride was added to dissolve the starting material. N-bromosuccinimide (7gm, 39.32 mmol) was added to the reaction mixture. The reaction mixture was illuminated with 500 W bulb for 6 hours. The course of the reaction mixture was monitored by T.L.C. After completion, the reaction mixture was filtered to remove succinimide, sodium metabisulfite was added, the reaction mixture was extracted with ethyl acetate and evaporated to yield brown solid. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1550, 1300 and 580. **MS**: $(M+1)^+$ 325.99, 326.99. **^{1}H-NMR** (DMSO-d_6): δ ppm 2.13(2H, p), 3.69(3H, s), 4.23(1H, t), 4.26(2H, t), 7.87(2H, t), 7.90(2H, t). **Elemental Analyses**

Calcd for $C_{13}H_{12}BrNO_4$ C: 47.87, H: 3.71, Br: 24.50, N: 4.29, O: 19.62. **Found**: C: 47.91, H: 3.77, Br: 24.46, N: 4.20, O: 19.66.

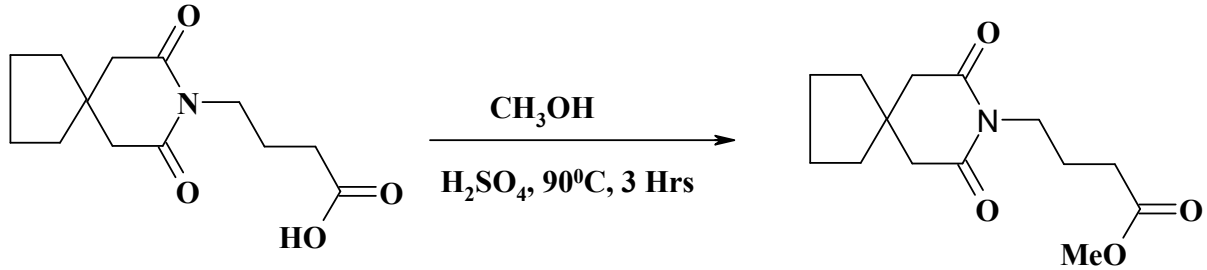
5. Synthesis of 2-bromo-4-(1, 3-dioxoisoindolin-2-yl)butanoic acid (BS-5):



BS-4 **BS-5**

Methyl 2-bromo-4-(1, 3-dioxoisoindolin-2-yl)butanoate **(BS-4,** 1 gm 3.08 mmol) was taken in a round bottom flask, dissolved in DMF, Potassium hydroxide (0.316 gm 7.71 mmol) was added, reaction mixture was refluxed for 3 hours, the course of reaction mixture was monitored by T.L.C. After the completion of reaction, the reaction mixture was extracted with ethyl acetate, the ethylacetate layer was evaporated and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3530, 3100, 2850, 1710, 1550, 1500, 1300 and 580. **MS**: $(M+1)^+$ 313.11, 314.11. **^{1}H-NMR** (DMSO-d_6): δ ppm 2.35(2H, q), 4.26(2H, t), 4.30(2H, t), 7.93(2H, t), 7.98(2H, t), 10.88 (s, 1H, OH). **Elemental Analyses Calcd for** $C_{12}H_{10}BrNO_4$ C: 46.18, H: 3.23, Br: 25.60, N: 4.49, O: 20.50. **Found**: C: 46.22, H: 3.27, Br: 25.58, N: 4.46, O: 20.47.

6. Synthesis of 4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoate (BS-6):

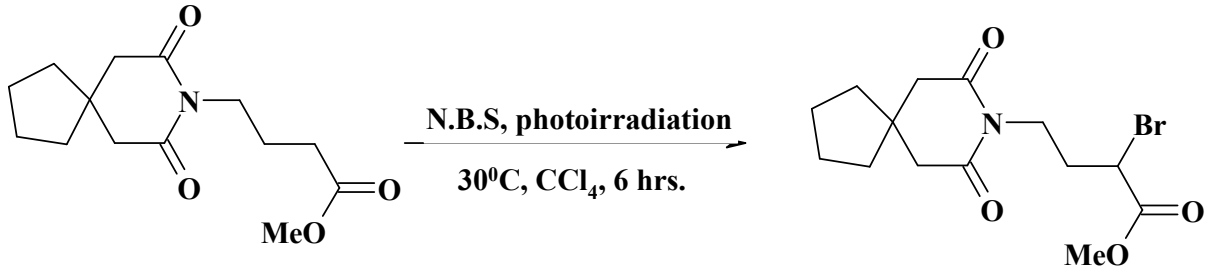


CP-2 **BS-6**

4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid **(CP-2,** 2 gm, 7.90 mmoles) obtained from scheme-4.3 was dissolved in methanol, concentrated 1 ml of concentrated sulphuric acid was added, the reaction mixture was refluxed for 3 hours, the course of

reaction mixture was followed by checking T.L.C, after completion, the reaction mixture was evaporated and then extracted with ethyl acetate The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1675, 1575 and 1550. **MS**: $(M+1)^+$ 268.32. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.44(4H, t), 1.46(4H, t), 2.49(4H, s), 2.11(2H, p), 2.17(2H, t), 3.71(3H, s), 4.31(2H, t). **Elemental Analyses Calcd for** $C_{14}H_{21}NO_4$ C: 62.90, H: 7.92, N: 5.24, O: 23.94. **Found**: C: 62.86, H: 7.97, N: 5.26, O: 23.91.

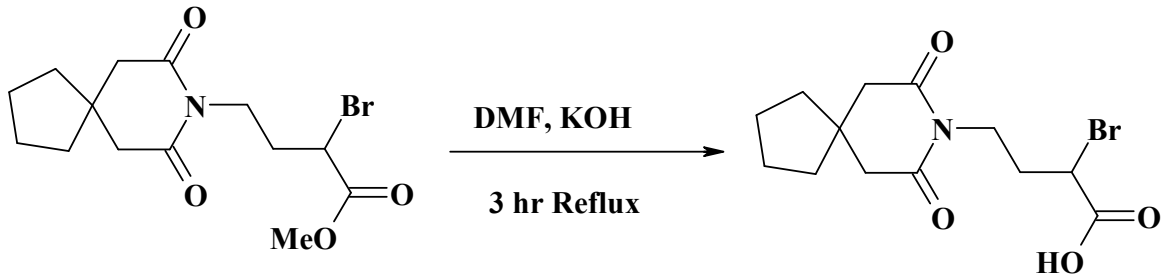
7. Synthesis of 2-bromo-4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoate (BS-7):



BS-6 **BS-7**

Methyl-4-(1, 3-dioxoisoindolin-2-yl)butanoate **(BS-3,** 1 gm 3.55 mmol) was taken in an round bottom flask carbon tetrachloride was added to dissolve the starting material. N-bromosuccinimide (7 gm, 39.52 mmol) was added to the reaction mixture. This reaction mixture was illuminated with 500 W bulb for 6 hours keeping the reaction mixture between 10-30 ^oC. The course of the reaction mixture was monitored by T.L.C. After completion, the reaction mixture was filtered to remove succinimide, sodium metabisulfite was added, the reaction mixture was extracted with ethyl acetate and evaporated to yield orange solid. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1675, 1575 and 580. **MS**: $(M+1)^+$ 347.21, 348.21. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.44(4H, t), 1.51(2H, t), 1.53(4H, s), 2.41(2H, p), 3.71(3H, s), 4.29(2H, t) 4.31(1H, t). **Elemental Analyses Calcd for** $C_{14}H_{20}BrNO_4$ C: 48.57, H: 5.82, Br: 23.08, N: 4.05, O: 18.48. **Found**: C: 48.53, H: 5.87, Br: 23.06, N: 4.13, O: 18.41.

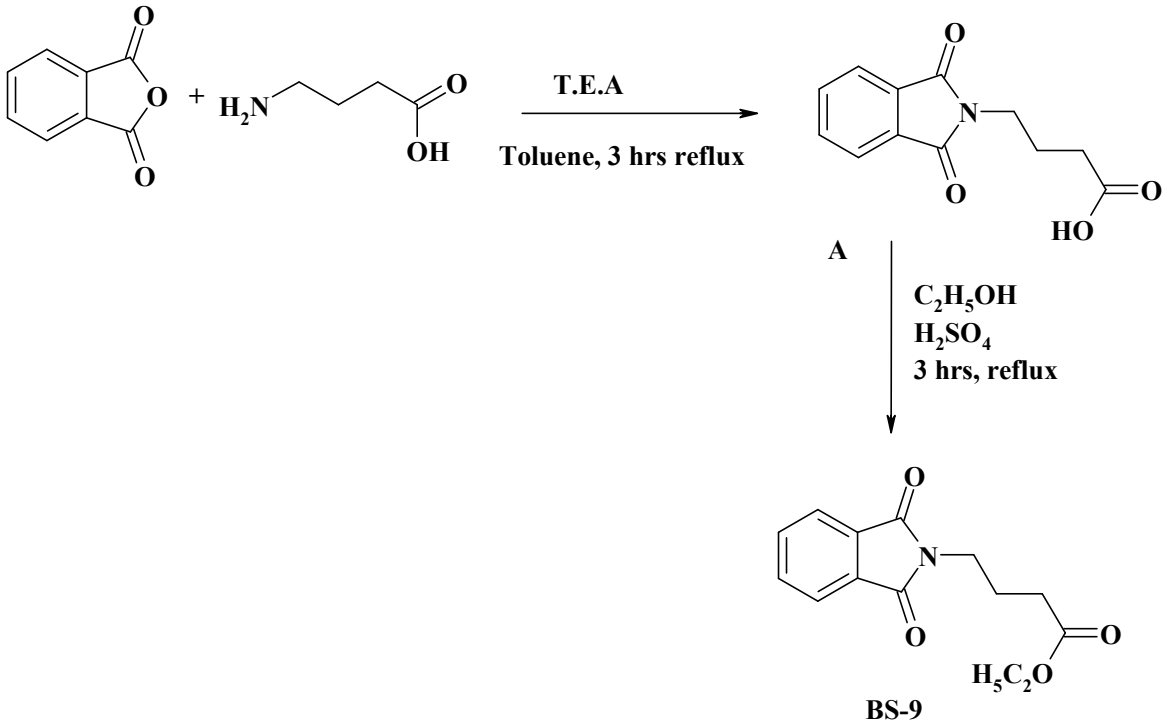
8. Synthesis of 2-bromo-4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid (BS-8):



BS-7 **BS-8**

2-Bromo-4-(7,9-dioxo-8-azaspiro[4.5]decan-8-yl)butanoic acid **(BS-7,** 1 gm 2.89 mmol) was taken in and round bottom flask, dissolved in DMF, Potassium hydroxide (0.296 gm 7.22 mmol) was added, reaction mixture was refluxed for 3 hours, the course of reaction mixture was monitored by T.L.C. After the completion of reaction, the reaction mixture was extracted with ethyl acetate, the ethylacetate layer was evaporated and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3300, 3200, 3100, 1710, 1675 and 580. **MS**: $(M+1)^+$ 333.19, 334.19. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.43(4H, t), 1.49(4H, t), 2.28(4H, s), 2.87(2H, p), 4.20(2H, t), 4.28(1H, t), 11.07(s, 1H, OH). **Elemental Analyses Calcd for** $C_{13}H_{18}BrNO_4$ C: 47.00, H: 5.46, Br: 24.05, N: 4.22, O: 19.27. **Found**: C: 46.92, H: 5.57, Br: 24.12, N: 4.16, O: 19.23.

9. Synthesis of ethyl-4-(1, 3-dioxoisoindolin-2-yl)butanoate (BS-9):



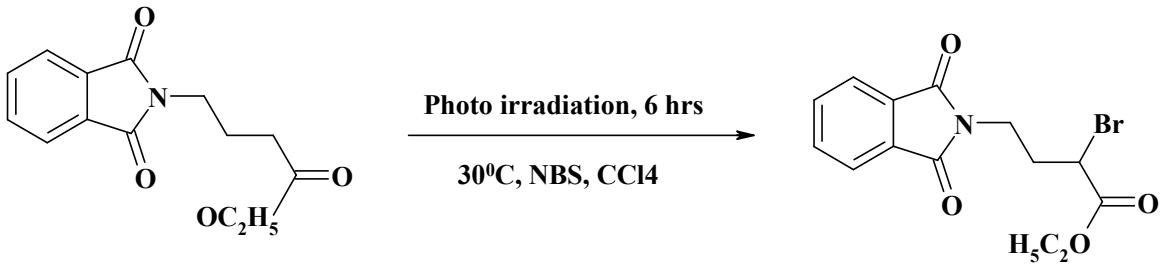
STEP: 1: Synthesis of 4-(1, 3-dioxoisoindolin-2-yl)butanoic acid (A):

As mentioned earlier for compound **BS-3**.

STEP: 2: Synthesis of ethyl 4-(1, 3-dioxoisoindolin-2-yl)butanoate.

4-(1, 3-Dioxoisoindolin-2-yl)butanoic acid was dissolved in ethanol, concentrated 1 ml of concentrated sulphuric acid was added, the reaction mixture was refluxed for 3 hours, the course of reaction mixture was followed by checking T.L.C, after completion, the reaction mixture was evaporated and then extracted with ethyl acetate The obtained precipitate was filtered, washed and dried to afford the title compound. **FT-IR** (cm^{-1} KBr): 3200, 3100, 3050, 1740, 1675 and 1550. **MS**: $(M+1)^+$ 262.27. **^{1}H-NMR** (DMSO-d_6): δ ppm 1.24(3H, t), 2.11(2H, p), 2.49(2H, t), 4.11(2H, q), 4.55(2H, t), 7.83(d, 2H, Aromatic), 7.88(d, 2H, Aromatic). **Elemental Analyses Calcd for** $C_{14}H_{15}NO_4$ C: 64.36, H: 5.79, N: 5.36, O: 24.49. **Found**: C: 64.33, H: 5.77, N: 5.43, O: 24.65.

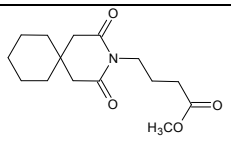
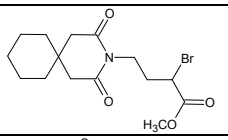
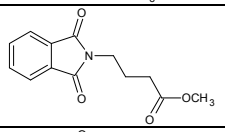
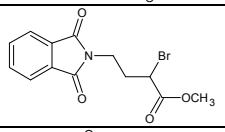
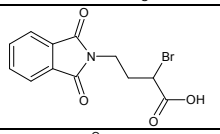
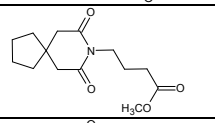
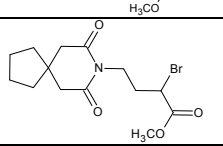
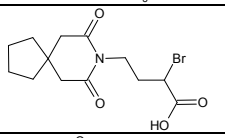
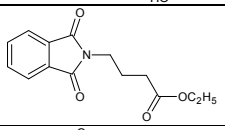
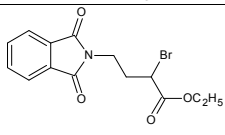
10. Synthesis of ethyl 2-bromo-4-(1, 3-dioxoisoindolin-2-yl)butanoate (BS-10):



BS-9 **BS-10**

Methyl-4-(1, 3-dioxoisoindolin-2-yl)butanoate **(BS-9,** 1 gm 3.81 mmol) was taken in an round bottom flask carbon tetrachloride was added to dissolve the starting material. N-bromosuccinimide(6.79 gm, 38.16 mmol) was added to the reaction mixture. This reaction mixture was illuminated with 500 W bulb for 6 hours keeping the reaction mixture between 10-30 ^{o}C. The course of the reaction mixture was monitored by T.L.C. After completion, the reaction mixture was filtered to remove succinimide, sodium metabisulfite was added and the reaction mixture was extracted with ethyl acetate, evaporated to yield orange solid. **FT-IR** (cm^{-1} KBr): 3200, 3100, 1740, 1675, 1575 and 1550. **MS**: $(M+1)^{+}$ 341.16, 342.16 **^{1}H-NMR** (DMSO-d_6): δ ppm 1.26(3H, t), 2.10(2H, p), 2.51(2H, t), 4.13(2H, q), 4.57(1H, t), 7.85(d, 2H, Aromatic), 7.87(d, 2H, Aromatic) **Elemental Analyses Calcd for** $C_{14}H_{14}BrNO_4$ C: 49.43, H: 4.15, Br: 23.49, N: 4.12, O: 18.81. **Found**: C: 49.33, H: 4.17, Br: 23.41, N: 4.23, O: 18.86.

TABLE: 5.4 PHYSICAL DATA OF BS COMPOUNDS:

Comp	STRUCTURE	MF	MW	LOGP[b]	MP (°C)	Rf[a]	%YIELD
BS-1		$C_{15}H_{23}N_4$	281.34	0.16	134-136	0.81	55.61
BS-2		$C_{15}H_{22}BrNO_4$	360.24	1.53	170-173	0.75	30.21
BS-3		$C_{13}H_{13}NO_4$	247.24	-0.03	95-97	0.79	60.36
BS-4		$C_{13}H_{12}BrNO_4$	321.14	0.49	Sticky solid	0.73	31.69
BS-5		$C_{12}H_{10}BrNO_4$	312.11	1.34	Sticky solid	0.28	35.50
BS-6		$C_{14}H_{21}NO_4$	267.32	-0.26	126-129	0.80	51.18
BS-7		$C_{14}H_{20}BrNO_4$	346.21	0.26	Sticky solid	0.77	33.28
BS-8		$C_{13}H_{18}BrNO_4$	332.19	1.11	Sticky solid	0.32	29.11
BS-9		$C_{14}H_{15}NO_4$	261.27	1.33	140-142	0.75	70.14
BS-10		$C_{14}H_{14}BrNO_4$	340.16	1.19	Sticky solid	0.71	41.12

[a]Mobile phase Ethyl acetate/Hexane (3:7).

[b]LogP was calculated using chem-biooffice software.

Results & Discussion: -

The design of miscellaneous GABA analogues was based on the literature report in which the 2-substitution increases the anticonvulsant potency.

All the compounds were found to be lipophilic indicated by their calculated partition coefficient value less than 2 [log P < 2].

With regard to the percentage yield of the synthesized compounds, esters have yields in the range of 51.18% to70.1% and for 2-substituted bromo derivatives have yields in the range of 29.11 to 41.12.

The IR spectrum of 2-bromo-4-(1, 3-dioxoisoindolin-2-yl)butanoic acid **(BS-5)** was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3530 cm^{-1} showed OH stretching 3100 cm^{-1} showed aromatic CH stretching, 2850 cm^{-1} showed aliphatic CH stretching, 1710 cm^{-1} showed C=O stretching, 1550 & 1500 cm^{-1} showed aromatic C=C stretching, 1300 cm^{-1} showed C–O stretching and 580 cm^{-1} showed C-Br stretching.

^{1}H-NMR spectrum revealed a quartet at δ 2.35, doublet at 4.26 and a triplet at 4.30 for CH_2 protons of GABA, two doublets at δ 7.93 and δ 7.98 for aromatic protons and 1 carboxylic acid proton at 10.88 was D_2O exchangeable.

Similarly, the structures of other compounds were confirmed according to their characteristic peaks depicted in Table-5.4.

The mass spectrum of compound **BS-5** showed peaks at m/z $(M+1)^{+1}$ 313.11 and $(M+1)^{+2}$ 314.11

Chapter-6
Pharmacological Intervention

CHAPTER 6
PHARMACOLOGICAL INTERVENTION

ANTICONVULSANT SCREENING

The anticonvulsant activity of all the synthesized compounds was established by electrical and chemical tests, using standard test protocols. In the preliminary studies, the electrical test employed was MES pattern test and the chemical test was scPTZ test. In order to elucidate the mechanism of anticonvulsant drug action, two second level tests namely, scSTY and scPIC tests were employed. Anticonvulsant activity was examined at 0.5 h and 4 h after drug injections in all the four tests. The minimum doses of the synthesized compounds where by bioactivity was demonstrated in half or more of the mice in MES, scPTZ, scSTY and scPIC tests were determined and compared with those of the standard drugs.

Neurotoxicity of all the synthesized compounds was evaluated by rotarod test after 0.5 h and 4 h of drug administration by i.p. route.

Table 6.1: Anticonvulsant and minimal impairment effects of SH compounds. [SH-(1-16)]

Compound	[a] Intraperitoneal injection in mice									
	MES		scPTZ		scSTY		scPIC		Neurotoxicity	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
SH-1	-	-	-	-	300	300	300	300	-	-
SH-2	-	-	-	-	300	300	300	300	-	-
SH-3	-	-	-	-	100	100	30	100	-	-
SH-4	-	-	-	-	100	300	100	100	-	-
SH-5	-	-	-	-	100	300	100	300	-	-
SH-6	-	-	-	-	300	-	-	-	-	-
SH-7	-	-	-	-	300	300	-	-	-	-
SH-8	-	-	-	-	100	100	30	30	-	-
SH-9	-	-	-	-	300	-	100	100	-	-
SH-10	300	-	-	-	300	-	30	30	300	300
SH-11	300	-	-	-	-	-	100	100	-	-
SH-12	300	-	-	-	300	-	100	100	300	-
SH-13	-	-	-	-	300	-	100	100	-	-
SH-14	-	-	-	-	100	100	10	30	-	-
SH-15	300	-	-	-	300	-	100	300	-	-
SH-16	-	-	-	-	-	-	300	300	-	-
Phenytoin	30	30	-	-	-	-	-	-	100	100
Ethosuximide	-	-	300	-	-	-	-	-	-	-
Diazepam	-	-	-	-	5	5	10	10	-	-

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose where by bioactivity is demonstrated in half or more mice. The animals were examined at 0.5 h and 4 h. The line (-) indicates an absence of effect at the maximum dose tested.

Results:

The series-I comprising of sixteen compounds, thirteen N-Spiro GABA (Spiro moiety is bicyclic with two six membered rings) acid hydrazones, one spiroanhydride, N-Spiro GABA and hydrazide were evaluated for anticonvulsant activity in four animal models of seizure. All of the synthesized compounds were ineffective in the scPTZ test at both the time-points of testing (0.5 h and 4 h). In the MES screen, compounds that showed protection include **SH-10, SH-11**, **SH-12** and **SH-15**.

Compound **SH-14** was found to be effective at the dose of 10 mg/kg at 0.5 hr and 30 mg/kg at 4^{th} hr in scPIC model.

SH-3 was found to be effective at the dose of 30 mg/kg body weight at 0.5 hr where as **SH-8** and **SH-10** were found to be effective at the dose of 30 mg/kg at both the points of testing, 0.5 and 4^{th} hr respectively.

All of the compounds except **SH-11** and **SH-16** were effective in the scSTY-induced seizure threshold test at 100 or 300 mg/kg.

In the neurotoxicity screening, the acid hydrazones except **SH-10** and **SH-12** showed no neurotoxicity at the maximum dose tested (300 mg/kg). **SH-10** was found to be neurotoxic at 0.5 h and 4 h of testing, **SH-14** was found to be neurotoxic at 0.5 h of testing.

Compound **SH-12** exhibited protection in three animal models of seizure, namely MES, scSTY and scPIC tests.

Compound **SH-10, SH-12 and SH-15** were active in the MES and scPIC and scSTY models, while **SH-10** showed neurotoxicity at the dose 300 mg/kg.

Discussion:

In the MES model, a model for grandmal epilepsy **SH-10, SH-11, SH-12** and **SH-15** were found to be active at 300mg/kg which were more potent than two comparators Ethosuximide and Diazepam but less effective when compared to phenytoin.

In scPTZ screen no compound showed protection, which means that the compounds have no affinity to GABA-B receptor.

In scSTY model (This test indicates the involvement of glycinergic pathway in the anticonvulsant action of these compounds) all the compounds except **SH-11** and **SH-16** showed protection. These compounds were effective than the standard drugs Phenytoin and Ethosuximide and less effective than Diazepam.

In scPIC model all the compounds except **SH-6** and **SH-7** were found to be effective. They were effective than the comparators Ethosuximide and Phenytoin but less potent than Diazepam. The efficacy of these compounds is probably due to the affinity to GABA-A receptor and the compounds may follow GABAergic pathway.

SH-14 was found to be equally effective with the standard comparator Diazepam, the compound does not possess neurotoxicity.

Three compounds **SH-10, SH-12** and **SH-15** showed activity in three models (MES, scSTY, scPIC) the potency of these compounds may be a result of isatin substitution.

Table 6.2: Anticonvulsant and minimal impairment effects of CP compounds. [CP-(1-16)]

Compound	[a] Intraperitoneal injection in mice									
	MES		scPTZ		scSTY		scPIC		Neurotoxicity	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
CP-1	-	-	-	-	-	-	300	300	-	-
CP-2	-	-	-	-	300	100	100	-	-	-
CP-3	-	-	-	-	300	300	300	300	-	-
CP-4	-	-	-	-	100	100	100	300	-	-
CP-5	-	-	-	-	100	300	100	30	-	-
CP-6	-	-	-	-	300	-	100	300	-	-
CP-7	-	-	-	-	300	300	30	30	30	30
CP-8	-	-	-	-	-	-	300	-	-	-
CP-9	300	-	-	-	100	100	30	30	100	-
CP-10	-	-	-	-	-	-	-	-	300	-
CP-11	-	-	-	-	300	300	300	-	100	300
CP-12	300	-	-	-	300	300	300	-	-	100
CP-13	-	300	-	-	100	300	30	30	300	100
CP-14	-	-	-	-	300	300	100	100	100	300
CP-15	300	-	-	-	300	300	100	300	30	30
CP-16	-	300	-	-	300	300	300	300	-	-
Phenytoin	30	30	-	-	-	-	-	-	100	100
Ethosuximide	-	-	300	-	-	-	-	-	-	-
Diazepam	-	-	-	-	5	5	10	10	-	-

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose where by bioactivity is demonstrated in half or more mice. The animals were examined at 0.5 h and 4 h. The line (-) indicates an absence of effect at the maximum dose tested.

Results:

The series-II comprising of sixteen compounds, thirteen N-spiro GABA (Spiro moiety is bicyclic with one six membered ring and one five membered ring) acid hydrazones, one spiroanhydride, N-spiro GABA and hydrazide were evaluated for anticonvulsant activity in five animal models of seizure. All of the synthesized compounds were ineffective in the scPTZ test at both the time-points of testing (0.5 h and 4 h). In the MES screen, a model for grandmal seizures compounds that showed protection included **CP-9, CP-12, CP-13, CP-15,** and **CP-16**.

Compounds **CP-7, CP-9** and **CP-13** were found to be effective at the dose of 30 mg/kg at both the points of testing 0.5 hr and 4^{th} hr30 mg/kg in scPIC model.

CP-5 was found to be effective at the dose of 30 mg/kg body weight at 4^{th} hr where in scPIC model.

All the compounds except **CP-1, CP-8** and **CP-10** were effective in the scSTY-induced seizure threshold test at 100 or 300 mg/kg.

In the neurotoxicity screening, More than half of the compounds in this were found to be neurotoxic, **CP-7, CP-9, CP-10, CP-11, CP-12, CP-13, CP-14** and **CP-15.**

CP-9, CP-12 and CP-15 exhibited protection in three animal models of seizure, namely MES, scSTY and scPIC tests.

CP-9, CP-12 and **CP-15** were active in the MES and scPIC and scSTY models, while SH-10 showed neurotoxicity at the dose 300 mg/kg.

Discussion:

In MES screen, a model for grandmal epilepsy, five compounds **CP-9, CP-12, CP-13, CP-15,** and **CP-16** were found to be effective than the standard comparators Ethosuximide and Diazepam. The efficacy of these compounds may be due to the blockade of excitatory pathway like sodium ion channel.

All these compounds except **CP-16** were found to be neurotoxic.

None of the compounds were found to be effective in scPTZ screen, indicating that there is no involvement of glycinergic pathway.

In scSTY screen all compounds except **CP-1, CP-8** and **CP-10** were found to be effective. Compounds **CP-3, CP-6, CP-7, CP-11, CP-12, CP-14, CP-15** and **CP-16** were found to be effective than one comparator phenytoin, equally effective than Ethosuximide but less effective than Diazepam.

CP-4, CP-5, CP-9 and **CP-13** showed good protection than Ethosuximide, **CP-4** and **CP-5** does not have neurotoxicity at the maximum dose tested.

In scPIC model, **CP-5, CP-9** and **CP-13** showed protection at 30mg/kg, they were effective than the comparators Phenytoin and Ethosuximide and less effective than Diazepam. **CP-5** with methyl isatin substitution is devoid of neurotoxicity.

CP-9, CP-12 and **CP-15** were active in three models (MES and scPIC and scSTY) this may be due to multiple mechanisms. The potency may be due to the mediation of GABA receptors or inhibition of excitatory neurotransmitters.

Table 6.3: Anticonvulsant and minimal impairment effects of MC compounds. [MC-(1-16)]

Compound	[a] Intraperitoneal injection in mice									
	MES		scPTZ		scSTY		scPIC		Neurotoxicity	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
MC-1	-	-	-	-	-	-	300	300	-	-
MC-2	-	-	-	-	300	300	100	100	-	-
MC-3	-	-	-	-	-	-	300	300	300	-
MC-4	-	-	-	-	100	300	100	100	-	-
MC-5	-	-	-	-	300	100	100	100	30	-
MC-6	-	-	-	-	-	-	100	100	-	-
MC-7	-	-	-	-	100	100	10	10	-	-
MC-8	-	100	-	-	-	-	300	300	-	-
MC-9	-	-	-	-	100	100	30	30	-	-
MC-10	300	300	-	-	-	-	300	300	300	-
MC-11	-	300	-	-	300	-	100	100	-	-
MC-12	-	-	-	-	300	300	100	100	300	-
MC-13	-	-	-	-	300	300	30	30	300	300
MC-14	-	-	-	-	-	-	30	100	100	-
MC-15	-	-	-	-	100	100	300	300	-	-
MC-16	-	-	-	300	-	-	300	300	300	-
Phenytoin	30	30	-	-	-	-	-	-	100	100
Ethosuximide	-	-	300	-	-	-	-	-	-	-
Diazepam	-	-	-	-	5	5	10	10	-	-

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose where by bioactivity is demonstrated in half or more mice. The animals were examined at 0.5 h and 4 h. The line (-) indicates an absence of effect at the maximum dose tested.

Results:

The series-III comprising of sixteen compounds, thirteen N-Spiro GABA (Spiro moiety is bicyclic with two six membered rings in which one ring has a methyl side chain) acid hydrazones, one spiroanhydride, N-Spiro GABA and hydrazide were evaluated for anticonvulsant activity in five animal models of seizure. All of the synthesized compounds were ineffective in the scPTZ test at both the time-points of testing (0.5 h and 4 h). In the MES screen, a model for grandmal seizures compounds that showed protection included **MC-8, MC-10 and MC-11.**

Compound **MC-7** was found to be effective at the dose of 10 mg/kg at 0.5 hr and 30 mg/kg at 4^{th} hr in scPIC model.

MC-14 was found to be effective at the dose of 30 mg/kg body weight at 0.5 hr where as **MC-9** and **MC-13** were found to be effective at the dose of 30 mg/kg at both the points of testing, 0.5 and 4^{th} hr respectively.

Half of the compounds **(MC-2, MC-4, MC-5, MC-7, MC-9, MC-11, MC-12, MC-13 and MC-15)** were effective in the scSTY-induced seizure threshold test at 100 or 300 mg/kg. This test indicates the involvement of glycinergic pathway in the anticonvulsant action of these compounds.

In the neurotoxicity screening, the acid hydrazones except **MC-3, MC-5, MC-10, MC-12, MC-13, MC-14** and **MC-16** showed no neurotoxicity at the maximum dose tested (300 mg/kg).

Compound **MC-11** exhibited protection in three animal models of seizure, namely MES, scSTY and scPIC tests.

Discussion:

In MES screen, a model for grandmal seizures three compounds (**MC-8, MC-10** and **MC-11**) were found to be effective than Ethosuximide and Diazepam but less effective than the comaparator Phenytoin. **MC-8** and **MC-10** has cyclic aliphatic ring substitution and **MC-11** has aromatic bicyclic substitution.

In scPTZ screen **MC-16** (fluoro-isatin substitution) was more effective than phenytoin and diazepam and equally effective to the other comparator Ethosuximide.

Half of the compounds in this series showed protection in scSTY model and were effective than the standards Phenytoin and Ethosuximide but less effective than Diazepam.

All the compounds in this series showed protection in scPIC model indicating that these compounds mediate GABA-A receptors and they follow GABAergic pathway. They were more effective than Phenytoin and Ethosuximide and less effective than Diazepam.

One compound in this series with 4-nitrophenyl substitution **(MC-7)** showed protection at 10mg/kg, equally effective to Diazepam.

Table 6.4: Anticonvulsant and minimal impairment effects of BS compounds. [BS-(1-10)]

Compound	[a] Intraperitoneal injection in mice									
	MES		scPTZ		scSTY		scPIC		Neurotoxicity	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
BS-1	-	-	-	-	300	300	100	100	300	-
BS-2	-	-	-	-	300	300	100	300	-	-
BS-3	-	-	-	-	100	100	100	100	100	-
BS-4	-	-	-	-	100	-	100	100	-	-
BS-5	-	-	-	-	100	300	100	100	300	-
BS-6	-	-	-	-	-	-	100	100	-	-
BS-7	-	-	-	-	300	-	300	100	-	-
BS-8	-	-	-	-	-	-	100	300	-	-
BS-9	-	-	-	-	100	100	100	300	-	-
BS-10	-	-	-	-	100	100	100	300	-	-
Phenytoin	30	30	-	-	-	-	-	-	100	100
Ethosuximide	-	-		300	-	-	-	-	-	-
Diazepam	-	-	-	-	5	5	10	10	-	-

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose where by bioactivity is demonstrated in half or more mice. The animals were examined at 0.5 h and 4 h. The line (-) indicates an absence of effect at the maximum dose tested.

Results:

The series-IV comprising of ten compounds, were evaluated for anticonvulsant activity in four animal models of seizures. All of the synthesized compounds were ineffective in the scPTZ and MES tests at both the time-points of testing (0.5 h and 4 h).

All of the compounds) were effective in the scPIC-induced seizure threshold test at 100 mg/kg or 300 mg/kg.

All of the compounds except **BS-6** and **BS-8** were effective in the scSTY-induced seizure threshold test at 100 or 300 mg/kg. This test indicates the involvement of glycinergic pathway in the anticonvulsant action of these compounds.

In the neurotoxicity screening, the acid compounds except **BS-1, BS-3** and **BS-5** showed no neurotoxicity at the maximum dose tested (300 mg/kg).

All Compounds except **BS-6** and **BS-8** exhibited protection in two animal models of seizure, namely scSTY and scPIC tests.

Discussion:

All the compounds in this series were ineffective in MES and scPTZ models at the maximum dose tested.

All the compounds except, **BS-6** and **BS-8** were found to be effective than Phenytoin and Ethosuximide in scPIC and scSTY models. They were less effective than Diazepam.

Overall with respect to effectiveness, the series-1 [**SH-1-16**] were more potent than other acid hydrazones in MES, scSTY and scPIC with less neurotoxicity.

Chapter-7
Structure-Activity Relationship studies

CHAPTER 7
STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

SERIES-I [(SH-(1-16)]:

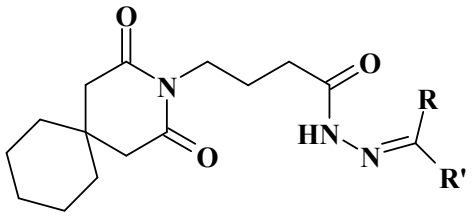


Fig. 14

The compounds with isatinimino group **(SH-10 & SH-12)** showed a broad spectrum of activity being protective against MES, scSTY and scPIC models. Similarly compound with p-aminoacetophenone **(SH-15)** also exhibited activity in the above three models. Hence **SH-10**, **SH-12** & **SH-15** were better than other compounds. With respect to neurotoxicity, **SH-10** was more neurotoxic, **SH-12** was neurotoxic for a shorter period and **SH-15** was devoid of neurotoxicity at the maximum dose tested. Thus **SH-15** emerges as the most effective compound in this series with respect to broad spectrum anticonvulsant action and no neurotoxicity.

At the imino terminal, the benzaldehyde derivatives **(SH-8 & SH-14)** were more effective than the acetophenone derivatives **(SH-9 & SH-6)**. This indicates that carbino hydrogen is crucial and substitution with methyl group is not favourable for anticonvulsant activity.

The general order for anticonvulsant activity with respect to the iminoterminal is as follows, benzaldehyde derivatives > isatinimino derivatives > acetophenone derivatives > cyclo alkanones.

SERIES-II [(CP-(1-16)]:

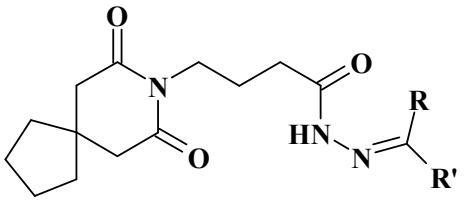


Fig. 15

The compounds **CP-9**, **CP-15** and **CP-16** with isatinimino group showed a broad spectrum of activity being protective against MES, scSTY and scPIC models. Similarly acetophenone derivatives 3-aminoacetophenone **(CP-12)**, 4-nitroacetophenone **(CP-13)** showed broad spectrum activity.

With respect to neurotoxicity **CP-15** was found to be neurotoxic at the lowest dose tested. **CP-9** was neurotoxic for a shorter period of time and **CP-16** was devoid of neurotoxicity.

CP-16 emerges as the most effective compound in this series with respect to broad spectrum anticonvulsant action and no neurotoxicity.

At the iminoterminal, acetophenone derivatives **(CP-4, CP-5 & CP-6)** were most effective than the benzaldehyde derivatives **(CP-7 & CP-14)** which indicates that carbino phenyl group is crucial and substitution with nitro or amino group is favourable for activity.

The general order for anticonvulsant activity with respect to the imino terminal is as follows, acetophenone derivatives > isatinimino derivatives > benzaldehyde derivatives >cyclo alkanones.

SERIES-III [(MC-(1-16)]:

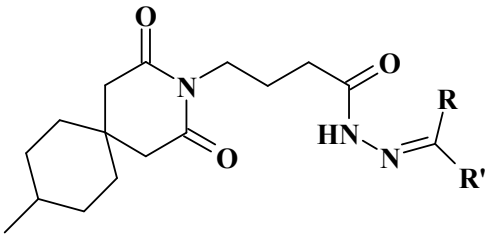


Fig. 16

The compound with benzophenone group **(MC-11)** showed broad spectrum anticonvulsant activity being protective against MES, scSTY and scPIC models.

Three compounds **MC-7** (4-nitrobenzaldehyde)**, MC-9** (isatin) & **MC-13** (4-nitroaceto phenone) were better than other compounds these compounds were active in two models at low doses. With respect to neurotoxicity **MC-13** was neurotoxic at the highest dose tested, **MC-7 & MC-9** were devoid of neurotoxicity.

Thus **MC-11** emerges as the most effective compound in this series with respect to broad spectrum anticonvulsant action and no neurotoxicity.

At the imino terminus benzaldehyde derivatives were more effective than the acetophenone derivatives. This indicates that the carbino hydrogen is crucial and substitution with methyl group is not favourable for activity.

The general order for anticonvulsant activity with respect to the iminoterminal is as follows,

benzaldehyde derivatives > isatinimino derivatives > acetophenone derivatives > cyclo alkanones.

SERIES-IV [(BS-(1-10)]:

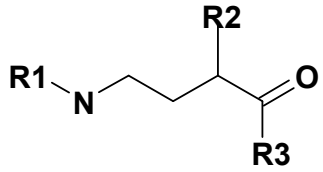


Fig. 17

The compounds with ethoxy group **(BS-9 & BS-10)** at the carboxylic acid terminal showed better anticonvulsant activity in two animal models (scSTY & scPIC) at both the time intervals. These two compounds emerged as the most active compounds in this series without neurotoxicity.

At the carboxy terminal, ethoxy substituted derivatives **(BS-9 & BS-10)** were more effective than the methoxy derivatives. **(BS-1 to BS-4 & BS-6 to BS-7)**

At the second position substitution with bromine found to be beneficial with **BS-2, BS-4** & **BS-7** as these compounds were effective in two animal models (scSTY & scPIC) without neurotoxicity.

The general order for anticonvulsant activity with respect to N-terminal is as follows.

N-phthaloyl derivatives > spiro [4, 5] decane derivatives > spiro [5, 5] undecane derivatives.

Over all the order of anticonvulsant activity was SH series > MC series > CP series > BS series.

Chapter-8
Summary and conclusion

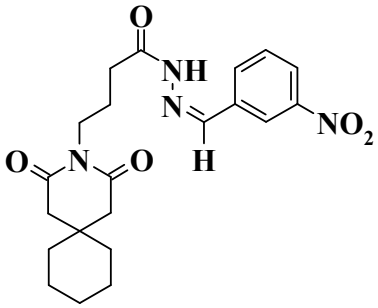
CHAPTER 8
SUMMARY & CONCLUSIONS

The present work was aimed at the design and synthesis of newer N-Spiro derivatives of the inhibitory neurotransmitter GABA effective in the treatment of epilepsy.

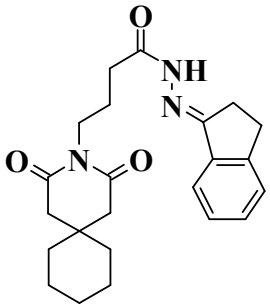
Various lipophilic analogues of N-spiroGABA amides and hydrazones were synthesized and evaluated for their anticonvulsant activities in animal models of epilepsy.

- The structures of the synthesized compounds were confirmed by the use of their spectral data besides elemental analysis.
- The present work provides the first report on Spiro GABA derivatives.
- Of a total of fifty eight compounds synthesized, only ten compounds exhibited activity in the MES test.
- In the scPTZ model, a test used to identify compounds that elevate seizure threshold, none of the compounds showed activity.
- In the scSTY-induced seizure threshold test, a total of fourty two compounds were found to be active.
- In the scPIC-induced seizure threshold test, most of the compounds exhibited activity indicative of the possible involvement of GABA-mediation in the anticonvulsant action. In particular, a total of fifty-five compounds were observed to be active in the scPIC model.
- In the acute neurotoxicity screen, most of the **CP**-series derivatives few compounds of **MC**- series were neurotoxic at the anticonvulsant dose.
- Overall, with respect to effectiveness and neurotoxicity **SH**-series derivatives **[(SH-1-16)]** were more potent and less neurotoxic than other series of compounds.

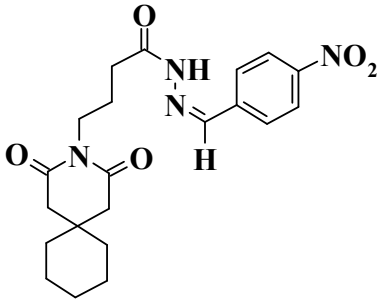
Structures of most active compounds:



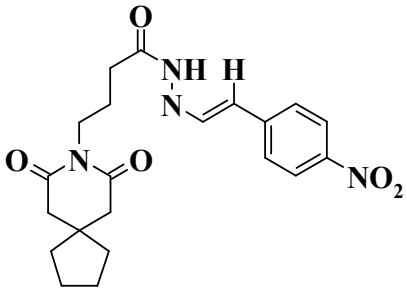
SH-8



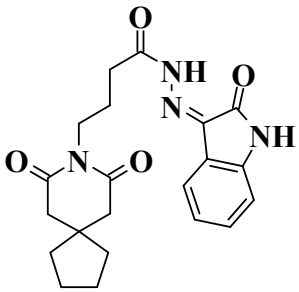
SH-10



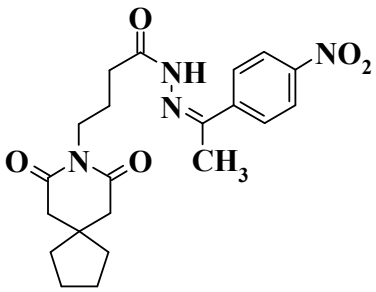
SH-14



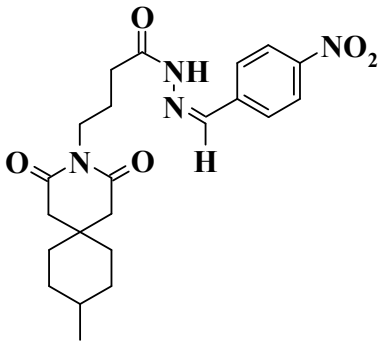
CP-7



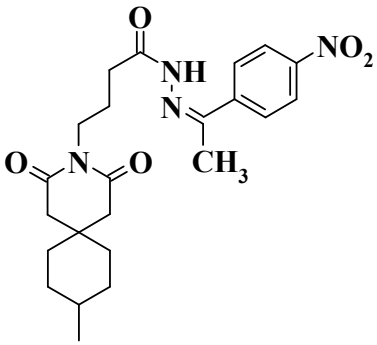
CP-9



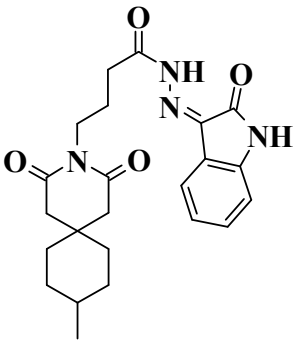
CP-13



MC-7



MC-13



MC-9

FUTURE PERSPECTIVES

The present work gave rise to novel Spiro-GABA derivatives possessing anticonvulsant activities in various animal models of epilepsy. Although all of the synthesized compounds have been found to possess a broad spectrum of activity, extensive studies are still required to confirm the hypothesized mechanisms of action of the compounds. In particular, molecular level studies involving whole-cell patch clamp experiments, radio-ligand binding studies and pharmacokinetic studies to determine bioavailability etc., should be undertaken.

Future studies should involve mechanism-specific models namely, scBIC-induced seizure model, NMDA and kainate-induced seizure models etc. Because the compounds are all derivatives of GABA, studies involving the estimation of the level of GABA in whole brain and in different parts of the brain tissue should be carried out.

Several other spiroderivatives can be synthesized employing the same scheme, or by slight changes in the synthetic procedures.

References

REFERENCES

1. Foye W., "Principles of medicinal chemistry" 5th ed. David A. Williams, Thomas l. lemke, 1996.
2. Jose E. C and **Frank L,** "Seizures and Epilepsy: Overview and Classification", 1996-2005, www. eMedicine.com.
3. Bernard S. Chang, M.D., and Daniel H. Lowenstein, M.D., "Epilepsy", N Engl J Med., 349, 1257- 1266, 2003.
4. Agarwal P, Mehndiratta M. M, Antony A. R, Kumar N, Dwivedi R. N, Sharma P, Kumar S., "Epilepsy In India: Nupitality behavior and fertility", Seizure., 15, 409-415, 2006.
5. Simon P. Aiken and William M. Brown., "Treatment of Epilepsy: Existing therapies and future developments", Frontiers in Bioscience. 5, e124-152, 2000.
6. Yogeeswari P, Ragavendran J. V, Thirumurugan R, Saxena A, Sriram D, "Ion Channels as Important Targets for Antiepileptic Drug Design", Current Drug Targets., 5, 1-13, 2004.
7. Acharya N. J., "Recent advances in epileptogenesis", Current science., 82, 679-688, 2002.
8. Browne T. R and Holmes G. L, "Handbook of Epilepsy" 4th ed. Lippincott Williams & Wilkins 2008.
9. *Bromfield E. B***,** Cavazos J. E, Sirven J. I. "An introduction to Epilepsy" American *Epilepsy* Society, 2006.
10. "Basic Mechanisms Underlying Epilepsy", American Epilepsy Society, http://www.aesnet.org/go/professional-development/educational-opportunities/epilepsy-education-program/basic-mechansims-of-epilepsy.
11. Nair D. R., "Epilepsy" The Cleveland Clinic Center for Continuing Education 2000-2008, http://www.clevelandclinicmeded.com/medicalpubs/disease management/neurology/epilepsy/
12. Gulli L.F., Gale A. M., Encyclopedia of Neurological Disorders, **2005,** http://www.healthline.com/adamcontent/seizures.

13. Engel J. R and Pedley T. A., “Epilepsy A Comprehensive Textbook”, Lippincott-Raven, Philadelphia; 1998.
14. Ferrendelli J.A, Aldenkamp A. P, Sander J. W, White H. S, Wilder B. J, “Emerging therapies for early seizure control in epilepsy”, Ortho-Mcneil Pharmaceuticals., 1-34, 2000.
15. Levy R. H, Meldrum B. S, Mattson R. H, Perucca E, “Antiepileptic drugs” Lippincott Williams and wilkins, 2006.
16. Benbadis S. R, “Epileptic seizures and syndromes”, Neurol. Clin., 19, 251-270, 2001.
17. Selim R. Benbadis, Pierre Thomas and Greg Pontone, “A prospective comparison between two seizure classifications”, Seizure., 10: 247–249, 2001.
18. Luders H, Acharya J, Baumgartner C, Benbadis S, Bleasel A, Burgess A, Dinner D. S, Ebner A, Foldvary N, Geller E, Hamer H, Holthausen H, Kotagal P, Morris H, Meencke H. J, Noachtar S, Rosenow F, Sakamoto A, Steinhoff B. J, Tuxhorn I and Wyllie E., “Semiological seizure classification”, Epilepsia., 39, 1006-1013, 1998.
19. Malawska B, “New Anticonvulsant Agents”, Current Topics in Med. Chem., 2005, 5, 69-85.
20. Brodie M. J and Dichter M. A, “New antiepileptic drugs”, N. Engl. J. Med., 334, 1583-1590, 1996.
21. Aldenkamp A. P, Krom M. D and Reijs R, “Newer Antiepileptic Drugs and Cognitive Issues”, Epilepsia., 44, 21-29, 2003.
22. White H. S., “Preclinical Development of Antiepileptic Drugs” Past, Present, and Future Directions”, Epilepsia, 44, 2-8, 2003.
23. Devinsky O, “Patients with refractory seizures”, N. Engl. J. Med., 340, 1565-1570, 1999.
24. Loscher W, “Current status and future directions in the pharmacotherapy of epilepsy”, Trends Pharmacol. Sci., 23, 113-118, 2002.
25. Bialer M, Johannessen S I, Kupferberg H. J, Levy R. H, Loiseau P, Perucca E., “Progress report on new antiepileptic drugs: a summary of the Fifth Eilat Conference (EILAT V)”, Epilepsy Research., 43, 11–58, 2001.

26. Bialer M, Kupferberg H. J, Levy R. H, Perucca E, "Progress report on new antiepileptic drugs: A summary of the seventh Eilat conference (EILAT VII)", Epilepsy Res., 61, 1-48, 2004.
27. Yogeeswari P, Ragavendran J. V and Sriram D., "An Update on GABA analogs for CNS drug discovery", "Recent patents on CNS drug discovery"., 1, 113-118, 2006.
28. Bowery N, Enna S. J, Olsen R. W, "Six decades of GABA", Biochempharm., 68, 1477-1478, 2004.
29. Sytinski I. A and Soldatenkov A. T, "The Conformation of γ-Aminobutyric acid and its Analogues., Russian Chemical Reviews, 48, 669-677, 1979.
30. Treiman D. M., "GABAergic Mechanisms in Epilepsy", Epilepsia, 42, 8-12, 2001.
31. Neilson E. B, Suzdak P. D, Andersen K. E, Knutsen L. J. S, Sonnewald U and Braestrup C, "Characterization of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. Eur J Pharmacol"., 257-266, 1991
32. Mengel H. "Tiagabine"., Epilepsia., 35(Suppl 5), S81-S84, 1994.
33. Ketter T. A, Post R. M, Theodore W. H, "Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders", Neurology., 53, S53-S67 1999.
34. A.L.Malizia, V. J. Cunningha M, Bell C. J, Liddle P. J, Jones T, Nutt D. J, "Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study"., Arch Gen Psychiatry., 55, 715-720, 1998.
35. Border L. A, Muralidhar T. G, Smith K. E , Weinshank R. L, Branchek T. A, Gluchowski C, "Tiagabine, SK&F 89976-A, CI-996, and NNC-711 are selective for the cloned GABA transporter GAT-1"., Eur J Pharmacol., 269, 219-224, 1994.
36. Petty F. "GABA and mood disorders: a brief review and hypothesis"., J Affect Disord. 34, 275-281, 1995.
37. Salzman C, Miyawaki E. K, le Bars P and Kerrihard, "Neurobiologic basis of anxiety and its treatment. Harv Rev Psychiatry"., 1, 197-206, 1993.
38. Zigmond M. J, Bloom F. E, Landis S.C, *Roberts J. L and Squire L. R, "Fundamental Neuroscience"., Academic press, 1999.*
39. Enna S. J, "GABA" Advances in Pharmacology, 54, Elseveir, 2006

40. Chebib M and Johnston G. A. R, "GABA-Activated Ligand Gated Ion Channels: Medicinal Chemistry and Molecular Biology", J. Med. Chem., 43, 1427-1447, 2000.
41. http://www.williams.edu/imput/IIIA5.html
42. http://www.williams.edu/imput/IIIA6.html
43. http://www.williams.edu/imput/index.html
44. *Bear M. F, Connors B.W and Paradisco M. A., "Neuroscience:Exploring the brain"., 2nd edition, Lippincott, Williams & Wilkins, 2001.*
45. Bormann J., "The 'ABC' of GABA receptors", Trends in pharmacol. Sciences., 21,17-18, 2000 .
46. Stein V and Nicoll R. A, "GABA Generates Excitement", Neuron., 37, 375–378, 2003.
47. Bowery N. G, Doble A, D. Hill D. R, Hudson A. L, Shaw J. S, Turnbull M. J and Warrington R, "Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals"., Eur. J. Pharmacol., 71, 53-70, 1981.
48. Rognan D, Boulanger T, Hoffmann R, Vercauteren D. P, Andre J. M, Durant F and Wermuth C. G., "Structure and molecular modeling of $GABA_A$ receptor antagonists", J. Med. Chem., 35, 1969-1977, 1992.
49. Yokogawa T, Seung U. Kim, Krieger C & Puil E, "Analysis of GABAA- and GABAB-receptor mediated effects on intracellular Ca2+ in DRG hybrid neurons", British Journal of Pharmacology., 134, 98 – 107, 2001.
50. Möhler H, Crestani F and Rudolph U, "$GABA_A$-receptor subtypes: a new pharmacology", Curr Opin Pharmacol., 1, 22-25, 2001.
51. Semyanov A and Kullmann D. M, "Modulation of GABAergic Signaling among Interneurons by Metabotropic Glutamate Receptors", Neuron., 25, 663–672, 2000.
52. Rochelle D. Schwartz-Bloom and Sah R, "γ-Aminobutyric acid neurotransmission and cerebral ischemia", J. Neurochem, 77, 353-71, 2001.
53. Chambon J. P, Feltz P, Heaulme H, Restle S, Schlichter R, Biziere K and Wermuth C.G, "An arylaminopyradazine derivative of γ-aminobutyric acid (GABA) is a selective and competitive antagonist at the $GABA_A$ receptor site" Proc. Natl. Acad. Sci. USA, 82, 1832-1836, 1985.

54. Allan R. D, Apostopoulos C and J. A. Richardson, "2-Imino-1,3,4-thiadiazole Derivatives of GABA as $GABA_A$ Antagonists", Aust J Chem., 43, 1767-1772, 1990.
55. Johnston G. A. R, "$GABA_A$ Receptor Pharmacology", Phamacol. Ther., 69, 173-198, 1996.
56. Hansen S. L, Ebert B, Fjalland B & Kristiansen U, "Effects of GABA-A receptor partial agonists in primary cultures of cerebellar granule neurons and cerebral cortical neurons reflect different receptor subunit compositions", Br J Pharmacol., 133, 539 - 549.
57. Cryan J. F and Kaupmann K., "Don't worry 'B' happy!: A role for GABA-B receptors in anxiety and depression", Trends in Pharmacol Sciences., 26, 36-43, 2005.
58. Bettler B, Kaupmann K, Mosbacher J and Gassmann M, "Molecular Structure and Physiological Functions of $GABA_B$ Receptors", Physiol. Rev., 84, 835-867, 2004.
59. Bowery N. G, Bettler B, Froestl W, Gallagher J. P, Marshall F, Raiteri M, Bonner T. I, and Enna S. J., "International Union of Pharmacology. XXXIII. Mammalian γ-Aminobutyric Acid B Receptors: Structure and Function", Pharmacol Rev., 54, 247-264, 2002.
60. Kaupmann K, Huggel H, Heid J, Flor P. J, Bischoff S, Mickel S. J, McMaster G, Angst C, Bittiger H, Froestl W and Bettler B, "Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors", Nature., 386, 239-46, 1997.
61. Costantino G, Macchiarulo A, Guadix A. E and Pellicciari R., "QSAR and Molecular Modeling Studies of Baclofen Analogues as $GABA_B$ Agonists. Insights into the role of the aromatic moiety in $GABA_B$ Binding and Activation", J. Med. Chem., 44, 1827-1832, 1991.
62. Galvez T, Parmentier M. L, Joly C, Malitschek B, Kaupmann K, Kuhn R, Bittger H, Froestl W, Bettler B and Pin J. P., "Mutagenesis and modeling of the $GABA_B$ Receptor extracellular domain support a venus flytrap mechanism for ligand binding", J. Biol. Chem., 274, 13362-13369, 1999.
63. Couve A, Filippov A. K, Connolly C.N, Bettler B, Brown D. A and Moss S. J., J. Biol. Chem., 273, 26361-26367, 1998.
64. Kerr D. I. B and J. Ong, "$GABA_B$ Receptors", Pharmacol. Ther., 67, 187-246, 1995.

65. Bowery N. G, "$GABA_B$ receptor Pharmacology", Annu. Rev. Pharmacol. Toxicol., 33, 109-147, 1993.

66. Jones K. A, Borowsky B, Tamm J. A, Craig D. A, Durkin M. M, Dai M, Yao W. J, Johnson M, Gunwaldsen C, Huang L. Y, Tang T, Shen Q, Salon J. A, Morse K, Laz T, Smith K. E, Nagarathnam D, Noble S. A, Branchek T. A and Gerald C, "**$GABA_B$** receptors function as a heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$", Nature., 396, 674-678, 1998.

67. White J. H, Wise A, Main M. J, Green A, Fraser N. J, Disney G. H, Barnes A. A, Emson P, Foord S. M and Marshall F. H, **"**Heterodimerization is required for the formation of a functional $GABA_B$ receptor"., Nature., 396, 679-682, 1998.

68. Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff J, Kulik A, Shigemoto R, Karschin A and Bettler B.,"**$GABA_B$-receptor** subtypes assemble into functional heteromeric complexes", Nature., 396, 683-686, 1998.

69. Kerr D. I. B, Ong J, Prager R. H, Gynther B. D and Curtis D. R., "Phaclofen: a peripheral and central baclofen antagonist", Brain Res., 405, 150-154, 1987.

70. Marshall F. H, Jones K. A, Kaupmann K and Bettler B, Trends Pharmacol. Sci., 20, 396-399, 1999.

71. Zhang D, Pan Z. H, Awobuluyi M and Lipton S. A., "Structure and function of $GABA_C$ receptors: a comparison of native versus recombinant receptors", Trends in Pharmacol Sciences., 22, 121-132, 2001.

72. McNamara J. O., "Emerging insights into the genesis of epilepsy", Nature, 339-341, A15-A22, 1999.

73. Graham D, Besnard F and Faure C., $GABA_A$ receptor subtype diversity implications for new generation hypnotic drug discovery", Sleep., 19, S43-S54, 1996.

74. en.wikipedia.org/wiki/Spirocompound.

75. Gupta R. R, Kumar M and Gupta V, "Nomenclature of Heterocycles", Heterocyclic chemistry., 1, 29-32, Springer 2005.

76. New J. S, Yevich J. P, Eisom M. S, Taylor D. P, Eison A. S, Riblet L. A, Maelen C. P. V and Temple D. L., "Buspirone Analogues. 2. Structure-Activity Relationships of Aromatic Imide Derivatives", J. Med. Chem., 29, 1476-1482, 1986.

77. Pradhan R, Patra M, Behera A. K, Mishra B. K and Behera R. K., "A Synthon approach to Spiro compounds", Tetrahedron., 62, 779-828, 2006.
78. Klioze S. S and Bauer V. J, "1,3-Dihydrospiro(Isobenzofuran-1,4-Piperidine)s", US 4024263 (1977).
79. Ong H. H and Profitt J. A., "Spiro[Dibenz(B,F)Oxepin-piperidine]s", US 4198418, 1980.
80. Ong H. H and Profitt J. A., "Spiro[Indoline-3,4-piperidine]s", US 4345081, 1982.
81. Borenstein M. R, Doukas P. H, "Mannich Bases of Spirosuccinimides", US 4925841, 1990.
82. Adam G, Cesura A, Galley C, Jenck F, Rover S, Wichnmann, "1,3,8-Traizaspiro(4,5)Decan-4-on Derivatives", US 6043366, 2000.
83. Rajopadhye M and Popp F. D, "Potential Anticonvulsants. 11. Synthesis and Anticonvulsant activity of Spiro[1,3-dioxolane-2,3'-indolin]-2'-ones and structural Analogues", J. Med. Chem., 31, 1001-1005, 1988.
84. Allan R. D and Johnston G. A. R, "Synthetic Analogs for the study of GABA as a Neurotransmitter", Med. Res. Rev., 3, 91-118, 1983.
85. Hansen N. S, Ebert B, Krogsgaard-Larsen P and Kehler J., "Synthesis and Pharmacology of Seleninic Acid Analogues of the Inhibitory Neurotransmitter γ-Aminobutyric Acid", Org. Lett., 2, 7-9, 2000.
86. Jacob J. N, Hesse G. W and Shashoua V. E., "Synthesis, Brain Uptake, and Pharmacological Properties of a Glyceryl Lipid Containing GABA and the GABA-T Inhibitor γ-Vinyl-GABA", J. Med. Chem., 33, 733-736, 1990.
87. Duke R. K, Chebib M, Hibbs D. E, Mewett K. N and Johnston G. A. R, "Synthesis and resolution of 2-methyl analogues of GABA", Tetrahedron: Asymmetry 15, 1745–1751, 2004.
88. Chebib M, Vandenberg R. J & Johnston G. A. R., "Analogues of γ-aminobutyric acid (GABA) and trans-4-aminocrotonic acid (TACA) substituted in the 2 position as GABA-C receptor antagonists", British J. of Pharmacol., 122, 1551 – 1560, 1997.
89. Vein J, Duke R. K, Mewett K. N, Johnston G. A. R, Shingai R & Chebib M, "trans-4-Amino-2-methylbut-2-enoic acid (2-MeTACA) and (+)-trans-2-aminomethylcyclopropanecarboxylic acid ((+)-TAMP) can differentiate rat ρ3 from

human ρ1 and ρ2 recombinant GABAC receptors", British J. of Pharmacol., 135, 883-890, 2002.

90. Silverman R. B, Durkee S. C and Invergo B. J., "4-Amino-2-(substituted methyl)-2-butenoic Acids: Substrates and Potent Inhibitors of γ-Aminobutyric Acid Aminotransferase", J. Med. Chem., 29, 764-770, 1986.
91. Silverman, R. B., Andruszkiewicz, R., Yuen, P.W., Sobieray, D. M., Franklin, L. C. Schwindt, M. A., "Update on GABA analogs for CNS drug discovery", US 5563175 1996.
92. Silverman, R. B., Andruszkiewicz, R., Yuen, P.W., Sobieray, D. M., Franklin, L. C. Schwindt, M. A., "Update on GABA analogs for CNS drug discovery", US 6117906 2000.
93. Justin S. Bryans and Wustrow D. J., "3-Substituted GABA Analogs with Central Nervous System Activity: A Review", Med. Res. Rev., 19, 149-177, 1999.
94. Hartenstein J, Satzinger G, Herrmann M. Cyclic amino acid derivatives. German patent 2626467. 1977.
95. Satzinger G, Hartenstein J, Herrmann M, Heldt W. Cyclic amino acids. German patent 2543821. 1977.
96. "Gabapentin" Drugs of the Future 9, 418–419, 1984.
97. Johnson D. S, and Li J. J, "The art of Drug Synthesis", Wiley interscience., 2007.
98. Belliotti T. R, Capiris T, Ekhato I. V, Kinsora J. J, Field M. J, Heffner T. G, Meltzer L. T, Schwarz J. B, Taylor C. P, Thorpe A. J, Vartanian M. G, Wise L. D, Ti Zhi-Su, Weber M. L, and Wustrow D. J, "Structure Activity Relationships of Pregabalin and Analogues That Target the α2-δ Protein", J. Med. Chem., 48, 2294-2307, 2005.
99. Hu S, Martinez C. A, Tao J, Tulley W. E, Kelleher P and Dummond Y, US 0283023 2005.
100. Andruszkiewicz and Silverman R. B., "A convenient synhesis of 3-alkyl-4-aminobutanoic acids", Synth. Commun,. 953-955, 1989.
101. Kopelevich V. M, "Advances in the search for medicinal drugs based on γ-Aminobutyric acid, Russian chem. Rev., 47, 1273-1296, 1979.

102. Allan R. D and Hue Tran, "Synthesis of Analogues of GABA. VII (Z) and (E)-4-Amino-3-(4-chlorophenyl)but-2-enoic acids as unsaturated Baclofen Derivatives", Aust. J. Chem., 34, 2641-2645, 1981.

103. Silverman R. B, Durkee S. C and Invergo B. J., "4-Amino-2-(substituted methyl) -2-butenoic Acids: Substrates and Potent Inhibitors of γ-Amino butyric Acid Aminotransferase", J. Med. Chem., 29, 764-770, 1986.

104. Wei Z. Y and Knaus E. E., "A Short Efficient Synthesis of (S)-4-Amino-5-hexenoic acid [(S)-Vigabatrin]", J. Org. Chem., 58, 1585-1588, 1983.

105. Paul D. R, Timothy J. B and Yelle E. Y., "Methods and compositions employing optically pure S(+) Vigabatrin", WO 00/10554.

106. Tapia R, Drucker-Colin R. R, Meza-Ruiz G, Duran L and Levi G., "Neurophysiological and Neurochemical Studies on the Action of the Anticonvulsant γ-Hydroxy, γ-Ethyl, γ-Phenyl-Butyramide", Epilepsia., 20, 135-145, 1979.

107. Yogeeswari P, Sriram D, Thirumurugan R, Ragavendran J. V, Sudhan K, Kumar R. P, and Stables J. P., "Discovery of N-(2,6-dimethylphenyl) substituted semicarbazones as anticonvulsants: hybrid pharmacophore based design", J. Med. Chem., 48, 6202-6211, 2005.

108. Bhowmick S, Pal M and Pal S. P., "Synthesis and anticonvulsant activity of N-phthaloyl GABA-a new GABA derivative", Ind. J. Exp. Biol., 27, 805-808, 1989.

109. Malawska B and Antkiewicz-Michaluk L "Synthesis, physicochemical properties, anticonvulsant activities and voltage-sensitive calcium channels affinity of N-substituted amides of α-(4-phenylpiperazino)-GABA", Pharmazie., 54, 239-243, 1999

110. Barbara Malawaska and A. Zejc, "search for new anticonvulsant compounds", Pharmazie., 50, 722-725, 1995.

111. Kaplan J. P, Raizon B. P "New Anticonvulsants: Schiff Bases of γ-Aminobutyric Acid and γ-Aminobutyramide", J. Med. Chem., 23, 702-704, 1980.

112. Ragavendran J. V, Sriram D, Patel S. K, Reddy I. V, Bharathwajan N, Stables J, Yogeeswari P., "Design and synthesis of anticonvulsants from a combined phthalimide-GABA-anilide and hydrazone pharmacophore", Eur. J. Med. Chem., 42, 146-151, 2007.

113. Yogeeswari P, Ragavendran J. V, Sriram D, Kavya R, Vanitha K and Neelakantan H., "Newer N-Phthaloyl GABA Derivatives with antiallodynic and Antihyperalgesic Activities in Both Sciatic Nerve and Spinal Nerve Ligation Models of Neuropathic Pain", Pharmacology., 81, 21-31, 2007.

114. Ragavendran J. V, Sriram D, Kotapati S, Stables J and Yogeeswari P., "Newer GABA derivatives for the treatment of epilepsy including febrile Seizures: A bioisosteric approach", Eur. J. Med. Chem., 43, 2650-2655, 2008.

115. Yogeeswari P, Sriram D, Sahithya P, Ragavendran J .V and Ranganadh V, "Synthesis and anticonvulsant activity of 4-(2-(2,6-dimethylphenylamino)-2-oxoethylamino)-N-(substituted) butanamides: A pharmacophoric hybrid approach", Bioorg. Med. Chem. Letters., 17, 3712-3715, 2007.

116. Qume M, Whitton P.S and Fowler L. J., "The Effect of Chronic Treatment with the GABA Transaminase γ-Vinyl-GABA and Ethanolamine-O-Sulphate on the In Vivo Release of GABA from Rat Hippocampus", J. Neurochem., 5, 2256-2261, 1995.

117. Yogeeswari P, Sriram D, Thirumurugan R, Jeewanlal L. R, SunilJit, Ragavendran J. V, Kavya R, Rakhra R and Saraswat V.," Synthesis of N4-(2,4-dimethylphenyl) semicarbazones as 4-aminobutyrate aminotransferase inhibitors", Acta Pharma., 56, 259-272, 2006.

118. Yogeeswari P, Sriram D, Thirumurugan R, Ragavendran J. V, Sudhan K, Kumar R, Pavana and Stables J, "Discovery of N-(2,6-Dimethylphenyl)-Substituted Semicarbazones as Anticonvulsants: Hybrid Pharmacophore-Based Design", J. Med. Chem., 48, 6202-6211, 2005.

119. Chande M. S and Khanwelkar R. R., "Michael addition approach for the synthesis of novel spiro compounds and 2-subsituted malonic acid derivatives from Meldrums acid", Tetrahedron Lett., 46, 7787-7792, 2005.

120. Poupaert J, Carato P and Colacino E, "2(3H)-Benzoxazolone and Bioisosters as "Privileged Scaffold" in the Design of Pharmacological Probes", Curr. Med. Chem., 12, 877- 885, 2005.

121. Evans B. E, Rittle K. E, Bock M. G, Dipardo R. M, Fridinger R. M, Whitner W. L, Lundell G. F, Veber D. F, Anderson P. S and Chang R. S., "Methods for drug

discovery, development of potent, selective, orally effective cholecystokinin antagonists", J. Med. Chem., 12, 2235-2246, 1988.

122.Patchett A. A and Nargund R. P., "Priveleged structure-An update", J. Am. Chem. Soc., 122, 9939-9953, 2000.

123.Scott K. R, Moore J. A, Zalucky T. B, Nicholson J.M, Lee J. A. M and Hinko C. N, "Spiro[4,5] and Spiro[4,6] carboxylic acids; cyclic analogues of valproic acid. synthesis and anticonvulsant evaluation", J. Med. Chem, 28, 413-417, 1985.

124.Ong H. H, Profitt J. A, Fortunato J, Glamkowski E. J, Ellis D.B, Geyer H. M, Wilker J. C, Burghard H., "Novel tetracyclic spiropiperidines. 3. 1-arylspiro[indoline-3,4-piperidine]s as potential antidepressants", J. Med. Chem., 26, 981-986, 1983.

125.Rajopadhye M and Popp F. D, "Potential anticonvulsants. 11. Synthesis and anticonvulsant activity of spiro [1, 3- dioxolane-2, 3'- indolin]-2'-ones and structural analogues", J. Med. Chem., 31, 1001-1005, 1988.

126.Blakemore D.C, and Bryans J.S, "Cyclic ketones, their preparation and their use in the synthesis of amino acids", U S 0215816, 2005.

127.Dolle R. E and Bourdonnec B. L, "Spirocyclic heterocyclic derivatives and methods of their use", US 7338962B2, 2008.

128.Lawrence M and Manfred W, "3-Phenylspiro[cyclohexane-1,1'(3H)-isobenzofuran]-4-one", US 4292245, 1981.

129.Bennani Y. L, Bunnelle W. H, Chang S, Chemburkar S. R, Chen J, Dart M. J, Fernando D. P, Yi-Yin KU, Lockwood M and LieWang, "Spirocyclopropylamides and acids and their therapeutic applications", US 0077616 A1, 2004.

130.Chafeev M, Chakka N, Chowdury S, Fraser R, Fu J, Hou D, Hsieh T, Kamboj R, Liu S, Raina V, Seid M, Bagherzadeh, Sun J, Sun S and Sviridov S, "Spiro-oxindole compounds and their uses as therapeutic agents", US 0252812 A1, 2006.

131.Rawson D. J and Swain N. A, "Spirocyclic deivatives", US 0129388 A1, 2007.

132.Rice L. M, Geschickter C. F and Grogan C. H, "Spiranes. III. Azaspiranes and Intermediates", J. Med. Chem. 6, 388-402, 1963.

133.Patel H. J, Sarra J, Caruso F, Rossi M, Doshi U and Stephani R. A, "Synthesis and anticonvulsant activity of new N-1', N-3'- disubstituted-2'H,3H,5' H-spiro-(2-

benzofuran-1,4'- imidazolidine)-2'3,5'-triones", Bioorg. Med. Chem Lett., 16, 4644-4647, 2006.

134. Rice L. M, Geschickter C. F and Grogan C. H, "Spiranes. III. Azaspiranes and Intermediates", J. Med. Chem., 6, 388-402, 1963.

135. Rice L. M, Sheth B. S and Scott K. R, "Spirans. XV. Spirans from 3-Trifluoromethylcyclohexanone", J. Med. Chem., 12, 126-130, 1969.

136. Stymiest J. L, Mitchelli B. F, Wong S and Vederas J. C, "Synthesis of Oxytocin Analogues with replacement of Sulfur by Carbon gives potent antagonists with increased stability", J. Org. Chem., 70, 7799-7809, 2005.

137. Chapman J. M, Cocolas G. H and Hall I. H, "Hypolipidemic activity of phthalimide derivatives. 1. N-Substituted phthalimide derivatives", J. Med. Chem., 22, 1399-1402, 1979.

138. Krall R. L, Penry J. K, White B. G, Kupferberg H. J and Swinyard E. A, "Antiepileptic drug development II: Anticonvulsant drug screening", Epilepsia., 19, 409- 428, 1978.

139. Conley J. P and Kohn H, "Functionalized DL-amino acid derivatives. Potent new agents for the treatment of epilepsy", J. Med. Chem., 30, 567-574, 1987.

140. Lapin I. P, "Antagonism of L-glycine to seizures induced by L-kynurenine, quinolinic acid and strychnine in mice", Eur. J. Pharmacol., 17, 495-502, 1981.

141. Mathis C, Barry J. D and Ungerer A, "NMDA antagonist properties of gamma-L-glutamyl-L-aspartate demonstrated on chemically induced seizures in mice", Eur. J. Pharmacol., 185, 53-59, 1990.

142. Dunham N. W and Miya T. A, "A note on a simple apparatus for detecting neurological deficit in rats and mice", J. Am. Pharm. Assoc. Sci., 46, 208-216, 1957.

143. Dimmock J. R, Vashishtha S. C and Stables J. P, "Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds", Eur. J. Med. Chem., 35, 241-248, 2000.

144. Pandeya S. N and Raja A. S, "Synthesis of isatin semicarbazones as novel anticonvulsants-role of hydrogen bonding", J. Pharm. Pharm. Sci., 5, 266-271, 2002.

Appendix

List of publications

Patent:

1. Perumal Yogeeswari, Dharmarajan Sriram, **Aaramadaka Sunil Kumar Reddy**, Jegadeesan Vaigunda Ragavendran, Semwal Arvind, Mishra Ram Kumar "Novel N-Spiro substituted compounds for the treatment of Epilepsy and Neuropathic pain" (an Indian patent under file, application number: 1138/CHE, 18th may 2009).

Publications:

1. ***Sunil Kumar Reddy****, J. V. Ragavendran, P.Yogeeswari, D. Sriram,* ***2006*** *Pharmacological screening of candidate Anticonvulsant in the animal model of febrile seizures. Medicinal chemistry research 15(1) 287-392.*

2. Kamaraj Balamurugan, Subbu Perumal, **A. Sunil Kumar Reddy**, Perumal Yogeeswari, Dharmarajan Sriram, "A facile domino protocol for the regioselective synthesis and discovery Of novel 2-amino-5-arylthieno-[2,3-b] thiophenes as antimycobacterial agents". *Tetrahedron Letters* 45 (11) 6191-6195.

3. Dalip Kumar, Swapna Sundaree, **A. Sunil K. Reddy**, P. Yogeeswari, D. Sriram, A facile synthesis and anticonvulsant activity of novel 2,4-disubstituted oxazoles. (*Bioorganic medicinal chemistry letters,* under revision)
4. P. Yogeeswari, V. Ragavendran, D. Sriram, A. priyanka, S. Ganguly, **A. Sunil K. Reddy,** Arvind Semwal, Effectiveness of GABA analogues for the treatment of Neuropathic pain. (*Indian journal of pharmacology*, under revision).

Conference Presentations:

1. Newer GABA Analogues as CNS agents: Synthetic integration of GABA in 1, 2, 4 –triazolo – 2H –3 onenucleus **A. Sunil Kumar Reddy**, Sravan Kumar Patel, Y. Nageswari, D. Sriram & P. Yogeeswari 58th Indian Pharmaceutical Congress, 1-3 Dec, 2006, Mumbai.
2. Synthesis of newer N-Spirosubstituted GABA derivatives as Anticonvulsants **A. Sunil Kumar Reddy**, D. Sriram, V. Ranganadh & P. Yogeeswari. 59th Indian Pharmaceutical Congress, 20- **23** Dec, 2007, Varanasi.
3. Effective new GABA analogues against febrile seizures in an rat immature model, Katiyar S. Viyogi S, **Reddy ASK**, Ragavendran J.V, Sriram D, Yogeeswari P. 41st annual conference of Indian Pharmacological Society, 18-20 Dec, 2008, New Delhi.

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(57) Abstract :
Disclosed herein are novel N-Spiro substituted compounds, which are derivatives of the inhibitory neurotransmitter GABA (γ-aminobutyric acid), represented by formula I, process for preparing these compounds and pharmaceutical use thereof as therapeutic agents in the treatment of epilepsy and neuropathic pain syndromes and their progression. Formula I wherein, n is 0,1,2 and 3 R is hydrogen, halo or straight or branched alkyl from 1 to 6 carbons. Ri is hydrogen or alkyl from 1 to 6 carbons, unsubstituted phenyl or halo, nitro, amino or alkyl substituted phenyl. R2 is hydrogen or alkyl from 1 to 6 carbons or unsubstituted phenyl or halo, nitro, amino or alkyl substituted phenyl, or Ri and R2 may form together unsubstituted isatinyl or halo or alkyl substituted isatinyl or cycloalkyl from 3 to 8 carbons.

Biography

BIOGRAPHY OF A. SUNIL KUMAR REDDY

Mr. A. Sunil Kumar Reddy has completed his Bachelor's degree in Pharmacy from Annai Veilankannis college of Pharmacy, Chennai, in the year 2000. He worked as Trainee at The Madras pharmaceuticals, Chennai, 2000-2001. He acquired his Masters degree in Pharmacy in Pharmaceutical chemistry from Manipal College of pharmaceutical sciences, Manipal, Karnataka in 2004. He was associated with New drug discovery division of Orchid chemicals and pharmaceuticals ltd from 2004-2006. He had been working as a research scholar at BITS, Pilani from 2006-2009 during which he worked on a CSIR project. He has few publications in international journals and a patent is under file.

BIOGRAPHY OF Dr. P.YOGEESWARI

Dr. P. Yogeeswari is presently working in the capacity of Assistant Professor and Group leader for Pharmacy Group, Birla Institute of Technology and Science, Pilani, Hyderabad Campus. She received her Ph.D. degree in the year 2001 from Banaras Hindu University; Varanasi. She has been involved in Research for the last 12 yrs and in teaching for 11 yrs. APTI honored her with YOUNG PHARMACY TEACHER AWARD for the year 2007. She has collaborations with various national and international organizations that include National Institute of Health, Bethesda, USA, National Cancer Institute, and USA, National Institute of Mental Health and Neurosciences, Bangalore, Indian Institute of Science, Bangalore, and Department of Ophthalmology & Visual Science, University of Illinois, Chicago, USA. She has to her credit more than 100 research publications and one patent under process. She is an expert reviewer of many international journals like Journal of Medicinal Chemistry (ACS), Bioorganic Medicinal Chemistry (Elsevier), Recent Patents on CNS Drug Discovery (Bentham), Current Enzyme Inhibition (Bentham), Acta Pharmacologica Sinica (Blackwell Publishing), European Journal of Medicinal Chemistry (Elsevier) and Natural Product Resources (Taylor and Francis). She is a lifetime member of Association of Pharmacy Teachers of India and Indian Pharmacological Society. She has successively completed three projects, one of DST SERC Fast Track under Young Scientist Scheme and other two of CSIR. In addition she is working on two another projects of UGC and DBT. She has guided two Ph.D students and currently she is guiding two students.