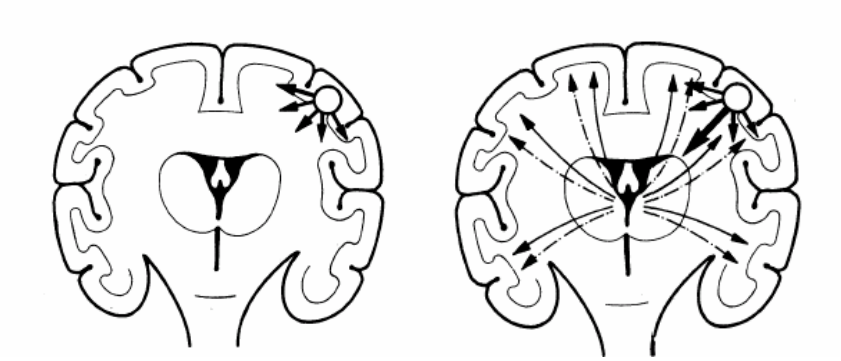
CHAPTER 1
INTRODUCTION

The term "epilepsy" encompasses a number of different syndromes whose cardinal feature is a predisposition to recurrent unprovoked seizures [1]. Seizures are the manifestations of abnormal, excessive, hypersynchronous discharges from an aggregate of central nervous system (CNS) neurons. Any acute disturbance like hypocalcaemia, or an infection such as meningitis, or a poisoning can lead to epileptogenesis (symptomatic or organic epilepsy). The incidence could also be due to an unknown cause (true, or idiopathic epilepsy).

The annual incidence rate of epilepsy on an average is around 500 cases per 100,000 of the population worldwide [2]. In all known surveys, the annual incidence rate is highest in the youngest age group, decreasing during the childhood, diminishing among adults and rising again in the aged. The lifetime likelihood of experiencing at least one epileptic seizure is about 9% and the lifetime likelihood of receiving a diagnosis of epilepsy is almost 3% [3].

Although seizures can be classified according to their clinical features and electroencephalographic (EEG) findings [4] (Table 1.1), epilepsy is classified according to the type of seizures, presence or absence of neurological changes and EEG changes. Epilepsy syndromes fall into two broad categories: **generalized** and **partial** (or localization-related) **syndromes** [5, 6] (Fig. 1). In generalized epilepsies, the predominant type of seizures begin simultaneously in both the cerebral hemispheres. In contrast, in partial epilepsies seizures originate in one or more localized foci, although they can spread to involve the entire brain. Most partial epilepsies are believed to be the result of one or more central nervous system insults, but in many cases the nature of the insult is never identified.



A B

Fig. 1 A-Partial seizure: discharge remains localized. B- Secondarily generalized seizure: epileptic discharge initially localized and then spreads to both the hemispheres

Table 1.1: Classification of seizures by International League Against Epilepsy, 1981

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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

1.1 MECHANISMS OF EPILEPSIES

The basic premise of epileptogenesis is the imbalance between the inhibitory and excitatory neurotransmission in the brain. Hence the mechanisms which lead to decreased inhibition (or) increased excitation (or) malformations in the ion channels, result in the generation of epileptogenic disorders. The excitatory and inhibitory effects for most of the neurotransmitters are mediated by transmembrane conductance of specific ions i.e. Na^+, K^+, Mg^{2+}, Cl^- etc.

A. Mechanisms Leading to Decreased Inhibition

1. Defective $GABA_A$ Inhibition: γ–Amino butyric acid (GABA) acts at inhibitory synapses in the brain and spinal cord, the inhibition resulting from a hyperpolarization of the transmembrane potential of the inhibited neuron, which is elicited by the binding of GABA molecules to specific receptors in the plasma membrane of both pre- and post-synaptic neurons. This binding opens ion channels to allow either the flow of chloride or potassium ions into or out of the cell. GABA binds to 2 major classes of receptors: $GABA_A$ and $GABA_B$. $GABA_A$ receptors maintain the neural transmembrane balance by two mechanisms, the first mechanism is coupled to chloride ion channel hyperpolarization, and the second is the inhibitory effect on the release of excitatory neurotransmitters i.e. glutamate. Properties of the chloride channels associated with the $GABA_A$ receptor are often clinically modulated by various anticonvulsant drugs either by increasing the frequency or duration of opening of chloride channels. Certain drugs (e.g.Topiramate) modulate the phosphorylation site of the chloride channel thus inducing a change in the normal electrophysiological behavior leading to an increased frequency of channel openings.

Some epilepsies may be due to mutations or lack of expression of the different $GABA_A$ receptor complex subunits, the molecules that govern their assembly, or the molecules that modulate their electrical properties. Changes in the distribution of subunits of the $GABA_A$ receptor complex have been demonstrated in several animal models of partial-onset epilepsy, such as the electrical-kindling, chemical-kindling, and pilocarpine model [7].

2. Defective $GABA_B$ Inhibition: $GABA_B$ receptors seem to be located predominantly in the presynaptic GABAergic neurons. They inhibit the post-synaptic neuron by means of two mechanisms: (a) direct induction of an inhibitory post-synaptic potential (IPSP), which a $GABA_A$ chloride current typically mediates, and (b) indirect inhibition of the release of excitatory neurotransmitters from the pre-synaptic afferent projections, typically associated with $GABA_B$ potassium current.

Once again, alterations or mutations in the different subunits or in the molecules that regulate their function might affect the seizure threshold or the propensity for recurrent seizures [7].

B. Mechanisms Leading To Increased Excitation

1. Defective NMDA (N-Methyl-D-Aspartate) Receptor-Mediated Activation: Glutamic acid is the most common and prevalent excitatory neurotransmitter in the central nervous system. Its action is mediated by two classes of receptors namely, NMDA, and non-NMDA receptors {2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl) propionic acid (AMPA) and kainate}, comprising the ionotropic class and metabotropic (G-protein coupled) receptors, via increasing calcium and sodium ion conductance. Intercellular recording in an epileptic focus during "spike discharge" revealed that its earlier component is due to the activation of AMPA receptors and the later component due to the NMDA receptor activation [8].

The contributing factors to hyperexcitability leading to epileptogenesis are increased density of NMDA receptors, differences in the NMDA subunit composition and activation kinetics, which result in reduced voltage-dependent Mg^+ blockade and longer receptor opening. Also, the mutated subunit composition of AMPA receptor in the early developmental stage, evoked seizures [9] enhancement *in vivo*, this glutamate release is generally not observed during acute phase, however in the chronic epilepsy models in rodents (amygdala-kindled rats, genetically epilepsy prone rats), there appears to be a consistent marked increase in glutamate release [8].

2. Defective Glycine-Mediated Activation: Glycine is an inhibitory neurotransmitter in the CNS, especially in the spinal cord. When glycine receptors are activated, Cl^- ions enter the neuron via ionotropic receptors, causing an IPSP. Strychnine is an antagonist at these ionotropic receptors. Its LD_{50} is 0.96 mg/kg in rats, and it usually causes death by hyperexcitability. Glycine is a required co-agonist along with glutamate in the CNS. In contrast to the inhibitory role of glycine in the spinal cord, this behavior is facilitated at the NMDA-glutaminergic receptors which are excitatory. Activation of NMDA receptors requires binding of both glutamate and the co-agonist glycine for the efficient opening of the ion channels [10].

3. Mutations in Nicotinic Acetylcholine Receptors: High doses of nicotine were found to induce tonic-clonic convulsions in animals after systemic and intracerebroventricular (i.c.v.) injections [11-13]. The convulsive effect of nicotine is mediated by central nicotinic receptors which belong to a family of excitatory ligand-gated ion channel receptors and blocked by different nicotinic antagonists such as mecamylamine and pempidine [11, 14]. In addition, seizures were also prevented by pretreatment with several non-nicotinic compounds, such as diazepam, haloperidol, and tricyclic antidepressants [15]. Finally, tolerance has been found to develop to nicotine-induced seizures after acute and chronic administration of nicotine.

The pharmacological mechanism behind the epileptogenic effect is as follows: nicotine either directly acts on the nicotinic acetylcholine receptors (nAch), which are permeable to calcium or indirectly opens L-type calcium channels resulting in membrane depolarization by increasing the intracellular free calcium ion concentration. This rise in intracellular calcium activates calcium-dependent events (calmodulin, calmodulin-dependent protein kinase, and so on), leading to the release of glutamate. The released glutamate can activate multiple post-synaptic receptors, of which the NMDA type is known to be involved in seizure processes. In addition, the influx of calcium through NMDA, ion channels stimulates nitric oxide synthase (NO synthase) to produce NO [16] and leads to seizure generation. Finally, the blockade of nicotine induced seizures by

diazepam and haloperidol, as previously reported [15] suggests the involvement of other neurotransmitter receptors, such as GABA and dopamine receptors.

C. Mechanisms Leading To Ion-Channel Impairments

Ion channels provide the basis for the regulation of excitability in the central nervous system and in other excitable tissues such as skeletal and heart muscle. Consequently, mutations in ion channel encoding genes are found in a variety of inherited diseases associated with hyper- or hypo-excitability of the affected tissue, and these ion channel defects are known as "channelopathies". An increasing number of epileptic syndromes belongs to this group of rare disorders eg., benign familial neonatal convulsions due to mutations in potassium channels constituting the M-current (KCNQ2, KCNQ3), generalized epilepsy with febrile seizures due to mutations in subunits of the voltage-gated sodium channel or the $GABA_A$ receptor (SCN1B, SCN1A, GABRG2), and episodic ataxia type-1, which is associated with epilepsy in a few patients by mutations within another voltage-gated potassium channel (KCNA1) [17].

1. Sodium Ion Channels: Voltage-gated sodium channels (VGSCs) play an important physiological role in excitable membranes, underlying the action potential initiation and propagation in nerves and muscles [18]. These are dynamic membrane proteins characterized by rapid conformational changes that switch the molecule among closed, resting, activated, and inactivated states ultimately regulating ion conductance through the intrinsic pore [19].

Because of their direct effect in neuronal firing the nervous system is intolerant of even minor variation in the properties of these channel proteins, leading to various kinds of seizure disorders namely infantile spasms, benign familial, neonatal-infantile, and intractable childhood epilepsy. All of the mentioned disorders result from the mutation in the sodium ion channel subunit composition. Nearly 200 sodium channel mutations in patients with epilepsy have been identified [19]. As a consequence, antagonists of these channels may confer beneficial antiepileptic properties and/or neuronal protection following ischemic events.

2. Calcium Ion Channels: Voltage-gated calcium channels (VGCCs) are key regulators of Ca^{2+} entry into neurons, and in this capacity they control a variety of Ca^{2+}-dependent functions, including neurotransmitter release, gene expression, mRNA stability, and neuronal excitability. VGCCs can be divided into two groups, high-voltage activated (HVA), subcategorized into L-, N-, P-, Q-, and R- types [20-21] and low-voltage activated (LVA) i.e. T-type, and both of these groups can affect membrane excitability. Most obvious is the role of HVA type VGCCs in controlling the release of neurotransmitters, such as the excitatory neurotransmitter glutamate [22]. Also, by controlling the entry of Ca^{2+} into neurons VGCCs indirectly influence the activity of Ca^{2+}-activated K^{+} channels and gene expression, which can result in both short and long-term changes in neuronal excitability. The T-type VGCCs, appear to be more directly involved in the control of membrane potential. These channels have been shown to be blocked by known antiepileptic drugs (AEDs) such as Ethosuximide [23-24], further validating them as potentially useful therapeutic targets. VGCCs are also implicated in epilepsy by the recent linkage of several mouse models of absence epilepsy to mutations in VGCC genes [25-26].

3. Potassium Ion Channels: Voltage-gated potassium channels (VGKCs) generally regulate neuronal excitability by influencing the resting membrane potential and shaping action potentials and post-synaptic potentials. Briefly, VGKCs serve diverse functions in the CNS, many of which can be considered in the context of controlling or eliminating epileptic seizure activity. Pre-synaptic VGKCs regulate the release of both excitatory and inhibitory neurotransmitters throughout the brain.

Thus, VGKC openers can be considered in a first approximation to be antiepileptic because they should -

1) Speed the repolarization of the pre-synaptic terminal, which will shorten the duration of the action potential and decrease the amount of transmitter released and

2) Dampen the post-synaptic response to transmitters by stabilizing the membrane potential.

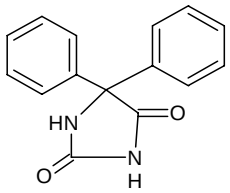
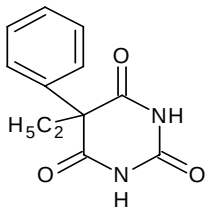
4. **Hydrogen Ion Channels:** The h-current (I_h) is a depolarizing, non-inactivating, mixed Na^+-K^+ current which is activated by hyperpolarization and is present in many neural and cardiac tissues [27-29]. At both single-cell and network levels, hydrogen ion channels are major regulators of neuronal excitability, and possess a rich diversity of subunit composition, distribution, modulation and function. They have also been found to be mechanistically linked with several plasticity processes that could contribute to the hyperexcitable states indicating the potential of hydrogen ion channels modulators as anticonvulsants [30-32]. Recent studies indicate that in complex febrile seizure model, the seizure leads to presynaptic potentiation of GABA release and long term slowing of kinetics of I_h. These changes limit the effect of potential inhibition and even convert the brain tissue to a hyperexcitability state [33].

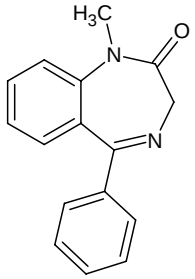
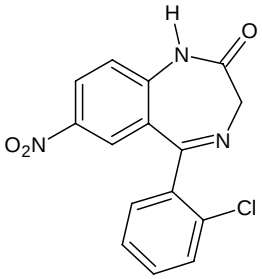
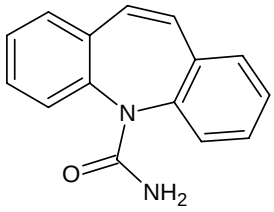
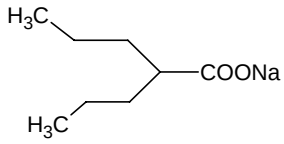
1.2 TREATMENT OF EPILEPSY

Antiepileptic Drug Therapy

Antiepileptic drug therapy is the mainstay of treatment for most patients with epilepsy. The currently available AEDs are a remarkably heterogeneous group of chemicals. They bear little resemblance to one another and exhibit a surprisingly broad array of biological effects. AEDs exert their pharmacologic effects by blocking one or more of the pathways that are involved in neuronal damage i.e. by acting on ion channels, enhancing inhibitory neurotransmission, inhibiting neuroexcitatory transmission, or acting at specific receptors in the brain. However there is considerable overlap between the mechanisms of actions of many AEDs. The following tables (Tables 1.2-1.4) summarize the various antiepileptic drugs according to their stages of development.

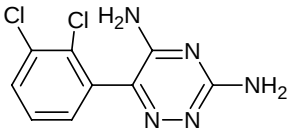
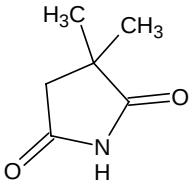
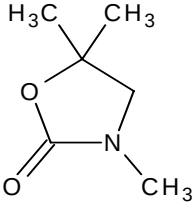
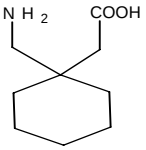
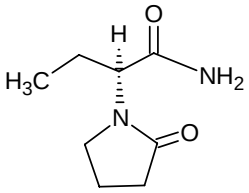
Table 1.2: First generation antiepileptic drugs

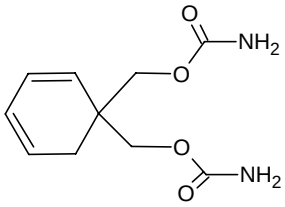
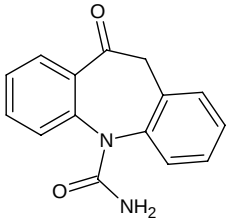
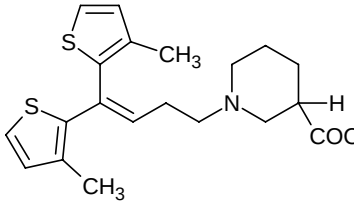
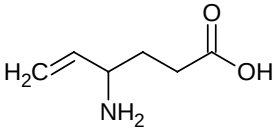
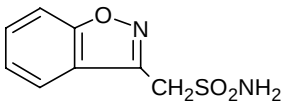
Conventional Drug	Mechanism of Action	Principal Uses	Side Effects
I-1: Phenytoin 	Voltage-dependent Na^+ channel blocker	Tonic-clonic, focal onset	Dizziness, ataxia, inco-ordination, gum hyperplasia, metabolism saturation
I-2: Phenobarbitone 	$GABA_A$ modulator	Tonic-clonic, focal onset	Sedation and tolerance, skin rashes, hepatic enzyme induction - drug interactions

I-3: Diazepam 	$GABA_A$ modulator	Absence, atypical absence, myoclonic, adjunctive for status epilepticus.	Sedation and tolerance
I-4: Clonazepam 	$GABA_A$ modulator	Absence, atypical absence, myoclonic, adjunctive for status epilepticus	Ataxia. sedation, lethargy, anorexia
I-5: Carbamazepine 	Voltage-dependent Na^+ channel blocker	Tonic-clonic focal onset	Ataxia, sedation, aplastic anemia, hepatotoxicity, worsen absence seizures
I-6: Sodium valproate 	Inhibits GABA transaminase and block Na^+ channel. (Synergistic effects).	Tonic-clonic, absence, atypical absence, myoclonic focal-onset	Ataxia, sedation, tremors, hepatotoxicity, gastric irritation, thrombocytopenia, weight gain, transient alopecia

Second generation antiepileptic drugs: This new generation drugs are used in add-on therapy, providing improvement in a significant number of patients suffering from previously refractory epilepsy whilst exhibiting a lower risk of unwanted side effects. They possess broad therapeutic indices, and lack of significant drug interactions [34].

Table 1.3: Second generation antiepileptic drugs

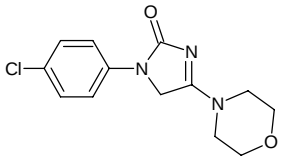
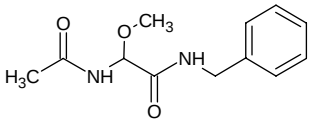
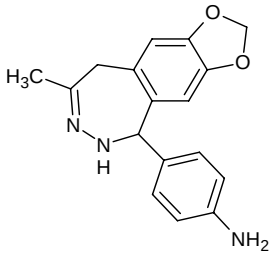
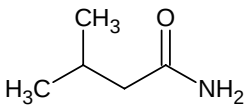
Conventional Drug	Mechanism of Action	Principal Uses	Side Effects
I-7: Lamotrigine 	Voltage-gated Na^+ and Ca^{2+} channel blocker, decrease release of glutamate and aspartate	Tonic-clonic, focal onset, atypical absence, myoclonic, Lennox-Gastaut syndrome	Sedation, ataxia, headache, skin rash, Stevens-Johnson syndrome
I-8: Ethosuximide 	T-type Ca^{2+} channel blocker	Absence –first line treatment	Ataxia, headache, skin rash, bone marrow suppression
I-9: Trimethadione 	T- type Ca^{2+} channel blocker	Absence – first line treatment	Sedation, ataxia, gastric irritation, weight gain
I-10: Gabapentin 	Ca^{2+} channel blocker	Adjunct to partial seizures	Sedation, ataxia, gastric irritation, weight gain
I-11: Levetiracetam 	N-type Ca^{2+} channel blocker AMPA/Kainate blocker	Adjunct for partial seizures and also for Absence seizures	Sedation, ataxia, psychoses, anemia, leucopenia

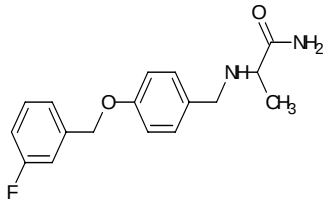
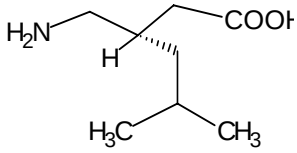
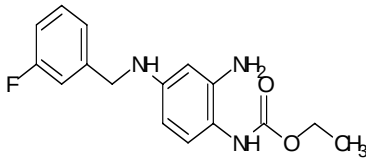
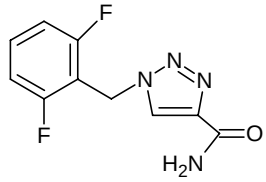
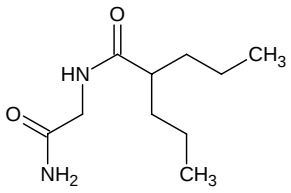
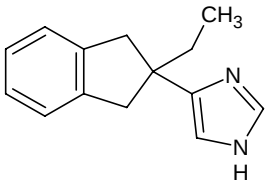
I-12: Felbamate 	Glutamate receptors (NMDA glycine site) antagonist	Partial seizures Lennox-Gastaut syndrome	Sedation, ataxia, gastric irritation, weight loss, hepatic failure, anemia
I-13: Oxcarbamazepine 	Voltage-dependent Na^+ channel blocker	Focal onset, primary generalized tonic–clonic seizures	Ataxia, sedation, aplastic anemia, hepatotoxicity, hyponatremia
I-14: Tiagabine 	Inhibits GABA reuptake	Tonic-clonic, focal onset	Sedation, ataxia, psychoses, worsen absence seizures
I-15: Vigabatrin 	Inhibits GABA transaminase	Adjunctive in partial seizures	Sedation, ataxia, psychoses, gastric irritation
I-16: Zonisamide 	Modifies low-threshold neural Ca^{2+} currents	Adjunctive in partial seizures	Sedation, ataxia, anorexia, renal stones

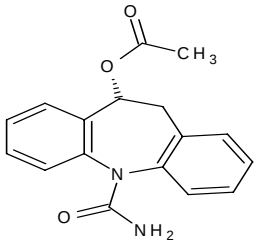
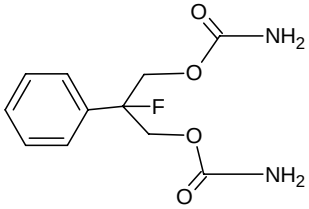
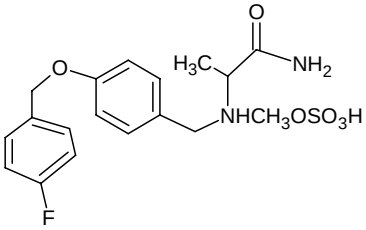
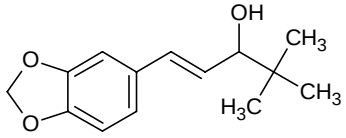
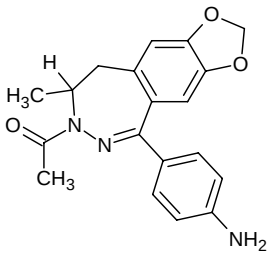
Newer antiepileptic drug moieties (under preclinical/clinical development stage)

To overcome the still existing problem with second generation AEDs, there are further lot many numbers of lead molecules, undergoing various stages of clinical/ preclinical development, which can serve as the promising prototype lead for future anticonvulsant drugs [35]

Table 1.4: Newer antiepileptic drug moieties (under preclinical/clinical development stage)

Drug Moiety	Mechanism of Action	Effective against	Side Effects
I-17: AWD-131 	Voltage-gated Ca^{2+} channel blocker	Maximal electroshock seizures (MES), and chemical e.g. pentylenetetrazole (PTZ), bicuculline (BIC)-induced seizures, audiogenic amygdala-kindling seizure models	Low neurotoxicity and high metabolic stability
I-18: Harkoseride 	Blocks glycine strychnine-insensitive recognition site of NMDA receptor	MES seizures, sound-induced seizures, animal models of status epilepticus	Good tolerability, no known histopathologic changes and drug interactions
I-19: LY 300164 	Selective, Non-competitive AMPA antagonist	MES seizures , PTZ-induced, chemical-kindles seizures, phenytoin-resistant status epilepticus models	Ataxia, depression recumbency, and developmental toxicity.
I-20: NPS 17 76 	Unknown	MES, and chemical induced seizures, corneal and amygdala-kindled rat models of epilepsy	No behavioral or genetic toxicity. no effect on cytochrome P450

I-21: NW- 1015 	Voltage-gated Na^+ and Ca^{2+} channel blocker, decreases release of glutamate	MES and chemical induced seizures, kainic acid model, primate model of partial complex seizures	Good tolerability
I-22: Pregabalin 	Increases GABA content, Voltage-gated Ca^{2+} channel blocker and enhances glutamic acid decarboxylase (GAD) activity	MES, and chemical induced seizures, audiogenic seizures models	Well tolerated
I-23: Retigabine 	Increasing K^+ ion conductance in neuronal cells	MES, and chemical induced seizures amygdala-kindled models, status epilepticus models	Well tolerated
I-24: Rufinamide 	Interacts with the inactivated state of the Na^+ channel	Various preclinical models MES, BIC, picrotoxin (PIC), strychnine (STY) and NMDA	High safety index and protective ratio
I-25: Valrocemide 	Inhibits GABA transaminase	Against MES and various chemoconvulsants, audiogenic and hippocampal-kindled seizures	Good tolerability over Sodium valproate
I-26: Atipamezole 	α_2-adrenoceptor Antagonist	Does not prevent epileptogenesis, but modifies to milder and non-progressive form.	Well tolerated

I-27: BIA 2-093 	Potent blocker of voltage-gated Na^+ channels	MES and amygdala kindling models, hence effective against generalized tonic-clonic and partial-onset seizures	Not known adverse effects
I-28: Fluorofelbamate 	Inhibit kainate and NMDA receptor activation, decrease voltage-dependent Na^+ currents	Effective in electrically & chemically induced seizures, audiogenic seizure models, and initial models of status epilepticus	No observed adverse-effect
I-29: Safinamide 	Reduces sodium, N-type and L-type calcium current amplitude and subsequent inhibition of glutamate release	MES and several chemoconvulsants including BIC, PTZ, 3-AMPA , PIC, STY and kainic acid, amygdaloid kindled seizures in rats	Favorable protective index, no observed adverse-effect and drug interactions
I-30: Stiripentol 	Inhibits the synaptosomal uptake of GABA	Broad spectrum of activity, about to become the first orphan AED for children	No conspicuous toxicity
I-31: Talampanel 	Non-competitive AMPA antagonism	Broad spectrum anticonvulsant properties	Well tolerated, mild dizziness, headache, and somnolence

1.3. Problems associated with the available antiepileptic drug therapy

1. Despite the remarkable effectiveness, the available drugs fail to produce complete seizure control. With the current drug therapy, prognosis of disease is good in at least 60% of patients, but up to 40% of individuals suffer from intractable pharmaco-resistant epilepsies [36-37]. Even in some circumstances, therapy leads to unwanted worsening of the seizures and causes tolerance and adverse pharmacodynamic effects of individual AEDs on seizure generating mechanisms [38].
2. These agents are also far from ideal in terms of side effects. Most of the conventional AEDs are associated with various kinds of toxicities, the most common being neurotoxic side effects, idiosyncratic reactions, metabolic disorders, serious liver damage and aplastic anemia. Children and pregnant women are more susceptible to these side effects. Major birth defects associated with anticonvulsants affect the heart, skeletal system, urogenital system and the neural tube. The conditions arising due to neural tube defects include spina bifida and anencephaly.
3. Furthermore, although some patients tolerate treatment with second generation AEDs better than the older drugs, the occurrence of either intractable/difficult to control seizures has not changed significantly following their introduction [39]. They just serve as add-on therapy and this multiple therapy further leads to the difficulty of differentiating the adverse effects of an added drug from those resulting from concomitant medications [40].
4. Until now it's not easy to correlate the known mechanism of conventional AEDs with the success of the treatment. The reasons include, a) treatment of epilepsy targets the symptoms and not the cause i.e. underlying mechanism of initiation and propagation of epileptogenesis b) Clinically relevant mechanism of the disorder in individual patients differs depending upon whether the initiating event is brain injury, status epilepticus or something else. As a result, there is no real consensus, as to which of the countless cellular effects actually contributes to the clinical activity of the individual AEDs in individual patients. c) Current seizure classification does not define the homogeneous population of patients. Most of the

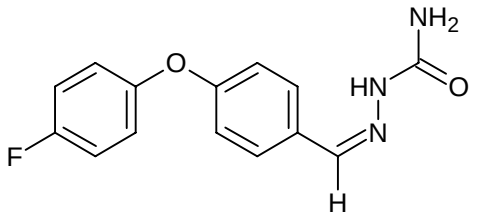
drugs used in the first line monotherapy treatment are not universally effective [38].

5. Lack of successful genetic models is another cause for many forms of epilepsy to go untreated. Immune mechanisms are involved in the pathogenesis of many forms of epilepsies, which transmit through complex polygenic inheritance rather than simple monogenic inheritance [41-43].
6. The mechanism of action of AEDs is commonly established in non-human species. There is little or no evidence to suggest that these mechanisms can be extrapolated to human beings. Without detailed investigations in human tissues, it's difficult to predict that any of the currently held perceptions of the AEDs mechanism of action are clinically relevant [38].

Although each new drug introduced has its unique advantage, disadvantages are still there. Thus none has proved to be the ultimate drug for epilepsies and the need for better, novel AEDs is still there. Application of rational drug design models has led to certain new leads, which are undergoing various preclinical and clinical trials [35]. On the basis of knowledge of the characteristics of different receptor sites, and by means of identification of the minimal requirements associated with the pharmacophoric pattern for a manifested activity at the targeted sites, several of them would undoubtedly become meaningful additions to the neurobiologist's pharmacological armamentarium. The discovery of these compounds was also based on the rational considerations of pathophysiological mechanisms of epileptic syndrome, in conjugation with the detailed understanding of central excitability mechanisms and logical principles of drug design. Unlike other classes these are all structurally and mechanically unique and not possible to discuss them as a single class of agents. Hence, the endeavor of the century is to develop antiepileptic drugs with 100% efficacy, safety and tolerability.

Recently aryl semicarbazones have been explored as newer chemical entities with potential anticonvulsant effects. The rationale behind the development of semicarbazones is their structural dissimilarity to the existing antiepileptic drugs. So it was hoped that such novel compounds would lack the side effects seen with many of the currently

available medications. The following 4-(4-fluorophenoxy) benzaldehyde semicarbazone (**I-32**) has undergone various stages of preclinical and clinical trials [44].



(I-32)

CHAPTER 2
LITERATURE REVIEW

2.1. SUBSTITUTED ARYL SEMICARBAZONES AS ANTICONVULSANTS

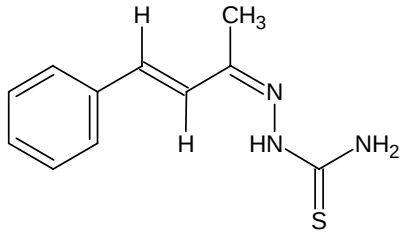
The structural requirements for activity in the MES test to detect compounds effective in treating generalized tonic-clonic seizures, have been claimed to be the presence of at least one phenyl ring or similar aromatic group in close proximity to two electron donor atoms [45]. Compounds displaying useful anticonvulsant properties in subcutaneous pentylenetetrazole test (scPTZ), often possess an alkyl substitution close to two electron donor atoms i.e. the hydrophobic moiety should be smaller than compounds effective in the MES test [45].

In view of these general requirements for activity and desire to produce novel synthetic anticonvulsants, that don't possess the dicarboximide group, which may be associated with toxicity and side effects [46] and also the strategy of incorporating the ureas, hydrazo and amidic functions into a single functional moiety as various ureas, hydrazones and amidic derivatives have been associated with good anticonvulsant activity [47-49], led to the development of some initial series of semicarbazones and thiosemicarbazones as reported by Dimmock *et al*.

2.1.1. Development of aldehyde/ketone substituted aryl semicarbazones

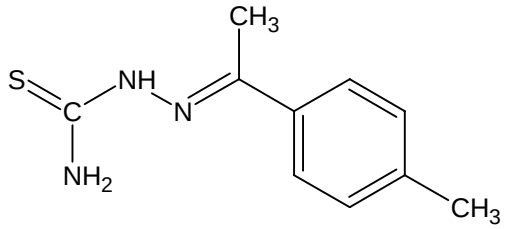
In the earliest reports, a series of thiosemicarbazones and semicarbazones of arylidene methyl ketones were prepared and evaluated in the MES, scPTZ and neurotoxicity screens by Dimmock *et al*. [50]. Seventeen compounds out of twenty-two, were active in MES and/or scPTZ screens when given by intraperitoneal (*i.p.*) route to mice. Both neurotoxicity and lethality in mice were higher with the thiosemicarbazones than semicarbazones. Compound 4-(4-methylphenyl)-3-butene-2-one thiosemicarbazones (**II-1**), showed an ED_{50} (effective dose in 50% animals) value of 6.96 mg/kg in the scPTZ induced threshold test and protection index (PI) (TD_{50} / ED_{50}) of 10.37. The compound

also afforded some protection against seizures induced by bicuculline and picrotoxin and had no effect on the activities of glutamic acid decarboxylase (GAD) and γ-aminobutyrate transaminase (GABA-T).



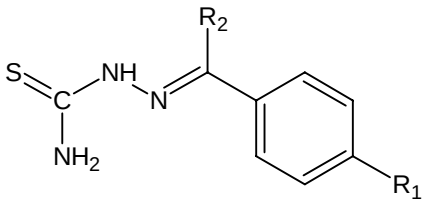
II-1

Dimmock *et al.*, [51] in 1990 evaluated a number of thiosemicarbazones of arylidene and aryl ketones as potential anticonvulsant agents. Most of the compounds displayed activity in the MES and scPTZ seizure models. Acetophenone thiosemicarbazone (**II-2)** exhibited good anticonvulsant activity when administered by the *i.p.* and oral routes. The X-ray crystallographic studies revealed that it possessed E-configuration to both olefinic and carbimino double bonds.



II-2

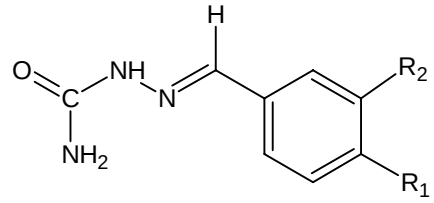
In 1993, Dimmock *et al*., [52] synthesized and evaluated few series of semicarbazones and thiosemicarbazones derived from aryl aldehydes, phenyl alkyl aldehydes and phenyl alkyl ketones as well as some related compounds for anticonvulsant activities [**II-(3-6)**]. Most of the compounds displayed activity in the MES, scPTZ, BIC and PIC-induced seizures models, when given intraperitoneally to mice.



II-3

R_1 = H, Cl, OCH_3

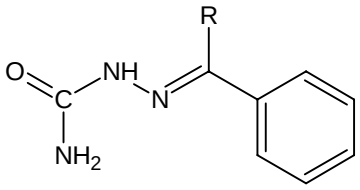
R_2 = H, CH_3



II-4

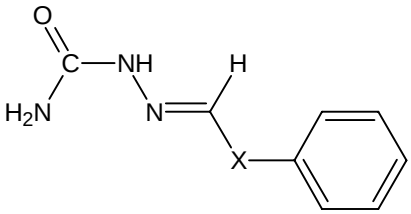
R_1 = H, Cl, OCH_3

R_2 = H, Cl



II-5

R = CH_3, C_2H_5, $CH(CH_3)_2$, $CH_3(CH_2)_3$ $CH_3(CH_2)_4$, C_6H_5



II-6

X = CH_2, CH_2CH_2, CH=CH

On oral administration to rats, two interesting features were observed. Firstly, a marked activity in the MES screen was noted whereby some of the compounds had ED_{50} values in 20-25 mg/kg range while activity in the scPTZ test was virtually abolished. Secondly neurotoxicity was diminished and high PI figures (~25) were observed in some of the semicarbazones, over the relative thiosemicarbazones. Most of the semicarbazones showed rapid onset of action and the data revealed a common mode of action with chloride ion channels.

Molecular modeling studies revealed a number of statistically significant descriptors. i.e. geometric distance between hydrogen bonding and lipophilic moiety (C4-C7), orientation between lipophilic and hydrogen bonding moiety (C7-N8-N9-C10 torsional), the geometry of hydrogen bonding moiety (C7-N8-N9 angle), charge on N12 and C6 atom, which contributed significantly to the anticonvulsant activity. Compounds with 3-Cl and 4-Cl substitutions displayed higher activity than 2-Cl, as the *ortho*-substitution would cause a large interplanar (ø) angle to be formed between aryl ring and the adjacent azomethine bond. Correlations were noted between the Hammett's constant (σ) and Taft constant (σ*) values of aryl substituents.

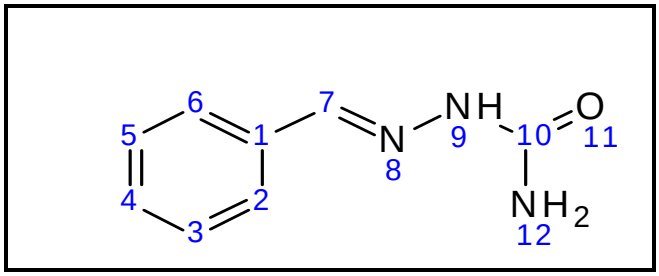
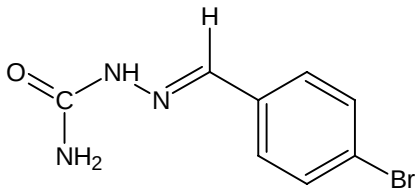


Fig. 2 Numbering scheme of the semicarbazones used in X-ray crystallography and molecular modeling studies

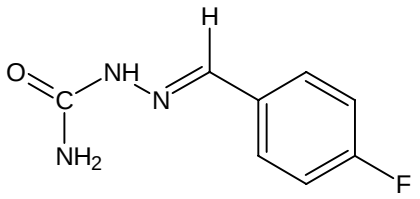
This study led to the identification of the first lead compound 4-bromo benzaldehyde semicarbazone, **II-7** (NC 1132) by Dimmock *et al*., [53] in 1994. The lead compound was bereft of neurotoxicity at the maximum dose administered orally to the rats (500mg/kg) yet displayed potent activity, showing an ED_{50} value of 22.3 mg/kg and high PI value of 44. This safety margin was compared with the established drugs such as Phenobarbital and Mephenytoin with PI indices of 6.7 and 4.7 respectively.



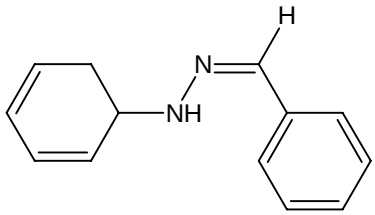
II-7

Dimmock *et al.*, [54] in 1995 synthesized and screened few series of aryl semicarbazones to develop structure activity relationships and also applied empirical and semi-empirical calculations to determine which portions of the molecules were important in conferring anticonvulsant properties. Introduction of electron-withdrawing substituents into the aryl ring produced compounds with promising activities in contrast to the use of electron-donating groups, as electronic properties of the aryl substituents (σ/σ*) were shown to influence anticonvulsant properties. Hence the compounds with 4-fluoro substituents (i.e. **II-8**) were most active in the series. Compounds with methyl group rather than a hydrogen atom adjacent to the carbimino group, showed comparatively low ED_{50} values. On replacement of the terminal amino group by a phenyl ring (**II-9, II-10**) decreased the

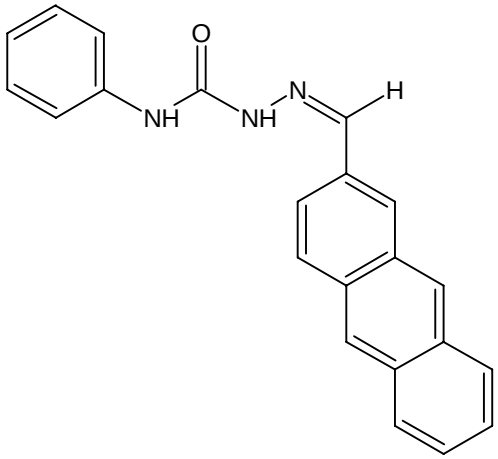
activity more than thirteen- and six-fold in the MES and scPTZ screens respectively i.e. increased Van der Waals' bonding at the receptor site on the loss of capacity of amino group to form hydrogen bonds, didn't lead to any increase in activity.



II-8

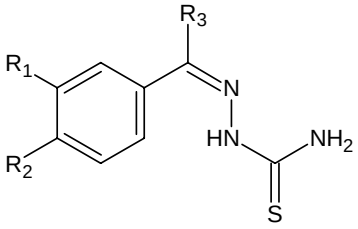


II-9



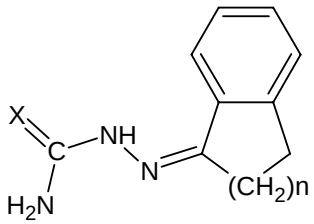
II-10

In continuation to this work Dimmock *et al*., [55] in 1995 synthesized a number of semicarbazones, thiosemicarbazones and bis-carbohydrazones of aryl alicyclic ketones [**II-**(**11-14**)]. X-ray crystallography had also been reported as an estimate of the shape of the synthesized molecules.



R_1, R_2 = H, Cl R_3 = H, Alkyl X= S

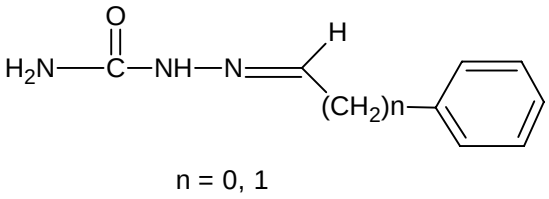
II-11



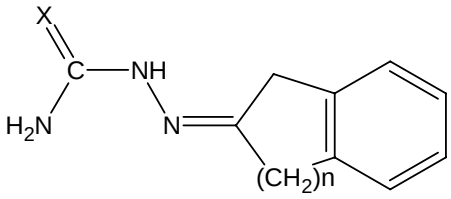
II-12

Most of the compounds displayed activity in the MES and scPTZ models, when administered *i.p.* to mice; however, upon oral administration the compounds displayed selective activity in the MES screen only. The important observations which emerged from this study were:

- Both semicarbazones and thiosemicarbazones displayed good anticonvulsant activity, but on certain occasions greater neurotoxicity was revealed with thiosemicarbazones than with the corresponding semicarbazones.
- It has been postulated that the semicarbazone group and aryl ring align at complimentary areas on the macromolecular complex *in vivo*, which has been referred to as hydrogen bonding area and aryl binding site respectively.
- The frozen molecules (**II-13** and **II-14**) exhibiting restricted rotation of the aryl ring showed lesser anticonvulsant activity than more flexible analogues i.e. the occupation of the different position by the aryl ring on the binding site depends upon the position occupied by the aminocarbonylamino group on the hydrogen bonding area.



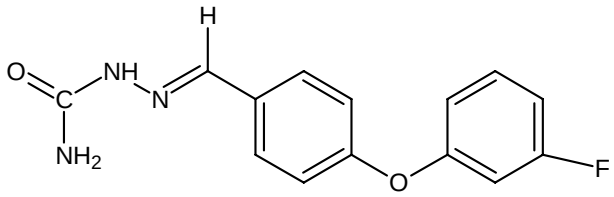
II-13



II-14

In 1996, Dimmock *et al.*, [56] investigated the area around the postulated aryl binding site, by attaching another phenyl ring to it at various positions *via* some spacer groups i.e. -O-, -NH- etc. This additional aryl ring could increase Van der Waals' bonding and so could increase the potency of the compounds. This resulted in the generation of a number of (aryloxy) aryl semicarbazones and related compounds. Initially, the second aryl ring designated as the distal aryl ring was attached with the proximal aromatic ring, nearest to the semicarbazono group, which resulted in the abolishment of the activity in the MES, scPTZ and neurotoxicity (NT) screens. So it was considered that the realignment of distal ring at some distance from the proximal ring i.e. placement of the spacer group between

two aryl rings. Compound **II-15** emerged as the most active compound showing greater protection in the MES test than the scPTZ screen, with no neurotoxicity at the maximum dose administered.



II-15

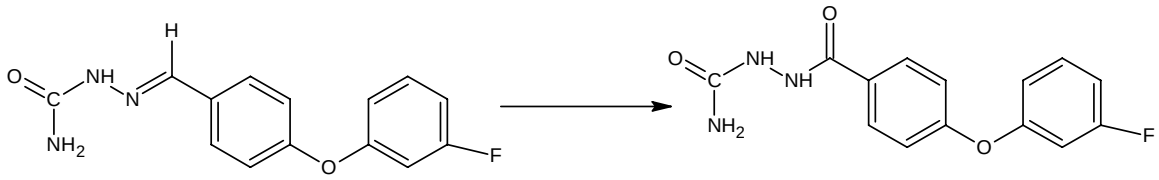
The conclusions drawn from this study were:

- The compounds with fluoro/ bromo group at the *para* position of the aryl ring showed high potency even greater than the leading drugs i.e. Phenytoin, Carbamazepine, and Sodium valproate.
- The placement of aryloxyaryl group at the *para* position of the proximal aryl ring is preferable to *ortho*/*meta* positions- no proper alignment at the aryl binding site.
- Highest activity was found with compounds having alkyl group in place of methine proton, indicating additional favorable hydrophobic area on the binding site.
- Also, further increase in the size of the spacer group other than -O-, -S-, -NH-, led to a remarkable decrease in the anticonvulsant activity.

X-ray crystallography studies of some of the compounds revealed the importance of certain interatomic distances and bond angles for activity in the mouse and rat MES screens.

The (aryloxy) aryl semicarbazones **II-15** (4-(4'-fluorophenoxy) benzaldehyde semicarbazone) was selected for full scale preclinical evaluation [44], which afforded protection in fringe audiogenic model, rat kindling hippocampal model (*i.p.*) and rat kindling corneal screen (oral). The compound didn't display proconvulsant properties, nor did it produce a significant effect on the liver weights and various hepatic enzymes and also found to be superior to the widely used antiepileptic drug Phenytoin.

Dimmock *et al.*, [57] also studied the metabolism of (aryloxy) aryl semicarbazones by examining the urinary metabolite of the compound **II-15**, after dosing orally to the rat at 50 mg/kg. This study revealed that most of the drug was converted into its metabolite **II-15a** (4-(4'-fluorophenoxy) benzoyl semicarbazide).



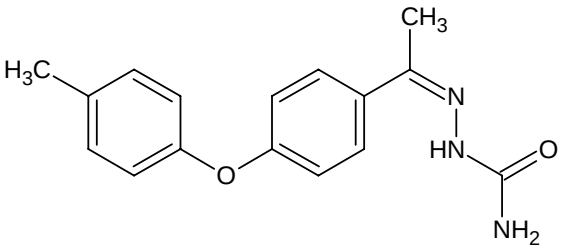
II-15 **II-15a**

The metabolite was devoid of activity in the rat oral MES screen. This provided strong evidence that the anticonvulsant activity of 4-(4'-fluorophenoxy) benzaldehyde semicarbazones and related compounds is due to the intact molecule.

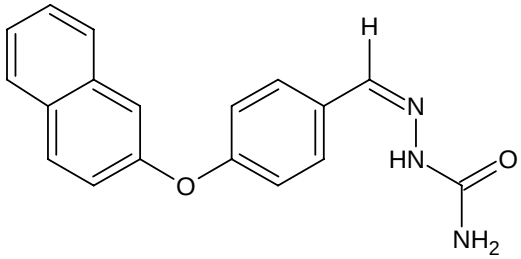
Recently, whole-cell patch-clamp techniques have been used to study the effects of 4-(4'-fluorophenoxy) benzaldehyde semicarbazones (**II-15**) on native and recombinant mammalian voltage-gated Na^+ channels [58]. **II-15** blocked Na^+ currents (I_{Na}) in acutely dissociated cultured rat hippocampal neurons and the potency increased with membrane depolarization, suggesting a state-dependent mechanism of inhibition. Compound **II-15** shifted the steady-state availability curve in the hyperpolarizing direction and significantly retarded the recovery of Na^+ channels from inactivation. These results suggested that inhibition of voltage-gated Na^+ channels was a major mechanism underlying the anticonvulsant properties of **II-15**.

Puthucode *et al.*, [59] evaluated a number of aryl, arylidene and aryloxy semicarbazones as candidate anticonvulsants. Various molecular modifications were undertaken which led to an increased potency. Firstly, insertion of an olefinic linkage between the aryl ring and carbimino carbon atom led to an increase in the activity and potency. The presence of electron rich atom at the *para* position of the aryl ring resulted in high potency in MES screen. Secondly, substitution in the aryl ring by halogen or methyl group led to a number of semicarbazones with low ED_{50} values in rat oral MES screen with high PI values. Compound **II-16,** showed an ED_{50} of 5.62 mg/kg, against MES-induced seizures. Further

increase in the size of distal aryl ring led to **II-17,** which was bereft of any neurotoxicity at the maximum dose administered to mice (500 mg/kg). Thus the distal binding site is capable of accommodating an aryl ring with at least two substituents on the distal ring.



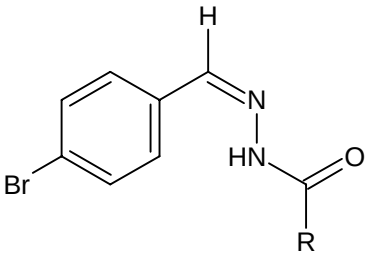
II-16



II-17

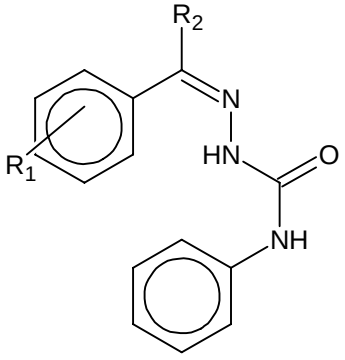
2.1.2. Development of alkyl/ phenyl substituted aryl semicarbazones

To evaluate the importance of the terminal amino group, Dimmock *et al.*, [60] in 1996, replaced it with different atoms and groups (**II-18, II-19**).



II-18

R = H, CH_3, $CH_2N(CH_3)_2$.HCl, $CH_2NC_6H_5$, Substituted Phenyl



II-19

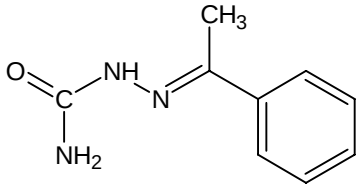
R_1 = H, Br, Cl, CH_3

R_2 = H, CH_3

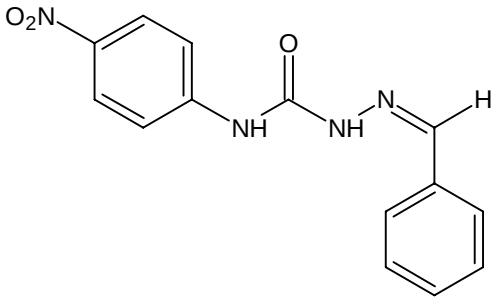
Semicarbazones possessing a phenyl amine group attached to the carbonyl function showed greater activity than the compounds in which an aryl group is adjacent to the carbonyl function. This complete removal of amino group led to the change in electronic

parameters (σ/σ*) of the ring. However small groups i.e. H, CH_3, with carbonyl function led to the retention of significant anticonvulsant activity.

A series of semicarbazones and thiosemicarbazones were synthesized and evaluated against various seizure models [61]. Most of the compounds provided significant protection against MES and subcutaneous strychnine (scSTY) induced seizures. The compound (**II-20**) was the most active in the series with activity at a dose of 30mg/kg in strychnine- induced seizure pattern test with an ED_{50} of 10 mg/kg in the MES test.



II-20



II-21

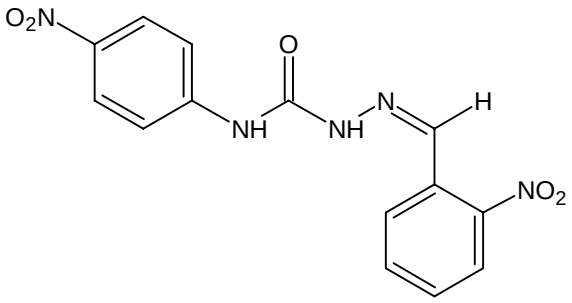
Comparison between the semicarbazones and the corresponding thiosemicarbazones revealed that the semicarbazones were active at the lower doses as compared to the corresponding thiosemicarbazones and in addition semicarbazones showed neurotoxicity at higher doses and lesser sedative-hypnotic potential. Further, compounds with *p*-nitro phenyl substitution in place of amino hydrogen (**II-21**) showed activity at 30 mg/kg and an ED_{50} of 83 mg/kg in the MES test.

Further, Pandeya *et al.*, [62] in 1999 evaluated a series of *p*-nitrophenyl substituted semicarbazones for anticonvulsant properties. Most of the compounds showed protection in the MES, scPTZ and scSTY seizure models and also exhibited neurotoxicity at higher doses. The compound (**II-22**) emerged as the most active compound.

The important results from the above studies were:

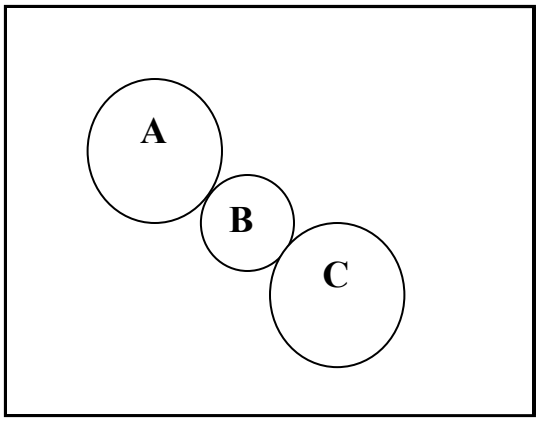
- Instead of primary amino function of the semicarbazone derivatives, the hydrogen bonding domain may be constituted by three atoms, that is -CONH-N= pharmacophore. Incidentally, this is present in many anticonvulsant drugs like the derivatives of diphenylhydantoin.

- The hydrophobic region i.e. aryl ring should have an electron withdrawing group at the *para* position.
- These compounds might also act through glycine receptors because of their profound activity in the scSTY test.



II-22

Previous studies led to the postulate that aryl semicarbazones displaying activity in the MES screen interact at the hydrophobic and hydrogen bonding area, designated as areas A and B (Fig. 2). Based on initial evidence that a second lipophilic group in the molecule was advantageous, Dimmock *et al.*, [47] in 2000 proposed the binding site hypothesis. It was suggested that a second lipophilic ring in the molecule will interact at the area C on the proposed binding site. Most of the compounds prepared with the view of interacting at sites A, B and C showed protection in mice against MES-induced seizures. These hydrophobic binding sites are capable of accommodating groups of various sizes as measured by their solvent accessible surface areas. It was also discovered that a linear group of up to four atoms capable of hydrogen bond formation can be accommodated well at area B of the binding site.

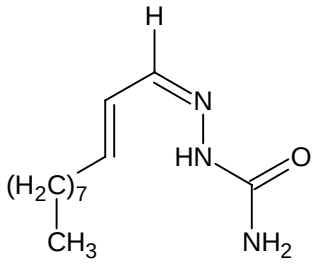


A and C = Hydrophobic binding sites

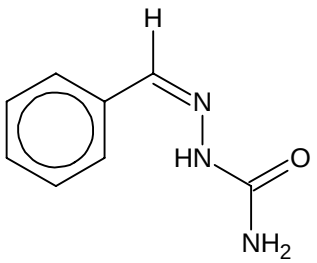
B = Hydrogen bonding area

Fig. 3 Proposed binding site of ureylene anticonvulsants

Various acetylhydrazones, oxamoylhydrazones and semicarbazones were synthesized in order to explore the effect of terminal amino group on the hydrogen bonding capabilities, and hence on the anticonvulsant activities [63]. Measurement of atomic charges showed that variation from amino to methyl and aminocarbonyl group affect the electronic properties of the compounds. Hence acetylhydrazones and semicarbazones afforded greater protection in the MES screen than the corresponding oxamoylhydrazones, which may have been due to the higher negative charge on the carbonyl oxygen with more active compounds. While semicarbazones afforded greatest protection in the rat oral MES screen, they showed unanticipated lower activity than acetylhydrazones in MES test when administered to mice by the *i.p.* route.



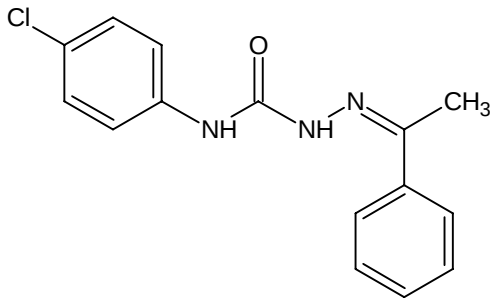
II-23



II-24

Compound **II-24** showed favorable ED_{50} and PI values of 69.7mg/kg and 2.93 respectively. Replacement of the phenyl ring of semicarbazones by an n-octyl group (**II-23**), led to reduced activity. However the preparation of a series of analogues with olefinic double bond led to an increase or retention of activity.

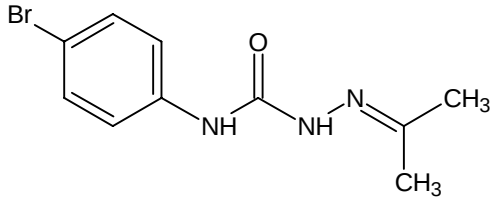
In 2000, Pandeya *et al.*, [64] reported a series of *p*-chlorophenyl substituted aryl semicarbazones and screened for anticonvulsant activity. Most of the compounds provided significant protection against MES at 100 mg/kg at 0.5 h and at 300 mg/kg after 4h in both MES and scPTZ seizure tests. In the scSTY test, most of the compounds showed protection at 30mg/kg, indicative of a broad spectrum of activity of the synthesized compounds. The compound (**II-25**) emerged as the most active compound of the series.



II-25

This study further supported the evidence that presence of an electron rich group (e.g. Cl) on the aryl ring is beneficial for anticonvulsant activity.

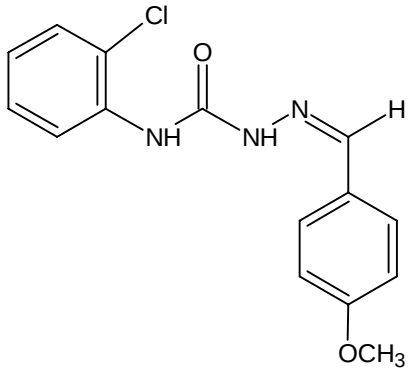
To further confirm the role of an electron rich group substituted at *para* position of the aryl ring, Pandeya *et al.*, synthesized a series of 4-bromophenyl substituted aryl semicarbazones [65]. After *i.p.* injection to mice, the semicarbazone derivatives were examined in the MES, scPTZ and scSTY and neurotoxicity (NT) screens. All the compounds showed activity in one or another model. The compound N^1-(4-bromophenyl)-N-(propan-2-one) semicarbazone (**II-26**), showed greatest activity, being active in all the screens with very low neurotoxicity and no sedative-hypnotic activity. Most of the compounds showed lesser neurotoxicity than Phenytoin and greater protection than Sodium valproate.



II-26

The distal aryl ring was supposed to be essential for the pharmacokinetic properties since variation at the distal aryl ring was found to affect the biological properties. The other important finding was that these substituted phenyl semicarbazones could emerge as the bioisosters of desmethyl diazepam as they largely resemble in structure.

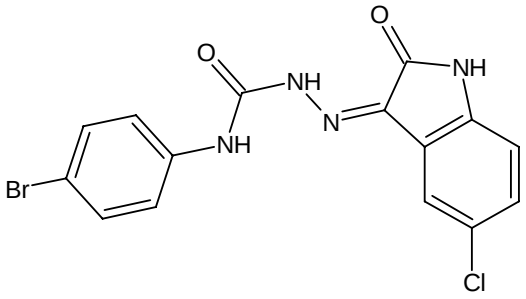
Further Pandeya *et al.*, in 2001, [66] synthesized a series of 2-chlorophenyl substituted semicarbazones and evaluated for the anticonvulsant activities. Compound N^1-(2-chlorophenyl)-N-(4-methoxy benzylidene) semicarbazone (**II-27**) emerged as the most potent compound showing activity at a dose of 30mg/kg in the scSTY-induced seizure model. Most of the compounds showed significant potentiation of sedative and hypnotic activity of Phenobarbitone sodium



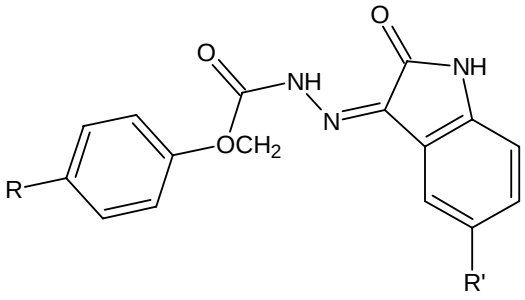
II-27

In 2002, Pandeya *et al.*, [67] designed a new series of substituted isatin semicarbazones and related bioisosteric hydrazones to meet the structural requirements essential for anticonvulsant activity. Most of the synthesized isatin semicarbazones exhibited protection at 100 and 300 mg/kg in MES and scPTZ test respectively, after intraperitoneal administration to mice. Some of them showed good anticonvulsant activity in the MES test in rats, after *per oral* (*p.o.*) administration at the dose of 30

mg/kg. Compound 6-chloro-isatin-3-(4-bromophenyl) semicarbazone (**II-28**) emerged as the most active analogue of the series.



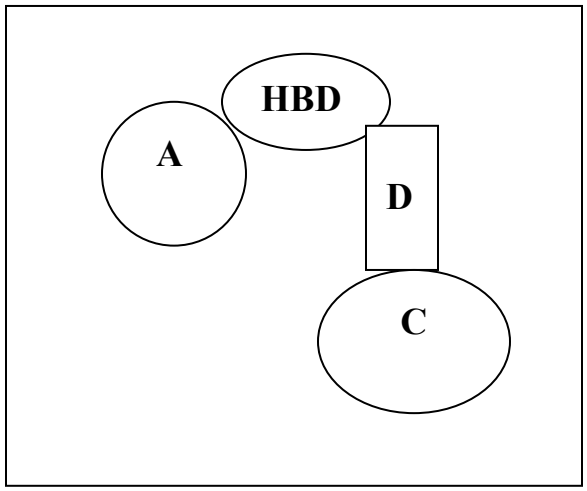
II-28



R = H, Br R’ = 4-Cl, 6-Cl

II-29

The study also revealed the importance of terminal amide linkage for the hydrogen bonding with the receptor, as the replacement of –NH group with non-hydrogen bonding –OCH_2 group (**II-29**), led to complete abolishment of anticonvulsant activity. The other important outcome of the study was the proposal for a new pharmacophoric model, which included not only the three binding site domains as mentioned earlier [47] but also an additional electron donor system D, for bioactivity.



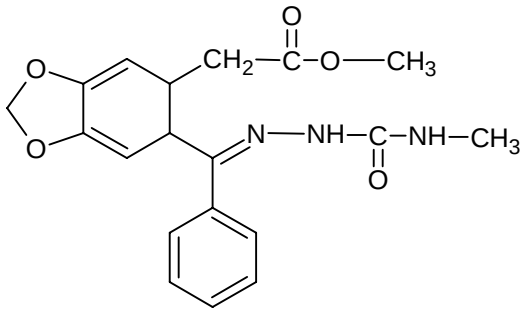
A and C = Hydrophobic binding sites

HBD = Hydrogen bonding domain

D = Electron donor group

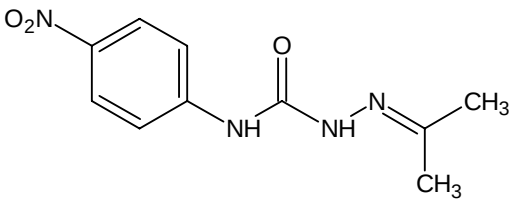
Fig. 4 Proposed pharmacophoric model for substituted aryl semicarbazones

Micale *et al*., [68] in 2002 described the synthesis of a series of novel 2-[(4-alkylsemicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxyphenylacetic acid alkyl esters, carrying an alkylsemicarbazono moiety at a benzylic site. The influence of this group on the biological activity was evaluated by testing the corresponding derivatives in which the 4-alkylsemicarbazono moiety was removed or its alkylureido portion shifted to other position. The anticonvulsant activity of all the compounds was evaluated against audiogenic seizures induced in DBA/2 mice. Within this series of derivatives, 2-[(4-aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxy-phenylacetic acid methyl ester (**II-30**), proved to be the most active compound. It displayed potency 5-fold higher than that shown by 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*- 2,3-benzodiazepine (GYKI 52466), a well-known noncompetitive AMPA receptor antagonist. It was also effective in suppressing seizures induced in swiss mice by MES or PTZ. Furthermore, it antagonized *in vivo* seizures induced by *i.c.v* administration of AMPA or kainate (KA).

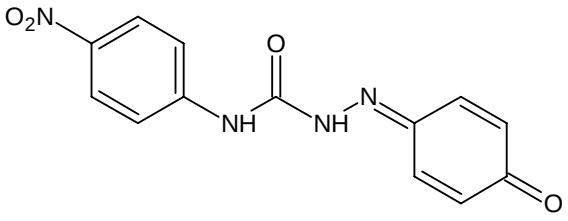


II-30

To reveal the essentiality of the hydrogen bonding domain in semicarbazones adjacent to the lipophilic aryl ring for anticonvulsant activity, Pandeya *et al*., in 2003 [69] further synthesized and evaluated a series of *p*-nitrophenyl substituted semicarbazones and phenoxy/*p*-bromophenoxy acetyl hydrazones. The compounds with the –NHCO- group (**II-31, II-32**) showed anticonvulsant activity in all the screens better than Valproic acid in MES and scPTZ tests.

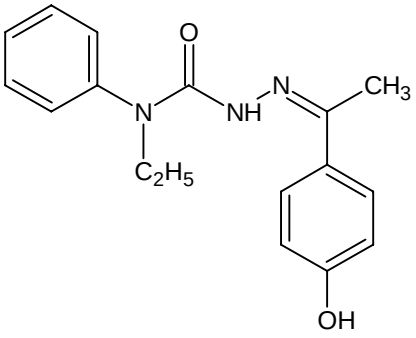


II-31

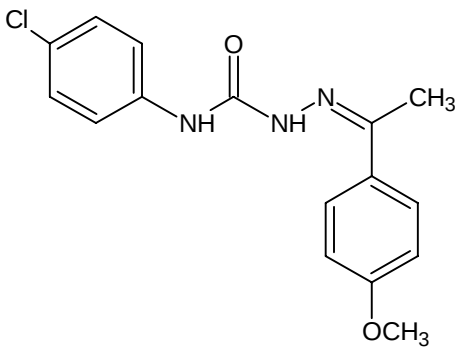


II-32

Pandeya *et al.*, in 2003, [70] synthesized a series of 4-N-substituted aryl semicarbazones and evaluated for anticonvulsant activity. The lipophilicity was increased by substituting alkyl (ethyl) moiety at the terminal nitrogen (4-N) of aryl semicarbazones and by synthesizing alkoxy (methoxy) derivatives at the distal aryl ring of aryl semicarbazones. These compounds were assumed to be dealkylated after metabolism and the alkyl (ethyl) and alkoxy (methoxy) groups were replaced by hydrogen, which is considered to be essential for activity. The compounds provided significant protection against MES and scPTZ-induced seizures at 300 mg/kg after 0.5 h. Compounds **II-33** and **II-34** showed activity in both MES and scPTZ models. This increased lipophilicity led to an increased activity and decreased toxicity.



II-33

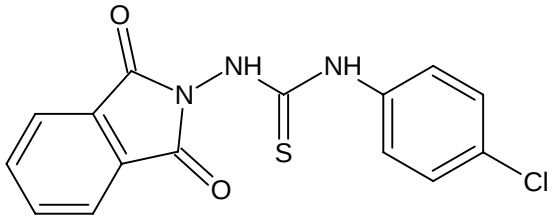


II-34

However, one disadvantage found to be associated with these semicarbazones was their low water solubility. As a strategy to circumvent this problem, a 1:1 inclusion compound of benzaldehyde semicarbazone and hydroxypropyl-β-cyclodextrin (HP-β-CD) was prepared and characterized [71]. The anticonvulsant activities of the free semicarbazone

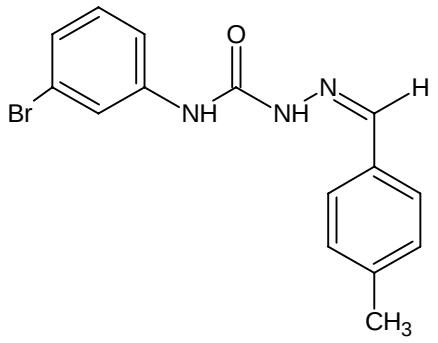
and of the inclusion compound were evaluated in rats using MES and audiogenic seizure models. In both tests the minimum dose of compound necessary to produce activity decreased from 100 mg/kg for the free semicarbazone to 35 mg/kg for the inclusion compound, indicating a significant increase in the bioavailability of the drug.

Yogeeswari *et al.*, in 2003 [72] synthesized and evaluated phenyl (thio) semicarbazide derivatives of the phthalimido pharmacophore for anticonvulsant and neurotoxic properties. Initial anticonvulsant screening was performed using intraperitoneal MES, scPTZ and scSTY tests in mice. Compound N^4-phthalimido-4-chlorophenyl-thio-semicarbazide (**II-35**) afforded protection in all the screens. Most of the compounds were bereft of neurotoxicity at the maximum administered (300 mg/kg) dose. Though the synthesized compounds exhibited CNS depression and behavioral side effects, they were lesser than that observed with the conventional antiepileptic drugs.



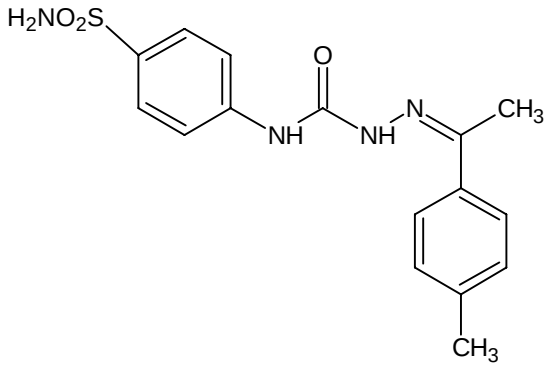
II-35

A series of 3-bromo phenyl semicarbazone derivatives has been synthesized and evaluated for anticonvulsant activity by Yogeeswari *et al*., further in 2003 [73]**.** Compound N^1-(3-bromo phenyl)-N^4-(4-methyl benzylidene) semicarbazone (**II-36**) was shown to possess anticonvulsant activity in both MES and scPTZ tests, the ED_{50} being 32.35 mg/kg and < 45.0 mg/kg., respectively. There was a significant increase of the GABA level in different regions of the rat brain after oral administration of aryl semicarbazones to rats. This report suggested for the first time that the effect of aryl semicarbazones on anticonvulsant activity was GABA-mediated.



II-36

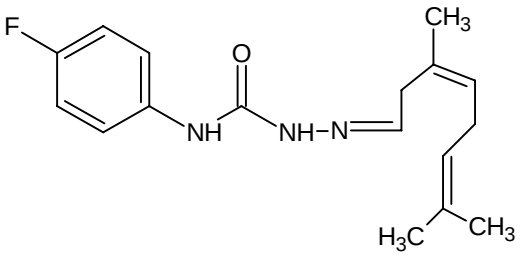
In 2004, a series of 4-sulphamoylphenyl semicarbazones derivatives were prepared starting from sulphanilamide and screened for anticonvulsant activity [74]. The results indicated that greater protection was obtained in MES screen and scSTY screen than the scPTZ screen. All the compounds showed low neurotoxicity when compared with the clinically used drugs. Some of the compounds possessed anticonvulsant activity even greater than Sodium valproate. Among the new derivatives evaluated the N^1-(4-sulphamoylphenyl)-N^4-(4-methyl benzylidene) semicarbazoes (**II-37**) emerged as the most active compound as indicated by its protection in the MES and scSTY screens with low neurotoxicity. Some of the compounds also possessed sedative-hypnotic activity.



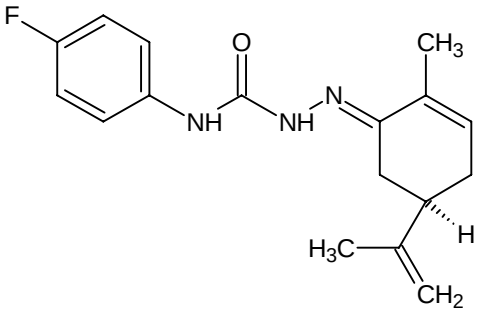
II-37

A series of 4-aryl substituted semicarbazones of citral and R- (-) carvone was synthesized to meet the structural requirements essential for anticonvulsant activity [75]. One cyclic terpene (R-(-) carvone) and one acyclic terpene with unsaturated hydrogen chain (citral) had been selected to increase the lipophilicity of the compounds and this increased

lipophilicity was expected to improve the anticonvulsant activity of the compounds. All the compounds were evaluated for anticonvulsant activity by the MES and scPTZ-induced seizure models and neurotoxicity by the rotarod test. The results showed that compounds with cyclic and acyclic terpenoid moiety retain the activity in the MES as well as scPTZ tests i.e. a broad spectrum of activity. So the terpenoid moiety fulfills the structural requirements of hydrophobic moiety for both the activities. The 4-fluoro aryl substituted semicarbazones (**II-38, II-39**) emerged as the most active in both cyclic and acyclic terpenes.

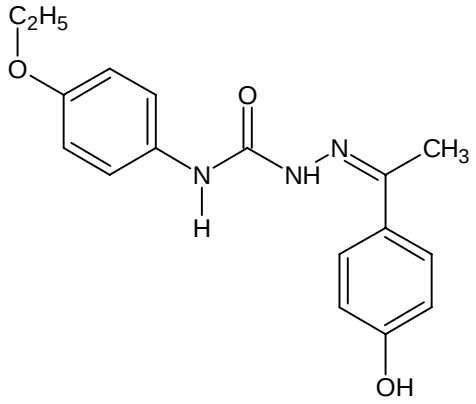


II-38



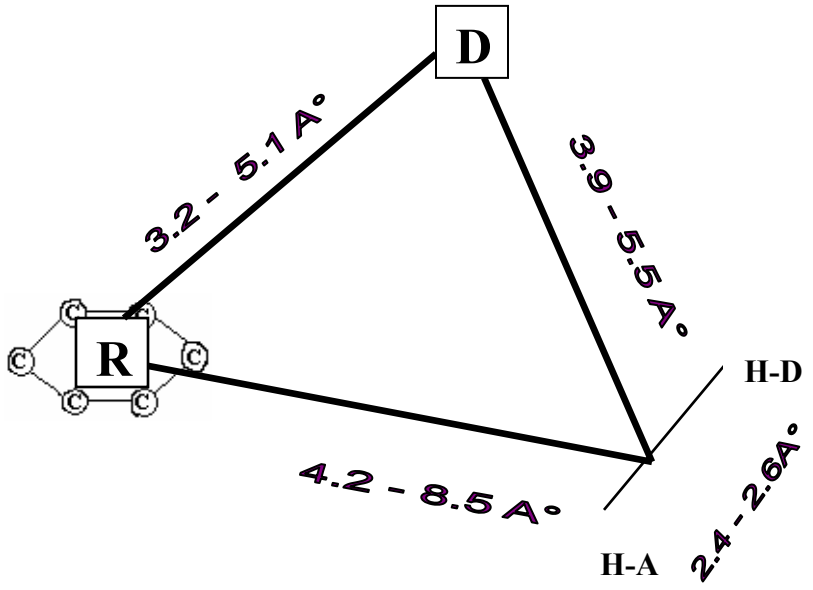
II-39

In 2005, Yogeeswari *et al.*, [76] reported a series of 4-ethoxyphenyl semicarbazones against MES and scPTZ seizure models and neurotoxicity screen in mice. Most of the compounds showed protection from the seizures in both the models. Among the compounds tested, compound **II-40** showed protection from seizures in both the animal models and was also found to increase the concentration of GABA in medulla oblongata region of the rat brain. They also reported that this effect was mediated by the inhibitory effect on GABA-T enzyme.



II-40

A three-point 3D pharmacophore model for anticonvulsants was proposed by using MM3 (Alchemy 2000) and CHARMm (ACD) parameterization (Fig. 5) to compared the structures of aryl semicarbazones with other conventional anticonvulsant drugs. Distance relationship analyses showed that the titled compounds fulfill the essential demands of pharmacophore when compared with Phenytoin (D-HAD), Carbamazepine (R-HAD), Denzinamide and Remacemide (R-D). In case of aryl semicarbazones, only the distance R-D is too long.



R = Hydrophobic binding site

H-D = Hydrogen Donor area

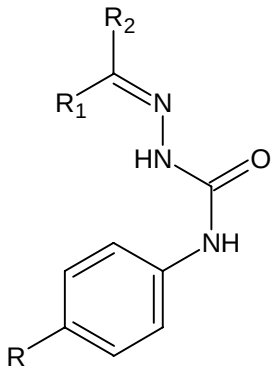
H-A = Hydrogen Acceptor area

D = Electron donor group

Fig. 5 Distance ranges between the essential structure elements R, D and HAD (Three point pharmacophore model)

Recently Yogeeswari *et al.*, [77] reported seven series of various substituted aryl semicarbazones as anticonvulsants in MES and scPTZ seizure threshold tests. A comprehensive structure-activity relationship was derived comparing the substituents on

the aryl ring and in the carbimino terminal. Generally the order of activity was found to be 4-F > 2-Br = 3-Br = 4-Cl > 4-CH_3 > 4-Br > 3-Cl > 3-CH_3 (**II-41**) with respect to the primary aryl group. Most of the compounds exhibited activity both in the MES and scPTZ screens. The 4-fluorophenyl substituted semicarbazones emerged as the most potent compounds exhibiting anticonvulsant activity in mouse *i.p.* and rat *p.o.* MES, scPTZ and psychomotor seizure (6 Hz) screens.



II-41

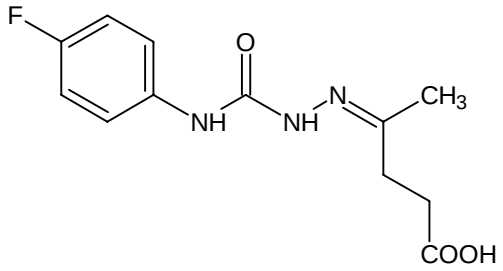
R = 2-Br, 3-Br, 3-Cl, 3-CH_3, 4-F, 4-Cl, 4-CH_3, 4-Br

R_1= H, CH_3

R_2= Substituted phenyl ring

A series of 4-aryl substituted semicarbazones of levulinic acid (4-oxo pentanoic acid) was designed and synthesized to meet the structural requirements essential for anticonvulsant activity [78]. All the compounds were evaluated for anticonvulsant activity. Anticonvulsant activity was determined after *i.p.* administration to mice by MES and scPTZ-induced seizure methods and minimal motor impairment was determined by rotarod test. A majority of the compounds exhibited significant anticonvulsant activity after *i.p.* administration. The compound 4-(4′-fluoro phenyl) levulinic acid semicarbazone (**II-42**) emerged as the most active molecule, showing broad spectrum of activity with low neurotoxicity. Unsubstituted levulinic acid semicarbazone was found to be inactive in all the screens. So this study further validated the hypothesis that presence of an aryl group near the semicarbazono moiety is essential for anticonvulsant activity. The results

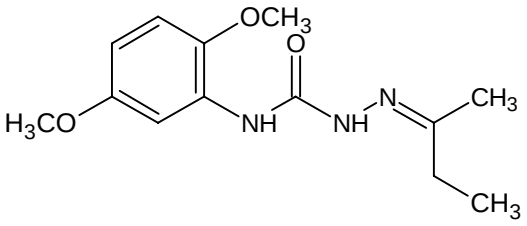
also indicated that the hydrophilic-hydrophobic site (distal aryl binding site) can accommodate hydrophilic groups.



II-42

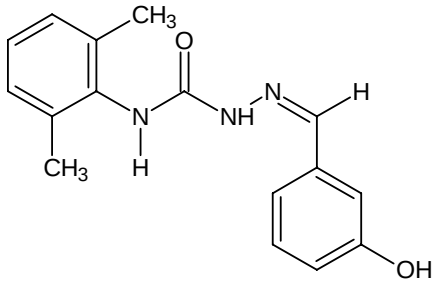
2.1.3. Development of disubstituted phenyl semicarbazones

Synthesis of disubstituted semicarbazones started with the work of Yogeeswari *et al* [80]. In 2004, a series of 3-chloro-2-methylphenyl-substituted semicarbazones was synthesized and evaluated for anticonvulsant and CNS activities. Substitution in the aryl ring by halogens had already been found to increase the potency and also the importance of *ortho*-methyl group substitution on aryl ring had been depicted very clearly in many studies including the one that gave rise to a recently marketed drug tiagabine [79]. Hence, merging of both the features yielded 3-chloro-2-methylphenyl-substituted semicarbazones. After *i.p.* injection to mice the semicarbazone derivatives were examined in the MES, scPTZ, and scSTY along with acute neurotoxicity screen. The semicarbazones showed good activity in the scSTY with moderate activity in the MES and scPTZ screens. Compound N^1-(3-chloro-4-methylphenyl)-N^4-(acetone) semicarbazone (**II-43**) emerged as the most active in all the three screens with lesser neurotoxicity. Some of the titled compounds exhibited lesser CNS depression and neurotoxicity when compared to Phenytoin and Carbamazepine.



II-43

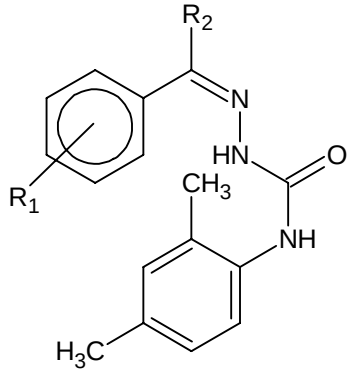
Further, recently N^4-(2,6-dimethylphenyl) semicarbazones were designed and reported as pharmacophoric hybrids between the aryl semicarbazones and Ameltolide by Yogeeswari *et al* [81]. These compounds were found to follow the three dimensional four-point pharmacophore model developed for anticonvulsants, and also matched with Ralitoline in pharmacophore mapping. All of the compounds exhibited anticonvulsant activity in the MES test when administered by both ***i.p.*** and oral routes. The compounds also showed protection in the scPTZ, scSTY screens and in the kindling models with lesser neurotoxicity. Compound N^1-(2,6-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde) semicarbazone (**II-44**), emerged as a prototype with wide spectrum of anticonvulsant activity in five models of seizure with no neurotoxicity and hepatotoxicity. Compounds were found to increase GABA level by 118% in different regions of the rat brain and also inhibited the GABA-T enzyme both *in vitro* and *ex vivo*.



II-44

In continuation to the study on various disubstituted semicarbazones, the next study was at N^4-(2,4-dimethylphenyl) semicarbazones [82]. All the compounds (**II-45**) were evaluated for anticonvulsant activity by using a series of test models including MES, scPTZ and scSTY seizure threshold tests and most of the compounds were found to

possess broad spectrum of activity. The preliminary studies also suggested that these compounds exhibit anticonvulsant activity via a GABA-mediated mechanism.

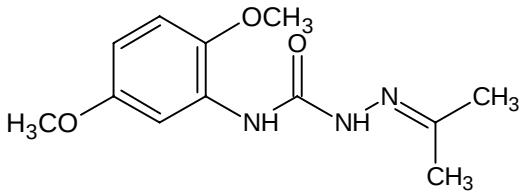


R_1 = H, 2-CH_3, 4-CH_3, 4-OH, 4-Cl etc.

R_2 = H, CH_3, C_6H_5

II-45

Similarly, various 2,4-dimethoxyphenylsemicarbazones were synthesized starting from 2,4-dimethoxyaniline via a phenyl carbamate intermediate. Nine compounds exhibited protection in all the three seizure models i.e MES, scPTZ and scSTY tests. Compound N^1-(2,4-dimethoxyphenyl)-N^4-(propan-2-one)semicarbazone (**II-46**) emerged as the most active compound with no neurotoxicity. These compounds were found to elevate GABA levels in the midbrain and medulla oblongata regions equipotent to Clobazam [83].

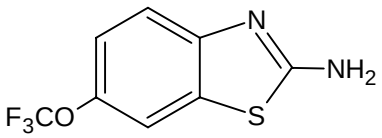


II-46

2.2. HETEROCYCLIC MOIETIES AS ANTICONVULSANTS

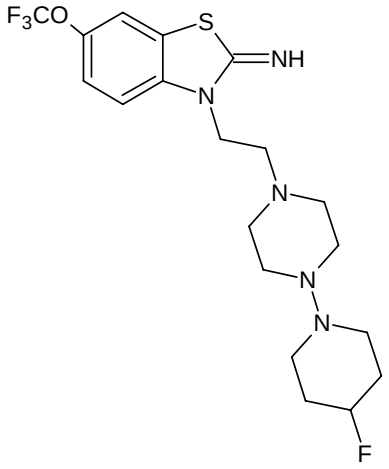
2.2.1. Development of benzthiazole derivatives

Anticonvulsant properties of the benzthiazole moiety were explored after the discovery of Riluzole [6-(trifluromethyoxy)-2-benzthiazolamine- **II-47**]. In 1991, Sylvie *et al*., [84] studied the anticonvulsive effect of Riluzole on the animal models of absence epilepsy. It has been found to decrease the number and spike-frequency of the discharges without inducing a sedative effect at even 3 mg/kg; hence this compound was thought to be of therapeutic interest in human absence epilepsy. It was found to act by reducing the K^+evoked release of glutamate and aspartate [85] and also by blocking the GABA-reuptake in neuronal cells [86]. Recently it has been discovered that Riluzole also modulated the Na^+ and the late K^+ dependent currents in cortical neurons. These phenomena may explain, at least in part, the anticonvulsant and neuroprotective properties of this compound [87].



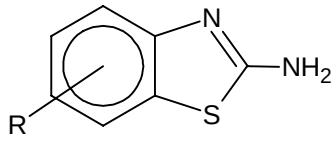
II-47

In 1994, anticonvulsant and neuroprotective activities of RP 66055 (**II-48**), a Riluzole derivative has been characterized by Jimonet *et al* [88]**.**



II-48

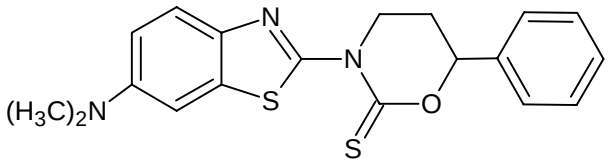
Further in 1994, Hay *et al.*, [89] studied thirty-two aryl-substituted 2-benzothiazolamines (**II-49**) for their ability to modulate Na^+ flux (NaFI) in rat cortical slices. A quantitative structure activity relationship (QSAR) study applied to these derivatives, showed a trend towards increasing the potency as sodium flux inhibitors with increasing lipophilicity, decreasing size, and increasing the electron withdrawing nature of the benzothiazole ring substituents. Additionally, substitution at the 4^{th} or 5^{th} position of the benzothiazole ring was found to decrease the potency. Nine of these compounds were found to be potent inhibitors of veratridine-induced NaFI. These compounds were also found to possess anticonvulsant activity in the MES assay. Fourteen additional 2-benzthiazolamines, exhibited activity in the MES screen, yet showed no activity in the NaFI assay. These derivatives may interact at the sodium channel but not discernible by the flux paradigm, or they act by an alternative mechanism *in vivo*. These benzthiazolamines also showed very low therapeutic index (<3) and would probably not be very effective anticonvulsants in human beings



II-49

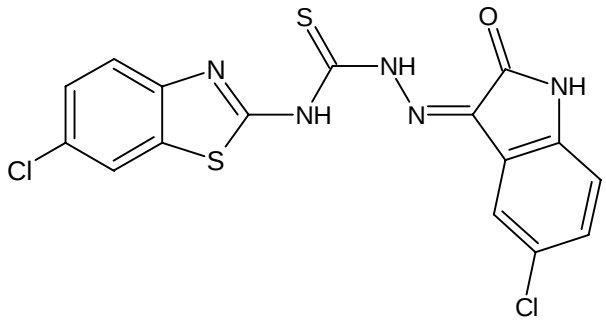
R= H, 6-Me, 6-SMe, 6-COOH, 6-SO_2Me, 6-Cl, 6-Br, 6-SO_2NH_2, 6-CF_3, 6-NO_2, 6-NH_2, 5-OMe, 5-Me, 5-CF_3, 5-COC_6H_5, 4-OMe, 4-Ph, 4,6-F_2

In 2002, Chopade *et al.*, [90] synthesized a new series of 3-(6-substituted-benzothiazol-2-yl)-6-phenyl-[1,3]-oxazinnane-2-thiones. The anticonvulsant activity of the synthesized compounds was evaluated against MES model and further the most potent compounds were evaluated against scPTZ-induced seizure model in mice. The neurotoxicity was evaluated by using rotarod procedure. Among the tested compounds, the 3-(6-dimethylaminobenzothiazol-2-yl)-6-phenyl-[1,3]-oxazinnane-2-thione (**II-50**) was found to be the most potent, showing ED_{50} of 9.85 and 14.8 in MES model and 12 and 17 in scPTZ model at 0.5h and 4h respectively, and TD_{50} of 42.8 and 44 at t = 0.5h and 4h, respectively.



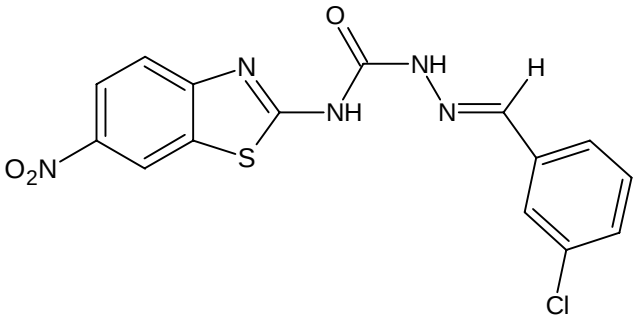
II-50

To overcome the problem of low therapeutic index of benzothiazolamines, Yogeeswari *et al* in 2002, [91] combined this benzothiazolamine moiety with the thiosemicarbazone group, which led to the development of a series of 6-chlorobenzthiazolyl-2-thiosemicarbazones. It has already been proposed that the aryl ring can be replaced by other hydrophobic moieties with the retention of activity [50]. Since various isatin derivatives were reported to possess anticonvulsant properties, so in the study the importance of investigating the effect of auxiliary heteroaryl ring was considered. Most of the compounds showed anticonvulsant activity in both MES and scPTZ screens and some of the compounds have shown good protection in rat *p.o* MES test at 30 mg/kg. Compound 4-(6-chlorobenzthothiazol-2-yl)-1-(3-isatinimino) thiosemicarbazone (**II-51**) emerged as the most promising one with an ED_{50} of 17.86 and 6.07 mg/kg in mice *i.p.* and rat *p.o.* respectively. This compound also displayed a weak potential of blocking the expression of fully kindled seizures.



II-51

In continuation to this earlier work, Yogeeswari *et al*., further in 2005 [92] reported various 6-substituted benzthiazolyl-2-thiosemicarbazones and screened for anticonvulsant activity in the MES and scPTZ-induced seizure models in mice. The neurotoxicity was assessed using the rotarod method. Most of the compounds showed activity in both mice *i.p.* and rat oral MES screen. The 6-nitro-benzthiazolyl thiosemicarbazone derivative (**II-52)** emerged as the most promising one exhibiting activity in the mice *i.p*. rat *i.p*. and rat *p.o.* evaluations. All the compounds exhibited lesser or no neurotoxicity compared to phenytoin.

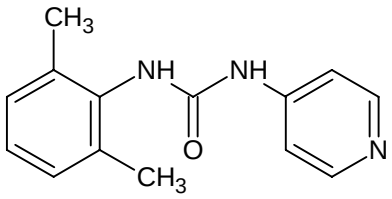


II-52

2.2.2. Development of pyridine derivatives

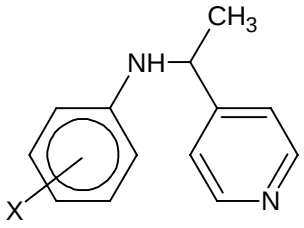
Structurally the simpler pyridine compounds which displayed anticonvulsant activity were N-phenyl-N'-(4-pyridinyl) ureas [93]. N-(2,6-dimethyl-phenyl)-N'-(4-pyridinyl) urea (**II-53**) emerged as the most potent compound of this series. Substitution in the 2nd

position of the phenyl ring with electron donating groups was generally beneficial to the activity and exception appears to be those capable of forming hydrogen-bonding interactions such as $–NH_2$ and –OH derivatives. Overall it appeared that small, lipophilic, non-hydrogen bonding groups at 2nd and 6th positions (CF_3, Cl, Br, F, CH_3, etc.) on the phenyl ring represented the optimal substitution pattern for this series of 4-pyridinyl ureas.



II-53

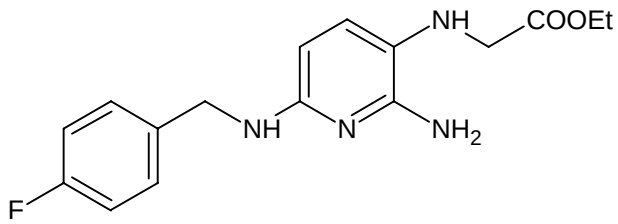
Elucidation of the metabolism and pharmacology of 1,2,3-triazolines (TRs) led to the evolution of aminoalkylpyridines (AAPs) [94]. The AAPs were found to be highly effective in the oral MES and kindling models of epilepsy, but showed no activity in the scPTZ test. They impair the glutamate release and afforded a high protection in kindled rats. They showed no affinity for the NMDA receptor sites. Variations of the heterocyclic unit, the alkyl chain and amino group in the AAPs indicated that the 4-pyridyl substituents along with a methyl (alkyl) group, and a 4-Cl, 3-Cl or 3, 4-dichloro substitution on the N-phenyl group, afforded the most active compounds (**II-54**). Amino group modification by acylation did not improve the activity.



X = 4-Cl, 3-Cl or 3, 4-dichloro

II-54

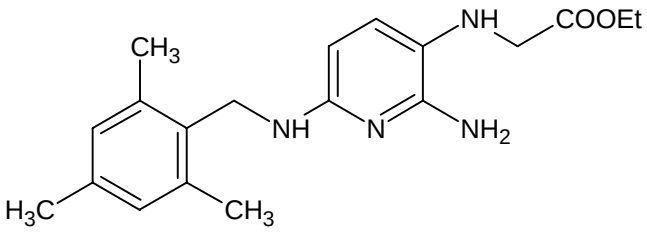
The synthesis and biological evolution of 2,3,6-triaminopyridine derivatives led to the development of Flupirtine (**II-55**), as a centrally acting analgesic, though later it was found to possess potent antiepileptic properties in MES and scPTZ tests [95]. Compared to the known anticonvulsants, the 2,3,6-triaminopyridines present a unique chemical structure wherein the only identifiable feature potentially in common is the presence of electron donor groups which can be oriented to fall within the intermolecular distance that may relate to the anticonvulsant activity. Later data derived from *in vitro* and *in vivo* studies suggested that Flupirtine functions as a weak NMDA antagonist with little evidence that it acts on AMPA–kainate type glutamate receptors. Studies on cultured cortical neurons showed that the NMDA-induced influx of Ca^{2+} is more readily decreased by Flupirtine [96].



II-55

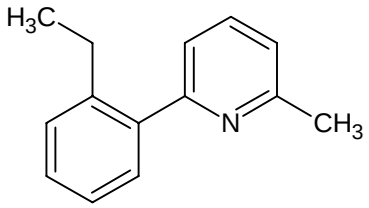
With these structural features, new Flupirtine derivatives were designed in 1994 [97]. A total of twenty-five analogs were designed and synthesized to determine the structural features controlling potency as well as neurotoxicity and continuous and concurrent QSAR techniques were also employed to increase the overall efficiency of the investigations. As a result, besides other structural properties, the overall molecular lipophilicity (log k', octanol-coated column) explained changes in anticonvulsant potency and neurotoxicity. Mimicking the interactions of the amphiphilic triaminopyridines with biological membranes, NMR (Neuclear Magnetic Resonance) experiments in the presence of lecithin vesicles were conduced to measure the phospholipid-binding parameter log Δ ($1/T_2$). The Replacement of log k' with log Δ ($1/T_2$) in the correlation analysis afforded a more significant equation describing the anticonvulsant activity of twenty-one derivatives. The synthesized compounds were tested in MES, scPTZ and Neurotoxicity screens. Compound **II-56** emerged as the most active and toxic compound

of the series. Preliminary QSAR study revealed that electron withdrawing groups such as $–CF_3$, -F or –Cl had little effect on anticonvulsant activity or neurotoxicity, whereas introduction of a lipophilic methyl group, especially in the *ortho*-position caused a significant increase in both types of activities.



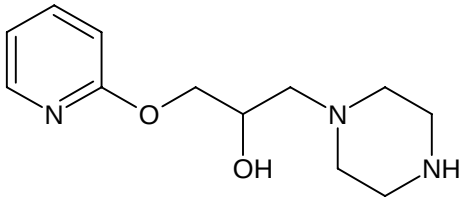
II-56

Another pyridine analog explored as a potent anticonvulsant was SIB 1893 [(E)-2-methyl-6-(2-phenylethynyl)-pyridine-**II-57**] [98]. The compound **II-57** was preferentially a selective mGluR5 receptor antagonist, also showing some agonistic activity towards mGluR4 (group III of mGluRs). This dual activity was advantageous for the protective activity of SIB 1893 in experimental models of epilepsy, since antagonists of group I and, generally, agonists of groups II and III, exert anticonvulsant action. There is evidence that **II-56** act as an anticonvulsant agent against electroconvulsions, audiogenic seizures and primary generalized nonconvulsive seizures in lethargic mice. Interestingly, in the electroconvulsive threshold test, SIB 1893 in the low dose range (0.5–2.0 mg/kg) lowered the threshold whilst at 40 mg/kg it evidently increased the threshold, showing an anticonvulsant activity.



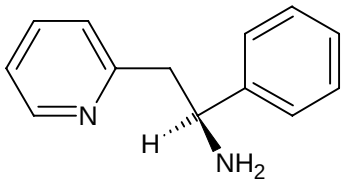
II-57

A series of 2-substituted pyridines were synthesized by Narendra *et al.*, in 2003 [99] and evaluated against seizures induced by MES and scPTZ. Neurologic deficit was evaluated by the rotarod test. Some of these derivatives showed high degree of protection against MES-induced seizures. But these were found to be less effective against scPTZ-induced seizures. Compound 2-(29-hydroxypropyloxy-39-piperazino) pyridine (**II-58**) was the most active in the MES test having an ED_{50} of 36.3 mg/kg.



II-58

A rational, chemical, synthetic effort to identify promising low affinity uncompetitive NMDA receptor antagonists for use as antiepileptic drugs led to the discovery of AR-R 15035AR (**II-59**) by Palmer *et al.* [100]. Chiral separation followed by intensive *in vivo* screening resulted in the selection of the [*S*] enantiomer, **II-59** as the best compound for further preclinical development. Compound **II-59** prevented tonic seizures in rodents for up to 6 to 8h in response to MES, 4-aminopyridine (4-AP), bicuculline or strychnine, as well as characteristic seizures following injections of NMDA or kainic acids. The compound **II-59** was ineffective in two kindling models of epilepsy, did not produce tolerance to MES, and was devoid of proconvulsant and phencyclidine-like properties in mice and rats, respectively. Therapeutic indices for **II-59** were comparable to or exceeded those for standard anticonvulsants. Orally administered **II-59** rapidly entered the rat brain and was eliminated in parallel from the plasma and plasma-free compartment.

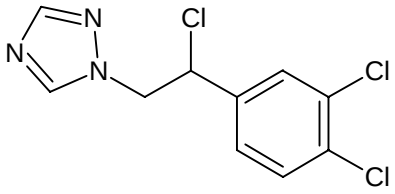


II-59

2.2.3. Development of 1,2,4-triazole derivatives

1,2,4-Triazole derivatives are known in the scientific literature for their wide pharmacological activity. They were known to possess antiviral, antibacterial, antifungal and CNS-related activities [101, 102].

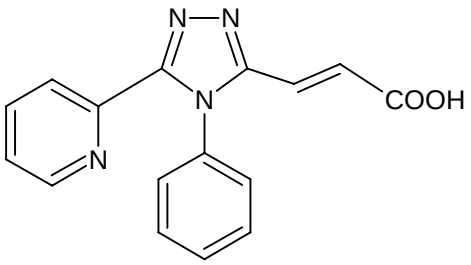
Loreclezole (**II-60**), the first marketed 1,2,4-triazole derivative as an anticonvulsant and antiepileptic compound, potentiates $GABA_A$ receptor function by interacting with a specific allosteric modulatory site on receptor β-subunits. The ability of Loreclezole to activate $GABA_A$ receptors directly, has been compared biochemically and electrophysiologically with that of Propofol. At concentrations of 50–100 μM, Loreclezole induced inward Cl^- currents in the absence of GABA in *Xenopus* oocytes expressing human recombinant $GABA_A$ receptors. At 100 μM, the current evoked by Loreclezole was 26% of that induced by 5 μM GABA. Currents induced by Loreclezole, like those evoked by Propofol were potentiated by Diazepam in a Flumazenil-sensitive manner and blocked by either bicuculline or picrotoxin. These data suggest that Loreclezole shares, with Propofol an agonistic action at $GABA_A$ receptors containing the β_2-subunit and that the different efficacies of the two compounds in this regard, may underlie the difference in their pharmacological profiles. [103].



II-60

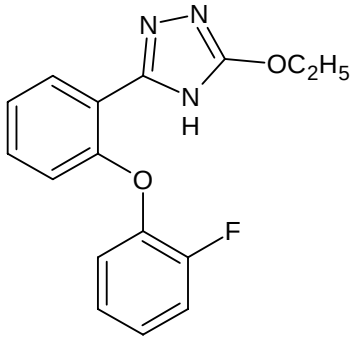
In 2004, Banachiewicz *et al.*, [104] synthesized new 3-(3,4-diaryl-1,2,4-triazole-5-yl) propenoic acid derivatives by condensation of N_3-substituted amidrazones with maleic anhydride. The influence of the compounds on the CNS of mice in some behavioral tests was also examined. The investigated compound showed anticonvulsive activity and a potent antinociceptive action. Compound **II-61** (50 and 100 mg/kg *i.p.*) produced a

significant anticonvulsant activity in the scPTZ screen. It significantly decreased the incidence of tonic seizures and lethality at both the dose levels.



II-61

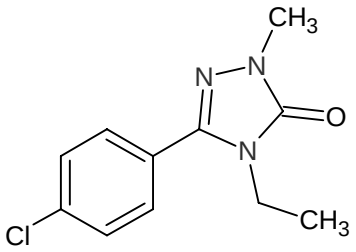
A series of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1,2,4-triazoles (e.g. **II-62**) has been synthesized and their anticonvulsant effects have been determined using PTZ-induced lethal convulsion and MES tests [105]. Compounds showed considerable anticonvulsant activity both in PTZ and MES models. To confirm the mode of action of the synthesized compounds, the effect of Flumazenil, a benzodiazepine antagonist on the anticonvulsant activity of the compounds were determined in MES model. The anticonvulsant activity of compounds was not reduced by Flumazenil. These interesting results showed that other mechanisms may be involved in their anticonvulsant effect and support the diversity of receptors that induce convulsion in MES and PTZ models.



II-62

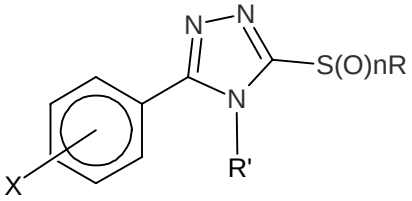
In 1990, John *et al.*, [106] synthesized a series of 5-aryl-2,4-dihydro-3H-1,2,4-trizol-3-ones and evaluated for their anticonvulsant activity. Approximately one-third of the compounds examined exhibited activity against both MES and scPTZ seizure models in

mice. Receptor binding studies suggested that this activity was not a consequence of activity at either benzodiazepine or NMDA-type glutamate receptors. From this series, compound **II-63** was selected for further evaluation where it was also found to be active against 3-mercaptopropionic acid, bicuculline, and quinolinic acid-induced seizures in mice.



II-63

In continuation to the work on 1,2,4-triazoles, John *et al*., further in 1994 [107] reported three series of isomeric (alkylthio)-1,2,4-triazoles and examined for their anticonvulsant activity against scSTY, MES, scPTZ, 3-MP-induced seizures in mice. The most potent antagonist of strychnine-induced convulsions was 5-(2-fluorophenyl)-4-methyl-3-(methylthio)-4H-l,2,4-triazole (**II-64a**), while the most selective antagonist was 5-(3-fluorophenyl)-4-methyl-3-(methylsulfonyl)-4H-l,2,4-triazole(**II-64b).** The anticonvulsant profiles of these 4H-l,2,4-triazoles suggested that they were acting functionally like glycine receptor agonists.. On the other hand, **II-64c** enhanced muscimol-stimulated $C1^-$ influx in a rat cerebellar membrane preparation, indicating a possible interaction of these triazoles with the $GABA_A$ receptor.



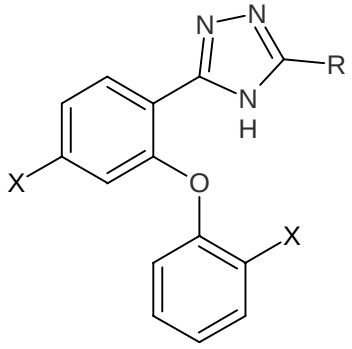
II-64

a. Ar = 2-FC_6H_4, R= CH3, R’ = CH3, n= 0

b. Ar = 3-FC_6H_4, R= CH3, R’ = CH3, n= 2

c. Ar = FC_6H_4, R= CH3, R’ = CH3, n= 2

A series of new 5-substituted analogues of 4H-3-(2-phenoxy)phenyl-1,2,4-triazole and its chlorinated derivatives (**II-65**) were designed and prepared by Akbarzadeh *et al*., in 2003 [108]. Conformational analysis and superimposition of energy minima conformers of the compounds on Estazolam, a known benzodiazepine receptor agonist, revealed that the main proposed benzodiazepine pharmacophores were well matched. Rotarod and pentylenetetrazole-induced lethal convulsion tests showed that the introduction of an amino group in position 5 of 1,2,4-triazole ring especially in chlorinated derivatives had the best effect which was comparable with Diazepam. The activity of the compounds was significantly reduced by Flumazenil, a benzodiazepine antagonist. The study indicated that some synthesized 1,2,4-triazoles with a simple non-rigid structure in which the proposed pharmacophores have a proper steric direction could show benzodiazepine activity comparable with Diazepam.

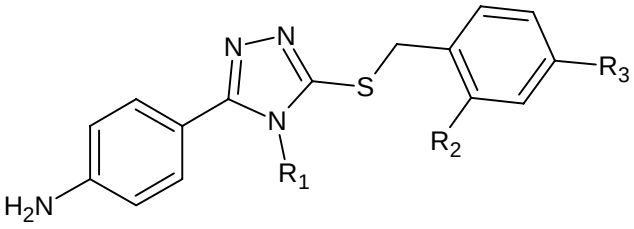


II-65

R = NH_2, SH, SMe, OH, OEt, SO_2Me X = H, Cl

A series of novel 3-{[(substituted phenyl)methyl]thio}-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazoles and several related schiff bases, 3-{[(substituted phenyl)-methyl]thio}-4-alkyl/aryl-5-{[[(substitutedphenyl/5-nitro-2-furyl)methylene]amino]-phenyl}-4H-1,2,4-triazoles were synthesized for evaluation of their biological properties [109]. All compounds were evaluated for their anticonvulsant activity in MES and scPTZ screens. A number of these triazole derivatives, exhibited protection after intraperitoneal administration at doses of 100 and 300 mg/kg in one or both models employed. Some of the compounds were subjected to oral MES screening in rats at 30 mg/kg and were

observed to protect 50% of the animals. Compounds **II-66** emerged as leads from the synthesized compounds.



II-66

a. R_1= CH_3, R_2= H, R_3= H

b. R_1= CH_3, R_2= Cl, R_3= Cl

CHAPTER 3

OBJECTIVE & PLAN OF WORK

3.1 Rationale for the design of proposed compounds

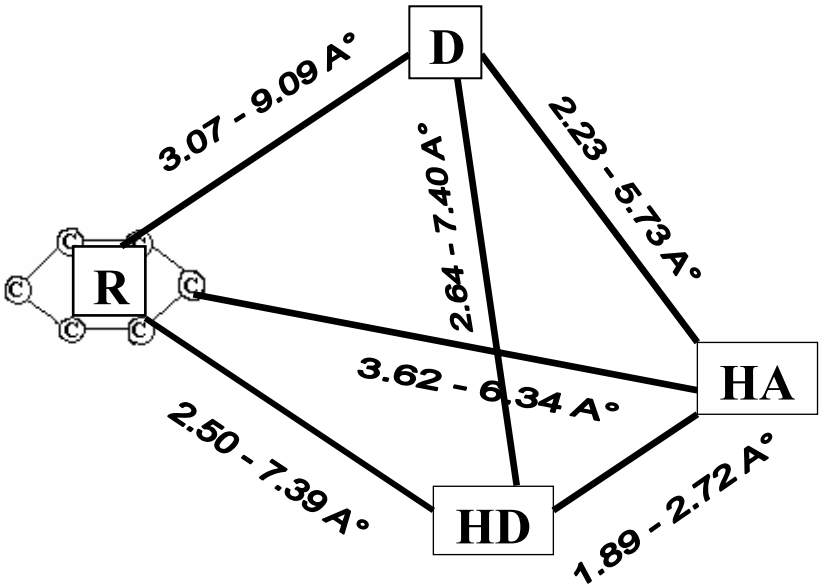
Like other chronic illnesses, AED therapy in patients with epilepsy is a continuously balancing process between disease control and side effects. Drug-related adverse events are well known to be one of the leading causes of treatment discontinuation by patients suffering from epilepsy. Conventional AEDs - Phenytoin, Carbamazepine, Valproic acid, Ethosuximide, Barbiturates, and Benzodiazepines provide some control of seizures but do that at the expense of various side effects i.e. blood dyscrasias, sedation, and cognitive impairment. Felbamate, Gabapentin, Lamotrigine, and Vigabatrin and new AEDs have shown fewer reported side effects than older generation medications. But while demonstrating promise for improved seizure control, the new AEDs are not a panacea. None of the new drugs seems to offer any marked advantage towards the old, first generation drugs with respect to the ultimate goal of drug treatment of epilepsy, i.e. complete control of seizures. Furthermore as epilepsy is a multifactorial disease, failure of several rational drug design strategies which aimed at developing selective drugs for selective targets, led to the postulation that combining mechanisms in a single drug, for example, combinatorial chemistry might prove a more successful strategy for treating epilepsy than the development of highly selective compounds. So a major goal in the epileptic research is to develop AEDs with broad spectrum of activity and with lesser side effects.

Considering rational drug design, the aryl/heteroaryl semicarbazones can serve as the prototype anticonvulsants with respect to both lesser side effects and broad spectrum of activity. The proposed aryl and heteroaryl semicarbazone leads are different from those of conventional AEDs as majority of them i.e. barbiturates, hydantoins, succinimides possess a dicarboximide moiety (-CO-NH-CO-) which contributes to various inherent

side effects. So an absence of ureylene moiety is expected to reduce the side effects in potential aryl and heteroaryl semicarbazone anticonvulsants.

Voltage-dependent blockage of sodium channels is a mechanism held by several structurally diverse compounds like Phenytoin, Topiramate and Lamotrigine. Conformational analysis of these older generation clinically active anticonvulsant drugs led to the proposal of a general model for anticonvulsant activity comprising two aromatic rings or their equivalent in a favored orientation and a third region, usually a cyclic ureide, containing a number of hydrogen bond forming functional groups [110, 111]. To support the suggested pharmacophore, Unverferth *et al*. in 1998, [112] performed a study on various anticonvulsants i.e. Carbamazepine, Phenytoin, Lamotrigine, Zonisamide and Rufinamide with sodium channel blockade activity. All the molecules possessed atleast one aryl ring, one electron donor atom, and a second donor atom in close proximity to the NH group forming a hydrogen bond donor/acceptor unit. Most of the compounds including the aryl semicarbazone were able to realize two alternative conformational orientations of the hydrogen bond acceptor/donor unit (HAD) unit, the one with H acceptor function, and the other with the H donor function toward the aryl ring. If this HAD unit is essential for sodium channel blocking activity, the receptor site for this group seems to be rather flexible.

Considering these previously reported models, Yogeeswari *et al.*, in 2005 [81] stressed on the relevance of an accurate distance between the essential pharmacophoric elements for the compounds possessing sodium channel blockade, which led to the design of a new four-point 3D pharmacophore model (Fig. 6).



R = Hydrophobic binding site

HAD = Hydrogen Acceptor/ Donor area

D = Electron donor group

Fig. 6 Distance ranges between the essential structure elements R, D, HA and HD (Four point pharmacophore model)

Similarly the proposed aryl/heteroaryl semicarbazones were, energy minimized by using both CHARMM (ACD 3D viewer) and MM3 (Alchemy 2000) parameterization. The distances between the 4-pharmacophoric points were calculated for a minimum of four different conformations represented as mean ± standard deviation (Table 3.1). Our analyses of the distance relationship showed that the proposed aryl/heteroaryl semicarbazones and analogues fulfill the essential demands of pharmacophores when compared with the standard drugs. With this as background, the present work highlights the importance of synthesis of prototypes of aryl semicarbazones and their analogues.

3.2 Objectives

The research work was aimed to design and synthesize novel aryl and heteroaryl semicarbazones as anticonvulsants with broad spectrum of activity and with lesser side effects like neurotoxicity, sedation and CNS depression. The main objective of the work is to perform a comprehensive SAR (Structure-Activity Relationship) on the following:

- Study the importance of various groups attached to the aryl ring.
- Decipher the size of aryl ring i.e. the effect of replacing the aryl ring with heteroaryl ring like pyridine, benzthiazole moiety.
- Study the importance of hydrogen bonding domain- effect of cyclization of the semicarbazono group on the anticonvulsant profile of aryl semicarbazones.

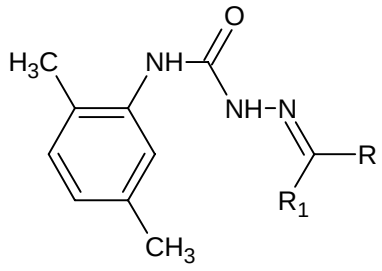
3.3 Plan of Work

The plan of work is broadly classified under following categories:

I. SYNTHESIS

1. Synthesis of disubstituted-phenyl semicarbazones

1.1. Synthesis of 2,5-dimethylphenyl semicarbazones

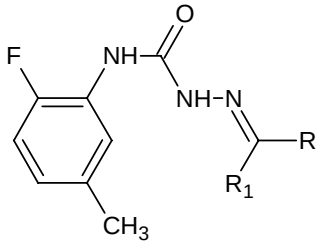


R, R_1 = H/ Alkyl/ Phenyl substitution

DM (1-19)

Various studies depicted the importance of methyl group substituted at various position of the aryl ring, in which *ortho*-CH_3 group was found to be more beneficial for anticonvulsant activity [73, 80]. Recently, Yogeeswari *et al.*, reported a series of 2,6-dimethylphenyl semicarbazones [81] as potential anticonvulsant agents. In continuation of the work on disubstituted semicarbazones, the present work focuses on the synthesis and anticonvulsant evaluation of new 2,5-dimethylphenyl semicarbazones towards exploring the effect of *meta*-substituted methyl group along with the *ortho* substitution on the anticonvulsant activity of aryl semicarbazones.

1.2. Synthesis of 2-fluoro-5-methylphenyl semicarbazones

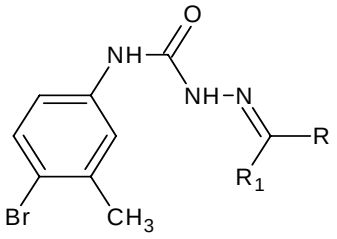


R, R_1 = H/ Alkyl/ Phenyl substitution

FM (1-27)

Based on the promising pharmacological results obtained with aryl semicarbazones having halogen substitution i.e. bromo/chloro/fluoro [65, 80] and also with those exhibiting highest activity by fluoro substitution [68, 77] we have designed and synthesized 2-fluoro-5-methylphenyl semicarbazones. So this study was conducted to study the effect of substitution with fluoro group along with *meta*-methyl group on the anticonvulsant activity of aryl semicarbazones.

1.3. Synthesis of 4-bromo-3-methyphenyl semicarbazones



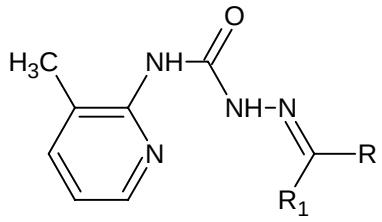
R, R_1 = H/ Alkyl/ Phenyl substitution

BM (1-26)

The 4-bromosubstituted phenyl semicarbazones have already been shown to possess promising anticonvulsant activity [65] and also various previous studies revealed that substitution with the methyl group on the aryl binding ring of semicarbazones led to an increase in the anticonvulsant activity when the compounds were administered by the *i.p.* route to mice [79, 81]. So to explore the effect of *meta*-methyl substitution on the anticonvulsant profile of 4-bromosubstituted phenyl semicarbazones, the present study was aimed at synthesizing and evaluating the pharmacological activity of 4-bromo-3-methyphenyl semicarbazones.

2. Synthesis of heteroaryl semicarbazones

2.1. Synthesis of N-(3-methylpyridin-2-yl)-substituted semicarbazones

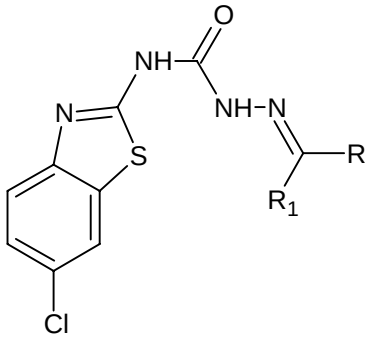


R, R_1 = H/ Alkyl/ Phenyl substitution

PY (1-20)

Starting from the development of pyridyl ureas, various pyridyl derivatives have been shown to possess a favorable anticonvulsant profile [93-99] and also substitution with small, lipophilic, non-hydrogen bonding groups at 2^{nd} and 6^{th} positions (CF_3, Cl, Br, F, CH_3, etc.) of the ring represented the optimal substitution [93]. So to merge these effects with aryl semicarbazones, N-(3-methylpyridin-2-yl)-substituted semicarbazones have been synthesized and evaluated for anticonvulsant and other CNS activities.

2.2. Synthesis of 6-chloro benzthiazol-2-yl semicarbazones

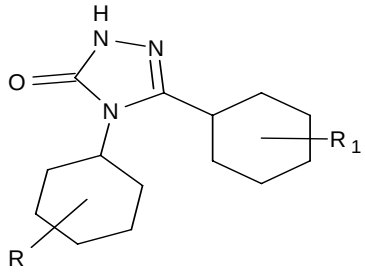


R, R_1 = H/ Alkyl/ Phenyl substitution

BZ (1-20)

Various benzthiazole derivatives were reported as anticonvulsants and the importance of substitution at the 6th position for anticonvulsant activity had also been depicted including the drug Riluzole [86, 89]. In earlier studies, Yogeeswari *et al.* reported some 6-substituted benzthiazoly-2-yl thiosemicarbazones as potential anticonvulsants, though they were found to be highly neurotoxic [91, 92]. So to lessen the neurotoxic effect of thiosemicarbazone derivatives, the present work focuses towards the synthesis and pharmacological evaluation of 6-chloro benzthiazol-2-yl semicarbazones.

3. Synthesis of cyclized aryl semicarbazones i.e. 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-one



R, R_1 = NH_2, NO_2, OH, CH_3, F

TR (1-18)

Many compounds bearing the 1,2,4-triazole nucleus have been earlier reported to possess anticonvulsant properties [104, 105]. Moreover, Loreclezole is a positive modulator of

$GABA_A$ receptors. The benzodiazepine agonist, Estazolam is an anticonvulsant drug which contains triazolobenzodiazepine ring [101]. As part of our drug design program, we were interested in the study of the effect of cyclization of these aryl semicarbazones i.e. effect of reducing the flexibility of the hydrogen bonding domain on their anticonvulsant activity. This led to the synthesis of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones.

II. PHARMACOLOGY

1. Anticonvulsant screening

    1.1. Maximal electro shock seizure test (MES).

    1.2. Subcutaneous pentylenetetrazole seizure threshold test (sc PTZ).

    1.3. Subcutaneous strychnine induced convulsive test (scSTY).and

    1.4. Subcutaneous picrotoxin seizure threshold test (scPIC)

2. Other CNS activities

    2.1. Neurotoxicity.

    2.2. Behavioral despair effect.

    2.3 CNS depression.

3. Neurochemical Study

    3.1. GABA level in different regions of rat brains/ rat whole brains.

III. QUANTUM MECHANICAL MODELING

1. 2,5-Disubstituted phenyl semicarbazones
2. 4-Bromo-3-methylphenyl semicarbazones
3. 3-Methylpyridin-2-yl semicarbazones
4. 6-Chloro benzthiazol-2-yl semicarbazones
5. 4,5-Diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones

CHAPTERS 4

MATERIALS & METHODS

4.1 Chemistry

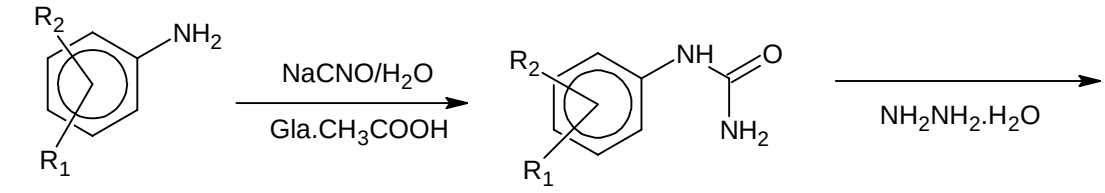
Melting points were determined in one end open capillary tubes on a Bűchi 530 melting point apparatus and are uncorrected. Some of the reactions were carried out using microwave oven of Matrix LG make, having input of 220V- 50 Hz, 980 W, 4.7 A, frequency 2450 MHz. Infra red spectra (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 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 addition of deuterated water (D_2O). Mass spectra of two compounds were carried out with Shimadzu GC-MS-QP5000 spectrophotometer. Elemental analyses (C, H, 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 aluminium plates and visualized by using iodine vapor. Developing solvents were chloroform - methanol (9:1) and petroleum ether - ethyl acetate (8:2). The log P values were determined using Scilog P software.

Synthesis of disubstituted phenyl semicarbazones

Synthesis of disubstituted phenyl semicarbazones was accomplished by either of two following methods:

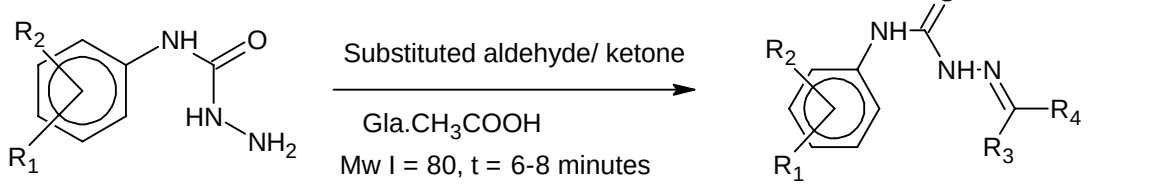
Method 1

The 2,5-dimethylphenyl semicarbazones and 4-bromo-3-methylphenyl semicarbazones have been prepared according to the earlier reported procedure [80]



Disubstituted aniline

Disubstituted phenyl urea



Disubstituted phenyl semicarbazide

Disubstituted phenyl semicarbazones

Where R_1 = 2-CH_3, 4-Br

R_2 = 3-CH_3, 5-CH_3

R_3 = H, CH_3, C_6H_5

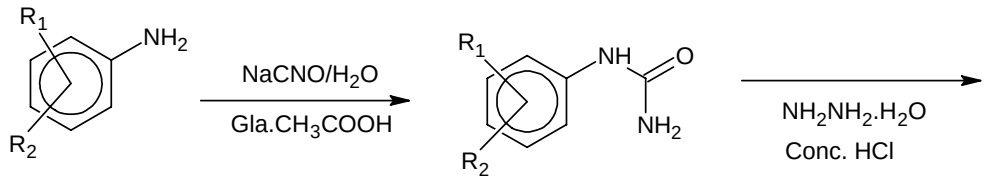
R_4 = CH_3, C_2H_5, C_6H_5, 4-Cl-C_6H_4, 4-CH_3-C_6H_4 etc.

The disubstituted aniline was treated with sodium cyanate in the presence of glacial acetic acid to yield disubstituted phenyl urea. The urea derivatives on condensation with hydrazine hydrate in ethanol in the presence of sodium hydroxide gave the disubstituted phenyl semicarbazides. In the final step, the dimethylphenyl semicarbazone derivatives were prepared by reaction of the appropriate aryl/alkyl/cycloalkyl aldehyde or ketone or

isatin with semicarbazides by irradiation in the microwave oven at a power setting of 80% with 30 seconds/cycle. The number of cycles in turn depended on the completion of the reaction, which was checked by TLC. The reaction time varied from 6 to 8 minutes.

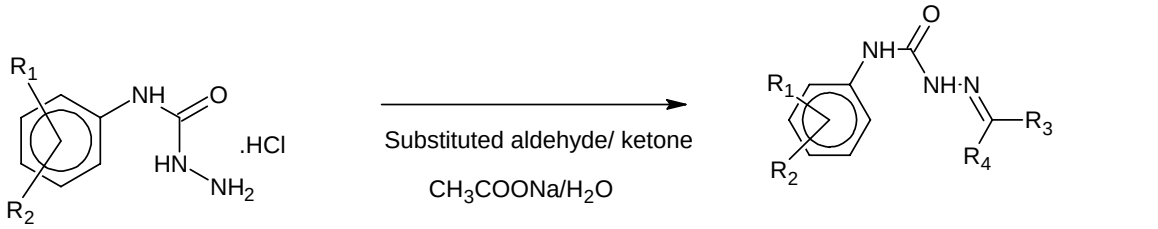
Method 2

The 2-fluoro-5-methyl phenyl semicarbazones have been synthesized from disubstituted aniline by the reported semicarbazide hydrochloride salt method [65].



Disubstituted aniline

Disubstituted phenyl urea



Disubstituted phenyl semicarbazide hydrochloride Salt

Disubstituted phenyl semicarbazones

Where R_1 = 2-F; R_2 = 5-CH_3

R_3 = H, CH_3, C_6H_5

R_4 = CH_3, C_2H_5, C_6H_5, 4-Cl-C_6H_4, 4-CH_3-C_6H_4 etc.

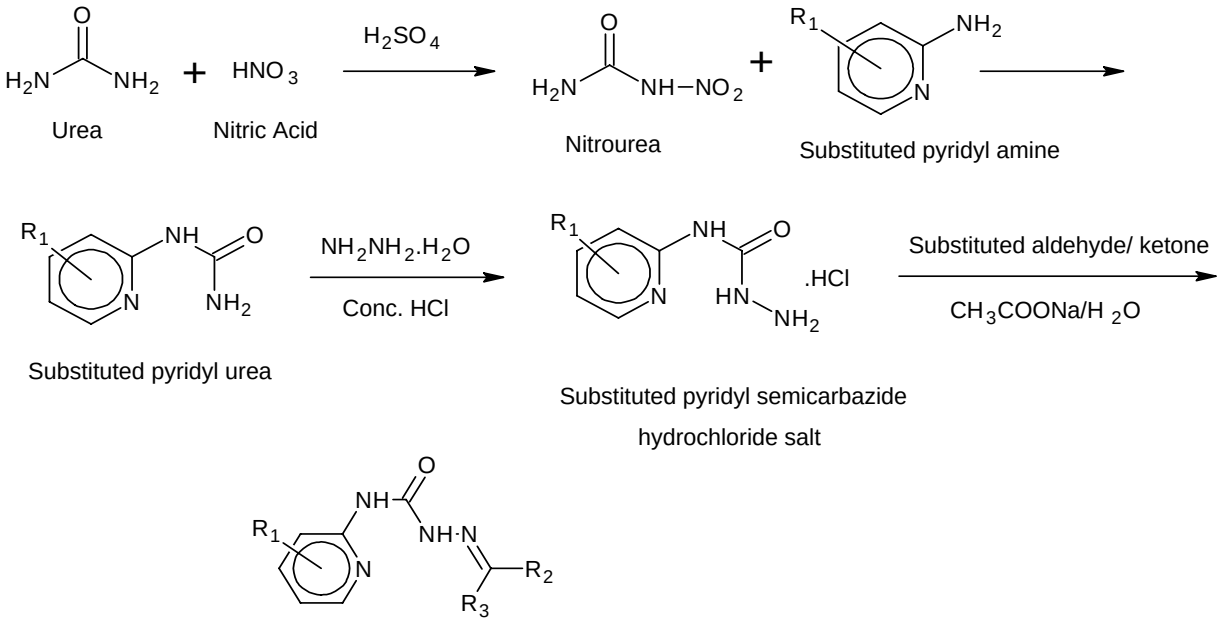
In a similar way as above, the disubstituted aniline was treated with sodium cyanate in the presence of glacial acetic acid to give the disubstituted phenyl urea and this urea on condensation with hydrazine hydrate in ethanol medium gave the disubstituted phenyl semicarbazide, which was further purified by converting it into its hydrochloride salt by the addition of concentrated hydrochloric acid. Finally, the required disubstituted phenyl semicarbazone derivatives were prepared by the reaction between the appropriate alkyl /

aryl aldehydes or ketones or isatins and disubstituted phenyl semicarbazide hydrochloride salt in the presence of sodium acetate in ethanol / water medium.

Synthesis of substituted pyridyl semicarbazones

Method 3

Substituted pyridyl semicarbazones have been synthesized by the known nitrourea method [113]. First of all, urea was converted to nitrourea in the presence of sulphuric acid and nitric acid. The reaction was done under ice-cold condition. This nitrourea was further treated with substituted pyridyl amine which resulted in the immediate precipitation of substituted pyridyl urea and then in a similar way as above, this substituted pyridyl urea was treated with hydrazine hydrate in ethanol to yield the corresponding semicarbazide, which was further purified by converting it into its hydrochloride salt by the addition of concentrated hydrochloric acid.. Finally substituted pyridyl semicarbazide on reaction with the appropriate aldehyde/ketone under acidic conditions, yielded the corresponding semicarbazones.



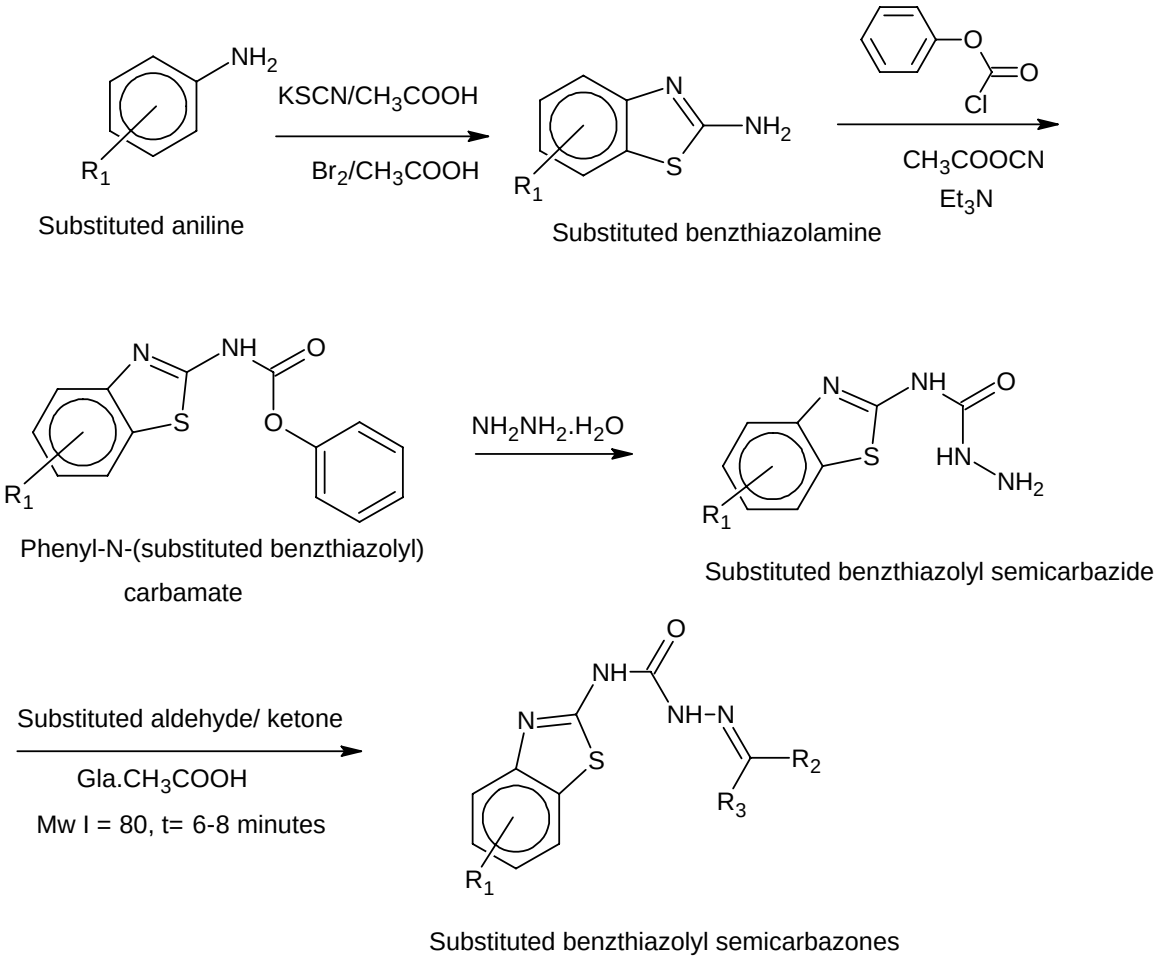
Where R_1 = 3-CH_3 ; R_2 = H; CH_3, C_6H_5

R_3 = CH_3, C_2H_5, C_6H_5, 4-Cl-C_6H_4, 4-CH_3-C_6H_4 etc.

Synthesis of benzthiazole substituted semicarbazones

Method 4

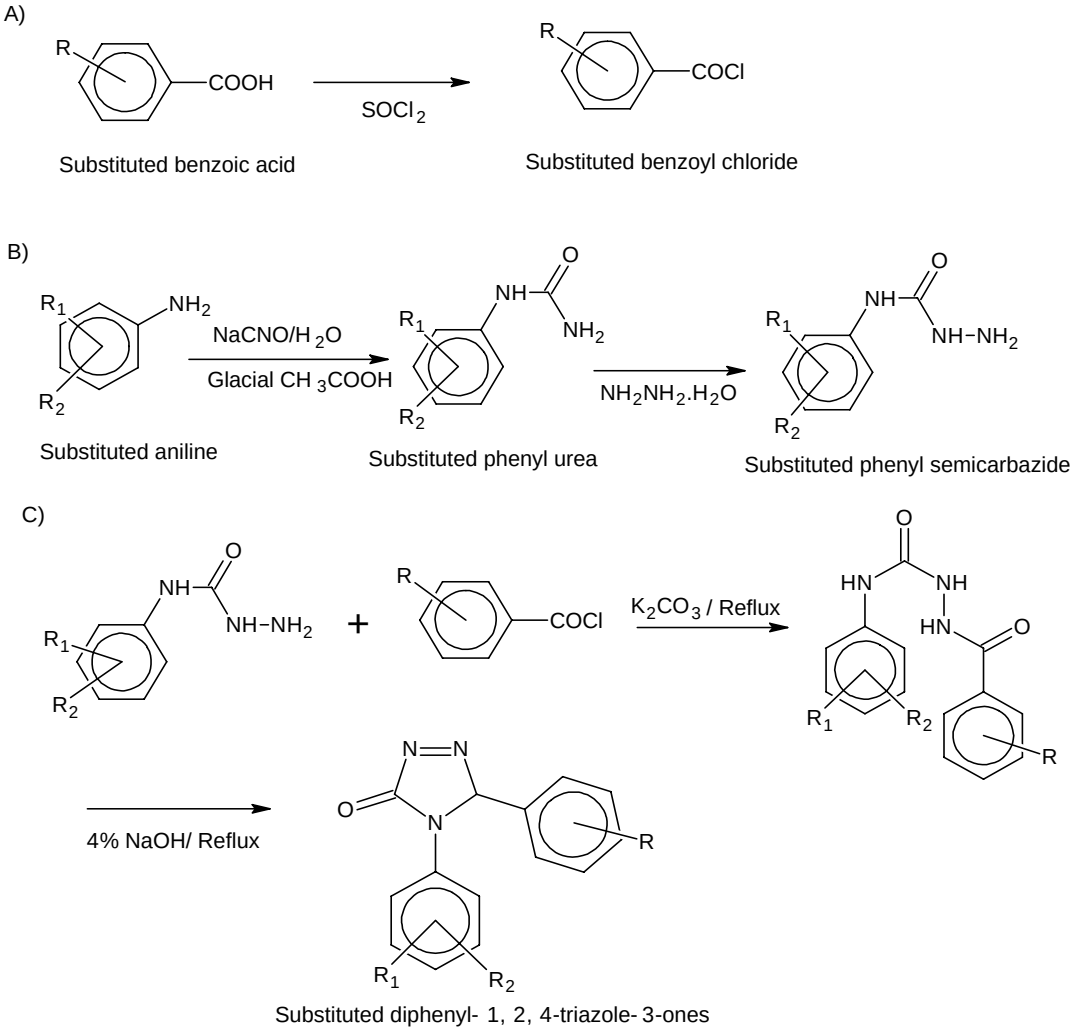
Substituted benzthiazolamine was synthesized from the corresponding aniline, by treating with potassium thiocyanate and bromine in glacial acetic acid according to the reported procedure [89]. Further reaction with phenyl chloroformate in the presence of acetonitrile and triethylamine and stirring at room temperature resulted in the formation of phenyl-N-(substituted-benzthiazolyl) carbamate [114]. This compound on condensation with hydrazine hydrate in ethanol, gave the disubstituted phenyl semicarbazide. Finally the required substituted benzthiazolyl semicarbazones were prepared by the reaction between the appropriate aryl/alkyl aldehydes or ketones and substituted benzthiazolyl semicarbazide in the presence of glacial acetic acid in ethanol.



Synthesis of substituted diphenyl 1,2,4-triazole-3-ones

Method 5

The synthesis of 1,2,4-triazole-3-ones was carried out in three steps. In the first step, substituted benzoic acids were converted to the corresponding acid chlorides in the presence of thionyl chloride, a well known method for the acylation of substituted acids. The next step involved the synthesis of substituted semicarbazides from the corresponding anilines by the urea method, which were then condensed with substituted acids in a slightly alkaline medium. Further cyclization of the resultant moiety in the presence of 4% NaOH generated substituted-diphenyl 1,2,4-triazole-3-ones [115]



Where R = H, 4-NO_2, 4-NH_2, 4-OH, 4-CH_3; R1 = 2-CH_3, 4-F; R2 = H, 4-CH_3, 5-CH_3, 6-CH_3

4.2 Pharmacology

The pharmacological studies were conducted on CF # 1 (Carworth Farms number-one) / Swiss albino mice (20-25g) or Sprague-Dawley / Wistar albino rats (130-160g) of either sex. The animals were obtained from Hisar Agricultural University and were housed under normal laboratory conditions (12-hour light-dark cycle) with free access to food and water. The animals were housed in groups of six in perspex cages in the laboratory three days prior to experimentation. The experimental sessions were conducted during the light phase of the cycle between 8 A.M and 4 P.M. The test drugs were dissolved in 30% polyethylene glycol 400. All procedures described in this thesis were reviewed and approved by the Institutional Animal Ethics Committee (Protocol No. IAEC/RES/3, 31-01-02).

The various tests done in the pharmacological study include.

1. Anticonvulsant activity: -
    a. Maximal Electroshock Seizure test (MES).
    b. Subcutaneous pentylenetetrazole seizure threshold test (scPTZ).
    c. Subcutaneous picrotoxin seizure threshold test (scPIC).
    d. Subcutaneous strychnine seizure pattern test (scSTY).
    e. Rat *p.o.* Identification.
2. CNS depressant activity: -
    a. Locomotor activity by using Photoactometer (INCO, Ambala).
    b. Porsolt's swim pool test.
3. Neurotoxicity study using rotarod test.

1. Anticonvulsant activity

Initially all the compounds were administered i.p. in a volume of 0.01 ml/g body weight for mouse and 0.004 ml/g body weight for rat 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.

(a) **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 sec 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. Test solutions of all compounds were prepared in 30% v/v polyethylene glycol 400 and the animals were dosed intraperitoneally 30 min prior to testing. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test [116].

(b) **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. The animals of control group received 0.2 mL saline (*i.p.*). The other groups were administered the experimental drug solution (*i.p.*). After 30 min pentylenetetrazole in 0.9% w/v sodium chloride, was administered subcutaneously in a loose fold of skin on the back of the neck 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 [117].

(c) **Subcutaneous picrotoxin seizure threshold test (scPIC)**

The test compounds were suspended in 30% v/v polyethylene glycol 400 and evaluated for their ability to antagonize scPIC-induced convulsions in mice after *i.p.* administration. After 30 min of drug administration the convulsive dose of picrotoxin (3.15 mg/kg) was injected subcutaneously in a volume of 0.01ml/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 and indicates that the test substance has the ability to elevate the picrotoxin seizure threshold [118].

(d) **Subcutaneous strychnine seizure pattern test (scSTY)**

Test solutions of all compounds were prepared in 30% v/v polyethylene glycol 400 and the animals 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 leg tonic extensor component of the seizure. Abolition of the hind leg tonic extensor component was taken as the end point and indicates that the test substance has the ability to prevent seizure spread [119].

(e) **Rat *p.o.* identification**

The onset and duration of action of a drug depends on the route of administration. For example, when a drug is given by intravenous route (*i.v.*) the effect is seen instantaneously as compared to oral administration of drug when it takes longer time to show the effect.

Some compounds were selected for oral evaluation for anti-MES and neurotoxicity in rats. The rat was held by its neck muscle and through the feeding needle the solution was gently pushed into the mouth. Care was taken that no drug came out of the mouth or nose. The anti-MES and neurotoxic effects were studied 0.25, 0.5, 1, 2 and 4 h after injection of either 30 mg/kg or 50 mg/kg of the experimental drug [120].

(2) CNS depressant activity

(a) **Behavioral test by using Photoactometer**

The test compounds (30, 100 and 300 mg/kg) were screened for their behavioral effects using Photoactometer (INCO- Ambala) at 0.5h and 1.0h after intraperitoneal injection to mice. The behavior of the animals inside the photocell was recorded as a digital score. The photoactometer was placed in a sound proof box and mice were placed inside. Duration of the experimental observation was up to 10 min (2+8). After an initial period of 2 min during which the animals got accustomed to the new environment, the counter was reset and the remaining

8 min reading was noted. After each trial, the base was cleaned with 20% v/v ethyl alcohol [121].

(b) **Porsolt's Swim Pool test**

The method adopted was a modification of that described by Porsolt *et al* [122]. Swimming sessions were conducted by placing individual mice or rats in plexiglass cylinders of 45 cm diameter (mice) and 60 cm diameter (rats) containing 25 cm and 35 cm of water (23-25°C) respectively (a volume deep enough so that the animal could not touch the bottom with its hind limbs or by its tail). The animals were subjected to a 15 min swimming session 24h prior to the conduct of a six-minute test. The animals were administered an *i.p.* injection (30 & 100 mg/kg) of the test compounds 30 min before the test session. The duration of immobility (Passive floating without struggling and making only those movements which are necessary to keep its head above the surface of water) was recorded during the last 4 min of the 6 min testing period. (For rats, the last 5 min of the total 7 min period).

(3) Neurotoxicity (NT) screen

Minimal motor impairment was measured in mice by the rotarod test [123, 124]. 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.

Neurochemical Studies

Determination of GABA level in rat brain

The disubstituted phenyl semicarbazone derivatives have shown good anticonvulsant activity. Hence neurochemical investigation was also carried out on the synthesized compounds, to study the effect on the GABA levels in various regions of rat brain.

Various methods for the estimation of GABA in tissue extracts have been reported. Some of the methods are,

i. Enzymatic UV method [125]

ii. Enzymatic fluorimetric method [126]

iii. Paper chromatographic method [127]

iv. Receptor inhibition method [128]

v. Column chromatographic method [129]

Among these methods, enzymatic methods are very selective, specific and can be used successfully to determine the concentration of GABA in tissue extracts. In the present study, the enzymatic ultraviolet (UV) method has been used.

Equipment: -

1.	Glass apparatus	: Borosil
2.	Micropipettes	: Accupipette
3.	Refrigerated centrifuge	: Remi cooling compufuge
4.	Refrigerator / deep freezer	: Vest frost
5.	Vacuum Centrifuge	: Maxi dry Iyo
6.	Water bath	: Remi
7.	U.V. Spectrophotometer	: Perkin Elmer Lambda EZ201
8.	Sonicator	: Biosonik I

Reagents (All the reagents used were of AR grade): -

1. Dipotassium hydrogen ortho phosphate; K_2HPO_4
2. Potassium dihydrogen phosphate; KH_2PO_4
3. Sodium Pyrophosphate; $Na_4P_2O_7$. $10H_2O$

4. 2-Mercapto ethanol
5. Nicotinamide Adenine Dinucleotide (β-form)
6. Nicotinamide Adenine Dinucleotide reduced (β-form)
7. α-ketoglutaric acid
8. γ-aminobutyric acid
9. Sodium hydroxide
10. Hydrochloric acid

Collection of samples:

Animals: Wistar Albino rats (130-160g) in groups of six.

Route of administration: *i.p.*

Schematic depictions of the UV enzymatic method for the determination of GABA in tissue samples are given in Fig. 7.

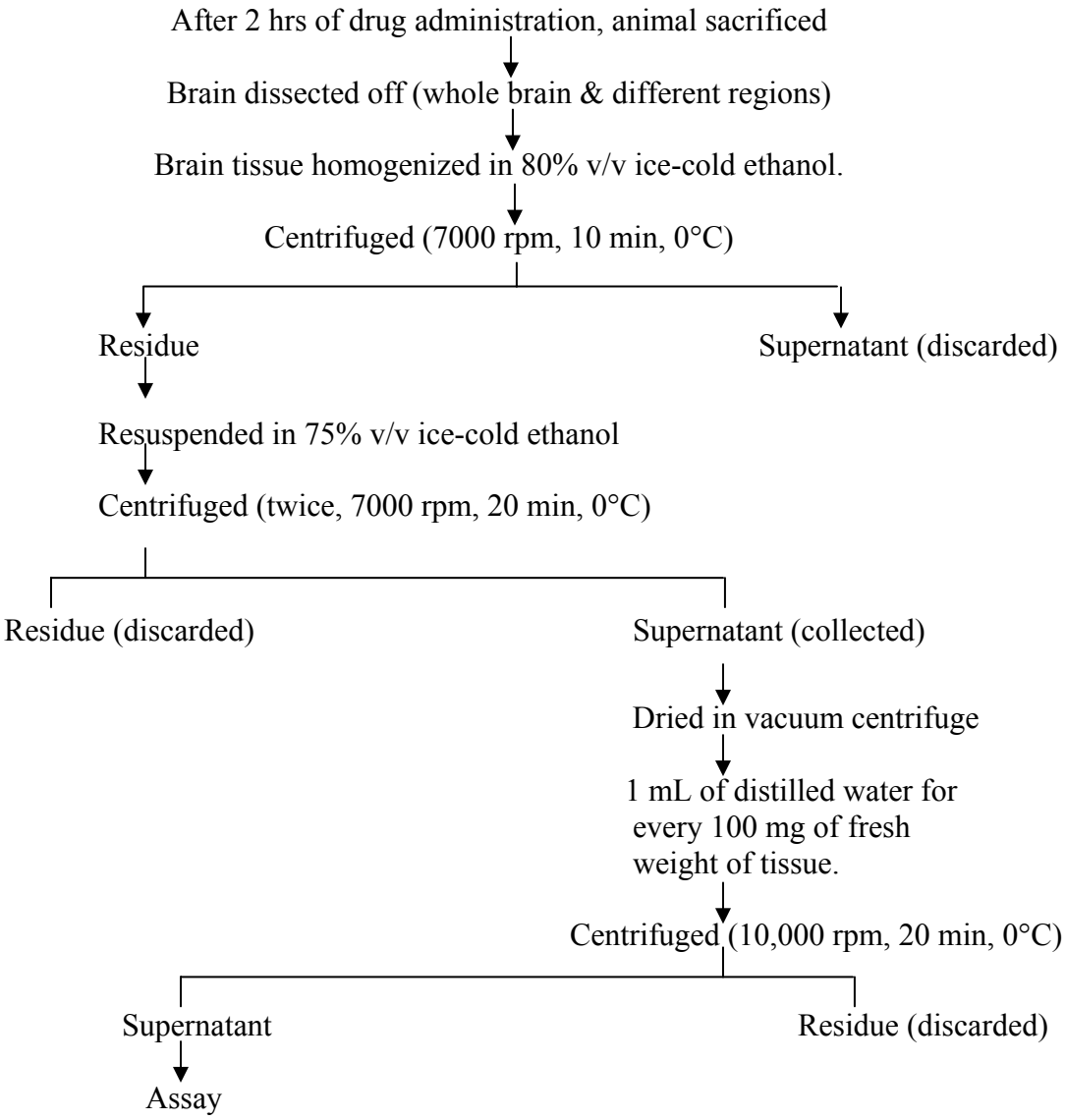


Fig. 7 Flow chart for the collection of samples from the rat brain tissue

UV method

The most rapid, specific and sensitive determination of GABA in biological extracts is based on the use of the UV enzymatic method. The procedure described below was employed for the determination of GABA in extracts from brain [125], but obviously can be modified for extracts from other types of tissues.

Composition of reagents

1. Sodium pyrophosphate buffer (0.1M, pH 8.1): -

   Weighed 4.46g of $Na_4P_2O_7.10H_2O$ in 70 mL of distilled water, adjusted to pH 8.1 and diluted to 100 mL with distilled water.

2. Mercaptoethanol (2 mg/ml): -

   70 µl of mercaptoethanol (20mg) was diluted to 10 mL in sodium pyrophosphate buffer.

3. NAD: -

   13.2 mg of NAD dissolved in 2 mL of distilled water

4. α-Ketogluarate: -

   0.146g of α-ketoglutaric acid was dissolved in 5 mL of distilled water. It was neutralized with 1N sodium hydroxide and diluted to 10 mL with distilled water.

5. Reagent mixture: - 1 mL of solution 2, 3 and 4 were mixed.

Stability of Solutions

Solution 5 was prepared freshly and stored in ice-cold condition. Also the enzyme solution was stored in ice-cold condition.

Procedure

For the study, wistar albino rats weighing 130-160g were used. The control group was treated only with the vehicle (30% v/v polyethylene glycol 400). The compounds were dosed at their minimal anticonvulsant activity doses. After 2h of drug administration, the animal was sacrificed by cervical dislocation and the brains were separated immediately and weighed. The whole brain or different brain regions like midbrain, cerebellum, medulla oblongata and olfactory lobe were dropped into separate vials containing 4-6 mL of 80% v/v ice-cold ethanol and processed further under frozen condition as presented in Fig. 7.

Calibration curve

Standard GABA solution

Standard GABA solutions were prepared to give a concentration range of 1-12µg / mL in distilled water and processed as follows. The reaction was started by the addition of 0.3 mL of pyrophosphate buffer (pH 8.1), 0.3 mL of the reagent mixture, and 1.53 mL of the enzyme solution and the sample was transferred to an optical tube. Optical density at 340nm was read immediately after transferring the reaction mixture.

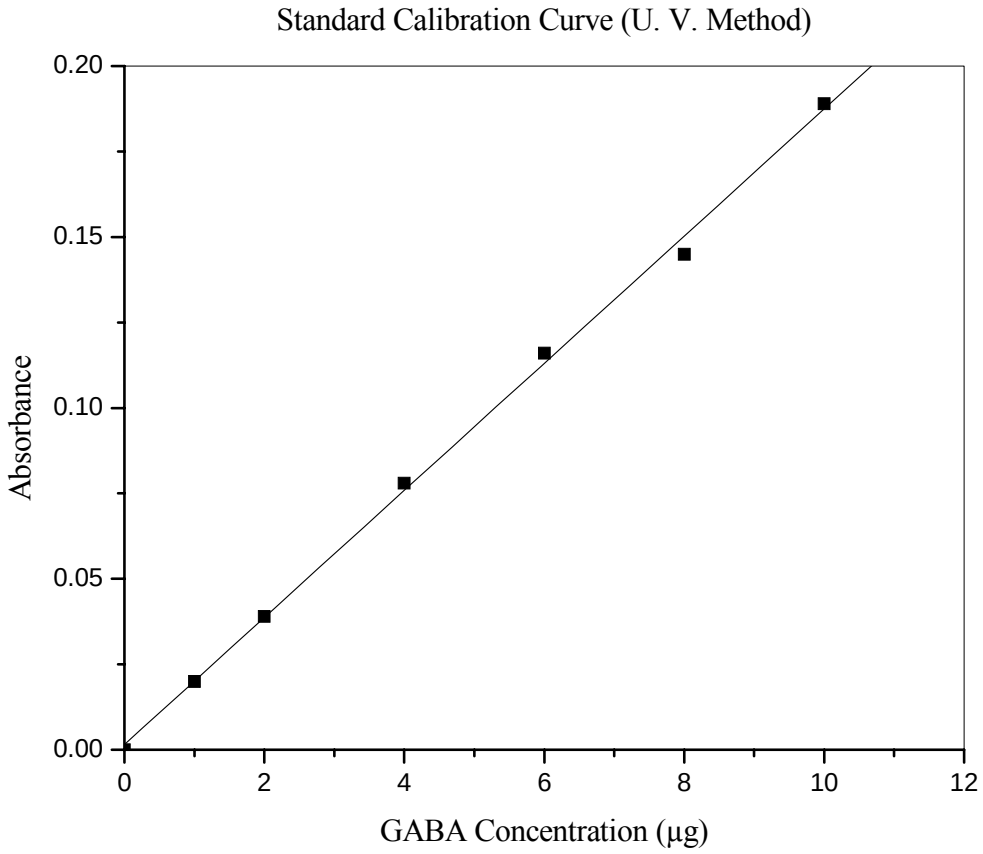
All spectrophotometric readings were made with the Perkin-Elmer UV spectrophotometer. A standard calibration curve was obtained by plotting different concentrations of standard GABA with the corresponding O.D. observed (Table 4.1, Fig. 8)

Observations and calculations

All the spectrophotometric readings were recorded at 340nm. The optical density was converted to concentration of GABA for the test samples from the standard calibration curve. The mean GABA concentrations of whole brain or in different regions of brain are presented in respective tables (Tables 5.9, 5.16, 6.6, 7.5)

Table 4.1 Preparation of standard curve for GABA level estimation in rat brain

Concentration (µg/mL)	Optical Density (O.D)
1	0.02
2	0.039
4	0.078
6	0.116
8	0.145
10	0.189



r = 0.99899386

y = 0.01846x + 0.00244

Fig. 8 Calibration curve for GABA level estimation in rat brain

4.3 Quantum Mechanical Modeling

Quantum Mechanical (QM) modeling methods predict the behavior of electrons. In the study, stress has been put on the study of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) surfaces and their corresponding energies (E_{HOMO} and E_{LUMO}). The quantum mechanical (QM) calculations were carried out using Argus Lab version 4.0.1. The three-dimensional structures of the compounds were geometry optimized using Hamiltonian PM3 (Parameterized Method 3) semi-empirical QM method [133]. For the estimation of HOMO and LUMO surfaces and energies (E_{HOMO} and E_{LUMO}), the single-point energy calculations using Hamiltonian ZINDO and RHF-SCF (Restricted Hartree-Fock-Single consistent Field) method (Basis set STO-6G) [137] were employed. The HOMO surfaces were visualized using a contour value of 0.05 in opaque mode using blue and red for positive and negative phase of the orbital in space.

CHAPTER 5

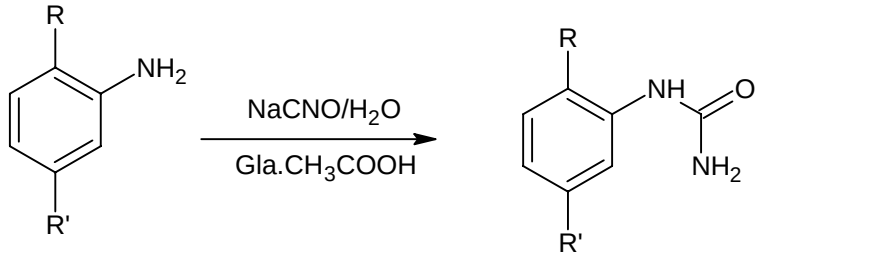
DISUBSTITUTED PHENYL SEMICARBAZONES

5.1 2,5-DISUBSTITUTED PHENYL SEMICARBAZONES

Under this study two series of semicarbazones namely 2,5-dimethyl and 2-fluoro-5-methylphenyl semicarbazones have been synthesized and evaluated for anticonvulsant activity.

5.1.1 Synthesis

Step-1: Synthesis of 2,5-disubstituted phenyl urea



2, 5-Disubstituted aniline

2, 5- Disubstituted phenylurea

a) R = CH_3; R' = CH_3
b) R = F; R' = CH_3

The 2,5-dimethyl or 2-fluoro-5-methyl aniline (0.1 M) was dissolved in 20 mL of glacial acetic acid and 10 mL of water. To this, equimolar amount of sodium cyanate in 80 mL of warm water was added with stirring. The reaction mixture was allowed to stand for 30 minutes, then cooled in ice and filtered with suction and dried. The product was then recrystallized from boiling water to yield the respective phenyl ureas.

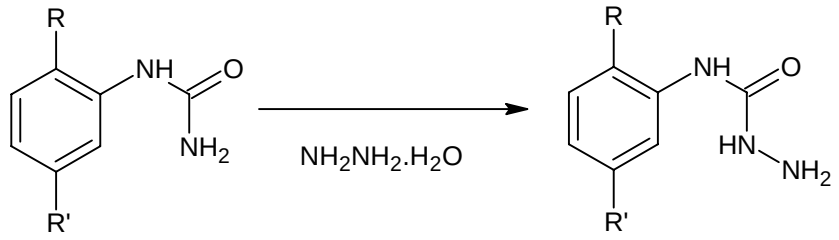
2,5-Dimethylphenyl urea

M.p. 172°C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3400, 1700, 780, 750, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.4 (s, 3H, $ArCH_3$), 2.24 (s, 3H, $ArCH_3$), 7.3-7.5 (m, 3H, ArH), 7.98 (s, 1H, ArNH, D_2O exchangeable), 9.88 (s, 2H, NH_2, D_2O exchangeable).

2-Fluoro-5-methylphenyl urea

M.p. 148°C; Yield: 62%; IR-KBr pellet (cm^{-1}) 3380, 1740, 780, 720, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.4 (s, 3H, $ArCH_3$), 7.3-7.5 (m, 3H, ArH), 7.98 (s, 1H, ArNH, D_2O exchangeable), 9.88 (s, 2H, $CONH_2$, D_2O exchangeable).

Step-2: Synthesis of 2,5-disubstituted phenyl semicarbazide



2, 5- Disubstituted phenylurea

2, 5-Disubstituted phenylsemicarbazide

a) R = CH_3; R' = CH_3
b) R = F; R' = CH_3

The 2,5-dimethyl and 2-fluoro-5-methylphenyl semicarbazides were prepared by heating equimolar quantities of the phenyl urea and hydrazine hydrate i.e. 0.05 M, in ethanol under reflux for 24h with stirring. The two-third volume of alcohol was distilled by vacuum distillation unit and then poured into ice. The resultant precipitate was filtered, washed with water and dried. The solid phenyl semicarbazide obtained was recrystallized from 50mL of 90% v/v alcohol

2,5-Dimethylphenyl semicarbazide

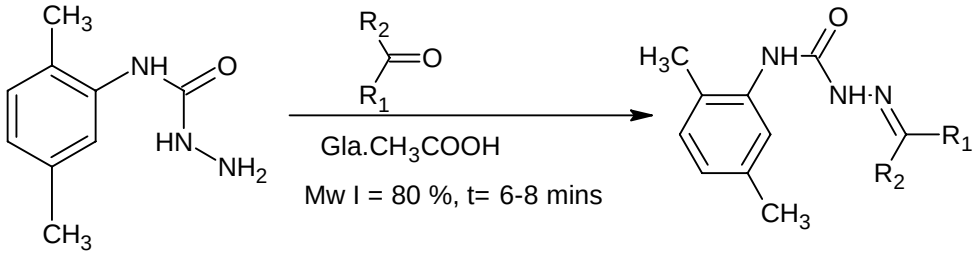
M.p. 192°C; Yield: 65%; IR-KBr pellet (cm^{-1}) 3400, 3280,1640, 760, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.18 (s, 3H, $ArCH_3$), 2.26 (s, 3H, $ArCH_3$), 7.4-7.5 (m, 3H, ArH), 5.60 (s, 2H, NH_2, D_2O exchangeable), 7.92 (s, 1H, ArNH, D_2O exchangeable), 9.94 (s, 1H, CONH, D_2O exchangeable).

2-Fluoro-5-methylphenyl semicarbazide

M.p. 178°C; Yield: 68%; IR-KBr pellet (cm^{-1}) 3370, 3300, 1620, 810, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.18 (s, 3H, $ArCH_3$), 7.4-7.5 (m, 3H, ArH), 5.60 (s, 2H, NH_2, D_2O exchangeable), 7.92 (s, 1H, ArNH, D_2O exchangeable), 9.94 (s, 1H, CONH, D_2O exchangeable).

Step-3:

Synthesis of 2,5-dimethylphenyl semicarbazones (DM 1-19)

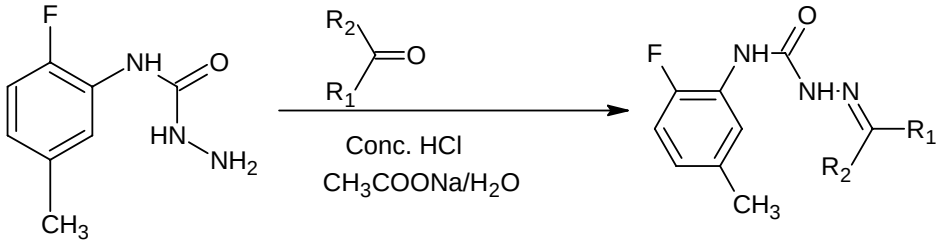


2, 5-Dimethylphenyl semicarbazide

2, 5-Dimethylphenyl semicarbazones

The conversion of 2,5-dimethylphenyl semicarbazide to semicarbazones was carried using microwave irradiation, as per the reported procedure. To a solution of 2,5-dimethylphenyl semicarbazide (0.003 M), in ethanol was added an equimolar quantity of the appropriate aldehyde or ketone. The pH of the reaction mixture was adjusted to 5-6 by adding glacial acetic acid, to facilitate the nucleophilic substitution. The reaction mixture was exposed to microwave irradiation for 6-8 minutes (Mw I = 80%). The product obtained after cooling was filtered and recrystallized from 95% v/v ethanol. IR-KBr pellet (cm^{-1}) 3450, 3300-3250, 1650, 1595, 840. ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) spectra of some representative compounds and physical data for the synthesized compounds are presented in Tables 5.1 and 5.3 respectively.

Synthesis of 2-fluoro-5-methylphenyl semicarbazones (FM 1-27)

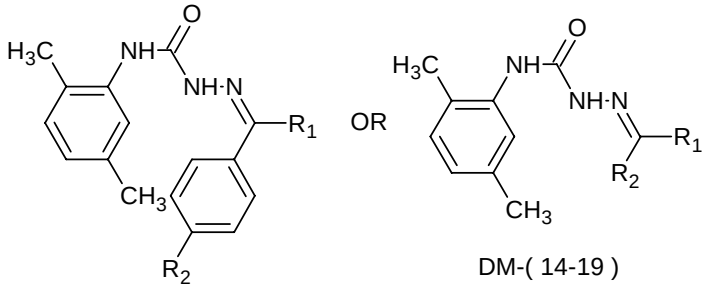


2-Fluoro- 5-methylphenyl semicarbazide

2-Fluoro- 5-methylphenyl semicarbazone

The 2-fluoro-5-methylphenyl semicarbazones were synthesized from the corresponding semicarbazide hydrochloride salt. The semicarbazide hydrochloride salt was prepared by the addition of conc. hydrochloric acid to the solution of semicarbazide in ethanol in 2:1 ratio (semicarbazide solution in ethanol: hydrochloric acid). To a solution of 2-fluoro-5-methylphenyl semicarbazide hydrochloride salt (0.001 M) in 25 mL of methanol was added sodium acetate solution in water (0.0005 M in 2 mL of water). This solution mixture was added to the appropriate aldehyde or ketone in alcohol, with stirring. The reaction was carried out for 5-10 minutes. Solid product was filtered, dried and recrystallized from hot alcohol. The IR spectra of the semicarbazone derivatives were identical in the following aspects; IR-KBr pellet (cm^{-1}) 3380, 3090, 2895, 2880, 1680, 1580-1540, 1340, 1210. ^{1}H-NMR (DMSO-$d_{6,}$ ppm, 300 MHz), spectra of some representative compounds are represented in Table 5.4.

Table 5.1: Physical data for 2,5-dimethylphenyl semicarbazones



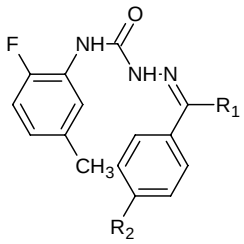
DM- (1-13) DM-(14-19)

Compound	R	R_1	Yield (%)	M.P. (°C)	Molecular formula	Mol. weight	R_f [a]	Log P[b]
DM-1	H	H	57	150	$C_{14}H_{14}N_4O$	254	0.78	2.39
DM-2	H	2-NO_2	58	60	$C_{14}H_{13}N_5O_3$	299	0.67	1.60
DM-3	H	2-OH	67	196	$C_{16}H_{17}N_3O_2$	283	0.77	2.06
DM-4	H	4-NO_2	70	190	$C_{16}H_{16}N_4O_3$	312	0.45	2.43
DM-5	H	4-CH_3	56	180	$C_{17}H_{19}N_3O$	281	0.50	3.21
DM-6	H	4-Br	62	172	$C_{16}H_{16}N_3OBr$	346	0.58	3.73
DM-7	H	4-OCH_3	64	174	$C_{17}H_{19}N_3O_2$	297	0.78	3.14
DM-8	H	4-OH 3-OCH_3	51	175	$C_{17}H_{19}N_3O_3$	313	0.62	3.06
DM-9	CH_3	3-NH_2	52	165	$C_{17}H_{20}N_4O$	296	0.52	2.20
DM-10	CH_3	4-OH	47	175	$C_{17}H_{19}N_3O_2$	297	0.38	2.29
DM-11	CH_3	4-NH_2	95	145	$C_{17}H_{20}N_4O$	296	0.52	1.54
DM-12	C_6H_5	H	72	153	$C_{22}H_{21}N_3O$	343	0.58	3.48
DM-13	C_6H_5	4-Br	91	165	$C_{22}H_{20}N_3OBr$	422	0.77	4.09
DM-14	CH_3	CH_3	63	185	$C_{12}H_{17}N_3O$	219	0.52	1.81
DM-15	CH_3	C_2H_5	72	184	$C_{13}H_{19}N_3O$	233	0.44	2.19
DM-16	CH_3	CH_2COCH_3	56	195	$C_{14}H_{19}N_3O_2$	275	0.58	3.62
DM-17	CH_3	C_5H_{11}	54	185	$C_{16}H_{25}N_3O$	261	0.56	1.88
DM-18	Cyclopentylene		61	165	$C_{14}H_{19}N_3O$	245	0.77	0.77
DM-19	Cyclohexylene		56	185	$C_{15}H_{21}N_3O$	259	0.79	0.79

[a] Mobile phase $CHCl_3$: CH_3OH (9:1)

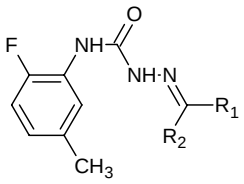
[b]Log P was generated using Alchemy 2000 and SciLog P softwares

Table 5.2: Physical data of 2-fluoro-5-methylphenyl semicarbazones



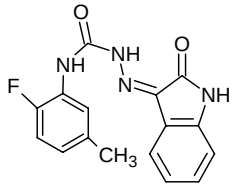
FM- (1-19)

OR



FM-(20-26)

OR



FM-27

Compound	R	R_1	Yield (%)	M.P (°C)	Molecular Formula	Mol. weight	Log P[a]	R_f[b]
FM-1	H	H	59	100	$C_{15}H_{14}N_3OF$	271	2.73	0.62
FM-2	H	2-NO_2	67	200	$C_{15}H_{13}N_4O_3F$	316	0.94	0.78
FM-3	H	2-Cl	72	178	$C_{15}H_{13}N_3OClF$	305	1.91	0.60
FM-4	H	2-CH_3	65	132	$C_{16}H_{16}N_3OF$	285	2.86	0.82
FM-5	H	3-NO_2	63	178	$C_{15}H_{14}N_3OF$	271	1.57	0.70
FM-6	H	4-NO_2	72	>260	$C_{15}H_{13}N_4O_3F$	316	1.93	0.63
FM-7	H	4-OH	78	210	$C_{15}H_{14}N_3O_2F$	210	2.25	0.66
FM-8	H	4-OCH_3	61	205	$C_{16}H_{16}N_3O_2F$	301	2.60	0.74
FM-9	H	4-Br	64	225	$C_{15}H_{13}N_3OFBr$	350	1.60	0.73
FM-10	H	4-CH_3	62	134	$C_{16}H_{16}N_3OF$	285	2.91	0.78
FM-11	H	4-$N(CH_3)_2$	74	198	$C_{17}H_{20}N_4OF$	315	2.26	0.80
FM-12	H	3-OCH_3 4-OH	60	165	$C_{16}H_{16}N_3O_3Br$	317	2.08	0.81
FM-13	CH_3	H	71	160	$C_{16}H_{16}N_3OF$	317	2.88	0.70
FM-14	CH_3	3-NH_2	68	182	$C_{16}H_{17}N_4OF$	300	0.06	0.82
FM-15	CH_3	4-NH_2	60	190	$C_{16}H_{17}N_4OF$	300	0.10	0.84
FM-16	CH_3	4-NO_2	62	238	$C_{16}H_{15}N_4O_3F$	330	1.51	0.71
FM-17	CH_3	4-OH	55	>260	$C_{16}H_{16}N_3O_2F$	301	3.09	0.80
FM-18	CH_3	4-CH_3	60	158	$C_{17}H_{18}N_3OF$	299	2.31	0.78
FM-19	C_6H_5	4-Br	90	200	$C_{21}H_{17}N_3OFBr$	426	3.00	0.74
FM-20	CH_3	CH_3	52	163	$C_{11}H_{14}N_3OF$	223	1.58	0.75
FM-21	CH_3	C_2H_5	54	175	$C_{12}H_{16}N_3OF$	237	1.98	0.66
FM-22	CH_3	CH_2COCH_3	52	144	$C_{13}H_{16}N_3O_2F$	265	-0.78	0.75
FM-23	CH_3	C_5H_{11}	50	100	$C_{15}H_{22}N_3OF$	279	3.81	0.67
FM-24	CH_3	$CH_2CH(CH_3)_2$	56	135	$C_{14}H_{20}N_3OF$	265	3.01	0.59
FM-25	CRR_1= cyclopentylene		61	184	$C_{13}H_{16}N_3OF$	249	0.89	2.09
FM-26	CRR_1= cyclohexylene		68	190	$C_{14}H_{18}N_3OF$	263	0.81	2.68
FM-27	-	-	68	215	$C_{13}H_{16}N_4O_2F$	275	0.68	3.13

[a]Log P was generated using Alchemy 2000 and SciLog P softwares.
[b]Mobile phase $CHCl_3$: CH_3OH (9:1).

Table 5.3: Spectral and elemental analyses data of 2,5-dimethylphenyl semicarbazones

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
DM-2	3400, 3308, 3100, 2860, 1650, 1600	2.20 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$) 6.58-7.58 (m, 7H, ArH), 7.78 (s, 1H, imine H), 8.60 (s, 1H, ArNH, D_2O exchangeable), 11.0 (s, 1H, CONH, D_2O exchangeable).	54.14 53.93	3.94 3.93	16.84 16.78
DM-3	3400, 3200, 3050, 2850, 1660, 1620	2.20 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$) 6.74-7.62 (m, 7H, ArH), 7.76 (s, 1H, imine H), 8.64 (s, 1H, ArNH, D_2O exchangeable), 9.47 (s, 1H, ArOH, D_2O exchangeable), 9.88 (s, 1H, CONH, D_2O exchangeable).	59.31 59.33	4.65 4.63	13.83 13.78
DM-5	3390, 3320, 3090, 2865, 1670, 1628	2.18 (s, 3H, $ArCH_3$), 2.22 (s, 6H, 2-$ArCH_3$) 6.87-8.16 (m, 7H, ArH), 8.06 (s, 1H, imine H), 8.54 (s, 1H, ArNH, D_2O exchangeable), 10.05 (s, 1H, CONH, D_2O exchangeable).	55.42 55.22	4.36 4.35	16.16 16.09
DM-7	3380, 3260, 3100, 2860, 1620	2.18 (s, 3H, $ArCH_3$), 2.20 (s, 3H, $ArCH_3$), 3.75 (s, 3H, OCH_3) 6.85-7.74 (m, 7H, ArH), 7.82 (s, 1H, imine H), 8.58 (s, 1H, ArNH, D_2O exchangeable), 11.00 (s, 1H, CONH, D_2O exchangeable)	60.47 60.24	5.08 5.06	13.22 13.18
DM-9	3460, 3390, 3070, 2880, 1710, 1620	2.02 (s, 3H,CH_3), 2.10 (s, 3H, $ArCH_3$), 2.24 (s, 3H, $ArCH_3$), 5.32 (s, 2H, NH_2, D_2O exchangeable), 7.14-7.64 (m, 7H, ArH), (8.36 (s, 1H, ArNH, D_2O exchangeable), 9.68 (s, 1H, CONH, D_2O exchangeable).	55.12 54.93	5.89 5.87	17.53 17.47
DM-10	3390, 3350, 3100, 2920, 1680, 1610	1.96 (s, 3H, CH_3), 2.12 (s, 3H, $ArCH_3$), 2.20 (s, 3H, $ArCH_3$), 7.12 (m, 7H, ArH), 8.16 (s, 1H, ArNH, D_2O exchangeable), 9.64 (s, 1H, CONH, D_2O exchangeable), 10.14(s, 1H, OH, D_2O exchangeable)	56.80 56.58	6.35 6.34	16.56 16.51

[a] Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

Table 5.4: Spectral and elemental analyses data of 2-fluoro-5-methylphenyl semicarbazones

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
FM-3	3400, 3308, 3210, 2855, 1706, 1620, 1140	2.20 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$) 6.74-7.62 (m, 7H, ArH), 7.76 (s, 1H, imine H), 8.64 (s, 1H, ArNH, D_2O exchangeable), 9.47 (s, 1H, ArOH, D_2O exchangeable), 9.88 (s, 1H, CONH, D_2O exchangeable).	54.14 53.93	3.94 3.93	16.84 16.78
FM-4	3410, 3250, 3100, 2860, 1710, 1610, 1160	2.20 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$) 6.58-7.58 (m, 7H, ArH), 7.78 (s, 1H, imine H), 8.60 (s, 1H, ArNH, D_2O exchangeable), 10.20(s, 1H, CONH, D_2O exchangeable).	59.31 59.23	4.65 4.63	13.83 13.78
FM-7	3430, 3210, 3070, 2865, 1680, 1620, 1250	2.18 (s, 3H, $ArCH_3$), 2.20 (s, 3H, $ArCH_3$), 6.85-7.74 (m, 7H, ArH), 7.82 (s, 1H, imine H), 8.58 (s, 1H, ArNH, D_2O exchangeable), 9.78 (s, 1H, ArOH, D_2O exchangeable), 10.20 (s, 1H, CONH, D_2O exchangeable).	55.42 55.22	4.36 4.35	16.16 16.09
FM-14	3380, 3210, 3080, 2850, 1700, 1620, 1260	1.82 (s, 3H,CH_3), 1.98 (s, 3H,CH_3), 2.20 (s, 3H, $ArCH_3$), 2.28 (s, 3H, $ArCH_3$), 6.80-7.12 (m, 3H, ArH), 8.31 (s, 1H, ArNH, D_2O exchangeable), 10.54 (s, 1H, CONH, D_2O exchangeable).	60.47 60.24	5.08 5.06	13.22 13.18
FM-15	3430, 3230, 3060, 2880, 1710, 1620, 1250	1.46-1.50 (t, 3H, CH_3), 1.90-1.94 (q, 2H, CH_2), 1.98 (s, 3H, CH_3), 2.20 (s, 6H, 2-$ArCH_3$) 7.19-7.26 (m, 3H, ArH), 8.46 (s, 1H, ArNH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable).	55.12 54.93	5.89 5.87	17.53 17.47

[a] Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

5.1.2. Pharmacology

Anticonvulsant activity

The anticonvulsant activity of 2,5-dimethylphenyl/2-fluoro-5-methylphenyl semicarbazones was established by electrical and chemical tests, using the standard protocol. In the preliminary studies, the electrical test employed was MES pattern test and the chemical test was scPTZ test. As 2,5-dimethylphenyl semicarbazones but not 2-fluoro-5-methylphenyl semicarbazones, exhibited good anticonvulsant activity in the preliminary seizure models, 2,5-dimethylphenyl semicarbazones were subjected to second level of tests i.e. scSTY and scPIC. Anticonvulsant activity was examined at 0.5h and 4.0h after the injections. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table 5.5 and 5.6, along with the data for standard drugs. Further to study the effect of route of administration of drug and animal species, on anticonvulsant activity profile some of the compounds were subjected to rats *p.o.* screen. The results are presented in Table 5.7.

Neurotoxicity of all the synthesized compounds was evaluated by rotarod test after 0.5h and 4h of drug administration by *i.p.* route (Table 5.6).

CNS depressant evaluation

All the active compounds were also examined for CNS depressant activity which included locomotor activity using actophotometer and porsolt's swim test, since many current frontline antiepileptic drugs are known to exhibit CNS depression effect [46].(Table 5.8)

Neurochemical study

To predict the mechanism for their anticonvulsant effect, the most active compounds (**DM-9**, **DM-14**, **FM-1**, **FM-20**) from both the series were subjected to study their effect on GABA level in different regions of the rat brain and results are presented in Table 5.10.

Table 5.5: Anticonvulsant and minimal motor impairment of 2,5-dimethylphenyl semicarbazones

Compound	Intraperitoneal injection in mice[a]									
	MES screen		scPTZ screen		scSTY screen		ScPIC screen		Neurotoxicity screen	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
DM-1	100	300	-	-	300	-	300	300	100	-
DM-2	-	-	-	-	300	300	300	300	-	-
DM-3	-	-	-	-	300	300	300	300	300	-
DM-4	300[b]	-	300[c]	-	300	300	-	-	300	-
DM-5	300[b]	-	-	-	300	300	300	300	300	-
DM-6	300	-	100	-	100	100	100	100	300	-
DM-7	300	-	100	300	100	100	100	100	-	-
DM-8	300	-	-	-	300	300	300	300	300	-
DM-9	300	300	-	-	300	-	100	100	300	-
DM-10	100	300	-	-	300	300	300	-	300	300
DM-11	300[b]	300	-	-	300	300	300	-	300	-
DM-12	-	-	-	-	300	-	300	-	100	-
DM-13	300	300	-	-	300	300	300	-	300	-
DM-14	100	300	300	-	100	100	100	100	300	-
DM-15	300[b]	300	300[c]	-	300	300	300	-	300	-
DM-16	300[b]	-	300	-	-	-	300	-	300	-
DM-17	300[b]	300	300[c]	300	-	-	-	-	300	-
DM-18	300[b]	300	300[c]	300	100	100	300	300	300	300
DM-19	300	300	-	-	300	300	-	-	300	300
Phenytoin	30	30	-	-	-	-	-	-	100	100
Ethosuximide	-	-	300	-	-	-	-	-	-	-
Sod. valproate	-	-	300	-	x	x	-	-	-	-

Table 5.6: Anticonvulsant and minimal motor impairment of 2-fluoro-5-methylphenyl semicarbazones

Compound	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicty screen	
	0.5h	4h	0.5h	4h	0.5h	4h
FM-1	300	300	-	-	-	-
FM-6	-	-	300	-	-	-
FM-8	300	-	-	-	-	-
FM-9	-	-	-	-	300	-
FM-14	-	300	-	-	-	-
FM-16	-	-	300	-	-	-
FM-19	300	-	300	-	300	-
FM-20	100	300	300	-	300	-
FM-21	-	300	-	-	-	-

[a]Doses of 30, 100 and 300mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (-) indicates an absence of activity at the maximum dose administered (300mg/kg).

Table 5.7: Evaluation of some selected compounds in the MES test after oral administration (30mg/kg) to rats.

Compound	Oral administration to rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
DM-10	-	-	-	-	1
DM-11	-	-	1	1	-
DM-14	-	-	-	-	1
Phenytoin[b]	1	4	3	3	3

[a]The figures indicate the number of rats out of four which were protected. The dash (-) indicates an absence of activity at the administered dose

[b]Compound administered at 100mg/kg.

Table 5.8: CNS studies of 2,5-dimethylphenyl semicarbazones

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec) [c]	
	0.5h	1h	Control	Test (After 1 h)
Control	318.00±13.68	228.50±11.31		
DM-1	174.50±4.22	133.00±3.21	96.00±3.02	132.50±4.77
DM-2	195.50±5.48	150.00±11.05	177.00±4.15	194.00±6.72 *
DM-3	260.00±62.57	196.50±15.11 NS	140.00±8.71	145.50±5.62 NS
DM-4	208.00±12.00	162.50±25.50	158.00±5.56	164.00±5.03 NS
DM-5	192.50±3.41	145.67± 4.68	156.00±.2.42	168.50±2.79
DM-6	230.50±7.98	192.00±4.72	167.00±6.01	176.00±2.93 NS
DM-7	200.00±11.00	145.50±5.06	186.00±10.60	206.50±5.60 NS
DM-8	173.00±3.52	149.00±3.53	120.00±17.91	165.00±9.29
DM-9	222.00±8.67	153.50±3.91	179.00±3.49	195.00±3.79
DM-10	230.00±4.77	173.00±5.16	180.50±3.75	193.00±4.10
DM-11	163.50±7.65	127.00±4.21	162.50±10.03	173.00±12.15 NS
DM-12	214.00±5.37	181.00±3.33	94.00±15.91	131.00±16.99 NS
DM-13	188.00±3.64	136.00±3.86	148.00±9.73	207.50±4.93
DM-14	270.00±17.78 NS	216.00±17.24 NS	179.00±4.12	179.50±3.94 NS
DM-15	233.33±8.67	184.57±8.59	100.50±18.12	141.50±9.49
DM-16	201.00±13.38	166.00±4.38	184.00±5.63	186.50±4.36 NS
DM-17	203.50±5.89	191.50±5.73	158.00±10.99	166.50±6.08 NS
DM-18	188.50±23.15	143.50±17.84	128.50±16.21	121.00±15.29 NS
DM-19	208.00±6.30	153.00±3.86	103.00±29.27	117.33±29.51 NS
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	-	-	131.50±9.32	207.33±08.49

[a]The compounds were tested at the dose level of 100mg/kg . [b] Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.05$ and NS denotes values, which were not significant (Student's t test). [c]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.005$, *$p < 0.05$ and NS denotes values which were not significant (Student's t test). [d]The compounds were tested at the dose level of 30mg/kg.

Table 5.9: CNS studies of 5-fluoro-2-methyl substituted phenyl semicarbazones

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec)[c]	
	0.5h	1h	Control	Test (after 1h)
Control	318.00±13.68	138.17±12.88		
FM-1	295.00±14.00 NS	205.50±5.50 NS	148.00±18.00	101.50±14.50 NS
FM-4	154.50±13.00.	113±12.00	135.00±1.00	192.00±4.00
FM-8	140.5±17.50	87.00±14.00	117.50±3.50	156.50±5.50
FM-9	173.50±12.50	121.00±14.00	154.50±4.50	125.00±13.67 NS
FM-14	285.00±6.67 NS	195.00±7.86 NS	185.00±9.00	156.50±18.50 NS
FM-16	236.00±14.00 *	202.50±5.50 NS	129.50±9.50	119.00±13.00 NS
FM-19	189.00±11.00	132.00±16.00 *	142.50±7.50	130.50±10.50 NS
FM-20	299.50±7.50 NS	207.00±13.00 NS	88.00±12.00	103.00±11.00 NS
FM-21	222.07±12.00	198.00±14.00 NS	97.00±14.00	86.00±11.00 NS
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	318.00±13.68	138.17±12.88	131.50±9.32	207.33±08.49

[a] The compounds were tested at the dose level of 100mg/kg. [b]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.0001$, *$p < 0.002$ and NS denotes values, which were not significant (Student's t test). [c]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.0001$ and NS denotes values, which were not significant (Student's t test).[d]The compounds were tested at the dose level of 30mg/kg.

Table 5.10: GABA concentration in µg/100mg weight of rat brain tissues

Compound[a]	GABA concentrations in different region of rat brain[b]			
	Olfactory lobe	Mid-brain	Cerebellum	Medulla oblongata
Control	40.5 ± 0.28	101.6 ± 0.30	39.3 ± 0.05	93.4 ± 0.22
DM-9	27.3 ± 0.04	64.8 ± 0.02	10.7 ± 0.04	93.9 ± 0.28 NS
DM-14	23.7 ± 0.15	248.5 ± 0.23	211.4 ± 0.33	159.5 ± 0.28
DM-15	47.9 ± 1.94*	149.0 ± 0.12	127.7 ± 0.19	169.5 ± 0.12
FM-1	61.5 ± 0.62	91.3± 0.16	150.0 ± 0.65	227.6±0.28
FM-20	129.9± 0.39	83.2 ± 0.37	153.1 ± 0.48	66.2± 0.57
Clobazam	143.0 ± 0.15	713.0 ± 0.47	235.0 ± 0.25	188.0 ± 0.30

[a]The compounds were administered at the dose level of 100mg/kg.

[b]Each value represents the mean ± SEM of three rats significantly different from the control at $p < 0.0001$, $*p < 0.02$ and NS denotes values which were not significant (Student's t test).

5.1.3. Results & Discussion

Chemistry

The 2,5-dimethylphenyl semicarbazones and 2-fluoro-5-methylphenyl semicarbazones were synthesized by a three-step process starting from substituted anilines which were converted first to substituted phenyl urea and then to substituted phenyl semicarbazide on refluxing with hydrazine hydrate in ethanol for 24h. The last step of conversion in to phenyl semicarbazones was carried out in microwave for 2,5-dimethylphenyl and resulted in higher yields compared to conventional reflux method. The 2-fluoro-5-methylphenyl semicarbazones were synthesized by converting the corresponding semicarbazide to its hydrochloride salt. The homogeneity of the compounds was monitored by performing TLC from which R_f values were calculated. Eluant for all the compounds was $CHCl_3$: CH_3OH (9:1) (Tables 5.1 and 5.2).

Most of the compounds were found to be more lipophilic indicated by their calculated log of partition coefficient value greater than 2 [log P > 2] except for compounds **DM-2**, **DM-11**, **DM-14**, **DM-(17-19), FM-(2-3), FM-(4-5), FM-9, FM-(14-16)** , **FM- (20-22)** and **FM-(25-27).** These compounds possessed log P < 2 because of the substitution with polar groups like OH, NO_2, and NH_2 etc. No correlation has been established between the calculated log P and anticonvulsant profile of the compounds, as compounds with log P less than 2 were also found to possess good anticonvulsant activities i.e. substituted acetone derivatives (**DM-20** and **FM-20**).

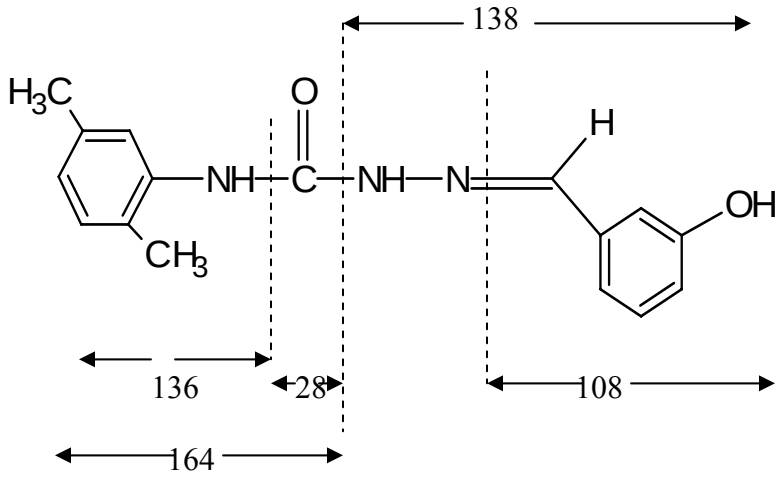
Compounds with substituted aryl / alkyl ketones and isatins showed yields in the range of 57-95 %, higher than substituted aryl/alkyl aldehydes i.e. 50-78 %, due to the higher weight of the ketone derivatives than the corresponding aryl/alkyl aldehydes.

The IR spectra for N^1-(2,5-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde) semicarbazone (**DM-3**) and N^1-(2-fluoro-5-methylphenyl)-N^4-(4-hydroxybenzaldehyde) semicarbazone (**FM-7**) was recorded in KBr pellet and absorption bands (ν_{max} cm^{-1}) confirmed the presence of following groups i.e. absorption band at 3430 and 3400 cm^{-1} showed NH stretching, 3070 and 3090 cm^{-1} showed aromatic CH stretching, 2850 and 2865 cm^{-1} showed aliphatic CH stretching, 1680 and 1660 cm^{-1} showed C=O stretching, , 1600-1525 cm^{-1} showed aromatic C=C stretching. The N^1-(2-fluoro-5-methylphenyl)-N^4-(4-

hydroxybenzaldehyde) semicarbazone (**FM-7**) showed additional band at 1220 cm^{-1} due to the aryl-fluoro stretching. The substituted semicarbazones showed in addition absorption band other than corresponding semicarbazides at 3200 and 3220 cm^{-1} due to OH stretching, 1620 and 1600 cm^{-1} due to C=N stretching, which confirmed the structure of substituted semicarbazones derivatives.

^{1}H-NMR spectrum revealed a singlet at δ 2.22 and δ 2.28 for 3H of CH_3 protons, singlet at δ 7.76 and δ 7.78 for 1H of imine proton and singlets at δ 8.64, δ 8.60 (1H, ArNH), δ 9.88 , δ 10.20 (1H, CONH) and δ 9.49, δ 9.78 (1H, OH) were D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristics peaks depicted in Table 5.3 and 5.4.

The mass spectrum of compound **DM-3** showed a molecular ion peak at m/z 299, base peak at m/z 164. The remaining major fragmentation peaks were at m/z 108, 136, and 138.



Pharmacological activity

1. MES test

In the preliminary MES screen, all the derivatives of 2,5-dimethylphenyl semicarbazones except **DM-2**, **DM-3**, and **DM-12** and six compounds (**FM-1**, **FM-8**, **FM-14**, **FM-19**, **FM-20**, **FM-21**) of 2-fluoro-5-methylphenyl semicarbazones showed protection, indicative of their ability to prevent seizure spread. Compounds that were active at

100 mg/kg include **DM-1**, **DM-10**, **DM-14**, and **FM-20** at 0.5h period, whereas other active compounds showed protection at the higher dose (300 mg/kg). Compounds **DM-1**, **DM-(9-11)**, **DM-(13-15), DM-(17-19), DM-20** and **FM-20** were active at both 0.5h & 4h time periods; hence these compounds exhibited prolonged duration of action. Other compounds showed rapid onset (0.5 h) with shorter duration of action except **FM-14** and **FM-21** which showed activity only at 4h time point (late onset of action).

2. scPTZ test

In the scPTZ screen, a test used to identify compounds that elevates seizure threshold, twelve compounds [**DM-4**, **DM-(6-7), DM-(14-18), FM-6**, **FM-16**, **FM-20**, and **FM-21**] showed protection. All the active compounds showed protection at 300 mg/kg except **DM-6** and **DM-7** (100 mg/kg) and the action was for a shorter duration (0.5h) except **DM-7**, **DM-(17-18**) in the pentylenetetrazole seizure model.

3. scSTY Screen

All 2,5-dimethylphenyl semicarbazone derivatives were subjected to scSTY test. All of the compounds except **DM-16** and **DM-17** showed protection in the scSTY model. Compounds **DM-6, DM-7**, **DM-14** and **DM-18** showed protection at 100mg/kg (0.5h and 4h) and the other compounds showed activity at 300mg/kg. Except compounds **DM-1**, **DM-9**, and **DM-12**, all the active compounds exhibited a longer duration (4h) of action. The results suggested that these compounds have the possibility of interacting with the glycinergic pathway.

4. scPIC Screen

In the scPIC screen, compounds except **DM-4, DM-17** and **DM-19** exhibited protection from seizures and death, which implicated that semicarbazones could also act through GABA-mediation as reported earlier. Compounds **DM-(6-7), DM-9**, and **DM-14** showed protection at 100 mg/kg and other compounds exhibited protection at 300 mg/kg. Except compounds **DM-10**, **DM-(11-13),** and **DM-(15-17),** all other compounds showed protection in both 0.5h and 4h intervals.

Neurotoxicity screen (NT)

In the acute neurological deficit test, compounds **DM-2**, **DM-7**, **FM-1, FM-6, FM-8**, **FM-(14-16),** and **FM-21** showed no neurotoxicity activity at the maximum dose administered (300mg/kg) and those 2-fluoro-5-methylphenyl substituted semicarbazones which did not exhibit anticonvulsant activity at the maximum dose administered did not possess neurotoxicity also. Compounds **DM-10**, **DM-14, FM-1**, **FM-6**, **FM-8**, **FM-16**, and **FM-21**, did not exhibit neurotoxicity at the anticonvulsive dose. Only compounds **DM-12** and **FM-9** were found to be more neurotoxic at the anticonvulsive dose.

The N^4-(2,5-dimethylphenyl)semicarbazones have shown the potential to treat a wide range of seizure types by their multiple mechanisms of action as indicated by their activity in four animal models of seizure. Five compounds [**DM-6**, **DM-7**, **DM-(14-15**), and **DM-18**] had shown activity in all the screens (MES, scPTZ, scSTY, scPIC), exhibiting a broad spectrum of anticonvulsant activity.

Rat *p.o.* identification

Out of all the compounds that were active in the *i.p.* anticonvulsant screen some were selected for activity in the oral MES screen and neurotoxicity tests in rats. The results are presented in Table 5.7. The compounds were tested at 30 mg/kg. Compound **DM-10**, **DM-11**, and **DM-14** showed 1/4 protections i.e. only one animal out of four exhibited protection from seizures, at 4h, 1-2h and 4h respectively. None of the compounds was found to be active when compared with the standard drug Phenytoin.

CNS depressant evaluation

In the behavioral despair test, all compounds except compounds **DM-3**, **DM-14**, **FM-1**, **FM-14**, **FM-16**, **FM-20** and **FM-21**, showed a significant decrease in motor activity as indicated by the actophotometer scores, in which the standard drug Phenytoin also showed behavioral despair side effect. The compounds with substituted acetone group (**DM-14** and **FM-20**) showed the least motor impairment in their respective series, with the maximum actophotometer scores of 270.00±17.78 (0.5 h), 216.00±17.24 (1h) and

299.50±7.50 (0.5h), 207.00±13.00 (1h) respectively. The semicarbazone derivatives were also studied for CNS depressant effect by porsolt's forced swim pool test and compared with Carbamazepine. In this study except compounds **DM-1**, **DM-2**, **DM-5**, **DM-(8-10),** **DM-13**, **DM-15**, **FM-4** and **FM-8**, all other compounds showed no significant increase in the immobility time with respect to control indicative of their lesser CNS depressant effect, as compared to the conventional AEDs (Tables 5.8 and 5.9).

Effect on the levels of GABA in different regions of rat brain

In order to explore the mechanism of anticonvulsant activity of these derivatives, five compounds (**DM-10**, **DM-14**, **DM-15**, **FM-1** and **FM-20**) were subjected to neurochemical investigation to determine GABA level in different regions of the brain, as there is regional difference in GABA concentration within the CNS. All the compounds were found to show significant changes in the GABA level, in different regions of rat brain, with respect to the control. Compound **DM-9** did not show a significant increase in GABA level concentration in all the rat brain regions except medulla oblongata, wherein there was no effect. Compound **DM-14** showed a significant increase in GABA level in mid brain, cerebellum and medulla oblongata region and compound **DM-15** showed a significant increase in all the brain regions. The 2-fluoro-5-methylphenyl substituted semicarbazone **FM-1** showed a significant increase in all the regions except mid brain and **FM-20** exhibited an increase in GABA level in olfactory lobe and medulla oblongata region with respect to the control. This shows that aryl semicarbazones exert anticonvulsant activity through a GABA-mediated mechanism (Table 5.10).

5.1.4. Structure-Activity Relationship

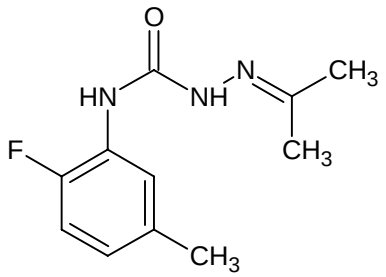
Forty-five variously substituted 2,5-dimethylphenyl and 2-fluoro-5-methylphenyl substituted semicarbazones were prepared with the substituents chosen to study the effects of electron donating and withdrawing groups, effect of bulky and lipophilic groups and also to study the effect of replacing the *ortho* methyl group with electronegative fluoro group on the anticonvulsant activity profile.

Comparison of anticonvulsant data for 2,5-dimethylphenyl and 2-fluoro-5-methylphenyl substituted semicarbazones, proved very clearly that replacement of the *ortho* methyl group with fluoro group is not beneficial for the anticonvulsant activity as out of twenty-seven synthesized 2-fluoro-5-methylphenyl substituted semicarbazones, only eight compounds were found to possess anticonvulsant activity, that too at higher doses in either of the anticonvulsant animal models. So the increase in the electronegativity of the *ortho* substitution i.e. decreased π value, resulted in decreased anticonvulsant activity. The common features, which emerged by comparing the two series, leading to potential anticonvulsant activity were:

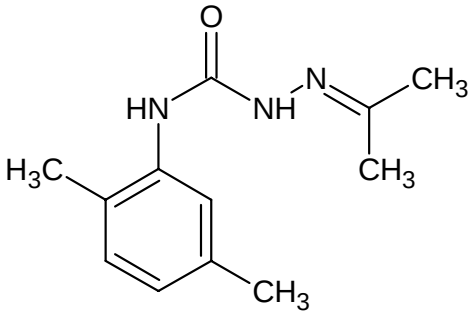
1. Unsubstituted benzylidene derivatives (**DM-1** and **FM-1**) were found to possess anticonvulsant activity in most of the screens and also with longer duration of action i.e. retention of activity up to 4h period.
2. Substitution at the 2^{nd} position of the phenyl ring with either electron donating or electron withdrawing groups was not found to be favorable for anticonvulsant activity.
3. Substitution at the 4^{th} position of the phenyl ring, with electron rich groups i.e. $-NH_2$, $-OCH_3$, -Br, led to increase in the activity for most of the animal models as compared to electron withdrawing groups.
4. Replacement of the carbimino hydrogen with methyl group i.e. **DM-(9-11)** and **FM-(14-18)**, led to the retention or increase in the anticonvulsant activity in all the animal models except scPTZ screen.
5. Replacement of the benzylidene proton with bulkier phenyl group (**DM-12**) led to a loss of anticonvulsant activity in the MES and scPTZ models with increase in neurotoxicity, but further substitution of the phenyl ring with *para*-bromo group (**DM-13** and **FM-19**) showed increased anticonvulsant activity in all the tested models, with subsequent lesser toxicity.
6. Replacement of the phenyl ring with alkyl groups i.e. CH_3, C_2H_5 showed activity in all the screens, but the order of activity was $\mathbf{CH_3 > C_2\ H_5 > C_5H_{11}}$ i.e. increase in carbon chain length resulted in decreased anticonvulsant activity, with increased neurotoxicity.

7. Replacement of phenyl ring with cycloalkyl groups like cyclopentyl (**DM-18**) and cyclohexyl (**DM-19**) showed retention of anticonvulsant activity in most of the seizure models, but cyclopentylene group was found to be more beneficial compared to cyclohexylene as it showed activity in all the models and also comparatively at lower doses.

From the above results, it could be concluded that the small lipophilic moiety like methyl was most beneficial for anticonvulsant activity that led to compounds with a broad spectrum of anticonvulsant activity and lesser neurotoxicity. The N^1-(2,5-dimethylphenyl)-N^4-(propan-2-one) semicarbazone (**DM-14**) and (2-fluoro-5-methylphenyl)-N^4-(propan-2-one) semicarbazone (**FM-20**)**,** emerged as the most active compounds**.** This was in confirmation with the earlier reports from this lab [80].



N^1-(2,5-Dimethylphenyl)-N^4-(propan-2-one) semicarbazone (DM-14)



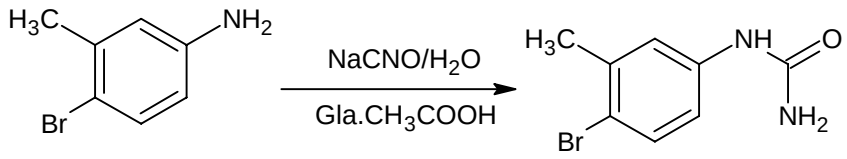
N^1-(2-Fluoro-5-methylphenyl)-N^4-(propan-2-one) semicarbazone (FM-20)

5.2 3,4-DISUBSTITUTED PHENYL SEMICARBAZONES

A series of 4-bromo-3-methylphenyl semicarbazones has been synthesized and evaluated for anticonvulsant activity.

5.2.1. Synthesis

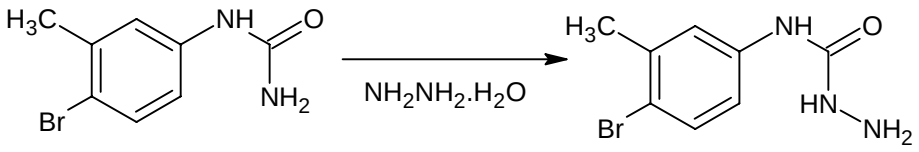
Step-1: Synthesis of 4-bromo-3-methylphenyl urea



4-Bromo-3-methyl aniline 4-Bromo-3-methylphenyl urea

4-Bromo-3-methyl aniline (0.1 M) was dissolved in 25 mL of glacial acetic acid and 12.5 mL of water. To this 0.1 M of sodium cyanate (6.5g) in 25 mL of warm water was added with stirring. This was allowed to stand for 30 minutes, then cooled in ice and filtered with suction and dried. Finally the product was recrystallized from boiling water to yield substituted phenyl urea. M.p. 172°C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3400, 1700, 780, 880, 750, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.32 (s, 3H, $ArCH_3$), 7.3-7.42 (m, 3H, ArH), 7.86 (s, 1H, ArNH, D_2O exchangeable), 9.67 (s, 2H, NH_2, D_2O exchangeable).

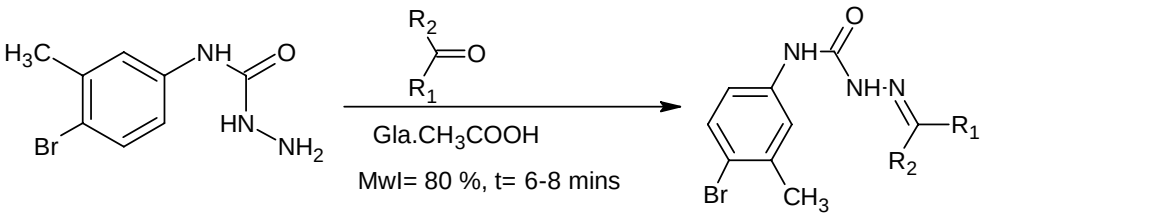
Step-2: Synthesis of 4-bromo-3-methylphenyl semicarbazide



4-Bromo-3-methylphenyl urea 4-Bromo-3-methyl phenyl semicarbazide

The 4-bromo-3-methylphenyl urea (0.05 M) and excess of hydrazine hydrate (0.1 M) in ethanol were refluxed for 24h. The two third volume of alcohol was distilled by vacuum distillation unit and poured into ice. The resultant precipitate was filtered, washed with water and dried. The solid was recrystallised with 90% v/v alcohol. M.p. 184°C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3400, 3280,1640,890,760, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.12 (s, 3H, $ArCH_3$), 7.14-7.30 (m, 3H, ArH), 5.78 (s, 2H, NH_2, D_2O exchangeable), 7.84 (s, 1H, ArNH, D_2O exchangeable), 9.96 (s, 1H, CONH, D_2O exchangeable).

Step-3: Synthesis of 4-bromo-3-methyl phenyl semicarbazones

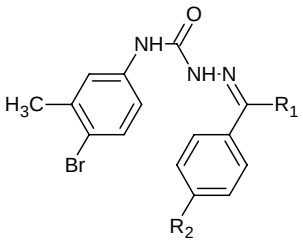


4-Bromo-3-methylphenyl semicarbazide

4-Bromo-3-methylphenyl semicarbazones

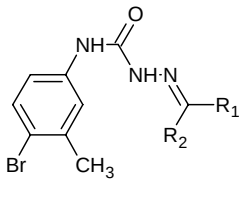
The conversion of semicarbazide to semicarbazones was carried out using microwave irradiation. To a solution of 4-bromo-3-methylphenyl semicarbazide (0.003 M), in ethanol was added an equimolar quantity of appropriate aldehyde or ketone. Glacial acetic acid was added to adjust the pH of the reaction mixture between 5-6, to facilitate the nucleophilic substitution. The reaction mixture was exposed to microwave irradiation for the time period of 6-8 minutes (Mw I = 80%). The product obtained after cooling was filtered and recrystallized from 95% v/v ethanol. The physical data for the synthesized compounds is presented in Table 5.12. The IR spectra of the semicarbazone derivatives were identical in the following aspects; IR (KBr) cm^{-1} 3450, 3300-3250, 1650, 1595, 840. ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) spectra of some representative compounds are presented in Table 5.12.

Table 5.11: Physical data for 4-bromo-3-methylphenyl semicarbazones



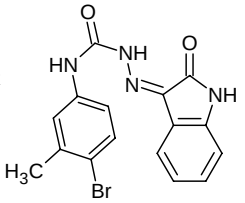
BM- (1-19)

OR



BM-(20-25)

OR



BM-26

Compound	R_1	R_2	Yield (%)	M.P. (°C)	Molecular formula	Mol. weight	R_f [a]	Log P[b]
BM-1	H	H	51	158	$C_{15}H_{14}N_3O.Br$	377	0.88	1.75
BM-2	H	2-NO_2	75	142	$C_{15}H_{13}N_4O_3.Br$	377	0.75	3.11
BM-3	H	2-OH	61	166	$C_{16}H_{19}N_5O.Br$	348	0.76	1.09
BM-4	H	2-OCH_3	58	138	$C_{16}H_{19}N_5O.Br$	348	0.76	1.09
BM-5	H	2-Cl	56	154	$C_{15}H_{13}N_3O.Cl.Br$	366	0.64	1.63
BM-6	H	2-CH_3	50	145	$C_{16}H_{16}N_3O.Br$	346	0.89	3.53
BM-7	H	3-NO_2	62	151	$C_{15}H_{13}N_4O_3.Br$	377	0.75	3.11
BM-8	H	4-NO_2	75	180	$C_{15}H_{13}N_4O_3.Br$	377	0.82	2.86
BM-9	H	4-OCH_3	67	155	$C_{16}H_{16}N_3O_2.Br$	362	0.84	3.69
BM-10	H	4-Br	62	172	$C_{15}H_{13}N_3O.Br$	411	0.73	4.05
BM-11	H	4-$N(CH_3)_2$	52	150	$C_{17}H_{19}N_4O.Br$	375	0.85	3.21
BM-12	H	3-OCH_3 4-OH	55	148	$C_{16}H_{16}N_3O_3.Br$	378	0.88	3.28
BM-13	CH_3	H	57	170	$C_{16}H_{16}N_3O.Br$	346	0.90	3.05
BM-14	CH_3	3-NH_2	62	185	$C_{16}H_{16}N_4O.Br$	361	0.82	2.69
BM-15	CH_3	4-NH_2	67	135	$C_{16}H_{17}N_4O.Br$	361	0.87	2.14
BM-16	CH_3	4-NO_2	82	189	$C_{16}H_{15}N_4O_3Br$	391	0.71	2.85
BM-17	CH_3	4-OH	57	145	$C_{16}H_{16}N_3O_2.Br$	362	0.80	2.86
BM-18	CH_3	4-CH_3	40	178	$C_{17}H_{18}N_3O.Br$	360	0.88	3.31
BM-19	C_6H_5	H	60	138	$C_{21}H_{18}N_3OBr$	408	0.74	3.91
BM-20	CH_3	CH_3	57	181	$C_{11}H_{14}N_3O.Br$	284	0.70	2.14
BM-21	CH_3	C_2H_5	53	161	$C_{12}H_{16}N_3OBr$	298	0.66	2.46
BM-22	CH_3	CH_2COCH_3	50	180	$C_{13}H_{16}N_3O_2Br$	326	0.71	-0.22
BM-23	CH_3	$CH_2CH(CH_3)_2$	56	160	$C_{14}H_{21}N_3OBr$	327	0.69	3.18
BM-24	CRR_1= Cylopentylene		54	138	$C_{13}H_{16}N_3OBr$	310	0.89	2.52
BM-25	CRR_1= Cylohexylene		58	185	$C_{14}H_{18}N_3OBr$	324	0.92	2.98
BM-26	-		68	142	$C_{13}H_{16}N_4O_2Br$	373	0.64	3.28

[a]Mobile phase $CHCl_3$: CH_3OH (9:1). [b]Log P was generated using Alchemy 2000 and SciLog P softwares.

Table 5.12: Spectral and elemental analyses data of the compounds

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
BM-3	3410, 3290, 3020, 2880, 1680, 1610, 1520, 1345	2.28 (s, 3H, $ArCH_3$), 6.81-7.80 (m, 7H, ArH), 8.25 (s, 1H, imine H), 8.67 (s, 1H, ArNH, D_2O exchangeable),10.06(s, 1H, OH, D_2O exchangeable), 10.71 (s, 1H, CONH, D_2O exchangeable)	59.31 59.33	4.65 4.63	13.83 13.78
BM-8	3400, 3320, 3040, 2860, 1665, 1630, 1500, 1355	2.26 (s, 3H, $ArCH_3$), 7.20-7.81 (m, 7H, ArH), 7.98 (s, 1H, imine H), 9.4 (s, 1H, ArNH, D_2O exchangeable), 10.6 (s, 1H, CONH, D_2O exchangeable).	54.14 53.93	3.94 3.93	16.84 16.78
BM-17	3390, 3320, 3020, 2890, 1714, 1628, 1520, 1340	2.24 (s, 3H, $ArCH_3$), 2.37 (s, 3H, $ArCH_3$), 7.24-8.41 (m, 7H, ArH), 8.98 (s, 1H, ArNH, D_2O exchangeable), 10.01(s, 1H, OH, D_2O exchangeable), 10.54 (s, 1H, CONH, D_2O exchangeable).	55.42 55.22	4.36 4.35	16.16 16.09
BM-18	3380, 3290, 3010, 2850, 1700, 1620, 1515, 1360	2.14 (s, 3H, $ArCH_3$), 2.30 (m, 6H, $ArCH_3$), 7.12-7.30 (m, 7H, ArH), 8.58 (s, 1H, ArNH, D_2O exchangea-ble), 10.01(s, 1H, CONH, D_2O exchangeable).	60.47 60.44	5.08 5.06	13.22 13.18
BM-20	3460, 3390, 3010, 1700, 1620, 1508, 1340	1.90 (s, 3H, CH_3), 1.95 (s, 3H, CH_3) 2.14 (s, 3H, $ArCH_3$, 6.80-7.13 (m, 3H, ArH), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.80 (s, 1H, CONH, D_2O exchangeable).	55.12 54.93	5.89 5.87	17.53 17.47

[a]Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

5.2.2. Pharmacology

Anticonvulsant activity

The anticonvulsant activity of the synthesized compounds was established in four seizure models after their *i.p.* administration to mice viz, MES test, scPTZ test, scSTY test and scPIC test. All the synthesized compounds were subjected to preliminary MES and scPTZ tests but only selected compounds were tested in scSTY and scPIC models. Anticonvulsant activity was examined at 0.5h and 4.0h after the injections. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table 5.13, along with the data for standard drugs.

Three compounds were evaluated in the rat oral MES screen (**BM-1**, **BM-4** and **BM-9**) and results are shown in Table 5.14.

Neurotoxicity of all the synthesized compounds was evaluated by rotarod test after 0.5h and 4h of drug administration by *i.p.* route (Table 5.13).

CNS depressant evaluation

All the active compounds were also studied for behavioral impairment and CNS depressant effect using actophotometer and porsolt's forced swim test. The compounds were administered at the dose level of 100 mg/kg by *i.p.* route to the animal. (Table 5.15)

Neurochemical study

GABA level study has been done for two most active compounds of the series i.e. **BM-20** and **BM-25**. Results are presented in Table 5.16.

Table 5.13: Anticonvulsant and minimal motor impairment of 4-bromo-3-methylphenyl semicarbazones

Compound	Intraperitoneal injection in mice[a]									
	MES screen		scPTZ Screen		scSty screen		scPIC screen		Neurotoxicity screen	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
BM-1	100	300	300[c]	300	300	300	300	300	300	-
BM-3	300	-	300	-	x	x	x	x	300	-
BM-5	300	-	300	-	300	-	300	300	300	-
BM-6	300[b]	300	300	-	x	x	x	x	300	-
BM-7	300	-	-	-	x	x	x	x	300	-
BM-8	300	-	-	-	-	-	x	x	-	-
BM-9	100[b]	-	300	-	300	300	300	300	300	-
BM-10	300	-	-	-	300	-	x	x	300	-
BM-11	300	-	300	-	-	-	x	x	300	-
BM-12	300[b]	-	300	-	300	300	300	-	300[b]	-
BM-13	300[b]	300	300	-	-	-	-	-	100	-
BM-14	300	-	300	-	x	x	x	x	300	-
BM-15	300	-	300[c]	-	100	100	100	100	300[b]	-
BM-16	100	-	300[c]	-	100	100	100	100	300	-
BM-17	100	-	300[c]	-	300	-	100	100	100	-
BM-18	100	-	300[c]	-	300	300	300	300	300	-
BM-19	300[b]	300	300[c]	-	100	100	300	300	300[b]	-
BM-20	100	300	300	300	100	100	300	300	300[b]	-
BM-21	300	-	300	-	-	-	300	300	100[b]	-
BM-22	300[b]	-	-	-	-	-	300	300	300	-
BM-23	300	-	300	-	100	100	300	-	100[b]	-
BM-24	300	-	300	-	x	x	x	x	300[b]	-
BM-25	100	300	300[c]	-	100	100	100	100	300	300
BM-26	100	300	300	-	100	100	300	300	100	-
Phenytoin	30	30	-	-	x	x	x	x	100	100
Ethsuximide	-	-	300	-	-	-	-	-	-	-
Sod. valproate	-	-	300	-	x	x	-	-	-	-

Table 5.14: Evaluation of selected compounds in the MES test after oral administration (30mg/kg) to rats

Compound	Oral administration to rats[a] (h)				
	0.25	0.5	1	2	4
BM-1	-	-	-	-	-
BM-4	1	-	-	-	-
BM-9	4	-	-	-	-
Phenytoin[b]	1	4	3	3	3

[a] The figures indicate the number of rats out of four which were protected. The dash (-) indicates an absence of activity at the administered dose

[b] Compound administered at 100mg/kg.

Table 5.15: CNS studies of 4-bromo-3-methylphenyl semicarbazones

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec) [c]	
	0.5h	1h	Control	Test (After 1 h)
Control	318.00±13.68	288.50±11.31		
BM-1	174.67±2.93	133.50±4.26	161.00±9.04	190.50±6.40*
BM-2	169.00±4.46	135.00±4.24	142.00±5.14	183.02±4.70
BM-3	181.50±3.82	150.00±4.94	103.00±16.68	174.50±4.71
BM-4	235.50±4.93	217.00±6.21	149.83±4.14	188.00±4.74
BM-5	209.50±7.34	193.33±3.91	142.50±17.55	143.00±18.93 NS
BM-6	181.00±4.72	161.00±4.46	151.00±17.58	144.00±11.95 NS
BM-7	170.01±4.08	130.00±5.03	206.00±4.32	217.83±1.17
BM-8	235.50±4.93	217.00±6.21	149.83±4.14	188.00±4.74
BM-9	200.00±11.00	170.50±5.06	186.00±10.60	206.50±5.60 NS
BM-10	194.50±5.59	140.57±3.94	174.50±5.59	204.17±3.94
BM-11	212.50±9.32	179.00±6.08	203.33±3.21	208.00±3.44 NS
BM-12	214.00±5.37	151.00±3.33	94.00±15.91	131.00±16.99 NS
BM-13	347.33±14.35 NS	280.50±17.49 NS	164.00±17.69	152.50±14.91 NS
BM-14	190.17±4.73	201.50±5.67	121.00±9.36	114.50±2.09 NS
BM-15	196.00±19.73	175.00±8.12	100.50±5.41	86.00±2.92*
BM-16	203.50±5.89	191.50±5.73	158.00±10.99	166.50±6.08 NS
BM-17	281.33±11.42NS	208.20±9.77	126.00±11.54	155.00±15.46 NS
BM-18	280.83±18.94 NS	274.50±11.77 NS	193.50±10.00	165.83±8.27 NS
BM-19	192.50±4.54	172.00±4.77	106.67±13.11	150.00±11.64*
BM-20	207.00±9.13	182.00±5.45	162.00±4.36	189.00±4.83
BM-21	228.50±16.57*	173.00±5.87	150.00±10.20	169.00±11.26 NS
BM-22	231.50±15.70**	263.00±15.70 NS	149.00±3.53	150.50±2.88 NS
BM-23	192.00±4.91	160.00±7.75	137.00±7.74	182.00±4.38
BM-24	178.00±6.65	158.00±9.27	94.00±11.32	121.50±17.35 NS
BM-25	255.67±16.68*	223.00±17.82**	187.00±3.12	183.00±5.34 NS
BM-26	206.00±4.47	175.50±4.88	139.00±14.36	186.83±5.16*
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	-	-	131.50±9.32	207.33±08.49

[a]The compounds were tested at the dose level of 100mg/kg . [b]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.0005$, * $p< 0.005$ and ** $p< 0.05$and NS denotes values which are not Significant (Student's t test). [c]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.005$ and *$p < 0.05$ and NS denotes values which are not significant (Student's t test). [d]The compounds were tested at the dose level of 30mg/kg.

Table 5.16: GABA concentration in µg/100mg weight of rat brain tissues

Compound[a]	GABA concentrations in different region of rat brain[b]			
	Olfactory lobe	Mid brain	Cerebellum	Medulla oblongata
Control	40.5 ± 0.28	101.6 ± 0.30	39.3 ± 0.05	93.4 ± 0.02
BM-20	112.4± 0.45	52.1 ± 0.40	108.3 ± 0.54	71.8± 0.35
BM-25	29.9 ± 0.09	74.0 ± 0.47	102.1 ± 0.24	72.1± 0.08
Clobazam	143.0 ± 0.15	713.0 ± 0.47	235.0 ± 0.25	188.0± 0.30

[a]The compounds were tested at the dose level of 100mg/kg .

[b]Each value represents the mean ± SEM of three rats significantly different from the control at $p< 0.0001$.

5.2.3. Results & Discussion

Chemistry

Twenty-six 4-bromo-3-methyl-substituted phenyl semicarbazones were synthesized starting from 4-bromo-3-methyl aniline, which was first treated with sodium cyanate in the presence of glacial acetic acid to yield 4-bromo-3-methyl-substituted phenyl urea. Substituted phenyl urea was converted to 4-bromo-3-methyl-substituted phenyl semicarbazide on treatment with hydrazine hydrate which was further treated with substituted aldehydes/ketones/isatins to yield 4-bromo-3-methyl-substituted phenyl semicarbazones via microwave irradiation method. The homogeneity of the compounds was monitored by TLC and R_f values were calculated. Eluant for all compounds was $CHCl_3$: CH_3OH (9:1) (Tables 5.11).

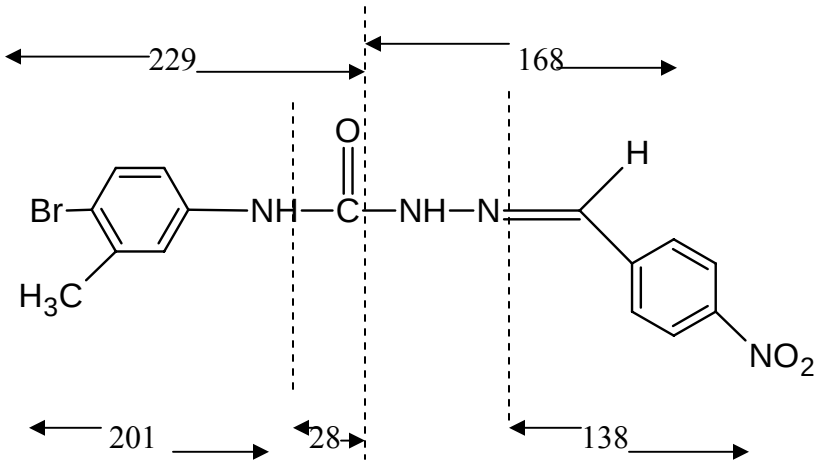
Most of the compounds were found to be more lipophilic indicated by their calculated log P values that were greater than 2 [log P > 2] except for compounds **BM-(3-5)**, and **BM-22.** No correlation has been established between the calculated log P and anticonvulsant profile of the compounds, as compounds with high log P i.e 3.11 (**BM-2**) was found to be bereft of anticonvulsant activity in all the animal models.

Compounds with substituted aryl ketones and isatins gave the yields in the range of 57-82 %, higher than substituted aryl aldehydes or alkyl ketones i.e. 50-75 %, which can be due to the higher molecular weight of the aryl ketone derivatives than the corresponding aryl aldehydes.

The IR spectrum for N^1-(2,5-dimethylphenyl)-N^4-(4-nitrobenzaldehyde) semicarbazone (**BM-8**) was recorded in KBr pellet and absorption bands (ν_{max} cm^{-1}) confirmed the presence of the following groups i.e. absorption band at 3400 cm^{-1} showed NH stretching, 3090 cm^{-1} showed aromatic CH stretching, 2860 cm^{-1} showed aliphatic CH stretching, 1665 cm^{-1} showed C=O stretching, , 1600-1525 cm^{-1} showed aromatic C=C stretching. The substituted semicarbazones also showed absorption bands other than the corresponding semicarbazides at 1500 cm^{-1}, 1350 cm^{-1} due to NO_2 stretching and 1630 cm^{-1} due to C=N stretching, which confirmed the synthesis of substituted semicarbazone derivatives.

^{1}H-NMR spectrum revealed a singlet at δ 2.26 for 3H of CH_3 protons, singlet at δ 7.98 for 1H of imine proton. Singlets at δ 9.4, (1H, ArNH) and δ 10.6 (1H, CONH) were D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristics peaks depicted in Table 5.12.

The mass spectrum of compound **BM-8** showed a molecular ion peak at m/z 368, base peak at m/z 201. The remaining major fragmentation peaks were at m/z 138,168 and 229.



Pharmacological activity

1. MES test

In the MES screen, all the compounds except **BM-2** and **BM-4**, all others (24 out of 26), exhibited protection. Compounds **BM-1, BM-9, BM-(16-18), BM-20, BM-26** and **BM-26** showed activity at 100 mg/kg of dose whereas other active compounds showed protection at 300 mg/kg. Compounds **BM-1**, **BM-6**, **BM-13**, **BM-19**, **BM-20**, **BM-25**, and **BM-26** were active at both 0.5h & 4h intervals; hence these compounds exhibited prolonged duration of action. With other active compounds the onset of action was rapid (0.5h), but the duration of action was short.

2. scPTZ test

In the scPTZ test, except compounds **BM-2**, **BM-4**, **BM-7**, **BM-8**, **BM-10**, and **BM-22**, all other compounds showed protection at 300mg/kg after 0.5h interval. Only compounds **BM-1** and **BM-20** showed protection in both the time periods (0.5h, 4h).

3. scSTY screen

Some selected compounds were screened in the scSTY model and out of these thirteen compounds showed protection. Compounds **BM-15, BM-16, BM-19, BM-2**, **BM-23** and **BM-26** showed protection at 100mg/kg whereas **BM-1, BM-5, BM-9, BM-10, BM-12, BM-17** and **BM-18** showed activity at 300mg/kg. Except compounds **BM-5, BM-10** and **BM-17,** other compounds showed activity in both 0.5h and 4h time periods.

4. scPIC screen

Some selected compounds were tested in scPIC screen and all of them except **BM-11**, **BM-13** exhibited protection against seizures. Compounds **BM-(15-17)** & **BM-25** showed protection at 100 mg/kg and other compounds exhibited activity at 300 mg/kg. Except compounds **BM-12** and **BM-23**, others showed protection in both 0.5h & 4h intervals.

Neurotoxicity screen (NT)

In the neurotoxicity evaluation by the rotarod method, compounds **BM-2, BM-4** and **BM-8** didn't exhibit any neurotoxity even at the maximum anticonvulsive dose (300mg/kg). Compounds **BM-13, BM-17, BM-21, BM-23** and **BM-26** were found to be more neurotoxic and others exhibited toxicity at the anticonvulsant dose.

The N^1-(4-bromo-3-methyl)-N^4-(propan-2-one) semicarbazone (**BM-20**) exhibited anticonvulsant activity in all the screens and lesser neurotoxicity and emerged as the most active compound in this series. Thirteen compounds (**BM-1**, **BM-5**, **BM-9**, **BM-12**, **BM-(15-20)**, **BM-23**, **BM-25** and **BM-26**) in this series have exhibited anticonvulsant activity against all the seizure models thereby indicating their potential as broad spectrum anticonvulsants covering epilepsies of the grand-mal and petit-mal type.

Rat *p.o.* identification

Compound **BM-4** showed 25% protection and compound **BM-9** exhibited 100% protection at 0.25h after the drug administration. Compound **BM-12** didn't exhibit any protection at the administered dose (30mg/kg).

CNS depressant evaluation

In the behavioral despair test, all compounds except compounds **BM-13**, **BM-17**, **BM-18**, and **BM-22**, showed a significant decrease in motor activity as indicated by the actophotometer scores, in which the standard drug Phenytoin also showed behavioral despair side effect. The compounds with substituted *para*-methyl acetophenone group (**BM-18)** showed the least motor impairment in their respective series, with the maximum actophotometer scores of 347.43±14.35 (0.5h) and 280.50±17.49 (1h). The semicarbazone derivatives were also studied for CNS depressant effect by porsolt's forced swim pool test and compared with Carbamazepine. In this study except compounds **BM-(1-4)**, **BM-7**, **BM-8**, **BM-10, BM-15**, **BM-20** and **BM-23**, all other compounds showed lesser or no CNS depressant effect, as compared to the conventional antiepileptic drugs (Table 5.15).

Effect on the levels of GABA in different regions of rat brain

In order to explore the mechanism of anticonvulsant activity of these derivatives, two compounds (**BM-20** and **BM-25**) were subjected to neurochemical investigation to determine GABA level, in different regions of the rat brain, as there is regional difference in GABA concentration within the CNS. All the compounds were found to show significant change in the GABA level in different regions of rat brain, with respect to the control. They were found to increase the GABA level significantly in the cerebellum region, while compound **BM-20** also showed significant increase in GABA level concentration for olfactory lobe region. (Table 5.16).

5.2.4. Structure-Activity Relationship

Twenty six variously substituted 4-bromo-3-methylphenyl semicarbazones were prepared. Among these the parent compound **BM-1,** [N^1-(4-bromo-3-methylphenyl)-N^4-(benzaldehyde) semicarbazone] showed activity against all the seizures models. The parent compound was substituted with different groups like electron donating or withdrawing groups in different position of the carbimino phenyl ring and the carbimino phenyl ring was replaced by alkyl / cycloalkyl groups and studied for their effect on anticonvulsant activity.

1. Substitution at the 2nd position of the phenyl ring with $–NO_2$, $-OCH_3$, showed no anticonvulsant activity and neurotoxicity, but –Cl substitution on phenyl ring led to activity in all the anticonvulsant screens, while $-CH_3$ substitution resulted in anti-MES and anti-scPIC activity. Lastly –OH substitution led to anti-MES and anti-scPTZ screen activity.
2. Substitution at the 3rd position of the phenyl ring, with electron donating groups i.e. $–NH_2$-, found to be beneficial in MES and scPTZ screens, but electron withdrawing substituents resulted in increased toxicity with loss of activity in the scPTZ screen.
3. Substitution at the 4th position of the phenyl ring, with electron rich groups i.e. $-OCH_3$, -Br, showed favorable anticonvulsant activity in most of the screens (**BM-9** and **BM-10**) than electron withdrawing groups (**BM-8**).
4. Replacement of the carbimino hydrogen, with bulky group i.e. phenyl group, showed increased anticonvulsant activity in all the models, with no subsequent increase in toxicity.
5. Replacement of the carbimino hydrogen, by methyl group showed increased activity in most of the screens but also resulted in increased toxicity.
6. Replacement of the phenyl ring with alkyl groups i.e. CH_3, C_2H_5 showed activity in all the screens, and the order of activity was,

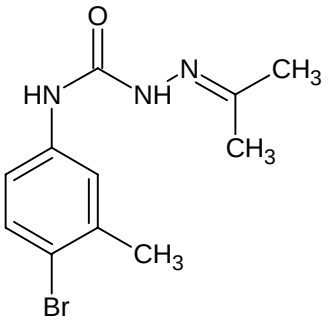
   $$\mathbf{CH_3 > C_2\ H_5 > C_5H_{11}}$$

   i.e. increase in carbon chain length resulted in decreased anticonvulsant activity,

and increased neurotoxicity.

7. Replacement of the phenyl ring with the cycloalkyl groups i.e. cyclopentylene, cyclohexylene resulted in anticonvulsant activity in all the screens but cyclohexylene substitution was found to be more beneficial than cyclopentylene substitution.
8. Replacements of the phenyl ring with the isatin group showed anticonvulsant activity in all the screens i.e. MES, scPTZ, scSTY, scPIC but also led to an increased neurotoxicity.

From the above results, it could be concluded that the small lipophilic moiety like methyl is most beneficial for anticonvulsant activity that led to compounds with broad spectrum of anticonvulsant activity and lesser neurotoxicity. The N^1-(4-bromo-3-methylphenyl)-N^4-(propan-2-one) semicarbazone (**BM-20**)**,** emerged as the most active compound.



N^1-(4-Bromo-3-methylphenyl)-N^4-(propan-2-one) semicarbazone (BM-20)

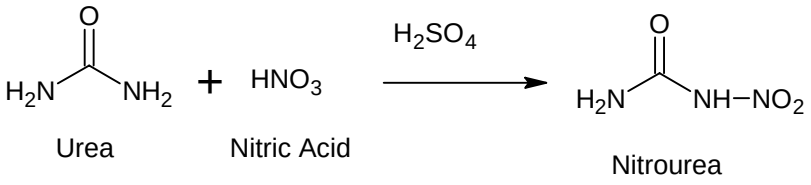
CHAPTER 6

HETEROARYL SUBSTITUED SEMICARBAZONES

6.1. 3-METHYLPYRIDIN-2-YL SEMICARBAZONES

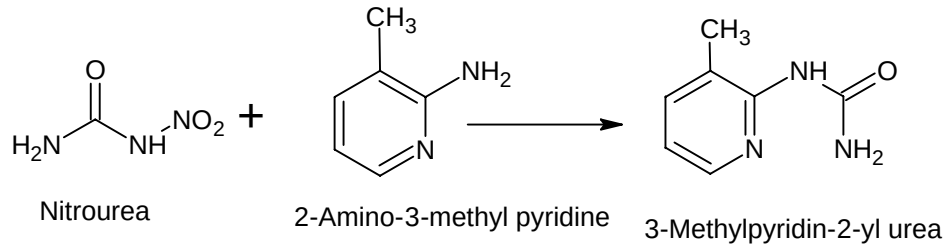
6.1.1. Synthesis

Step-1: Synthesis of N-nitrourea



Concentrated nitric acid (25 mL) was added dropwise to an ice-cold solution of 5 g urea in 25 mL water. The white precipitate of urea nitrate was collected and washed with ice-cold nitric acid. Then to 0.01 M of urea nitrate precipitate was added 32 mL of ice-cold concentrated sulphuric acid. A precipitate was obtained by pouring the reaction mixture into ice, washed with ice-cold water and air dried. M.p. 132°C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3330, 1690, 1640, 1560, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 7.98 (s, 1H, CONH, D_2O exchangeable), 9.88 (s, 2H, NH_2, D_2O exchangeable).

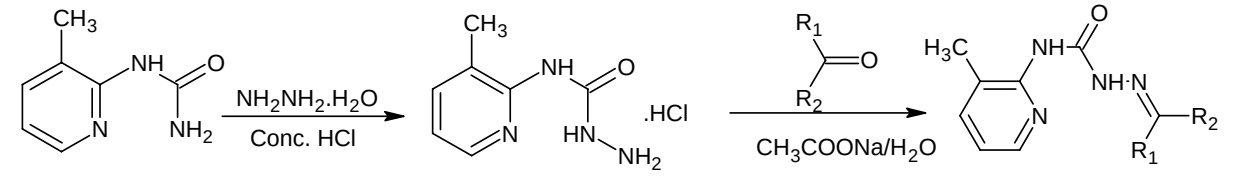
Step-2: Synthesis of 3-methylpyridin-2-yl urea



To a saturated solution of nitrourea, 2-amino-3-methyl pyridine was added. An immediate precipitate of 3-methylpyridin-2-yl urea was obtained that was collected and air dried. M.p. 154°C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3400, 3350, 3290, 3050, 1640,

750, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 2.28 (s, 3H, $ArCH_3$), 8.3-8.5 (m, 3H, ArH), 8.06 (s, 1H, ArNH, D_2O exchangeable), 9.78 (s, 2H, NH_2, D_2O exchangeable).

Step-3: Synthesis of 3-methylpyridin-2-yl semicarbazones



3-Methylpyridin-2-yl urea

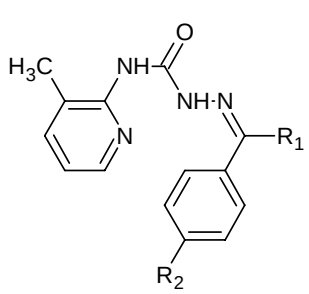
3-Methylpyridin-2-yl semicarbazide hydrochloride Salt

3-Methylpyridin-2-yl semicarbazone

The 3-methylpyridin-2-yl urea (0.05M) was refluxed in ethanol with double the quantity of hydrazine hydrate (0.1M) for 24h with stirring. The two-third volume of alcohol was distilled under vacuum and poured into ice. The resultant precipitate was filtered, washed with water and dried. The solid was recrystallised from ethanol (95% v/v) to which concentrated hydrochloric acid was added in the ratio of 1:10:5 (solid-ethanol-conc. HCl). The precipitate of 3-methylpyridin-2-yl semicarbazide hydrochloride was filtered under vacuum and dried. Then it was converted into free semicarbazide by alkaline hydrolysis in which the semicarbazide hydrochloride was dissolved in minimum quantity of alcohol and neutralized with 10% w/v sodium carbonate solution. At neutral pH, 3-methylpyridin-2-yl semicarbazide appeared as precipitate, which was separated by vacuum filtration and dried. M.p. 172 °C, IR-KBr pellet (cm^{-1}) 3450, 3340, 3320, 3100, 1650, 1240, 780, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) δ 2.23 (s, 3H, CH_3), 5.50 (s, 2H, NH_2, D_2O exchangeable), 8.30-8.45 (m, 3H, ArH), 8.54 (s, 1H, ArNH, D_2O exchangeable), 9.68 (s, 1H, $NHNH_2$, D_2O exchangeable). To a solution 3-methylpyridin-2-yl semicarbazide hydrochloride (0.005M) in 25mL of water, was added sodium acetate (0.005 M) in 2 mL of water. About 25 mL of ethanol was added to clear turbidity. This solution mixture was added to an equimolar quantity of the appropriate aldehyde or ketone in alcohol with stirring on a magnetic stirrer for 30 minutes to 2h until the completion of reaction. The resultant precipitate was filtered,

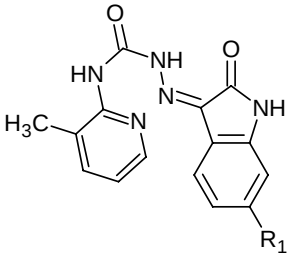
dried and recrystallised from hot ethanol. The physical data of the semicarbazones are given in Table 6.1. The IR spectra of the semicarbazone derivatives were identical in the following aspects: 3450, 3300-3250, 3120, 1650, 1240, 770. ^{1}H-NMR (DMSO-$d_{6,}$ ppm, 300 MHz) spectra of some representative compounds are given in Table 6.2.

Table 6.1: Physical data for 3-methylpyridin-2-yl semicarbazones



PY- (1-18)

OR



PY-(19-20)

Comp	R_1	R_2	Yield (%)	M.P. (°C)	Molecular Formula	Mol. weight	R_f[a]	Log P[b]
PY-1	H	H	57	150	$C_{14}H_{14}N_4O$	254	0.75	2.39
PY-2	H	2-NO_2	38	60	$C_{14}H_{13}N_5O_3$	299	0.64	1.60
PY-3	H	2-OH	62	185	$C_{14}H_{14}N_4O_2$	270	0.54	0.78
PY-4	H	2-CH_3	67	90	$C_{15}H_{16}N_4O$	268	0.66	2.36
PY-5	H	2-Cl	56	210	$C_{14}H_{13}N_4OCl$	288	0.66	2.60
PY-6	H	3-NO_2	49	145	$C_{14}H_{13}N_5O_3$	299	0.76	1.29
PY-7	H	4-NO_2	58	95	$C_{14}H_{13}N_5O_3$	299	0.62	1.55
PY-8	H	4-CH_3	48	220	$C_{15}H_{16}N_4O$	268	0.63	2.58
PY-9	H	4-OCH_3	64	185	$C_{15}H_{16}N_4O_2$	284	0.64	2.45
PY-10	H	4-$N(CH_3)_2$	58	125	$C_{16}H_{19}N_5O$	297	0.52	1.96
PY-11	H	4-Cl	65	135	$C_{14}H_{13}N_4OCl$	288	0.78	3.08
PY-12	H	4-OH 3-OCH_3	44	250	$C_{15}H_{16}N_4O_3$	300	0.70	1.71
PY-13	CH_3	H	72	115	$C_{15}H_{16}N_4O$	268	0.50	2.08
PY-14	CH_3	4-OH	40	130	$C_{15}H_{16}N_4O_2$	284	0.67	1.51
PY-15	CH_3	4-NH_2	54	72	$C_{15}H_{17}N_5O$	283	0.70	0.91
PY-16	CH_3	4-CH_3	73	184	$C_{16}H_{18}N_4O$	282	0.84	2.34
PY-17	C_6H_5	H	72	85	$C_{20}H_{18}N_4O$	330	0.59	3.19
PY-18	C_6H_5	4-Br	61	65	$C_{20}H_{17}N_4OBr$	409	0.73	3.69
PY-19	H	-	48	180	$C_{15}H_{13}N_5O_2$	295	0.62	1.04
PY-20	F	-	78	135	$C_{15}H_{12}N_5O_2.F$	313	0.66	2.97

[a] Mobile phase $CHCl_3$: CH_3OH (9:1)

[b] Log P was generated using Alchemy 2000 and SciLog P softwares

Table 6.2: Spectral and elemental analyses data of the compounds

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
PY-1	3400, 3290, 3080, 3050, 2880, 1690, 1610, 1230	2.28 (s, 3H, $ArCH_3$), 7.20-7.81 (m, 8H , ArH), 8.60 (s, 1H, imine H), 9.24 (s, 1H, ArNH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable)	66.13 65.93	5.55 5.33	22.03 21.78
PY-8	3420, 3290, 3100, 3030, 2880, 1660, 1600, 1220	2.14 (s, 3H, $ArCH_3$), 2.24 (s, 3H, $ArCH_3$), 6.74-7.96 (m, 7H, ArH), 8.52 (s, 1H, imine H), 8.75 (s, 1H, ArNH, D_2O exchangeable), 9.56 (s, 1H, CONH, D_2O exchangeable)	67.15 66.13	6.01 5.83	20.88 19.78
PY-12	3400, 3280, 3090, 3050, 2860, 1690, 1620, 1270, 1060	2.12 (s, 3H, $ArCH_3$), 3.64 (s, 3H, OCH_3), 7.14-7.78 (m, 6H, ArH), 8.24 (s, 1H, imine H), 8.42(s, 1H, ArNH, D_2O exchangeable), 8.64 (s, 1H, CONH, D_2O exchangeable) 10.28 (s, 1H, ArOH, D_2O exchangeable).	59.99 60.19	5.37 5.87	18.66 19.08
PY-14	3410, , 3220, 3080, 3045, 2880, 1680, 1620, 1220, 1040	2.02 (s, 3H, CH_3), 2.14 (s, 3H, $ArCH_3$), 7.04-7.86 (m, 7H, ArH), 8.60 (s, 1H, ArOH, D_2O exchangeable) 9.26(s, 1H, ArNH, D_2O exchangeable),9.84 (s, 1H, CONH, D_2O exchangeable)	63.37 64.22	5.67 5.35	19.71 19.09
PY-15	3380, 3340, 3030, 3010, 2885, 1705, 1610, 1245	1.98 (s, 3H, CH_3), 2.27 (s, 3H, $ArCH_3$), 5.34 (s, 2H, $ArNH_2$, D_2O exchangeable), 7.18-7.56 (m, 7H, ArH), 8.68 (s, 1H, ArNH, D_2O exchangeable), 10.04 (s, 1H, CONH,D_2O exchangeable)	63.59 63.24	6.05 6.06	24.72 23.18
PY-17	3350, 3200, 3160, 3040, 2860, 1680, 1610, 1220, 820	2.22 (s, 3H, $ArCH_3$), 7.18-8.16 (m, 13H, ArH), 8.54 (s, 1H, ArNH, D_2O exchangeable), 10.20 (s, 1H, CONH,D_2O exchangeable)	72.71 73.93	5.49 5.87	16.96 16.47

[a]Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

6.1.2. Pharmacology

Anticonvulsant activity

All the synthesized compounds were subjected to preliminary MES and scPTZ tests and from those which showed promising activity were selected for scSTY and scPIC models. The compounds were administered at the dose level of 30, 100 and 300mg/kg dose level by *i.p.* route and anticonvulsant activity was examined at 0.5h and 4.0h after the injections. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table 6.3, along with the data for standard drugs.

Some selected compounds, showing good activity in the preliminary seizure models were also evaluated in the rat oral MES screen (**PY-5, PY-15, PY-16**) and results are shown in Table 6.4.

Neurotoxicity of all the synthesized compounds was evaluated by rotarod test after 0.5h and 4h of drug administration (Table 6.3).

CNS depressant evaluation

All the compounds were examined for CNS depressant activity which included locomotor activity using actophotometer and porsolt's swim test. (Table 6.5)

Neurochemical study

GABA level study has been done for two most active compounds of the series i.e. **PY-4** and **PY-16** in rat whole brain. Results are presented in Table 6.6.

Table 6.3: Anticonvulsant and minimal motor impairment of 3-methylpyridin-2-yl semicarbazones

Compound	Intraperitoneal injection in mice[a]									
	MES screen		scPTZ screen		scSTY screen		scPIC screen		Neurotoxicity screen	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
PY-1	-	300	100	-	-	-	300	300	-	-
PY-2	300	-	300	-	300	-	300	-	300	-
PY-5	300	300	100	-	300	300	300	300	-	-
PY-7	-	-	300	-	x	x	x	x	100	-
PY-8	100	300	-	300	x	x	x	x	100	300
PY-11	-	-	300	-	-	-	300	-	-	-
PY-12	300	-	300	-	300	-	300	-	-	300
PY-15	300	-	300	-	300	-	100	100	300	-
PY-16	100	-	100	-	300	300	300	300	100	-
PY-17	300	300	-	-	300	-	300	-	300	-
PY-19	100	300	-	-	100	100	300	300	300	-
Phenytoin	30	30	-	-	-	-	x	x	100	100
Ethsuximide	-	-	300	-	300	-	-	-	-	-
Sodium valproate	-	300	100	-	100	-	-	-	-	-

[a]Doses of 30, 100 and 300mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (-) indicates an absence of activity at the maximum dose administered (300mg/kg).The cross (x) indicates that the compound were not tested.

Table 6.4: Evaluation of selected compound in the MES test after oral administration (30mg/kg) to rats.

Compound	Oral administration to rats[a]				
	0.25 (h)	0.5 (h)	1 (h)	2 (h)	4 (h)
PY-5	1	-	-	-	-
Phenytoin[b]	1	4	3	3	3

[a]The figures indicate the number of rats out of four which were protected. The dash (-) indicates an absence of activity at the administered dose
[b]Administered at 100mg/kg.

Table 6.5: CNS studies of 3-methylpyridin-2-yl semicarbazones

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec) [c]	
	0.5h	1h	Control	Test (After 1 h)
Control	318.00±13.68	288.50±11.31		
PY-1	184.67±3.13	142.50±5.18	102.00±4.13	144.50±5.65
PY-2	182.00±4.16	145.00±4.14	158.00±4.04	196.00±7.14
PY-3	201.56±4.02	150.00±6.18	148.00±.4.49	168.50±2.78 *
PY-4	231.50±5.98	192.00±4.72	154.00±5.03	166.00±2.73 **
PY-5	272.00±5.06 *	170.50±4.86	184.00±6.47	206.00±6.42 **
PY-6	159.01±4.88	160.50±5.11	158.00±5.56	182.00±8.12 **
PY-7	198.50±3.35	196.00±7.04	162.00±5.26	195.00±5.16 *
PY-8	308.00±8.00 NS	272.50±5.06 NS	192.00±8.58	206.50±7.64 NS
PY-9	209.50±6.34	188.33±4.42	120.00±7.93	165.00±9.34 *
PY-10	188.50±5.54	156.00±3.84	160.00±3.48	193.00±7.69 *
PY-11	200.00±7.42	179.00±6.18	161.50±8.03	171.00±10.15NS
PY-12	216.00±5.34	150.00±3.92	194.00±15.98	232.00±6.94 NS
PY-13	286.53±12.64 NS	271.00±12.00 NS	198.00±11.73	207.50±6.58 NS
PY-14	188.17±4.93	205.50±6.90	179.00±4.12	179.50±3.94 NS
PY-15	184.00±12.43	167.00±8.64	100.50±18.12	141.50±9.49 NS
PY-16	295.00±6.78 NS	268.00±8.48 NS	179.00±4.12	179.50±3.94 NS
PY-17	281.33±11.42NS	194.20±8.55	205.00±5.13	212.50±4.16 NS
PY-18	207.33±4.35	200.50±7.49	128.50±16.21	131.00±15.29 NS
PY-19	272.50±4.84 *	212.00±4.59	100.50±18.12	111.50±9.58 NS
PY-20	217.00±8.16	187.00±5.11	139.00±14.36	186.83±5.16 *
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	-	-	131.50±9.32	207.33±08.49

[a]The compounds were tested at the dose level of 100mg/kg . [b]Each value represents mean ± SEM of six mice significantly different from the control at $p < 0.0005$ and * $p < 0.05$ and NS denotes values which were not significant (Student's t test). [c]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.0005$, *$p < 0.005$ and **$p < 0.05$ and NS denotes values which were not significant (Student's t test) [d]The compounds were tested at the dose level of 30mg/kg.

Table 6.6: Effect on GABA level in rat whole brain

Group	GABA concentration in μg/100mg[b]
Control	22.24 ± 1.91
PY-4[a]	28.01±1.31 NS
PY-16[a]	24.07±5.65 NS

[a]The compounds were tested at a dose of 100 mg/kg (oral).

[b]Each value represents mean ± SEM of six rats and NS denotes values which are not significant (Student's t test)

6.1.3. Results & Discussion

Chemistry

The required 3-methylpyridin-2-yl semicarbazones were synthesized by the nitro urea method starting from 2-amino-3-methyl-pyridine, which was converted to corresponding urea and semicarbazide as reported earlier. The 3-methylpyridin-2-yl semicarbazones were synthesized from the semicarbazide hydrochloride salt due to the high solubility of 3-methylpyridin-2-yl semicarbazide in ethanol. The structures were identified on the basis of elemental analyses, IR and ^{1}H-NMR spectra. All the compounds were obtained in moderate yields ranging from 54-78 %. The homogeneity and R_f of the compounds were monitored by TLC using $CHCl_3$: CH_3OH (9:1) as eluant. (Tables 6.1).

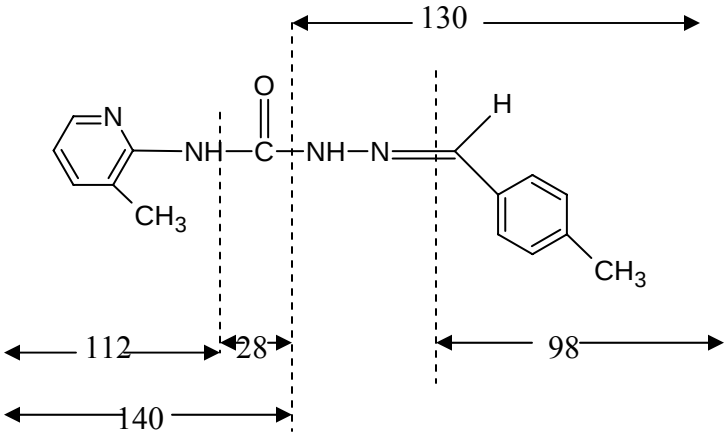
Most of the compounds were found to be more lipophilic indicated by their calculated log of partition coefficient value greater than 2 [log P > 2] except for compounds **PY-2**, **PY-3**, **PY-6**, **PY-7**, **PY-10**, **PY-12**, **PY-14**, **PY-15**, and **PY-19.** These compounds possessed lesser log P values, because of the substitution with polar groups like OH, NO_2 and NH_2 etc. No correlation has been established between the calculated log P and anticonvulsant profile of the compounds, as compounds with log P less than 2 were also found to possess good anticonvulsant activities

The IR spectrum for N^1-(3-methyl-pyridyl)-N^4-(4-methylbenzaldehyde) semicarbazone (**PY-8**) showed the presence of NH group at 3420 cm^{-1}, the band in the aromatic region at 3080 cm^{-1} and 3040 cm^{-1} were due to aromatic C-H stretch, 2890 cm^{-1} showed aliphatic CH, 1680 cm^{-1} showed C=O and 1230 cm^{-1} showed pyridine C=C stretching. The substituted semicarbazones showed in addition an absorption band other than the corresponding semicarbazides at 1610 cm^{-1} due to C=N stretching, which confirmed the synthesis of substituted semicarbazone derivatives.

^{1}H-NMR spectrum in dimethyl sulfoxide (DMSO) revealed a singlet at δ 2.14 and δ 2.24 for 3H of CH_3 protons and also the singlets at δ 8.52 for 1H of imine proton and at δ 8.75 for aromatic NH group which was D_2O exchangeable. Proton of –CONH group at δ 9.56 was also D_2O exchangeable. Presence of these characteristics peaks confirmed the

structure of compound **PY-8**. Similarly, the structures of other compounds were confirmed according to their characteristics peaks depicted in Table 6.2.

The mass spectrum of compound **PY-8** showed a molecular ion peak at m/z 299, base peak at m/z 140. The remaining major fragmentation peaks were at m/z 98, 112, and 130.



Pharmacological activity

2. MES test

Nine compounds showed activity in the preliminary MES screen. Compounds **PY-8, PY-16,** and **PY-19** showed protection at 100 mg/kg (0.5h) and other active compounds showed protection at 300 mg/kg dose. Compounds **PY-2, PY-12, PY-15,** and **PY-16** exhibited shorter duration of action (i.e. activity at 0.5h interval only) whereas compound **PY-1** showed late onset of action. Other compounds showed activity at both 0.5h & 4h intervals.

2. scPTZ test

Nine compounds (**PY-1**, **PY-2, PY-7**, **PY-8**, **PY-(10-12)**, **PY-15,** and **PY-16**) showed protection in scPTZ screen. The compounds **PY-1, PY-5,** and **PY-16** showed protection at 100mg/kg up to 0.5h time period.

3. scSTY screen

Some selected compounds (**PY-1**, **PY-2**, **PY-(10-12),** **PY-(15-17),** and **PY-19**) were examined in the scSTY animal model. All the tested compounds except **PY-1** and **PY-11** showed protection in the scSTY model. Compound **PY-19** showed activity at 100 mg/kg and other compounds showed activity at 300mg/kg. Only compounds **PY-5**, **PY-16**, and **PY-19** exhibited a longer duration (0.5h and 4h) of action.

4. scPIC screen

In the scPIC screen, only compound **PY-15** showed protection at 100 mg/kg, all others showed at 300mg/kg. Except compounds **PY-2**, **PY-11**, **PY-12**, and **PY-17**, others exhibited protection for a longer duration of time i.e. up to 4h time interval.

Neurotoxicity screen (NT)

In the acute neurological toxicity screen, the compounds **PY-1, PY-5** and **PY-11**, showed no neurotoxicity at the maximum dose administered (300 mg/kg), and compounds **PY-3**, **PY-4**, **PY-6**, **PY-9**, **PY-10**, **PY-13**, **PY-14**, **PY-18**, and **PY-20** did not exhibit anticonvulsant activity as well as neurotoxicity. Compound **PY-19** showed no neurotoxicity at the anticonvulsive dose and compounds **PY-2**, **PY-8**, and **PY-(15-17)** exhibited neurotoxicity at the anticonvulsive dose. Compound **PY-7** was found to be more neurotoxic at the anticonvulsive dose.

The MES and scPTZ have become the most widely employed seizures models for the early identification and high-throughput screening of investigational antiepileptic drugs and only a very few standard anticonvulsants exhibit a broad spectrum of activity in all the threshold models. In this respect, it is clear that 3-methylpyridin-2-yl semicarbazones have the potential to treat a wide range of seizure types by their multiple mechanisms of action as indicated by their activity in four animal models of seizures, viz. MES, scPTZ, scSTY, and scPIC tests. Five compounds (**PY-2**, **PY-5**, **PY-12**, **PY-15** and **PY-16**) had shown activity in all the four screens, exhibiting a broad spectrum of anticonvulsant activity. The compounds were found to be equipotent or more potent as compared to some of the conventional AED i.e. Phenytoin, Ethosuximde, Sodium valproate, in one or other anticonvulsant screens.

Rat *p.o.* identification

Out of all the compounds that were active in the *i.p.* anticonvulsant screen some were selected for activity in the oral screen in the MES and neurotoxicity tests in rats. The result is presented in Table 6.4. Only compound **PY-5** showed 25 % protection at 0.25h.

CNS depressant evaluation

In the behavioral despair test, compounds except **PY-8**, **PY-13, PY-16**, and **PY-17** showed significant decrease in motor activity as indicated by the actophotometer scores. The standard drug Phenytoin also showed significant decrease in the locomotor activity i.e. possessed behavioral despair side effect. The compound with 4-chloro benzylidene substituents (**PY-11**) showed no motor impairment with the maximum actophotometer scores of 308.00±8.00 (0.5h) and 272.50±5.06 (1h). The semicarbazone derivatives were also studied for CNS depressant effect by porsolt's forced swim pool test and compared with Carbamazepine. Compounds except **PY-(1-7), PY-(9-10)** and **PY-20,** showed a significant increase in immobility time as compared to the control, indicating their significant CNS depressant effect than the conventional drugs.

Effect on the levels of GABA in different regions of rat brain

In order to explore the mechanism of anticonvulsant activity of these derivatives, two compounds (**PY-4** and **PY-11**) were subjected to neurochemical investigation to determine GABA level in the whole rat brain, but they showed no significant increase in the GABA level concentration in rat brain as compared to the control, suggesting a different mechanism for their anticonvulsant activity (Table 6.6).

6.1.4. Structure-Activity Relationship

Table 6.1 lists twenty variously substituted 3-methylpyridin-2-yl semicarbazones. The unsubstituted derivative (**PY-1**) showed potent anticonvulsant activity in three of the seizures models and exhibited no neurotoxicity. A series of various substituents was prepared to study the effects of electron donating and withdrawing groups and effect of

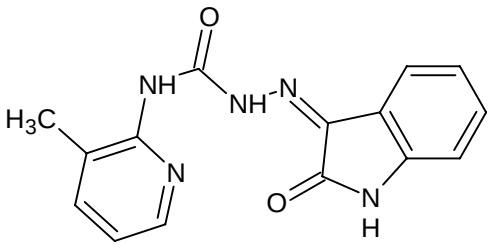
bulky and lipophilic groups on the anticonvulsant activity profile of the parent compound.

The common features which emerged for potential activity, were:

1. Substitution at the 2nd position of the phenyl ring with electron withdrawing groups i.e. -NO_2, leads to potent anticonvulsant activity in all the four animal seizure models (**PY-2**), with lesser neurotoxicity, while the substitution with electron-rich groups other than -Cl group (**PY-5**) resulted in complete loss of activity as well as neurotoxicity.
2. Substitution at the 3rd position of the phenyl ring, with electron withdrawing group i.e. -NO_2 (**PY6**) also led to totally inactive compounds with no neurotoxicity. This might be due to their low log P values that restrained the compound to cross blood brain barrier (BBB).
3. Substitution at the 4th position of the phenyl ring with electron withdrawing groups i.e. -NO_2 (**PY-7**), resulted in potent anticonvulsant activity only for scPTZ models, with high neurotoxicity whereas substitution with -CH_3 group, showed activity in most of the animal models but also resulted in high neurotoxicity (**PY-8**). Substitution with other electron rich groups i.e. -Cl, -OCH_3, led to the loss of anticonvulsant activity (**PY-9**, and **PY-10**).
4. Replacement of the carbimino hydrogen, by methyl group i.e. **PY-(13-16)** led to the retention or increase in the anticonvulsant activity in all the animal models.
5. Replacement of the benzylidene proton with bulkier phenyl group **(PY-17)** led to loss of anticonvulsant activity in the scPTZ model and further substitution of the phenyl ring with *para*-bromo group (**PY-18**) showed complete loss of anticonvulsant activity with no subsequent toxicity also.
6. Replacement of phenyl ring with isatinimino groups led to potent anticonvulsant activity against most of the seizures models with lesser neurotoxicity but further substitution of isatinimino group with –F group was not found to be beneficial for anticonvulsant activity.

In conclusion, the present work indicated that the aryl ring of the semicarbazones can be replaced by other lipophilic heteroaryl ring i.e. pyridine ring, which led to a series of

compounds with broad spectrum of activity in the anticonvulsant screens with lesser neurotoxicity. The compound with isatinimino substitution (**PY-19**) emerged as the most effective compound with broad spectrum of activity and lesser neurotoxicity.

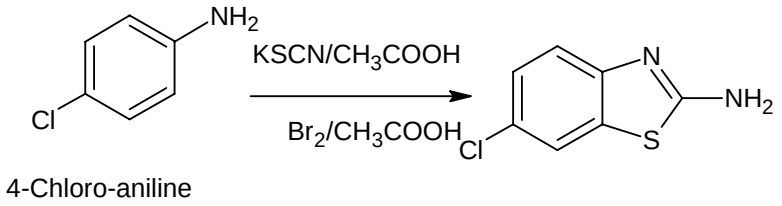


N^1-(3-Methyl-pyridin-2-yl)-N^4-(isatinimino) semicarbazone (PY-19)

6.2. 6-CHLORO SUBSTITUTED BENZTHIAZOLYL SEMICARBAZONES

6.2.1. Synthesis

Step-1: Synthesis of 6-chloro benzthiazol-2-amine

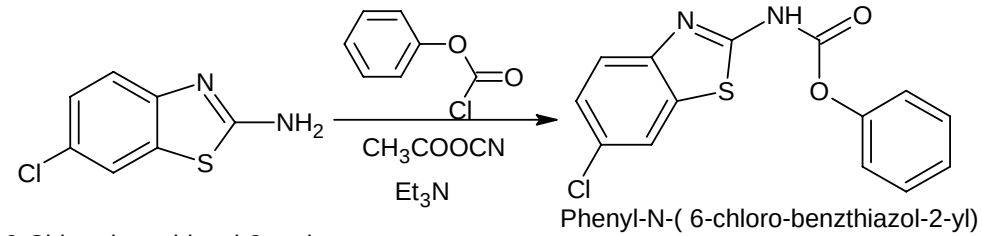


4-Chloro-aniline

6-Chloro-benzthiazol-2-amine

To a solution of 4-chloro aniline (0.078 M) in 150 mL of glacial acetic acid, 0.156 M of potassium thiocyanate was added. The reaction mixture was stirred vigorously and a solution of bromine (0.078 M) in glacial acetic acid was added slowly over a period of 30 minutes. The reaction mixture was stirred continuously for 24h and poured into water. The aqueous mixture was neutralized over a period of 3h with concentrated ammonium hydroxide to pH 10. The reaction was stirred for 18h and the precipitated product was collected by filtration. The product was recrystallized from toluene. M.p. 190 °C; Yield: 60%; IR-KBr pellet (cm^{-1}) 3300, 3050, 2150, 1080, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 7.45-7.67 (m, 3H, ArH), 9.76 (s, 2H, NH_2, D_2O exchangeable).

Step-2: Synthesis of N-(6-chloro-benzthiazolyl) phenyl carbamate



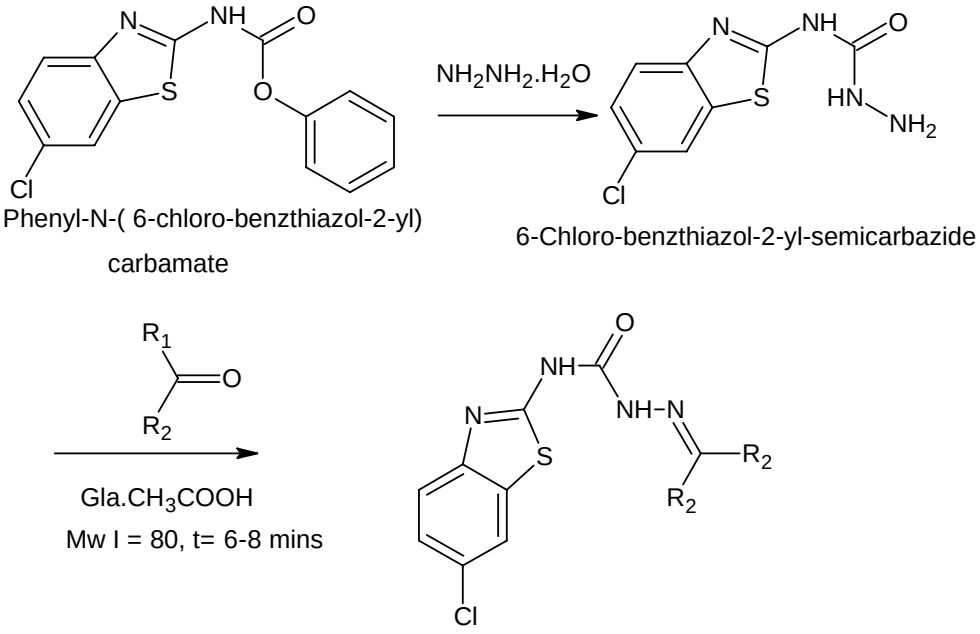
6-Chloro-benzthiazol-2-amine

Phenyl-N-(6-chloro-benzthiazol-2-yl) carbamate

Phenylchloroformate (0.1 M) was dissolved in 40mL of acetonitrile and taken in a 3-necked round-bottomed flask. To this solution, 6-chloro-benzthiazolamine (0.1 M) and triethylamine (0.1 M, 13.9 mL) were added slowly and stirred at room temperature for 5h. Then the reaction mixture was concentrated to one-third volume and 100 mL of

petroleum ether was added to the above solution. The precipitate appeared immediately which was filtered, washed with large quantity of water and again filtered and dried, M.p. 106°C; IR-KBr pellet (cm^{-1}) 3290, 3040, 2150, 1760, 1140 cm^{-1}; ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 7.3- 7.4 (m, 3H, ArH), 7.72-7.87 (m, 5H, ArH), 8.44 (s, 1H, ArNH, D_2O exchangeable).

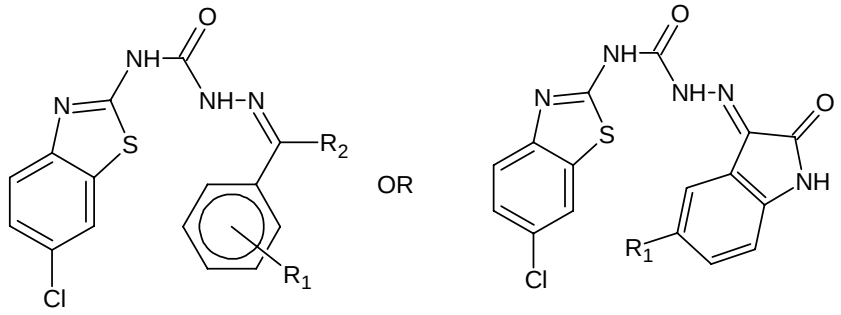
Step-3: Synthesis of 6-chloro benzthiazol-2-yl semicarbazones



6-Chloro benzthiazol-2-yl phenyl carbamate (0.05 M) was dissolved in 100 mL of dichloromethane. To this solution, 4.85 mL of hydrazine hydrate (0.1 M) was added and stirred at room temperature for 24h. The precipitate of 6-chloro benzthiazol-2-yl semicarbazide was separated out, filtered, washed with dichloromethane and dried. M.p. 220 °C; IR-KBr pellet (cm^{-1}) 3450, 3300, 3050, 2980, 2150, 1660, 1620, 1590-1540, 1360, 1110 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) 7.38-7.76 (m, 3H, ArH), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.46 (s, 1H, $NHNH_2$, D_2O exchangeable). Then to a solution of 6-chloro benzthiazol-2-yl semicarbazide (0.003 M, 0.54g) in ethanol, an equimolar quantity of appropriate alkyl/aryl aldehydes or ketones was added and stirred for 1h to 3h until the completion of the reaction. The resultant precipitate was

filtered, dried and recrystallized from 95% v/v ethanol. The physical data of the compounds are presented Table 6.7. The IR spectra of the compounds were identical in the following aspects 3420, 3300, 3050, 2150, 1680-1660, 1620, 1350, 1110, 840 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) spectra of the some of the representative compounds are given in the Table 6.8.

Table 6.7: Physical data for 6-chloro benzthiazol-2-yl semicarbazones



BZ-(1-17)

BZ-(18-20)

Compound	R_1	R_2	Yield (%)	M.P. (°C)	Molecular Formula	Mol. weight	R_f[a]	Log P[b]
BZ-1	H	2-NO_2	58	>250	$C_{15}H_{10}N_5O_3S.Cl$	376	0.72	2.40
BZ-2	H	3-NO_2	56	>250	$C_{15}H_{10}N_5O_3S.Cl$	376	0.61	3.32
BZ-3	H	3-Cl	55	>250	$C_{15}H_{10}NOS.Cl_2$	366	0.54	4.30
BZ-4	H	4-NO_2	67	>250	$C_{15}H_{10}N_5O_3S.Cl$	376	0.51	3.75
BZ-5	H	4-CH_3	56	>250	$C_{16}H_{13}N_4OS.Cl$	345	0.62	4.29
BZ-6	H	4-OH	55	>250	$C_{15}H_{11}N_4O_2S.Cl$	346	0.76	4.24
BZ-7	H	4-OCH_3	54	>250	$C_{16}H_{13}N_4O_2S.Cl$	360	0.52	4.00
BZ-8	H	4-Cl	53	>250	$C_{15}H_{10}N_4OS.Cl_2$	365	0.58	2.37
BZ-9	CH_3	3-NH_2	56	>250	$C_{16}H_{14}N_5OS.Cl$	358	0.70	1.43
BZ-10	CH_3	4-Cl	58	>250	$C_{16}H_{12}N_4OS.Cl_2$	379	0.62	2.72
BZ-11	CH_3	4-NO_2	54	>250	$C_{16}H_{12}N_5O_3S.Cl$	390	0.58	2.30
BZ-12	CH_3	4-CH_3	54	>250	$C_{17}H_{15}N_4OS.Cl$	358	0.61	1.85
BZ-13	CH_3	4-OH	52	>250	$C_{16}H_{13}N_4O_2S.Cl$	360	0.50	2.86
BZ-14	CH_3	4-NH_2	55	>250	$C_{16}H_{14}N_5OS.Cl$	360	0.47	2.50
BZ-15	CH_3	CH_3	58	>250	$C_{11}H_{11}N_4OS.Cl$	283	0.62	0.99
BZ-16	CH_3	C_2H_5	53	>250	$C_{12}H_{13}N_4OS.Cl$	296	0.74	1.12
BZ-17	CRR_1=Cyclohexylene		52	>250	$C_{14}H_{15}N_4OS.Cl$	322	0.54	2.37
BZ-18	H	-	54	>250	$C_{16}H_{10}N_4O_2S.Cl$	371	0.63	2.00
BZ-19	F	-	57	>250	$C_{16}H_9N_5O_2S.Cl.F$	390	0.62	2.85
BZ-20	Cl	-	68	>250	$C_{16}H_9N_5O_2S.Cl_2$	406	0.64	2.42

[a] Mobile phase $CHCl_3$: CH_3OH (9:1)

[b] Log P was generated using Alchemy 2000 and SciLog P softwares

Table 6.8: Spectral and elemental analyses data of the compounds

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
BZ-1	3400, 3280, 3050, 2880, 2130, 1690, 1610, 1130	7.20-7.81 (m, 7 H,ArH), 858(s, 1H, imine H), 9.64 (s, 1H, ArNH, D_2O exchangeable), 9.86 (s, 1H, CONH, D_2O exchangeable)	51.14 51.43	3.43 3.26	14.86 15.18
BZ-7	3420, 3290, 3040, 2885, 2145, 1660, 1600, 1120	3.64 (s, 3H, OCH_3), 6.74-7.96 (m, 7H, ArH), 8.52 (s, 1H, imine H), 8.75 (s, 1H, ArNH, D_2O exchangeable), 9.56 (s, 1H, CONH, D_2O exchangeable)	53.26 53.13	3.63 3.67	15.83 15.78
BZ-13	3400, 3280, 3010, 2840, 2120, 1690, 1620, 1110	2.12 (s, 3H, CH_3), 7.44-7.65 (m, 7H, ArH), 8.42(s, 1H,ArNH, D_2O exchangeable), 8.78 (s, 1H, CONH, D_2O exchangeable), 10.08 (s, 1H, ArOH, D_2O exchangeable).	53.26 53.01	3.63 3.83	15.53 15.96

[a]Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

6.2.2. Pharmacology

Anticonvulsant activity

The 6-chloro benzthiazol-2-yl semicarbazones were tested only in the preliminary seizure models of MES and scPTZ. The anticonvulsant evaluation data of the 6-chloro benzthiazol-2-yl semicarbazones in MES, scPTZ, and neurotoxicity screens are summarized in Table 6.3, along with the data on Phenytoin, Ethosuximide and Sodium valproate.

Some compounds (**BZ-1**, **BZ-8**, **BZ-13**) were selected for activity in the oral MES screen and neurotoxicity tests in rats.

CNS depressant evaluation

Only the active compounds have been tested for the CNS depression effect and the results are shown in Table 6.10.

Neurochemical study

Mechanistic study has not been done for 6-chloro benzthiazol-2-yl semicarbazones as they exhibited very low anticonvulsant activity.

Table 6.9: Anticonvulsant and minimal motor impairment of 6-chloro benzthiazol-2-yl semicarbazones

Compound	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicty screen	
	0.5h	4h	0.5h	4h	0.5h	4h
BZ-13	-	100	-	-	-	300
BZ-14	-	300	-	-	-	-
Phenytoin	30	30	-	-	100	100
Ethosuximide	-	-	300	-	-	-
Sodium valproate	-	300	100	-	-	-

[a]Doses of 30, 100 and 300mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (-) indicates an absence of activity at the maximum dose administered (300mg/kg).

Table 6.10: CNS studies of 6-chloro benzthiazol-2-yl semicarbazones

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec)[c]	
	0.5h	1h	Control	Test after 1 h
Control	318.00±13.68	138.17±12.88		
BZ-13	260.00±62.57	196.50±15.11 NS	140.00±8.71	145.50±5.62 NS
BZ-14	163.50±7.65	127.00±4.21	162.50±10.03	173.00±12.15 NS
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	-	-	131.50±9.32	207.33±08.49

[a]The compounds were tested at the dose level of 100mg/kg . [b]Each value represents the mean ± SEM of six mice significantly different from the control at $p< 0.05$ and NS denotes values which are not significant (Student's t test) [c]Each value represents the mean ± SEM of six mice and NS denotes values which are not significant (Student's t test) [d]The compounds were tested at the dose level of 30mg/kg.

6.2.3. Results & Discussion

Chemistry

The 6-chloro benzthiazol-2-yl semicarbazones were designed to ascertain the influence of the size of aryl ring on the biological activity. Twenty semicarbazones were prepared by following method 4. The structures were identified on the basis of elemental, IR and 1H-NMR analysis. All the compounds were obtained in yields ranging from 53-68 %. The homogeneity and R_f of the compounds were monitored by TLC using $CHCl_3$: CH_3OH (9:1) as eluant. (Table 6.7).

Most of the compounds were found to be more lipophilic except for compounds **BZ-9, BZ-12, BZ-15,** and **BZ-16** No correlation has been established between the calculated log P and anticonvulsant profile of the compounds.

The IR spectrum for N^1-(6-chloro-benzthiazolyl)-N-(4-methoxybenzaldehyde) (**BZ-7**) semicarbazone showed absorption band at 3420 cm^{-1} of NH stretching, absorption band at 3040 cm^{-1} confirmed aromatic CH stretching and 2885 cm^{-1} showed aliphatic CH stretching. Two absorption bands at 1660 cm^{-1} and 1600 cm^{-1} appeared due to amidic C=O and C=N stretching vibrations. A strong band at 1120 cm^{-1} confirmed the presence of aromatic –Cl group.

^{1}H-NMR spectrum revealed a singlet at δ 2.22 and δ 3.64 for 3H of OCH_3 protons, Two singlets were observed at δ 8.75 and δ 9.56 for one proton each of ArNH and CONH which were exchangeable with D_2O. One singlet for imine proton also appeared at of δ 8.52. Thus the NMR spectrum also confirmed the structure of **BZ-7**. Similarly, the structures of other compounds were confirmed according to their characteristic peaks depicted in Table 6.8.

Pharmacological activity

1. MES test

In the preliminary MES screen, only two compounds **BZ-13** and **BZ-14**. Compound **BZ-13** showed protection at 100 mg/kg while **BZ-14** exhibited at a dose of 300 mg/kg of dose. Both the compounds exhibited late onset of action, i.e. showed protection only at 4h time interval.

3. scPTZ Test

None of the compounds exhibited protection in the scPTZ screen.

Neurotoxicity screen (NT)

Only compound **BZ-13** exhibited neurotoxicity at 4h period but not at the anticonvulsive dose of 100 mg/kg. Other compounds which did not show protection did not exhibit neurotoxicity. Compound **BZ-14** though being slight active did not possess any neurotoxicity.

Rat *p.o.* identification

None of the compounds showed protection in the rat *p.o* screen.

CNS depressant evaluation

In the behavioral despair test, compound **BZ-14** showed a significant decrease in motor activity as indicated by the actophotometer scores, but compound **BZ-13** did not show significant motor impairment. Both the compounds showed no significant increase in the immobility time with respect to control in porsolt's forced swim pool test, indicative of their lesser CNS depressant effect, as compared to the conventional antiepileptic drug Carbamazepine.

6.2.4. Structure-Activity Relationship

Totally twenty variously substituted 6-chloro benzthiazol-2-yl semicarbazones were synthesized with various electron donating and withdrawing substitutions, to study the effect of bulky benzthiazole moiety and effect of various substitutions on the anticonvulsant profile of semicarbazones.

From the above data, it could be concluded that the replacement of the phenyl ring of the substituted phenyl semicarbazones with a bulkier benzthiazolyl moiety led to tremendous fall in the anticonvulsant activity, which may be due to steric hindrance in binding with the receptor sites. In this series, replacement of benzylidene proton with the methyl group along with the substitution on the phenyl ring with *para*-hydroxy and *para*-amino group

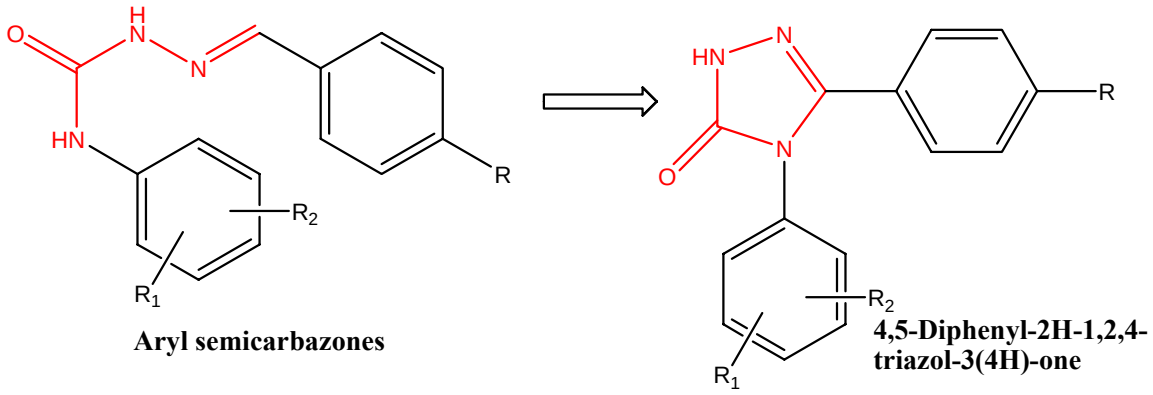
were found to be beneficial for anticonvulsant activity. Further increase in the size of the auxiliary aryl ring with isatinimino substituents led to a loss of activity.

CHAPTER 7

CYCLIZED ARYL SEMICARBAZONES

SUBSTITUTED DIPHENYL-1,2,4-TRIAZOLE-3-ONES

The substituted diphenyl-1,2,4-triazole-3-ones were designed as the cyclized derivatives of corresponding open chain aryl semicarbazones (Fig. 9). In this study, eighteen substituted diphenyl-1,2,4-triazole-3-ones were synthesized and evaluated for anticonvulsant activity.



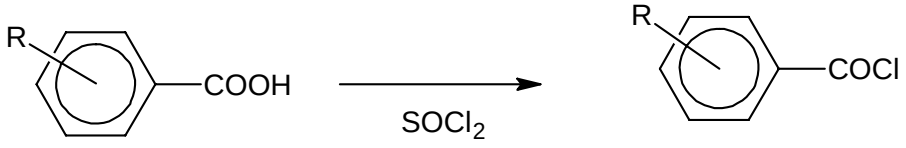
Aryl semicarbazones **4,5-Diphenyl-2H-1,2,4-triazol-3(4H)-one**

1-18a **TR-(1-18)**

Fig. 9 Cyclization of aryl semicarbazones to substituted diphenyl-1,2,4-triazole-3-ones

7.1. Synthesis

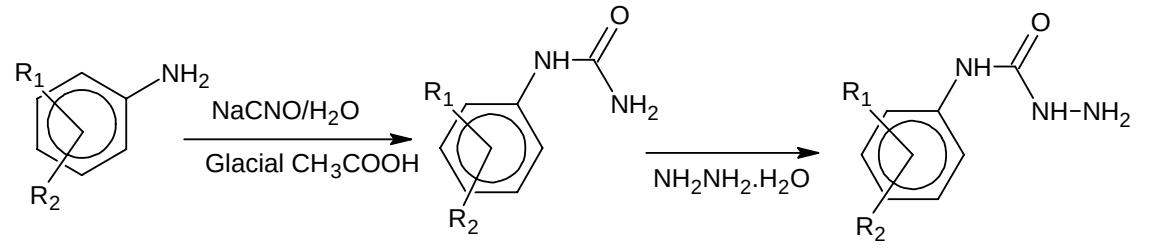
Step-1: Synthesis of substituted benzoyl chlorides



Substituted benzoic acid Substituted benzoyl chloride

Substituted benzoic acid (0.01 M) was refluxed with a slight excess of thionyl chloride (0.013 M). The reaction was carried out for 20 minutes. Then excess of thionyl chloride was distilled off and an oily residue left behind was evaporated at room temperature. The IR and NMR spectra of the substituted benzoyl chlorides were identical in the following aspects: IR-KBr pellet (cm^{-1}) 3450, 1730, 690, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 7.3-7.5 (m, 3H, ArH).

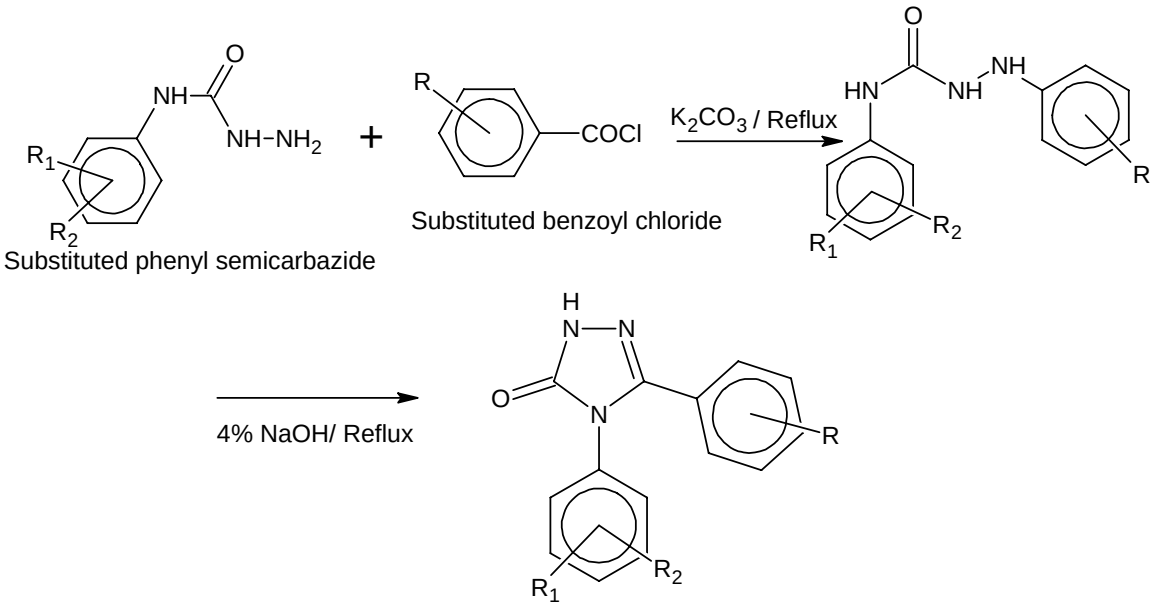
Step-2: Synthesis of substituted phenyl semicarbazides



Substituted aniline — Substituted phenyl urea — Substituted phenyl semicarbazide

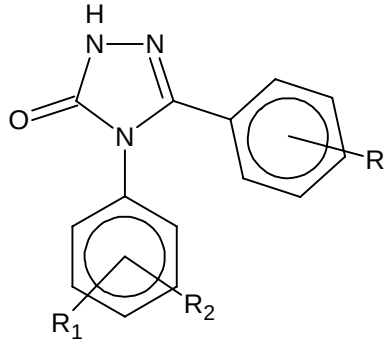
The substituted aniline (0.1 M) was dissolved in 20 mL of glacial acetic acid and 10 mL of water. To this, equimolar amount of sodium cyanate in 80 mL of warm water was added with stirring. This was allowed to stand for 30 minutes, then cooled in ice and filtered with suction and dried. The product was then recrystallized from boiling water to yield the respective phenyl ureas. Substituted semicarbazides were prepared by treating equimolar quantities of the phenyl urea and hydrazine hydrate i.e. 0.05 M, in ethanol under reflux for 24h with stirring. The two-third volume of alcohol was distilled by a vacuum distillation unit and then poured into ice. The resultant precipitate was filtered, washed with water and dried. The solid phenyl semicarbazide obtained was recrystallized from 50mL of 90% v/v alcohol. The IR and NMR spectra of the substituted benzoyl chlorides were identical in the following aspects: IR-KBr pellet (cm^{-1}) 3420, 3280,1620, 890, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) 7.24-7.45 (m, 3H, ArH), 5.58 (s, 2H, NH_2, D_2O exchangeable), 7.68 (s, 1H, ArNH, D_2O exchangeable), 9.86 (s, 1H, CONH, D_2O exchangeable).

Step-3: Synthesis of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones



Equimolar amounts of the semicarbazide and substituted benzoyl chlorides were dissolved in ethanol and to this added 10% w/v solution of potassium carbonate in water. The reaction mixture was refluxed for 2-3h. Excess of alcohol was distilled off by vacuum distillation and the resultant precipitate was filtered, washed with water and dried. Then 0.05 M of the synthesized compound was dissolved in 4% w/v aqueous NaOH and heated on water bath for 3h. The resultant mixture was cooled; precipitate was dried and recrystallized with boiling alcohol. IR-KBr pellet (cm^{-1}) 3400, 3300-3250, 1620, 1580, 1480, 840, 690. ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz) spectra of some representative compounds and physical data for the synthesized compounds are presented in Table 7.3.

Table 7.1: Physical data of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones



Compound	R	R_1	R_2	Yield (%)	M.p. (°C)	Molecular Formula[b]	Mol. Wt.	R_f[a]	Log P[b]
TR-1	NO_2	2-CH_3	4-CH_3	59	68	$C_{16}H_{14}N_4O_3$	310	0.61	3.09
TR-2	NH_2	2-CH_3	4-CH_3	53	195	$C_{16}H_{16}N_4O$	280	0.73	2.31
TR-3	CH_3	2-CH_3	4-CH_3	54	>250	$C_{17}H_{17}N_3O$	279	0.58	3.00
TR-4	H	2-CH_3	4-CH_3	60	>250	$C_{16}H_{15}N_3O$	265	0.82	1.58
TR-5	OH	2-CH_3	4-CH_3	53	>250	$C_{16}H_{15}N_3O_2$	281	0.54	1.98
TR-6	NO_2	2-CH_3	5-CH_3	54	195	$C_{16}H_{14}N_4O_3$	310	0.63	0.78
TR-7	NH_2	2-CH_3	5-CH_3	61	>250	$C_{16}H_{16}N_4O$	280	0.56	3.81
TR-8	CH_3	2-CH_3	5-CH_3	53	>250	$C_{17}H_{17}N_3O$	279	0.74	3.01
TR-9	H	2-CH_3	5-CH_3	65	>250	$C_{16}H_{15}N_3O$	265	0.73	0.89
TR-10	NO_2	2-CH_3	6-CH_3	61	200	$C_{14}H_9N_4O_3F$	310	0.67	0.81
TR-11	NH_2	2-CH_3	6-CH_3	63	149	$C_{16}H_{16}N_4O$	280	0.80	0.68
TR-12	CH_3	2-CH_3	6-CH_3	55	169	$C_{17}H_{17}N_3O$	279	0.81	2.20
TR-13	H	2-CH_3	6-CH_3	64	>250	$C_{16}H_{15}N_3O$	265	0.70	2.29
TR-14	NO_2	H	4-F	88	176	$C_{16}H_{16}N_4O$	314	0.82	1.54
TR-15	NH_2	H	4-F	48	>250	$C_{14}H_{11}N_4OF$	284	0.84	3.48
TR-16	CH_3	H	4-F	59	202	$C_{15}H_{12}N_3OF$	284	0.71	4.09
TR-17	H	H	4-F	65	>250	$C_{14}H_{10}N_3OF$	269	0.80	1.81
TR-18	OH	H	4-F	52	174	$C_{14}H_{10}N_3O_2F$	285	0.56	2.19

[a]Mobile phase $CHCl_3$: CH_3OH (9:1)

[b]Log P was generated using Alchemy 2000 and SciLog P softwares

Table 7.2: Spectral and elemental analyses data of the compounds

Compound	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)[a]		
			C	H	N
TR-1	3400, 3300, 3004, 1620, 1580, 1480, 1355,820, 690	2.35 (s, 3H, $ArCH_3$), 2.24 (s, 3H, $ArCH_3$) 6.98-8.31 (m, 7H, ArH), 8.31 (s, 1H, ArNH, D_2O exchangeable).	61.93 60.98	4.55 4.30	18.06 17.94
TR-5	3400, 3280, 2940, 1640, 1550, 840, 760	2.12 (s, 6H, 2-$ArCH_3$) 7.19-7.24 (m, 7H, ArH), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.68 (s, 1H, ArOH, D_2O exchangeable).	68.31 67.78	5.37 5.34	14.94 14.51
TR-9	3410, 3390, 3040, 2950, 1620, 1585, 800, 740.	2.20 (s, 6H, 2-$ArCH_3$) 7.19-7.26 (m, 8H, ArH), 8.46 (s, 1H, ArNH, D_2O exchangeable).	72.43 72.03	5.70 5.87	15.84 15.47
TR-11	3390, 3310,3010, 1714, 1628, 1590, 1500, 840, 690	2.28 (s, 3H, $ArCH_3$), 2.40 (s, 3H, $ArCH_3$), 6.85-7.74 (m, 7H, ArH), 8.26(s, 1H, ArNH, D_2O exchangeable), 8.58 (s, 1H, NH_2, D_2O exchangeable),	68.55 67.96	5.75 5.35	19.99 19.09
TR-12	3390, 3260, 3010, 2940, 1650, 1540, 820, 750	2.20 (s, 3H, $ArCH_3$), 2.28 (s, 6H, $ArCH_3$), 6.80-7.12 (m, 7H, ArH), 8.31 (s, 1H, ArNH, D_2O exchangeable).	60.47 60.24	6.13 6.06	15.04 14.78
TR-14	3410, 3290, 3025, 1710, 1610, 1510,1360, 810, 700	6.58-7.58 (m, 7H, ArH), 7.84 (s, 1H, ArNH, D_2O exchangeable).	56.00 56.65	3.02 3.00	18.66 18.08

[a]Elemental analyses for C, H, N were within ± 0.4 % of the theoretical values.

7.2. Pharmacology

Anticonvulsant activity

All the synthesized compounds were tested in four animal seizure models i.e. MES, scPTZ, scSTY, and scPIC at the dose levels of 30, 100 and 300 mg/kg. The anticonvulsant evaluation data of the substituted diphenyl-1,2,4-triazole-3-ones for MES, scPTZ, scSTY, scPIC and neurotoxicity screens are summarized in Table 7.3, along with the data on corresponding open chain aryl semicarbazones. From the compounds which displayed good anticonvulsant activity in the *i.p.* seizure models, some were selected for activity in the oral MES screen and neurotoxicity tests in rats.

CNS depressant evaluation

All the compounds were examined for CNS depressant activity which included locomotor activity using actophotometer and porsolt's swim test. The results are displayed in Table 7.4.

Neurochemical study

Some selected compounds (**TR-10** and **TR-18**) were studied fore their effect on the GABA level in whole rat brain and results are presented in Table 7.5.

Table 7.3: Anticonvulsant activity and minimal motor impairment of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-ones *vs.* corresponding open chain aryl semicarbazones

Compound	Intraperitoneal Injection in mice[a]									
	MES screen		scPTZ screen		scSTY screen		scPIC screen		Neurotoxicity screen	
	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h	0.5h	4h
TR-1	300	-	-	-	300	300	100	100	300	-
1a[b]	-	-	-	-	-	-	-	-	-	-
TR-2	300	-	-	-	300	300	100	100	300	-
2a[b]	-	-	-	-	-	-	-	-	-	-
TR-3	-	-	-	-	100	100	100	100	-	-
3a[b]	300	300	-	-	100	300	x	x	-	
TR-4	300	-	100	-	100	100	300	300	300	-
4a[b]	-	-	-	-	-	-	-	-	-	-
TR-5	300	300	-	-	300	300	300	300	300	-
5a[b]	-	-	-	-	-	-	-	-	-	-
TR-6	300	-	-	-	30	30	100	100	-	-
6a[c]	300	-	300	-	300	300	-	-	300	-
TR-7	-	-	-	-	100	100	100	100	-	-
7a	300	300	-	-	300	300	300	-	300	-
TR-8	-	-	300	-	100	100	100	100	-	-
8a[c]	300	-	-	-	300	300	300	300	300	-
TR-9	300	-	300	-	300	300	300	300	300	-
9a	100	300	-	-	300	-	300	300	100	-
TR-10	-	-	-	-	300	300	300	-	100	-
10a[b]	100	300	300	-	30	100	x	x	300	-
TR-11	300	-	-	-	30	30	100	100	-	-
11a[b]	100	300	300	-	30	100	30	-	300	-
TR-12	-	-	300	-	300	300	-	-	100	-
12a[b]	100	300	300	-	100	100	x	x	-	300
TR-13	300	300	-	-	300	300	100	100	-	-
13a[b]	100	100	300	-	30	100	x	x	300	-
TR-14	100	-	300	-	300	300	300	300	300	-
14a[b]	-	-	-	-	x	x	x	x	100	-
TR-15	300	-	300	-	300	300	300	300	300	-
15a	-	-	-	-	x	x	x	x	-	-
TR-16	300	300	300	-	300	300	300	300	300	-
16a	300	300	-	-	x	x	x	x	-	-
TR-17	100	300	300	-	300	300	300	300	300	-
17a	300	300	-	-	x	x	x	x	300	-
TR-18	300	-	300	-	30	30	100	100	300	-
18a	300	-	300	-	x	x	x	x	300	300

[a]Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5h and 4.0h after injection were made. The dash (-) indicates the absence of activity at the maximum dose administered (300 mg/kg). The cross (x) indicates that the compounds were not tested in animals. [b]From references [77], [81], [82].

Table 7.4: CNS studies of (substituted diphenyl)-2H-1,2,4-triazole-3(4H)-one derivatives

Compound[a]	Actophotometer (Locomotor activity score) [b]		Immobility Time (Sec) [c]	
	0.5h	1h	Control	Test (After 1 h)
Control	318.00±13.68	228.50±11.31		
TR-1	174.50±4.22 NS	133.00±3.21	181.00±9.40	190.00±9.81 NS
TR-2	229.50±7.58	97.00± 3.52	179.00±.6.27	189.50±5.32
TR-3	341.50±11.20 NS	264.00±10.07 NS	39.00±5.42	28.00±4.70 NS
TR-4	203.00±11.31	70.17±3.18	148.50±8.92	179.50±12.25
TR-5	353.00±14.10 NS	177.00±8.70	106.00±15.71	81.00±9.48 NS
TR-6	348.00±17.10 NS	215.00±10.01	52.00±6.59	103.00±13.22
TR-7	478.00±18.60	336.00±14.54	112.00±17.80	144.50±10.55 NS
TR-8	269.50±12.14	127.83±17.98	102.00±17.38	119.00±16.45 NS
TR-9	270.00±17.78 NS	216.00±17.24 NS	74.67±5.68	94.00±7.98
TR-10	330.50±15.51	295.50±1.88 NS	28.17±6.76	18.00±1.81 NS
TR-11	400.50±14.95	267.50±11.93 NS	173.00±8.52	192.00±7.26
TR-12	287.00±9.04 NS	184.00±13.76	142.50±4.22	152.00±4.40
TR-13	397.50±12.66 NS	170.50±5.43	104.00±16.16	117.00±15.96 NS
TR-14	256.50±8.13	93.50±3.73	114.67±16.45	118.50±14.73 NS
TR-15	310.00±14.45 NS	101.50±4.81	182.50±8.40	193.00±10.01 NS
TR-16	261.00±14.91	247.50±17.56 NS	163.33±10.78	180.00±13.02
TR-17	324.00±18.32 NS	267.17±17.51 NS	66.00±17.15	71.00±7.49 NS
TR-18	346.00±17.46 NS	283.00±17.55 NS	119.50±7.91	129.33±8.82
Phenytoin[d]	104.11±14.56	106.23±12.44	-	-
Carbamazepine[d]	-	-	131.50±9.32	207.33±08.49

[a]The compounds were tested at the dose level of 100mg/kg .

[b]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.05$ and NS denote values, which were not significant (Student's t test).

[c]Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.005$, *$p < 0.05$ and NS denotes values which were not significant (Student's t test).

[d]he compounds were tested at the dose level of 30mg/kg.

Table 7.5: Effect on GABA level in rat whole brain

Group	GABA concentration in μg/100mg[b]
Control	22.24 ± 1.91
TR-10[a]	139.59±1.49
TR-18[a]	235.34 ± 14.67

[a]The compounds were tested at a dose of 100 mg/kg (oral).
[b]Each value represents the mean ± SEM of six rats significantly different from the control at *P < 0.0001.

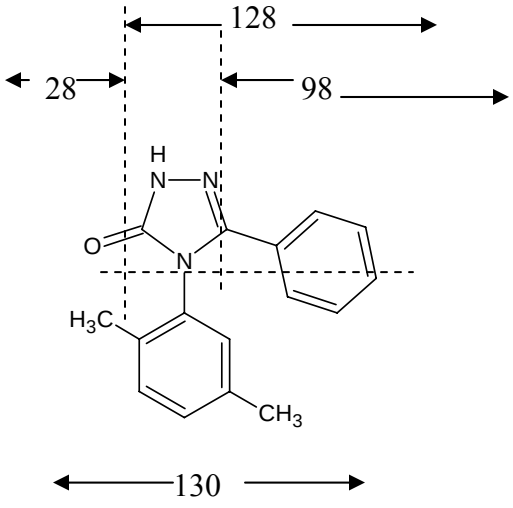
7.3. Results & Discussion

Chemistry

The substituted diphenyl-1,2,4-triazole-3-ones were synthesized by the condensation of substituted benzoyl chlorides and substituted phenyl semicarbazides, which were then cyclized in slightly alkaline condition to the corresponding 1,2,4-triazole-3-ones. The structures were identified on the basis of elemental, IR and ^{1}H-NMR analyses. All the compounds were obtained in moderate yields ranging from 53-88%. The homogeneity and R_f values of the compounds were monitored by TLC using $CHCl_3$: CH_3OH (9:1) as eluant. (Table 7.1).

The IR spectrum for (2,5-dimethylphenyl)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**TR-9**) showed the presence of NH group at 3410 cm^{-1}. Aromatic C-H stretch was observed at 3050 cm^{-1} and aliphatic C-H stretch at 2950 cm^{-1}. The bands corresponding to C=O stretch and C=N stretch appeared at 1620 cm^{-1} and 1535 cm^{-1} respectively. The spectrum also showed bending vibrations at 850 cm^{-1} and 690 cm^{-1}, due to the presence of substituted benzene ring.

^{1}H-NMR spectrum for **TR-9**, in DMSO revealed a singlet at δ 2.20 for 3H of CH_3 protons, and the singlet at δ 8.46 for 1H of imine proton was D_2O exchangeable. Proton of –CONH group at δ 9.56 was also D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristic peaks depicted in Table 7.2. The mass spectrum of compound **TR-9** showed a molecular ion peak at m/z 285, base peak at m/z 158. The remaining major fragmentation peaks were at m/z 98, 128 and 130.



Pharmacological activity

3. MES test

Most of the compounds showed activity in the preliminary MES screen except compounds **TR-3, TR-7**, **TR-8, TR-10**, and **TR-12**. Compounds **TR-14,** and **TR-17** showed protection at 100 mg/kg (0.5h) and other active compounds showed protection at 300 mg/kg dose. Most of the compounds exhibited shorter duration of action (i.e. activity at 0.5h time period only) except compounds **TR-16**, **TR-13, TR-17,** and **TR-18**.

2. scPTZ test

In the scPTZ screen, nine compounds (**TR-4**, **TR-8**, **TR-9**, **TR-12**, **TR-16**, **TR-14, TR-15, TR-17** and **TR-18**) showed protection. Except compound **TR-4** others showed protection at 300mg/kg up to 0.5h time period.

3. scSTY screen: -

All the compounds were screened in this model and were found to show protection. Compound **TR-6**, **TR-11** and **TR-18** showed protection even at 30mg/kg. Compounds **TR-3**, and **TR-(7-9**) exhibited protection at 100 mg/kg, and others showed activity at 300 mg/kg. All the compounds exhibited a longer duration (0.5h and 4h) of action. The results are presented in Table 7.3.

4. scPIC Screen

In the scPIC screen, all the compounds except **TR-12** exhibited protection from seizures and death. Compounds **TR-(1-3), TR-6**, **TR-7, TR-8, TR-11, TR-13,** and **TR-18** showed protection at 100 mg/kg, all others showed activity at 300mg/kg. Except compound **TR-10,** others exhibited protection for longer duration of time i.e. up to 4h time interval.

Neurotoxicity screen (NT)

In the acute neurological toxicity screen, the compounds **TR-3**, **TR-5, TR-7, TR-11, TR-8,** and **TR-13** showed activity but no neurotoxicity at the maximum dose administered (300 mg/kg). Compounds **TR-1, TR-2, TR-4, TR-5, TR-9, TR-14, TR-15, TR-16,** and **TR-(17-18**) exhibited neurotoxicity at the anticonvulsive dose. Compounds **TR-10** and **TR-12** were found to be more neurotoxic at the anticonvulsive dose.

As triazole derivatives were designed by the cyclization of the parent aryl semicarbazones (Fig. 9), the anticonvulsant activity of cyclized aryl semicarbazones i.e. substituted diphenyl-1,2,4-triazole-3-ones were also compared with that of the parent open chain aryl semicarbazones to evaluate the effect of this cyclization, as shown in Table 7.3 and the following observations were made:

1. In the 2,4-dimethylphenyl substituted derivatives {**TR-(1-5)** and **1a-5a**}, except compound **TR-3**, other compounds were effective in at least three out of the four animal models in which the parent compound was inactive. The compounds except **TR-3** showed neurotoxicity at the anticonvulsive dose.
2. With respect to the 2,5-dimethylpheny substituted derivatives, compound **TR-6** and **TR-7** were more effective in scSTY, and scPIC, but was ineffective in the scPTZ model compared to the parent derivatives **6a** and **7a**, and compound **TR-7** was ineffective the MES model. Compound **TR-8** showed better protection in the scPTZ, scSTY and scPIC and was inactive in the MES screen compared to the parent derivative **8a** which showed anticonvulsant property in the MES, scSTY and scPIC models. Hence compounds **TR-(6-8)** were more effective and not neurotoxic compared to the parent derivatives **6a-8a**. Compound **TR-9** showed protection in all the four animal models tested but there was no separation between the anticonvulsant and neurotoxic doses.
3. The 2,6-dimethylphenyl semicarbazones were reported earlier [81] to be more effective among the disubstituted aryl semicarbazones. In this study, cyclization of these derivatives reduced the activity profile of the compounds as seen with compounds **TR-(10-13)**. With regard to neurotoxicity, compounds **TR-10** and **TR-12** were more neurotoxic, while compounds **TR-11** and **TR-13** did not show any neurotoxicity.
4. Lastly with respect to the 4-fluorophenyl compounds **TR-(14-18)**, the compounds were more effective or equipotent with the parent derivatives **14a-18a**, but were neurotoxic at the anticonvulsive dose.

CNS depressant evaluation

In the behavioral despair test, except compounds **TR-2**, **TR-4**, **TR-7**, **TR-8**, and **TR-14**, others did not show significant decrease in motor activity as indicated by the actophotometer scores. In porsolt's forced swim pool test, compounds **TR-1**, **TR-3**, **TR-5**, **TR-7**, **TR-8**, **TR-10**, **TR-(13-15)**, and **TR-17** did not show depression, when compared to the control, indicating their lesser CNS depressant effect than the conventional drugs (Table 7.4).

Effect on the levels of GABA in whole rat brain

Compounds (**TR-10** and **TR-18**) showed a significant increase in the GABA level concentration in rat brain as compared to the control ($p < 0.0001$), which led to the conclusion that substituted diphenyl-1,2,4-triazole-3-ones also act by a GABA-mediated mechanism. (Table 7.5).

7.4. Structure-Activity Relationship

The anticonvulsant data of the synthesized compounds indicated that, the cyclization of semicarbazone template of aryl semicarbazones was beneficial and yielded a series of more potent/equipotent derivatives of substituted diphenyl-1,2,4-triazole-3-ones, showing a broad spectrum of activity with lesser neurotoxicity.

Further to investigate the similarities and differences between the assessable conformations of the parent open chain aryl semicarbazones and substituted diphenyl-1,2,4-triazole-3-ones with respect to the spatial positions of their pharmacophoric elements, representative compounds of the proposed 1,2,4-triazoles (**TR-3**, **TR-6**, **TR-11**, and **TR-18**) were superimposed with the parent aryl semicarbazones (**3a**, **6a**, **11**a, and **18a**) by merging the energy-minimized structures generated by MM3 force field (Figure 8 and 9). The proposed 1,2,4-triazoles showed a root mean square (RMS) deviation in the range of 0.228 Å to 0.296Å from the parent aryl semicarbazones. This ~ 30% deviation in superimposition deduced that the restricted movement about the hydrogen bonding domain due to cyclization, might be responsible for proper fitting of the triazole derivatives on the receptor sites and led to the beneficial anticonvulsant profile of the synthesized triazole derivatives over parent semicarbazone derivatives.

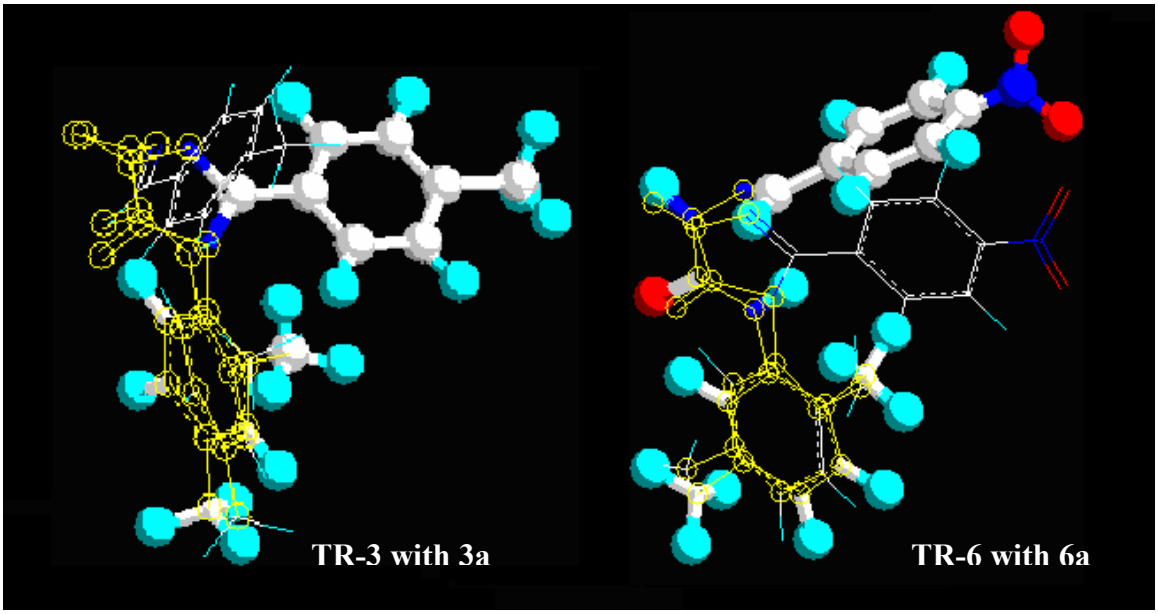


Fig. 10 Pharmacophore matching of 1,2,4-triazole derivatives (TR-3, and TR-6 ball and stick model) with the aryl semicarbazones (3a, and 6a wire frame)

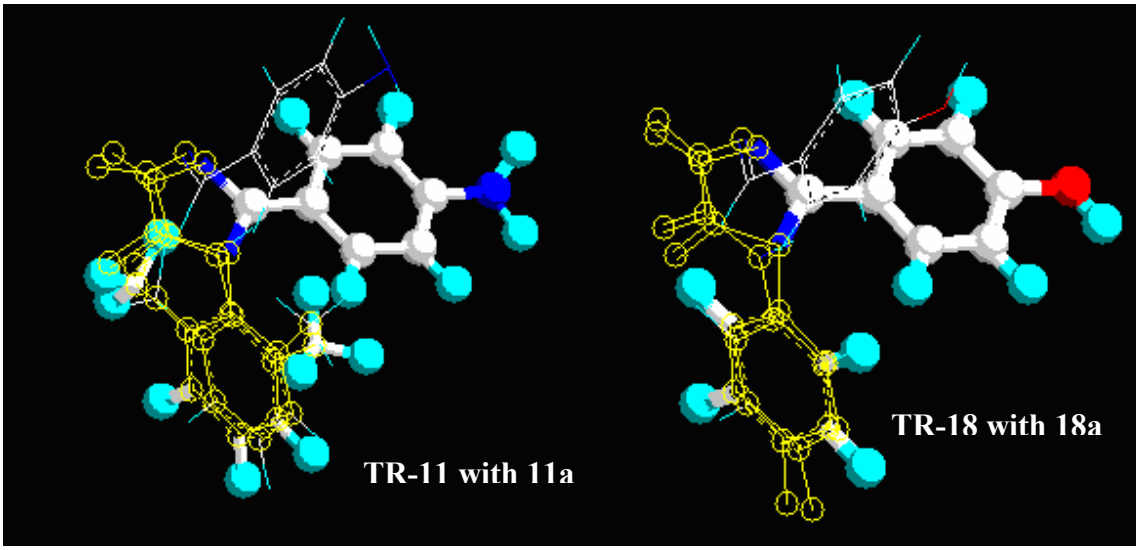
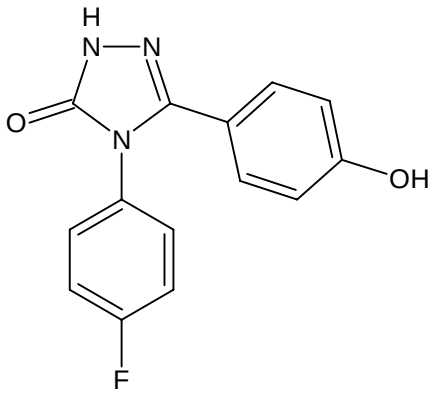


Fig. 11 Pharmacophore matching of 1,2,4-triazole derivatives (TR-11, and TR-18 ball and stick model) with the aryl semicarbazones (11a, and 18a wire frame)

Structure-activity relationship study has also been done. The important features which emerged as a result of the study were:

2. For MES screen, substitution by an electron withdrawing group i.e. *para*-fluoro group was found to be more beneficial [**TR-**(**14-18**)]. Similar is the case for the auxiliary aryl ring i.e. substitution with *para*-nitro group on phenyl ring or unsubstituted phenyl ring was more favorable for activity (**TR-1**, **TR-5**, **TR-9**, **TR-14**).
3. For scPTZ screen, *para*-fluoro substituted phenyl derivatives were found to be most active (**TR-14-18**), while for the auxiliary aryl binding site presence of electron donating groups i.e. CH_3 or unsubstituted phenyl ring (**TR-4**, **TR-8**, **TR-9**, and **TR-12**) showed better protection than electron withdrawing groups.
4. For the compounds possessing activity in the scSTY screen, electron donor substitutions i.e. -CH_3 (**TR-3**, **TR-4**, **TR-7**, and **TR-11**), led to more active compounds than electron withdrawing substituents.
5. Most of the compounds showed protection in the scPIC screen, and no clear cut correlation between the substitution pattern and anticonvulsant activity could be pointed out though compounds possessing one or more electron donating groups showed slightly better anticonvulsant activity in the scPIC screen, than the presence of electron withdrawing groups.

Compound with *para*-fluoro substitution (**TR-18**) emerged as the most effective compound with broad spectrum of activity and lesser neurotoxicity.



4-(4-Fluorophenyl)-5-(4-hydroxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (TR-18)

CHAPTER 8
QUANTUM MECHANICAL MODELING

8.1 PRINCIPLE

Quantum mechanical modeling involves the study of the electrons and revolves around "electronic theory". In the electronic theory, the static and dynamic behaviors of molecules are explained by the electronic effects which are based on the distribution of electrons in an atom or a molecule. It also considers that the electron distribution in an orbital is directly connected to chemical observations and this fact was certainly felt to be interesting by many chemists [130].

A molecular orbital (MO) is a composite of weighted atomic obitals (AO's) which collectively define the shape and spatial density of the electrons in a molecular species [131]. In most quantum chemistry calculations, the starting point is a collection of atoms which each possesses one or more atomic orbital functions. Taking notice of the principal role played by the valence electrons in the case of the molecule formation from atoms, the distribution of the electrons occupying the highest energy orbital was studied most extensively. The two particular orbitals, which act as the essential part in a wide range of chemical reactions of various compounds, saturated or unsaturated, were referred to under the general term of "frontier orbitals", and abbreviated frequently by HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) (Fig. 12). The HOMO is the orbital that could act as an electron donor, since it is the outermost (highest energy) orbital containing electrons. The LUMO is the orbital that could act as the electron acceptor, since it is the innermost (lowest energy) orbital that has room to accept electrons. In accordance with the above definitions, a single orbital may be both the LUMO and the HOMO.

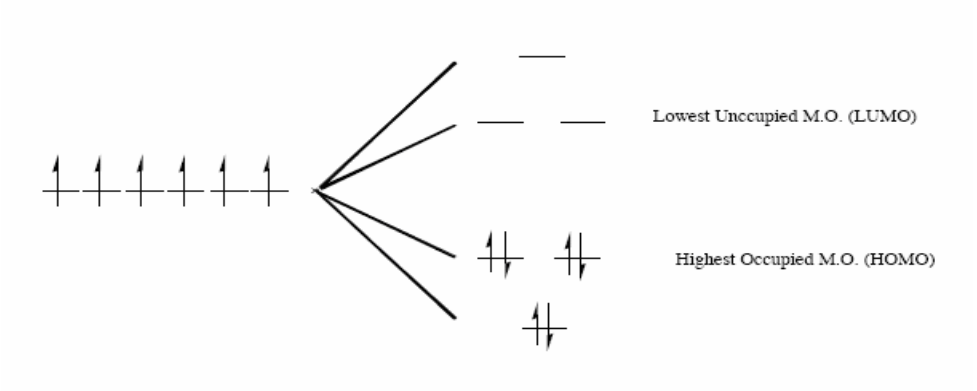


Fig. 12 Molecular orbital and energy diagram

A number of reactions can be rationalized by examining the distribution of the frontier molecular orbitals, both with regard to mechanism (such as Diels-Alder reactions and sigmatropic rearrangements), and preferential reaction sites when examination of steric constraints does not provide the answer. Analysis of the molecular orbitals also reveals the extent of conjugation, and elucidates the nature of extended pi-systems in particular. Electron-rich and electron-poor species tend to reveal the localization or delocalization of the partial or full charge by the shape of the HOMO or LUMO. Molecular orbitals, when viewed in a qualitative graphical representation, can provide insight into the nature of reactivity, and some of the structural and physical properties of molecules. Well known concepts such as conjugation, aromaticity and lone pairs are well illustrated by molecular orbitals [130].

The other important observation drawn from a molecular orbital calculation is the absolute energy of the system, which in turn provides useful information about the molecule's reactivity. In particular, from the energy associated with HOMO (E_{HOMO}) and LUMO (E_{LUMO}) molecular orbitals, it is possible to estimate how powerful electrophile/ nucleophile a molecule will be and which part of the molecule will participate in the bond formation with the reacting species. The greater E_{HOMO} is, the greater the electron-donating capability; conversely, the smaller E_{LUMO} is, the smaller the resistance to accept electrons. Compounds that present larger values of E_{HOMO} are more electron donors and the compounds that present smaller values of E_{LUMO} are more electron acceptors. These variables are interpreted as measures of molecular reactivity and stability. As E_{HOMO} increases (relative to other molecules), the molecule is less stable and more reactive. For E_{LUMO}, the situation is the opposite [132].

8.2 METHODS USED IN QUANTUM MECHANICAL MODELING

Quantum mechanics is a set of molecular property calculations based on the Schrödinger equation, which takes into account the interactions between electrons in the molecule. Schrödinger equation describes the wave function, the square of which corresponds to the probability of finding an electron at a particular point. This shows that the orbitals do not have clearly defined edges which contain their electrons, but the probability of finding an electron diminishes gradually with distance. One way of calculating their structures is to assume that molecular orbitals can be expressed as linear combinations of atomic orbitals, as shown in equation:

$$\Psi = c_1\phi_1 + c_2\phi_2 + \ldots\ldots\ldots + c_i\phi_i \ldots\ldots\ldots + c_n\phi_n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8.1)$$

Where, ϕ_i's are the atomic orbitals; Ψ is the molecular orbital; c_i's are the coefficients.

The energy of the orbital can be found from its wave function, by the use of the Schrödinger equation (equation 8.2)

$$H\Psi = E\Psi \qquad \text{or}$$

$$E = \int \Psi H \Psi.dt \,/\, \int \Psi\Psi.dt = (\Psi \mid H \mid \Psi) \,/\, (\Psi)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8.2)$$

So, all that needs to be done is to calculate the coefficients c_i's in the equation 8.1, which help in calculating the Hamiltonian operator $\int \Psi H \Psi.dt$, and later to obtain a value for the energy from the wave function by adding up the contribution from the kinetic energy of the corresponding particle and all of the potential energy terms, which arise from the electrostatic repulsion between each electron and all the other electrons and the nuclei. This is difficult, because it suggests it is only possible to solve the equation for one electron if the positions of all the other electrons are already known. This rather fundamental problem can be solved by using Huckel theory, Born-Oppenheimer approximation and Hartree-Fock method.

Huckel theory- This theory takes into account only the π-electrons in the delocalized system. This can make the Schrödinger equation more straightforward but it uses extreme approximations.

Born-Oppenheimer approximation- This is the idea that electrons move very fast than the nuclei, and so the movement of the electrons can be considered independently of the movement of nuclei. So the nucleus is treated as unmoving object, and only the effect of electrons is considered.

Hartree-Fock method- The energy of the particular electron depends on the electric fields produced by the atomic nuclei and by all the other electrons. If the wavefunctions for all the electrons except one are known, then it is possible to calculate the wave function of the remaining electron. This approach uses this phenomenon and the process is repeated until a self-consistent solution is obtained. A further approximation is often used. If a system has a multiplicity of zero, means that all the electrons are paired, then the electrons may be restricted to moving in pairs. This is called as restricted Hartree-Fock (RHF), and is the best method. If unpaired electrons are present, unrestricted Hartree-Fock (UHF) method must be used [133].

Semi-empirical approach: From the above discussion it is clear that the number of arithmetical operations which are required to investigate a simplest of the system would be very large. Hence, a drastic approximation would be required. One approach is to neglect all electrons except the valence electrons, and to consider some of the orbitals which only just overlapped but do not overlap and that way calculate the interactions of other orbitals through parameters. This is called as the semi-empirical approach. The first semi-empirical approach was CNDO (Complete Neglect of Differential Overlap) for which the required parameters were developed from the *ab initio* calculations (quantum chemical calculations using exact equations with no approximations, which involve the whole electronic population of the molecule). Similar approaches, following the alternative philosophy of parameterising the method from the experimental data rather than from *ab initio* calculation led to the development of MNDO [134] and then AM1 [135], and PM3 [136] semi-empirical methods. The MNDO, AM1, and PM3 methods are

all based on the Neglect of Diatomic Differential Overlap (NDDO) approximate Hamiltonian. These methods have been very successful in treating a wide array of organic systems for structures, properties, heats of formation, and describing reactions. MNDO, AM1, and PM3 differ in their parameterization. Generally, AM1 and PM3 have the smallest overall errors with regard to experiment. Similarly the other popular method is the ZINDO semi-empirical approach. The ZINDO method is based on an Intermediate Neglect of Diatomic Differential Overlap Hamiltonian (INDO), first implemented in the ZINDO program developed by the Zerner group [137].

Basis set - A set of basis functions employed for the representation of molecular orbitals. Slater-type atomic orbital (STO) and Gaussian type orbital functions are used very frequently. Gaussian type atomic functions have now largely superseded Slater orbitals, which are rather difficult to manipulate. Some simple Gaussian basis sets mimic slater-type atomic orbitals, for example STO-3G is a basis set which uses three Gaussian functions to form each slater-type orbital. Similarly STO-4 G and STO-6 G use four and six Gaussians to form each slater-type orbital, and is so likely to give somewhat higher energies for any system [133].

So the Quantum mechanical (QM) calculations are molecular property calculations based on the Schrödinger equation, which take into account the interactions between electrons in the molecule and modeling involves the display of these interactions of electrons.

In the present work, QM calculations were carried out using Argus Lab version 4.0.1. The three-dimensional structures of the compounds were geometry optimized using Hamiltonian PM3 (Parameterized Method 3) semi-empirical QM. For the estimation of HOMO and LUMO energy and surfaces, the single-point energy calculation using Hamiltonian ZINDO and RHF-SCF (Restricted Hartree-Fock-Single consistent Field) method (Basis set STO-6G) [137] was employed. The HOMO surfaces were visualized using a contour value of 0.05 in opaque mode using blue and red for positive and negative phase of the orbital in space.

8.3 RESULTS AND DISCUSSION

The concept of quantum mechanical modeling was applied to all the active series of synthesized aryl semicarbazones and their analogues, which could provide better insights into the nature of reactivity, and some of the structural and physical properties of synthesized molecules. The parameters which are studied extensively include:

- Single-point energy calculation of HOMO, LUMO energies (E_{HOMO} and E_{LUMO}) and the difference (ΔE) in E_{HOMO} and E_{LUMO}.
- HOMO surfaces analysis of the basic anticonvulsant pharmacophore of aryl semicarbazones and their analogues.

The study was done extensively for the disubstituted-phenyl semicarbazones and heteroaryl and cyclized analogues of aryl semicarbazones were studied very briefly.

8.3.1 2,5-Disubstituted phenyl semicarbazones

To explore the favorable effect of *ortho*-methyl substitution on the phenyl ring i.e. to predict the lesser anticonvulsant activity of the 2-fluoro-5-methylphenyl semicarbazones over 2,5-dimethylphenyl semicarbazones, preliminary quantum mechanical studies have been performed on some selected common derivatives from both the series. The single-point energy calculation of E_{HOMO}, E_{LUMO} and the difference (ΔE) in E_{HOMO} and E_{LUMO} were studied and presented in Table 8.1. The important observations drawn from the study are:

1. For both series higher ΔE value, led to greater anticonvulsant activity (**DM-1** and **FM-1**). High value of ΔE, shows the rigidity of the molecule i.e. the larger energy gap between the HOMO and LUMO. This outcome favors the finding that intact semicarbazone molecule is responsible for activity [57].
2. In both the series, higher energy values (E_{HOMO} and E_{LUMO}) for alkyl ketone compounds [**DM-(14-17)** and **FM-(20-24]** as compared to aryl aldehyde/ketone compounds could be due to the absence of resonance stability which is seen with the former.
3. 2-Fluoro-5-methylphenyl semicarbazones showed higher values of E_{HOMO}, E_{LUMO} and ΔE, than the corresponding 2,5-dimethylphenyl semicarbazones derivatives, which could be due to ring deactivating effect of fluoro group on the phenyl ring.

Table 8.1: E_{HOMO}, E_{LUMO} and ΔE values for 2,5-dimethyl/2-fluoro-5-methylphenyl semicarbazones

R_1	R_2	2, 5-dimethylphenyl-semicarbazones			2-Fluoro-5-methylphenyl-semicarbazones		
		E_{HOMO} (eigenvalues)	E_{LUMO} (eigenvalues)	ΔE	E_{HOMO} (eigenvalues)	E_{LUMO} (eigenvalues)	ΔE
H	H	-0.310	-0.033	-0.277	-0.316	-0.041	-0.275
H	2-NO_2	-0.317	-0.144	-0.174	-0.327	-0.147	-0.180
H	4-NO_2	-0.317	-0.056	-0.261	-0.325	-0.157	-0.278
H	4-OCH_3	-0.305	-0.041	-0.264	-0.310	-0.044	-0.266
H	4-CH_3	-0.303	-0.039	-0.263	-0.307	-0.042	-0.265
H	4-OH 3-OCH_3	-0.283	-0.050	-0.233	-0.311	-0.048	-0.263
CH_3	3-NH_2	-0.289	-0.043	-0.247	-0.291	-0.042	-0.249
CH_3	4-NH_2	-0.279	-0.044	-0.235	-0.286	-0.042	-0.244
CH_3	4-OH	-0.304	-0.034	-0.270	-0.291	-0.053	-0.237
CH_3	CH_3	-0.305	-0.009	-0.300	-0.325	-0.013	-0.312
CH_3	C_2H_5	-0.301	-0.005	-0.294	-0.320	-0.011	-0.309
CH_3	CH_2COCH_3	-0.306	-0.027	-0.279	-0.324	-0.037	-0.288
CH_3	C_5H_{11}	-0.304	-0.011	-0.294	-0.321	-0.013	-0.308
Cyclopentylene		-0.304	-0.006	-0.295	-0.321	-0.021	-0.301
Cyclohexylene		-0.300	-0.008	-0.292	-0.319	-0.020	-0.299

E_{HOMO}, E_{LUMO} and ΔE calculations using Hamiltonian ZINDO and RHF-SCF (Restricted Hartree Fock-Single consistent Field) method

HOMO surfaces were visualized for both the series for comparison (Fig. 13) and the following observations were made.

1. As proposed earlier, the anticonvulsant pharmacophore requirement for aryl semicarbazones include an aryl ring, a hydrogen-bonding domain and an electron donor system [81, 112]. The HOMO surface analysis also confirmed this hypothesis wherein compounds **DM-5** and **DM-7** showed a delocalized orbital surface over an aryl ring, imine group (electron donor), and the amide moiety (hydrogen acceptor-donor unit) (Fig. 12). Similar surfaces were observed with all the active compounds. Compound **DM-2** which did not comply with this hypothesis was found to be inactive.
2. Another observation made with compound **DM-2** is that the electron cloud of the carbimino aryl ring is shifted to the 2,5-dimethylphenyl ring due to the presence of deactivating nitro group.
3. Undelocalized or splitting of electron cloud on the aryl ring was found to be not favorable for activity as represented in figure for compound **FM-8** and **FM-10**. Similar observations were made with other inactive or lesser active compounds of the series.
4. It was also seen that unsymmetrical molecular orbital surfaces resulted in a decrease in activity. This was observed with other less active and inactive compounds of 2-fluoro-5-methylphenyl semicarbazones.
5. The fluorine group because of its electron withdrawing nature might deactivate the ring when compared to the *ortho*-methyl group in 2,5-dimethylphenyl semicarbazones, which could be responsible for lower anticonvulsant activity of 2-fluoro-5-methylphenyl semicarbazones.
6. The exceptionally active acetone derivatives **DM-14** and **FM-20** also showed HOMO surface in accord to the pharmacophore hypothesis with an aryl binding site contributed by the disubstituted phenyl ring, electron donor unit (imine group), hydrogen donor-acceptor unit (amidic hydrogen and lone pair in nitrogen). Similar observations were made with the compounds **DM-15** and **FM-21**.

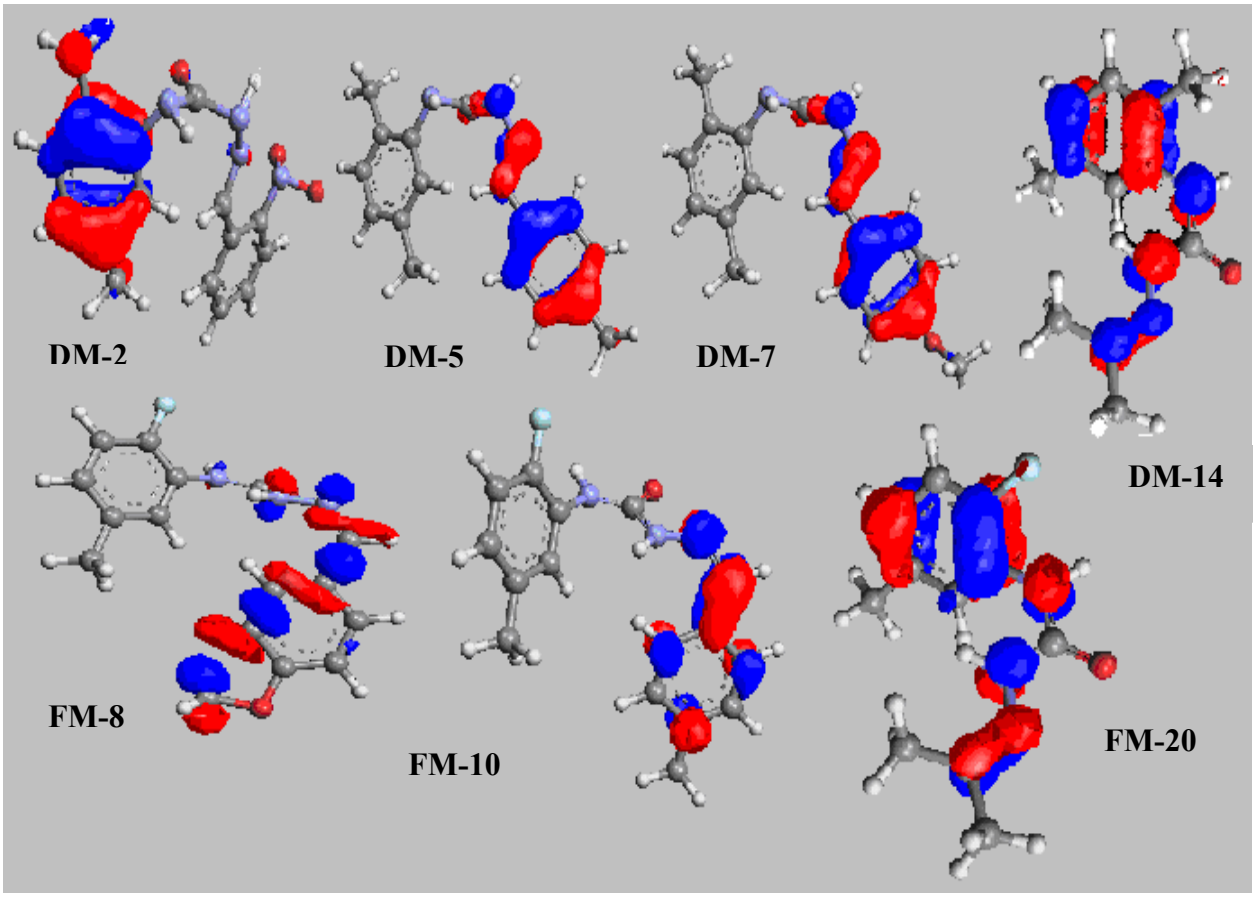


Fig. 13 HOMO surface visualization (Contour value = 0.05) of some representative compounds of 2,5-disubstituted phenyl semicarbazones in opaque mode. The colors indicate the phase of the orbital in space (blue for positive and red for negative).

8.3.2. 4-Bromo-3-methylphenyl semicarbazones

The E_{HOMO}, E_{LUMO} and ΔE calculations of 4-Bromo-3-methylphenyl semicarbazones are presented in Table 8.2, which showed the following trends, as were seen with 2,5-disubstituted phenyl semicarbazones:

1. Higher ΔE value, led to greater anticonvulsant activity (**BM-1**, **BM-9**, **BM-16**, **BM-20**).
2. The other similar observation was that alkyl ketone derivatives of the 4-Bromo-3-methylphenyl semicarbazones (**BM-20** – **BM-23**) showed greater E_{HOMO}, E_{LUMO} and ΔE values than aryl aldehyde/ketone compounds that could be again due to the absence of resonance stability which is seen with the former.

Similarly HOMO surface analysis of 4-Bromo-3-methylphenyl semicarbazones also found to show the same pattern as observed with the 2,5-disubstituted phenyl semicarbazones. The HOMO surface analysis led to the following observations (Fig. 14):

1. Again the HOMO surface analysis confirmed the hypothesis that the compounds with a delocalized orbital surface over an aryl ring, imine group (electron donor), and the amide moiety (hydrogen acceptor-don donor unit) (**BM-1** and **BM-9**) showed higher activity, while compounds which did not comply with this hypothesis were found to be inactive (i.e. **BM-2** and **BM-4**).
2. Undelocalized or splitting of electron cloud on the aryl ring was found not to be favorable for activity as represented in figure for compound **BM-2** and **BM-4**. and the unsymmetrical molecular orbital surfaces resulted in decrease in activity. This observation was confirmed with other less active and inactive compounds also.

Table 8.2: E_{HOMO}, E_{LUMO} and ΔE values for 4-bromo-3-methylphenyl semicarbazones

Compound	E_{HOMO} (eigenvalues)	E_{LUMO} (eigenvalues)	ΔE
BM-1	-0.342	-0.033	-0.291
BM-2	-0.338	-0.144	-0.194
BM-3	-0.339	-0.056	-0.283
BM-4	-0.344	-0.061	-0.233
BM-5	-0.339	-0.052	-0.287
BM-6	-0.337	-0.048	-0.233
BM-7	-0.359	-0.073	-0.286
BM-8	-0.361	-0.077	-0.284
BM-9	-0.340	-0.051	-0.289
BM-10	-0.345	-0.069	-0.275
BM-11	-0.332	-0.052	-0.280
BM-12	-0.342	-0.057	-0.284
BM-13	-0.330	-0.044	-0.286
BM-14	-0.326	-0.052	-0.274
BM-15	-0.335	-0.049	-0.286
BM-16	-0.357	-0.062	-0.293
BM-17	-0.316	-0.047	-0.279
BM-18	-0.335	-0.044	-0.291
BM-19	-0.334	-0.047	-0.287
BM-20	-0.346	-0.011	-0.335
BM-21	-0.346	-0.021	-0.325
BM-22	-0.352	-0.025	-0.327
BM-23	-0.348	-0.020	-0.327
BM-24	-0.348	-0.021	-0.326
BM-25	-0.349	-0.019	-0.330
BM-26	-0.350	-0.056	-0.304

E_{HOMO}, E_{LUMO} and ΔE calculations using Hamiltonian ZINDO and RHF-SCF (Restricted Hartree-Fock-Single consistent Field) method

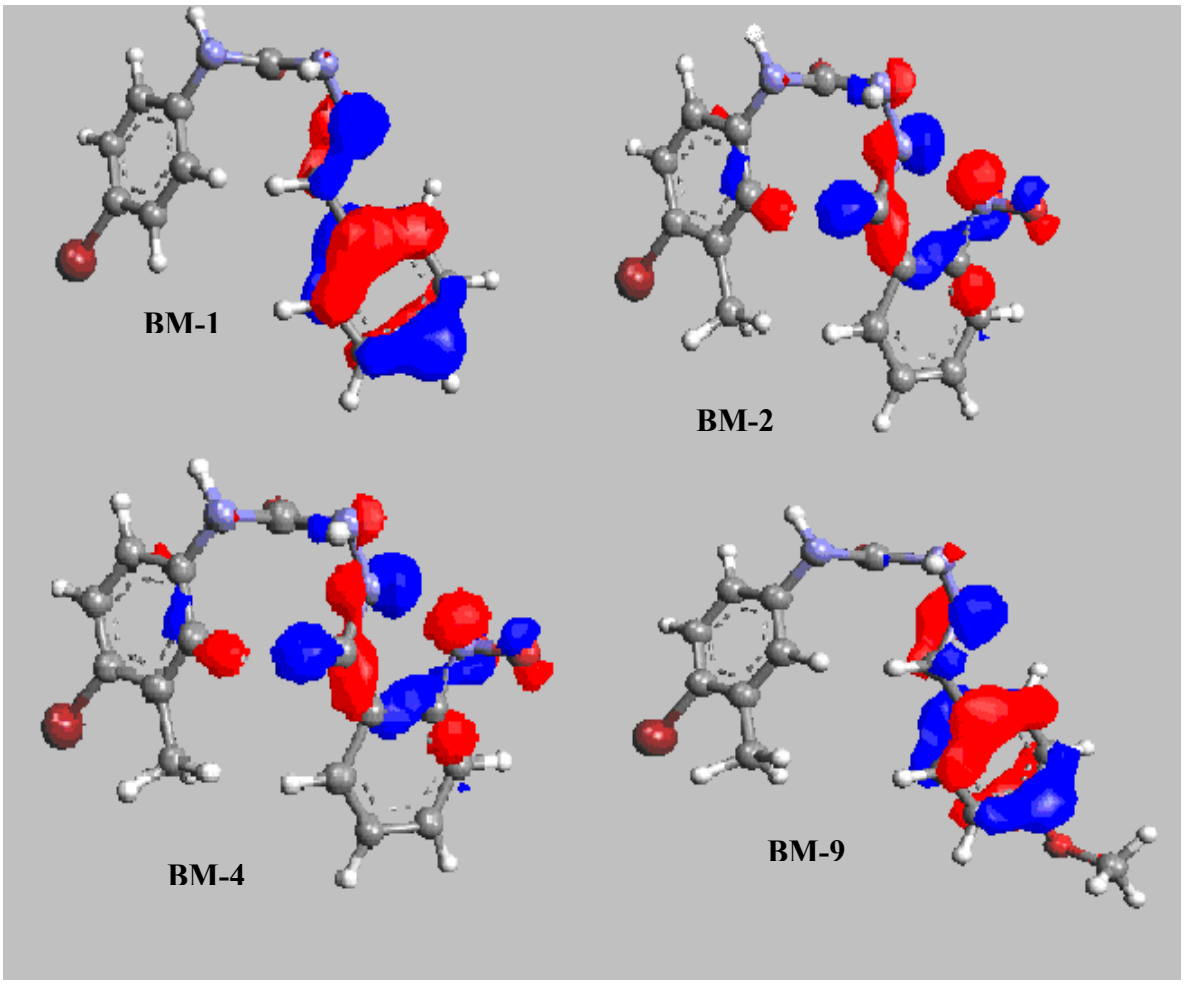


Fig. 14 HOMO surface visualization (Contour value = 0.05) of some representative compounds of 4-Bromo-3-methylphenyl semicarbazones in opaque mode. The colors indicate the phase of the orbital in space (blue for positive and red for negative).

8.3.3. 3-Methylpyridin-2-yl semicarbazones

Preliminary HOMO surface analysis for 3-methylpyridin-2-yl semicarbazones (Fig. 15) led to the following observations.

1. Presence of a delocalized orbital surface over an aryl ring along with the involvement of imine group (electron donor) and the amide moiety (hydrogen acceptor-donor unit) in binding with the receptor are essential for potent anticonvulsant activity. Similar surfaces were observed with all active compounds i.e. **PY-8** and **PY-16**. Compounds which did not comply with this hypothesis were found to be inactive i.e. compound **PY-9** and **PY-13**.
2. The ring deactivating nitro group caused a shift in electron cloud of the carbimino aryl ring as was observed with compound **PY-2**. Ring activating group i.e. CH_3 showed the opposite trend, retaining the electron cloud on the same aryl ring. i.e. **PY-8** and **PY-16**.
3. Unsymmetrical molecular orbital surfaces resulted in decrease in activity, as was observed with less active and inactive compounds of the series. i.e. **PY-2**.

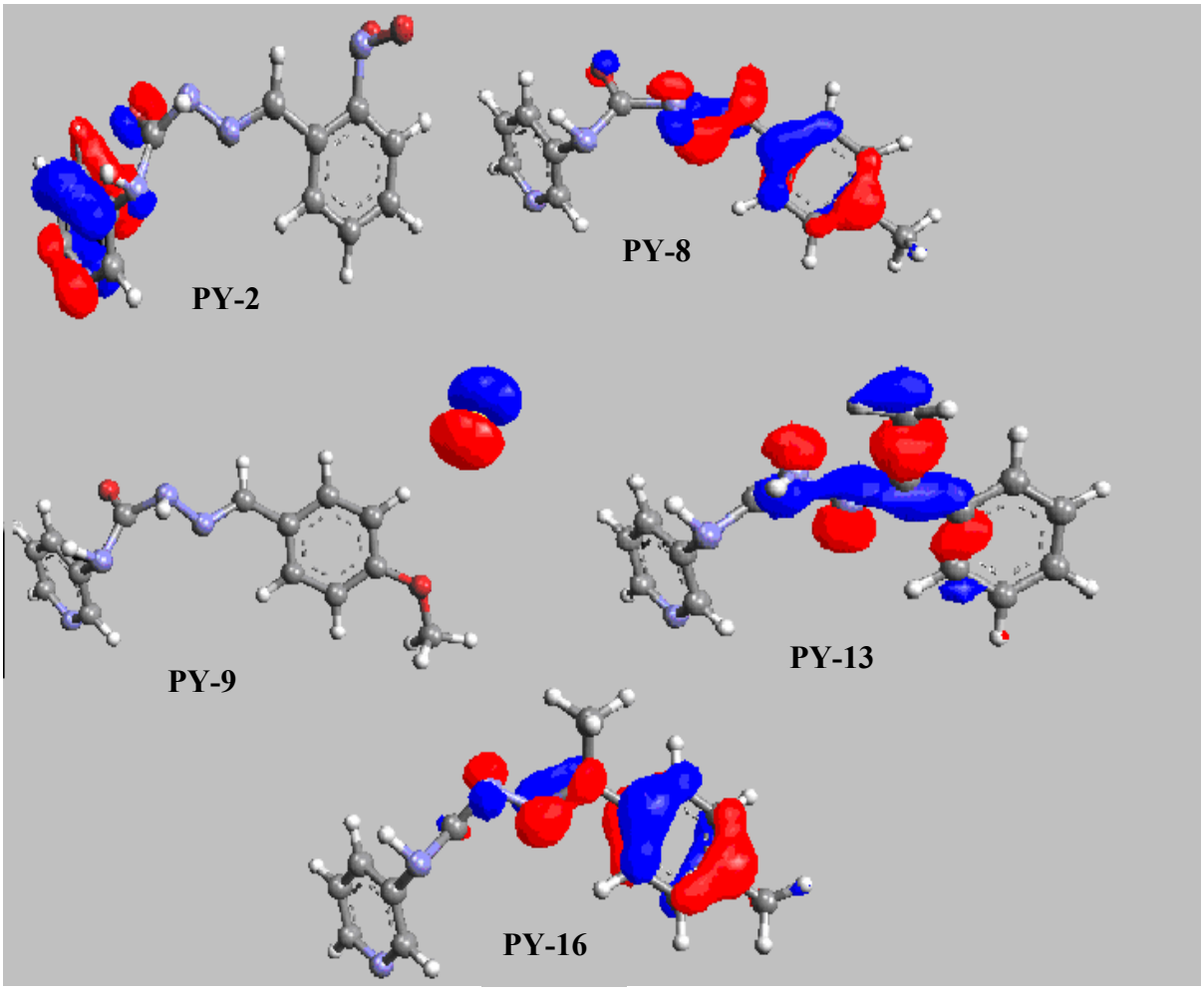


Fig. 15 HOMO surface visualization (Contour value = 0.05) of some representative compounds of 2-Amino-3-methylpyridyl semicarbazones in opaque mode. The colors indicate the phase of the orbital in space (blue for positive and red for negative).

8.3.4. 6-Chloro benzthiazol-2-yl semicarbazones

HOMO surface analysis of 6-chloro benzthiazolyl semicarbazones (Fig. 16) also further confirmed the proposed pharmacophoric model requirements for anticonvulsant activity i.e. presence of a delocalized orbital surface over an aryl ring along with the involvement of imine group (electron donor) and the amide moiety (hydrogen acceptor-donor unit). Most of the synthesized derivatives of 6-chloro benzthiazolyl semicarbazones showed high electron density over the hydrophobic area (benzthiazole ring) with no subsequent involvement of electron donor and hydrogen donor/acceptor unit, and in accordance with the pharmacophoric model, they did not displayed anticonvulsant activity in any of the seizure model.

Further the active derivatives of 6-chloro benzthiazolyl semicarbazones, **BZ-14** displayed better anticonvulsant activity in both MES and scPTZ model than **BZ-13**,that could be again due to the presence of comparative symmetrical and delocalized orbital surface over the aryl ring of **BZ-14**

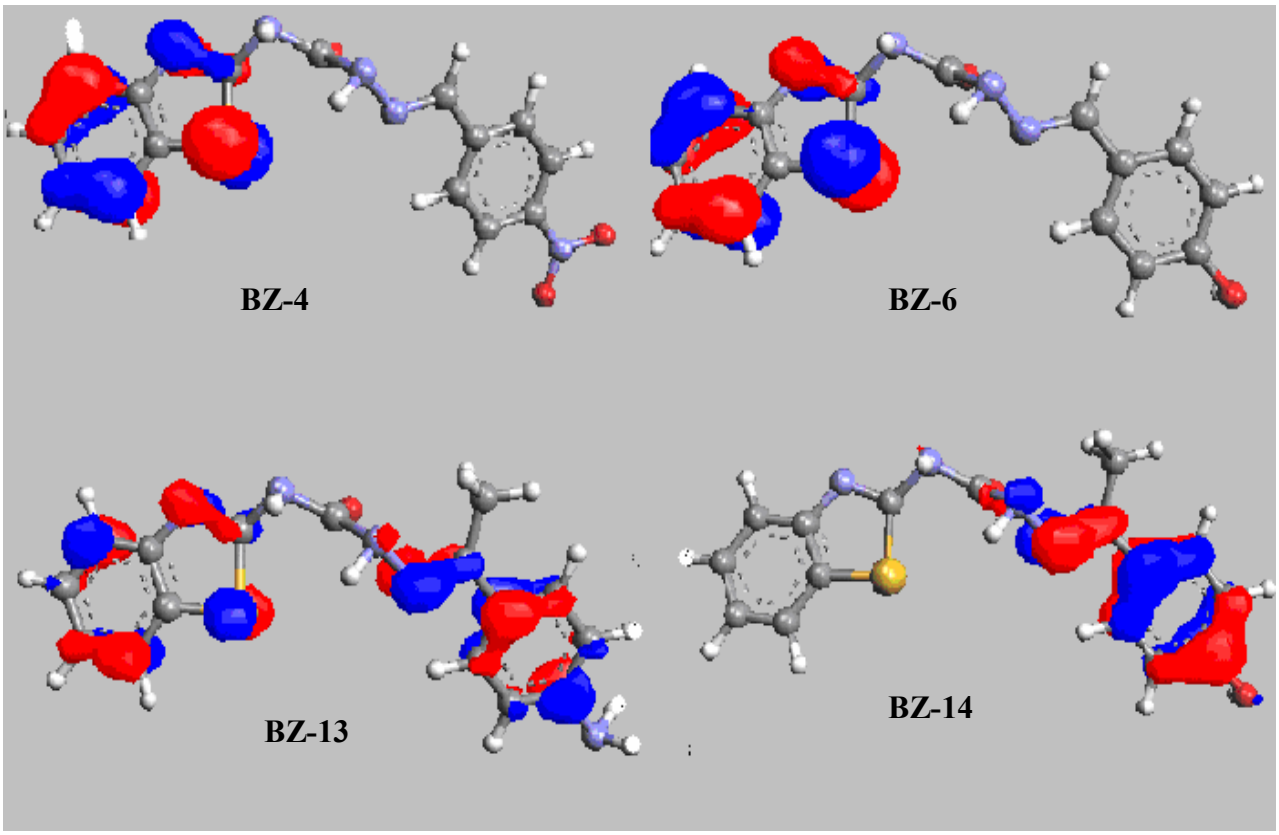


Fig. 16 HOMO surface visualization (Contour value = 0.05) of some representative compounds of 6-chloro benzthiazolyl semicarbazones in opaque mode. The colors indicate the phase of the orbital in space (blue for positive and red for negative).

8.3.5. Substituted diphenyl-2H-1,2,4-triazole-3-(4H)-one derivatives

Comparative HOMO surface analysis was done for some of the active and inactive triazole derivatives (Fig.17). This study also proved very clearly that the presence of delocalized orbital surfaces over an aryl ring and imine group (electron donor) and the amide moiety (hydrogen acceptor-donor unit) are very much essential for potent anticonvulsant activity. The triazole derivatives accomplishing these pharmacophoric requirements were found to possess near about similar anticonvulsant activity profile i.e. compound **TR-6** and **TR-11**. Further, in accordance with the proposed hypothesis unsymmetrical/undelocalized molecular orbital surfaces on the aryl ring resulted in decreased activity, as was observed with inactive derivative **TR-7** and also absence of electron cloud on hydrogen bonding domain and electron donor system led to other inactive compound **TR-10.** Similar trends for anticonvulsant activity and HOMO surfaces were observed for other active/ inactive analogues of 1,2,4-triazole-3-ones and their corresponding open chain analogues.

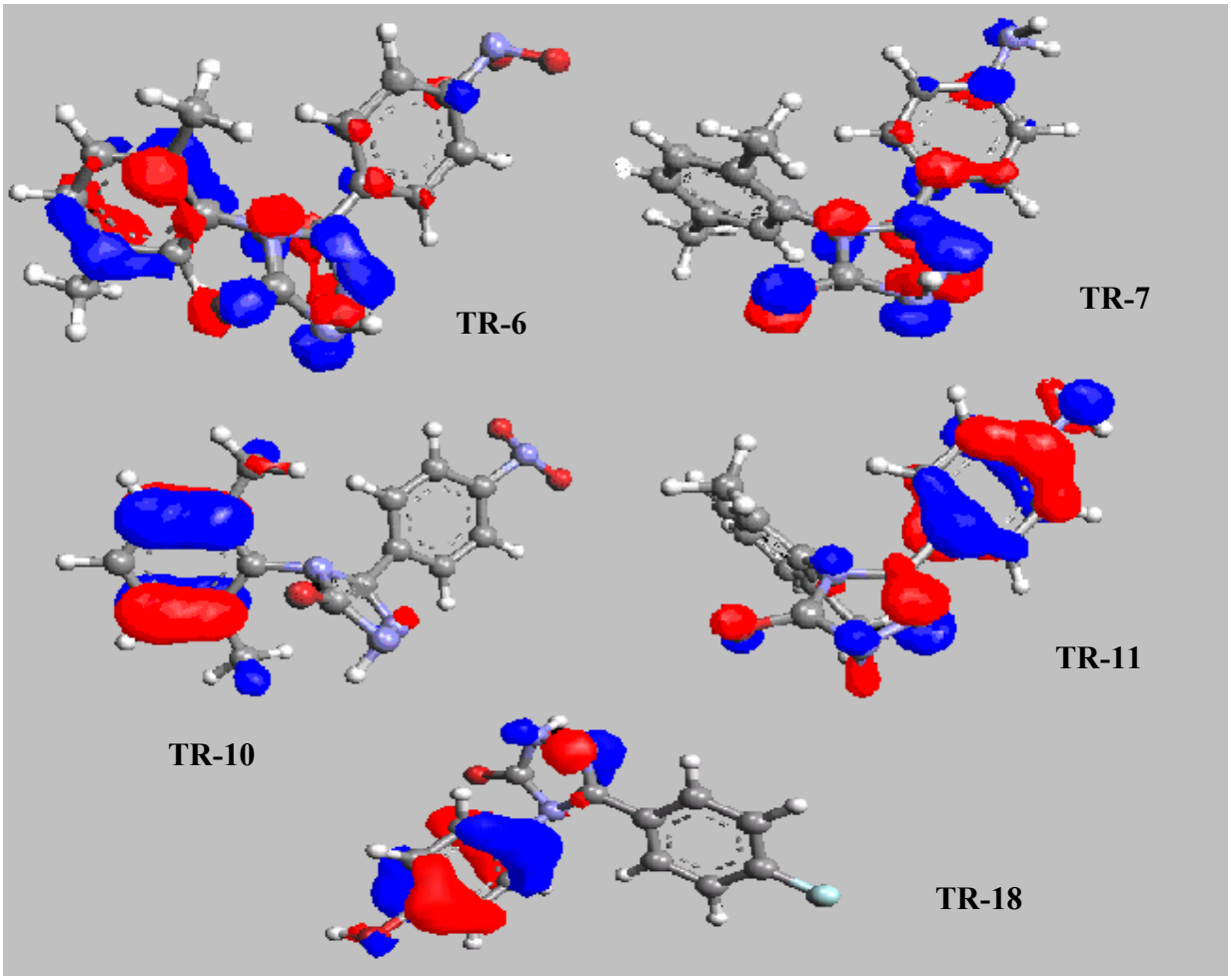


Fig. 17 HOMO surface visualization (Contour value = 0.05) of some representative compounds of substituted diphenyl-2H-1,2,4-Triazole-3(4H)-one derivatives and their open chain analogues in opaque mode. The colors indicate the phase of the orbital in space (blue for positive and red for negative).

CHAPTER 9

SUMMARY AND CONCLUSIONS

Totally six series of compounds (130 compounds), three of disubstituted phenyl semicarbazones, two of heteroaryl semicarbazones and one of cyclized derivatives of aryl semicarbazones i.e. 1,2,4-triazoles, were designed and synthesized.

2,5-Dimethylphenyl semicarbazones (**DM** series) and 4-bromo-3-methylphenyl semicarbazones (**BM** series) were synthesized by microwave-assisted synthesis. 2-fluoro-5-methylphenyl semicarbazones (**FM** series) were synthesized by semicarbazide hydrochloride salt method. The **PY** series (3-methylpyridin-2-yl semicarbazones) was prepared by nitrourea method, while **BZ** series (6-chloro benzthiazolyl semicarbazones) was prepared by phenyl carbamate method. The **TR** series (substituted phenyl-1,2,4-triazole-3-ones) was derived by the condensation of the substituted benzoyl chlorides and substituted phenyl semicarbazides.

Purity of the compounds was ascertained by TLC and their structures were elucidated by spectral (IR, ^{1}H-NMR, Mass) and elemental analyses.

The synthesized compounds were evaluated for anticonvulsant activity in MES, scPTZ, scSTY and scPIC seizure models as well as for other CNS side effects like neurotoxicity, CNS depression, behavioral impairment etc.

In the MES test, compounds were screened after *i.p.* and oral administration. Most of the compounds exhibited high potency after *i.p.* administration but not in the oral MES screen.

Out of the synthesized one hundred and thirty compounds, seventy and fifty compounds exhibited protection in MES and scPTZ-induced seizures respectively. Some selected (64) compounds were screened in the scSTY and scPIC-induced seizure models and fifty-five compounds showed protection.

Most of the compounds exhibited lesser or no neurotoxicity in the rotarod test but showed marked CNS depression and behavioral side effects. Substituted pyridine derivatives (**PY** series) and triazole derivatives (**TR** series) exhibited least CNS depressant effects, even lesser than the standard drug Carbamazepine.

Neurochemical studies of the most active compounds, revealed a marked increase in the GABA level in different regions/whole of rat brain.

Most of the designed compounds exhibited broad spectrum of activity i.e. showed protection in more than one seizure model.

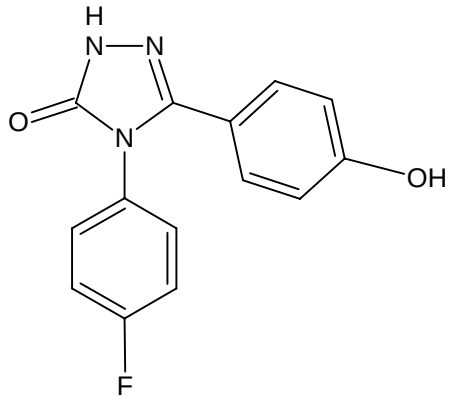
The anticonvulsant activity of the synthesized compounds revealed that substitution at the *ortho* and *para* position of the phenyl ring with electron rich group i.e. –Br or -CH_3, was beneficial and resulted in a marked increase in the anticonvulsant activity of the synthesized compounds.

Replacement of the aryl ring of the phenyl semicarbazones with heteroaryl ring i.e. with pyridine resulted in equipotent derivatives, but bulkier benzthiazolyl ring showed tremendous decrease in anticonvulsant activity. Furthermore, the cyclization of the semicarbazone moiety was found to be more beneficial for anticonvulsant activity, which might be due to the restricted rotation about the hydrogen bonding domain. Substituted triazole derivatives (**TR**-series), emerged as the most active among the designed compounds.

The quantum mechanical modeling of the synthesized compounds also established the above drawn conclusions and further confirmed the anticonvulsant pharmacophore requirements for aryl semicarbazones to be an aryl ring, a hydrogen-bonding domain and an electron-donor system, by the HOMO surface analysis, wherein compounds with delocalized orbital surface over an aryl ring, imine group (electron donor), and the amide moiety (hydrogen acceptor-donor unit) exhibited higher anticonvulsant activity.

The most potent compound was found to be compound **TR-18** (4-(4-fluorophenyl)-5-(4-hydroxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one), which exhibited a broad spectrum

of activity i.e. protection in all the four seizure models with lesser neurotoxicity and behavioral side effects. The compound resulted in a 10-fold increase in the GABA level in rat brain when compared to the control.



TR-18

4-(4-Fluorophenyl)-5-(4-hydroxyphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one

FUTURE PERSPECTIVES

Although all the synthesized compounds have been found to possess a broad spectrum of activity, exhaustive studies are still required to confirm the pharmacodynamic/pharmacokinetic properties such as deliverable formulation, bioavailability, attainment of effective cerebral to plasma concentration ratio and low toxicity which render them as useful anticonvulsants.

Furthermore, exhaustive QSAR studies and QM modeling should also be done to predict the effect of various factors imparting beneficial anticonvulsant properties to the designed compounds.

Future studies should extend to other seizure models like bicuculline, NMDA etc., to study their effects on these seizure models and also further neurochemical investigation to study their effects on other neurotransmitter levels in the brain i.e. glycine, to explore other mechanisms behind their anticonvulsant effects. Extension of the preclinical studies to different age groups of the animals is also required to be studied as epilepsy is a disorder of all age groups.

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APPENDIX

LIST OF PUBLICATIONS

- **From thesis work**

1. **M. Shalini**, P. Yogeeswari, D. Sriram, P. Kudyar, P. Roheeth Kumar, S. Induja and J.P. Stables , "Quantum Mechanical Modeling of N^4-(2, 5-Disubstituted phenyl) semicarbazones: Synthesis and anticonvulsant activity of N^4-(2, 5-dimethylphenyl/-2-fluoro-5-methyl phenyl) semicarbazones", Med. Chem., Revised version communicated. (Bentham Publication).
2. **M. Shalini**, P. Roheeth, P.Yogeeswari, D. Sriram and J. Stables, "Heteroaryl-Substituted Semicarbazones: Synthesis and Anticonvulsant activity of *N*-(3-methylpyridin-2-yl)-substituted semicarbazones", J. Hetero. Chem., In press. (Genamics Publication)
3. **M. Shalini**, P. Yogeeswari, D. Sriram and J.P. Stables, "Cyclization of the semicarbazone template of aryl semicarbazones: Synthesis and anticonvulsant activity of 4,5-diphenyl-2*H*-1,2,4-triazol-3(4*H*)-one", Eur. J. Med. Chem., Communicated. (Elsevier Publication)
4. **M. Shalini**, P.Yogeeswari, D. Sriram, S. Induja and J. Stables, "Microwave-assisted Synthesis, Anticonvulsant activity and Quantum mechanical modeling of N-(4-Bromo-3-Methylphenyl) semicarbazones", J. Zhej. Uni. Sci., Communicated. (Zhejiang University Science-Chinese Publication)

- **Other publications**

5. P.Yogeeswari, D.Sriram, V.Veena, R.Kavya, K.Rakhra, J.V.Ragavendran, **M.Shalini**, R. Thirumurugan and J.P.Stables, "Synthesis of aryl semicarbazones as potential anticonvulsant agents", Biomed. Pharmacother., 59, 51-55, 2005.
6. P.Yogeeswari, D.Sriram, **M.Shalini**, D.Nigam, M.M.Kumar, S.Murugesan and J.P.Stables, "Anticonvulsant and neurotoxicity evaluation of some 6-substituted benzthiazolyl-2-thiosemicarbazones", Farmaco, 60, 1-5, 2005.

Abstracts in Symposium/conferences:

1. **M. Shalini**, P.Yogeeswari, D. Sriram, J. P. Stables, "Synthesis of some 4,5-(substituted diphenyl)-2H-1,2,4-triazole-3(4H)-one derivatives and their anticonvulsants properties", International symposium on Recent Advances in Drug Design and Drug Delivery System, Pilani, February 26-27, 2005.
2. J.V. Ragavendran, **M. Shalini**, D. Sriram, P.Yogeeswari, "Neuropharmacology of new leads", 6th EMBL International PhD students symposium on Animal Model-Tips and Tricks from Nature, Rome, May 12-14, 2005.
3. **M. Shalini**, P.Yogeeswari, D. Sriram and J. P. Stables, "Effect of cyclization of semicarbazone moiety on the anticonvulsant activity of aryl semicarbazones", 57th Indian Pharmaceutical Congress, Hyderabad, December 2-5, 2005.
4. **M. Shalini**. J. V. Ragavendran, K. Roheeth, D. Sriram, P. Yogeeswari, "Quantum mechanical modeling of aryl semicarbazones as anticonvulsants", 2nd International symposium on Drug Discovery and Process Research, Belgaum-590 010, February, 10-12, 2006.
5. **M.Shalini,** P.Yogeeswari, D. Sriram, S.Induja, "Microwave-assisted synthesis, snticonvulsant activity and quantum mechanical modeling of N-(4-bromo-3-methylphenyl) semicarbazones", National symposium on Challenges in Drug Discovery Research: Networking Opportunities between Academia and Industry, BITS, Pilani, April 7-8, 2006 (accepted).

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BIOGRAPHY OF SHALINI MEHTA

Miss Shalini Mehta, has completed her Bachelors degree in Pharmacy from Guru Jambheshwar University, Hissar, Haryana, in the year 2001 and post graduation from Birla Institute of Technology and Science, Pilani, Rajasthan in 2003. She had been working as a research scholar at BITS, Pilani from 2003-2006 during which she worked on DST SERC Fast Track project. She has few publications in well renowned international journals.

BIOGRAPHY OF Dr. P.YOGEESWARI

Dr. P. Yogeeswari is presently working in the capacity of Assistant Professor at Pharmacy Group, Birla Institute of Technology and Science, Pilani, Rajasthan. She received her Ph.D. degree in the year 2001 from Banaras Hindu University; Varanasi. She has been Involved in Research for the last 9 yrs and in teaching for 8 yrs. She has collaborations with various national and international organizations that include National Institute of Health, Bethesda, USA, National Cancer Institute, 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 50 research publications and also is the 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 Rresources (Taylor and Francis). She is a lifetime member of Association of Pharmacy Teachers of India and Indian Pharmacological Society. As a result of her research accomplishment, she is presently handling two projects, one of DST SERC Fast Track under Young Scientist Scheme and other of CSIR, as principal investigator. In addition she is working on two another projects of CSIR and ICMR. Currently she is guiding two Ph.D students