Design and Synthesis of Novel Disubstituted Phenyl Semicarbazones and their Analogues as Anticonvulsants

THESIS

Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

By
R.THIRUMURUGAN

Under the supervision of
Dr.D.SRIRAM

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN) INDIA
2004

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN) INDIA

CERTIFICATE

This is to certify that the thesis entitled "**Design and Synthesis of Novel Disubstituted Phenyl Semicarbazones and their Analogues as Anticonvulsants**" and submitted by **R.Thirumurugan, ID.No. 2001PHXF020**, for the award of Ph.D. degree of the Institute, embodies the original work done by him under my supervision.

Signature in full of the supervisor

Name in capital block letters: **D.Sriram.**

Date: 17.11.2004

Designation: Lecturer.

Dedicated to my beloved parents

Acknowledgments

First I offer my prayers to Shri Ghanshyam Das Birla, Founder Chairman of this esteemed Birla Institute of Technology and Science for his altruism.

I am grateful to Prof. S. Venkateswaran, Vice-Chancellor, BITS, Pilani, (formerly Director) for allowing me to carryout my doctoral research work in the Institute.

My sincere thanks are due to Prof. L.K. Maheswari, Director, BITS, Pilani, for the encouragement and support during the course of my thesis work.

Often, it gives me immense pleasure to remember his ever smiling face that imparted me the courage to enjoy the cumbersome work and no words could adequately express the deep sense of gratitude I owe to my beloved supervisor, Dr. D. Sriram, Lecturer, Pharmacy Group, BITS, Pilani. He has been wonderful, being a consistent source of vast guidance and inspiration throughout the deal by sparing his valuable time for numerous discussions on the subject at every stage of its progress.

I am thankful to Prof. Ravi Prakash, Dean, Research and Consultancy Division, BITS, Pilani, for his co-operation and encouragement during various stages of this work.

I am happy to express my sincere thanks to Prof. R.N. Saha, Dean, Educational Development Division and Faculty Division III, BITS, Pilani, for his constructive suggestions and patience, which are inexpressible in a few words.

I would like to express my gratitude to Dr. R. Mahesh, Group Leader, Pharmacy Group, BITS, Pilani, for his support, encouragement and constructive criticism and for providing various laboratory facilities of the Group.

I sincerely acknowledge the encouragement and support lent by Dr. P. Yogeeswari, Lecturer, Pharmacy Group, BITS, Pilani, who has been kind enough to guide me in all possible ways at any time of need without hesitation.

I am very much grateful to Dr. S.M. Ray, Assistant Professor, Pharmacy Group, BITS, Pilani, for his more valuable and dexterous suggestions during my experimental work.

I am thankful to all the staff members of the Pharmacy Group, BITS, Pilani for their wholesome co-operation, extra-ordinary patience and voluntary help extended to me.

I am grateful to Dr. James P. Stables, Program Director, Epilepsy Unit, National Institutes Health, Bethesda, Maryland, U.S.A., for his kindness in evaluating my samples for anticonvulsant activity.

I appreciate Mr. A.R. Subramanian, PS Faculty, CDRI, Lucknow, for his magnanimous help in the NMR sample analysis.

Lines are insufficient to express my special thankfulness to my friends Dr. Ajay Pratap Singh, Dr. Satyan, S. Lakshmana Prabhu, C. Selvam, and Meenakshi Sharma for their avuncular support. Their constant source of encouragement and inspiration had given me omnipotence during my work.

I would like to express my heartfelt thanks to Suniljit, Vaigunda Raghavendran, Jaffer Sadik Basha, Murugesan, Mohan Kumar, Induja Sridharan, R. Kavya, Selwin Samuel, Kavya Rakhra, Jyothi Mallika, Pavan Kumar, M.S. Muthu, T.G. Ashok Kumar, Amrita Saxena and Roheeth Kumar for their help and co-operation during my experimental work.

My sincere appreciation goes to my friends P. Vijaya Pandi, R. Venkatesa Perumal, Venugopal, Snehalatha, S. Kumaran, Tanushree Ratan Bal, Shalini Mehta, A. Ashok Kumar, K.V.G. Chandra Shekar, Joy Scaria, Ramachandran, Vishal Saxena, Praveen S. Hiremath, Balasubramanian, Anand, Kanagaraj, Sheshadri, Harmeet Dhillon, Rajendra Khapre, Ragul V. Ralegaonkar, Vijay Kumar, Siva Prakash and Girish S. Bende who had been a moral support during my stay here at Pilani.

Thanks are due to all the office staff of Pharmacy Group, BITS, Pilani, M.P. Soniji, Sita Ramji, Matu Ramji, K.M. Sharmaji, Gokul Prasadji, Hari Ramji, Yasin Nilgarji and Mr. Navin Rana for their help throughout the course of work.

Special thanks are due to Dr. S.D. Pohekar and the office staffs of Research and Consultancy division, BITS, Pilani, Raghuveer Singhji, Soniji and Mahipalji for various help at different point of time during the course of this work.

I highly acknowledge the assistantship provided by the institute and the Senior Research Fellowship of Council of Scientific and Industrial Research.(CSIR), for providing financial support during my research tenure.

I will be a sinner, if I don't sympathize the sacrifices, though for the betterment of mankind, of the animals made during my studies.

I highly appreciate Mr. Ramesh Sharma for typing this thesis meticulously.

Above all, I bow before the feet of my parents, family members for their understanding and affection towards me, which have been my rock of strength in keeping my morale high.

Date: 17. 11. 04

R. Thirumurugan

List of figures

List of tables

List of abbreviations

ADD - Antiepileptic drug development/After discharge duration
AED - *Antiepileptic* drug
CHARMM - Chemistry at Harvard Macromolecular Mechanics
CNS - Central nervous system
DMSO - Dimethyl sulphoxide
DNPH - 2,4-dinitro phenyl hydrazine
EAA - Excitatory amino acid
ED_{50} - Effective dose in 50 % population
Eqn - Equation
Fig - Figure
GABA - Gamma amino butyric acid
GABA-T - Gamma amino butyric acid transaminase
GAD - Glutamate decarboxylase
^{1}H-NMR - Proton nuclear magnetic resonance
Hz - Hertz
i.p - intra peritoneal
IR - Infra red
IU - International unit
MAO - Mono amine oxidase
MES - Maximal electro shock seizure
mg/kg - milligram/kilogram
MM3 - Molecular mechanics 3
MS - Mass spectroscopy
n - Number of animals
NAD - Nicotinamide adenine dinucleotide
NADH - Nicotinamide adenine dinucleotide hydride
NMDA - N-methyl-D-aspartate
NS - not significant

NT	- Neurotoxicity
P/p	- Partition coefficient / probability
PEG	- Polyethylene glycol
PI	- Protective index
p.o	- per oral
ppm	- parts per million
PS	- Protective score
R	- Correlation coefficient
RMS	- root mean square
s	- Standard deviation
s.c	- subcutaneous
scPTZ	- subcutaneous pentylenetetrazole
scPIC	- subcutaneous picrotoxin
scSTY	- subcutaneous strychnine
SEM	- standard error in mean
SGOT	- Serum glutamate oxaloacetate transaminase
SGPT	- Serum glutamate Pyruvate transaminase
SSADH	- Succinic semialdehyde dehydrogenase
TD_{50}	- Toxic dose in 50 % population
TLC	- Thin layer chromatography
TMS	- Tetra methyl silane
TOX	- Toxicity
TPE	- Time of peak effect
UV	- Ultra violet

Table of contents

CHAPTER-I

INTRODUCTION

Epilepsy is a chronic and often-progressive disorder characterized by the periodic and unpredictable occurrence of seizures that is caused by abnormal discharge of cerebral neurons (1). Epilepsy is not a disease, but a syndrome of different cerebral disorders of the central nervous system (CNS), which is characterized by paroxysmal, excessive and hypersynchronous discharges of large number of neurons (2). These discharges disrupt normal brain functions by creating fluctuations in electrochemical balance. Seizures are sudden and transitory episodes of abnormal phenomena of motor, sensory, automatic or psychic origin. The motor episodes are termed convulsions. These seizures are nearly always correlated with abnormal and excessive discharges in the brain, which can be recorded on an electroencephalogram (EEG) (3). In addition, epileptic seizures are seen in many illnesses that directly or indirectly involve the brain, including ionic and electrolyte imbalances, disorders of carbohydrate, amino acid and lipid metabolism, infections, brain tumors, brain trauma and elevation of body temperature in the young (2, 4, 5).

Types of Seizure: -

Seizures are conventionally categorized into four major classes: -

- a) Partial seizures
- b) Generalized seizures
- c) Undetermined seizures and
- d) Special syndromes

(a) Partial Seizures: -

Partial seizures are epileptic episodes in which excessive neuronal discharges remain localized within a focal area of the brain. These seizures may or may not impair consciousness. Traditionally, partial seizures include temporal lobe seizures and focal motor seizures. Partial seizures are generally characterized by convulsions confined to a single limb; muscle group or specific localized sensory disturbances usually without impairment of consciousness. This type of seizure may become secondarily generalized (6).

(b) Generalized Seizures: -

Generalized seizures impair consciousness. By convention, this class includes those seizures known as grand mal, petit mal, and minor seizures.

6. Juvenile myoclonic epilepsy (impulsive petit mal)
7. Epilepsy with grand mal (GTCS) seizures on awakening
8. Other generalized idiopathic epilepsies not defined above
9. Epilepsies with seizures precipitated by specific modes of activation

B. *Cryptogenic* or *symptomatic* (in order of age)

1. West syndrome (infantile spasms)
2. Lennox- Gastuat syndrome
3. Epilepsy with myoclonic absences

C. *Symptomatic*

1. Nonspecific etiology
    a) Early myoclonic encephalopathy
    b) Early infantile epileptic encephalopathy with suppression burst
    c) Other symptomatic generalized epilepsies not defined above
2. Specific syndromes

III. Epilepsies and syndromes undetermined whether focal or general

A. *With both generalized and focal seizures*

1. Neonatal seizure
2. Severe myoclonic epilepsy in infancy
3. Epilepsy with continuous spike-waves during slow wave sleep
4. Acquired epileptic aphasia (Landau-Kleffner syndrome)
5. Other undetermined epilepsies not defined above.

B. *Without unequivocal generalized or focal features*

IV. Special syndromes

A. *Situation-related seizures* (Gelegenheitsanfalle)

1. Febrile convulsions
2. Isolated seizures or local status epilepticus
3. Seizures occurring only when there is an acute metabolic or toxic event due to alcohol, drugs, eclampsia, or nonketotic hyperglycemia.

Approximately 50 million people worldwide have epilepsy; making this condition the second leading neurological disorder (8). In India, studies have reported the prevalence rate varying from 171 to 978 cases per 1,00,000 population (9). It is estimated that 25% of the epileptic population have seizures that are not responsive to presently available medical therapies (8). The incidence of epilepsy is higher within the first decade of life and more particularly, in the first year of life (6). Males tend to have a higher prevalence and incidence of epilepsy than females (6). Despite the optimal use of available antiepileptic drugs, many patients with epilepsy fail to experience seizure control and others do so only at the expense of significant toxic side effects. Estimates suggest that the available medication controls the seizures in only 50% of patients or decreases the incidence in only 75% of patients (10).

Although many new antiepileptic drugs (AEDs) have been introduced in the last decade, but still approximately about 30% of patients remain pharmaco-resistant (11) and have not benefited significantly from the introduction of several new AEDs since 1993. Furthermore, none of the currently marketed AEDs have been shown to prevent the development of epilepsy in susceptible individuals after a variety of central nervous system (CNS) insults including infection, trauma, or febrile seizures (12-15). The poor success in identifying novel therapies that may prevent the development of epilepsy and pharmaco-resistance has been partially the result of lack of experimental models that closely approximate chronic human epilepsy syndromes as well as our poor understanding of the pathophysiological basis for pharmaco-resistance and epileptogenesis.

Epileptogenesis is the process by which normal central nervous system tissue is transformed into brain tissue prone to the manifestations of spontaneous recurrent seizures (16). Neuronal injury in the form of stroke is the most common factor associated with epileptogenesis and acquired epilepsy (17).

Twenty years ago, the selection of AEDs for individual patients relied upon a combination of clinical experience, personal reference and serendipity. The last two decades have witnessed unprecedented progress in the pharmacotherapy of epilepsy with the introduction of nine new drugs and considerable advance in our understanding of how AEDs exert their effect at the cellular level.

Anticonvulsant's mechanism of Action (Fig. I.1):

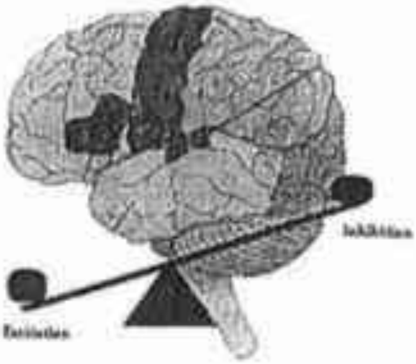


Figure I.1: Representation of epileptic condition.

At the cellular level, three major mechanisms of action have been recognized,

1. Modulation of voltage gated ion channel
2. Enhancement of γ-amino butyric acid (GABA) mediated inhibitory neurotransmission
3. Attenuation of glutamate mediated excitatory transmission (18, 19).

(1) Modulation of Voltage gated ion channels: -

The intrinsic excitability of the nervous system is ultimately controlled by voltage gated ion channels, which regulate the flow of cations across surface and internal cell membranes. Voltage dependent sodium channels are responsible for depolarization of the cell membrane and the characteristic upstroke of the neuronal action potential. Voltage sensitive calcium channels are similarly involved in depolarization, often recruited in response to initial sodium dependent action potential generation. Calcium channels are distributed throughout the nervous system on dendrites, cell bodies and nerve terminals. The N, P and Q-type calcium channels have been implicated in the control of neurotransmitter release at the synapse, whereas the T-type channel, expressed predominantly in thalamocortical relay neurons, is believed to play a role in the distinctive rhythmic discharges of generalized absence seizures. (18-20).

Blockade of voltage gated sodium channels is the most common mechanism of action amongst the currently available AEDs. The established agents Phenytoin and Carbamazepine are archetypal sodium channel blockers and the newer drugs Lamotrigine, Felbamate, Topiramate and Oxcarbazepine also share this mechanism. There is also evidence that Sodium Valproate and Gabapentin have effects on sodium channels. In the majority of cases, these drugs bind to the inactivated state of the sodium channel and produce a voltage and frequency dependent reduction in channel conductance, resulting in a limitation of repetitive neuronal firing with little or no effect on the generation of single action potentials (19, 21).

Voltage gated calcium channels represent a major target for AEDs. The efficacy of Ethosuximide against generalized absence seizures is believed to mediate by blockade of T-type calcium channel. Anecdotal evidence suggests that Sodium Valproate may have similar effects. In addition, Lamotrigine has been reported to limit neurotransmitter release by blockade of the N-and P-subtypes of voltage sensitive calcium channel, while Gabapentin binds to the $\alpha_2 \delta$ subunit of the L-type channel. Felbamate and Topiramate also appear to influence calcium channel conductance, although these effects are less well characterized in terms of specificity for individual channel subtypes.

(2) **Enhancement of Inhibitory Neurotransmission: -**

GABA is the predominant inhibitory neurotransmitter in the mammalian central nervous system where it is released at up to 40% of all synapses. Following synaptic release, GABA acts on three specific receptors, $GABA_A$, $GABA_B$, and the newly characterized $GABA_c$, which are distinguished by their pharmacology, function and anatomical distribution. The $GABA_A$ receptor, with its pentameric subunit array and central chloride ion pore, is perhaps the best characterized. It belongs to the ligand-gated ion channel super family and responds to GABA binding by increasing chloride conductance, resulting in neuronal hyperpolarisation. GABA is removed from the synaptic cleft into localized nerve terminals and glial cells by specific transport molecules, four of which (GAT-1; GAT-2; GAT-3 & BGT-1) have been described. Thereafter, GABA is either recycled to the readily releasable neurotransmitter pool or metabolized by the action of the mitochondrial enzyme GABA-Transaminase (GABA-T) thereby completing the cycle (18, 19) (Fig I.2)

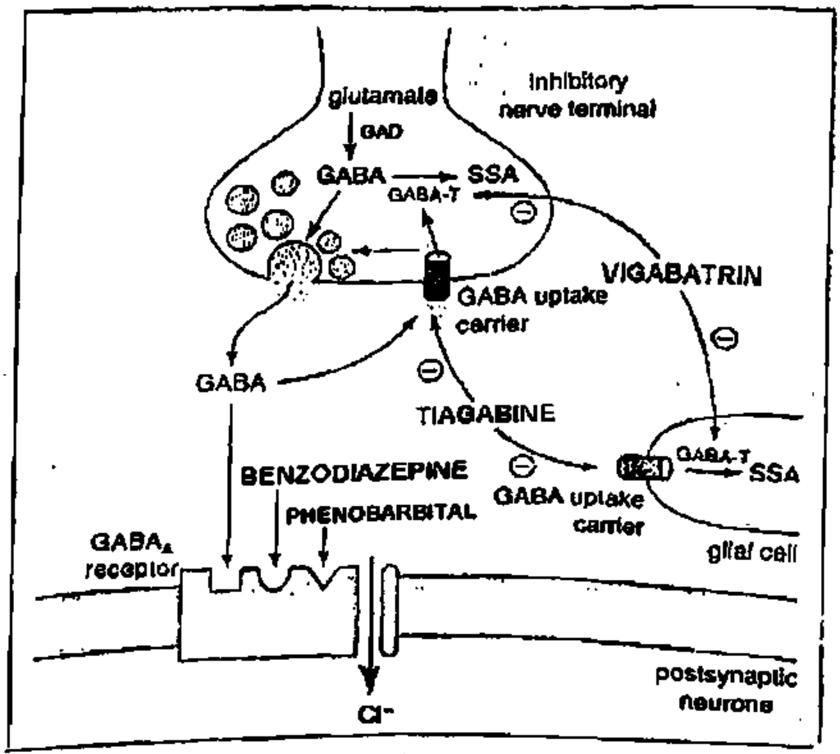


Fig I.2: Methods of augmenting GABAergic transmission.

Several AEDs exert their pharmacological effects on the GABA system. The established agents Phenobarbital and the benzodiazepines bind to distinct sites on the $GABA_A$ receptor complex and exert an allosteric influence on the opening of the chloride ion channel in response to GABA. Phenobarbital increases the duration of channel opening, while the benzodiazepines increase the frequency of opening. Felbamate and Topiramate also activate the $GABA_A$ receptor and these effects are less well characterized. Current evidence suggests that they have similarly distinct binding sites and different effects on chloride channel kinetics. The contemporary AEDs, Vigabatrin and Tiagabine exert their antiepileptic actions by selective effects at the GABA synapse. Vigabatrin is an irreversible inhibitor of the enzyme GABA-T, while Tiagabine prevents the uptake of GABA from the synaptic cleft by blockade of the GAT-1 transporter. These mechanisms are importantly distinguished by their neurochemical consequences.

Inhibition of GABA metabolism results in the global elevation of brain GABA concentrations, whereas blockade of GABA uptake temporarily prolongs the presence of neuronally released GABA in the synapse. Other AEDs including Sodium Valproate, Gabapentin and Topiramate, have also been proposed to influence GABA- ergic neurotransmission by increasing the synthesis and/or release of GABA (18, 19).

(3) **Attenuation of Excitatory Neurotransmission: -**

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following synaptic release, it exerts its effects on both ionotropic and metabotropic receptor types. The ionotropic glutamate receptors are arguably the best characterized and are classified into three specific subtypes, AMPA, Kainate and NMDA, which form Ligand-gated ion channels permeable to sodium and depending on subtype and subunit composition, calcium ions (18). The AMPA and Kainate subtypes are implicated in fast excitatory neurotransmission, whereas the NMDA receptor, quiescent at resting membrane potential, is recruited during periods of prolonged depolarization (19).

Despite attracting considerable attention in the ongoing search of novel AEDs none of the currently available agents exert their pharmacological effects solely by an action on the glutametergic system. Nevertheless, blockade of NMDA subtype of glutamate receptor has been reported to contribute to the antiepileptic effects of Felbamate. Topiramate is similarly distinguished by an inhibitory action on the

AMPA receptor. In addition, several AEDs have been reported to reduce glutamate release, although this effect may be more indicative of their actions on calcium channels than a direct effect on the glutamate system. (18, 19).

Table 1.1: Proposed Pharmacological targets of Antiepileptic drugs (19, 20): -

S.No.	Drugs	Na^+ channels	Ca^{2+} channels	GABA receptors	GABA synapse	Glutamate receptors
1	Phenobarbital	-	-	+++	-	-
2	Phenytoin	+++	-	-	-	-
3	Ethosuximide	-	+++	-	-	-
4	Carbamazepine	+++	-	-	-	-
5	Sodium Valproate	+	+	-	++	-
6	Benzodiazepines	-	-	+++	-	-
7	Vigabatrin	-	-	-	+++	-
8	Lamotrigine	+++	++	-	-	-
9	Felbamate	++	++	++	-	++
10	Gabapentin	+	+	-	++	-
11	Topiramate	++	++	++	+	++
12	Tiagabine	-	-	-	+++	-
13	Oxcarbazepine	+++	+	-	-	-
14	Leviracetam	?	?	?	?	?

+++ ⇒ Primary target

++ ⇒ Probable target

\+ ⇒ Possible target

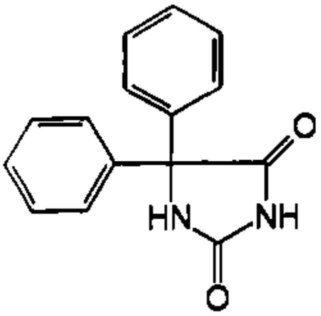
? ⇒ Unknown

Most of the antiepileptic drugs identified by screening methods have more than one molecular mechanism of action. Among the recently evaluated agents, Topiramate and Felbamate possess two or three key mechanisms of action. Topiramate and Felbamate potentiate GABA, prolong inactivation of sodium channels and also block AMPA or NMDA receptors (21). Models of acquired epilepsy are associated with multiple changes in receptors and ion channels. All synaptic systems are subject to modulatory control systems that tend to nullify and sustain shift in activity. Therefore, it is probable that an optimal therapeutic effect will be achieved by simultaneous actions at several targets.

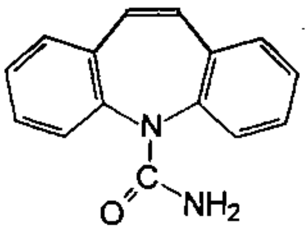
Anticonvulsant drug classes: -

The anticonvulsant agents may be conveniently grouped into *three* general categories.

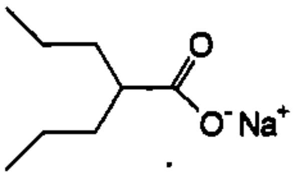
(1) **"First Generation"** or older agents as exemplified by Phenytoin **(I-1)**, Carbamazepine **(I-2)**, Valproate **(I-3)**, the Benzodiazepines **(I-4, I-4a and I-4b)**, Ethosuximide **(I-5)**, Phenobarbital **(I-6)**, Primidone **(I-7)** and Trimethadione **(I-8)**, all of which were introduced between 1910 and 1970.



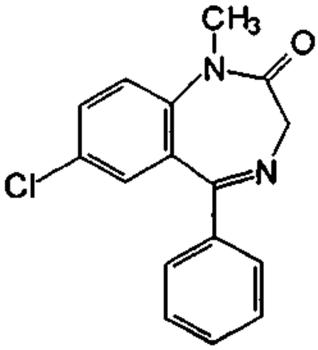
(I-1)



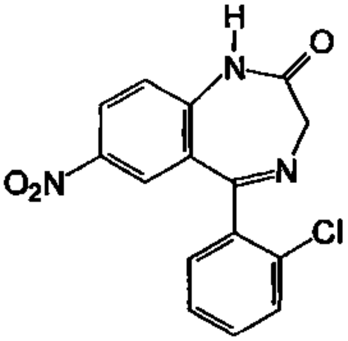
(I-2)



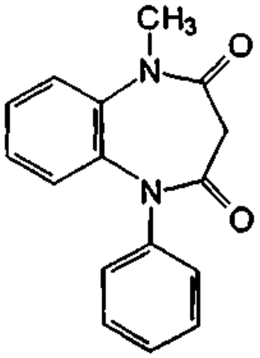
(I-3)



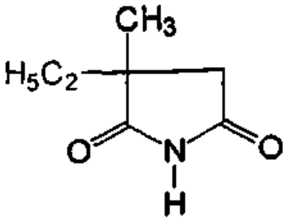
(I-4)



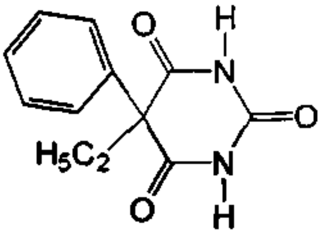
(I-4a)



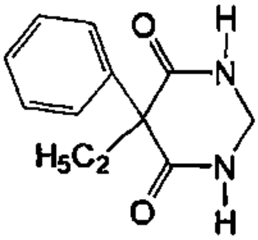
(I-4b)



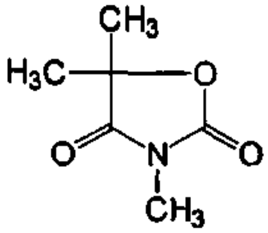
(I-5)



(I-6)

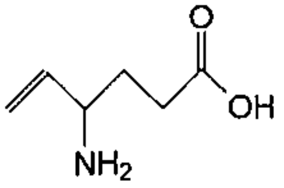


(I-7)

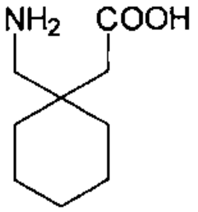


(I-8)

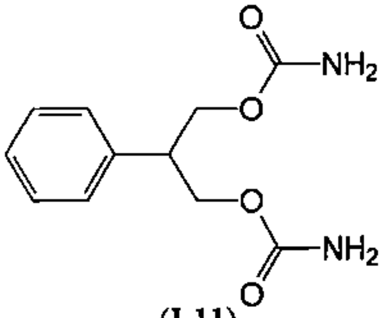
(2) **"Second Generation"** or newer agents consisting of Vigabatrin (**I-9**), Gabapentin (**I-10**), Felbamate (**I-11**), Lamotrigine (**I-12**), Oxcarbazepine (**I-13**), Zonisamide (**I-14**), Tiagabine (**I-15**), Topiramate (**I-16**) and Leviteracetam (**I-17**).



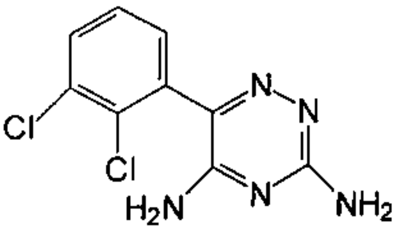
(I-9)



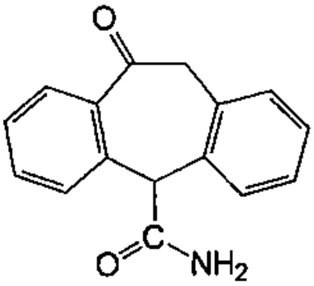
(I-10)



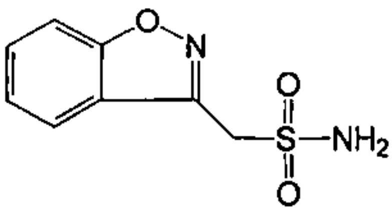
(I-11)



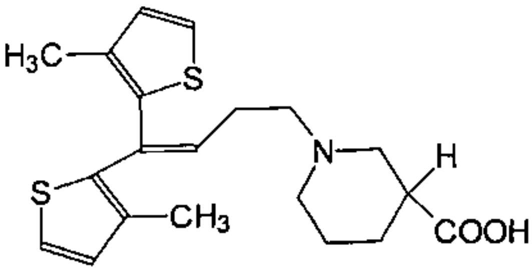
(I-12)



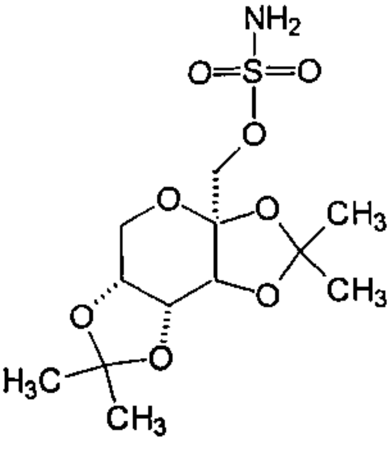
(I-13)



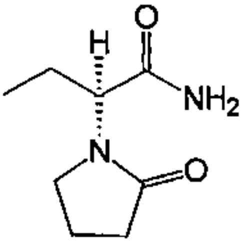
(I-14)



(I-15)



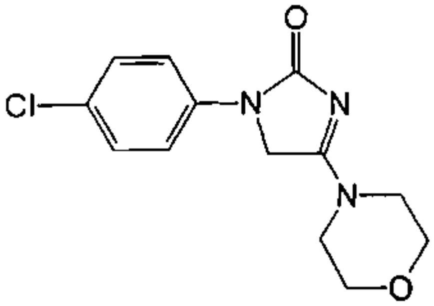
(I-16)



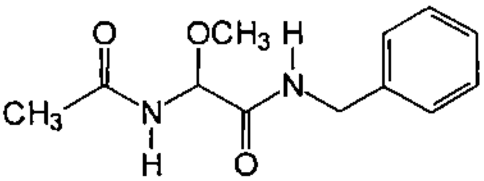
(I-17)

(3) **"Third Generation"** agents are those agents that are in preclinical or clinical development which is exemplified by AWD-131 (**I-18**), Harkoseride (**I-19**), LY-300164 (**I-20**), NPS-1776 (**I-21**), NW-1015 (**I-22**), Pregabalin (**I-23**), Remacemide (**I-24**), Retigabine (**I-25**), Rufinamide (**I-26**), Losigamone (**I-27**), Stiripentol (**I-28**),

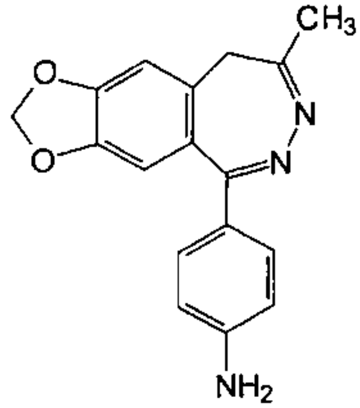
Ganaxolone **(I-29)**, Soretolide **(I-30)**, 4-(4-fluorophenoxy)benzaldehyde semicarbazone **(I-31)**, AMP-397A **(I-32)**, U-594494A **(I-33)**, SB-204269 **(I-34)**.



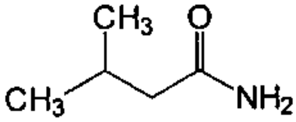
(I-18)



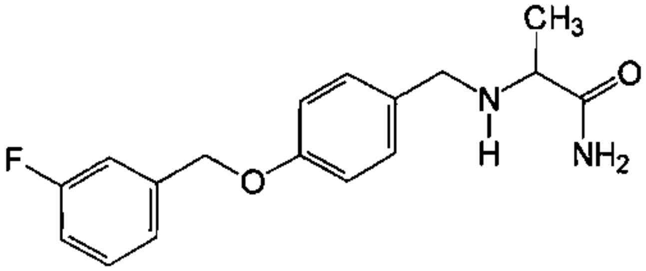
(I-19)



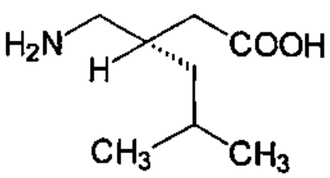
(I-20)



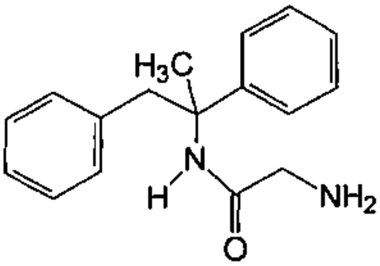
(I-21)



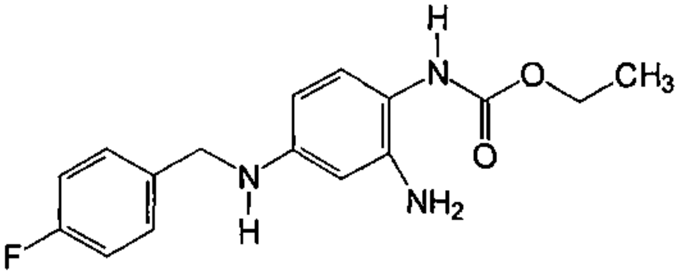
(I-22)



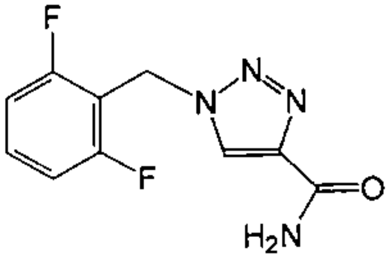
(I-23)



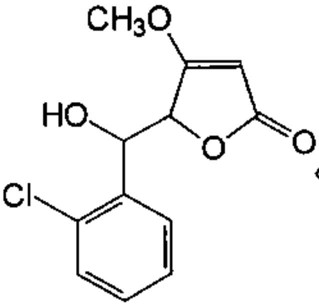
(I-24)



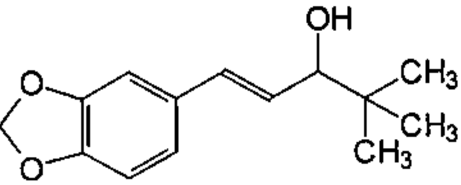
(I-25)



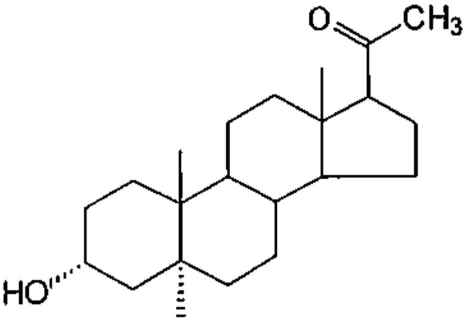
(I-26)



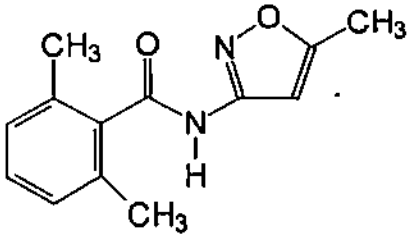
(I-27)



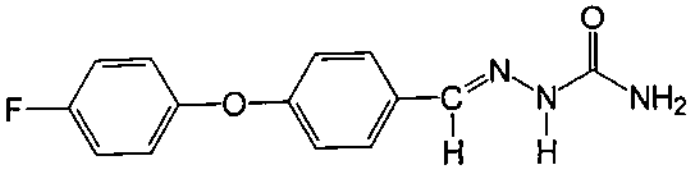
(I-28)



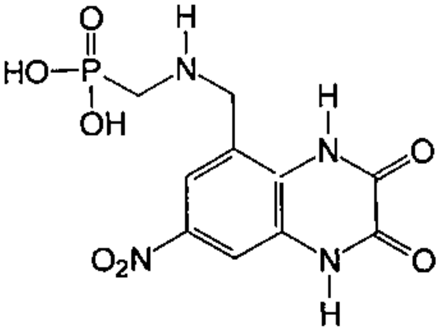
(I-29)



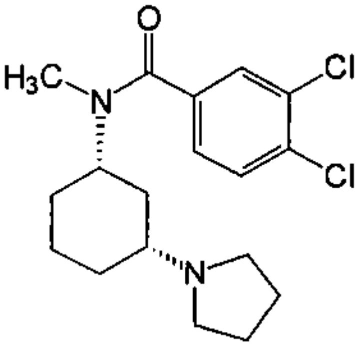
(I-30)



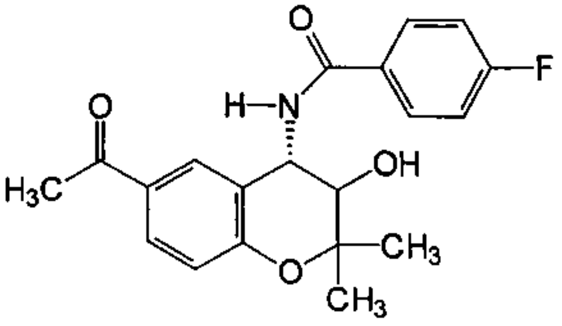
(I-31)



(I-32)



(I-33)



(I-34)

Adverse Effects of Marketed AEDs: -

The efficacy of many of the marketed drugs is largely compromised by notable adverse effect in patients (22-24). Barbiturates such as Phenobarbital and Mephobarbital (N-Methyl phenobarbital), marketed early in the 20th century and used mainly in the treatment of generalized and partial seizures, cause severe drowsiness and sedative-hypnotic effect that greatly limit their use at present (25-27).

Phenytoin, a member of the hydantoin family, although widely used in controlling generalized and partial seizures, possess a range of CNS related side effects such as nystagmus, ataxia, mental confusion, disturbances of equilibrium etc (28, 29). Ethosuximide, a succinimide drug, is regarded as the most effective and least toxic in the treatment of absence seizures, and yet causes drowsiness, ataxia, skin rashes, and possibly hepatic and renal dysfunction (30, 31).

Benzodiazepines such as Diazepam and Clonazepam used in the treatment of partial and generalized seizures and status epilepticus, although clinically effective, are unsuitable for chronic treatment because of the development of drug-tolerance and drug-dependence in patients (32, 33). Valproic acid has a wide spectrum of anticonvulsant action and is used clinically for the treatment of generalized, partial and absence seizures. However, its use is also limited by a potential for fatal hepatotoxicity and teratogenicity. In addition, gastro- intestinal disturbances are frequently observed with this drug (34, 35).

Gabapentin, Lamotrigine, Vigabatrin and Tiagabine are among the major newly marketed antiepileptic drugs, which are effective in the treatment of refractory adult patients with complex partial seizures. Some of the undesirable side effects of CNS origin that accompany the use of these drugs are somnolence, fatigue, dizziness, headache, ataxia, diplopia, and mental confusion. (36-39).

These limitations with the conventional AEDs highlighted the need for developing newer agents for epilepsies and the AED search has come a long way, particularly over the last two decades.

Table 1.2: Side effect profile of Anticonvulsants: -

Drug	Dose related (Predictable)	Non-dose related (Idiosyncratic)
Carbamazepine	Diplopia, drowsiness, headache, nausea, orofacial dyskinesia, arrhythmias	Photosensitivity, Stevens-Johnson syndrome, agranulocytosis, aplastic anaemia, hepatotoxicity
Sodium valproate	Dyspepsia, nausea, vomiting, hair loss, anorexia, drowsiness	Acute pancreatitis, aplastic anaemia, thrombocytopenia, hepatotoxicity
Phenytoin	Ataxia, nystagmus, drowsiness, gingival hyperplasia, hirsutism, diplopia, asterixis, orofacial dyskinesia, folate deficiency	Blood dyscrasias, rash, Dupuytren's contracture, hepatotoxicity
Phenobarbitone	Fatigue,listlessness, depression, poor memory, impotence, hypocalcaemia, osteomalacia, folate deficiency	Macropopular rash, exfoliation, hepatotoxicity
Ethosuximide	Nausea, vomiting, drowsiness, headache, lethargy	Rash, erythema multiforms, Stevens-Johnson syndrome
Clonazepam	Fatigue, drowsiness, ataxia	Rash, thrombocytopenia
Lamotrigine	Headaches, drowsiness, diplopia, ataxia	Liver failure, disseminated intravascular coagulation
Gabapentin	Drowsiness, diplopia, ataxia, headache	Not reported
Topiramate	Dizziness, drowsiness, nervousness, fatigue, weight loss	Not reported
Vigabatrin	Drowsiness, dizziness, weight loss	Behavioral disturbances, severe psychosis

LITERATURE REVIEW

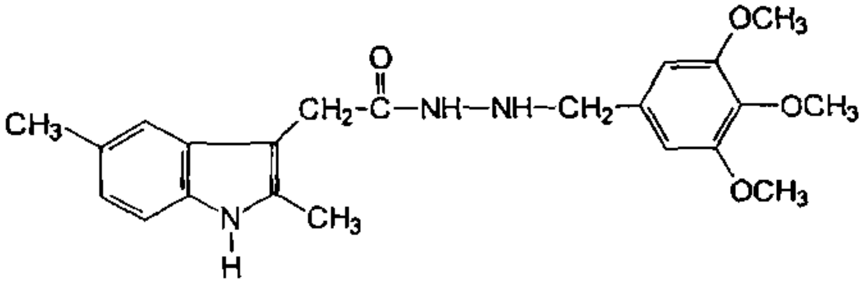
The conventional antiepileptic drugs are unable to control seizures effectively in as many as 25% of patients (40), their dose related neurotoxicity and other side effects, at times, become a major limitation in their clinical use (41). Furthermore, since AED therapy is for a long duration, there is a risk of drug interactions, with concomitant administration of other drugs (42). This problem is further compounded by the high protein bindings for some of the drugs and potential for inducing hepatic enzymes. The limitations with the conventional AEDs highlighted the need for developing newer agents for epilepsies and the AED search has come a long way, particularly over the last two decades.

Hydrazides and Hydrazones: -

The hydrazine derivatives are capable of exerting either convulsant or anticonvulsant actions depending upon the compound and the conditions employed. Hydrazine induced convulsions have been attributed to their ability to interact with pyridoxal or pyridoxal-5-phosphate. Dixon et al. (43) demonstrated that pyridoxal hydrazone and pyridoxal-5-phosphate hydrazone of semicarbazide and other hydrazides were much more effective convulsants than the parent compounds. Since hydrazones of the phosphorylated form of the vitamin were active convulsants, but not inhibitors of pyridoxal phosphokinase, the authors concluded that neither pyridoxal phosphate binding nor phosphokinase inhibition could completely explain the mode of action in producing seizures.

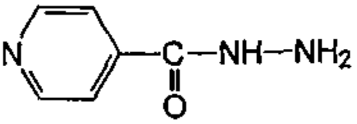
Hydrazine derivatives (44) have been shown to inhibit monoamine oxidase enzyme. Prockop et al. (45, 46) first observed that the MAO inhibitors exhibit anticonvulsant activity. In mice, they were found to be effective against electro shock and metrazole induced seizures, but not against strychnine model; and the highest anticonvulsant activity corresponded to the time when brain amine levels were elevated.

Agarwal et al. (47) synthesized a series of N^1-substituted indole-3-acetyl-N^3-substituted hydrazines and hydrazones and evaluated for anticonvulsant activity against subcutaneous pentylene tetrazole (scPTZ) induced seizure. Compound **II-1** showed 50 % protection.



(II-1)

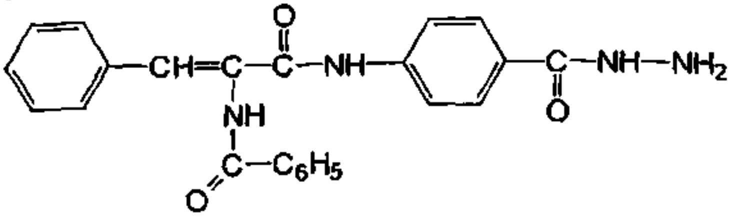
Wood et al. (48) studied sequential lowering and raising of GABA levels by the intramuscular administration of Isonicotinic acid hydrazide (II-2) to chicks. Initially a rapid and significant decrease in the concentration of brain GABA, followed by a reversal of the effect in which the GABA level increased was observed. It elevates GABA levels due to a strong inhibitory action on GABA-α-ketoglutarate transaminase (GABA-T), but a concomitant inhibition of glutamic acid decarboxylase (GAD) activity occurs, which may reduce the potential of the compound as an anticonvulsant agent.



(II-2)

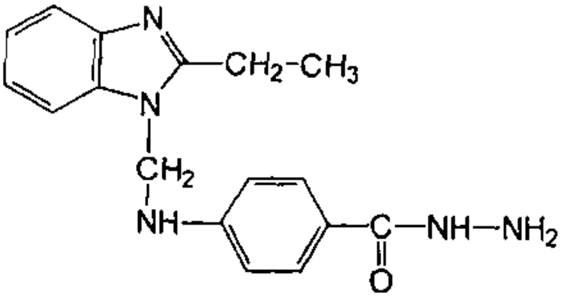
Wood et al. (49) developed an anticonvulsant agent based on its effect on GABA metabolism. The administration of INH and pyridoxine in chicks 14-18 h prior exposure to hyperbaric oxygen provided excellent protection against the onset of oxygen-induced seizures.

Parmar et al. (50) synthesized a series of substituted cinnamides to investigate their *in vitro* MAO inhibitory properties and their ability to protect against scPTZ convulsions produced in mice. The degree of protection offered by these compounds did not correlate with their enzyme inhibitory properties. The compound **II-3** showed 50 % protection against scPTZ-induced seizure.



(II-3)

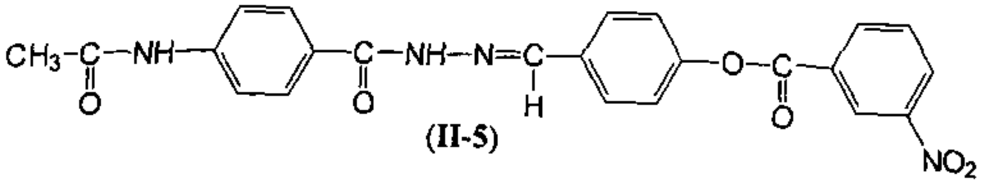
Parmar et al. (51) synthesized 2-alkyl-1-(4'-benzhydrazide)aminomethylbenzimidazoles and evaluated for their MAO inhibitory property and anticonvulsant activity. The anticonvulsant activity exhibited by these compounds against scPTZ-induced seizure was found to be unrelated to the MAO inhibitory ability of these compounds. In this series, the compound **II-4** showed 50% protection against sc PTZ induced convulsion.



(II-4)

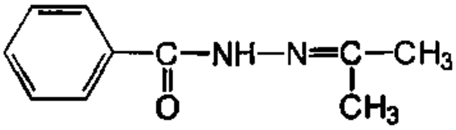
Wood et al. (52) studied the anticonvulsant properties of **II-2** and associated changes in GABA metabolism. The administration of INH and pyridoxine to chicks prior to being exposed to oxygen at high pressure brought about a delay in the onset of the hyperbaric-oxygen induced seizures in the birds. The hydrazide was the active anticonvulsant component of the drug mixture but pyridoxine was necessary to prevent seizures induced by the hydrazide itself shortly after its administration. The anticonvulsant action of the drug mixture developed slowly but lasted for several hours and correlated well with concomitant changes in the concentration of GABA in the brain. No similar correlation was observed between the anticonvulsant action and the activity of either glutamic acid decarboxylase or GABA-α-oxoglutarate aminotransferase.

Parmar et al. (53) prepared some N^1-(4-acetamidobenzoyl)-N^2-(substituted phenyl carboxylate)benzylidinohydrazines and evaluated for their ability to inhibit monoamine oxidase activity of rat brain homogenates. These compounds possessed anticonvulsant activity, which was reflected by the protection afforded against scPTZ induced convulsion and also potentiated pentobarbital-induced hypnosis in mice. Monoamine oxidase inhibitory effectiveness of these substituted benzylidino hydrazines was unrelated to their anticonvulsant activity and their ability to potentiate pentobarbital-induced hypnosis. The compound N^1-(4-acetamidobenzoyl)-N^2-(3-nitrophenylcarboxylate)benzylidinohydrazine (**II-5**) showed 50% of protection against scPTZ-induced seizure.



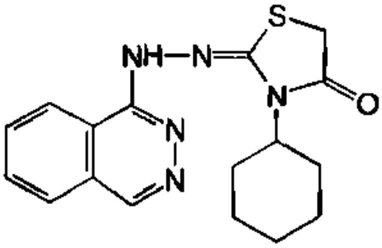
(II-5)

Wood et al. (54) synthesized several aryl / heteroaryl hydrazides and hydrazones and evaluated for their inhibitory effects on GAD, GABA-T and MAO enzyme systems in chick brain 24 h after their intramuscular administration. All compounds produced reduction in GAD, GABA-T and MAO activity. Structure activity relationships indicated that the ring structure had a greater influence on the degree of GAD and GABA-T inhibition than the N^1-terminal group. In contrast, structural requirements for MAO inhibition were much more restrictive. The intramuscular administration of benzoic acid hydrazones to chicks 24 h prior exposure to oxygen at high pressure provided significant protection against the onset of the hyperbaric oxygen induced seizures. The compound II-6 showed GABA-T inhibitory activity and anticonvulsant activity against scPTZ-induced convulsion.



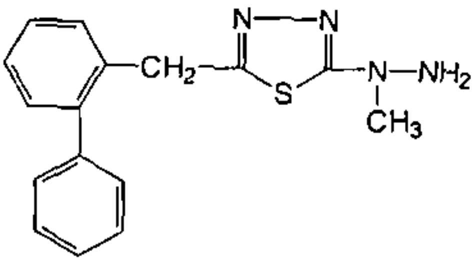
(II-6)

Soliman et al. (55) prepared 3-substituted-4-oxothiazolin-2yl-(1-Phthalazinyl)hydrazones, 3-substituted-4-oxo-5,6-dihydro-1,3-thiazin-2yl-(1-phthalazinyl)hydrazones and 2-substituted-amino-5-oxo-4-(1-phthalazinyl)-6-hydro-1,3,4-thiadiazines and tested for their anticonvulsant activity. Some of the compounds showed weak to moderate anticonvulsant activity. The compound II-7 showed 70% protection against scPTZ-induced convulsion.



(II-7)

Chapleo et al (56) synthesized a series of 2-aryl-5-hydrazino-1,3,4–thiadiazoles and evaluated for anticonvulsant activity. The combination of preferred aromatic substituents in the 2-position coupled with alkyl substitution on the hydrazine moiety led to a number of potent compounds lacking sedation, ataxia, or lethality. 2-(2'-biphenyl)methyl-5-(1"-methyl hydrazino)-1,3,4-thiadiazole (II-8) represents a new class of anticonvulsant agent. It showed ED_{50} of 18 mg/kg against MES induced seizure.

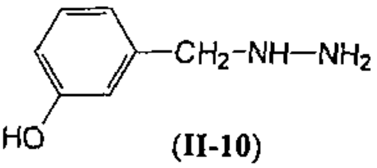


(II-8)

Lightcap et al. (57) found methylhydrazine (**II-9**) and (3-hydroxybenzyl)hydrazine (**II-10**) to be potent slow binding inhibitors of the pyridoxal-5-phosphate (PLP)-dependent enzyme GABA-T. The rate constant for formation of the enzyme inhibitor complex determined from the slow binding kinetics was 2.08×10^{3} and 1.98×10^{4} $m^{-1}min^{-1}$ for methyl hydrazine and (3-hydroxybenzyl)hydrazine, respectively. The rate constant for dissociation of the enzyme-inhibitor complex determined for the slow binding kinetics was 4.6×10^{-3} and 5×10^{-3} min^{-1}, respectively. The inhibition constants calculated from the slow binding inhibition kinetics are 2.2 μm for methylhydrazine and 0.3 μm for (3-hydroxybenzyl)hydrazine.

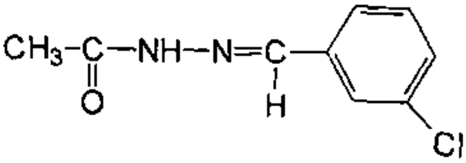
$CH_3-NH-NH_2$

(II-9)

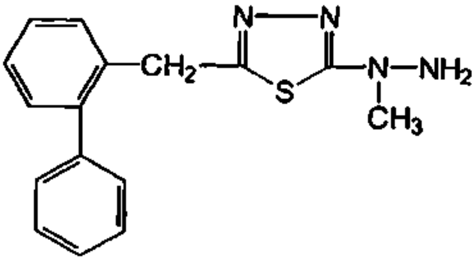


(II-10)

Dimmock et al. (58) synthesized various acetylhydrazones and oxamoylhydrazones as candidate anticonvulsants with a view to examine the viability of a putative binding site hypothesis. Atomic charge calculations were undertaken to determine the hydrogen bonding capacities of various molecules. The biological results obtained revealed that in general the acetylhydrazones (**II-11**) afforded good protection against convulsions while the oxamoyl hydrazones were significantly less active. These data suggest that terminal electron donating groups enhanced the hydrogen bonding capabilities and anticonvulsant properties of these molecules.



(II-11)

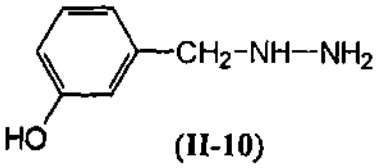


(II-8)

Lightcap et al. (57) found methylhydrazine (II-9) and (3-hydroxybenzyl)hydrazine (II-10) to be potent slow binding inhibitors of the pyridoxal-5-phosphate (PLP)-dependent enzyme GABA-T. The rate constant for formation of the enzyme inhibitor complex determined from the slow binding kinetics was 2.08×10^3 and 1.98×10^4 $m^{-1}min^{-1}$ for methyl hydrazine and (3-hydroxybenzyl)hydrazine, respectively. The rate constant for dissociation of the enzyme-inhibitor complex determined for the slow binding kinetics was 4.6×10^{-3} and 5×10^{-3} min^{-1}, respectively. The inhibition constants calculated from the slow binding inhibition kinetics are 2.2 μm for methylhydrazine and 0.3 μm for (3-hydroxybenzyl)hydrazine.

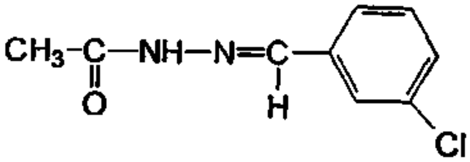
$CH_3-NH-NH_2$

(II-9)



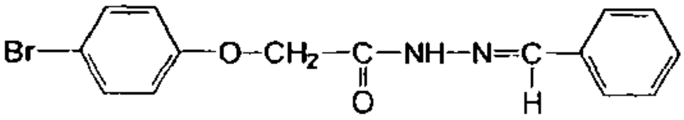
(II-10)

Dimmock et al. (58) synthesized various acetylhydrazones and oxamoylhydrazones as candidate anticonvulsants with a view to examine the viability of a putative binding site hypothesis. Atomic charge calculations were undertaken to determine the hydrogen bonding capacities of various molecules. The biological results obtained revealed that in general the acetylhydrazones (II-11) afforded good protection against convulsions while the oxamoyl hydrazones were significantly less active. These data suggest that terminal electron donating groups enhanced the hydrogen bonding capabilities and anticonvulsant properties of these molecules.



(II-11)

Pandeya et al. (59) synthesized a series of phenoxy / p-bromophenoxy acetylhydrazones (II-12) and their anticonvulsant activity was screened against MES, scPTZ and scSTY tests. Compounds with $-OCH_2-$ were devoid of anticonvulsant activity. The studies reveled that the hydrogen-bonding domain in semicarbazones, adjacent to the lipophilic aryl ring, is essential for the anticonvulsant activity.

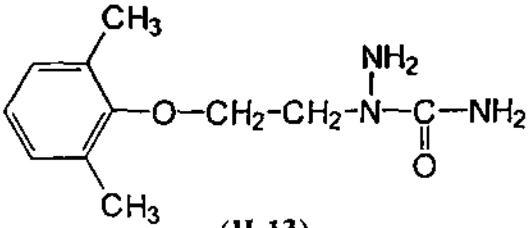


(II-12)

Semicarbazides and Thiosemicarbazides: -

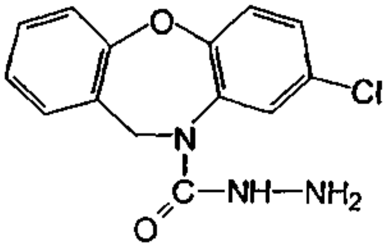
Semicarbazides and thiosemicarbazides (60, 61) have been shown to inhibit monoamine oxidase enzyme. Prockop et al. (45, 46) first observed that the MAO inhibitors exhibit anticonvulsant activity. In mice, these were found to be effective against electro shock and metrazole induced seizures, but not against strychnine model; and the highest anticonvulsant activity corresponded to the time when brain amine levels were elevated.

Durant et al. (62) prepared the series of aryloxyalkyl semicarbazides, in which 2,6-dimethylphenoxyethylsemicarbazide (II-13) showed marked anticonvulsant activity against MES model of seizures.



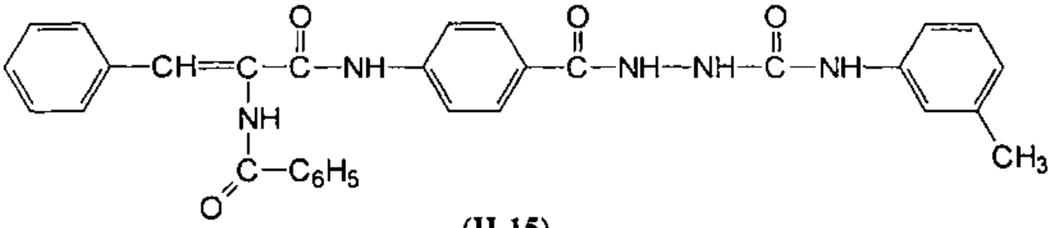
(II-13)

Coyne et al. (63) synthesized a series of semicarbazides from various tricyclic amines and the structure-activity relationship of their anticonvulsant activity was investigated. In that 2-chloro substituted tricyclic amine (II-14) had shown a ED_{50} of 14.8 mg kg^{-1}



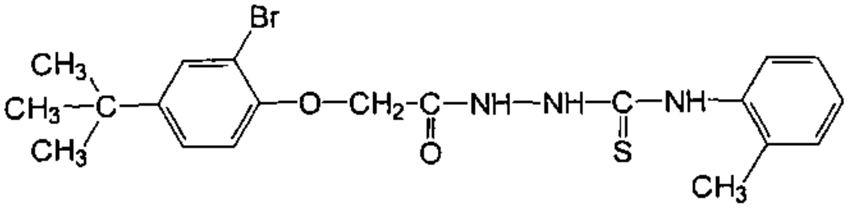
(II-14)

Parmar et al. (50) reported compound **II-15** to show 70 % protection against scPTZ-induced seizure.



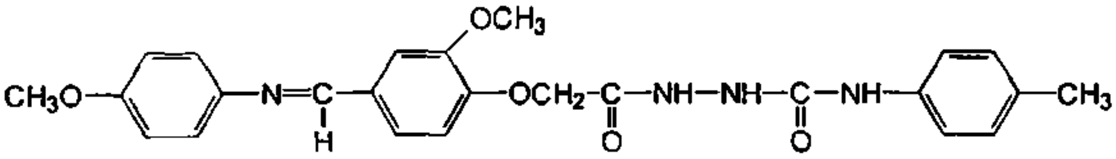
(II-15)

Parmar et al. (64) prepared several 2,5-disubstituted-1,3,4-oxadiazoles and tested for their effectiveness in inhibiting the respiratory activity of rat brain homogenate. All substituted oxadiazoles and their precursors, thiosemicarbazides were found to inhibit nicotinamide adenine dinucleotide (NAD) dependent oxidations of pyruvate and α-ketoglutarate as well as the NAD-independent oxidation of succinate. Anticonvulsant activity, as exhibited by protection against scPTZ induced seizures, with substituted thiosemicarbazides and the corresponding cyclized oxadiazoles ranged from 30 to 90% at a dose of 100 mg/kg. The degree of protection afforded by these compounds, however, was unrelated to their ability to inhibit oxidation of pyruvate, α-ketoglutarate and succinate. The compound **II-16** showed 90% protection against scPTZ-induced convulsion.



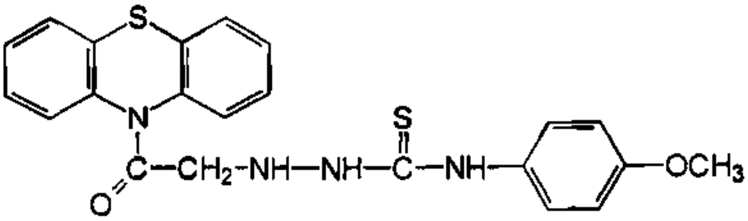
(II-16)

Dwivedi et al. (65) synthesized several substituted anilino-[3-methoxy-4-(4-aryl semicarbazido carbonyl methylene oxy)] benzylidene compounds. Their ability to inhibit monoamine oxidase and to afford protection against hypo-osmotic hemolysis in dog erythrocytes was found to bear no relationship with their anticonvulsant activity against scPTZ induced seizures in mice. The compound 4-methoxy anilino-[3-methoxy-4-(4-methyl phenyl semicarbazido carbonyl methylene oxy)] benzylidene (**II-17**) showed 70% protection against scPTZ-induced seizure.



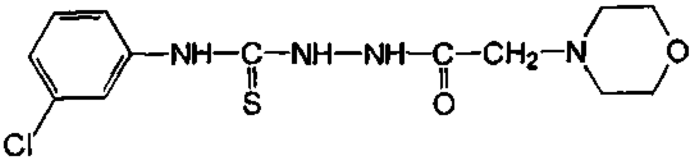
(II-17)

Singh et al. (66) synthesized several 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and evaluated for the inhibition of nicotinamide adenine dinucleotide (NAD)- dependent oxidation of pyruvate and α-ketoglutarate. All phenothiazine derivatives exhibited anticonvulsant activity, which was reflected by the 20-60% protection observed against scPTZ-induced convulsions in mice. The anticonvulsant activity possessed by these substituted phenothiazines bore no relationship with their ability to selectively inhibit the NAD-dependent oxidations. The compound 10-[1-acetyl-4-(4-methoxyphenyl)thiosemicarbazido]phenothiazine (II-18) showed 60% protection against scPTZ-induced convulsion.



(II-18)

Sengupta et al. (67) prepared N^1-(N-morpholinoacetyl)-N^4-aryl-3-thiosemicarbazide derivatives, in which, the compound N^1-(N-morpholinoacetyl)-N^4-(3-chlorophenyl)-thio semicarbazide (II-19) showed 83% protection against scPTZ-induced convulsion.

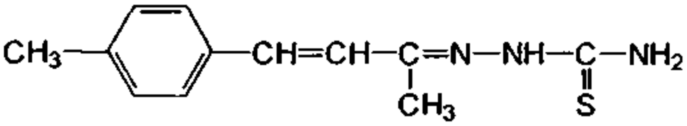


(II-19)

Semicarbazones and Thiosemicarbazones: -

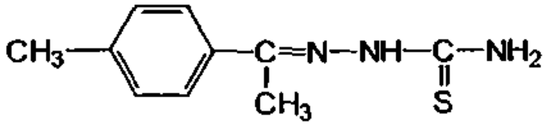
Dimmock et al. (68) prepared a number of thiosemicarbazones and semicarbazones derived from 4-aryl-3-buten-2-ones and most of the compounds were found to have antiepileptic activity. In particular, 4-(4-methylphenyl)-3-buten-2-one thiosemicarbazone (II-20) had an ED_{50} of 6.96 mg/kg in the scPTZ induced seizure threshold test when administered by the intraperitoneal route to mice and a protection index (PI) of 10.37.

When given orally, the activity of this compound was retained albeit diminished. In addition, this compound afforded some protection against seizures induced by bicuculine and picrotoxin but had no effect on the activities of glutamic acid decarboxylase and GABA-T.



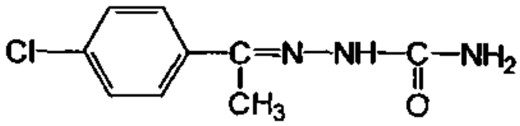
(II-20)

Dimmock et al. (69) synthesized a number of thiosemicarbazones of arylidene and aryl ketones as candidate anticonvulsant agents. X-ray crystallography of 4-(4-methylphenyl)-3-buten-2-one thiosemicarbazone revealed that it had the E-configuration with respect to both olefinic and carbimino double bonds. Most of the compounds displayed activity in the MES and/or scPTZ tests and of particular interest was acetophenone semicarbazone (**II-21**), which exhibited good activity when administered by the intraperitoneal and oral routes.



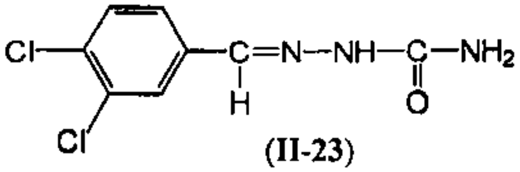
(II-21)

Dimmock et al. (70) carried out the systemic chemical modification of thiosemicarbazone of acetophenone. The activities of these analogues were studied in the MES and scPTZ tests along with their neurotoxicity screen in the mice when administered by i.p. route. Most of the compounds were active and the representative compounds examined for oral activity in rats revealed that in most of the cases protection against seizures induced in the MES screen but not the scPTZ test was achieved at a dose of 50mg/kg. Some correlations between chemical structures and anticonvulsant properties were reported. The compound **II-22** showed ED_{50} of 12.84 mg/kg against MES induced seizure.



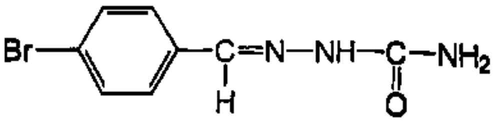
(II-22)

Dimmock et al. (71) prepared various semicarbazones derived from aryl aldehydes, phenyl alkyl aldehydes and phenyl alkyl ketones as well as some related compounds and evaluated for anticonvulsant activity. Most of the compounds displayed anticonvulsant activity in the MES and scPTZ screens accompanied by neurotoxicity when given to mice by the i.p. route. However, quantitative data revealed Protective Index (PI=TD_{50}/ED_{50}) of less than 4 in general. Oral administration of the compounds to rats led to excellent potency in the MES screen accompanied by high protection indices while virtually no activity in the scPTZ test. These observations support the theory that one large hydrophobic group (in this case the aryl ring) and two electron donor systems (present in the semicarbazono group) are the structural requirements for protection in the MES screen. In general, the semicarbazones had rapid onset of action, and one of the ways in which these compounds displayed their anticonvulsant activity is likely to be interaction with chloride channels. Empirical and semiempirical conformational calculations indicated that certain molecular fragments and hydrophobicity of these molecules affects bioactivity. The compound **II-23** showed ED_{50} of 20.18 mg/kg against MES induced seizure.



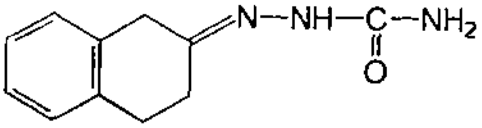
(II-23)

Further, Dimmock et al. (72) studied some of the properties of 4-bromobenzaldehyde semicarbazone **(II-24)**, a prototype molecule of a new class of anticonvulsants. 4-bromobenzaldehyde semicarbazone demonstrated activity in the MES and scPTZ tests in mice, with low neurotoxicity. When given orally to rats, it displayed high potency in the MES test and very low neurotoxicity, resulting in a high protection index (PI). It displayed no proconvulsant properties, and development of rapid tolerance was not noted. When administered i.p. at doses of 100, 300 or 600 mg/kg to rats, it had no effect on levels of GABA or GABA-T activity in whole brain. When tested *in vitro*, this compound had no effect on the brain GABA-T at a drug concentration of 100 μM. This compound showed ED_{50} of 22.0 mg/kg against MES induced seizure.



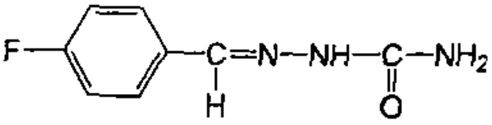
(II-24)

Dimmock et al. (73) converted a number of aryl alicyclic ketones to their corresponding semicarbazones, thiosemicarbazones and bis-carbohydrazones. Anticonvulsant activity was displayed by most of the compounds in the MES and scPTZ screens, when given by i.p. route to mice. However, upon oral administration to rats, a marked selective activity in the MES screen only was noted. The compound **II-25** showed ED_{50} of 14.35 mg/kg against MES induced convulsion.



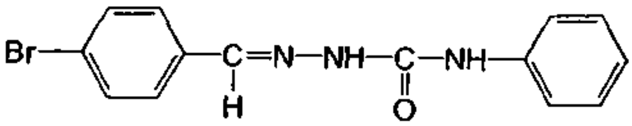
(II-25)

Dimmock et al. (74) studied a number of aryl semicarbazones, which displayed anticonvulsant activity in the MES and scPTZ screens when administered intraperitoneally to mice. When given by the oral route to rats, protection was afforded in the MES but not in the scPTZ test. Correlations were noted between the σ and σ* values of the aryl substituents, the interplanar angles made by the aryl rings with the adjacent carbimino groups, the shapes of certain semicarbazones were determined by X-ray crystallography and the activities in the rat oral MES screen. Molecular modeling studies revealed a number of statistically significant descriptors, which contributed to anticonvulsant activity. The compound 4-fluorophenyl semicarbazone (**II-26**) showed ED_{50} of 8.77 mg/kg against MES induced seizure.



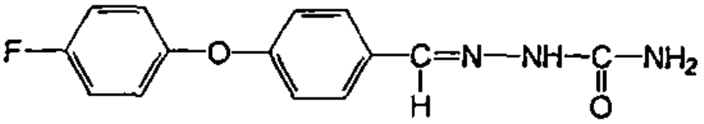
(II-26)

Dimmock et al. (75) studied the importance of the primary amino group in the aryl semicarbazones, which had been shown previously to have significant anticonvulsant property, by replacing it with other substituents. The results indicated that the amino group was not essential for anticonvulsant property. However its replacement by an aryl ring generally abolished activity while a terminal phenyl amino function (**II-27**) was better tolerated. Thus both the size of the group and its hydrogen bonding capabilities appear to influence the bioactivity. Alteration of the oxygen atom of the semicarbazones by isosteres did not enhance anticonvulsant properties.



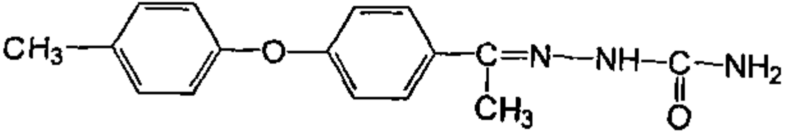
(II-27)

Dimmock et al. (76) synthesized a number of (aryloxy)arylsemicarbazones and related compounds and evaluated for anticonvulsant activities. After i.p. injections to mice, the semicarbazones were examined in the MES, scPTZ and NT screens. Quantitation of approximately one third of the compounds revealed an average protection index of approximately 9. After oral administration to rats, a number of compounds displayed significant potency in the MES screen (ED_{50} of 1-5 mg/kg) that accompanied by very high protection indices. In fact over half of the compounds had PI greater than 100, and two were in excess of 300. The compounds were essentially inactive in the scPTZ and NT screens after oral administration to rats. The compound 4-(4-Flurophenoxyphenyl)semicarbazone **(II-28)** showed ED_{50} of 1.59 mg/kg (PI >315) against MES induced seizure.



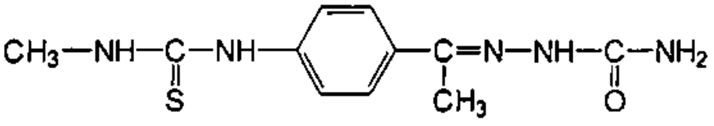
(II-28)

Puthucode et al. (77) evaluated a number of aryl, arylidene and aryloxyaryl semicarbazones as candidate anticonvulsants. In particular, insertion of an olefinic group between the carbimino carbon atom and an aryl ring (referred to as the proximal ring) led to a series of new compounds in which there was retention in activity and two compounds were shown to be useful lead molecules. At the doses utilized, neurotoxicity was absent in these compounds when given orally to rats. The aryloxyaryl semicarbazones showed oral activity in MES screen substantially greater than that of phenytoin and PI of over 100. A binding site hypothesis as a result of the biodata generated was in accord with the informations obtained by X-ray crystallography. The compound **II-29** showed ED_{50} of 5.62 mg/kg against MES induced seizure.



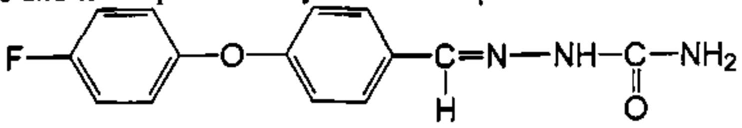
(II-29)

Pandeya et al. (78) synthesized a series of thioureido derivatives of acetophenone semicarbazone and evaluated for anticonvulsant activity. Some compounds provided significant protection against MES and scPTZ induced seizures. The compound methyl thioureido derivative of acetophenone semicarbazone (**II-30**) was the most active compound with ED_{50} 23.5 mg kg^{-1} and equipotent to phenytoin (ED_{50} = 23.2 mg/kg).

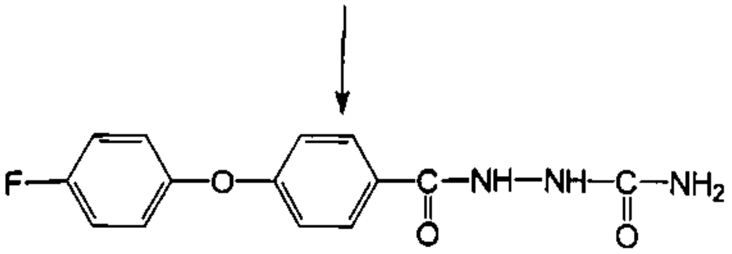


(II-30)

Dimmock et al. (79) studied the 4-(4-fluorophenoxy)benzaldehyde semicarbazone (**II-28**) as a novel anticonvulsant affording excellent protection in the rat oral MES screen as well as having an apparent PI over 300. The metabolism of this compound was studied by examining the urine of rats dosed orally with 50 mg/kg of 4-(4-fluorophenoxy)benzaldehyde semicarbazone which revealed that most of the drug was converted into a metabolite 1-[4-(4-fluorophenoxy)benzoyl]semicarbazide (**II-31**), which was confirmed by an independent synthesis. This compound was bereft of activity in the rat oral MES screen. This datum provided strong evidence that the anticonvulsant activity of 4-(4-fluorophenoxy) benzaldehyde semicarbazone and related compounds is due to the intact molecules and is not produced by breakdown products *in vivo*.

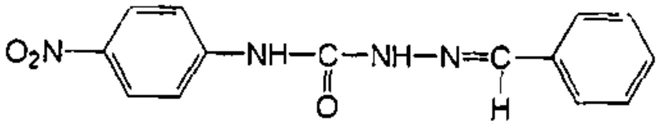


(II-28)



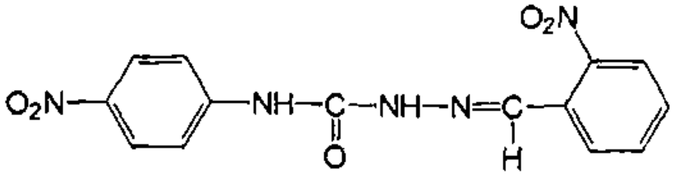
(II-31)

Pandeya et al. (80) synthesized a series of semicarbazones and thiosemicarbazones and evaluated for anticonvulsant activity. Some compounds provided significant protection against MES and scSTY induced seizures. The acetophenone semicarbazone was the most active in the series with activity in a dose of 30 mg/kg in the strychnine seizure pattern test and ED_{50} of 10 mg/kg in the MES test. Hence it could serve as a prototype molecule for future developments. Also compounds with a p-nitrophenyl substitution (**II-32**) in place of the amino hydrogen of semicarbazone moiety showed activity in a dose of 30 mg/kg and an ED_{50} of 83 mg/kg in the MES test.



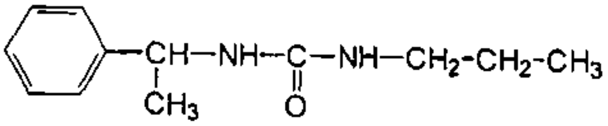
(II-32)

Pandeya et al. (81) prepared a series of p-nitrophenyl substituted semicarbazones and their anticonvulsant activity was screened against MES, scPTZ, and scSTY tests. 4-(4'-nitrophenyl)-2-nitrobenzaldehyde semicarbazone (**II-33**) has been found to be the most active in all these tests. This study revealed that a primary amino function is not essential for anticonvulsant activity in the semicarbazone series of compounds. Presumably these compounds also act on glycine receptors.

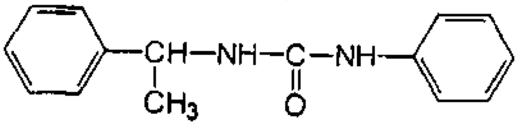


(II-33)

Dimmock et al. (82) obtained a result from a previous study which led to the postulate that a number of aryl semicarbazones displaying anticonvulsant activity in the MES screen interacted at both a hydrophobic and a hydrogen bonding areas on a specific binding site. The two parts of the binding site may be referred to as areas A and B, respectively (Fig II.1). In order to circumvent the possible problems of the carbimino group in semicarbazones such as toxicity and acid lability, some related ureylenes (**II-34, II-35**) were considered. Initial evidences suggested that a second lipophilic group in the molecule was advantageous; this group may interact at area 'C' on the proposed binding site. Most of the compounds prepared with a view to interact at areas A, B and C showed protection in mice against MES induced seizures. A number of compounds were active when given orally to rats and devoid of neurotoxicity at the doses utilized.



(II-34)



(II-35)

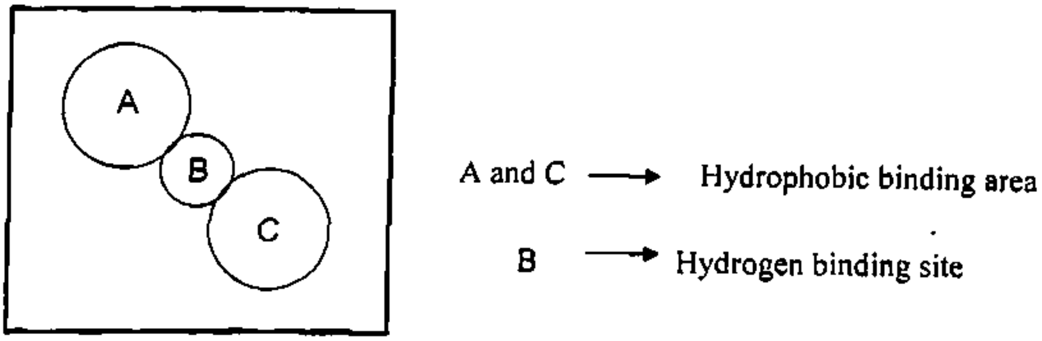
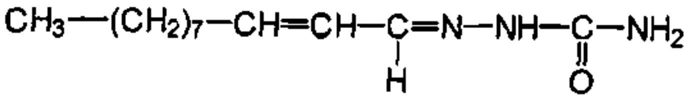


Fig II.1: Proposed binding site of Ureylene Anticonvulsants.

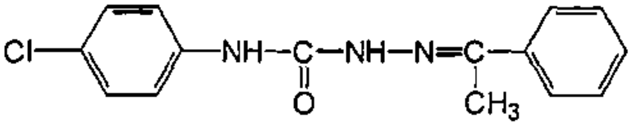
Dimmock et al. (58) synthesized various semicarbazones as candidate anticonvulsants with a view to examine the viability of a putative binding site hypothesis. Atomic charge calculations were undertaken to determine the hydrogen bonding capacities of various molecules. The biological results obtained revealed that in general the semicarbazones (**II-36**) afforded good protection against convulsions. These data suggest that terminal electron donating groups enhanced the hydrogen bonding capabilities and anticonvulsant properties of these molecules. Compound **II-36** showed ED_{50} of 21.3 mg/kg against MES induced seizure.



$$CH_3-(CH_2)_7-CH=CH-\underset{H}{C}=N-NH-\underset{O}{\overset{}{C}}-NH_2$$

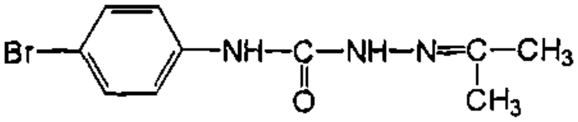
(II-36)

Pandeya et al. (83) synthesized a series of p-chlorophenyl substituted aryl semicarbazones and evaluated for anticonvulsant activity. Most of the compounds provided significant protection against MES induced seizures at 100 mg kg^{-1} after 0.5h and at 300 mg kg^{-1} after 4h in both MES and scPTZ induced seizures. The majority of compounds showed protection against scSTY induced seizures at 30 mg kg^{-1}. The compound p-Chlorophenyl semicarbazone with acetophenone (**II-37**) was active in both MES and scPTZ tests. The study has shown that the terminal primary amino group is not necessary for anticonvulsant activity.



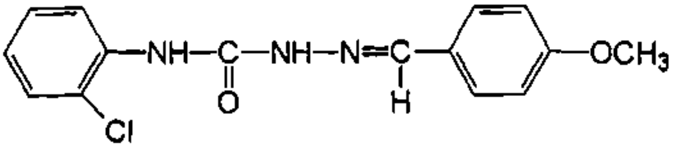
(II-37)

Pandeya et al. (84) further synthesized a number of 4-bromophenyl semicarbazones and evaluated for anticonvulsant and sedative-hypnotic activities. After intraperitoneal injection to mice, the semicarbazone derivatives were examined in the MES, scPTZ, scSTY and NT screens. All the compounds showed anticonvulsant activity in one or more test models. 4-(4-bromophenyl) acetone semicarbazone (**II-38**) showed greatest activity, being active in all the screen with very low neurotoxicity and no sedative hypnotic activity. The essential structural features responsible for interaction with receptor site were established within a suggested pharmacophore.



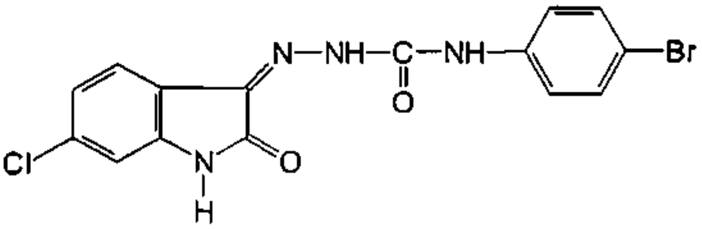
(II-38)

Pandeya et al. (85) also reported a series of semicarbazones and hydrazones and evaluated for anticonvulsant activity. Some compounds provided significant protection against MES and scSTY induced seizures. N^1-(2-chlorophenyl)-N^4-(4-methoxybenzylidene) semicarbazone (**II-39**) emerged as the most active compound at a dose of 30 mg kg^{-1} in scSTY test. This compound showed significant potentiation of sedative and hypnotic activity of pentobarbitone sodium and could serve as a prototype of future development.



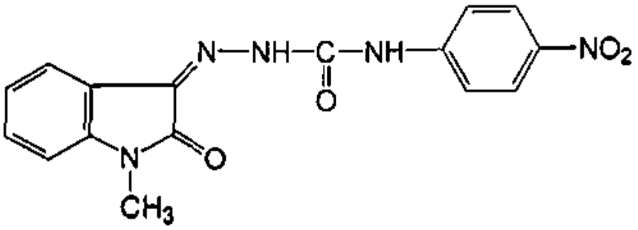
(II-39)

Pandeya et al. (86) designed and synthesized a series of substituted isatin semicarbazones and related bioisosteric hydrazones to meet the structural requirements essential for anticonvulsant properties. A number of isatin semicarbazones exhibited significant protection after intraperitoneal administration at the dose of 100 and 300 mg kg^{-1} and some of them showed good anticonvulsant activity in MES test in rats after oral administration at the dose of 30 mg kg^{-1}. The bioisosteric hydrazone derivatives were inactive in all the tests. The compound 6-chloroisatin-3-(4-bromophenyl) semicarbazone (**II-40**) has emerged as the most active analogues of the series showing good activity in all the three tests and was more active than phenytoin and valproic acid.



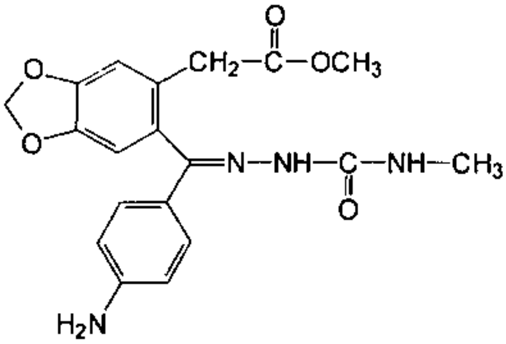
(II-40)

Pandeya et al. (87) screened a series of N-methyl/acetyl, 5-(un)-substituted isatin-3-semicarbazones for anticonvulsant and sedative-hypnotic activities. The results revealed that protection was obtained in all the screens i.e., MES, scPTZ, and scSTY. Compound N-methylisatino-3-(4-nitrophenyl)semicarbazone (**II-41**) showed good activity in the rat oral screen. Among all the compounds, **II-41** emerged as the most active compound as indicated by the protection it exhibited in MES, scSTY and scPTZ screens. All the compounds showed significant sedative-hypnotic activity.



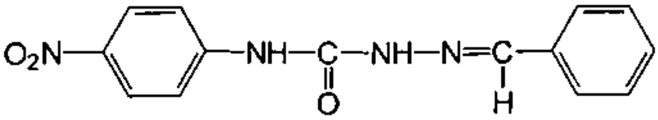
(II-41)

Micale et al. (88) prepared a series of novel 2-[(4-alkyl semicarbazono)-(4-aminophenyl)-methyl]-4,5-methylenedioxyphenyl acetic acid alkyl esters carrying an alkyl semicarbazono 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-alkyl semicarbazono moiety had been removed. The anticonvulsant activity of all compounds was assayed against audiogenic seizures induced in DBA/2 mice. Within this series of derivatives, 2-[(4-aminophenyl)-(4-methylsemicarbazono)-methyl]-4,5-methylenedioxy phenylaceticacid methylester (**II-42**) proved to be most active compound. It displayed a potency 5-fold higher than that shown by 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI-52466), a well known non-competitive 2-amino-3-(3-hydroxy-5methyl-isoxazol-4yl)propionicacid (AMPA) receptor antagonist.



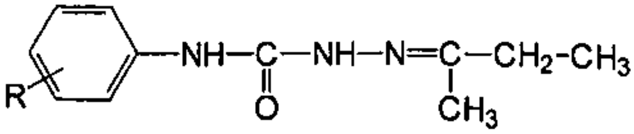
(II-42)

Pandeya et al. (59) synthesized a series of p-nitrophenyl substituted semicarbazones (**II-43**) and their anticonvulsant activity was screened against MES, scPTZ and scSTY tests. Compounds with –NHCO-were found to be the most active in the MES test after oral administration in rats. The studies revealed that the hydrogen-bonding domain in semicarbazones, adjacent to the lipophilic aryl ring, is essential for the anticonvulsant activity.



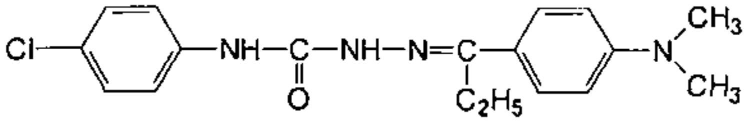
(II-43)

Pandeya et al. (89) synthesized a series of 4-N-substituted arylsemicarbazones with increased lipophilicity and evaluated for anticonvulsant activity. The compounds provided significant protection against MES and scPTZ induced seizures at 300 mg/kg after 0.5 h. The study has shown that introduction of alkyl (ethyl) at the terminal amino group (**II-44**) led to increased activity and decreased toxicity.



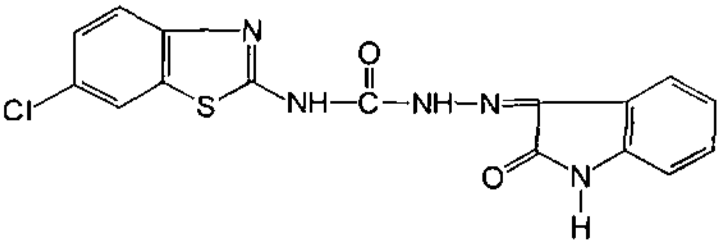
(II-44)

Pandeya et al. (90) reported a series of semicarbazones in which p-Chlorophenyl semicarbazone of N,N-dimethylamino propiophenone (**II-45**) has been found to be the most active compound.



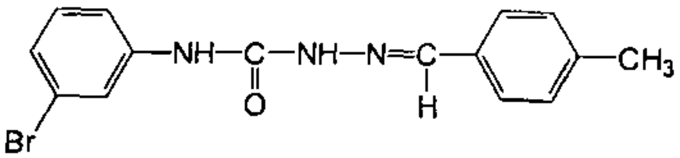
(II-45)

Yogeeswari et al. (91) synthesized ten 6-chlorobenzothiazolyl-2-thiosemicarbazones and screened for anticonvulsant and neurotoxic properties. Most of the compounds showed anticonvulsant activity against both MES and scPTZ screens. Compound [4-(6-chlorobenzothiazol-2-yl)-1-(3-isatimino)thiosemicarbazone] (**II-46**) emerged as the most promising one with an ED_{50} of 17.86 and 6.07 mg kg^{-1} in mice and rat p.o., respectively. Compound **46** showed a weak ability to block the expression of fully kindled seizures.



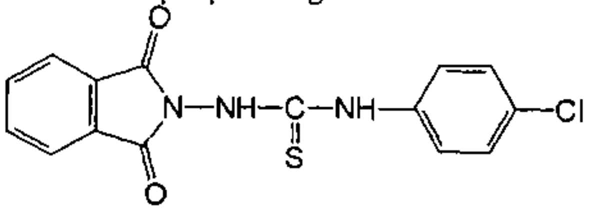
(II-46)

Yogeeswari et al. (92) synthesized a series of 3-bromophenylsemicarbazone derivatives and evaluated for anticonvulsant activity. Compound N^1-(3-bromophenyl)-N^4-(4-methyl benzylidene)semicarbazone (**II-47**) was demonstrated to have anticonvulsant activity in the MES and scPTZ tests, the ED_{50} being 32.35 mg/kg and <45.0 mg/kg, respectively. The quantitative measurement of γ-amino butyric acid (GABA) levels in different regions of the rat brain after administration of the aryl semicarbazone had shown a significant increase in GABA level. This result was the first of its kind to report aryl semicarbazone to act through GABA mediation.



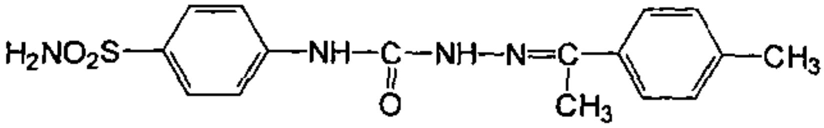
(II-47)

Yogeeswari et al. (93) prepared phenyl(thio)semicarbazide derivatives of phthalimido pharmacophore and evaluated for their anticonvulsant and neurotoxic properties. Initial anticonvulsant screening was performed using intraperitoneal (i.p) route, MES induced seizure, scPTZ and scSTY induced seizure threshold tests in mice. N^4- phthalimido-4-chloro phenyl(thio)semicarbazide (**II-48**) compound afforded protection in all the three screens. The compounds exhibited CNS depression and behavioral despair side effects, lesser than the conventional antiepileptic drugs.



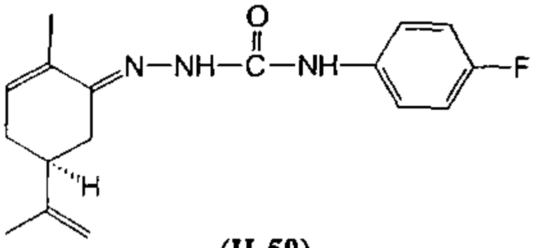
(II-48)

Yogeeswari et al. (94) prepared a series of 4-sulphamoyl phenyl semicarbazone derivatives and screened for anticonvulsant activity. The results indicated that greater protection was obtained in the MES screen and scSTY than the scPTZ tests. All the compounds showed low neurotoxicity when compared to the clinically used drugs. Compounds with substituted acetophenone showed good activity in the rat oral MES screen. Among the new derivatives evaluated, compound 4-sulphamoyl phenyl semicarbazone with 4-methyl acetophenone (**II-49**), emerged as the most active compound as indicated by its protection in the MES and sc STY screens and with low neurotoxicity.

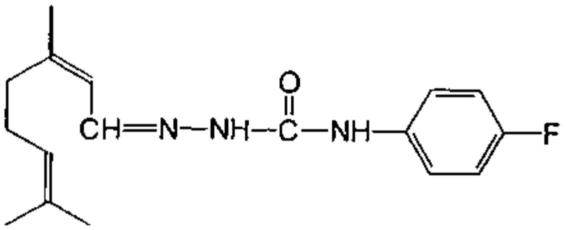


(II-49)

Aggarwal et al. (95) designed and synthesized a series of 4-aryl substituted semicarbazones of citral and R (-) carvone to meet the structural requirements essential for anticonvulsant activity. All the compounds were evaluated for anticonvulsant activity by MES and scPTZ induced seizure models and NT by rotorod test. The results showed that anticonvulsants with cyclic and acyclic terpenoid moiety retain activity in MES as well as scPTZ test. The 4-Fluro aryl substituted semicarbazones (**II-50, II-51**) emerged as the most active in both cyclic and acyclic terpenes.

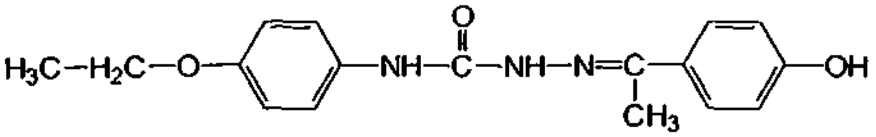


(II-50)



(II-51)

Yogeeswari et al. (96) synthesized a series of 4-ethoxyphenyl semicarbazone derivatives and screened for anticonvulsant activity. All the test compounds were administered to mice at doses of 30, 100 and 300 mg kg^{-1} body weight and the anticonvulsant activity was noted at 0.5h and 4h time intervals after the drug administration. Among the compounds tested, compound **II-52** showed protection from seizures in both the animal models and also found to increase γ-aminobutyric acid (GABA) levels in the medulla oblongata region of the rat brain.



(II-52)

These literature reports gave an impetus to design and develop newer aryl semicarbazone derivatives, which has emerged as a newer generation of anticonvulsants in the recent years.

CHAPTER-III

OBJECTIVE & PLAN OF WORK

Objective: -

(1) The aim of the research work was to design and synthesize novel disubstituted phenyl semicarbazones and their analogues with potential broad spectrum anticonvulsant activity with no or lesser neurotoxicity and devoid of side effects like sedation, CNS depression and hepatotoxicity.

(2) The research work would also attempt to study the mechanism of action by carrying out neurochemical study in rat brain.

Plan of work: -

The plan of work is broadly classified into 3 headings

1. **Molecular Design: -**

   Comparing the structures of the well-known and recently approved antiepileptic drugs led to a common pharmacophore model comprising of one aryl ring, a hydrogen bond acceptor / donor system and an electron donor atom / group. With this as basic, in the present study pharmacophore mapping and matching including superimposition of the pharmacophores and estimation of the root mean square deviation has been carried out.

2. **Synthesis: -**

   Various disubstituted phenyl semicarbazones and its analogues have been synthesized either by conventional urea method or by phenyl carbamate method. The present work comprises of the synthesis of the following

   (A) 3-chloro-2-methylphenylsemicarbazones.

   (B) 2,4-dimethylphenylsemicarbazones.

   (C) 2,4-dimethoxyphenylsemicarbazones.

   (D) 2,6-dimethylphenylsemicarbazones.

   (E) 2,6-dimethylhydrazonoacetanilide derivatives.

3. **Pharmacology: -**

All the synthesized compounds were estimated for anticonvulsant activity using a battery of tests which includes

1. Maximal electroshock seizure test (MES).
2. Subcutaneous pentylenetetrazole seizure threshold test (sc PTZ).
3. Subcutaneous strychnine induced convulsive test (scSTY).

Selected compounds that showed promising activity were tested for their ability to protect against

4. Subcutaneous picrotoxin seizure threshold test (scPIC) and
5. Hippocampal kindling test.

Some representative compounds from each series were estimated for the side effects and toxicity that includes

1. Neurotoxicity.
2. Behavioral despair effect.
3. CNS depression.
4. Sedative-hypnotic property.
5. Hepatotoxicity.

In order to determine the possible mechanism of action, representative compounds from each series were evaluated in the neurochemical study.

CHAPTER-IV

MOLECULAR DESIGN

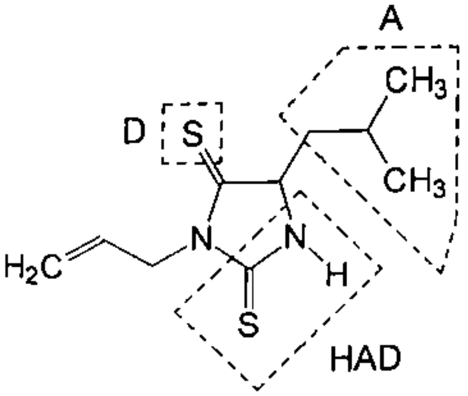
A very important part of drug design is prediction of small molecule binding to a target macromolecule. A reasonable qualitative prediction of binding can be made by specifying the spatial arrangement of a small number of atoms or functional groups. Such arrangement is called pharmacophore. The pharmacophore search finds molecule with different overall chemistry, but which have the functional groups in the correct geometry. Once a pharmacophore has been isolated, it can be used to further improve the activity of pharmaceutical drugs (97).

The pharmacophore is an important and unifying concept in rational drug design that embodies the notion that molecules are active at a particular receptor because they possess a number of key features (i.e. functional groups) that interact favourably with the receptor and which possess geometry complimentary to it (98).

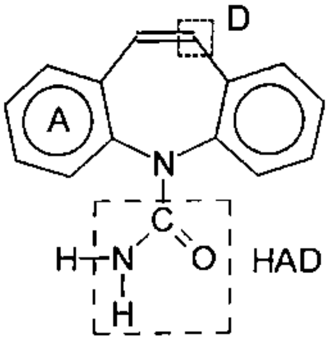
The process of deriving a pharmacophore, called pharmacophore mapping consists of three steps

(i) Identifying common binding elements that are responsible for biological activity.
(ii) Generating potential conformations that active compound may adopt and
(iii) Determining the 3D relationship between the pharmacophore elements in each conformation generated.

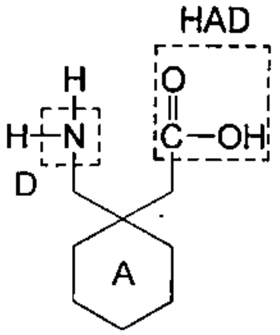
Earlier 2D-modeling on anticonvulsants have identified that atleast one aryl unit, one or two electron donor atoms, and / or an NH group in a special spatial arrangement to be recommended for anticonvulsant activity (99-103). On the basis of some ideas of Camerman and Camerman (99) and Wong et al. (100), Jones and Woodbury (101,102) defined a model with two electron donors in some proximity to a bulky hydrophobic moiety. Selecting other compounds as those of Jones and Woodbury, Codding et al. (103) postulated a pharmacophore consisting of a linear arrangement of a rotated phenyl ring, an electron donor atom, and a hydrogen donor site, which partially agrees with the model of Jones and Woodbury. Bruillette et al. (104-106) investigated the sodium channel blocking activity of several mono- and bicyclic phenytoin analogues and concluded that high log P values, a free imide group, and a specific aromatic ring orientation are optimal for high binding affinity to the sodium channel. These criteria were well fulfilled in monocyclic hydantoins. However, the conclusions of these studies were not related to other substance classes acting at the same receptor site. Since only the primary structures of several types of voltage-dependent sodium channels are known (107-110), a study of the various anticonvulsants with sodium channel blockade activity may define structural elements, which are essential for activity.



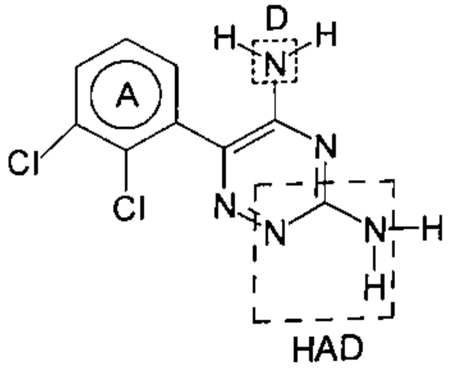
Albutoin



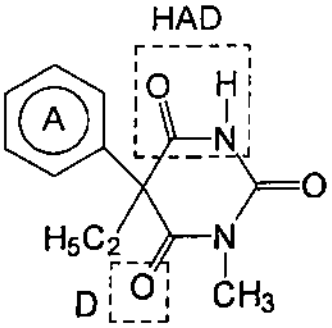
Carbamazepine



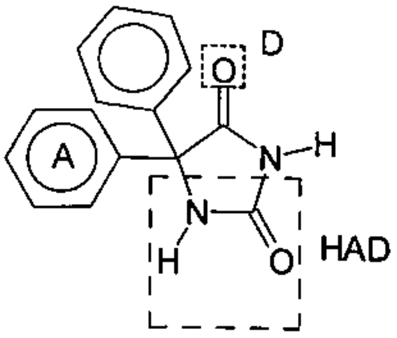
Gabapentin



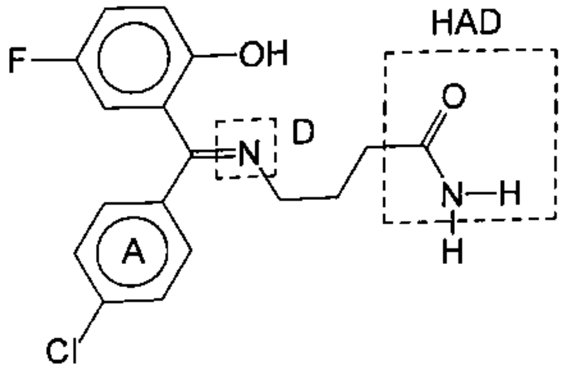
Lamotrigine



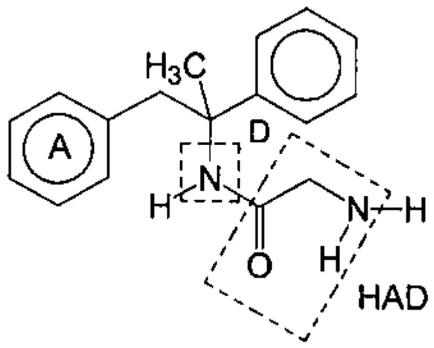
Mephobarbital



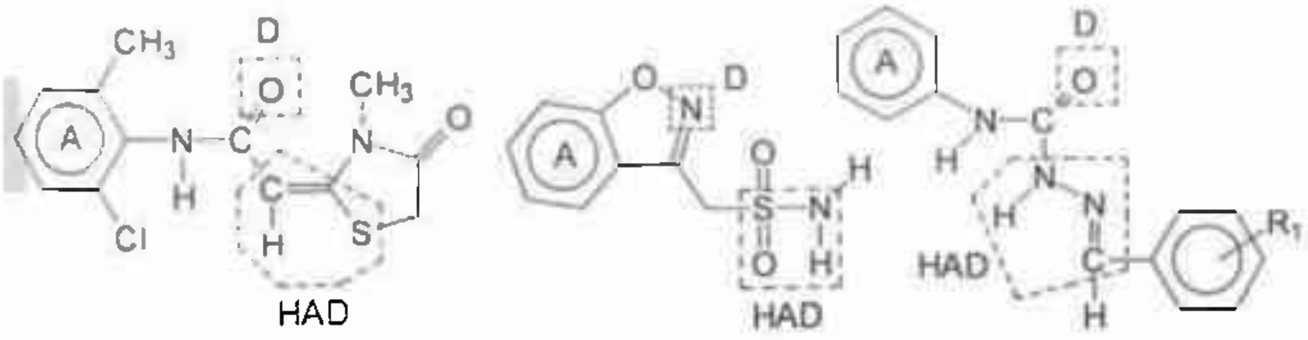
Phenytoin



Progabide



Remacemide



Ralitoline Zonisamide Aryl semicarbazone

A → Hydrophobic unit
D → Electron donor group
HAD → Hydrogen bond acceptor / donor unit

Fig IV.1: Selected Anticonvulsants for the development of a pharmacophore model.

Development of a pharmacophore model and design of aryl semicarbazones

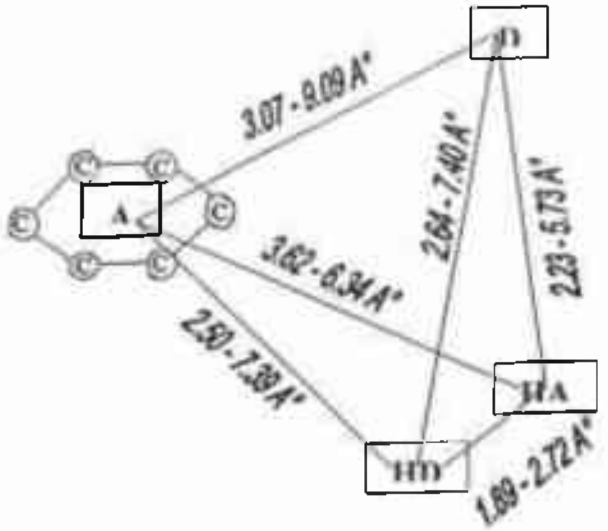


Fig IV.2: Suggested pharmacophore model for Anticonvulsants on the basis of MM3 and CHARMM simulations.

In the present study, the ten well-known and structurally different compounds with anticonvulsant activity, Albutoin, Carbamazepine, Gabapentin, Lamotrigine, Mephobarbital, Phenytoin, Progabide, Ralitoline, Remacemide and Zonisamide were selected.

To build a pharmacophore based on the structures of anticonvulsant compounds, two methods were applied. In the first method a set of minimum energy conformation for each structure was generated and common structural features were noted. In another method all possible conformations for each structure were considered to evaluate shared orientations of the common functional groups. The pharmacophore group's distance estimation was done by molecular mechanics calculations with the force fields based on both CHARMM force fields and MM3 parameterization (15). In the present work, energy minimization was performed on Albutoin, Carbamazepine, Gabapentin, Lamotrigine, Mephobarbital, Phenytoin, Progabide, Ralitoline, Remacemide, Zonisamide, and the Aryl semicarbazones using both CHARMM (ACD 3D views) and MM3 (Alchemy 2000) parameterization. A systemic conformational search was performed using Alchemy 2000 program.

The crucial structural components that were included in the 4-point pharmacophore model (Figure IV.1) were the aryl ring center or the lipophilic group (A), an electron donor atom (D), a hydrogen bond acceptor (HA) and a hydrogen bond donor (HD).

Pharmacophore generation through conformational analysis and manual alignment is a very time consuming task, especially when the list of active compounds is large. In agreement with all the compounds, we determined the centre of the aromatic ring as the reference point for A. The distance between the 4-pharmacophoric points were calculated for minimum four different conformations and represented as mean ± standard deviation (Table IV.1). An optimized range for every point were obtained and compared for aryl semicarbazones.

Now it may be interesting to examine whether the aryl semicarbazones reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that the aryl semicarbazones 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.

The proposed aryl semicarbazones were also found to comply within the optimal range between the pharmacophoric elements. A representative of the proposed aryl semicarbazone was superimposed by merging the two energy minimized structures by MM3 force fields and fast RMS (Root mean square) fit was calculated with Carbamazepine, Lamotrigine, and Phenytoin by superimposing the aryl ring and the electron donor atoms and are presented in Figures IV.3-5. The proposed aryl semicarbazone was also superimposed with Ralitoline due to structural similarity and presented as Figure IV.6. Comparing the RMS error, it was found that the proposed aryl semicarbazone would fit more with Ralitoline and Lamotrigine.

***Table IV.1:* Distance ranges between the essential structure elements A, D, HA and HD of ten anticonvulsants by molecular mechanics (MM 3) and Chemistry at Harvard Macromolecular Mechanics (CHARMM) force fields: -**

S.No.	Compound	A - HA		A - HD		A - D		HA - HD		HD - D		HA - D	
		CHARMM	MM3	CHARMM	MM3	CHARMM	MM3	CHARMM	MM3	CHARMM	MM3	CHARMM	MM3
1	Albutoin	4.87 ± 0.00	5.06 ± 0.00	2.50 ± 0.00	2.53 ± 0.00	4.69 ± 1.61	4.81 ± 1.66	2.64 ± 0.00	2.72 ± 0.00	4.21 ± 1.52	4.16 ± 1.45	4.45 ± 1.27	4.35 ± 1.65
2	Carbamazepine	3.62 ± 0.99	3.73 ± 1.02	4.54 ± 0.70	4.41 ± 0.09	3.39 ± 0.62	3.86 ± 0.68	2.32 ± 0.11	2.28 ± 0.06	5.30 ± 0.21	5.24 ± 0.03	4.15 ± 1.20	3.88 ± 1.03
3	Gabapentin	4.77 ± 0.00	4.78 ± 0.00	4.61 ± 0.00	4.64 ± 0.00	3.88 ± 0.00	4.00 ± 0.00	2.23 ± 0.00	2.26 ± 0.00	2.66 ± 0.00	2.64 ± 0.00	3.52 ± 0.00	3.36 ± 0.00
4	Lamotrigine	5.14 ± 0.00	5.17 ± 0.00	5.62 ± 1.97	5.70 ± 1.94	4.46 ± 0.85	4.50 ± 0.88	2.42 ± 0.00	2.42 ± 0.01	3.47 ± 0.73	3.48 ± 0.74	2.58 ± 0.26	2.53 ± 0.26
5	Mephobarbital	5.46 ± 1.72	5.50 ± 1.69	5.03 ± 0.00	5.04 ± 0.00	4.64 ± 0.56	4.64 ± 0.53	2.24 ± 0.00	2.26 ± 0.01	3.20 ± 1.15	3.19 ± 1.18	3.85 ± 1.11	3.88 ± 1.12
6	Phenytoin	4.88 ± 0.05	4.82 ± 0.06	4.94 ± 1.24	4.97 ± 1.22	4.43 ± 0.01	4.51 ± 0.03	2.30 ± 0.05	2.26 ± 0.12	4.04 ± 0.82	4.15 ± 0.82	2.36 ± 0.00	2.43 ± 0.00
7	Progabide	3.92 ± 0.21	4.02 ± 0.28	4.20 ± 2.06	4.32 ± 2.14	9.09 ± 0.76	8.96 ± 0.68	2.40 ± 0.00	2.67 ± 0.00	6.81 ± 1.25	7.40 ± 1.12	5.63 ± 0.85	5.73 ± 0.72
8	Ralitoline	4.02 ± 0.04	4.24 ± 0.05	4.49 ± 1.51	4.43 ± 1.54	6.50 ± 1.48	6.89 ± 1.42	2.21 ± 0.00	2.29 ± 0.00	2.82 ± 0.00	2.84 ± 0.00	3.34 ± 1.11	3.47 ± 1.13
9	Remacemide	6.34 ± 1.01	5.55 ± 1.51	6.61 ± 1.33	7.39 ± 1.64	4.50 ± 0.92	4.54 ± 0.95	2.68 ± 0.00	3.01 ± 0.00	3.69 ± 0.00	3.40 ± 0.00	2.23 ± 0.00	2.29 ± 0.00
10	Zonisamide	4.42 ± 0.06	4.51 ± 0.13	6.03 ± 0.00	5.82 ± 0.00	3.07 ± 0.47	3.29 ± 0.49	2.22 ± 0.51	1.89 ± 0.53	5.24 ± 1.05	5.02 ± 1.09	4.02 ± 0.84	4.07 ± 0.89
11	Aryl semicarbazone	4.09 ± 0.13	4.19 ± 0.18	3.95 ± 1.62	3.96 ± 1.58	6.90 ± 0.73	6.59 ± 0.91	2.25 ± 0.02	2.26 ± 0.03	3.06 ± 1.46	2.95 ± 1.43	3.46 ± 0.92	3.20 ± 0.85

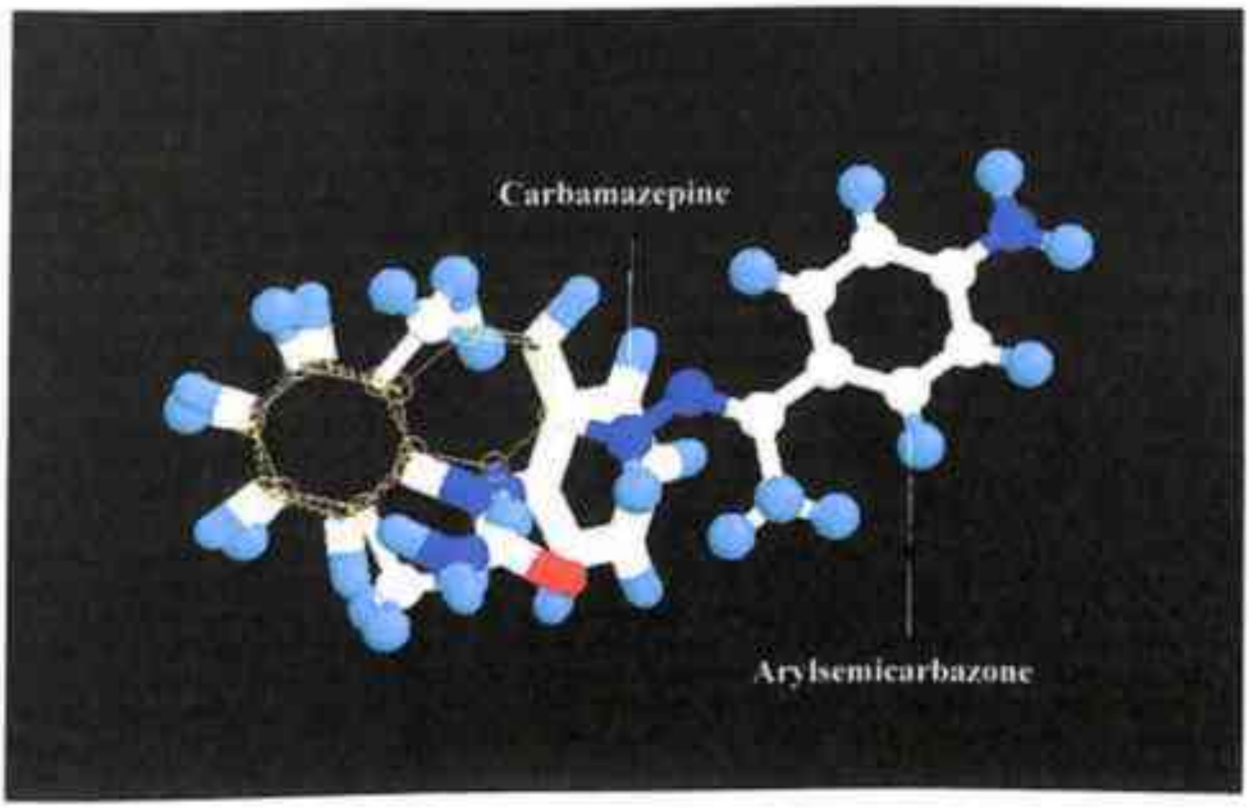


Figure IV. 3. Superimposition and Fast RMS fit of the proposed arylsemicarbazone with Carbamazepine (RMS error = 0.166).

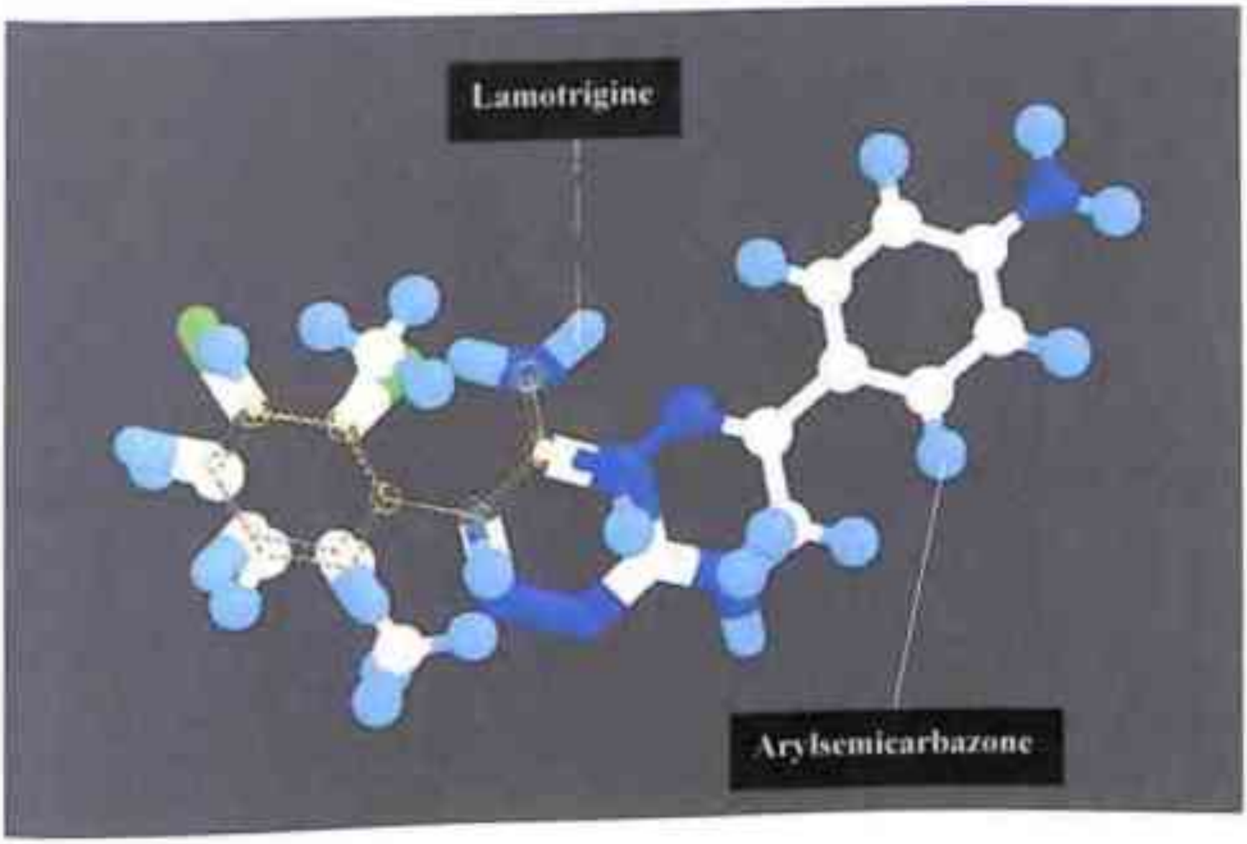


Figure IV. 4. Superimposition and Fast RMS fit of the proposed arylsemicarbazone with Lamotrigine (RMS error = 0.097).

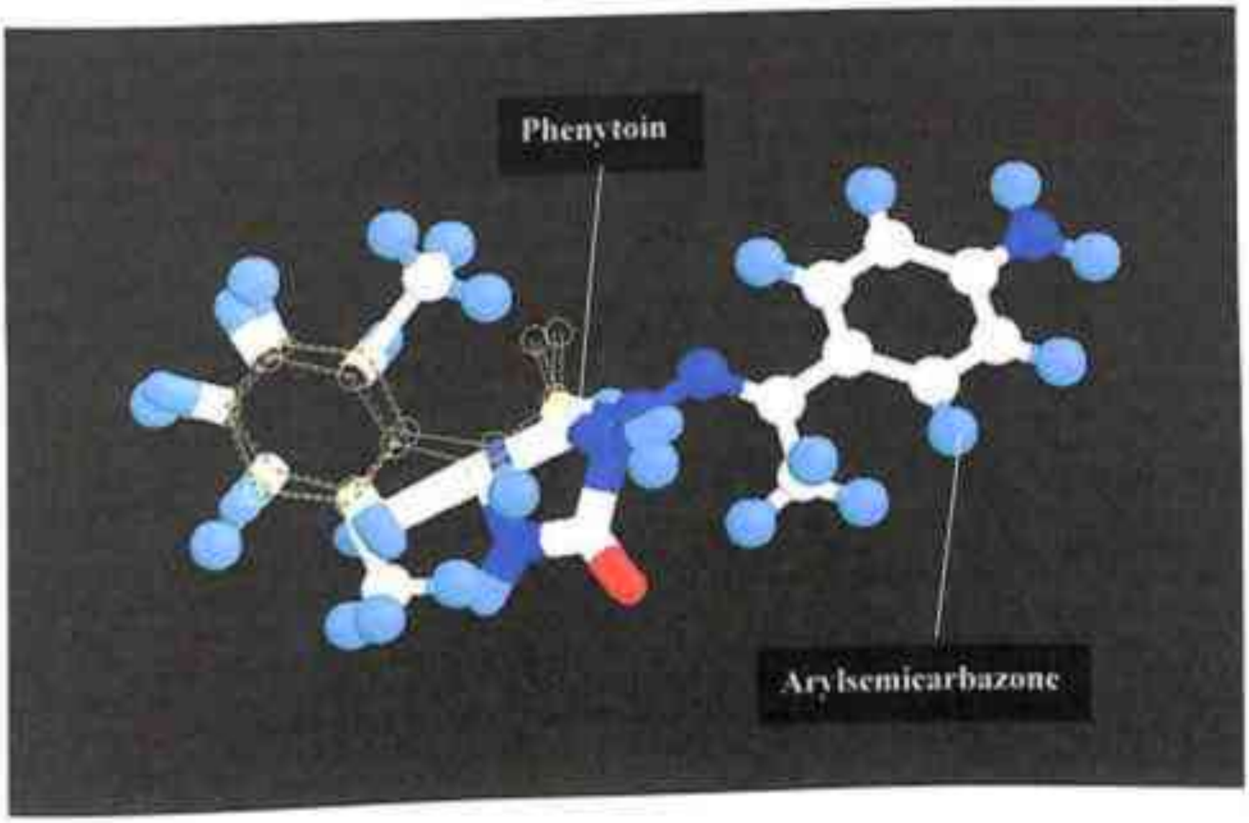


Figure IV. 5. Superimposition and Fast RMS fit of the proposed arylsemicarbazone with Phenytoin (RMS error =0.142).

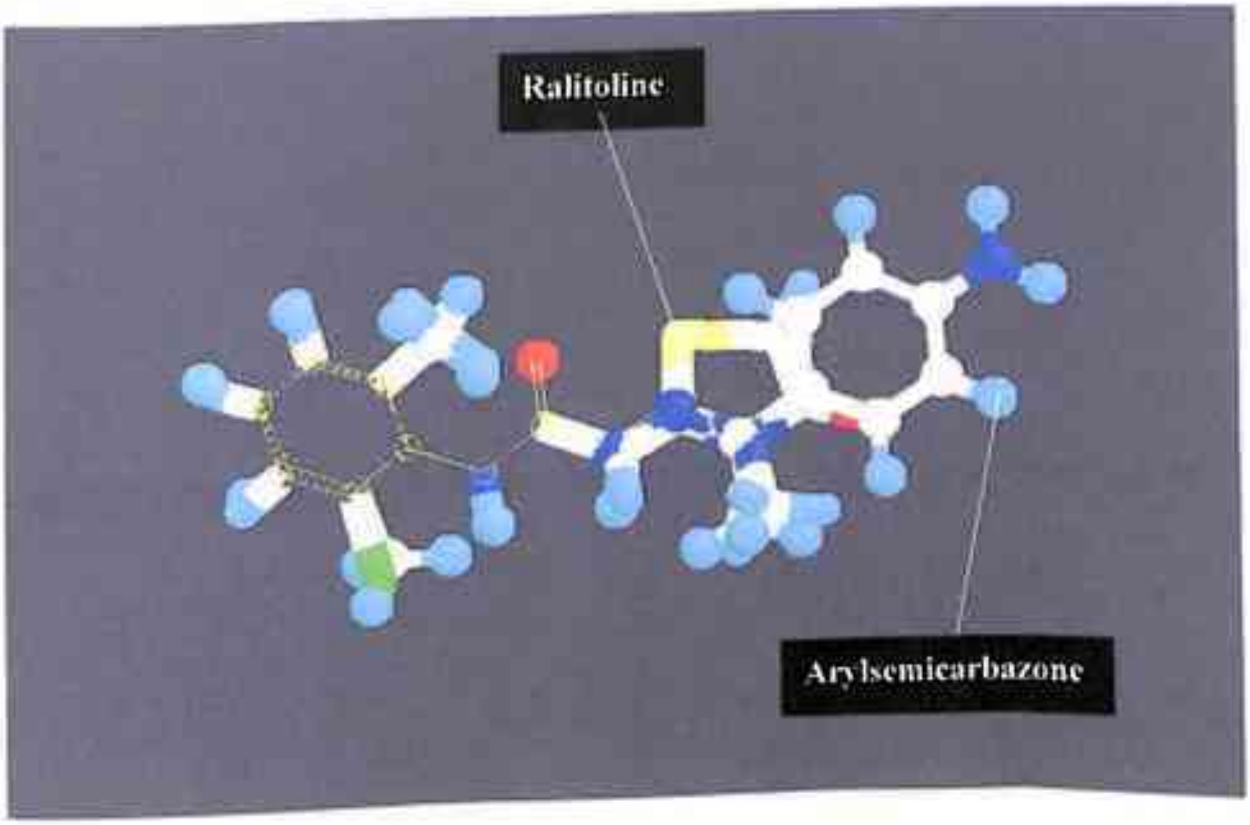


Figure IV. 6. Superimposition and Fast RMS fit of the proposed arylsemicarbazone with Ralitoline (RMS error =0.036).

CHAPTER-V

MATERIALS AND METHODS

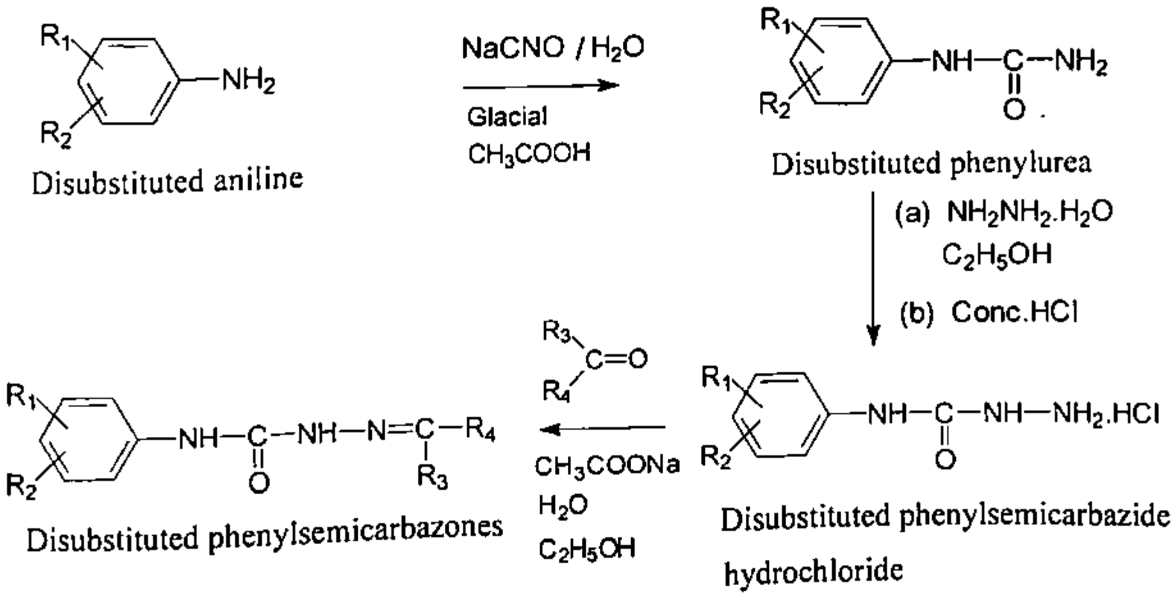
Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus and are uncorrected. Infra red spectra (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) spectrophotometers, 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 Shimatzu 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 aluminum 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.

I. Synthesis: -

Synthesis of disubstituted phenyl semicarbazones was accomplished by either of the following methods.

Method I: -

The syntheses of the 3-chloro-2-methyl substituted phenylsemicarbazones and 2,4-dimethyl substituted phenylsemicarbazones have been prepared according to the earlier reported procedure (84).



Where (A) $R_1 = 3\text{-Cl}; R_2 = 2\text{-CH}_3$

(B) $R_1 = 2\text{-CH}_3; R_2 = 4\text{-CH}_3$

$R_3 = H; CH_3; C_6H_5$

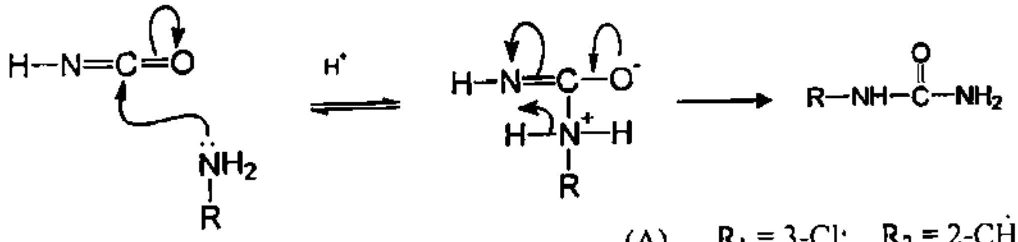
$R_4 = CH_3; C_2H_5; C_6H_5; 4\text{-Cl } C_6H_4; 4\text{-}CH_3 C_6H_4$ etc.

The disubstituted aniline was the starting material, which was treated with sodium cyanate in the presence of glacial acetic acid to give the disubstituted phenyl area. The urea on condensation with hydrazine hydrate in ethanol medium gave the disubstituted phenyl semicarbazide, which was purified by converting it into its hydrochloride salt by the addition of concentrated hydrochloric acid. Finally, the required disubstituted phenyl semicarbazone derivatives was prepared by the reaction between the appropriate alkyl / aryl aldehydes or ketones and disubstituted phenyl semicarbazide hydrochloride in the presence of sodium acetate in ethanol / water medium.

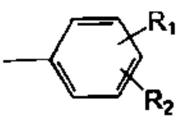
Mechanism of reaction: -

Step-1: Preparation of Disubstituted phenylurea: -

In this step, first the sodium cyanate reacts with glacial acetic acid to from sodium acetate and isocyanic acid. Then the disubstituted aniline attacking the positively polarized carbon of the isocyanic acid (HNCO) to form a Zwitter-ionic intermediate. Finally it takes a simple proton shift to form disubstituted phenyl urea (nucleophilic addition reaction).



Where R =

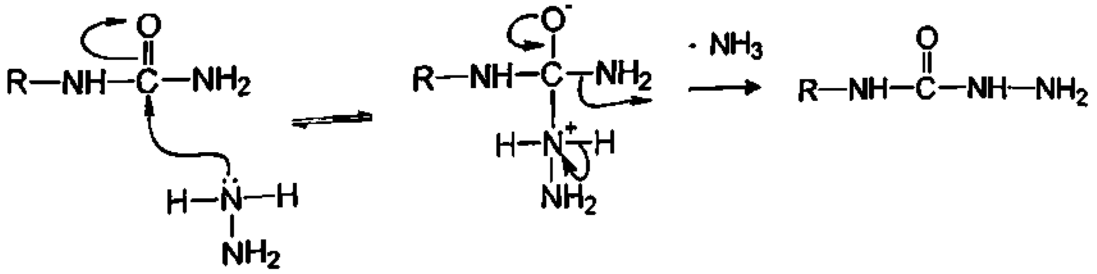


(A) R_1 = 3-Cl; R_2 = 2-CH_3

(B) R_1 = 2-CH_3; R_2 = 4-CH_3

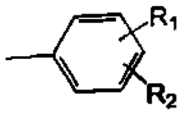
Step-2: Preparation of Disubstituted phenylsemicarbazide: -

Nucleophilic substitution at a carbonyl group most often proceeds by a second-order mechanism, which is called the tetrahedral mechanism. In the present reaction mechanism, the hydrazine moiety attacks the positively polarized carbon of the disubstituted phenyl urea to give an intermediate containing both hydrazine and amine moieties and then the amine released as ammonia.



(A) R_1 = 3-Cl; R_2 = 2-CH_3

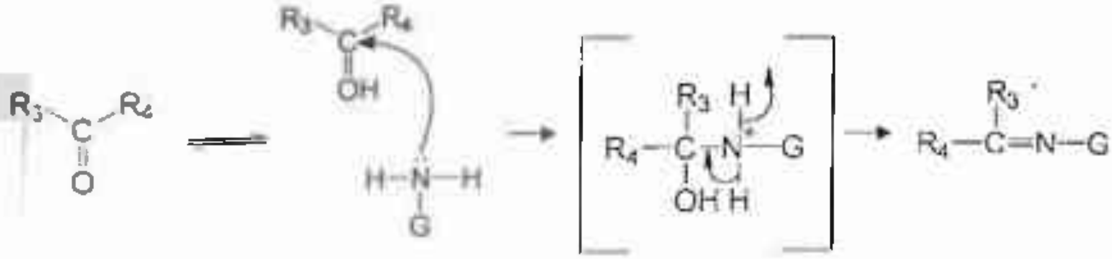
Where R =



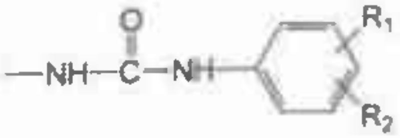
(B) R_1 = 2-CH_3; R_2 = 4-CH_3

Step-3: Preparation of Disubstituted phenylsemicarbazones: -

The reaction of formation of semicarbazones takes place by nucleophilic addition-elimination type reaction. In this step first the sodium acetate reacts with disubstituted phenyl semicarbazide hydrochloride to form free semicarbazide, sodium chloride and acetic acid. The formed acetic acid adjusts the reaction medium to the right acidic pH. Addition involves nucleophilic attack by the basic nitrogen compound on carbonyl carbon. Protonation of carbonyl oxygen makes carbonyl carbon more susceptible to nucleophilic attack; in so far as the carbonyl compound is concerned, then, addition will be favoured by high acidity. But the semicarbazide can also undergo protonation to form the ion, which lacks unshared electrons and is no longer nucleophilic; in so far as the nitrogen compound is concerned, then, addition is favoured by low acidity. The exact condition used depends upon the basicity of the reagent and upon the reactivity of carbonyl compound.



Where G =



(A) R_1 = 3-Cl; R_2 = 2-CH_3

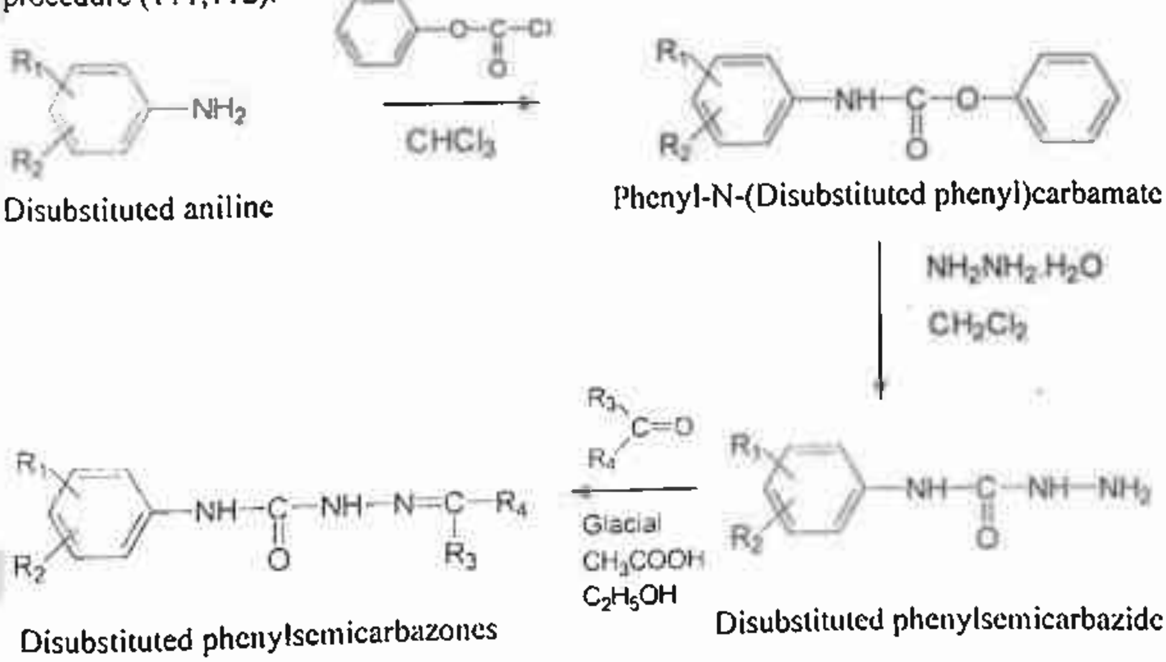
(B) R_2 = 2-CH_3; R_2 = 4-CH_3

R_3 = H, CH_3, C_6H_5

R_4 = CH_3, C_2H_5, C_6H_5, 4-CH_3-C_6H_5

Method II: -

The syntheses of the 2,4-dimethoxy substituted phenylsemicarbazones and 2,6-dimethyl substituted phenylsemicarbazones have been prepared according to the earlier reported procedure (111,112).



Where (C) $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$

(D) $R_1 = 2\text{-}CH_3$; $R_2 = 6\text{-}CH_3$

$R_3 = H$; CH_3; C_6H_5

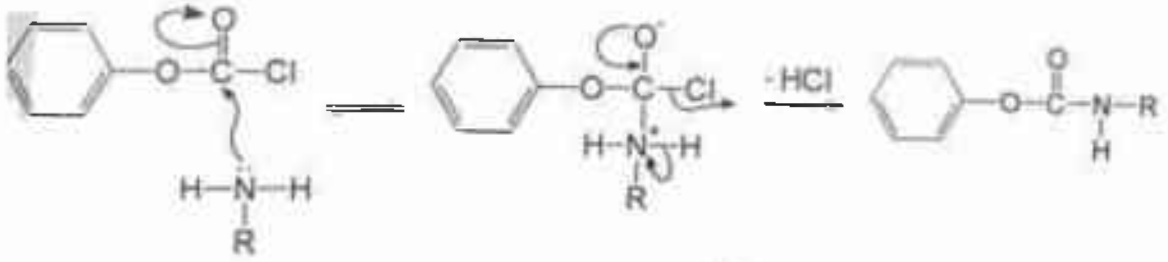
$R_4 = CH_3$; C_2H_5; C_6H_5; 4-Cl C_6H_4; 4-CH_3 C_6H_4 etc...

The disubstituted aniline was treated with phenyl chloroformate in the presence of chloroform and stirred at room temperature to get Phenyl-N-(disubstituted phenyl) carbamate. This compound on condensation with hydrazine hydrate in methylene dichloride, gave the disubstituted phenyl semicarbazide. Finally the required disubstituted phenyl semicarbazones were prepared by the reaction between the appropriate aryl/alkyl aldehydes or ketones and disubstituted phenyl semicarbazide in the presence of glacial acetic acid in ethanol.

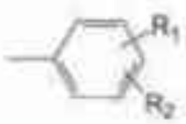
Mechanism of reaction: -

Step-1: Preparation of Phenyl-N-(Disubstituted phenyl)carbamates: -

This is a nucleophilic substitution reaction. Nucleophilic substitution at a carbonyl group often proceeds by a second order mechanism, which is called tetrahedral mechanism. In this reaction mechanism, disubstituted aniline moiety attacking the positively polarized carbon of the phenyl chloroformate containing both aniline and chlorine moieties and then the chlorine released as HCl.



Where R =

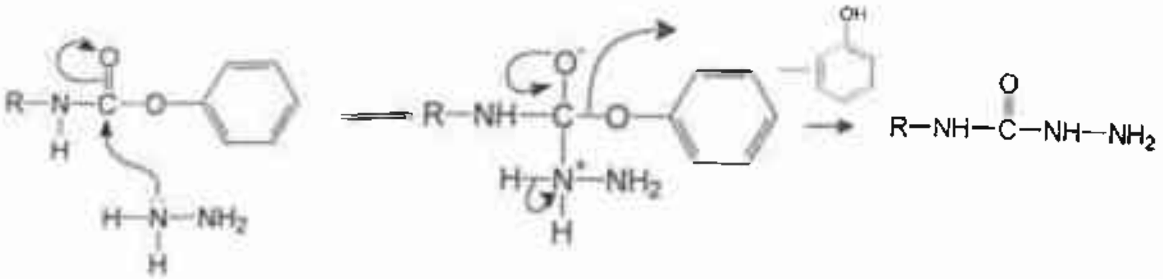


(C) $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$

(D) $R_1 = 2\text{-}CH_3$; $R_2 = 6\text{-}CH_3$

Step-2: Preparation of Disubstituted phenylsemicarbazide: -

This reaction is also nucleophilic substitution reaction. In this reaction mechanism, hydrazine hydrate moiety attacking the positively polarized carbon of the Phenyl-N-(disubstituted phenyl)carbamate containing both hydrazine and phenoxy moieties and then the phenoxy group released as phenol.



Where R =

(C) $R_1 = 2\text{-}OCH_3$; $R_2 = 4\text{-}OCH_3$

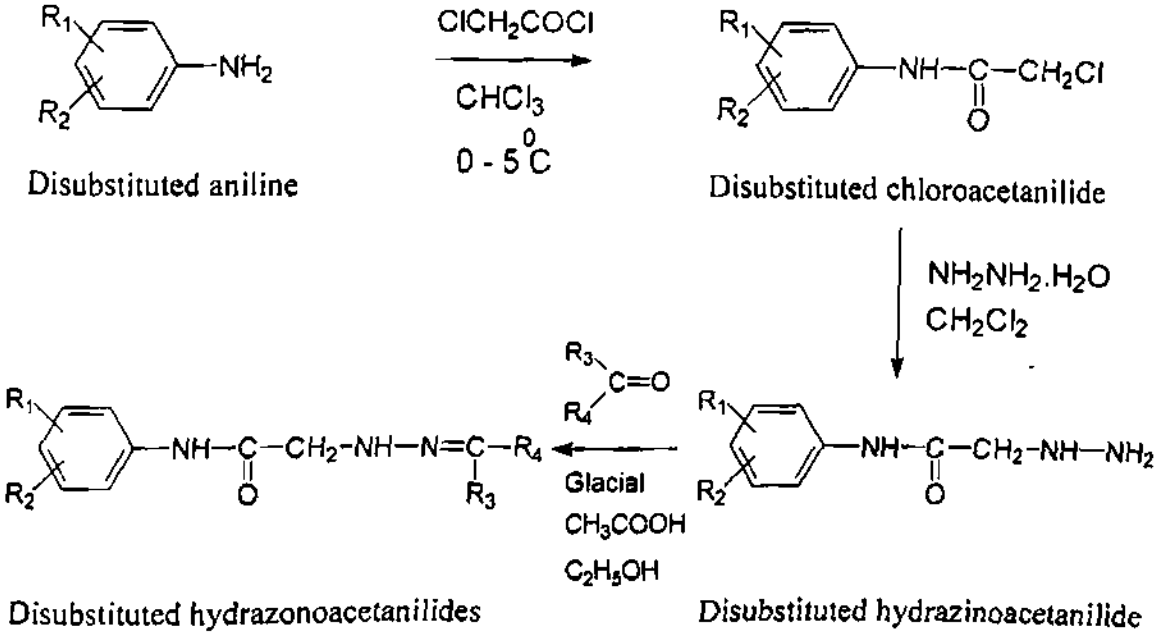
(D) $R_1 = 2\text{-}CH_3$; $R_2 = 6\text{-}CH_3$

Step-3: Preparation of Disubstituted phenylsemicarbazones: -

This reaction is nucleophilic addition-elimination type reaction. As per the description presented earlier (Page No. 50).

Modification at the semicarbazone group was studied by the synthesis of disubstituted hydrazonoacetanilide derivatives, which is presented in the following scheme. The synthetic protocol was based on the earlier reported procedure (113).

Synthesis of disubstituted hydrazonoacetanilides: -



Where $R_1 = 2\text{-}CH_3$ $R_2 = 6\text{-}CH_3$

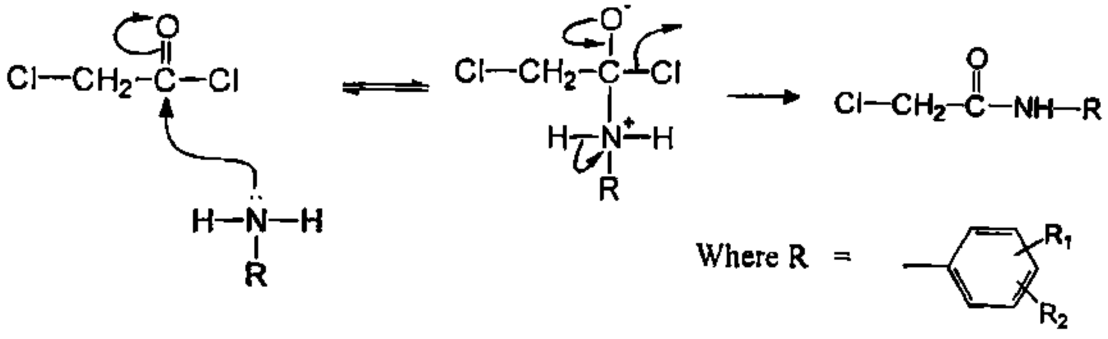
$R_3 = H; CH_3$ $R_4 = CH_3; C_6H_5;$ 4-Cl C6H4; 4-CH_3 C_6H_4 etc

The disubstituted aniline was reacted with chloro acetyl chloride at 0-5 °C to get disubstituted chloro acetanilide, which was further treated with hydrazine hydrate, to get disubstituted hydrazino acetanilide. This compound on reaction with appropriate alkyl / aryl aldehydes or ketones in ethanol in the presence of glacial acetic acid, gave the required disubstituted hydrazonoacetanilides.

Mechanism of reaction: -

Step-1: Preparation of Disubstituted chloroacetanilide: -

This is a nucleophilic substitution reaction. In this reaction, the substitution at a carbonyl group often proceeds as a second order mechanism, which is called tetrahedral mechanism. In this mechanism 2,6-dimethyl aniline moiety attacking the positively polarized carbon of the chloro acetyl chloride containing both aniline and chlorine moieties and then the chlorine released as HCl.

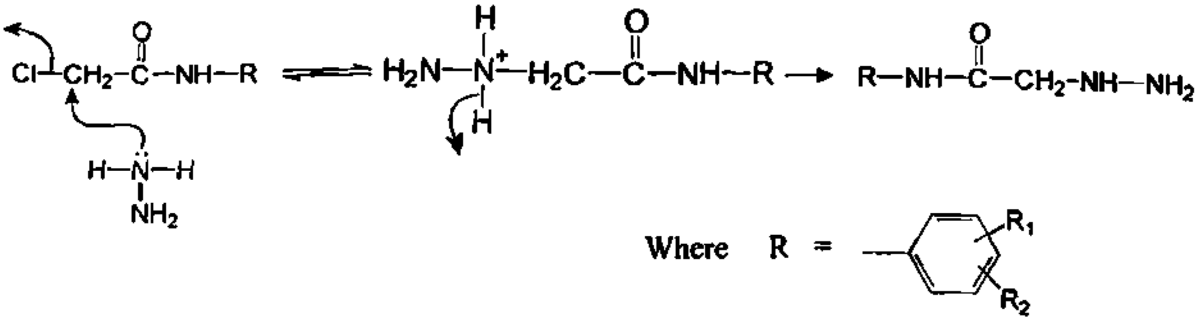


$R_1 = 2\text{-}CH_3$: $R_2 = 6\text{-}CH_3$

Step-2: Preparation of Disubstituted hydrazinoacetanilide: -

This is an aliphatic nucleophilic substitution reaction (S_N2 mechanism).

The organic halide is attacked by the nucleophilic hydrazine molecule form a transition state and then the chlorine atom is leaved as HCl.



$R_1 = 2\text{-}CH_3$: $R_2 = 6\text{-}CH_3$

Step-3: Preparation of Disubstituted hydrazonoacetanilides: -

This reaction is nucleophilic addition-elimination type reaction. As per the description presented earlier (Page No. 50).

II. Pharmacological Activities: -

The studies were conducted on CF # 1 (Carworth Farms number-one) / Swiss albino mice (20-25 gm) or Sprague-dawley / Wistar albino rats (130-160 gm) of either sex. The animals were obtained from Hissar Agricultural University and were housed under normal Laboratory conditions (12 hours light-dark cycle) with free access to food and water. The animals were housed in groups of six in perplex 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 or 2% methylcellulose / water. All procedures described in this report were reviewed and approved by the Institutional Animal Ethical Committee (Protocol No. IAEC/RES/3, 31-01-02).

The parameters selected for the pharmacological study include.

1. Anticonvulsant activity: -
    - a. Maximal Electroshock seizure test (MES).
    - b. Subcutaneous pentylene tetrazole seizure threshold test (sc PTZ).
    - c. Subcutaneous picrotoxin seizure threshold test (scPIC).
    - d. Subcutaneous strychnine seizure pattern test (sc STY).
    - e. Rat p.o. Identification.
    - f. Quantification studies (ED_{50}, TD_{50} and PI).
    - g. Hippocampal kindling test.
2. CNS depressant activity: -
    - a. Locomotor activity by using Photoactometer (INCO, Ambala).
    - b. Porsolt's swim test.
    - c. Pentobarbitone induced narcosis test.
3. Neurotoxicity study using rotorod test.
4. Hepatotoxicity study.

1. Anticonvulsant activity: -

Initially all the compounds were administered intraperitoneally (i.p) in a volume of 0.01 ml/g body weight for mouse and 0.004 ml/10g 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 after i.p. injection by one electrical and three chemical tests. The electrical test employed was the maximal electroshock seizure (MES) pattern test. The chemical tests employed were the subcutaneous pentylenetetrazole (scPTZ) seizure threshold test. Some selected compounds were evaluated in the subcutaneous picrotoxin (scPIC) seizure threshold test and subcutaneous strychnine (scSTY) seizure pattern test.

(a) *Maximal Electroshock Seizure test* (MES)(114): -

Maximal seizures were elicited by a 60 Hz alternating current of 50 mA (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% 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% 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.

(b) *Subcutaneous pentylenetetrazole seizure threshold test* (scPTZ)(115): -

This test produces minimal clonic seizures. Compounds were tested for their ability to antagonize scPTZ-induced convulsions in mice after i.p. injection as discussed elsewhere (107). The animals of control group received 0.2 ml saline (i.p.). The other groups were administered experimental drug solution (i.p). After 30 min and 4.0h, pentylenetetrazole as 0.85% solution in 0.9% sodium chloride, was administered subcutaneously in a loose fold of skin on the back of the neck in a dose of 85 mg / kg. The animals were placed in individual cages and observed for 30 min after pentylene tetrazole administration. A threshold convulsion was defined as one episode of clonic convulsion, which persisted for at least 5 seconds. Absence of a single 5 seconds episode of clonic spasm was taken as the end point in this test.

(c) ***Subcutaneous picrotoxin seizure threshold test*** **(scPIC)**(116): -

The test compounds were suspended in 30% polyethylene glycol 400 and evaluated for their ability to antagonize scPIC-induced convulsion in mice after i.p. administration. After 30 min 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 minutes 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.

(d) ***Subcutaneous strychnine seizure pattern test*** **(scSTY)**(117): -

Test solutions of all compounds were prepared in 30% polyethylene glycol 400 and 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 weights 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 indicate that the test substance has the ability to prevent seizure spread.

(c) ***Rat p.o Identification*** (118): -

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 up to longer hours to show the effect.

Some compounds were selected for oral evaluation for anti-MES and neurotoxicity activity 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 experimental drug.

(f) *Quantitative studies* (119-123): -

For the determination of the median effective dose (ED_{50}) and median neurotoxicity dose (TD_{50}), groups of 6-12 CF # 1 albino mice were given a range of i.p. doses of the test drug until at least three points were established in the range of 0-100% seizure protection or minimal observed neurotoxicity. The observed percentage was converted into probits by referring to the appropriate probit table and the values thus obtained were plotted against log dose. Before plotting, the percent for the 0 and 100 are corrected according to the formula,

$$0\% \text{ activity} = 100\ (0.25/n)$$

$$100\% \text{ activity} = 100\ ((n-25)/n)$$

The dose corresponding to probit 5 or 50% was found to be representating ED_{50}. TD_{50} values and the 95% confidence intervals were calculated. The protective index (*PI*) was then determined by taking the ratio of TD_{50} to ED_{50}.

(g) ***Kindling experiment*** **(Hippocampal kindling test)**(124): -

The test compounds were evaluated in the hippocampal-kindling seizure model in rats. Briefly a bipolar electrode was placed sterotaxically into the ventral hippocampus (AP-3.6, ML-4.9, VD-5.0 from dura, incisor bar ± 5.0) of adult male Sprague-Dawley rats (275-300g) under ketamine-xylazine anaesthesia as reported in the literature (125). After 1-week of recovery period, animals were kindled to stage 5 behavior seizure by using a stimulus consisting of a 50 Hz, 10 sec train of 1 msec biphasic 200 μA pulses delivered every 0.5 for 6h (12 stimulations per day) on alternating days for a total of 60 stimulations (5 stimulus days). Five seizure scores were assigned as follows: 1 (mouth and facial clonus); 2 (stage 1 plus head nodding); 3 (stage 2 plus forelimb clonus); 4 (stage 3 plus rearing) and 5 (stage 4 plus repeated rearing and falling). During the subsequent 7 days period, the animals were not electrically stimulated. After this 1-week recovery period, the animals received two to three suprathreshold stimulations delivered every 0.5h before administration of the test compound. During these control blocks, the stability of the behavioral seizure stage and after discharge duration (ADD) was recorded. A 130 mg/kg dose of compound 8 and 100 mg/kg dose of compound 6 were administered intraperitoneally 0.25 h after the last control block. Each of the two rats was stimulated after 0.25 h and the individual scores and after discharge durations (ADD) were recorded after each stimulation. The control rats received the vehicle only. The behavioral seizure latency was the time span between the application of stimulus and the appearance of the first sign of a motor seizure. Behavioral seizure duration was the length of time that the motor seizure was observed.

(2) CNS depressant activity: -

(a) ***Behavioral test by using Photoactometer*** (126): -

The test compounds (30 mg & 100 mg/kg) were screened for their behavioral effects using Photoactometer (INCO- Ambala) at 30 min and 1.0 h 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 was 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 gets 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.

(b) ***Porsolt's Swim test: -***

The method adopted was a notification of that described by Porsolt et al (127). 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 24 hours prior to the conduct of a six-minute test. The animals were administered an i.p. injection (30 mg & 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, last 5 min of the total 7 min period)

Pentobarbitone induced narcosis study: -

The method of barbiturate hypnosis potentiation activity by Forney et al. (123) was employed. Swiss albino mice of either sex (20-25 g) were divided into groups of six and food was withdrawn 12 hr before the start of the experiment. Each animal of the control group was injected pentobarbitone sodium (40 mg / kg, i.p) and sleeping time of each mouse was noted as the interval between loss and gain of righting reflex. The criteria for loss and gain of righting reflex were taken as the inability or ability respectively of the mice to right themselves within 30 sec, in 3 successive trials when placed on its back.

In the other group, each animal was injected intraperitoneally the test drug at a dose of 30 mg/kg. After 30 min, each animal was injected pentobarbitone sodium (40 mg/kg, i.p) and the time of loss and gain of righting reflex were taken as the end point. The significance of the data was determined by performing Student's t-test in comparison with the control group (128).

(3) Neurotoxicity (NT) screen: -

Minimal motor impairment was measured in mice by the rotorod test (129, 130). The mice were trained to stay on an accelerating rotorod 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.

(4) Hepatotoxicity Studies: -

Wistar albino rats (120-160 gm) of either sex were reared in a quiet room with an ambient temperature of 25 ± 2°C, on a standard normal diet and water *ad libitum*. The animals were divided into groups of 6 and the control group received a basal diet and vehicle. Other groups were administered the test drug in a dose of 30 or 100 mg / kg / day p.o (in PEG 400 or 2% methyl cellulose) for 14 days. After the stipulated period, each animal was anaesthetized by anesthetic ether and blood was collected by cardiac puncture to assess the transaminase activity. The *in-vitro* determination of transaminase activity was carried out by the 2,4-dinitro-phenyl hydrazine method (131, 132) using SPAN diagnostic reagents kits.

Principle: -

SGPT (ALT) catalyses

α - Ketoglutarate + L-Alanine $\xrightleftharpoons{\text{SGPT}}$ L-Glutamate + Pyruvate.

SGOT (AST) catalyses

α - Ketogluarate + L-Alanine $\xrightleftharpoons{\text{SGOT}}$ L-Glutamate + Oxaloacetate.

Pyruvate and oxaloacetate so formed is coupled with 2, 4-dinitro-phenyl hydrazine (2, 4 - DNPH) to give the corresponding hydrazone, which gives brown color in alkaline medium and this can be measured colorimetrically or spectrophotometrically.

Reagents: (Supplied in the kit)

Reagent-1: Buffered Alanine - α - Ketogluarate substrate, pH 7.4

Reagent-2: DNPH color reagent.

Reagent-3: Sodium hydroxide, 4N

Reagent-4: Working Pyruvate standard, 2 mM.

Reagent-5: working oxaloacetate standard, 2mM

Preparation of working solutions:

1 ml of Reagent 3 was diluted to 10 ml with distilled water (solution I). Other reagents were ready for use.

Procedure:

Standard curve: -

As the reaction proceeds with time, more amounts of products are formed and since the end products inhibit the enzyme, there is more of inhibition. Because of this problem, it is necessary to standardize any spectrophotometric method against a standard kinetic method. With the use of the working standards provided with the kit, standard calibration curves were obtained by taking enzyme activity on X-axis and O.D on Y-axis. The standard curve is presented in Fig-V.3 and V.4.

Test for hepatotoxicity: -

0.1 ml of the serum samples were dissolved in 0.5 ml buffered substrate (pH 7.4) and incubated at 37°C for 30 min. To this, Reagent-2 (DNPH color reagent) was added and mixed well. The mixture was allowed to stand at room temperature for 10 min and the 0.D was read against a blank on a Jasco UV spectrophotometer at an absorption maxima of 505nm. The enzyme activity corresponding to the O.D was obtained from the standard curve.

Neurochemical Work: -

Determination of GABA level in rat brain: -

The disubstituted phenyl semicarbazone derivatives have shown good anticonvulsant activity. Hence neurochemical investigation was carried out to study the effect of the compounds on the GABA levels in rat brain. Various methods for estimation of GABA in tissue extracts have been reported. Some of the methods are

i. Enzymatic UV method

ii. Enzymatic fluorimetric method

iii. Paper chromatographic method (133)

iv. Receptor inhibition method (134)

v. Column chromatographic method (135)

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 UV method have been used.

Equipments: -

1. Glass apparatus : Borosil
2. Micropipettes : Accupipette
3. Incubator shaker : MAC Orbital shaking incubator
4. Refrigerated centrifuge : Remi cooling compufuge
5. Refrigerator / deep freezer : Vest frost
6. Vacuum Centrifuge : Maxy dry Ivo

7. Water bath : Remi
8. Incubator : SEW India
9. U.V. Spectrphotometer : Perkin Elmer Lamda EZ201
10. Sonicator : Biosonik I

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

1. Dipotassium hydrogen ortho phosphate; K_2HPO_4
2. Potassium dihydrogen phosphate; $K H_2PO_4$
3. Sodium Pyrophosphate; $Na_4P_2O_7$ $10H_2O$
4. Magnesium sulphate; $MgSO_4 . 7H_2O$
5. Protease peptone
6. Ammonium nitrate; NH_4NO_3
7. 2-Mercapto ethanol
8. Nicotinamide Adenine dinucleotide (β-form)
9. Nicotinamide Adenine dinucleotide hydride (β-form)
10. α-ketoglutaric acid
11. γ-aminobutyric acid
12. Sodium hydroxide
13. Hydrochloric acid
14. Glycerol
15. Agar

The following steps were involved for the estimation of GABA level in rat brain.

Step 1: GABAse enzyme preparation.

Step 2: Assay of GABAse enzyme

Step 3: Neurochemical determination of GABA level.

Step 1: GABAse enzyme preparation (136, 137): -

The presence of relatively high concentration of γ-aminobutyric acid in mammalian brain led to the discovery of a reaction utilizing this amino acid by what appeared to be transamination with α-ketoglutaric acid. Studies with brain preparations were, however, handicapped by the particulate nature of the system catalyzing the reaction. The most rapid, specific and sensitive determination of GABA in biological extracts is based on the use of an enzyme system found in the bacterium *Pseudomonas fluorescens* grown on a medium containing pyrrolidine which converts GABA to succinate via transamination and oxidation coupled to NAD reduction.

Process I: -

Growth conditions: -

The strain of *Pseudomonas fluorescens* (NCIM 2638) was first grown on King's B medium, the specific medium for this strain, which composed of

King's B medium (138): -

Protease peptone	- 20 g
K_2HPO_4	- 2.5 g
Glycerol	- 15 ml
$MgSO_4.7H_2O$	- 6 g
Agar	- 15 g
Distilled water	- 1000 ml

The medium was prepared and sterilized by autoclaving. Then in aseptic condition the strain was transferred to the medium and the medium was incubated at 28°C for 24hr in an incubator.

The grown strain was then incubated similarly in partially GABA containing medium. The King's B medium was prepared in which 50% of peptone was substituted with GABA as sole carbon source.

This adopted strain was then finally transferred to the medium containing GABA as the sole carbon source. The liquid medium consists of the following (in grams per liter):

GABA - 4g

K_2HPO_4 - 1.15g

KH_2PO_4 - 0.625g

$MgSO_4$ - 0.02 g

NH_4NO_3 - 1g

The broth was sterilized in autoclave and the strain was transferred to it aseptically. The medium was incubated for 24 h with vigorous mechanical shaking in an incubator shaker at 28°C.

Process – II: -

Extraction (Fig-V.1):

The cells were collected in log phase and stored at –20°C for 24 hrs. The medium was thawed, to which 2 volumes of 0.1M potassium phosphate buffer (pH 7.35) containing 0.01% of mercaptoethanol was added and centrifuged at 7000 rpm at 0°C for 10 min. After centrifugation the supernatant was discarded. This freezing, thawing and washing procedure was found to remove large amounts of nucleic acid. The residue was then dispersed in an equal volume of 0.1M phosphate buffer and oscillated for 5 min in a sonicator. Then the entire suspension was centrifuged at 7000 rpm at 0°C for 20 min. The supernatant was collected and the residue was again suspended in phosphate buffer and oscillated in a sonicator for 5 min. The turbid solution was again centrifuged at 7000 rpm at 0°C for 20 min. The supernatant was collected. The collected supernatants were mixed with each other and they represent the crude extract of the enzyme system. The extract was transferred to small tubes and stored at –20°C.

Extraction of GABAse enzyme: -

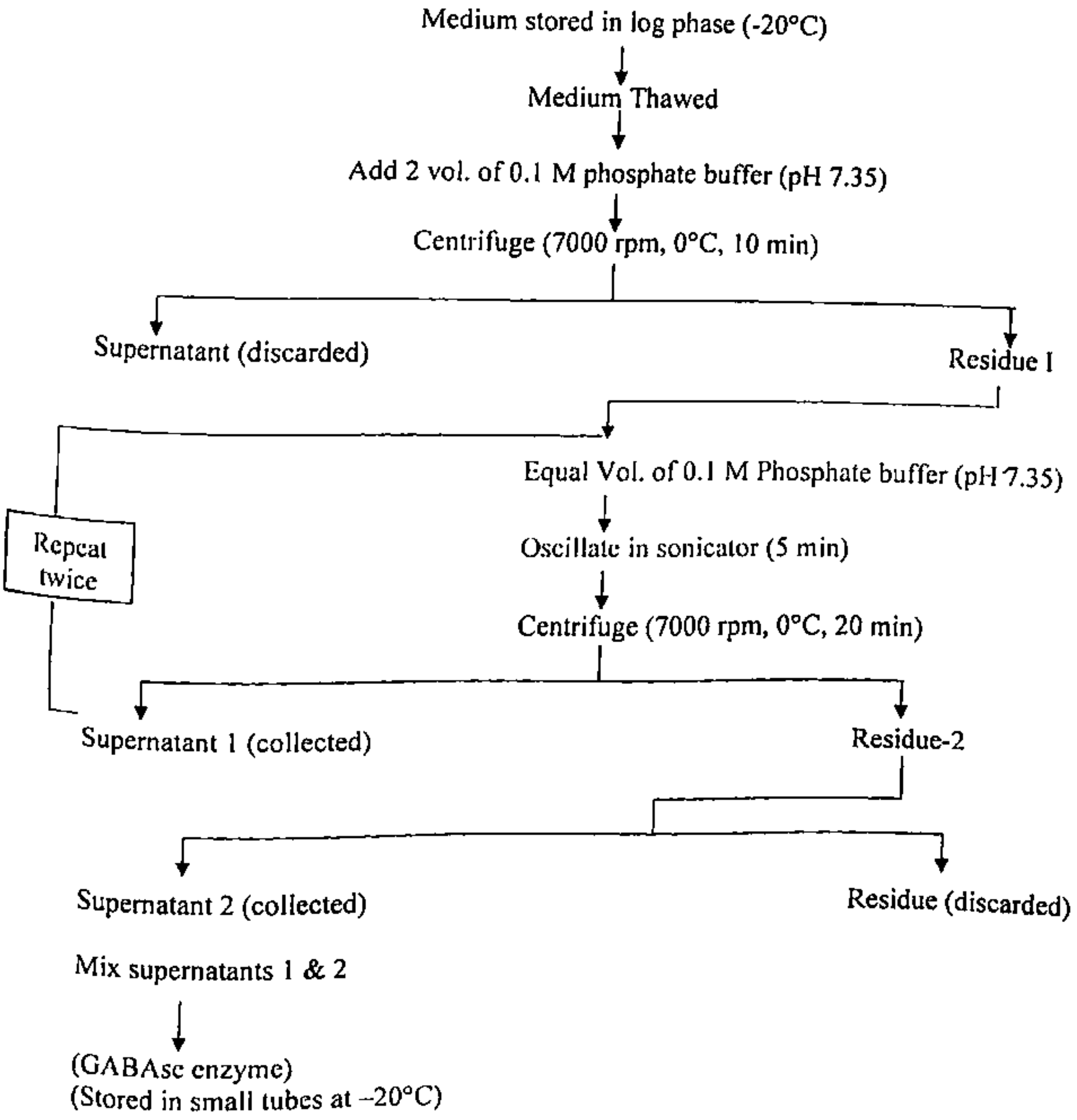


Fig-V.1. Flow chart for the extraction of GABAse enzyme

Step-2: **<u>Assay of GABAse enzyme</u>** (139): -

<u>Method</u>: UV spectrophotometric method at λ_{max} <u>340 nm</u>.

$$\gamma\text{-aminobutyrate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GABA-T}} \text{succinic semialdehyde} + \text{Glutamate} \quad \text{— Eqn.1}$$

$$\text{succinic semialdehyde} + NAD^{+} \xrightarrow{\text{SSADH}} \text{succinic acid} + NADH + H^{+} \quad \text{— Eqn. 2}$$

Enzyme activity was determined by coupling reaction 1 with an excess of succinic semialdehyde dehydrogenase (Reaction 2) present in the extract so that the formation of reduced pyridine nucleotide was a function of transaminase activity.

Reagents: -

1. Sodium pyrophosphate buffer (0.1M) at pH 8.1
2. NAD (0.01M) adjusted to pH 6.5
3. Mercaptoethanol (0.1 M), 0.07 ml per 10 ml of water
4. α-ketoglutarate (0.02M) adjusted to pH 7.0
5. γ-amino butyrate (0.02M) adjusted to pH 8.1
6. Enzyme preparation

The reaction was followed spectrophotometrically at 340nm in an incubation system, which included the following:

Sodium pyrophosphate buffer, 0.3 ml; mercaptoethanol, 0.1 ml; NAD, 0.1 ml; α-ketoglutarate, 0.1 ml; γ-aminobutyrate, 0.1 ml; required quantity of enzyme preparation (contains both γ-amino butyric acid transaminase + succinic semialdehyde dehydrogenase).

A unit of activity is defined as that amount of transaminase allowing the formation of 1 μmole of reduced pyridine nucleotide per minute in the standard assay system. Specific activity was expressed in terms of unit per mg of protein. So for the determination of the unit of activity, the enzyme extract was added in increasing volumes upto 1.8 ml.

NADH calibration curve was drawn in UV at λ_{max} 340 nm (Fig-V.5). Then a curve between volume of enzyme and the O.D was drawn in UV at λ_{max} 340 nm (Fig-V.6). These two curves were correlated and a third curve was drawn between concentration of NADH and ml of enzyme. 1 μM concentration of NADH is 664.4 μg. At this concentration, the enzyme volume was found out to be 1.53 ml, which is taken as the unit of activity (Fig-V.7).

Step-3: <u>Determination of GABA level in the rat brain regions</u>: -

Schematic depictions of the UV enzymatic method for the determination of GABA in tissue sample are given in Fig-V.2.

Collection of sample:

Animals: Wistar Albino rats (130-160gm) in groups of 6.

Route of administration: - intraperitoneal (i.p)

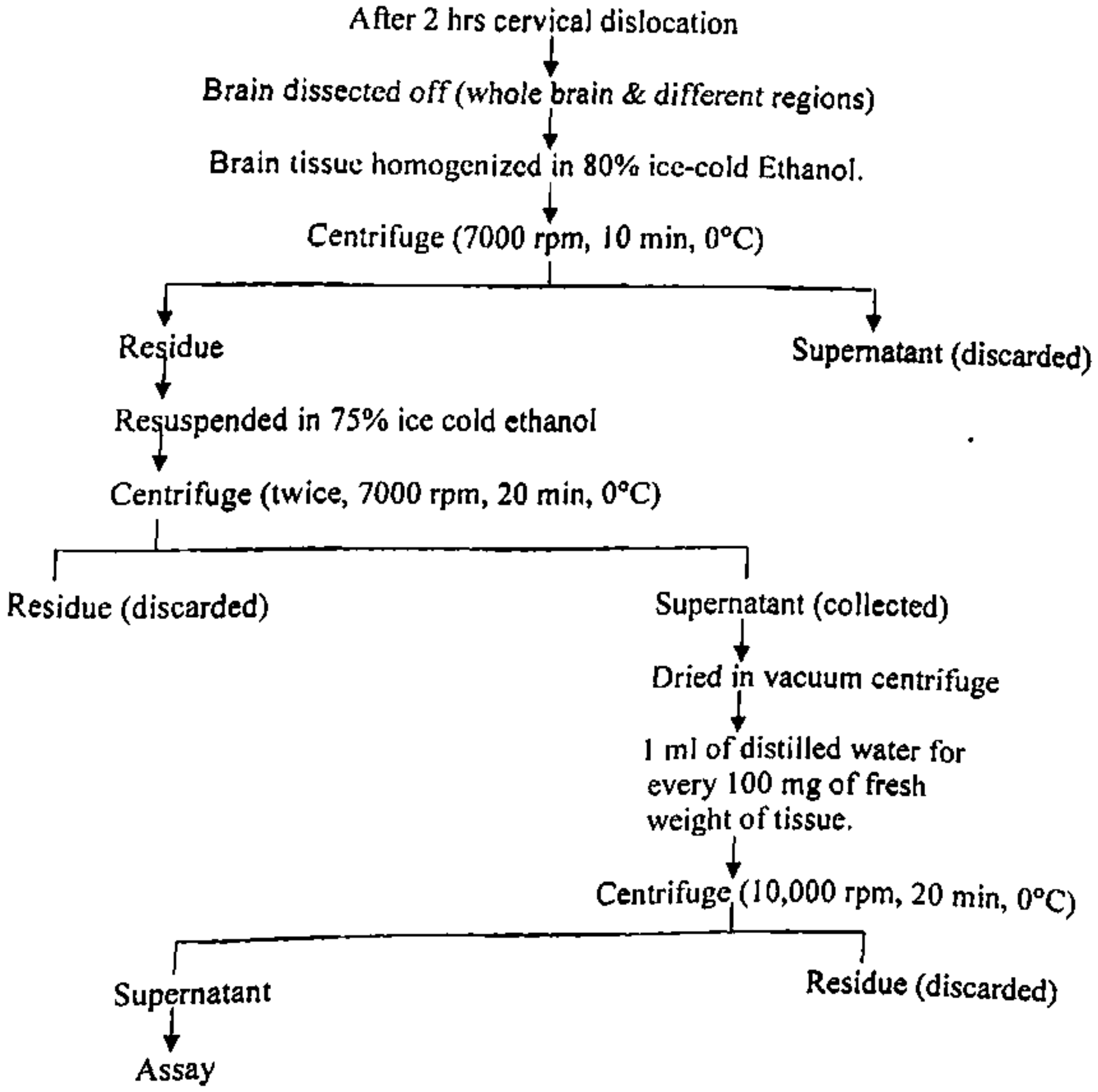


Fig-V.2. Flow chart for the estimation of GABA from the rat brain tissue

Assay: -

Reagents: - Sodium pyrophosphate (0.1M, pH 8.1)

NAD (0.01M, pH 6.5)

Mercaptoethanol (0.1M, 0.07 ml in 10 ml of H_2O)

α - ketoglutarate (0.02 M, pH 7.0)

λ_{max} → 340 nm.

UV method (140): -

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 (136), but obviously can be modified for extracts from other types of material.

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

   Weighed 4.46g of $Na_4P_2O_7.10\ H_2O$ 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 1 N 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 conditions.

Procedure:

For the study, wistar albino rats weighing 130-160gm were used. The control group was treated only with vehicle (30% polyethyleneglycol 400). The compounds were dosed at their minimal anticonvulsant activity doses. After 2h of drug administration, the animal was sacrificed 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% ice-cold ethanol and processed further under frozen condition as presented in Fig-V.2.

Calibration curve: -

Standard GABA solution: -

Standard GABA solutions were prepared in the concentration of 10-120 µg / 0.1 ml of distilled water and processed as follows. The reaction was started by addition of 0.3 ml of pyrophosphate buffer (pH 8.1), 0.3 ml of reagent mixture, 1.53 ml of the enzyme solution and sample contained in the optical tube. Optical density at 340nm was read immediately after the reaction mixture has been stirred.

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

Observation and calculations: -

All the spectrophotometric reading was taken at 340 nm. 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-VI-9; VII-7; VIII-7; IX-14, 15).

x axis 1 cm = 20 Units/ml
y axis 1 cm = 0.05 A

x axis Units/ml	y axis A
28	0.095
57	0.180
97	0.265
150	0.345

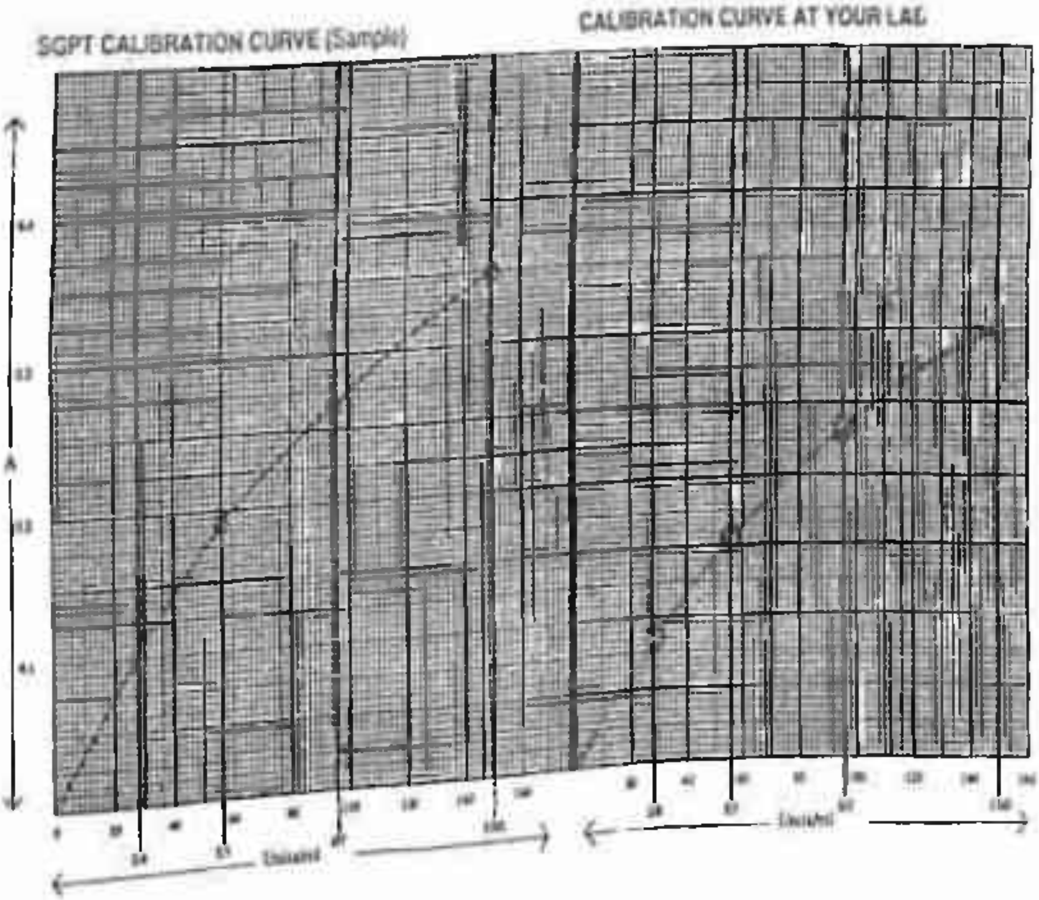
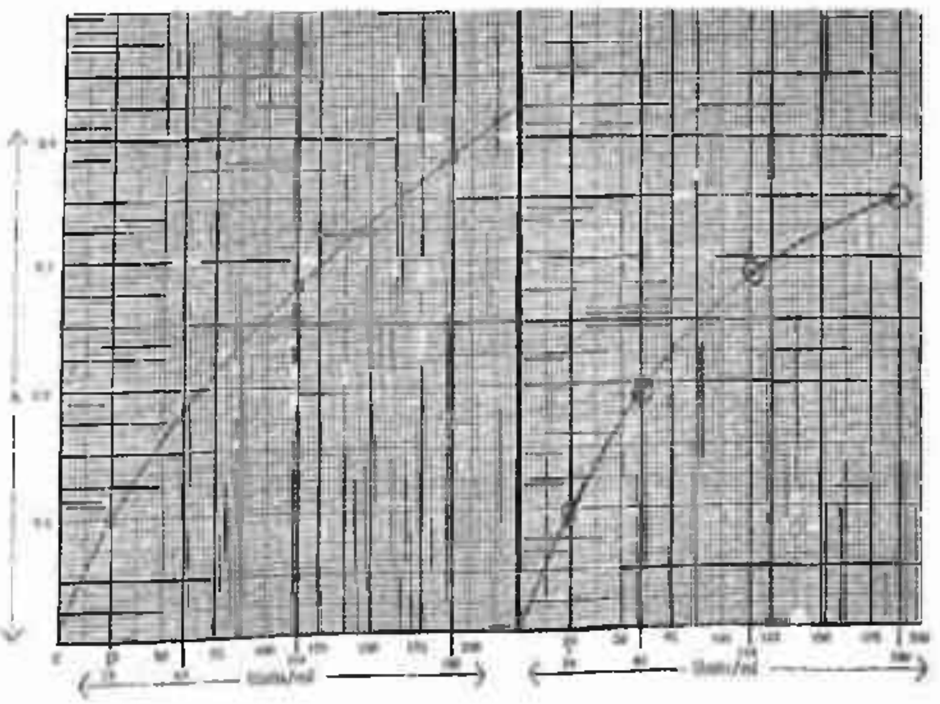


Fig V.3. Calibration curve for SGPT determination.



SGOT CALIBRATION CURVE (Sample)

CALIBRATION CURVE AT YOUR LAB.

x axis 1 cm = 25 Units/ml
y axis 1 cm = 0.05 A

x axis	y axis
Units/ml	A
24	0.100
61	0.183
114	0.275
190	0.391

Fig V.4. Calibration curve for SGOT determination.

Calibration curve for NADH determination

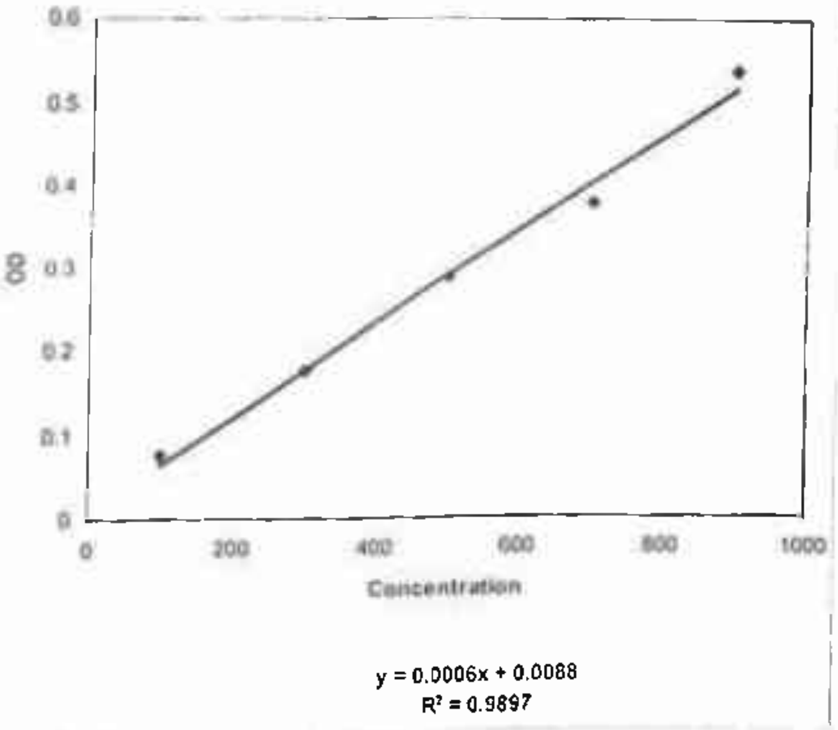


Fig-V.5. O.D vs Concentration of NADH (μg / ml) graph.

Calibration curve for GABAse enzyme

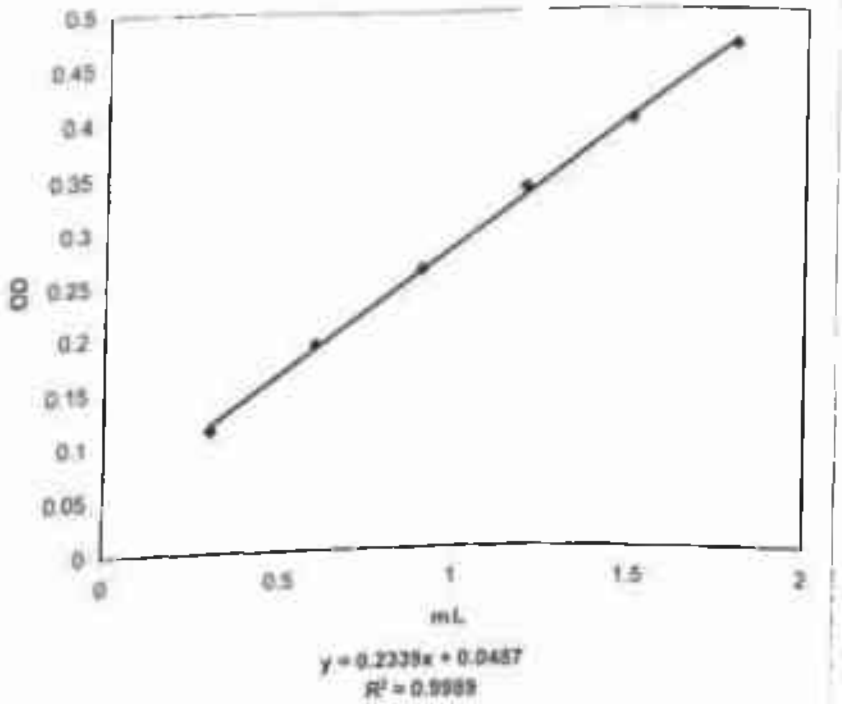


Fig-V.6. O.D vs Volume of enzyme (ml) graph.

Unit of activity determination of GABAse enzyme

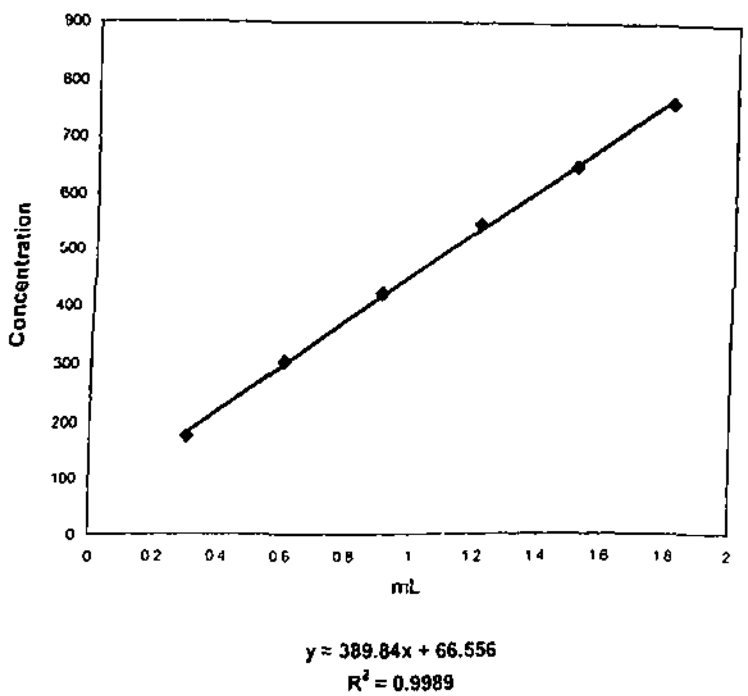


Fig-V.7. Concentration of NADH (µg / ml) vs Volume of enzyme (ml) graph

Calibration curve for GABA determination

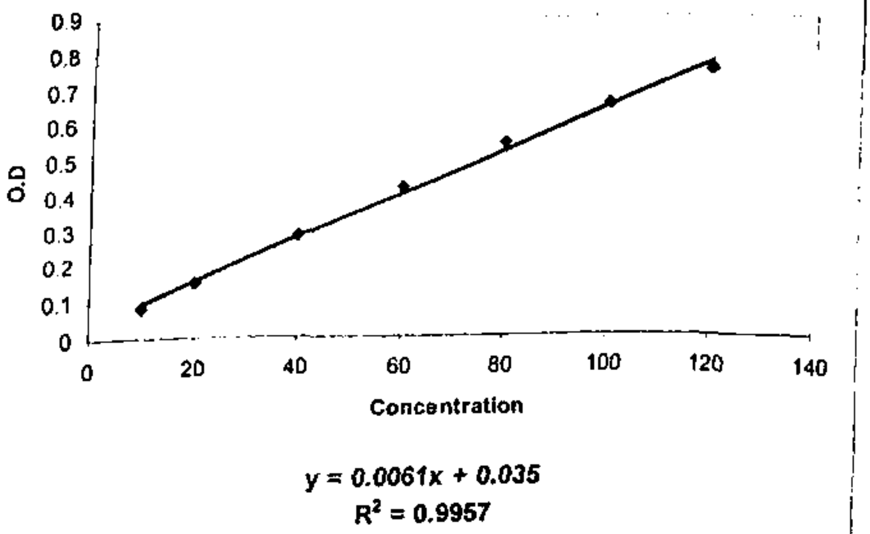
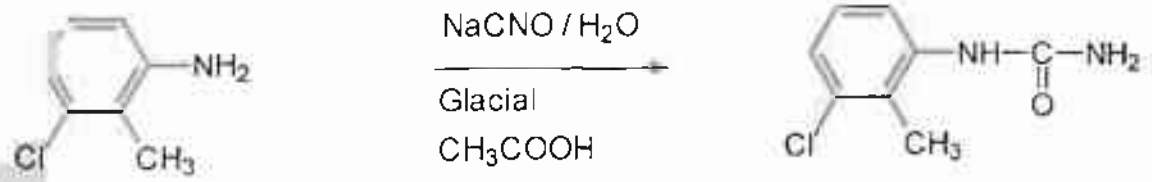


Fig-V.8. O.D vs Concentration of GABA (µg / 0.1 ml) graph.

e present work focuses on synthesis and anticonvulsant evaluation of 3-chloro-2-methyl enyl substituted semicarbazones, since substitution in the 2-position of the phenyl ring with tron-donating groups was generally beneficial to activity as reported elsewhere (141) and importance of the ortho-methyl group for anticonvulsant activity had been depicted in ny studies (142-147) including the recently marketed drug tiagabine.

nthesis: -

tep - 1: ***Synthesis of 3-chloro-2-methylphenylurea: -***

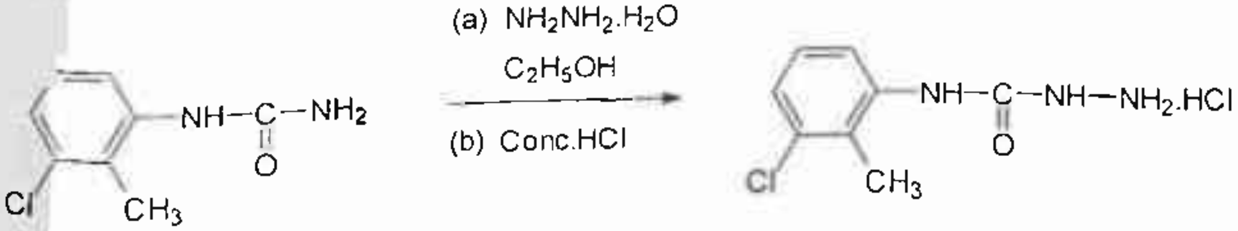


-chloro-2-methylaniline

3-chloro-2-methylphenylurea

-chloro-2-methylaniline (0.1M, 14.1g, 11.8 ml) was dissolved in 20 ml of glacial acetic acid nd 10 ml of water. To this 0.1 mole of sodium cyanate (6.5g) in 80 ml of warm water was dded with stirring. Allowed to stand for 30 min, then cooled in ice and filtered with suction nd dried. Recrystallisation was done with boiling water to yield 3-chloro-2-methyl phenylurea (**A-32**), with m.p. 201°C, IR (KBr) ν_{max} 3450, 1650, 840 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.4 (s, 3H, CH_3), 7.2-7.4 (m, 3H, ArH), 8.28 (s, 1H, ArNH, D_2O exchangeable), 9.33 (s, 2H, $CONH_2$, D_2O exchangeable).

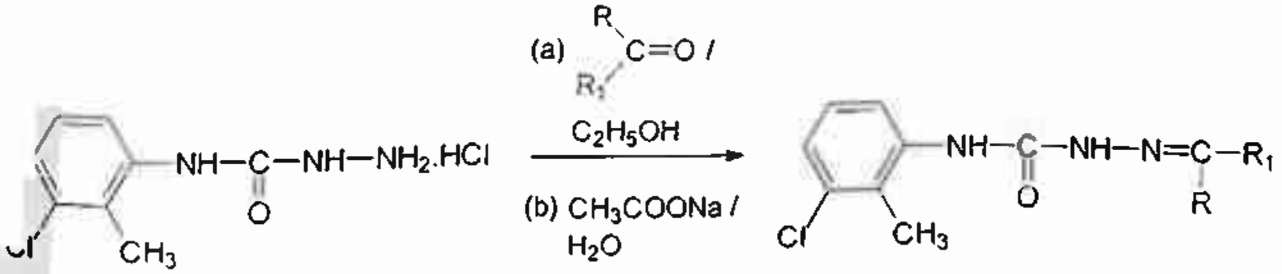
Step-2: ***Synthesis of 3-chloro-2-methylphenylsemicarbazide hydrochloride: -***



3-chloro-2-methylphenylurea

3-chloro-2-methylphenylsemicarbazide hydrochloride chloro-2-methylphenylurea (0.05M, 9.2 gm) was refluxed in ethanol with double the antity of hydrazine hydrate (0.1M, 4.85 ml) for 24 hr with stirring. The two-third volume alcohol was distilled by vacuum distillation and poured into ice. The resultant precipitate was filtered, washed with water and dried. The precipitate was recrystallised from ethanol 95%) to which concentrated hydrochloric acid was added in the ratio of 1:10:5 (precipitate-thanol-conc. HCl). The precipitate of 3-chloro-2-methylphenylsemicarbazide hydrochloride was filtered by vacuum and dried. Then it was converted into free semicarbazide in which semicarbazide hydrochloride was dissolved in minimum quantity of alcohol and neutralized with 10% sodium carbonate solution. At neutral pH, the semicarbazide (**A-33**) appeared as precipitate, which was separated by vacuum filtration and dried, m.p. 163 °C, IR (KBr) ν_{max} 3450, 3269, 1640, 840 Cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 5.56 (s, 2H, NH_2, D_2O exchangeable), 7.20-7.45 (m, 3H, ArH), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.6 (bs, 1H, $NHNH_2$, D_2O exchangeable).

Step-3: ***General method for the synthesis of 3-chloro-2-methylphenylsemicarbazones: -***

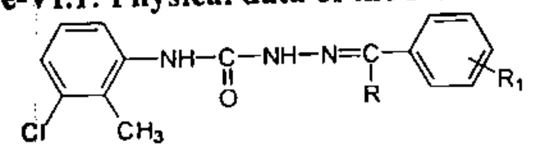


3-chloro-2-methylphenylsemicarbazide hydrochloride

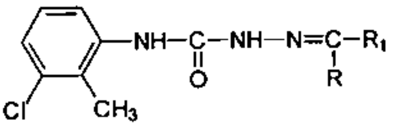
3-chloro-2-methylphenylsemicarbazones

To a solution of 3-chloro-2-methylphenylsemicarbazide hydrochloride (0.005M, 1.175g) in 25ml of water, was added sodium acetate (0.005M, 0.41g) 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 min to 2 hr 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-VI.1 and VI.2. The IR spectra of the semicarbazone derivatives were identical in the following aspects: 3450, 3300-3250, 1650, 1595, 840 cm^{-1}. ^{1}H-NMR (300 MHz, δ) spectra of some representative compounds are given in Table-VI.3.

e-VI.1: Physical data of the 3-chloro-2-methylphenylsemicarbazones: -



A-(1-18)



A-(19-26)

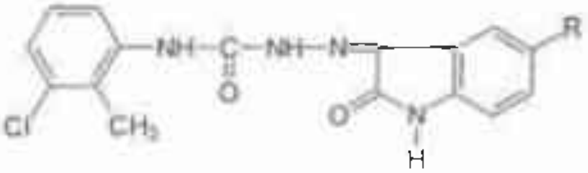
ound o.	Substituents		Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
	R	R_1						
-1	H	H	75	193	$C_{15}H_{14}N_3OCl$	287.75	0.91	2.53
-2	H	4-CH_3	83	199	$C_{16}H_{16}N_3OCl$	301.77	0.88	2.71
-3	H	4-OCH_3	83	187	$C_{16}H_{16}N_3O_2Cl$	317.77	0.92	1.98
-4	H	4-OH	53	239	$C_{15}H_{14}N_3O_2Cl$	303.75	0.82	1.88
-5	H	4-$N(CH_3)_2$	79	216	$C_{19}H_{19}N_4OCl$	330.82	0.87	2.17
-6	H	4-NO_2	86	257	$C_{15}H_{13}N_4O_3Cl$	332.75	0.85	2.78
-7	H	3-OCH_3 4-OH	62	232	$C_{16}H_{16}N_3O_3Cl$	333.77	0.75	1.39
-8	H	3-NO_2	71	214	$C_{15}H_{13}N_4O_3Cl$	332.75	0.82	2.81
-9	H	2-Cl	80	209	$C_{15}H_{13}N_4OCl_2$	322.19	0.90	2.90
-10	H	2-OH	86	224	$C_{15}H_{14}N_3O_2Cl$	303.75	0.74	2.19
-11	H	2-NO_2	84	211	$C_{15}H_{13}N_4O_3Cl$	332.75	0.81	2.83
-12	CH_3	H	63	220	$C_{16}H_{16}N_3OCl$	301.77	0.92	2.54
-13	CH_3	4-Cl	66	227	$C_{16}H_{15}N_3OCl_2$	336.22	0.90	2.63
-14	CH_3	4-NH_2	54	189	$C_{16}H_{17}N_4OCl$	316.79	0.83	2.03
-15	CH_3	4-OH	54	191[c]	$C_{16}H_{16}N_3O_2Cl$	317.77	0.85	1.84
-16	CH_3	4-NO_2	52	265	$C_{16}H_{15}N_4O_3Cl$	346.77	0.93	2.72
-17	CH_3	3-NH_2	61	184	$C_{16}H_{17}N_4OCl$	316.79	0.64	1.87
-18	CH_3	2-OH	59	215	$C_{16}H_{16}N_3O_2Cl$	317.77	0.77	2.15
-19	CH_3	CH_3	57	166	$C_{11}H_{14}N_3OCl$	239.71	0.88	2.03
-20	CH_3	C_2H_5	74	175	$C_{12}H_{16}N_3OCl$	253.73	095	2.34
-21	CH_3	$CH_2CH(CH_3)_2$	53	144	$C_{14}H_{20}N_3OCl$	281.78	0.87	2.70
-22	CH_3	CH_2COCH_3	56	251	$C_{13}H_{16}N_3O_2Cl$	281.74	0.74	2.14
-23	H	2-furfuryl	51	186	$C_{13}H_{12}N_3O_2Cl$	277.71	0.89	2.42
-24	$C_6H_5CH_2$	$C_6H_5CH_2$	78	160	$C_{23}H_{22}N_3OCl$	391.90	0.90	2.57
-25	CRR_1=Cyclopentylene		75	191	$C_{13}H_{16}N_3OCl$	265.74	0.72	2.30
-26	CRR_1=Cyclohexylene		82	182	$C_{14}H_{18}N_3OCl$	279.77	0.77	2.40

mental analyses for C, H, N were within ± 0.4 % of the theoretical values.

; P was generated using Alchemy 2000 and SciLog P softwares.

lting point of the compounds at their decomposition.

able-VI.2: Physical data of the 3-chloro-2-methylphenylsemicarbazones: -



A-(27-31)

Compound No.	R	Yield (%)	m.p. (°C)	Molecular formula[a]	Mol. Weight	R_f	Log P[b]
A-27	H	77	266	$C_{16}H_{13}N_4O_2Cl$	328.76	0.78	1.28
A-28	F	56	271[c]	$C_{16}H_{12}N_4O_2ClF$	346.75	0.69	1.75
A-29	Cl	62	255	$C_{16}H_{12}N_4O_2Cl_2$	363.20	0.88	1.65
A-30	Br	51	231[c]	$C_{16}H_{12}N_4O_2ClBr$	407.65	0.80	1.68
A-31	CH$_3$	71	267[c]	$C_{17}H_{15}N_4O_2Cl$	342.78	0.79	1.35

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

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

[c] Melting point of the compounds at their decomposition.

Table-VI.3: Spectral and Elemental analyses data of the compounds: -

Compound No.	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)*		
			C	H	N
A-6	3370, 3350, 3090, 2870, 1690, 1610, 1600-1520, 1450, 1340, 1310	2.35 (s, 3H, ArCH$_3$), 7.20-8.31 (m, 7H, ArH), 8-10 (s, 1H, imine H), 9.10 (s, 1H, ArNH, D$_2$O exchangeable), 11.19 (s, 1H, CONH, D$_2$O exchangeable).	54.14 53.93	3.94 3.93	16.84 16.78
A-10	3440, 3200, 3090, 1680, 1620, 1600-1530, 1300, 1260	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$_2$O exchangeable), 10.71 (s, 1H, CONH, D$_2$O exchangeable), 10.06 (s, 1H, ArOH, D$_2$O exchangeable).	59.31 59.13	4.65 4.63	13.83 13.78
A-16	3380, 3100, 2850, 1710, 1580-1510, 1460, 1340, 1300, 860	2.24 (s, 3H, CH$_3$), 2.37 (s, 3H ArCH$_3$), 7.24-8.41 (m, 7H, ArH), 8.98 (s, 1H, ArNH, D$_2$O exchangeable), 11.05 (s, 1H, CONH, D$_2$O exchangeable).	55.42 55.22	4.36 4.35	16.16 16.09
A-18	3420, 3210, 3090, 2840, 1680, 1620, 1600-1530, 1310, 1260	2.18 (s, 3H, CH$_3$), 2.30 (s, 3H ArCH$_3$), 6.74-7.78 (m, 7H, ArH), 8.62 (s, 1H, ArNH, D$_2$O exchangeable), 10.01 (s, 1H, ArOH, D$_2$O exchangeable), 10.64 (s, 1H, CONH, D$_2$O exchangeable).	60.47 60.24	5.08 5.06	13.22 13.18
A-19	3390, 3100, 2880, 1695, 1580-1540, 1320, 1240	2.14 (s, 3H, CH$_3$), 2.24 (s, 3H, CH$_3$), 2.34 (s, 3H ArCH$_3$), 7.12-7.30 (m, 3H, ArH), 8.58 (s, 1H, ArNH, D$_2$O exchangeable), 10.54 (s, 1H, CONH, D$_2$O exchangeable).	55.12 54.93	5.89 5.87	17.53 17.47
A-20	3380, 3090, 2860, 1690, 1590-1540, 1325, 1220	1.32-1.36 (t, 3H, CH$_3$, J = 7.6 Hz), 1.82-1.88 (q, 2H, CH$_2$, J = 7.6 Hz), 2.20 (s, 3H, CH$_3$), 2.32 (s, 3H, ArCH$_3$), 7.08-7.24 (m, 3H, ArH), 8.62 (s, 1H, ArNH, D$_2$O exchangeable), 10.01 (s, 1H, CONH, D$_2$O exchangeable).	56.80 56.58	6.35 6.34	16.56 16.51
A-29	3350, 3250, 1720, 1700, 1610, 1590-1510, 1350, 1180	2.31 (s, 3H, ArCH$_3$), 6.88-8.14 (m, 6H ArH), 8.74 (s, 1H, ArNH, D$_2$O exchangeable), 10.82 (s, 1H, NH isatinyl, D$_2$O exchangeable), 10.90 (s, 1H, CONH, D$_2$O exchangeable).	47.14 46.96	2.97 2.96	13.74 13.70
A-30	3360, 3240, 1725, 1700,1605, 1590-1510, 1320, 1160	2.30 (s, 3H, ArCH$_3$), 6.94-8.28 (m, 6H ArH), 9.32 (s, 1H, ArNH, D$_2$O exchangeable), 10.38 (s, 1H, NH, isatinyl, D$_2$O exchangeable), 10.92 (s, 1H, CONH, D$_2$O exchangeable).	52.91 52.79	3.33 3.32	15.42 15.37

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

Pharmacological activity: -

Table-VI.4: Anticonvulsant activity and minimal motor impairment of 3-chloro-2-methylphenylsemicarbazones: -

Compound No.	Intraperitoneal Injection in mice[a]							
	MES Screen		scPTZ Screen		scSTY Screen[b]		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	2.0h	0.5h	4.0h
A-1	-	300	-	-	×	×	-	-
A-2	-	-	300	-	300[e]	-	-	-
A-6	-	300[c]	-	-	100	100	100	100
A-9	-	300[c]	-	-	100	300	300[f]	300
A-10	-	-	-[d]	-[d]	100	300	300	300
A-11	-	-	300	-[d]	100	100	300	300
A-17	-	300[c]	-	-	100	300	-	-
A-19	300[c]	300	300	-	100	100	300	300
A-20	-	300	-	-	300[e]	300	-	-
A-21	-	300	-	-	-[e]	-	-	-
A-25	-	300	-	-	×	×	-	-[f]
A-26	-	300	-	-	×	×	-	-
A-28	-	-[c]	-	-	×	×	-	-
A-29	-	-	-[d]	-	×	×	300	-
A-32	100	-	100	-	×	×	100	-
A-33	100	-[c]	300	-	×	×	300	100
Phenytoin	30	30	-	-	-	-	100	100
Ethosuximide	-	-	100	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. (scSTY test, 0.5h and 2.0h period study). The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg) and (×) indicates not tested.

[b] In the scSTY screen compounds A-3 and A-27 showed protection at 300 mg/kg for 0.5h.

[c] In the MES screen at 30 mg/kg compound A-6 (1/4, 4h) and at 100 mg/kg compounds A-9 (1/3, 4h), A-17 (1/3, 4.0h), A-19(2/3, 1.0h) and A-28 (1/7, 4.0h).

[d] In the scPTZ screen, at 100mg/kg, compounds A-10 (1/5, 4.0h), A-11 (1/5, 4.0h), A-29 (1/5, 0.5h) and at 300 mg/kg compound A-10 (1/5, 0.5h & 4.0h).

[e] In the scSTY screen, mild clonic jerks were observed with compounds A-2 and A-20 (300 mg/kg, 1/3) compound A-21 caused loss of righting reflex and death (2/2, 300 mg/kg).

[f] In the neurotoxicity screen, at 100 mg/kg, compounds A-9 (2/3, 2h) and A-25 (2/3, 2.0h).

Table-VI.5: Evaluation of some compounds in the MES test after oral administration (30 mg/kg) to rats: -

Compound No.	Oral administration to Rats[a]				
	0.25h	0.5h	1.0h	2.0h	4.0h
A-11	1	0	1	0	0
A-17	0	0	0	0	0
A-19	0	2	0	1	0
A-32	1	0	0	0	0
A-33	2	1	1	1	1
Phenytoin	1	4	3	3	3

[a] *The figures indicate the number of rats out of four, which were protected.*

Table-VI.6: Behavioral study on some selected compounds using Actophotometer: -

Compound No[a]	Activity Score[b]		
	Control (24.0h Prior)	Post treatment	
		0.5h after	1.0h after
A-2	408.23 ± 32.96	48.67 ± 04.66	33.17 ± 02.11
A-6	154.67 ± 10.90	113.32 ± 10.30**	159.32 ± 06.77 NS
A-9	320.00 ± 27.73	531.17 ± 17.22	343.67 ± 21.11 NS
A-10	519.17 ± 13.38	421.00 ± 26.78*	359.00 ± 29.87*
A-11	180.83 ± 30.72	61.33 ± 09.69*	164.17 ± 11.02 NS
A-17	303.17 ± 22.66	41.67 ± 02.21	27.33 ± 01.02
A-19	373.33 ± 32.75	70.23 ± 04.12	12.83 ± 02.35
A-20	247.11 ± 15.66	11.32 ± 03.12	12.67 ± 03.35
Phenytoin	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

[a] The compounds were tested at a dose of 30 mg/kg (i.p).

[b] Each score represents the mean ± SEM of six mice, significantly different from the control score at $p < 0.0001$, $*p < 0.008$, $**p < 0.02$ and NS at $p < 0.02$ denotes not significant (Student's t-test).

Table-VI.7: CNS study on selected compounds in Porsolt's swim test: -

Compound No[a]	Immobility time[b] (sec)	
	Control (24.0h prior)	Post treatment (60 min after)
PEG	164.67 ± 11.69	168.53 ± 12.32 NS
A-1	125.67 ± 11.89	190.30 ± 12.35
A-2	200.00 ± 11.63	137.30 ± 12.49
A-6	78.65 ± 12.62	104.00 ± 12.94 NS
A-9	57.00 ± 12.16	130.30 ± 11.25
A-11	134.00 ± 12.70	205.00 ± 11.63
A-17	115.67 ± 12.05	150.60 ± 12.06 NS
A-21	104.33 ± 13.29	187.30 ± 13.09
Carbamazepine	138.40 ± 17.30	240.60 ± 14.10

[a] The compounds were tested at a dose of 30 mg/kg (i.p).

[b] Each value represents mean ± SEM of six rats significantly different from the control at $p < 0.05$ and NS denotes not significant at $p < 0.05$ (Student's t-test).

Table-VI.8: Effect of some 3-chloro-2-methylphenylsemicarbazones on serum levels of transaminase in six rats: -

Compound No[a]	**SGPT[b] (Units / ml)**	**SGOT[b] (Units / ml)**
Control	45.00 ± 6.24	73.17 ± 5.98
A-6	69.17 ± 5.46	94.33 ± 5.18*
A-9	46.67 ± 5.47	64.17 ± 7.26
A-17	54.33 ± 3.18	67.08 ± 4.80
A-19	49.33 ± 4.38	73.58 ± 4.48
Phenytoin[c]	52.67 ± 3.48	84.16 ± 6.50

[a] The compounds were tested at a dose of 100 mg/kg (p.o) for 14 days.

[b] Each value represents mean ± SEM of six rats, significantly different from the control at $^*p < 0.05$ and remaining values are not significantly different (Student's t-test).

[c] Tested at 25mg/kg (p.o) for the treatment of 14 days.

Table-VI.9: Effect of compounds on GABA systems: -

Compound No[a]	Concentration of GABA (µg /100 mg tissue)[b]			
	Olfactory lobe	Mid brain	Medulla oblongata	Cerebellum
Control	12.01 ± 1.74	44.31 ± 1.78	33.28 ± 2.04	21.93 ± 2.52
A-6	14.34 ± 2.81	62.75 ± 2.57*	39.72 ± 2.26	28.91 ± 2.08
A-9	13.22 ± 3.05	59.82 ± 2.29*	37.89 ± 2.03	28.55 ± 3.35
A-17	13.50 ± 2.78	62.83 ± 2.39*	41.55 ± 3.96	30.59 ± 4.16
A-19	15.59 ± 3.38	60.95 ± 3.59*	46.89 ± 2.69*	25.47 ± 2.47
Clobazam[c]	17.67 ± 2.62	63.57 ± 2.83*	45.91 ± 2.16*	28.73 ± 3.12

[a] The compounds were tested at a dose of 100 mg/kg (i.p) for 2 hrs.

[b] Each value represent mean ± SEM of six rats, significantly different from the control at *$p < 0.05$ and remaining values are not significantly different (Student's t-test).

[c] Tested at 30 mg/kg (i.p).

Results & Discussion: -

Chemistry: -

The design of 3-chloro-2-methylphenylsemicarbazones was based on the literature report in which the introduction of lipophilic (π), electron-withdrawing substituents ($+\sigma$) in the meta position (3^{rd} position) of the phenyl ring was found to significantly increase the anticonvulsant potency compared to the unsubstituted benzene (142).

The required 3-chloro-2-methylphenylsemicarbazones were synthesized by a three-step process starting from 3-chloro-2-methylaniline. The homogeneity of the compounds was monitored by performing thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were $CHCl_3 : CH_3OH$ (9:1)(Tables-VI.1 and VI.2).

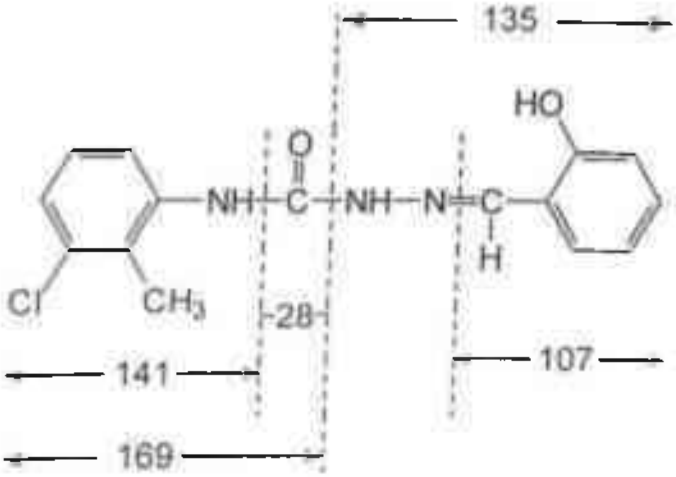
All the compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 [log $P > 2$] except for compounds **A-4**, **A-7**, **A-15**, **A-17** and **A-(27-31)**. These compounds possessed log $P < 2$ since all substituted with polar groups like OH, NH_2 and substituted isatin **A-(27-31)** derivatives.

With regard to the percentage yield of the synthesized compounds, the compounds with substituted aryl aldehydes have yields in the range of 62-86%, and those with substituted aryl / alkyl ketones and isatins gave 52-66% and 51-77% respectively.

The IR spectrum of N^{1}-(3-chloro-2-methylphenyl)-N^{4}-(2-hydroxybenzaldehyde) semicarbazone (**A-10**) was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3440 cm^{-1} showed NH stretching, 3200 cm^{-1} showed OH stretching, 3090 cm^{-1} showed aromatic CH stretching, 2850 cm^{-1} showed aliphatic CH stretching, 1680 cm^{-1} showed C=O stretching, 1620 cm^{-1} showed C=N stretching, 1600-1525 cm^{-1} showed aromatic C=C stretching, 1240 cm^{-1} showed C–O stretching were observed.

^{1}H-NMR spectrum revealed a singlet at δ 2.28 for 3H of CH_3 protons, singlet at δ 8.25 for 1H of imine proton and singlets at δ 8.67 (1H, ArNH), 10.71 (1H, CONH) and 10.06 (1H, OH) were D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristics peaks depicted in Table-VI.3.

The mass spectrum of compound **A-10** showed a molecular ion peak at m/z 303, base peak at m/z 169. The remaining major fragmentation peaks were at m/z 141, 135 and 107



Pharmacological activity: -

The anticonvulsant activity of the synthesized compounds was established after their i.p administration in three seizure models in mice viz MES, scPTZ and scSTY screens. The animals were dosed with 30, 100 and 300 mg/kg body weight of the test drugs and anti convulsant activity was examined at 0.5h and 4.0h and scSTY screen 0.5h and 2.0h, after the injections.

This provided a profile of the anticonvulsant activity due to absorption of compounds or due to a possible metabolite. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table-VI.4, along with the data of phenytoin and ethosuximide.

(i) MES test: -

The compounds **A-1, 6, 9, 17, 19, 20, 21, 25** and **26** were active in the MES test at a dose of 300 mg/kg and the compounds **A-32** and **A-33** were active at 100 mg/kg, indicative of their ability to prevent seizure spread. At a dose of 300 mg/kg, most of the compounds showed protection only at 4.0h except compound **A-19** which showed activity at both 0.5h and 4.0h period hence this compound showed a rapid onset of action and also had prolonged action. The compounds **A-32** and **A-33** showed protection only at 0.5h, so these compounds had rapid onset and short duration of action and it may have immediately metabolized to an inactive compound. The introduction of methyl group in the place of carbimino hydrogen **A-(12-18)** showed no activity except **A-17**, which showed activity at 300 mg/kg body weight at 4.0 h.

(ii) **scPTZ Test: -**

In this screen, except **A-6, 11, 19, 32** and **33** none of the compounds were active. This test is used to identify compounds that elevate the seizure threshold. All these five compounds showed protection at 300 mg/kg at 0.5h period, which indicated that the compounds showed a shorter duration of action.

(iii) **scSTY Screen: -**

Out of the nine compounds (**A-2, 6, 9, 10, 11, 17** and **19-21**) screened, eight compounds showed protection against scSTY-induced seizures. Compound **A-2** showed protection at 0.5h and **A-20** showed protection in both 0.5 and 2.0h. All other compounds showed activity at 100 mg/kg in both 0.5h and 4.0h except compounds **A-9, 10, 17** and **20**, which showed protection only at 300 mg/kg for 2.0h period. This indicated that these compounds have the ability to prevent seizure spread and also these compound may have a role via inhibitory glycine receptors.

Neurotoxicity screen (NT): -

In the NT Screen, compounds **A-6, 9, 11, 19, 25, 29, 32** and **33** showed neurotoxicity at higher dose level (300 mg/kg) except **A-6, 9, 25, 32** and **33**. Compound **A-6, 9** and **25** (100mg/kg) showed neurotoxicity at 0.5h, 2.0h and 2.0h respectively. Compounds **A-32** and **A-33** showed neurotoxicity at 100 mg/kg at 0.5h & 4.0h period. When compared with phenytoin, compounds **A-11, 19** and **29** showed lesser neurotoxicity. All other compounds exhibited neither anticonvulsant activity nor neurotoxicity (Table-VI.4).

The N^1-(3-chloro-2-methylphenyl)-N^4-(propan-2-one)semicarbazone (**A-19**) exhibited anticonvulsant activity in all three screen and emerged as the most active compound in this series. The compounds in this series have exhibited anticonvulsant activity against MES, scPTZ and scSTY induced seizures thereby indicating their potential as broad spectrum anticonvulsants covering epilepsies of grandmal and petitmal.

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 results are presented in Table-VI.5. The compounds were tested at 30 mg/kg in which none of the compounds showed protection except compound **A-33**, which exhibited anti-MES protection throughout the time period, compared with the standard drug phenytoin.

CNS depressant evaluation: -

Some selected 3-choloro-2-methylphenylsemicarbazone derivatives were 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 side effect (39).

In the behavioral study using actophotometer compound **A-9** showed no behavioral despair effect when compared to phenytoin as represented in Table-VI.6. Compounds **A-6** and **11** did show decreased locomotor activity in the 30 min interval but did not show any significant behavioral despair effect in 1h time period. All other compounds were found to decrease the motor activity of the animals.

In a similar study using Porsolt's swim test the immobility time after the administration of the test compounds were compared with Carbamazepine (Table-VI.7). The compounds **A-6** and **A-17** were found to show no significant CNS depression compared with the control at $P<0.04$. All other compounds tested were found to emerge as CNS depressants as they increased the immobility time. The compounds facilitated depression at a dose of 30 mg/kg i.p. The doses were lower than the anticonvulsant dose, which suggest that the mechanism involved in the anticonvulsant action and in the facilitation of depression could be different.

Hepatotoxicity Studies:

The effect of some selected 3-chloro-2-methylphenylsemicarbazones on the serum levels of transaminases (SGPT and SGOT) were determined as some drugs like Phenytoin, Phenobarbital and Sodium Valproate exhibit liver toxicity (148, 149).

The serum SGOT and SGPT levels in animals administered with vehicle (PEG) were taken as the control. All the tested compounds except **A-6** did not show any significant increase in the SGOT and SGPT level. N^1-(3-Chloro-2-methylphenyl)-N^4-(4-nitrobenzaldehyde) semicarbazone **(A-6)** showed a significant increase in the level of SGOT, though it did not reach the hepatotoxic level (SGOT >150 Units / ml)(Table-VI.8).

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

In order to explore the mechanism of anticonvulsant activity, some selected compounds that were highly active were subjected to neurochemical investigation to study their effects on the levels of GABA in different regions of rat brain, viz. olfactory lobe, midbrain, medulla oblongata and cerebellum. All the compounds subjected to neurochemical study were found to increase the GABA level in the mid brain region significantly, when compared to standard drug Clobazam. In all other regions there is no significant increase in the level of GABA except compound **A-19** which increased the GABA level in medulla oblongata region significantly. From this result it can be concluded that the 3-chloro-2-methylphenyl semicarbazones act as anticonvulsants through GABA-mediated mechanism (Table-VI.9).

Structure Activity Relationship: -

Tables-VI.1 and VI.2 lists thirty-one N^1-(3-chloro-2-methylphenyl)-N^4-(substituted benzaldehyde / acetophenone / benzophenone)semicarbazones. The unsubstituted derivative **A-1** showed activity in the MES test at a dose of 300 mg/kg.

Various compounds of 2-, 3- and 4-substituted phenyl analogues **[A-(2-11, 13-18)]** were prepared with the substituents chosen to examine the effects of electron donating and withdrawing groups as well as changes in bulk and lipophilicity **[A-(19-31)]**.

(i) Substitution in the 2-position with chloro **(A-9)** group showed anticonvulsant activity against MES and scSTY test. Substitution with hydroxyl **(A-10)** and nitro **(A-11)** groups showed anticonvulsant activity against scPTZ and scSTY tests. All three compounds showed neurotoxicity at a maximum dose of 300 mg/kg.

(ii) Substitution at 3-position with amino **(A-17)** group showed anticonvulsant activity against both MES and scSTY tests and no neurotoxicity.

(iii) Similarly, substitution at 4-position with electron donating group, viz. methyl group **(A-2)** exhibited activity both in scPTZ and scSTY tests and was not neurotoxic.

(iv) The carbimino hydrogen atom when replaced with methyl group showed diminished anticonvulsant activity. Only compound **A-17** showed anticonvulsant activity.

(v) Substitution in the carbimino hydrogen atom by bulky group like phenyl **(A-24)** group showed total abolition of anticonvulsant activity.

(vi) Replacement of phenyl ring with furan moiety **(A-23)** also showed loss of activity.

(vii) Replacement of phenyl ring with cycloalkyl groups like cyclopentyl **(A-25)** and cyclohexyl **(A-26)** showed anticonvulsant activity against MES test with absence of neurotoxicity.

(viii) Replacements of carbimino phenyl ring with alkyl group like methyl **(A-19)** showed activity against all the three screens *viz.* MES, scPTZ and scSTY tests.

(ix) Replacement of benzylidene group with substituted isatins **[A-(27-31)]** exhibited diminished anticonvulsant activity.

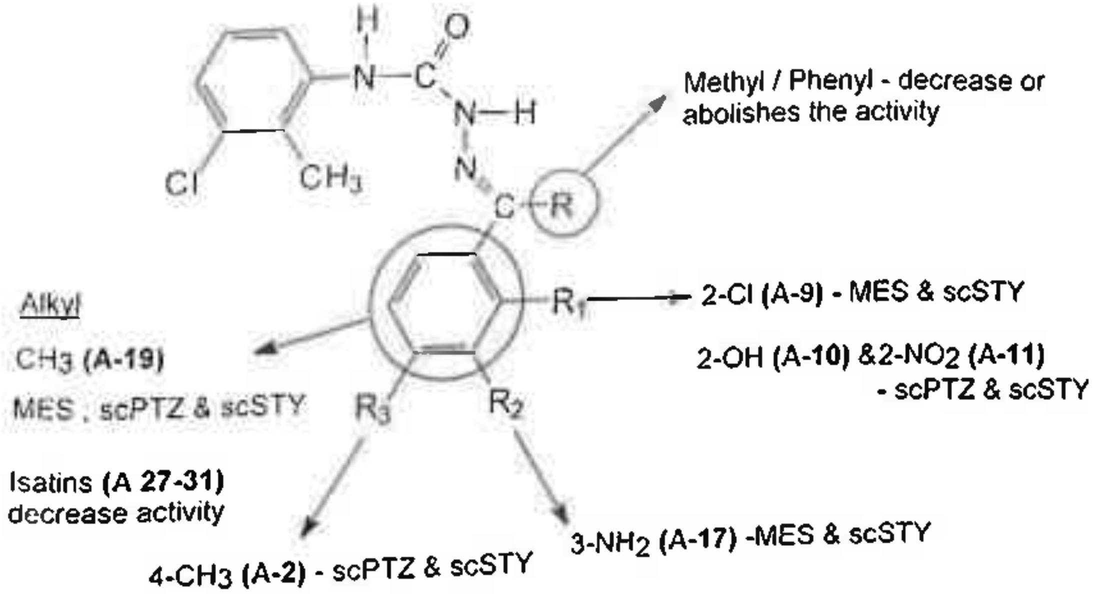
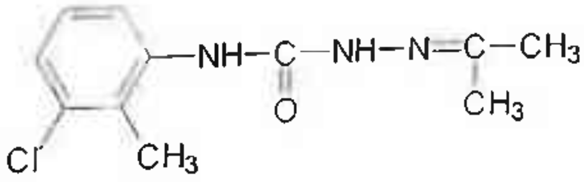


Figure VI.1. Schematic representation of SAR of 3-chloro-2-methylphenylsemicarbazones.

From the above results, it could be concluded that the small lipophilic moiety like methyl (A-19) substituted semicarbazone showed broad spectrum of anticonvulsant activity. The most active compound of this series was **N^1-(3-chloro-2-methylphenyl)-N^4-(propan-2-one)semicarbazone (A-19).**

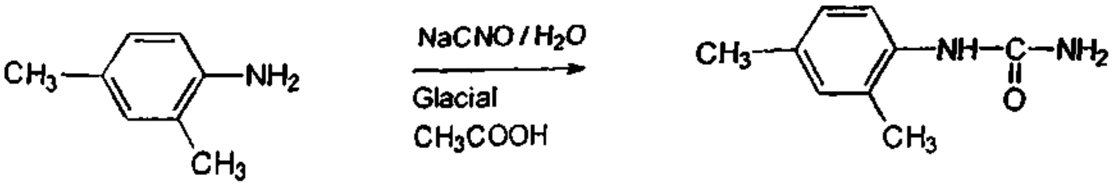


MES : 100% protection at 300 mg / kg
scPTZ : 100% protection at 300 mg / kg
scSTY : 100% protection at 100 mg / kg
Toxicity: No hepatotoxicity
GABA : Increased the level of GABA in mid brain and medulla oblongata of the rat brain by 37% and 40% compared to the control respectively.

The design of 2,4-dimethylphenylsemicarbazones was based on the importance of the ortho methyl group for anticonvulsant activity had been depicted in many studies (142-146) including the recently marketed drug Tiagabine. So the present series was an attempt to study the effect of dimethyl substitution in aryl semicarbazones.

Synthesis: -

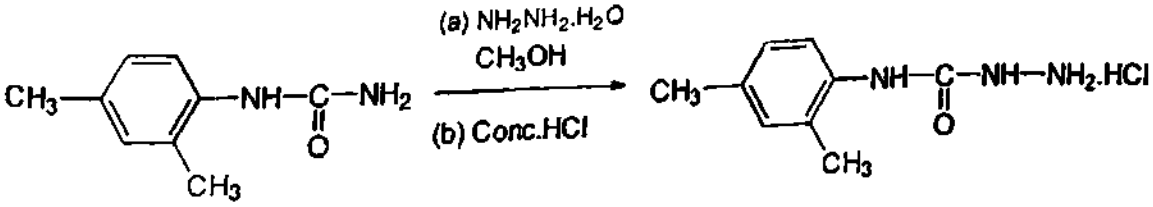
Step-1: ***Synthesis of 2,4 - dimethylphenylurea: -***



2,4-dimethylaniline **2,4-dimethylphenylurea**

2,4-dimethylaniline (0.1M, 12.1 gm) was dissolved in 20 ml of glacial acetic acid and 20 ml of water. To this solution, 0.1 M of sodium cyanate (6.5g) in 80 ml of warm water was added with stirring. Allowed to stand for 45 min and then cooled in ice and filtered with suction and dried. Recrystallisation from boiling water yielded 2,4-dimethylphenylurea (**B-29**) with m.p 210 °C. IR (KBr) ν_{max} 3430, 2940, 1650, 845 cm^{-1}; ^{1}H-NMR (DMSO-d_6, ppm) δ 2.16 (s, 3H, ArCH$_3$), 2.20 (s, 3H, ArCH$_3$), 6.80-7.24 (m, 3H, ArH), 8.68 (s, 1H, ArNH, D$_2$O exchangeable), 9.74 (s, 2H, CONH$_2$, D$_2$O exchangeable).

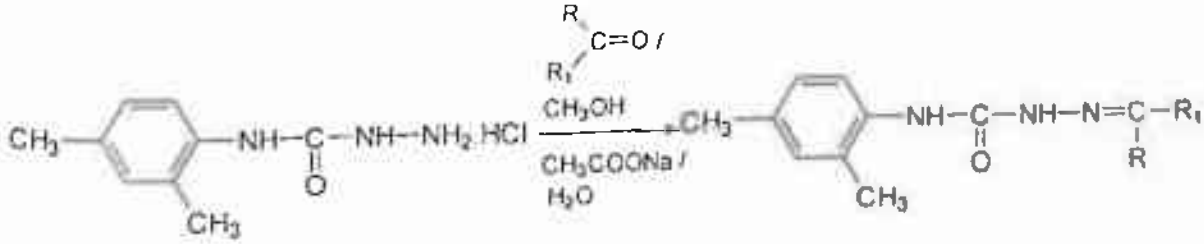
Step-2: ***Synthesis of 2,4 - dimethylphenylsemicarbazide hydrochloride: -***



2,4-dimethylphenylurea 2,4-dimethylphenylsemicarbazide hydrochloride

2,4-dimethylphenylurea (0.07M, 11.48 gm) was refluxed in methanol with double the quantity of hydrazine hydrate (7.0 ml) for 24 hr. The resultant precipitate was filtered and dried. The dried precipitate (solid) was recrystallised from methanol to which concentrated hydrochloric acid was added in the ratio of 1:10:5 (solid-methanolconc.HCl) and cooled in a freezer for 2hr. The precipitate of 2,4-dimethylphenylsemicarbazide hydrochloride was filtered by vacuum filtration and dried. Then it was converted into its free semicarbazide (**B-30**) by using 10% sodium carbonate solution, filtered and dried, m.p.158 °C, IR (KBr) ν_{max} 3450, 3300-3250, 2940, 1670-1650, 1400-1300, 845-835 cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm) 2.17 (s, 3H, $ArCH_3$), 2.22 (s, 3H, $ArCH_3$), 5.80 (s, 2H, NH_2, D_2O exchangeable), 6.84-7.26 (m, 3H, ArH), 9.02 (s, 1H, ArNH, D_2O exchangeable), 10.08 (bs, 1H, $NHNH_2$, D_2O exchangeable).

Step-3: *General procedure for the synthesis of 2,4 - dimethylphenylsemicarbazones: -*

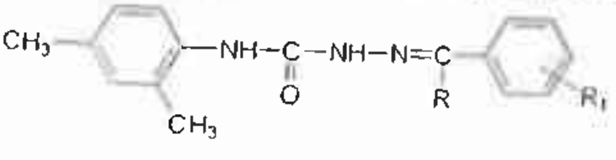


2,4-dimethylphenylsemicarbazide hydrochloride

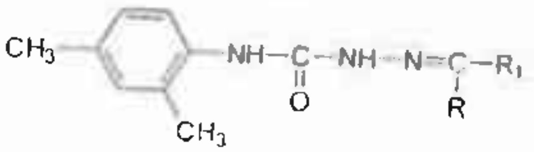
2,4-dimethylphenylsemicarbazones

To a solution of 2,4-dimethylphenylsemicarbazide (0.003M, 0.55g) in methanol was added an equimolar quantity of sodium acetate in water and appropriate aldehyde or ketone in methanol. The mixture was stirred with heating on a magnetic stirrer for 30 min to 2.0 hr until the completion of the reaction and the resultant precipitate was filtered and dried. The physical data of the semicarbazones are presented in Table-VII.1. The IR spectra of the compounds were identical in the following aspects: 3450, 3300-3260, 3040, 2940, 1670-1650, 1570-1550, 1400-1300, 845-835 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) spectra and the elemental analyses of some representative compounds are given in the Table-VII.2.

Table-VII.1: Physical data of the 2,4-dimethylphenylsemicarbazones: -



B-(1-23)



B-(24-28)

Compound No.	Substituents		Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
	R	R_1						
B-1	H	H	91	211	$C_{16}H_{17}N_3O$	267.33	0.85	2.11
B-2	H	4-Br	97	225	$C_{16}H_{16}N_3OBr$	346.22	0.87	3.06
B-3	H	4-CH_3	89	196	$C_{17}H_{19}N_3O$	281.35	0.86	2.84
B-4	H	4-OCH_3	77	140	$C_{17}H_{19}N_3O_2$	297.35	0.90	2.03
B-5	H	4-OH	86	210	$C_{16}H_{17}N_3O_2$	283.32	0.79	1.93
B-6	H	4-$N(CH_3)_2$	68	209	$C_{18}H_{22}N_4O$	310.39	0.82	2.30
B-7	H	4-NO_2	80	252	$C_{16}H_{16}N_4O_3$	312.32	0.84	2.88
B-8	H	4-OH 3-OCH_3	75	192	$C_{17}H_{19}N_3O_3$	313.35	0.72	1.44
B-9	H	3-Cl	67	195	$C_{16}H_{16}N_3OCl$	301.77	0.81	2.91
B-10	H	3-NO_2	57	230	$C_{16}H_{16}N_4O_3$	312.32	0.82	2.92
B-11	H	2-Cl	67	194	$C_{16}H_{16}N_3OCl$	301.77	0.88	2.99
B-12	H	2-OH	88	212	$C_{16}H_{17}N_3O_2$	283.32	0.70	2.26
B-13	H	2-NO_2	73	217	$C_{16}H_{16}N_4O_3$	312.32	0.82	3.11
B-14	CH_3	H	75	208	$C_{17}H_{19}N_3O$	281.36	0.81	2.32
B-15	CH_3	4-Cl	62	225	$C_{17}H_{18}N_3OCl$	315.80	0.87	2.71
B-16	CH_3	4-CH_3	68	176	$C_{18}H_{21}N_3O$	295.38	0.78	2.76
B-17	CH_3	4-NH_2	55	185	$C_{17}H_{20}N_4O$	296.37	0.78	2.17
B-18	CH_3	4-OH	53	178	$C_{17}H_{19}N_3O_2$	297.35	0.89	1.90
B-19	CH_3	4-NO_2	86	248	$C_{17}H_{18}N_4O_3$	315.80	0.92	2.82
B-20	CH_3	3-NH_2	73	220	$C_{17}H_{20}N_4O$	296.37	0.62	2.03
B-21	CH_3	2-OH	68	218	$C_{17}H_{19}N_3O_2$	297.35	0.71	2.25
B-22	C_6H_5	H	59	190	$C_{22}H_{21}N_3O$	343.42	0.92	2.61
B-23	C_6H_5	4-Br	51	68	$C_{22}H_{20}N_3OBr$	422.32	0.91	2.77
B-24	CH_3	CH_3	55	159	$C_{12}H_{17}N_3O$	219.28	0.79	2.14
B-25	CH_3	C_2H_5	57	148	$C_{13}H_{19}N_3O$	233.31	0.85	2.44
B-26	CH_3	$CH_2CH(CH_3)_2$	60	103	$C_{15}H_{23}N_3O$	261.36	0.82	2.89
B-27	CH_3	CH_2COCH_3	56	85	$C_{14}H_{19}N_3O_2$	261.32	0.71	2.28
B-28	CRR_1–Cyclohexylene		71	174	$C_{15}H_{21}N_3O$	259.35	0.73	2.51

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

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

Table-VII.2: Spectral and Elemental analyses data of the compounds: -

Compound No.	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)*		
			C	H	N
B-3	3390, 3360, 2840, 1680, 1620, 1595-1540, 1450, 1340, 1250	2.16 (s, 3H, CH_3), 2.20 (s, 3H, ArCH_3), 2.24 (s, 3H, ArCH_3), 6.76-7.84 (m, 7H, ArH), 7.90 (s, 1H, imine H), 8.48 (s, 1H, ArNH, D_2O exchangeable), 9.72 (s, 1H, CONH, D_2O exchangeable).	72.57 72.29	6.81 6.79	14.94 14.89
B-7	3400, 2910, 1680, 1610, 1590-1540, 1340,1440, 1245	2.20 (s, 3H, ArCH_3), 2.24 (s, 3H, ArCH3), 6.96-8.23 (m, 7H ArH), 8.01 (s, 1H, imine H), 8.63 (s, 1H, ArNH, D_2O exchangeable), 11.00 (s, 1H, CONH, D_2O exchangeable).	61.53 61.29	5.16 5.14	17.94 17.87
B-17	3400, 3200, 3090, 2950, 2850, 1690, 1620, 1590-1540, 1340, 1280, 1140	2.14 (s, 3H, CH_3), 2.20 (s, 3H ArCH_3), 2.22 (s, 3H, ArCH_3), 5.32 (s, 2H, ArNH_2, D_2O exchangeable), 6.54-7.67 (m, 7H, ArH), 8.44 (s, 1H, ArNH, D_2O exchangeable), 9.55 (s, 1H, CONH, D_2O exchangeable).	68.89 68.62	6.80 6.77	18.90 18.82
B-24	3380, 3090, 2880, 1680, 1580-1545, 1310, 1230	1.85 (s, 3H, CH_3), 1.95(s, 3H, CH_3), 2,18(s, 3H, ArCH_3), 2.24(s, 3H, ArCH_3), 6.80-7.12 (m, 3H, ArH), 8.24 (s, 1H, ArNH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable).	65.73 65.47	7.81 7.78	19.16 19.10
B-25	3390, 3100, 2840, 1680, 1590-1535, 1320, 1210	1.46-1.50 (t, 3H, CH_3, J=7.6 Hz), 1.84-1.90 (q, 2H, CH_2, J=7.6Hz), 1.98 (s, 3H, CH_3), 2.22 (s, 3H, ArCH_3), 2.26 (s, 3H, ArCH_3), 6.82-7.18 (m, 3H, ArH), 8.20 (s, 1H, ArNH, D_2O exchangeable), 9.44 (s, 1H, CONH, D_2O exchangeable).	66.92 66.67	8.21 8.18	18.01 17.94

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

Pharmacological activity: -

Table-VII.3: Anticonvulsant activity and minimal motor impairment of 2,4-dimethylphenylsemicarbazones: -

Compound No.	Intraperitoneal Injection in mice[a]							
	MES Screen		scPTZ Screen		scSTY Screen		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	2.0h	0.5h	4.0h
B-2	-	-	300	-	100	300	300	300
B-3	300	300	-	-	100	100	-	-
B-16	-	-	-	-	100	100	-	300
B-23	300	-	300[c]	-	300	300	300	-
B-24	100	100	300[c]	300[c]	300	300	100	300
B-25	300	-	300[c]	-	100	-	300	-
B-26	100	300	100	300	300	300	100	300
B-27	-	-	-[c]	-	-	-	-	-
B-28	300	300	300	-	300	300	300	300
B-29	100	-	300[c]	-	-	-	300	-[e]
B-30	100	-	300[d]	-[d]	-	-	100	100
Phenytoin	30	30	-	-	-	-	100	100
Carbamazepine	30	100	100	300	-	-	100	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 dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg).

[b] In the MES screen the compound showed protection at 100mg/kg in 1/3 mice.

[c] In the scPTZ screen at a dose of 100 mg/kg, compounds that showed protection were **B-23, 25, 29** and **30** (0.25h), **B-24** (0.25h, 1h), **B-27** (0.5h) and **B-28** (1h).

[d] The compound **B-30** at 100 mg/kg caused death following continuous seizures at 1 h period after administration.

[e] Compound **B-29** showed neurotoxicity at 100 mg/kg after 1h of administration.

Table-VII.4: Evaluation of some compounds in the psychomotor seizure (6 Hz MES test) after i.p. administration (100mg/kg) to mice: -

Compound No	Intraperitoneal administration to mice[a]				
	0.25h	0.5h	1.0h	2.0h	4.0h
B-3	1	-	-	-	-
B-7	-	-	-	1	-
B-13	-	1	-	-	-
Levetiracetam[b]	-	4	4	4	3

[a] The figures indicate the number of mice out of four which were protected. The dash (-) indicates not protected.

[b] Tested at 30 mg/kg (i.p).

Table-VII.5: Behavioral study on some selected compounds using Actophotometer: -

Compound No.[a]	Activity score[b]		
	Control (24h prior)	Post treatment	
		0.5h after	1.0h after
B-23	197.67 ± 13.24	120.33 ± 11.35	41.33 ± 03.25
B-24	282.33 ± 29.08	100.33 ± 13.11	51.17 ± 06.80
B-25	301.50 ± 27.67	137.33 ± 11.33	93.17 ± 05.93
B-26	154.83 ± 16.11	70.33 ± 06.43	55.00 ± 05.99
B-28	155.17 ± 26.98	60.17 ± 05.04	71.00 ± 07.45
Phenytoin[c]	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

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

[b] Each score represents the mean ± SEM of six mice, significantly different from the control score at $p < 0.05$ (Student's t-test).

[c] Tested at 30 mg/kg (i.p).

Table-VII.6: CNS study on selected compounds in Porsolt's swim test: -

Compound No.[a]	Immobility time[b] (sec)	
	Control (24 h prior)	Post treatment (60 min after)
PEG	143.50 ± 11.42	157.83 ± 10.04 NS
B-23	163.33 ± 08.53	208.33 ± 09.92
B-24	151.00 ± 06.39	212.33 ± 06.89
B-25	142.17 ± 06.27	197.83 ± 08.44
B-26	147.33 ± 05.82	225.50 ± 06.99
B-28	139.33 ± 06.67	192.17 ± 06.47
Carbamazepine[c]	131.50 ± 09.32	207.33 ± 08.49

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

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

[c] Tested at 30 mg/kg (i.p).

Table-VII.7: Effects of compounds on GABA system: -

Compound No.[a]	Concentration of GABA (µg/100 mg tissue)[b]			
	Olfactory Lobe	Mid Brain	Medulla Oblongata	Cerebellum
Control	12.01 ± 1.74	44.31 ± 1.78	33.28 ± 2.04	21.93 ± 2.52
B-24	19.79 ± 3.22	63.39 ± 2.77*	37.64 ± 6.79	19.36 ± 2.22
B-25	17.85 ± 2.89	36.09 ± 5.05	46.87 ± 3.34*	28.92 ± 3.34
B-26	18.71 ± 2.63	72.27 ± 4.37*	41.08 ± 2.96	25.82 ± 3.46
B-30	29.79 ± 0.93*	48.91 ± 3.77	38.77 ± 5.25	45.54 ± 2.20*
Clobazam[c]	17.67 ± 2.62	63.57 ± 2.83*	45.91 ± 2.16*	28.73 ± 3.12

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

[b] Each value represents the mean ± SEM of six rats significantly different from the control at

*$p < 0.05$ and the remaining values are not significant at $p < 0.05$ (Student's t-test).

[c] Tested at 30 mg/kg (i.p).

Results & Discussion: -

Chemistry: -

The 2,4-dimethylphenylsemicarbazones have been prepared starting from 2,4-dimethyl aniline. The homogeneity of the compounds was monitored by thin layer chromatography (TLC) by which *R_f values were calculated. Eluent* for all compounds were $CHCl_3$: CH_3OH (9:1).

All the compounds were found to be more *lipophilic* indicated by their calculated partition coefficient value greater than 2 (log $P > 2$) except for compounds **B-5**, **8** and **18**. These compounds had log $P < 2$ which were substituted with polar group like OH (Table-VII.1).

With *regard to the percentage yield* of the synthesized compounds, the compounds with substituted aryl aldehydes have yielded in the range of 73-97% except for compounds **B-9**, **10** and **11** whose percentage yields were 67, 57 and 67% respectively. The compounds with substituted aryl/alkyl ketones yielded about 53-86% (Table-VII.1).

The IR *spectrum* of N^1-(2,4-dimethylphenyl)-N^4-(4-nitrobenzaldehyde)semicarbazone (B-7) was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3400, 3350 cm^{-1} showed NH stretching, 1680 cm^{-1} showed C=O stretching, 1620 cm^{-1} showed C=N stretching, 1600-1540 cm^{-1} showed aromatic C=C stretching, 1340 cm^{-1} showed aryl C-N stretching.

^{1}H-NMR spectrum of compound *B-7 revealed singlets* at δ 2.20 for 3H of aryl CH_3 protons and at δ 2.24 for another 3H of aryl CH_3 protons, singlet at δ 8.00 for 1H of imine proton and singlets at δ 8.63 (1H, ArNH) and δ 11.00 (1H, CONH), which were D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristic peaks depicted in Table-VII.2.

Pharmacological activity: -

The anticonvulsant activity of the synthesized compounds was established after their i.p. administration in three seizure models in mice viz, MES, scPTZ and scSTY screens. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table-VII.3 along with the data of Phenytoin and Ethosuximide.

(i) *MES test: -*

The compounds **B-3**, **23-26**, and **28-30** showed anti-MES activity at a dose of 300 mg/kg, indicative of their ability to prevent seizure spread. At a dose of 100 mg/kg, compounds **B-26**, **29** and **30** showed protection only at 0.5h but compound **B-24** which showed activity at both 0.5h and 4.0h period. Hence this compound showed a rapid onset of action and also had prolonged action at 100 mg/kg. But the compounds **B-3**, **26** and **28** were capable of preventing seizure spread over a period of 4h only at a dose of 300 mg/kg. The compounds **B-29** and **B-30** were 2,4-dimethylphenylurea and semicarbazide respectively which showed protection only at 0.5h at a dose of 100 mg/kg and it could not prevent seizure spread over a period of 4h even at a maximum dose of 300 mg/kg administered. So these compounds had rapid onset and shorter duration of action, which might be due to metabolism to inactive compounds.

(ii) **scPTZ test: -**

The compounds **B-2**, **23-26** and **28-30** were found to be active in the scPTZ test, a test used to identify compounds that elevate seizure threshold. Compound **B-26** showed activity at a dose of 100 mg/kg comparable with Carbamazepine. Compounds that showed moderate protection at a dose of 100 mg/kg include **B-23** (0.25h, 2/5), **B-24** (0.25h, 1h, 5/5), **B-25** (0.25h, 3/5), **B-28** (1h, 1/5), **B-29** (0.25h, 2/5) and **B-30** (0.25h, 3/5).

(iii) ***scSTY test: -***

Promising compounds were carried on to the scSTY tests. The activity of compounds **B-2**, **3**, **23**, **24** and **26-28** in the scSTY test indicated that semicarbazones could also act through inhibitory glycine receptors as reported earlier (83). The compound **B-3** was able to prevent seizure at a dose of 75mg/kg over a 2.0h period. Compounds that showed anti-scSTY activity at 100 mg/kg include **B-2** (0.5h), **B-25** (0.5h) and **B-23** at 150 mg/kg. Compounds **B-24**, **26** and **28** showed protection at 300 mg/kg and compounds **B-26** and **B-28** were found to show loss of righting reflex after 0.25h of drug administration.

Neurotoxicity screen (NT): -

In the neurotoxicity test, compounds **B-3** and **B-27** did not show toxicity in the highest administered dose (300 mg/kg) and the compounds **B-12**, **16**, **23**, **25**, **28** and **29** showed less toxicity than standard drugs Phenytoin and Carbamazepine. On the other hand, compounds **B-2** (4h), **B-24** (0.5h), **B-26** (0.5h) and **B-30** (4h) were more toxic (100 mg/kg). Mice were unable to grasp rotorod after administration of the following compounds viz. **B-3** (300 mg/kg, 0.5h), **B-12** (300 mg/kg, 0.5h), **B-24** (100 mg/kg, 0.5h, 1h and 300 mg/kg 0.5h, 4h), **B-28** (300 mg/kg, 0.5h, 4h) and **B-30** (300 mg/kg, 0.5h). With compound **B-26**, loss of righting reflex was observed at 0.5h and 4h after 300 mg/kg dose was administered and with compound **B-28** (300 mg/kg) slight tremors were noted. Compounds **B-4-11, 13, 15** and **17-22** did not exhibit anticonvulsant activity and neurotoxicity (Table-VII.3).

In the rat i.p MES model, compounds **B-5** and **B-12** at 30 mg/kg showed protection at 1h and 0.25h respectively. And in the rat oral MES screen, compounds that were active include **B-24** (0.5h) and **B-25** (0.5h, 1h and 2h).

The MES and scPTZ tests have become the two most widely employed seizure models for the early identification and high throughput screening of investigational antiepileptic drugs. These tests, albeit extremely effective in identifying new antiepileptic drugs that may be useful for the treatment of human generalized tonic-clonic and generalized myoclonic seizures respectively, may miss novel antiepileptic drugs that may be useful for the treatment of therapy resistant partial seizures. Results obtained with Leviteracetam in the 6 Hz test suggest that it may identify a compound, which is not active by either the MES or scPTZ test and thus may detect active substances that would have been missed by the more traditional identification procedure (150, 151). In the present study, some selected compounds **B-(3, 5, 7, 12, 13** and **17)** were screened in 6 Hz psychomotor seizure model. Compounds **B-3**, 7 and **13** showed protection in 25% mice at 100 mg/kg at 0.25h, 2.0h and 0.5h respectively (Table-VII.4). Recent studies completed by the anticonvulsant screening program, NIH, have found that 6Hz seizures appear to be somewhat resistant to phenytoin and other sodium channel blockers.

CNS depressant Evaluation: -

Some selected 2,4-dimethylphenylsemicarbazone derivatives were 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 side effect (39).

In the behavioral study using actophotometer, all the compounds were found to decrease the behavior of the animals in both 0.5h and 1.0h period, which was similar to standard drug Phenytoin as represented in Table-VII.5.

In a similar study using Porsolt's swim test the immobility time after the administration of the test compounds were compared with Carbamazepine (Table-VII.6). All the compounds tested were found to show CNS depression which were significantly different from the control at $P<0.05$. The present study showed an increase in immobility time by the tested compounds and hence indicating facilitation of depression.

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

In order to explore the mechanism of anticonvulsant activity, some selected compounds that were highly active were subjected to neurochemical investigation to study their effects on the levels of GABA in different regions of rat brain viz olfactory lobe, midbrain, medulla oblongata and cerebellum (Table-VII.7). Compound **B-30** showed an increase of GABA level in olfactory lobe and cerebellum while compounds **B-24** and **B-26** showed a significant increase in GABA levels in mid brain region of rat brain. The compound **B-25** has significant effect on the GABA level in medulla oblongata when compared to standard drug Clobazam. From this result it can be concluded that the 2,4-dimethylphenylsemicarbazones acts as anticonvulsants by influencing GABA mediated mechanism.

Structure Activity Relationship: -

Table-VII.1 lists twenty-eight N^1-(2,4-dimethylphenyl)-N^4-(substituted benzaldehyde/acetophenone/benzophenone)semicarbazones. Various compounds of 2-, 3- and 4-substituted phenyl analogues [**B-(2-13, 15-21 and 23)**] were prepared with the substituents chosen to examine the effects of electron donating and withdrawing groups as well as changes is bulk and lipophilicity [**B-(22, 24-28)**].

(i) Substitution in the 2- and 3-position of the phenyl ring showed no anticonvulsant activity.

(ii) Substitution in 4-position of the phenyl ring with electron withdrawing group (**B-2**) showed anticonvulsant activity against scPTZ and scSTY test.

(iii) Substitution in 4-position of the phenyl ring with electron donating group (**B-3**) exhibited activity in MES and scSTY tests.

(iv) Replacement of carbimino hydrogen by methyl group showed no change in the anticonvulsant effect. The compound **B-16** {N^1-(2,4-dimethylphenyl)-N^4-[1-(4-methylphenyl)ethan-1-one]semicarbazone} showed activity against scSTY test similar to compounds possessing carbimino hydrogen atom like **B-2** and **B-3**.

(v) Similarly, the carbimino hydrogen atom when replaced with bulky group like phenyl (**B-22**, **B-23**) showed activity as equal to **B-3**. The compound **B-3** showed activity in the MES and scSTY tests but compound **B-23** exhibited anticonvulsant activity against all screens *viz.* MES, scPTZ and scSTY.

(vi) Introduction of small lipophilic group like alkyl groups [**B-(24-26)**] instead of carbimino phenyl ring showed broad spectrum of anticonvulsant activity. These compounds showed activity in all the screens *viz.* MES, scPTZ and scSTY.

(vii) Similarly, replacement of carbimino phenyl ring with cyclohexyl group (**B-28**) exhibited broad spectrum of anticonvulsant activity.

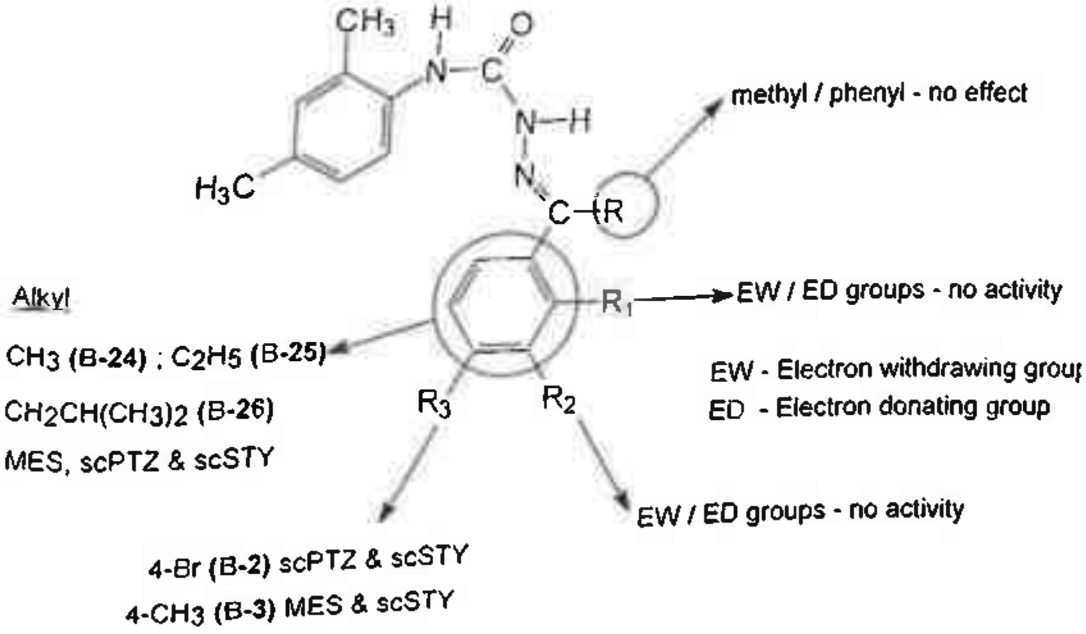
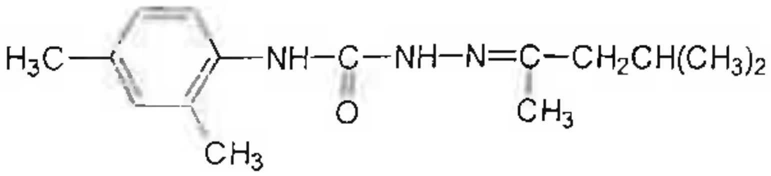


Figure VII.1. Schematic representation of SAR of 2,4-dimethylphenylsemicarbazones.

It could be concluded that small lipophilic group like alkyl groups at the carbimino end showed broad spectrum of anticonvulsant activity. The most active compound of this series is **N^1-(2,4-dimethylphenyl)-N^4-(4-methylpentan-2-one)semicarbazone (B-26).**



MES : 100% protection at 100 mg / kg

scPTZ : 100% protection at 100 mg / kg

scSTY : 100% protection at 300 mg / kg

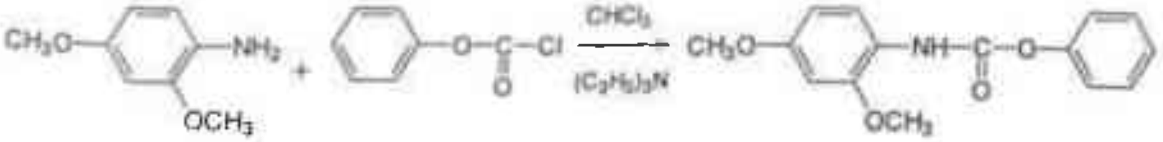
Toxicity: Neurotoxicity at 300 mg / kg

GABA : Increased the level of GABA in the mid brain region of the rat brain by 63% when compared to the control.

The idea behind the synthesis of 2,4-dimethoxy substituents was based on the literature report (141), in which 2, 4 and 5th position of the molecule was substituted with methoxy group and also that substitution in the 2-position of phenyl ring with electron donating groups was generally beneficial to anticonvulsant activity (142).

Synthesis: -

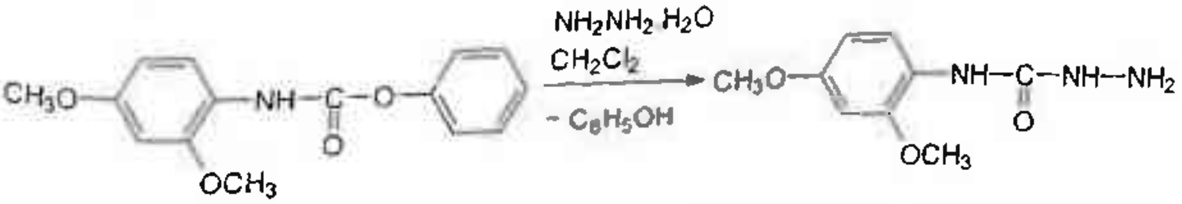
Step-1: ***Synthesis of Phenyl-N-(2,4-dimethoxyphenyl)carbamate: -***



2,4-dimethoxyaniline Phenylchloroformate Phenyl-N-(2,4-dimethoxyphenyl) carbamate

Phenylchloroformate (0.1M, 12.6 ml) was dissolved in 40 ml of chloroform and equimolar quantities of 2,4-dimethoxyaniline (0.1M, 12.3ml) and triethylamine (0.1M, 13.9 ml) were added drop wise and stirred in the room temperature for 5hr. The reaction mixture was concentrated to one-third volume and 100 ml of petroleum ether was added. The precipitate which appeared was washed with water, filtered and dried, m.p. 126°C, IR (KBr) ν_{max} 3400, 3100-3060, 2940, 1700, 1320, 845 cm^{-1}; ^1H-NMR ($CDCl_3$, δ ppm) 3.74 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 6.86-7.67 (m, 8H, ArH), 8.32 (s, 1H, ArNH, D_2O exchangeable).

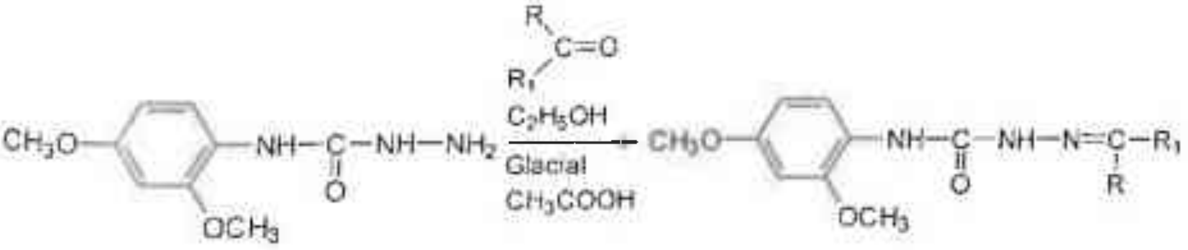
Step-2: ***Synthesis of 2,4-dimethoxyphenylsemicarbazide: -***



Phenyl-N-(2,4-dimethoxyphenyl) carbamate 2,4-dimethoxyphenylsemicarbazide

Phenyl-N-(2,4-dimethoxyphenyl)carbamate (0.05M, 12.05 gm) was dissolved in 100 ml of dichloromethane. To this solution, 4.85 ml of hydrazine hydrate (0.1M) was added and refluxed with stirring for 24hr. The precipitate of 2,4-dimethoxyphenylsemicarbazide (C-21) was separated by vacuum filtration and washed with dichloromethane and dried. m.p.190 °C, IR (KBr) ν_{max} 3390, 3090, 2860, 1680, 1320, 840 cm^{-1} ; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm) 3.68 (s, 3H, $ArOCH_3$), 3.72 (s, 3H, $ArOCH_3$), 5.42 (s, 2H, NH_2, D_2O exchangeable), 7.12-7.37 (m, 3H, ArH), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.48 (bs, 1H, $NHNH_2$, D_2O exchangeable).

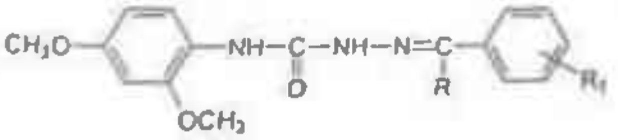
Step-3: *General procedure for the synthesis of 2,4-dimethoxyphenylsemicarbazones: -*



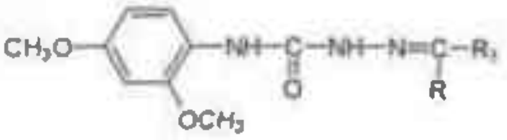
2,4-dimethoxyphenylsemicarbazide 2,4-dimethoxyphenysemicarbazones

To a solution of 2,4-dimethoxyphenylsemicarbazide (0.003M, 0.84g) in ethanol, an equimolar quantity of appropriate aldehyde or ketone in ethanol and glacial acetic acid were added. The mixture was stirred with heating for 1hr to 4hr until the completion of the reaction and the resultant precipitate was filtered and dried. The product was recrystallised from 95% ethanol. The physical data of the compounds are presented in Table-VIII.1. The IR spectra of the compounds were identical in the following aspects. 3400-3320, 2900-2850, 1690-1670, 1620-1540, 1320, 840 cm^{-1}; ^{1}H-NMR (300MHz, δ ppm) spectra and the elemental analyses of some representative compounds are given in the Table-VIII.2.

Table-VIII.1: Physical data of the 2,4-dimethoxyphenylsemicarbazones: -



C-(1-15)



C-(16-20)

Compound No.	Substituents		Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
	R	R_1						
C-1	H	H	62	188	$C_{16}H_{17}N_3O_3$	299.33	0.71	1.88
C-2	H	4-CH_3	55	159	$C_{17}H_{19}N_3O_3$	313.36	0.62	2.07
C-3	H	4-OCH_3	52	155	$C_{17}H_{19}N_3O_4$	329.35	0.64	1.48
C-4	H	4-N-$(CH_3)_2$	50	137	$C_{18}H_{22}N_4O_3$	342.40	0.64	1.27
C-5	H	4-NO_2	66	173	$C_{16}H_{16}N_4O_5$	344.33	0.72	2.21
C-6	H	3-Cl	64	139	$C_{16}H_{16}N_3O_3Cl$	333.77	0.70	2.21
C-7	H	3-NO_2	69	200	$C_{16}H_{16}N_4O_5$	344.33	0.74	2.19
C-8	H	2-Cl	60	179	$C_{16}H_{16}N_3O_3Cl$	333.77	0.68	2.19
C-9	H	2-CH_3	61	127	$C_{17}H_{19}N_3O_3$	313.35	0.69	1.97
C-10	H	2-OH	57	156	$C_{16}H_{17}N_3O_4$	315.33	0.65	1.52
C-11	H	2-NO_3	65	145	$C_{16}H_{16}N_4O_5$	344.32	0.74	2.13
C-12	CH_3	H	61	161	$C_{17}H_{19}N_3O_3$	313.35	0.71	1.85
C-13	CH_3	4-Cl	58	166	$C_{17}H_{18}N_3O_3Cl$	347.80	0.74	1.95
C-14	CH_3	4-CH_3	53	151	$C_{18}H_{21}N_3O_3$	327.38	0.63	2.06
C-15	CH_3	4-NO_2	67	118	$C_{17}H_{18}N_4O_5$	358.35	0.77	2.05
C-16	CH_3	CH_3	54	159	$C_{12}H_{17}N_3O_3$	251.28	0.69	1.51
C-17	CH_3	C_2H_5	52	138	$C_{13}H_{19}N_3O_3$	265.31	0.62	1.79
C-18	CH_3	$CH_2CH(CH_3)_2$	52	141	$C_{15}H_{23}N_3O_3$	293.36	0.61	2.13
C-19	CH_3	CH_2COCH_3	50	156	$C_{14}H_{19}N_3O_4$	293.32	0.66	1.62
C-20	CRR_1=Cyclohexylene		51	147	$C_{15}H_{21}N_3O_3$	291.35	0.67	1.76

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

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

Table-VIII.2: Spectral and elemental analyses data of the compounds: -

Compound No.	IR Spectroscopy (cm^{-1}; KBr)	^{1}H-NMR Spectra & ppm (DMSO-d_6)	Elemental analyses (Calculated/ found)*		
			C	H	N
C-2	3340, 2890, 1670, 1610, 1590-1530, 1320	2.24 (s, 3H, ArCH$_3$), 3.64 (s, 3H, ArOCH$_3$), 3.68 (s, 3H, ArOCH$_3$), 6.78-7.86 (m, 7H, ArH), 7.92 (s, 1H, imine H), 8.46 (s, 1H, ArNH, D$_2$O exchangeable), 9.70 (s, 1H, CONH, D$_2$O exchangeable).	65.16 64.91	6.11 6.09	13.41 13.36
C-5	3350, 2900, 1690, 1605, 1590-1540, 1435, 1330, 1310, 1240	3.70 (s, 3H, ArOCH$_3$), 3.74 (s, 3H, ArOCH$_3$), 6.84-7.96 (m, 7H, ArH), 8.02 (s, 1H, imine H), 8.62 (s, 1H, ArNH, D$_2$O exchangeable), 10.94 (s, 1H, CONH, D$_2$O exchangeable).	55.81 55.60	4.68 4.66	16.27 16.21
C-16	3370, 2890, 1670, 1590-1545, 1320, 1200	1.82 (s, 3H, CH$_3$), 1.93 (s, 3H, CH$_3$), 3.66 (s, 3H, ArOCH$_3$), 3.70 (s, 3H, ArOCH$_3$), 6.72-6.84 (m, 3H, ArH), 8.22 (s, 1H, ArNH, D$_2$O exchangeable), 9.54 (s, 1H, CONH, D$_2$O exchangeable).	57.36 57.14	6.82 6.80	16.72 16.66

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

Pharmacological activity: -

Table-VIII.3: Anticonvulsant activity and minimal motor impairment of 2,4-dimethoxyphenylsemicarbazones: -

Compound No.	Intraperitoneal Injection in mice[a]							
	MES Screen		scPTZ Screen		scSTY Screen		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	2.0h	0.5h	4.0h
C-1	300	-	-	-	×	×	300	-
C-2	300	-	-	-	×	×	100	100
C-3	300[b]	300	300	-	100	300	300	-
C-4	300[b]	300	-	-	300	300	-	-
C-6	300[b]	-	-	-	×	×	-	-
C-7	-	300	100	-	×	×	100	300
C-8	-	-	300[c]	-	×	×	100	100
C-9	300[b]	-	-	-	×	×	300	-
C-12	300	-	-	-	300	-	-	-
C-13	300	-	300	-	×	×	300	-
C-14	300	300	300[c]	-	300	300	300	-
C-15	-	-	300[c]	-	×	×	300	-
C-16	300	300	300	-	100	100	-	-
C-17	300[b]	-	300[c]	-	100	300	300	-
C-18	300[b]	-	300	-	300	-	300	-
C-19	300[b]	-	300	-	300	300	300	-
C-21	300[b]	-	300[c]	-	×	×	-	-
Phenytoin	30	30	-	-	-	-	100	100
Ethosuximide	-	-	100	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 (scSTY test, 0.5h and 2.0h period study) after injection were made. The dash (-) indicates the absence of activity at max dose administered (300 mg/kg) and (x) indicates not tested.

[b] In the MES screen at a dose of 100 mg/kg, compounds that showed protection were C-9 and C-19 (0.25h, 1/3), C-3, 6, 7 and 18 (0.5h, 1/3), C-21 (1h, 1/3) and C-4 (2.0h, 1/3).

[c] In the scPTZ screen, at a dose of 100 mg/kg, compounds that showed protection were C-3 (0.5h, 1/5), C-8 (0.25h, 1/5), C-14 (0.25h, 2/5), C-15 (0.25, 1h, 1/5), C-17 (0.25h, 1/5) and C-21 (0.25h, 1/5).

Table-VIII.4: Evaluation of some compounds in the scPTZ test after oral administration (50mg/kg) to rats: -

Compound No	Oral administration to rats[a]				
	0.25h	0.5h	1.0h	2.0h	4.0h
C-21	1	2	0	0	0
Ethosuximide	0	3	4	3	3

[a] The figures indicate the number of rats out of four which were protected.

Table-VIII.5: Behavioral study on some selected compounds using Actophotometer:-

Compound No.[a]	Activity Score[b]		
	Control (24h before)	Post treatment	
		0.5h after	1.0h after
C-3	350.67 ± 17.43	207.00 ± 09.56	305.67 ± 11.64 NS
C-4	406.67 ± 19.71	282.00 ± 11.08	350.33 ± 15.75
C-12	395.33 ± 17.48	231.17 ± 12.68	264.50 ± 10.90
C-14	417.50 ± 15.68	268.67 ± 07.53	326.00 ± 11.94
C-16	488.00 ± 12.57	276.50 ± 08.09	308.33 ± 13.12
C-17	414.17 ± 17.59	323.33 ± 07.91	340.33 ± 09.20
C-18	322.00 ± 10.58	240.67 ± 07.85	303.33 ± 09.28 NS
C-19	338.00 ± 11.43	154.17 ± 06.44	225.50 ± 09.63
Phenytoin[c]	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

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

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

[c] Tested at 30 mg/kg (i.p).

Table-VIII.6: CNS study on selected compounds in Porsolt's swim test: -

Compound No.[a]	Immobility time[b] (sec)	
	Control (24 h prior)	Post treatment (60 min after)
PEG	151.50 ± 3.77	163.67 ± 5.08 NS
C-3	154.33 ± 6.06	209.50 ± 6.23
C-4	157.33 ± 7.97	197.17 ± 8.03
C-12	170.67 ± 7.57	204.33 ± 7.87
C-14	162.33 ± 3.88	218.67 ± 6.08
C-16	158.50 ± 4.90	219.00 ± 5.01
C-17	165.50 ± 6.53	187.33 ± 7.32
C-18	176.00 ± 6.94	202.33 ± 7.80
C-19	171.67 ± 8.44	193.33 ± 6.09 NS
Carbamazepine[c]	131.50 ± 9.32	207.33 ± 8.49

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

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

[c] Tested at 30mg/kg (i.p).

Table-VIII.7: Effects of compounds on GABA system: -

Compound No.[a]	Concentration of GABA (μg/100 mg tissue)[b]			
	Olfactory Lobe	Mid Brain	Medulla Oblongata	Cerebellum
Control	12.01 ± 1.74	44.31 ± 1.78	33.28 ± 2.04	21.93 ± 2.52
C-3	13.00 ± 2.56	58.87 ± 2.61*	39.51 ± 2.74	29.25 ± 3.40
C-14	12.71 ± 1.80	63.77 ± 2.82*	42.18 ± 2.54*	29.79 ± 3.08
C-16	14.58 ± 1.78	64.72 ± 3.48*	44.32 ± 2.92*	27.99 ± 2.83
Clobazam[c]	17.67 ± 2.62	63.57 ± 2.83*	45.91 ± 2.16*	28.73 ± 3.12

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

[b] Each value represents the mean ± SEM of six rats significantly different from the control at *$P < 0.05$ and the remaining values are not significant at $P < 0.05$ (Student's t-test).

[c] Tested at 30mg/kg (i.p).

Results & Discussion: -

Chemistry: -

The required 2,4-dimethoxyphenylsemicarbazones were prepared by a three-step process starting from 2,4-dimethoxyaniline. The homogeneity of the compounds was monitored by thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were $CHCl_3$: CH_3OH (9:1). Out of the twenty compounds, nine compounds were found to be more lipophilic (i.e. **C-2, 5-8, 11, 14, 15** and **18**) indicated by their calculated partition coefficient values greater than 2 [log $P > 2$]. The remaining compounds had shown log $P < 2$ which were all substituted with polar groups like OH, NH_2 and simple alkyl ketones (Table-VIII.1).

With regard to the percentage yield of the synthesized compounds, most of the compounds have yielded in the range of 50-58% except for compounds **C-1, 5-8, 11, 12** and **15**, which have yielded in the range of 60-69% (Table-VIII.1).

The IR spectrum of N^1-(2,4-dimethoxyphenyl)-N^4-(4-nitrobenzaldehyde)semicarbazone (**C-5**) was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption bands at 3410, 3300 cm^{-1} showed NH stretching, 1660 cm^{-1} showed C=O stretching, 1610 cm^{-1} showed C=N stretching, 1600-1535 cm^{-1} showed aromatic C=C stretching, 1340 cm^{-1} showed aryl C-N stretching.

^{1}H-NMR spectrum of compound **C-5** revealed singlets at δ 3.82 for 3H of aryl OCH_3 protons and at δ 3.86 for another 3H of aryl OCH_3 protons, singlet at δ 8.12 for 1H of imine proton and singlets at δ 8.43 (1H, ArNH) and δ 10.86 (1H, CONH), which were D_2O exchangeable. Similarly, the structures of other compounds were confirmed according to their characteristic peaks depicted in Table-VIII.2.

Pharmacological activity: -

The anticonvulsant activity of the synthesized compounds was established after their i.p administration in three seizure models in mice viz, MES, scPTZ and scSTY screen. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table-VIII.3 along with the data of Phenytoin and Ethosuximide.

(i) MES test: -

The compounds **C-1-6, 9-14** and **16-21** showed anti-MES activity at a dose of 300 mg/kg, indicative of their ability to prevent seizure spread. The compounds **C-3,**

4, 14 and 16 showed activity both at **0.5h** and **4.0h** periods and other compounds like **C-1, 2, 5, 6, 9-13** and **17-21** showed activity only at 0.5h. The compounds that showed activity till 4.0h period was mainly due to substitution of electron donating group in the carbimino phenyl ring. But most of the compounds showed activity only at 0.5h indicating that they have rapid onset and shorter duration of action.

(ii) scPTZ screen: -

Compounds **C-3**, 7, **8** and **13-21** were found to be active in the scPTZ test, a test used to identify compounds that elevate seizure threshold. The compound **C-7** showed activity at a dose of 100 mg/kg comparable with Ethosuximide. **Compounds that showed moderate protection at a dose of 100 mg/kg include C-3** (0.5h, 1/5), **C-8** (0.25h, 1/5), **C-14** (0.25h, 2/5), **C-15** (0.25h, 1h, 1/5), **C-17** (0.25h, 1/5) and **C-21** (0.25h, 1/5). **All these compounds showed 100% protection** at a dose of 300 mg/kg at 0.5h. So these compounds have quick onset of action but for shorter duration.

(iii) scSTY test: -

Promising compounds were carried on to the scSTY pattern test. Compounds C-3, 4, 12, 14 and 16-19 showed protection against scSTY induced seizures indicative of their ability to prevent seizure spread and also these compounds might play a role in the inhibitory glycine receptors. Compounds **C-3, 16** and **17** showed **activity at 100 mg/kg** at 0.5h in which compound **C-16** showed activity till 4.0h in the same dose **but compounds C-3 and C-16 showed activity at a dose of 300 mg/kg at 4.0h. All the other compounds C-4, 12, 14, 18 and 19 showed activity at** 300 mg/kg both in the 0.5h and **4.0h periods.**

Neurotoxicity screen: -

In the NT screen, compounds C-4, 5, 12, 16 and 21 did not show neurotoxicity and the **compounds C-1, 3, 9, 13-15 and** 17-19 were **less neurotoxic than standard drug Phenytoin. On the other hand, compounds C-2 (0.5h, 1h), C-7 (0.5h), C-8 (0.5h, 4h),** showed neurotoxicity at a dose of 100 **mg/kg.** The compounds **C-5, 10 and 11 exhibited** neither anticonvulsant activity nor neurotoxicity (Table-VIII.3).

Rat p.o. Identification: -

Among the compounds that were active in the i.p anticonvulsant screen, only and 2,4-dimethoxyphenylsemicarbazide (**C-21**) was selected for activity in the oral screen in the scPTZ and neurotoxicity tests. The results are presented in Table-VIII.4. The compound was tested at 50mg/kg and compared with standard drug Ethosuximide. Compound **C-21** showed protection only at 0.25h and 0.5h [**C-21** (1/4, 2/4)] periods and the oral activity was comparatively lesser than the standard drug Ethosuximide. This compound had shown no neurotoxicity at 50 mg/kg.

CNS depressant evaluation: -

Some selected, 2,4-dimethoxyphenylsemicarbazone derivatives (**C-3, 4, 12, 14** and **16-19**) were 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 depressant side effect (39).

In the behavioral study using actophotometer, the compounds **C-3** and **C-18** showed no behavioral despair effect after 1.0h when compared to Phenytoin as represented in Table-VIII.5. But these compounds showed behavioral despair effect at 0.5h period. All other compounds were found to decrease the behavior of the animals.

In a similar study using Porsolt's swim test, the immobility time after the administration of the test compounds were compared with Carbamazepine (Table-VIII.6). The compound **C-19** was found to show no significant CNS depression compared with the control at $p < 0.05$. All other compounds tested were found to emerge as CNS depressants as they increased the immobility time.

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

In order to explore the mechanism of anticonvulsant activity, some selected compounds which were highly active were subjected to neurochemical investigation to study their effects on the levels of GABA in different regions of rat brain viz. olfactory lobe, mid brain, medulla oblongata and cerebellum (Table-VIII.7). All the compounds subjected to neurochemical study were found to increase the GABA level in the mid brain region significantly. In the other brain regions, there was no significant increase in the level of GABA except for compounds **C-14** and **C-16**, which increased the GABA level in the medulla oblongata region similar to Clobazam.

Structure Activity Relationship: -

In the structure activity relationship study of 2,4-dimethoxyphenylsemicarbazones, among twenty compounds the parent compound **C-1** [N^1-(2,4-dimethoxyphenyl)-N^4-(benzaldehyde) semicarbazone] showed activity against MES test at a dose of 300 mg/kg. 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.

(i) Substitution at 2-position of carbimino phenyl ring with chloro (**C-8**) and methyl (**C-9**) groups showed anticonvulsant activity against both MES and scPTZ tests. But, the compounds substituted with nitro group (**C-11**) and hydroxyl group (**C-10**) exhibited loss of anticonvulsant activity.

ii) Substitution in the 3-position with chloro (**C-6**) and nitro (**C-7**) groups showed anticonvulsant activity in MES and scPTZ tests respectively.

iii) Substitution in the 4-position of carbimino phenyl ring with electron donating groups [**C-(2-4)**] showed activity against MES and scSTY tests but no anticonvulsant activity was observed with electron withdrawing substituents (**C-5**).

(iv) Replacement of carbimino hydrogen by small lipophilic group like alkyl groups [**C-(12-15)**] showed anticonvulsant activity similar to unsubstituted compounds.

(v) When the carbimino phenyl ring was replaced with small lipophilic alkyl moieties, [**C-(16-19)**] a broad spectrum of anticonvulsant activity viz. MES, scPTZ and scSTY was observed. With regard to neurotoxicity, the compound **C-16** did not show neurotoxicity even at the maximum dose (300 mg/kg) tested. But other compounds **C-17, 18** and **19** showed neurotoxicity at a dose of 300 mg/kg at 0.5h period.

(vi) Compounds **C-3, 16** and **17** (4-methoxyphenyl and alkyl groups like dimethyl and ethylmethyl groups) showed potent anticonvulsant activity against scSTY screen when compared to standard drug ethosuximide.

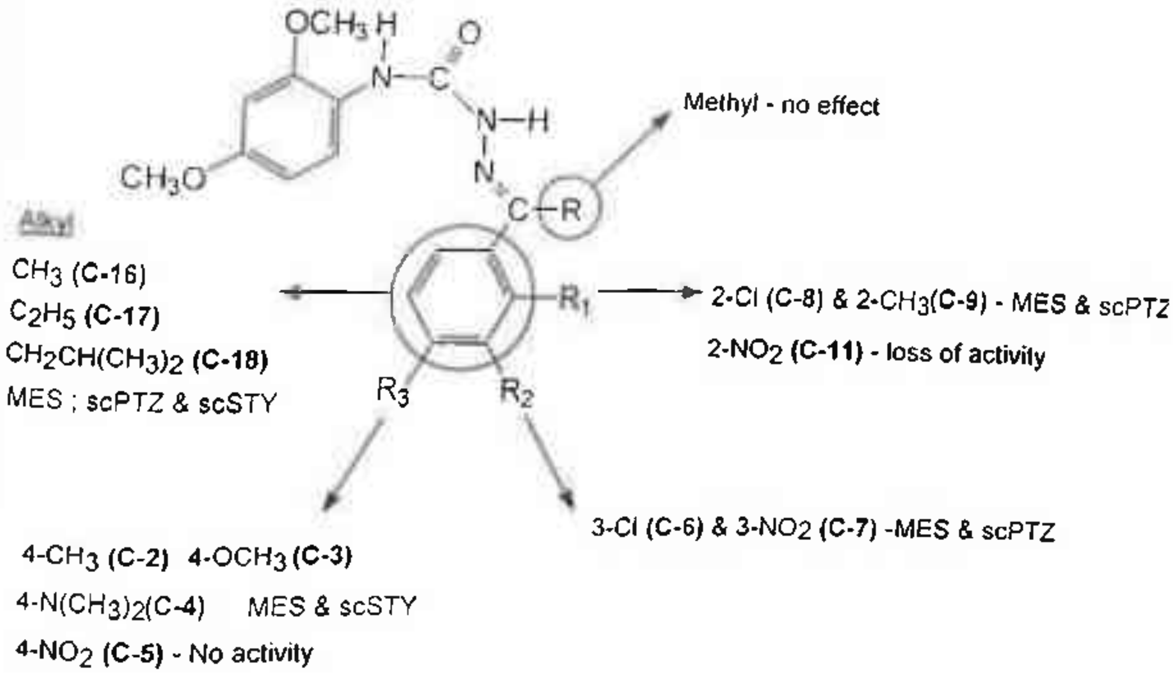
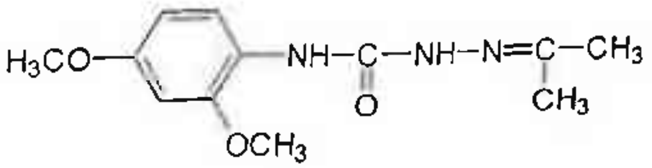


Figure VIII.1. Schematic representation of SAR of 2,4-dimethoxyphenylsemicarbazones.

From the above results, it could be concluded that the small lipophilic moiety like methyl **(C-16)** substituted semicarbazone showed broad spectrum of anticonvulsant activity. The most active compound of this series is **N^1-(2,4-dimethoxyphenyl)-N^4-(propan-2-one)semicarbazone (C-16)**



<u>MES</u> : 100% protection at 300 mg / kg

<u>scPTZ</u> : 100% protection at 300 mg / kg

<u>scSTY</u> : 100% protection at 100 mg / kg

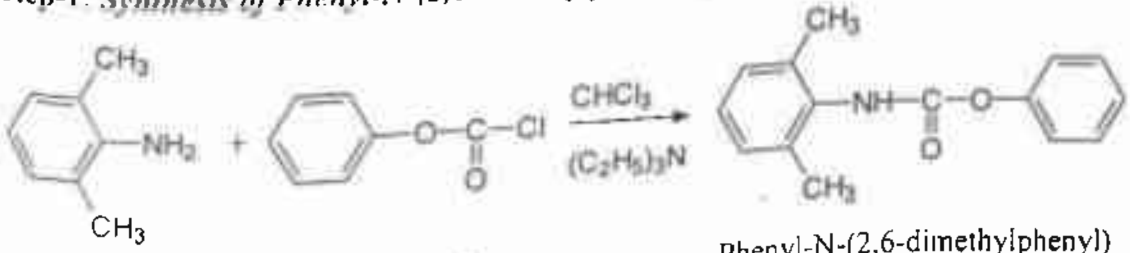
<u>Toxicity</u>: No Neurotoxicity

<u>GABA</u> : Increase the level of GABA in the region of mid brain and medulla oblongata of the rat brain by 46% and 33% compared to the control respectively.

The selection for the synthesis of 2,6-dimethylphenylsemicarbazones was based on the literature report in which the substitution in the 2,6 position of phenyl ring with small lipophilic (π) had generally showed potent activity when compared to other substitutions (142, 143). Clark et al. (152, 153) synthesized a number of benzamides of amino benzoic acids having potent activity against MES seizures in mice. The 2,6-dimethylanilide, ameltolide proved to be the most potent compound arising from these studies with an ED_{50} of 2.6 mg/kg when administered by an i.p. route in mice. 4-Amino-N-(2,6-dimethylphenyl)phthalimide was previously designed from the models of ameltolide and thalidomide (154).

Synthesis: -

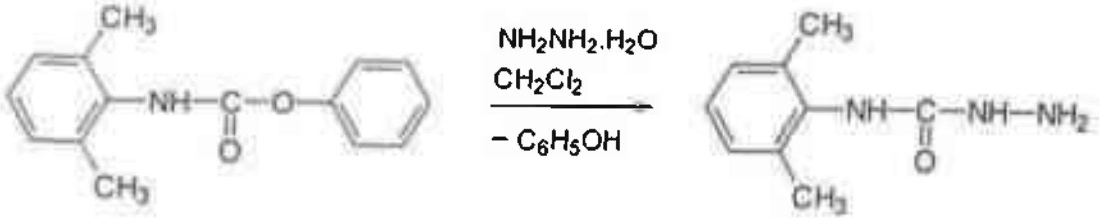
Step-1: ***Synthesis of Phenyl-N-(2,6-dimethylphenyl)carbamate: -***



2,6-dimethylaniline Phenylchloroformate Phenyl-N-(2,6-dimethylphenyl) carbamate

Phenylchloroformate (0.1M, 12.1 ml) was dissolved in 40ml of chloroform and taken in a 3-necked round bottom flask. To this solution, 2,6-dimethylaniline (0.1M, 12.3 ml) and triethylamine (0.1M, 13.9 ml) were added slowly and stirred at room temperature for 5 hr. 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) ν_{max} 3260, 3040, 2850, 1710, 1600-1540, 1240 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz, δ ppm) 2.28 (s, 6H, Ar-$2CH_3$), 7.02-7.87 (m, 8H, ArH), 8.44 (s, 1H, ArNH, D_2O exchangeable).

Step-2: ***Synthesis of 2,6-dimethylphenylsemicarbazide: -***

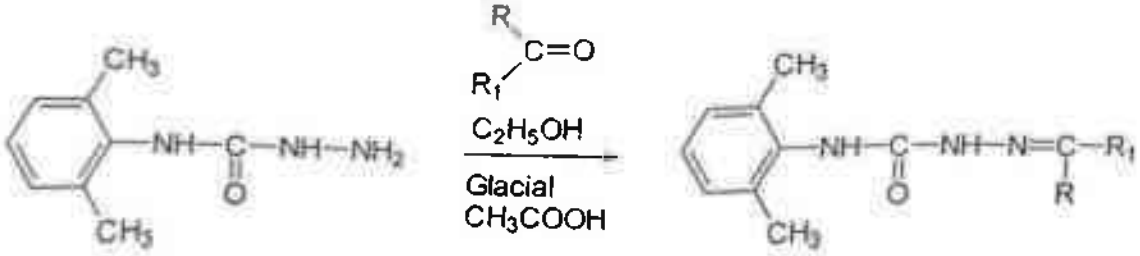


Phenyl-N-(2,6-dimethylphenyl) carbamate

2,6-dimethylphenylsemicarbazide

Phenyl-N-(2,6-dimethylphenyl)carbamate (0.05M, 12.05g) was dissolved in 100 ml of dichloromethane. To this solution, 4.85 ml of hydrazine hydrate (0.1M) was added and stirred at room temperature for 24 hr. The precipitate of 2,6-dimethylphenylsemicarbazide (**D-31**) was separated out, filtered, washed with dichloromethane and dried. m.p. 184 °C; IR (KBr) ν_{max} 3450, 3300, 2980, 1660, 1620, 1590-1540, 1360, 1310, 1210 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz, δ ppm) 2.24 (s, 6H, Ar-2CH_3), 5.46 (s, 2H, NH_2, D_2O exchangeable), 7.18-7.36 (m, 3H, ArH), 8.38 (s, 1H, ArNH, D_2O exchangeable), 9.52 (bs, 1H, $NHNH_2$, D_2O exchangeable).

Step-3: ***General procedure for the synthesis of 2,6-dimethylphenylsemicarbazones: -***

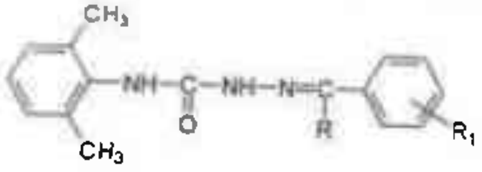


2,6-dimethylphenylsemicarbazide

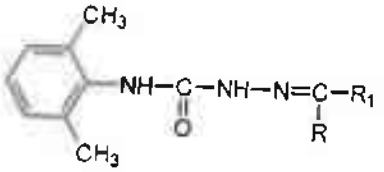
2,6-dimethylphenylsemicarbazones

To a solution of 2,6-dimethylphenylsemicarbazide (0.003M, 0.54g) in ethanol, an equimolar quantity of appropriate alkyl/aryl aldehydes or ketones was added and stirred for 1 hr to 3 hr until the completion of the reaction. The resultant precipitate was filtered, dried and recrystallised from 95% ethanol. The physical data of the compounds are presented Tables-IX.1 and IX.2. The IR spectra of the compounds were identical in the following aspects 3420-3200, 2950-2900, 1680-1660, 1620-1520, 1350, 1210, 840 cm^{-1}; ^1H-NMR (DMSO-d_6, 300 MHz, δ ppm) spectra of the some of the representative compounds are given in the Table-IX.3.

Table-IX.1: Physical data of the 2, 6-dimethylphenylsemicarbazones: -



D-(1-16)



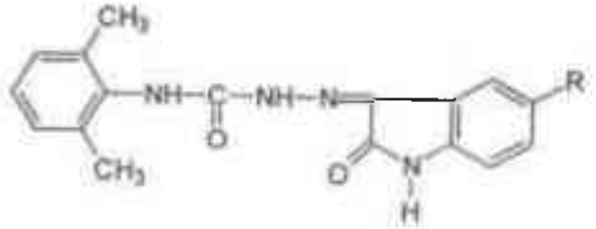
D-(17-25)

Compound No.	Substituents		Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
	R	R_1						
D-1	H	H	57	213	$C_{16}H_{17}N_3O$	267.33	0.82	2.57
D-2	H	4-CH_3	52	199	$C_{17}H_{19}N_3O$	281.36	0.80	2.75
D-3	H	4-OCH_3	62	201	$C_{17}H_{19}N_3O_2$	297.36	0.78	1.98
D-4	H	4-N-$(CH_3)_2$	64	209	$C_{18}H_{22}N_4O$	313.39	0.81	2.22
D-5	H	4-NO_2	85	256	$C_{16}H_{16}N_4O_3$	312.33	0.90	2.80
D-6	H	4-OH 3-OCH_3	77	236	$C_{17}H_{19}N_3O_3$	313.36	0.77	1.35
D-7	H	3-NO_2	83	224	$C_{16}H_{16}N_4O_3$	312.33	0.91	2.84
D-8	H	2-Cl	53	208	$C_{16}H_{16}N_3OCl$	301.78	0.85	2.90
D-9	H	2-OH	81	214	$C_{16}H_{17}N_3O_2$	283.33	0.81	2.22
D-10	H	2-NO_2	83	233	$C_{16}H_{16}N_4O_3$	312.33	0.93	3.02
D-11	CH_3	H	60	205	$C_{17}H_{19}N_3O$	281.36	0.79	2.55
D-12	CH_3	4-OH	50	200	$C_{17}H_{19}N_3O_2$	297.36	0.82	1.82
D-13	CH_3	4-NH_2	53	194	$C_{17}H_{20}N_4O$	296.37	0.76	2.08
D-14	CH_3	4-NO_2	64	256	$C_{17}H_{18}N_4O_3$	326.35	0.90	2.76
D-15	CH_3	3-NH_2	51	198	$C_{17}H_{20}N_4O$	296.37	0.77	1.92
D-16	CH_3	2-OH	52	230	$C_{17}H_{19}N_3O_2$	297.36	0.75	2.17
D-17	H	2-furfuryl	59	>270	$C_{14}H_{15}N_3O_2$	257.29	0.70	1.85
D-18	CH_3	CH_3	60	>270	$C_{12}H_{17}N_3O$	219.29	0.71	2.06
D-19	CH_3	CH_2CH_3	57	>270	$C_{13}H_{19}N_3O$	233.31	0.72	2.37
D-20	CH_3	$CH_2CH(CH_3)_2$	54	>270	$C_{15}H_{23}N_3O$	261.37	0.69	2.74
D-21	CH_3	CH_2COCH_3	56	250	$C_{14}H_{19}N_3O_2$	261.32	0.68	2.37
D-22	C_6H_5	C_6H_5	82	202	$C_{22}H_{21}N_3O$	343.43	0.88	2.52
D-23	$C_6H_5CH_2$	$C_6H_5CH_2$	90	205	$C_{24}H_{25}N_3O$	371.48	0.76	2.57
D-24	CRR_1=Cyclohexylene		58	>270	$C_{15}H_{21}N_3O$	259.35	0.82	2.44
D-25	CRR_1=Cyclopentylene		57	>270	$C_{14}H_{19}N_3O$	245.32	0.81	2.33

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

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

Table-IX.2: Physical data of the 2,6-dimethylphenylsemicarbazones: -



D-(26-30)

Compound No.	R	Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
D-26	H	77	190	$C_{17}H_{16}N_4O_2$	308.34	0.87	1.79
D-27	F	63	182	$C_{17}H_{15}N_4O_2F$	326.33	0.89	1.84
D-28	Cl	64	191	$C_{17}H_{15}N_4O_2Cl$	342.78	0.90	1.92
D-29	Br	60	222	$C_{17}H_{15}N_4O_2Br$	387.24	0.89	2.11
D-30	CH_3	66	183	$C_{18}H_{18}N_4O_2$	322.37	0.84	1.89

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

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

Table-IX.3: Spectral and Elemental analyses data of the compounds: -

Comp. No.	IR Spectroscopy (cm^{-1}, KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)*		
			C	H	N
D-2	3390, 2890, 1690, 1610, 1600-1525, 1330, 1230	2.14 (s, 6H, Ar-2CH_3), 2.32 (s, 3H, ArCH_3), 6.56-6.58 (d 2H, J=7.6 Hz), 7.04 (s, 3H, ArH), 7.23-7.27 (d, 2H, J=7.6 Hz) 8.02 (s, 1H, imine-H), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable).	72.57 72.47	6.81 6.79	14.93 14.91
D-4	3380, 2900, 1685, 1620, 1600-1535, 1320, 1210	1.82 (s, 6H, Ar-N(CH_3)$_2$), 2.16 (s, 6H, Ar-2CH_3), 6.54-6.56 (d, 2H, J=7.5 Hz) 7.07 (s, 3H, ArH) 7.18-7.21 (d, 2H, J=7.5 Hz), 8.20 (s, 1H, imine-H), 8.32 (s, 1H, ArNH, D_2O exchangeable), 9.62 (s, 1H, CONH, D_2O exchangeable)	69.65 69.57	7.14 7.13	18.05 18.04
D-5	3410, 2900, 1680, 1605, 1595-1540, 1430, 1315,1305, 1200	2.18 (s, 6H, Ar-2CH_3), 7.14 (s, 3H, ArH), 7.42-7.44 (d, 2H, J=7.5 Hz), 7.87 (s, 1H, imine-H), 8.13-8.16 (d, 2H, J=7.5 Hz), 8.57 (s, 1H, ArNH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable).	61.53 61.96	5.16 5.15	17.94 17.91
D-6	3400, 3210, 3090, 2885, 1665, 1600-1535, 1335, 1220, 1190	2.20 (s, 6H, 2-CH_3), 3.81 (s, 3H, OCH_3), 6.75-6.78 (d, 1H, J=7.5 Hz), 7.03-7.08 (m, 4H, Aryl-H), 7.48 (s, 1H, Aryl-H), 7.80 (s, 1H, imine-H), 8.41 (s, 1H, Aryl NH D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable), 10.34 (s, 1H, OH, D_2O exchangeable).	65.16 65.13	6.11 6.09	13.41 13.39
D-9	3420, 3300, 2900, 1660, 1610, 1600-1540, 1345, 1220	2.21 (s, 6H, 2-CH_3), 6.79-6.88 (m, 2H, Aryl-H), 7.08 (s, 3H, Aryl H), 7.16-7.21 (m, 1H, Aryl H), 7.91-7.94 (d, 1H, Aryl-H, J=7.5 Hz), 8.24 (s, 1H, imine-H), 8.42 (s, 1H, Aryl NH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable), 10.49 (s, 1H, OH, D_2O exchangeable).	67.83 67.69	6.05 6.04	14.83 14.80
D-12	3390, 3190, 2895, 1660, 1615, 1590-1540, 1320, 1230, 1205	2.14 (s, 3H, CH_3), 2.20 (s, 6H, 2-CH_3), 6.74-6.76 (d, 2H, J=7.6 Hz), 7.05 (s, 3H, Aryl-H), 7.63-7.65 (d, 2H, J=7.6 Hz), 8.30 (s, 1H, Aryl NH, D_2O exchangeable), 9.72 (s, 1H, CONH, D_2O exchangeable), 10.34 (s, 1H, OH, D_2O exchangeable).	68.67 68.52	6.44 6.43	14.13 14.12
D-13	3410, 3205, 3090, 2890, 1690, 1605, 1595-1535, 1330, 1160	2.14 (s, 3H, CH_3), 2.19 (s, 6H, 2-CH_3), 5.32 (s, 2H, NH_2, D_2O exchangeable), 6.52-6.54 (d, 2H, J=7.5 Hz), 7.07 (s, 3H, Aryl-H), 7.61-7.64 (d, 2H, J=7.5 Hz), 8.30 (s, 1H, Aryl-NH, D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable).	68.89 68.75	6.80 6.79	18.90 19.86
D-18	3400, 3100, 2850, 1680, 1590-1530, 1310, 1205	1.76 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.18 (s, 6H, 2-CH_3), 7.07-7.13 (t, 1H, J=7.5Hz), 7.20-7.22 (d, 2H, J=7.5 Hz), 8.42 (s, 1H, Aryl NH, D_2O exchangeable), 9.52 (s, 1H, CONH, D_2O exchangeable)	65.73 65.58	7.81 7.80	19.16 19.15
D-19	3395, 3100, 2860, 1685, 1605, 1595-1540, 1320, 1185	1.01-1.03 (t, 3H, CH_3, J=7.6 Hz), 2.14 (s, 3H, CH_3), 2.73-2.77 (q, 2H, J=7.6Hz), 2.20 (s, 6H, 2-CH_3), 7.11-7.14 (m, 3H, Aryl-H), 8.40 (s, 1H, Aryl NH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable).	66.92 66.78	8.21 8.19	18.01 17.92
D-21	3400, 2870, 1700, 1665, 1600-1535, 1300, 1190	1.64 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.12 (s, 2H, CH_2), 2.21 (s, 6H, 2-CH_3), 7.07-7.11 (m, 3H, Aryl-H), 8.38 (s, 1H, Aryl NH, D_2O) exchangeable), 9.64 (s, 1H, CONH, D_2O exchangeable).	64.34 64.29	7.33 7.32	16.08 16.06

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

Pharmacological activity: -

Table-IX.4: Anticonvulsant activity and minimal motor impairment of 2,6-dimethyl phenylsemicarbazones: -

Compound No.	Intraperitoneal Injection in mice[a]							
	MES Screen		scPTZ Screen		scSTY Screen		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	2.0h	0.5h	4.0h
D-1	100	100	300	-	30	100	300	-
D-2	100	300	300	-	100	100	-	300
D-3	100	300	300	-	30	100	300	-
D-4	100	300	300	-	100	100	300	-
D-5	100	300	300	-	30	100	300	-
D-6	100	300	300	-	30	100	-	-
D-7	100	300	300	-	30	100	300	-
D-8	100	300	300	-	30	100	300	-
D-9	100	300	300	-	30	100	-	-
D-10	300	300	300	-	100	-	300	-
D-11	100	300	300	-	30	100	-	-
D-12	100	300	300	-	30	100	-	-
D-13	100	300	300	-	30	100	300	-
D-14	300	300	300	-	100	300	300	-
D-15	100	300	-	-	100	300	300	-
D-16	100	300	300	-	100	300	-	-
D-17	100	300	300	-	100	300	300	-
D-18	100	300	300	-	100	100	300	-
D-19	100	300	300	-	30	100	-	-
D-20	100	300	-	-	30	100	-	-
D-21	100	300	300	-	100	300	300	-
D-22	100	300	300	-	300	-	300	-
D-23	300	300	-	-	300	-	300	-
D-24	100	300	-	-	100	300	300	-
D-25	100	300	300	-	-	-	300	-
D-26	100	300	-	-	30	100	300	-
D-27	100	300	300	-	30	100	300	-
D-28	100	300	300	-	30	100	300	-
D-29	100	300	-	-	30	100	300	-
D-30	100	300	300	-	30	100	300	-
D-31	100	300	300	-	×	×	300	-
Phenytoin	30	30	-	-	-	-	100	100
Carbamazepine	30	100	100	300	-	-	100	300

[a] Doses of 30, 100, 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. (For scSTY test 0.5h and 2.0h) were made. The line (-) indicates an absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg) and (×) indicates not tested.

Table-IX.3: Spectral and Elemental analyses data of the compounds: -

Comp. No.	IR Spectroscopy (cm^{-1}, KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)*		
			C	H	N
D-2	3390, 2890, 1690, 1610, 1600-1525, 1330, 1230	2.14 (s, 6H, Ar-2CH_3), 2.32 (s, 3H, ArCH_3), 6.56-6.58 (d 2H, J=7.6 Hz), 7.04 (s, 3H, ArH), 7.23-7.27 (d, 2H, J=7.6 Hz), 8.02 (s, 1H, imine-H), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable).	72.57 72.47	6.81 6.79	14.93 14.91
D-4	3380, 2900, 1685, 1620, 1600-1535, 1320, 1210	1.82 (s. 6H, Ar-N$(CH_3)_2$), 2.16 (s, 6H, Ar-2CH_3), 6.54-6.56 (d, 2H, J=7.5 Hz), 7.07 (s, 3H, ArH), 7.18-7.21 (d, 2H, J=7.5 Hz), 8.20 (s, 1H, imine-H), 8.32 (s, 1H, ArNH, D_2O exchangeable), 9.62 (s, 1H, CONH, D_2O exchangeable).	69.65 69.57	7.14 7.13	18.05 18.04
D-5	3410, 2900, 1680, 1605, 1595-1540, 1430, 1315, 1305, 1200	2.18 (s, 6H, Ar-2CH_3), 7.14 (s, 3H, ArH), 7.42-7.44 (d, 2H, J=7.5 Hz), 7.87 (s, 1H, imine-H), 8.13-8.16 (d, 2H, J=7.5 Hz), 8.57 (s, 1H, ArNH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable).	61.53 61.96	5.16 5.15	17.94 17.91
D-6	3400, 3210, 3090, 2885, 1665, 1600-1535, 1335, 1220, 1190	2.20 (s, 6H, 2-CH_3), 3.81 (s, 3H, OCH_3), 6.75-6.78 (d, 1H, J=7.5 Hz), 7.03-7.08 (m, 4H, Aryl-H), 7.48 (s, 1H, Aryl-H), 7.80 (s, 1H, imine-H), 8.41 (s, 1H, Aryl NH D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable). 10.34 (s, 1H, OH, D_2O exchangeable).	65.16 65.13	6.11 6.09	13.41 13.39
D-9	3420, 3300, 2900, 1660, 1610, 1600-1540, 1345, 1220	2.21 (s, 6H, 2-CH_3), 6.79-6.88 (m, 2H, Aryl-H), 7.08 (s, 3H, Aryl H), 7.16-7.21 (m, 1H, Aryl H), 7.91-7.94 (d, 1H, Aryl-H, J=7.5 Hz), 8.24 (s, 1H, imine-H), 8.42 (s, 1H, Aryl NH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable), 10.49 (s, 1H, OH, D_2O exchangeable).	67.83 67.69	6.05 6.04	14.83 14.80
D-12	3390, 3190, 2895, 1660, 1615, 1590-1540, 1320, 1230, 1205	2.14 (s, 3H, CH_3), 2.20 (s, 6H, 2-CH_3), 6.74-6.76 (d, 2H, J=7.6 Hz), 7.05 (s, 3H, Aryl-H), 7.63-7.65 (d, 2H, J=7.6 Hz), 8.30 (s, 1H, Aryl NH, D_2O exchangeable), 9.72 (s, 1H, CONH, D_2O exchangeable), 10.34 (s, 1H, OH, D_2O exchangeable).	68.67 68.52	6.44 6.43	14.13 14.12
D-13	3410, 3205, 3090, 2890, 1690, 1605, 1595-1535, 1330, 1160	2.14 (s, 3H, CH_3), 2.19 (s, 6H, 2-CH_3), 5.32 (s, 2H, NH_2, D_2O exchangeable), 6.52-6.54 (d, 2H, J=7.5 Hz), 7.07 (s, 3H, Aryl-H), 7.61-7.64 (d, 2H, J=7.5 Hz), 8.30 (s, 1H, Aryl-NH, D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable).	68.89 68.75	6.80 6.79	18.90 19.86
D-18	3400, 3100, 2850, 1680, 1590-1530, 1310, 1205	1.76 (s. 3H, CH_3), 1.85 (s, 3H, CH_3), 2.18 (s, 6H, 2-CH_3), 7.07-7.13 (t, 1H, J=7.5Hz), 7.20-7.22 (d, 2H, J=7.5 Hz), 8.42 (s, 1H, Aryl NH, D_2O exchangeable), 9.52 (s, 1H, CONH, D_2O exchangeable).	65.73 65.58	7.81 7.80	19.16 19.15
D-19	3395, 3100, 2860, 1685, 1605, 1595-1540, 1320, 1185	1.01-1.03 (t, 3H, CH_3, J=7.6 Hz), 2.14 (s, 3H, CH_3), 2.73-2.77 (q, 2H, J=7.6Hz), 2.20 (s, 6H, 2-CH_3), 7.11-7.14 (m, 3H, Aryl-H), 8.40 (s, 1H, Aryl NH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable).	66.92 66.78	8.21 8.19	18.01 17.92
D-21	3400, 2870, 1700, 1665, 1600-1535, 1300, 1190	1.64 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.12 (s, 2H, CH_3), 2.21 (s, 6H, 2-CH_3), 7.07-7.11 (m, 3H, Aryl-H), 8.38 (s, 1H, Aryl NH, D_2O) exchangeable), 9.64 (s, 1H, CONH, D_2O exchangeable).	64.34 64.29	7.33 7.32	16.08 16.06

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

Pharmacological activity: -

Table-IX.4: Anticonvulsant activity and minimal motor impairment of 2,6-dimethyl phenylsemicarbazones: -

Compound No.	Intraperitoneal Injection in mice[a]							
	MES Screen		scPTZ Screen		scSTY Screen		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	2.0h	0.5h	4.0h
D-1	100	100	300	-	30	100	300	-
D-2	100	300	300	-	100	100	-	300
D-3	100	300	300	-	30	100	300	-
D-4	100	300	300	-	100	100	300	-
D-5	100	300	300	-	30	100	300	-
D-6	100	300	300	-	30	100	-	-
D-7	100	300	300	-	30	100	300	-
D-8	100	300	300	-	30	100	300	-
D-9	100	300	300	-	30	100	-	-
D-10	300	300	300	-	100	-	300	-
D-11	100	300	300	-	30	100	-	-
D-12	100	300	300	-	30	100	-	-
D-13	100	300	300	-	30	100	300	-
D-14	300	300	300	-	100	300	300	-
D-15	100	300	-	-	100	300	300	-
D-16	100	300	300	-	100	300	-	-
D-17	100	300	300	-	100	300	300	-
D-18	100	300	300	-	100	100	300	-
D-19	100	300	300	-	30	100	-	-
D-20	100	300	-	-	30	100	-	-
D-21	100	300	300	-	100	300	300	-
D-22	100	300	300	-	300	-	300	-
D-23	300	300	-	-	300	-	300	-
D-24	100	300	-	-	100	300	300	-
D-25	100	300	300	-	-	-	300	-
D-26	100	300	-	-	30	100	300	-
D-27	100	300	300	-	30	100	300	-
D-28	100	300	300	-	30	100	300	-
D-29	100	300	-	-	30	100	300	-
D-30	100	300	300	-	30	100	300	-
D-31	100	300	300	-	×	×	300	-
Phenytoin	30	30	-	-	-	-	100	100
Carbamazepine	30	100	100	300	-	-	100	300

[a] Doses of 30, 100, 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. (For scSTY test 0.5h and 2.0h) were made. The line (-) indicates an absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg) and (×) indicates not tested.

Table-IX.5: Evaluation of 2,6-dimethylphenylsemicarbazones in the MES test after oral administration (30 mg/kg) to rats: -

Compound No	Oral administration to rats*				
	0.25h	0.5h	1.0h	2.0h	4.0h
D-1	1	3	3	3	4
D-2	0	2	3	4	3
D-3	1	4	3	3	3
D-4	1	2	2	3	4
D-5	1	1	2	1	1
D-6	3	3	4	3	4
D-7	1	1	3	1	3
D-8	2	1	1	1	4
D-9	1	3	3	4	4
D-10	2	2	2	2	2
D-11	1	2	4	3	3
D-12	1	2	4	3	4
D-13	1	3	4	2	4
D-14	1	2	2	3	1
D-15	1	3	1	2	3
D-16	3	1	2	3	4
D-17	0	2	1	4	3
D-18	3	4	4	3	2
D-19	1	4	4	3	3
D-20	1	2	2	4	2
D-21	2	4	2	4	4
D-22	1	1	1	1	0
D-23	0	1	1	0	0
D-24	1	1	2	4	3
D-25	2	2	1	3	1
D-26	2	2	1	1	3
D-27	1	1	1	1	3
D-28	1	1	0	1	1
D-29	1	1	0	2	0
D-30	1	1	3	3	3
D-31	0	0	1	3	2
Phenytoin	1	4	3	3	3

* The figures in the screen indicate the number of rats out of four which were protected.

Compound No.	MES Screen					Neurotoxicity Screen					PI[a] (MES)
	t	ED_{50} mg/kg	95% CI	Slope	SE	t[b]	TD_{50} mg/kg	95% CI	Slope	SE	
D-2	2	33.19	20.18-54.78	3.84	1.34	0.25-24	>120	-	-	-	>03.62
D-9	4	19.85	13.04-29.03	3.98	1.11	0.25-24	>500	-	-	-	>25.19
D-13	4	29.14	18.32-44.44	3.29	1.00	0.25-24	>464	-	-	-	>15.92
D-19	4	29.05	15.66-51.08	2.48	0.81	0.25-24	>500	-	-	-	>17.21
D-21	2	19.89	11.75-30.82	2.25	0.60	0.25-24	>216	-	-	-	>10.86
Phenytoin	2	23.2	21.5-25.4	15.1	4.28	0.25-24	>500	-	-	-	>21.60

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}.

[b] The compound was examined 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration.

Table-IX.7: Quantification study of 2,6-dimethylphenylsemicarbazones in the MES and Neurotoxicity test after intraperitoneal injection to rats: -

Compound No.	MES Screen					Neurotoxicity Screen					PI[a] (MES)
	t	ED_{50} mg/kg	95% CI	Slope	SE	t	TD_{50} mg/kg	95% CI	Slope	SE	
D-4	2.0	18.33	7.41-34.01	2.01	0.69	0.25-24[b]	>250	-	-	-	>13.64
D-6	1.0	26.22	17.9-35.06	3.83	1.08	1	>117	-	-	-	>4.46
D-7	1.0	29.77	20.76-37.74	5.68	1.80	0.25-6	>120	-	-	-	>4.03
D-10	0.5	23.26	14.36-31.15	4.09	1.27	0.25-6	>100	-	-	-	>4.30
D-12	0.25	41.64	22.33-61.77	3.04	0.94	0.25-24[b]	>68	-	-	-	>1.63
Phenytoin	2.0	6.48	5.65-7.24	12.4	3.60	0.5	42.8	-	-	-	6.60

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}.

[b] The compound was examined 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration.

Table-IX.10: Behavioral study of 2,6-dimethylphenylsemicarbazones using Actophotometer: -

Compound No.[a]	Activity Score[b]		
	Control (24h before)	Post Treatment	
		0.5h after	1.0h after
D-1	228.67 ± 27.39	154.17 ± 19.63	146.00 ± 18.23
D-2	268.74 ± 25.54	175.67 ± 16.61	133.50 ± 14.62
D-3	231.75 ± 16.39	177.50 ± 09.72	166.17 ± 09.28
D-4	245.50 ± 14.95	147.11 ± 08.13	124.50 ± 08.06
D-5	255.33 ± 28.03	158.00 ± 13.68	159.83 ± 07.53
D-6	238.78 ± 16.83	157.32 ± 12.67	130.78 ± 16.05
D-7	202.23 ± 25.71	129.23 ± 14.39	131.50 ± 10.65
D-8	274.17 ± 17.09	236.17 ± 17.21	166.17 ± 13.38
D-9	217.00 ± 19.41	155.50 ± 14.35	135.23 ± 12.55
D-10	281.50 ± 28.36	177.00 ± 21.24	152.33 ± 17.46
D-11	323.11 ± 23.08	138.67 ± 09.76	117.75 ± 10.57
D-12	260.23 ± 25.98	205.75 ± 11.07	198.67 ± 12.55
D-13	258.50 ± 26.55	202.83 ± 08.49	187.17 ± 05.31
D-14	252.17 ± 16.92	171.50 ± 08.31	117.23 ± 06.28
D-15	212.25 ± 19.58	149.67 ± 07.97	137.25 ± 06.49
D-16	208.75 ± 20.10	135.83 ± 16.90	106.25 ± 17.75
D-17	202.67 ± 18.71	150.23 ± 11.16	107.32 ± 09.52
D-18	227.00 ± 20.05	159.33 ± 11.89	148.67 ± 16.98
D-19	224.32 ± 20.82	188.00 ± 12.13	195.50 ± 18.48
D-20	256.33 ± 20.12	182.78 ± 07.92	161.23 ± 07.63
D-21	214.00 ± 26.97	127.23 ± 12.24	144.25 ± 15.25
D-22	246.75 ± 16.73	162.50 ± 18.43	134.32 ± 13.73
D-23	202.83 ± 25.19	146.23 ± 12.02	121.50 ± 10.79
D-24	308.17 ± 31.49	207.67 ± 20.88	148.17 ± 09.06
D-25	290.11 ± 29.79	138.23 ± 09.03	143.11 ± 13.07
D-26	270.32 ± 20.96	224.17 ± 16.77 NS	217.50 ± 17.60 NS
D-27	224.23 ± 27.13	204.32 ± 16.57 NS	194.83 ± 19.68 NS
D-28	280.83 ± 31.45	263.23 ± 17.06 NS	267.00 ± 21.49 NS
D-29	201.33 ± 17.71	172.23 ± 16.11 NS	176.50 ± 12.09 NS
D-30	372.67 ± 28.22	355.50 ± 20.67 NS	368.67 ± 24.35 NS
Phenytoin	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

[a] The compounds were tested at a dose of 30mg/kg (i.p).

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

Table-IX.5: Evaluation of 2,6-dimethylphenylsemicarbazones in the MES test after oral administration (30 mg/kg) to rats: -

Compound No	Oral administration to rats[a]				
	0.25h	0.5h	1.0h	2.0h	4.0h
D-1	1	3	3	3	4
D-2	0	2	3	4	3
D-3	1	4	3	3	3
D-4	1	2	2	3	4
D-5	1	1	2	1	1
D-6	3	3	4	3	4
D-7	1	1	3	1	3
D-8	2	1	1	1	4
D-9	1	3	3	4	4
D-10	2	2	2	2	2
D-11	1	2	4	3	3
D-12	1	2	4	3	4
D-13	1	3	4	2	4
D-14	1	2	2	3	1
D-15	1	3	1	2	3
D-16	3	1	2	3	4
D-17	0	2	1	4	3
D-18	3	4	4	3	2
D-19	1	4	4	3	3
D-20	1	2	2	4	2
D-21	2	4	2	4	4
D-22	1	1	1	1	0
D-23	0	1	1	0	0
D-24	1	1	2	4	3
D-25	2	2	1	3	1
D-26	2	2	1	1	3
D-27	1	1	1	1	3
D-28	1	1	0	1	1
D-29	1	1	0	2	0
D-30	1	1	3	3	3
D-31	0	0	1	3	2
Phenytoin	1	4	3	3	3

[a] The figures in the screen indicate the number of rats out of four which were protected.

Compound No.	MES Screen					Neurotoxicity Screen					PI[a] (MES)
	t	ED_{50} mg/kg	95% CI	Slope	SE	t[b]	TD_{50} mg/kg	95% CI	Slope	SE	
D-2	2	33.19	20.18-54.78	3.84	1.34	0.25-24	>120	-	-	-	>03.62
D-9	4	19.85	13.04-29.03	3.98	1.11	0.25-24	>500	-	-	-	>25.19
D-13	4	29.14	18.32-44.44	3.29	1.00	0.25-24	>464	-	-	-	>15.92
D-19	4	29.05	15.66-51.08	2.48	0.81	0.25-24	>500	-	-	-	>17.21
D-21	2	19.89	11.75-30.82	2.25	0.60	0.25-24	>216	-	-	-	>10.86
Phenytoin	2	23.2	21.5-25.4	15.1	4.28	0.25-24	>500	-	-	-	>21.60

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}.

[b] The compound was examined 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration.

Table-IX.7: Quantification study of 2,6-dimethylphenylsemicarbazones in the MES and Neurotoxicity test after intraperitoneal injection to rats: -

Compound No.	MES Screen					Neurotoxicity Screen					PI[a] (MES)
	t	ED_{50} mg/kg	95% CI	Slope	SE	t	TD_{50} mg/kg	95% CI	Slope	SE	
D-4	2.0	18.33	7.41-34.01	2.01	0.69	0.25-24[b]	>250	-	-	-	>13.64
D-6	1.0	26.22	17.9-35.06	3.83	1.08	1	>117	-	-	-	>4.46
D-7	1.0	29.77	20.76-37.74	5.68	1.80	0.25-6	>120	-	-	-	>4.03
D-10	0.5	23.26	14.36-31.15	4.09	1.27	0.25-6	>100	-	-	-	>4.30
D-12	0.25	41.64	22.33-61.77	3.04	0.94	0.25-24[b]	>68	-	-	-	>1.63
Phenytoin	2.0	6.48	5.65-7.24	12.4	3.60	0.5	42.8	-	-	-	6.60

[a] PI indicates the protection index, i.e. TD_{50}/ED_{50}.

[b] The compound was examined 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after administration.

Table-IX.8: Anticonvulsant activity of some selected compounds against scPIC seizure threshold test after intraperitoneal injection into mice: -

Compound No.	Intraperitoneal Injection in mice[a]	
	scPIC Screen	Neurotoxicity Screen
D-9	30	-
D-12	30	-
D-13	30	300
D-21	30	300
Phenytoin	-	100
Phenobarbital	30	100

[a] Doses of 30 and 100 mg were administered. The figures in the table indicate the minimum dose whereby bioactivity was determined. The line (-) indicates no activity at the maximum dose of 100mg/kg.

Table-IX.9: Hippocampal kindling screen: -

Compound No.	Animal	Dose mg/kg	Seizure Score		ADD[a] (sec)	
			Predrug	Drug	Predrug	Drug
D-7	Rat # 1	100	5	1	26-42	52
	Rat # 2		4-5	5	11-39	59
D-9	Rat # 1	130	5	1	26-39	59
	Rat # 2		5	1	30-39	25

[a] ADD = After discharge duration (time of maximum effect : 45 min). Result suggests ability to prevent/modify fully kindled seizures.

Table-IX.10: Behavioral study of 2,6-dimethylphenylsemicarbazones using Actophotometer: -

Compound No.[a]	Activity Score[b]		
	Control (24h before)	Post Treatment	
		0.5h after	1.0h after
D-1	228.67 ± 27.39	154.17 ± 19.63	146.00 ± 18.23
D-2	268.74 ± 25.54	175.67 ± 16.61	133.50 ± 14.62
D-3	231.75 ± 16.39	177.50 ± 09.72	166.17 ± 09.28
D-4	245.50 ± 14.95	147.11 ± 08.13	124.50 ± 08.06
D-5	255.33 ± 28.03	158.00 ± 13.68	159.83 ± 07.53
D-6	238.78 ± 16.83	157.32 ± 12.67	130.78 ± 16.05
D-7	202.23 ± 25.71	129.23 ± 14.39	131.50 ± 10.65
D-8	274.17 ± 17.09	236.17 ± 17.21	166.17 ± 13.38
D-9	217.00 ± 19.41	155.50 ± 14.35	135.23 ± 12.55
D-10	281.50 ± 28.36	177.00 ± 21.24	152.33 ± 17.46
D-11	323.11 ± 23.08	138.67 ± 09.76	117.75 ± 10.57
D-12	260.23 ± 25.98	205.75 ± 11.07	198.67 ± 12.55
D-13	258.50 ± 26.55	202.83 ± 08.49	187.17 ± 05.31
D-14	252.17 ± 16.92	171.50 ± 08.31	117.23 ± 06.28
D-15	212.25 ± 19.58	149.67 ± 07.97	137.25 ± 06.49
D-16	208.75 ± 20.10	135.83 ± 16.90	106.25 ± 17.75
D-17	202.67 ± 18.71	150.23 ± 11.16	107.32 ± 09.52
D-18	227.00 ± 20.05	159.33 ± 11.89	148.67 ± 16.98
D-19	224.32 ± 20.82	188.00 ± 12.13	195.50 ± 18.48
D-20	256.33 ± 20.12	182.78 ± 07.92	161.23 ± 07.63
D-21	214.00 ± 26.97	127.23 ± 12.24	144.25 ± 15.25
D-22	246.75 ± 16.73	162.50 ± 18.43	134.32 ± 13.73
D-23	202.83 ± 25.19	146.23 ± 12.02	121.50 ± 10.79
D-24	308.17 ± 31.49	207.67 ± 20.88	148.17 ± 09.06
D-25	290.11 ± 29.79	138.23 ± 09.03	143.11 ± 13.07
D-26	270.32 ± 20.96	224.17 ± 16.77 NS	217.50 ± 17.60 NS
D-27	224.23 ± 27.13	204.32 ± 16.57 NS	194.83 ± 19.68 NS
D-28	280.83 ± 31.45	263.23 ± 17.06 NS	267.00 ± 21.49 NS
D-29	201.33 ± 17.71	172.23 ± 16.11 NS	176.50 ± 12.09 NS
D-30	372.67 ± 28.22	355.50 ± 20.67 NS	368.67 ± 24.35 NS
Phenytoin	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

[a] The compounds were tested at a dose of 30mg/kg (i.p).

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

Table-IX.11: CNS study on selected compounds in Porsolt's swim test: -

Compound No.[a]	Immobility time[b] (sec)	
	Control (24 h prior)	Post treatment (60 min after)
PEG 400	164.67 ± 11.69	168.53 ± 12.32 NS
D-2	146.33 ± 21.37	225.66 ± 07.88
D-4	182.00 ± 14.26	207.00 ± 08.54 NS
D-6	143.23 ± 16.78	207.32 ± 10.14
D-7	173.33 ± 15.93	231.00 ± 11.15
D-9	190.25 ± 16.55	191.25 ± 10.17 NS
D-10	132.00 ± 15.71	190.50 ± 13.06
D-12	159.00 ± 11.59	223.16 ± 12.00
D-13	184.25 ± 17.23	204.50 ± 15.17 NS
D-19	176.66 ± 22.69	199.33 ± 11.63 NS
D-21	164.25 ± 21.53	198.75 ± 17.03 NS
Carbamazepine	131.50 ± 09.32	207.33 ± 08.49

[a] The compounds were tested at a dose of 30mg/kg (i.p).

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

Table-IX.12: Evaluation of compounds for the potentiation or antagonism of pentobarbitone induced narcosis: -

Compound No.[a]	Mean sleeping time[c] (min)
Pentobarbital[b]	54.25 ± 15.73
D-2	150.50 ± 19.50
D-4	-[d]
D-6	68.42 ± 12.61 NS
D-7	147.00 ± 13.00
D-9	-[d]
D-10	141.00 ± 17.50
D-12	108.00 ± 15.71
D-13	-[d]
D-19	61.33 ± 14.90 NS
D-21	-[d]

[a] Compounds were tested at a dose of 30mg/kg (i.p).

[b] Compound was tested at a dose of 40mg/kg (i.p).

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

[d] Reversal of pentobarbitone induced narcosis.

Table-IX.13: Effects of 2,6-dimethylphenylsemicarbazones on serum levels of transaminases in six rats: -

Compound No.[a]	SGPT[c] (units /ml)	SGOT[c] (units/ml)
Control	45.00 ± 6.24	73.17 ± 5.98
D-2	47.39 ± 3.19	70.28 ± 4.78
D-9	43.33 ± 4.81	64.83 ± 5.30
D-13	44.67 ± 6.57	71.33 ± 4.06
D-19	49.32 ± 4.32	79.63 ± 5.71
Phenytoin[b]	52.67 ± 3.48	84.16 ± 6.50

The compounds were tested at a dose of 30mg/kg (p.o) for the treatment of 14 days.

[b] Tested at 25mg/kg (p.o) for the treatment of 14 days.

[c] Each value represents the mean ± SEM of six rats, not significantly different from the control value at $p < 0.05$ (Student's t-test).

Table-IX.14: Effect of compounds on GABA system (2hr study): -

Compound No.[a]	Concentration of GABA (µg/100mg of tissue)[b]
	Whole rat brain
Control	48.44 ± 5.05
D-2	90.26 ± 4.30
D-4	75.58 ± 6.18**
D-6	91.24 ± 7.67*
D-7	79.84 ± 5.37*
D-9	92.16 ± 8.75**
D-10	84.76 ± 6.39**
D-12	81.18 ± 7.61**
D-13	100.46 ± 8.31*
D-19	87.37 ± 9.90**
D-21	83.35 ± 9.35**
Clobazam[c]	101.17 ± 6.77

[a] The compounds were tested at a dose of 30 mg/kg (i.p).

[b] Each value represents the mean ± SEM of six rats, significantly different from the control at $p < 0.001$, $*p < 0.004$ and $**p < 0.01$ (Student's t-test).

[c] **Tested at 30 mg/kg (i.p).**

Table-IX.15: Effect of compounds on GABA system (7 days study): -

Compound No[a]	Concentration of GABA (µg/100mg of tissue)[b]
	Whole rat brain
Control	50.59 ± 2.68
D-2	100.43 ± 3.73
D-4	68.17 ± 4.77*
D-6	106.27 ± 3.68
D-7	87.97 ± 6.87
D-9	110.43 ± 2.73
D-10	81.65 ± 1.88
D-12	93.65 ± 4.52
D-13	112.32 ± 3.29
D-19	101.86 ± 5.14
D-21	93.55 ± 2.51
Clobazam[c]	110.67 ± 4.83

[a] The compounds were tested at a dose of 30 mg/kg (p.o) for 7 days.

[b] Each value represents the mean ± SEM of six rats, significantly different from the control at *$p < 0.008$ and remaining values are significantly different from the control at $p < 0.001$ (Student's t-test).

[c] Tested at 30 mg/kg (p.o).

Results & Discussion: -

Chemistry: -

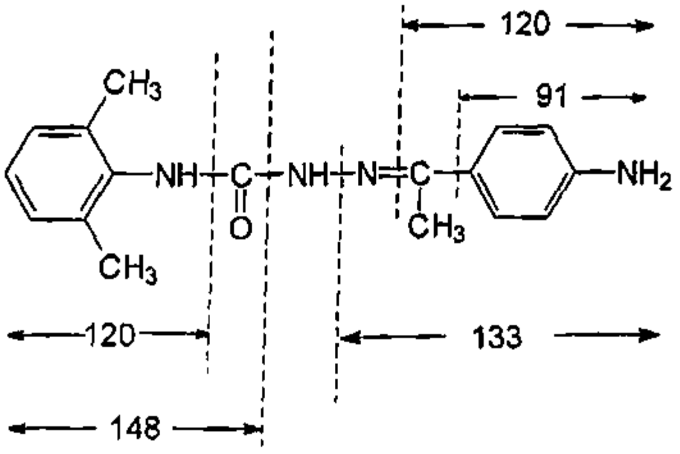
The required 2,6-dimethylphenylsemicarbazones were prepared by a three-step process starting from 2,6-dimethylaniline. The homogeneity of the compounds was monitored by thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were $CHCl_3$: CH_3OH (9:1).

All the compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 (log $P > 2$) except for compounds **D-6, 12, 15, 17, 26-28** and **30**. These compounds had log $P < 2$ which were all substituted with polar groups like OH, NH_2 and isatin derivatives (Tables-IX.1 and IX.2). With regard to percentage yield of the synthesized compounds, the compounds with substituted aryl aldehydes have yielded in the range of 62-84% and those with substituted aryl/alkyl ketones and isatins gave 50-60% and 50-77% respectively.

The IR spectrum of N^1-(2,6-dimethylphenyl)-N^4-[1-(4-aminophenyl)ethan-1-one] semicarbazone (**D-13**) was recorded in KBr pellet and the following bands (v_{max} cm^{-1}) were observed. Absorption band at 3410 and 3205 cm^{-1} showed NH stretching and 3090 cm^{-1} (Ar CH str); 2890 cm^{-1} (alk CH str) ; 1690 cm^{-1} (C=O str); 1605 cm^{-1} (C=N str); 1595-1535 cm^{-1} (C=C str); 1330 cm^{-1} (Ar C-N str); 1190 cm^{-1} (Alk C-N str) were observed.

^{1}H-NMR spectrum of compound **D-13** {N^1-(2,6-dimethylphenyl)-N^4-[1-(4-aminophenyl) ethan-1-one]semicarbazone}revealed a singlet at δ 2.14 for 3H of imino CH_3 protons; singlet at δ 2.19 for 6H of Ar-2CH_3 protons; singlet at δ 5.32 for 2H for NH_2 which was D_2O exchangeable; doublets at δ 6.52-6.54 and 7.61-7.64 for 2H of carbimino ArH protons of 2,6 and 3,5 respectively; singlet at δ 7.07 for 3H of ArH of 2,6 dimethyl phenyl ring; singlets at δ 8.30 and 9.34 for 1H of ArNH and CONH respectively which were D_2O exchangeable (Table-IX.3).

The mass spectrum of compound **D-13** showed a molecular ion peak at m/z 296, base peak at m/z 148. The remaining major fragmentation peaks were at m/z 133, 120 and 91.



Pharmacological activity: -

The anticonvulsant activity of the synthesized compounds was established after their i.p administration in three seizure models in mice *viz.* MES, scPTZ and scSTY screens. The animals were dosed with 30, 100 and 300 mg/kg body weight of the test drugs and anticonvulsant activity was examined at 0.5h and 4.0h and scSTY screen at 0.5h and 2.0h, after the injections. The minimum dose whereby bioactivity was demonstrated in half or more of the mice is presented in Table-IX.4, along with the data of Phenytoin and Ethosuximide.

(i) **MES test: -**

All the synthesized compounds showed activity at 0.5h in the MES screen at a dose of 100 mg/kg except compounds **D-10**, **14** and **23**, which were active at a dose of 300 mg/kg, indicative of their ability to prevent seizure spread. In the 4.0h period, most of the compounds showed activity at a dose of 300 mg/kg except compound **D-1**, which showed activity at 100 mg/kg. Hence, these compounds showed a quick onset and longer duration of action.

(ii) **scPTZ test: -**

In this screen, all the compounds showed activity at the maximum dose of 300 mg/kg at 0.5h period except compounds **D-15**, **20**, **23**, **24**, **26** and **29**, which did not exhibit protective activity. This test is used to identify the compounds that elevate the seizure threshold. These compounds possessed quick onset and short duration of action.

(iii) scSTY test: -

In this series, all the synthesized compounds were examined for their ability to prevent seizure induced by scSTY model. The results of this evaluation are presented in Table-IX.4. All the synthesized compounds showed potent activity in this model at a dose of 30 mg/kg at 0.5h except compounds **D-10, 14-18, 21** and **22-23**, which were active at 100 mg/kg and 300 mg/kg respectively. So these results indicated that the compounds have an ability to prevent seizure spread and possessed quick onset of action. At 2.0h period most of the compounds showed activity at 100 mg/kg except **D-14-17, 21** and **24** that were active at 300 mg/kg. The compounds **D-10, 22, 23** and **25** did not show protective activity at 2.0h period even at the maximum dose administered (300 mg/kg).

(iv) scPIC test: -

Four semicarbazones of this series were examined for their ability to prevent seizures induced by picrotoxin, an epileptic model that relates the activity with GABA-mediation. The result of this evaluation is presented in Table-IX.8. All the four compounds showed protection at 30 mg/kg. During the 45-minute observation, the animals did not show clonic convulsion continuously for 5 seconds. This indicated that the compounds have the ability to increase the threshold level of convulsion and also these compounds might be acting through inhibitory neurotransmitter pathway like GABA.

Neurotoxicity Screen: -

In the NT screen, all the compounds showed neurotoxicity at 0.5h but no toxicity was observed at 4.0h even at the maximum dose of 300 mg/kg administered, except compound **D-2** which showed neurotoxicity at 300 mg/kg at 4.0h which might be due to its metabolite or due to delayed absorption of the compound. The compounds **D-2, 6, 9, 11, 12, 19** and **20**, did not show neurotoxicity at the maximum dose administered. (Table-IX.4).

Rat p.o. Identification: -

All the compounds that was active in the i.p. anticonvulsant screen were selected for activity in the oral screen in the MES and neurotoxicity tests. The results are presented in Table-IX.5. The compounds were tested at 30 mg/kg in which most of the compounds showed protection equal to or more than the standard drug Phenytoin and exhibited anti-MES protection throughout the period i.e. up to 24 h.

Out of the thirty-one compounds, **D-5, 7, 10** and **14** showed lesser activity when compared to Phenytoin. All these four compounds showed 25-50% of protection in all the time intervals. In these compounds, the carbimino phenyl ring was substituted with nitro group. This was consistent with the results obtained in the i.p. screen. Compound **D-22** showed 25% protection at 0.5, 1 and 2h and compound **D-23** showed 25% protective activity only at 0.5 and 1.0h. In both these compounds (**D-22** and **D-23**), the carbimino hydrogen was substituted with bulky group like phenyl and benzyl respectively. This indicated that the bulkier groups caused a reduction in activity due to either steric effect or poor absorption. The compounds substituted with isatins (**D-(26-30)**) showed 25-50% activity at 0.25, 0.5 1 & 2h and 75% protection at 4h, except compound **D-30** which showed 75% protection at 1, 2 and 4h.

The compound **D-31** (2,6-dimethyl phenyl semicarbazide) showed activity at 1, 2 and 4h in the range of 25-75% protection. At 0.25h and 0.5h this compound had shown no activity. This indicated that this compound did not have rapid onset of action.

Compounds **D-6** and **D-18** showed 100% protection in all time periods i.e. 0.25, 0.5, 1, 2 and 4h. But the standard drug Phenytoin showed 100% protection only at 0.5h and at 0.25h it showed 25% protection. Hence the compounds **D-6** and **D-18** have quick onset of action when compared to Phenytoin and also showed 75-100% protection in the entire time interval, which was similar to Phenytoin. So these two compounds have better properties than Phenytoin because these showed both quick onset and longer duration of action. Compounds **D-1-4, 9, 11-13, 16, 17** and **19-21** showed protection similar to standard drug Phenytoin.

Quantitative Evaluation of 2,6-dimethylphenylsemicarbazones: -

More complete data were obtained from the quantitative MES evaluation in mice and rats dosed intraperitoneally and orally, respectively. Results of the quantitative test for selected compounds, along with the data on standard drug phenytoin are reported in tables IX.6 and IX.7.

The compounds that were active in the preliminary anticonvulsant screening were quantified for the anticonvulsant and toxic effects. The ED_{50} values were determined against MES induced convulsion and TD_{50} values were measured by the rotorod procedure for evaluating neurological deficit.

The compounds **D-2, 9, 13, 19** and **21** were administered orally to rats. The ED_{50} values for these compounds in the MES test were 33.19, 19.85, 29.14, 29.05 and 19.89 mg/kg respectively. The TD_{50} figures were > 120, >500, >464, >500 and >216 mg/kg respectively. The quantitation of representative compounds have been undertaken in rats in order to get a precise indication of their potencies and to compare their activities with that of existing drugs, and to evaluate the protective index (PI) values of the compounds. Phenytoin had an ED_{50} value of 23.2 mg/kg in the MES test in rats after p.o. administration. The compounds **D-9** and **D-21** exhibited greater potency than Phenytoin. The PI value for phenytoin was > 21.6. Only compound **D-9** showed more potency than standard drug phenytoin in which the PI was > 25.19 and Time of peak effect (TPE) was 4h, which indicated, that this compound has longer duration of action. The remaining compounds **D-13, 19** and **21** have shown PI nearly around 11-17 and compound **D-2** showed PI of 4.

The TPE (Time of peak effect) determination revealed that the compounds **D-10** and **D-12** had shown TPE at 0.5h and 0.25h respectively. Other compounds **D-(4, 6** and **7)** exhibited TPE at 2h, 1h and 1h respectively.

Compounds **D-4, 6, 7, 10** and **12** were administered intraperitoneally to rats. The ED_{50} values for those compounds in the MES test were 18.33, 26.22, 29.77, 23.26 and 41.64 mg/kg respectively. The TD_{50} figures were >250, >117, >120, >100 and >68 mg/kg respectively. Phenytoin, drug used in treating generalized tonic-clonic seizures, had an ED_{50} value in the MES in rats after i.p injection of 6.48 mg/kg. The PI value for phenytoin was 6.60: hence compared with this data, the compound **D-4** showed more potency than standard drug phenytoin in which the PI was > 13.64. Other compounds **D-4, 6** and 7 were equipotent to Phenytoin whose PI ranged from 4-5. The compound **D-12** showed PI > 1.63 indicating that its therapeutic window was very narrow. In the mice i.p. MES screen, among the selected compounds **D-(4, 6, 7, 10,** and **12)**, compound **D-4** gave an ED_{50} of 18.33 mg/kg and TD_{50} greater than 250 mg/kg, resulting in a high protection index (PI), i.e.TD_{50}/ED_{50}, of >13.64 compared to clinically used drug. The time to peak effect for compound **D-4** was found to be 2h similar to Phenytoin. With an ED_{50} of 41.64 mg/kg and a TD_{50} >68, compound **D-12** was less active and toxic than other compounds

Kindling experiment: -

Compounds **D-7** and **D-9** were examined in the hippocampal kindling test in rats and results are summarized in Table-IX.9. Significant reduction in the seizure scores of 80% occurred after 0.75 h, which suggests ability to prevent/modify fully kindled seizures. This screen provides a good model for focal seizures as well as a means of studying complex brain networks that may contribute to seizure spread and generalization from a focus (124).

CNS depressant Evaluation: -

All the compounds were examined for CNS depressant activity, which included locomotor activity using actophotometer, Porsolt's swim test and pentobarbital induced narcosis since many current frontline antiepileptic drugs were known to exhibit CNS depression side effect (39).

In the behavioral study using actophotometer all the synthesized compounds were found to decrease the behavior of the animals except compounds **D-26-30** which did not show any behavior despair effect. These compounds substituted with isatin derivatives generally act as MAO inhibitors and hence act as antidepressants (Table-IX.10).

In a similar study using Porsolt's swim test, the immobility time after the administration of the test compounds were compared with Carbamazepine. The compounds **D-4, 9, 13, 19** and **21** were found to show no significant CNS depression compared with the control at $p<0.05$. All other compounds **D-2, 6, 7, 10** and **12** tested were found to emerge as CNS depressants as they increased the immobility time. The compounds that showed depression mainly had NO_2 substitution in the carbimino aryl ring (Table-IX.11).

Some selected compounds of 2,6-dimethyl phenyl semicarbazones were examined for barbiturate hypnosis potentiation activity at a dose of 30 mg/kg i.p. The control animals lost the righting reflex and slept for 54.25 min. Compounds **D-4, 9, 13** and **21** did not induce sleep. Compounds **D-6** and **19** did not show any effect when compared to control animals. But the compounds **D-2, 7, 10** and **12** exhibit sedation. The mean sleeping time for the compounds are given in Table-IX.12.

Hepatotoxicity studies: -

The effect of some selected 2,6-dimethyl phenyl semicarbazones on the serum levels of transaminases (SGOT and SGPT) were determined as some drugs like Phenytoin, Phenobarbital and Sodium Valproate exhibited liver toxicity (149, 155)

The serum SGOT and SGPT levels in animals administered with vehicle (PEG 400, Control) were found to be 73.17 ± 5.98 Units/ml and 45.0 ± 6.24 Units/ml, respectively. All the screened compounds did not show any significant increase in the SGOT and SGPT levels when compared to control animals (Table-IX.13).

Determination of the levels of GABA in the whole rat brain: -

In order to explore the mechanism of anticonvulsant activity, some selected compounds that were highly active were subjected to neurochemical investigation to study their effects on the levels of GABA in the whole rat brain. The study was conducted in groups of six animals, at two time points.

In the first set of study the brain was removed after 2h administration of the drug, which was given by i.p. route. In another set of animals, the drug was given by oral route for 7 days and their brain was removed after 4 hr of the 7th day administration of drug. The GABA level were measured in the whole brain which were compared with control animals.

All the compounds subjected to neurochemical study were found to increase the GABA level significantly when compared to control animals at $p<0.05$ in both set of animals (Tables-IX.14 and IX.15). From this result, it can be concluded that the 2,6-dimethylphenyl semicarbazones act as anticonvulsants by influencing GABA mediated mechanism.

Structure Activity Relationship: -

Compound **D-1** possessed broad spectrum anticonvulsant activity against MES, scPTZ and scSTY models.

Table-IX.1 and IX.2 lists thirty N^1-(2,6-dimethylphenyl)-N^4-(substituted benzaldehyde/acetophenone/benzophenone)semicarbazones. The unsubstituted derivative, **D-1** showed no activity at 30 mg/kg but robust anticonvulsant effect at 100 mg/kg. Series of 2-, 3- and 4- substituted phenyl analogues [**D-(2-10, 12-16)**] were prepared with the substituents chosen to examine the effects of electron donating and withdrawing groups as well as changes in the bulk and lipophilicity [**D**-(**17**-**30**)].

All the compounds showed anticonvulsant activity in the preliminary screening of mice i.p model including both MES and scPTZ test.

According to rat oral study

(i) Compounds substituted with electron withdrawing groups [**D**-(**5**, **7**, **8**, **10** and **14**)] showed lesser anticonvulsant activity compared to compounds with electron donating groups.

(ii) Compounds substituted with electron withdrawing groups showed 25-50% of protection in the MES test but the compounds bearing electron donating groups showed 50-100% of protection at all time points (0.25, 0.5, 1, 2, 4, 6, 8 and 24h).

(iii) Replacement of carbimino hydrogen atom by bulky groups like phenyl (**D-22**) and benzyl (**D-23**) group exhibited loss of anticonvulsant activity.

(iv) Similarly, the carbimino phenyl ring when replaced with isatins [**D**-(**26**-**30**)] and cyclopentyl group (**D-25**) showed diminished anticonvulsant activity.

(v) Replacement of carbimino phenyl ring with small lipophilic alkyl moieties [**D**-(**18**-**21**)] showed enhancement of anticonvulsant activity.

(vi) With regard to onset of action, most of the compounds showed 50-75% protection only at 0.5h or 1.0h period except compounds **D-6**, **16** and **19**, (Vanillyl, 2-hydroxyl phenyl and ethylmethyl groups) which showed 75% protection at 0.25h. So these 3 compounds had a quick onset of action.

(vii) Compounds other than **D-5**, **7**, **8**, **10**, **14**, **25** and **26**-**30** (nitro group and isatinyl group) showed longer duration of action i.e. most of these compounds showed 75-100% protection till 4h period.

(viii) Quantification study of 2,6-dimethylphenylsemicarbazones revealed that the compounds **D-9** and **D-21** (2-hydroxyphenyl and acetylacetone groups) showed more potency than the standard drug phenytoin. ED_{50} values of compounds of **D-9** and **D-21** are 19.85 and 19.89 mg/kg respectively against phenytoin ED_{50} value 23.2 mg/kg. The compound **D-9** exhibited PI value (>25.19), which is more than standard drug phenytoin (21.6). It indicated that the compound exhibited a wide therapeutic window.

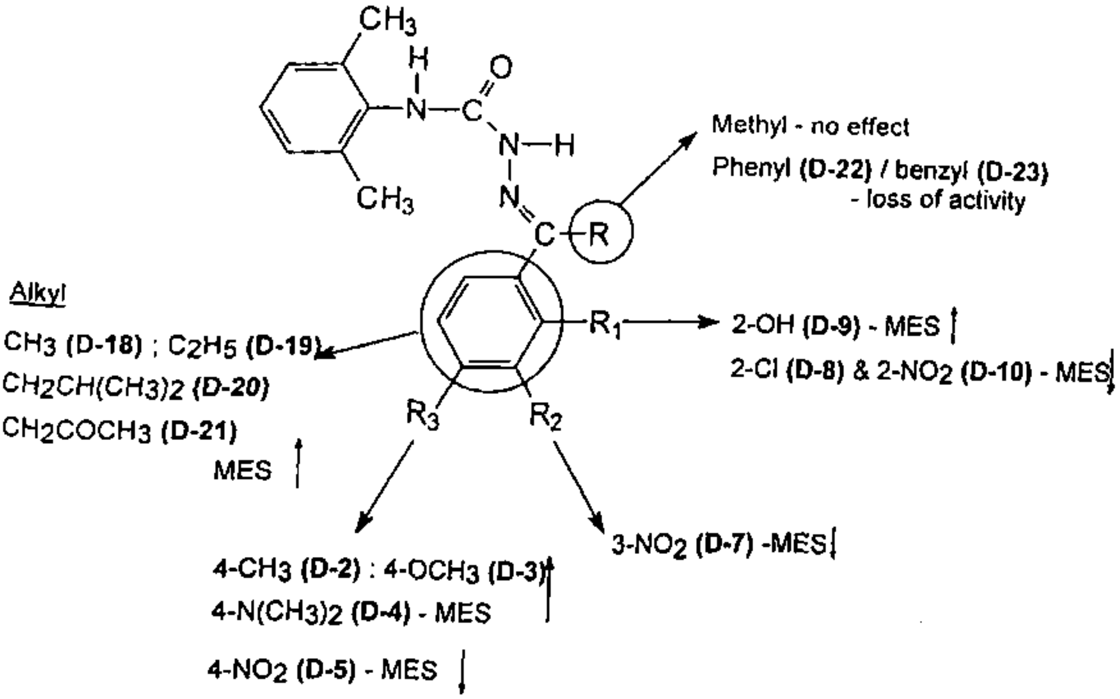
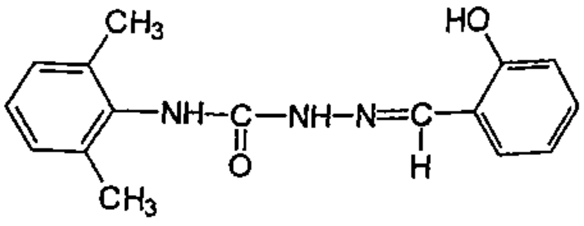


Figure IX.1. Schematic representation of SAR of 2,6-dimethylphenylsemicarbazones.

The above results revealed that 2,6-dimethylphenylsemicarbazones possessed broad spectrum of anticonvulsant activity with lesser neurotoxicity. In the 2,6-dimethylphenylsemicarbazone series, compound **D-9** emerged as the most active compound with an ED_{50} and TD_{50} of 19.85 and >500 mg/kg respectively.

N^1-(2,6-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde)semicarbazone (D-9)



MES (p.o): ED_{50} **19.85** mg / kg
PI = **> 25.19**

scPTZ : 100% protection at 300 mg / kg

scSTY : 100% protection at 30 mg / kg

scPIC : 100% protection at 30 mg / kg

Kindling : Active in Hippocampal kindling test.

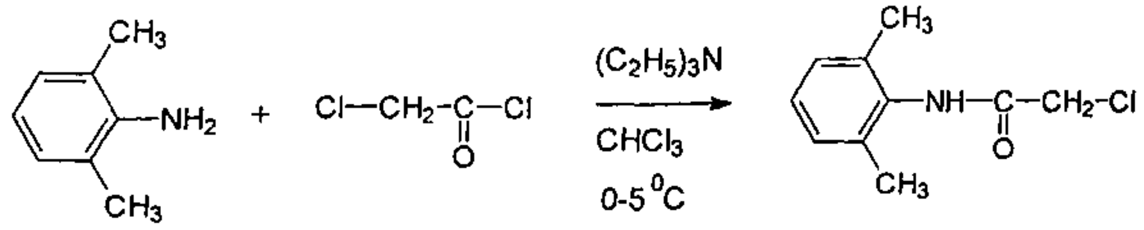
Toxicity : No hepatotoxicity & Neurotoxicity

GABA : Increased the level of GABA in the rat brain by 118% compared to control.

The effect of hydrogen bonding on anticonvulsant activity has been studied by modification of the semicarbazone moiety by introducing CH_2 group.

Synthesis: -

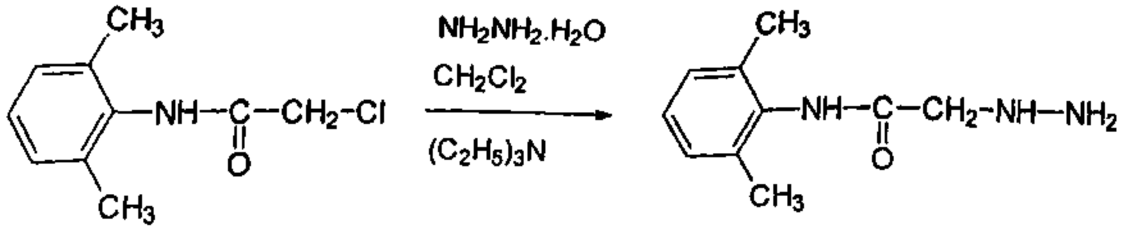
Step-1: ***Synthesis of (2,6-dimethyl)chloroacetanilide: -***



2,6-dimethylaniline Chloroacetylchloride (2,6-dimethyl)chloroacetanilide

Equimolar quantities of 2,6-dimethylaniline (0.1M, 12.35 ml) and triethylamine (0.1M, 13.9 ml) were dissolved in 40ml of chloroform and stirred in the magnetic stirred under cold condition (0-5°C). Chloro acetyl chloride (0.1 M, 8.0 ml) was dissolved in 20 ml of chloroform and added to the above solution slowly while maintaining the temperature (0-5°C). The stirring was continued for half an hour and 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, dried and analyzed. m.p 141°C, IR (KBr) ν_{max} 3440, 3100, 2860, 1665, 1600, 1540, 1300, 1210 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz, δ ppm) 2.26 (s, 6H, Ar-$2CH_3$), 4.26 (s, 2H, CH_2), 7.12-7.46 (m, 3H, ArH), 8.72 (s, 1H, ArNH, D_2O exchangeable).

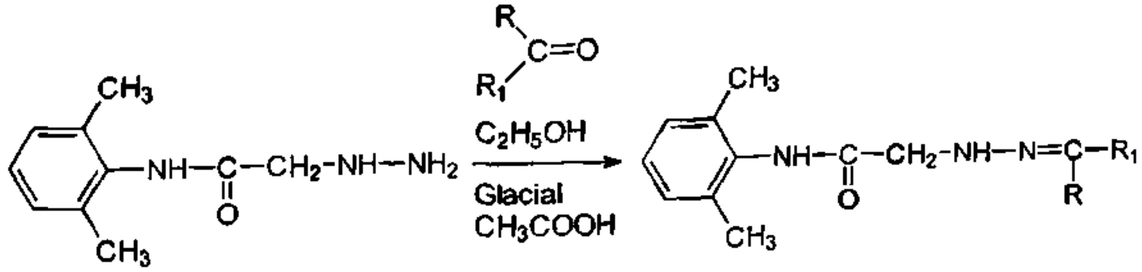
Step-2: ***Synthesis of 2,6-dimethylhydrazinoacetanilide: -***



(2,6-dimethyl)chloroacetanilide 2,6-dimethylhydrazinoacetanilide

Equimolar quantities of (2,6-dimethyl)chloroacetanilide (0.05M, 9.88g) and triethylamine (0.05M, 6.95 ml) were dissolved in 80ml of dichloromethane and stirred with heating. To this solution, double the quantity of hydrazine hydrate (0.1M, 4.85ml) was added and continued the stirring with heating. Then the reaction mixture was concentrated to one-third volume and kept in room temperature and the precipitate appeared immediately. The resultant precipitate was recrystallised from 95% ethanol, m.p. 193°C, IR (KBr) ν_{max} 3420, 3100, 2860, 1670, 1600, 1535, 1305, 1200 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300 MHz, δ ppm) 2.24 (s, 6H, Ar-2CH$_3$), 3.41 (s, 2H, CH$_2$), 5.16 (s, 2H, NH$_2$, D$_2$O exchangeable), 5.24 (bs, 1H, NHNH$_2$, D$_2$O exchangeable), 6.82-7.06 (m, 3H, ArH), 8.26 (s, 1H, ArNH, D$_2$O exchangeable).

Step-3: ***General procedure for the synthesis of 2,6-dimethylhydrazonoacetanilides: -***

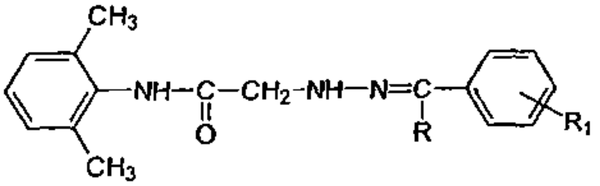


2,6-Dimethylhydrazinoacetanilide

2,6-Dimethylhydrazonoacetanilides

To a solution of 2,6-dimethylhydrazinoacetanilide (0.03M, 0.58g) in ethanol was added an equimolar quantity of appropriate aldehyde or ketone in ethanol. The mixture was stirred with heating for 30 min to 4 hr until the completion of the reaction. The resultant precipitate was filtered, dried and recrystallised from 95% ethanol. The physical data of the compounds are presented in Table-X.1. The IR spectra of the compounds were identical in the following aspects 3420-3380, 3100-3060, 3000-2850, 1690-1670, 1600-1540, 1305, 1210-1190 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$, 300MHz, δ ppm) spectra and elemental analyses of the two compounds are given in the Table-X.2.

Table-X.1: - Physical data of the 2,6-dimethylhydrazonoacetanilides: -



E-(1-12)

Compound No.	Substituents		Yield (%)	m.p. (°C)	Molecular Formula[a]	Mol. Weight	R_f	Log P[b]
	R	R_1						
E-1	H	4-CH_3	58	166	$C_{18}H_{21}N_3O$	295.38	0.72	2.49
E-2	H	4-NO_2	56	>270	$C_{17}H_{18}N_4O_3$	326.35	0.84	2.55
E-3	H	4-OH 3-OCH_3	67	189	$C_{18}H_{21}N_3O_3$	327.38	0.76	1.11
E-4	H	3-Cl	59	136	$C_{17}H_{18}N_3OCl$	315.80	0.78	2.43
E-5	H	3-NO_2	51	187	$C_{17}H_{18}N_4O_3$	326.35	0.80	2.52
E-6	H	2-Cl	52	139	$C_{17}H_{18}N_3OCl$	315.80	0.73	2.33
E-7	H	2-OH	59	215	$C_{17}H_{19}N_3O_2$	297.36	0.67	1.59
E-8	H	2-NO_2	57	198	$C_{17}H_{18}N_4O_3$	326.35	0.70	2.52
E-9	CH_3	4-CH_3	52	155	$C_{19}H_{23}N_3O$	309.41	0.64	2.50
E-10	CH_3	4-OH	53	208	$C_{18}H_{21}N_3O_2$	311.38	0.74	1.77
E-11	CH_3	4-NO_2	60	201	$C_{18}H_{20}N_4O_3$	340.38	0.82	2.50
E-12	C_6H_5	4-Br	59	164	$C_{23}H_{22}N_3OBr$	436.35	0.81	2.25

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

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

Table-X.2: Spectral and elemental analyses data of the compounds: -

Compound No.	IR Spectroscopy (cm^{-1}, KBr)	^{1}H-NMR (δ ppm, DMSO-d_6)	Elemental Analyses (Calculated/Found)*		
			C	H	N
E-1	3420, 3100, 2910, 1700, 1602, 1595-1520, 1300, 1205	2.18 (s, 6H, Ar-2CH$_3$), 2.24 (s, 3H, ArCH$_3$), 3.39 (s, 2H, CH$_2$), 5.18 (s, 1H, CH$_2$NH, D$_2$O exchangeable), 6.97 (s, 1H, imine H), 6.72-7.06 (m, 7H, ArH), 8.18 (s, 1H, ArNH, D$_2$O exchangeable).	72.57 72.29	6.81 6.78	14.94 14.89
E-11	3440, 3090, 2890, 1710, 1610, 1600-1525, 1310, 1200	2.14 (s, 3H, CH$_3$), 2.20 (s, 6H, Ar-2CH$_3$), 3.37 (s, 2H, CH$_2$), 5.20 (s, 1H, CH$_2$NH, D$_2$O exchangeable), 6.84-7.21 (m, 7H, ArH), 8.34 (s, 1H, ArNH, D$_2$O, exchangeable).	62.57 62.34	5.56 5.54	17.17 17.11

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

Pharmacological activity: -

Table-X.3: Anticonvulsant activity and minimal motor impairment of 2,6-dimethyl hydrazonoacetanilides: -

Compound No.[a]	Intraperitoneal Injection in mice[b]					
	MES Screen		scPTZ Screen		Neurotoxicity Screen	
	0.5h	4.0h	0.5h	4.0h	0.5h	4.0h
E-1	-	-	-	-	-	-
E-2	-	-	-	-	-	-
E-3	-	-	-	-	-	-
E-4	-	-	-	-	-	-
E-5	-	-	-	-	-	-
E-6	-	-	-	-	-	-
E-7	-	-	-	-	-	-
E-8	-	-	-	-	-	-
E-9	-	-	-	-	-	300
E-10	-	-	-	-	100	-
E-11	-	-	-	-		-
Phenytoin	30	30	-	-	100	100
Ethosuximide	-	-	100	300	-	-

[a] Doses of 30, 100 and 300 mg/kg were administered.

[b] 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 an absence of activity at maximum dose administered.

Results & Discussion: -

Chemistry: -

Out of four series of compounds, 2,6-dimethylphenylsemicarbazone derivatives exhibited potent anticonvulsant activity when compared to other series. So we planed to modify the semicarbazone moiety of 2,6-dimethylphenylsemicarbazone by introducing CH_2 group to 2,6-dimethylhydrazonoacetanilide.

The required 2,6-dimethylhydrazonoacetanilides were prepared by a three-step process in which 2,6-dimethylaniline was taken as the starting material. In this series eleven compounds were synthesized and the homogeneity of the compounds was monitored by thin layer chromatography (TLC) by which R_f values were calculated. Eluent for all compounds were $CHCl_3$: CH_3OH (9:1) (Table-X.1).

All the compounds were found to be more lipophilic indicated by their calculated partition coefficient value greater than 2 (log P>2) except for compounds **E-3**, **6** and **7**. These compounds had log P<2 which were all substituted with polar group like OH in the imine phenyl ring.

With regard to percentage yield of the synthesized compounds, most of the compounds yielded in the range of 50-60% except compound **E-3**, which was yielded 67%.

The IR spectrum of the N^1-(2,6-dimethylacetanilido)-N^2-(4-methylbenzaldehyde)hydrazone (**E-1**) was recorded in KBr pellet and the following bands (ν_{max} cm^{-1}) were observed. Absorption band at 3420 cm^{-1} showed NH stretching, 3100 cm^{-1} showed aryl CH stretching, 2910 cm^{-1} showed alkyl-CH stretching, 1700 cm^{-1} showed C=O stretching, 1602 cm^{-1} showed C=N stretching, 1595-1520 cm^{-1} showed aryl C=C stretching, 1300 cm^{-1} showed aryl C-N stretching.

^{1}H-NMR spectrum of compound **E-1** revealed singlets at δ 2.14 for 6H of aryl 2,6-CH_3 protons, and at δ 2.24 for 3H for aryl CH_3 protons, singlet at δ 6.97 for 1H of imine proton, singlets at δ 5.18 (1H, CH_2NH) and δ 8.18 (1H, ArNH) which were D_2O exchangeable and multiplet at δ 6.72-7.06 for 7H of ArH. Similarly, the spectrum of other compounds was confirmed according to their characteristic peaks depicted in Table-X.2.

Pharmacological activity: -

Stage I screening consisted of administering doses of 30, 100 and 300 mg/kg of the compounds in this series **E-1-11** to mice by i.p route and examination in the MES and scPTZ screens occurred and the neurotoxicity test.

All the eleven compounds exhibited neither anticonvulsant activity nor neurotoxicity except for **E-9** and **E-10**. The compound **E-9** (4-methylphenyl group) showed neurotoxicity at a dose of 300 mg/kg at 4.0h. Compound **E-10** (4-hydroxyphenyl group) showed neurotoxicity at 0.5h at a dose of 100 mg/kg (Table-X.3).

Structure Activity Relationship: -

Table-X.1 showed the lists of eleven compounds of 2,6-dimethylhydrazonoacetanilides, in which none of the compounds showed anticonvulsant activity. It indicated that the arylsemicarbazone moiety is essential for anticonvulsant activity.

(i) If the number of spacer atom was increased from 5 to 6 it showed a loss of anticonvulsant activity.

(2) Substitution in the phenyl ring did not influence the anticonvulsant activity but hydrogen bonding area (semicarbazono group) played a major role in the anticonvulsant activity.

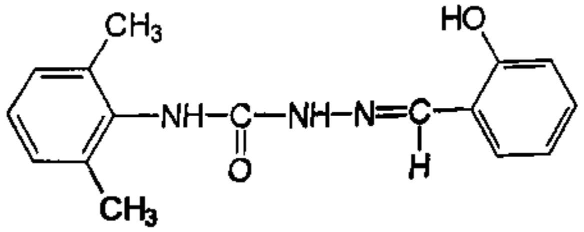
From the above result, it could be concluded that increase in the chain length of the linker atom (hydrogen bonding area) causes loss of anticonvulsant activity.

SUMMARY AND CONCLUSION

- Totally 5 series of 121 compounds in which 4 series (**A-D**) of disubstituted phenyl semicarbazones and 1 series (**E**-series) of disubstituted hydrazono acetanilides with different substituents in the carbimino phenyl ring and different alkyl groups in the carbimino carbon atom were designed and synthesized.
- Series **A** and **B** like 3-chloro 2-methyl phenyl semicarbazones and 2,4-dimethyl phenyl semicarbazones were prepared via urea formation.
- Series **C** and **D** like 2,4-dimethoxy phenyl semicarbazones and 2,6-dimethyl phenyl semicarbazones were prepared via phenyl carbamate formation.
- The series **E** (2,6-dimethyl hydrazono acetanilides) was prepared via chloroacetyl chloride formation.
- The purity of the compounds was ascertained by TLC and elemental analyses and their structures were elucidated by spectral data.
- Synthesized compounds were screened for anticonvulsant activity, which included MES, scPTZ, scSTY and scPIC test.
- In the MES screen, the activity was estimated after i.p and p.o administration of tested compounds.
- Quantification studies for 10 compounds of 2,6-dimethyl phenyl semicarbazones were carried out. Among the 10 compounds, 2 compounds (**D-4** and **D-9**) were more potent than standard drug phenytoin with respect to protective index (PI).
- With regard to toxicity studies, most of the compounds had exhibited lesser neurotoxicity or no neurotoxicity.
- CNS depressant activity studies were carried out in mice by using actophotometer and Porsolt's swim test.
- In the Porsolt's swim test, most of the compounds showed slight CNS depressant effect except some compounds of 2,6-dimethylphenylsemicarbazones, which did not show any CNS depressant effect.

- **Hepatoxicity studies of some selected compounds showed that these compounds were not hepatotoxic after chronic administration.**
- Neurochemical studies of most active compounds were performed in the rat brain. The results showed that there was a significant increase in the level of GABA in the midbrain region.
- The results from the above screening procedure showed that the compounds have a broad spectrum of anticonvulsant activity.
- **Structure activity relationship studies on the disubstituted phenyl semicarbazones suggest that the carbiminophenyl group with alkyl groups showed broad spectrum of anticonvulsant activity. All other modifications were found to show difference in activity with respect to different disubstituted phenyl semicarbazones.**
- Among all the synthesized compounds, the compound D-9 [N^1-(2,6-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde)semicarbazone] has emerged as the most active lead compound as it showed promising results viz broad spectrum anticonvulsant activity with less neurotoxicity and no hepatotoxicity. The compound ED_{50} value was 19.85 mg/kg and PI is >25.17 that are more than standard drug Phenytoin.

N^1-(2,6-dimethyl phenyl)-N^4-(2-hydroxybenzaldehyde)semicarbazone (D-9)



MES : ED_{50} **19.85 mg / kg ; PI = > 25.19**

scPTZ : **100% protection at 300 mg / kg at 0.5 hr period**

scSTY : **100% protection at 30 mg / kg at 0.5 & 4.0 hr period**

scPIC : 100% protection at 30 mg / kg at 0.75 hr period

Kindling: Active in Hippocampal kindling test.

Toxicity: No hepatotoxicity & Neurotoxicity

GABA : Increased the GABA level in the rat brain by 118% compared to control.

FUTURE PERSPECTIVES

Thus potentially active compounds of disubstituted phenyl semicarbazones with broad spectrum anticonvulsant activity were successfully synthesized. However, further toxicological screening of these compounds has to be carried out and more attention has to be paid in the lines of developing a suitable formulation of these synthesized compounds. Extensive pharmacodynamic and pharmacokinetic studies of the safer compounds (from toxicological screening) will have to be undertaken in various animal models. Further, the feasibility, cost effectiveness and reproducibility of synthesizing these compounds in bulk has to be tried, so as to convert these compounds in to life saving drugs for the betterment of mankind.

****

1. Loscher, W.; Eur. J. Pharmacol., **342**, 1-13, 1998.
2. Wasterlain, C. in Siegel, G., Agranoff, G., Albers, R.W., Molinoff, P., Eds., Basic Neurochemistry : Molecular, Cellular and Medical Aspects, 4th ed., Raven Press, New York, 1989, pp. 797-810.
3. Rall, T.W., Schleifer, L.S., in Goodman, A.G., Rall, T.W., Nies, A.S., Taylor, P., Eds., The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, New York, 1990, pp 436-462.
4. Delgado-Escueta, A.V., Ward, Jr. A.A., Woodbury, D.M., Porter, R.J., Eds., Basic Mechanisms of the epilepsies, Advances in Neurology, Vol. 44, Raven Press, New York, 1986, pp 3-55.
5. Wasterlain, C.G., Morin, A.M., Dwyer, B.E., in Lajtha, A., Eds., Handbook of Neurochemistry, Vol. 10, Raven Press, New York, 1985, pp 407-409.
6. Fukuzako, H., Izumi, K., in Tunnicliff, G., Raess, B.U., Eds., GABA mechanisms in Epilepsy, Wiley-Liss, Inc., New York, 1991, pp 1-30.
7. Commission on Classification and Terminology of the International League Against Epilepsy; Epilepsia, **26**, 268-278,1985.
8. Porter, R.J., Rogaswski, M.A.; Epilepsia, **33**, (Suppl. 1), S1-S6, 1992.
9. Gupta, Y.K., Malhotra, J.; Indian J. Physiol. Pharmacol., **44** (1), 8-23, 2000.
10. Coatsworth, J.J.; Studies on the Clinical Efficacy of Marketed Antiepileptic Drugs, NINDS monograph No. 12, HEW publication No. (NIH) 73-15. U.S. Government Printing Office, 1971.
11. Leppik, I.E.; Epilepsy Res., (Suppl 5), 7-11, 1992.
12. Salazar, A.M., Jabbari, B., Vance, S.C., et al.; Neurology, **35**, 1406-14, 1985.
13. Marks, D.A., Kim, J., Spencer, D.D., et.al; Neurology, **42**, 1513-18, 1992.
14. Marks, D.A., Kim, J., Spencer, D.D., et.al; Neurology, **45**, 2051-57, 1995.
15. Temkin, N.R.; Epilepsia, **42**, 515-24, 2001.
16. DeLorenzo, R.J.; Epilepsy Res., **4**, 3-17, 1991.
17. Hauser, W.A., Annegers, J.F., Kurland, L.T.; Epilepsia, **32**, 429-45, 1991.
18. Kwan, P., Brodie, M.J.; Epilepsia, **42**, 1255-1260, 2001.

19. Kwan, P., Sills, G.J., Brodie, M.J.; Pharmacol. Ther., **90**, 21-34, 2001.

20. Yogeeswari, P., Vaigunda Raghavendran, J., Thirumurugan, R., Saxena, A., Sriram, D.; Curr. Drug Targets, **5**, 553-568, 2004.

21 Meldrum, B.S.; Epilepsia, **37** (Suppl. 6), S 4-11, 1996.

22. Pellock, J.M.; Epilepsia, **35**, (Suppl. 4), S 11, 1994.

23. Shin, C., McNamara, J.O.; Am. Rev. Med., **45**, 379, 1994.

24. Macdonald, R.L., Kelly, K.M.; Epilepsia, **35**, (Suppl. 4), S 41, 1994.

25. Lilly Research Laboratories, "Phenobarbital", in Physician's Desk reference, Sifton, D.W., Eds., Medical Economics Data Production Company, Montvale, NJ, 1994, pp. 1255.

26. Maltson, R.H., Cramer, J.A., "Phenobarbital"; Toxicity in Antiepileptic Drugs, Woodbury, D.M., Penry, J.K., Pippenger, C.E., Eds., Raven Press, New York, 1982, pp. 851-863.

27. Hooper, W.D., Eadie, M.J., "Mephobarbital" in the Medical treatment of Epilepsy; Resor, Jr., S.R., Kutt, H., Eds., Marcel Dekker, New York, 1992, pp. 363-369.

28. Parke-Davis, "Dilantin" (prescription drug labeling), in physician's desk reference, Sifton, D.W., Eds., Medical Economics Data Production Company, Montvale, NJ, 1994, pp. 1730-1735.

29. Dam, M., "Phenytoin : Toxicity", in Antiepileptic drugs, Woodbury, D.M., Penry, J.K., Pippenger, C.E., Eds., Raven Press, New York, 1982, pp. 247-256.

30. Dreifuss, F.E., "Ethosuximide : Toxicity", in Antiepileptic Drugs, Woodbury, D.M., Penry, J.K., Pippenger, C.E., Eds., Raven Press, New York, 1982, pp. 647-653.

31. Sherwin, A.L., "Ethosuximide", in The Treatment of Epilepsy: Principles and Practices, Wyllie, E., Eds., Lea and Febiger, Philadelphia, 1993, pp. 923-929.

32. Homan, R.W., Rosenberg, H.C., "Benzodiazepines", in The Treatment of Epilepsy : Principles and Practices, Wyllie, E., Eds., Lea and Febiger, Philadelphia, 1993, pp. 932-944.

33. Kutt, H., "Diazepam", in the Medical Treatment of Epilepsy, Resor, Jr, S.R., Kutt, H., Eds., Marcel Dekker, New York, 1992, pp. 341-343.

34. Abbot Laboratories, "Depakene" (Prescription drug labeling), in Physician's Desk reference, Sifton, D.W., Eds., Medical Economics Data Production Company, Montvale, NJ, 1994, pp. 411-415.
35. Dean, J.C., "Valproate", in The Treatment of Epilepsy: Principles and Practices, Wyllie, E., Eds., Lea and Febiger, Philadelphia, 1993, pp. 915-921.
36. Ramsay, R.E.; Neurology, **44**, (Suppl. 5) S 23-26, 1994.
37. Matsuo, F., Bergen, D., Faught, E., Messenhermin, J.A., Dren, A.T., Rudd, G.D., Line berry, C.G.; Neurology, **43**, 2284-2288, 1993.
38. Sander, J.W., Hart, Y.M., Trimble, M.O., Shorvon, S.D.; Epilepsia, **82**, (Suppl.1), 12-17, 1992.
39. Sachdev, R., Leroy, R., Krauss, G., Green, P., Drake, M., Leppik, I., Shu, V., Ringhani, G., Sommerville, K.; Neurology, **45**, (Suppl. 4), 202-207, 1995.
40. Mattson, R.H.; Neurology, **44**, 54-59, 1994.
41. Gupta, Y.K., Malhotra, J.; J. Physiol. Pharmacol., **41**, 329-343, 1997.
42. Malhotra, J.; Drugs: News and Views, **2**, 73-74, 1994.
43. Dixon, R.H., Williams, H.L.; Federation Proc., **21**, 338-339, 1962.
44. Biel, J.H., Horita, A., Drukker, A.E., in "Psychopharmacological Agent", Vol. 1, Gorden, M., Eds., Academic, New York, N.Y., 1964, 359-360.
45. Prockop, D.J., Shore, P.A., Broidie, B.B.; Ann. N.Y. Acad. Sci., **80**, 643-653, 1959.
46. Prockop, D.J., Shore, P.A., Broidie, B.B.; Experientia, **15**, 145-149, 1959.
47. Agarwal, V.K., Gupta, T.K., Parmar, S.S.; J. Med. Chem., **15**, 1000-1001, 1972.
48. Wood, J.D., Peesker, S.J.; Can. J. Physiol. Pharmacol., **49**, 780-781, 1971.
49. Wood, J.D., Peesker, S.J., Urton, J.I.M.; Can. J. Physiol. Pharmacol., **50**, 1217-1218, 1972.
50. Parmar, S.S., Chaturvedi, A.K., Chaudhari, A., Misra, R.S.; J. Pharm. Sci., **61**, 78-81, 1972.
51. Parmar, S.S., Misra, R.S., Chaudhari, A., Gupta, T.K.; J. Pharm. Sci., **61**, 1322-1324, 1972.

52. Wood, J.D., Peesker, S.J.; Can. J. Physiol. Pharmacol., **51**, 859-875, 1973.

53. Parmar, S.S., Gupta, A.K., Gupta, T.K., Stenberg, V.I.; J. Pharm. Sci., **64**, 154-157, 1975.

54. Wood, J.D., Gorecki, D.K., Dimmock, J.R., Hawes, E.M.; Can. J. Physiol. Pharmacol., **53**, 47-55, 1975.

55. Soliman, R., Gabr, M., Abouzeit-Har, M.S., Sharabi, F.M.; J. Pharm. Sci., **70**, 94-96, 1981.

56. Chapleo, C.B., Myers, M., Myers, P.L., Saville, J.F., Smith, A.C.B., Stilling, M.R., Tulloch, I.F., Walter, D.S., Welbourn, A.P.; J. Med. Chem., **29**, 2273-2280, 1986.

57. Lightcap, E.S., Silverman, R.B.; J. Med. Chem., **89**, 686-694, 1996.

58. Dimmock, J.R., Vashishtha, S.C., Stables, J.P.; Eur. J. Med. Chem., **35**, 241-248, 2000.

59. Pandeya, S.N., Agarwal, A.K., Singh, A., Stables, J.P.; Acta Pharm., **53**, 15-24, 2003.

60. Biel, J.H., Horita, A., Drukker, A.E., in "Psychopharmacological Agent", Vol. 1, Gorden, M., Eds., Academic, New York, N.Y., 1964, 414-416.

61. Carter, L.A.; J. Med. Chem., **10**, 925-936, 1967.

62. Durant, G.J. Wright, S.H.B.; J. Med. Chem., **9**, 247-248, 1966.

63. Coyne, W.E., Cusic, J.W.; J. Med. Chem., **11**, 1158-1160, 1968.

64. Parmar, S.S., Joshi, P.C., Ali, B., Cornatzer, W.E.; J. Pharm. Sci., **63**, 872-875, 1974.

65. Dwivedi, C., Harbison, R.D., Ali, B., Parmar, S.S.; J. Pharm. Sci., **63**, 1124-1128, 1974.

66. Singh, S.P., Ali, B., Auyong, T.K., Parmar, S.S., Boer, B.D.; J. Pharm. Sci., **65**, 391-396, 1976.

67. Sengupta, A.K., Agarwal, K.C.; Ind. J. Chem., **17B**, 184-185, 1979.

68. Dimmock, J.R., Smith, D.C., Brenner, J.M., Jonnalagadda, S.S., Sardessai, M.S., Wood, J.D., Bigam, G.E.; Eur. J. Med. Chem., **21**, 187-192, 1986.

69. Dimmock, J.R., Jonnalagadda, S.S., Hussein, S., Tewari, S., Quail, J.W., Reid, R.S., Delbaere, L.T.J., Prasad, L.; Eur. J. Med. Chem., **25**, 581-588, 1990.

70. Dimmock, J.R., McColl, J.N., Wonko, S.L., Thayer, R.S., Hancock, D.S.; Eur. J. Med. Chem., **26**, 529-534, 1991.

71. Dimmock, J.R., Sidhu, K.K., Thayer, R.S., Mack, P., Duffy, M.J., Reid, R.S., Quail, J.W., Pugazhenthi, U., Ong, A., Bikker, J.A., Weaver, D.F.; J. Med. Chem., **36**, 2243-2252, 1993.

72. Dimmock, J.R., Baker, G.B.; Epilepsia, **35**, 648-655, 1994.

73. Dimmock, J.R., Pandeya, S.N., Quail, J.W., Pugazhenthi, U., Allen, T.M., Kao, G.Y., Balzarini, J., clercq, E.De.; Eur. J. Med. Chem., **30**, 303-314, 1995.

74. Dimmock, J.R., Sidhu, K.K., Tumber, S.D., Basran, S.K., Chen, M., Quail, J.W., Yang, J., Rozas, I., Weaver, D.F.; Eur. J. Med. Chem., **30**, 287-301, 1995.

75. Dimmock, J.R., Puthucode, R.N., Lo, M.S., Quail, J.W., Yang, J., Stables, J.P.; Pharmazie, **51**, 83-88, 1996.

76. Dimmock, J.R., Puthucode, R.N., Smith, J.M., Hetherington, M., Quail, J.W., Pugazhenthi, U., Lechler, T., Stables, J.P.; J. Med. Chem., **39**, 3984-3997, 1996.

77. Puthucode, R.N., Pugazhenthi, U., Quail, J.W., Stables, J.P., Dimmock, J.R.; Eur. J. Med. Chem., **33**, 595-607, 1998.

78. Pandeya, S.N., Mishra, V., Singh, P.N., Rupainwar, D.C.; Pharmacol. Res., **37**, 17-22, 1998.

79. Dimmock, J.R., Semple, H.A., John, S., Beazely, M.A., Abrams, G.D.; Pharmazie, **54**, 260-262, 1999.

80. Pandeya, S.N., Aggarwal, N., Jain, J.S.; Pharmazie, **54**, 300-302, 1999.

81. Pandeya, S.N., Ponnilavarasan, I., Pandey, A., Lakhan, R., Stables, J.P.; Pharmazie, **54**, 923-925, 1999.

82. Dimmock, J.R., Vashishtha, S.C., Stables, J.P.; Pharmazie, **55**, 490-494, 2000.

83. Pandeya, S.N., Mishra, V., Ponnilavarasan, I., Stables, J.P.; Pol. J. Pharmacol., **52**, 282-290, 2000.

84. Pandeya, S.N., Yogeeswari, P., Stables, J.P.; Eur. J. Med. Chem., **35**, 879-886, 2000.

85. Pandeya, S.N., Manjula, H., Stables, J.P.; Pharmazie, **56**, 121-124, 2001.

86. Pandeya, S.N., Raja, A.S., Stables, J.P.; J. Pharm. Pharm. Sci., **5**, 275-280, 2002.

87. Pandeya, S.N., Smitha, S., Stables, J.P.; Arch. Pharm. (weinheim), **335**, 129-134, 2002.

88. Micale, N., Zappala, M., Grasso, S., Puja, G., Sarro, G.D., Ferreri, G., Sarro, A.D., Toma, L., Micheli, C.D.; J. Med. Chem., **45**, 4433-4442, 2002.

89. Pandeya, S.N., Kohli, S., Siddique, N., Stables, J.P.; Pol. J. Pharmacol., **55**, 565-571, 2003.

90. Pandeya, S.N., Sowmyalakshmi, V., Panda, S.S., Pandeya, A., Stables, J.P.; Ind. J. Chem., **42B**, 2657-2661, 2003.

91. Yogeeswari, P., Sriram, D., Sunil Jit, L.R.J., Sathish Kumar, S., Stables, J.P.; Eur. J. Med. Chem., **37**, 231-236, 2002.

92. Yogeeswari, P., Sriram, D., Brahmandam, A., Sridharan, I., Thirumurugan, R., Stables, J.P.; Med. Chem. Res., **12**, 57-68, 2003.

93. Yogeeswari, P., Sriram, D., Saraswat, V., Vaigunda Ragavendran, J., Stables, J.P.; Eur. J. Pharm. Sci., **20**, 341-346, 2003.

94. Yogeeswari, P., Sriram, D., Pandeya, S.N., Stables, J.P.; Farmaco, **59**, 609-613, 2004.

95. Aggarwal, N., Mishra, P.; J. Pharm. Pharm. Sci., **7**, 260-264, 2004.

96. Yogeeswari, P., Sriram, D., Veena, V., Kavya, R., Rakhra, K., Vaigunda Raghavendran, J., Mehta, S., Thirumurugan, R., Stables, J.P.; Biomed. Pharmacother., **5**, Article in press.

97. Glen, R., Martin, G., Hill, A., Hyde, R., Wollard, P., Salmon, J., Buckingham, J., Robertson, A.; J. Med. Chem., **38**, 3566-3580, 1995.

98. Marriotte, D.P., Dougall, I.G., Meghani, P., Liu, Y.J., Flower, D.R.; J. Med. Chem., 42, 3210-3216, 1999.
99. Camerman, A., Camerman, N.; "Stereochemical similarities in chemically different antiepileptic drugs", In Antiepileptic Drugs: Mechanism of Action; Glaser, G.H., Pentry, J.K., Woodbury, D.M., Eds.; Raven Press: New York, 1980, pp 223-231.
100. Wong, M.G., Defina, J.A., Andrews, P.R.; J. Med. Chem., 29, 562-572, 1986.
101. Jones, G.L., Woodbury, D.M.; "Principles of drug action: structure activity relationships and mechanisms. In Antiepileptic Drugs, Woodbury, D.M., Penry, J.K., Pippenger, C.E., Eds.,Raven Press: New York, 1982, pp 83-109.
102. Jones, G.L., Woodbury, D.M.; Drug Dev. Res., 2, 333-355, 1982.
103. Codding, P.W., Duke, N.E., Aha, L.J., Palmer, L.Y., McClurg, D.K., Szkaradzinska, M.B.; "Structural and computational studies of anticonvulsants: a search for correlation between molecular systematics and activity. In Crystallogr. Model Methods. Mol. Des. [Pap. Symp] Meeting Date 1989; Bugg, E., Ealick, M., Eds., Springer: New York, 1989, pp 151-160.
104. Brouillette, W.J., Brown, G.B., DeLorey, T.M., Liang, G.; J. Pharm. Sci., 79, 871-874, 1990.
105. Brouillette, W.J., Brown, G.B., DeLorey, T.M., Shirali, S.S., Grunewald, G.L.; J. Med. Chem., 31, 2218-2221, 1988.
106. Brouillette, W.J., Jestkov, V.P., Brown, M.L., Akhtar, M.S., DeLorey, T.M., Brown, G.B.; J. Med. Chem., 37, 3289-3293, 1994.
107. Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., Numa, S.; Nature, 320, 188-192, 1986.
108. Trimmer, J.S., Cooperman, S.S., Tomiko, S.A., Zhou, J., Crean, S.M., Boyle, M.B., Kallen, R.G., Sheng, Z., Barchi, R.L., Sigworth, F.J., Goodman, R.H., Agnew, W.S., Mandel, G.; Neuron, 3, 33-49, 1988.
109. Salkoff, L., Butler, A., Wie, A., Scavarda, N., Giffen, K., Ifune, C., Goodman, R., Mandel, G.; Science, 237, 744-748, 1987.
110. Catterall, W.A.; Science, 242, 50-61, 1988.
111. Hegarty, A.F., Frost, L.N.; J. Chem. Soc., 1719-1728, 1973.

112. Beukers, M.W., Wanner, M.J., Von Frijtag Drabbe Kunzel, J.K., Klaasse, E.C., Ijzerman, A.P., Gerrit-Jan Koomen; J. Med. Chem., **46**, 1492-1503, 2003.

113. Mohamed, E.A., El-Deen., I.M., Ismail, M.M., Mohamed, S.M.; Ind. J. Chem., **32B**, 933-937, 1993.

114. Krall, R.L., Penry, J.K., White, B.G., Kupferbeng, H.J., Swinyard, E.A.; Epilepsia, **19**, 409-428, 1978.

115. Conley, J.P. Kohn, H.; J. Med. Chem., **30**, 567-574, 1987.

116. Lapin, I.P.; Eur. J. Pharmacol., **17**, 495-504, 1981.

117. Barrada, O, Oftedal, S.I.; Electroencephalogr, Clin. Neurophysiol., **29**, 220-229, 1970.

118. Kulkarni, S.K.; "Handbook of Experimental Pharmacology", Vallabh Prakashan, Delhi, 1st edition, 1987, 51-53.

119. Turner, K.E.; "Screening methods in Pharmacology", Academic Press, 1st edition, 1965, 60-65.

120. Miller, L.C., Tainter, M.L.; Proc. Soc. Exptl. Biol. Med., **57**, 261-266, 1944.

121. Fischer, J.W.; "Statistical Tables for Biological, Agricultural and Medical Research", Oliver and Boyd, Edinburgh, 1st edition, 1957, 50-52.

122. Litchfield, J.T., Wilcoxon, F.; J. Pharmacol. Exp. Therap., **96**, 99-113, 1949.

123. Forney, R.B., Halpien, H.R., Hughes, F.W.; Experientia, **18**, 468-469, 1962.

124. Lothman, E.W., Williamson, J.W.; Brain Res., **649**, 71-84, 1994.

125. Lothman, E.W., Salerno, R.A., Perlin, J.B., Kaiser, D.L.; Epilepsy Res., **2**, 366-379, 1988.

126. Boissier, J.R., Simon, P.; Arch. Int. Pharmacodyn. Ther., **158**, 212-214, 1965.

127. Porsolt, R.D., Anton, G., Blanet, N., Jalbre, M.; Eur. J. Pharmacol., **47**, 379,-386 1978.

128. Crossland, J.; "Lewis Pharmacology", 5th edition, 1982, 32-37.

129. Dunham, N.W., Miya., T.A.; J. Am. Pharm. Assoc., Sci. Ed., **46**, 208-211, 1957.

130. Vida, J.A., in Principles of Medicinal Chemistry, Second Edn., Foye, W.O., Eds., Philadelphia, Lea & Febiger, 1981, .

131. Nydick, I., Wrobelewski, F., Due, J.S.; Circulation, **22**, 161-163, 1955.

132. Reitman, S., Frankel, S.; Amer. J. Clin. Path., **28**, 56-58, 1957.

133. Roberts, E., Franzel, S.; J. Biol. Chem., **187**, 55-67, 1950.

134. Florey, E.; J. Physiol., **144**, 220-227, 1950.

135. Tallen, H., Moore, S., Stein, W.H.; J. Biol. Chem., **211**, 927-938, 1954.

136. Scott, E.M., Jakoby, W.B.; J. Biol. Chem., **234**, 932-941, 1959.

137. Scott, E.M., Jakoby, W.B.; Science, **128**, 361-363, 1958.

138. Dhingra, O.P., Sinclair, J.B.; "Basic Plant Pathology Methods", Raven Press, 1952, 300-302.

139. Jakoby, W.B., in colowick, S.P., Kaplan, N.O.; 'Methods in Enzymology", Academic Press, New York, Vol. V, 1962, 771-775.

140. Roberts, E.; "Methods in Enzymology", Academic Press, New York, Vol. III, 1962, 612-618.

141. Baxter, R.M., Dandiya, P.C., Kandel, S.J., Okany, A., Walker, G.L.; Nature, **185**, 466-467, 1960.

142. Pavia M.R., Lobestael S.J., Taylor C.P., Hershenson F.M., Miskell D.L.; J. Med. Chem., **33**, 854-861, 1990.

143. Moreau S., Coudert P., Rubat C., Gardette D., Goyet D.V., Couquelet J., Bastide P., Tronche P.; J. Med. Chem., **37**, 2153-2160, 1994.

144. Bernard C.G., Bohm E.; Experientia, **10**, 474-483, 1954.

145. Bailleux V., Valtee L., Nuyts J.P., Vamecq J.; Biomed. Pharmacother., **48**, 95-101, 1994.

146. Frederichs E.; Arzneim. Forsch. / Drug Res., **32**, 613-626, 1982.

147. Pandeya S.N., Dimmock J.R.; Pharmazie, **48**, 659-666, 1993.

148. Baxer, C.F., Roberts, E.; Proc. Soc. Exptl. Biol. Med., **101**, 811-824, 1959.

149. Lee, T.J., Carney, C.N., Lapis, J.L.; Gastroenterology, **70**, 422-425, 1976.

150. Loscher, W., Fassbender, C.P., Nolting, B.; Epilepsy Res., **8**, 79-89, 1991.

151. Piredda, S.G., Woodhead, J.H., Swinyard, E.A.; J. Pharmacol. Exp. Ther., **232**, 741-751, 1985.

152. Clark, C.R., Sanson, R.T., Lin, C.M., Norris, G.N.; J. Med. Chem., **28**, 1259-1262, 1985.

153. Clark, C.R., Lin, C.M., Sanson, R.T.; J. Med. Chem., **29**, 1534-1537, 1986.

154. Vamecq, J., Bac, P., Herrenknecht, C., Maurois, P., Delcourt, P., Stables, J.P.; J. Med. Chem., **43**, 1311-1319, 2000.

155. Wassner, J.J.; Pediatr., **88**, 134-136, 1976.

List of Publications

1. P. Yogeeswari, D. Sriram, A. Brahmandam, I. Sridharan, **R. Thirumurugan**, J.P. Stables; "Synthesis of novel aryl semicarbazones as Anticonvulsants with GABA-mediated mechanism", *Med. Chem. Res.*, **12**, 57-68, 2003.
2. P. Yogeeswari, D. Sriram, **R. Thirumurugan**, R. Kavya, S. Samuel, J.P. Stables; "3-chloro-2-methylphenylsemicarbazones; synthesis and anticonvulsant activity", *Eur. J. Med. Chem.*, **39**, 729-734, 2004.
3. D. Sriram, P. Yogeeswari, **R. Thirumurugan**; "Antituberculous activity of some aryl semicarbazone derivatives", *Bio org. Med. Chem. Lett.*, **14**, 3923-3924, 2004.
4. P. Yogeeswari, J. Vaigunda Raghavendran, **R. Thirumurugan**, A. Saxena, D. Sriram; "Ion channels as important targets for antiepileptic drug design", *Curr. Drug Targets*, **5**, 553-568, 2004.
5. P. Yogeeswari, D. Sriram, V. Veena, R. Kavya, K. Rakhra, S. Mehta, J. Vaigunda Ragavendran, **R. Thirumurugan**, J.P. Stables; "Synthesis of novel aryl semicarbazones as potential anticonvulsant agents", *Biomed. Pharmacother.*, (in Press), 2004.
6. **R. Thirumurugan**, D. Sriram, L.R.J. Sunil Jit, J. Vaigunda Ragavendran, R. Kavya, K. Rakhra, J.P. Stables, P. Yogeeswari; "Synthesis of some new semicarbazone derivatives as anticonvulsants with GABA-mediated mechanism", *Eur. J. Med. Chem.*, (in Press), 2004.
7. P. Yogeeswari, D. Sriram, **R. Thirumurugan**, J.V. Raghavendran, K. Sudhan, J. Stables; "Discovery of N-(2,6-dimethylphenyl)-substituted Semicarbazones as Anticonvulsants effective against various animal models of seizure with GABA-T Inhibitory Activity", *J. Med. Chem.*, Communicated, 2004.
8. P. Yogeeswari, D. Sriram, **R. Thirumurugan**, J.P. Stables; "Synthesis and anticonvulsant evaluation of 2,4-dimethoxyphenylsemicarbazones", *Chem. Pharm. Bull.*, Communicated, 2004.

Abstracts in Conference / symposium:

1. I. Sridharan, B. Archana, **R. Thirumurugan**, P. Yogeeswari, D. Sriram; "γ-aminobutyric acid (GABA) level studies on novel anticonvulsant compounds in rat brain tissue preparation", 54th Indian Pharmaceutical Congress, Pune, December 13-15, 2002.
2. **R. Thirumurugan**, D. Sriram, P. Yogeeswari; "Design and synthesis of novel aryl semicarbazone derivatives as anticonvulsants with GABA and glycine mediated mechanism", International symposium on drug discovery and process research, Shivaji University, Kolhapur – 416004, January 23-25, 2003.
3. J. Vaigunda Ragavendran, **R. Thirumurugan**, I. Sridharan, D. Sriram, P. Yogeeswari; "4-amino butyrate aminotransferase (GABA-T) inhibition as a possible mechanism of some novel anticonvulsant aryl semicarbazones", 55th Indian Pharmaceutical Congress, Chennai, December, 19-21, 2003.
4. R. Kavya, **R. Thirumurugan**, D. Sriram, P. Yogeeswari; "Pharmacological Evaluation of aryl(heteroaryl)semi(thio)carbazones for CNS activity", 55th Indian Pharmaceutical congress, Chennai, December 19-21, 2003.

BIOGRAPHY OF Dr. D. SRIRAM

Dr. D.Sriram is presently working as Lecturer, at Pharmacy group, Birla Institute of Technology, Pilani. He received his Ph.D. degree in 2000 from Banaras Hindu University, Varanasi. He has been involved in teaching for 8 years and in research for 9 years. Dr. Sriram has to his credit 35 peer reviewed research publications and 5 popular review articles in international journals. He is a life member of Association of Pharmacy Teachers of India, and Association of Microbiologists of India and member of Canadian Society of Pharmaceutical Sciences. He has been collaborating with various national and international organizations that include National Institute of Health, USA, Southern Research Institute, USA, Rega Institute for Medical research, Belgium, Indian Institute of Science, Bangalore, India, and National Institute of Mental Health and Neurosciences, Bangalore, India. As a result of his academic and research achievements, his biographical profile has been included in the prestigious registry of the 6th edition of "*Marquis Who's Who in Science and Engineering*" published in USA, 2002-2003 and been regularly updated.

BIOGRAPHY OF Mr. R. THIRUMURUGAN

Mr. R. Thirumurugan completed his basic degree B.Pharmacy from Madurai Medical College, Madurai, Tamil Nadu, and completed his post graduation (M.Pharmacy) in 2000, from Banaras Hindu University, Varanasi, Uttar Pradesh. He had been working as a Research Chemist at Sami Labs Ltd., Bangalore for 18 months. Mr. R. Thirumurugan has been a research fellow working for his Ph.D. at BITS, Pilani from 2001-2004 during which he was a recipient of the Senior Research Fellowship (SRF) granted by the Council of Scientific and Industrial Research (CSIR). He has published seven scientific publications in well renowned international journals.