# Menthone Semicarbazides and Thiosemicarbazides as Anticonvulsant Agents

Jainendra Jain[1,*], Y. Kumar[2], James Stables[3] and Reema Sinha[1]

[1]*Department of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education, 3, Knowledge Park I, Greater Noida, Gautam Budh Nagar, Uttar Pradesh-201308, India;* [2]*ITS Paramedical College (Pharmacy), Muradnagar, Ghaziabad, Uttar Pradesh-201206, India;* [3]*Preclinical Pharmacology, National Institute of Neurological Disorders and Stroke (NINDS), NIH, Bethesda, MD 20892-9020, USA*

**Abstract:** A series of novel (±) 3-menthone semicarbazides (1-7) and thiosemicarbazides (8-14) were synthesized using an appropriate synthetic route and characterized by thin layer chromatography and spectral analysis. The anticonvulsant activity of synthesized compounds was established after intraperitoneal administration in three seizure models in mice which include maximal electroshock seizure (MES), subcutaneous pentylene tetrazole (scPTZ) induced seizure and minimal neurotoxicity test. Seven compounds exhibited protection in both models and $N^1$ – (4-fluorophenyl) – $N^4$- (menth-3-one) semicarbazide (4) emerged as the most active compound with MES $ED_{50}$ of 44.15mg/kg and scPTZ $ED_{50}$ of 38.68mg/kg at 0.25h duration. These compounds were found to elevate γ-amino butyric acid (GABA) levels in the midbrain region, thus indicating that (±) 3-menthone semicarbazides could be considered as a lead molecule in designing of a potent anticonvulsant drug.

**Keywords:** (±) 3-menthone, semicarbazides, thiosemicarbazides, anticonvulsant, Maximal Electroshock Seizure (MES), subcutaneous pentylene tetrazole (scPTZ), Antiepileptic drug development (AED).

## INTRODUCTION

Epilepsy is a neurological disorder of varied etiology, characterized by paroxysmal, excessive and hyper synchronous discharges of large number of neurons [1]. Approximately 50 million people worldwide suffer from epilepsy, making this condition, the second leading neurological disorder [2]. 25% of the epileptic populations have seizures that are not responsive to presently available therapies. Despite the optimal use of available antiepileptic drugs (AEDs), many patients fail to experience seizure control and others do so only at the expense of significant toxic effects that range in severity from minimal brain impairment to death from aplastic anemia or hepatic failure [3]. It is estimated that available medication controls seizures in only 50% of patients or decrease incidence in only 75% of patients.

These facts make the field of anticonvulsant drug discovery, a high priority. The search continues for the ideal drug that should be potent, selective in raising seizure threshold and preventing seizure spread without causing serious side effects.

Semicarbazones presents a wide range of bioactivities and their pharmacological applications have been extensively investigated [4]. Semicarbazones can be considered as a new class of anticonvulsants with oral activity [5, 6]. A number of (aryl-oxy) aryl semicarbazones possessed greater protection in the MES screen [7]. Aryl semicarbazones displaying activity in the MES screen interact at a specific bind ing site referred as the hydrogen bonding area and aryl binding site respectively [8]. Terminal amino group of aryl semicarbazones was shown to affect the binding properties at the hydrogen bonding area [9, 10]. However Pandeya *et al* have suggested a new pharmacophore model for semicarbazones displaying anticonvulsant activity (Fig. **1**). They proposed that terminal amino function of semicarbazones could be substituted with a lipophilic substituted aryl ring. Proposed pharmacophore model contain four binding sites for interaction with a macromolecular complex *in vivo*.

1. An aryl hydrophobic binding site (A) with halogen substituent preferably at para position.
2. A hydrogen bonding domain (HBD).
3. An electron donor group (D).
4. Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C).

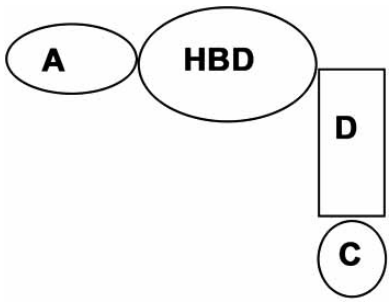



**Fig. (1).** Suggested Pharmacophore model for semicarbazones displaying anticonvulsant activity.

*Address correspondence to this author at the Department of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education, 3, Knowledge Park I, Greater Noida,Gautam Budh Nagar, Uttar Pradesh-201308, India; Tel: +91 121 6454514; Fax: +91 121 2439058;
E-mail: jainendrem@yahoo.com

1573-4064/10 $55.00+.00 

These new aspects might be useful for designing prototypic molecules with potential anticonvulsant activity. In the recent study halogen substituents showed low $ED_{50}$ values in the rat oral MES screen with high protective index values, particularly when they are present at para position of phenyl ring [11]. The effect of other substituents at para position has also been studied. In this study the major change has been to explore the size and functional requirements of the hydrophobic-hydrophilic site controlling pharmacokinetic properties. In this regard, a cyclic terpene (±) 3-menthone has been selected because it is expected to increase the lipophilicity of the molecule and this may result in improved anticonvulsant activity.

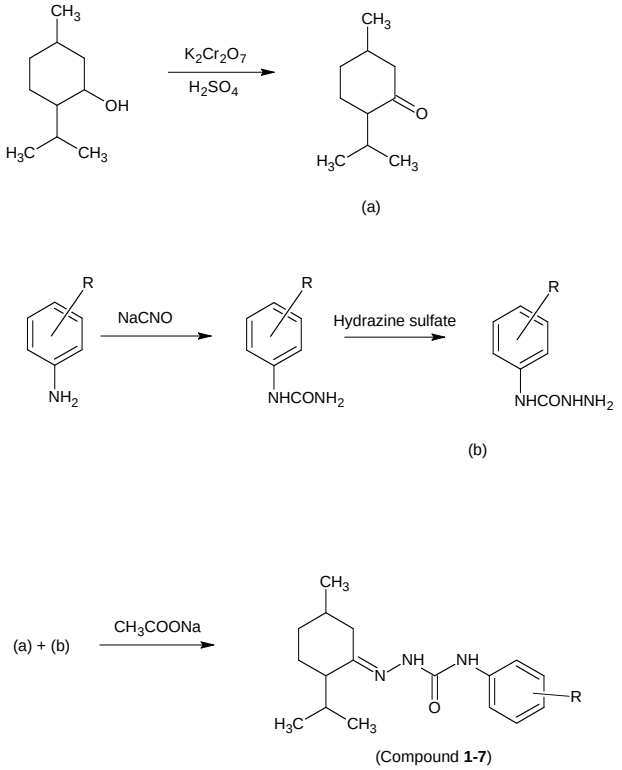
Earlier studies of (±) 3-menthone and its derivatives have shown marked anticonvulsant activity. The essential oil containing menthone from flower heads of *Egletes viscosa*, showed significant protection in scPTZ screen [12]. A ten carbon amino acid derived from hydrolysis of (-) menthone oxime exhibits structural similarities with gamma amino butyric acid (GABA) and has shown potent inhibition of GABA neuroreceptor [13]. Schiff bases of (±) 3-menthone with aryl amines were found to show significant protection in MES screen [14]. In addition, compound **6, 8, 11, 13** and **14** from present scheme have been reported to possess significant analgesic, sedative hypnotic and anti-HIV activity [15, 16]. Hence (±) 3-menthone aryl semicarbazides and thiosemicarbazides are expected to possess potent anticonvulsant properties with enhanced lipophilicity for better penetration across biological membranes.

## 2. RESULTS AND DISCUSSION

### 2.1. Synthesis

The synthesis of (±) 3-menthone aryl semicarbazides and thiosemicarbazides was accomplished as presented in Scheme **1** and Scheme **2.** (±) 3-menthone **(a)** was prepared from oxidation of (-) menthol by chromic acid oxidation method. Substituted aryl semicarbazides **(b)** were prepared from aryl ureas by reaction with hydrazine sulfate. Aryl ureas were prepared from reaction of substituted primary aromatic amines with Sodium cyanate. (±) 3-menthone aryl semicarbazides **(1-7)** were synthesized by reaction between (a) and (b) in presence of anhydrous Sodium acetate.

(±) 3-menthone aryl thiosemicarbazides **(8-14)** were prepared from reaction between aryl isothiocyanates **(c)** and (±) 3-menthone hydrazone **(d)**. Aryl isothiocyanates were synthesized from primary aromatic amines by reaction with carbon disulfide whereas (±) 3-menthone hydrazone was prepared from reaction between (±) 3-menthone and hydrazine hydrate. The details of physicochemical properties of the synthesized compounds are presented in Tables **1** and **2.** Chemical structures of synthesized compounds were characterized by spectral analysis (IR and 1H NMR) and the data were found to be consistent with proposed structures. The details of spectral analysis are shown in Table **3**. Compounds **1-14** were injected intraperitoneally into mice and evaluated for anticonvulsant activity by MES, scPTZ and neurotoxicity screens using doses of 30, 100 and 300mg/kg. These observations were carried out at two different time intervals (0.5h and 4.0h) and the results are compared with standard drug Phenytoin. These data are presented in Table **4.** Seven compounds (**1, 2, 4, 6, 9, 10, and 12)** showed marked protection in MES epilepsy model which is indicative of their ability to prevent seizure spread. Most of the compounds showed protection at 0.5h, indicating rapid onset and shorter duration of action. Similarly seven compounds (**1, 2, 3, 4, 9, 10, and 12)** showed significant protection in scPTZ test at 0.5h time interval which indicates properties of the molecule to elevate seizure threshold. Compounds (**4, 6, 9, 10, and 12)** showed activity in MES model at 100mg/kg whereas compounds (**1** and **2)** were found to be active at 300mg/kg. In scPTZ screen, compound (**4)** has shown protection at 100mg/kg whereas compounds (**1, 2, 3, 9, 10** and **12)** were found to be active at 300mg/kg.



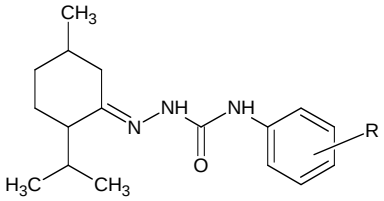

**Scheme 1.** Synthesis of menthone aryl semicarbazides.

In the neurotoxicity screen, most of the compounds except (**8)** were found to be neurotoxic at 300mg/kg. Compounds (**1**, **6**, **9, 10, 11, 13** and **14)** have also shown toxicity at 100mg/kg dose.

Compound (**4)** was found to be the most emerging derivative in both MES and scPTZ test with MES $ED_{50}$ of 44.15 ± 1.5 mg/kg and scPTZ $ED_{50}$ of 38.68 ± 1.54 mg/kg at 0.25h time interval. However it has also shown MES $TD_{50}$ of 73.5 ± 1.57 mg/kg and scPTZ $TD_{50}$ of 125.62 ± 1.62 mg/kg at 0.5h time interval, indicating that (±) 3-menthone aryl semicarbazides and thiosemicarbazides are required to be explored further to improve MES $ED_{50,}$ scPTZ $ED_{50}$ and corresponding $TD_{50}$ values. These data are presented in Tables **5** and **6**.

Among the compounds, aryl substituted semicarbazides were found to be more active than the corresponding thiosemicarbazides and better protection was observed in scPTZ screen than MES test. Halogen substitutents at para position of phenyl ring in semicarbazides showed more

**Table 1. Physical Data of the (±) 3-Menthone–Aryl Semicarbazides**

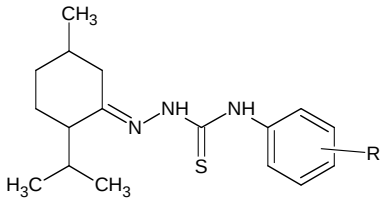


(Compound **1-7**)

| Compound | Substituent R | Yield (%) | m.p (°C) | Molecular Formula | Molecular Weight | $R_f$[a] | logP[b] |
|---|---|---|---|---|---|---|---|
| 1. | H | 78 | 182-4 | $C_{17}H_{25}N_3O$ | 287 | 0.67 | 3.78 |
| 2. | 4-Br | 65 | 162-4 | $C_{17}H_{24}N_3OBr$ | 365 | 0.52 | 4.95 |
| 3. | 2-$CH_3$ | 80 | 176-8 | $C_{18}H_{27}N_3O$ | 301 | 0.84 | 4.24 |
| 4. | 4-F | 78 | 181-3 | $C_{17}H_{24}N_3OF$ | 305 | 0.80 | 4.23 |
| 5. | 4-$CH_3$ | 85 | 159-61 | $C_{18}H_{27}N_3O$ | 301 | 0.65 | 4.24 |
| 6. | 4-Cl | 53 | 172-4 | $C_{17}H_{24}N_3OCl$ | 321 | 0.41 | 4.77 |
| 7. | 4-$NO_2$ | 43 | 245-7 | $C_{17}H_{24}N_4O_3$ | 332 | 0.31 | 4.24 |

[a] Solvent system: $CHCl_3$: MeOH (9:1).
[b] logP was generated using Chemsketch 12.0 software.

**Table 2. Physical Data of the (±) 3-Menthone–Aryl Thiosemicarbazides**



(Compound **8-14**)

| Compound | Substituent R | Yield (%) | m.p (°C) | Molecular Formula | Molecular Weight | $R_f$[a] | logP[b] |
|---|---|---|---|---|---|---|---|
| 8. | H | 61 | 171-3 | $C_{17}H_{25}N_3S$ | 303 | 0.49 | 5.17 |
| 9. | 4-$NO_2$ | 71 | 249-52 | $C_{17}H_{24}N_4O_2S$ | 348 | 0.32 | 3.74 |
| 10. | 4-Br | 51 | 219-21 | $C_{17}H_{24}BrN_3S$ | 381 | 0.62 | 6.00 |
| 11. | 4-Cl | 67 | 189-92 | $C_{17}H_{24}ClN_3S$ | 337 | 0.52 | 5.73 |
| 12. | 4-F | 52 | 201-4 | $C_{17}H_{24}N_3FS$ | 321 | 0.82 | 5.33 |
| 13. | 4-$OCH_3$ | 61 | 189-91 | $C_{18}H_{27}N_3OS$ | 333 | 0.68 | 5.05 |
| 14. | 4-$CH_3$ | 69 | 179-81 | $C_{18}H_{27}N_3S$ | 317 | 0.76 | 5.66 |

[a] Solvent system: $CHCl_3$: EtOAc (4:1).
[b] logP was generated using Chemsketch 12.0 software.

activity than other substituents. Fluoro substituted derivative (compound **4**) had shown better protection than chloro and bromo derivatives. The corresponding halogen substituted thiosemicarbazides, in which oxygen was replaced with bio isoster sulfur, were found to possess mild protection in both MES and scPTZ screen.

**Table 3. IR and 1H NMR Data of Synthesized Compounds**

| Compound No. | IR and 1H NMR Analysis Data |
|---|---|
| 1. | IR (KBr): 3415, 3324, 2872, 1672, 1552 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) δ (ppm): 0.75-0.82 (6H, t), 1.05-1.11 (3H, d), 1.65-1.73 (4H, m), 1.84-2.00 (3H, m), 2.14-2.25 (1H, m), 2.57-2.62 (1H, m), 3.12 (1H, m), 7.03-7.25 (5H, m), 8.15 (1H, s). |
| 2. | IR (KBr): 3413, 3324, 2852, 1672, 610 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) δ (ppm): 0.79-0.92 (6H, t), 1.09-1.12 (3H, d), 1.60-1.71 (4H, m), 1.86-2.00 (3H, m), 2.12-2.30 (1H, m), 2.62-2.81 (1H, m), 3.64 (1H, s), 6.15-6.21 (2H, d), 7.26-7.34 (2H, d), 8.21 (1H, s). |
| 3. | IR (KBr): 3436, 3315, 2954, 1650 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) δ (ppm): 0.81-0.87 (6H, t), 1.02-1.07 (3H, d), 1.55-1.60 (4H, m), 1.84-1.95 (3H, m), 2.12-2.18 (1H, m), 2.24 (3H, s), 2.51-2.58 (1H, m), 4.12 (1H, s), 7.10-7.57 (4H, m), 7.72 (1H, s). |
| 4. | IR (KBr): 3450, 3310, 2857, 1680, 1220 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) δ (ppm): 0.91-1.01 (6H, t), 1.05-1.09 (3H, d), 1.13-1.38 (4H, m), 1.64-1.71 (3H, m), 2.05-2.10 (1H, m), 2.56-2.59 (1H, m), 3.96 (1H, s), 6.86-6.95 (2H, d), 7.36-7.45 (2H, d), 8.39 (1H, s). |
| 8. | IR (KBr): 3210, 2952, 1593, 1502, 1199, 750 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ (ppm): 0.89-0.94 (6H, t), 0.99-1.01 (3H, d), 1.25-1.33 (4H, m), 1.84-2.00 (3H, m), 2.14-2.25 (1H, m), 2.57-2.62 (1H, m), 3.34 (1H, s), 6.90-7.56 (5H, m), 9.84 (1H, s). |
| 11. | IR (KBr): 3210, 2952, 1593, 1502, 1199, 750, 690 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ (ppm): 0.82-0.91 (6H, t), 1.05-1.11 (3H, d), 1.18-1.26 (4H, m), 1.84-2.00 (3H, m), 2.14-2.25 (1H, m), 2.57-2.62 (1H, m), 3.65 (1H, s), 6.37- 6.41 (2H, d), 7.42 – 7.49 (2H, d), 9.84(1H, s). |
| 12. | IR (KBr): 3350, 2821, 1620, 1502, 1210, 852, 712 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ (ppm): 0.89-0.96 (6H, t), 1.05-1.11 (3H, d), 1.69-1.73 (4H, m), 1.95-2.12 (3H, m), 2.24-2.37 (1H, m), 2.57-2.62 (1H, m), 3.85 (1H, s), 6.42- 6.58 (2H, d), 6.98–7.10 (2H, d), 9.14 (1H, s). |
| 14. | IR (KBr): 3317, 2923, 2867, 1640, 1540, 1490, 1271, 1078, 840 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ (ppm): 0.89-0.94 (6H, t), 0.99-1.01 (3H, d), 1.65-1.73 (4H, d), 1.84-2.00 (3H, m), 2.14-2.25 (1H, m), 2.28 (3H, s), 2.57-2.62 (1H, m), 3.34 (1H, s), 7.11-7.14 (2H, d), 7.37-7.40 (2H, d), 9.60-9.79 (1H, br s). |

**Table 4. Anticonvulsant Activity and Minimal Motor Impairment of (±) 3-Menthone–Aryl Semicarbazides and Thiosemicarbazides**

| Compound No. | Intraperitoneal Injection in Mice[a] | | | | | |
|---|---|---|---|---|---|---|
| | MES Screen | | scPTZ Screen | | Neurotoxicity | |
| | 0.5h | 4.0h | 0.5h | 4.0h | 0.5h | 4.0h |
| 1. | 300 | - | 300 | - | 100 | 300 |
| 2. | 300 | 300 | 300 | - | 300 | 300 |
| 3. | - | - | 300 | - | 300 | 300 |
| 4. | 100 | - | 100 | - | 300 | - |
| 5. | - | - | - | - | 300 | - |
| 6. | 100 | 300 | - | - | 100 | 300 |
| 7. | - | - | - | 300 | 300 | 300 |
| 8. | - | - | - | - | - | - |
| 9. | 100 | 100 | 300 | - | 100 | - |
| 10. | 100 | 100 | 300 | - | 100 | - |
| 11. | - | - | - | - | 100 | - |
| 12. | 100 | 100 | 300 | - | 300 | - |
| 13. | - | - | - | - | 100 | - |
| 14. | - | - | - | - | 100 | 100 |
| Phenytoin | 30 | 30 | - | - | 100 | 100 |

[a] Doses of 30, 100 and 300mg/kg were administered. The figures in the table indicate.
the minimum dose whereby bioactivity was demonstrated in half or more of the mice.
The animals were examined at 0.5 and 4.0h after injections were administered.
The dash (-) indicates the absence of activity at maximum dose administered (300mg/kg).

**Table 5. Evaluation of Compound 4 for MES $ED_{50}$ and $TD_{50}$ in Mice**

| Dose (mg/kg) | MES $ED_{50}$[a] | Dose (mg/kg) | MES $TD_{50}$[b] |
|---|---|---|---|
| | 0.25h | | 0.5h |
| 25 | 1/8 | 25 | 0/8 |
| 38 | 3/8 | 50 | 2/8 |
| 50 | 6/8 | 75 | 4/8 |
| 75 | 6/8 | 125 | 7/8 |

[a] The figures indicate the number of mice out of eight which were protected.
[b] The figures indicate the number of mice out of eight which had shown toxicity.

**Table 6. Evaluation of Compound 4 for scPTZ $ED_{50}$ and $TD_{50}$ in Mice**

| Dose (mg/kg) | scPTZ $ED_{50}$[a] | Dose (mg/kg) | scPTZ $TD_{50}$[b] |
|---|---|---|---|
| | 0.25h | | 0.5h |
| 18.8 | 0/8 | 25 | 2/8 |
| 28.2 | 2/8 | 75 | 3/8 |
| 37.5 | 5/8 | 150 | 8/8 |
| 75 | 7/8 | - | - |

[a] The figures indicate the number of mice out of eight which were protected.
[b] The figures indicate the number of mice out of eight which had shown toxicity.

In order to explore the mechanism of anticonvulsant activity, two compounds (**4** and **12**) were further selected for 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. These data are presented in Table **7**. These compounds were found to increase the GABA level in mid brain region significantly. In other brain regions, the increase in GABA level was insignificant. These results are compared with standard drug Clobazam.

No significant correlation was observed between logP value and anticonvulsant activity. Thus the present study revealed that halogen substituted (±) 3-menthone aryl semicarbazides possessed a significant anticonvulsant activity in both MES and scPTZ screens, indicating that (±) 3-menthone could be considered as a lead compound in anticonvulsant drug design. Further modifications are however necessary to improve $ED_{50}$ and $TD_{50}$ values in both MES and scPTZ tests.

## 3. EXPERIMENTAL

### 3.1. Chemistry

Melting points were determined with a Veego India Digital Melting point apparatus and are uncorrected. Infrared (IR) spectra for the compounds were recorded in KBr discs on Shimadzu FTIR spectrophotometer 8400s. $^1$H NMR spectra were recorded in $CDCl_3$ and DMSO $d_6$ on a Brucker Avance 300MHz spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane as internal standard. The purity of the compounds was monitored by as-

**Table 7. Effect of Selected Compounds on GABA System**

| Compound[a] | Concentration of GABA (mg/100mg tissue)[b] | | | |
|---|---|---|---|---|
| | Olfactory lobe | Midbrain | Medulla oblongata | Cerebellum |
| Control. | 12.52 ± 2.15 | 41.42 ± 2.91 | 31.43 ± 1.91 | 19.94 ±3.15 |
| 4. | 12.91 ± 2.61 | 59.11 ± 2.21 | 36.59 ± 2.14 | 22.41 ± 3.49 |
| 12. | 14.58 ± 1.71 | 53.68 ± 1.77 | 35.64 ± 2.12 | 23.81 ± 2.72 |
| Clobazam[c] | 17.97 ± 2.62 | 65.57 ± 2.83 | 43.91 ± 2.16 | 29.12 ± 3.19 |

[a] The compounds were tested at a dose of 100mg/kg.
[b] Each value represents the means ± SEM of six rats significantly different from the control at $p < 0.05$ (student's t test).
[c] Tested at 30mg/kg (i.p).

cending thin layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapors. Developing solvent used in TLC was chloroform:methanol (9:1). The logP values for all synthesized compounds were calculated using ACD ChemSketch 12.0 software.

### *3.1.1. Synthesis of (±) 3-Menthone (a)*

Potassium dichromate (60.0g, 0.2mol) was dissolved in 300ml of distilled water and to it concentrated Sulfuric acid (27.0ml, 0.27mol) was added with continuous stirring. (-) Menthol crystals (50g, 0.32mol) were added in portions with continuous stirring. The reaction mixture was heated at 55$^{o}$C for 15min and after cooling, menthone was extracted with solvent ether (100ml, 3 times). The ethereal layer was separated and washed with 5% Sodium Hydroxide solution followed by distilled water. The ethereal layer was dried over anhydrous Sodium Sulfate and menthone was purified by distillation under reduced pressure, yield 75%, b.p 207-9$^{o}$C, IR (KBr) cm$^{-1}$ : 2950, 1710, 1450, 1360; $^{1}$H NMR ($CDCl_3$) δ: 0.79 (6H, d, *J*=6.2 Hz), 0.95 (3H, d, *J*=6.6 Hz ), 1.27 (2H, m), 1.79 (2H, m), 1.97 (2H, m), 2.06 (2H, m), 2.27 (1H, m).

### *3.1.2. Synthesis of Substituted Aryl Semicarbazides (b)*

Aryl semicarbazides were prepared by reacting substituted aryl ureas with hydrazine sulfate. Substituted aryl ureas were prepared according to the prescribed procedure [17]. Substituted aryl ureas (0.05mol) and hydrazine sulfate were dissolved in 95% ethanol. The solution was made alkaline with sodium hydroxide and the mixture was refluxed on water bath for 24.0h. The excess solvent was evaporated under vacuum and the reaction mixture was poured in ice cold water. The precipitate was collected, washed with excess water and dried at 100$^{o}$C. The compounds were recrystallized from 50% alcohol.

### *3.1.3. Synthesis of (±) 3-Menthone–Aryl Semicarbazides (1-7)*

Compounds (1-7) were synthesized as per the synthetic Scheme **1**. To a solution of substituted aryl semicarbazides (0.05mol) in 20ml of 95% ethanol, (±) 3-menthone (7.7ml, 0.05mol) and anhydrous sodium acetate (4.1g, 0.05mol) were added and the reaction mixture was refluxed on water bath for 12.0h. The reaction was monitored by TLC using silica gel G layers. The solution was concentrated under vacuum and the reaction mixture was poured in ice cold water. The precipitate was collected, washed with petroleum ether (60-80ºC) and dried at 100$^{o}$C. The compounds were recrystallized from absolute ethanol.

### *3.1.4. Synthesis of Aryl Isothiocyanates (c)*

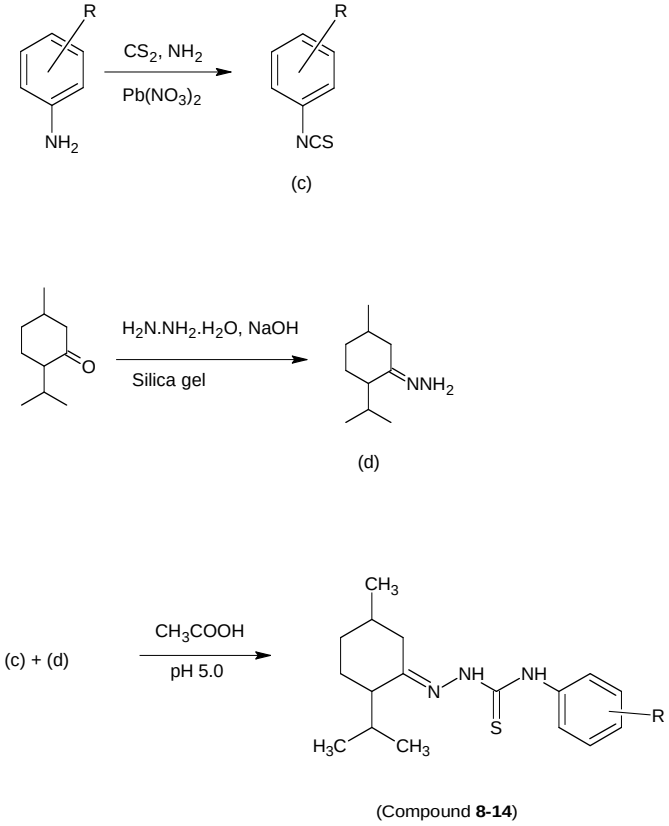
The aryl isothiocyanates were prepared according to the procedure reported in literature [17], in which aryl amines and carbon disulfide were dissolved in 95% ethanol and the ammonia solution was added to it with constant stirring. The aqueous solution of lead nitrate was added and the resulting solution was steam distilled to yield corresponding aryl isothiocyanates.

### *3.1.5. Synthesis of (±) 3-Menthone Hydrazone (d)*

(±) 3-menthone hydrazone was synthesized according to the procedure reported in literature [18]. A mortar was charged with (±) 3-menthone (0.05mol), hydrazine hydrate (100%, 0.05mol), sodium hydroxide (0.05mol) and silica gel G (2.0g). The reaction mixture was ground with a pestle in the mortar for 1.0 hr, when the TLC showed no spot of remaining menthone. The reaction mixture was poured into a mixture of dichloromethane (50ml) and 5% HCl (25ml). The organic layer was washed with saturated sodium bicarbonate, dried over anhydrous sodium sulphate and evaporated under reduced pressure to give the pure product, yield 64%, IR (KBr) cm$^{-1}$: 3350, 3210, 2970, 2850, 1640, 1520; $^{1}$H NMR ($CDCl_3$), 0.89-0.94 (6H, d, *J*=6.2Hz), 0.99-1.01 (3H, d, 6.62Hz), 1.22-1.26 (2H, m), 1.67-1.72 (1H, m), 1.79-1.86 (2H, m), 1.97-2.02 (2H, m), 2.06-2.13 (2H, m), 7.26 (2H, s).

### *3.1.6. Synthesis of (±) 3-Menthone Aryl Thiosemicarbazides (8-14)*

(±) 3-menthone thiosemacarbazides were synthesized according to the procedure specified in literature with some modifications [15] as shown in synthetic Scheme **2**. An appropriate aryl isothiocyanate (0.02mol) was added to (±) 3-menthone hydrazone (0.02mol) in absolute ethanol (30ml). The solution was acidified with glacial acetic acid (pH 4-5) and the reaction mixture was refluxed for 5.0 hrs. The precipitated solid was filtered off, washed with petroleum ether (60-80$^{o}$C) and recrystallized from absolute ethanol to give pure product.




**Scheme 2.** Synthesis of menthone aryl thiosemicarbazides.

## 3.2. Pharmacology

### *Animals*

Male albino mice (CF-1, strain, 18-25g) and male albino rats (Sprague-Dawley/Wistar, 100-150g) were used as ex-

perimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The title compounds (**1-14**) were suspended in 0.5% methyl cellulose/water mixture or in polyethylene glycol (PEG 200).

### *3.2.1. Anticonvulsant Screening*

The anticonvulsant evaluations were undertaken by the Anticonvulsant Screening Program, National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Health, using their reported procedures [19, 20]. Initially all compounds were administered intraperitoneally in doses of 30, 100 and 300mg/kg to one to four mice. Activity was established using MES and scPTZ tests.

One of the compounds (**4)** was also examined for MES $ED_{50}$, MES $TD_{50}$, scPTZ $ED_{50}$ and scPTZ $TD_{50}$ studies.

### *3.2.2. Neurotoxicity Screening*

The rotorod test was used to evaluate neurotoxicity. The animal was placed on a 3.2 cm diameter knurled rod rotating at 10 rpm. Normal mice can remain on a rod rotating at this speed indefinitely. Neurological toxicity is defined as the failures of the animal to remain on the rod for 1.0min. Trained animals were given intraperitoneal injections of the test compounds in doses of 30, 100 and 300mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1.0 min in each of three trials. Results are expressed as number of animals exhibiting toxicity/ number of animals tested [21].

### *3.2.3. Isolation of Rat Brain Regions and GABA Assay*

The GABA assay for compounds **4** and **12** was performed in brain tissue extracts enzymatically as described in the literature [22]. Adult Wistar rats were used for this purpose. After 2.0 hrs of drug administration intraperitoneally, the animal was sacrificed by decapitation and the brain regions, midbrain, olfactory lobe, cerebellum and medulla oblongata were dropped into separate vials containing 4-6ml of ice cold 80% ethanol and processed further as described in the literature [23].

## ACKNOWLEDGEMENTS

The authors are thankful to the Director, Department of Pharmaceutical Sciences, Meerut Institute of Engineering and Technology, Meerut, Uttar Pradesh, India for providing the infrastructure for undertaking the project. One of the authors James Stables deeply acknowledges NINDS, NIH, Bethesda, MD, USA for undertaking the pharmacological screening of synthesized compounds. Authors are also thankful to Mr. M.N. Lal, IIT, Delhi, for the generation of $^1$H NMR data.

## REFERENCES

[1] Wasterlain, C.; Siegel, G.; Albers, R. W.; Molinoff, P. *Basic Neurochemistry. Molecular, Cellular and Medical Aspects*, Raven Press: New York, **1989**.

[2] Porter, R. J.; Rogawski, M. A. New Antiepileptic drugs: from serendipity to rational discovery. *Epilepsia*, **1992**, *33*(Suppl.1), S1-S6.

[3] McNamara, J. O. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Hardman, J. G.; Limbird, L.E.; Gilman, A. G., Ed.; The McGraw-Hill: New York, **2001**; pp. 521-547.

[4] Beraldo, H.; Gambino, D. preface: hot topic: metal compounds in medicinal chemistry. *Mini Rev. Med. Chem*., **2004**, *4*(1), 31-39.

[5] Yogeeswari, P.; Sriram, D.; Pandeya, S. N.; Stables, J. P. 4-Sulphamoyl phenyl semicarbazones with anticonvulsant activity. *Farmaco*, **2004**, *59*(8), 609-613.

[6] Yogeeswari, P.; Sriram, D.; Veena, V.; Kavya, R.; Rakhra, K.; Mehta, S.; Raghvendran, J. V.; Thirumurugan, R.; Stables, J. P. Synthesis of aryl semicarbazones as potential anticonvulsant agents. *Biomed. Pharmacother*., **2005**, *59*(1-2), 51-55.

[7] Dimmock, J. R.; Puthukode, R. N.; Smith, J. M.; Hetherington, M.; Quail, J. W.; Pugazhenthi, U.; Lechler, T.; Stables J. P. (Aryl oxy) aryl Semicarbazones and related compounds: a novel class of anticonvulsant agents possessing high activity in the maximal electroshock screen. *J. Med. Chem.*, **1996**, *39*(20), 3984-3997.

[8] Dimmock, J. R.; Pandeya, S. N.; Quail, J. W.; Pugazhenthi, U.; Allen, T. M.; Kao, G. Y.; Bulzarini, J.; De Clercq, E. Evaluation of the semicarbazones, thiosemicarbazones and biscarbohydrazones of some aryl alicyclic ketones for anticonvulsant and other biological properties. *Eur. J. Med. Chem*., **1995**, *30*, 303-314.

[9] Dimmock, J. R.; Sample, H. A.; John, S.; Beazely, M. A.; Abrams, G. D. Isolation and identification of the major urinary metabolite of 4-(4-fluorophenoxy) benzaldehyde semicarbazone after oral dosing to rats. *Die Pharmazie*, **1999**, *54*, 260-262.

[10] Pandeya, S. N.; Mishra, V.; Singh, P. N.; Rupainwar, D. C. Anticonvulsant activity of thioureido derivatives of acetophenone semicarbazone. *Pharmacol. Res*., **1998**, *37*, 17-22.

[11] Pandeya, S. N.; Raja, A. S.; Stables, J. P. Synthesis of isatin semicarbazones as potential anticonvulsants: Role of hydrogen bonding. *J. Pharm. Pharm. Sci*., **2002**, *5*(3), 266-271.

[12] Souza, M. F.; Santos, F. A.; Sidrim J. J. C. Antinociceptive, anticonvulsant and antibacterial effects of the essential oil from flower heads of *Egletes Viscosa* L. *Phytother. Res.*, **1998**, *12*, 28-31.

[13] Stanislaw, L.; Kuldo, J.; Frackowiak, B.; Holband, J.; Wojcik, G. Stereochemistry of terpene derivatives, Part 2[1]: Synthesis of new chiral amino acids with potential neuroactivity. *Tetrahedron*: *Asymmetry*, **2000**, *11*, 1295-1302.

[14] Jain, J. S.; Srivastava, R. S.; Aggrawal, N.; Sinha, R. Synthesis and evaluation of Schiff bases for anticonvulsant and behavioral depressant properties. *Cent. Nerv. Syst. Agents Med. Chem.*, **2007**, *7*, 200-204.

[15] Mishra, V.; Pandeya, S. N. Analgesic activity and hypnosis potentiation effect of (±) 3-menthone semicarbazone and thiosemicarbazone derivatives. *Acta. Pharm*., **2001**, *51*, 83-88.

[16] Mishra, V.; Pandeya, S. N.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. Anti HIV activity of thiosemicarbazone and semicarbazone derivatives of (±) 3-menthone. *Arch. Pharm. Pharm. Med. Chem*., **2002**, *5*, 183-186.

[17] Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry;* Wesley Longman Limited: England, **1998**.

[18] Hajipour, A. R.; Baltrok, I. M.; Bigdeli, M. A convenient and mild procedure for the synthesis of hydrazones and semicarbazones from aldehydes or ketones under solvent free conditions. *J. Chem. Res*., **1999**, *9*, 570.

[19] Krall, R. I.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. Antiepileptic drug development. II: Anticonvulsant drug screening. *Epilepsia*, **1978**, *19*, 409-428.

[20] Loscher, W.; Schimdt, D. Strategies in antiepileptic drug development: Is rational drug design superior to random screening and structural variation. *Epilepsy Res*., **1994**, *17*, 95-134.

[21] Siddiqui, N.; Rana, A.; Khan, S. A.; Bhat, M. A.; Haque, S. E. Synthesis of benzothiazole semicarbazones as novel anticonvulsants- The role of hydrophobic domain. *Bioorg. Med. Chem. Lett*., **2007**, *17*, 4178-4182.

[22] Baxter, C. F.; Roberts, E. Elevation of Gamma amino butyric acid in brain: Selective inhibition of γ-aminobutyric-α-ketoglutaric acid transaminase. *J. Biol. Chem*., **1961**, *236*, 3287-3294.

[23] Roberts, E. *Methods in Enzymology*, Academic Press: New York, **1962**.

**Received: August 27, 2009 Revised: December 23, 2009 Accepted: December 23, 2009**