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Synthesis of 4 – aryl substituted semicarbazones and their terpenes derivatives : A newer scaffold as an anticonvulsant agents

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ABSTRACT

*A series of 4 – aryl substituted semicarbazones of some terpenes ie , citral (acyclic terpene) , camphor (bicyclic terpene) and menthone (monocyclic terpene) were synthesized from substituted anilines , to meet the structural requirements essential for anticonvulsant activity . The structures of the synthesized terpene semicarbazones were confirmed by I.R. , ^{1}H-NMR and elemental analysis. The synthesized semicarbazone derivatives were evaluated for anticonvulsant and sedative – hypnotic activity . After intraperitoneal injection to mice , the semicarbazone derivatives were examined by three chemoshock models for a single dose study . These three chemoshock model includes were , Isoniazid (INH) induced convulsion model , Thiosemicarbazide (TSC) induced convulsion model and 4 – aminopyridine (4-AMP) induced convulsion model . All the synthesized semicarbazone derivatives were also evaluated for neurotoxicity (N T) screen by rotorod test and sedative – hypnotic activity by using pentobarbitone induced narcosis model . All the compounds showed anticonvulsant activity in one or more test models . The preliminary result showed that all of the tested compounds were protective against INH and TSC screen at a dose of 30 mg kg $^{-1}$ at 0.5 h. Compounds (**2a, 4a and 4c)** were found to be most active against INH screen at a dose of 30 mg kg^{-1} showed prolonged duration of action for 4h . compound **4c** showed prolong activity at a dose of 30 mg kg^{-1} against TSC screen . In 4 – AMP screen all of the compounds except **(2a , 4a and 4c)** exhibited proconvulsion rather protection at a dose of 30mg kg^{-1} at 0.5 h. , they potentiate the convulsion at a dose of 30 mg kg^{-1} . None of the compound was found to be neurotoxic at a dose of 30 mg kg^{-1} . Compound **4c** showed most sedative activity in pentobarbitone induced narcosis model . compound 4a was found to be most potent anticonvulsant that showed activity in all sreens with no neurotoxicity and no sedative – hypnotic activity . In conclusion semicarbazones with terpenoid as the lipophilic moiety resulted in compounds with broad spectrum of anticonvulsant activity and therefore, they may be utilized for the future development of novel anticonvulsants with broad spectrum of anticonvulsant activity with no neurotoxicity and lesser sedative – hypnotic activity .*

Key words: Semicarbazone derivatives, Terpenes, Anticonvulsant activity, Sedative–hypnotic activity.

INTRODUCTION

Epilepsy is a common neurological disorder of central nervous system characterized by recurrent seizures of cerebral origin , presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness . Approximately 0.4 – 1 % of the population worldwide suffers from this disorder [1] . Around 75- 80 % of epileptic patients may be provided with adequate seizure controls with the help of conventional antiepileptic drugs such as phenytoin , carbamazepine, ethosuximide, primidone and valproate . These conventional anti-epileptic drugs suffers from a range of adverse effects such as drowsiness, ataxia, gastro-intestinal disturbances, hepatotoxicity, megaloblastic anaeimia and even life threatening condition . Furthermore the convulsion of 25% of epilepsies are inadequately controlled by currently available medication [2] . In the recent years, much efforts has been devoted to explore the novel approaches by elucidating the cellular and molecular mechanisms of the hyperexcitability to provide specific target for novel therapies and as a result several new drugs such as vigabatrin , lamotrigine , gabapentin, tiagabine, fosphenytoin , eslecarbamazepine have appeared in the market [3] . However all these drugs are not ideal and able to completely eradicate the disease as they can be associated with chronic and adverse side effects . Hence there is an urgent need to explore newer anticonvulsants with broad spectrum, lower neurotoxicity and lesser sedative activity.

Semicarbazones are of considerable interest due to their biological activity [4,5,6] . Dimmock et al have reported semicarbazones as potential anticonvulsant agents . A number of aryloxy aryl semicarbazones have shown significant anticonvulsant activity [7] . Further Padeya and his co-workers have also reported the synthesis of several analogs of semicarbazones and thiosemicarbazones [8 , 9, 10] .

Recently Pandeya et al. [11] have suggested a new pharmacophore model for semicarbazones displaying an anticonvulsant activity (Fig. 1). They proposed that the terminal amino function of semicarbazones was not essential for activity and could be substituted with a lipophilic aryl ring. Proposed pharmacophore model contain four binding sites for interaction with a macromolecular complex in vivo . These binding sites include:

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

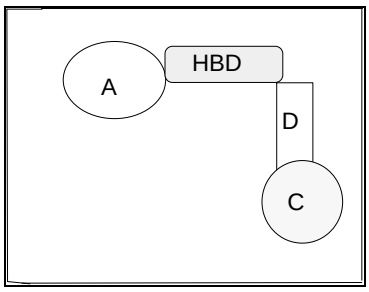

Fig. 1 : Suggested pharmacophore model for semicarbazone displaying anticonvulsant activity .

In present study a series of novel 4- phenyl substituted semicarbazones of some terpenes ie , acyclic terpene (citral) , monocyclic terpene (menthone) and bicyclic terpene (camphor) were synthesized and evaluated for their anticonvulsant activity , neurotoxicity screening and sedative hypnotic activity . The anticonvulsant activity of the synthesized terpenoidal semicarbazones were evaluated by using various chemoshock models ie , isoniazid (INH) screen model , thiosemicarbazide (TSC) screen model and 4- aminopyridine (4- AMP) induced convulsion model . The purpose behind the using of various chemical induced convulsion model was that all the three chemical precipitates convulsions in patients through different mechanism . Isoniazid (INH) can precipitate convulsions in patients by inhibiting GABA synthesis thus it lowers GABA level and the activity of glutamate decarboxylase (GAD) [12 , 13] . Thiosemicarbazide (TSC) can precipitate convulsion by inhibiting the GABA synthesis via cofactor antagonism through impairment of the synthesis or coenzyme action of pyridoxal phosphate [14, 15] . The 4- AMP (Amino pyridine) is the K^+ channel antagonist and it is a powerful convulsant in animals and in man .The drug readily penetrates the blood – brain barrier and is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release [16 , 17] . The synthesized semicarbazones derivatives act against these chemoshocks in one or more models and exhibited anticonvulsant activity .

EXPERIMENTAL SECTION

Chemistry

A series of 4 – Aryl substituted semicarbazones and their terpenes derivatives were synthesized according to the synthetic schemes as shown in figure 2 . Melting points (m.p.) were determined in open capillary tubes on a Jindal melting point apparatus and are uncorrected . Proton nuclear magnetic resonance (1H-NMR) spectra were recorded on Bruker model DRX 300 NMR spectrometer in DMSO – d_6 using tetramethyl silane(TMS) as an internal standard . Infra – red spectra (IR) were recorded on BIO – RAD FTS 135 spectrometer using KBr pellets . Elemental analysis (C , H and N) were undertaken with a Perkin – Elmer model 240C analyser , and all analysis were consistent with theoretical value (within 0.4%) unless indicated . The homogeneity of the compounds was monitored by ascending thin – layer chromatography (TLC) using silica gel G as stationary phase and visualized by iodine vapours . Solvent system was Chloroform : Methanol (9 : 1) . All the chemicals and solvents used were procured from E. Merck (India) , S.D. Fine Chemicals (India) . & Rankem (India.) .

Synthesis of para-substituted aromatic ureas (1a-c)

Different para- substituted aniline (0.1mol) were dissolved in 10 ml of glacial acetic acid and diluted to 100ml with water. Equimolar quantity (0.1mol) of sodium cyanate in 50 ml of warm water was added in previous solution with stirring. The reaction mixture was allowed to stand for 30 min, and then it was filtered, washed, dried and recrystallized from ethanol.

Synthesis of para-substituted aryl semicarbazide derivatives (1d-f)

To a solution of para- substituted phenyl urea (0.1mol) in 20 ml ethanol, an equimolar (0.1mol) quantity of hydrazine hydrate was added. The reaction mixture was made alkaline by adding sodium hydroxide and heated under reflux for 1-2 hr and then cooled in ice. The resultant product was filtered and recrystallized from ethanol.

Synthesis of 4 - substituted aryl semicarbazones (2a-c),(3a-c),(4a-c)

To a solution of p-phenyl semicarbazide (0.01mol) in 20 ml of ethanol, an equimolar (0.01mol) quantity of appropriate terpenes was added and pH of the reaction mixture was adjusted between 4-5 by adding glacial acetic acid. The mixture was refluxed for 45min to 1.5h and then cooled in

ice bath .In some cases; the solution was poured on crushed ice to induce crystallization. The resultant precipitates were filtered, dried and recrystallized from ethanol.

Fig. 2 : Synthetic Schemes

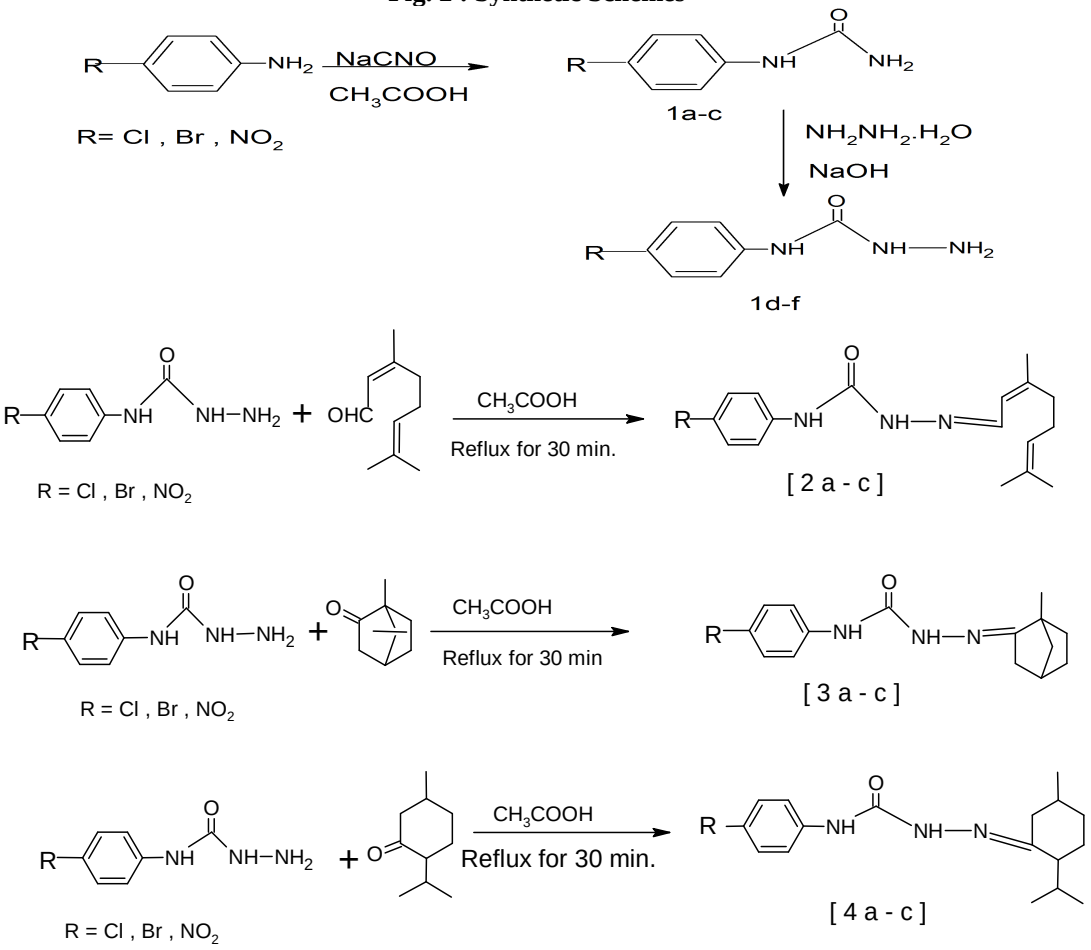

Table 1. Physicochemical properties of synthesized compounds

Compd.	Mol. formula	M. wt.	M.P. (Ċ)	R_f Value	% Yield	Log P
1a	$C_7H_7ClN_2O$	170.596	208-210	0.56	64	1.17
1b	$C_7H_7BrN_2O$	215.047	216-218	0.62	72	1.44
1c	$C_7H_7N_3O_3$	181.148	170-172	0.83	76	0.50
1d	$C_7H_8ClN_3O$	185.610	228-230	0.65	67	-
1e	$C_7H_8BrN_3O$	230.061	240- 242	0.70	75	1.20
1f	$C_7H_8N_4O_3$	196.163	190-192	0.64	70	0.34
2a	$C_{17}H_{22}ClN_3O$	319.829	258-260	0.57	67	4.00
2b	$C_{17}H_{22}BrN_3O$	364.280	286-288	0.68	78	4.27
2c	$C_{17}H_{22}N_4O_3$	330.381	230-232	0.63	73	3.00
3a	$C_{17}H_{24}ClN_3O$	321.844	198-200	0.54	72	4.73
3b	$C_{17}H_{24}BrN_3O$	366.295	168-170	0.76	75	5.00
3c	$C_{17}H_{24}N_4O_3$	332.397	150-152	0.65	78	3.57
4a	$C_{17}H_{24}ClN_3O$	321.845	218-220	0.56	68	4.89
4b	$C_{17}H_{24}BrN_3O$	366.295	268-270	0.65	64	5.16
4c	$C_{17}H_{24}N_4O_3$	332.397	206-208	0.77	78	3.23

Characterization of the synthesized semicarbazone derivatives :

(2z)-3, 7-dimethyl octa-2, 6-dienal N-(4-chlorophenyl)semicarbazone [2a]

IR(KBr) cm^{-1} 3420 (secondary NH), 3315 (amide NH), 1608 (C=N), 1645 (NH-CO-NH), 2919 (Ar CH stretch). ^1H NMR (DMSO-d_6, δ ppm) 1.71(s, 3H, CH_3), 1.9(s, 6H, $2CH_3$), 2.24(m, 4H, $2CH_2$), 4.8(t, 1H, =CH), 5.46(d, 1H, =CH-CH=N), 5.7(s, 1H, CONH), 7.2-7.5(m, 4H, p-chlorophenyl), 7.8(d, 1H, CH=N), 8.75(s, 1H, =NNH). Elemental analysis calculated for $C_{17}H_{22}ClN_3O$. Calculated C 63.84; H 6.93; N 13.14; Found C 63.81; H 6.91; N 13.12.

(2z)-3, 7-dimethyl octa-2, 6-dienal N-(4-bromophenyl)semicarbazone [2b]

IR(KBr) cm^{-1} 3425 (secondary NH), 3310 (amide NH), 1610 (C=N), 1655 (NH-CO-NH), 2929 (Ar CH stretch). ^1H NMR (DMSO-d_6, δ ppm) 1.71(s, 3H, CH_3), 1.9(s, 6H, $2CH_3$), 2.24(m, 4H, $2CH_2$), 4.8(t, 1H, =CH), 5.46(d, 1H, =CH-CH=N), 5.7(s, 1H, CONH), 7.4-7.5(m, 4H, p-bromophenyl), 7.8 (d, 1H, CH=N), 8.75(s, 1H, =NNH). Elemental analysis calculated for $C_{17}H_{22}BrN_3O$. Calculated C 56.05; H 6.09; N 11.54;. Found C 56.02; H 6.07; N 11.52.

(2z)-3, 7-dimethyl octa-2, 6-dienal N-(4-nitrophenyl)semicarbazone [2c]

IR(KBr) cm^{-1} 3428 (secondary NH), 3308 (amide NH), 1619 (C=N), 1658 (NH-CO-NH), 1533(asymmetric Ar-NO_2 stretch), 1355 (symmetric NO_2 Stretch). ^1H NMR (DMSO-d_6, δ ppm) 1.71(s, 3H, CH_3), 1.9(s, 6H, $2CH_3$), 2.24(m, 4H, $2CH_2$), 4.8(t, 1H, =CH), 5.46(d, 1H, =CH-CH=N), 5.7(s, 1H, CONH),7.6-7.8(m, 4H, p-nitrophenyl),7.8(d, 1H, CH=N) , 8.75(s, 1H, =NNH). Elemental analysis calculated for $C_{17}H_{22}N_4O_3$ Calculated C 61.80; H 6.71; N 16.96; Found C 61.78; H 6.70; N 16.94.

(2E)-1-methyl bicyclo [2,2,1]heptan-2-one N-(4-chlorophenyl)semicarbazone ethane [3a]

IR(KBr) cm^{-1} 3420 (secondary NH), 3315 (amide NH), 1608 (C=N), 1645 (NH-CO-NH), 2919 (Ar CH stretch). ^1H NMR (DMSO-d_6, δ ppm) 7.25-7.58(m, 4H, p-chlorophenyl) 6.1(s, 1H, CONH), 7.1(s, 1H, =NNH), 0.86-1.5(m, 16H, camphor ring). Elemental analysis calculated for $C_{17}H_{24}ClN_3O$ Calculated C 63.44; H 7.52; N 13.06, Found C 63.42; H 7.51; N 13.02.

(2E)-1-methyl bicyclo [2,2,1]heptan-2-one N-(4-bromophenyl) semicarbazone ethane (1:1). [3 b]

IR(KBr) cm^{-1} 3425 (secondary NH), 3310 (amide NH), 1610 (C=N), 1655 (NH-CO-NH), 2929 (Ar CH stretch). ^1H NMR (DMSO-d_6, δ ppm) 7.41-7.53(m, 4H, p-bromophenyl), 6.1(s, 1H, CONH), 7.1(s, 1H, =NNH), 0.86-1.5(m, 16H, camphor ring). Elemental analysis calculated for $C_{17}H_{24}BrN_3O$ Calculated C 55.74; H 6.60; N 11.47, Found C 55.72; H 6.58; N 11.45.

(2E)-1-methyl bicyclo [2,2,1]heptan-2-one N-(4-nitrophenyl)semicarbazone ethane (1:1). [3c]

IR(KBr) cm^{-1} 3428 (secondary NH), 3308 (amide NH), 1619 (C=N), 1635 (NH-CO-NH), 1533(asymmetric Ar NO_2 stretch), 1355 (symmetric NO_2 Stretch). ^1H NMR (DMSO-d_6, δ ppm) 7.90-8.17(m, 4H, p-nitrophenyl), 6.1(s, 1H, CONH), 7.1(s, 1H, =NNH), 0.86-1.5(m, 16H, camphor ring). Elemental analysis calculated for $C_{17}H_{24}N_4O_3$ Calculated C 61.43; H 7.28; N 16.86, Found C 61.42; H 7.25; N 16.84.

2-isopropyl-5-methyl cyclohexan-1-one N-(4-chlorophenyl) semicarbazone [4a]

IR(KBr) cm^{-1} 3380 (secondary NH), 3315 (amide NH), 1425 (C=N str), 1655 (NH-CO-NH), 2919 (Ar-CH stretch). ^1H NMR (DMSO-d_6, δ ppm) 7.25-7.58(m, 4H, p-chlorophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02(m, 9H, $3CH_3$), 2.08-2.5 (m, 3H, CH), 0.76-0.99 (m, 6H, $3CH_2$). Elemental analysis calculated for $C_{17}H_{24}ClN_3O$ Calculated C 63.44; H 7.52; N 13.06 Found C 63.42; H 7.51; N 13.02.

2-isopropyl-5-methyl cyclohexan-1-one N-(4-bromoophenyl) semicarbazone [4b]
IR(KBr) cm^{-1} 3365 (secondary NH), 3320 (amide NH), 1415 (C=N str), 1660 (NH-CO-NH), 2915 (Ar-CH stretch). ^{1}H NMR (DMSO-d_6, δ ppm) 7-7.6(m, 4H, p-bromophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02(m, 9H, $3CH_3$), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, $3CH_2$). Elemental analysis calculated for $C_{17}H_{24}BrN_3O$ Calculated C 55.74; H 6.60 N 11.47 Found C 55.72; H 6.58; N 11.45.

2-isopropyl-5-methyl cyclohexan-1-one- N-(4-nitrophenyl) semicarbazone [4c]
IR(KBr) cm^{-1}3260(N-H str)NH), 1450 (C=N str), 1685 (C=Ostr). ^{1}H NMR (DMSO-d_6, δ ppm) 7.2-7.5 (m, 4H, p-chlorophenyl), 3.5(s, 1H, CONH), 9.7(s, 1H, =NNH), 1.52-2.02 (m, 9H, $3CH_3$-), 2.08-2.5 (m, 3H, CH), 0.76-0.99(m, 6H, $3CH_2$). Elemental analysis calculated for $C_{17}H_{24}N_4O_3$ Calculated C 61.43; H 7.28; N 16.86; Found C 61.41; H 7.25; N 16.84.

Pharmacology

Animals

The healthy Swiss albino mice of both sexes weighing 25-30 g were taken for the study. The animals were kept in large spacious hygienic cages during the course of experimental period. The animals had free access to standard commercial diet and water ad libitum and were kept in rooms maintained at 22 ± 1^0C with 12h light dark cycle.

Anticonvulsant screening

The anticonvulsant activities were established by the anticonvulsant drug development (ADD) programme protocol . [18]

Isoniazid-induced convulsions

Procedure

Mice of either sex with a weight of 18 to 22 g are treated with the test compounds or the standard (e.g. diazepam 10 mg/kg i.p.) by oral or intraperitoneal administration. Controls receive the vehicle only. 30 min after i.p. or 60 min after p.o. treatment the animals are injected with a subcutaneous dose of 300 mg/kg isoniazid (isonicotinic acid hydrazide). During the next 120 min the occurrence of clonic seizures, tonic seizures and death is recorded. [12] [13]

Thiosemicarbazide Induced Convulsion Model

Procedure

mice of either sex with a weight of 25-30g were treated with the test compounds or the standard (e.g. diazepam 10 mg/kg b.w.) by intraperitoneal administration. Controls received the vehicle only. 30 min after i.p. treatment the animals were injected with a subcutaneous dose of 20 mg/kg thiosemicarbazide. During the next 120 min. the occurrence of clonic seizures, tonic seizures and death were recorded. [14] [15]

4 – Aminopyridine Induced Convulsion Model

Procedure

Male NIH Swiss mice weighing 25–30 g were allowed to acclimatize with free access to food and water for a 24-h period before testing. Test drugs were administered at a dose of 30mg/kg b.w. intraperitoneally, 30 min prior to S.C. injection of 4-aminopyridine at a dose of 13.3 mg/kg. Controls treated with 4-aminopyridine only exhibit characteristic behavioral signs, such as hyper reactivity, trembling, intermitted forelimb/hindlimb clonus followed by hindlimb extension, tonic seizures, opisthotonus and death. The standard drug phenytoin at a dose of 30mg/kg body weight was taken for comparison. [16] [17] [19]

Neurotoxicity Screening

Rotorod test

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotate at 6 revolutions per minute. The rod diameter was 3.2 cm. neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials. The dose at which the animals was unable to grasp the rotorod, was determined . [18] [20]

Sedative – Hypnotic Activity

Pentobarbitone induced narcosis model

This test was performed using the test substance at a dose of 30 mg/kg only . The drug was administered to a group of six animals . The animals were injected with a solution of pentobarbitone sodium (in PEG 200) at a dose of 30mg/kg after 30 min . The animals were then placed on their back and the loss of righiting reflex was taken as the onset of sleep . The time taken by the animals to awake was noted . a control was also performed after pretreatment with the test substance vehicle (PEG – 200) . [21]

Table 2. Anticonvulsant and neurotoxicity data of compounds

Comp. code	INH Screen		TSC screen		4-AMP screen		Neurotoxicity screen	
	0.5 h	4h	0.5h	4h	0.5h	4h	0.5h	4h
2a	30	30	30	(-)	30	(-)	---	----
2b	30	(-)	30	(-)	Proconvulsion	(-)	---	---
2c	30	(-)	30	(-)	Proconvulsion	(-)	---	----
3a	30	(-)	30	(-)	Proconvulsion	(-)	----	----
3b	30	(-)	30	(-)	Proconvulsion	(-)	----	----
3c	30	(-)	30	(-)	Proconvulsion	(-)	----	----
4a	30	30	30	30	30	(-)	-----	----
4b	30	(-)	30	(-)	Proconvulsion	(-)	----	---
4c	30	30	30	(-)	30	(-)	----	----
Diazepam	10	10	10	(-)	Proconvulsion	(-)	----	----
Phenytoin					30		----	----

[a]Figures in the table indicates the dose in mg kg^{-1} were administered intraperitoneal injection in mice . The animals were examined 0.5h and 4h after injections were made .

[b]The dash (-) indicates an absence of activity at a dose of 30 mg kg^{-1} administered i.p..

[c]The dash ------ indicates the absence of neurotoxicity at a dose of 30 mg kg^{-1} administered i.p.

Table 3. Sedative – Hypnotic activity data of compounds

COMPOUND CODE	DOSE (mg kg^{-1})	MEAN SLEEPING TIME MEAN ± S.E.M.
Pentobarbitone (Control)	30	56 ± 11.47
2a	30	65 ± 9.00
2b	30	58 ± 12.04
2c	30	63 ± 10.98
3a	30	68±12.6
3b	30	114.34±2.51**
3c	30	148±12.15**
4a	30	69.66±2.51
4b	30	157±12.09**
4c	30	237.27±1.50**

[a]Compounds were tested at a dose of 30 mg kg^{-1} (i.p.)

[b]Each value represents the mean SEM of six rats significantly different from the control (P <0.5) (student's t – test)

RESULTS AND DISCUSSION

The results of anticonvulsant screening and neurotoxicity data are given in table 2 . The anticonvulsant screening were performed in mice at dose of 30 mg kg^{-1}(single dose study) by 3 chemoshock methods ie. , Isoniazid (INH) induced convulsion model , Thiosemicarbazide(TSC) induced convulsion model and 4-Amino pyridine (4 –AMP) induced convulsion model . All of the compounds showed activity at a dose of 30 mg kg^{-1} at 0.5 h against Isoniazid and Thiosemicarbazide screen . The compounds 2a , 4a and 4c showed activity at a dose of 30 mg kg^{-1} at 0.5h in 4 – aminopyridine screen , other compounds showed proconvulsions rather protection . In isoniazid screen the compounds 2a , 4a and 4c showed protection for a longer duration of action at a dose of 30 mg kg^{-1} at 4h. In thiosemicarbazide screen the only compound 4a was found to be active for a longer duration at a dose of 30 mg kg^{-1} at 4h. No any compound was found to be active at a dose of 30 mg kg^{-1} at 4h in 4-aminopyridine screen . The compounds 4a and 4c were found to be most potent anticonvulsants because they showed activity in all the three screening model . The compound 2a was found to be most active at a dose of 30 mg kg^{-1} only in isoniazid screen model . The reference compound, Diazepam was active in isoniazid screen at a dose of 10 mg kg^{-1} at 0.5h and 4h. However the diazepam as reference drug was active at a dose of 10 mg kg^{-1} only at 0.5h in thiosemicarbazide screen . The reference compound phenytoin was active only in 4- Aminopyridine screen at a dose of 30 mg kg^{-1} at 0.5 h. only . All the synthesized semicarbazone derivatives were also evaluated for neurotoxicity screen using rotorod test . The result of neurotoxicity screen showed that no compound was found to be neurotoxic at a dose of 30 mg kg^{-1} at 0.5 h and 4h. In general menthone semicarbazone exhibited potent anticonvulsant activity in comparision to the corresponding citral and camphor semicarbazones . The p-chloro phenyl substituted semicarbazone of both cyclic and acyclic terpene were most active , showing broad spectrum of activity at a dose of 30 mg kg^{-1} and at the same time show no neurotoxicity . Most of the compounds in isoniazid and thiosemicarbazide screen exhibited anticonvulsant activity at 30 min rather than 4h. Therefore it can be said that the onset of action for the compounds is rapid .

In the sedative hypnotic evaluation (table 3), compounds 3b , 3c , 4b and 4c were found to potentiate the pentobarbitone induced narcosis and other compounds showed not significant increase in sedative – hypnotic activity . Hence compounds 2a and 4a emerged as the most promising anticonvulsant agents with no neurotoxicity and less sedative activity .

CONCUSION

The series of 4 – aryl substituted terpenes semicarbazones were found to posess significant anticonvulsant activities . In terms of interaction at the binding site, as proposed previously by Dimmock et al.[7] , the pharmacophoric descriptors were thought to be a lipophilic aryl ring and a hydrogen bonding semicarbazone moiety . The attachment of a second aryl ring designated as the distal ring to the proximal aryl ring to increase the vanderwalls bonding at the binding site and to increase potency have been studied [7] . In all these studies the terminal amino function was found to be free . In present study , terminal amino group was substituted with terpene moiety in contrast to dimmock study , to reveal the importance of the terminal amino function for anticonvulsant activity . Substitution at the terminal amino groups with terpene moiety increases the lipophilicity of the molecules and improves pharmacokinetic properties of the molecules . The pharmacophore model of these aryl semicarbazones resemble that of the standard anticonvulsants as shown in figure , and the 4 – arylsubstituted terpenes semicarbazones largely resemble the structure of desmethyl diazepam . Hence these semicarbazones could emerge as bioisosters of desmethyl diazepam (CH_2 replaced with NH) .

Overall , our results validated the pharmacophore model proposed by Pandeya et al . with four binding sites essential for anticonvulsant activity . Thus 4- aryl substituted terpene semicarbazones emerged as an anticonvulsants with broad spectrum of anticonvulsant with no neurotoxicity and less sedative activity .

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REFERENCES

[1] Ho, B. , Crider , A.M. , Stables , J.P. *Eur.J.Med Chem.* , **2001** 36 : 265 – 286 .
[2] Craig, C. R. Anticonvulsant Drugs . In : Craig , C. R. , Stitzel , R.E. (Eds.), Modern Pharmacology with Clinical Application . 5th edition , Little Brown and Company , New York, , **1997**, 391 – 405 .
[3] Willmore , L.J. *Epilepsy Behav.* **2005**; 7 (suppl. 3) : 25 – 28 .
[4] Pandeya , S.N. , Mishra , V. , *Acta Pharm* . **2001** 51 , 183 – 188.
[5] Singhal, M.; Paul, A; Singh, H.P; Dubey, S.K.; Gaur,K; *J. Chem. Pharm. Res*., **2011**, 3 (3): 639 – 645 .
[6] Rajasekaran, S.; Rao, G.K. ; Pai, S. ; Sodhi, G.S. ; *J.Chem.Pharm.Res.* , **2010** , 2 (1), 482 – 488 .
[7] Dimmock , J.R. , Puthucode , R.N. , Smith , J.M. , *J. Med Chem.* **1996** , 39(20) , 3984-3997
[8] Pandeya , S.N. , Mishra , V. , Singh , P.N. *Pharmacological Res.***1998**, 37, 17 – 22 .
[9] Pandeya , S.N. , Mishra , V., Stable , J.P, *Polish J. Pharmacol.* **2000,** 52 , 283 – 290 .
[10] Pandeya , S.N. , Sowmayalakshmi , V. , Panda , S.S. , Pandey, A. , Stable , J.P. Anticonvulsant activity of semicarbazone derivatives of Mannich bases . *Indian J Chem.* **2003**, 42B , 2657 – 2661 .
[11] Pandeya , S.N. , Raja , A.S. and Stable , J.P. *J. Pharm. Pharmaceut. Sci.* ,**2002**, 5 (3), 266 – 271 .
[12] Vogel , H.G. , Drug discovery and evaluation : pharmacological assays , Berlin : Springer Verlag , New York , **2002** , 696 – 716 .
[13] Lioscher , U. , Breg , H.H. , *Arch. Pharmacol.* , **1977** , 296 (3) , 263 – 269 .
[14] Nishie , K. , Weary , M. , Berger , A. *The J. Pharmacol . Exp. Thera.* , **1996** , 153 (3) , 387 – 395 .
[15] Wada, J.A. , Asakura, T. , *Exp. Neuro* . , **1969** , 24 (1) , 19 – 37 .
[16] Yamaguchi , S.I. , Rogawski , M.A. *Epilepsy Res* , **1992** , 11 , 9 – 16 .
[17] Oyama , Y. , *Brain Res.* , **1987** , 409 (2) , 243 – 249 .
[18] Krall, R.L. , Penry, J.K. , White, B.G. , Kupferberg, H.J. , Swinyard, E.A. Antiepileptic drug development II ; Anticonvulsant drug screening . *Epilepsia*, **1978**; 19 , 409 – 28.
[19] Grafe , P. , Galvan , M. , Bruggencate , G, *Brain Res.* , **1982** , 241 , 75 – 86 .
[20] Bhragual, D.D. , Kumar , N. , Drabu, S. *J.Chem.Pharm.Res*., **2010**, 2 (2), 345 – 349.
[21] Pandeya , S.N. , Aggarwal , N. , Jain , J.S. , *Pharmazie* ,**1999**, 54 , 4 , 300 – 302 .