European Journal of Medicinal Chemistry 45 (2010) 1536–1543

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Triazole incorporated thiazoles as a new class of anticonvulsants: Design, synthesis and *in vivo* screening

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ARTICLE INFO

Article history:
Received 15 April 2009
Received in revised form
14 December 2009
Accepted 23 December 2009
Available online 13 January 2010

Keywords:
Thiazoles
Triazoles
Anticonvulsants
Neurotoxicity
Distance mapping

ABSTRACT

Various 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones (**7a**–**t**) were designed keeping in view the structural requirements suggested in the pharmacophore model for anticonvulsant activity. Thiazole and triazole moieties being anticonvulsants were clubbed together to get the titled compounds and their *in vivo* anticonvulsant screening were performed by two most adopted seizure models, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ). Two compounds **7d** and **7f** showed significant anticonvulsant activity in both the screens with ED_{50} values 23.9 mg/kg and 13.4 mg/kg respectively in MES screen and 178.6 mg/kg and 81.6 mg/kg respectively in scPTZ test. They displayed a wide margin of safety with Protective index (PI), median hypnotic dose (HD_{50}) and median lethal dose (LD_{50}) much higher than the standard drugs.

1. Introduction

Epilepsy is a group of disorders of the central nervous system characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbances of consciousness, with or without characteristic body movements (convulsions), sensory or psychiatric phenomenon [1]. Estimates suggest that available medication controls the seizures in only 50% of patients or decreases the incidence in only 75% of patients [2]. The search for antiepileptic agents with more selectivity and lower toxicity continues to be an area of investigation in medicinal chemistry.

In the effort to get those agents we have reported [3–6] several five membered heterocyclic compounds, which have shown considerable anticonvulsant activities. As part of our continuous research in this area we have designed and synthesized several new 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones (**7a**–**t**) by incorporating triazole moiety into the thiazole ring.

Since the past few decades, the literature has been enriched with progressive findings about the anticonvulsant activities of various substituted thiazole derivatives [7,8]. In addition many triazoles have attracted much attention due to their prominent utilization as anticonvulsants [9,10]. The present series has been designed by clubbing both the pharmacologically active moieties viz. thiazole and triazole and the resulting compounds are expected to have the synergistic effect in dealing with the epilepsy.

All the titled compounds comprised of the four pharmacophoric elements (Fig. 1) that are necessary for good anticonvulsant activity as suggested by Pandeya et al. [11]. These elements are present in many currently used antiepileptic drugs. These are hydrophobic domain **A**, Hydrogen bonding domain **HBD**, electron donor moiety **D**, and distal hydrophobic domain **R**.

The attachment of a second aryl ring designated as the distal ring to the proximal aryl ring to increase the Van der Waal's bonding at the binding site and to increase potency have also been reported [12]. The present work further gives impetus to these observations.

Our work also highlights the distance mapping and matching of the synthesized compounds with the help of the given model.

2. Chemistry

The synthesis of the titled compounds (**7a**–**t**) was carried out as presented in Scheme 1. Appropriate substituted acetophenones were brominated and refluxed with thiourea in acetic acid to get the substituted thiazoles (**2a**–**d**). These thiazole derivatives on refluxing with ethylchloroformate and triethylamine afforded the carbamate derivatives (**3a**–**d**). On the other hand, the substituted phenylthioureas (**4a**–**e**) were obtained by treating the substituted anilines with ammonium thiocyanates in presence of

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doi:10.1016/j.ejmech.2009.12.062

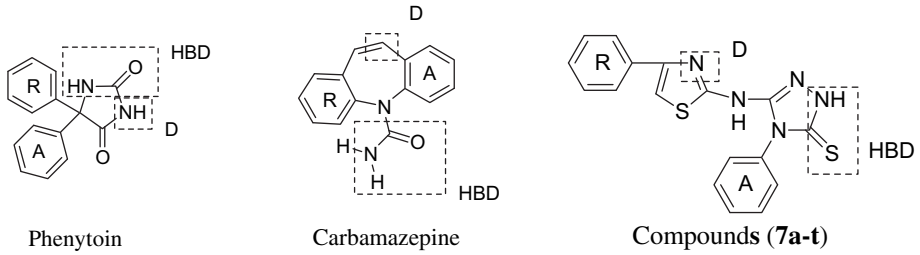


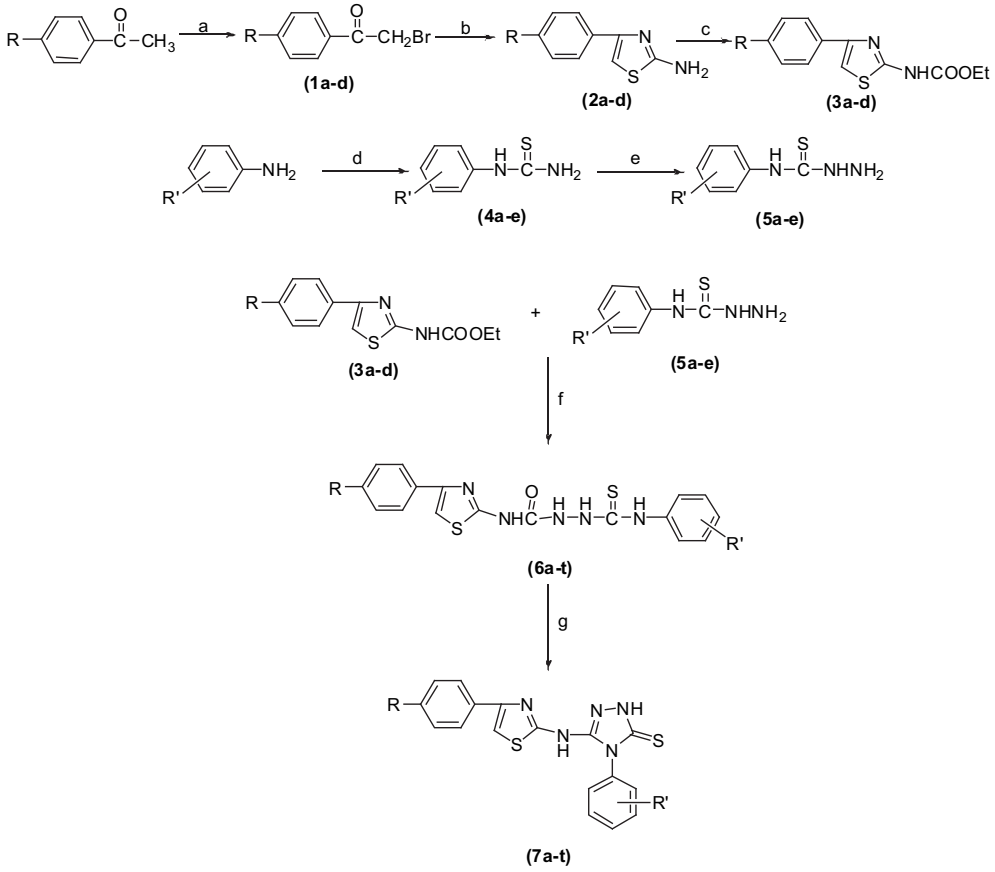
Fig. 1. Anticonvulsant agents showing the required pharmacophoric elements.

dilute hydrochloric acid. These phenylthioureas were then refluxed with hydrazine hydrate to afford hydrazinecarbothioamides (**5a**–**e**). The hydrazinecarbothioamides on condensation with different substituted carbamates (**3a**–**d**) in presence of ethanol gave substituted hydrazinecarboxamides (**6a**–**t**). Finally the substituted hydrazinecarboxamides were cyclized in presence of aqueous sodium hydroxide to yield the titled compounds (**7a**–**t**). The physicochemical parameters of the synthesized compounds are presented in Table 1. The structures and purity of the final compounds were confirmed in the basis of spectral and elemental analyses and the data were within ±0.4% of the theoretical values.

3. Pharmacology

The pharmacological testing of all the final compounds were performed according to the standard protocol given by epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program [13].

The Phase I pharmacological screening comprised MES, scPTZ and neurotoxicity. Compounds were administered intraperitoneally as a solution in polyethylene glycol (PEG). The most active compounds were evaluated quantitatively in phase II screening in which the ED_{50} and TD_{50} of the compounds were determined.



Reactions and conditions: a) Br_2, Chloroform, r. t. b) Thiourea, glacial acetic acid, reflux
c) Ethylchloroformate, triethylamine, benzene, reflux d) NH_4SCN, HCl e) $NH_2.NH_2.H_2O$, ethanol, reflux
f) ethanol, reflux g) 2N NaOH, reflux

Scheme 1. Synthetic route to the synthesized compounds (**7a**–**t**).

Table 1
Physicochemical parameters of the titled compounds (**7a–t**).

Compound	R	R′	Mol. formula[a]	Log *P*[b] found (calculated)	R_f[c] value	Elemental analyses % found (calculated)		
						C	H	N
7a	Cl	2-CH_3	$C_{18}H_{14}ClN_5S_2$	4.39 (4.67)	0.69	54.10 (54.06)	3.50 (3.53)	17.47 (17.51)
7b	Cl	4-CH_3	$C_{18}H_{14}ClN_5S_2$	4.43 (4.67)	0.78	54.12 (54.06)	3.55 (3.53)	17.49 (17.51)
7c	Cl	2-OCH_3	$C_{18}H_{14}ClN_5OS_2$	4.32 (4.09)	0.54	51.93 (51.98)	3.32 (3.39)	16.81 (16.84)
7d	Cl	4-OCH_3	$C_{18}H_{14}ClN_5OS_2$	4.39 (4.09)	0.61	51.91 (51.98)	3.35 (3.39)	16.87 (16.84)
7e	Cl	H	$C_{17}H_{12}ClN_5S_2$	4.46 (4.17)	0.68	52.96 (52.91)	3.16 (3.13)	18.21 (18.15)
7f	Br	2-CH_3	$C_{18}H_{14}BrN_5S_2$	5.08 (4.82)	0.74	48.59 (48.65)	3.23 (3.18)	15.71 (15.76)
7g	Br	4-CH_3	$C_{18}H_{14}BrN_5S_2$	4.98 (4.82)	0.79	48.71 (48.65)	3.19 (3.18)	15.70 (15.76)
7h	Br	2-OCH_3	$C_{18}H_{14}BrN_5OS_2$	4.48 (4.24)	0.72	46.91 (46.96)	3.01 (3.07)	15.26 (15.21)
7i	Br	4-OCH_3	$C_{18}H_{14}BrN_5OS_2$	4.41 (4.24)	0.64	46.92 (46.96)	3.12 (3.07)	15.29 (15.21)
7j	Br	H	$C_{17}H_{12}BrN_5S_2$	4.49 (4.32)	0.71	47.51 (47.45)	2.78 (2.81)	16.35 (16.27)
7k	NO_2	2-CH_3	$C_{18}H_{14}N_6O_2S_2$	3.52 (3.71)	0.50	52.61 (52.67)	3.49 (3.44)	20.49 (20.47)
7l	NO_2	4-CH_3	$C_{18}H_{14}N_6O_2S_2$	3.59 (3.71)	0.66	52.64 (52.67)	3.39 (3.44)	20.41 (20.47)
7m	NO_2	2-OCH_3	$C_{18}H_{14}N_6O_3S_2$	3.44 (3.13)	0.70	50.71 (50.70)	3.29 (3.31)	19.74 (19.71)
7n	NO_2	4-OCH_3	$C_{18}H_{14}N_6O_3S_2$	3.40 (3.13)	0.77	50.74 (50.70)	3.29 (3.31)	19.77 (19.71)
7o	NO_2	H	$C_{17}H_{12}N_6O_2S_2$	3.09 (3.21)	0.63	51.48 (51.51)	3.12 (3.05)	21.19 (21.20)
7p	H	2-CH_3	$C_{18}H_{15}N_5S_2$	4.21 (3.94)	0.49	59.19 (59.16)	4.09 (4.14)	19.22 (19.16)
7q	H	4-CH_3	$C_{18}H_{15}N_5S_2$	4.11 (3.94)	0.52	59.09 (59.16)	4.16 (4.14)	19.11 (19.16)
7r	H	2-OCH_3	$C_{18}H_{15}N_5OS_2$	3.24 (3.37)	0.57	56.69 (56.68)	3.91 (3.96)	18.41 (18.36)
7s	H	4-OCH_3	$C_{18}H_{15}N_5OS_2$	3.19 (3.37)	0.64	56.71 (56.68)	3.89 (3.96)	18.34 (18.36)
7t	H	H	$C_{17}H_{13}N_5S_2$	3.59 (3.44)	0.53	58.07 (58.10)	3.71 (3.73)	19.96 (19.93)

[a] Solvent of crystallization–Ethanol.
[b] Log *P* was determined by octanol:phosphate buffer method; *C*Log *P* was calculated using software ChemOffice 6.0.
[c] Solvent system- Toluene: Ethyl acetate: Formic acid (5:4:1).

These compounds were also tested for their median hypnotic dose (HD_{50}) and median lethal dose (LD_{50}) in phase III screening. The promising nature of the compounds prompted us to undertake the phase IV screening in which the ED_{50} and TD_{50} values were determined after the oral administration (p.o.) of the compounds.

4. Results and discussion

4.1. Anticonvulsant activity

The anticonvulsant activity was assessed by two most adopted animal models electroshock (MES) and chemoshock (scPTZ) methods. All the synthesized compounds were administered intraperitoneally into mice using doses of 30, 100 and 300 mg/kg and the observations were taken at two different time intervals (0.5 h and 4.0 h). Neurological impairment was evaluated by rotorod method and the data are presented in Table 2.

In the phase I preliminary anticonvulsant screening, all the compounds showed some degree of protection in MES screen which was the indicative of the good ability of these compounds to prevent the seizure spread. Among these compounds, **7d** and **7f** showed protection from seizure at the lowest dose 30 mg/kg after 0.5 h. Interestingly, compound **7f** continued the anticonvulsant activity at 4.0 h also which indicated the promising nature of the compound having quick onset and long duration of action. Compound **7d** was also active at 4.0 h but at a higher dose of 100 mg/kg. These compounds were also devoid of any sign of neurotoxicity. Majority of the compounds were active at a dose of 100 mg/kg after 0.5 h. These include compounds **7a**, **7c**, **7g**, **7k**, **7l**, **7m**, **7n**, **7p**, **7r** and **7s**. Among these compounds, **7c**, **7k**, **7m** and **7s** were also found to be active at the same dose after 4.0 h.

In the scPTZ screen, compounds that were found to be active include **7c**, **7d**, **7f**, **7g**, **7k**, **7l** and **7q**. Among these compounds, **7c**, **7f** and **7k** were active at 100 mg/kg after 0.5 h. Compounds **7f** and **7k** were also found to be active after 4.0 h but at a higher dose 300 mg/kg. Compounds that were active at 300 mg/kg dose after 0.5 h were **7d**, **7g**, **7l** and **7q**. Compound **7g** was also active at the same dose after 4.0 h.

In the neurotoxicity screening, compounds that were devoid of minimal motor impairment at any dose were **7d**, **7e**, **7f**, **7i**, **7q**, **7r** and **7s**. Rest all the compounds showed some degree of neurotoxicity but were less neurotoxic than the standard drug phenytoin.

The design of compounds was in such a way that the phenyl ring attached to the thiazole moiety was substituted with electron withdrawing groups and the phenyl ring attached to the triazole moiety was substituted with different electron releasing groups at different positions. The structure activity relationship was studied

Table 2
Anticonvulsant activity and minimal motor impairment of the synthesized compounds (**7a–t**).

Compound	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Neurotoxicity screen	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
7a	100	–[b]	–	300	300	300
7b	300	300	–	–	–	300
7c	100	100	100	–	300	–
7d	30	100	300	–	–	–
7e	–	300	–	–	–	–
7f	30	30	100	300	–	–
7g	100	300	300	300	–	100
7h	300	300	–	–	100	300
7i	300	300	–	–	–	–
7j	–	300	–	–	–	300
7k	100	100	100	300	300	100
7l	100	300	300	–	100	100
7m	100	100	–	–	100	300
7n	100	300	–	300	–	300
7o	–	300	–	–	300	300
7p	100	–	–	–	–	300
7q	–	300	300	–	–	–
7r	100	300	–	–	–	–
7s	100	100	–	300	–	–
7t	300	–	–	300	–	300
Phenytoin	30	30	–	–	100	100
Ethosuximide	–	–	100	300	–	–
Phenobarbital	100	30	–	–	100	300

[a] Number of animals used = 6; Solvent used- Polyethylene glycol; Dose of 30, 100 and 300 mg/kg were administered i.p. 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 h and 4 h after injections were administered.
[b] The dash (–) indicates an absence of activity at maximum dose administered (300 mg/kg).

and found that the derivatives with nitro group attached to the phenyl ring were most active of the series but were most neurotoxic too. The effect of electron releasing groups was found to be uncertain on the anticonvulsant activity. Derivatives having methyl group attached to the second position of the phenyl ring were comparatively more effective than the derivatives having methyl group at the fourth position. The unsubstituted derivatives were found to be least neurotoxic.

In the phase II anticonvulsant screening, the two most active compounds **7d** and **7f** were quantitatively evaluated for their anticonvulsant activity (ED_{50}) and neurotoxicity (TD_{50}). The results are presented in Table 3. Both the compounds showed comparable anticonvulsant activity and higher protective index than the standard drugs. Compound **7f** possessed strong anti-MES activity with ED_{50} of 13.4 mg/kg, which was close to currently used antiepileptic drugs phenytoin and carbamazepine and better than that of phenobarbital and valproate. The motor impairment caused by it was minimal and was markedly lower than all the drugs compared. It showed a protective index of 51.0, which was many folds higher than the current antiepileptic drugs having the PI values in the range of 1.6–8.1. Compound **7d** also displayed a better profile of anticonvulsant activity with lesser neurotoxicity (PI = 18.3). Since the activity of both the compounds were very promising in both phase I and phase II screening, therefore they were chosen to be further evaluated in phase III and phase IV screening.

In phase III pharmacological testing the toxicity profile of compounds **7d** and **7f** was determined and the results are shown in Table 4. The test compounds were administered intraperitoneally to mice at different doses in the multiple of TD_{50} ($1TD_{50}$, $2TD_{50}$ and $4TD_{50}$) and the toxicity induced by them was characterized by decreased motor activity, ataxia, sedation, muscular relaxation, loss of righting reflex and decreased respiration. At higher doses, animals were also seen to experience hypnosis, analgesia and anesthesia.

The median hypnotic dose (HD_{50}) of compound **7d** was found to be 867 mg/kg, which is nearly twice the TD_{50} of the compound. It also showed the 24 h median lethal dose (LD_{50}) of 946 mg/kg. Compound **7f** displayed the HD_{50} value 632 mg/kg with LD_{50} 834 mg/kg. Both the compounds showed wide range of safety profile as the HD_{50}/ED_{50} values of **7d** and **7f** were found to be 36.27 and 47.16 against MES induced seizures. These values are much higher than that showed by phenytoin. They showed a significant safety profile in PTZ induced seizure also indicative of the effectiveness of both the compounds as broad spectrum anticonvulsants.

In the phase IV pharmacological screening, the ED_{50} and TD_{50} values were determined after administering the test compounds orally (p.o.). In order to increase the bioavailability of the compounds they were converted to their respective hydrochloride salts by treating them with dilute hydrochloric acid. The salts of the compounds **7d** and **7f** were dissolved in water and given orally to mice and the results are shown in Table 5. Both the compounds showed the time to peak effect at 2 h, which was comparable to the standard drug phenytoin. The ED_{50} values were higher than the ED_{50} values found in phase II screening when the compounds were administered intraperitoneally. It indicates the decrease in the bioavailability of the compounds when given orally. But, nevertheless the ED_{50} values and the TD_{50} values were still comparable to the standard drugs showing the adequate absorption of the compounds in mice orally with lesser neurotoxic effects in both the modes of administration.

Table 4
Phase III quantitative toxicity profile of selected compounds.

Compound	HD_{50}[a]	LD_{50}[b]	HD_{50}/ED_{50}	
			MES	scPTZ
7d	867 (645–1078)[c]	946 (773–1087)	36.27	4.85
7f	632 (578–702)	834 (639–1092)	47.16	7.74
Phenytoin	182 (136–229)	224 (202–251)	19.15	>0.60

[a] Median hypnotic dose (HD_{50}) in mg/kg; determined by loss of righting reflex.
[b] Median lethal dose (LD_{50}) in mg/kg; mortality was determined 24 h after i.p. injection.
[c] 95% confidence interval in parentheses.

4.2. Log P determination

The dependence of biological activity in the set of congeneric agents or lipophilic character has been shown in many types of drug action in particular, the reports by Lien and co-workers indicate that the anticonvulsant activity of different types of compounds were correlated with lipophilicity [14]. However it has been observed that the maximum potency of the drugs that act on the central nervous system (CNS) is obtained with congeners having an optimum lipophilicity (log Po). In this study, we attempted to correlate the anticonvulsant activity of synthesized compounds with their log *P* value. The experimental log *P* values were determined using octanol-phosphate buffer method and the calculated log *P* values were taken from the software ChemOffice 6.0 and the results are shown in Table 1. Almost all the compounds are found to be lipophilic and having potent anticonvulsant activity in both MES as well as scPTZ test. Due to their higher lipophilicity they are expected to have rapid onset and shorter duration of action.

4.3. In silico studies

4.3.1. Distance mapping

Further the present work involves the comparison of the structures for molecular modeling of well known and structurally different compounds and the synthesized compounds. Comparison of the structures of the synthesized compounds and other

Table 3
Phase II quantitative anticonvulsant evaluation in mice.

Compound	ED_{50}[a]		TD_{50}[b]	PI[c]	
	MES	scPTZ		MES	scPTZ
7d	23.9 (19.2–28.4)[d]	178.6 (126.3–244.6)	439.3 (311.4–564.3)	18.3	2.4
7f	13.4 (9.8–17.1)	81.6 (53.4–113.3)	684.1 (451.3–908.5)	51.0	8.3
Phenytoin	9.5 (8.1–10.4)	>300	65.5 (52.5–72.9)	6.9	<0.22
Carbamazepine	8.8 (5.5–14.1)	>100	71.6 (45.9–135)	8.1	<0.22
Phenobarbital	21.8 (21.8–25.5)	13.2 (5.8–15.9)	69 (62.8–72.9)	3.2	5.2
Valproate	272 (247–338)	149 (123–177)	426 (369–450)	1.6	2.9

Number of animals used = 10; Solvent used: polyethylene glycol (0.1 mL, i.p.).
[a] Dose in milligrams per kilogram body mass.
[b] Minimal toxicity which was determined by rotorod test 30 min after the test drug was administered.
[c] PI = Protective index (TD_{50}/ED_{50}).
[d] Data in parentheses are the 95% confidence limits.

Table 5
Phase IV pharmacological evaluation of selected compounds given orally (p.o.) in mice.

Compound	TPE (h)	ED_{50} (MES)[a]	TD_{50}[a]	PI[b]
7d	2	54.8 (43.9–67.1)	734.7 (683.1–793.9)	13.40
7f	2	49.9 (40.1–60.4)	883.6 (739.1–998.3)	17.70
Phenytoin	2	9.16 (7.9–11.4)	87.6 (78.3–98.4)	9.56

95% confidence interval in parentheses.
[a] ED_{50} and TD_{50} values are in mg/kg units and determined at the indicated time.
[b] PI (Protective index) was determined by TD_{50}/ED_{50}.

molecules with anticonvulsant activity were performed to find out the structural elements essential for action. The compounds selected for this comparison have at least one aryl (R) hydrophobic domain, one electron donor (D) and a hydrogen bond acceptor/donor unit (HBD). In an initial study, calculations on the basis of molecular mechanics, with the force field based on CHARMM parameterization were performed to obtain an overview on their minimum conformation for bioactivity. Table 6 shows the distances between the various groups postulated as essential for anticonvulsant action. The synthesized compounds were examined to check whether they reflect the conditions of the derived pharmacophore model. Analyses of the distance relationship showed that synthesized compounds (**7a**–**t**) fulfil the essential demands of pharmacophore when compared with other known anticonvulsant drugs. In case of the titled compounds the distances R-D, R-HBD and D-HBD were in conformity with the distances of active anticonvulsant drugs.

4.3.2. Three dimensional structure analysis

The ORTEP diagram (50% probability) was drawn using the software Ortep3v2 and the characteristics were studied. As noted in Fig. 2 of the compound **7t**, strong hydrogen bonding occurred between the vinyl protons and the nitrogen atoms as well as with the sulphur atoms. The N···H distance was calculated to be 1.89 Å and that of S···H was found to be 2.49 Å. These hydrogen bondings ensure the rings are planar. It was reported that the planarity of molecules is an essential factor in determination of biological activity [15].

5. Conclusions

A new series of anticonvulsants 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones has been identified. Two compounds showed better anticonvulsant activity as compared to the standard drugs when they were subjected to preliminary anticonvulsant screenings. They also showed marked lower neurotoxicity and therefore a higher protective index. These can be regarded as strong candidates for future investigations.

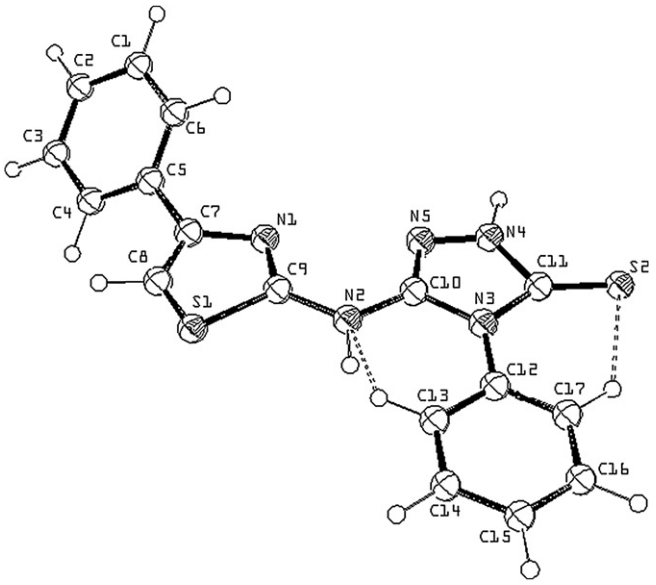


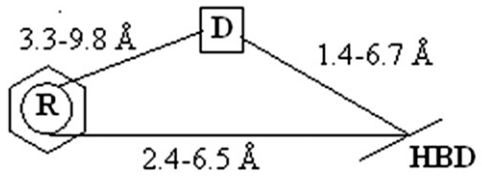
Fig. 2. ORTEP diagram (50% probability) of 4-phenyl-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (7t) showing hydrogen bonding interactions (N···H and S···H) as dotted line.

6. Experimental protocols

6.1. Chemistry

The melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. The homogen elemental analyses (C, H, N) of all compounds were performed on the CHNS Elimentar (Analysen systime, GmbH) Germany Vario EL III. All the Fourier transform infra red (FTIR) spectra were recorded in KBr pellets on a Jasco FT/IR 410 spectrometer. The ^{1}H NMR

Table 6
Distance ranges between the essential structure elements R, D and HBD.



R = Aryl ring
D = Electron donor atom
HBD = Hydrogen bond acceptor/donor atom

Compound	R-HBD[a]	R-D[a]	D-HBD[a]
7t	7.799	2.619	5.304
Carbamazepine	6.517	3.931	5.554
Phenytoin	3.042	3.868	2.497
Lamotrigine	5.807	3.301	4.598
Zonisamide	4.058	5.651	6.729
Rufinamide	2.407	7.474	5.209
Dezinamide	4.481	5.909	2.948
Remacemide	3.211	9.811	6.635
Diazepam	4.793	4.827	1.497

[a] Distance calculated for 3D optimized structures using ACD/Chemsketch/3-D viewer 2.0 version program.

spectra were taken on a Bruker 400 Ultra shield™ (400 MHz) NMR spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. The homogeneity of the compounds was checked by thin layer chromatography (TLC) on silica gel G (Merck) coated plates by using toluene: ethyl acetate: formic acid (5:4:1) as solvent system. Iodine chamber and UV lamp were used for the visualization of TLC spots. For the molecular mechanics calculations, the ACD/Chemsketch/3-D viewer Freeware version program was used for employing the Chemistry at Harvard Macromolecular Mechanics (CHARMM) force field. The three-dimensional structural analysis of the synthesized compounds was performed using the software Ortep3v2.

6.1.1. General procedure for the synthesis of titled compounds (7a–t)

*6.1.1.1. 2-Bromo-1-(substituted phenyl)ethanones (**1a–d**).* To the solution of substituted acetophenones (0.1 mol) in chloroform (50 mL), was added bromine previously dissolved in chloroform (0.11 mol in 50 mL) dropwise for a period of 15 min. The reaction mixture was stirred for an additional 2 h at room temperature. When the reaction was complete, the reaction mixture was concentrated and cooled to get the crystals of brominated acetophenones (**1a–d**).

*6.1.1.2. 4-(Substituted phenyl)-1,3-thiazol-2-amines (**2a–d**).* A solution of compound (**1a–d**, 0.1 mol) in 150 mL of acetic acid was refluxed with thiourea (0.1 mol) for 2 h and cooled to get thiazoles as crystals, which were filtered, washed with water and recrystallized with ethanol.

*6.1.1.3. Ethyl [4-(substituted phenyl)-1,3-thiazol-2-yl]carbamates (**3a–d**).* To the substituted thiazoles (**2a–d**, 0.1 mol) in benzene were added ethylchloroformate (0.11 mol) and triethylamine (25 mL), and the reaction mixture was refluxed for 3 h. After cooling the reaction mixture was poured into cold dil. HCl (50%) and the carbamate thus formed was recrystallized from benzene.

*6.1.1.4. 1-(Substituted phenyl)thioureas (**4a–e**).* Substituted anilines (0.1 mol) were taken in water and warmed with dilute hydrochloric acid (5 mL) until a clear solution was obtained. To this solution was added ammonium thiocyanate (0.11 mol) dissolved in water (25 mL) gradually. The reaction mixture was boiled and evaporated to less than half of the volume. It was then cooled to get the precipitate of phenyl thiourea which were filtered, washed with water and recrystallized from ethanol to get the target compounds.

*6.1.1.5. N-(substituted phenyl)hydrazinecarbothioamides (**5a–e**).* To the solution of substituted phenylthioureas (**4a–e**, 0.1 mol) in ethanol was added hydrazine hydrate (0.11 mol) and the reaction mixture was refluxed for 16 h. It was then concentrated, cooled and poured over crushed ice to get the precipitate which was filtered, washed with water and recrystallized from ethanol to get hydrazinecarbothioamides (**5a–e**).

*6.1.1.6. 2-[(Substituted phenyl)carbamothioyl]-N-[4-(substituted phenyl)-1,3-thiazol-2-yl]hydrazinecarboxamides (**6a–t**).* The solution of carbamates (**3a–d**, 0.01 mol) and hydrazinecarbothioamides (**5a–e**, 0.01 mol) in ethanol (25 mL) was refluxed for 4 h. The residue was concentrated, cooled and poured over crushed ice to the precipitate which was filtered, washed with water, dried and recrystallized from ethanol to get the targeted compounds (**6a–t**).

*6.1.1.7. 4-(Substituted phenyl)-5-[{4-(substituted phenyl)-1,3-thiazol-2-yl}amino]-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**7a–t**).* A mixture of substituted hydrazinecarboxamides (**6a–t**, 0.01 mol) and 30 mL of 2% aq. NaOH solution was refluxed for 6 h. After completion of reaction, the reaction mixture was filtered and the filtrate was neutralized with conc. HCl dropwise till pH was adjusted to 7. The mixture was kept aside for few minutes. A distinctive precipitate thus obtained was filtered, washed with water, and recrystallized from ethanol to get the titled compounds (**7a–t**).

*6.1.1.7.1. 3-[4-(4-Chlorophenyl)-1,3-thiazol-2-ylamino]-4-(2-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7a**).* Yield 84%, mp 238 °C. IR (KBr) ν_{max} cm^{-1}: 3316 (NH str.), 2889 (Ar-CH str.), 1395 (C═N), 1064 (C═S), 819 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 5.35 (s, 1H, ArH-thiazole), 6.95–7.73 (m, 8H, ArH), 10.61 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.2. 3-[4-(4-Chlorophenyl)-1,3-thiazol-2-ylamino]-4-(4-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7b**).* Yield 76%, mp 251 °C. IR (KBr) ν_{max} cm^{-1}: 3341 (NH str.), 2991 (Ar-CH str.), 1405 (C═N), 1051 (C═S), 807 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 5.39 (s, 1H, ArH-thiazole), 6.98–7.86 (m, 8H, ArH), 10.55 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.3. 3-[4-(4-Chlorophenyl)-1,3-thiazol-2-ylamino]-4-(2-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7c**).* Yield 62%, mp 209 °C. IR (KBr) ν_{max} cm^{-1}: 3343 (NH str.), 2893 (Ar-CH str.), 1415 (C═N), 1059 (C═S), 831 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 3H, OCH_3), 5.42 (s, 1H, ArH-thiazole), 6.89–7.89 (m, 8H, ArH), 10.57 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.4. 3-[4-(4-Chlorophenyl)-1,3-thiazol-2-ylamino]-4-(4-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7d**).* Yield 74%, mp 245 °C. IR (KBr) ν_{max} cm^{-1}: 3314 (NH str.), 2994 (Ar-CH str.), 1407 (C═N), 1084 (C═S), 864 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 3.52 (s, 3H, OCH_3), 5.49 (s, 1H, ArH-thiazole), 6.99–7.79 (m, 8H, ArH), 10.51 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.5. 3-[4-(4-Chlorophenyl)-1,3-thiazol-2-ylamino]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7e**).* Yield 73%, mp 194 °C. IR (KBr) ν_{max} cm^{-1}: 3319 (NH str.), 2987 (Ar-CH str.), 1426 (C═N), 1087 (C═S), 846 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 1H, ArH-thiazole), 6.81–7.71 (m, 9H, ArH), 10.43 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.6. 3-[4-(4-Bromophenyl)-1,3-thiazol-2-ylamino]-4-(2-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7f**).* Yield 65%, mp 179 °C. IR (KBr) ν_{max} cm^{-1}: 3318 (NH str.), 3015 (Ar-CH str.), 1478 (C═N), 1135 (C═S), 563 (C–Br); ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H, CH_3), 5.40 (s, 1H, ArH-thiazole), 6.98–7.95 (m, 8H, ArH), 10.37 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.7. 3-[4-(4-Bromophenyl)-1,3-thiazol-2-ylamino]-4-(4-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7g**).* Yield 71%, mp 186 °C. IR (KBr) ν_{max} cm^{-1}: 3334 (NH str.), 2918 (Ar-CH str.), 1454 (C═N), 1054 (C═S), 556 (C–Br); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 5.51 (s, 1H, ArH-thiazole), 7.08–7.96 (m, 8H, ArH), 10.41 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.8. 3-[4-(4-Bromophenyl)-1,3-thiazol-2-ylamino]-4-(2-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7h**).* Yield 76%, mp 226 °C. IR (KBr) ν_{max} cm^{-1}: 3345 (NH str.), 2934 (Ar-CH str.), 1405 (C═N), 1085 (C═S), 559 (C–Br); ^{1}H NMR ($CDCl_3$) δ 3.51 (s, 3H, OCH_3), 5.65 (s, 1H, ArH-thiazole), 7.15–8.03 (m, 8H, ArH), 10.43 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.9. 3-[4-(4-Bromophenyl)-1,3-thiazol-2-ylamino]-4-(4-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7i**).* Yield 69%, mp 238 °C. IR (KBr) ν_{max} cm^{-1}: 3351 (NH str.), 2884 (Ar-CH str.), 1398 (C═N), 1074 (C═S), 564 (C–Br); ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3H, OCH_3), 5.66 (s, 1H, ArH-thiazole), 7.09–7.89 (m, 8H, ArH), 10.14 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.10. 3-[4-(4-Bromophenyl)-1,3-thiazol-2-ylamino]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7j**).* Yield 61%, mp 198 °C. IR (KBr) ν_{max} cm^{-1}: 3325 (NH str.), 2875 (Ar-CH str.), 1385 (C═N), 1081 (C═S), 561 (C–Br); ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 1H, ArH-thiazole), 6.95–7.83 (m, 9H, ArH), 10.39 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.11. 4-(2-Methylphenyl)-3-[4-(4-nitrophenyl)-1,3-thiazol-2-ylamino]-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7k**).* Yield 62%, mp 209 °C. IR (KBr) ν_{max} cm^{-1}: 3315 (NH str.), 2956 (Ar-CH str.), 1449 (C=N), 1339 (NO_2), 1041 (C=S); ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 5.49 (s, 1H, ArH-thiazole), 7.21–8.29 (m, 8H, ArH), 10.69 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.12. 4-(4-Methylphenyl)-3-[4-(4-nitrophenyl)-1,3-thiazol-2-ylamino]-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7l**).* Yield 76%, mp 245 °C. IR (KBr) ν_{max} cm^{-1}: 3364 (NH str.), 3016 (Ar-CH str.), 1467 (C=N), 1347 (NO_2), 1029 (C=S); ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3), 5.51 (s, 1H, ArH-thiazole), 7.16–8.12 (m, 8H, ArH), 10.71 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.13. 4-(2-Methoxyphenyl)-3-[4-(4-nitrophenyl)-1,3-thiazol-2-ylamino]-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7m**).* Yield 58%, mp 216 °C. IR (KBr) ν_{max} cm^{-1}: 3316 (NH str.), 3048 (Ar-CH str.), 1451 (C=N), 1336 (NO_2), 1045 (C=S); ^1H NMR ($CDCl_3$) δ 3.54 (s, 3H, OCH_3), 5.46 (s, 1H, ArH-thiazole), 7.19–8.24 (m, 8H, ArH), 10.69 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.14. 4-(4-Methoxyphenyl)-3-[4-(4-nitrophenyl)-1,3-thiazol-2-ylamino]-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7n**).* Yield 73%, mp 263 °C. IR (KBr) ν_{max} cm^{-1}: 3349 (NH str.), 3013 (Ar-CH str.), 1464 (C=N), 1329 (NO_2), 1019 (C=S); ^1H NMR ($CDCl_3$) δ 3.57 (s, 3H, OCH_3), 5.41 (s, 1H, ArH-thiazole), 7.02–8.16 (m, 8H, ArH), 10.58 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.15. 3-[4-(4-Nitrophenyl)-1,3-thiazol-2-ylamino]-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7o**).* Yield 59%, mp 186 °C. IR (KBr) ν_{max} cm^{-1}: 3346 (NH str.), 3046 (Ar-CH str.), 1449 (C=N), 1329 (NO_2), 1037 (C=S); ^1H NMR ($CDCl_3$) δ 5.51 (s, 1H, ArH-thiazole), 7.27–8.16 (m, 9H, ArH), 10.57 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.16. 4-(2-Methylphenyl)-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7p**).* Yield 67%, mp 194 °C. IR (KBr) ν_{max} cm^{-1}: 3306 (NH str.), 3008 (Ar-CH str.), 1445 (C=N), 1045 (C=S); ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 5.43 (s, 1H, ArH-thiazole), 6.74–7.79 (m, 9H, ArH), 10.41 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.17. 4-(4-Methylphenyl)-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7q**).* Yield 76%, mp 229 °C. IR (KBr) ν_{max} cm^{-1}: 3318 (NH str.), 3026 (Ar-CH str.), 1458 (C=N), 1034 (C=S); ^1H NMR ($CDCl_3$) δ 2.27 (s, 3H, CH_3), 5.41 (s, 1H, ArH-thiazole), 6.69–7.62 (m, 9H, ArH), 10.39 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.18. 4-(2-Methoxyphenyl)-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7r**).* Yield 73%, mp 209 °C. IR (KBr) ν_{max} cm^{-1}: 3371 (NH str.), 2976 (Ar-CH str.), 1429 (C=N), 1017 (C=S); ^1H NMR ($CDCl_3$) δ 3.53 (s, 3H, OCH_3), 5.51 (s, 1H, ArH-thiazole), 6.86–7.83 (m, 9H, ArH), 10.29 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.19. 4-(4-Methoxyphenyl)-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7s**).* Yield 68%, mp 267 °C. IR (KBr) ν_{max} cm^{-1}: 3356 (NH str.), 2937 (Ar-CH str.), 1415 (C=N), 1048 (C=S); ^1H NMR ($CDCl_3$) δ 3.46 (s, 3H, OCH_3), 5.47 (s, 1H, ArH-thiazole), 6.81–7.76 (m, 9H, ArH), 10.37 (bs, 1H, NH, D_2O exchangeable).

*6.1.1.7.20. 4-Phenyl-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1H-1,2,4-triazole-5-thione (**7t**).* Yield 79%, mp 174 °C. IR (KBr) ν_{max} cm^{-1}: 3319 (NH str.), 2961 (Ar-CH str.), 1467 (C=N), 1039 (C=S); ^1H NMR ($CDCl_3$) δ 5.47 (s, 1H, ArH-thiazole), 6.81–7.78 (m, 10H, ArH), 10.46 (bs, 1H, NH, D_2O exchangeable).

6.2. Pharmacology

The investigations were conducted on albino mice of either sex (25–30 g). The albino mice were kept under standard conditions at an ambient temperature of 25 ± 2 °C and allowed free access to food and water except at the time they were brought out of the cage. All the experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC), form no. 502. Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-62. Registration no. and date of registration is 173/CPCSEA, 28 Jan., 2000.

6.2.1. Anticonvulsant activity

6.2.1.1. Maximal electroshock test (MES). The maximal electroshock seizure test was carried out according to the standard protocol [16]. Albino mice were stimulated through corneal electrodes to 50 mA current at a pulse of 60 Hz applied for 0.25 s. Animals were previously administered with the test drug i.p. Abolition of hind limb tonic extension spasm was recorded as the anticonvulsant activity. The test compounds were dissolved in polyethylene glycol (PEG). In the preliminary screening, each compound was administered as an i.p. injection at three dose levels (30, 100 and 300 mg/kg body mass) and the anticonvulsant activity assessed after 0.5 h and 4.0 h intervals of administration.

6.2.1.2. Subcutaneous pentylenetetrazole induced seizure test (scPTZ). The subcutaneous pentylenetetrazole test was performed according to the known protocol [17]. This method utilizes pentylenetetrazole (75 mg/kg) that produces seizures in >95% of animals as a 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min, failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

The pharmacological parameters estimated in phase I screening was quantified in phase II screening (Table 3). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For the determination of ED_{50} and TD_{50} values, groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plot of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of the computer program by Litchfield and Wilcoxon's method [18].

Phase III screening comprises of the assessment of general behavior of mice at regular time intervals up to 24 h following the i.p. administration of the test compounds at doses equivalent to TD_{50}, $2TD_{50}$ and $4TD_{50}$. The median hypnotic dose was assessed by loss of righting reflex and the 24 h median lethal dose was determined by using the procedure described previously for evaluation of ED_{50} and TD_{50} values in phase II screening.

In phase IV screening the same procedure was followed as discussed in phase II screening except the fact that the test compounds and standard drugs are administered orally (p.o.). The test compounds were converted to their respective hydrochloride salt, dissolved in water and given orally to groups of mice and the ED_{50} and TD_{50} values of the compounds were determined.

6.2.2. Neurotoxicity screening (NT)

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were given i.p. injection of the test compounds 30, 100 and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials [19].

6.3. Log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature (29 °C) using a procedure

described elsewhere [20]. 10 mL of octanol and 10 mL phosphate buffer (pH = 7.4) were taken in a glass stoppered graduated tube and 5 mg of accurately weighed drug was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature. The mixture was then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy.

Partition coefficient was calculated by;

$PC = (Ct - Ca)/Ca$.

PC = Partition coefficient, Ct = Concentration of total drug, Ca = Concentration of the drug in aqueous phase.

6.4. In silico studies

6.4.1. Distance mapping

In the conformational analysis of the older generation clinically active anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigene, rufinamide, remacemide and phenobarbitone, a molecular model was suggested on the basis of molecular dynamics distance estimations [21]. According to which an electron donor (D) should be in a distance range of 3.2—5.1 Å to an aryl ring or any other hydrophobic unit (R) and of 3.9—5.5 Å to the hydrogen bonding domain (HBD). For the molecular mechanics calculations, the ACD/Chemsketch/3-D viewer 2.0 version program was used for employing the CHARMM force field [22].

6.4.2. Three-dimensional structure analysis

This is performed to define the role of three-dimensional structures of synthesized compounds to their anticonvulsant activity. Compound 4-phenyl-3-(4-phenyl-1,3-thiazol-2-ylamino)-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**7t**) which represents the prototype structure of the synthesized compounds was chosen for the study. It was performed using software Ortep3v2.

Acknowledgments

One of the authors (W.A.) is thankful to Council of Scientific and Industrial Research (CSIR), Government of India for the Senior Research Fellowship (SRF) as financial assistance. We are also thankful to Shrenik Pharma Ltd., Mumbai, India for providing pentylenetetrazole as gift sample.

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