EXCLI Journal 2014;13:225-240 – ISSN 1611-2156
Received: December 31, 2013, accepted: February 16, 2014, published: March 10, 2014

Original article:

DESIGN, SYNTHESIS AND ANTICONVULSANT ACTIVITY OF SOME NEW 6,8-HALO-SUBSTITUTED-2*H*-[1,2,4]TRIAZINO[5,6-B]INDOLE-3(5*H*)-ONE/-THIONE AND 6,8-HALO-SUBSTITUTED 5-METHYL-2*H*-[1,2,4]TRIAZINO[5,6-B]INDOL-3(5*H*)-ONE/-THIONE

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ABSTRACT

A new series of 6,8-halo-substituted-2*H*-[1,2,4]triazino[5,6-b]indole-3(5*H*)-one/-thione and 6,8-halo-substituted 5-methyl-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one/-thione (**5a**-**5l**) were designed and synthesized keeping in view of the structural requirement of pharmacophore. The above compounds were characterized by thin layer chromatography and spectral analysis. Anticonvulsant activity of the synthesized compounds was evaluated by the maximal electroshock (MES) test. Neurotoxicity and CNS depressant effects were evaluated by the rotarod motor impairment and Porsolt's force swim tests, respectively. A computational study was carried out, for calculation of pharmacophore pattern, prediction of pharmacokinetic properties and toxicity properties. The above study revealed that the compounds 8-chloro-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one (**5e**), 6,8-dibromo-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one (**5i**) and 6,8-dibromo-5-methyl-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one (**5k**) possess excellent anticonvulsant activity in the series with little CNS depressant effect and no neurotoxicity as compared to standard drugs phenytoin and carbamazepine.

Keywords: Anticonvulsant activity, computational study, neurotoxicity, 1,2,4-triazine

INTRODUCTION

Epilepsy is a heterogeneous group of disorders characterized by the neuronal hyperexcitability and hypersynchronous neuronal firing presented with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness (Stafstrom, 2006). The cellular mechanism of human epilepsy is still uncertain and hence the present drug therapy is rather concerned only with control of epilepsy symptoms than cure (Kenda et al., 2004). Epilepsy is one of the most common disorders of the brain, affecting 60 million people worldwide according to epidemiological studies (Husain et al., 2010; Scheurer and Pedley, 1990). Every year approximately 250,000 new cases are added to this figure (Husain et al., 2009). Now there are more than 40 different anti-epileptic drugs (AEDs) in clinical use, but still about 30 % of patients continue to experience uncontrolled seizures, and they are pharmaco-resistant to the available

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therapy (Picot et al., 2008). Further, today's treatment for seizures requires continuous medication for a long period, which is in turn associated with many adverse effects, such as nausea, ataxia, drowsiness, gastro-intestinal disturbance, hyperplasia, anaemia etc. (Naithani et al., 2010).

It is observed that most of the antiepileptic drugs, which are in clinical use are neither linked with any particular site of action nor with a known mechanism of action (Bialer et al., 2010). Many AEDs exhibit their potency via many possible mechanisms of action. The lack of understanding and complexity in mechanism of action certainly affect the development of new candidates as possible AEDs through mechanism-driven designs. So, presently the antiepileptic research mainly focuses on investigation of new anticonvulsant agents through conventional screening and structural modifications rather than mechanism based drug design. Therefore, drug identification is usually conducted via *in vivo* screening tests on the basis of seizure type rather than etiology. Anticonvulsant activity is mainly attributed to the presence of aryl binding site with aryl/alkyl hydrophobic group, hydrogen bonding domain and electron donor group, which are the essential requirements for the molecules to show potential activity as proposed by Dimmock et al. (2000a, b). Pandeya et al. (2002) have suggested a new pharmacophore model for semicarbazones displaying anticonvulsant activity (Figure 1). Proposed pharmacophore model contains 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).

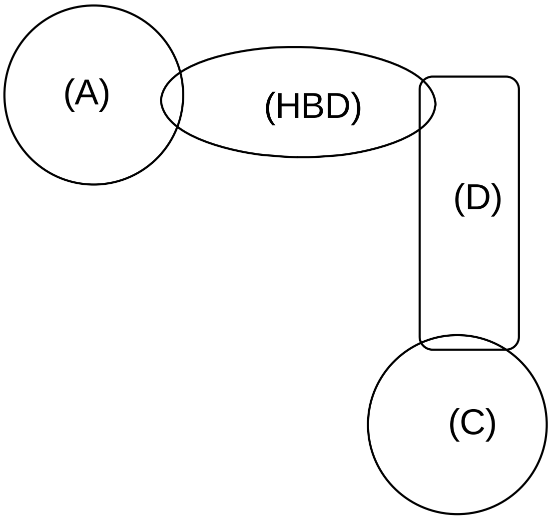

Figure 1: Suggested pharmacophore model for designed triazines displaying anticonvulsant activity

These new aspects might be useful for designing prototypic molecules with potential anticonvulsant activity (Figure 2).

Lamotrigine, a phenyl-1,2,4-triazine analog is used as anticonvulsant agent and in the treatment of bipolar disorder. Lamotrigine acts by prolonging inactivation of voltage-sensitive Na^+ channels and suppression of high frequency firing. It may also directly block voltage sensitive Na^+ channels by stabilizing the presynaptic membrane and preventing release of excitatory neurotransmitters, mainly glutamate and aspartate (Taylor, 1996; Tripathi, 2010). 3-(4-Chloro-phenylimino)-5-methyl-1,3-dihydro-indol-2-one showed 87 % protection at 100 mg kg^{-1} with an ED_{50} value of 53.61 mg kg^{-1} in the metrazol-induced convulsion model. This new agent was less neurotoxic than phenytoin, and showed greater protection against maximal electroshock method and metrazol-induced convulsion models than standard drug (Sodium Valproate) (Sridhar et al., 2002). Replacement of hydrogen (from -NH in indole nucleus) by methyl group enhanced the lipophilicity of the compounds (Smitha et al., 2008). Aryl semicarbazones and 4-(Aryloxy) phenyl semicarbazones showed potent anticonvulsant activity in the MES screening (Dimmock et al., 1995; Wang et al.,

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1998). Several investigations have recognized aryl thiosemicarbazones as structurally novel class of anticonvulsants (Karki et al., 2009; Kshirsagar et al., 2009; Ragab et al., 2010).

Intrigued by the above observations, and in an attempt to design and develop new potential anticonvulsant agents, a hybrid pharmacophoric approach was adopted in which the 1,2,4-triazine, indole and semicarbazone or thiosemicarbazone nucleus were hybridized in one structure hoping to synergize the anticonvulsant potential of these nucleus (explained diagrammatically in Figure 3). The validity of this design was assessed through anticonvulsant screening of the target compounds.

Chemistry

The reaction sequence leading to the synthesis of titled compounds (**5a-l**) is shown in Scheme 1. All chemicals and solvents for synthesis were supplied by Spectrochem chemicals (India) and S.D. Fine Chemicals (India). Synthesized compounds were purified by recrystallized and column chromatography. The progress of reaction was monitored by Silica gel-GF coated aluminum plate using iodine vapors or UV light as visualizing agents. Developing solvent

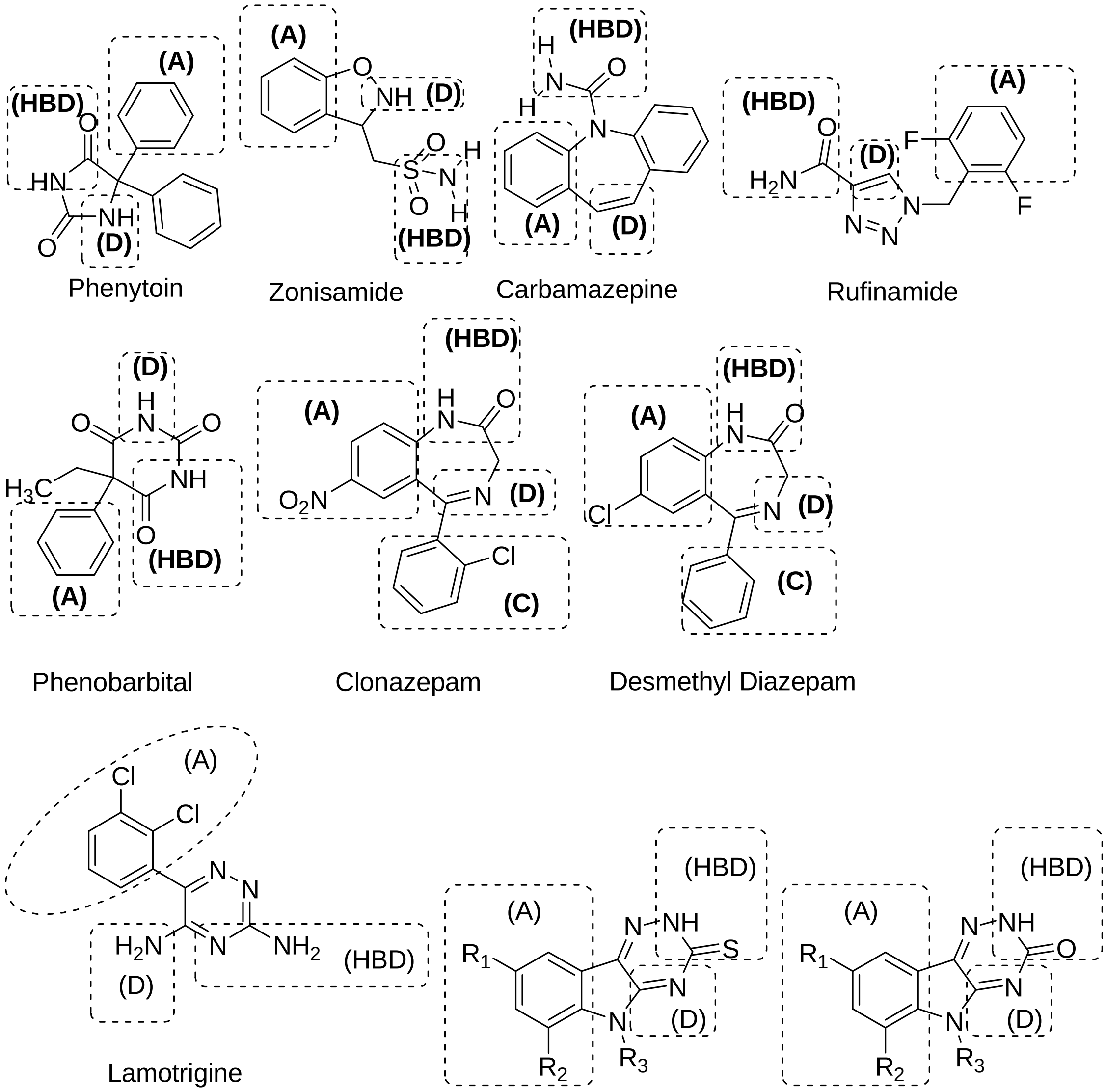


Figure 2: Structures of proposed general pharmacophore model of the synthesized compound and reported chemical drugs

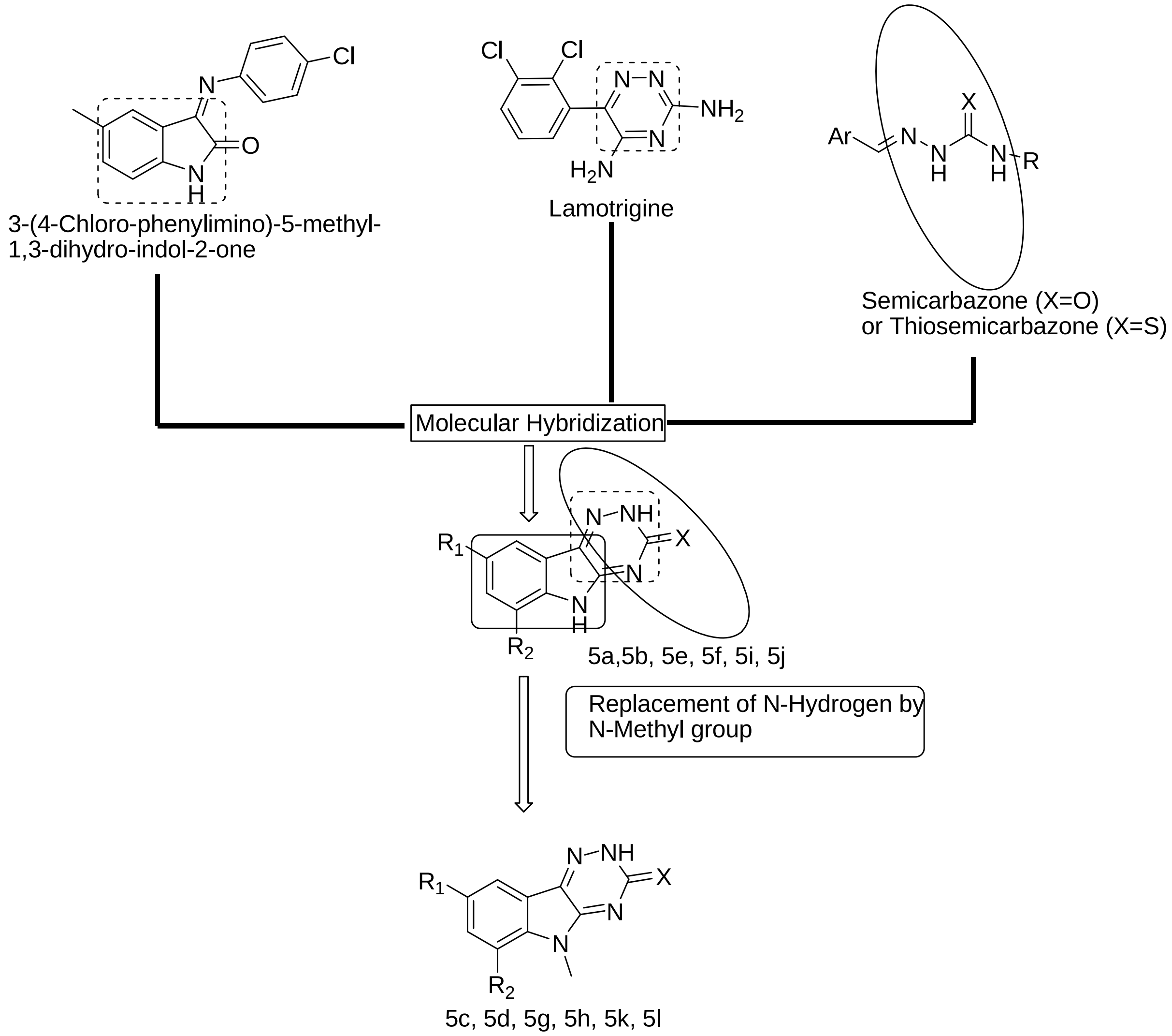


Figure 3: Rational concept to new 6,8-halo substituted-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-one/-thione and 6,8-halo-substituted 5-methyl-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one/-thione (5a-l)

for TLC was ethyl acetate and n-hexane (50 %). The melting points of newly synthesized compounds were determined by digital melting point apparatus and are uncorrected. IR (KBr) spectra of the synthesized compounds were on a Nicolet 5PC FTIR spectrophotometer (λ-max in cm^{-1}) and ^1H NMR spectra were recorded on a Brucker Model-300 NMR Spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as the internal reference (chemical shifts in δ ppm). Chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). The physical characterization data of the synthesized compounds are given in Table 1.

Pharmacology

Anticonvulsant screening

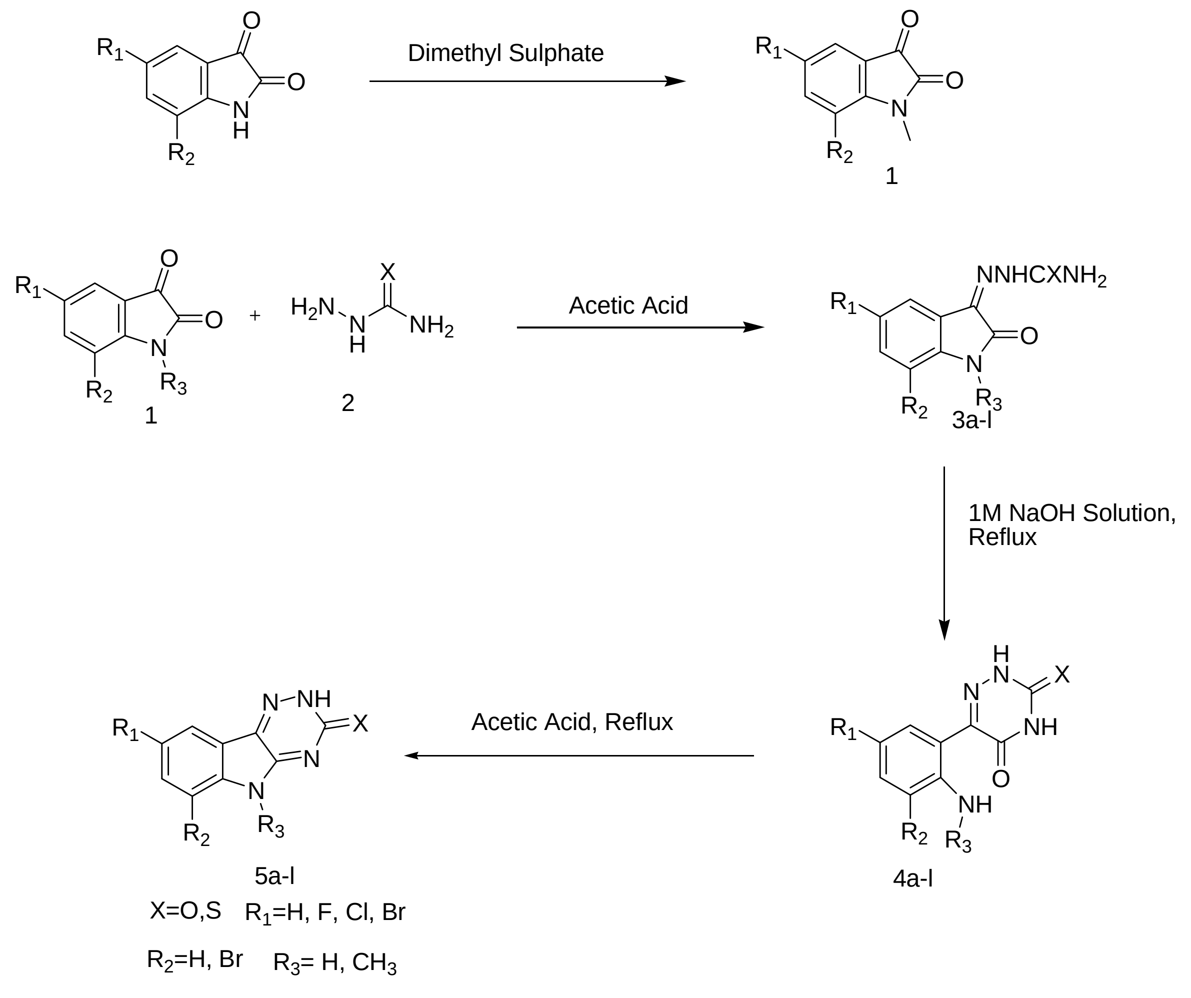
The anticonvulsant evaluation of the synthesized compounds (**5a-l**) was performed using reported procedures (Krall et al., 1978; Porter et al., 1984). Male albino mice (CF-1 strain, 18-25 g) were used as experimental animal. The synthesized compounds were suspended in polyethylene glycol (PEG-400). All experimental protocols were carried out with permission from the Institutional Animal Ethics Committee (IAEC). Animals were obtained from the Central Animal House Facility, Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, India.

Maximal electroshock seizure method (MES)

Anticonvulsant activity of titled compounds **5a-l** was measured by maximal electroshock seizure (MES) test following the methods of Krall et al. (1978) and Porter et al. (1984). For all tests based on MES convulsions, 60 Hz of alternating current (50 mA) was delivered for 0.2 s by electrodes which had been primed with an electrolyte solution containing an anaesthetic agent (0.5 % tetracaine HCl). In preliminary screening, titled compounds were administered intraperitoneal in volume 0.01 mL/kg body weight at the dose of 30, 100, 300 mg/kg and anticonvulsant activity was assessed after 0.5 and 4 h intervals administration. Abolition of the hind limb tonic extensor spasm was recorded as a measurement of anticonvulsant activity.

Neurotoxicity screening

Motor impairment of all synthesized compounds (**5a-l**) was measured in mice by the rotarod test (Dunham and Miya, 1957). The mice were trained to stay on an accelerating rotarod (INCO, Ambala, India) that rotated at six revolutions per minute. The rod diameter is 3.2 cm. Motor impairment was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.



Scheme 1: Synthesis of 6,8-halo-substituted-2*H*-[1,2,4]triazino[5,6-b]indole-3(5*H*)-one/-thione and 6,8-halo-substituted 5-methyl-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one/-thione derivatives

Table 1: Physical data of synthesized compounds **5a-l**

Code No.	R_1	R_2	R_3	X	Molecular formula	R_f	% yield	M.P. (^{0}C)$^\#$
5a	F	H	H	O	$C_9H_5FN_4O$	0.56	45	261-64
5b	F	H	H	S	$C_9H_5FN_4S$	0.39	48	274-77
5c	F	H	CH_3	O	$C_{10}H_7FN_4O$	0.58	52	253-56
5d	F	H	CH_3	S	$C_{10}H_7FN_4S$	0.49	56	267-70
5e	Cl	H	H	O	$C_9H_5ClN_4O$	0.43	56	209-12
5f	Cl	H	H	S	$C_9H_5ClN_4S$	0.31	61	238-41
5g	Cl	H	CH_3	O	$C_{10}H_7ClN_4O$	0.41	56	> 300
5h	Cl	H	CH_3	S	$C_{10}H_7ClN_4S$	0.33	63	> 300
5i	Br	Br	H	O	$C_9H_4Br_2N_4O$	0.34	65	> 300
5j	Br	Br	H	S	$C_9H_4Br_2N_4S$	0.27	67	> 300
5k	Br	Br	CH_3	O	$C_{10}H_6Br_2N_4O$	0.52	59	> 300
5l	Br	Br	CH_3	S	$C_{10}H_6Br_2N_4S$	0.30	64	> 300

***** TLC solvent was ethyl acetate: n-hexane=50 %

CNS depression study

The CNS depression study of synthesized compounds is performed by the forced swim model (Porsolt's swim pool test) (Porsolt et al*.,* 1978). Albino mice were placed in a chamber (diameter 45 cm, height 20 cm) containing water up to a height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted, an initial 15 min pre-test, followed by a 5 min test session 24 h later. The animals were administered with an i.p. injection (100 mg/kg) of the test compounds 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which were necessary to keep its head above the surface of water) during the 5 min test period were measured.

Computational study

Calculation of physicochemical parameters

A computational study of titled compounds (**5a-l**) was performed for prediction of ADME properties. Polar surface area (TPSA) (Ertl et al., 2000), milog P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five (Lipinski et al., 2001) were calculated using Molinspiration online property calculation toolkit (Molinspiration, 2013). Absorption (% ABS) was calculated by: % ABS =109-(0.345×TPSA) (Zhao et al., 2002). The druglikeness, drugscore and theoretical toxicity risks (mutagenic, tumorigenic, irritant and reproductive effects)

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were also calculated using Osiris Property Explorer (http://www.organic-chemistry.org).

RESULTS AND DISCUSSION

Anticonvulsant and CNS depressant activities

The newly synthesized 6,8-halo substituted-2*H*-[1,2,4]triazino[5,6-b]indole-3(5*H*)-one/-thione and 6,8-halo-substituted 5-methyl-2*H*-[1,2,4]triazino[5,6-b]indol-3(5*H*)-one/-thione compounds **5a-l** were subjected to anticonvulsant screening by using standard model MES (Maximal Electroshock Seizure test) for their ability to reduce seizure spread (Table 2). Motor impairment screening of the synthesized compounds was also carried out by rotarod test and CNS depressant effect of the compounds was determined by Porsolt's force swim pool model. The anticonvulsant activity was tested after 0.5 and 4.0 h time intervals at doses of 30, 100, and 300 mg/kg body weight, after i.p. administration. The CNS depressant activity was studied at a dose level of 100 mg/kg body weight (Table 3). Phenytoin and carbamazepine were used as standard drugs.

The anticonvulsant data of titled compounds **(5a-l)** was summarized in Table 2, compounds **5a** and **5b** showed protection at maximum dose level of 300 mg/kg after 0.5 and 4.0 h. Both compounds were found to be inactive at dose levels of 30 and 100 mg/kg. Compound **5c** displayed protection at maximum dose level (300 mg/kg) after 0.5 h. Compounds **5f**, **5g**, and **5j** exhibited activity at a dose level of 100 mg/kg after 0.5 h. These compounds were found to be inactive at 30 mg/kg dose. Compound **5i** containing R_1=R_2=Br, R_3=H, X=O groups, showed protection against induced seizures at lower dose level (30 mg/kg) after 4.0 h. Compound **5k** reflected protection at 100 and 300 mg/kg after 0.5 and 4.0 h. This compound was inactive at the dose level of 30 mg/kg after 0.5 and 4.0 h. Compounds **5d**, **5h** and **5l** were inactive at all dose levels (30, 100 and 300 mg/kg) after 0.5 and 4.0 h. Compound **5e** having R_1=Cl, R_2=H, R_3=H, X=O groups, was found to be most active of the series showing activity both at 100 mg/kg and 30 mg/kg after 0.5 and 4.0 h. The neurotoxicity screening data revealed that both compounds **5a** and **5f** having R_1=F, R_2=H, R_3=H, X=O and R_1=Cl, R_2=H, R_3=H, X=S substitutions respectively, displayed neurotoxicity at a dose level of 300 mg/kg after 4.0 h, whereas the rest compounds were without any neurotoxicity.

Some selected compounds (**5e, 5i** and **5k**) exhibiting significant anticonvulsant activity were also tested for their CNS depressant activity. These compounds showed 54.39, 37.68 and 45.96 % increase in immobility time with respect to control where as standard drug carbamazepine showed 58.63 % increase in the immobility time (Table 3). Thus CNS depressant screening data showed that compounds **5i** and **5k** having R_1=R_2=Br, R_3=H, X=O and R_1=R_2=Br, R_3=CH_3, X=O groups respectively, exhibited less CNS depressant activity in comparison to carbamazepine. Compound **5e** containing R_1=Cl, R_2=H, R_3=H and X=O groups, showed approximate equal CNS depressant activity in comparison to carbamazepine. The most active compound of this series was found to be **5e** as it was effective in both 0.5 and 4.0 h time interval at dose levels of 100 and 30 mg/kg showing no motor impairment effect. The compound **5e** also showed approximate equal CNS depressant activity in comparison to standard drug carbamazepine. The activity may be due to the presence of chloro (R_1=Cl) and oxo (X=O) groups that provide adequate lipophilicity and well fitted to receptor site.

Using the putative binding site theory proposed by Dimmock et al. (2000a, b) subsequently used by others (Gibson et al., 2009; Pandeya et al., 2002) in postulating the interaction of anticonvulsant compounds at a specific binding site, the molecule observed to interact with the protein receptor as shown in Figure 4.

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Table 2: Anticonvulsant and motor impairment screening of synthesized titled compounds (**5a-l**) using maximal electroshock seizure (MES) and rotarod models

Code No.	**R_1**	**R_2**	**R_3**	**X**	**MES[a]**		**Motor Impt.[a]**	
					0.5h	**4.0h**	**0.5 h**	**4.0 h**
5a	F	H	H	O	300	300	-	300
5b	F	H	H	S	300	300	-	-
5c	F	H	CH_3	O	300	-	-	-
5d	F	H	CH_3	S	-	-	-	-
5e	Cl	H	H	O	100	30	-	-
5f	Cl	H	H	S	100	-	-	300
5g	Cl	H	CH_3	O	100	-	-	-
5h	Cl	H	CH_3	S	-	-	-	-
5i	Br	Br	H	O	-	30	-	-
5j	Br	Br	H	S	100	-	-	-
5k	Br	Br	CH_3	O	100	300	-	-
5l	Br	Br	CH_3	S	-	-	-	-
Control					-	-	-	-
Phenytoin[b]					30	30	100	100
Carbamazepine[b]					30	100	100	300

[a] Doses of 30, 100, and 300 mg/kg were administered to albino mice through intraperitoneal (i.p.) route. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of mice. The animals were examined 0.5 and 4 h after the drug administration. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg).
[b] Data of Phenytoin and Carbamazepine were used as reference drugs and were obtained from the reference Dimmock et al. (1995) and White et al. (1995).

Table 3: Data of CNS depressant activity of the selected compounds performed in mice using forced swim test

Compound	**Duration of immobility (sec) (mean ± SEM)**	**% Increased of immobility**
5e	81.89 ± 3.12	54.39
5i	73.03 ± 5.53	37.68
5k	77.42 ± 2.82	45.96
Carbamazepine	84.14 ± 1.33	58.63
Control	53.04 ± 2.47	-

The compounds were tested at a dose of 100 mg/kg (i.p. in PEG 400). Control animals were administered with PEG 400 (i.p.). Each value represents the mean ± SEM of six mice. The CNS depressant effect was compared with respect to standard drug. $^*p < 0.0001$. Data was analyzed by unpaired student’s *t* test.

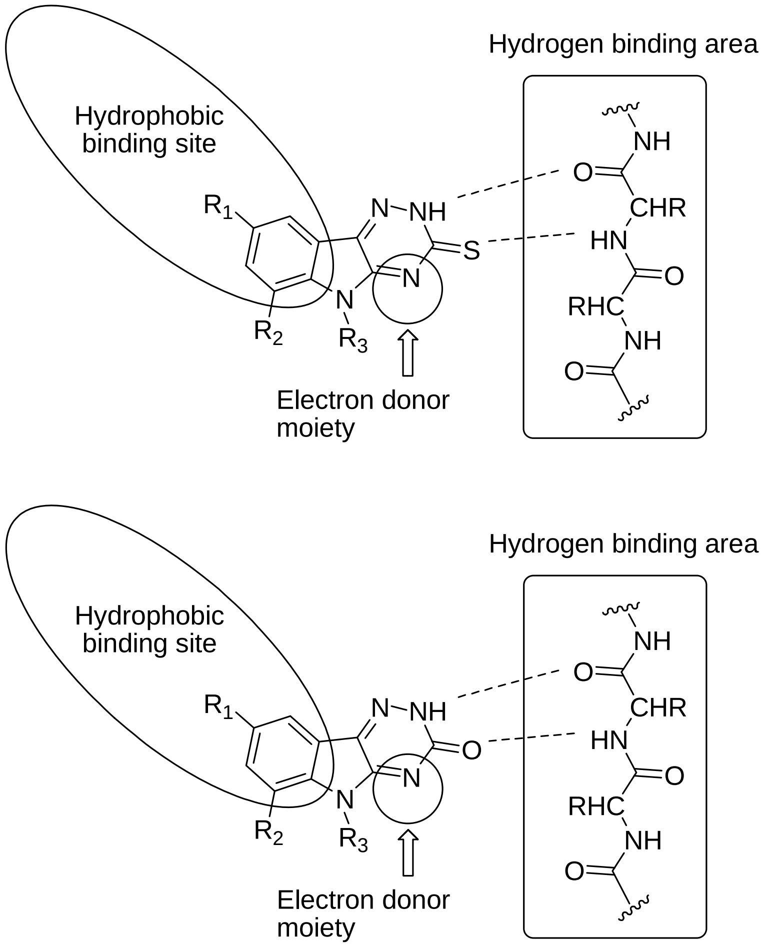

Figure 4: Proposed binding interactions of the title compounds using putative binding site theory (Dimmock et al., 2000a, b; Gibson et al., 2009; Pandeya et al., 2002).

Prediction of ADME properties

A computational study of titled compounds (**5a-l**) was performed for prediction of ADME properties such as absorption (% ABS), polar surface area (TPSA), miLog P, number of rotatable bonds, and violations of Lipinski's rule of five by using Molinspiration online property calculation toolkit. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood-brain barrier crossing (Ertl et al., 2000). The percentage of absorption (% ABS) was calculated using TPSA. From all these parameters, it can be observed that all titled compounds (**5a-l**) exhibited a great % ABS ranging from 83.31 to 92.95 % (Table 4). None of the titled compounds violated Lipinski's parameters and making them potentially promising agents for epilepsy therapy.

Herein, we also calculated the druglikeness and drugscore values for some compounds (**5e**, **5i** and **5k**) to analyze their overall potential to qualify for a drug including the comparison with some drugs currently in use in therapy against epilepsy (i.e., phenytoin, lamotrigine and carbamazepine) (Table 5, Figure 5). The druglikeness value is calculated based on the occurrence frequency of every one of the fragments of the analyzed molecules and compared to commercial drugs and non-drug like compounds. In the Osiris program, the occurrence frequency of each fragment is determined within the collection created by shredering 3300 traded drugs as well as 15,000 commercially available chemicals (Fluka) yielding a complete list of all avail-

Table 4: Pharmacokinetic parameters important for good oral bioavailability of titled compounds **(5a-l)**

Code No.	R_1	R_2	R_3	X	% ABS	TPSA (A^2)	n-ROTB	MW	MV	miLogP	n-OHNH donors	n-ON acceptors	Lipinski's violations
Rule					-	-	-	<500	-	<5	<5	<10	<1
5a	F	H	H	O	83.319	74.437	0	204.164	157.600	0.332	2	5	0
5b	F	H	H	S	89.208	57.366	0	220.232	166.478	0.675	2	4	0
5c	F	H	CH_3	O	87.064	63.58	0	218.191	174.543	0.400	1	5	0
5d	F	H	CH_3	S	92.954	46.509	0	234.259	183.421	0.742	1	4	0
5e	Cl	H	H	O	83.319	74.437	0	220.619	166.205	1.819	2	5	0
5f	Cl	H	H	S	89.208	57.366	0	236.687	175.083	2.162	2	4	0
5g	Br	Br	H	O	83.319	74.437	0	343.966	188.44	2.688	2	5	0
5h	Br	Br	H	S	89.208	57.366	0	360.034	197.318	3.030	2	4	0
5i	Cl	H	CH_3	O	87.064	63.58	0	234.646	183.147	1.887	1	5	0
5j	Cl	H	CH_3	S	92.954	46.509	0	250.714	192.025	2.230	1	4	0
5k	Br	Br	CH_3	O	87.064	63.58	0	357.993	205.382	2.755	1	5	0
5l	Br	Br	CH_3	S	92.954	46.509	0	374.061	214.26	3.098	1	4	0

% ABS, percentage of absorption; TPSA, topological polar surface area; n-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; n-OHNH, number of hydrogen bond donors; n-ON, number of hydrogen bond acceptors; miLogP, logarithm of compound partition coefficient between n-octanol and water

Table 5: Druglikeness, drugscore and in silico toxicity risks of titled compounds **5e**, **5i**, **5k** and **standard drugs**

Code No.	R_1	R_2	R_3	**X**	**Druglikeness**	**Drugscore**	**Mutagenic**	**Tumorigenic**	**Irritant**	**Reproductive Effective**
5e	H	Cl	H	O	2.38	0.50	3	1	1	1
5i	H	Br	Br	O	3.85	0.71	1	1	1	1
5k	CH_3	Br	Br	O	2.93	0.65	1	1	1	1
Lamotrigine					-0.88	0.51	1	1	1	1
Phenytoin					4.20	0.87	1	1	1	1
Carbamazepine					2.80	0.22	3	1	1	3

Toxic (3), less toxic (2) and no toxicity (1)

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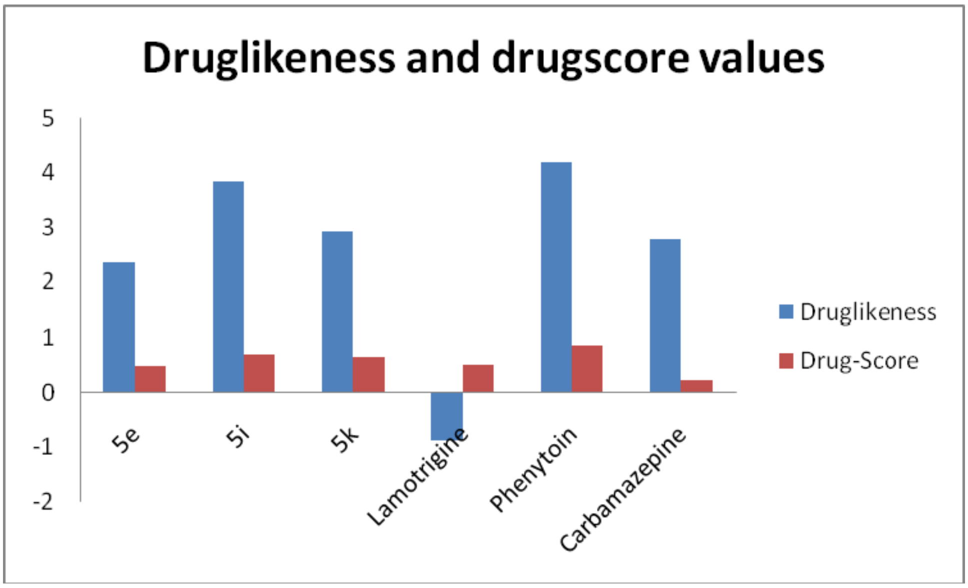

Figure 5: Graphical representation of Druglikeness and drugscore values of **compounds 5e, 5i, 5k, lamotrigine, phenytoin** and **carbamazepine**

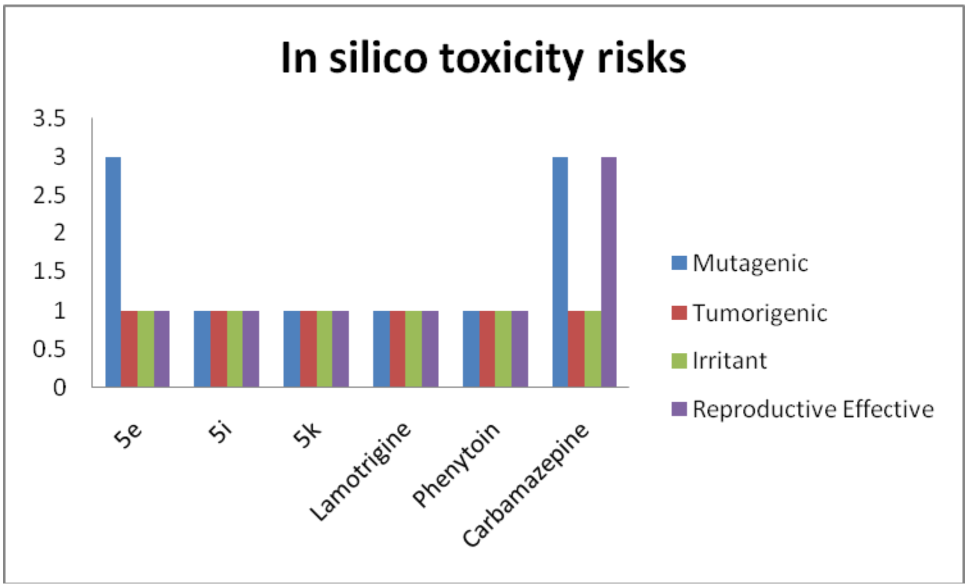

Figure 6: Graphical representation of in silico toxicity risks of **compounds 5e, 5i, 5k, lamotrigine, phenytoin** and **carbamazepine**

able fragments. In this case, positive values point out that the molecule contains predominantly the better fragments, which are frequently present in commercial drugs but not in the non-druglike collection of Fluka compounds. The drugscore combines druglikeness, clogP, logS, molecular weight and toxicity risks in one handy value that may be used to judge the drug potential of a compound. Interestingly we noticed that the compounds (**5e**, **5i** and **5k**) with positive druglikeness (2.38-3.85) and drugscore (0.50-0.71) values were similar or even better than some of the drugs currently used on the market (Table 5, Figure 5).

Theoretical toxicity risks (mutagenic, tumorigenic, irritant and reproductive effects) were also calculated by Osiris program. Only one compound **5e** displayed mutagenic toxicity. Tumorigenic, irritant risks and reproductive effects were not reported by compound **5e**. Theoretical toxicity profile (mutagenic, tumorigenic, irritant and reproductive effective) of the compound **5i** and **5k** was found to be equal to that of the standard drugs Phenytoin and Lamotrigine (Table 5 and Figure 6). Theoretically no-toxicity profile of compounds **5i** and **5k** reinforces further synthetic and pharmacological exploration for the development of new anticonvulsant drugs.

CONCLUSION

In present study, the compounds (**5a-l**) were designed, synthesized, characterized and their anticonvulsant activity was evaluated by using MES model. The compounds were also screened for neurotoxicity by using rotarod model and CNS depressant activity by forced swim test. The some titled compounds displayed the significant anticonvulsant activity with little or no neurotoxicity as compared to standard drug (phenytoin and carbamazepine). A computational study showed that none of the compounds violated Lipinski's parameters, hence making them potentially promising agent for epilepsy therapy. It was concluded that halogen substitution (i.e. F, Cl and Br) on the phenyl ring and thio group on 1,2,4-triazine ring in compounds **5a-l** increase the lipophilicity of compound that lead to gradual augmentation of compounds' ability to cross the blood brain barrier (BBB). Dibromo substitution on phenyl ring (i.e. Dibromophenyl) also increases the lipophilicity of compound. Compounds **5e**, **5i** and **5k** containing electron withdrawing groups (i.e. Cl, and Br), displayed excellent protection against MES model. Hence, these compounds may be regarded as a potential anticonvulsant candidate for further investigation.

EXPERIMENTAL PROTOCOL

Chemistry

Synthesis of 5,7-Dibromoisatin

Isatin (5.0 g, 34 mmol, 1 equiv) was warmed in ethanol (95 %, 100 mL) with stirring until it dissolved. Bromine (16.3 g, 102 mmol, 5.2 mL, 3.0 equiv) was added dropwise to the stirred isatin solution whilst maintaining the temperature of the reaction mixture between 70-75 °C. The solution was cooled to room temperature and placed on ice for 30 min. The solid product was washed with water and cold ethanol and then recrystallized from ethanol to yield bright orange-red crystals of 5,7-dibromoisatin (60 %), mp 251-254 °C (lit. Vine et al., 2007, 248-250 °C).

Synthesis of N-Methyl isatin derivatives

To a suspension of (0.1 mol) of isatin derivatives, in 200 mL of anhydrous methanol, 100 mL of 10 % methanolic potassium hydroxide solution was added in portions with stirring for 30 min. To this mixture, 15 mL of dimethyl sulfate was added and after 1 h, the solution was filtered to remove potassium methyl sulfate. After removal of about 250 mL of solvent under reduced pressure, 40 mL of warm water was added to the obtained residue. On cooling, orange precipitate occurred which was filtered and dried to obtain N-Methyl isatin derivatives (Gupta et al., 2010).

Synthesis of isatin semicarbazones or isatin thiosemicarbazones (3a-l)

Semicarbazones and thiosemicarbazones (**3a-l**) were synthesized according to the procedure reported in literature (Hlavac et al., 2003; Aanandhi et al., 2008). Substituted isatin (1 mole) was dissolved in boiling acetic acid in 100 mL beaker on hot plate. Semicarbazide hydrochloride (1 mole) was dissolved in distilled water (10 mL) in another 100 mL beaker. Then this solution was added in boiling isatin solution. The mixture was boiled for 20-30 min with stirring. After cooling, the solid formed was filtered off, washed with acetic acid followed with water. The dried product was recrystallized from acetic acid affording yellow crystals.

Substituted isatin (1 mole) was dissolved in boiling acetic acid (50 mL) in 250 mL round bottom flask. Thiosemicarbazide (1 mole) was dissolved in distilled water (10 mL) in 100 mL beaker. Then this solution was added in boiling isatin solution. The mixture was refluxed for about 10 hours with stirring. After cooling the solid formed was filtered off, washed with acetic acid followed with water. The dried product was recrystallized from ethanol-chloroform (50 %).

Synthesis of 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazines (4a-l)

6-(2-Amino-3,5-substituted phenyl)-1,2,4-triazines (**4a-l**) were synthesized according to the procedure reported in literature (Hlavac et al., 2003). An appropriate isatin semicarbazone or isatin thiosemicarbazone (**3a-l**, 2.00 g) was dissolved in a boiling solution of sodium hydroxide (1 M, 100 mL) in 250 mL conical flask. This mixture was refluxed for 3-4 hours. The progress of reaction was monitored by aluminum coated TLC plate using 50 % ethyl acetate and n-hexane solvent system. After completion of reaction, the mixture was cooled and then acidified with acetic acid. The solid formed was immediately filtered off, washed with water and dried.

Synthesis of 6,8-halo- substituted-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-one/-thione and 6,8-halo-substituted 5-methyl-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one/-thione (5a-l)

Triazine derivatives (**5a-l**) were synthesized according to the procedure reported in literature (Hlavac et al., 2003). An appropriate 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazine (**4a-l,** 4 mmol) was dissolved in acetic acid (100 mL) in 250 mL round bottom flask. The mixture was boiled. The progress of reaction was monitored by aluminum coated Merck TLC plate using 50 % ethyl acetate and n-hexane solvent system. After completion of reaction, the solid formed was filtered off, washed with water and dried.

*8-fluoro-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one **5a***

IR (KBr): ν_{max} = 3257 (NH), 1655 (C=O, amide), 1601 (C=N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ= 7.41-7.42 (m, 3H, ArH), 11.60 (s, 1H, =N-NH), 12.72 (s, 1H, NH) ppm. Anal. calcd for $C_9H_5FN_4O$: C 52.95, H 2.47, N 27.44; found C 52.99, H 2.52, N 27.40.

*8-fluoro-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione **5b***

IR (KBr): ν_{max} = 3134 (NH), 1613 (C=N), 1043 (C=S) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ= 7.62-7.63 (m, 3H, ArH), 12.10 (s, 1H, NH), 12.83 (s, 1H, =N-NHCS) ppm. Anal. calcd for $C_9H_5FN_4S$: C 49.08, H 2.29, N 25.44; found C 49.03, H 2.34, N 25.48.

*8-fluoro-5-methyl-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one **5c***

IR (KBr): ν_{max} = 3178 (NH), 1663 (C=O, amide), 1612 (C=N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ= 2.19 (s, 3H, N-CH$_3$), 7.51-7.53 (m, 3H, ArH), 13.04 (s, 1H, =N-NH), ppm. Anal. calcd for $C_{10}H_7FN_4O$: C 55.05, H 3.23, N 25.68; found C 55.09, H 3.28, N 25.72.

*8-fluoro-5-methyl-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione **5d***

IR (KBr): ν_{max} = 3268 (NH), 1618 (C=N), 1018 (C=S) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ= 2.26 (s, 3H, N-CH$_3$), 7.50-7.55 (m, 3H, ArH), 12.82 (s, 1H, =N-NHCS) ppm. Anal. calcd for $C_{10}H_7FN_4S$: C 51.27, H 3.01, N 23.92; found C 51.31, H 2.71, N 23.97.

*8-chloro-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one **5e***

IR (KBr): ν_{max} = 3321 (NH), 1650 (C=O, amide), 1606 (C=N) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ= 7.81-7.86 (m, 3H, ArH), 11.85 (s, 1H, =N-NH), 12.92 (s, 1H, NH), ppm. Anal. calcd for $C_9H_5ClN_4O$: C 49.00, H 2.28, N 25.40; found C 48.94, H 2.36, N 25.34.

*8-chloro-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione **5f***

IR (KBr): ν_{max} = 3298 (NH), 1615 (C=N), 1070 (C=S) cm^{-1}. ^{1}H NMR (300 MH$_Z$, DMSO-d_6) δ=7.02-7.15 (m, 3H, ArH), 12.52 (s, 1H, NH), 12.86 (s, 1H, =N-NHCS) ppm. Anal. calcd for $C_9H_5ClN_4S$: C 45.67, H 2.13, N 23.67; found C 45.72, H 2.19, N 23.63.

8-chloro-5-methyl-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one ***5g***

IR (KBr): ν_{max} = 3328 (NH), 1679 (C=O, amide), 1609 (C=N) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 2.23 (s, 3H, N-CH_3), 7.68-7.77 (m, 3H, ArH), 11.65 (s, 1H, =N-NH) ppm. Anal. calcd for $C_{10}H_7ClN_4O$: C 51.19, H 3.01, N 23.88; found C 51.24, H 3.07, N 23.84.

8-chloro-5-methyl-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione ***5h***

IR (KBr): ν_{max} = 3248 (NH), 1635 (C=N), 1040 (C=S) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 2.21 (s, 3H, N-CH_3), 7.50-7.78 (m, 3H, ArH), 12.89 (s, 1 H, =N-NHCS) ppm. Anal. calcd for $C_{10}H_7ClN_4S$: C 47.91, H 2.81, N 22.35; found C 47.97, H 2.76, N 22.39

6,8-dibromo-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one ***5i***

IR (KBr): ν_{max} = 3317 (NH), 1659 (C=O, amide), 1588 (C=N) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 7.31 (d, 1H, *J*= 8.1 Hz, ArH), 7.43 (d, 1H, *J*= 8.1 Hz, ArH), 11.85 (s, 1H, =N-NH), 12.89 (s, 1H, NH) ppm. Anal. calcd for $C_9H_4Br_2N_4O$: C 31.43, H 1.17, N 16.29; found C 31.37, H 1.22, N 16.34.

6,8-dibromo-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione ***5j***

IR (KBr): ν_{max} = 3286 (NH), 1622 (C=N), 1046 (C=S) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 7.36 (d, 1H, *J*= 7.5 Hz, ArH), 7.79 (d, 1H, *J*= 7.5 Hz, ArH), 11.79 (s, 1H, NH), 13.12 (s, 1H, =N-NHCS) ppm. Anal. calcd for $C_9H_4Br_2N_4S$: C 30.02, H 1.12, N 15.56; found C 29.95, H 1.15, N 15.63

6,8-dibromo-5-methyl-2H-[1,2,4]triazino[5,6-b]indol-3(5H)-one ***5k***

IR (KBr): ν_{max} = 3271 (NH), 1661 (C=O, amide), 1610 (C=N) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 2.14 (s, 3H, N-CH_3), 7.27 (d, 1H, *J*= 7.8 Hz, ArH), 7.47 (d, 1H, *J*= 7.8 Hz, ArH), 11.93 (s, 1H, =N-NH) ppm. Anal. calcd for $C_{10}H_6Br_2N_4O$: C 33.55, H 1.69, N 15.65; found C 33.59, H 1.63, N 15.71.

6,8-dibromo-5-methyl-2H-[1,2,4]triazino[5,6-b]indole-3(5H)-thione ***5l***

IR (KBr): ν_{max} = 3247 (NH), 1627 (C=N), 1025 (C=S) cm^{-1}. ^1H NMR (300 $MH_{Z,}$ DMSO-d_6) δ= 2.26 (s, 3H, N-CH_3), 7.26 (d, 1H, *J*= 7.7 Hz, ArH), 7.68 (d, 1H, *J*= 7.7 Hz, ArH), 12.39 (s, 1H, C =N-NHCS) ppm. Anal. calcd for $C_{10}H_6Br_2N_4S$: C 32.11, H 1.62, N 14.98; found C 32.06, H 1.68, N 15.05.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar, Ghaziabad, Uttar Pradesh, for providing research facilities. The authors are also really grateful to CDRI, Lucknow and IIT, New Delhi for providing the spectral analysis of the compounds.

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