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Synthesis, preliminary anticonvulsant and toxicity screening of substituted {1-[4-Methyl-2-substitutedphenyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine

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ABSTRACT

1,5-benzothiazepine moiety is a very important pharmacophore bioactive compound that exhibits different biological activities. The basic constituent of benzodiazepine nucleus is diltiazem. 1,5-benzothiazepine nucleus shows different potential biological activities in various field. Our research mainly focuses on synthesis of new substituted benzodiazepine nucleus which retained anticonvulsant biological activities with less toxic effect. We synthesized successfully various substituted {1-[4-Methyl-2-substitutedphenyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (**6a**–**6j**) and chemical structures confirmed by chromatographic, electro analytical, and physiochemical methods. The preliminary screening of novel synthesized compounds anticonvulsant activity by maximal electroshock model. Based on anticonvulsant screening result, the most potent derivative was found **6c**. Furthermore, preliminary safety profile of most active compound **6c** was evaluated by the neurotoxicity and acute oral toxicity testing.

INTRODUCTION

The medicinal chemistry is an essential tool for development and discovery of new drug for prevention of cure and disease. The new drug design is very complex method for the discovery of new pharmacophore moiety (Jeyaprakash *et al*., 2009). The benzodiazepines are the class of compound having benzodiazepine type nucleus the only difference between then is of sulfur atom in place of the nitrogen atom in heterocyclic ring system (Jeyaprakash *et al*., 2009).

Epilepsy is the one of the oldest diseases which found in human being population according to the World Health Organization. The distinctive inclination to recurring seizures and is definite by one or more malicious seizures. Seizure may differ from the least period of time or muscle twitching to chronic and elongated convulsions. They may also diverge in regularity from several months to several days (Ameta *et al*., 2013).

World Health Organization, epilepsy is a result of cellular dysfunction in the brain region due to excessive firing of a hyper excitability of neurons, and leads to neuronal dysfunction (Pandeya *et al*., 2003).

1, 5-benzothiazepine nucleus containing drug molecules is used for the lead discovery because it acts against various target receptor site. 1, 5-benzothiazepine nucleus containing first molecule diltiazem that is used as clinical interest, then after clentiazem and clothiapine were used in CNS as well as cardiovascular disorders. Substituted 1, 5-benzothiazepine is an identical crucial agent in the field of new drug development and research (Raja *et al*., 2007).

1,5-benzothiazepine and its derivatives are significant class of compounds in organic as well as medicinal chemistry. 1,5-benzothiazepine nucleolus is an important pharmacophore ring system which contains various pharmacological biological activities (Ansari *et al*., 2008).

Literature survey revealed that various substitution at 3 and 5 positions yield a compound of clinical interest. In the past studies,

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the substitution has been done at various positions in the ring system. Our consideration is mainly at the position 3 and 5. At position number 3, the substitution is the done at the side chain and at position number 5, the H atom is replaced by different suitable substituent.

The newly synthesize molecules were identified by elemental and spectroscopic methods.

The newly synthesized molecules (**6a–6j**) were preliminary screen as anticonvulsant agent by MES model. Rota rod study was performed for evaluation of neurotoxicity of compounds. Hence, to ascertain and establish the safety for its use, acute toxicity studies as per OCED guidelines 420.

MATERIALS AND METHODS

The determination of melting point of synthesized compound was confirmed by open tube capillary technique. Chemical reaction was monitor through TLC and R_f value determine on silica gel G (E. Merck) plate using chloroform: methanol (9:1) as a mobile phase. Infrared was identified on PerkinElmer FTIR-8400S spectrometer (SHIMADZU, Japan) by pressed pellet technique. Proton NMR spectra were characterized by Bruker DRX-300 spectrophotometer in solvent DMSO-D_6. Molecular mass was characterized by electro spray ionization (ESI)-MS (Schimadzu-2010 AT) under ESI technique. Microanalysis was determined by Carlo Erba EA 1108 elemental analyzer. Log *P* calculated by using octanol-phosphate buffer, and C log *P* value was identified by software Chem Draw Ultra 8.

All the solvents and reagents were obtained by Qualigens® Fine chemical.

Synthesis of substituted 1, 5-benzothiazepine (6a–6j)

2, 4-Pentadione and piperidine were dissolve in benzene, at the room temperature substituted benzaldehyde was mixed drop wise drop over 20 minutes. The chemical reaction mixture reflux for 2 hours with continuous stirring. Cool the reaction mixtures, organic layer was wash with aqueous cold 10% sodium carbonate, collect the product, and treat with *o*-aminothiophenol in methanol with stirring about 1 hour, collect the solid product, and washed with water and methanol.

Collected solid product was taken in methanol then acetic acid added until pH reaches 4 and stirred the reaction mixture for 12 hours. Collect the solid product, wash, and recrystallized by methanol. Schiff's bases (**6a–6j**) were synthesized by reacting compound (**5**) with hydrazine hydrate for about 3 hours refluxing. The scheme is given in Figure 1.

Partition coefficient (log P) determination

Partition coefficient (log *P*) of synthesized compound determine by flask shake method by using octanol and phosphate

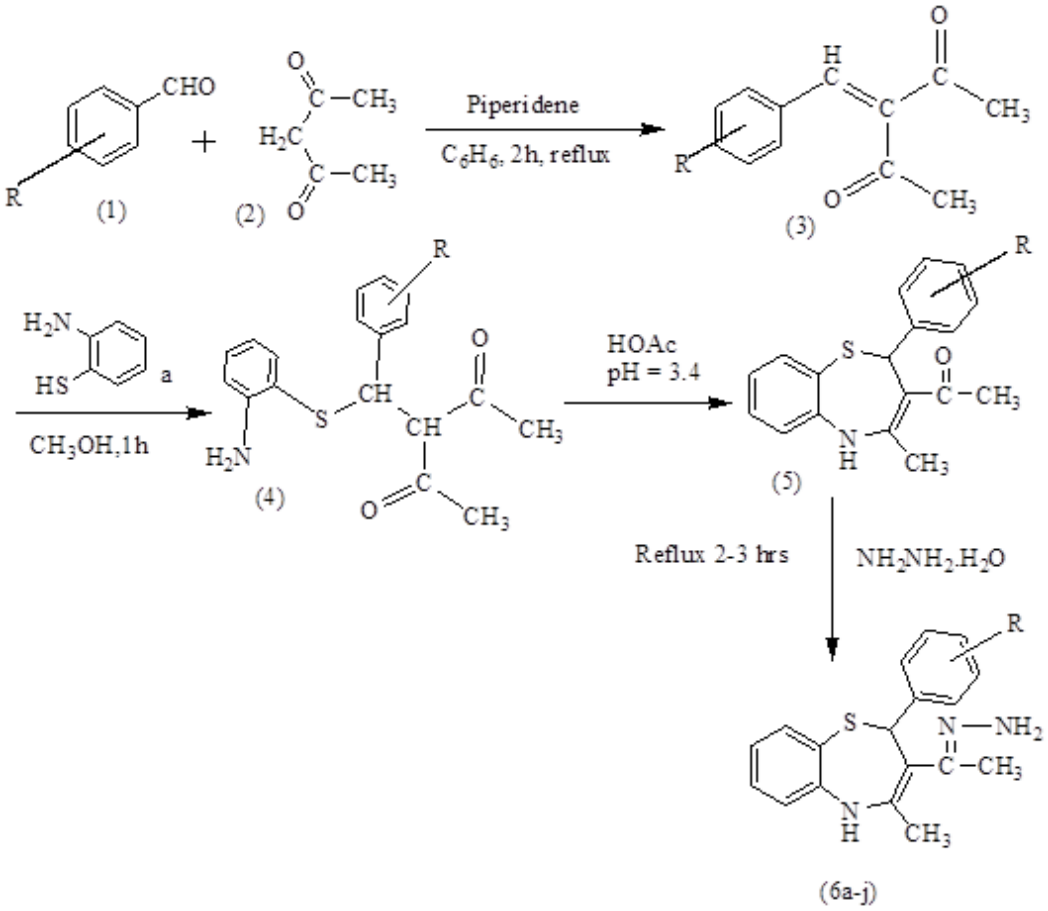

Figure 1. Scheme for Synthetic route of the Schiff's base (**6a–6j**).

Table 1. Physicochemical data of compound (**6a–6j**).

Compound code	*R*	Molecular formula	Log P value	C Log *p* value	Melting point (°C)	[a]R_f value	Yield (%)
6a	H	$C_{18}H_{19}N_3S$	1.97	1.92	65	0.84	49
6b	4-Cl	$C_{18}H_{18}ClN_3S$	2.68	2.58	55–56	0.78	46
6c	3-Cl	$C_{18}H_{18}ClN_3S$	2.16	2.13	60–62	0.80	49
6d	2-Cl	$C_{18}H_{18}ClN_3S$	2.35	2.27	90–91	0.76	51
6e	4-NO_2	$C_{18}H_{18}N_4O_2S$	0.94	0.82	87–89	0.64	55
6f	3- NO_2	$C_{18}H_{18}N_4O_2S$	0.86	0.87	80–82	0.59	62
6g	2-NO_2	$C_{18}H_{18}N_4O_2S$	0.82	0.84	75–76	0.85	39
6h	4-OCH_3	$C_{19}H_{21}N_3OS$	2.28	2.18	98–101	0.48	67
6i	3-OCH_3	$C_{19}H_{21}N_3OS$	2.45	2.54	58–59	0.72	48
6j	2-OCH_3	$C_{19}H_{21}N_3OS$	2.67	2.62	68–70	0.51	62

[a]Mobile phase: Chloroform: Methanol (9:1).

buffer. The calculate log *P* (C log *P*) determine by using software chem. Draw ultra 8 version.

COMPOUND DETAIL

{1-[4-Methyl-2-phenyl-2, 5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6a)

Yield 49%; FTIR (KBr) ν, cm^{-1}): 3,380 (N-H_{str}), 3,080 (Ar. C-H_{str}), 2,958 (Ali. C-H_{str}), 1,685 (Ar. C⋯C$_{str}$), 1,602 (C = N_{str}), 1,554 (Ar. C-C_{str}), 1,326 (Ar. C-N_{str}), 703 (C-S_{str}); ^{1}H NMR, δ ppm: 1.210 (s, 3H, CH_3), 2.300 (s, 3H, CH_3), 4.207 (s, 2H, NH_2, D_2O exchange), 6.103 (s, 1H, Ar-H), 7.103–7.179 (t, 2H, Ar-H), 7.210–7.243 (d, 2H, Ar-H), 7.501–7.584 (m, 3H, Ar-H), 7.605–7.641 (d, 2H, Ar-H), 8.174 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 310 $[M + 1]^+$; Elemental analysis: C, 68.92, H, 6.36, N, 12.31, S, 09.23%.

{1-[4-Chlorophenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6b)

Yield 46%; FTIR (KBr) ν, cm^{-1}): 3,386 (N-H_{str}), 3,089 (Ar. C-H_{str}), 2,887 (Ali. C-H_{str}), 1,686 (Ar. C-C_{str}), 1,598 (C = N_{str}), 1,531 (Ar. C-C_{str}), 1,363 (Ar. C-N_{str}), 1,064 (Ar. C-Cl_{str}), 858 (C-H *p*-disub. benzene), 686 (C-S_{str}); ^{1}H NMR, δ ppm: 1.201 (s, 3H, CH_3), 2.700 (s, 3H, CH_3), 4.100 (s, 2H, NH_2, D_2O exchange), 5.951 (s, 1H, Ar-H), 7.203–7.244 (t, 2H, Ar-H), 7.410–7.425 (d, 2H, Ar-H), 7.501–7.527 (d, 2H, Ar-H), 7.854–7.895 (m, 3H, Ar-H), 8.901 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 344 $[M + 1]^+$; 345 $[M + 2]^+$; Elemental analysis: C, 64.83, H, 6.27, N, 12.41, S, 10.01, Cl, 11.05%.

{1-[3-Chlorophenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6c)

Yield 49%; FTIR (KBr) ν, cm^{-1}): 3,414 (N-H_{str}), 3,093 (Ar. C-H_{str}), 2,977 (Ali. C-H_{str}), 1,618 (Ar. C-C_{str}), 1,548 (C = N_{str}), 1,461 (Ar. C-C_{str}), 1,274 (Ar. C-N_{str}), 1,074 (Ar. C-Cl_{str}), 721 (C-H *m*-disub. benzene), 645 (C-S_{str}); ^{1}H NMR, δ ppm: 1.220 (s, 3H, CH_3), 2.200 (s, 3H, CH_3), 4.122 (s, 2H, NH_2, D_2O exchange), 6.167 (s, 1H, Ar-H), 7.123–7.169 (t, 2H, Ar-H), 7.363–7.367 (d, 1H, Ar-H), 7.500–7.534 (t, 1H, Ar-H), 7.705–7.721 (d, 2H, Ar-H), 7.902 (s, 1H, Ar-H), 7.910–8.032 (d, 1H, Ar-H), 9.140 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 344 $[M + 1]^+$; 345 $[M + 2]^+$; Elemental analysis: C, 62.82, H, 5.31, N, 13.83, S, 10.22, Cl, 10.21%.

{1-[2-Chlorophenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6d)

Yield 51%; FTIR (KBr) ν, cm^{-1}): 3,365 (N-H_{str}), 3,051 (Ar. C-H_{str}), (Ali. C-H_{str}), 1,677 (C = N_{str}), 1,630 (Ar. C-C_{str}), 1,419 (Ar. C-C_{str}), 1,315 (Ar. C-N_{str}), 1,091 (Ar. C-Cl_{str}), 761 (C-H *o*-disub. benzene), 678 (C-S_{str}); ^{1}H NMR, δ ppm: 1.310 (s, 3H, CH_3), 2.202 (s, 3H, CH_3), 4.100 (s, 2H, NH_2, D_2O exchange), 4.402 (s, 1H, Ar-H), 7.043–7.103 (d, 2H, Ar-H), 7.216–7.243 (d, 1H, Ar-H), 7.401–7.435 (m, 3H, Ar-H), 7.509–7.524 (d, 2H, Ar-H), 9.162 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 344 $[M + 1]^+$; 345 $[M + 2]^+$; Elemental analysis: C, 62.45, H, 6.75, N, 11.39, S, 10.69, Cl, 11.03%.

{1-[4-Nitrophenyl-4-methyl--2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6e)

Yield 55%; FTIR (KBr) ν, cm^{-1}): 3,320 (N-H_{str}), 3,076 (Ar. C-H_{str}), (Ali. C-H_{str}), 1,722 (Ar. C-C_{str}), 1,613 (C=N_{str}), 1,510 (Ar. C-C_{str}), 1,450 (N-O str.), 1,348 (Ar. C-N_{str}), 847 (C-H *p*-disub. benzene), (C-S_{str}); ^{1}H NMR, δ ppm: 1.321 (s, 3H, CH_3), 2.102 (s, 3H, CH_3), 4.000 (s, 2H, NH_2, D_2O exchange), 5.410 (s, 1H, Ar-H), 7.053–7.109 (d, 2H, Ar-H), 7.210–7.235 (d, 2H, Ar-H), 7.421–7.439 (d, 2H, Ar-H), 7.654–7.690 (d, 2H, Ar-H), 9.102 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 355 $[M + 1]^+$; Elemental analysis: C, 62.37, H, 6.62, N, 16.92, S, 08.39%.

{1-[3-Nitrophenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6f)

Yield 62%; FTIR (KBr) ν, cm^{-1}): (N-H_{str}), 3,097 (Ar. C-H_{str}), 2,913 (Ali. C-H_{str}), 1,647 (Ar. C-C_{str}), 1,612 (C=N_{str}), 1,579 (Ar. C-C_{str}), 1,483 (N-O str.), 1,337 (Ar. C-N_{str}), 698 (C-H *m*-disub. benzene), 624 (C-S_{str}); ^{1}H NMR, δ ppm: 1.240 (s, 3H, CH_3), 2.212 (s, 3H, CH_3), 4.100 (s, 2H, NH_2, D_2O exchange), 6.107 (s, 1H, Ar-H), 7.103–7.189 (t, 2H, Ar-H), 7.303–7.342 (d, 1H, Ar-H), 7.510–7.574 (t, 1H, Ar-H), 7.705–7.731 (d, 2H, Ar-H), 7.907 (s, 1H, Ar-H), 7.915–8.035 (d, 1H, Ar-H), 9.240 (s, 1H, N-H, D_2O exchange); MS (*m*/*z*): 355 $[M + 1]^+$; Elemental analysis: C, 63.82, H, 6.18, N, 14.30, S, 10.72%.

{1-[2-Nitrophenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6g)

Yield 39%; FTIR (KBr) ν, cm^{-1}): 3,370 (N-H_{str}), 3,075 (Ar. C-H_{str}), 2,925 (Ali. C-H_{str}), 1,635 (Ar. C-C_{str}), 1,623 (C=N_{str}), 1,500 (Ar. C-C_{str}), 1,421 (N-O str.), 1,325 (Ar. C-N_{str}), 806 (C-H *o*-disub. benzene), 700 (C-S_{str}); ^{1}H NMR, δ ppm: 1.150 (s, 3H, CH_3), 2.003 (s, 3H, CH_3), 4.000 (s, 2H, NH_2, D_2O exchange), 5.502 (s, 1H, Ar-H), 7.215–7.269 (d, 2H, Ar-H), 7.304–7.325 (d, 1H, Ar-H), 7.610–7.630 (d, 2H, Ar-H), 7.904–7.923 (m, 3H, Ar-H), 9.213 (s, 1H, N-H, D_2O exchange); MS (*m/z*): 355 $[M + 1]^+$; Elemental analysis: C, 62.83, H, 6.26, N, 14.29, S, 9.71%.

{1-[4-Methoxyphenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6h)

Yield 67%; FTIR (KBr) ν, cm^{-1}): 3,246 (N-H_{str}), 3,114 (Ar. C-H_{str}), 2,947 (Ali. C-H_{str}), 1,608 (Ar. C-C_{str}), 1,564 (C=N_{str}), 1,500 (Ar. C-C_{str}), 1,346 (Ar. C-N_{str}), 1,064 (C-O-C_{str}), 769 (C-H *p*-disub. benzene), 690 (C-S_{str}); ^{1}H NMR, δ ppm: 1.304 (s, 3H, CH_3), 2.241 (s, 3H, CH_3), 3.730 (s, 3H, OCH_3), 4.000 (s, 2H, NH_2, D_2O exchange), 5.408 (s, 1H, Ar-H), 6.110–6.133 (d, 2H, Ar-H), 6.940–6.953 (d, 2H, Ar-H), 7.310–7.364 (t, 2H, Ar-H), 7.733–7.765 (d, 2H, Ar-H), 8.802 (s, 1H, N-H, D_2O exchange); MS (*m/z*): 340 $[M + 1]^+$; Elemental analysis: C, 66.54, H, 5.27, N, 11.03, S, 9.83%.

{1-[3-Methoxyphenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6i)

Yield 48%; FTIR (KBr) ν, cm^{-1}): 3,386 (N-H_{str}), 3,089 (Ar. C-H_{str}), 2,970 (Ali. C-H_{str}), 1,686 (Ar. C-C_{str}), 1,598 (C=N_{str}), 1,531 (Ar. C-C_{str}), 1,363 (Ar. C-N_{str}), 1,145 (C-O-C_{str}), 730 (C-H *m*-disub. benzene), 686 (C-S_{str}); ^{1}H NMR, δ ppm: 1.264 (s, 3H, CH_3), 2.320 (s, 3H, CH_3), 3.734 (s, 3H, OCH_3), 4.122 (s, 2H, NH_2, D_2O exchange), 5.601 (s, 1H, Ar-H), 6.080–6.093 (d,1H, Ar-H), 6.408 (s, 1H, Ar-H), 6.700–6.777 (t, 1H, Ar-H), 6.940–6.957 (d, 1H, Ar-H), 7.260–7.283 (t, 2H, Ar-H), 7.509–7.525 (d, 2H, Ar-H), 8.942 (s, 1H, N-H, D_2O exchange); MS (*m/z*): 340 $[M + 1]^+$; Elemental analysis: C, 66.72, H, 7.95, N, 13.83, S, 08.11%.

{1-[2-Methoxyphenyl-4-methyl-2,5-dihydro-1,5-benzothiazepin-3-yl]-ethylidene}-hydrazine (6j)

Yield 62%; FTIR (KBr) ν, cm^{-1}): 3,290 (N-H_{str}), 3,070 (Ar. C-H_{str}), 2,937 (Ali. C-H_{str}), 1,708 (Ar. C-C_{str}), 1,610 (C=N_{str}), 1,510 (Ar. C-C_{str}), 1,348 (Ar. C-N_{str}), 1,180 (C-O-C_{str}), 744 (C-H *o*-disub. benzene), 692 (C-S_{str}); ^{1}H NMR, δ ppm: 1.208 (s, 3H, CH_3), 2.203 (s, 3H, CH_3), 3.734 (s, 3H, OCH_3), 4.312 (s, 2H, NH_2, D_2O exchange), 6.280 (s, 1H, Ar-H), 6.503–6.548 (m, 3H, Ar-H), 6.957–7.060 (d, 1H, Ar-H), 7.269–7.299 (t, 2H, Ar-H), 7.305–7.359 (d, 2H, Ar-H), 8.350 (s, 1H, N-H, D_2O exchange); MS (*m/z*): 340 $[M + 1]^+$; Elemental analysis: C, 66.02, H, 7.32, N, 13.59, S, 08.96%.

PHARMACOLOGY

In this study, we take both sexes of albino mice 25–30 gm in my protocol. The animals were housed protocol standard condition at ambient temperature 25°C ± 2°C and tolerable free access to food and water except at time they brought out of cage. Experimental protocol was done as per institutional animal ethical committee of the Mahatma Gandhi Institute of Pharmacy, Lucknow (1957/PO/Re/S/17CPCSEA), who approved the protocol.

Anticonvulsant screening

Anticonvulsant screening was performed on laboratory experimental animal albino mice weight 20–25 g. All required experimental animal acclimatized to the experimental condition 1 day prior to initiation of biological activity. All experimental animals were fasted overnight. The test compounds mixed in PEG400 aq. Solution (30% *v/v*) and administered intraperitoneally to experimental animals for phase first pharmacological screening by maximum electroshock and neurotoxicity study.

Maximum electroshock (MES) test

Weight all the experimental animals, marked, and divided into three groups with six animals in each group named as control, standard, and test. At the first day of experiment, the control group was administered with 30% *v/v* PEG400 aq. Solution and to the standard group Phenytoin (30 mg/kg) in 30% *v/v* PEG400) was administered intraperitoneally and test compound administered intraperitoneally to test group at three different doses 30, 100, and 300 mg/kg in 30% *v/v* PEG400. Next day, the same procedure was followed for second and third group with others test compounds (Bhrigu *et al*., 2012; Garg, 2010; Siddiqui *et al*., 2015).

Handling the animal properly then corneal electrodes placed the upper eye lid and 150 mA current was given for 0.2 second time. We note down that the time spends at 0.5 and 4 hours after drug administration in different phases of seizures (a) tonic flexion, (b) tonic extensor, (c) clonic convulsion, (d) stupor, and (e) recovery or death was observed in each group of animals.

The above procedure repeated with control and standard group of the animals. We observed that the reduce time or abolish extensor phase after given electroshock and data presented in Tables 2 and 3 (Kulkarni, 2011).

Neurotoxicity studies

Motor impairment was screen in rats by using rotarod apparatus. Before the experiment start all the experimental animals trained to stay on accelerating rotarod at rotational speed up to 10 rpm. The test drug administered to animals then placed the animals on a knurled rotating rod. Neurotoxicity became visible by absence of rats to hold rod for 1 minute at least in every of three trials (Kulkarni, 2011).

Acute oral toxicity studies

Most potent active compound was evaluated for acute oral toxicity in albino mice weight 20–25 g according to the OECD guidelines 420. The animals were randomly divided into two groups with six mice (both sex) in each. Group-I (Control) receive distal water orally. Group-II was administered **6c** compound at single dose of 2,000 mg/kg, orally, respectively. Food and water was hold for about 2 hours after the administration of test drug. We notice that related to clinical sign and symptoms, for initially 24 hours, with special courtesy given starting 4 hours duration and daily subsequently 14 days after the test drug administration. Furthermore, factors like righting reflux, gripping, pupils, pain response, tremors, convulsion, skin color, corneal

Table 2. Anticonvulsant evaluation after 0.5 hours administration of synthesized compounds (**6a–6j**) using MES model.

Compound No.	Doses (mg/kg)	Time in second of various phases after 0.5 hours				Death/recovery	Neurotoxicity screen
		Flexion	Extensor	Clonus	Stupor		
6a	30	3.43 ± 0.16**	16.27 ± 0.26***	14.80 ± 0.06***	115.4 ± 0.32**	Recovery	Absent
	100	3.21 ± 0.19**	14.31 ± 0.17*	9.24 ± 0.17***	48.40 ± 0.19***	Recovery	Absent
	300	4.45 ± 0.19ns	23.78 ± 0.99***	34.63 ± 0.13***	118.4 ± 0.17***	Recovery	Absent
6b	30	2.92 ± 0.21**	5.58 ± 0.16***	8.74 ± 0.18***	45.86 ± 0.28***	Recovery	Absent
	100	2.68 ± 0.32**	4.48 ± 0.97***	8.59 ± 0.07***	46.45 ± 0.16***	Recovery	Absent
	300	3.51 ± 0.05***	21.38 ± 0.29***	32.70 ± 0.15***	112.8 ± 0.30**	Recovery	Absent
6c	30	1.97 ± 0.12***	3.04 ± 0.13***	7.67 ± 0.31***	38.59 ± 0.50***	Recovery	Absent
	100	2.20 ± 0.09***	3.19 ± 0.10***	7.96 ± 0.19***	43.55 ± 1.35***	Recovery	Absent
	300	2.43 ± 0.15***	9.81 ± 0.32***	18.22 ± 0.23***	113.8 ± 0.40***	Recovery	Absent
6d	30	2.29 ± 0.09***	3.83 ± 0.24***	7.73 ± 0.26**	42.45 ± 0.24***	Recovery	Absent
	100	2.69 ± 0.07***	4.40 ± 0.20***	8.42 ± 0.15***	44.11 ± 0.30***	Recovery	Absent
	300	3.48 ± 0.13*	20.23 ± 0.20***	34.57 ± 0.10***	118.8 ± 0.29***	Recovery	Absent
6e	30	7.42 ± 0.21***	22.65 ± 0.16***	39.05 ± 0.22***	190.7 ± 0.22***	Recovery	Absent
	100	7.39 ± 0.13***	22.91 ± 0.18***	38.25 ± 0.20***	189.4 ± 0.24***	Recovery	Absent
	300	6.62 ± 0.10***	20.50 ± 0.18***	36.31 ± 0.14***	188.1 ± 0.26***	Recovery	Absent
6f	30	6.19 ± 0.28**	17.63 ± 0.10***	35.72 ± 0.06***	164.9 ± 0.26***	Recovery	Absent
	100	6.45 ± 0.08***	17.78 ± 0.24***	34.90 ± 0.16***	163.2 ± 0.43***	Recovery	Absent
	300	6.40 ± 0.14***	21.82 ± 0.25***	38.16 ± 0.24***	187.4 ± 0.40***	Recovery	Absent
6g	30	8.42 ± 0.08***	22.70 ± 0.11***	42.43 ± 0.14***	194.0 ± 0.17***	Recovery	Absent
	100	7.95 ± 0.21***	21.80 ± 0.20***	41.99 ± 0.23***	192.7 ± 0.15***	Recovery	Absent
	300	7.43 ± 0.09***	21.78 ± 0.24***	38.05 ± 0.20***	192.4 ± 0.24***	Recovery	Absent
6h	30	5.53 ± 0.12*	22.43 ± 0.30***	21.92 ± 0.45***	121.4 ± 0.25***	Recovery	Absent
	100	3.56 ± 0.16*	14.26 ± 0.27**	17.46 ± 0.16***	52.17 ± 0.24***	Recovery	Absent
	300	5.64 ± 0.11*	23.57 ± 0.30***	34.75 ± 0.18***	128.0 ± 0.28***	Recovery	Absent
6i	30	4.74 ± 0.17ns	20.82 ± 0.20***	18.11 ± 0.42***	120.7 ± 0.30***	Recovery	Absent
	100	3.84 ± 0.13**	12.82 ± 0.25**	11.44 ± 0.37***	51.41 ± 0.35***	Recovery	Absent
	300	5.70 ± 0.14*	22.36 ± 0.28***	39.44 ± 0.22***	127.0 ± 0.29***	Recovery	Absent
6j	30	4.50 ± 0.13ns	18.34 ± 0.16***	15.42 ± 0.17***	115.6 ± 0.15**	Recovery	Absent
	100	3.42 ± 0.09**	5.05 ± 0.20***	9.09 ± 0.22***	48.40 ± 0.12**	Recovery	Absent
	300	5.41 ± 0.07*	21.88 ± 0.18***	38.51 ± 0.06***	124.7 ± 0.22***	Recovery	Absent
Control	30% v/v PEG-400	4.86 ± 0.21	15.97 ± 0.15	22.67 ± 0.36	108.7 ± 1.15***	Recovery	Absent
Phenytoin	30	2.45 ± 0.12***	3.35 ± 0.15***	7.98 ± 0.30***	41.40 ± 0.37***	Recovery	Absent

$n = 6$.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

reflex, salivation, torch response, water intake, food intake, sleep, diarrhea, grooming, urination, alertness, lethargy, touch response, coma, and mortality were observed (Ali and Siddiqui, 2014; Jonsson *et al.*, 2013; OECD 2000, 2000; Porwal *et al*., 2017) and presented in Table 4.

Statistical analysis

All the statistical analysis were analyzed and collect the data by using graph pad prism 5 version software. All the values represented in the form of Mean ± SEM, and these all values analyzed by analysis of variance then multiple comparison test followed by Dunnett's.

RESULTS AND DISCUSSION

Chemistry

All newly synthesized compounds **(6a–6j)** were identified by physicochemical parameters and related data described in Table 1. Finally, synthesized substituted Schiff's bases identified by elemental and spectroscopic analytical methods.

Anticonvulsant activity

The newly synthesized substituted antiepileptic drug containing activity of Schiff's bases carried out research under the antiepileptic drug development program. Classified active drug into three subsequent classes: class I drug active at 100 mg/kg or

Table 3. Anticonvulsant evaluation after 4 hours administration of synthesized compounds (**6a–6j**) using MES model.

Compound No.	Doses (mg/kg)	Time in second of various phases after 4 hours				Death/ recovery	Neurotoxicity screen
		Flexion	Extensor	Clonus	Stupor		
6a	30	3.05 ± 0.17**	15.73 ± 0.21ns	14.17 ± 0.14***	115.0 ± 0.29**	Recovery	Absent
	100	2.61 ± 0.10***	3.58 ± 0.11***	8.47 ± 0.10***	42.37 ± 0.30***	Recovery	Absent
	300	5.39 ± 0.25*	24.32 ± 0.20***	35.00 ± 0.63***	118.2 ± 0.23***	Recovery	Absent
6b	30	2.78 ± 0.30**	5.32 ± 0.29***	8.25 ± 0.15***	44.71 ± 0.24***	Recovery	Absent
	100	2.53 ± 0.24**	4.10 ± 0.10***	8.35 ± 0.11***	45.67 ± 0.42***	Recovery	Absent
	300	3.49 ± 0.10**	22.27 ± 0.23***	33.29 ± 0.17***	114.6 ± 0.56*	Recovery	Absent
6c	30	1.85 ± 0.17***	2.87 ± 0.10***	7.49 ± 0.16***	37.41 ± 0.36***	Recovery	Absent
	100	1.61 ± 0.19***	2.84 ± 0.08***	7.75 ± 0.23***	40.80 ± 0.65***	Recovery	Absent
	300	2.52 ± 0.20***	12.29 ± 0.24***	19.62 ± 0.35*	118.0 ± 0.25***	Recovery	Absent
6d	30	2.18 ± 0.06***	3.67 ± 0.29***	7.55 ± 0.32***	41.61 ± 0.31***	Recovery	Absent
	100	2.37 ± 0.04***	4.16 ± 0.17***	7.62 ± 0.28***	43.73 ± 0.36***	Recovery	Absent
	300	3.24 ± 0.11***	20.17 ± 0.40***	35.68 ± 0.09***	120.1 ± 0.26***	Recovery	Absent
6e	30	6.89 ± 0.18***	22.14 ± 0.26***	38.14 ± 0.30***	189.8 ± 0.63***	Recovery	Absent
	100	7.09 ± 0.26***	22.23 ± 0.36***	37.69 ± 0.29***	188.4 ± 0.40***	Recovery	Absent
	300	6.78 ± 0.21***	16.71 ± 0.18**	36.57 ± 0.28***	187.4 ± 0.40***	Recovery	Absent
6f	30	5.74 ± 0.19**	16.99 ± 0.10**	35.04 ± 0.08***	164.3 ± 0.24***	Recovery	Absent
	100	5.83 ± 0.11**	17.07 ± 0.17**	34.58 ± 0.18***	162.7 ± 0.48***	Recovery	Absent
	300	5.64 ± 0.17**	21.40 ± 0.21***	37.06 ± 0.39***	186.1 ± 0.73***	Recovery	Absent
6g	30	8.00 ± 0.16***	21.97 ± 0.19***	42.35 ± 0.16***	193.6 ± 0.22***	Recovery	Absent
	100	7.69 ± 0.22***	21.35 ± 0.20***	41.62 ± 0.38***	192.6 ± 0.28***	Recovery	Absent
	300	7.52 ± 0.19***	18.50 ± 0.13***	37.59 ± 0.11***	188.1 ± 0.24***	Recovery	Absent
6h	30	4.97 ± 0.19ns	21.95 ± 0.29***	21.62 ± 0.72 ns	121.0 ± 0.28***	Recovery	Absent
	100	3.47 ± 0.12**	5.59 ± 0.19***	10.94 ± 0.19***	48.96 ± 0.15***	Recovery	Absent
	300	4.88 ± 0.24ns	23.02 ± 0.28***	33.84 ± 0.23***	126.1 ± 0.29***	Recovery	Absent
6i	30	4.24 ± 0.30ns	19.55 ± 0.25***	17.53 ± 0.48**	120.2 ± 0.41***	Recovery	Absent
	100	2.74 ± 0.11***	4.79 ± 0.12***	8.93 ± 0.24***	48.59 ± 0.20***	Recovery	Absent
	300	5.16 ± 0.12**	21.94 ± 0.25***	38.66 ± 0.15***	126.1 ± 0.55***	Recovery	Absent
6j	30	3.97 ± 0.07**	17.76 ± 0.17**	14.89 ± 0.20***	115.0 ± 0.21**	Recovery	Absent
	100	2.42 ± 0.12***	4.36 ± 0.07***	8.69 ± 0.26***	44.98 ± 0.28***	Recovery	Absent
	300	4.86 ± 0.16ns	21.14 ± 0.23***	37.65 ± 0.27***	124.2 ± 0.18***	Recovery	Absent
Control	30% v/v PEG-400	4.67 ± 0.26	15.57 ± 0.21	21.26 ± 0.65	109.1 ± 1.46	Recovery	Absent
Phenytoin	30	1.83 ± 0.10***	2.92 ± 0.08***	7.13 ± 0.23***	38.41 ± 0.57***	Recovery	Absent

$n = 6$.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

less, class II drug active more than 100 mg/kg, and class III drug inactive at 300 mg/kg body weight of the animal.

Mostly compounds exhibit potent activity at 30 and 100 mg/kg dose without neurotoxin in nature except compounds **6e** and **6g**. Most of the compounds produced neurotoxicity at a maximum dose 300 mg/kg. Limited dose range (30–100 mg/kg) for activity showed the concept of therapeutic window phenomena. Compound **6c** was found to be most potent compound having large range of dose for activity and produces less neurotoxicity at 300 mg/kg. Compounds **6e, 6f,** and **6g** (nitro substituted) showed poor or less activity due to low log *P*.

From the above results, it was found that the chloro derivatives exhibit better activity and the *o*-substituted derivative was found to possess more significant activity, alteration at *p*-position affect the activity. All the nitro substituted compounds showed poor activity due to low CNS penetration. The introductions of the chloro group in ring enhance the anticonvulsant activity. Graphical data given in Figures 2 and 3.

Acute oral toxicity studies

Compound **6c** with a dose of 2,000 mg/kg delivered no harmful impact on the social reactions of the treated rodents (dosed once) and watched for 14 days. All the animals show neither any poisonous nor deadly impact. My current investigation shows that the administration of test compound up to 2,000 mg/kg did not any indication of harmfulness toxicity or mortality in animals at the time of experimental period.

Table 4. Effect of compound 6c for acute toxicity study.

S.N.	Response	Animals	
		Prior to treatment	Later to treatment
1.	Skin color	Normal	Normal
2.	Pain response	Normal	Normal
3.	Grooming	Absent	Absent
4.	Food intake	Normal	Normal
5.	Alertness	Normal	Normal
6.	Righting reflux	Normal	Normal
7.	Corneal reflex	Present	Present
8.	Tremors	Absent	Absent
9.	Pupils	Normal	Normal
10.	Convulsion	Absent	Absent
11.	Urination	Normal	Normal
12.	Sleep	Normal	Normal
13.	Diarrhea	Absent	Absent
14.	Torch response	Normal	Normal
15.	Lethargy	Absent	Absent
16.	Water intake	Normal	Normal
17.	Salivation	Normal	Normal
18.	Coma	Absent	Absent
19.	Gripping	Normal	Normal
20.	Mortality	Not applicable	Nil
21.	Touch response	Normal	Normal

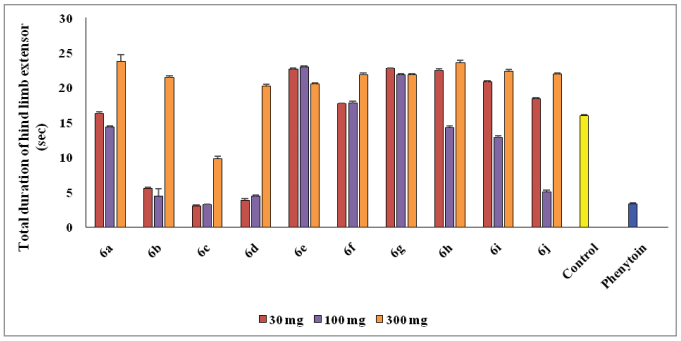

Figure 2. Graphical representation of anticonvulsant evaluation after 0.5 hours administration of synthesized compounds (**6a–6j**) using MES model.

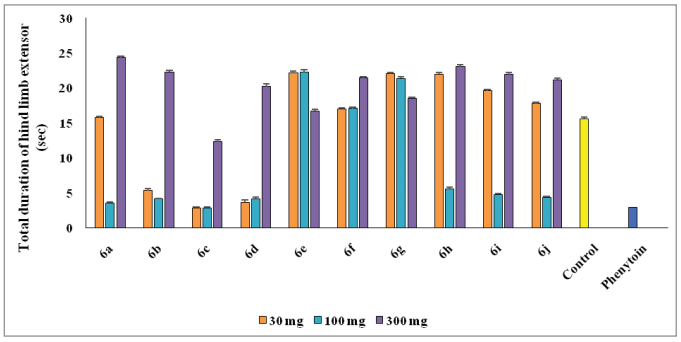

Figure 3. Graphical representation of anticonvulsant evaluation after 4 hours administration of synthesized compounds (**6a–6j**) using MES model.

CONCLUSION

In conclusion, a series of substituted Schiff's bases synthesized successful and every compound screen for anticonvulsant activity by utilizing MES model. The most potent compound **6c** was found as a primary class of anticonvulsants that have shown practically identical anticonvulsant action with uniquely lower neurotoxicity. The non-toxic nature of compound **6c** was determined by acute toxicity study according to the OECD 420 guidelines. Total 14 days observation normal behavior of animals that suggest the safety and innocuous nature of newly synthesized potent compound up to dose 2,000 mg/kg. Compound **6c** found as a lead molecule for further modification and improvement in field of antiepileptic drug development.

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ETHICAL APPROVALS

Experimental protocol was done as per institutional animal ethical committee of the Mahatma Gandhi Institute of Pharmacy, Lucknow (1957/PO/Re/S/17CPCSEA), approved the protocol.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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