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SYNTHESIS OF SOME NOVEL 4-(4-CHLOROPHENYL)-6-*p*-TOLYL-+-PYRIMIDINE DERIVATIVES AND THEIR ANTICONVULSANT ACTIVITY

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The term epilepsy is a collective term that includes disorders of the brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsies are common and frequently devastating and affect around 1–2% of the world population (1). It has been estimated that about 20% of the patients with epilepsy using the first generation of antiepileptic drugs (phenobarbital, phenytoin, carbamazepine, sodium valproate and diazepam) were not able to acquire adequate control of seizures. Current drug therapy for epilepsy suffers from a number of disadvantages, including the fact that the convulsions of approximately 25% of epileptics are inadequately controlled by medication (2). In recent years, the field of antiepileptic drug development has become quite dynamic, affording many promising research opportunities. In this paper, we present the synthesis of the semicarbazones (Scheme 1) and evaluation of their anticonvulsant activity.

EXPERIMENTAL

Chemistry

3-(4-Chlorophenyl)-1-(4-methyl phenyl)-2-propen-1-one (III)

The synthesis was accomplished by cross aldol condensation of 4-methylacetophenone (**I**) and 4-chlorobenzaldehyde (**II**).

Aqueous solution of sodium hydroxide (10% w/v, 10 mL) was added to a solution of 4-chlorobenzaldehyde (0.02 mol) and 4-methylacetophenone (0.02 mol) in ethanol (6 mL). The reaction mixture was stirred at room temperature overnight and poured into water (100 mL). After neutralization with hydrochloric acid (10% w/v), a yellow solid was obtained, which was recrystallized from water-ethanol, m.p. 114-116°C, yield 70% and R_f value 0.67 [silica gel-G, mobile phase: $CHCl_3$: $(C_2H_5)_2O$ (2:3, v/v)].

4-(4-Chlorophenyl)-6-(4-methylphenyl)-2-aminopyrimidine (IV)

A mixture of 3-(4-chlorophenyl)-1-(4-methylphenyl)-2-propen-1-one (**III**, 0.01 mol) and guanidine hydrochloride (0.015 mol) was added to sodium hydroxide (0.045 mol in 2 mL of water) and ethanol (50 mL). The reaction mixture was refluxed for 6 h. The mixture was concentrated under reduced pressure and cooled; a yellow solid was obtained which was recrystallized from ethanol, m. p. 174-176°C, yield 70.35% and R_f value 0.78 [silica gel-G, mobile phase: $CHCl_3$: $(C_2H_5)_2O$: C_2H_5OH (2:1:1, v/v/v)].

Phenyl 4-(4-chlorophenyl)-6-*p*-tolylpyrimidin-2-ylcarbamate (V)

Phenylchloroformate 12.6 mL (0.1 mol) was dissolved in 40 mL of chloroform and equimolar quantities of compound (**IV**), 19.41 mL (0,1 mol) and triethylamine 13.9 mL (0.1 mol) were added dropwise and stirred at room temperature for 5 h. The reaction mixture was concentrated to one-third volume and 100 mL of petroleum ether was added. The precipitate which appeared (**V**) was washed with water, filtered and dried, m.p. 124-126°C, yield 74% and R_f value 0.68 [silica gel-G, mobile phase: $CHCl_3$: dioxane : CH_3OH (2:1:1, v/v)].

4-[4-(4-Chlorophenyl)-6-*p*-tolylpyrimidin-2-yl] semicarbazide (VI)

Compound (**V**), 12.05 g (0,05 mol) was dissolved in 100 mL of dichloromethane. To this solution, 4.8 mL of hydrazine hydrate (0.1 mol) was added

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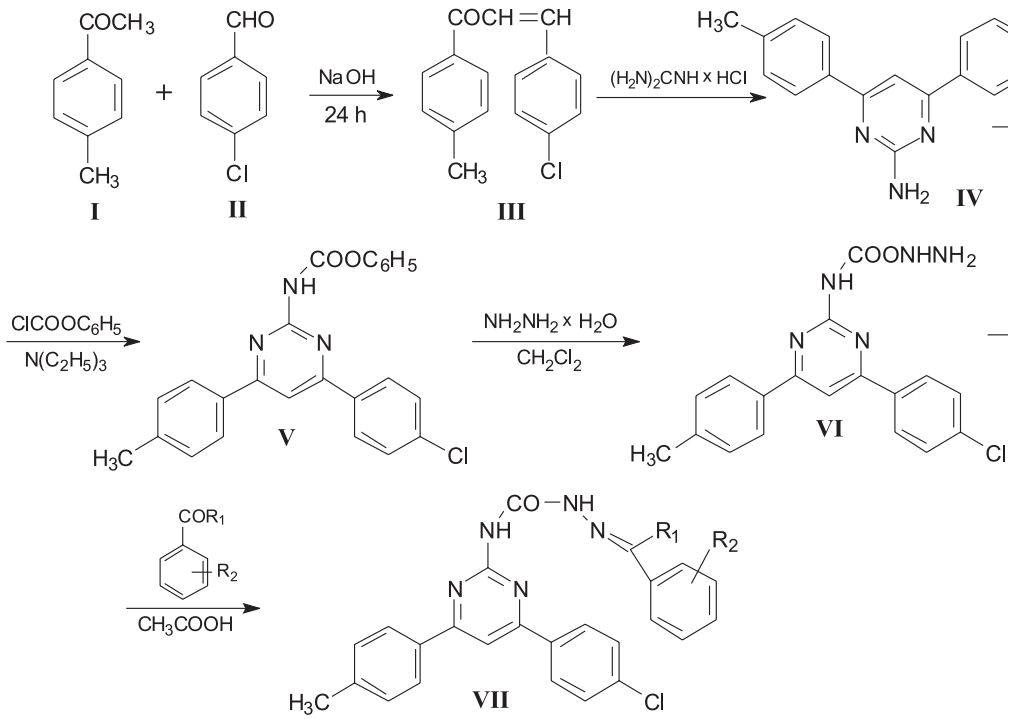


Scheme 1. Synthesis of semicarbazones **VII**

and refluxed with stirring for 24 h. The precipitate of compound **VI** was separated by filtration and washed with dichloromethane and dried, m.p. 184-186°C, yield 70%, R_f value 0.63 [(silica gel-G, mobile phase: $CHCl_3$: dioxane : CH_3OH (2:2:1, v/v/v)].

Preparation of final compounds (VII – DST 1–10)

To a solution of compound **(VI)** (0.84 g, 0.003 mol) in 25 mL of ethanol equimolar quantity of appropriate aldehyde or ketone in 5 mL of ethanol and 1–2 drops of glacial acetic acid were added. The mixture was stirred with heating for 3–4 h until the completion of the reaction and the resultant precipitate was filtered and dried. The products were recrystallized from 95% ethanol.

Biological evaluation of synthesized compounds

Maximal electroshock (MES) and neurotoxicity (rotarod method) models were used. The MES induced convulsions in animals represent grand mal type of epilepsy. In MES, electric shock is applied through the corneal electrodes.

MES method-induced seizures

Male albino mice (weight 20–25 g) were used to test the compounds. Female animals were excluded because of the fact that menstrus cycle influences the seizures threshold. Animals were housed in polypropylene cage with dust free rice husk as bedding material under laboratory conditions with controlled environment of temperature 25 ± 2°C, humidity 60 ± 10% and before subjecting them to experimentation, the animals were given a week of time to get acclimatized with laboratory condition. The animals were fasted overnight before the experiment. The animals used in the testing were numbered and weighed and were divided into three groups each containing six mice. One group was used as the control, the second one as the standard, and the third one was test group.

The animal was firmly held in the hand and corneal electrodes were placed on the cornea and a current of 60 mA was applied for 0.2 s. The different stages of convulsions i.e., (a) tonic flexion, (b) tonic extensor phase, (c) clonic convulsions, (d) stupor, and (e) recovery, or death were properly figured out and noted. The time (in s) spent by the animal in each phase of the convulsions was also noted. Similar steps were repeated with other animals of the control group.

Phenytoin (dose 25 mg/kg) was used as the standard drug. Its stock solution contained 5 mg/mL of the drug and 0.5 mL/100 g b.w. of the animal was injected intraperitoneally (*i.p.*) to each mice in a

group of six mice. After 30 min. the animals were subjected to electroconvulsions Saline solution (0.9%) was used as a control. Test samples were suspended in Tween-80 (50 mg/kg).

The recovery of the animal from convulsions was analyzed using one-way ANOVA (followed by Dunnett's multiple comparisons test) and Fisher's test, respectively; p values < 0.05 were considered significant.

Neurotoxicity screening (3–5)

The animals used in the testing were numbered and weighed. They were placed individually on the rod for at least 5 s and it was rotated at the speeds of 10 rpm. Mice were tested on two trials per day for 2 consecutive days. Trained animals were given *i.p.* injection of the test compound in the doses of 50 mg/kg. Noted down was the fall off time when the mice falls from the rotating rod.

RESULTS

Physical properties and solubilities of synthesized [4-(4-chloro-phenyl)-6-p-tolylpyrimidine] derivatives are presented in Table 1. FTIR spectral analysis of pyrimidine derivatives gave the following results (KBr, cm^{-1}):

DST-1: 1-Benzylidene-3-[4-(4-chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-urea:
3336.85 (N-H of amide) 3064.89 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1681.93 (C=O of amide), 1624.48 (C=N stretching), 1512.19 (Ar-C=C stretching), 848.68 (*p*-substituted aromatic compound).

DST-2: 1-[4-(4-Chlorophenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(4-chlorobenzylidene)-urea:
3384.48 (N-H stretching in amine), 3064.89 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1681.93 (C=O of amide), 1627.92 and 1505.13 (C=C stretching), 1602.36 (C=N stretching), 848.68 (*p*-substituted aromatic compound), 802.36 (Ar-Cl stretching).

DST-3: 1-[4-(4-Chlorophenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(4-nitrobenzylidene)-urea:
3311.78 (N-H of amide), 3034.03 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1660.71 (C=O of amide), 1608.03 (C=N stretching), 1603.63 and 1514.12 (Ar-C=C stretching), 1544.98 and 1336.67 (Ar-NO_2, N=O stretching).

DST-4: 1-[4-(4-Chlorophenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(4-methylbenzylidene)-urea:
3336.85 (N-H of amide), 3012.81 (Ar-C-H stretching) 2839.22 (C-H stretching in CH_3), 1694.30 and 1512.19 (Ar-C=C stretching), 1687.71 (C=O of amide), 1615.52 (C=N stretching)

DST-5: 1-[4-(4-Chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(4-dimethylaminobenzylidene)-urea.
3334.92 (N-H of amide), 3051.39 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1668.43 (C=O of amide), 1560.41 (Ar-C=C stretching).

DST-6: 1-[4-(4-Chlorophenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(1-p-tolylethylidene)-urea:
3339.22 (N-H of amide), 3062.96 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1649.14 (C=O of amide), 1607.06 and 1509.06 (Ar-C=C stretching), 1411.89 (C-N stretching).

DST-7: 1-[4-(4-Chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-3-[1-(4-hydroxyphenyl)-ethylidene]-urea:
3309.85 (OH stretching), 2999.31 (Ar-C-H stretching), 2922.16 (C-H stretching in CH_3), 1660.71 (C=O of amide), 1600.71 and 1512.19 (Ar-C=C stretching).

DST-8: 1-[4-(4-Chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(3-chlorobenzylidene)-urea:
3384.48 (N-H stretching in amine), 3064.89 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1681.93 (C=O of amide), 1627.92 and 1505.13 (C=C stretching, aromatic compound), 1624.48 (C=N stretching), 802.36 (Ar-Cl stretching).

DST-9: 1-[4-(4-Chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(3-nitrobenzylidene)-urea:
3311.78 (N-H of amide), 3034.03 (Ar-C-H stretching), 2839.22 C-H stretching in CH_3), 1660.71 (C=O of amide), 1608.03 (C=N stretching), 1603.63 and 1514.12 (Ar-C=C stretching), 1544.98 and 1336.67 (Ar-NO_2, N=O stretching).

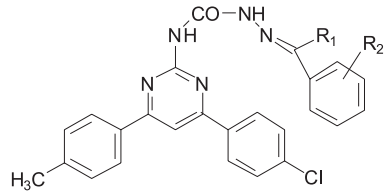
DST-10: 1-[4-(4-Chloro-phenyl)-6-p-tolyl-pyrimidin-2-yl]-3-(4-hydroxy-benzylidene)-urea:
3309.85 (OH stretching), 3062.96 (Ar-C-H stretching), 2839.22 (C-H stretching in CH_3), 1683.86 (C=O of amide), 1598.99 (Ar-C=C stretching).

The results of biological tests evaluation are presented in Table 2.

DISCUSSION AND CONCLUSION

The conformational exploration of the clinically active anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigine, rufinamide and phenobarbital has resulted in the proposal of a general model for anticonvulsant activity (6). This pharmacophore model is based on the semicarbazones comprised of the following four essential binding sites: (i) An aryl hydrophobic binding site (A) with halo substituent preferably at para position; (ii) A hydrogen bonding domain (HBD); (iii) An electron donor group (D) and (iv) Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C) (Fig. 1).

Table 1. Physical properties and solubilities[a] of synthesized [4-(4-chloro-phenyl)-6-p-tolylpyrimidine] derivatives.



Code	R_1	R_2	Yield (%)	m.p. (°C)	R_f value[b]	H_2O	HCl (5%)	NaOH (5%)	CCl_4 (5%)	$NaHCO_3$ (5%)
DST-1	H	H	61.53	192–194	0.83	–	+	–	–	+
DST-2	H	3-Cl	65.54	183–185	0.82	–	++	+	++	+
DST-3	H	3-NO_2	54.43	183–185	0.78	–	++	–	+	++
DST-4	H	3-CH_3	61.54	152–155	0.75	–	++	+	+	++
DST-5	H	3-$N(CH_3)_2$	64.45	159–160	0.81	–	++	+	–	–
DST-6	CH_3	3-CH_3	67.00	165–166	0.79	–	++	+	–	–
DST-7	CH_3	3-OH	66.65	178–181	0.73	–	+	+	–	–
DST-8	H	2-Cl	64.43	179–183	0.65	–	++	–	+	–
DST-9	H	2-NO_2	65.42	182–186	0.72	–	+	–	++	++
DST-10	H	3-OH	69.08	176–179	0.75	–	++	–	+	++

[a]Approximately 100 mg of synthesized compound was taken and dissolved in 2 mL of solvent: (–) insoluble, (+) sparingly soluble, (++) soluble; [b]$CHCl_3$:MeOH (9:1, v/v).

In the present studies, we have designed and synthesized substituted semicarbazones with keeping two major facts in mind. Firstly, a number of clinically active anticonvulsants possess a nitrogen hetero atomic system with one or two phenyl rings and at least one carbonyl group in their structure. Secondly, several investigations concluded that at least one aryl group, one or two-electron donor atom and/or an NH group in a special spatial arrangement appears to be vital for anticonvulsant activity.

The structure of the title compounds fulfilled all the pharmacophoric structural requirements i.e., the presence of [4-(4-chloro-phenyl)-6-*p*-tolyl-pyrimidine] moiety as hydrophobic portion, N as electron donor system and another hydrophobic aryl ring responsible for metabolism.

All the synthesized compounds were screened for their anticonvulsant potential through maximal electroshock seizure (MES) model. The majority of the compounds showed anticonvulsant activity except compound **DST-2**. The most active compounds were **DST-6, DST-7,** and **DST-9,** while compounds with lesser activity were **DST-3, DST-4, DST-5** and **DST-8**. Compounds **DST-1, DST-3, DST-4** and **DST-8** exhibited considerable neurotoxicity while compound **DST-9** showed lesser neurotoxicity at the administered dose of 50 mg/kg. The most active compound **DST-9** was the most potent compound among all the synthesized semicarbazone with lesser neurotoxicity. Compound **DST-2** was not tested for anticonvulsant evaluation due to its significant neurotoxic potential (Table 2).

In general, compounds bearing the groups like nitro, hydroxy on distant phenyl ring showed high potency in MES test. The replacement of these groups with methyl group on the distant phenyl ring has resulted in compounds with a decrease in anticonvulsant activity.

Replacement of the proton on the carbimino carbon atom by methyl group i.e., **DST-6** and **DST-7** has demonstrated variation in activity due to increase in the dimension of the group at this position of the molecule.

The results obtained showed that the majority of the compounds exhibited anticonvulsant activity. The results confirmed that the pharmacophoric model elements are necessary for the anticonvulsant activity, which may account for bioactivity of majority of compounds. These new data might be beneficial in the future development of semicarbazones as novel anticonvulsants.

Table 2. Anticonvulsant and neurotoxicity evaluation of synthesized semicarbazones

Treatment	Dose	Duration of tonic hind limb extension (s)	Recovery	Mean of time (s) spent on the rod ± SD
Control (saline)	2 mL / kg	16.84 ± 1.84	yes	136.0 ± 22.6
Phenytoin	50 mg/kg	6.34 ± 1.76*	yes	Not tested
DST-1		12.63 ± 1.28	yes	84.5 ± 26.6*
DST-2		Not tested due to toxicity		3 out of 6 dead
DST-3		8.17 ± 0.34*	yes	70.5 ± 29.6*
DST-4		14.94 ± 0.39	yes	75.6 ± 16.4*
DST-5		14.53 ± 0.83	yes	82.6 ± 13.8*
DST-6		9.34 ± 0.9*	yes	110.8 ± 20.9
DST-7		8.62 ± 1.93*	yes	102.7 ± 18.4
DST-8		11.62 ± 1.93*	yes	80.6 ± 16.4*
DST-9		11.16 ± 1.50*	yes	106.6 ± 3.4
DST-10		7.59 ± 0.73*	yes	91 ± 25.6*

* $p < 0.01$. One way ANOVA followed by Dunnet's *t*-test.

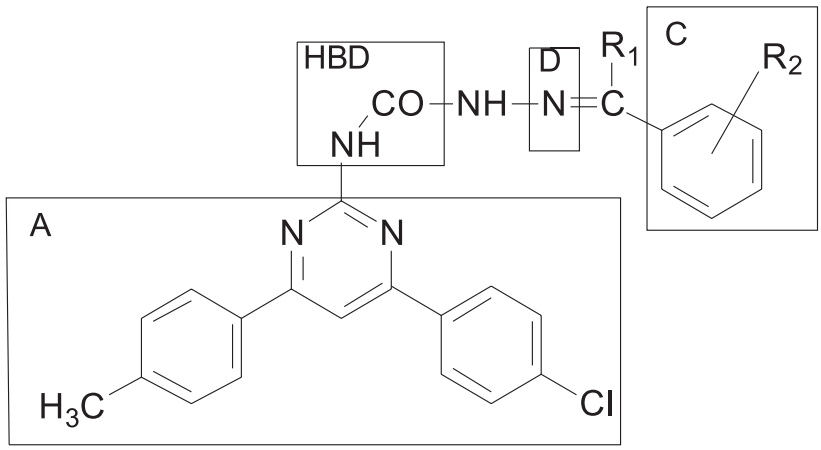


Figure 1. Semicarbazone based pharmacophoric model and its vital structural features present in title compounds: (**A**) hydrophobic aryl ring system, (**HBD**) hydrogen binding domain, (**D**) electron donor moiety and (**C**) distal aryl ring

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