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Anticonvulsant and neurotoxicity evaluation of 5-(un)-substituted isatin-imino derivatives

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Various Schiff bases were prepared by reacting 5-(un)-substituted isatin with some heterocyclic compounds, viz., *N*-[4-(4′-chlorophenyl-thiazol-2-yl] semicarbazide, 3-amino-2-methylmercaptoquinazolin-4-one, 3-(4′-pyridyl)-4-amino-5-mercapto-4(*H*)-1,2,4-triazole and 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-aminopyrimidine. The compounds were evaluated for anticonvulsant and neurotoxic properties. The compound 3-(3′,4′-dihydro-2′-methylmercapto-4′-oxoquinazolin-3′-yl) iminoisatin (**3**) emerged as the most active analogue showing anti-MES and anti-PTZ activities better than valproic acid. All the compounds showed lower neurotoxicity than phenytoin and carbamazepine.

1. Introduction

In a review, Bruni [1] pointed out that with the drugs available, significant seizure control can be achieved in 70–80% of persons with epilepsy, and complete control can be obtained in 60%. Infantile spasms and complex partial seizures pose the most difficult therapeutic problems. Gradual and orderly changes in antiepileptic drug therapy are often required, and there is still a strong need for new anticonvulsants with more selective action and fewer toxic effects. Recently a binding site hypothesis for anticonvulsant semicarbazones was established within a suggested pharmacophore [2, 3] in which the structural features essential to interact at the binding site were a lipophilic aryl ring, a hydrogen bonding domain and a distal aryl ring. In the present study, an attempt was made with isatin as the hydrophobic aryl ring to interact at the aryl binding site, which itself possesess significant anticonvulsant activity [4, 5]. Isatin has also been found to reduce the seizure severity and percentage of generalized seizure ($P < 0.01$) in kindled rats [6]. Various isatin-3-thiosemicarbazones and 3-(4-thiazolidine-2-hydrazon)-2-indolines were reported to possess anticonvulsant properties [7]. The presence of an electron rich group as in 4-(4′-nitrophenyl)-1-(7-nitroisatin-3-yl) semicarbazone showed excellent protection in both MES and scPTZ screens [8]. The present investigation was carried out to evaluate the anticonvulsant activity of some of the biologically active Schiff bases of isatin reported earlier [9–12].

2. Investigations, results and discussion

The synthesized compounds were screened for anticonvulsant activity after i.p. injections by an electrical and a chemical test. The electrical test employed was the maximal electroshock seizure (MES) pattern test and the chemical test was the subcutaneous pentylenetetrazole (scPTZ) seizure threshold test. Minimal motor impairment was measured by the rotorod (neurotoxicity, NT) test. The results are summarized along with the literature data of clinically used drugs and isatin in Table 1. Compounds **3** and **8** showed protection at 100 mg/kg in the MES screening 0.5 h after administration and compound **3** showed protection after 4 h at 300 mg/kg. Compounds **2**, **10**, and **11** showed protection at 300 mg/kg after 4 h. In the scPTZ screening, only compounds **3**, **5**, and **10** showed protection at 300 mg/kg. Compounds **3** and **10** thus emerged as more potent compounds than valproic acid being active in both the screenings (MES, scPTZ). All the compounds showed only a margin of neurotoxicity at 0.5 h interval and no neurotoxicity at the maximum dose administered (300 mg/kg). Though compounds **1**, **6**, and **7** were inactive in all the anticonvulsant screenings, they were devoid of neurotoxicity at the maximum doses tested.

Table 1: Compounds displaying anticonvulsant activity and neurotoxicity in mice

Compd.[a]	Intraperitoneal injection in mice[b]				
	MES screen		scPTZ screen	NT screen	
	0.5 h	4 h	0.5 h	0.5 h	4 h
2	300	300	–	–	–
3	100	300	300	300	–
4	–	–	–	300	–
5	–	–	300	–	–
8	100	–	–	300	–
9	300	–	–	300	–
10	–	300	300	300	–
11	–	300	–	300	–
Phenytoin	30	–	–	100	100
Carbamazepine	30	100	100	100	300
Valproic acid	–	–	300	–	–
Isatin	400	–	–	–	–

[a] Doses of 30, 100 and 300 mg/kg of each compound were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 h and 4 h after injections were made. The dash (–) indicates an absence of activity

[b] Compounds **1**, **6** and **7** were inactive in all the screenings with no neurotoxicity at the maximum dose tested (300 mg/kg)

The compound 3-(3′,4′-dihydro-2′-methylmercapto-4′-oxoquinazolin-3′-yl) imino-isatin (**3**) was further evaluated in the MES test upon oral administration to rats (Table 2). At a dose of 30 mg/kg, the compound showed 50% protection until 1 h and a maximum of 75% protection at 0.25 h. The compound exhibited no acute neurotoxicity at this dose throughout the interval tested (4 h).

The bioevaluation led to an understanding that the 5-bromoisatin derivatives of thiazolyl thiosemicarbazide (**2**) and triazole analogues (**8**) have shown better anticonvulsant activity than the 5-unsubstituted derivatives (**1**, **6**). Thus electron rich atoms/groups like bromo-, thiomethyl- (**3**–**5**), mercaptomethyl- (**6**–**8**) and chloro-groups (**9**–**11**) substi-

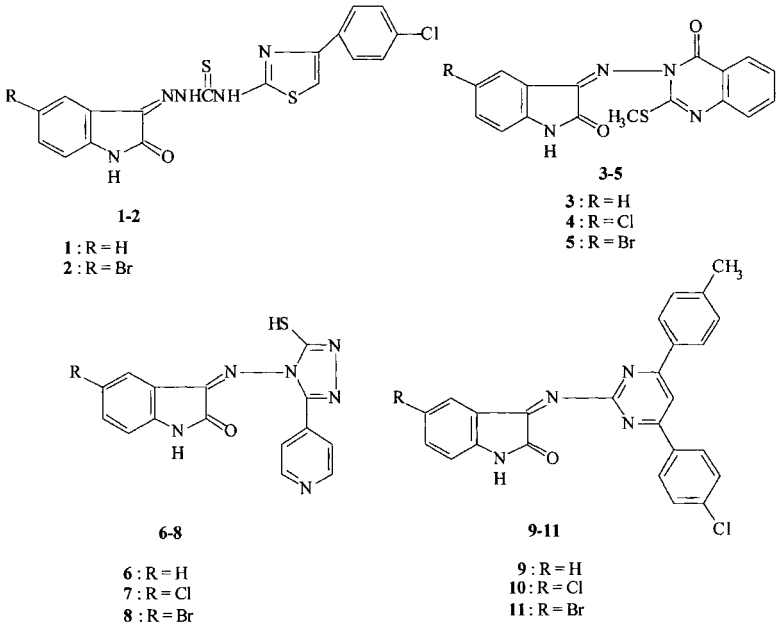

Table 2: Evaluation of compound 3 in the MES test after oral administration (30 mg/kg) in rats

Test	0.25 h	0.5 h	1 h	2 h	4 h
MES	3/4	2/4	2/4	0/4	0/4
TOX	0/4	0/4	0/4	0/4	0/4

tuted in the heteroaryl ring afforded active compounds. In terms of interaction at the binding site proposed previously by Pandeya et al. [2, 3] for the aryl semicarbazones, the introduction of a heteroaryl ring like isatin in the present study also afforded active compounds with low neurotoxicity. It is likely that the thiosemicarbazone moiety, heteroatoms in the quinazolone, triazole and pyrimidine moieties along with the amide function (−CONH) in the isatin ring, all contribute to the hydrogen bonding domain at the binding site.

Thus, the present study has thrown up a new light on the anticonvulsant activity of isatinimino compounds. Further molecular modification of this molecule are in progress and may lead to the discovery of novel anticonvulsants.

3. Experimental

3.1. Compounds

Schiff bases were synthesized by reacting 5-(un)-substituted isatin with some heterocyclic analogues like *N*-[4-(4′-chlorophenyl-thiazol-2-yl] semicarbazide, 3-amino-2-methylmercaptoquinazolin-4-one, 3-(4′-pyridyl)-4-amino-5-mercapto-4(*H*)-1,2,4-triazole and 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-aminopyrimidine according to the methods reported previously [9–12].

3.2. Anticonvulsant screening

Initially all the compounds were administered i.p. in a volume of 0.01 ml/g body weight in mice at doses of 30, 100 and 300 mg/kg to one to four animals respectively. Activity was established by following the anticonvulsant drug development (ADD) program protocol [13, 14] and the data are presented in Table 1. Compound **3** was examined for oral activity in the MES screening (Table 2).

3.3. Neurotoxicity (NT) screening

Minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions per min. The rod diameter was 3.2 cm^{-1}. Trained animals were given i.p. injection of the test compounds in doses of 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 three trials.

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