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Pharmacological Screening of Isatin-[N-(2- alkylbenzoxazole-5-carbonyl)] hydrazones

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Fifteen different isatin-[N-(2-alkylbenzoxazole-5-carbonyl)] hydrazones prepared earlier were screened for analgesic, antidepressant and H_1-antihistaminic activities, and also for their effect on pentobarbitone-induced narcosis, by standard methods. Results revealed that three compounds h, n and e possessing a methyl substituent at 7-position of the benzoxazole system could exhibit good analgesic activity, in relation to standard. Five of the test compounds, h, g, j. n and i were to potentiate the pentobarbitone-induced narcosis more powerfully than Imipramine HCl. The antidepressant and H_1-antihistaminic activities of these compounds were not comparable to those of the standards imipramine HCl and diphenhydramine. HCl, employed in the investigation. Out of all, only compound k was found to exhibit all the tested activities, though to varied extents.

IT is evident from literature that isatins possess a variety of biological and pharmacological properties si ch as antimicrobial[1,2], analgesic[3], MAO inhibitory[4] and anti-inflammatory activities[5]. The bezoxazole system is also known for its broad pharmacological profile. Therefore, in continuation of our work on potent isatin derivatives[6,7] and in view of the importance of both the indolinone and benzoxazole systems, it has been felt worthwhile to screen the title compounds for their possible pharmacolgical properties, since the synthesis and antimicrobial properties are reported previously from our laboratories[6]. It could be noted from literature that some isatins such as 5-bromoisatin, N-methylisation, isatin itself and isatin-3-oxime stimulated the contractions of isolated guinea pig ileum. All these four compounds also blocked both serotonin and acetylcholine - stimulated contractions of the guinea pig ileum; most active being 5-bromoisatin[8]. Keeping this in view, the title compounds were screened against histamine-induced contractions of the guinea pig ileum. As a few isatins are known to exhibit MAO inhibitory property[4], the antidepressant activity was evaluated. In order to know their mode of action, the effect of the title compounds on the sodium pentobarbitone-induced sleeping time (narcosis) was also studied. On the basis of our earlier observations,[7] the title compounds were also evaluated for analgesic activity. The results of these investigations are presented in this communication.

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EXPERIMENTAL

Pharmacology

Analgesic activity

It was assayed by the acetic acid-induced writhing method[9]. Overnight fasted, healthy and adult male albino swiss mice weighing between 20 to 30 g in groups of six each were used in this investigation. Sodium carboxymethyl cellulose suspensions (0.08%) of the test compounds were administered, intraperitoneally, at a dose of 100 mg/kg. The control group of animals was given only sodium CMC suspension and one group of animals was adminis-

Table 1
Pharmacological Activities of Isatin-[N-(2-alkylbenzoxazole-5-carbonyl)]hydrazones(IV)

Compound IV	Substituents		Analgesic Activity (% protection)	Antidepressant Activity ED_{50} mg/kg)	Effect on Pentobarbitone-Induced narcosis (% potentiation)
	R	R'			
a	H	H	46.56	265.4	116.41
b	H	Me	51.73	282.3	127.17
c	H	Et	37.94	279.4	108.46
d	5-Me	H	53.45	251.3	111.28
e	5-Me	Me	56.90	138.3	154.53
f	5-Me	Et	34.49	195.2	143.10
g	7-Me	H	41.38	125.3	168.87
h	7-Me	Me	67.25	135.5	196.97
i	7-Me	Et	27.59	201.5	163.46
j	7-Cl	H	37.94	105.6	166.20
k	7-Cl	Me	50.00	101.5	172.71
l	7-Cl	Et	29.32	158.8	157.74
m	5-Br	H	53.45	225.3	139.89
n	5-Br	Me	58.63	255.6	163.61
o	5-Br	Et	24.14	186.9	146.02
Aspirin			55.18	–	–
Imipramine HCl			–	14.9	123.70

tered with aspirin as a standard, intraperitoneally at a dose of 8 mg/kg. After 30 min of the administration of the test compounds or the standard drug, all the groups of the mice were given the writhing agent, 3% aqueous acetic acid, at a dose of 2 ml/kg, intraperitioneally. The writhings produced in these animals were counted for 30 min and compared with those in the control group. The percentage protection for each compound was calculated as shown below:

$$\% \text{ Protection} = 100 - \frac{\text{No, of wriths in test}}{\text{No, of wriths in Control}} \times 100$$

The values of percentage protection of test compounds are presented in Table 1.

Antidepressant Activity

The antidepressant activity of the test compounds was determined by the method of reversal of reserpine-induced ptosis[10]. Healthy, adult male albino swiss mice weighing between 20 to 30 g were used as experimental animals taking six animals in each group. Reserpine in the form of 0.8% sodium CMC suspension was administered, intraperitoneally to all groups at a dose of 5 mg/kg. Two hours after the reserpine injection, the test compounds in 0.8% sodium CMC suspensions was administered, intraperitoneally, in graded doses. The control group received only 0.8% sodium CMC solution. The degree

of ptosis developed after an hour was evaluated and the prevention of ptosis for 30 min was used as a criterion for the drug action. The ptosis rated as 4 representing a palepebral opening and scores 3, 2, 1 and 0 representing various degrees of response from slight to complete closure of eye-lids. The ED_{50} which is the dose at which 50% of the animals have eye-lid opening with scores of 3 or above, was determined graphically by the method of Litchfield and Wilcoxon[11]. Imipramine. HCl was used as a standard and the results are presented in Table 1.

Potentiation of Pentobarbitone-induced Narcosis[12,13]

Healthy and adult male albino swiss mice weighing between 20 and 30 g fasted for 24 h before the experiment and were divided into groups of eight animals, each. The test compounds were administered in the form of 0.8% sodium CMC suspensions, intraperitoneally, at a dose of 100 mg/kg. After 30 min pe ıtobarbitone sodium was administered, intraperitc ıeally, to all the groups at a dose of 30 mg/kg. The average duration of loss of righting reflex was noted and the percentage potentiation of pentobarbitone-induced narcosis by the test compounds was calculated using the formula given below, considering righting reflex in control as 100%.

$$\% \text{ Effect} = \frac{\text{Average duration of loss of righting reflex in test group}}{\text{Average duration of loss of righting reflex in control}} \times 100$$

Imipramine. HCl was employed as the reference drug and the results are presented in Table 1.

Antihistaminic Activity

The antihistaminic activity of the test compounds was assayed by the isolated gunea-pig ileum method[14]. A submaximal dose (10 μg) of agonist was selected and the response of the tissue to this dose in presence of increasing concentrations (logarithemic doses) of the test compounds, was observed. Diphenhydramine. HCl was employed as the standard and the results are presented in Table 2.

Table 2
H_1-Antihistaminic Activity Data of Isatin-[N-(2-alkylbenzoxazole-5-carbonyl)]hydrazones(IV)

Sl. No.	Compound IV	$IC_{50}^{(M)}$
1.	a	1.38×10^{-4}
2.	b	7.26×10^{-5}
3.	c	4.86×10^{-3}
4.	d	–
5.	e	2.44×10^{-4}
6.	f	3.38×10^{-4}
7.	g	3.30×10^{-4}
8.	h	2.72×10^{-4}
9.	i	3.12×10^{-3}
10.	j	3.12×10^{-4}
11.	k	2.35×10^{-4}
12.	l	3.26×100^{-4}
13.	m	1.21×10^{-4}
14.	n	8.30×10^{-5}
15.	o	9.14×10^{-5}
Diphenhydramine.HCl		4.90×10^{-7}

RESULTS AND DISCUSSION

The results of analgesic activity of the title compounds, presented in Table 1 reveal that compound h was the most potent analgesic in comparison with the standard (Aspirin) with 67.3% protection against the acetic acid-induced writhings. Compound n with a 2-methyl substitutent in benzoxazole system and 5-bromo substituent in indolinone system was found to be the next to h in its analgesic action with 58.63% protection. Compound e with a 2- methyl and 5-methyl substituent in bezoxazole and in indolinone systems, respectively could be noticed as the third effective analgesic with 57% protection. It is interesting to note that all these three compounds uniquely possess a methyl group at 2- position of

the bezoxazole system. Compound e, f, i, l and o were mildy active and rest were moderately active.

It could be noted that all the test compounds exhibited antidepressant activity. But, ofcourse, the results prove them to be quite inferior and not comparable with that of the standard. Out of the fifteen compounds tested in the present investigation, as many as five compounds e, g, h, j and k were found to be effective antidepressant with lower ED_{50} values. Compound k, with 7-chloro and 2-methyl substituents in indolinone and benzoxazole moieties, respectively, was found to be the most effective compound with ED_{50} 101.5 mg/kg. Compound j with 7-chloro substituent in indolinone was falling next in its antidepressant potency with ED_{50} 105.6 mg/kg. These were followed by compounds g, h and e in the order of their antidepressant potency. However, the rest of the compounds were also found to exhibit some degree of antidepressant activity.

Perusal of Table 1, reveals that a majority of the title compounds were relative in potentiating the pentobarbitone- induced narocis. Among the compounds tested, compounds c and d showed very mild potentiation, whereas, all others were found to be comparable even to imipramine. HCl, in potentiating the narcosis. Compound h with a 2-methyl substituent in benzoxazole and 7-methyl substituent in indolinone moieties was found to be most effective with about 197% potentiation. Compound k with a 2- methyl substituent in bezoxazole and a 7-chloro substituent in indolinone system was the second in order with 172% potentiation. This was followed by compound g, with 169% potentiation.

The antihistaminic activity data of the title compounds as presented in Table 2 show that none of the compounds are comparable with that of standard. But among them, compound b with a 2-methyl substituent in benzoxazole moiety was relatively more potent with an IC_{50} value of 7.26×10^{-5}M. The next compound, in order of its antihistaminic potency was n with a 2- methyl substituent in benzoxazole and a bromine at 5-position of indolinone system, with an IC_{50} of 8.3×10^{-5} M. Compound o with 2-ethyl substituent in benzoxazole moiety and a bromine at 5-position of indolinone was the third most potent antihistaminic with an IC_{50} of 9.14×10^{-5}M. The rest of the compounds do not possess significant antihistaminic activity.

Thus, it could be concluded that some of these test compounds (**h, n, e, d, m, c, b and k**) exhibited a comparable analgesic property and a few (**k, j, g, e** and **l**), weak antidepressant activity. Five compounds **h, g, j, n** and **i** were found to be superior in potentiating the pentobarbitone-induced narcosis. The antihistaminic activity of the title compounds is not at all comparable with the standard employed. Only compound k with a chloro substituent at 7-position of the indolinone and a methyl substituent at 2-position of the benzoxazole systems was found to exhibit all the phamacological activities for which it was tested, though to varied degrees. It is interesting to note that the compound h with 7- and 2-methyl groups was found to be quite potent analgesic agent and a good CNS depressant.

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