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Research Article

EFFECTS OF 6-FLUORO-3-(PIPERIDIN-4-YL) BENZO[D]ISOXAZOLE DERIVATIVES ON DOPAMINE-D2 AND SEROTONIN-5HT2 RECEPTORS MEDIATED BEHAVIOURS IN ALBINO MICE AND OTHER ANTIPSYCHOTIC STUDIES

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ABSTRACT
Benzisoxazole derivatives are well known for their antipsychotic properties. Efforts were made to synthesis derivatives of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole (S1, S2, S3 and S4) to study their efficacy as a promising antipsychotic drug producing menial Extrapyramidal symptoms (EPS). The pathogenesis of many psychotic disorders are due to hyperactivity of central Dopamine D2 and serotonin 5HT2 receptors and antagonism against these receptors have been associated with lowering the negative symptoms and decreased tendency to cause EPS. In the present study we have investigated for D2 receptor and serotonin receptor antagonism activity by *in vivo* methods wherein 5-Hydroxytryptophan (5-HTP) induced head twitches and apomorphine induced climbing behaviour are analysed. Treatment of synthesized molecules (1-5mg/kg) to albino mice showed a dose-dependent decrease in the apomorphine-induced climbing behaviour and 5-hydroxytryptamine (5-HTP)-induced head twitch activities. The molecules also revealed significant results for L-dopa induced hyperactivity, Clonidine induced aggression behaviour.
Key words: Antipsychotic, Benzisoxazole, EPS, Dopamine, Serotonin, L-Dopa

INTRODUCTION

Dopamine D2 and Serotonin 5HT2 receptors are important in effecting the efficacy of Atypical Antipsychotic drugs[1]. These antipsychotics are known to be antagonist to Dopamine D2 and Serotonin 5HT2 receptors, and are effective against negative symptoms of psychotic disorders and decrease the causes that lead to Extrapyramidal symptoms[2,3]. Even though antipsychotic drugs available in the market are regularly used in the treatment are successful in preventing the psychotic disorders, they are usually associated with Extrapyramidal symptoms[4]. An appropriate Dopamine-Serotonin model has been selected for the present study. In this direction we have synthesized[5] 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole derivatives, namely 4-(6-fluorobenzo[d]isoxazole-3-yl)-N-(3-methoxyphenyl)piperidine-1-carbothiamide (S1), N-(2-chlorophenyl)-4--(6-fluorobenzo[d] isoxazole-3-yl) piperidine-1-carbothiamide (S2), 4-(6-fluorobenzo[d]isoxazole-3-yl)-N-(2-fluorophenyl)) piperidine-1-carbothiamide (S3), N-(4-chlorophenyl)-4-(6-fluorobenzo[d] isoxazole-3-yl) piperidine-1-carbothiamide (S4) which can exhibit antagonistic activity against D2 and 5HT2 receptors thus confirming antipsychotic properties along with menial extrapyramidal symptoms(EPS)[6]. These Synthesized molecules were screened for Atypical Antipsychotic potentials such as Inhibition of 5-HTP (5-hydroxytryptophan) induced head twitches (5-HT2A antagonism)[7], inhibition of apomorphine induced climbing behaviour (D2 antagonism)[8], L-dopa induced hyperactivity and Clonidine induced aggression behaviour [9]

MATERIALS AND METHODS

Animals

Swiss Albino mice (25-30g) were approved by Institutional Animal Ethics Committee (IAEC), under the rules 5(a) of the "Breeding and Experiments on Animal (control and supervision) rules 1998" [Ref: HSK Cp/IAEC, Clear/12013-14/121] after observing the usual formalities lay down by IAEC as per provisions made by CPCSEA. All the animals were placed in laboratory cages in a animal house maintained at temperature 23±2°C under 24 hr standard light/dark cycle. All the animals had free access to standard food pellets and filtered water.

Drugs

Apomorphine, Pargyline, 5 HTP, Clonidine and L-DOPA were procured from Sigma Aldrich, Sodium dihydrogen phosphate and Disodium hydrogen phosphate were obtained from SD Fine, and all the other solvents and chemicals used were of analytical grade and procured from commercial suppliers. All the drugs including Apomorphine (4mg/kg) and 5-HTP (50mg/kg) were administered subcutaneously, per oral or intraperitoneally in a constant volume of 10ml/kg normal saline.

Apomorphine induced climbing behaviour

All animals were placed in a cylindrical cage (18x19cm) composed of vertical (1 cm apart) and horizontal (4.5 cm apart) metal bars (2 mm), with wire mesh, for 1hr prior to experiments. Animals were pre-treated with various doses of standard drugs (1-3mg/kg) and test compounds (1-3mg/kg), 30 minutes before the treatment of apomorphine (4mg/kg). Inhibition of apomorphine induced climbing behaviour was recorded based on the time spent in minutes by the animals climbing.

Antagonism of 5-hydroxytryptophan (5-HTP) induced head twitches

Swiss mice in the control group (n=6) were injected with Pargyline (75mg/kg) intraperitoneally in order to prevent the

rapid degradation of 5-HTP. Thirty minutes later, the standard drugs (1mg/kg) and test compounds were administered (1-3mg/kg). After a period of 30 min, the mice received 5-HTP (50mg/kg) subcutaneously. The mice were returned to the test cages and then head twitches were assessed starting 20 min after the 5-HTP treatment at 10 min intervals for 30min. Head twitches were monitored using the following scoring system, 0-absent, 1-moderate, 2-marked. A maximum of 8 score is possible. An observer made all the observations unaware of the specific drug treatments.

Clonidine-induced aggression in mice

Swiss albino mice were divided in 7 groups of 6 each (n=6), each group containing 3 pairs of mice. Doses (1-3mg/kg) were administered for 7 consecutive days, and on the 7th day, Clonidine was injected 1 hr after the administration of the standard drugs and the test compounds. All the animals were than caged in jar with a floor area of around 18cm^2. The fighting/biting were observed unaware of the specific drug treatment over a period of 30min, in each pair.

L-DOPA induced hyperactivity

The experiments were performed according to the method of Serra et al[10]. Swiss albino mice were treated intraperitoneally with L-DOPA (100mg/kg) by diving into 7 groups of 6 each (n=6), each group with 3 pairs of mice. Doses were administered for 7 days, and on the 7th day, L-DOPA was administered 1 hr after the injection of standard drugs and test compounds. Different stages of activity and aggressive behaviour were observed for every 10 min for 30 min after administration of L-DOPA. Different variables of observation were jumping, squeaking, salivation, piloerection, irritability, increase in motor activity and aggressive fighting. The scores were awarded according to the following criteria: : 0-No effect; 1-Piloerection, slight salivation, slight increase in motor activity; 2-Piloerection, salivation, marked increase in motor activity and irritability; 3-Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting[11].

7days, and on the 7th day , 1 hr after the injection of standard drugs and test compounds, mice were placed on the edge of a table 70cm above the floor by the adhesive tape placed approximately 1.5cm form the tip of the tail. Time of immobility exhibited by preteated and control mice was recorded for 6 min[12]. Animals with no movement of the body with passive behaviour were considered to be immobile.

Statistical Analysis

In our studies, all the data were expressed as mean ± Standard Error of Mean (S.E.M) and analysed using one-way analysis of variance(ANOVA) followed by turkey`s test. A probability of $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The effect of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives on Dopamine (D2) and Serotonin (5HT2a) receptors is to elucidate the CNS active properties in comparison with Standard drugs[13,14]. Psychotic disorders have been associated with variations in neurotransmission of 5HT, Dopamine and norepinephrine[15]. Apomorphine is a non-selective dopamine agonist which is believed to directly act on D2 receptors, and the competence of the drugs and test molecules to alleviate the changes in stereotype behaviour of animals induced by apomorphine is considered as a standard test of potential antipsychotic activity[16], indicated through the antagonistic activity of the test molecules on central dopamine D2 receptors. Antagonistic activity of drugs against Serotonin 5-HT2a receptors have been associated with efficiency against negative symptoms of psychotic disorders and decreased frequency to cause Extrapyramidal symptoms[17,18].

The head twitch behaviour is observed in mice as a result of increased activity of central 5-hydroxytryptamine (5-HTP) in the CNS[19], which is attributed to the response from 5-HT2a receptors[20]. The above stereotype behaviour is due to the synthesis of serotonin from 5-HTP. In the above results we can observe that all the synthesized molecules (S1-S4) have decreased the head twitching, S2 (0.95±0.16) is seen to be equally potent as those of standard drugs Resperidone (0.93±0.43) and Haloperidol (0.81±0.16) at similar doses (1mg/kg). However all the molecules including standard drugs showed no head twitching at higher concentration (3mg/kg). The results suggest the fact that, all the synthesized compounds (S1-S4) have central 5-HT2a receptor antagonistic activity comparable to the established drugs[21]. (Table 1)

All synthesized molecules (S1-S4) showed dose dependent (1-3mg/kg) inhibition of apomorphine-induced climbing behaviour (table 2). S2, Resperidone, and Haloperidol showed complete inhibition, with animals showing zero climbing tendency at 3mg/kg, confirming 100% inhibition of apomorphine induce stereotype behaviour, S1 (0.16±0.36 min) and S3 (0.12±0.28 min) molecules showed moderate activity, however S4 (0.76±0.54 min) had comparably lower activity at 3mg/kg. The above data suggests that all molecules are indicating antagonistic property for central dopamine D2 receptors. (Table 2)

According to the literature[22] combined 5-HT and Norepinephrine (NE) reuptake inhibitor is more effective than used alone, depending on these findings, we investigated the role of NE in the antipsychotic studies with Standard drugs haloperidol, resperidone and S1-S4. According to the results it is clear that Haloperidol and resperidone showed no bouts at dosage 3mg/kg and S2 (0.66±0.29) also had very ignorable values.S1, S3 and S4 also showed control of aggression in animals at higher concentrations (3mg/kg). Thus, the antipsychotic activities indicate role of S1-S4 molecules in both central serotonergic and noradrenergic systems. (Table 3)

Results in Table 4 indicates that antipsychotic drugs as well as synthesized molecules (S1-S4) show remarkable inhibition of L-Dopa induced hyperactivity at all concentrations compared to the control, however significant values were observed at 3mg/kg. Resperidone (0.26±0.21), Haloperidol (0.57±.18) and S2 (0.78±0.34) showed better results, compared to other test candidates, Treatment of S1, S3 and S4 also showed decrease in hyperactivity. Consequently, indicating their role in dopaminergic system.

Table 1: **Effect of Haloperidol, Resperidone and Synthetic molecules (S1-S4) on 5-HTP induced head twitches**

Treatment	Dose[mg/kg]	Total head twitching
Control	10mL/kg, normal saline	6±0.12
Haloperidol	1	0.81±0.16***
	2	0.0±0.0
	3	0.0±0.0
Resperidone	1	0.93±0.43***
	2	0.0±0.0
	3	0.0±0.0
S1	1	1.22±0.22
	2	0.35±0.31*
	3	0.0±0.0
S2	1	0.95±0.16***
	2	0.0±0.0
	3	0.0±0.0
S3	1	1.13±0.25**
	2	0.24±0.44**
	3	0.0±0.0
S4	1	1.36±0.22**
	2	0.42±0.17**
	3	0.12±0.18***

The values were expressed as Each value mean ±SEM and the values were statistically show significant at levels $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to the control group.

Table 2: Effect of Haloperidol, Resperidone and S1-S4 on Apomorphine induced climbing behaviour

Treatment	Dose [mg/kg]	Time spent [min]	Time spent [%]
Control	10ml/kg Normal saline	24.52±1.24	81.73
Haloperidol	1	02.64±0.96***	08.80
	2	0.21±0.63***	0.79
	3	0.0±0.0	0.0
Resperidone	1	2.82±0.63***	09.12
	2	0.29±0.57***	0.85
	3	0.0±0.0	0.0
S1	1	03.21±1.36***	10.70
	2	01.52±1.62***	04.52
	3	0.16±0.36***	0.43
S2	1	02.83±0.88***	09.14
	2	0.27±1.31***	0.83
	3	0.0±0.0	0.0
S3	1	03.15±0.214***	10.63
	2	01.22±1.35***	04.06
	3	0.12±0.28***	0.39
S4	1	05.51±1.57**	18.36
	2	02.13±2.52***	08.43
	3	0.76±0.54***	2.24

One way ANOVA followed by turkey`s test. All the values were statistically significant at $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared to the control group.

Table 3: **Effect of Haloperidol, Resperidone and S1-S4 on Clonidine-induced aggression in mice**

Treatment	Dose[mg/kg]	MEAN ± SEM	
		Latency to 1st attack	No. of bouts
Control	10mL/kg, normal saline	341±12.7	21.5±1.19
Haloperidol	1	423±66.2***	9.3±2.72***
	2	531±33.1**	2.7±1.83***
	3	609±84.6***	0.0±0.0
Resperidone	1	407±48.5**	10.1±2.8***
	2	554±61.8***	1.9±1.29***
	3	621±55.9**	0.0±0.0
S1	1	395±19.3***	16.4±1.72***
	2	439±46.3**	8.2±2.18**
	3	464± 25.7***	2.3±1.06***
S2	1	403±28.7**	10.8±2.2**
	2	517±43.5**	3.1±1.32**
	3	598±14.6***	0.66±0.29***
S3	1	398±31.8***	15.9±1.46**
	2	451±28.5**	7.7±1.62***
	3	477±43.4**	2.1±0.68**
S4	1	388±11.5***	17.3±1.23***
	2	415±27.5**	9.2±1.21**
	3	484±48.9***	2.3±2.43***

The values were expressed Mean ± S.E.M. (n = 6). a = $p < 0.001$ compared to induced group.

Table 4: Effect of Haloperidol, Resperidone and S1-S4 on L-DOPA-induced hyperactivity and aggressive behaviour in mice

Treatment	Dose[mg/kg]	Behavioural score(MEAN ± SEM)
Control	10ml/kg, normal saline	07.67±2.82
Haloperidol	1 2 3	2.25±0.87*** 1.23±0.61*** 0.57±.18**
Resperidone	1 2 3	2.36±1.05** 1.08±0.62*** 0.26±0.21***
S1	1 2 3	2.89±1.13** 1.66±0.55*** 0.97±0.28***
S2	1 2 3	2.44±0.31** 1.19±1.21*** 0.78±0.34**
S3	1 2 3	2.63±0.68*** 1.59±1.05*** 0.92±0.33**
S4	1 2 3	3.32±1.85** 2.19±1.24* 1.14±0.91***

The values were expressed Mean ± S.E.M. (n = 6). a = $p < 0.001$ compared to induced group.

CONCLUSION

Based on the results observed from the above antipsychotic studies, it can be concluded that all the synthesized molecules (S1-S4), have shown significant roles in serotonin, dopaminergic and noradrenalin neuronal systems. Thus, confirming they are potential drug candidates, however more clinical and molecular mechanisms are required to support the cause.

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