



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 drugs¹. 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 symptoms⁴. An appropriate Dopamine-Serotonin model has been selected for the present study. In this direction we have synthesized⁵ 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 behaviour⁹

“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

MATERIALS AND METHODS**Animals**

Swiss Albino mice (25-30g) were approved by Institutional Animal Ethics Committee (IAEC), under the rules 5(a) of 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 18cm². 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 al¹⁰. 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 fighting¹¹. 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 from the tip of the tail. Time of immobility exhibited by pretreated and control mice was recorded for 6 min¹². 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 \pm 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 norepinephrine¹⁵. 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 activity¹⁶, 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 Extrapryramidal 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 CNS¹⁹, which is attributed to the response from 5-HT2a receptors²⁰. 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 \pm 0.16) is seen to be equally potent as those of standard drugs Resperidone (0.93 \pm 0.43) and Haloperidol (0.81 \pm 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 drugs²¹. (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 \pm 0.36 min) and S3 (0.12 \pm 0.28 min) molecules showed moderate activity, however S4 (0.76 \pm 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 literature²² 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 \pm 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 \pm 0.21), Haloperidol (0.57 \pm 0.18) and S2 (0.78 \pm 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, Risperidone 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 |
| Risperidone | 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, Risperidone 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 |
| Risperidone | 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, Risperidone and S1-S4 on Clonidine-induced aggression in mice

| Treatment | Dose[mg/kg] | MEAN ± SEM | |
|-------------|------------------------|-----------------------------------|--------------|
| | | Latency to 1 st 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 |
| Risperidone | 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, Risperidone 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 | 2.25±0.87*** |
| | 2 | 1.23±0.61*** |
| | 3 | 0.57±.18** |
| Risperidone | 1 | 2.36±1.05** |
| | 2 | 1.08±0.62*** |
| | 3 | 0.26±0.21*** |
| S1 | 1 | 2.89±1.13** |
| | 2 | 1.66±0.55*** |
| | 3 | 0.97±0.28*** |
| S2 | 1 | 2.44±0.31** |
| | 2 | 1.19±1.21*** |
| | 3 | 0.78±0.34** |
| S3 | 1 | 2.63±0.68*** |
| | 2 | 1.59±1.05*** |
| | 3 | 0.92±0.33** |
| S4 | 1 | 3.32±1.85** |
| | 2 | 2.19±1.24* |
| | 3 | 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.

REFERENCES

- Meltzer HY, Nash JF. Effects of antipsychotic drugs on serotonin receptors. *Pharmacological Reviews* 1991; 43:587-604.
- Anden, N.E., Butcher, S.G., Corrodi, H., Fuxe, K., and Ungerstedt, U.; Receptor activity & turnover of dopamine and noradrenaline after neuroleptics. *European Journal of Pharmacology*.1970; 11, 303-314.
- Carlsson A, et al; Effect of chlorpromazine or haloperidol on formation of 3 methoxytyramine and nor metanephrine in mouse brain. *Acta Pharmacologica et Toxicologica*. 1985; 20: 140-144.
- Carlsson, A. and Lindquist, M.; Effect of chlorpromazine or haloperidol on formation of 3 methoxytyramine and nor metanephrine in mouse brain. *Acta Pharmaol Toxicol*. 1985; 20: 140-144.
- Sharath Chandra S P, Raghava B, Sharada A C ; Synthesis, Characterisation, Molecular property prediction and Antipsychotic activity of Novel 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole Derivatives. *International Journal of Pharmaceutical Sciences Review and Research*, eISSN: 0976-044X (in press)
- Meltzer HY. Clinical studies on the mechanism of action of clozapine: the dopamine – serotonin hypothesis of schizophrenia. *Psychopharmacology* 1989; 99:S18-27.
- Rowley M, Bristow L J & Huston P H, *Journal of Medicinal Chemistry*, 44, 2001, 481.
- Costa B, Naylor R & Nohria V, *European Journal of Pharmacology* 50, 1978, 39
- Morpurgo C. Aggressive behaviour induced by large doses of 2-(2, 6-dichlorophenyl amino)-2 imidazoline hydrochloride (ST 155) in mice. *European Journal of Pharmacology* 1968; 3 (4):374-7
- Serra G, Collu M, D'Aquila PS, De Montis GM, Gessa GL. Possible role of dopamine D1 receptor in the behavioural supersensitivity to dopamine agonists induced by chronic treatment with antidepressants. *Brain Research* 1990; 527(3):234-43.
- Suresh D, Madhu M, Saritha Ch, Raj Kumar V And Shankaraiah P, Antidepressant activity of spirulina platensis in experimentally induced depressed mice, *International journal of pharmaceutical archive-3(2)*, 2014, 317-326.
- Rodrigues AS, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, et al. Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. *Life Sciences* 2002; 70:1347- 58.
- Vogel, G.H. and Vogel,G.H.: Drug discovery and evaluation: Pharmacological assays. Springer Verlag, Berlin, pp. 292-316, 413(1997).
- Kumar, V., Jaiswal, A.K., Singh, P.N. and Bhattacharya, S.K.: *Indian journal of experimental biology*. 38: 36-41(2000).
- Maes M, Meltzer HY. The serotonin hypothesis of major depression. In: Bloom FE, et al, editor. *Psychopharmacology: the third generation of progress*. New York: Raven Press; 1995. p. 993-1044.
- Guy M. Gudwin, Richard Green et al; Assessment of 5-HTP induced head twitch activity in mice. *British Journal of Pharmacology*. 1985; 735-743.
- Nakamura M. Fukushima H; Central serotonergic neurotransmission & negative symptoms of Schizophrenia. *The Journal of Pharmacology & Pharmacotherapeutics* 1978 a; 30:56-65.
- Niemgeers CJE, P. Janssen ; Extrapyramidal symptoms with neuroleptics. *Industrial Phramacology part II* 1975; 25:125-141.
- Peroutka SJ, Lebovitz RM, Snyder SH.; Serotonin induced head twitch activity in mice. *Science*. 1981; 30: 212-827.
- Singleton, Marsden CA et al; 5-HT receptor mediated animal behaviours. *Psychopharmacology*.1981. 74:173-178.
- Richelson E; Serotonin mediated behaviours. *Clinical Psychiatry*. 1984; 45: 331-345.
- Anderson IM. SSRIs versus tricyclic antidepressants in depressed inpatients: a metaanalysis of efficacy and tolerability. *Depression and Anxiety* 1998; 7:11-7.

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