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RESEARCH ARTICLE

IN VITRO AND IN SILICO SCREENING OF 6-FLUORO-3-(PIPERIDIN-4-YL) BENZO[D]ISOXAZOLE DERIVATIVES FOR BLOOD BRAIN BARRIER PERMEABILITY

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ABSTRACT

Blood Brian Barrier (BBB) is a physiologically and metabolically significant membrane that enables and limits the uptake of specific molecules by the brain, thus preserving the homeostasis within the Central nervous System (CNS).All the Antipsychotic drugs are permeable across the BBB, however most of First/Second generation antipsychotic drugs employed in the medical field are known to be associated with Extrapyramidal symptoms (EPS). Hence our interest is devoted in the development of novel antipsychotic drugs that ameliorate psychosis with least frequency of EPS. In our previous study the newly synthesized molecules, 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives (S1-S4) which have shown positive alterations in chemical properties and biological activities demonstrating significant neuroleptic properties. However possibility and efficiency of these synthesized molecules to penetrate through the Blood Brain Barrier will be of utmost importance to determine its treatment efficacy. In this line of investigation, in the present study, *in silico* and *in vitro* methods are employed to evaluate the permeability in comparison with that of standard antipsychotic drugs, Resperidone and Haloperidol. *In vitro* analysis was done by Parallel artificial Membrane Permeability Assay (PAMPA) in PBS, where in permeability of S2($8.3x10^{-6}$) and S3($6.6x10^{-6}$) molecules showed higher permeability than known standards Warfarin($1x10^{-6}$), Resperidone($2.3x10^{-6}$) and Haloperidol($3.5x10^{-6}$), and the molecules exhibited recovery above 50% and *in silico* studies also showed high permeability rate with all the synthesized molecules. Thus we can conclude that, all the synthesized molecules have the potential to develop into promising Antipsychotic drugs.

Key words: Antipsychotics, Blood Brain Barrier, EPS, PAMPA, *In silico*

INTRODUCTION

In the process of designing new drug molecules, permeation of drugs through the Blood Brain Barrier (BBB) is elementary for the antipsychotics to interact with the specific site of action inside the Central nervous system (CNS). The BBB is composed of distinctly specialised microvascular endothelial cells along with astrocytes, neurons, pericytes, microglia and basement membrane [1,2]. The endothelial capillary cells are interconnected by claudins, occludins (proteins) and adhesion molecules resulting in tight junctions, which shut the intercellular space, thus limiting the permeability of many CNS active molecules[3,4]However large number of molecules can enter the Central nervous system(CNS) through transcellular passive diffusion, resulting due to concentration gradient between brain and blood[5]. Off the available drugs, only 2% are able to cross the BBB especially antipsychotics. Drugs due to their physiological and molecular properties, polar surface area, hydrophilicity, hydrophilicity, molecular charge and size can easily cross the BBB, hence BBB permeability for the prospective drug molecules at different conditions would be of paramount importance in the design and development of these molecules as drugs[6]. In the present study we are assessing the Blood Brain Barrier (BBB) permeability potentials of 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole derivatives[7], 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-fluorobenz [d] isoxazole-3-yl) piperidine-1-carbothiamide (S4) along with the standard antipsychotic drugs Haloperidol and Resperidone , through *in vitro* and *in silico* methods. All the above molecules in our earlier studies have satisfied Lipinski`s rule of five parameters suggesting that they are orally bioavailable, non-toxicity, and indicated significant bioactive scores, thus making them suitable candidates for further investigation as potential Antipsychotic drugs, in this regard BBB permeability studies is an very important and essential criteria for the molecules to qualify for further antipsychotic studies. Hence *in vitro* screening of the molecules was conducted using Parallel artificial membrane permeability assay (PAMPA) in PBS and *in silico* screening was done using rodent model.

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MATERIAL AND METHODS

Materials

All the chemicals were procured form Fluka, Merck and Sigma Aldrich. Permeability assay was performed in Phosphate buffer (PBS, K_2HPO_4 and KH_2PO_4, pH 7) in Multiscreen Millipore TM, plate and PTFE acceptor plate. L-α-Phosphatidylcholine, and Dimethyl Sulfoxide was purchased from (Sigma,USA) and Dodecane was purchased from (Fluka,India) The entire reagents used in the investigation were of analytical grade. The drug analysis was done on LC-MS/MS (AB Sciex Triple Quad 5500).

Methods

PAMPA Assay: The experiment was performed in a 96 well sandwich plate, according to Schmidt and Lynch[8] with slight modifications. 1% lecithin solution (w/v) was prepared in dodecane (~500 µL/plate) and the mixture was sonicated to ensure complete dissolution. Then 5 µL of Lecithin/dodecane mixture was carefully spiked into each donor plate well with the help of a 10 µL adjustable-volume pipette, avoiding pipette tip contact with the membrane. Immediately after the application of the artificial membrane (within 10 min maximum), 150 µL of 50 µM drug containing donor solutions (drug dissolved in PBS) was added to each well of the donor plate. 300 µL of aqueous buffer was added to each well of the acceptor plate. Slowly and carefully the drug filled donor plate was placed into the acceptor, making sure that the underside of the membrane is in contact with the buffer in all wells. Finally the lid was replaced and incubated at room temperature for 16h.The samples were than analysed on LC-MS/MS (AB Sciex Triple Quad 5500) with gradient elution mobile phase (Acetinitril:0.2% Formic acid in water) with a flow of 0.85mL/min, 2 µL wer injected with a total run time of 4.5 min using the column Atlantis C_{18} (50 x 4.6 mm, 3 µm-Waters, Ireland).

Permeability calculations: The apparent permeability Co-efficient (P_{app}) for the Uni-transport direction (A to B) was calculated [9] according to the following equation:

$$P_{app} = (\Delta Q \tilde{} \Delta t) \tilde{} (AxC_D) \text{ [cm/s]}$$

Where ($\Delta Q \tilde{} \Delta t$) [cm/s] is the cumulative amount of test compound transported overtime, A is the surface area of the monolayer membrane (cm^2) and C_D is the average drug concentration in the donor chamber over the period ($\Delta Q \tilde{} \Delta t$) was determined.

Recovery during the experiment was calculated as:

$$\text{Recovery [\%]} = [(QD_{end} + QR_{end}) \tilde{} QD_0] \times 100$$

Where (QD_{end} + QR_{end}) is the final amount of the compound found in both donor and receiver sides and QD_{0min} is the amount in the donor side at start (0 min).

Computational studies: All computational studies were carried in the ACD/ILAB property predictions. BBB parameters such as Rate of Brain penetration (logPS), Extent of Brain penetration (logPB), Brain Plasma equilibrium constant (log PS^* fu, brain) and LogBB were measured *in silico*.

RESULTS AND DISCUSSION

In the present study Apparent permeability (P_{app}) and Recovery % of the synthesized 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives (S1-S4), Standards Warfarin, Propranolol HCl and standard Antipsychotic drugs Haloperidol and Resperidone were determined by PAMPA studies.

Table 1: Apparent permeability (P_{app}) and Recovery % values of S1-S4, Warfarin, Propranolol HCl, Haloperidol and Resperidone determined through PAMPA studies.

COMPOUND	A→B(10^{-6} cm/sec)	RECOVERY[%]
Warfarin	1	62
Propranolol HCl	3.6	76
Haloperidol	3.2	72
Resperidone	4.4	81
S1	1.4	65
S2	8.3	79
S3	6.6	77
S4	0.8	59

The Apparent permeability (P_{app}) values for any molecule to be considered permeable across BBB will be compared to following criteria :< 1 x 10^{-6} cm/sec indicates low permeability; 1 to 3 x 10^{-6} cm/sec indicates medium permeability and > 3 x 10^{-6} cm/sec indicates high permeability. In Table 1, P_{app} values of the molecules are recorded and the S2 and S3 molecules showed high permeability with 8.3 x 10^{-6} cm/sec and 6.6 x 10^{-6} cm/sec respectively which is found to be much higher than normally used standard compounds Propranolol HCl (3.6 x 10^{-6} cm/sec) and Warfarin (1 x 10^{-6} cm/sec). The values obtained for S2 and S3 is also significant as they also exhibited P_{app} values much higher than antipsychotic drugs Haloperidol (3.2 x 10^{-6} cm/sec) and resperidone (4.4 x 10^{-6} cm/sec). However P_{app} values of S1 (1.4 x 10^{-6} cm/sec) and S4 (0.8 x 10^{-6} cm/sec) were medium and low. The percentage of the administered drug that is actually penetrating the Blood Brain Barrier (BBB) [10] is another important factor when considering the interaction potentials of Antipsychotics within the Central nervous system (CNS) is. Thus recovery % across the membrane, of the above molecules assumes a significant factor. From the above table it is clear that the synthesized molecules S2 (79%) and S3 (77%) almost have very similar values that of Haloperidol (72%) and Resperidone (81%), predicting the positive intactness of

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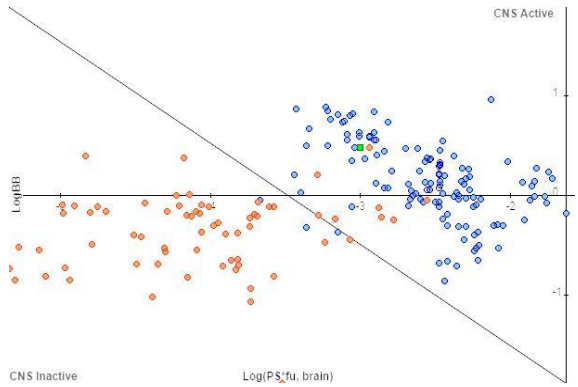
the molecules. Even S1 (65%) and S4 (59%) showed almost similar values like that of standard Warfarin (62%). The above results clearly indicated that the synthesized molecules have very good permeability like that of the established antipsychotic drugs and standard compounds.

***In silico* permeability predictions:**

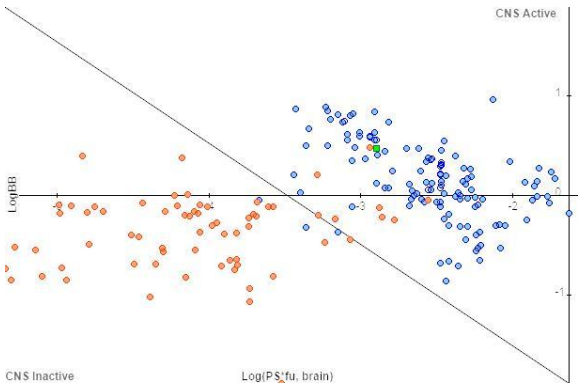
Table 2: Blood Brain Barrier permeability predictions of S1-S4, standard compounds, Haloperidol and Resperidone

COMPOUND	LogPS	LogPB	Log (PS*fu, brain)	LogBB
Haloperidol	-1.5	0.47	-2.9	0.61
Resperidone	-1.6	0.48	-3	0.57
S1	-1.7	0.16	-3.2	0.42
S2	-1.2	0.56	-3.2	0.58
S3	-1.1	0.46	-3.2	0.49
S4	-1.6	0.51	-3.1	0.44

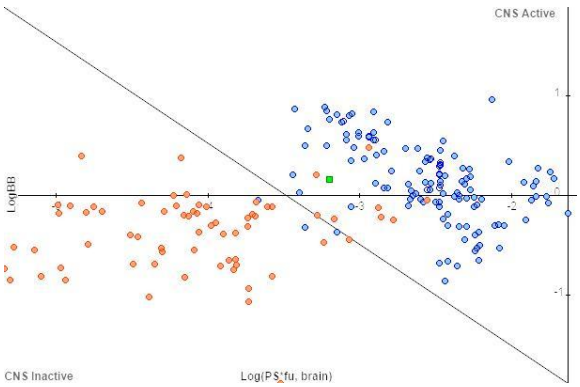
The information given in the Table 2 provides a comprehensive assessment of the permeation potential of candidate molecules based on their passive transport across the BBB. From the above Table 2 it is clearly evident that extent of brain penetration determined by ratio of total drug concentrations in tissue to the plasma at steady-state conditions (LogBB values) indicate [11] very high values for Haloperidol (0.61), resperidone (0.57) and S2 (0.58) molecules, even the other molecules S1, S3, S4 also showed high values indicating good BBB permeation. Rate of Brain penetration (LogPS) values for the standard antipsychotic drugs and the synthesized test molecules did not show significant difference and it ranged from -1.1 to -1.7. LogPB (extent of Brain penetration) values were remarkably and high for the molecules S2 (0.56) and S4 (0.51), which was higher than the standard antipsychotics Haloperidol (0.47) and Resperidone (0.48). High values were also recorded for S3 (0.46), but a comparatively lesser value was seen with S1 (0.16) molecule. Brain/plasma equilibration rate [Log (PS*fu, brain)] for all the molecules tested ranged from -2.9 to -3.2 indicating permeability potentials of the test compounds are similar to that of standard drugs. From the above findings it is obvious that the synthesized fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives (S1-S4) have shown very promising results like that of standard antipsychotic drugs.



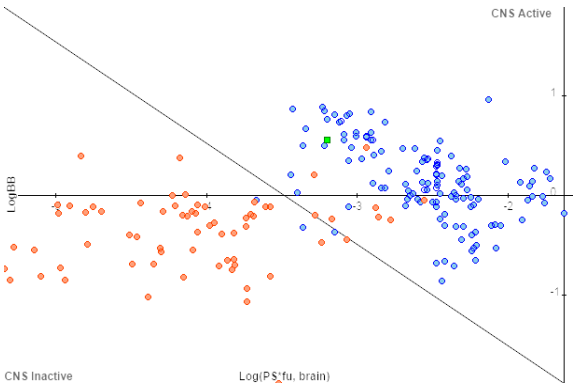
Haloperidol



Resperidone



S1



S2

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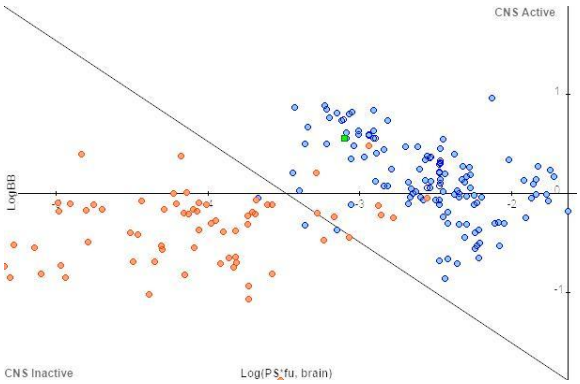
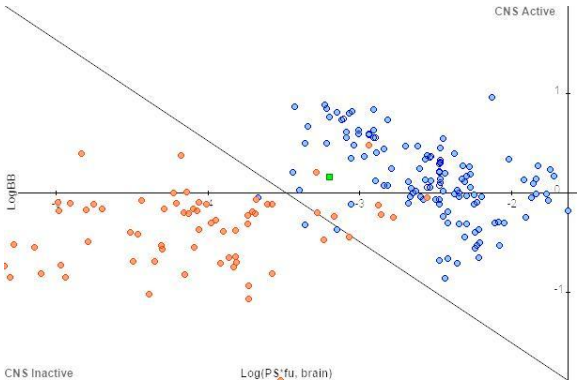


S3



S4

In the above graphs compounds in the green represent CNS activity of the respective molecules

CONCLUSION

The *in vitro* and *in silico* studies have indicated that the synthesized molecules (S1-S4) have very high Blood Brain Barrier (BBB) permeability. The comparison of the results with the standard antipsychotics Haloperidol and Resperidone reveal that the values are very significant and encouraging. PAMPA studies, Log PB and Log BB values were higher or similar to the standards concludes by itself that the synthesized molecules may be potential antipsychotic drug candidates. Thus it can be concluded that fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives (S1-S4) can be investigated further for its Antipsychotic properties and may lead to design of new promising antipsychotic drugs.

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