Research Article

Synthesis, Characterisation, Molecular property prediction and Antipsychotic activity of Novel 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole Derivatives

Sharath Chandra SP[1,3], Raghava B[2], Sharada AC*[1]
[1]Department of Biochemistry, Yuvaraja`s College, Mysore, Karnataka, India.
[2]Department of Studies in Chemistry, University of Mysore, Mysore, Karnataka, India.
[3]Department of Biochemistry, Government Science College, Hassan, Karnataka, India.
***Corresponding author's E-mail:** sharadaac@gmail.com

Accepted on: 20-09-2014; **Finalized on:** 31-10-2014.

ABSTRACT

Our present study focuses on Synthesis, Characterisation, Molecular Property predictions and Structure-Toxicity studies to choose the molecule possessing Antipsychotic properties. A new series of benzisoxazole derivatives were synthesized, which are close structural analogs of resperidone, a known Second generation Antipsychotic Drug. The structures of the newly synthesized molecules were confirmed by 1) NMR, LC MS, FTIR and elemental analysis. *In silico* analysis of the parameters of Lipinski`s Rule of five, along with Polar surface area (PSA), absorption%(%ABS), drug likeness indicated that all the synthesized compounds may have prospective role as antipsychotic molecules. *In silico* structure-toxicity predictions and bioactive scores of synthesized molecules showed identical properties as that of known antipsychotic drugs Haloperidol and Resperidone. The antipsychotic behavioural studies also indicate the antagonistic property of synthesized molecules (S1-S4) to dopamine D2 and serotonin receptors and also lower propensity to caused extra pyramidal symptoms (EPS). These encouraging results of the synthesized molecules have led to further analysis for *In vivo* and *In vitro* Antipsychotic Properties.

Keywords: Antipsychotic, Benzisoxazole, Molecular Property, Resperidone, Toxicity.

INTRODUCTION

Psychotic disorders (CNS), is reflected as significant psychological and social repercussions for everyday living.[1] Around three percent experience psychosis, more frequent than diabetes. Unlike infection, where the cause and effect are clear, most CNS ailments follow a complex biology, and have differing outcomes depending on predisposing factors. Even though the available drugs fulfil the requirements in the segment, there is need for more effective drugs that are better tolerated and cost effective to enhance the long term compliance.[2] Moreover the available herbal medicines have failed to show early recovery and many Antipsychotic therapies are known to produce extra pyramidal side effects (EPS) such as Parkinsonism, dystonia, and tardive dyskensia (TD) mainly due to the production of free radicals.[3] Our efforts have been devoted to the development of novel antipsychotic drugs that ameliorate psychosis without causing EPS and 1, 2 benzisoxazole derivatives are known to be precursors for Antipsychotic drugs like Resperidone.[4]

The derivatives of 6-fluoro-3-(piperidin-4-yl)benzo [d]isoxazole and corresponding heterocyclic analogs have been used as valuable intermediates in the synthesis of Second generation antipsychotic drugs such as Respridone, palperidone and iloperidone.[5] The drugs have shown affinity for serotonergic and dopaminergic receptor[6], however they are also known to produce extrapyramidal side effects (EPS). In this direction we have extended our effort to synthesis novel 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole analogs as potent antipsychotic agents exhibiting menial EPS.[7] The molecules were evaluated *in silico* for their Molecular Property predictions, involving Lipinski`s rule of five, polar surface area (PSA), absorption %, drug likeness, and toxicity values for these molecules as candidates for new drugs.[8] *In vivo* Antipsychotic studies of the novel molecules *in vivo* are one of the important ways of determining the neurological potentials of the molecules. Therefore antipsychotic studies such as rota-rod test, catalepsy tests, forced swim test and tail suspension tests were conducted.

MATERIALS AND METHODS

Instrumentation

The melting points were determined in a Selaco melting point apparatus and are uncorrected. FT-IR spectra were recorded as KBr pellet on Shimadzu FT-IR model 8300 spectrophotometer.^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$ on 500MHz Bruker instrument using Tetramethylsaline (TMS) as the internal standard. Purity and Mass analysis were recorded on a Thermo LCQ Deca XP MAX (Range: m/z = 1-2000) LC-MS. The C, H and N analysis were performed using CE-400 CHN analyzer The Homogeneity of the molecules was monitored by TLC on silica gel coated plates of 0.25mm thickness. All the chemicals were procured from Aldrich, Fluka and Merck Chemicals.

Animals

Swiss Albino mice of both genders weighing 25±5g and studies on them 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 housed in laboratory cages in animal house maintained at 23±2°C under standard light/dark cycle. All the animals had free access to standard food pellets and filtered water.

Drugs

Resperidone (Risdon, Intas Lab, India), Haloperidol (Senorm, Sun, India), were used as standard drugs. All the drugs were dissolved in normal saline and administered intraperitoneally (ip) in volumes of 10 mL/Kg body weight. Control animals were treated with distilled water in the same period.

Synthesis of 4-(6-fluorobenzo[d] isoxazole-3-yl)-N-(3-methoxyphenyl) piperidine-1-carbothiamide (S1)

A mixture of 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole(0.5g) and 3 methoxy phenyl Isothiocyanate(0.38g) were refluxed in the presence of powdered potassium carbonate(0.7g) in Dichloromethane at 60°C overnight. The reaction was stopped once a single spot in TLC was observed indicating presence of a single molecule, the compound thus formed was dissolved in Hexane to remove the liquid layer and the liquid was dried in flash evaporator and left in the flask overnight to remove the colour. Yield (89%), M.P. (164-166)°C. CHN analysis for $C_{20}H_{20}FN_3O_2S$; C 62.34; H 5.23; N 10.90 Found C 62.38; H 5.27; N 10.85, FT-IR spectra (KBr, ν,cm-1) 1601(C=S str) 2143(C=C str) 3055 (Ar. CH str.) 3624(OH str) 1540 (NH str). ^{1}H NMR (500 MHz, CDCl3, δ, ppm) 7.55(q,J=4.5,H) 7.16 (m,2H), 7.00(td,J=9.4,2.2Hz,1H), 6.61(s,1H),6.60(t,J=2.25Hz,2H),4.47(d,J=13Hz,1H),3.71(s,3 H), 3.29(m,4H),2.03 (m,4H) ^{13}C NMR (500 MHz, CDCl3, δ, ppm) 185.74, 165.23, 163.23, 160.37, 141.35, 130.06, 122.78, 119.42, 117, 114.42, 112.82, 97.72, 77.28, 55.38, 49.66, 33.74, 29.88. MS (ESI, *m/z*):385.34

Synthesis of N-(2-chlorophenyl)-4-(6-fuorobenzo[d] isoxazol-3-yl) piperidine-1-carbothiamide (S2)

A mixture of 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole(0.5g) and 2 Chlorophenyl Isothiocyanate (0.35g) were refluxed in the presence of powdered potassium carbonate(0.7g) in Dichloromethane at 60°C overnight. The reaction was stopped once a single spot in TLC was observed indicating presence of a single molecule, the compound thus formed was dissolved in Hexane to remove the liquid layer and the liquid was dried in flash evaporator and left in the flask overnight to remove the colour. Yield (84%), M.P. (148-151)°C. CHN analysis for $C_{19}H_{17}ClFN_3OS$; C 58.55; H 4.40; N 10.78 Found C 58.52; H 4.46; N 10.81, FT-IR spectra (KBr, ν,cm-1) 1610 (C=S str) 2144(C=C str) 3038 (Ar. CH str.) 3625(OH str) 1524 (NH str). ^{1}H NMR (500 MHz, CDCl3, δ, ppm) 7.65(dd,J=8, 1.5Hz, 1H),7,27(m,2H), 7.10(m,2H), 4.64(d,J=13.5Hz,1H), 3.45(m,4H), 2.18(m,4H). ^{13}CNMR (500MHz,CDCl3,δ,ppm) 182.43, 165.2, 163.20, 159.81, 136.71, 129.58, 126, 36 122.20,116.96, 112.84, 109.96, 97.70,77.26, 48.95, 29.65. MS (ESI, *m/z*):389.08

Synthesis of 4-(6-fluorobenzo[d] isoxazole-3-yl)-N-(3-fluorophenyl) piperidine-1-carbothiamide (S3)

A mixture of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole (0.5g) and 2 fluorophenyl Isothiocyanate (0.32g) were refluxed in the presence of powdered potassium carbonate(0.7g) in Dichloromethane at 60°C overnight. The reaction was stopped once a single spot in TLC was observed indicating presence of a single molecule, the compound thus formed was dissolved in Hexane to remove the liquid layer and the liquid was dried in flash evaporator and left in the flask overnight to remove the colour. Yield (87%), M.P. (160-162)°C. CHN analysis for $C_{19}H_{17}ClF_2N_3OS$; C 55.83; H 4.19; N 10.28 Found C 55.88; H 4.24; N 10.43, FT-IR spectra (KBr, ν,cm-1) 1611 (C=S str) 2144(C=C str) 3043 (Ar. CH str.) 3625(OH str) 1523 (NH str). ^{1}H NMR (500 MHz, CDCl3, δ, ppm) 7.64(m,2H), 7.38(dd,J=8,2.0Hz,1H), 7.28(m,2H), 7.12(m,2H), 4.65 (d,J=1.0Hz,1H), 3.45(m,4H), 2.17(m,2H). ^{13}C NMR (500MHz,CDCl3,δ,ppm) 182.77, 165.23, 163.90, 159.84, 155.83, 153.88, 127.83, 124.16, 122.14, 116.98, 112.65, 97.51, 77.25, 76.75, 48.88, 33.67, 29.65.183, LCMS (ESI,*m/z*):373.12

Synthesis of N-(4-chlorophenyl)-4-(6-fluorobenzo[d] isoxazol-3-yl) piperidine-1-carbothiamide (S4)

A mixture of 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole (0.5g) and 4 Chlorophenyl Isothiocyanate (0.35g) were refluxed in the presence of powdered potassium carbonate(0.7g) in Dichloromethane at 60°C overnight. The reaction was stopped once a single spot in TLC was observed indicating presence of a single molecule, the compound thus formed was dissolved in Hexane to remove the liquid layer and the liquid was dried in flash evaporator and left in the flask overnight to remove the colour. Yield (85%), M.P. (147-150)°C. CHN analysis for $C_{19}H_{17}ClFN_3OS$; C 58.55; H 4.40; N 10.78 Found C 58.52; H 4.46; N 10.81, FT-IR spectra (KBr, ν,cm-1) 1609 (C=S str) 2142(C=C str) 3034 (Ar. CH str.) 3627(OH str) 1522 (NH str). ^{1}H NMR (500 MHz, CDCl3, δ, ppm) 7.64(m, 2H), 7.38(dd,J=8.0,2.0Hz,1H), 7.32(dd,J=5.6,2.0Hz,2H), 7.12 (dd,J=5.8,2.0Hz,2H), 4.59 (d,J=13.0Hz,1H), 3.42(m,4H), 2.17(m,4H). ^{13}C NMR (500MHz,CDCl3,δ,ppm) MS (ESI, *m/z*) .33, 165.26, 163.91,159.82,138.57,130.65,129.3, 124.40, 122.47, 117.01, 112.69, 97.70, 89.77, 77.26, 49.15, 46.40, 33.60, 31.43, 29.67 LCMS (ESI, *m/z*).:389.16

Computational methods

All *in silico* work were performed using the computational methods implemented using *in silico* animal models. Molecular Property predictions were performed using MOLSOFT and ACD/I-LAB and Structure-Toxicity was performed in ADMETexp and ACD/I-LAB.

Acute Toxicity Study

The dose selection will be carried out according to safe dose calculation as per the acute toxicity studies (OECD

TG420) for the given sample S1 to S4. Acute toxicity studies were similar that of antipsychotic drugs. With this above basis, we selected the following dose 1 mg/kg for further studies.

Rota-Rod experiment

Before conduction of experiment animals were trained by placing them on a scraped rotating rod (25 rpm) of the Rota rod assembly to remain there on the rod at least for 3 minutes. After 30 minutes of treatment with different doses of standard drug or test compound, time of fall from the rotating rod for animals was observed.

Catalepsy test

Catalepsy was performed by administering haloperidol (1mg/kg, i.p), resperidone (1mg/kg) and synthesized molecules (S1-S4) (n=6) into different groups of albino mice, and was assessed at 30 min intervals until 120 min. and at the end of 240 min. by means of a standard bar test. Catalepsy was assessed in terms of the time (sec.) for which the mouse maintained an imposed position with both front limbs extended and resting on a 4 cm high wooden bar (1.0 cm diameter). The endpoint of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. Severity of the cataleptic behaviour was scored as 1 if maintained the imposed posture for at least 20 sec. and every additional 20 sec. one extra point was given. A cut-off time of 1100 sec. was applied during the recording of observations. The animals were returned to their individual home cages in between determinations. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25ºC.

Forced swim test

Swimming periods were conducted by placing the animals in glass cylinders (30x22.5cm) at temperature 24±2ºC water 40 cm deep, ensuring animals do not support themselves by the floor with their feet, thus making only their head above the water. This was performed for 2min which makes mice assume immobile position which is characterised by passive floating in water. The period of immobility indicates the state of depression and was recorded for 6 min. The mice were trained for 15 min, 24hr before the tests.

Tail Suspension test

The mobility observed in rodents when exposed to inescapable and unavoidable stress has been speculated to reflect behavioural despair which sequentially may reflect depressive disorders in animals. Swiss albino mice were divided into 7 groups (n=6), doses (1mg/kg) were administered once daily for 7 consecutive days. On the last day of the treatment schedule 1 hr after administration of drugs and test compounds, the animals were suspended on the edge of a table 75cm above the floor with a tape, and place approximately 2cm from the tip of the tail. Time of immobility was recorded for 5 min duration. Animals were assumed to be immobile if they did not exhibit any restlessness and were passive in hanging position.

Statistical Analysis

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

Molecular properties, Toxicity and Drug likeness is generally affiliated to some elemental molecular descriptors, like log P (Partition Coefficient), PSA (Polar Surface Area) and others. Lipinski`s rule of five uses these basic parameters to determine structure-activity properties such as Oral Bioavailability, if they fulfil the following norms: MW ≤500, hydrogen bond acceptors≤10, logP ≤5 and hydrogen bond donors ≤5. This form of theoretical description is extensively used in designing new drugs. Therefore we determined to perform *in silico* using Lipinski`s rule, including Absorption percentage (%ABS), Polar surface Area (PSA) and also predicting the Drug Likeness which indicates the physiochemical and pharmacodynamic properties of the molecules. All the Tests leading to the establishment of the synthesized molecules as orally bioavailable were performed taking Haloperidol and Resperidone drugs as standards.[9]

The Lipophilicity (log P), aqueous solubility, Polar surface area, Drug likeness were determined using the online software MOLSOFT[10] and ACD/I-LAB.[11] The Structure-activity studies were MOLSOFT uses fragment-based contributions. A method for splitting a molecule into a set of linear or non-linear fragments of different length and representation levels and counting the number of occurrences of each chemical pattern found. A Partial Least Squares (PLS) regression model was used to study the optimization of a particular property using a leave-50%-out cross-validation calculation. The Absorption Percentage was calculated applying the equation: %ABS= (PSA x 0.345) as reported by Zhao *et al.*[12,13]

All the results obtained in the above table fulfil the Lipinski's rule of five, indicating that the synthesized molecules have similar molecular properties like that of established drugs thus would qualify as orally bioavailable. All the synthesized molecules (S1-S4) had values less than 5 and similar to standard Antipsychotic drug haloperidol ranging between the values 4.43-4.85 for the compounds S1-S4. All the tested molecules had PSA less than 140 A^2, suggesting satisfactory cellular plasma membrane permeability properties, and also the calculated absorption percentage (%ABS) of all the derivatives and standard drugs ranged between 91.2 and 97.95 %, indicating that these molecules possess good permeability across the cellular plasma membrane.

From the literature survey it is observed that most of the established Antipsychotic drugs have logS higher than -

4.00.[14] All molecules particularly synthesized compounds S1-S4 have logS values ranging from -6.41to -7.14 (Table 1) higher than the standard antipsychotic drugs. Drug likeness for the synthesized molecules S1-S4 has similar positive values as that of standard antipsychotic drugs Haloperidol and Resperidone. The data clearly indicates that these compounds have in them fragments that are commonly available in most of the second generation antipsychotic drugs.[15] All the above parameters obtained are showing encouraging factors for the synthesized molecules S1-S4 to be investigated further for their *in vivo* and *in vitro* properties to confirm their likeness to become new potent antipsychotic drugs.

Table 1: Molecular property Predictions along with Lipinski Parameters and Drug Likeness of test compounds S1, S2, S3, S4 and standard drugs Haloperidol, Resperidone.

Lipinski`s Parameters						Mol PSA (A^2)	Mol Vol (A^3)	Mol LogS	% ABS	Drug Likeness
Compound	HBA	HBD	MW	Mol LogP	Violations					
Haloperidol	3	1	375.14	4.58	0	32.03	372.03	-4.90	97.95	1.28
Resperidone	5	0	410.21	3.62	0	51.68	452.78	-4.53	91.2	1.21
S1	4	1	385.13	4.43	0	40.16	375.27	-6.41	95.2	0.95
S2	3	1	389	4.63	0	35.37	359.73	-6.75	96.93	1.17
S3	3	1	373.11	4.48	0	38.37	349.29	-6.60	95.9	1.09
S4	3	1	389.08	4.85	0	43.06	360.54	-7.14	94.17	0.92

*HBA:Hydrogen bond acceptor, HBD: Hydrogen bond donor, MW: Molecular weight, LogP: Lyophilicity, PSA: Polar surface area, ABS: Absorption

Table 2: Toxicity Profile of test compounds S1, S2, S3, S4 compared with the standard drugs haloperidol and resperidone

Compound	hERG inhibition Probability	AMES Toxicity	Carcinogens	Acute Oral Toxicity	Rat Acute Toxicity LD 50, mol/kg
Haloperidol	0.52	Non Toxic	Non-Carcinogens	Moderately toxic Class-II	3.3376
Resperidone	0.56	Non Toxic	Non	Moderately Toxic Class-II	3.1071
S1	0.59	Toxic	Non	Slightly Toxic Class-III	2.974
S2	0.51	Non Toxic	Non	Slightly Toxic Class-III	3.450
S3	0.57	Non Toxic	Non	Slightly Toxic Class-III	3.016
S4	0.61	Toxic	Non	Slightly Toxic Class-III	2.893

Table 3: Bioactivity score of Haloperidol, Resperidone and test compounds S1-S4

Name	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Enzyme inhibitor
Resperidone	0.91	-0.01	-0.42	-0.16	-0.04
Haloperidol	0.43	0.19	-0.31	-0.13	0.14
S1	0.35	-0.23	-0.22	-0.21	-0.19
S2	0.38	-0.07	-0.29	-0.29	-0.12
S3	0.33	-0.16	-0.19	-0.05	-0.14
S4	0.37	-0.13	-0.13	-0.16	-0.20

Predicted toxicity values for Antipsychotic drugs Haloperidol, Resperidone and synthesized molecules S1-S4 are given in table 2. All the molecules exhibited moderate inhibition for Human ether-a-go-go-related gene (hERG) potassium channels, which are better than other Antipsychotic drugs used in medication, which remarkably show strong hERG inhibition.[16] All the synthesized molecules indicated nil AMES toxicity similar to the standard antipsychotic drug. The Drug likeness of the synthesized molecules were further supported by the absence any carcinogenic fragments in the molecules S1-S4.However most encouraging result of the above study is that of acute oral toxicity which is suggesting that the synthesized molecules are less toxic (Class-III) may be more tolerable without any side effects than the Antipsychotic drugs(Class-II). LD50 values also indicated that the newly synthesized molecules specially S2 and S3 showed almost similar values like that of antipsychotic drugs, however S1 and S4 molecules values were also satisfactory. Based on the above toxicity studies we can conclude that the synthesized molecules were less toxic than Antipsychotic drugs. Furthermore non AMES toxicity results, absence of carcinogens and moderate hERG inhibition, further supports to continue the study on the

in vitro and *in vivo* models to evaluate their properties, which may lead to the development of new Antipsychotic drug.[17]

In Table 3 calculated scores of synthesized molecules S1-S4 and antipsychotic drugs for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, and enzyme inhibitory activity is presented. According to standard norms, higher the bioactivity score, higher will be the probability of the molecule being active. Accordingly molecules with positive values indicate probability of being more active than molecules with negative values, however values between -0.5 to 0.00 indicate moderate activity and scores less than -0.5 is considered to be inactive. The results obtained have indicated that the synthesized molecules S1-S4 are biologically active compounds and will play a significant role in physiological interactions with GPCR ligands, nuclear receptor ligands, kinase inhibition and enzyme inhibition. All the molecules have exhibited almost similar score for GPCR ligand more than >0.00, and the highest score (0.91) was observed for resperidone. Bioactivity score for Ion channel modulator for all the molecules ranged from -0.23 to 0.19, with haloperidol exhibiting highest score of 0.19, followed by resperidone with -0.01 and S2 with -0.07. However in kinase inhibition, synthesized molecules seemed to be on the higher side of the bioactivity score with S4 indicating value of -0.13. Bioactive scores for nuclear receptor ligand activity was found to be moderate for all synthesized molecules and standard drugs ranging between -0.05(S3) to -0.29(S2). All the molecules exhibited bioactivity for enzyme inhibition however haloperidol being most active (0.14) and S4 (-0.20) was least active. From the present findings we observed that the molecules tested for bioactive scores exhibited activity with values ranging from moderate to very active, thus indicating potential pharmacological activity.

In the rota-rod test [18] Haloperidol (10.7 ± 1.43) and resperidone (14.2 ± 1.56) decreased the mean fall time compared to the normal animals (95 ± 1.34). However better results were observed in S2 (15.1 ±1.21) and S3 (14.3 ±1.53) when compared to the standard drugs, S1 also decreased mean fall time compared to haloperidol. The findings indicate a better motor coordination in animals administered with synthesized molecules (S2 and S3) when compared to standard antipsychotic drugs. According to the literature the above data suggest acute blockade of Dopamine D2 receptors in the striatum.[19,20]

Table 4: Effect of standard drugs and synthesized molecules (S1-S4) on Mean-off time in Rota-rod test.

Treatment	Mean fall-off time after drug Treatment [sec]
Normal	95 ± 1.34
Haloperidol	11.5 ± 1.43
Resperidone	14.2 ± 1.56
S1	13.8 ± 1.32
S2	15.1 ±1.21
S3	14.3 ±1.53
S4	8 ±1.22

Table 5: Effect of standard drugs and synthesized molecules (S1-S4) on induction of catalepsy in mice

Treatment/ Dose [1mg/kg i.p]	Mean Catalepsy score				
	30 min	60 min	90 min	120 min	240 min
Haloperidol	4.60±0.341	6.91±0.457	7.81±0.16	9.81±0.171	5.31±0.125
Resperidone	4.33±0.281	6.02±0.125	5.92±0.37	7.32±0.168	4.42±0.111
S1	5.01±0.241	6.93±0.125	7.23±0.321	6.21±0.164	5.86±0.157
S2	3.10±0.11*	4.10±0.647**	6.02±0.64	5.98±0.245	5.12±0.752
S3	3.21±0.45	4.98±0.487*	5.92±0.37*	6.41±0.425	3.82±0.314
S4	4.38±0.167	5.61±0.356	6.94±0.16	7.32±0.168	6.21±0.164

The results of catalepsy in the Table 5 show decreased manifestation of catalepsy in S2 and S3 treated mice in most of the periods compared to the standard drugs. Antipsychotic drugs are known to cause extra pyramidal side effects (EPS) due to the blockade of dopamine D2 receptors. Thus from the present findings we can conclude that there is decreased blocking of D2 receptors by synthesized molecules (S2 and S3) when compared to standard drugs. These observations may indicate the potential antipsychotic activities of the synthesized molecules.

Forced swim test (FST) [21] results showed comparable values for synthesized molecules S2 (68 ±0.22) and S3 (71 ±0.13) as that of standard drugs haloperidol (78 ± 0.12) and resperidone (66 ± 0.71). The synthesized molecules showed good antidepressant property compared to the antipsychotic drugs.

In Table 7 S2 (223 ±0.121) and S3 (238 ±0.153) significantly reduced the duration of immobility compared to standard drugs haloperidol (261 ± 0.143) and resperidone (248 ± 1.56). The results demonstrate increased production of serotonin and norepinephrine in animals administered with test molecules compared to animals administered with standard drugs [22]. The above conclusion is due to possible blockade of NMDA (N-methyl D-aspartate) receptors by antipsychotic drugs [23].

Thus the results indicate lower blockade of the NMDA receptors by synthesized molecules (S2 and S3) and hence more concentration of serotonin and norepinephrine, resulting in better neurotransmission.

Table 6: Effect of standard drugs and synthesized molecules (S1-S4) on Forced swim test

Treatment	Immobility period [sec]
Normal	163 ± 0.231
Haloperidol	78 ± 0.12
Resperidone	66 ± 0.71
S1	91 ± 0.154
S2	68 ± 0.22
S3	71 ± 0.13
S4	82 ± 0.116

Table 7: Effect of standard drugs and synthesized molecules (S1-S4) on tail suspension test.

Treatment	Duration of immobility(sec)
Normal	321 ± 0.134
Haloperidol	261 ± 0.143
Resperidone	248 ± 1.56
S1	255 ± 0.132
S2	223 ± 0.121
S3	238 ± 0.153
S4	260 ± 0.122

CONCLUSION

The treatment with antipsychotic drugs is usually associated with significant side effects. The molecules synthesized (S1-S4) in the present study have shown promising results and appears to be safe for treatment. The *in silico* studies gave significant understanding of the synthesized molecules as biologically potential compounds possessing desirable molecular characteristics with respect to bioavailability, toxicity and bioactivity. Synthesized molecules were less toxic compared to standard drugs and showed high values for oral bioavailability and antipsychotic studies also revealed antagonistic effects for dopamine D2 receptors and serotonin 5HT2a receptors. Thus we can conclude that the synthesized molecules are suitable to investigate further as potential antipsychotic drugs.

REFERENCES

1. Nestor, Paul G. Matthew K, Ileana B, Joel H, Psychosis, Psychopathy, and Homicide: A Preliminary Neuropsychological Inquiry, American J Psychiatry, 159, 138–40.
2. Mancuso MR, Kuhnert F, Kuo CJ, Developmental angiogenesis of the central nervous system, Lymphat Res Biol., 6, 2008, 173-80.
3. Meltzer HY, Stahl SM, The dopamine hypothesis of schizophrenia:a review, Schizophr Bull, 2, 1976, 19–76.
4. Meltzer HY, McGurk SR, The effect of clozapine, resperidone and olanzapine on cognitive function in schizophrenia, Schizophr Bull, 25, 1999, 233–255.
5. Caccia S, Pasina L, Nobili A. New atypical antipsychotics for schizophrenia: iloperidone. Laboratory of Drug Metabolism, Mario Negri Institute for Pharmacological Research, 20156, Milan.
6. Nuhrich A, Varache-Lembege M, Vercauteren J, Dokhan R, Renard P, Devaux G, Synthesis and binding affinities of a series of 1,2-benzisoxazole-3-carboxamides to dopamine and serotonin receptors, Eur.J. Med. Chem., 31, 1996, 957-964.
7. Hrib NJ, Jurcak JG, Burgher KL, Conway PG, Hartman HB, Kerman LL, Roehr JE, Woods AT, Benzisoxazole and benzisothiazole-3-carboxamides as potential atypical antipsychotic agents, J. Med.Chem., 37, 1994, 2308-2314.
8. Kalani K, Yadav DK, Khan F, Srivatsava SK, Suri N, Pharmacophore, QSAR and ADME based semi-synthesis and *in vitro* evaluation of ursolic acid analogs for anti-cancer acitivty, J Mol Model, 8, 2011, 3389-3413.
9. Salomon CJ, First century of Chagas' disease: An overview on novel approaches to nifurtimox and benznidazole delivery systems, J. Pharm. Sci., 101, 2012, 888–894.
10. Filikov AV, Mohan V, Vickers TA, Griffey RH, Cook PD, Abagyan RA, James TL, Identification of ligands for RNA targets via structure-based virtual screening: HIV-1 TAR, J Comput Aided Mol Des, 14(6), 2000, 593-610.
11. ACD/Structure Elucidator, version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada.
12. Zhao MY, Abraham MH, Le J, Hersey A, Luscombe CN, Beck G, Sherborne B, Rate-limited steps of human oral absorption and QSAR studies, Pharm. Res., 19, 2002, 1446–1457.
13. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ, Adv. Drug Delivery Rev, 23, 1997, 3.
14. Schlecker R, Thieme PC, The synthesis of antihypertensive 3-(1,3,4-oxadiazol-2-yl) phenoxy propanolahines, Tetrahedron, 44, 1988, 3289–3294.
15. Organic Chemistry Portal. Available online: http://www.organic-chemistry.org/prog/peo/(accessed on 10 February 2012).
16. Thomas D, Karle CA, Kiehn J. Curr Pharm Des, The cardiac hERG/IKr potassium channel as pharmacological target: structure, function, regulation, and clinical applications. 2006; 12(18):2271-83.
17. Verma A, Lead finding from Phyllanthus debelis with hepatoprotective potentials, Asian Pac J Trop Biomed, 2012, S1735-S1737.
18. Dunham NW, Miya TS, A note on a simple apparatus for detecting neurological deficits in rats and mice, J Am Pharm Assoc Am Pharm Assoc., 46, 1957, 208–209. PMid:13502156.
19. Vasconcelos SM, Nascimento VS, Nogueira CR, Vieira CM, Sousa FC, Fonteles MM, Viana GS, Effects of haloperidol on rat behavior and density of dopaminergic D2-like receptors. Behav Processes, 63, 2003, 45–52. doi:10.1016/S0376-6357(03)00028-7.

20. Hou Y, Wu CF, Yang JY, Guo T, Differential effects of haloperidol, clozapine and olanzapine on learning and memory functions in mice. Prog Neuro-Psychoph, 30, 2006, 1486–1495. doi:10.1016/j.pnpbp.2006.06.001

21. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M, Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity, European Journal of Pharmacology, 57(2–3), 1979, 201–210.

22. Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, Dunn RW, Antipsychotic agents antagonize noncompetitive N-methyl-D-aspartate antagonist-induced behaviours, Psychopharmacology, 120, 1995, 67–74. PMid:7480537.

23. Hoffmann DC, Typical and atypical neuroleptics antagonize MK-801-induced locomotion and stereotypy in rats, J Neural Transm Gen Sect, 89, 1992, 1–10. PMid:1358122.

Source of Support: Nil, **Conflict of Interest:** None.