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ORIGINAL RESEARCH ARTICLE

COMPARATIVE EVALUATION OF 6-FLUORO-3-(PIPERIDIN-4-YL) BENZO [D] ISOXAZOLE DERIVATIVES AND ATYPICAL ANTIPSYCHOTICS FOR THEIR ANTI-DIABETIC PROPERTIES

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Abstract: Atypical antipsychotics used to treat psychotic disorders are known to be associated with extrapyramidal side effects when chronically administered. However there is also evidence of other metabolic side effects which includes type II diabetes mellitus. In the present study we have investigated the anti-diabetic properties of standard antipsychotic resperidone and synthesized molecules S1-S4, which showed significant antipsychotic properties in earlier studies. Studies were performed for α amylase inhibition, α glucosidase inhibition, sucrose inhibition, glucose estimation by GOD-POD method by *in vitro* gluconeogenesis analysis. In the all the studies performed we observed significant results for synthesized molecules S2 and S4 compared to atypical antipsychotic resperidone. Thus the study concludes that the synthesized molecules have fewer tendencies to cause type II diabetes mellitus when compared to atypical antipsychotic drug.

Key Words: α Amylase; Antipsychotics; Diabetes mellitus; GOD-POD; Gluconeogenesis

INTRODUCTION

Atypical antipsychotic drugs used in the treatment of psychotic disorders are limited by its tendency to cause a range of extrapyramidal symptoms (EPS) like parkinsonism, akathisia, dystonia and tardive dyskensia (TD)[1]. EPS may be manifested after prolong treatment of antipsychotics and may persevere even after the withdrawal of the drug treatments, however chronic administration of these antipsychotic drugs also result in several metabolic abnormalities[2]. In general there is increased risk of diabetes in patients with psychotic disorders and this threat is augmented by some atypical antipsychotic drugs [3,4]. Though weight gain may be an instrument for the development of diabetes, a direct effect of these antipsychotic drugs on insulin mechanism in muscle may also be a significant contributor**.** These metabolic undesirable effects are reported to be associated with receptor mediated mechanisms, mainly by antagonising the histamine (H_1) and serotonin HT_{2C} receptors[5] and possibly by interacting with neurochemical pathways in the central nervous system[6]. In our earlier studies we had synthesized derivatives of 6-fluoro-3-(piperidin-4-yl) benzo [d] isoxazole 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). The designated molecules S1, S2, S3, and S4 were designed with piperidine moiety to interact with dopamine D2 receptor with lesser affinity and serotonin 5HT2a receptors with comparatively higher affinity as possible drug molecules with fewer propensities to cause EPS[7]. The studies revealed antipsychotic properties of synthesized molecules S1-S4, particularly S2 and S3 showing significant activity with lower propensity to cause EPS[8].The interaction of these synthesized molecules with the metabolic mechanisms, particularly type II diabetes mellitus will be evaluated in the current investigation for, α glucosidase inhibition, sucrose inhibition, glucose estimation by GOD-POD method and *in vitro* gluconeogenesis analysis.

MATERIALS AND METHODS

All chemicals and solvents were purchased from Himedia chemicals, Mumbai, India and were of analytical grade. The enzyme α-amylase (EC 3.2.1.1) (Type IIA from Bacillus species), and HBSS media were obtained from Sigma-Aldrich Co, Bengaluru, India. p-nitrophenyl-α-D glucopyranoside (PNDG) was obtained from SRL, India. Insulin was procured from Torrent Pharmaceuticals Ltd under the license from Novo nordisk, India. God-pod reagent was obtained from Aspen Laboratories, India.

The inhibitory effect of the compounds S1-S4 against α-amylase, intestinal α-glucosidase and sucrase were evaluated according to the method described with a few modifications [9].

α-amylase inhibition assay

The α-amylase inhibition assay was performed by dissolving 1 unit/ml of enzyme α-amylase from

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Bacillus species in 0.1M phosphate buffered saline, pH 6.9. The different concentrations of the S1-S4 compounds (100-400 µM) were pre-incubated with the enzyme solution for 10 min at 37˚C. The reaction was initiated by the addition of starch solution (0.1%) to the incubation medium and allowed for the enzymatic reaction for 30 min at 37˚C. The reaction was terminated by the addition of DNS reagent to the reaction mixture and the tubes are kept in boiling water bath for 10 min. The colour obtained was stabilized by the addition of 40% sodium potassium tartarate solution and cooled to room temperature. The absorbance was measured at 540 nm. Acarbose was used as positive control. The percentage inhibitory effect of compounds was calculated by the formula:

$$\% \text{ Inhibition} = \frac{(\text{control absorption} - \text{sample absorption})}{(\text{control absorption})} \times 100$$

α-Glucosidase inhibition assay

The inhibitory studies of the compounds S1-S4 against intestinal α-glucosidase and sucrase were performed[10]. Briefly, the rat intestinal acetone powder was homogenized in 0.9% saline and the suspension was centrifuged at 10,000 g for 30 min at 4˚C and the supernatant was used as an enzyme source for α-glucosidase and sucrase. The enzyme solution was pre-incubated with compounds S1-S4 at different concentrations (100-400 µM) for 10 min at 37˚C. The reaction was initiated by the addition of p-nitrophenyl-α-D glucopyranoside solution in phosphate buffer (100 mM, pH 6.9). The reaction mixture was incubated for 30 min at 37˚C for the enzymatic reaction. The reaction was terminated by the addition of 2M NaOH solution. The activity of the enzyme was measured at 400 nm using Shimadzu UV-1800 spectrophotometer. Acarbose was used as positive control. The percentage inhibitory effect of compounds was calculated by the formula:

$$\% \text{ Inhibition} = \frac{(\text{control absorption} - \text{sample absorption})}{(\text{control absorption})} \times 100$$

Sucrase Inhibition assay

The inhibitory action of compounds S1-S4 against sucrase was determined by measuring the amount of glucose hydrolyzed from sucrose. Briefly, the enzyme solution was pre-incubated with compounds S1-S4 at different concentrations (100-400 µM) in phosphate buffer (100mM, pH 6.9) for 10 min at 37˚C. The reaction was initiated by the addition of sucrose solution (60mM). The reaction mixture was incubated for 30 min at 37˚C for the enzymatic reaction. After incubation, the reaction is terminated by incubating the mixture in a water bath for 10 min. The amount of glucose released in the reaction mixture was determined using the GOD-POD method as described below.

Estimation of glucose by GOD-POD method

Glucose formed in the reaction mixture was estimated by the GOD-POD assay kit protocol. Briefly, 50 µl of the incubated medium was taken in a 96 well ELISA plate. The GOD-POD color reagent (200 µl) was added to each well and incubated for the color formation in the dark at 37˚C for 30 min. The optical density was measured at 505 nm. The percentage production of glucose was calculated by using the formula:

$$\% \text{ glucose production} = \frac{(\text{glucose in control} - \text{glucose in sample})}{(\text{glucose in control})} \times 100$$

In vitro gluconeogenesis assay in isolated rat liver slices

The antidiabetic ability of the different compounds (S1-S4) was studied by *In vitro* gluconeogenesis assay[11]. Adult male albino rats approved by institutional animal ethics Committee (IAEC) of University of Mysore (letter no. UOM/IAEC/18/2012) were fasted overnight and were killed by cervical dislocation. The liver was excised and washed in ice cold saline and stored on ice. Compounds (S1-S4) dissolved in DMSO at different concentrations (50-200 µM) were transferred to different wells in different 24 well plates containing Hank's Balanced Salt Solution (HBSS). Sodium pyruvate (10 mmol/l) prepared in HBSS was added to the 24 well plates such that the final concentration of pyruvate should be 5 mmol/l. Liver slices were cut as described with few modifications. The slices were weighed in a digital balance. The weights of tissue slices were between 100 and 150 mg and are added to plates containing HBSS medium and pyruvate with compounds at different concentrations. DMSO treated plates served as control and Insulin (1mmol/l) was taken as standard drug. The culture plates were incubated at ambient temperature (27˚C) for up to 60 min. Aliquots were taken from the plates at 0, 30 and 60 min. The amount of glucose formed in the culture plate was assayed using the GOD-POD method as described above.

Statistical analysis

The data obtained were analyzed using excel software. The data were expressed as mean ± standard deviation and all experiments were compared with control and performed in triplicates.

RESULTS AND DISCUSSION

Diabetes mellitus is a widespread metabolic disorder which in due course results in multiple organ failures[12]. This condition may be enhanced by administration of antipsychotic drugs[13]. Management of blood glucose level during chronic administration of these antipsychotic drugs is a crucial part in the control of diabetes and its complications. Thus in the present study we have investigated the anti-diabetic properties

of synthesized molecules S1-S4, which have demonstrated antipsychotic properties, in comparison with standard drug resperidone.

α-Amylase inhibition assay

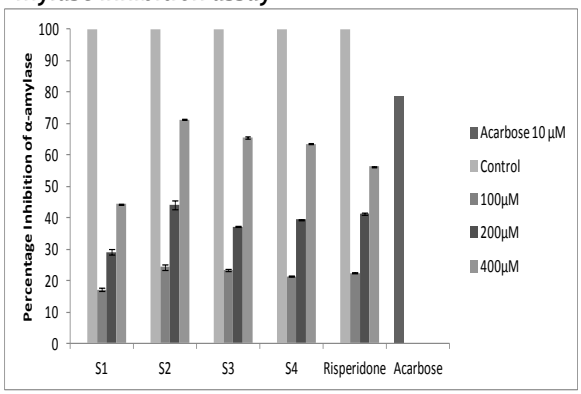

Figure 1: α amylase inhibitory effects of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives in comparison with standard drugs Acarbose and resperidone

In the above studies all the samples showed α amylase inhibitory activity at all concentration tested. However at higher concentration the inhibitory potentials of all the samples were significant. Synthesized molecules S2 and S4 showed significant α amylase inhibitory activity of 71.23% and 65.47% at 400µM concentration when compared to standard atypical antipsychotic drug resperidone (56.25%) at the same concentration. However S1 and S4 also indicated strong inhibitory activity at 44.32% and 63.45% at the above said concentration. All the studies were performed using acarbose as standard. The results indicate the compounds to be potent hypoglycaemic agents which bring forth their pharmacological properties with lesser side effects. It investigation reveals that the active factor of the synthetic molecules binds to the site other than the active site of the enzymes and interact with either enzyme substrate complex or free enzyme possibly interfering with mechanism of action of the both[14].

α-Glucosidase inhibition assay

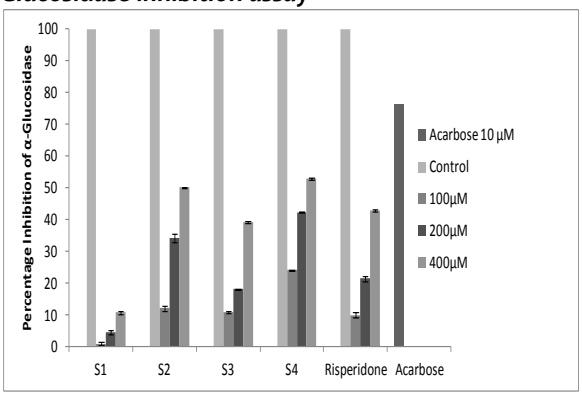

Figure 2: α-Glucosidase inhibition effects of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives in comparison with standard drugs acarbose and resperidone

The figure 2 demonstrates the α-glucosidase inhibitory potentials of synthesized molecules S1-S4 and antipsychotic drug resperidone when compared to standard drug acarbose. However higher inhibitory potential of 50.03% and 52.83% at 400 µM were observed in S2 and S4, whereas inhibitory values of resperidone was 42.65% lesser than the synthesized molecules. S3 also showed inhibitory potentials with 39.13% at 400µM however there was negligible results for S1. The above results imply that the synthetic molecules compete with the substrates to bind with the active site of the enzymes, by so doing preventing or slowing down the breakdown of polysaccharides / oligosaccharides to disaccharides[15].

Sucrase Inhibition assay

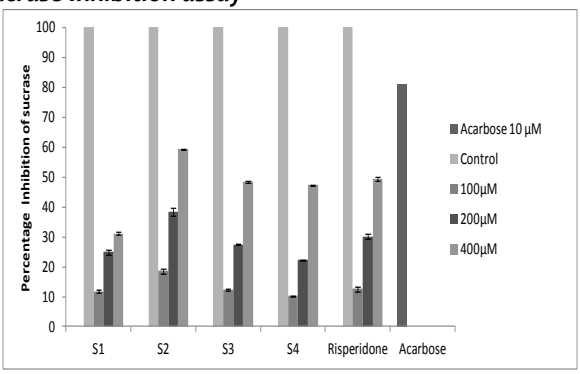

Figure 3: Sucrase inhibition effects of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives in comparison with standard drugs acarbose and resperidone

Figure 3 reveals the sucrase inhibitory activity of synthesized compounds and antipsychotic drug resperidone in comparison with acarbose. Compound S2 (59.30%) has shown better inhibition of sucrase when compared to S3 (48.34%), S4 (47.30%) and resperidone (49.52%) in dose dependent concentrations. Thus, indicating lesser propensity of synthesized compounds S2 and S3 in particular to cause type II diabetes mellitus when compared to the drug resperidone.

In vitro gluconeogenesis assay

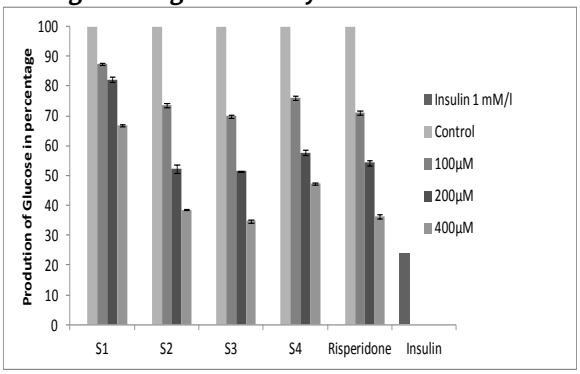

Figure 4: Hypoglycemic studies of insulin, synthetic compounds (S1-S4) and resperidone on gluconeogenesis in rat liver slices showing production of glucose in percentage.

Compounds (S1-S4) were screened for hypoglycemic effects using gluconeogenesis inhibition studies in rat liver slices. 0.145 units of insulin (1 mmol/l) inhibited gluconeogenesis and showed 16.84 % glucose production with reference to the production of glucose in DMSO treated plates. Compound S2 showed 73.67, 52.19 and 38.56% production of glucose at 100, 200 and 400µmol/l concentrations respectively. Compound S3 exhibited 69.88, 51.38 and 34.71% of glucose production at 100, 200 and 400 µmol/l concentrations respectively. Compounds S1 and S4 were not significant in inhibiting gluconeogenesis and showed 66.68 and 47.16 % of glucose production at 400 µmol/l concentration respectively. Standard drug risperdone showed 71.05, 54.23 and 36.25 % of glucose production at 100, 200 and 400 µmol/l concentrations respectively, whereas 1 mmol/l of insulin exhibited 23% of glucose production in the rat liver slices. Thus from the above data we an conclude that synthesized molecules S2 and S3 in particular show a slightly better potential to inhibit gluconeogenesis when compared to standard antipsychotic drug rispridone, thus having lesser tendency to cause diabetes mellitus.

CONCLUSION

The present study concludes that the synthesized molecules S1-S4 have significant anti-diabetic properties. Particularly S2 and S3 have shown promising hypoglycemic properties when compared to standard drug resperidone. Thus if the synthesized molecules if established as a drug, will have less chances to cause metabolic disorders, particularly type II diabetes mellitus.

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