IN SILICO MODELLING, SYNTHESIS, AND ANTI-DIABETIC EVALUATION OF BENZOTHIAZOLE SUBSTITUTED OXADIAZOLE DERIVATIVES

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ABSTRACT

Objective: The study contemplates in silico modeling, synthesis and in-vitro anti-diabetic evaluation of benzothiazole substituted oxadiazole derivatives. [5-[(1, 3-benzothiazol-2-ylsulfanyl) methyl]-1, 3, 4-oxadiazol-2-yl] sulfanyl] methyl] derivatives were synthesized by a conventional method.

Methods: All the newly synthesized derivatives were characterized by determining their melting point, retention factor from thin-layer chromatography, and spectral methods (Infrared, 1H NMR spectroscopy, 13C NMR spectroscopy, Mass spectroscopy) and evaluated for their anti-diabetic activity.

Results: [5-[(1, 3-benzothiazol-2-ylsulfanyl) methyl]-1, 3, 4-oxadiazol-2-yl] sulfanyl] methyl] derivatives have been made and characterized using physical and spectral methods. The in-vitro anti-diabetic screening study revealed that BZT1 and BZT4 exhibited high inhibition against glucose uptake assay and alpha-amylase enzyme. But only the derivative BZT4 showed inhibition against alpha-glucosidase enzyme.

Conclusion: Various benzothiazole substituted oxadiazole derivatives were synthesized, characterized by spectral studies. The anti-diabetic studies revealed that the synthesized derivatives have significant anti-diabetic properties and further structure-activity relationship studies may develop more potent and less toxic molecules.

Keywords: Diabetes mellitus, Benzothiazole, Oxadiazole, Anti-diabetic.

INTRODUCTION

Diabetes mellitus is a critical metabolic disorder characterized by high blood glucose levels and impairment in insulin action or both [1]. It has many sub-classifications including type-1, type-2, gestational diabetes, and other specific types. Type-1 diabetes is mainly due to autoimmune destruction of pancreatic β cells through T- cell mediated inflammatory response as well as humoral response. Type-2 may range from predominant insulin deficiency to secretory defect with insulin resistance [2]. The chronic hyperglycemia of diabetes is associated with long-term microvascular complications affecting the eyes, kidneys, and nerves, as well as an increased threat for cardiovascular disease. Currently, available drugs for the treatment of diabetes include Sulphonylureas (Tolbutamide and Glibenclamide), Biguanides (Metformin), Thiazolidinediones (Pioglitazone), Meglitinide derivatives (Repaglinide and Nateglinide), α- glucosidase inhibitors (Acarbose and Voglibose), DPP-4 inhibitors (Sitagliptin) [3].

Peroxisome proliferator-activated receptors (PPARs) constitute a group of nuclear receptors (NRs) that play crucial roles in the regulation of several physiological processes such as cellular differentiation and development, whole-body energy homeostasis (carbohydrate, lipid, and protein metabolism) [4]. PPARs are ligand-activated transcription factors and encompass a DNA binding domain in its N-terminus and ligand binding area in C-terminus [5]. The family of PPARs accommodates three isoforms: PPAR-α, PPAR-β, PPAR-γ. Among these subtypes, PPAR-γ agonist shows a prominent role in the orally effective anti-hyperglycemic agent. Thiazolidinediones act via PPAR-γ influences free fatty acid reflux, reduction of insulin resistance, and blood glucose level. Hence, they are widely used in the treatment of Diabetes mellitus [6].

A heterocyclic compound having nitrogen, oxygen, sulfur show wide applications in fields of medicinal chemistry. Benzothiazole is a privileged bicyclic ring system. The versatile biological functions exhibited by the benzothiazole nucleus include anti-diabetic, anti-microbial, analgesics, anti-convulsant, anti-inflammatory, etc. [7]. Oxadiazole is recognized as a promising class of bioactive heterocycle. Thereby it is considered as an important construction motif for the drug discovery and development process [8].

METHODS

ACD Lab Chemsketch ver 12.0 was used to draw chemical structures including organics, organometals, polymers, and also for the calculation of molecular properties [9].

Molinspiration molecular viewer allows the visualization of molecules which is encoded as SMILES or SD file for the calculation of important molecular descriptors as well as prediction of bioactivity score of important drug targets [10].

Pharmacokinetic study by Swiss ADME
Swiss ADME revealed pharmacokinetic properties, drug-likeness (Table 1) of a potent molecule through predictive models such as BOILED-Egg (Fig. 1) iLOGP, and Bioavailability Radar (Fig. 2) [11].

PASS online
Prediction of Activity Spectra for Substances (PASS) is a computer program that allows to estimating the probable profile of biological activity of a drug-like organic compound based on its structural formula [12].

Protein data bank (PDB)
PDB provided three-dimensional structural data for large biological molecules such as proteins, and nucleic acids. Each structure published PDB receives a four-character alphanumeric identifier called PDB ID,
for example,: 4YT1 (Human PPAR-γ1 ligand-binding domain in complex with Gamma Selective Synthetic Partial Agonist MEKT76) [13].

**Molecular docking**

Molecular docking is achieved by Autodock Vina (Table 2). The 3D crystallographic structures of proteins were uncovered from the (PDB ID- 4YT1). PyMOL produces a high-quality 3D image of protein as well as its visualization. PyRx is for docking analysis (Fig. 3) [14,15,16].

**Synthetic procedure**

**STEP 1: Synthesis of ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate**

A mixture of 2-mercaptobenzothiazole (0.01 mol) and ethyl-2-chloroacetate (0.01 mol) in dry acetone in the presence of anhydrous potassium carbonate (2 g) was allowed to reflux with stirring for 5 h at 70°C. Then it poured into ice-cold water with rapid stirring. The solid residue obtained is filtered and washed with water, dried, and recrystallized using absolute ethanol. Thin-layer chromatography (TLC) was carried out using ethyl acetate:petroleum ether (1:4). Spots visualized using ultraviolet cabinet.

**STEP 2: Synthesis of 2-(1, 3-benzothiazol-2-ylsulfanyl) acetohydrazide**

A solution of ethyl (1, 3-benzothiazol-2-ylsulfanyl) acetate (0.01 mol) and hydrazine hydrate 99% (0.02 mol) in ethanol (20 mL) was stirred well and refluxed for 8 h. The cooled product was filtered, dried, and recrystallized from methanol. TLC was carried out using ethyl acetate:petroleum ether (1:4).

**STEP 3: Synthesis of 5-[(1,3-benzothiazol-2-ylsulfanyl)methyl]-1,3,4-oxadiazole-2-thiol**

2-(1, 3-benzothiazol-2-ylsulfanyl) acetohydrazide (0.02 mol) in solution of potassium hydroxide (0.22 g) in ethanol (20 mL) of carbon disulphide (2 mL) with stirring. It is then refluxed at 40°C for 4 h. The filtrate is then neutralized with dilute hydrochloric acid and the solid residue is filtered, dried, and recrystallized with ethanol. TLC was carried out using ethyl acetate:petroleum ether (1:4).

**STEP 4: Synthesis of [[5-[[1,3-benzothiazol-2-ylsulfanyl)methyl]-1,3,4-oxadiazol-2-ylsulfanyl)methyl] derivatives**

A mixture of 5-[1, 3-benzothiazol-2-ylsulfanyl] methyl]-1,3,4-oxadiazole-2-thiol (0.01 mol) in dioxane and absolute ethanol (1:1, 20 mL), formaldehyde 37% (0.05 mol) was refluxed for 1–6 h at 30°C. To this solution, primary or secondary amine (0.01 mol) in absolute ethanol (5 mL) was added dropwise. The obtained product is filtered, dried, and recrystallized with ethanol. TLC was carried out using ethyl acetate:petroleum ether (1:4).

The synthetic scheme is given below:

**Characterization**

The synthesized benzothiazole substituted oxadiazole derivatives were characterized by various analytical techniques are as follows:

1. Melting point determination
2. TLC
3. Spectroscopy (Infrared, $^1$H NMR, $^{13}$C NMR, MASS)

Glucose uptake assay

Glucose uptake activity in L6 cells was estimated by the methods described by Gupta et al. with slight modifications. Cells were cultured on 12 well plates and incubated for 24 h at 37°C in a CO$_2$ incubator. When a semi-confluent monolayer was formed, the culture was renewed with serum-free DMEM containing 0.2% BSA and incubated for 18 h at 37°C in the CO$_2$ incubator. After 18 h, the medium was discarded and cells were washed with phosphate buffer solution (pH 7.4) buffer once and treated with 1000 $\mu$g/mL glucose along with test compound (25, 50, and 100 $\mu$g/mL) and insulin standard (2.35, 4.7 and 9.4 $\mu$g/mL) for 1 h. The cells treated only with 1000 $\mu$g/mL glucose were kept as control. Glucose uptake was calculated as the difference between the initial (1000 $\mu$g/mL) and final glucose content in the incubated medium. The final glucose concentration was estimated by the anthrone method with the aid of a glucose standard graph. The glucose uptake in L6 cells treated with test compounds was compared with that of control cells (untreated). If the treated cells showed improved glucose uptake compared to control cells indicates the compound has medicinal value [17].

Fig. 1: BOILED Egg model to predict Passive diffusion by Swiss ADME
Alpha-glucosidase inhibitory assay

The effect of the sample on α-glucosidase activity was determined according to the method described by Shai et al., (2011) with slight modification. 400 μL of α-glucosidase (0.067 U/mL) was preincubated with different concentrations of the sample for 30 min. Then 200 μL of 3.0 mM (pNPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of 0.1 M Na₂CO₃. The α-glucosidase activity was determined by measuring the yellow-colored para-nitrophenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition. The same procedure was done with Acarbose (1 mg/ml stock) which was used as standard [18].
Table 3: Synthesis of benzothiazole substituted oxadiazole derivatives

| Comp code | Substitution | Final product |
|-----------|--------------|---------------|
| BZT₁      | N            | ![BZT1](image) |
| BZT₂      | O            | ![BZT2](image) |
| BZT₃      | NH           | ![BZT3](image) |
| BZT₄      | NO₂          | ![BZT4](image) |
| BZT₅      | NH₂          | ![BZT5](image) |

*Comp code: Compound code

Table 4: Analysis of Lipinski’s rule of five by Molinspiration

| Comp code | MW (g/mol) | HA | HD | LogP | nrotb | Violations |
|-----------|------------|----|----|------|-------|------------|
| BZT₁      | 420.97     | 5  | 1  | 4.46 | 6     | 0          |
| BZT₂      | 362.48     | 6  | 0  | 2.17 | 6     | 0          |
| BZT₃      | 360.51     | 5  | 0  | 3.24 | 7     | 0          |
| BZT₄      | 382.51     | 5  | 1  | 4.23 | 6     | 0          |
| BZT₅      | 375.52     | 6  | 1  | 1.96 | 6     | 0          |
| STD       | 367.8      | 5  | 1  | 4.12 | 7     | 0          |

STD (Pioglitazone) 367.8 5 1 4.12 7 0

MW: Molecular weight, HD: Hydrogen bond Donors, Nrotb: No. of rotatable bonds, HA: Hydrogen bond Acceptor

Table 5: Drug-likeness score evaluation by molinspiration

| Comp code | Gpcr ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|-----------|-------------|-----------------------|-----------------|------------------------|------------------|-----------------|
| BZT₁      | -0.82       | -1.35                 | -0.28           | -0.88                  | -0.45            | -0.50           |
| BZT₂      | -0.55       | -0.90                 | -0.04           | -1.13                  | -0.20            | -0.31           |
| BZT₃      | -0.46       | -0.80                 | -0.07           | -1.09                  | -0.16            | -0.26           |
| BZT₄      | -0.59       | -0.68                 | -0.13           | -1.06                  | -0.20            | -0.28           |
| BZT₅      | -0.64       | -0.79                 | -0.22           | -1.05                  | -0.26            | -0.30           |

Table 6: Physical characterization of synthesized compounds

| Compound code | Molecular formula | Melting point (°C) | R<sub>f</sub> value |
|---------------|-------------------|--------------------|--------------------|
| BZT₁          | C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>O<sub>3</sub>S | 189–192           | 0.58               |
| BZT₂          | C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S | 185-188           | 0.63               |
| BZT₃          | C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>S | 158-161           | 0.74               |
| BZT₄          | C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>O<sub>3</sub>S | 164-167           | 0.57               |
| BZT₅          | C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>O<sub>3</sub>S | 148-152           | 0.61               |

% inhibition = \[\frac{OD \text{ of Test} - OD \text{ of Control}}{OD \text{ of test}}\] × 100

Alpha-amylose inhibitory assay

Screening of alpha-amylose inhibitors was performed using Xiao et al. method in test tubes with slight modifications based on the starch iodine test. The assay mixture was about 120 μL of 0.02M sodium phosphate buffer (pH 6.9), 1.5 mL of salivary alpha amylose, and plant extracts at a concentration from 0.5 to 1.5 mg/mL incubated at 37°C for 10 min. After that, soluble 1% starch was added to each reaction mixture and incubated at 37°C for 15 min. Then 60 μL of 1 M HCl was added to the reaction mixture to stop the enzymatic reaction and immediately 300 μL of iodine reagents was added. If any color change was noted and at 625 nm the absorbance was read. Inhibition [19,20]. The absorbance was measured at 625 nm and the percentage inhibitory activity was calculated using the following equation:

RESULTS AND DISCUSSION

The benzothiazole substituted oxadiazole derivatives synthesized were depicted in Table 3. The estimation of molecular descriptors and pharmacokinetic parameters of the proposed derivatives were done by ACD Lab Chemsketch ver. 12.0 and Molinspiration Online Software, respectively. From all these parameters enlisted in Table 4, the compounds obeying Lipinski’s rule of five were selected for docking studies. The drug-likeness score which is used to determine their
Table 8: $^1$H NMR spectral values of synthesized derivatives

| Compound code | $^1$H NMR (ppm) |
|---------------|-----------------|
| BZT$_1$      | 7.418 (d, Ar-H, 1H), 7.421 (t, Ar-H, 1H), 7.830 (d, Ar-H, 1H), 7.752 (d, Ar-H, 1H), 4.161 (s, S-CH$_2$, 2H), 4.263 (s, -NH$_2$, 2H), 7.850 (t, -NH, 1H) |
| BZT$_2$      | 7.936 (t, Ar-H, 1H), 7.825 (d, Ar-H, 1H), 7.440 (d, Ar-H, 1H), 7.409 (d, Ar-H, 1H), 4.441 (s, S-CH$_2$, 2H), 9.579 (d, S-H, 1H) |
| BZT$_3$      | 7.803 (d, Ar-H, 2H), 7.337 (d, Ar-H, 2H), 4.153 (s, S-CH$_2$, 2H), 4.441 (s, S-CH$_2$, 2H), 2.264 (d, Ar-C, 4H), 3.570 (d, Ar-C, 4H) |
| BZT$_4$      | 7.312 (d, Ar-H, 2H), 7.804 (d, Ar-H, 2H), 4.150 (s, S-CH$_2$, 2H), 4.466 (s, S-CH$_2$, 2H), 7.993 (s, -NH, 1H), 6.925 (d, Ar-H, 2H), 6.998 (d, Ar-H, 2H), 2.166 (s, Ar-CH$_3$, 3H) |
| BZT$_5$      | 7.012 (d, Ar-H, 2H), 7.194 (d, Ar-H, 2H), 4.218 (s, S-CH)$_2$, 4.473 (s, S-CH$_2$, 2H), 8.113 (s, -NH, 1H), 7.79 (d, Ar-H, 2H), 8.071 (d, Ar-H, 2H) |

affinity toward certain receptors is shown in Table 5. All the proposed derivatives showed a high score for NR ligand. The biological activity of derivatives was predicted by PASS as an anti-diabetic with a p<0.5. Pharmacokinetic prediction of the derivatives by Swiss ADME is illustrated in Table 5. All the derivatives exhibited high gastrointestinal absorption except BZT$_1$. All the derivatives were found to be non-permeant of the Blood-brain barrier and zero alert for PAINS. Docking results revealed a high negative docking score (Table 6). It indicates very good interaction and affinity with the binding site of protein 4YT1. The designed derivatives and standard exhibited polar interaction such as hydrogen bonding with amino acids.

Synthetic methodology

[[5-[[1, 3-benzothiazol-2-ylsulfanyl] methyl]-1, 3, 4-oxadiazol-2-yl] sulfonyl] methyl] derivatives were synthesized through a four-step conventional method. Five synthesized derivatives were named as BZT$_1$, BZT$_2$, BZT$_3$, BZT$_4$, and BZT$_5$. The characterization of synthesized derivatives carried out by TLC and melting point determination is presented in Table 6. Spectral characterization was done by Infrared.
Table 9: $^{13}$C NMR Spectral values of synthesized derivatives

| Compound code | $^{13}$C NMR (ppm) |
|---------------|-------------------|
| BZT$_1$       | a (122.28-1C, s), b (128.95-1C, s), c (152.87-1C, s), d (135.38-1C, s), e (165.27-1C, s), f (36.11-1C, s), g (159.17-1C, s), h (52.14-1C, s), i (135.14-1C, s), j (121.83-1C, s), k (128.95-1C, s), l (128.95-1C, s), m (129.07-1C, s) |
| BZT$_2$       | a (121.83-1C, s), b (128.95-1C, s), c (152.87-1C, s), d (135.38-1C, s), e (165.27-1C, s), f (36.11-1C, s), g (159.17-1C, s), h (52.14-1C, s), i (135.14-1C, s), j (121.83-1C, s), k (128.95-1C, s), l (128.95-1C, s), m (129.07-1C, s) |
| BZT$_3$       | a (122.35-1C, s), b (128.95-1C, s), c (152.89-1C, s), d (135.14-1C, s), e (165.27-1C, s), f (36.11-1C, s), g (159.17-1C, s), h (52.14-1C, s), i (135.38-1C, s), j (19.08-1C, s), k (129.07-1C, s), l (129.07-1C, s), m (147.51-1C, s), n (27.11-1C, s) |
| BZT$_4$       | a (119.45-1C, s), b (131.76-1C, s), c (147.85-1C, s), d (131.96-1C, s), e (66.99-1C, s), f (39.30-1C, s), g (107.70-1C, s), h (67.11-1C, s), i (40.55-1C, s), j (111.96-1C, s), k (116.44-1C, s), l (116.23-1C, s), m (147.23-1C, s) |

Table 10: Mass spectral values of synthesized derivatives

| Compound code | Molecular mass | Molecular ion peak | Parent peak |
|---------------|----------------|-------------------|-------------|
| BZT$_1$       | 420.97         | 420.95            | 279.95      |
| BZT$_2$       | 382.51         | 382.74            | 279.95      |
| BZT$_3$       | 420.97         | 420.95            | 279.95      |
| BZT$_4$       | 382.51         | 382.74            | 279.95      |

Table 11: Percentage of glucose uptake of synthesized derivatives and pioglitazone

| Sample      | Concentration (μg) | OD at 630 nm | % Glucose uptake |
|------------|--------------------|--------------|-----------------|
| Blank      |                    |              |                 |
| BZT$_1$    | 25                 | 0.0591       | 23.73           |
|            | 50                 | 0.7471       | 51.27           |
|            | 100                | 0.6510       | 89.07           |
| BZT$_2$    | 25                 | 0.9158       | 20.52           |
|            | 50                 | 0.8806       | 25.87           |
|            | 100                | 0.8243       | 35.46           |
| BZT$_4$    | 25                 | 0.8057       | 36.97           |
|            | 50                 | 0.7455       | 51.69           |
|            | 100                | 0.6369       | 81.54           |
| Pioglitazone| 25                 | 0.7723       | 45.73           |
|            | 50                 | 0.7105       | 60.15           |
|            | 100                | 0.6619       | 83.68           |

Table 12: Alpha-glucosidase inhibitory activity of synthesized derivatives and acarbose

| Sample      | IC$_{50}$ (μg) |
|------------|----------------|
| BZT$_1$    | 58.11          |
| BZT$_2$    | 72.13          |
| BZT$_3$    | 81.03          |
| BZT$_4$    | 48.80          |
| Acarbose   | 92.48          |

Table 13: Alpha-amylase inhibitory activity of synthesized derivatives and acarbose

| Sample      | IC$_{50}$ (μg) |
|------------|----------------|
| BZT$_1$    | 51.46          |
| BZT$_2$    | 100.08         |
| BZT$_3$    | 100.66         |
| BZT$_4$    | 63.74          |
| Acarbose   | 34.10          |

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