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# *IN-SILICO* DESIGN AND SYNTHETIC STRATEGY OF BENZIMIDAZOLE-OXADIAZOLE SCAFFOLDS AS POTENT ANTIDIABETIC AGENTS

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## ABSTRACT

**Objective:** To design and synthesize benzimidazole bearing 1,3,4 oxadiazole derivatives followed by their *invitro* evaluation as antidiabetic agents. **Methods:** In this study, *in-silico* screening were done and six derivatives were selected on the basis of docking. Their structures have been established on the basis of their spectral analysis (IR, NMR, MASS). Then compounds have been tested for their *invitro* alpha glucosidase inhibition and glucose uptake activity. Docking has been predicted using Schrodinger Maestro 13.3 software. **Results:** All the synthesized compounds were shown to exhibit significant alpha glucosidase inhibitory property when compared with standard drug acarbose. The samples show gradual increase in inhibition, when the sample concentration increased from 100 to 500 µg/ml. In glucose uptake assay, all the synthesized compounds showed enhancing the glucose uptake in L6 cells compared to untreated cells (Control). The glucose uptake was found to be increasing in concentration dependant manner also. The highest glucose uptake activity was shown by the sample at the concentration of 100µg/ml. **Conclusion:** According to the studies, it is observed that the synthesis and antidiabetic activities of benzimidazole-oxadiazole derivatives have been shown significant activity. In particular, compounds with electron withdrawing substituents were more potent. The newly synthesized compounds appears to be promising leads for future design for antidiabetic treatment.

**KEYWORDS:** Benzimidazole, Oxadiazole, Anti-diabetic activity, *In-silico* studies, *Invitro* studies, ∝- glucosidase, Glucose uptake.

## INTRODUCTION

Heterocycles are a typical and essential component of many natural products and therapeutic medicines. It plays an important role in biochemical processes because of the side groups of the most typical and essential constituents of living cells, DNA and RNA, are based on aromatic heterocycles. Most of the drugs belong to the class of heterogenous compounds.[1] All living cells metabolism depends heavily on heterocyclic compounds, many of which are five and six-membered molecules with one to three heteroatoms in the nucleus.[2] Benzimidazole is a heterocyclic aromatic organic compounds formed by the fusion of benzene and imidazole rings. It is an important pharmacophore structure in medicinal chemistry. Benzimidazole possess many pharmacological activities out of many N-ribosyldimethyl benzimidazole is one of the most well-known benzimidazole derivatives and is used as an axial ligand for cobalt in vitamin $B_{12}$. They are also known as benzoglyoxalines.[3] Benzimidazole containing medicines bind to a wide range of therapeutic targets with a wide range of biological activities because of their distinctive structural characteristics and electron-rich environments. They are also called derivatives of o-phenylenediamine.[4] Various biological activities of benzimidazole derivatives includes anticancer, antiviral, anti-HIV, anthelmintic, antiprotozoal, antihypertensive, anti-inflammatory, analgesic, anxiolytic, antiallergic, anticoagulant, antidiabetic activity etc.[5] Oxadiazoles are five-membered heterocyclic rings with one oxygen and two nitrogen atoms each. It is derived from furan by substitution of two methylene groups (=CH) with two pyridine type nitrogens (-N=). There are four distinct isomers: 1,2,3-oxadiazole, 1,2,5-oxadiazole, 1,2,4-oxadiazole, and 1,3,4-oxadiazole. Among heterocyclic compounds, 1,3,4-oxadiazole has become a key building motif for the development of new drugs. They are the bioisosteres for carboxylic acids, esters and carboxamides. A wide range of biological activities, including antibacterial, antifungal, analgesic, anti-inflammatory, antiviral, anticancer, antihypertensive, anticonvulsant, and anti-diabetic characteristics, are exhibited by compounds with 1,3,4-oxadiazole cores.[6]

Diabetes mellitus (DM) is an emerging persistent endocrine disease that modifies the metabolism of carbohydrates, fats and proteins. It is considered as a global burden, since it is associated with the failure and dysfunction of many organs, such as the heart, blood vessels,

and kidneys.[7] Postprandial hyperglycemia (PPHG) reduction is one of the therapeutic strategy for the treatment of type 2 diabetes. This can be accomplished by blocking carbohydrate enzymes.[8] Mammalian α-glucosidases inhibitors, which prevent enzymatic activity in the small intestine, may delay the release of D-glucose from oligosaccharides and disaccharides, resulting in a decrease in postprandial blood glucose levels and a delay in the absorption of glucose. These are first line oral sugar lowering agents.[9] The lack of efficacy of currently available medications emphasises the need for new therapies. Due to its pleiotropic effects on protein, lipid, and carbohydrate metabolism as well as its capacity to treat vascular dysfunction, AMP-activated protein kinase (AMPK) is of particular relevance in this respect. The physiological advantages of AMPK activation on metabolism have led to the perception of AMPK as a critical therapeutic target for the prevention and management of human diseases. AMP-activated protein kinase (AMPK), which regulates the metabolic pathways, balances the production and consumption of nutrients and energy. AMP-activated protein kinase (AMPK) provides a potential target capable of mediating the metabolic effects of metformin[10]. Keeping in view of the above mentioned importance of benzimidazole and 1,3,4 oxadiazole moieties in the field of antidiabetic activity, herein, we designed, synthesized, and screened benzimidazole-oxadiazole scaffolds for α-glucosidase inhibitory assay and glucose uptake activity. Molecular docking studies are also performed for understanding the binding of ligand to receptor (α-glucosidase and AMPK).

## MATERIALS AND METHODS

### General

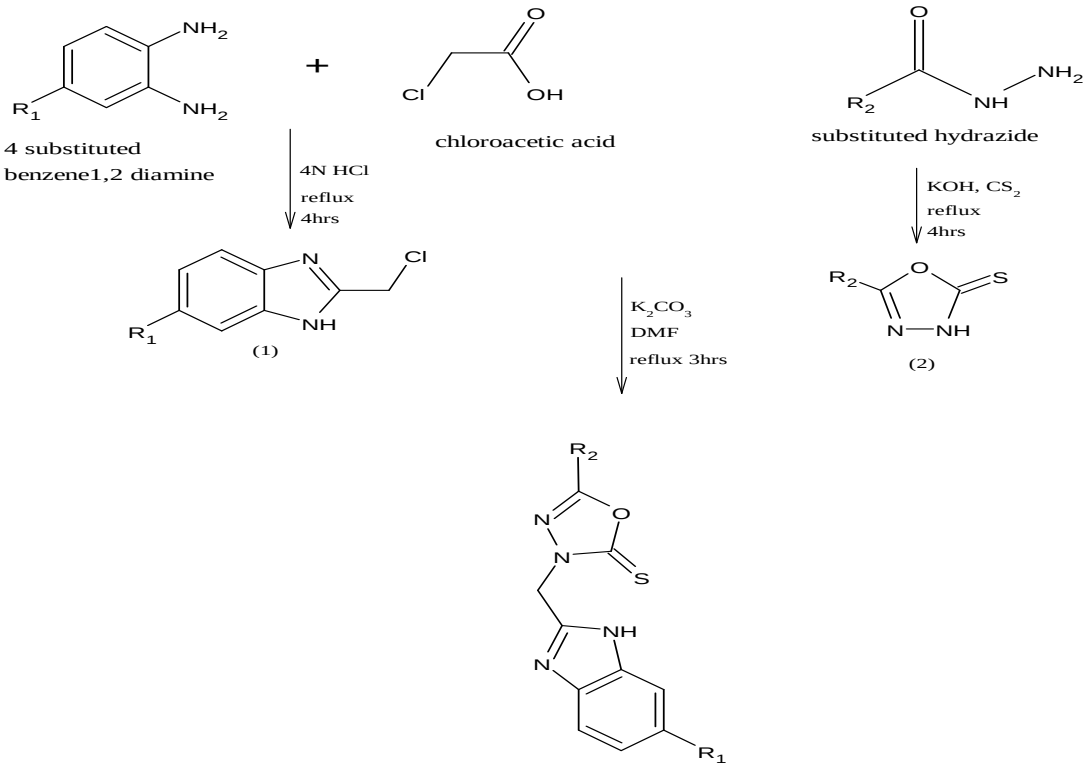
Solvents and materials were purchased from Sigma Aldrich Merck and Nice Chemicals Pvt Ltd were of analytical and synthetic grade. Progress of the reactions were observed by thin layer chromatography (TLC) using Merck precoated silica gel G plates. Melting points were determined on digital melting point apparatus. Spectroscopic data were measured on the following instruments: The IR spectra were recorded on an using Perkin Jasco FT- IR spectrophotometer in the range of 3999.64 to 649.893 $cm^{-1}$. $^{1}H$ NMR and $^{13}C$ NMR spectra of all the prepared compounds were recorded on a JEOL NMR JNM ECZ4000S/L1 spectrometer in deuterated methanol. Chemical shift values are reported in parts per million (ppm). The splitting pattern abbreviations used are as follows: s for singlet; m for multiplet. Mass spectra have been recorded on Mass spectrophotometer (SHIMADZU LC MS-8045) by LC-MS, using methanol as a solvent. The *In silico* drug properties calculation was performed using Chemsketch, molinspiration (drug likeness properties), QikProp (ADME), LAZAR

1.4.2 (toxicity) softwares. *Invitro* antidiabetic activity was carriedout against α-glucosidase and L6 cells.

**Molecular docking**

One of the most used virtual screening techniques in drug design is molecular docking. This method investigates the best ligand-target binding position. The Schrodinger Maestro 2022-4 programme was used in this work to carry out molecular docking. Steps involved in docking are protein preparation, ligand preparation, receptor grid generation, molecular docking analysis and selection of the best scored pose. Based on the docking scores compounds were selected for wet lab synthesis. The selected target and their PDB id includes, α-glucosidase enzyme- 3TOP, AMPK- 2UV4.

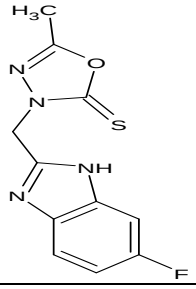
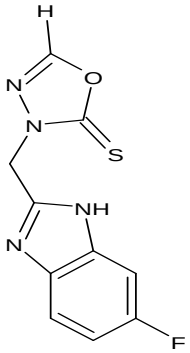
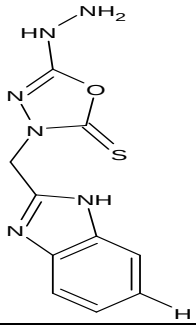
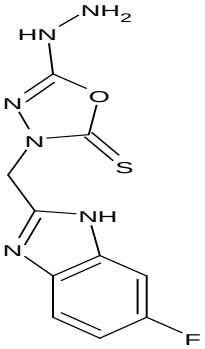
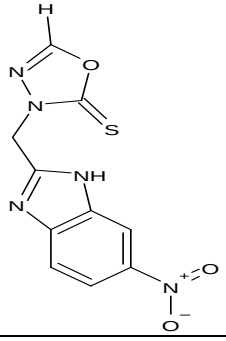
**Synthesis**

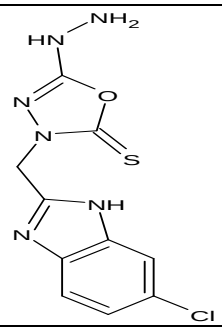



**$R_1$- H, Cl, F, $NO_2$**
**$R_2$-H, $CH_3$, NH-$NH_2$**

**Fig. 1: Scheme of the work.**

**Table 1: List of synthesized compounds.**

| Compound code | IUPAC name | Structure | Smile notation |
|---|---|---|---|
| BOX 8 | 3-[(6-fluoro-1H-benzimidazol-2-yl)methyl]-5-methyl-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC(C)=NN1Cc1[NH]c2cc(F)ccc2n1 |
| BOX 9 | 3-[(6-fluoro-1H-benzimidazol-2-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC=NN1Cc1[NH]c2cc(F)ccc2n1 |
| BOX 14 | 3-[(1H-benzimidazol-2-yl)methyl]-5-hydrazinyl-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC(=NN1Cc1[NH]c2ccccc2n1)NN |
| BOX 22 | 3-[(6-fluoro-1H-benzimidazol-2-yl)methyl]-5-hydrazinyl-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC(=NN1Cc1[NH]c2cc(F)ccc2n1)NN |
| BOX 23 | 3-[(6-nitro-1H-benzimidazol-2-yl)methyl]-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC=NN1Cc1[NH]c2cc(ccc2n1)[N+]([O-])=O |

| | | | |
|---|---|---|---|
| BOX 28 | 3-[(6-chloro-1H-benzimidazol-2-yl)methyl]-5-hydrazinyl-1,3,4-oxadiazole-2(3H)-thione |  | S=C1OC(=NN1Cc1[NH]c2cc(Cl)ccc2n1)NN |

**General procedure for synthesis of 1, 3, 4-oxadiazole derivatives**

**Step 1**

To a solution of 4 substituted orthophenylene diamine (1.0 mmol) in 4N HCl (10ml), chloroacetic acid (2.0 mmol) in 4N HCl (7ml) was added. The reaction mixture was refluxed for 4hrs. The completion of reaction was monitored by TLC. The reaction mixture was cooled to room temperature then it is basified with sodium bicarbonate. The precipitate so obtained was filtered, dried and recrystallized with ethanol.

**Step 2**

A mixture of substituted hydrazides (0.1 mol), KOH (0.1 mol) in absolute alcohol (50 ml) and $CS_2$ (0.2 mol) was taken in a round bottom flask and refluxed for about 4 hrs till evolution of hydrogen sulfide was ceased. The reaction mixture was cooled to room temperature and diluted with water. The product precipitated out on acidification with dilute hydrochloric acid was filtered, thoroughly washed with cold water and recrystallized from ethanol.

**Step 3**

The obtained 5 substitued oxadiazole thione (2) (0.2g) and 2.5eq (0.00223mol) of $K_2CO_3$ are stirred in DMF for 30 min. Then 1.2 eq of substituted 2-(chloromethyl)1H benzimidazole (1) is added to the mixture , followed by reflux for 3hrs. A 10% HCl solution is added to the reaction medium after cooling to room temp. The organic phase is extracted with ethylacetate and washed with salt water. Solvent is evaporated under reduced pressure. The crude product recrystallized from a mixture of solvent.

**Biological evaluation**

The designed and synthesized molecules need to be screened for their anti-diabetic activity.

***Invitro* studies**

1. α-glucosidase inhibition assay: reaction mixture containing 50µl phosphate buffer (100mM, pH = 6.8), 10 µl α-glucosidase and 20µl of varying concentration of test sample (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml) was taken and pre incubated at $37^0$C for 15 minutes. Then 20µl pNPG (5mM) was added as a substrate and incubated further at $37^0$C for 20 minutes. Finally, the reaction was stopped by adding $Na_2CO_3$ (0.1 M). Absorbance of released p-nitrophenol was measured at 405 nm. Same procedure followed for Acarbose at various concentrations as standard. Control were also prepared without the test sample. Percentage inhibition was calculated using following equation,

$$\% \text{ Inhibition} = \frac{(\text{Abs of control} - \text{Abs of derivatives})}{\text{Abs of control}} \times 100$$

2. Glucose uptake assay: Cells were cultured on 48 well plates and incubated for 48 hours at $37^o$C in a $CO_2$ incubator. When semi-confluent monolayer was formed, the culture was renewed with serum free DMEM containing 0.2% BSA and incubated for 18 hours at 37°C in the $CO_2$ incubator. After 18 hours, the medium was discarded and cells were washed with PBS (pH 7.4) buffer once and treated with 1000 µg/ml glucose along with test compound (25, 50, 100µg/ml) for 1 hour. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium. The final glucose concentration was estimated by anthrone method with the aid of a glucose standard graph. The glucose uptake in L6 cells treated with test compounds were compared with that of control cells (untreated). If the treated cells showed improved glucose uptake compared to that of control cells we can assume that the compound have medicinal value.

Standard Curve- Anthrone Method

Procedure: Prepare the standards by taking different concentrations (200-1000 µg/ml) in each test tube and make up to 1ml with water. Add 4ml of anthrone reagent to each tube. Heat for eight minutes in a boiling water bath. Cool rapidly and read the intensity of green to dark green color at 630 nm. Similarly after glucose uptake assay the remaining glucose present in the control and treated wells were assayed by anthrone method with the aid of standard curve.

$$\% \text{ Glucose uptake} = \frac{\text{Initial glucose - final glucose}}{\text{Initial glucose}} \text{ X } 100$$

## RESULTS AND DISCUSSION

### *In-silico* Molecular Modelling

Benzimidazole -oxadiazole derivatives derivatives were designed by Chemsketch software. *In-silico* analysis of the designed compounds were done including drug likeness properties and ADME and toxicity prediction. The derivatives obeying Lipinski's rule of five and having good ADMET properties were selected and the other derivatives were excluded. Based on the *in-silico* evaluation, 30 compounds were selected for further studies. The chemical structure of the selected compounds was drawn in Chemsketch software. The IUPAC name and smile notations were generated.

The designed compounds were undergone Lipinski's rule analysis by molinspiration cheminformatics software. Lipinski's rule of five is used in drug design and development to predict oral bioavailability of potential lead or drug molecules.

**Table 2: Lipinski's rule analysis of proposed derivatives.**

| Compound code | logP | Molecular weight | nON | nOHNH | nrotb | nviolations |
|---|---|---|---|---|---|---|
| BOX 1 | 3.51 | 342.81 | 5 | 1 | 3 | 0 |
| BOX 2 | 1.59 | 266.71 | 5 | 1 | 2 | 0 |
| BOX 3 | 1.81 | 280.74 | 5 | 1 | 2 | 0 |
| BOX 4 | 2.79 | 353.36 | 8 | 1 | 4 | 0 |
| BOX 5 | 0.87 | 277.26 | 8 | 1 | 3 | 0 |
| BOX 6 | 1.09 | 291.29 | 8 | 1 | 3 | 0 |
| BOX 7 | 3.00 | 326.36 | 5 | 1 | 3 | 0 |
| BOX 8 | 1.30 | 264.29 | 5 | 1 | 2 | 0 |
| BOX 9 | 1.08 | 250.26 | 5 | 1 | 2 | 0 |
| BOX 10 | 2.86 | 308.37 | 5 | 1 | 3 | 0 |
| BOX 11 | 0.94 | 232.27 | 5 | 1 | 2 | 0 |
| BOX 12 | 1.16 | 246.29 | 5 | 1 | 2 | 0 |
| BOX 13 | 3.28 | 322.39 | 5 | 1 | 3 | 0 |
| BOX 14 | 0.30 | 262.30 | 7 | 4 | 3 | 0 |
| BOX 15 | 1.58 | 260.32 | 5 | 1 | 2 | 0 |
| BOX 16 | 1.50 | 354.35 | 9 | 1 | 4 | 0 |
| BOX 17 | 1.71 | 327.34 | 6 | 1 | 3 | 0 |
| BOX 18 | 3.67 | 387.26 | 5 | 1 | 3 | 0 |
| BOX 19 | 1.57 | 309.35 | 6 | 1 | 3 | 0 |
| BOX 20 | 1.99 | 323.38 | 6 | 1 | 3 | 0 |
| BOX 21 | 1.94 | 325.19 | 5 | 1 | 2 | 0 |
| BOX 22 | 0.44 | 280.29 | 7 | 4 | 3 | 0 |
| BOX 23 | 0.87 | 277.26 | 8 | 1 | 3 | 0 |
| BOX 24 | 3.64 | 387.26 | 5 | 1 | 3 | 0 |
| BOX 25 | 2.72 | 402.28 | 6 | 3 | 3 | 0 |
| BOX 26 | 2.35 | 388.25 | 6 | 1 | 3 | 0 |
| BOX 27 | 0.73 | 276.32 | 7 | 4 | 3 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| BOX 28 | 0.96 | 296.74 | 7 | 4 | 3 | 0 |
| BOX 29 | 0.87 | 277.26 | 8 | 1 | 3 | 0 |
| BOX 30 | 2.91 | 338.39 | 6 | 1 | 4 | 0 |

The selected 30 derivatives exhibited no violations of Lipinski rule of five.

**Molecular docking**

The molecular docking studies of 30 derivatives were carried out to explore the possible binding interaction as well as to compare the binding pattern of these designed compounds to the standard ligand using Glide Maestro 13.3 Schrodinger 2022-4 software. Docking studies were conducted on α-glucosidase (PDB ID: 3TOP), AMPK (PDB ID: 2UV4) in which the selected derivatives were placed into the potent binding sites of the target specific region of the receptor forming a stable complex of potential efficacy and more specificity. The docking score were given in the table 3.

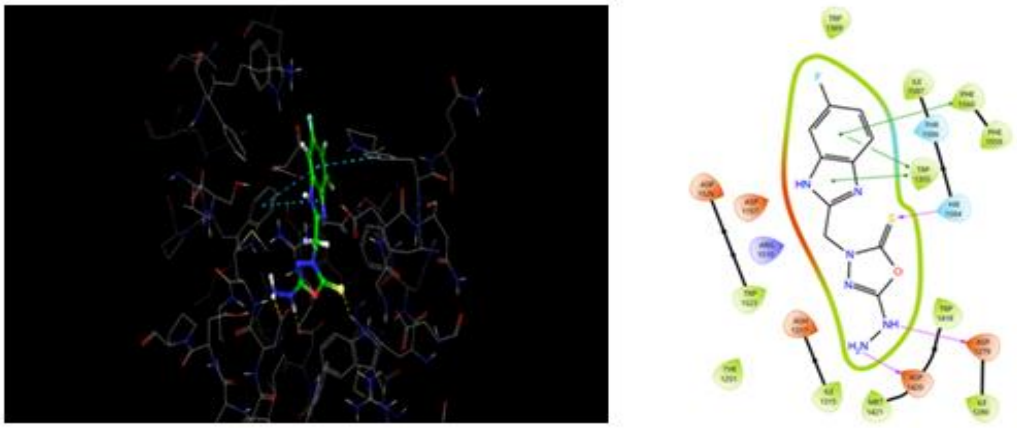
**Table 3: Docking scores of proposed derivatives.**

| **3TOP** | | **2UV4** | |
|---|---|---|---|
| Compound code | Energy (kcal/mol) | Compound code | Energy (kcal/mol) |
| BOX 22 | -7.59 | BOX 23 | -9.41 |
| BOX 28 | -7.42 | BOX 8 | -8.85 |
| BOX 14 | -7.21 | BOX 9 | -8.60 |
| BOX 18 | -6.37 | BOX 6 | -8.45 |
| BOX 1 | -6.19 | BOX 7 | -7.98 |
| BOX 10 | -6.15 | BOX 3 | -7.95 |
| BOX 30 | -6.14 | BOX 26 | -7.89 |
| BOX 25 | -6.08 | BOX 19 | -7.66 |
| BOX 24 | -5.98 | BOX 27 | -7.45 |
| BOX 11 | -5.94 | BOX 4 | -7.35 |
| BOX 29 | -5.82 | BOX 20 | -7.15 |
| BOX 16 | -5.80 | BOX 13 | -6.96 |
| BOX 2 | -5.76 | BOX 17 | -6.82 |
| BOX 5 | -5.68 | BOX 21 | -6.78 |
| BOX 12 | -5.64 | BOX 15 | -6.69 |
| Acarbose | -7.56 | Metformin | -5.65 |

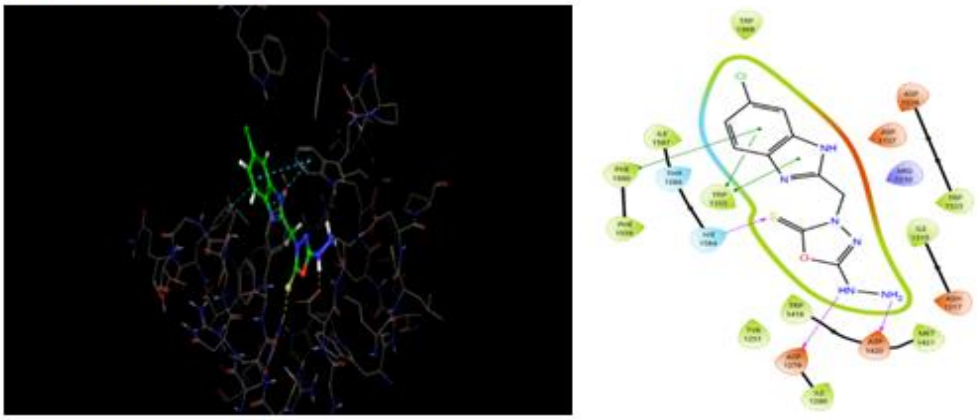
Compounds BOX 22, BOX 28, BOX 14 showed high docking score on alpha glucosidase. Compounds BOX 23, BOX 8, BOX 9 showed good docking score on AMPK. Binding interactions of the selected compounds are given below in figures. These compounds are selected for wet lab synthesis.

**Interaction with α-glucosidase receptor**

•**Compound BOX 22**



•**Compound BOX 28**



•**Compound BOX 14**

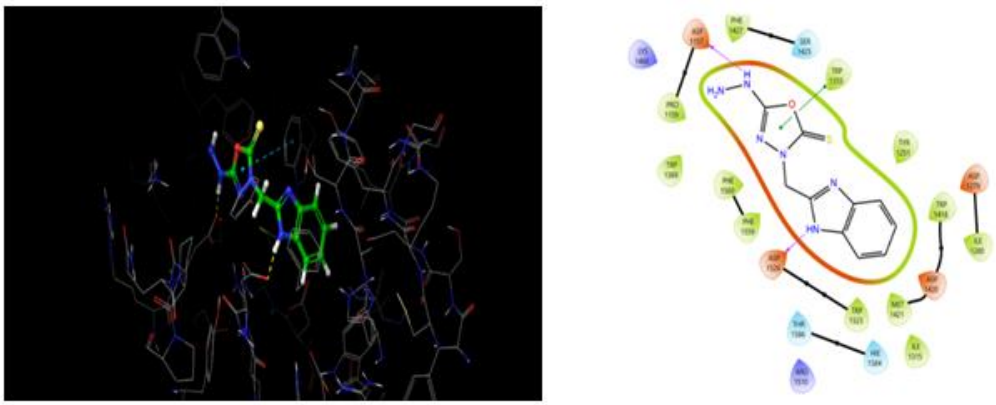
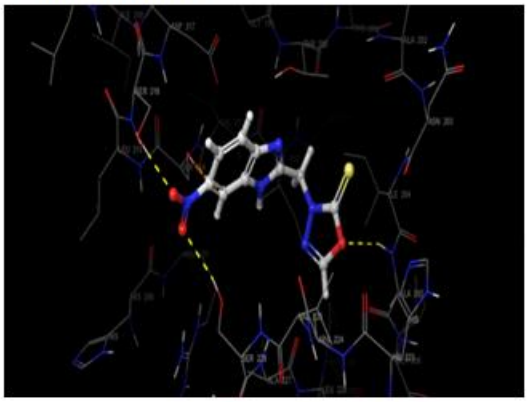
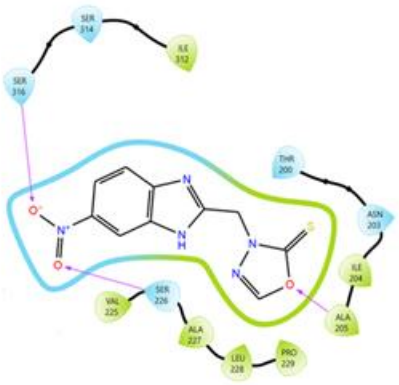


**Figure 2: 3D and 2D binding interactions of compound BOX 22, BOX 28, BOX 14.**

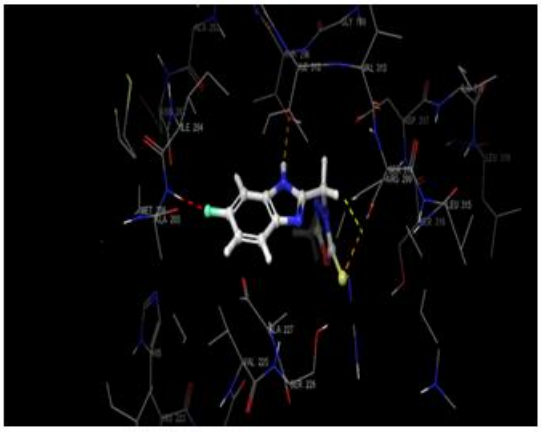
**Interaction with AMPK receptor**

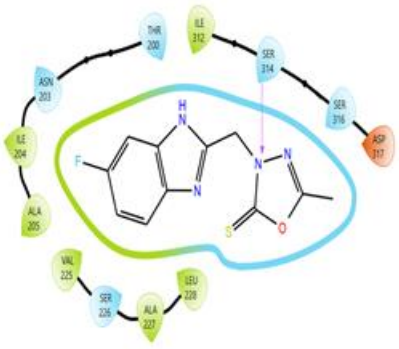
• **Compound BOX 23**



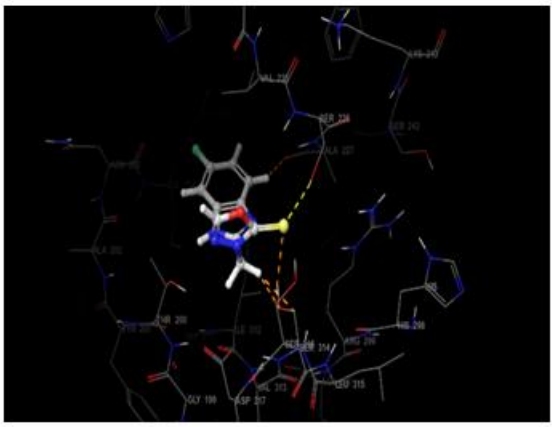


• **Compound BOX 8**





• **Compound BOX 9**



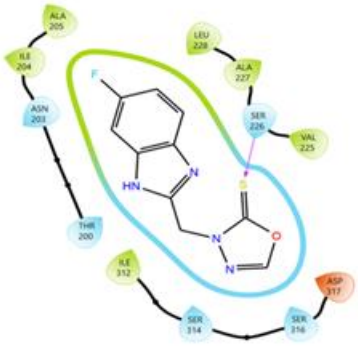


**Figure 3: 3D and 2D Binding interaction of compound BOX 23, BOX 8, BOX 9.**

**Table 4: ADME prediction of proposed derivatives.**

| Parameters | Compound code | | | | | |
|---|---|---|---|---|---|---|
| | BOX 22 | BOX 28 | BOX 14 | BOX 23 | BOX 8 | BOX 9 |
| Caco-2cell permeability (nm) | 984 | 796 | 617.30 | 970.1 | 851.69 | 534 |
| Metabolic Reactions | 3 | 2 | 3 | 3 | 3 | 2 |
| Protein Binding (HSA) | 0.06 | 0.13 | 0.15 | 0.08 | 0.14 | 0.28 |
| Human Oral Absorption | 3 | 3 | 3 | 3 | 3 | 3 |
| Percent Human Oral Absorption (%) | 100 | 100 | 95.5 | 100 | 100 | 92.8 |

**Table 5: Toxicity prediction of proposed derivatives.**

| Sl.no | Compound code | Toxicity | |
|---|---|---|---|
| | | Mutagenicity | Carcinogenicity |
| 1 | BOX 22 | No risk | No risk |
| 2 | BOX 28 | No risk | No risk |
| 3 | BOX 14 | No risk | No risk |
| 4 | BOX 23 | No risk | No risk |
| 5 | BOX 8 | No risk | No risk |
| 6 | BOX 9 | No risk | No risk |

**Table 6: Physical characteristics of synthesized compounds.**

| Compound Code | Physical appearance | Melting point (ºC) | Percentage yield | $R_f$ value | TLC solvents |
|---|---|---|---|---|---|
| BOX 22 | Light brown powder | $210^0$C | 54% | 0.88 | Chloroform: Ethanol |
| BOX 28 | Dark brown powder | $209^0$C | 58% | 0.82 | Chloroform: Ethanol |
| BOX 14 | Pale brown powder | $208^0$C | 58% | 0.81 | Chloroform: Ethanol |
| BOX 23 | Light brown powder | $210^0$C | 55% | 0.83 | Chloroform: Methanol |
| BOX 8 | Brown powder | $214^0$C | 58% | 0.87 | Chloroform: Ethanol |
| BOX 9 | Light orange powder | $204^0$C | 57% | 0.86 | Chloroform: Ethanol |

**Table 7: IR spectral analysis of synthesized compounds**

| Compound Code | IR peak in $cm^{-1}$ |
|---|---|
| BOX 22 | 1127.52 (C-F), 1335.79 (Ar C-N), 1523.22 (C=S), 1069.69 (Ar C-O-C), 1272.52 (Ar C-C stretch), 1682.62 (Ar C=N), 1635.62 (NH bend), 3371.79 (NH stretch), 728.28 (C-H bend), 3162.73 (C-H stretch), 1482.12 (Ar C=C) |
| BOX 28 | 1483.21 (Ar C=C), 1352.1 (Ar C-N), 1503.01 (C=S), 1124.3 (Ar C-O-C), 1005.7 (Ar C-C stretch), 1783.71 (Ar C=N), 1633.41 (NH bend), 3378.67(NH stretch), 800.198 (C-H bend), 3178.64 (C-H stretch), 700.98(C-Cl) |
| BOX 14 | 1461.78 (Ar C=C), 1338.36 (Ar C-N), 1061.62 (C=S), 1519.68 (Ar C-O-C), 1273.28 (Ar C-C stretch), 1681.73 (Ar C=N), 1624.73 (NH bend), 3384.46 (NH stretch), 747.281 (C-H bend), 2916.81(C-H stretch) |
| BOX 23 | 1496.49 (Ar C=C), 1356.33 (Ar C-N), 1546.49 (C=S), 1053.91 (Ar C-O-C), 828.81 (Ar C-C stretch), 1687.41 (Ar C=N), 1627.73 (NH bend), 3412.08 (NH stretch), 744.201 (C-H bend), 3023.41 (C-H stretch), 1546.49 (N=O), 1235.31 (N-O) |
| BOX 8 | 1580.33 (Ar C=C), 1348.33 (Ar C-N), 1467.73 (C=S), 1063.72 (Ar C-O-C), 1263.27 (Ar C-C stretch), 1688.33 (Ar C=N), 1636.77 (NH bend), 3386.73 (NH stretch), 737.361 (C-H bend), 3164.46 (C-H stretch), 2863.27 ($CH_3$), 1172.36 (C-F) |
| BOX 9 | 1588.38 (Ar C=C), 1467.73 (Ar C-N), 1273.27 (C=S), 1073.72 (Ar C-O-C), 957.38 (Ar C-C stretch), 1685.35 (Ar C=N), 1635.27 (NH bend), 3486.77 (NH stretch), 738.361 (C-H bend), 3064.46 (C-H stretch), 1165.36 (C-F) |

**Table 8: $^1$H NMR analysis of synthesized compounds.**

| Compound code | Molecular formula | $^1$H NMR (δ value in ppm) |
|---|---|---|
| BOX 22 | $C_{10}H_9FN_6OS$ | 4.991(2H, s, $CH_2$), 11.362(1H, s, NH) 6.125(2H, s, $NH_2$), 7.412(1H, m, Ar-H) 7.565(1H, m, Ar-H), 7.255(1H, m, H) 10.21(1H, m, NH) |
| BOX 28 | $C_{10}H_9ClN_6OS$ | 5.001(2H, s, $CH_2$), 11.242(1H, s, NH) 6.235(2H, s, $NH_2$), 7.412(1H, m, Ar-H) 7.615(1H, m, Ar-H), 7.33(1H, m, H) 10.211(1H, m, NH) |
| BOX 14 | $C_{10}H_{10}N_6OS$ | 5.041(2H, s,$CH_2$), 11.262(1H, s, NH) 6.225(2H, s, $NH_2$), 6.925(1H, m, Ar-H) 7.105(1H, m, Ar-H), 7.552(1H, m, Ar-H), 7.715(1H, m, H), 10.41(1H, m, NH) |
| BOX 23 | $C_{10}H_7N_5O_3S$ | 5.051(2H, s, $CH_2$), 11.362(1H, s, NH) 7.752(1H, m, Ar-H), 7.915(1H, m, Ar-H) 8.471(1H, m, Ar-H), 8.484(1H,s,H) |
| BOX 8 | $C_{11}H_9FN_4OS$ | 5.001(2H, s, $CH_2$), 11.362(1H, s, NH) 7.412(1H, m, Ar-H), 7.665(1H, m, Ar-H) 7.165(1H, m, Ar-H), 2.262(3H,s,$CH_3$) |

| | | |
|---|---|---|
| BOX 9 | $C_{10}H_7FN_4OS$ | 5.041(2H, s, $CH_2$), 11.242(1H, s, NH) 7.412(1H, m, Ar-H), 7.615(1H, m, Ar-H) 7.135(1H, m, Ar-H), 8.484(1H,s,H) |

**Table 9: $^{13}$C NMR analysis of synthesized compounds.**

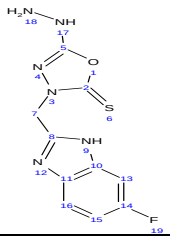
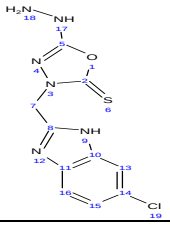
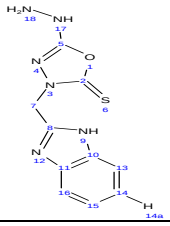
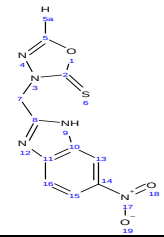
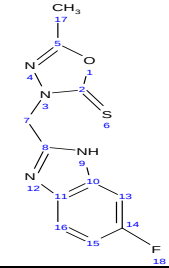
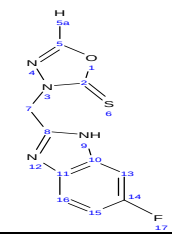
| Compound code | Molecular formula | $^{13}$C NMR( δ value in ppm) | Structure |
|---|---|---|---|
| BOX 22 | $C_{10}H_9FN_6OS$ | 49.186-1C;$CH_2$($C_7$),150.912 1C;C=N($C_8$),137.212,138.212-2C; Ar-C($C_{10}$,$C_{11}$),108.452,160.27,115.312, 117.271 4C;Ar-CH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$) ,158.98-1C;C=N($C_5$),175.859-1C;C=S($C_2$) |  |
| BOX 28 | $C_{10}H_9ClN_6OS$ | 49.186-1C;$CH_2$($C_7$),150.912 1C;C=N($C_8$),137.212,137.912-2C;Ar-C($C_{10}$,$C_{11}$),117.981,132.312,128.452,120.4524C;ArCH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$)158.98-1C;C=N($C_5$),175.859-1C; C=S($C_2$) |  |
| BOX 14 | $C_{10}H_{10}N_6OS$ | 49.085-1C;$CH_2$($C_7$), 150.9129 1C;C=N($C_8$),137.212,138.212-2C;Ar C($C_{10}$,$C_{11}$),114.312,128.27,118.452-4C; Ar-CH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$),158.98-1C; C=N($C_5$),175.859-1C; C=S($C_2$) |  |
| BOX 23 | $C_{10}H_7N_5O_3S$ | 49.186-1C;$CH_2$($C_7$),150.981-1C;C=N($C_8$), 137.912,137.212-2C;ArC($C_{10}$,$C_{11}$), 115.312,138.45,117.271,114.254C; Ar-CH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$),158.98-1C;C=N($C_5$),175.859-1C; C=S($C_2$) |  |
| BOX 8 | $C_{11}H_9FN_4OS$ | 12.452-1C;$CH_3$($C_{17}$),49.271-1C;$CH_2$($C_7$),150.981-1C;C=N($C_8$),137.912,137.212-2C;ArC($C_{10}$,$C_{11}$),108.452,160.27,115.31,117.27-4C;Ar-CH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$),158.91 1C;C=N($C_5$),175.859-1C; C=S($C_2$) |  |
| BOX 9 | $C_{10}H_7FN_4OS$ | 49.271-1C;$CH_2$($C_7$),158.981-1C;C=N($C_8$), 137.912,137.212-2C;Ar-C($C_{10}$,$C_{11}$), 108.452,160.27,115.31,117.27-4C;ArCH($C_{13}$,$C_{14}$,$C_{15}$,$C_{16}$),143.98-1C;C=N($C_5$), 175.859-1C; C=S($C_2$) |  |

**Table 10: Mass spectral analysis of synthesized compounds.**

| Compound code | Molecular Formula | Peaks (m/z) | Molecular mass (Da) |
|---|---|---|---|
| BOX 22 | $C_{10}H_9FN_6OS$ | Base peak: 249.02 Molecular ion peak: 280.05 | 280.29 |
| BOX 28 | $C_{10}H_9ClN_6OS$ | Base peak : 264.99 Molecular ion peak: 296.02 | 296.74 |
| BOX 14 | $C_{10}H_{10}N_6OS$ | Base peak: 231.03 Molecular ion peak: 262.06 | 262.30 |
| BOX 23 | $C_{10}H_7N_5O_3S$ | Base peak: 233.01 Molecular ion peak: 277.06 | 277.26 |
| BOX 8 | $C_{11}H_9FN_4OS$ | Base peak: 151.06 Molecular ion peak: 264.04 | 264.29 |
| BOX 9 | $C_{10}H_7FN_4OS$ | Base peak: 206.01 Molecular ion peak: 250.03 | 250.26 |

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