IN SILICO DESIGN, SYNTHESIS AND EVALUATION OF IN VITRO GLUCOSE UPTAKE, GENE EXPRESSION, AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF NOVEL 2-AMINOBENZIMIDAZOLE DERIVATIVES

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Received: 14 March 2019, Revised and Accepted: 18 June 2019

ABSTRACT

Objective: The present study was aimed to design and evaluate the antidiabetic potential of novel 2-aminobenzimidazole derivatives by in silico method.

Materials and Methods: Various in silico tools such as Chemsketch, Molinspiration, Prediction of activity spectra for substances, OpenBabel, Discovery Studio was used in the designing and evaluation of the biological activity. The retrieved hits were further filtered by absorption, distribution, metabolism, and excretion descriptors. The designed molecules having required physiochemical properties, drug-likeness, and obeying Lipinski’s rule of five were selected for the synthesis. The synthesized compounds were subjected to determination of yield, melting point and characterized by infrared, HNMR, 13CNMR, and mass spectroscopic methods. The selected derivatives were subjected to in vitro glucose uptake, 50% lethal dose (LD50) determination, gene expression analysis, and α-glucosidase inhibitory assay.

Results: Totally, 32 novel analogs of 2-aminobenzimidazole were designed and 17 compounds were selected for docking analysis; and finally, five derivatives (3a, 3c, 3e, 3f, and 3h) were selected for synthesis. Among them, the compounds 3a and 3f were selected for in vitro glucose uptake analysis. Finally, the compound 3f was selected for LD50 determination, gene expression analysis, and α-glucosidase inhibitory assay. The selected derivative 3f showed a significant α-glucosidase inhibitory activity compared with the standard drug acarbose.

Conclusion: These results are useful for further investigation in the future, and hopefully, these studies could discover a new specific lead in antidiabetic category as α-glucosidase inhibitor.

Keywords: In silico design, 2-aminobenzimidazole derivatives, Antidiabetic activity, α-glucosidase inhibitor.

INTRODUCTION

Globally, diabetes is one of the major health problems, and it is one of the top five leading causes of death in developed countries. A report of the World Health Organization predicted that the diabetes become the 7th leading cause of death worldwide by the year 2030 [1-3].

Defects in secretion and sensitivity of insulin lead to type 2 diabetes mellitus. Some receptors and enzymes also have a significant role in the development of diabetes. Peroxisome proliferator-activated receptor (PPARγ), glycosidases, and dipeptidyl peptidase IV (DPP4) are few among them. PPARγ acts as one of the regulators of the metabolism of glucose and lipids. Glycosidases are a group of enzymes leads to the hydrolysis of glycosidic bonds in complex carbohydrates. DPP4 is a serine protease that brings rapid cleaving and inactivation of the incretin glucagon-like peptide 1 (GLP-1) in the blood. The inhibition of DPP4 leads to the increase of circulating GLP-1, thus causing an increase of insulin secretion and therefore can control the blood glucose level effectively. Recently, α-glucosidase, PPARγ, and DPP4 received attention as potential targets for the development of antidiabetic drugs. Importantly, the 2-aminobenzimidazole agents exhibit antidiabetic activity [4-6]. Nowadays, in silico molecular modification studies are one of the important preliminary steps in the rational designing of novel drugs [7-11].

Our research directed toward the in silico design, synthesis and characterization of some new 2-aminobenzimidazole derivatives and investigates their antidiabetic potential by in vitro glucose uptake, gene expression analysis, and α-glucosidase inhibitory assay, an attempt to provide a direction for further research.

MATERIALS AND METHODS

In silico molecular modification

In the present study, different proposed 2-aminobenzimidazole derivatives were subjected to in silico evaluation using different softwares. The three-dimensional (3D) drawing of the proposed compounds was done using ACD Lab Chemsketch 12.0 and Marvin Sketch. Lipinski’s rule of five and drug-likeness properties such as G protein-coupled receptors ligand score, ion channel modulator, kinase inhibitor, nuclear receptor ligand protease inhibitor, and enzyme inhibitor scores were analyzed by Molinspiration Cheminformatic software. The general biological activity of the proposed derivatives was screened by prediction of activity spectra for substances (PASS) software. Three targets, α-glucosidase, PPARγ, and DPP4 were selected for docking study (Table 1). Miglitol, rosiglitazone, and sitagliptin were used as standards.

For docking, the structure of targets and ligands was converted into protein data bank format using OPENBABEL program. Molecular docking of proposed molecules was done by Discovery Studio Ligand Binding Program. The affinity of selected compounds with protein target of interest was analyzed by prediction of activity spectra for substances (PASS) software.
metabolism, and excretion (ADME) descriptors. Various parameters, such as aqueous solubility, blood-brain barrier penetration, absorption level, hepatotoxicity, and ALogP scores were evaluated. Five 2-amino benzimidazole derivatives were selected for synthesis with the help of in silico evaluation. They are, 1. \([2-(1H\text{-benzimidazol-2-ylamino})-2\text{-oxoethyl} \text{amino}]\text{acetic acid. (3a)}\]
2. \(4-[2-(1H\text{-benzimidazol-2-ylamino})-2\text{-oxoethyl} \text{amino}]\text{benzoic acid. (3c)}\]
3. \(N-(1H\text{-benzimidazol-2-yl})-2\text{-}(2\text{-phenylhydrazinyl}) \text{acetamide. (3e)}\]
4. \(N-(1H\text{-benzimidazol-2-yl})-2\text{-}(2\text{-pyridin-2-ylamino}) \text{acetamide. (3f)}\]
5. \(N-(1H\text{-benzimidazol-2-yl})-2\text{-}(2\text{-pyridin-4-ylcarbonyl} \text{) hydrazinyl} \text{acetamide. (3h)}\)

**Synthesis of selected derivatives by the conventional method**

**Step-1: Synthesis of 2-amino benzimidazole**

O-phenylenediamine (0.4 mole) was stirred with water (400 ml) in an ice bath and cyanogens bromide (0.33 mole) was added drop wise during 1 h. The mixture was stirred for 3 h, filtered and made basic with aqueous sodium hydroxide, the base being precipitated and finally with water. Yield and melting point of the product were recorded. The melting point was determined by Digital auto-melting point apparatus (Labtronics, India).

**Step-2: Synthesis of N-(1H-benzimidazol-2-yl) 2-chloroacetamide**

Chloroacetyl chloride (6 mole) was slowly added to the solution of the compound obtained in step 1 (3 mole) in dry benzene which kept at 0–5°C. The reaction mixture was refluxed for 6 h, and the excess solvent was removed under vacuum. The residue obtained was washed with 5% sodium bicarbonate and then recrystallized from ethanol. Yield and melting point of the product were recorded.

**Step-3: Synthesis of 2-amino benzimidazole derivatives**

\(N-(1H\text{-benzimidazol-2-yl})\text{2-Chloroacetamide (0.001 mole) was dissolved in ethanol (20 ml). To these different compounds, containing amino group (0.001 mole) and 5 ml of pyridine were added and heated under reflux for 6 h. After that, excess ethanol was recovered by distillation. The residue obtained was washed with sodium bicarbonate to remove the acid impurities and finally with water. The product thus obtained was recrystallized from ethanol. Yield and melting point of the product were recorded.}

For the synthesis of compound 3a, glycine, the compound containing amino group is used in the step 3. Similarly, phenyldrazine and 2-amino pyridine were used for the synthesis of 3c and 3e, respectively. Para-aminobenzoic acid and isoniazid were used for the synthesis of 3f and 3h, respectively.

**Characterization of synthesized compounds**

**Infrared (IR) spectrum**

IR spectra were recorded using KBr pellets in the range of 4000–5000 cm\(^{-1}\) on Jasco Fourier transform IR Model 600 Type A to elucidate the structure of the compounds.

**\(^1\)H NMR spectrum**

\(^1\)H NMR spectra were recorded in D,O. Chemical shifts were recorded in parts per million with reference to internal standard Tetra Methyl Silane on JEOLECSX500 NMR.

**\(^13\)C NMR spectrum**

\(^13\)C NMR spectra of synthesized compounds were scanned with JEOLECSX500. Chemical shifts were reported in ppm (δ).

**Mass spectrum**

Mass spectra were recorded with Thermo Exactive Orbitrap. The absorbance of reaction mixture was recorded by Bio Tek microplate reader.

**Determination of in-vitro glucose uptake**

Based on the in silico evaluation, two derivatives 3a and 3f were selected for the in vitro glucose uptake evaluation. For this evaluation, L6 rat myoblast cell line was purchased from the National Centre for Cell Science, Pune, Maharashtra, India, and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO\(_2\) in a humidified atmosphere at CO\(_2\) incubator. After attaining the confluence, the cells were trypsinized (500 ml trypsin 0.025% in phosphate buffered saline/0.5 mM ethylenediaminetetraacetic acid [EDTA] solution) for 2 min and passage to T flasks in complete aseptic conditions.

The cells were then sub-cultured in 24 well plates. After attaining 80% confluence, cells were kept in DMEM without glucose for 24 h. Then, the selected derivatives 3a and 3f, in the concentration of 25 μg, 50 μg, and 100 μg/ml were added individually and incubated for the next 24 h in DMEM containing 300 mM glucose. An untreated control with high glucose was also maintained. After incubation cells were isolated by spinning at 6000 rpm for 10 min. The supernatant was discarded, and 200 μl of cell lysis buffer was added. The incubation was done for 30 min at 4°C and the glucose uptake was estimated by glucose oxidase kit method [12,13]. All experiments were repeated in triplicates, and mean average was used for calculation using the following formula.

\[
\text{% Glucose Uptake} = \frac{(\text{Glucose (g/dl) of test} - \text{Glucose (g/dl) of control})}{\text{Glucose (g/dl) of control}} \times 100
\]

Based on the results of in vitro glucose uptake, the compound 3f was selected for the 50% lethal dose (LD\(_{50}\)) determination, gene expression analysis, and α-glucosidase inhibitory assay. LD\(_{50}\) of the selected compound was determined using ED\(_{50}\) plus software.

**Gene expression analysis**

**Isolation of total RNA (TRizol method)**

Total RNA was isolated using total RNA isolation kit by following the manufacturer instruction. 70% confluent cells in 6-well plate (approximately 4×10\(^6\) cells) were treated with the synthesized benzimidazole derivative 3f (38.502 μg/ml) and incubated for 24 h in a CO\(_2\) incubator with untreated control. After incubation DMEM was removed aseptically and 200 μl of TRizol reagent was added to the culture well plate and incubated for 5 min. The contents were then transferred to a fresh sterile Eppendorf tube. 200 μl of chloroform was added and subjected to vigorous shaking for 15 s and incubated for 2–3 min at room temperature, followed by centrifugation at 14000 rpm for 15 min at 4°C. The aqueous layer was collected, and 500 μl of 100% isopropanol was added to it and incubated for 10 min at room temperature and again centrifuged at 14000 rpm for 15 min at 4°C. Supernatant was discarded, and pellet thus obtained was washed with 200 μl of 75% of ethanol. It was then centrifuged at 14000 rpm for 5 min at 4°C in a cooling centrifuge. The RNA pellet was dried and suspended in Tris base EDTA buffer (TE buffer).

**Reverse transcriptase polymerase chain reaction (PCR) analysis**

The cDNA synthesis was performed using Thermo scientific verso cDNA synthesis kit. About 4 μl of 5× cDNA synthesis buffer, 2 μl of deoxynucleotide triphosphate mix, 1 μl of anchored oligo-deoxycytidine, 1 μl of reverse transcriptase enhancer, 1 μl of Verso enzyme mix and 5 μl of RNAtemplate (1 g of total RNA) were added to an RNAase-free tube and made the volume up to 20 μl with the addition of

### Table 1: Targets selected for docking studies

| Protein          | PDB ID | Classification | Resolution | Chain |
|------------------|--------|----------------|------------|-------|
| α-glucosidase    | 3L4T   | Hydrolase      | 1.9Å       | A     |
| PPARγ            | 4HEE   | Transcription inhibitors | 2.5Å | X     |
| DPP4             | 4CDC   | Hydrolase      | 2.4Å       | A, D, G, J |
sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling conditions were employed, 30 min at 42°C and 2 min at 95°C. The amplification process was done using the Thermo Scientific Amplification kit which was illustrated in Table 2.

Separation and visualization

After the amplification, the DNA fragments were separated and visualized by Agarose gel electrophoresis and gel documentation system (E-Gel imager).

α-glucosidase inhibitory assay

This evaluation was employed to screen the α-glucosidase inhibitory effect of the synthesized compounds. 1 mg protein equivalent to 10 units of α-glucosidase was inoculated with different concentrations of the sample and incubated for 5 min. To this added 37 mM of sucrose, 1 ml of 0.1 M phosphate buffer (pH 7.2). The reaction mixture was incubated for 20–30 min at 37°C and kept in boiling water bath for 2 min. A tube with phosphate buffer and enzyme was maintained as control. The tubes were added with 250 μl of glucose reagent (Glucose oxidase kit Erba) and incubated for 10 min and followed by measuring absorbance at 540 nm and measured by a microplate reader. Acarbose was used as the standard drug and compare the values. Alpha-glucosidase inhibitor activity was expressed as percentage of inhibition and was calculated as

\[
\text{% inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

RESULTS AND DISCUSSION

In the present study, in silico molecular modifications of proposed derivatives were done by using different softwares. Totally, 32 novel 2-amino benzimidazole derivatives were designed. ACD Lab Chemsketch 12.0 and Marvin Sketch were used for the structural designing of proposed derivatives. The structure of few proposed compounds is shown in Table 3.

Physicochemical properties which facilitate the membrane permeability, absorbability, and lipophilicity of the ligands were analyzed based on Lipinski’s rule of five by Molinspiration software. According to Lipinski’s rule, the ligand should have molecular weight <500, hydrogen bond donor <5, hydrogen bond acceptor <10, and the LogP value below 5 [14]. All the selected ligands obeyed the parameters of Lipinski’s rule which means the selected ligands may have good absorbability. The results of the evaluation of drug-likeness properties and Lipinski’s rule of five scores of proposed compounds are shown in Tables 4 and 5, respectively.

The PASS software was used to predict the general biological activities of proposed molecules. The result of prediction is presented as the list of activities with appropriate Pa (Probability to be active) and Pi (Probability to be inactive) sorted in descending order of the difference (Pa-Pi)>0. Pa and Pi are the estimates of probability for the compound to be active or inactive, respectively, for each type of activity from the biological activity spectrum. Their values vary from 0.000 to 1.000.

If Pa>0.7, the compound is very likely to reveal this activity in experiments, but in this case, the chance of being the analog of the known pharmaceutical agents for this compound is also high.

If 0.5<Pa<0.7, the compound is likely to reveal its activity in experiments, but this probability is less, and the compound is not so similar to the known pharmaceutical agents.

If Pa<0.5, the compound is unlikely to reveal its activity in experiments, but if the presence of this activity is confirmed in the compound, it might be a new chemical entity.

Based on the scores of drug-likeness properties, Lipinski’s rule of five analysis, and scores of PASS evaluation, 17 compounds were selected for docking analysis. The best docking possibility was interpreted from the data table (Tables 6-8). The docking visualizations were shown in Fig. 1.

From the results of docking studies, it was found that five-hit compounds named as 3a, 3c, 3e, 3f, 3h had shown good binding energy and good hydrogen bonding interactions with the receptors such as 3L4T, 4HEE, and 4CDC which is shown in Table 9.

The same five-hit compounds showed significant positive results in the evaluation of ADME descriptors which were selected for the wet laboratory synthesis by conventional method through a series of three steps. The general scheme for the synthesis is presented in Fig 2.

2-amino benzimidazole formed in the step 1 of the synthesis showed 70% total yield and 228°C as melting point. Its characterization revealed IR (KBr, cm⁻¹): 3178 and 3060 (NH-NH₂), 1168 and 1568 (NH bend), 1269 and 1313 (C-N); 1H NMR (500MHz, D₂O) δ (ppm): 5.6 (2H,NH), 125 MHz δ:154.63, 6.7 (1H,NH), 7.10–7.28 (4H,Ar-H); 13C NMR (D₂O) 125 MHz 6:154.63, 138.48, 134.89, 122.67, 116.29, 118.54, 145.89. High resolution mass spectrometry calculated for C₇H₇N₂: M 133.1541 found 133.1592.

In step 2, synthesis of N-(1-H-benzoimidazole-2-yl) 2-chloroacetamide gave 85% total yield and showed the melting point in the range of

![Fig. 1: Docking visualizations of ligands and standards](image-url)
### Table 3: Structure of few proposed compounds

| S. No. | Structure | Name |
|--------|-----------|------|
| 1      | ![Structure 1](image1) | \([2-(1H-benzimidazol-2-ylamino)-2-oxoethyl] amine\) acetic acid |
| 2      | ![Structure 2](image2) | \(N-(1H-benzimidazol-2-yl)-2-[(benzylcarbamoyl) amino] acetamide\) |
| 3      | ![Structure 3](image3) | \(4-\{[2-(1H-benzimidazol-2-ylamino)-2-oxoethyl]amino\} benzolic acid\) |
| 4      | ![Structure 4](image4) | \(4-\{[2-(1H-benzimidazol-2-ylamino)-2-oxoethyl]amino\}2-hydroxybenzoic acid\) |
| 5      | ![Structure 5](image5) | \(N-(1H-benzimidazol-2-yl)-2-(2-phenylhydrazinyl) acetamide\) |
| 6      | ![Structure 6](image6) | \(N-(1H-benzimidazol-2-yl)-2-(pyridin-2-ylamino) acetamide\) |
| 7      | ![Structure 7](image7) | \(N-[2-(1H-benzimidazol-2-ylamino)-2-oxoethyl] benzamide\) |
| 8      | ![Structure 8](image8) | \(N-(1H-benzimidazol-2-yl)-2-[2-(pyridin-4-ylcarbonyl) hydrazinyl] acetamide\) |

(Contd...)
### Table 3: Continued

| S. No. | Structure | Name | Non-proprietary name |
|--------|-----------|------|----------------------|
| 9      | ![Structure](image) | N-(1H-benzimidazol-2-yl)-2-(piperidin-3-ylamino) acetamide |
| 10     | ![Structure](image) | N-(1H-benzimidazol-2-yl)-2-(pyrimidin-4-ylamino) acetamide |

### Table 4: Drug-likeness properties of selected compounds

| S. No. | Compound code | GPCR ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|--------|---------------|-------------|-----------------------|-----------------|------------------------|------------------|-----------------|
| 1      | 3a            | 0.12        | 0.06                  | -0.02           | -0.66                  | 0.00             | 0.09            |
| 2      | 3b            | 0.19        | 0.01                  | 0.18            | -0.55                  | 0.10             | -0.03           |
| 3      | 3c            | 0.07        | -0.05                 | 0.14            | 0.14                   | -0.06            | 0.02            |
| 4      | 3d            | 0.09        | -0.04                 | 0.19            | 0.13                   | -0.05            | 0.05            |
| 5      | 3e            | -0.08       | -0.13                 | 0.13            | 0.13                   | -0.24            | -0.10           |
| 6      | 3f            | 0.14        | 0.03                  | 0.36            | 0.13                   | -0.10            | 0.08            |
| 7      | 3g            | 0.18        | 0.00                  | 0.28            | -0.71                  | -0.01            | 0.02            |
| 8      | 3h            | 0.03        | -0.26                 | 0.23            | -0.84                  | -0.16            | -0.09           |
| 9      | 3i            | 0.36        | 0.18                  | 0.25            | -0.77                  | 0.18             | 0.05            |
| 10     | 3j            | 0.26        | 0.11                  | 0.62            | -0.87                  | -0.09            | 0.25            |

GPCR: G protein-coupled receptor

### Table 5: Lipinski’s rule analysis of selected compounds

| Compound | M.W | n. HDO | n. HDA | Log p | n. violation |
|----------|-----|--------|--------|-------|-------------|
| 3a       | 248.24 | 4 | 7 | -0.94 | 0 |
| 3b       | 310.31 | 4 | 7 | 2.69 | 0 |
| 3c       | 326.31 | 5 | 8 | 2.69 | 0 |
| 3d       | 281.32 | 6 | 6 | 2.54 | 0 |
| 3e       | 267.29 | 3 | 6 | 1.88 | 0 |
| 3f       | 259.30 | 3 | 7 | 0.88 | 0 |
| 3g       | 310.34 | 4 | 8 | 0.58 | 0 |
| 3h       | 273.34 | 4 | 6 | 0.91 | 0 |
| 3i       | 268.28 | 3 | 7 | 0.98 | 0 |

M.W: Molecular weight, n. HDO: Number of hydrogen bond donor, n. HDA: Number of hydrogen acceptor

### Table 6: -CDocker energy of selected compounds and standard drug with 3L4T

| Ligand | PDB ID | -CDocker energy of ligand | -CDocker energy of standard (Miglitol) |
|--------|--------|---------------------------|---------------------------------------|
| 3a     | 3L4T   | -15.5652                  | 13.7435                                |
| 3b     |        | -12.0385                  |                                        |
| 3c     |        | -10.2408                  |                                        |
| 3d     |        | -27.2196                  |                                        |
| 3e     |        | -13.992                   |                                        |
| 3f     |        | -9.3277                   |                                        |
| 3g     |        | -13.992                   |                                        |
| 3h     |        | -9.3277                   |                                        |
| 3i     |        | -10.0413                  |                                        |
| 3j     |        | -11.1379                  |                                        |

PDB: Protein data bank, -CDocker energy: -Protein-ligand interaction energy

In the step 3, synthesis of the 2-aminobenzimidazole derivative, (2-[2-(1H-benzimidazol-2-ylamino)-2-oxoethyl] amino) acetic acid (3a) showed 75% total yield and melting point in the range of 173–175°C. Its characterization data revealed that IR (KBrυcm⁻¹): 3377 (OH), 1557 (C=O amide), 1463 (hetero aromatic), 662 (aromatic) 821 (NH); ¹HNMR (500MHz,D₂O) δ (ppm): 7.03–7.218 (4H,Ar-H) 9.15 (1H NH) 3.22 (2H CH₂) 3.5 (2H CH₂) 5.0 (1H Ar NH); ¹³CNMR (D₂O) 125 MHzδ: 145.82, 136.8 (C4,C5), 121.02 (C7,C8), 115.2 (C6,C9), 65.5, 45.59, 50.8, 170.83; high resolution mass spectroscopy calculated for C₁₁H₁₄N₄O₃ 248.238 found 248.4179.

The 2-amino-5-benzimidazol-2-yl(2-aminobenzimidazol-2-ylamino)-2-oxoethyl] amino) benzoic acid gave 70% total yield and it melting point is 188°C. The characterization data revealed that IR (KBrυcm⁻¹): 3377 (OH), 1557 (C=O amide), 1463 (hetero aromatic), 662 (aromatic) 821 (NH); ¹HNMR (500MHz,D₂O) δ (ppm): 7.020–8.473 (8H,Ar-H) 8.640 (1H NH) 3.0 (2H CH₂) 5.6 (1H NH) 4.8 (1H Ar NH); ¹³CNMR (D₂O) 125 MHzδ: 149.70, 130.82 (C16 C18), 126.20 (C7 C8), 115.04 (C6 C9), 120.90, 164.98, 145.72, 112.22 (C15 C19), 40.25, 175.65, 135.6 (C4 C5); high resolution mass spectroscopy calculated for C₆₃H₆₄N₂O₂ 310.307 found 309.915.

The total yield of 2-aminobenzimidazole derivative (3e), N-(1H-benzimidazol-2-yl)-2-[2-(phenylhydrazinyl) acetamide - 75%;
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melting point - 183°C; IR(KBrυcm⁻¹): 3426 (NH), 1689 (C=O), 1452 (heteroaromatic);
HNMR (500MHz,D₂O) (ppm): 8.345 (1HNHCO), 7.7657.021 (9H,ArH), 5.203 (1HNH) 4.7 (1HNH) 3.82 (2HCH₂);

CNMR: (D₂O); 168.11, 150.00, 141.50, 136.50, 129.00, 129.01, 115.00, 115.04, 122.22, 55.20. High resolution mass spectroscopy calculated for C₁₅H₁₅N₅O 281.312 found 282.417.

Fig. 2: General scheme for the synthesis of 2-aminobenzimidazole derivatives. 3a: R=C₁₁H₁₂N₄O₃; 3c: R=C₁₆H₁₄N₄O₃; 3e: R=C₁₅H₁₅N₅O; 3f: R=C₁₄H₁₃N₅O; 3h: R=C₁₅H₁₄N₆O₂

Table 7: -CDocker energy of selected compounds and standard drug with 4HEE

| Ligand | PDB ID | -CDocker energy of ligand | -CDocker energy of standard (Rosiglitazone) |
|--------|--------|---------------------------|---------------------------------------------|
| 3a     | 4HEE   | −31.4344                  | −23.7593                                    |
| 3b     |        | −29.4891                  |                                             |
| 3c     |        | −23.1043                  |                                             |
| 3d     |        | −42.2766                  |                                             |
| 3e     |        | −31.4054                  |                                             |
| 3f     |        | −27.0464                  |                                             |
| 3g     |        | −25.1519                  |                                             |
| 3i     |        | −33.1826                  |                                             |
| 3j     |        | −14.4648                  |                                             |

Table 8: -CDocker energy of selected compounds and standard drug with 4CDC

| Ligand | PDB ID | -CDocker energy of ligand | -CDocker energy of standard (Sitagliptin) |
|--------|--------|---------------------------|------------------------------------------|
| 3a     | 4CDC   | −31.7986                  | −25.1509                                 |
| 3b     |        | −30.9102                  |                                           |
| 3c     |        | −30.645                   |                                           |
| 3d     |        | −49.5603                  |                                           |
| 3e     |        | −25.7454                  |                                           |
| 3f     |        | −29.5999                  |                                           |
| 3g     |        | −28.0454                  |                                           |
| 3h     |        | −37.0777                  |                                           |
| 3i     |        | −35.4451                  |                                           |
| 3j     |        | −27.6802                  |                                           |

PDB: Protein data bank, -CDocker energy: -Protein-ligand interaction energy

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The total yield of 2-aminobenzimidazole derivative (3f), N-[1H-benzimidazol-2-yl]-2-(pyridin-2-ylamino) acetamide - 89%: melting point - 180°C; IR (KBr cm⁻¹): 3442 (NH), 1638 (C=O), 846 (aromatic); 1H NMR: (D₂O) (ppm): 8.601 (1HNHCO), 8.5347.621 (8H,ArH), 5.033 (2HCH₃) 1.80 (1HNH); 13C NMR: (D₂O) 165.25, 154.50, 148.10, 145.75, 138.00, 136.10, 125.00, 118.02, 115.50, 106.00, 55.20. High-resolution mass spectroscopy calculated for C₁₁₃H₇₉N₂O₂ 267.285 found 267.987.

The total yield of 2-aminobenzimidazole derivative (3h), N-[1H-benzimidazol-2-yl]-2-(pyridin-4-ylcarboxy) hydrazinylacetamide - 80%: Melting point - 185°C; IR (KBr cm⁻¹): 3389 (NH), 1601 (C=O), 1454 (pyridine) 844 (aromatic); 1H NMR: (500MHz, D₂O) (ppm): 8.654 (1HNHCO), 8.5347.621 (8H,ArH), 5.033 (2HCH₃) 1.80 (1HNH); 13C NMR: (D₂O) 173.44, 168.56, 164.56, 149.21, 149.14, 145.75, 145.13, 138.00, 127.83, 122.97, 121.73, 120.89, 110.52, 55.21. High-resolution mass spectroscopy calculated for C₁₁₃H₇₉N₂O₂ 310.310 found 311.167.

Regarding with the in-vitro glucose uptake evaluation, the percentage of glucose uptake of test agents is shown in Table 10. From this, it was found that the selected derivative 3f showed maximum glucose uptake. In the LD₅₀ determination using ED₅₀ Plus software, the synthesized derivative 3f showed 38.502 µg/ml as the LD₅₀ value. In the gene expression analysis, fluorescence produced by the GLUT4 in control and sample was visualized and showed high glucose uptake which clearly indicated the presence of GLUT4 in the cDNA and amplified. GLUT4 is a gene which is responsible for the glucose transporter type 4 (GLUT4) in control and sample was visualized and compared which revealed that the fluorescence produced in control is less than the sample 3f. The fluorescence by GAPDH in control and sample was same which revealed that the presence of GLUT4 in the sample is more than that of control. It indicated that the forward and reverse primer which added was attached on the GLUT4 in the cDNA and amplified. GLUT4 is a gene which is responsible for the glucose uptake. From the PCR analysis, it was found that the sample 3f showed high glucose uptake which clearly indicated the presence of GLUT4, which permits the facilitated diffusion of circulating glucose down its concentration gradient into muscle and fat cells. Once within cells glucose rapidly phosphorylated by glucokinase in the liver and hexokinase in the other tissue to form glucose-6-phosphate which then enters glycolysis or polymerized into glycogen. Glucose-6-phosphate cannot diffuse back out of cells, which also serves to maintain the concentration gradient for glucose to passively enter the cells. Glut 4 is a primary glucose transporter. Increase in GLUT4 expression in adipose which allows for increased glucose uptake. α-glucosidase inhibitors prevent the decrease in skeletal muscle GLUT4 transporter. In case of α-glucosidase inhibitor assay, the percentage inhibition of standard drug and synthesized derivative 3f is shown in Table 11.

In this evaluation, it was observed that the α-glucosidase inhibitor activity was increased with increase of concentration and the sample

### Table 9: Active binding sites of selected hit ligands with targets

| Ligand | 3L4T | 4HEE | 4CDC |
|--------|------|------|------|
| 3a     | His50, Cys15, Arg29, Ser47, Val177 | Leu330, Ile326, Arg288, Cys28, Ala292, Met364 | Phen278, Cys234, Asp1, Glu275 |
| 3e     | Arg29, Val177, His50, Ser47, Tyr46 | Leu330, Ile341, Gly284, Arg288, Met364 | Ala349, Cys234, Asp1, Gly232, Phen278 |
| 3f     | Arg29, Cys15, His50, Ser47, Tyr46, Pro137 | Leu330, Ile341, Arg288, Ala292, Met364 | Tyr164, Ala349, Cys234, Pro274, Gly232, Asp1 |
| 3c     | Arg29, His50, Ser47, Val177, Pro158 | Leu330, Ile341, Met329, Cys285, Arg288, Ala292 | Ala349, Cys234, Asp1, Gly232, Cys274 |
| 3h     | Arg29, His50, Cys15, Ser47, Pro158, Tyr46 | Leu330, Ile341, Met329, Cys285, Arg288, Ala292 | Asp1, Gly232, Ala349, Cys273 |

| 3L4T: α-glucosidase, 4HEE: Peroxisome proliferator-activated receptor, 4CDC: Dipeptidyl peptidase-4 |
ACKNOWLEDGMENT

We would like to thank Mr. J. Kumaran, M. Pharm., (Pharmaceutical Biotechnology) for his assistance in the preparation of this manuscript.

AUTHORS’ CONTRIBUTION

S. Sreeja: Carried out the whole experiment, Dr. A. Anton Smith: Design the whole research work, Dr. S. Mathan: carried out the supervision of experiments.

CONFLICTS OF INTERESTS

None.

FUNDING

None.

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