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Synthesis and Biological Evaluation of Benzimidazoles as Target for α-Glucosidase Inhibitors

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ABSTRACT

Diabetes mellitus is rising globally touching more than 180 million people worldwide. This is prevailing mostly in type 2 diabetes and according to WHO report the incidence is likely to be more than doubled by 2030. α-glucosidase inhibitors work by reducing the amount of glucose that the intestines absorb from food. In our previous work, forty-five benzimidazoles analogues were studied using 3D QSAR, HQSAR, and Pharmacophore mapping and based on their results 60 compounds were designed. Docking studies of those designed compounds showed that most of the compounds are bonding with important amino acids LEU 520, ARG 335 and ASP 69 through hydrogen bonds and steric interaction. In this work, synthesis of eleven compounds was done on the basis of molecular docking studies. Compounds containing hydroxyl and alkyl groups (compound no. 3, 9 and 10) were found to be five to eight folds more active with IC_{90} values in the range of 6.02 ± 1.10 to 33.25 ± 1.20 µg/ml, in comparison with the standard drug, Acarbose (IC_{90}= 290.55 ± 0.081 µg/ml). Thus, these compounds after the toxicity studies could be of therapeutic use in treating diabetes.

Keywords: Acarbose, Alpha-glucosidase inhibition, Benzimidazoles, Docking, Molecular modelling, Post-prandial hyperglycemia

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INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder diagnosed by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia. Over 18 years of age the international occurrence of diabetes among adults has ascended from 4.7% in 1980 to 8.5% in 2014 (Global report on diabetes. WHO, Geneva, 2016). In 2030 diabetes would be the seventh leading cause of death as per the World Health Organization (WHO) (Mathers CD, Loncar D, 2006). The effects of diabetes mellitus include long term damage, dysfunction and of various organs. Adult-onset blindness, lower limb amputations and kidney failure are the major outcomes of diabetes (Alberti K, Zimmet Z.1999).

A common and severe difficulty confronted by many people with type 2 diabetes is postprandial hyperglycemia, α-glucosidase inhibitors (AGIs) are well suited for its treatment (Vats Rajesh.2005). Furthermore, α-glucosidase may also be used as therapeutic target for other carbohydrate mediated diseases including cancer, HIV and hepatitis. Alpha-glucosidase is located in the brush border of the small intestine that acts upon 1, 4- alpha bonds. Most of the carbohydrates are present as oligo- or poly- saccharides, and have to be broken down to disaccharides, before sucrose, isomaltase, maltase, glycol-amylase, and lactase break them into digestible mono-saccharides (Kalra S., 2014). AGIs inhibits both alpha amylase and the other α-glucosidases, thus preventing absorption of starch and other carbohydrates from the brush border of the intestine.

Benzo-hydrazides had been reported to possess various biological activities, which includes anti-leishmanial (Taha et al., 2014b), anti-oxidant (Aziz et al., 2014, Khan et al., 2012), anti-glycation (Khan et al., 2013a, Khan et al., 2013b, Khan et al., 2015, Jamil et al., 2015), anti-bacterial (Imran et al., 2014), and α-glucosidase inhibition (Taha et al., 2015f) activities. Benz-imidazole nucleus is an important pharmacophore with unique chemical and biological properties. Benzimidazoles have been found to possess anti-inflammatory (Kohno T, et. al. 1990), anti-diabetic (Bhise UN, et. al. 2008), antispasmodic (Francisco AC, et. al. 2006), diuretics (Pashinski VG, et. al. 1978), analgesic (Kohno T, et. al. 1990), antimicrobial (Durmaz R, et. al. 2003), anti-helminthic (Solominova PS, et. al. 2004), anti-HIV (Gardiner JM, et. al. 1995), antiulcer (Bariwal JB, 2008), anticancer (Gellis A, et. al. 2008), and anticonvulsant activities (Chimrri A, et al. 2001).

Furthermore, recent studies have shown that some benzimidazole derivatives have been identified to exhibit α-glucosidase inhibitory activity. In our work, we have

combined benzohydrazide and benz-imidazole nucleus, and developed SAR based on 3D-QSAR, HQSAR studies and Pharmacophore Mapping. Further from docking results, 11 compounds were synthesized based on their dock scores. The synthesized compounds were tested for their *in vitro* α-glucosidase inhibitory activity.

EXPERIMENTAL

Chemistry

Melting points were determined by Veego model VMP-D melting point apparatus and were uncorrected. Thin layer chromatography was performed on precoated silica gel-G (0.2 mm thick) using different solvent system (petroleum ether: acetone 7:3, n-hexane: ethyl acetate 5:5) to ascertain the purity of the synthesized compounds. UV light and iodine vapors were used as the detecting agents. Infrared spectrum was taken on FT-IR-SP 10 STD at School of Chemical Sciences, Devi Ahilya Vishwavidyalaya, Indore. ^{1}H-NMR and ^{13}C-NMR spectrums were obtained on Bruker DRX-400 (400 MHz FT-NMR) at sophisticated analytical instrument facility, Panjab University, Chandigarh. Mass spectrum were obtained on Water UPLC-TQD (electron ionization spray and ACPI-MS) at sophisticated analytical instrument facility, CDRI, Lucknow.

General Procedure for the Synthesis of Compounds 1-11

Initially 0.001 moles of o-phenylenediamine (for first five compounds) and 2, 3-diamino naphthalene (for next six compounds) was taken into a round bottom flask and to it methyl-4-formyl benzoate (0.001 moles) was added. Ceric ammonium nitrate and hydrogen peroxide in catalytic amount was carefully added to the above mixture. This mixture was carefully assembled over heating mantle maintained at 50 °C. The TLC was taken at the regular interval and it is observed that the completion of reaction took 60 min. The reaction mixture was allowed to dry at room temperature. This intermediate product (IM 1) was taken into next step.

The intermediate was purified and 0.001 mole was taken in a round bottom flask, hydrazine hydrate (10 ml, 95%) was added to it along with 25 ml of ethanol and magnetically stirred for 9 h maintaining a temperature of 120 °C. the completion of reaction is confirmed by TLC. This reaction mixture was allowed to cool at room temperature, to this mixture ice cold water added and filtered. The intermediate formed was collected on Whatmann filter paper, which was further purified and crystallized using n-hexane and methanol. This intermediate (IM 2) was then taken into next step.

To this intermediate different benzaldehydes were added (equimolar) into round bottom flask and to this mixture, glacial acetic acid in catalytic amount was added and to this mixture 1-butanol (25 ml) was added. This mixture was again refluxed for 3 h. The completion of reaction was confirmed by TLC. The obtained mixture was allowed to cool at room temperature; ice cold water was added to the mixture and the obtained product was filtered using Whatmann filter paper and collected product was dried, recrystallized and purified by using n-hexane and ethanol. The obtained product was allowed to air dry (Panda et. al. 2016, Taha et al. 2015, Rostamizadeh S et al.2009, Kiumars Bahrami.2008).

(E)-N'-(4-diethylamino) benzlidene)-4-(1H-benzo[d] imidazol-2-yl)benzohydrazide

Yield 97.83%; M.P. 275-276 °C; FTIR (KBr) 3422 (NH of hydrazide), 3197 (NH of imidazole), 1680 (C=O), 1591, 1567 (NH bend.), 1354, 1311, 1291 (C-N), 1073 (N-N), 861, 835 (p-disubstituted rings); ^{1}H-NMR (400 MHz, DMSO) δ ppm: δ 11.6 ppm (s, 1H, NH), δ 8.34 ppm (s, 1H, CH=N), δ 6.68-8.32 (m, 13H, Ar-H), δ 1.0-1.11 (m, 6H, CH_3), δ 3.33-3.38 (m, 4H, CH_2); ^{13}C-NMRδ 161.97 (C=O), δ 148.98 (C=N), δ 43.69 (CH_2), δ 12.39 (CH_3), δ 150.29 (N-C), δ 111.05-134.59 (Ar-C); MS (ESI): m/z 412.3$(M+H)^+$.

(E)-N'-(4-isopropylbenzlidene)-4-(1H-benzo[d]imidazol-2-yl) benzohydrazide

Yield 80.3%; M.P. 280-282 °C; FTIR (KBr) 3476 (NH of hydrazide), 3221 (NH OF imidazole), 3053 (CH3), 2870 (=CH2 str.), 1682 (C=O), 1632 (NH bend.), 1613 (C=N), 1177 (C-N), 1451-1511 (ring breathing), 1425 (CH2), 1365 (CH3), 824 (p-disubs.), 764-666 (CH bend oop); ^{1}H-NMR (400 MHz, DMSO) δppm:δ 13.1 ppm (s, 1H, NH), δ 11.93 ppm (s, 1H, CH=N),δ 7.22-8.48 (m, 13H, Ar-H), δ 1.18-1.21 (m, 6H, CH_3), δ 2.98 (m, 1H, CH); ^{13}C-δ 162.46 (C=O), δ 150.76 (C=N),δ 33.35 (CH), δ 23.60 (CH_3),δ 111.51-150.25 (Ar-C); MS (ESI): m/z 383.3$(M+H)^+$.

(E)-N'-(4-isopropylbenzlidene)-4-(1H-naphtho [2,3-d]imidazol-2-yl)benzohydrazide

Yield 65.48%; M.P. 275 °C; FTIR (KBr) 3485 (NH of hydrazide), 3211 (NH of imidazole), 3053 (=CH str.), 2961 (-CH3 str.), 1660 (C=O), 1628 (C=N bend.), 1612 (NH bend.), 1173 (C-N str.), 1449-1511 (ring breathing), 1363 (CH3 bend.), 1011-1289 (CH bend. In-plane), 827 (p-disubs. ring), 708-856 (CH bend. oop); ^{1}H-NMR (400 MHz, DMSO) δppm: δ 13.15 ppm (s, 1H, NH), δ 11.97 ppm (s, 1H, CH=N), δ 7.31-8.49 (m, 15H, Ar-H), δ 1.17-1.21 (m, 6H, CH_3), δ 2.87-2.92 (m, 1H, CH); ^{13}C-δ 162.41 (C=O), δ 154.51 (C=N), δ 33.36 (CH), δ 23.60 (CH_3), δ 106.51-154.37 (Ar-C);MS (ESI): m/z 433.3$(M+H)^+$.

(E)-4-(1H-benzo[d]imidazol-2-yl)-N'-((2-hydroxynaphthalen-7-yl)methylene) benzohydrazide

Yield 90.9%; M.P. 308-309 °C; FTIR (KBr) 3253 (NH of hydrazide), 3070 (NH of imidazole), 1693 (C=O), 1623 (C=N), 1595 (NH bend.), 1456-1498 (ring breathing), 1470 (-CH2 bend.), 1324 (C-N str.), 1244 (C-O str.), 1020-1297 (CH bend. In-plane), 814 (p-disubs. ring), 711-940 (CH bend. oop); ^{1}H-NMR (400 MHz, DMSO) δppm: δ 12.82 ppm (s, 1H, NH), δ 9.93 ppm (s, 1H, CH=N), δ 7.53-8.41 (m, 10H, Ar-H), δ 7.2-7.5 (m, 4H, Ar-H), δ 12.32 (m, 1H, OH); ^{13}C-δ 172.01 (C=O), δ 158.07 (C=N), δ 108.5-120.5 (Ar-C), δ 161.87 (OH), δ 122.52-150.18 (Ar-C); MS (ESI): m/z 407.3$(M+H)^+$.

(E)-N'-(3, 5-dimethoxybenzlidene)-4-(1H-benzo[d] imidazol-2-yl) benzohydrazide

Yield 84%; M.P. 282 °C; FTIR (KBr) 3407 (NH of hydrazide), 3087 (NH of imidazole), 1660 (-C=O), 1616 (-C=N), 1587 (NH bend.), 1459-1500 (ring breathing), 1337, 1372 (CH3 bend.), 1305 (C-N str.), 1125-1281 (C-H bend. In-plane), 1113, 1055 (C-O str.), 838 (p-disubs. ring), 855 (p-disubs. ring), 706-891 (C-H bend. oop); ^{1}H-NMR (400 MHz, DMSO) δppm:δ 12.0 ppm (s, 1H, NH), δ 8.4 ppm (s, 1H, CH=N), δ 6.58-8.35 (m, 12H, Ar-H), δ 3.79 (m, 6H, OCH_3); ^{13}C-δ 162.63 (C=O), δ 160.68 (C=N), δ 55.29 (OCH_3), δ 111.51-150.14 (Ar-C); MS (ESI): m/z 401.3$(M+H)^+$.

(E)-N'-(2,5-dimethoxybenzlidene)-4-(1H-naphtho[2,3-d] imidazol-2-yl) benzohydrazide

Yield 91.6%; M.P. 166-168 °C; FTIR (KBr) 3154 (NH of hydrazide), 2827 (NH of imidazole), 1652 (-C=O), 1467-1493 (ring breathing), 1361 (C-N str.), 1220.11, 1199.77 (C-O str.), 1021-1277 (C-H bend. In-plane), 706-894 (C-H bend. oop); ^{1}H-NMR (400 MHz, DMSO) δppm: δ 13.2 ppm (s, 1H, NH), δ 12.05 ppm (s, 1H, CH=N), δ 6.98-8.86 (m, 14H, Ar-H),

δ 3.76-3.82 (m, 6H, OCH_3); ^{13}C-δ 171.99 (C=O), δ 162.30 (C=N), δ55.41-56.19 (OCH_3), δ 109.21-154.37 (Ar-C);MS (ESI): m/z 451.3$(M+H)^+$.

(E)-N'-(3, 4, 5-trimethoxybenzlidene)-4-(1H-naphtho [2, 3-d] imidazol-2-yl) benzohydrazide

Yield 78%; M.P. 167 °C; FTIR (KBr) 3503.31 (NH of hydrazide), 2947 (NH of Imidazole), 1707 (-C=O), 1614 (-C=N), 1587 (NH bend.), 1461- 1551 (ring breathing), 1327 (C-N str.), 1188-1276 (C-H bend. in-plane), 1125, 1108, 1125 (-C-O str.), 827 (p-disubs. ring), 710-855 (C-H bend. oop); ^1H-NMR (400 MHz, DMSO) δppm:δ 13.17 ppm (s, 1H, NH), δ 9.89 ppm (s, 1H, CH=N), δ 7.24-8.44 (m, 13H, Ar-H), δ 3.45 - 3.90 (m, 9H, OCH_3); ^{13}C-δ 165.70 (C=O), δ 154.31 (C=N), δ 52.26 (OCH_3), δ 106.69 -154.07 (Ar-C);MS (ESI): m/z 481.3$(M+H)^+$.

(E)-N'-(2, 4-dimethoxybenzlidene)-4-(1H-naphtho [2, 3-d] imidazol-2 yl) benzohydrazide

Yield 84.8%; M.P. 289-291 °C; FTIR (KBr) 3491.84 (NH for hydrazide), 3203 (NH of imidazole), 3080 and 3049 (-CH3 str.), 1659 (-C=O), 1626 (C=N), 1601 (NH bend.), 1466-1520 (ring breathing),1372 (-CH3 bend.), 1353 and 1311 (C-N str.), 1011-1289 (C-H bend. in-plane), 811 and 855 (p-disubs. ring), 710-786 (C-H bend. oop);^1H-NMR (400 MHz, DMSO) δppm:δ 13.12 ppm (s, 1H, NH), δ 11.84 ppm (s, 1H, CH=N), δ 6.63-8.77 (m, 12H, Ar-H), δ 3.45 - 3.90 (m, 6H, OCH_3); ^{13}C-δ 172.03 (C=O), δ 162.50 (C=N), δ 55.39 (OCH_3), δ 106.39 -159.21 (Ar-C);MS (ESI): m/z 401.3$(M+H)^+$.

(E)-N'-(4-(diethylamino) benzlidene)-4-(1H-naphtho [2, 3-d] imidazol-2-yl) benzohydrazide

Yield 64.20%; M.P. 203-205 °C; FTIR (KBr) 3230.2 (NH of hydrazide), 3090 (NH of Imidazole), 3023 (CH3 str.), 2968, 2941 and 2840 (CH str.), 1653 (C=O), 1631 (C=N), 1615 (NH bend.), 1450-1466 (ring breathing), 1243 (C-O str.), 1013-1277 (CH bend. In-plane), 815, 839 (p-disubs. ring), 735-893 (CH bend. oop); ^1H-NMR (400 MHz, DMSO) δppm: δ 13.06 ppm (s, 1H, NH), δ 11.64 ppm (s, 1H, CH=N), δ 6.58-8.38 (m, 14H, Ar-H), δ 0.96-1.00 (m, 6H, CH_3), δ 3.22-3.37 (m, 4H, CH_2); ^{13}C-δ 161.98 (C=O), δ 149.16 (C=N), δ43.67 (CH_2), δ12.36 (CH_3), δ 154.46 (N-C), δ 106.77-148.90 (Ar-C);MS (ESI): m/z 462.4 $(M+H)^+$.

(E)-N'-(2-hydroxy-3-ethoxybenzlidene)-4-(1H-naphtho[2,3-d]imidazol-2-yl) benzohydrazide

Yield 94.41%, M.P. 200°C; FTIR (KBr) 3221 (NH of hydrazide), 3063 (NH of Imidazole), 1664(C=O), 1611(C=N), 1555 (NH bend.), 1465 and 1491 (ring breathing), 1433 (C-O-H bend.), 1357 (C-N str.), 1019-1308 (CH bend. In-plane), 1130 (C-O str.), 838 (p-disubs. ring), 705- 893 (CH bend. oop); ^1H-NMR (400 MHz, DMSO) δppm: δ 10.28 ppm (s, 1H, NH), δ 7.53-8.98 (m, 10H, Ar-H), δ 4.0 (m, 4H, OCH_2), δ 10.28 (m, 1H, OH), δ 1.3 ppm (m, 6H, CH_3); ^{13}C-δ 192.51 (C=O), δ 166.81 (C=N), δ 64.3 (OCH_2), δ 14.65 (CH_3), δ 118.34-154.03 (Ar-C);MS (ESI): m/z 451.3$(M+H)^+$.

(E)-N'-(2-hydroxy-3-ethoxybenzlidene)-4-(1H-benzo[d]imidazol-2-yl) benzohydrazide

Yield 91.44%; M.P. 272 °C; FTIR (KBr) 3502 (NH of hydrazide), 3053 (NH of imidazole), 2963 (CH3 str.), 2925 (CH str.), 1708 (C=O), 1615 (C=N), 1455-1468 (Ring breathing), 1275 (C-O-H bend.), 1071-1249 (CH in-plane bend.), 1109 (C-O str.), 1089 (C-N str.), 800-850 (p disubs. ring), 711-780 (CH oop bend.);^1H-NMR (400 MHz, DMSO) δppm:δ 13.12 ppm (s, 1H, NH), δ 12.21 ppm (s, 1H, CH=N), δ 7.14-8.36 (m, 6H, Ar-H), δ 4.0 (m, 4H, OCH_2), δ 11.08 (m, 1H, OH), δ 1.3 ppm (m, 6H, CH_3); ^{13}C-δ 162.20 (C=O), δ 150.27 (C=N), δ 14.70 (CH_3), δ 148.87 (OH), 64.13 (CH_2), δ 115.27-147.58 (Ar-C);MS (ESI): m/z 401.3$(M+H)^+$.

Assay for α-Glucosidase Inhibitory Activity

The α-glucosidase inhibition activity was carried out at School of Biochemistry, Devi Ahilya Vishwavidyalaya, Indore, M.P. Potassium dihydrogen phosphate (4.35 g) and 3.55 g of disodium hydrogen phosphate was dissolved in 100 ml distilled water and sufficient water was added to produce 500 ml of phosphate buffer pH 6.8, 50 mM. The substrate (p-Nitrophenyl-α-D-glucopyranoside) solution 0.7 mM was prepared in buffer solution. 0.14 mg of the enzyme α-glucosidase. Test compounds 10 mg, 20 mg, 30 mg, 40 mg and 50 mg were dissolved in the DMSO and further dilutions were made with distilled water. Enzyme (20 µl), 20 µl of test samples and 135 µl of the buffer solution was added to the 96-well plates. The solution was pre-incubated for 15 minutes at 37 °C. Instead of test samples 20 µl of DMSO is added in case of control (Zawawi et al. 2016). Substrate (25) was added and incubated for 30 min. After that the shaking of the 96-well plate is done for 15 s and absorbance was taken at 405 nm. All experiments were triplicated and the results were expressed as the percentage inhibition of three determinations. The enzyme inhibitory rates of samples were calculated as follows:

Percentage inhibition = [(absorbance of control - absorbance of the test sample)/ absorbance of control] × 100

RESULT AND DISCUSSION

Chemistry

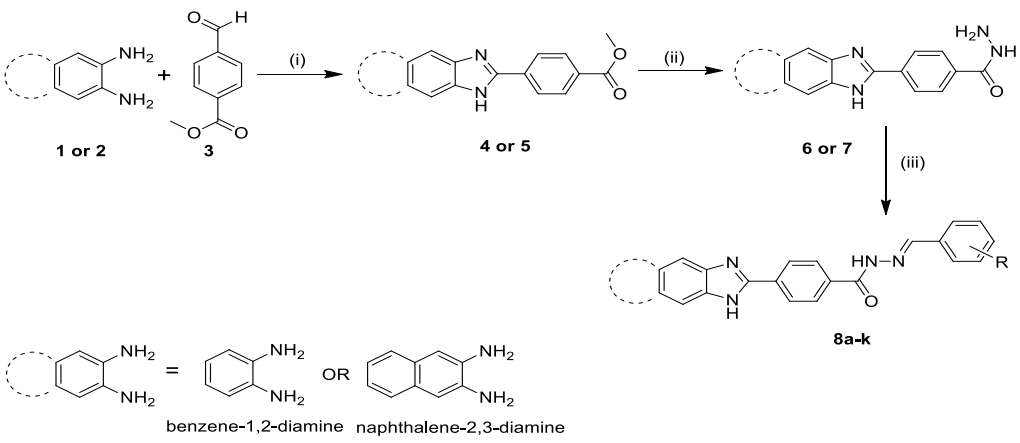


Figure 1: Reaction conditions for synthesis of substituted benzimidazoles: (i) 30% hydrogen peroxide or ceric ammonium nitrate; (ii) hydrazine hydrate; (iii) substituted benzaldehyde.

The designed 11 benzimidazoles having different substitutions were synthesized from good to excellent yields. First intermediate (IM 1) was formed in oxidation reaction using ceric ammonium nitrate, the carbonyl carbon of the aldehyde was attacked by the nitrogen atom of either o-phenylenediamine or 2, 3-diamino naphthalene followed by the removal of the water molecule, ring cyclization and oxidized in presence of ceric ammonium nitrate. In the intermediate so formed nucleophilic addition of the hydrazine moiety took place in the second step after the removal of methanol as good leaving group. This intermediate (IM 2) undergoes the Mannich reaction to form the final product in good yield as shown in Scheme 1. All these compounds (1-11) were characterized by spectroscopic methods (IR, NMR, and MS).

Enzyme Inhibitory Studies

The novel synthesized compounds were evaluated for the α-glucosidase inhibitory activity using commercially available α-glucosidase inhibitor acarbose as positive control. Compounds 1, 3, 4, 6, 7, 9, 10 and 11 showed significant activity as compared with the standard drug acarbose. From these tested compounds, three compounds were found to be most active. Compound 9, 10 and 3 inhibited the enzyme α-glucosidase by 90.84%, 78.67% and 89.96% respectively at the minimum dose concentration that is 10 µg/ml. The standard drug acarbose inhibited 55.95% of the enzyme α-glucosidase at the same concentration.

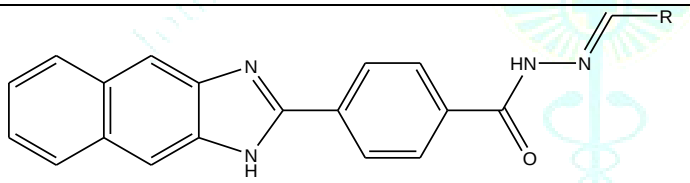
The most active analog among the series is 9 which was having benzyl group at R_1 and 4-diethyl amino group at R_2. The second most active compound is 10 which was having benzyl group at the R_1 and 2-hydroxy-3-ethoxy at R_2. Similarly, the third potent analog was 3 which was having benzyl group at the $R_1$and 4-isopropyl group at R_2. All these substituents resulted in hydrogen bonding with the crucial amino-acids having significant role in the activity.

Based on the percentage inhibition results of these compounds the IC_{90} values were calculated by plotting the graph of the concentration versus the percentage inhibition of the enzymatic activity extrapolating the 90% inhibition to the concentration. It was found that the IC_{90} value of the standard drug acarbose was found to be 290.55 ± 0.081 µg/ml, and that of 9, 3 and 10 it was found at 6.02 ± 1.10 µg/ml, 12.94 ± 1.41 µg/ml and 33.25 ± 1.20 µg/ml.

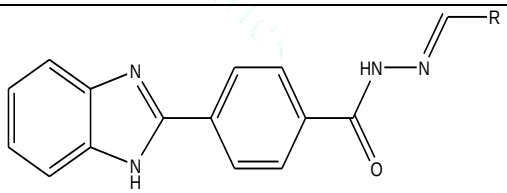
The IC_{90} value of the synthesized compounds were found to be lower than the standard drug acarbose means they will have even less IC_{50} value concluding they could have a considerable inhibitory activity against the standard drug acarbose.

On the basis of the *in vitro* activity of the α-glucosidase inhibition it was found that nearly all compounds were more potent than standard drug acarbose so further these compounds can be proceeded for the toxicity studies and pharmacodynamics studies.

Table 1. α-glucosidase inhibitory activity of benzohydrazide benzimidazoles derivatives (1-11).



3*, 6*, 7*, 8*, 9*, 10*



1, 2, 4, 5, 11

Compound	R	% Inhibition (Mean ± SEM)
1	4-diethyl amino benzylidene	90.58 ± 0.40
2	4-isopropyl benzylidene	82.93 ± 1.24
3*	4-isopropyl benzylidene	95.48 ± 1.10
4	2-hydroxy naphthalene	96.66 ± 0.10
5	3,5-dimethoxy benzylidene	84.56 ± 1.10
6	2,5-dimethoxy benzylidene	84.31 ± 0.60
7*	3,4,5-trimethoxy benzylidene	81.05 ± 2.60
8*	2,4-dimethoxy benzylidene	82.68 ± 0.10
9*	4-diethyl amino benzylidene	98.26 ± 0.65
10*	2-hydroxy-3-ethoxy benzylidene	93.72 ± 0.35
11	2-hydroxy-3-ethoxy benzylidene	86.44 ± 0.42
Standard drug	Acarbose	72.54 ± 0 .81

Molecular Docking

In compound 9, the tertiary amino group was one of the strong electron donating and also contributed in formation of hydrogen bond with amino acid Glu 332 which was found to be very important for the activity.

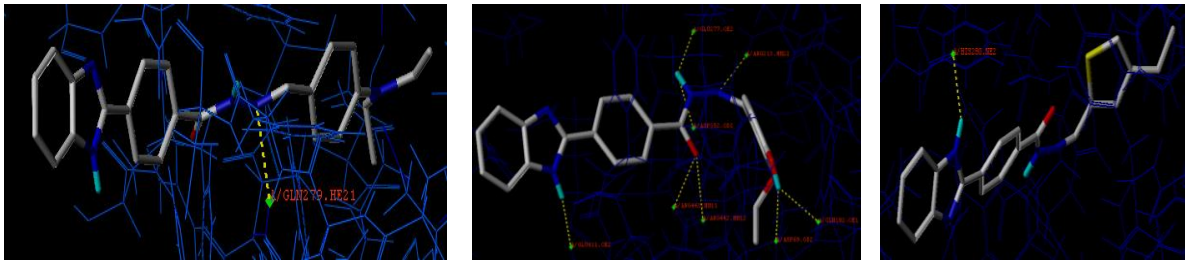


Figure 2. Docking pose of compound 9, 10 and 3 in the active site.

The second most active compound 10 had a benzyl group at R_1 and 2-hydroxy-3-ethoxy at R_2. The hydroxyl and alkoxy groups were individually among the strong electron donating groups and also contribute to the important interactions with the active site of enzyme. Arg 335 was found to be critical for the binding of the substrate at the active site of enzyme and Asp 519 forms hydrogen bond at the distance between 2.7-2.5 A° with the enzyme. Compound 3 had a benzyl group at R_1 and 4-isopropyl at R_2. 4-isopropyl was considered to be weak electron donating group but formed hydrogen bond with the oxygen molecule of the amino acid residue Asp 69. It was observed that the marketed α-glucosidase inhibitors were bulky molecules. The cavity of the active site should be large that might be the reason for the increased activity of the naphtho-imidazoles than benzimidazoles. It can be concluded that the hypotheses of the SAR made from computational studies was found to be correct as per the *in vitro* activity results. A bulky group should be placed at R_1 and stronger electron donating group needs to be substituted at R_2 for enhanced α-glucosidase inhibitory activity.

Table 11: Docking score of designed compounds.

S.N.	R	Total score	Crash	Polar	D score	PMF score	G score	ChemScore	CS score
1.	3,4-OC_2H_5	3.81	-6.24	2.44	-193.62	-152.33	-241.75	-39.03	1
2.	3-ethoxy-4-hydroxy	4.40	-2.45	4.24	-146.21	-158.65	-209.26	-33.30	1
3.	4-dimethyl amino	2.77	-1.28	0.93	-134.45	-61.370	-188.77	-30.48	1
4.	4-diethyl amino	5.31	-2.59	0.01	-183.44	-110.25	-260.77	32.54	2
5.	4-ethyl	4.20	-2.10	0.70	-143.07	-114.35	-255.61	-28.42	0
6.	2,5-dimethoxy	5.53	-2.34	1.54	-172.44	-159.95	-176.77	-31.97	3
7.	2,4-dimethoxy	4.63	-5.02	1.64	-199.71	-152.83	-270.99	-39.55	1
8.	2-ethoxy-4-hydroxy	5.53	-4.12	2.71	-196.27	-153.80	-237.78	-43.27	1
9.	2-hydroxy-3-methoxy	5.10	-5.26	4.19	-191.03	-160.26	-295.48	-42.86	0
10.	3-ethoxy-2-hydroxy	5.03	-4.32	1.22	-207.77	-160.03	-315.82	-35.14	0
11.	3,4,5- trimethoxy	5.47	-2.57	2.01	-190.93	-77.444	-247.700	-34.3814	0
12.	4-isopropyl	5.78	-2.27	0.001	-165.30	-103.38	-256.098	-31.4411	1
13.	4-acetamido	3.89	-1.53	1.49	-143.93	-107.27	-196.830	-27.3129	2
14.	2,6- dimethoxy	2.41	-2.80	0.0001	-180.18	-135.46	-267.256	-33.3888	1
15.	4-hydroxy-2-methoxy	4.51	-5.71	3.2449	-193.29	-150.07	-271.467	-42.2919	0
16.	2-hydroxy-6-methoxy	4.67	-4.21	1.5344	-202.13	-140.97	-288.026	-34.6364	1
17.	2,3-dimethoxy	4.99	-1.79	1.08	-158.54	-119.97	-199.31	-30.44	0
18.	2,3,4- trimethoxy	3.05	-6.25	1.46	-208.62	-176.35	-292.61	-35.25	3
19.	3,5-dimethoxy	6.81	-5.34	-2.73	-213.70	-169.8	-299.4	-44.16	1
20.	2,4,6- trimethoxy	3.95	-5.32	6.65	-175.80	-182.48	-292.39	-43.23	3

21.	1-methoxy naphthalene	2.30	-4.59	1.09	-163.26	-84.639	-227.51	-36.62	1
22.	2-methoxy naphthalene	2.93	-4.06	0.02	-235.77	-56.110	-301.99	-59.72	2
23.	2-ethoxy naphthalene	2.40	-4.15	1.79	-184.36	-85.596	-269.44	-43.11	2
24.	2-hydroxy naphthalene	4.48	-3.87	1.21	-208.84	-6.0921	-327.25	-53.92	1
25.	1, 4-dimethoxy naphthalene	3.03	-4.20	0.91	-197.77	-116.78	-253.07	-40.56	2
26.	1-indol-2-yl	3.82	-7.31	4.04	-185.33	-138.15	-275.25	-45.22	2
27.	4-ethylthiophen-2-yl	4.41	-3.77	1.87	-181.85	-8.7379	-254.40	-44.54	1
28.	Quinolin-3-yl	4.87	-1.75	0.04	-165.85	-114.18	-240.56	-31.44	2
29.	2-hydroxy-4-methoxy	4.87	-2.36	3.59	-169.39	-181.02	-227.96	-35.82	0
30.	3,4,5- trimethoxy	3.22	-3.00	0.03	-174.48	-106.45	-212.32	-25.58	0
31.*	3,4-OC_2H_5	5.97	-3.01	0.06	-189.57	-146.58	-247.28	-29.09	3
32.*	3-ethoxy-4-hydroxy	4.18	-5.25	3.85	-173.60	-165.93	-243.86	-37.08	1
33.*	4-dimethyl amino	5.25	-3.37	0	-152.04	-116.51	-270.76	-31.62	2
34.*	4-diethyl amino	6.69	-2.43	0.19	-178.50	-115.03	-252.62	-29.47	4
35.*	4-ethyl	4.87	-0.82	0.28	-143.55	-121.10	-219.30	-30.15	0
36.*	2,5-dimethoxy	4.64	-3.74	0.92	-179.33	-147.07	-200.01	-29.57	3
37.*	2,4-dimethoxy	5.34	-4.03	2.47	-186.16	-153.46	-219.65	-37.37	3
38.*	2-ethoxy-4-hydroxy	4.76	-5.64	4.46	-186.19	-173.36	-307.90	-40.80	2
39.*	2-hydroxy-3-methoxy	5.07	-2.91	1.93	-176.96	-160.67	-243.93	-33.93	0
40.*	3-ethoxy-2-hydroxy	4.47	-4.52	1.18	-191.29	-156.10	-304.55	-30.38	2
41.*	3,4,5- trimethoxy	5.15	-4.94	3.13	-181.05	-95.176	-276.14	-40.44	2
42.*	4-isopropyl	5.88	-1.60	0.01	-162.37	-99.421	-268.22	-30.47	2
43.*	4-acetamido	3.86	-1.64	0.089	-156.27	-125.77	-229.04	-28.04	4
44.*	2,5- dimethoxy	6.08	-1.13	1.24	-147.49	-136.61	-174.16	-32.08	4
45.*	4-hydroxy-2-methoxy	5.98	-2.76	1.42	-175.23	-157.46	-274.95	-31.85	1
46.*	2-hydroxy-6-methoxy	5.45	-3.47	3.89	-179.01	-180.18	-225.42	-39.15	4
47.*	2,3-dimethoxy	6.43	-1.16	1.85	-150.56	-56.386	-203.44	-30.82	3
48.*	2,3,4- trimethoxy	4.44	-2.06	1.14	-162.98	-140.08	-191.51	-33.43	5
49.*	3,5-dimethoxy	5.67	-1.42	0.24	-150.44	-125.93	-192.15	-29.73	0
50.*	2,4,6- trimethoxy	5.88	-2.55	3.18	-168.55	-191.29	-227.57	-32.03	1
51.*	1-methoxy naphthalene	4.49	-5.67	1.43	-175.35	-70.719	-251.55	-37.44	2
52.*	2-methoxy naphthalene	5.60	-1.67	0.33	-161.26	-115.30	-252.93	-31.18	0
53.*	2-ethoxy naphthalene	4.95	-3.38	1.17	-180.89	-114.44	-276.61	-38.52	4
54.*	2-hydroxy naphthalene	5.70	-1.73	2.10	-160.00	-131.73	-243.35	-39.25	4
55.*	1,4-dimethoxy naphthalene	5.36	-2.59	2.82	-167.87	-139.38	-217.17	-38.41	2
56.*	1-indol-2-yl	6.65	-1.84	0.02	-163.33	-119.67	-263.51	-30.38	1
57.*	4-ethylthiophen-2-yl	3.20	-4.36	1.11	-152.52	-119.87	-255.72	-31.19	1
58.*	Quinolin-3-yl	5.58	-1.52	2.07	-160.56	-134.22	-256.59	-32.76	2
59.*	2-hydroxy-4-methoxy	5.29	-2.76	3.74	-160.00	-175.18	-193.79	-33.74	2
60.*	3,4,5- trimethoxy	3.83	-2.72	0.013	-172.81	-137.03	-232.94	-26.83	0

CONCLUSION

Eleven benzimidazoles derivatives had been synthesized and evaluated for their α-glucosidase inhibitory activities. Based on results obtained from docking and biological evaluation, benzyl group substitution on benzimidazole and electron donating groups like diethyl amino, hydroxyl played an important role in this activity as they were found to be five to eight folds more active as compared to standard drug Acarbose. Compound 9, 10 and 3 bonding with significant amino acids LEU 520, ARG 335 and ASP 69 which were located at the entrance of active site pocket, and if they don't move, the substrate cannot bind at the active site. It can further be concluded from the results that the good inhibitory activity of the compounds may be due to bulky substitution of benzimidazole ring and existence of electron donating group on R.

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