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*Scientific paper*

# Synthesis, Molecular Docking Studies and ADME Prediction of Some New Albendazole Derivatives as α-Glucosidase Inhibitors

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## Abstract

A series of novel 2-(substituted arylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide derivatives **3a**–**i** were synthesized *via* condensation of *N*-(5-(propylthio)-1*H*-benzo[*d*]imidazol-2-yl) hydrazinecarboxamide (**2**), with the corresponding ketone or aldehydes. The chemical structures of the compounds prepared were confirmed by analytical and spectral data. The compounds were screened for their α-glucosidase inhibitory activity and all of them showed better inhibition than acarbose, except **3h**. In particular, compound **3a** proved to be the most active compound among all synthetic derivatives having $IC_{50}$ value 12.88 ± 0.98 μM. Also, molecular docking studies were carried out for the compounds to figure out the binding interactions. Compound **3a** has exhibited the highest binding energy (Δ*G* = –9.4 kcal/mol) and the most hydrogen bond interactions with active sites. Eventually, *in silico* studies were in good agreement with *in vitro* studies.

**Keywords:** Benzimidazole; antidiabetic; albendazole; α-glucosidase; semicarbazone; docking study

## 1. Introduction

Diabetes Mellitus (DM), known simply as diabetes, is a major health problem as a metabolic disease and is characterized by a failure of insulin production. It can be classified into two broad categories; type 1 and type 2 diabetes. Type 2 diabetes ranks as the most common type of diabetes worldwide among all reported cases.[1] This form of diabetes results from a combination of insulin resistance and insulin secretion defects.[2] Insulin plays an important role in the regulation of blood glucose levels and energy metabolism.[3] If diet and exercise fail to adequately control blood glucose levels, it is recommended to start oral drug therapy such as α-glucosidase inhibitors.

α-Glucosidase is a key enzyme in carbohydrate digestion, released from mucosal cells and plays a significant role in carbohydrate metabolism.[4] The enzyme has important functions in diabetes, viral infections, and cancer. α-Glucosidase inhibitors delay the hydrolysis of carbohydrates and this action reduces the glucose absorption. Acarbose, miglitol, and voglibose are used in the clinic as α-glucosidase inhibitors. Nevertheless, side effects such as diarrhea, hepatotoxicity and flatulence are observed in the long-term treatment with these inhibitors.[5,6] Hence, developing novel α-glucosidase inhibitors with minimum side effects is always a promising medicinal chemistry effort.

Benzimidazoles are important nitrogen-containing heterocyclic compounds and literature review showed that there are a great number of studies on α-glucosidase inhibitory activity of benzimidazole derivatives. Zawawi *et al.*[7] and Ozil *et al.*[8] have reported that a novel series of benzimidazole derivatives **I**–**II** (Figure 1) act as a new class of α-glucosidase inhibitors.

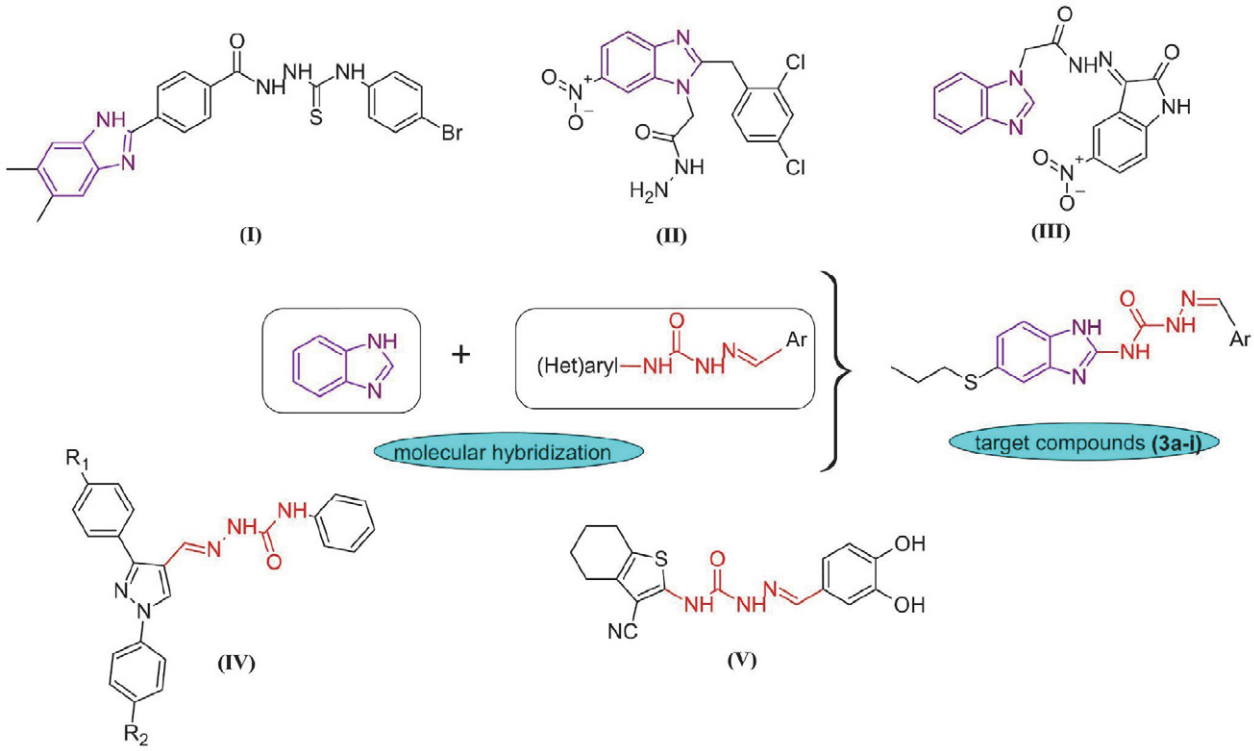



**Figure 1.** Designing of target molecules *via* molecular hybridization strategy

Ahmad *et al.*[9] provided an overview about the α-glucosidase inhibitory potential of a variety of benzimidazole derivatives **III** (Figure 1).

On the other hand, semicarbazones, as medicinally significant scaffolds, are imine derivatives formed by condensation reaction between aldehyde/ketone functional groups and the -$NH_2$ group of semicarbazides. These derivatives are known to have a broad range of biological properties including antidiabetic activity.[10–14] For instance, pyrazole-phenyl semicarbazone derivatives were reported by Azimi *et al.* as potent α-glucosidase inhibitors **IV** (Figure 1) with $IC_{50}$ values in the range of 65.1–695.0 μM comparing with acarbose ($IC_{50}$ = 750.0 μM).[14] More recently, a (*E*)-2-benzylidene-*N*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl) hydrazine-1-carboxamide derivative (**V**) has been reported for its α-glucosidase inhibitory potential[15] (Figure 1).

From the literature survey, we revealed that molecules containing benzimidazole and semicarbazone moiety have gained a huge interest as potent α-glucosidase inhibitors.

## 2. Experimental

### 2. 1. General

All the reagents used were analytical reagent grade. All melting points were determined on a Thermo Scientific 9300 melting point apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu FTIR 8400S spectrophotometer. NMR spectra were measured on a Bruker Avance 300 spectrometer in DMSO-$d_6$ solutions using TMS as the internal standard. Elemental analyses were determined on CHNS-932 (LECO) analyzer. The liquid chromatographic system consists of an Agilent Technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G1315 A photodiode array detector. The chromatographic data were collected and processed using Agilent Chemstation Plus software. Chromatographic separation was performed at ambient temperature using a reverse phase Zorbax C8 (4.0×250 mm) column. All experiments were performed using acetonitrile-water gradient mobile phase (50:50 from 0 to 3 min; 75:25 to 50:50 from 3 to 6 min; 100:0 to 75:25 from 6 to 12 min; the flow rate was 1.0 mL/min).

### 2. 2. Chemistry

#### 2. 1. 1. Synthesis of *N*-(5-(Propylthio)-1*H*-benzo[*d*]imidazol-2-yl) Hydrazinecarboxamide (2)

*N*-(5-(Propylthio)-1*H*-benzo[*d*]imidazol-2-yl) hydrazinecarboxamide (**2**) was prepared by heating hydrazine hydrate and Albendazole (**1**) in methanol. The product was purified by recrystallization from methanol yielding white solid.

### 2. 1. 2. General Procedure for the Synthesis of 2-(Substituted Arylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamides 3a–i

A mixture of *N*-(5-(propylthio)-1*H*-benzo[*d*]imidazol-2-yl) hydrazinecarboxamide (**2**) (0.001 mol) and various aromatic aldehydes or ketones (0.001 mol) in absolute ethanol (20 mL), in the presence of a catalytic amount of glacial acetic acid, was refluxed for 6–7 hours. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The precipitated compound was filtered and washed with water and recrystallized from absolute ethanol.

**2-(5-Chloro-2-oxoindolin-3-ylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3a)**

Yield 79%; m.p. 296–297 °C; HPLC $t_R$ (min): 6.32; FT-IR: ν 3327 (NH), 1681 (C=O), 1630 (C=N), 1608 (C=C) $cm^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.56 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.88 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.15–8.34 (m, 6H, Ar-**H**), 10.92 (s, 1H, NH), 11.24 (s, 1H, NH), 11.61 (s, 1H, NH), 11.65 (s, 1H, NH); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.5 (**C**$H_2$), 36.9 (**C**$H_2$), 112.5, 117.1, 124.8, 126.2, 128.1, 132.0, 135.6, 136.2, 142.6, 148.8 (**C**=N), 153.6 (**C**=O), 165.0 (**C**=O). Anal. calcd for $C_{19}H_{17}ClN_6O_2S{\cdot}4/3H_2O$: C, 50.39; H, 4.38; N, 18.56; S, 7.08. Found: C, 50.51; H, 4.69; N, 18.65; S, 7.02. LC/MS (ESI) *m/z* 429 $[M+H]^+$.

**2-(2-Fluorobenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3b)**

Yield 72%; m.p. 196–197 °C; HPLC $t_R$ (min): 5.27; FT-IR: ν 3321 (NH), 1678 (C=O), 1631 (C=N), 1556 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.56 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.86 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.24–8.41 (m, 8H, Ar-**H** and C**H**=N), 10.65 (s, 1H, NH), 11.34 (s, 1H, NH), 11.93 (s, 1H, NH); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.6 (**C**$H_2$), 37.2 (**C**$H_2$), 116.2, 122.1, 122.3, 124.5, 125.0, 125.1, 127.1, 127.6, 131.9, 132.0, 135.5, 135.6, 148.5 (**C**=N), 153.8 (**C**=O), 159.7 and 162.2 (**C**-F, *J* = 248 Hz). Anal. calcd for $C_{18}H_{18}FN_5OS$: C, 58.21; H, 4.88; N, 18.86; S, 8.63. Found: C, 58.60; H, 4.92; N, 18.74; S, 8.58. LC/MS (ESI) *m/z* 372 $[M+H]^+$, 394 $[M+Na]^+$.

**2-(3-Fluorobenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3c)**

Yield 85%; m.p. 188–190 °C; HPLC $t_R$ (min): 4.94; FT-IR: ν 3348 (NH), 1674 (C=O), 1627 (C=N), 1552 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.54 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.86 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.11–8.01 (m, 8H, Ar-**H** and C**H**=N), 11.32 (s, 1H, NH), 11.89 (s, 1H, NH), 11.92 (s, 1H, NH); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.6 (**C**$H_2$), 37.2 (**C**$H_2$), 113.2, 113.4, 116.7, 116.9, 124.5, 124.6, 125.9, 127.0, 131.0, 137.3, 141.7, 148.5 (**C**=N), 153.7 (**C**=O),161.8 and 164.2 (**C**-F, *J* = 242 Hz). Anal. calcd for $C_{18}H_{18}FN_5OS$: C, 58.21; H, 4.88; N, 18.86; S, 8.63. Found: C, 58.40; H, 4.72; N, 18.14; S, 8.11. LC/MS (ESI) *m/z* 372 $[M+H]^+$, 394 $[M+Na]^+$.

**2-(2,5-Difluorobenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3d)**

Yield 74%; m.p. 202–204 °C; HPLC $t_R$ (min): 5.20; FT-IR: ν 3335 (NH), 1674 (C=O), 1631 (C=N), 1552 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.56 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.86 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.11–8.45 (m, 7H, Ar-**H** and C**H**=N), 10.88 (s, 1H, N**H**), 11.44 (s, 1H, N**H**), 11.98 (s, 1H, N**H**); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.5 (**C**$H_2$), 37.2 (**C**$H_2$), 113.5, 117.7, 117.9, 118.0, 118.3, 118.5, 123.9, 124.0, 124.5, 127.1, 134.4, 148.4 (**C**=N), 153.7 (**C**=O), 155.9 and 158.4 (**C**-F, *J* = 243 Hz), 157.8 and 160.2 (**C**-F, *J* = 238 Hz). Anal. calcd for $C_{18}H_{17}F_2N_5OS$: C, 55.52; H, 4.40; N, 17.98; S, 8.23. Found: C, 55.91; H, 4.76; N, 17.80; S, 8.22. LC/MS (ESI) *m/z* 390 $[M+H]^+$, 428 $[M+K]^+$.

**2-(4-Methylbenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3e)**

Yield 82%; m.p. 199–200 °C; HPLC $t_R$ (min): 3.19; FT-IR: ν 3335 (NH), 1681 (C=O), 1625 (C=N), 1556 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.53 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.35 (s, 3H, C$\mathbf{H_3}$), 2.86 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.10–7.99 (m, 8H, Ar-**H** and C**H**=N), 10.29 (s, 1H, N**H**), 11.15 (s, 1H, N**H**), 11.91 (s, 1H, N**H**); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.6 (**C**$H_3$), 21.5 (**C**$H_2$), 22.6 (**C**$H_3$), 37.5 (**C**$H_2$), 124.5, 126.6, 127.0, 127.1, 127.8, 128.8, 129,6, 129.9, 131.9, 139.9, 143.3, 148.3 (**C**=N), 153.3 (**C**=O). Anal. calcd for $C_{19}H_{21}N_5OS{\cdot}1/4H_2O$: C, 61.35; H, 5.83; N, 18.83; S, 8.62. Found: C, 61.52; H, 6.24; N, 18.56; S, 8.69. LC/MS (ESI) *m/z* 368 $[M+H]^+$, 390 $[M+Na]^+$.

**2-(2,4-Dichlorobenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3f)**

Yield 78%; m.p. 177–179 °C; HPLC $t_R$ (min): 4.22; FT-IR: ν 3317 (NH), 1680 (C=O), 1631 (C=N), 1556 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.95 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.53 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.51 (t, 2H, -S-$C\mathbf{H_2}CH_2CH_3$), 7.05–8.53 (m, 7H, Ar-**H** and C**H**=N), 10.56 (s, 1H, N**H**), 11.17 (s, 1H, N**H**), 11.45 (s, 1H, N**H**); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.5 (**C**$H_2$), 37.2 (**C**$H_2$), 114.5, 115.8, 116.2, 124.5,127.2,

128.0, 129.5, 131.0, 133.7, 135.0, 137.8, 138.5, 148.5 (**C**=N), 154.6, 156.5 (**C**=O). Anal. calcd for $C_{18}H_{17}Cl_2N_5OS$: C, 51.19; H, 4.06; N, 16.79; S, 7.59. Found: C, 51.04; H, 4.04; N, 16.79; S, 7.59. LC/MS (ESI) *m/z* 422 $[M+H]^+$.

**2-(3,4-Dimethylbenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3g)**

Yield 81%; m.p. 191–193 °C; HPLC $t_R$ (min): 6.02; FT-IR: ν 3332 (NH), 1681 (C=O), 1627 (C=N), 1556 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.54 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.25 (s, 3H, -C$\mathbf{H_3}$), 2.26 (s, 3H, -C$\mathbf{H_3}$), 2.85 (t, 2H, -S-C$\mathbf{H_2}CH_2CH_3$), 7.11–7.96 (m, 7H, Ar-**H** and C**H**=N), 10.35 (s, 1H, N**H**), 11.16 (s, 1H, N**H**), 11.93 (s, 1H, N**H**); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 19.7 (**C**$H_2$), 19.9 (**C**$H_3$), 22.6 (**C**$H_3$), 37.2 (**C**$H_2$), 124.4, 125.4, 126.9, 127.1, 127.4, 128.5, 130.2, 130.7, 132.2, 137.0, 138.7, 139.6, 143.6, 148.4 (**C**=N), 153.4 (**C**=O). Anal. calcd for $C_{20}H_{23}N_5OS$: C, 62.71; H, 6.24; N, 17.92; S, 8.21. Found: C, 63.04; H, 5.96; N, 17.67; S, 7.99. LC/MS (ESI) *m/z* 382 $[M+H]^+$, 404 $[M+Na]^+$.

**2-(5-Bromo-2-methoxybenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3h)**

Yield 81%; m.p. 198–200 °C; HPLC $t_R$ (min): 5.86; FT-IR: ν 3348 (NH), 1681 (C=O), 1627 (C=N), 1556 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.54 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.86 (t, 2H, -S-C$\mathbf{H_2}CH_2CH_3$), 3.84 (s,3H, -OC$\mathbf{H_3}$), 7.04–8.50 (m, 7H, Ar-**H** and C**H**=N), 10.65 (s, 1H, N**H**), 11.26 (s, 1H, N**H**), 11.95 (s, 1H, N**H**); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.6 (**C**$H_2$), 37.2 (**C**$H_2$), 56.7 (O**C**$H_3$), 113.3, 114.4, 116.6, 124.5, 124.8, 127.0, 128.8, 130.3, 130.4, 133.6, 136.9, 138.9, 148.4 (**C**=N), 153.4 (**C**-$OCH_3$) 156.9 (**C**=O). Anal.calcd for $C_{19}H_{20}BrN_5O_2S$: C, 49.36; H, 4.36; N, 15.15; S, 6.94. Found: C, 49.56; H, 4.36; N, 14.35; S, 6.55. LC/MS (ESI) *m/z* 462 $[M+H]^+$, 484 $[M+Na]^+$.

**2-(4-Trifluoromethylbenzylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamide (3i)**

Yield 75%; m.p. 208–210 °C; HPLC $t_R$ (min): 6.72; FT-IR: ν 3348 (NH), 1681 (C=O), 1614 (C=N), 1539 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$) δ 0.96 (t, 3H, -S-$CH_2CH_2C\mathbf{H_3}$), 1.54 (m, 2H, -S-$CH_2C\mathbf{H_2}CH_3$), 2.86 (t, 2H, -S-C$\mathbf{H_2}CH_2CH_3$), 7.11–8.15 (m, 8H, Ar-**H** and C**H**=N), 11.38 (s, 1H, N**H**, other NH proton not observed). $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 13.5 (**C**$H_3$), 22.6 (**C**$H_2$), 37.2 (**C**$H_2$), 115.6, 120.6, 123.3, 125.8, 125.9, 126.0, 126.2, 127.2 (q, $CF_3$, $J$ = 265 Hz), 128.3, 128.7, 129.2, 130.1, 138.7, 141.3, 148.6 (**C**=N), 153.9 (**C**=O). Anal. calcd for $C_{19}H_{18}F_3N_5OS$: C, 54.15; H, 4.30; N, 16.62; S, 7.61. Found: C, 54.12; H, 4.33; N, 16.62; S, 7.43. LC/MS (ESI) *m/z* 422 $[M+H]^+$, 444 $[M+Na]^+$.

## 2. 3. α-Glucosidase Assay

The α-glucosidase inhibitor activity was evaluated as described by Ramakrishna *et al.* with slight modifications described by Sen *et al.*[16] Each concentration had three replicates when preliminary screened and inhibition percentages were calculated using the following formula.

$$\%\ \text{Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \cdot 100$$

where $A$ is the absorbance.

Finally, $IC_{50}$ values were also determined for the compounds having 50% or above inhibition values.

## 2. 4. Molecular Docking

Molecular docking simulations were performed against α-glucosidase by using AutoDock Vina software.[17] The protein data of α-glucosidases (PDB ID: 4J5T) was referenced from Protein Data Bank.[18]

The target protein was prepared in three steps using the AutoDock Tools program[19] for docking studies: (1) were removed water molecules; (2) were added polar hydrogen to α-glucosidase macromolecule; (3) the obtained structure was energy-minimized.

The synthesized compounds **3a–i** and reference ligands were prepared in two steps for docking studies: (1) were drawn with the Spartan 04 software (SPARTAN 04, Wavefunction, Inc., Irvine, USA)[20] and optimized for each compound by using the semi-empirical PM3 method; (2) the docking input files of the most stable conformation were generated using the AutoDock Tools program.

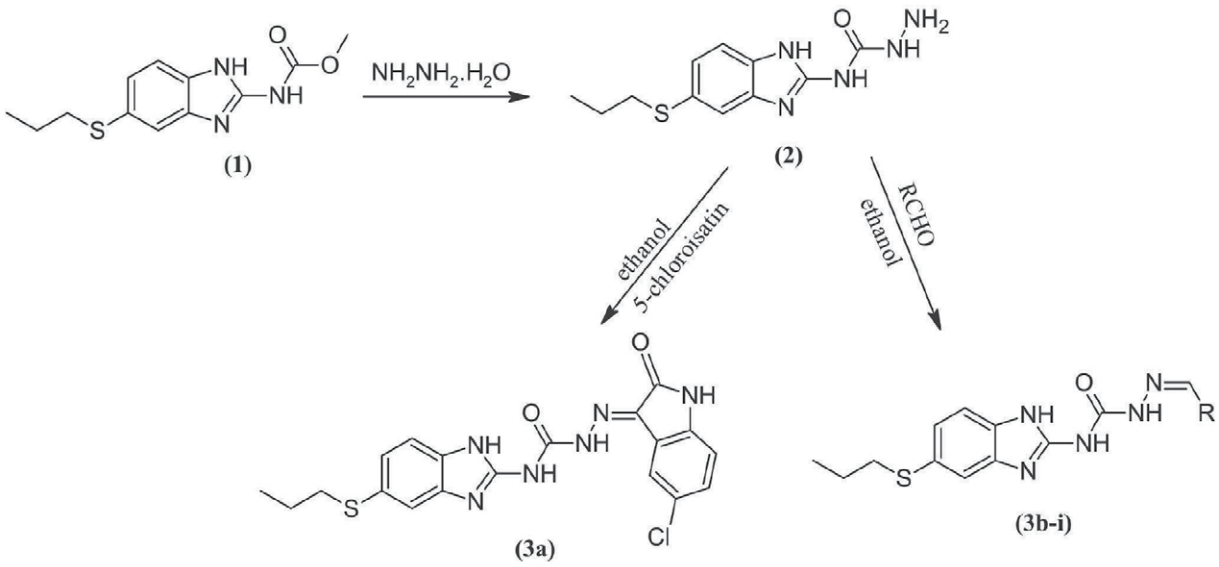
The grid box size was determined as 78 Å × 69 Å × 104 Å within 0.375 Å grid spacing and center_x = −18.44, center_y = −20.91, center_z = 8.22 dimensions were used in α-glucosidase enzyme docking studies, appropriate to the literature.[18] The Vina parameter "exhaustiveness" was set to the value of 10.

The reference ligands used in validation studies include sugars that form part of the biological substrate, as well as known inhibitors (acarbose, glucose, miglitol, deoxynojirimycin, and kojibiose). During study of validations as well as our docking of ligands were used flexible ligands in rigid protein. The resulting files were analyzed using Accelrys Discovery Studio Visualizer 4.0 program.

# 3. Results and Discussion

## 3. 1. Chemistry

Scheme 1 shows the synthetic route for the target compounds. Albendazole (**1**) was reacted with hydrazine hydrate, affording **2** according to the similar method reported in the literature.[21] The new derivatives **3a–i** were obtained by the reaction of **2** with appropriate aromatic




**Scheme 1.** Synthetic route to target derivatives **3a–i**

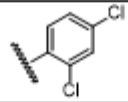
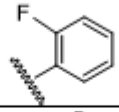
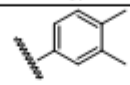
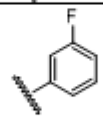
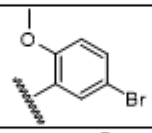
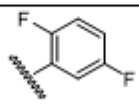
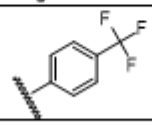
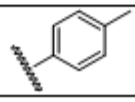
aldehydes or ketone in the presence of acid according to the literature procedures.[22,23] The structures of the desired target compounds were confirmed by FT-IR, $^1$H NMR, and elemental analysis.

In the IR spectra of the studied compounds, the less intense broad bands around 3317–3348 cm$^{-1}$ are assigned to ν(N–H) vibrations of the NHCO group. The band around 1674–1681 cm$^{-1}$ corresponds to the carbonyl group of CONH. The azomethine band is observed at 1625–1631 cm$^{-1}$. $^1$H NMR spectrum revealed three signals around 10.29–11.98 ppm assigned to three NH protons. In the $^1$H NMR spectrum of compound **3i**, the NH proton was seen as a singlet at δ 11.38 ppm and two NH protons were not observed.

## 3.2. α-Glucosidase Inhibitory Activity

We have synthesized benzimidazoles bearing semicarbazones **3a–i** and evaluated them for α-glucosidase inhibitory potential. All derivatives showed excellent inhibitory activities having $IC_{50}$ values ranging between 12.88–44.35 μM as compared to the standard acarbose

**Table 1.** α-Glucosidase inhibitory activity ($IC_{50}$) of albendazole derivatives **3a–i**

| Compound | -Ar | $IC_{50}$ (μM)[a] | Compound | -Ar | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **3a** | | 12.88±0.98 | **3f** |  | 31.16±0.17 |
| **3b** |  | 30.80±0.27 | **3g** |  | 28.72±1.32 |
| **3c** |  | 13.68±0.65 | **3h** |  | 44.35±0.21 |
| **3d** |  | 30.30±0.89 | **3i** |  | 28.36±0.50 |
| **3e** |  | 14.54±0.25 | **Acarbose**[b] | | 40.06±2.14 |

[a]Data represents means SD of triplicate samples obtained from the dose inhibition curve, [b]Standart drug

($IC_{50}$ = 40.06 μM) (Table 1). Their possibly potent inhibitory potential may be due to the benzimidazole core bearing a semicarbazone entity.

The isatin moiety of the most active compound **3a**, was determined to be important for biological activity, suggesting intramolecular hydrogen bond formation.

## 3. 3. ADME Prediction

The potent inhibitors **3a–i** were evaluated *in silico* for selected ADME properties using the SwissADME online tool (http://www.swissadme.ch/). Table 2 shows the ADME prediction results of the compounds. Topological Polar Surface Area (TPSA) shows the surface belonging to polar atoms in the compound and lower TPSA values are appropriate for drug-likeness properties. The absorption percentage was also calculated by using the following formula:

$$\%\text{Abs} = 109 - [0.345 \cdot \text{TPSA}]$$

as given in the literature[24,25] and showed a good absorption profile.

According to Lipinski's rule of five, drug candidate should have log *P* less than 5, its polar surface area within 140 Å², it should have less than 10 H bond acceptors, it should have less than 5 H bond donors and its molecular weight should be below 500 Dalton.[26] Also, the more negative the skin permeability (log *K*p) value is, the less possible for the compound to penetrate the skin barrier. The tool predicted that derivatives have suitable skin permeability with log *K*p values of –5.24 to –5.90 cm/s.

Eventually, all of the molecules were shown to comply best with these properties used to predict drug-likeness (Table 2).

To reveal the capability of intestinal absorption and permeability of the blood–brain barrier (BBB), the boiled-egg model of the molecules was predicted using SwissADME (Figure 2). Molecules that fall in the yellow field de-

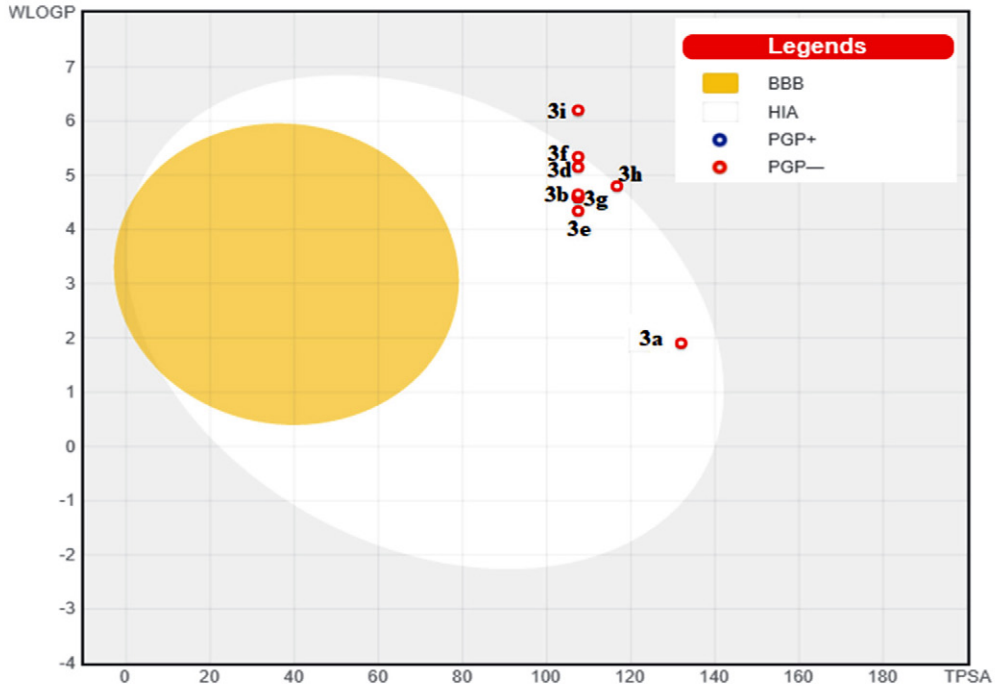



**Figure 2.** The boiled-egg plot of compounds **3a–i**. HIA: Human Intestinal Absorption; BBB: Blood–Brain Barrier; PGP: Permeability Glycoprotein.

**Table 2.** ADME results of the albendazole derivatives 3a–i

| ADME properties | 3a | 3b | 3c | 3d | 3e | 3f | 3g | 3h | 3i |
|---|---|---|---|---|---|---|---|---|---|
| Molecular weight (g/mol) | 428.903 | 371.439 | 371.439 | 389.429 | 367.476 | 422.339 | 381.503 | 462.371 | 421.446 |
| Num.H-bond acceptors | 8 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 6 |
| Num.H-bond donors | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Num.rotatable bonds | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 9 | 9 |
| logP | 2.86 | 3.63 | 3.63 | 4.01 | 3.47 | 4.24 | 3.70 | 3.55 | 4.08 |
| TPSA (Å²) | 131.95 | 107.47 | 107.47 | 107.47 | 107.47 | 107.47 | 107.47 | 116.70 | 107.47 |
| Absorption (%) | 63.48 | 71.92 | 71.92 | 71.92 | 71.92 | 71.92 | 71.92 | 68.73 | 71.92 |
| Log $K_p$ (skin permeation (cm/s) | -5.65 | -5.75 | -5.75 | -5.79 | -5.54 | -5.24 | -5.37 | -5.90 | -5.50 |

pict the BBB permeation, whereas the white eclipse region symbolizes gastrointestinal absorption. According to the boiled-egg plot, all of the compounds are BBB-impermeable and have good absorption, except compound **3i**. P-glycoprotein (P-gp) plays a significant role in drug absorption and disposition. Compounds **3a–i** are non substrates of

**Table 3.** Types of interactions of the compounds **3a–i** and small reference ligands with the binding site residues of α-glucosidase enzyme.

| Compounds[a] | Binding energy Δ*G* (kcal/mol) | Binding site | Distance (Å) | Hydrogen bond Interactions (A/D)[b] |
|---|---|---|---|---|
| **3a** | –9.3 | A | 2.80 | Asp392 (A) |
| | | | 1.80 | Arg428 (D) |
| | | | 2.59 | Arg428 (D) |
| | | | 2.66 | Arg428 (D) |
| **3b** | –7.7 | A | 2.50 | Arg428 (D) |
| **3c** | –8.4 | A | 2.25 | Arg428 (D) |
| **3d** | –7.9 | A | 2.88 | Trp391 (D) |
| | | | 2.49 | Arg428 (D) |
| | | | 2.25 | Arg428 (D) |
| **3e** | –8.4 | A | 2.31 | Arg428 (D) |
| | | | 2.97 | Val446 (A) |
| | | | 2.78 | Gln447 (D) |
| **3f** | –7.3 | A | 2.87 | Glu429 (A) |
| **3g** | –7.9 | A | 2.87 | Arg428 (D) |
| **3h** | –7.3 | A | 2.84 | Arg428 (D) |
| **3i** | –8.4 | A | 2.71 | Trp391 (D) |
| | | | 2.27 | Asp568 (A) |
| | | | 3.02 | Glu771(A)[c] |
| **ACB** | –8.1 | A | 3.09 | Glu443 (A) |
| | | | 3.16 | Val446 (A) |
| | | | 3.09 | Asp568 (A) |
| | | | 1.97 | Trp710 (D) |
| | | | 3.33 | Glu771 (A) |
| **GLC** | –5.5 | A | 1.84 | Trp391 (D) |
| | | | 2.26 | Arg428 (D) |
| | | | 2.73 | Arg428 (D) |
| | | | 2.46 | Gly566 (A) |
| | | | 2.72 | Asp568 (D) |
| **DNJ** | –5.4 | A | 2.54 | Trp391 (D) |
| | | | 2.13 | Gly566 (A) |
| | | | 2.69 | Gly566 (A) |
| | | | 1.98 | Trp710 (D) |
| | | | 2.37 | Trp710 (D) |
| **MGL** | –5.5 | A | 2.27 | Gly566 (A) |
| **KJB** | –6.1 | A | 2.41 | Ile362 (A) |
| | | | 2.75 | Glu429 (A) |
| | –6.4 | B | 2.66 | Leu50 (A) |
| | | | 2.21 | His51 (A) |
| | | | 2.44 | Phe56 (A) |
| | | | 2.46 | Asp61 (A) |
| | | | 1.96 | Arg209 (D) |
| | | | 2.33 | Arg209 (D) |
| | | | 2.62 | Arg209 (D) |

[a] ACB: Acarbose; GLC: Glucose; DNJ: Deoxynojirimycin; MGL: Miglitol; KJB: Kojibiose. [b] A: H-bond acceptor; D: H-bond donor. [c] Halogen bond.

P-gp, therefore it can be theorized that they may possibly act as inhibitors of P-gp.

## 3. 3. Molecular Modeling

The molecular docking analysis was carried out to investigate the binding mode of novel α-glucosidase inhibitors compounds **3a–i** within the binding pocket of the target enzyme, and to further understand their structure-activity relationship. Firstly, we examined the interactions of the known inhibitors (acarbose, glucose, miglitol, deoxynojirimycin, and kojibiose) with α-glucosidase active site to compare with our synthesized compounds **3a–i**. The binding energies and interactions with the active site of the reference ligands and compounds **3a–i** are given in Table 3.

As shown in Figure 3, one of the two binding sites of α-glucosidase has been defined as "site A" containing a glutamate (Glu771) and an aspartate (Asp568), while the other is "site B".[18] It was determined that among the known

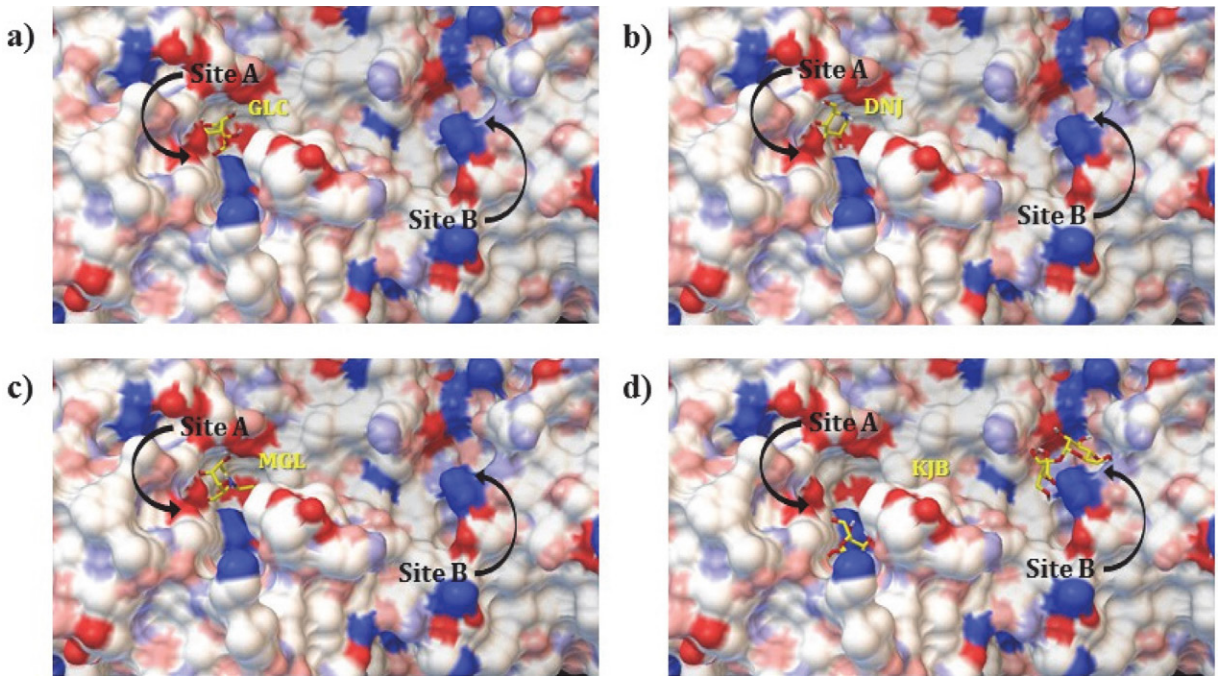



**Figure 3**. For α-glucosidase enzyme small references ligands a) GLC is in binding site A, b) DNJ is in binding site A, c) MGL is in binding site A, and d) KJB is in both binding sites A and B. A surface representation of α-glucosidase shown as the DG color scheme (neutral oxygen (pink), nitrogen atoms (light blue), charged oxygen (red) and nitrogen atoms (dark blue)).

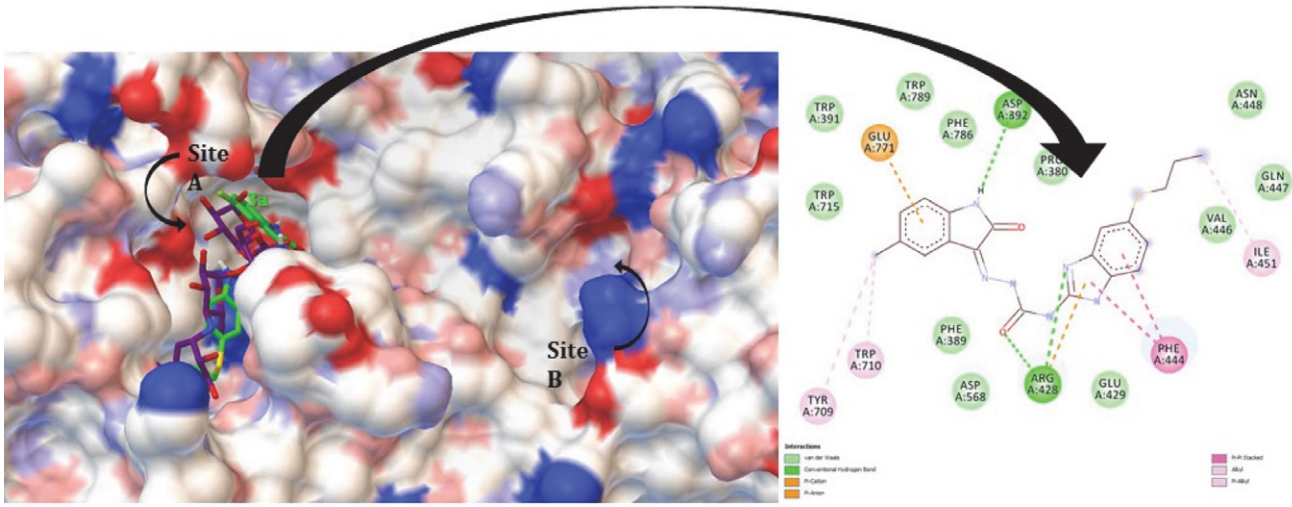



**Figure 4**. A surface representation of α-glucosidase shown as the DG color scheme (neutral oxygen (pink), nitrogen atoms (light blue), charged oxygen (red) and nitrogen atoms (dark blue)). Positions of compound **3a** (green) and acarbose (purple) in the binding site A of α-glucosidase enzyme and 2D interactions of the compound **3a** with active site residues.

ligands ACB, GLC, DNJ and MGL are docked into "site A" of α-glucosidase, while KJB docked into both of the sites.

According to the *in silico* molecular modeling studies, it has been determined that the synthesized compounds **3a–i** interact with the binding site A. Binding energies of these compounds have been determined to be between –7.3 kcal/mol and –9.3 kcal/mol as higher than the known inhibitors GLC, DNJ, MGL and KJB. Compared with the binding energy of ACB (–8.1 kcal/mol) used in *in vitro* inhibition studies, compound **3a** was found to be remarkable with the value of –9.3 kcal/mol, while the other synthesized compounds showed similar results. These findings supports the results of *in vitro* enzyme inhibition studies. All of the synthesized novel benzimidazole derivatives, except **3f** and **3i** have exhibited hydrogen bond (H-bond) interaction with Arg428, like reference ligand GLC. Also, it was detected that compound **3a** exhibited π–anion interaction with active site residue Glu771, while compound **3i** exhibited halogen bond interaction.

Compound **3a**, which was found to be the most effective compound according to both *in vitro* and *in silico* studies, exhibited three H-bond interactions with Arg428. One of the three H-bond interactions were detected with =N– atom of benzimidazole moiety and the others with CO group of semicarbazone moiety. Moreover, a H-bond interaction between the NH group of 2-oxoindolin structure and Asp392 has been detected. Additionally, hydrophobic interactions have been determined between Arg428, Glu771 residues and benzimidazole (π-cation), 2-oxoindolin (π-anion) rings, respectively. These hydrophobic interactions were supported by the π–π stacking interactions of the benzimidazole ring with Phe444 besides other hydrophobic interactions between compound **3a** and Ile451, Leu563, Trp709 and Trp710 (Figure 4). Finally, it has been detected that the compound **3a** exhibits more hydrophobic interactions than all reference ligands and synthesized compounds and these findings contribute to having the highest binding energy.

## 4. Conclusions

The targeted 2-(substituted arylidene)-*N*-(5-(propylthio)-2,3-dihydro-1*H*-benzo[*d*]imidazol-2-yl)hydrazine-1-carboxamides **3a–i** were synthesized in good yields. All of the molecules demonstrated encouraging inhibitory activity against α-glucosidase which was also supported by molecular docking studies. Docking studies revealed that compound **3a** is the most active compound with the highest binding energy value of –9.3 kcal/mol. As a result, it has been determined that the findings obtained from *in vitro* and *in silico* α-glucosidase enzyme inhibition studies were compatible. These compounds revealed also reasonable i*n silico* physicochemical and pharmacokinetic parameters (ADME). The present findings may invite researchers to work in the area of development of the α-glucosidase inhibitors.

**Conflict of Interest**

Authors declare no conflict of interest.

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## Povzetek

S pomočjo kondenzacije *N*-(5-(propiltio)-1*H*-benzo[*d*]imidazol-2-il) hidrazinkarboksamidov (**2**) z ustreznimi ketoni ali aldehidi smo sintetizirali serijo novih 2-(substituiranih ariliden)-*N*-(5-(propiltio)-2,3-dihidro-1*H*-benzo[*d*]imidazol-2-il)hidrazin-1-karboksamidnih derivatov **3a**–**i**. Kemijske strukture pripravljenih spojin smo potrdili z analitskimi in spektroskopskimi metodami. Za pripravljene spojine smo določili njihovo inhibitorno aktivnost na α-glukozidazo; vse spojine, razen **3h**, so se izkazale kot boljši inhibitorji od akarboze. Še posebej zanimiva je spojina **3a**, ki je pokazala največjo aktivnost ($IC_{50}$ vrednost 12.88±0.98 μM) izmed vseh sintetiziranih derivatov. Da bi raziskali vezavne interakcije, smo izvedli tudi študije molekulskega sidranja. Spojina **3a** je pokazala največjo vezno energijo ($\Delta G$ = –9.4 kcal/mol) in največje število interakcij z aktivnim mestom z vodikovimi vezmi. Tudi rezultati *in silico* študij se dobro ujemajo z rezultati *in vitro* raziskav.

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