Synthesis and Antidiabetic Evaluation of *N'*-Benzylidenebenzohydrazide Derivatives by *In Silico* Studies

Yusuf Syaril Alam[1], Pratiwi Pudjiastuti[2*], Saipul Malulana[3], Nur Rahmayanti Affifah[1], Fahimah Martak[1], Arif Fadlan[1], Tutik Sri Wahyuni[3], and Syukri Arief[4]

[1]*Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Jl. Arif Rahman Hakim, Surabaya 60115, Indonesia*

[2]*Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia*

[3]*Department of Pharmaceutical Science, Faculty of Pharmacy, Airlangga University, Surabaya 60115, Indonesia*

[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis Campus, Padang 25163, Indonesia*

Corresponding author:

email: pratiwi-p@fst.unair.ac.id

Received: February 6, 2023
Accepted: June 7, 2023

DOI: 10.22146/ijc.82073

Abstract: *Three new* N'*-benzylidenebenzohydrazide (NBB) derivatives were successfully synthesized and yielded 50–58%. FTIR, ESI-MS,* ^{1}*H- and* ^{13}*C-NMR were used to investigate the characteristic of NBB derivates. The structure and relationship of NBB derivatives into α-glucosidase and α-amylase as good targets for diabetes treatment were evaluated using* in silico *screening. Molecular mechanics-Poisson Boltzmann/generalized born surface area (MM-PB/GBSA) was used to calculate the free binding energy (*$\Delta G_{bind\ (MM\text{-}GBSA)}$*) of NBB to α-glucosidase and α-amylase receptors showed that the results of −0.45 and −20.79 kcal/mol, respectively. In the* ortho *position, NBB derivatives exhibited electron donating groups (EDG like -*OCH_3*, -OH and -Cl with binding free energies of −21.94, −6.71, and 21.94, respectively, and acarbose, a native ligand energy of −32.62 kcal/mol. In addition, the binding free energy of N-2-(-*OCH_3*, -OH and -Cl)-NBB to the α-amylase receptor showed the number of −39.33, −43.96, −42.81, respectively and −46.51 kcal/mol in comparing with a native ligand. As a result, it was found that all the NBB derivatives were able to interact with several amino acids in the α-glucosidase cavity as well as the native ones, including Ala281, Asp282, and Asp616. NBB and native ligand showed similar interaction between α-amylase with Gly110 amino acid residue.*

Keywords: N'*-benzylidenebenzohydrazide; α-amylase; derivatives; antidiabetic;* in silico

■ INTRODUCTION

Diabetes, in general, is a chronic metabolic disease characterized by elevated levels of blood glucose, which is divided into several types. Specifically, type-2 diabetes is a metabolic disorder distinguished by chronic hyperglycemia and either complete or partial deficiencies of insulin secretion [1]. Over the past few decades, there has been a rise in the prevalence of type-2 diabetes in many countries of the world from a wide range of income levels. An alternative therapeutic approach for controlling hyperglycemia associated with type-2 diabetes is to target α-glucosidase and α-amylase enzymes that catalyze starch hydrolysis in the intestine. Inhibition of α-glucosidase and α-amylase can decrease hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM) and retard the absorption of glucose [2]. Acarbose, miglitol, and voglibose are the three α-glucosidase inhibitors that have been approved for use in clinical trials at this time [3]. Voglibose comes from a microbial origin, whereas miglitol is synthetically derived

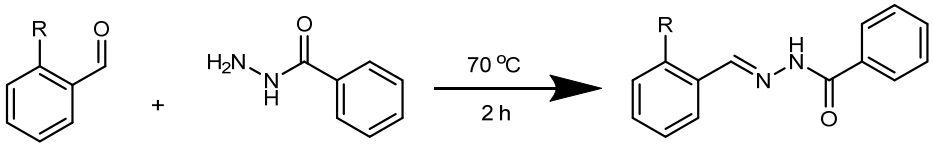


Fig 1. Synthesis of NBB and derivatives

from 1-deoxynojirimycin [4]. The α-amylase is a calcium metalloenzyme that helps in the digestion of polysaccharide molecules into small saccharides. As same as α-glucosidase, the α-amylase enzyme causes postprandial hyperglycaemia and increased blood glucose levels. With these characteristics, α-amylase is a well-known therapeutic target for the treatment and maintenance of elevated postprandial blood glucose [5].

N'-benzylidenebenzohydrazide (NBB) and its derivatives are important to the development of a significant class of new drugs [6]. Taha et al. [7] recently reported benzothiazole NBB derivatives containing benzohydrazide as α-glucosidase inhibitor with a wide range of IC_{50} values. Ullah et al. [8] also reported that benzohydrazide-based imine and thiazolidine-4-one inhibit α-glucosidase and α-amylase enzymes. According to Fan et al. [9] chromone-based NBB derivatives may have the potential to perform as α-glucosidase inhibitors. The core of benzohydrazide is crucial to the inhibition of their enzymes. Therefore, the research aims to synthesize and evaluate NBB-derived with electron-donating groups (-OCH_3, -OH and -Cl) (Fig. 1) as antidiabetic to target α-glucosidase and α-amylase enzymes by using molecular docking.

■ EXPERIMENTAL SECTION

Materials

Analytical grades of benzohydrazide, benzaldehyde, salicylaldehyde, *o*-anisaldehyde, and *o*-chlorobenzaldehyde were purchased from Sigma Aldrich. Analytical grades of hexane, dimethylformamide, dimethyl sulfoxide, dichloromethane, sodium acetate and glacial acid were purchased from Merck. The pure solvents of ethanol 99.9%, methanol 99.8%, chloroform, and ethyl acetate were prepared from Fulltime.

Instrumentation

NMR measurement was used TMS as an internal reference, NMR spectra were recorded on a JEOL Resonance 400 MHz spectrometer, and the chemical shifts were reported in δ (ppm). TLC was performed using silica gel 60 F254 aluminium sheet, while ESI-MS data were obtained using water mass spectrometer Q-TOF XEVO. At last, functional groups were analyzed using FTIR Bruker Opus with KBr pellet preparation. Software for docking analysis**:** the computing study in this research was performed under a Dell WorkStation Personal Computer, Linux Ubuntu 20.04.3 LTS OS, Intel® Xeon(R) W-2223 CPU @3.60 GHz octa-core; RAM 16 GB and GPU NVIDIA Quadro P2200. Meanwhile, molecular docking was conducted with Maestro Schrödinger 2022-1 software (Schrödinger, New York, NY, USA).

Procedure

Synthesis of NBB derivatives

NBB synthesis was conducted by using the reflux process and several modified procedures from Jubie et al. [10]. The ligands were prepared by adding 6 mmol (0.8169 g) of benzohydrazide in 30 mL of ethanol. Then, 6 mmol of the *o*-benzaldehyde derivative and 30 mL of ethanol were added to the flask with a small amount of acetic acid. The mixture was refluxed for 2 h at 70 °C temperature. After that, the product was cooled overnight at 4 °C and separated by using a funnel. As the last step, an aluminium sheet with TLC gel 60 F_{254} was used for product tracing. This procedure was repeated for the synthesis of the other four derivates, such as *N*-2-(-Cl, -OH, and -OMe)benzohydrazide.

***N*'-(2-chlorobenzylidene)-benzohydrazide 1.** Yield: 53.40%. FTIR (KBr, cm^{-1}): 3181 (-C_{sp2}H aromatic); 1643 (-C=O); 1555 (-N=N-). ^{1}H-NMR (DMSO-d_6): 12.05 (*s*, 1H, -NH); 8.84 (*s*, 1H, -CH=N); 8.00 (*m*, 1H); 7.91 (*d*, 2H); 7.57 (*t*, 1H); 7.50 (*dd*, 3H); 7.42 (*m*, 2H). ^{13}C-NMR (DMSO-d_6): 163.8 (-C=O); 144.2 (-CH=N); 133.8; 133.7; 132.5; 132.1; 132.1; 130.5; 129.1; 128.2; 128.2; 127.4. ESI-MS: 259.0636 (100%) $[L+H]^+$; 281.0360 (100%) $[L+Na]^+$.

***N'*-(2-hydroxybenzylidene)-benzohydrazide 2.** Yield: 50.12%. FTIR (KBr, cm^{-1}): 3268 (-NH); 3268 (-OH); 3057 (-C_{sp2}H aromatic); 1672 (-C=O); 1538 (-N=N); 1271 (-C-O). ^{1}H-NMR (DMSO-d_6): 12.1 (*s*, 1H, -NH); 11.37 (*s*, 1H, -CH=N); 8.61 (*s*, 1H); 7.90 (*m*, 2H); 7.58 (*t*, 1H); 7.51 (*ddd*, 3H); 7.27 (*td*, 1H); 6.90 (*m*, 2H). ^{13}C-NMR (DMSO-d_6): 163.4 (-C=O); 158.0 (-C-OH); 148.9 (-CH=N)-; 133.4; 132.5; 132.1 130.1; 129.1; 128.2; 119.9; 119.2; 117.0. ESI-MS: 241.0979 (100%) $[L+H]^+$.

***N'*-(2-methoxybenzylidene)-benzohydrazide 3.** Yield: 57.93%. FTIR (KBr, cm^{-1}) 3184 (-C_{sp2}H aromatic); 2988 (-C_{sp3}H of methyl); 1640 (C=O); 1556 (-N=N); 1251 (-C-O). ^{1}H-NMR (DMSO-d_6): 11.81 (*s*, 1H, -NH); 8.79 (*s*, 1H, -CH=N); 7.89 (*m*, 2H); 7.85 (*dd*, 1H); 7.55 (*dd*, 1H); 7.48 (*t*, 2H), 7.38 (*m*, 1H); 7.07 (*d*, 1H); 6.99 (*t*, 1H); 3.83 (*s*, 3H, -OCH_3). ^{13}C-NMR (DMSO-d_6): 163.5 (-C=O); 158.3 (-C=OCH_3); 143.8 (-CH=N); 133.9; 132.3; 132.1; 129.0; 128.2; 126.1; 122.9; 121.3; 112.4; 56.2 (-OCH_3). ESI-MS: 255.1136 (100%) $[L+H]^+$.

Ligands and receptor preparation

ChemDraw was used to generate the ligand structures, which were then converted into a 3D model using LigPrep [11] module in Schrodinger 2022-1 as well as protonated at pH 7.4 with Epik [12] and OPLS4 forcefield [13]. These processes aim to restore improper or missing bonds, assign protonation, possible ionization, and tautomeric states [14-16]. Moreover, α-glucosidase (PDB ID: 5NN8) protein was prepared by removing the residual solvent, optimizing the hydrogen bond, and protonating using ProtAssign [17] and PROPKA [18]. At the same time, in the "protein preparation wizard" that is incorporated into Maestro Schrodinger 2022-1 [19-20], the partial charge was also added using OPLS4 forcefield.

Molecular docking

The docking study was performed using Glide [21] Maestro Schrodinger's 2022-1 to predict the binding affinities and molecular interactions of synthesized compounds against two receptor targets: α-glucosidase (PDB ID: 5NN8) and α-amylase (PDB ID: 6GXV). Acarbose, an inhibitor that acts as the native ligand, was used for comparing to compounds. A grid box was placed at the center of acarbose position with similar dimensions for both receptors (20 × 20 × 20 Å) for setting the docking region. The docking grid box for α-glucosidase and α-amylase were assigned based on the coordinates of the native ligand at (x = −13.92, y = −38.29, z = 95.23) and (x = 44.03, y = 22.72, and z = −11.87), respectively. Redocking the native ligand was conducted to validate docking protocols and calculated their root mean square deviation (RMSD) with 100 conformations limit numbers. The docking protocol was deemed valid if the RMSD value was < 2 Å [22]. The docking process was carried out with Glide in extra precision (XP) mode under rigid receptors and flexible ligand conditions. The molecular mechanics-generalized Born surface area (MM-GBSA) was calculated to assess the docking pose of the ligands and determine the potency of each compound [23-26]. The "ligand interactions panel" on Maestro Schrodinger was used to visualize the molecular interactions. The results provide valuable insights into the binding modes and mechanisms of these receptors. It could help to guide the design of new compounds with improved efficacy and specificity.

■ RESULTS AND DISCUSSION

Computer-Aided Drug Discovery

Computer-aided drug discovery is the most important tool to predict drug activity through computational structure-based drug discovery. The relationship between sites of protein action and compounds acting as ligands can be explained using a variety of software. In point of fact, a physics-based equation is used to determine the binding free energy [27]. The α-glucosidase is an essential enzyme that is found on the luminal surface of enterocytes that functions to regulate blood glucose by converting complex carbohydrates into absorbable glucose, which is required for energy metabolism [28-29]. The removal of the anomeric carbon from the glucosyl group and the glycosidic oxygen (C_1–O) is the first step in the hydrolysis reactions carried out by α-glucosidases. Then, the glucosyl group is replaced by a proton from water resulting in the process of hydrolysis and transglycosylation exchange process between glucosyl residues and the protons [30]. Acarbose (Fig. 2) inhibits

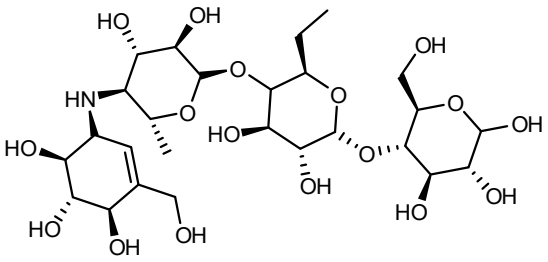


Fig 2. Acarbose

intestinal α-glucosidases, the enzymes responsible for the metabolism of complex carbohydrates into absorbable monosaccharide units in a reversible manner. This mechanism might be used to identify and develop new diabetic medications that are useful for showing diabetes progression [31-34].

Molecular Docking

Molecular docking is an important and helpful tool to predict the binding affinity of molecules to proteins [6]. In this study, the docking protocol was validated by internal validation after redocking the native ligand in its original positions resulting in RMSD values of 1.812 and 1.165 Å for α-glucosidase and α-amylase receptors. Based on these findings, it is possible to determine the test compound's activity against α-glucosidase as well as α-amylase receptors using either docking protocol [22]. Prior to docking the molecule, RMSD was used to determine the native acarbose ligand's docking position, as depicted in Fig. 3.

Compounds NBB **1**, **2**, and **3** with aromatic parts in their structures were evaluated for the protein-ligand complexes in the structure-activity relationship. It was discovered that a more negative score of binding affinity indicated a stronger binding. This ligand-protein binding process is correlated with this score which is also known as the change of the free energy. The measurement of how strong the interaction between the ligand and the protein is often directly related to the potential for ligand activity [35-36]. NBB without substituents resulted in an MMGBSA score of −0.45 on the binding affinity of complexes ligand-receptors. The *ortho* positions of the NBB derivatives with EDG (-Cl, -OH and -OMe) were −18.28, −6.71, and −21.94 kcal/mol, respectively. As can be seen in Table 1 and Fig. 4, the results of the ligand-receptor binding indicated that these compounds were bound to the same residues (Ala284, Asp282 and Asp616) in the entry area of the α-glucosidase active site.

The native acarbose inhibitor was validated by a redocking process on α-amylase in its original positions, which resulted in an RMSD value of 1.165 Å. In Table 2, the docking result between NBB derivatives and the α-amylase receptors showed that the value of MMGBSA as a binding affinity score for the ligand-receptors complex had a nearly equal range between −43.09 to −39.42, with native ligand acarbose −46.51 kcal/mol. Similar to α-glucosidase, the NBB grid score, −20.78 kcal/mol, was found to be greater than the NBB derivatives. Through the use of a hydrogen bond, NBB derivatives were able to interact with the same amino acids in the cavity of α-amylase Gly110 in a strong π-π interaction [37]. It should

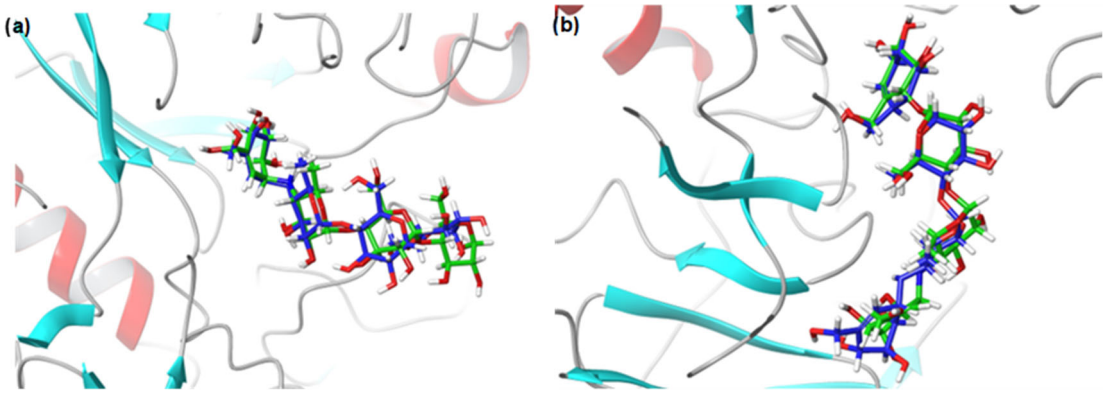


Fig 3. The acarbose native ligand on (a) α-glucosidase and (b) α-amylase receptors (Blue = original position and green = native ligand after redocking)

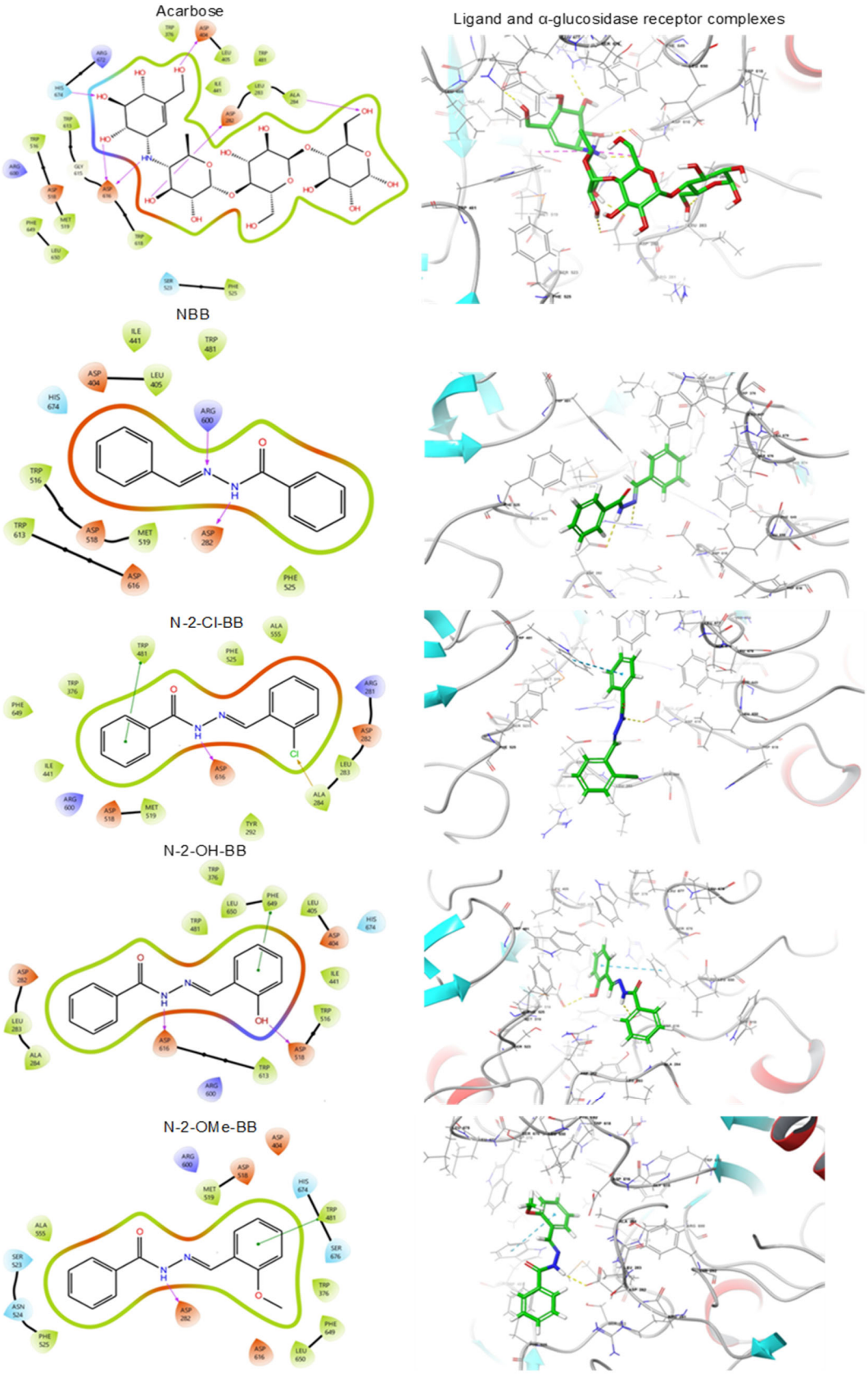


Fig 4. The binding of NBB derivatives and α-glucosidase

Table 1. Molecular docking result against α-glucosidase receptor

No.	Compound	MMGBSA (kcal/mol)	Amino acid interaction
1	*N*′-benzylidenebenzohydrazide	−0.45479	**Asp282**, Arg600
2	*N*′-(2-chlorobenzylidene)benzohydrazide, **1**	−17.2886	**Ala284**, Trp481, **Asp616**
3	*N*′-(2-hydroxybenzylidene)benzohydrazide, **2**	−6.71190	Asp518, **Asp616**, Phe649
4	*N*′-(2-methoxybenzylidene)benzohydrazide, **3**	−21.9442	**Asp282**, Trp481
5	Acarbose (Native ligand)	−32.6238	**Asp 282**, **Ala284**, Asp404, **Asp616**, His674

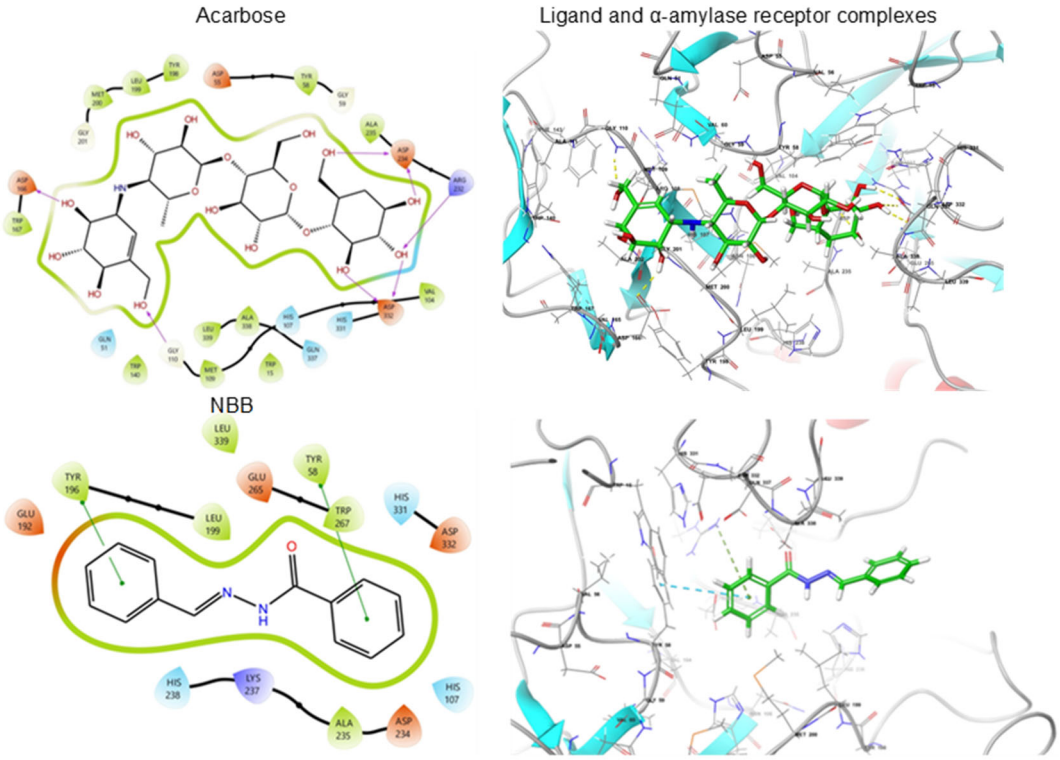
Table 2. Molecular docking results against α-amylase receptor

No.	Compound	MMGBSA (kcal/mol)	Amino acids interaction
1	Acarbose (Native ligand)	−46.5148	**Gly110**, Asp166, Asp234, Arg232, Asp332
2	*N*′-benzylidenebenzohydrazide	−20.7892	Tyr58, Tyr196
3	*N*′-(2-chlorobenzylidene)benzohydrazide, **1**	−42.8137	**Gly110**, Ala111
4	*N*′-(2-hydroxybenzylidene)benzohydrazide, **2**	−43.0944	**Gly110**, Gln51
5	*N*′-(2-methoxybenzylidene)benzohydrazide, **3**	−39.3278	**Gly110**, Ala111

be noted that NBB was not involved in binding interactions with Gly110 and other amino acid residues that include the native ligand (Table 2).

Through a variety of hydrophobic and hydrogen bonds, the active site of residue α-amylase receptor with NBB and its derivates can be followed in Fig. 5.

The compounds synthesized exhibit affinity energy values that are suboptimal compared to native ligands. However, some of the synthesized compounds show interactions with the catalytic site of the α-glucosidase receptor, specifically *N*′-(2-chlorobenzylidene)benzo hydrazide, *N*′-(2-hydroxybenzylidene)benzohydrazide,



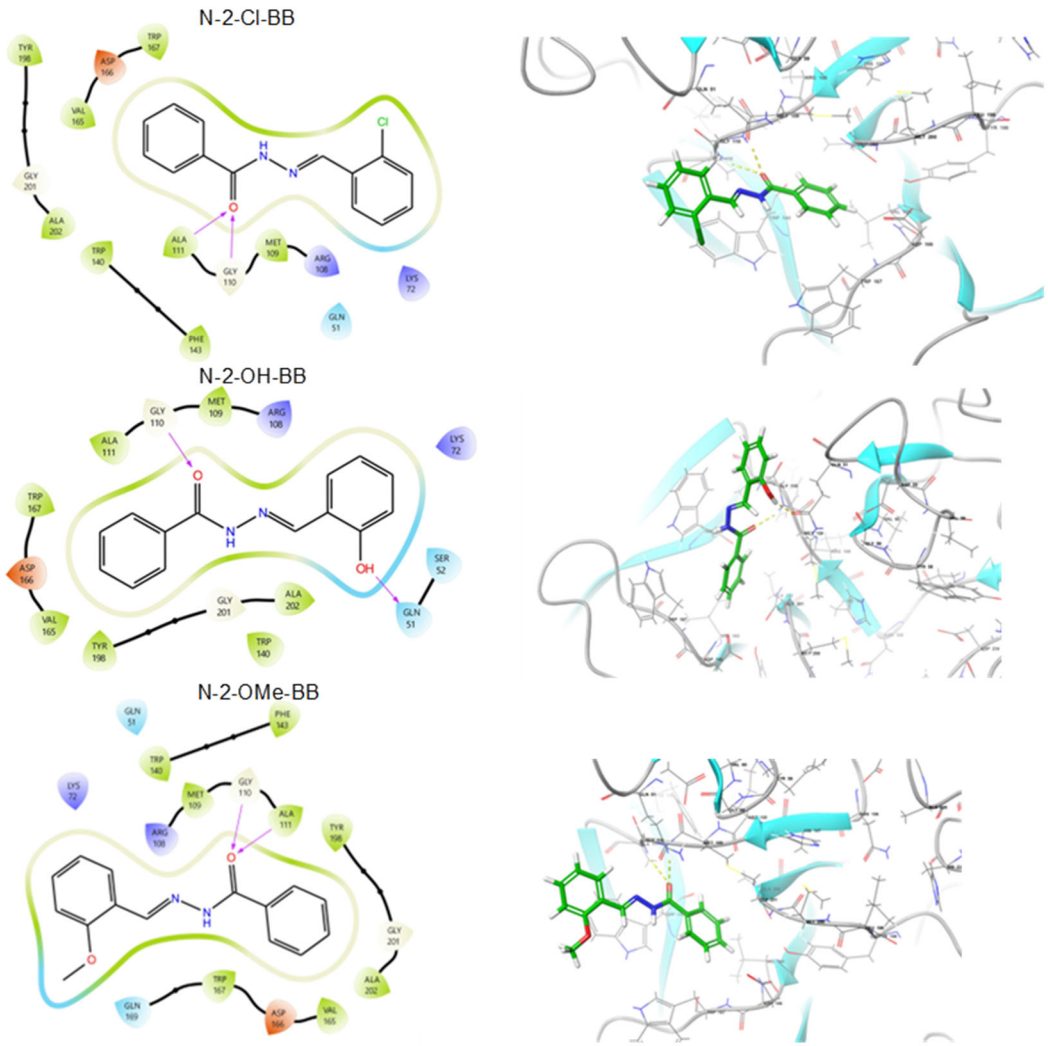


Fig 5. The binding of NBB and derivatives toward α-amylase receptor

and *N*'-(3-nitrobenzylidene)benzohydrazide on amino acids Asp518 and Asp616. Although these interactions can inhibit the activity of the α-glucosidase enzyme, they do not perform as well as the native ligand due to the lack of other molecular interactions that could enhance the affinity of the interaction. Despite this limitation, these three compounds hold the potential for further development as α-glucosidase inhibitor agents [38].

■ CONCLUSION

In drug design, the molecular docking technique has been used extensively to predict the ligands-receptor interactions. Substituents on the benzene ring play a role in antidiabetic activity. NBB derivatives ligand with electron-donating groups at the *ortho* position has the potential to increase the activity of antidiabetic target receptors for both α-glucosidase and α-amylase. Numerous functional groups and para positions will be performed in the subsequent experiment.

■ ACKNOWLEDGMENTS

The authors would like to thank Research, Innovation and Community Development and LPPM, Airlangga University for funding this research number 1076/UN3.15/PT/2022 through the "*Riset Kolaborasi Indonesia*" Research Grant organized by Ministry of Education, Culture, Research and Technology, Republic Indonesia in FY 2022.

■ AUTHOR CONTRIBUTIONS

Yusuf Syarif Alam, Nur Rahmayanti Afifah, Arif Fadlan conducted the synthesis experiment, Tutik Sri Wahyuni and Saipul Maulana performed docking analysis. Pratiwi Pudjiastuti, Fahimah Martak and Arif Syukri wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

■ REFERENCES

[1] Ramadhan, R., and Phuwapraisirisan, P., 2015, Arylalkanones from *Horsfieldia macrobotrys* are effective antidiabetic agents achieved by α-glucosidase inhibition and radical scavenging, *Nat. Prod. Commun.*, 10 (2), 325–328.

[2] Poovitha, S., and Parani, M., 2016, *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.), *BMC Complementary Altern. Med.*, 16 (1), 185.

[3] Dirir, A.M., Daou, M., Yousef, A.F., and Yousef, L.F., 2022, A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes, *Phytochem. Rev.*, 21 (4), 1049–1079.

[4] Tan, K., Tesar, C., Wilton, R., Jedrzejczak, R.P., and Joachimiak, A., 2018, Interaction of antidiabetic a-glucosidase inhibitors and gut bacteria α-glucosidase, *Protein Sci.*, 27 (8), 1498–1508.

[5] Bashary, R., Vyas, M., Nayak, S.K., Suttee, A., Verma, S., Narang, R., and Khatik, G.L., 2020, An Insight of alpha-amylase inhibitors as a valuable tool in the management of type 2 diabetes mellitus, *Curr. Diabetes Rev.*, 16 (2), 117–136.

[6] Kaur, N., Kumar, V., Nayak, S.K., Wadhwa, P., Kaur, P., and Sahu, S.K., 2021, Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review, *Chem. Biol. Drug Des.*, 98 (4), 539–560.

[7] Taha, M., Ismail, N.H., Lalani, S., Fatmi, M.Q., tul-Wahab, A., Siddiqui, S., Khan, K.M., Imran, S., and Choudary, M.I., 2015, Synthesis of novel inhibitors of α-glucosidase based on the benzothiazole skeleton containing benzohydrazide moiety and their molecular docking studies, *Eur. J. Med. Chem.*, 92, 387–400.

[8] Ullah, H., Uddin, I, Rahim, F., Khan, F., Sobia, S., Taha, S.M., Khan, M.U., Hayat, S., Ullah, M., Gul, Z., Ullah, S., Zada, H., and Hussain, J., 2022, *In vitro* α-glucosidase and α-amylase inhibitory potential and molecular docking studies of benzohydrazide based imines and thiazolidine-4-one derivatives, *J. Mol. Struct.*, 1251, 132058.

[9] Fan, M., Yang, W., Peng, Z., He, Y., and Wang, G., 2023, Chromone-based benzohydrazide derivatives as potential α-glucosidase inhibitor: Synthesis, biological evaluation and molecular docking study, *Bioorg. Chem.*, 131, 106276.

[10] Jubie, S., Ashish, W., Sabaritha, K., Nishanthini, P., Thomas, A., and Antony, J., 2016, Synthesis and *in-vitro* anti-cancer screening of N^1 [(substituted phenyl)benzylidene]benzohydrazides, *J. Pharm. Sci. Res.*, 8 (7), 582–585.

[11] Lanka, G., Bathula, R., Bhargavi, M., and Potlapally, S.R., 2019, Homology modeling and molecular docking studies for the identification of novel potential therapeutics against human PHD3 as a drug target for type 2 diabetes mellitus, *J. Drug Delivery Ther.*, 9 (4), 265–273.

[12] Johnston, R.C., Yao, K., Kaplan, Z., Chelliah, M., Leswing, K., Seekins, S., Watts, S., Calkins, D., Chief Elk, J., Jerome, S.V., Repasky, M.P., and Shelley, J.C., 2023, Epik: pK_a and protonation state prediction through machine learning, *J. Chem. Theory Comput.*, 19 (8), 2380–2388.

[13] Lu, C., Wu, C., Ghoreishi, D., Chen, W., Wang, L., Damm, W., Ross, G.A., Dahlgren, M.K., Russell, E., Von Bargen, C.D., Abel, R., Friesner, R.A., and Harder, E.D., 2021, OPLS4: Improving force field accuracy on challenging regimes of chemical space, *J. Chem. Theory Comput.*, 17 (7), 4291–4300.

[14] Choudhary, M.I., Shaikh, M., tul-Wahab, A., and ur-Rahman, A., 2020, *In silico* identification of potential inhibitors of key SARS-CoV-2 3CL hydrolase (Mpro) *via* molecular docking, MMGBSA predictive binding energy calculations,

and molecular dynamics simulation, *PLoS One*, 15 (7), e0235030.

[15] Khairul Ikram, N.K., Durrant, J.D., Muchtaridi, M., Zalaludin, A.S., Purwitasari, N., Mohamed, N., Abdul Rahim, A.S., Lam, C.K., Normi, Y.M., Abd Rahman, N., Amaro, R.E., and Wahab, H.A., 2015, A virtual screening approach for identifying plants with anti H5N1 neuraminidase activity, *J. Chem. Inf. Model.*, 55 (2), 308–316.

[16] Schrödinger Release 2022-1, 2021, *Desmond Molecular Dynamics System*, D.E. Shaw Research, New York.

[17] Fusani, L., Palmer, D.S., Somers, D.O., and Wall, I.D., 2020, Exploring ligand stability in protein crystal structures using binding pose metadynamics, *J. Chem. Inf. Model.*, 60 (3), 1528–1539.

[18] Rostkowski, M., Olsson, M.H.M., Søndergaard, C.R., and Jensen, J.H., 2011, Graphical analysis of pH-dependent properties of proteins predicted using PROPKA, *BMC Struct. Biol.*, 11 (1), 6.

[19] Schrödinger Release 2022-1, 2022, *Protein Preparation Wizard*, Epik, Schrödinger, LLC, New York.

[20] Jokinen, E.M., Niemeläinen, M., Kurkinen, S.T., Lehtonen, J.V., Lätti, S., Postila, P.A., Pentikäinen, O.T., and Niinivehmas, S.P., 2023, Virtual screening strategy to identify retinoic acid-related orphan receptor γt modulators, *Molecules*, 28 (8), 3420.

[21] Friesner, R.A., Banks, J.L., Murphy, R.B., Halgren, T.A., Klicic, J.J., Mainz, D.T., Repasky, M.P., Knoll, E.H., Shelley, M., Perry, J.K., Shaw, D.E., Francis, P., and Shenkin, P.S., 2004, Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, *J. Med. Chem.*, 47 (7), 1739–1749.

[22] Hevener, K.E., Zhao, W., Ball, D.M., Babaoglu, K., Qi, J., White, S., and Lee, R.E., 2009, Validation of molecular docking programs for virtual screening against dihydropteroate synthase, *J. Chem. Inf. Model.*, 49 (2), 444–460.

[23] Schrödinger Release 2022-1, 2022, *Glide*, Schrödinger, LLC, New York.

[24] Genheden, S., and Ryde, U., 2015, The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities, *Expert Opin. Drug Discovery*, 10 (5), 449–461.

[25] Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C., and Mainz, D.T., 2006, Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes, *J. Med. Chem.*, 49 (21), 6177–6196.

[26] Schrödinger Release 2022-1, 2022, *Prime*, Schrödinger, LLC, New York.

[27] Al-Karmalawy, A.A., Dahab, M.A., Metwaly, A.M., Elhady, S.S., Elkaeed, E.B., Eissa, I.H., and Darwish, K.M., 2021, Molecular docking and dynamics simulation revealed the potential inhibitory activity of ACEIs against SARS-CoV-2 targeting the *h*ACE2 receptor, *Front. Chem.*, 9, 661230.

[28] Kumar, S., Narwal, S., Kumar, V., and Prakash, O., 2011, α-Glucosidase inhibitors from plants: A natural approach to treat diabetes, *Pharmacogn. Rev.*, 5 (9), 19–29.

[29] Kazmi, M., Zaib, S., Ibrar, A., Amjad, S.T., Shafique, Z., Mehsud, S., Saeed, A., Iqbal, J., and Khan, I., 2018, A new entry into the portfolio of α-glucosidase inhibitors as potent therapeutics for type 2 diabetes: Design, bioevaluation and one-pot multi-component synthesis of diamine-bridged coumarinyl oxadiazole conjugates, *Bioorg. Chem.*, 77, 190–202.

[30] Feng, Y., Nan, H., Zhou, H., Xi, P., and Li, B., 2022, Mechanism of inhibition of α-glucosidase activity by bavachalcone, *Food Sci. Technol.*, 42, e123421.

[31] Hakim, R.W., Fadilah, F., Tarigan, T.J.E., Jusman, S.W.A., and Purwaningsih, E.H., 2021, Molecular study of *Acalypha indica* to leptin, alpha glucosidase, and its antihyperglycemic effect on alpha alucosidase, *Pharmacogn. J.*, 13 (6s), 1639–1647.

[32] Li, D.Q., Qian, Z.M., and Li, S.P., 2010, Inhibition of three selected beverage extracts on α-glucosidase and rapid identification of their active compounds using HPLC-DAD-MS/MS and biochemical detection, *J. Agric. Food Chem.*, 58 (11), 6608–6613.

[33] Kim, K.T., Rioux, L.E., and Turgeon, S.L., 2014, Alpha-amylase and alpha-glucosidase inhibition is

differentially modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*, *Phytochemistry*, 98, 27–33.

[34] Papoutsis, K., Zhang, J., Bowyer, M.C., Brunton, N., Gibney, E.R., and Lyng, J., 2021, Fruit, vegetables, and mushrooms for the preparation of extracts with α-amylase and α-glucosidase inhibition properties: A review, *Food Chem*., 338, 128119.

[35] Lahlou, M., 2007, Screening of natural products for drug discovery, *Expert Opin. Drug Discovery*, 2 (5), 697–705.

[36] Xue, Q., Liu, X., Russell, P., Li, J., Pan, W., Fu, J., and Zhang, A., 2022, Evaluation of the binding performance of flavonoids to estrogen receptor alpha by Autodock, Autodock Vina and Surflex-Dock, *Ecotoxicol. Environ. Saf.*, 233, 113323.

[37] Peng, W., Shen, H., Lin, B., Han, P., Li, C., Zhang, Q., Ye, B., Rahman, K., Xin, H., Qin, L., and Han, T., 2018, Docking study and antiosteoporosis effects of a dibenzybutane ligan isolated from *Litsea cubeba* targeting Capthepsin K and MEK1, *Med. Chem. Res.*, 27 (9), 2062–2070.

[38] Roig-Zamboni, V., Cobucci-Ponzano, B., Iacono, R., Ferrara, M.C., Germany, S., Bourne, Y., Parenti, G., Morraci, M., and Sulzenbacher, G., 2017, Structure of human lysosomal acid α-glucosidase–a guide for the treatment of Pompe disease, *Nat. Commun.*, 8 (1), 1111.