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Synthesis, molecular docking, and pharmacological evaluation of halobenzodithiophene derivatives against alpha-glucosidase, urease, and free radical production

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Abstract: Benzodithiophenes are heterocyclic compounds that have various medicinal and industrial applications. In the present study, halobenzodithiophene, the simplest benzofused thiophene, and its derivatives were synthesized and evaluated against alpha-glucosidase, urease, and free radical production. In the alpha-glucosidase inhibition assay, compound 2,2-bisbenzothiophne (**1**) exhibited potent activity with IC_{50} = 135 ± 0.51 μM, while its derivative 2,7-bis(butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*']dithiophene (**2**) exhibited promising inhibition with IC_{50} = 263 ± 0.32 μM. In the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, compound **2** exhibited promising activity with IC_{50} = 33 ± 0.42 μM, while compound **1** showed moderate inhibition in the urease inhibition assay. Molecular docking studies determined the possible interaction of benzodithiophene and alpha-glucosidase on the basis of binding energy and scoring function. Structure–activity relationship analysis revealed that compound 2,7-bis (butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*'] dithiophene (**2**) containing two chlorine substitutions exhibited more alpha-glucosidase inhibition (IC_{50} = 263 ± .0.32 μM) than other derivatives. Moreover, compound 2,7-bis (butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*'] dithiophene (**2**) with two chlorine substitutions exhibited potent DPPH radical scavenging activity compared to other derivatives.

Key words: Halobenzothiophenes, alpha-glucosidase enzyme, urease enzyme, radical scavenging assay, molecular docking, structure–activity relationship

1. Introduction

At present, enzymes are excellent targets for pharmacological intervention due to their imperative roles in biological systems and life processes. Enzyme inhibition studies have attracted much interest, particularly in pharmaceutical research, and several useful drugs have been discovered. Since small halogenated organic compounds have shown great potential for inhibiting various biological disorders, various enzymes have attracted the attention of pharmaceutical companies for new drug discovery. It is therefore convincing that specific enzyme inhibition will play a key role in pharmaceutical research in future.[1]

Alpha-glucosidase is an enzyme under extensive investigation due to its potential role in the management of diabetes mellitus. It is released in the small intestine and involved in the process of carbohydrate digestion

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and absorption. It converts poly- and oligosaccharides into monomers by hydrolysis, and as a result the concentration of glucose in the blood increases.[2] Alpha-glucosidase inhibitors slow down the digestion process and carbohydrate absorption. As a result, a rapid increase in blood glucose level after a meal can be controlled independent of insulin.[3] Another important enzyme, urease (EC 3.5.1.5), is a nickel-dependent metalloenzyme that produces ammonia and carbamate by catalyzing the hydrolysis of urea. In the next step, carbamate is converted to carbonic acid and ammonia. As a result, the pH of the stomach solution is changed, becoming more suitable for dangerous pathogens to survive and grow. Urease is involved in major pharmacological disorders, such urinary and gastrointestinal tract infections and stomach ulcers. One useful approach to curing duodenal ulcers is killing *H. pylori* with antibiotics; however, antibiotic resistance and its adverse effects are important challenges that make the situation worse. In these circumstances, urease inhibitors have attracted much attention. Discovery of new and safer potential antiulcer drugs is highly desirable.[4–6]

Reactive oxygen species (ROS), such as free radicals, peroxides, and oxygen ions, are produced as a result of several biological processes in the body. These ROS cause many health problems such as carcinogenesis, coronary heart disease, and ageing problems.[7,8] Synthetic inhibitors of ROS, also known as antioxidants, have been used to inhibit the production of free radicals by either retarding their formation or scavenging them to prevent serious health problems.[9]

Benzodithiophenes are pharmaceutically significant heterocyclic compounds that have attracted the interest of medicinal chemists and biochemists over the last two decades.[10] In recent years, synthesis of annulated thiophene-fused aromatic compounds with an extended conjugation and their biological properties have been focused on extensively.[11] These compounds are of great industrial and pharmacological importance due to symmetry pattern, molecular structure, and their mechanism of formation. Among the various developed methodologies, palladium-catalyzed reactions play a special role not only in the formation of a new bond but also the direct insertion of a variety of active functional groups of pharmacological importance in modern day science.[12]

The significant effect of benzothiophene derivatives on various biological disorders motivated us to synthesize new benzodithiophene derivatives and investigate them against pharmacologically important targets such as alpha-glucosidase and urease as well as free radical production. It is a part of our continuous efforts towards the synthesis and discovery of new heterocyclic compounds with expected biological activities.

2. Results and discussion

Previously, benzothiophene derivatives have been used extensively for their antiallergic, analgesic, antiinflammatory, and ocular hypotensive activities and in the treatment of osteoporosis.[13] Some benzothiophene derivatives have shown anticoagulant and selective estrogen receptor modulator activities.[14]

2.1. Synthesis outcome

In the present work, we examined the selective synthesis and key cyclization pattern of highly strained benzodithiophenes ring formation that was overlooked in several earlier reports, as shown in Figure 1a.

We have previously reported structural correctness and reassignments of this particular cyclization pattern of hindered benzodithiophene ring formation that was overlooked for almost 40 years and reported incorrectly in several earlier publications. The proposed mechanism of the formation of 3,8-dichlorobenzo[1,2-*b*;6,5-*b*']dithiophene is shown in Figure 1b, while details related to the synthesis, mechanism, NMR spectroscopy, crystallography, and computational quantum studies were also discussed in our previous publications.[15,16]

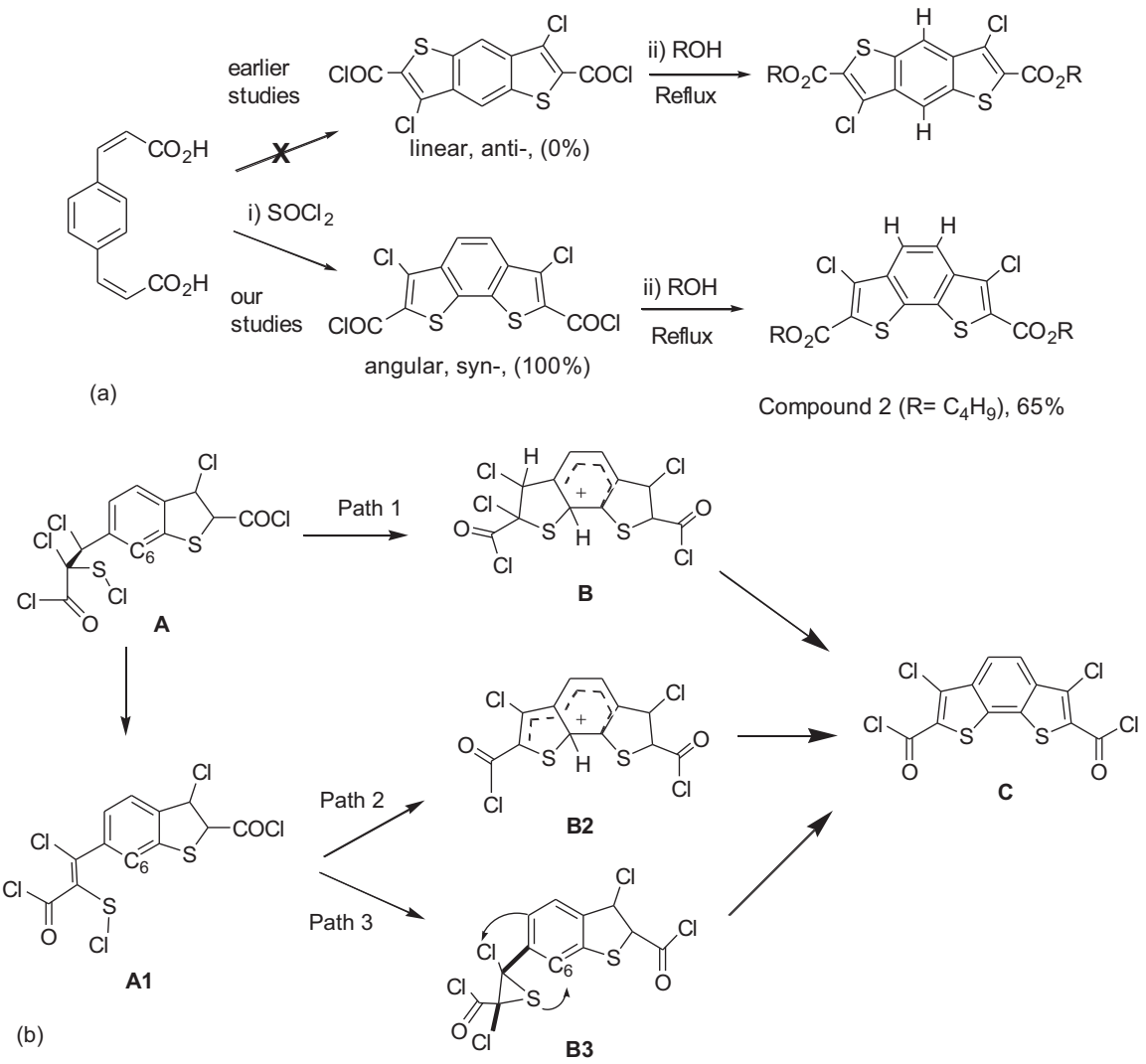


Figure 1. Scheme for the synthesis of compound **2.** Reagents and conditions: (i) $SOCl_2$, pyridine, 5 h, 140 °C; (ii) ROH (R = Bu) 2 h, reflux. b. Scheme for the proposed mechanism for the formation of 3,8-dichlorobenzo[1,2-*b*;6,5-*b*']dithiophene **C**. Adopted from our previous publications.[15,16]

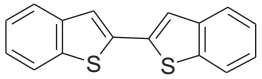
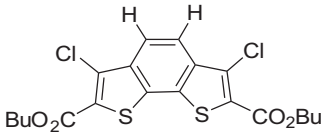
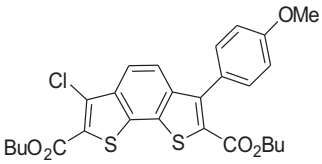
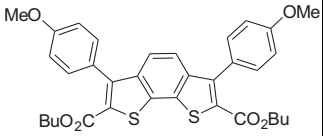
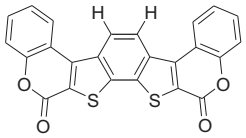
Various benzodithiophene derivatives were synthesized and separated via column chromatography. In this study, benzothiophenes derivatives were investigated for their inhibitory potential against alpha-glucosidase and urease as well as for their free-radical–scavenging activity.

2.2. Alpha-glucosidase inhibition

In this study, compound 2,2-bisbenzothiophne (**1**) exhibited potent activity with $IC_{50} = 135 \pm 0.5$ μM in the alpha-glucosidase inhibition assay, while 2,7-bis(butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*'] dithiophene (**2**) exhibited promising inhibition $IC_{50} = 263 \pm 0.32$ μM, as shown by and in Figure 2, respectively. Similarly, derivatives such as 2,7-bis(butoxycarbonyl)-3-(4-methoxyphenyl)-6-chlorobenzo[1,2-*b*;6,5-*b*'] dithiophene (**3**) and 2,7-bis(butoxycarbonyl)-3,6-bis(2-methoxyphenyl)benzo[1,2-*b*;6,5-*b*']dithiophene (**4**) showed good inhibition, with $IC_{50} = 705 \pm 1.0$ μM and 789 ± 1.0 μM, respectively. In addition, compound benzo [1,2-*b*:6,5-*b*']

dithienobis[2,3-c]chromen-6-one (**5**) showed moderate activity with 48 ± 0.5% inhibition at 1 mM, as shown in Table 1. In this assay, acarbose (IC_{50} = 789 ± 1.5 μM) was used as a standard inhibitor against alpha-glucosidase.

Table 1. Inhibitory potential of benzodithiophene and its derivatives.

Compound name	Structure	Alpha-glucosidase inhibition (1 mM)	Urease inhibition (1 mM)	Antioxidant activity (1 mM)
2,2-Bisbenzothiophne (**1**)		86 ± 0.5% (IC_{50} = 135 ± 0.51 µM)	45 ± 1%	NA
2,7- Bis(butoxycarbonyl) -3,6-dichlorobenzo[1,2-*b*;6,5-*b*']dithiophene (**2**)		65 ± 1.5% (IC_{50} = 263 ± 0.32 µM)	NA	88.25% (IC_{50} = 33 ± 0.42 µM)
2,7-Bis(butoxycarbonyl)-3-(4-methoxyphenyl)-6-chlorobenzo[1,2-*b*;6,5-*b*']dithiophene (**3**)		56 ± 1% (IC_{50} = 705 ± 1.0 µM)	NA	20.11%
2,7-Bis(butoxycarbonyl)-3,6-bis(2-methoxyphenyl)benzo[1,2-*b*;6,5-*b*']dithiophene (**4**)		53 ± 0.5% (IC_{50} = 789 ± 1.0 µM)	NA	NA
Benzo[1,2-*b*:6,5-*b*']dithienobis[2,3-c]chromen-6-one (**5**)		48 ± 0.5%	NA	NA

NA = Not active

2.3. Urease inhibition

In the urease inhibition assay, only compound **1** showed a moderate inhibition (45 ± 1%), while all other samples were inactive. The moderate inhibition of compound **1** can be attributed to the structural features and binding interactions of compound **1** with active sites of urease, which work under a lock-and-key mechanism, while other derivatives (inactive) lack these structural features and binding interactions.

2.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity

In the DPPH-radical scavenging assay, compound **2** exhibited promising activity (IC_{50} = 33 ± 0.42 μM), as shown in Figure 3, while other compounds were inactive. Moreover, in the urease inhibition assay, only compound **1** showed moderate activity (43 ± 1% inhibition at 1 mM). Butylated hydroxy anisole (IC_{50} = 0.22 mM) and thiourea (IC_{50} = 21.01 ± 0.51 μM) were used as standard inhibitors in the radical-scavenging assay and the urease inhibition assay, respectively.

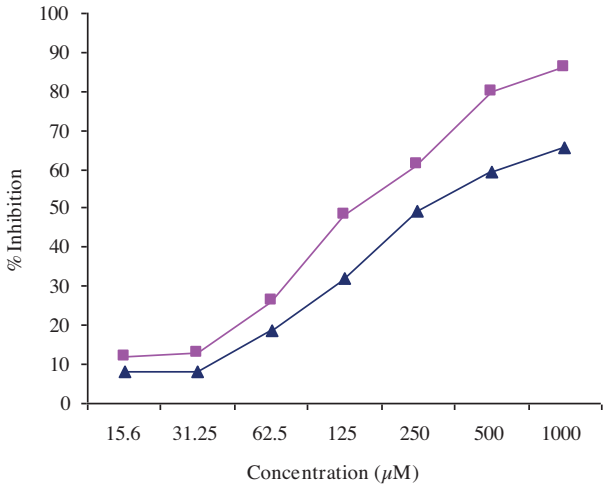


Figure 2. Alpha-glucosidase inhibition (IC_{50}) of compound **1** (■) and compound **2** (▲).

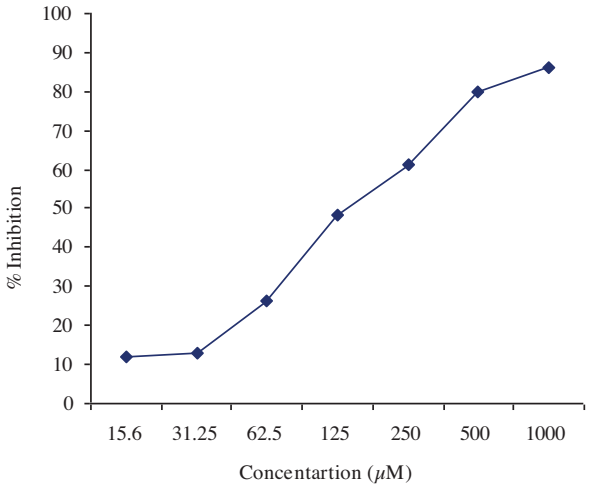


Figure 3. Free-radical–scavenging activity (IC_{50}) of compound **2**.

2.5. Docking studies outcome

During molecular docking studies, Molecular Operating Environment (MOE) software (Chemical Computing Group; Montreal, QC, Canada) was employed to analyze binding sites of the target (alpha-glucosidase). The possible binding sites of ligands molecules in alpha-glucosidase are: ALA338, THR339, GLY342, ALA343, ASP344, GLU345, ASP346, PRO347, HIS348, ALA349, PRO351, LYS352, LYS352, LEU373, GLY374, LEU375, PRO376, ARG429, GLU432, LEU433, ARG437, GLN438, ASP441, ASN443, ALA444, THR445, HIS515, THR517, THR517, PRO528, ALA529, TYR530, GLN531 (Figure 4). Protein geometric arrangement of amino acid residues in allowed and disallowed regions indicates the quality of target proteins.

In the present study, the association strength of protein and ligand is determined on the basis of minimum binding energy and scoring function. In order to determine target protein specificity on structural basis, we utilized the ligand–target binding approach to analyze structural complexes of alpha-glucosidase with selected compounds. Finally, these ligands were docked with the potential active sites of target molecules and binding energies were calculated. Briefly, atomic affinity potentials were utilized at each step of simulation to measure the interaction energy of ligands and protein. The lowest binding energy cluster reflected the representative binding state in docking results, as shown in Figure 5. The lowest binding energies indicated that target proteins were docked successfully with ligand molecules. Here, the MOE ligand interaction analysis feature was used to obtain binding interaction diagrams. These results revealed that compound **3** (–6.4082346 kcal/mol), compound **4** (–6.770165 kcal/mol), and compound **5** (–6.1879964 kcal/mol) are effective binders of alpha-glucosidase (Table 2).

2.6. Structure–activity relationship analysis

Structure–activity relationship studies showed that compound 2,7-bis(butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*'] dithiophene containing two chlorine substitutions exhibited more alpha-glucosidase inhibition (IC_{50} = 263 ± 2.0 μM) than other derivatives having one chlorine substitute, such as compound **3** (IC_{50} = 789 ± 1.5 μM) and compound **4** (IC_{50} = 705 ± 1.5 μM), and with no chlorine substitution, such as compound **5** (35.2 ± 1.5% inhibition at 1 mM), as shown in Table 1. Interestingly, compound 2,7-bis(butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*;6,5-*b*'] dithiophene (**2**), containing two chlorine substitutions, exhibited potent DPPH-

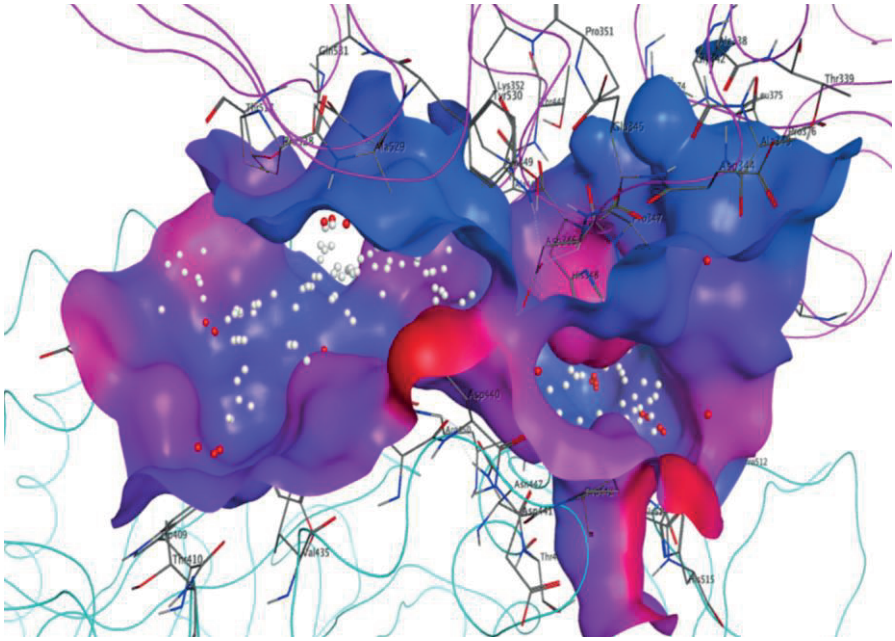


Figure 4. Isolated active binding sites of alpha-glucosidase enzyme target analyzed by Molecular Operating Environment.

Table 2. Energy values obtained during docking analysis of compounds as ligand molecules against alpha-glucosidase as target molecules.

Compounds	Compound **1**	Compound **2**	Compound **3**	Compound **4**	Compound **5**
RSEQ	1	1	1	1	1
MSEQ	1	2	3	5	6
S	–4.9972	–5.68209	–6.40823	–6.77017	–6.188
RMSD_REFINE	1.9475	4.0576119	3.251621	3.2581472	2.9393084
E_CONF	43.3460	15.545682	57.65239	82.496132	117.51772
E_PLACE	–23.5115	–66.9648	–9.64047	0.772133	–65.3354
E_SCORE1	–11.016631	–14.328066	–11.014072	–13.242846	–12.520577
E_REFINE	–11.420643	–3.4923279	–17.8018	–10.011946	–10.15156
E_SCORE2	–5.6820898	–6.4082346	–5.7810273	–6.770165	–6.1879964

radical–scavenging activity (IC_{50} = 33 ± 1.5 μM) compared to other derivatives, which remained inactive against this assay. The possible action mechanism of these inhibitors can be attributed to the fact that inhibition in compounds **1** and **2** is an allosteric type, where inhibitors bind to the protein on site other than its active ligand-binding site. Compounds **3**–**5** exhibited inhibition by binding to the active site of alpha-glucosidase and thus blocked the normal substrate from bonding with the enzyme's active binding site during molecular docking studies, as shown in Figures 3 and 4.

2.7. Conclusion

In brief, we described the synthesis of various halobenzodithiophene derivatives, their enzyme inhibition activity against alpha-glucosidase and urease, and their free-radical–scavenging activity. In this work, two new potent

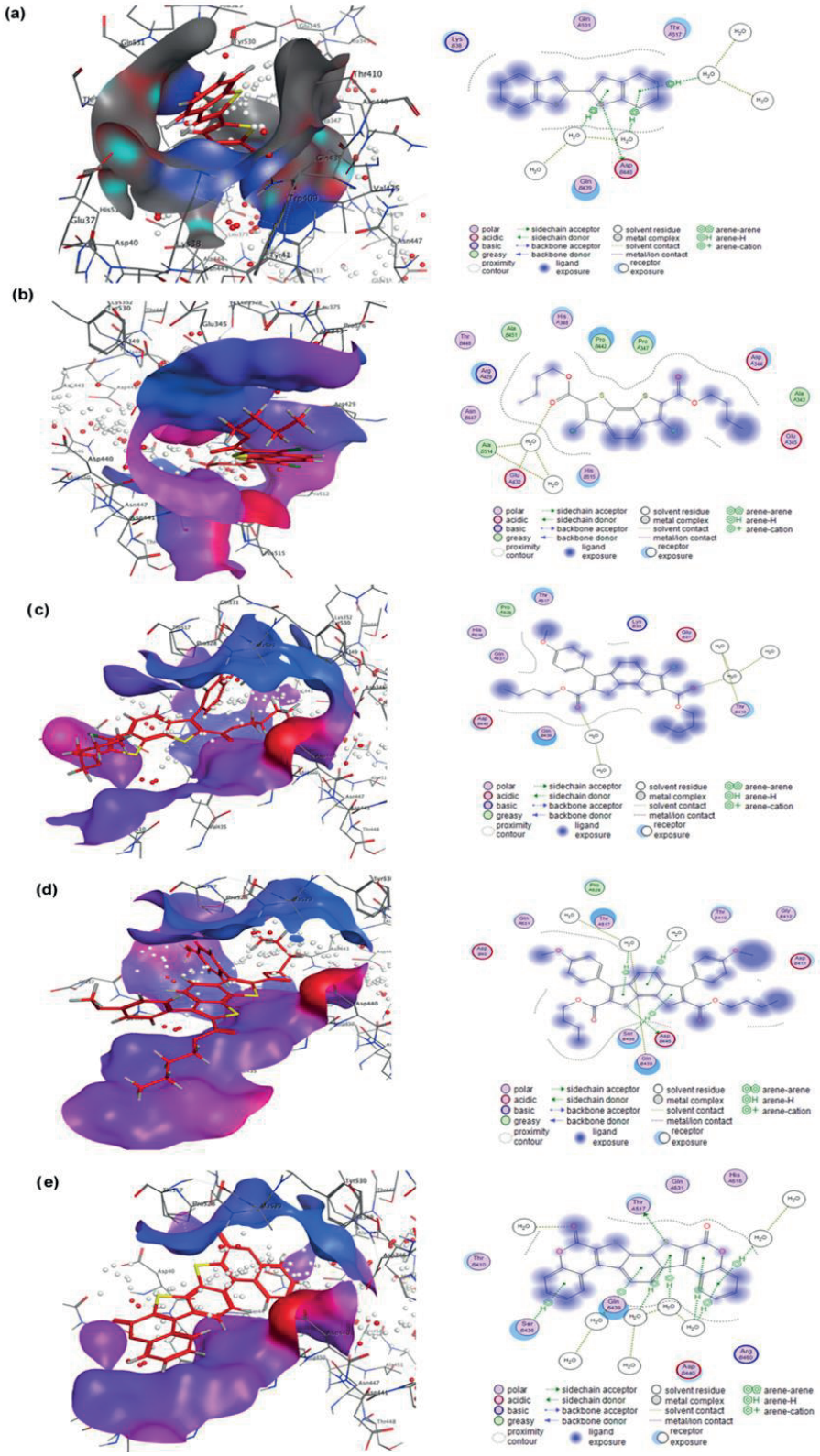


Figure 5. Docking visual and interaction analysis of ligands molecules with target enzyme from **1**–**5** (a–e), respectively.

inhibitors of alpha-glucosidase, compounds **1** and **2,** were identified. Moreover, compound **2** also exhibited promising antioxidant activity against free radical production. Our docking MD-based protocol effectively described stable and unstable binding modes and a careful comparison of different inhibitors. The structure–activity relationship studies derived from biochemical and computational data can significantly contribute to the identification and design of highly selective inhibitors as possible leads for the treatment of carbohydrate-mediated diseases and free radical production. Moreover, these inhibitors may serve as templates for new drug candidates in the future.

3. Experimental

3.1. Chemicals

Required chemicals and reagents such as alpha-glucosidase enzyme (E.C. 3.2.1.20), p-nitrophenyl α-D-glucopyranoside, acarbose, Na_2CO_3, K_2HPO_4, KH_2PO_4, dimethyl sulfoxide (DMSO), and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) were purchased from Sigma Aldrich (St. Louis, MO, USA). Absorbance was measured using a spectrophotometer (Molecular Devices; San Jose, CA, USA).

3.2. Research methodology for synthesis

The following research methodology was adopted for the synthesis of the below mentioned compounds.

3.2.1. Procedure for the synthesis of compound 2: 2,7-Bis(butoxycarbonyl)-3,6-dichlorobenzo[1,2-*b*:6,5-*b*'] dithiophene

Initially, 1,4-phenylene-diacrylic acid (2.00 g, 9.16 mmol) and pyridine in catalytic amounts (0.2 mL) were mixed while thionyl chloride (6 mL, 80 mmol) was added portionwise into it. This reaction mixture was heated at 140 °C for 5 h. The product solidified upon cooling as a greenish solid, and the excess amount of thionyl chloride was removed using reduced pressure. This product was then dissolved in 50 mL of benzene and n-BuOH (10 mL) was added to the benzene solution using inert conditions. Crude butyl ester **2** (white crystalline solid) was obtained (2.73 g, 65%); mp 95–97 °C after refluxing for 2 h.

IR (KBr): 2872, 2931, 2956, 3418 (s), 2736 (w), 1726, 1494, 1510, 1708(s), 1301, 1380, 1406, 1476 (m) 964, 1017, 1045, 1060, 1082, 1095, 1211, 1235 (s), 756, 804, 850, 932 (m), 715, 734, 756 (s) cm^{-1}. H NMR (300 MHz, $CDCl_3$): $\delta = 0.94$ (t, $J = 7.3$ Hz, 6 H, CH_3), 1.68–1.77 (m, 4 H, CH_2), 1.39–1.46 (m, 4 H, CH_2), 4.34 (t, $J = 6.5$ Hz, 4 H, OCH_2), 7.91 (s, 2 H, Ar).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 121.2$ (CH), 19.2, 30.6 (CH_2), 13.7 (CH_3), 65.9 (OCH_2), 160.8, 137.1, 132.9, 128.2, 126.2 (C).

GC-MS (EI, 70 eV): m/z (%) = 458 (100) ([M] +, 2 × 35 Cl), 257 (33), 329 (41) 346 (78).

HRMS (EI, 70 eV): m/z [M] + calcd. for $C_{20}H_{20}{}^{35}Cl_2O_4S_2$: 458.0180; found: 458.0171.

3.2.2. Procedure for the synthesis of compound 3: 2,7-Bis(butoxycarbonyl)-3-chloro-6-(4-methoxyphenyl)ben-zo[1,2-*b*:6,5-*b*']dithiophene

Starting with **2** (0.43 mmol, 200 mg), $Pd(PPh_3)_4$ (5 mol %, 25 mg), 4-methoxyphenylboronic acid (0.52 mmol, 78 mg, 1.2 equiv), K_3PO_4 (0.85 mmol, 185 mg, 2.0 equiv), and 1,4-dioxane (5 mL), as a result light greenish oil (compound **3**) was obtained (170 mg, 74%).

IR (KBr): 1716, 2872, 2930 (s), 1311, 1337, 1413, 1486, 1501, 1531 (m), 1071, 1114, 1175, 1274 (s), 875, 906 (s) cm^{-1}.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.77 (d, J = 8.8 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 4.37 (t, J = 6.2 Hz, 2H), 4.19 (t, J = 6.3 Hz, 2H), 3.87 (s, 3 H, OCH_3), 1.75–1.80 (m, 2 H), 1.55–1.64 (m, 2 H), 1.50–1.54 (m, 2 H), 0.99 (t, J = 7.1 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3 H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 13.6, 13.7 (CH_3), 19.1, 19.2 (CH_2), 30.4, 30.5 (CH_2), 55.2 (OCH_3), 65.3, 65.6 (OCH_2), 113.5, 120.3, 122.8 (C), 128.1, 128.6 (C), 125.3, 126.1 (CH), 130.9 (CH), 134.1 (CH),132.8 (C), 162.1, 160.9, 159.6, 144.3, 140.2, 136.2

3.2.3. Procedure for the synthesis of compound 4: 2,7-Bis(butoxycarbonyl)-3,6-bis(4-methoxyphenyl)benzo[1,2-*b*:6,5-*b*']dithiophene

Starting with **2** (0.21 mmol, 100 mg), 4-methoxyphenylboronic acid (0.53 mmol, 80 mg, 2.5 equiv), $NiCl_2$ (dppe) (5 mol %, 6.0 mg), K_3PO_4 (0.87 mmol, 185 mg, 4.0 equiv), and toluene (5 mL), as a result a yellowish viscous solid (compound 4) was obtained (115 mg, 88%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.4 Hz, 6H, CH_3), 1.26–1.32 (m, 4H), 1.56–1.60 (m, 4H), 3.86 (s, 6H, OCH_3), 4.20 (t, J = 6.5 Hz, 4H,OCH_2), 7.41 (s, 2H), 7.32 (d, J = 8.4 Hz, 4H), 7.01 (d, J = 8.4 Hz, 4H).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 13.7 (CH_3), 19.1 (CH_2), 30.5 (CH_2), 55.2(OCH_3), 65.3 (OCH_2), 126.5, 122.1, 113.5 (CH), 162.4, 159.6, 144.5, 139.5, 134.3, 131.0, 128.0 (C).

HRMS (EI, 70 eV): m/z [M] + calcd. for $C_{34}H_{34}O_6S_2$: 602.1764; found: 602.1764.

3.2.4. Procedure for the synthesis of compound 5: Benzo[1,2-*b*:6,5-*b*']dithienobis[2,3-c]chromen-6-one

Starting with **2** (0.21 mmol, 100 mg), 2-hydroxyphenylboronic acid (0.53 mmol, 73 mg, 2.5 equiv), $NiCl_2$ (dppe) (5 mol, 6.0 mg), K_3PO_4 (0.87 mmol, 185 mg, 4.0 equiv), and toluene (5 mL), as a result of this reaction, a white solid (compound 5) was isolated (50 mg, 54%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.81 (s, 2H), 7.59 (dd, J = 8.4, 1.6 Hz, 2H), 7.34–7.37 (m, 2H), 6.85–6.88 (m, 4H)

^{13}C NMR (150 MHz, $CDCl_3$): δ = 119.5, 115.4, 115.3 (CH), 120.5 (C), 135.7, 133.8, 129.6 (CH), 163.5 (C).

3.3. Biological activities

The following biological assays were performed for evaluation of the above-mentioned compounds.

3.3.1. Alpha-glucosidase enzyme inhibition assay protocol

A general procedure was used in vitro with slight modifications.

A solution of alpha-glucosidase (E.C. 3.2.1.20) was freshly prepared by dissolving α-glucosidase (0.1 mg) in 50 mM phosphate buffer (10 mL) with a pH 6.8. A 20-μL aliquot of inhibitor sample (1 mM) was premixed in a 96-well plate with 120 μL of phosphate buffer (pH 6.8), followed by 20 μL of 5 mM p-nitrophenyl α-D-glucopyranoside. The reaction mixture was mixed gently and then preincubated at 37 °C for 5 min. After preincubation, 20 μL of α-glucosidase enzyme solution was added to the reaction wells and the reaction plate was incubated again for 15 min at the same temperature. The control sample wells contained DMSO (20 μL)

in place of the inhibitor sample (20 μL). In order to terminate the reaction, 100 μL of Na_2CO_3 (200 mM) was added to each well after 15 min of incubation Enzyme inhabitation activity was measured at 405 nm via spectrophotometer. In this assay, acarbose was used as standard inhibitor.[17,18] The following formula was used to calculate percent inhibition:

$$\text{Inhibition } \% = 1 - \text{ (absorbance of blank sample/absorbance of control) } \times 100$$

More promising inhibitors were subjected to IC_{50} (concentration at which 50% inhibition is observed) studies.

3.3.2. DPPH-radical scavenging assay protocol

The spectrophotometric method was used to determine the free-radical–scavenging potential of the tested compounds. In this method, the DPPH absorbance change at 515 nm was measured.[19] The measurements were performed in triplicate.

3.3.3. Urease inhibition assay protocol

In this assay, urease inhibition activity was determined using the same procedure as mentioned in the literature, with slight modifications.[20] In this assay, thiourea was used as the standard inhibitor.

In the present study, we used EZ-fit software (Perrella Scientific Inc., Amherst, MA, USA) to calculate the IC_{50} values (μg/mL) by using different concentrations of active samples.

3.4. Docking procedure

In order to perform molecular docking studies, chemical structures of benzodithiophene derivatives were arranged by ChemBioDraw (Cambridgesoft; Cambridge, MA, USA) and the MOL format of these ligands was generated.

3.4.1. Accession of targets proteins and active binding site analysis

The chemical structures of benzodithiophenes derivatives were prepared by employing ChemBioDraw and the MOL format of these ligands was generated.

The 3D structures of alpha-glucosidase (PDB ID: 3WY) were acquired from the Protein Data Bank (PDB) database. MOE was used to analyze the active sites of target protein.

The active sites of target protein were analyzed using MOE. An active site was defined from the coordinates of the ligand in the original target protein sites.

3.4.2. Protein–ligand docking

A computational ligand–target interaction analysis was used to determine structural complexes of the alpha-glucosidase with ligand molecules to identify the structural basis of this protein target's specificity. Finally, MOE was used to carry out the docking study. The energy of interaction of these compounds with the protein target was assigned as "grid point."

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