Bioorganic Chemistry 86 (2019) 151–158

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design, molecular docking and synthesis of novel 5,6-dichloro-2-methyl-1*H*-benzimidazole derivatives as potential urease enzyme inhibitors

Emre Menteşe[a,*], Mustafa Emirik[a], Bahar Bilgin Sökmen[b]

[a] Department of Chemistry, Art and Science Faculty, Recep Tayyip Erdogan University, Rize, Turkey
[b] Department of Chemistry, Faculty of Arts and Sciences, Giresun University, 28049 Giresun, Turkey

ARTICLE INFO

Keywords:
Benzimidazole
Urease inhibition
Docking study
Triazole

ABSTRACT

A novel series of 5,6-dichloro-2-methyl-1*H*-benzimidazole derivatives was synthesized and then screened for their urease inhibitory activity. All compounds showed more potent inhibitory activity in the range of IC_{50} = 0.0294 ± 0.0015–0.1494 ± 0.0041 μM than thiourea (IC_{50} = 0.5117 ± 0.0159 μM), as a reference inhibitor. Among all the tested compounds, the compound **15** (IC_{50} = 0.0294 ± 0.0015 μM) having strong electron-withdrawing nitro group on the phenyl ring was recorded as the most potent inhibitor of urease. All compounds were docked at the active sites of the *Jack bean* urease enzyme to investigate the reason of the inhibitory activity and the possible binding interactions of enzyme-ligand complexes.

1. Introduction

The non-redox metalloenzyme urease (urea amidohydrolase EC 3.5.1.5) is a nickel-containing enzyme located in the body structure of many organisms like plants, algae, fungi and variousmicroorganisms [1,2]. Urease enzyme causes diseases such as pyelonephritis, urolithiasis, duodenal ulcer, chronic gastritis, gastric ulcerin human and animal [3]. Especially, this enzyme has a major role in the virulence of *Helicobacter pylori*. Control of the urease activity via utilize of effective inhibitors could counteract the negative effects of this enzyme [4]. For this reason, there is an immediate need for the development of potent new antiurease agents. The urease inhibitors can be extensively classified into organic compounds, imidazoles, hydroxamicacid, phosphorodiamidates, humic acid, and 1,4-benzoquinone [5–7], and some heavy metal ions (like Cu^{2+}, Zn^{2+}, Pd^{2+}, Cd^{2+}) [8–10]. The synthesis of urease inhibitors is a very important area in medicinal chemistry.

Medicine has been greatly influenced by heterocyclic synthesis, with the result that today drugs play an important role in medical practice. Here drugs played a supporting role. This situation has changed in many areas of modern medicine. Drugs must first be obtained as compounds or synthesized biological activities [11]. Among these heterocyclic compounds, benzimidazoles are efficient sources for designing new bioactive molecules due to their diverse biological properties like anticancer [12], antiurease [13], antimicrobial [14], antioxidant [15], antiviralagents [16], anthelmintic [17], α-glucosidase [18], and lipase inhibition [19]. In addition, benzimidazole derivatives are represented by a wide variety of medicines, such as omeprazole, albendazole, carbendazim, thiabendazole, timoprazole, and mebendazole [20,21]. It is structurally similar to purine and is found in the structure of vitamin B_{12} [22,23]. Moreover, previous studies show that 5,6-dichloro benzimidazole derivatives are remarkable compounds for their inhibitory properties and their favorable selectivity ratio [12,13,24–26]. Recently, some benzimidazole derivatives were reported as potential urease inhibitors. These compounds containphenyl or benzyl groups at position-2 on the benzimidazole nucleus (Scheme1) [13,27–30]. Previously, we have synthesized 4,5-dichlorobenzimidazole derivatives containing cyclopropyl group at position C-2 that showed strong urease inhibition activities, when compared to previously reported benzimidazoles [13]. This indicated that small groups at the position-2 on the benzimidazole nucleus greatly influence the inhibitory activity of this class of compounds.

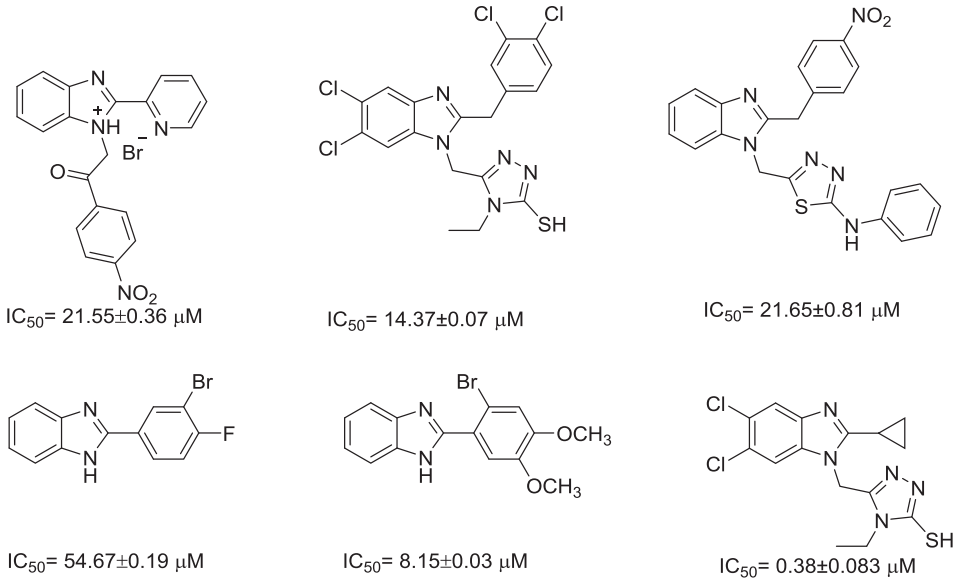
Therefore, prompted by these observations and in continuation to our interest in benzimidazoles as potent urease inhibitor, herein we synthesized novel 2-methylbenzimidazole derivatives and investigated their antiurease activities. Furthermore, in silico molecular docking studies were performed to further investigate the interactions of these compounds with the active site of *jack bean* urease (JBU) enzyme.

2. Result and discussion

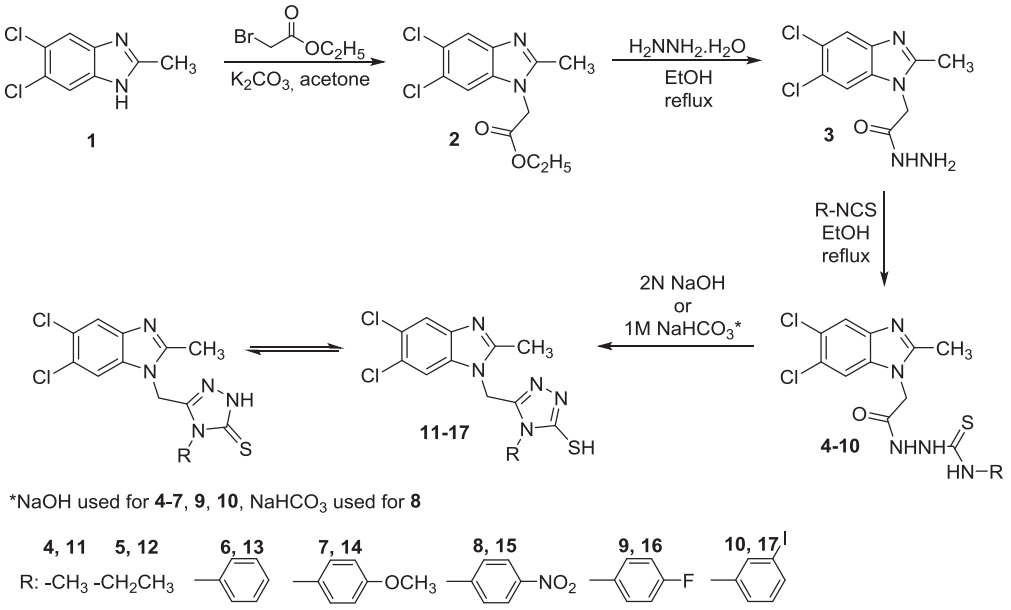
In this study, a series of 5,6-dichloro-2-methyl-1*H*-benzimidazole derivatives was synthesized according to the pathways outlined in

* Corresponding author.
E-mail address: emre.mentese@erdogan.edu.tr (E. Menteşe).

https://doi.org/10.1016/j.bioorg.2019.01.061
Received 6 November 2018; Received in revised form 12 January 2019; Accepted 27 January 2019
Available online 28 January 2019



Scheme 1. Previously synthesized anti-urease active 2-substituted benzimidazole derivatives with their IC_{50} values.

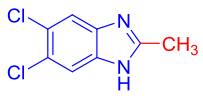
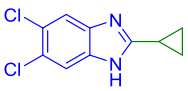
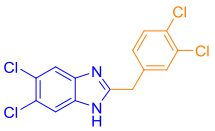
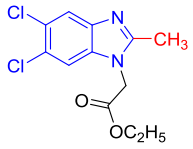
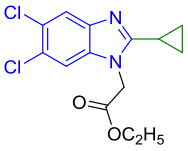
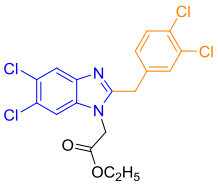
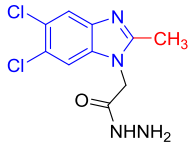
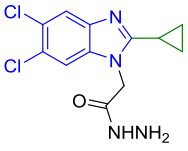
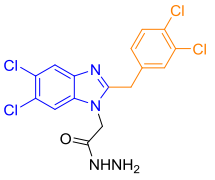
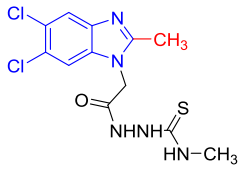
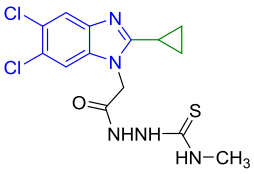
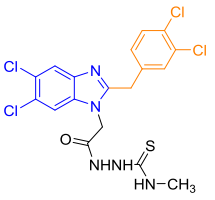
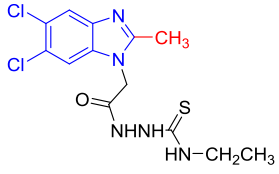
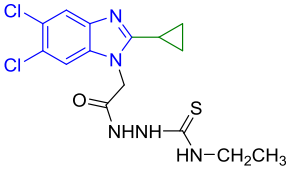
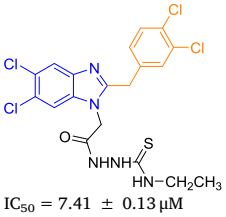
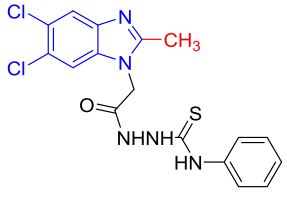
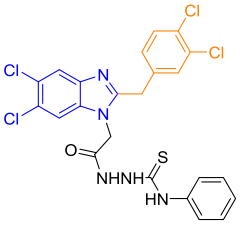
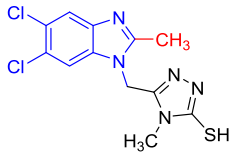
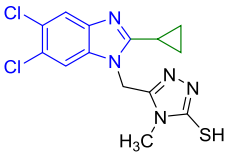
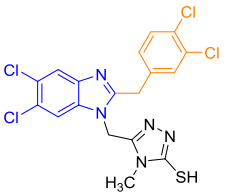


Scheme 2. The synthetic pathway for target compounds.

Scheme 1. 5,6-Dichloro-2-methyl-1*H*-benzimidazole (**1**) was synthesized from the reaction of 4,5-dichloro-1,2-phenylenediamine with ethyl acetimidate hydrochloride. The reaction of compound **1** with ethyl bromoacetate in the presence of K_2CO_3 yielded molecule **2**. The compound **3** was prepared by reacting **2** with hydrazine monohydrate in ethanolic solution. 2-(5,6-Dichloro-2-methyl-1*H*-benzimidazol-1-yl) acetohydrazide (**3**) was reacted with commercially available isothiocyanates in ethanol under reflux to give the corresponding thiosemicarbazides (**4–10**). Finally, intramolecular cyclization of compounds **4–10** in the presence of 2NNaOH or 2 N $NaHCO_3$ (for **8**) solutions to afford the title 1,2,4-triazole derivatives (**11–17**). Structures of all the synthesized compounds were identified by ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis (see Scheme 2).

All newly synthesized compounds **1–17** were evaluated for their *in vitro* urease inhibitory activity (Table 2). Lower IC_{50} values indicate higher enzyme inhibitor activity. The results indicated that all benzimidazole derivatives exhibited potent urease inhibitory activity in the range of IC_{50} = 0.0294 ± 0.0015–0.1494 ± 0.0041 μM, when compared to the standard inhibitor thiourea. Among the series, compounds **8** and **15**having strong electron-withdrawing nitro group on the phenyl ring proved to be the most potent showing an enzyme inhibition activity with an IC_{50} = 0.0354 ± 0.0017 and 0.0294 ± 0.0015 μM, respectively. The least active compound **1** had an IC_{50} = 0.1494 ± 0.0041 μM. Compounds **4** and **5** having alkyl substituent like methyl and ethyl, which is a thiosemicarbazide derivative, showed more potent activity as compared to the molecules **11** and **12**. This result indicated that benzimidazoles bearing open-chain alkyl-substituted thiosemicarbazide group (**4**, **5**), are more potent than their mercapto-1,2,4-triazole counterparts (**11** and **12**). Among the triazole derivatives (**13–17**), compounds **15–17** displayed a better activity, as compared to compound **13** and **14**. The greater potential of these compounds seem to be because of having electron withdrawing group such as nitro and halogen. If we compare the current study having methyl group 2-position on benzimidazol ring with previously reported compounds having cyclopropyl, phenyl or benzyl groups at 2-position on the benzimidazole nucleus, compounds **1–17** are found to be more

Table 1
Comparison of structure activity relationship between compounds **1–6**, **11–13** (A) and previously reported analogs (B).

A	B [13,28]	
		
IC_{50} = 0.1494 ± 0.0041 μM	IC_{50} = 0.17 ± 0.011 μM	–
		
IC_{50} = 0.1222 ± 0.0028 μM	IC_{50} = 1.12 ± 0.08 μM	–
		
IC_{50} = 0.1299 ± 0.0018 μM	IC_{50} = 1.92 ± 0.33 μM	–
		
IC_{50} = 0.0425 ± 0.0015 μM	IC_{50} = 0.05 ± 0.002 μM	IC_{50} = 18.51 ± 0.39 μM
		
IC_{50} = 0.0921 ± 0.0028 μM	IC_{50} = 0.28 ± 0.006 μM	IC_{50} = 7.41 ± 0.13 μM
		
IC_{50} = 0.0635 ± 0.0013 μM	–	IC_{50} = 10.48 ± 0.15 μM
		
IC_{50} = 0.1081 ± 0.0042 μM	IC_{50} = 0.17 ± 0.072 μM	IC_{50} = 46.79 ± 0.28 μM

(*continued on next page*)

Table 1 (*continued*)

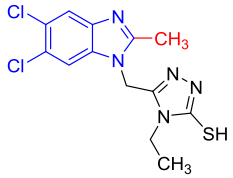
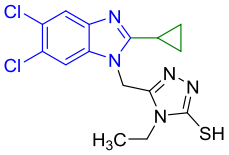
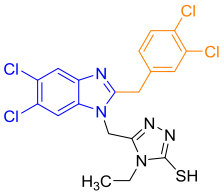
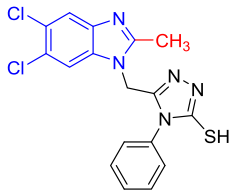
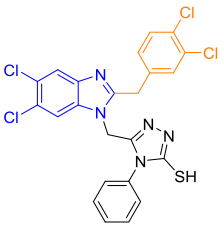
A	B [13,28]	
		
IC_{50} = 0.1384 ± 0.0048 μM	IC_{50} = 0.38 ± 0.083 μM	IC_{50} = 14.37 ± 0.07 μM
		
IC_{50} = 0.0723 ± 0.0011 μM	–	IC_{50} = 45.31 ± 0.47 μM

Table 2
The urease enzyme inhibition and Docking scores of benzimidazole derivatives (**1–17**).

Compounds	IC_{50} (μM)*	Docking scores (kcal/mol)
1	0.1494 ± 0.0041	−6.263
2	0.1222 ± 0.0028	−7.403
3	0.1299 ± 0.0018	−7.851
4	0.0425 ± 0.0015	−8.634
5	0.0921 ± 0.0028	−5.718
6	0.0635 ± 0.0013	−8.224
7	0.0653 ± 0.0014	−6.355
8	0.0354 ± 0.0017	−8.344
9	0.0771 ± 0.0019	−6.486
10	0.0759 ± 0.0015	−6.055
11	0.1081 ± 0.0042	−7.701
12	0.1384 ± 0.0048	−7.153
13	0.0723 ± 0.0011	−8.818
14	0.0720 ± 0.0017	−7.267
15	0.0294 ± 0.0015	−9.061
16	0.0549 ± 0.0020	−7.544
17	0.0357 ± 0.0015	−8.676
Thiourea	0.5117 ± 0.0159	−3.711

* Values were the means of three replicates ± Standard deviation (SD).

active than the previously reported analogs. These compounds have the same groups at 1-position on the benzimidazole ring, but differences of the urease inhibition properties responsible for substituents at 2-position on the benzimidazoles. Comparison of urease inhibition activity for compounds **1–17** and previously synthesized benzimidazoles are presented in Table 1 [13,28]. It is clear that small groups at the position-2 on the benzimidazole nucleus greatly influence the inhibitory activity of 5,6-dichloro-2-substituted-1*H*-benzimidazole derivatives.

3. In-silico Docking study

To screen for potential inhibitor of the urease enzyme, the molecular docking studies were performed using Maestro Molecular Modeling platform (version10.5) by Schrödinger, LLC [32]. The crystal structure of JBU was retrieved from the Protein Data Bank using PDBID 3LA4 [33]. The docking procedures were performed as described in our previous articles [34]. ADME properties of all the compounds were predicted using Qikprop module of Schrödinger. The receptor-ligand complexes obtained after docking study of the newly synthesized compounds were analyzed in terms of docking scores and orientation of the docked compounds in the active site of target enzyme structures. The docked poses of the compounds were ranked based on the docking scores and the best ones were given in Table 2**.** Docking scores showed a good agreement with experimentally determined inhibition results.

Since compounds **4**, **8**, **15** and **17** demonstrated as the most active ligands based on the experimental result, the best fitted conformations and ligand interactions of these compounds were illustrated in Fig. 1 and analyzed in detail.

According to the in silico docking results, it was clearly observed that compound **15** could form hydrogen bonding interactions with ARG439 and pi-pi stacking interaction with HIS593 residue of JBU. The salt bridge between the amino acid residues ASP494 and nitro group of compound **15** was contributed to the binding energy of protein-ligand complex as well. The corresponding docking score is −9.06 kcal/mol. While ARG609, HIS593 and Ni842 residues construct pi-cation stacking interactions, ASP494 and Ni841 at the binding cavity form salt bridge interactions with the compound **17**. Both Ni cations at the catalytic side of enzyme contribute to the formation of enzyme-ligand complex. The corresponding docking score of compound **17** is −8.676 kcal/mol. The similar interactions could be seen for compound **4** and **8** in Fig. 1 with docking score of 8.634 kcal/mol and 8.344 kcal/mol, respectively.

The absorption, distribution, metabolism, and excretion (ADME) properties of the synthesized compounds were calculated with QikProp module of Schrodinger which is a quick and accurate ADME related properties prediction program. The selected properties summarized in Table 3 are mostly influence the drug metabolism, cell permeation, and bioavailability which are important descriptor in drug discovery, namely MW: Molecular Weight, LogPo/w: octanol/water partition coefficient, logS: aqueous solubility, PCaco: the gut-blood barrier, % HOA: Percent Human Oral Absorption, RO5: Number of violations of Lipinski's rule of five. The selected ADME properties of the compounds **4**, **8** and **15** were in the range for 95% of known oral drugs without violation of the Lipinski's rules to be an evidence of drug like potential. Qualitative Model for human oral absorptions were also satisfy the drug like potential of these compounds.

4. Experimental section

4.1. Chemistry

The chemicals were supplied from Merck, Aldrich and Fluka. Melting points were uncorrected and determined in open capillary tubes on a Büchioil-heated melting point apparatus. Reactions were monitored by thin-layer chromatography (TLC) using precoated

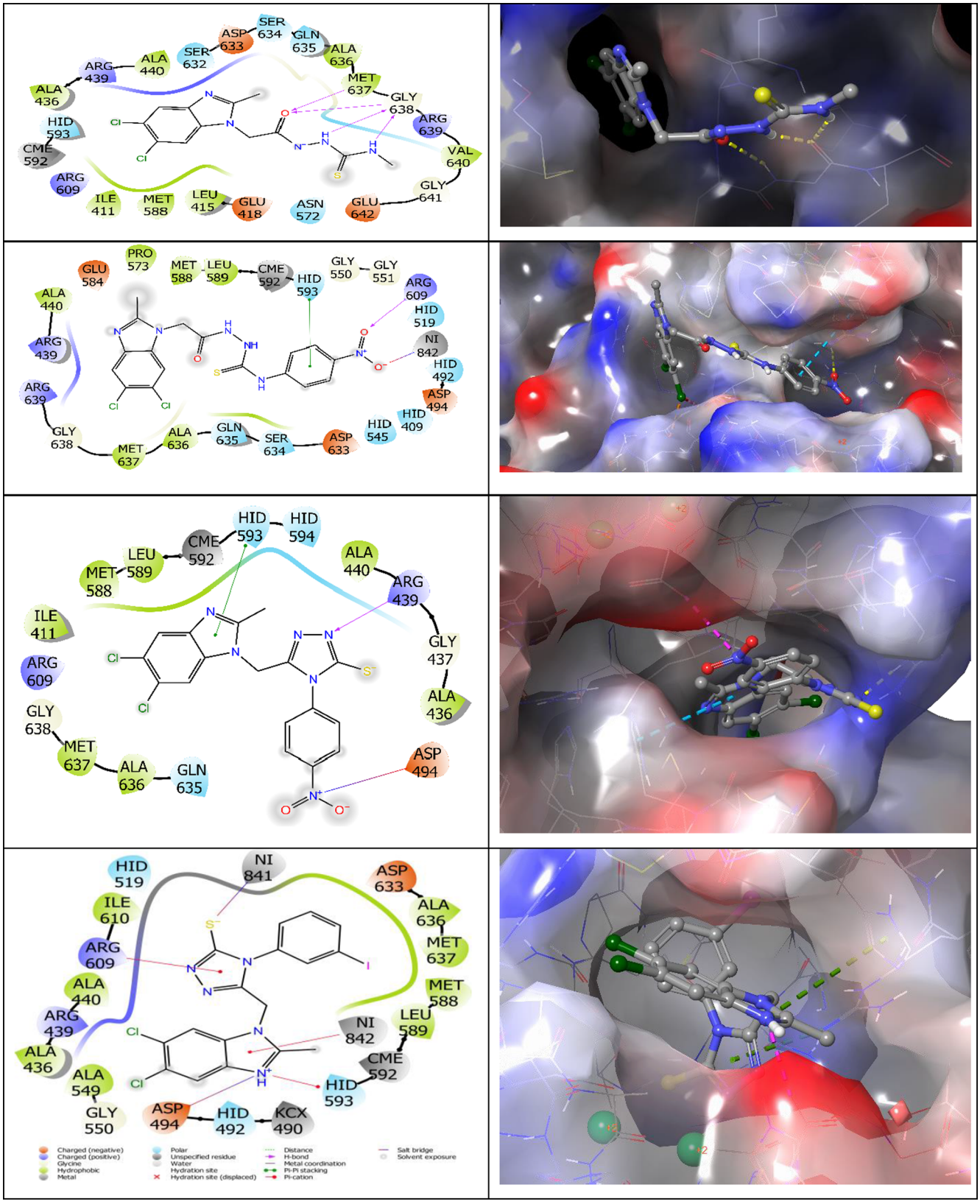


Fig. 1. Ligand interaction diagrams and the binding pose of the compounds **4**, **8**, **15** and **17** at the active site of studied enzyme structures.

aluminum sheets (silicagel60 F 2.54 0.2 mm thickness). The mobile phase was ethyl acetate and hexane (2:1 or 3:1) and detection was made using UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer using DMSO-d_6 as solvent and TMS as internal standard. All chemical shifts were reported in ppm. Elemental analyses were performed on a Carla Erba 1106 CHN analyzer (Heraeus, Hanau, Germany); the experimental values were in agreement (± 0.4%) with calculated ones. The

Table 3
The ADME properties of all the studied compounds.

Title	mol MW	[a]HBD	[b]HBA	[c]logPo/w	[d]PCaco	[e]RO5	[f]%HOA
1	201.055	1	1.5	2.66	3492.075	0	100
2	287.145	0	3.5	3.523	2394.101	0	100
3	273.121	3	4.5	1.664	454.28	0	84.247
4	346.234	2.25	5.75	2.853	720.623	0	94.798
5	360.26	2.25	5.75	3.076	791.993	0	96.836
6	408.304	2.25	5.75	4.188	774.393	0	100
7	438.331	2.25	6.5	3.997	742.176	0	100
8	453.302	2.25	6.75	3.508	80.777	0	81.622
9	426.295	2.25	5.75	4.414	775.069	0	100
10	534.201	2.25	5.75	4.84	809.708	1	94.383
11	328.218	0.8	3.5	3.977	1991.276	0	100
12	342.245	0.8	3.5	4.261	2060.733	0	100
13	390.289	0.8	3.5	5.153	1992.47	1	100
14	420.315	0.8	4.25	5.2	1992.47	1	100
15	435.287	0.8	4.5	4.461	207.834	0	94.547
16	408.28	0.8	3.5	5.388	1992.55	1	100
17	516.186	0.8	3.5	5.819	1992.316	2	94.155
Thiourea	76.116	4	2.5	−0.957	481.218	0	69.349

[a] Number of average Hydrogen Bond Donor.
[b] Number of average Hydrogen Bond Donor.
[c] Predicted octanol/water partition co-efficient (acceptable range from −2 to 6.5).
[d] Predicted Caco-2 cell permeability in nm/s (acceptable range: < 25 and > 500).
[e] Number of violations of Lipinski's rule of five (maximum 4).
[f] Percentage human oral absorption (< 25% is poor and > 80% is high).

compound**1**was synthesized by the methods reported in our previous studies [13,31].

*4.1.1. Synthesis of ethyl 2-(5,6-dichloro-2-methyl-1H-benzimidazol-1-yl) acetate (**2**)*

To a solution of compound **1** (0.010 mol) in dry acetone, K_2CO_3 (0.025 mol) was added and the mixture was stirred at room temperature for 20 min. Then, methyl bromoacetate (0.011 mol) was added to the mixture and stirred at room temperature for one night. Afterwards, the reaction was completed (monitored by TLC, ethylacetate/hexane, 3:1). The product was precipitated by addition of water. It was filtrated off, and recrystallized from ethanol/water, 1:2.

Yield: 92%, mp: 166–167 °C. ^{1}H NMR (DMSO-d_6) ppm: 1.20 (3H, t, J = 6.8 Hz, CH_3), 2.44 (3H, s, CH_3), 4.17 (2H. q, J = 6.8 Hz, OCH_2), 5.20 (2H, s, N-CH_2), 7.78 (1H, s, Ar-H), 7.91 (1H, s, Ar-H). ^{13}C NMR (DMSO-d_6) ppm: 13.78, 14.42 (CH_3), 45.12 (NCH_2), 61.90 (OCH_3), ArC: [112.25, 119.83, 124.45, 124.64, 135.65, 142.19], 155.72 (C═N), 168.35 (C═O). Anal. calcd. (%) for $C_{12}H_{12}Cl_2N_2O_2$: C, 50.20; H, 4.21; N, 9.76. Found: C, 50.28; H, 4.26; N, 9.69.

*4.1.2. Synthesis of 2-(5,6-dichloro-2-methyl-1H-benzimidazol-1-yl) acetohydrazide (**3**)*

Hydrazine monohydrate (0.025 mol) was added to the solution of compound **2** (0.01 mol) in ethanol (10 mL). Then, it was refluxed for 5 h. The end of the reaction was monitored by TLC (ethyl acetate/hexane = 2:1). After cooling the mixture to room temperature a white solid appeared. This crude product was filtrated, dried and recrystallized from ethanol to obtain the desired product **3**.

Yield: 84%, mp: 265–266 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.47 (3H, s, CH_3), 4.34 (2H, s, NH_2, exchanged with D_2O), 4.81 (2H, s, N-CH_2), 7.76 (1H, s, Ar-H), 7.78 (1H, s, Ar-H), 9.47 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.02 (CH_3), 45.22 (NCH_2), ArC:[112.01, 119.77, 124.22, 124.41, 135.76, 142.23], 155.86 (C═N), 166.07 (C═O). Anal. calcd. (%) for $C_{10}H_{10}Cl_2N_4O$: C, 43.98; H, 3.69; N, 20.51. Found: C, 44.05; H, 3.74; N, 20.45.

*4.2. Synthesis of compounds **4–10***

A mixture of an acid hydrazide **3** (0.01 mol) in ethanol (15 mL) and corresponding isothiocyanate (0.011 mol) was refluxed for 2 h. The solution was cooled and a white solid appeared. The precipitated product was filtrated and recrystallized from ethanol to obtain the desired pure products **4–10**.

*4.2.1. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-methylhydrazine-1-carbothioamide (**4**)*

Yield: 95%, mp: 239–240 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.53(3H, s, CH_3), 2.88 (3H, s, CH_3), 4.92 (2H, s, NCH_2), 7.75 (m, 2H, Ar-H), 8.06, 8.60 (1H, s, NH), 9.29, 9.44 (1H, s, NH), 9.68, 10.21 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.06 (CH_3), 31.36 (NCH_3), 45.18 (NCH_2), ArC: [112.15, 119.77, 124.30, 124.47 135.76, 142.27], 155.95 (C═N), 166.70 (C═O), 170.46 (C═S). Anal. calcd. (%) for $C_{12}H_{13}Cl_2N_5SO$: C, 41.63; H, 3.78; N, 20.23; S, 9.26. Found: C, 41.70; H, 3.83; N, 20.29; S, 9.32.

*4.2.2. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-ethylhydrazine-1-carbothioamide (**5**)*

Yield: 98%, 237–238 °C. ^{1}H NMR (DMSO-d_6) ppm: 1.06 (3H, t, J = 7.2 Hz, CH_3), 2.53 (3H, s, CH_3), 3.46 (2H, q, J = 7.2 Hz, CH_2), 4.90 (2H, s, NCH_2), 7.76 (1H, s, Ar-H), 7.81 (1H, s, Ar-H), 8.06, (1H, s, NH), 9.22 (1H, s, NH), 10.21 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.06, 14.87 (CH_3), 38.97 (NCH_2), 45.18 (NCH_2), ArC:[112.05, 119.70, 124.17, 124.48, 135.86, 142.26], 155.93 (C═N), 166.58 (C═O), 170.44 (C═S). Anal. calcd. (%) for $C_{13}H_{15}Cl_2N_5SO$: C, 43.34; H, 4.20; N, 19.44; S, 8.90. Found: C, 43.40; H, 4.25; N, 19.49; S, 8.97.

*4.2.3. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-phenylhydrazine-1-carbothioamide (**6**)*

Yield: 95%, 204–205 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.51 (3H, s, CH_3), 5.00 (2H, s, NCH_2), 7.32–7.43 (5H, m, Ar-H), 7.78 (1H, s, Ar-H), 7.84 (1H, s, Ar-H), 9.67, (1H, s, NH), 9.76 (1H, bs, NH), 10.48 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.09 (CH_3), 45.28 (NCH_2), ArC: [112.09, 119.77, 124.16, 124.33, 124.52, 125.86, 128.72, 135.78, 139.35, 142.24], 155.98 (C═N), 166.59 (C═O), 170.60 (C═S). Anal. calcd. (%) for $C_{17}H_{15}Cl_2N_5SO$: C, 50.01; H, 3.70; N, 17.15; S, 7.85. Found: C, 50.11; H, 3.75; N, 17.19; S, 7.91.

*4.2.4. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (**7**)*

Yield: 93%, 185–186 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.29 (3H, s, OCH_3), 2.51 (3H, s, CH_3), 4.99 (2H, s, NCH_2), 7.14 (2H, d, J = 8.0 Hz, Ar-H), 7.26 (2H, d, J = 8.0 Hz, Ar-H), 7.78 (1H, s, Ar-H), 7.86 (1H, s, Ar-H), 9.60, (1H, s, NH), 9.69 (1H, bs, NH), 10.45 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.09 (CH_3), 21.01 (OCH_3), 45.28 (NCH_2), ArC: [112.23, 119.77, 124.15, 124.23, 124.50, 129.17, 135.80, 135.92, 136.76, 142.25], 155.98 (C═N), 166.67 (C═O). Anal. calcd. (%) for $C_{18}H_{17}Cl_2N_5SO_2$: C, 49.32; H, 3.91; N, 15.98; S, 7.31. Found: C, 49.39; H, 3.97; N, 16.03; S, 7.37.

*4.2.5. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-(4-nitrophenyl)hydrazine-1-carbothioamide (**8**)*

Yield: 94%, 196–197 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.52 (3H, s, CH_3), 5.03 (2H, s, NCH_2), 7.75 (1H, s, Ar-H), 7.86 (2H, d, J = 7.2 Hz, Ar-H), 7.88 (1H, s, Ar-H), 8.21 (2H, d, J = 7.2 Hz, Ar-H), 10.08, (1H, s, NH), 10.55 (1H, bs, NH), 10.68 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.09 (CH_3), 45.28 (NCH_2), ArC:[112.24, 119.78, 121.54, 124.19, 124.37, 124.54, 125.11, 135.75, 142.21, 145.82], 155.95 (C═N), 166.82 (C═O). Anal. calcd. (%) for $C_{17}H_{14}Cl_2N_6SO_3$: C, 45.04; H,3.11; N, 18.54; S, 7.07. Found: C, 45.13; H, 3.16; N, 18.49; S, 7.13.

*4.2.6. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-(4-fluorophenyl)hydrazine-1-carbothioamide (**9**)*

Yield: 90%, 152–154 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.51 (3H, s, CH_3), 4.99(2H, s, NCH_2), 7.38–7.41 (2H, m, ArH), 7.38–7.41 (2H, m, ArH), 7.78 (1H, s, Ar-H), 7.84 (1H, s, Ar-H), 9.71, (1H, s, NH), 9.76 (1H, s, NH), 10.47 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.09 (CH_3), 45.28 (NCH_2), ArC:[112.20, 115.40 (d, $J_{C\text{-}F}$ = 22 Hz), 119.78, 124.33, 124.51, 135.67, 135.70, 135.79, 135.90, 142.25], 155.97 (C═N), 161.40 (C-F, d, J = 240 Hz), 166.57(C═O), 170.42 (C═S). Anal. calcd. (%) for $C_{17}H_{14}Cl_2FN_5SO$: C, 47.90; H, 3.31; N, 16.43; S, 7.52. Found: C, 47.98; H, 3.36; N, 16.38; S, 7.58.

*4.2.7. 2-(2-(5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)acetyl)-N-(3-iodophenyl)hydrazine-1-carbothioamide (**10**)*

Yield: 92%, 200–201 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.51 (3H, s, CH_3), 5.00 (2H, s, NCH_2), 7.11–7.52 (4H, m, ArH), 7.78 (1H, s, Ar-H), 7.88 (1H, s, Ar-H), 9.81, (2H, s, 2NH), 10.48 (1H, s, NH). ^{13}C NMR (DMSO-d_6) ppm: 14.10 (CH_3), 45.28 (NCH_2), ArC:[112.25, 119.79, 124.16, 124.33, 124.53, 128.87, 130.51, 130.69, 135.78, 140.84, 142.26], 155.95 (C═N), 166.58 (C═O), 170.47 (C═S). Anal. calcd. (%) for $C_{17}H_{14}Cl_2IN_5SO$: C, 38.22; H, 2.64; N, 13.11; S, 6.00. Found: C, 38.28; H, 2.69; N, 13.06; S, 6.09.

*4.3. Synthesis of compounds **11–17***

2 N NaOH (10 mL) (1 M $NaHCO_3$ for **8**) was added to the solution of compounds **4–10** (0.01 mol) in ethanol (10 mL). Then, the mixture was refluxed for 4 h. After completion of the reaction, the formed mixture was cooled at room temperature and acidified to pH 5–6 with 37% HCl. The formed precipitated product was filtrated off, washed with water and recrystallized from ethanol.

*4.3.1. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-methyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**11**)*

Yield: 88%, mp: 294–295 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.48 (3H, s, CH_3), 3.51 (3H, s, NCH_3), 5.61 (2H, s, NCH_2), 7.79 (1H, s, Ar-H), 7.94 (1H, s, Ar-H), 13.55 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.87 (CH_3), 30.37 (NCH_3), 39.49 (NCH_2), ArC:[112.40, 119.87, 124.53, 124.68, 135.56, 142.30], 148.67 (Triazol-C_3), 155.84 (Benzimidazole, C═N), 168.12 (Triazol-C_5). Anal. calcd. (%) for $C_{12}H_{11}Cl_2N_5S$: C, 43.91; H, 3.38; N, 21.34; S, 9.77. Found: C, 43.99; H, 3.43; N, 21.27; S, 9.84.

*4.3.2. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-ethyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**12**)*

Yield: 85%, mp: 293–294 °C. ^{1}H NMR (DMSO-d_6) ppm: 1.19 (3H, t, J = 7.2 Hz, CH_3), 2.48 (3H, s, CH_3), 4.04 (3H, q, J = 7.2 Hz, NCH_2), 5.68 (2H, s, NCH_2), 7.80 (1H, s, Ar-H), 7.94 (1H, s, Ar-H), 13.62 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.52 (CH_3), 13.91 (NCH_3), 39.09, 39.22 (NCH_2), ArC:[112.35, 119.95, 124.62, 124.78, 135.52, 142.26], 147.99 (Triazol-C_3), 155.77 (Benzimidazole, C═N), 167.68 (Triazol-C_5). Anal. calcd. (%) for $C_{13}H_{13}Cl_2N_5S$: C, 45.62; H, 3.83; N, 20.46; S, 9.37. Found: C, 45.68; H, 3.88; N, 20.40; S, 9.41.

*4.3.3. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (**13**)*

Yield: 80%, mp: 297–298 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.27 (3H, s, CH_3), 5.34 (2H, s, NCH_2), 7.38–7.47 (5H, m, Ar-H), 7.55 (1H, s, Ar-H), 7.71 (1H, s, Ar-H), 13.94 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.66 (CH_3), 39.30 (NCH_2), ArC: [112.25, 119.71, 124.45, 124.55, 128.65, 129.90, 130.21, 133.33, 135.20, 142.04], 147.82 (Triazol-C_3), 155.39 (Benzimidazole, C═N), 169.35 (Triazol-C_5). Anal. calcd. (%) for $C_{17}H_{13}Cl_2N_5S$: C, 52.32; H, 3.36; N, 17.94; S, 8.21. Found: C, 52.39; H, 3.40; N, 17.88; S, 8.29

*4.3.4. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-(4-methoxyphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**14**)*

Yield: 83%, mp: 305–306 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.30 (6H, s, CH_3 + OCH_3), 5.35 (2H, s, NCH_2), 7.15 (2H, d, J = 8 Hz, Ar-H), 7.20 (2H, d, J = 8 Hz, Ar-H), 7.44 (1H, s, Ar-H), 7.69 (1H, s, Ar-H), 13.91 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.77 (CH_3), 21.17 (OCH_3), 39.45 (NCH_2), ArC: [112.20, 119.59, 124.33, 124.47, 128.27, 130.28, 130.67, 135.18, 139.93, 141.92], 147.92 (Triazol-C_3), 155.28 (Benzimidazole, C═N), 169.45 (Triazol-C_5). Anal. calcd. (%) for $C_{18}H_{15}Cl_2N_5SO$: C, 51.44; H, 3.60; N, 16.66; S, 7.63. Found: C, 51.61; H, 3.72; N, 16.58; S, 7.70

*4.3.5. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-(4-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**15**)*

Yield: 79%, mp: 266–267 °C.^{1}H NMR (DMSO-d_6) ppm: 2.41 (3H, s, CH_3), 5.43 (2H, s, NCH_2), 7.50 (1H, s, Ar-H), 7.70 (1H, s, Ar-H), 8.20 (2H, d, J = 8.2 Hz, Ar-H), 8.27 (2H, d, J = 8.2 Hz, Ar-H), 11.59, 14.17 (1H, s, SH).^{13}C NMR (DMSO-d_6) ppm: 13.93 (CH_3), 39.55 (NCH_2), ArC: [112.12, 119.65, 124.42, 124.49, 124.95, 125.10, 130.55, 138.30, 141.89, 143.38], 145.62, 148.18 (Triazol-C_3), 155.55 (Benzimidazole, C═N), 169.45, 188.11 (Triazol-C_5). Anal. calcd. (%) for $C_{17}H_{12}Cl_2N_6SO_2$: C, 46.91; H, 2.78; N, 19.31; S, 7.37. Found: C, 46.99; H, 2.84; N, 19.24; S, 7.43

*4.3.6. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-(4-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**16**)*

Yield: 82%, mp: 291–292 °C. ^{1}H NMR (DMSO-d_6) ppm: 2.34 (3H, s, CH_3), 5.36 (2H, s, NCH_2), 7.30–7.44 (4H, m, Ar-H), 7.54 (1H, s, Ar-H), 7.71 (1H, s, Ar-H), 13.94 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.78 (CH_3), 39.51 (NCH_2), ArC: [112.12, 116.78 (d, $J_{C\text{-}F}$ = 22.7 Hz), 119.68, 124.43, 124.54, 129.58, 129.61, 131.15 (d, $J_{C\text{-}F}$ = 9.1 Hz), 135.21, 141.99], 147.88 (Triazol-C_3), 155.41 (Benzimidazole, C═N), 162.89 (C-F, d, J = 246.5 Hz), 169.49, (Triazol-C_5). Anal. calcd. (%) for $C_{17}H_{12}Cl_2FN_5S$: C, 50.01; H, 2.96; N, 17.15; S, 7.85. Found: C, 50.11; H, 3.01; N, 17.09; S, 7.91

*4.3.7. 5-((5,6-Dichloro-2-methyl-1H-benzimidazol-1-yl)methyl)-4-(3-iodophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**17**)*

Yield: 82%, mp: 291–292 °C. IR (υ max/cm^{-1}): ^{1}H NMR (DMSO-d_6) ppm: 2.35 (3H, s, CH_3), 5.38 (2H, s, NCH_2), 7.25 (1H, t, J = 8 Hz, Ar-H), 7.41 (1H, d, J = 8 Hz, Ar-H), 7.50 (1H, s, Ar-H), 7.71 (2H, s, Ar-H), 7.80 (1H,d, J = 8 Hz, Ar-H), 13.95 (1H, s, SH). ^{13}C NMR (DMSO-d_6) ppm: 13.86 (CH_3), 39.35–40.61 (DMSO-d_6 + NCH_2), ArC: [95.14 (C-I), 112.02, 119.73, 124.47, 124.63, 128.30, 131.53, 134.36, 135.22, 136.98, 138.96, 141.91], 147.66 (Triazol-C_3), 155.41 (Benzimidazole, C═N), 169.32, (Triazol-C_5). Anal. calcd. (%) for $C_{17}H_{12}Cl_2IN_5S$: C, 39.56; H, 2.34; N, 13.57; S, 6.21. Found: C, 39.63; H, 2.40; N, 13.50; S, 6.29

4.4. Urease inhibition assay

The urease inhibitory activities of the newly synthesized compounds were determined spectrophotometrically according to the method of Van Slyke and Archibald [35]. Briefly, 500 μL of urease solution (16 mg/mL urease solution was prepared in 100 mM pH 6.8 phosphate buffer) was added to 0.5 mL of the compounds and standard (0.01–0.00001 μg/mL). The mixture was incubated for 15 min at room temperature. After incubation, 400 μL phenol red solution which was prepared in urea-phosphate buffer (pH: 6.8) was transferred to the mixture. The absorbance values were read at 570 nm. The assays were done in triplicate. The percentage of anti-urease activity was calculated as:

$$\text{Inhibition } (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})(\text{Abs}_{\text{control}})] \times 100$$

Thiourea was utilized for standard urease inhibitor, and the values are expressed as IC_{50}, the concentration of the samples that causes 50% inhibition.

5. Conclusion

The current study involved design, synthesis, *in vitro* evaluation and molecular docking studies of novel 5,6-dichloro-2-methyl-1*H*-benzimidazole derivatives as potential antiurease agents. All the synthesized compounds displayed significant inhibitory potential when compared with the standard inhibitor thiourea. Among the series, compounds **8** and **15** having strong electron-withdrawing nitro group on the phenyl ring proved to be the most potent showing an enzyme inhibition activity with an $IC_{50} = 0.0354 \pm 0.0017$ and 0.0294 ± 0.0015 μM, respectively. Based on these studies, structure–activity relationship indicated that small groups at the position-2 on the benzimidazole nucleus greatly influence the inhibitory activity of this class of compounds. In-silico docking results support that all the compounds were located in active site of the JBU and directly interact with the active amino acids residues. This can be interpreted the reason of inhibition against JBU.

Conflict of interest

The authors confirm that this article has no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.061.

References

[1] J.B. Sumner, J. Biol. Chem. 69 (1926) 435–441.
[2] M.J. Maroney, S. Ciurli, Chem. Rev. 114 (2014) 4206–4228.
[3] P.A. Karplus, M.A. Pearson, R.P. Hausinger, Acc. Chem. Res. 30 (1997) 330–337.
[4] B.J. Krajewska, Mol. Catal. B Enzym. 59 (2009) 9–21.
[5] Z. Amtul, A.U. Rahman, R.A. Siddiqui, M.I. Choudhary, Curr. Med. Chem. 9 (2002) 1323–1348.
[6] W. Zaborska, M. Kot, K. Superata, J. Enzym. Inhib. Med. Chem. 17 (2002) 247–253.
[7] M.A. Pearson, L.O. Michel, R.P. Hausinger, P.A. Karplus, Biochemistry 36 (1997) 8164–8172.
[8] W. Zaborska, B. Krajewska, Z. Olech, J. Enzym. Inhib. Med. Chem. 19 (2004) 65–69.
[9] W. Zaborska, B. Krajewska, M. Leszko, Z. Olech, J. Mol. Catal. B Enzym. 13 (2001) 103–108.
[10] A.Y. Louie, T.J. Meade, Chem. Rev. 99 (1999) 2711–2734.
[11] Jürgen Drews, Quest of Tomorrow's Medicines, Springer, 2003.
[12] B. Kahveci, E. Menteşe, M. Özil, S. Ülker, M. Ertürk, Monatsh. Chem. 144 (2013) 993–1001.
[13] E. Menteşe, H. Bektaş, M. Emirik, B.B. Sökmen, B. Kahveci, Bioorg. Med. Chem. Lett. 27 (2017) 3014–3018.
[14] E. Menteşe, S. Ülker, B. Kahveci, Chem. Heterocycl. Comp. 50 (12) (2015) 1671–1682.
[15] F. Yılmaz, E. Menteşe, N. Baltaş, Lett. Drug Design Discov. 14 (2017) 201–208.
[16] T.M.A. Eldebss, A.M. Farag, M.M. Abdulla, R.K. Arafa, Mini-Rev. Med Chem. 16 (2016) 67–83.
[17] L. Stuchlíková, R. Jirásko, L. Skálová, et al., Chemosphere 157 (2016) 10–17.
[18] N.K.N. Abdullah Zawawi, M. Taha, N. Ahmat, A. Wadood, N.H. Ismail, F. Rahim, S.S. Azam, N. Abdullah, Bioorg. Chem. 64 (2016) 29–36.
[19] E. Menteşe, F. Yılmaz, M. Emirik, S. Ülker, B. Kahveci, Bioorg. Chem. 76 (2018) 478–486.
[20] J. Velik, V. Baliharova, J. Fink-Gremmels, S. Bull, J. Lamka, L. Skalova, Res. Vet. Sci. 76 (2004) 95–98.
[21] M. Gaba, C. Mohan, Med. Chem. Res. 25 (2016) 173–210.
[22] H.A. Barker, R.D. Smyth, H. Weissbach, J.I. Toohey, J.N. Ladd, B.E. Volcani, J. Biol. Chem. 235 (1960) 480–488.
[23] B.V.S. Kumar, S.D. Vaidya, R.V. Kumar, S.B. Bhirud, R.B. Mane, Eur. J Med. Chem. 41 (2006) 599–604.
[24] E. Menteşe, H. Bektaş, S. Ülker, O. Bekircan, B. Kahveci, Enzyme Inhib. Med. Chem. 29 (1) (2014) 64–68.
[25] A. Dittmer, I. Woskobojnik, R. Adfeldt, J.C. Drach, L.B. Townsend, S. Voigt, E. Bogner, Antiviral Res. 137 (2017) 102–107.
[26] R.V. Devivarar, E. Kawashima, G.R. Revankarar, J.M. Breitenbach, E.D. Kreske, J.C. Drach, L.B. Townsend, J. Med. Chem. 37 (1994) 2942–2949.
[27] T. Arshad, K.M. Khan, N. Rasool, U. Salar, S. Hussain, H. Asghar, M. Ashraf, A. Wadood, M. Riaz, S. Perveen, M. Taha, N.H. Ismail, Bioorg. Chem. 72 (2017) 21–31.
[28] N. Karaali, N. Baltaş, E. Menteşe, Ind. J. Chem. 57B (2018) 374–384.
[29] N. Baltaş, F. Yılmaz, E. Menteşe, Hacettepe J. Biol. & Chem. 44 (3) (2016) 293–305.
[30] Z.S. Saify, A. Kamil, S. Akhtar, M. Taha, A. Khan, K.M. Khan, S. Jahan, F. Rahim, S. Perveen, M.I. Choudhary, Med. Chem. Res. 23 (2014) 4447–4454.
[31] H. Antaki, V. Petrow, J. Chem. Soc. (1951) 2873–2877.
[32] Schrödinger Suite 2009 Induced Fit Docking protocol; Glide version 5.5, S., LLC, New York, NY, 2009; Prime version 2.1, Schrödinger, LLC, New York, NY, 2009.
[33] A. Balasubramanian, K.J. Ponnuraj, Mol. Biol. 400 (2010) 274–283.
[34] G. Akyüz, E. Menteşe, M. Emirik, N. Baltaş, Bioorg. Chem. 80 (2018) 121–128.
[35] D.D. Van Slyke, R.M. Archibald, J. Biol. Chem. 154 (1944) 623–642.