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

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ABSTRACT
A novel series of 5,6-dichloro-2-methyl-1H-benzimidazole derivatives was synthesized and then screened for their urease inhibitory activity. All compounds showed more potent inhibitory activity in the range of IC₅₀ = 0.0294 ± 0.0015–0.1494 ± 0.0041µM than thiourea (IC₅₀ = 0.5117 ± 0.0159µM), as a reference inhibitor. Among all the tested compounds, the compound 15 (IC₅₀ = 0.0294 ± 0.0015µM) 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 various microorganisms [1,2]. Urease enzyme causes diseases such as pyelonephritis, uriculitis, duodenal ulcer, chronic gastritis, gastric ulcer in human and animal [3]. Especially, this enzyme has a major role in the virulence of Helicobacter pylori. Control of the urease activity via utilization 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, hydroxamic acid, phosphorodiamidates, humic acid, and 1,4-benzoquinone [5–7], and some heavy metal ions (like Cu²⁺, Zn²⁺, Cd²⁺, Cd³⁺) [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], antiviral agents [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, carbidazin, thiabendazole, timoprazole, and mebendazole [20,21]. It is structurally similar to purine and is found in the structure of vitamin B₁₂ [22,23]. Moreover, previous studies show that 5,6-dichlorobenzimidazole 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 contain phenyl 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.

2. Result and discussion
In this study, a series of 5,6-dichloro-2-methyl-1H-benzimidazole derivatives was synthesized according to the pathways outlined in
Scheme 1. 5,6-Dichloro-2-methyl-1H-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 K₂CO₃ yielded molecule 2. The compound 3 was prepared by reacting 2 with hydrazine monohydrate in ethanolic solution. 2-(5,6-Dichloro-2-methyl-1H-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 2N NaOH or 2 N NaHCO₃ (for 8) solutions to afford the title 1,2,4-triazole derivatives (11–17). Structures of all the synthesized compounds were identified by ¹H NMR, ¹³C 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 IC₅₀ values indicate higher enzyme inhibitor activity. The results indicated that all benzimidazole derivatives exhibited potent urease inhibitory activity in the range of IC₅₀ = 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 IC₅₀ = 0.0354 ± 0.0017 and 0.0294 ± 0.0015 µM, respectively. The least active compound 1 had an IC₅₀ = 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).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B [13,28]</th>
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<tr>
<td></td>
<td>IC\textsubscript{50} = 0.1494 ± 0.0041 μM</td>
<td>IC\textsubscript{50} = 0.17 ± 0.011 μM</td>
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<tr>
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<td>IC\textsubscript{50} = 0.1222 ± 0.0028 μM</td>
<td>IC\textsubscript{50} = 1.12 ± 0.08 μM</td>
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<td>IC\textsubscript{50} = 0.1299 ± 0.0018 μM</td>
<td>IC\textsubscript{50} = 1.92 ± 0.33 μM</td>
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<tr>
<td></td>
<td>IC\textsubscript{50} = 0.0425 ± 0.0015 μM</td>
<td>IC\textsubscript{50} = 0.05 ± 0.002 μM</td>
</tr>
<tr>
<td></td>
<td>IC\textsubscript{50} = 0.0921 ± 0.0028 μM</td>
<td>IC\textsubscript{50} = 0.28 ± 0.006 μM</td>
</tr>
<tr>
<td></td>
<td>IC\textsubscript{50} = 0.0635 ± 0.0013 μM</td>
<td>IC\textsubscript{50} = 10.48 ± 0.15 μM</td>
</tr>
<tr>
<td></td>
<td>IC\textsubscript{50} = 0.1081 ± 0.0042 μM</td>
<td>IC\textsubscript{50} = 46.79 ± 0.28 μM</td>
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(continued on next page)
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-1H-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 HISS93 residue of JBU. The salt bridge between the amino acid residues ASP494 and Ni841 at the binding cavity form salt bridge 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 Büchiol-heated melting point apparatus. Reactions were monitored by thin-layer chromatography (TLC) using precoated

Table 1 (continued)

<table>
<thead>
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<th>B [13,28]</th>
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<tr>
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<td><img src="image2.jpg" alt="Image" /></td>
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<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 0.1384 ± 0.0048μM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 0.38 ± 0.083μM</td>
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<td><img src="image4.jpg" alt="Image" /></td>
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<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 0.0723 ± 0.0011μM</td>
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Table 2

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)*</th>
<th>Docking scores (kcal/mol)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.1494 ± 0.0041</td>
<td>−6.263</td>
</tr>
<tr>
<td>2</td>
<td>0.1222 ± 0.0028</td>
<td>−7.403</td>
</tr>
<tr>
<td>3</td>
<td>0.1299 ± 0.0018</td>
<td>−7.851</td>
</tr>
<tr>
<td>4</td>
<td>0.0425 ± 0.0015</td>
<td>−8.634</td>
</tr>
<tr>
<td>5</td>
<td>0.0921 ± 0.0028</td>
<td>−5.718</td>
</tr>
<tr>
<td>6</td>
<td>0.0635 ± 0.0013</td>
<td>−8.224</td>
</tr>
<tr>
<td>7</td>
<td>0.0653 ± 0.0014</td>
<td>−6.355</td>
</tr>
<tr>
<td>8</td>
<td>0.0354 ± 0.0017</td>
<td>−8.344</td>
</tr>
<tr>
<td>9</td>
<td>0.0771 ± 0.0019</td>
<td>−6.486</td>
</tr>
<tr>
<td>10</td>
<td>0.0759 ± 0.0015</td>
<td>−6.055</td>
</tr>
<tr>
<td>11</td>
<td>0.1081 ± 0.0042</td>
<td>−7.701</td>
</tr>
<tr>
<td>12</td>
<td>0.1384 ± 0.0048</td>
<td>−7.153</td>
</tr>
<tr>
<td>13</td>
<td>0.0723 ± 0.0011</td>
<td>−8.818</td>
</tr>
<tr>
<td>14</td>
<td>0.0720 ± 0.0017</td>
<td>−7.267</td>
</tr>
<tr>
<td>15</td>
<td>0.0294 ± 0.0015</td>
<td>−9.061</td>
</tr>
<tr>
<td>16</td>
<td>0.0549 ± 0.0020</td>
<td>−7.544</td>
</tr>
<tr>
<td>17</td>
<td>0.0357 ± 0.0015</td>
<td>−8.676</td>
</tr>
<tr>
<td>Thiourea</td>
<td>0.0101 ± 0.0059</td>
<td>−3.617</td>
</tr>
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</table>

* Values were the means of three replicates ± Standard deviation (SD).
aluminum sheets (silicagel 60 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. 1H NMR and 13C NMR spectra were recorded on a Varian-Mercury 400 (1H, 400 MHz; 13C, 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

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.
4.2. Synthesis of compounds 4–10

A mixture of an acid hydrizade 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. 1H NMR (DSMO-d6) ppm: 2.53 (3H, s, CH3), 2.88 (3H, s, CH3), 4.92 (2H, s, NCH3), 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). 13C NMR (DSMO-d6) ppm: 14.06 (CH3), 31.36 (NCH3), 45.18 (NCH3), 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. calcld. (%) for C16H15Cl2N5SO: 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. 1H NMR (DSMO-d6) ppm: 1.06 (3H, t, J = 7.2 Hz, CH3), 2.53 (3H, s, CH3), 3.46 (2H, q, J = 7.2 Hz, CH2), 4.90 (2H, s, NCH3), 7.76 (1H, s, Ar-H), 8.17 (1H, t, Ar-H), 8.06 (1H, s, NH), 9.22 (1H, s, NH), 10.21 (1H, s, NH). 13C NMR (DSMO-d6) ppm: 14.06, 14.87 (CH3), 38.97 (NCH3), 45.18 (NCH3), 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. calcld. (%) for C16H15Cl2N5SO: C, 34.34; 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. 1H NMR (DSMO-d6) ppm: 2.51 (3H, s, CH3), 5.00 (2H, s, NCH3), 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). 13C NMR (DSMO-d6) ppm: 14.09 (CH3), 45.28 (NCH3), ArC: [112.09, 119.77, 124.16, 124.33, 124.52, 125.86, 127.82, 137.78, 139.35, 142.24], 155.98 (C=N), 166.59 (C=O), 170.60 (C=S). Anal. calcld. (%) for C17H15Cl2N5SO: 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. 1H NMR (DSMO-d6) ppm: 2.29 (3H, s, OCH3), 2.51 (3H, s, CH3), 4.99 (2H, s, NCH3), 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), 8.16 (1H, s, NH), 9.60 (1H, s, NH), 10.45 (1H, s, NH). 13C NMR (DSMO-d6) ppm: 14.09 (CH3), 45.28 (NCH3), 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. calcld. (%) for C18H16Cl2N5OSO2C: 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. 1H NMR (DSMO-d6) ppm: 2.52 (3H, s, CH3), 5.03 (2H, s, NCH3), 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). 13C NMR (DSMO-d6) ppm: 14.09 (CH3), 45.28 (NCH3), 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. calcld. (%) for C18H16Cl2N5SO2C: 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-(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\(_{2}\)), 7.36–7.41 (2H, m, ArH), 7.38–7.41 (2H, m, ArH), 7.78 (1H, Ar-H), 7.84 (1H, s, NH), 9.71 (1H, s, NH), 9.76 (1H, s, NH), 10.47 (1H, s, NH). \(^{13}C\) NMR (DMSO-\(d_{6}\)) ppm: 60.92, 44.28, 158.00, 158.05, 158.46, 158.47, 158.48, 158.49 (C=N), 161.40 (C-F, d, J = 240 Hz), 166.57 (C=O), 170.42 (C=S). Anal. calcld. (%) for C\(_{27}\)H\(_{22}\)Cl\(_{2}\)N\(_{4}\)S\(_{2}\): C, 43.36; H, 3.36; N, 14.03; S, 7.58.

4.2.7. 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\(_{3}\)), 7.00 (2H, s, NCH\(_{2}\)), 7.11–7.52 (4H, m, ArH), 7.78 (1H, Ar-H), 7.88 (1H, Ar-H), 9.81 (2H, s, 2NH), 10.48 (1H, s, NH). \(^{13}C\) NMR (DMSO-\(d_{6}\)) ppm: 14.10 (NCH\(_{2}\)), 45.28 (NCH\(_{2}\)), ArC: [122.15, 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. calcld. (%) for C\(_{17}\)H\(_{12}\)Cl\(_{2}\)N\(_{5}\)SO\(_{2}\): C, 43.82; H, 2.64; N, 13.11; S, 6.00. Found: C, 43.82; 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)acetyl)-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\(_{2}\)), 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 (SH), 30.37 (NCH\(_{2}\)), 39.49 (NCH\(_{2}\)), ArC: [112.40, 119.87, 124.53, 126.56, 142.30], 148.67 (Triazol-C), 155.84 (Benzimidazole, C=N), 168.12 (Triazol-C). Anal. calcld. (%) for C\(_{17}\)H\(_{12}\)Cl\(_{2}\)N\(_{4}\)S: 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)acetyl)-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\(_{2}\)), 39.09, 39.22 (NCH\(_{2}\)), ArC: [112.35, 119.95, 124.62, 124.78, 135.52, 142.26], 147.99 (Triazol-C), 155.77 (Benzimidazole, C=N), 167.68 (Triazol-C). Anal. calcld. (%) for C\(_{18}\)H\(_{13}\)Cl\(_{2}\)N\(_{5}\)S: C, 45.62; H, 3.83; N, 20.40; S, 9.41. 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)acetyl)-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\(_{2}\)), 7.36–7.47 (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, 141.12], 147.82 (Triazol-C), 155.39 (Benzimidazole, C=N), 169.35 (Triazol-C). Anal. calcld. (%) for C\(_{18}\)H\(_{13}\)Cl\(_{2}\)N\(_{5}\)S: C, 45.2; H, 3.36; N, 17.94; S, 8.21. Found: C, 45.39; H, 3.40; N, 17.88; S, 8.29.

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 g/mL). The mixture was incubated for 15 min at room temperature. After incubation, 400 μL mol 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:

Inhibition (%) = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}}\right) \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-1H-benzimidazole derivatives as potential antiiurease 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 IC₅₀ = 0.0354 ± 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