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Synthesis of Some New Benzimidazole Derivatives Containing Chlorine and Investigation of Their Antioxidant and Anti-urease Activities

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Abstract: In this work, the synthesis of a 2-substituted benzimidazole compound containing chlorine (**1**) was carried out and then converted to the ester (2) and hydrazide (3) derivatives respectively. In the next steps, the hydrazide compound (**3**) was reacted with the appropriate reagents to give the thiadiazole (**8-10**) and triazole (**11-13**) derivatives. Synthesis of the compound **4** was carried out as a result of the interaction of the ester derivative compound (**2**) with morpholine. The structures of all new synthesized compounds were determined by FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis spectroscopic methods. Also, the antioxidant and urease enzyme inhibition properties of these compounds were also investigated.

Keywords: Benzimidazole, triazole, thiadiazole, urease inhibition, antioxidant.

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INTRODUCTION

Nitrogen-containing heterocycles are unique pharmacophoric units found in bioactive natural products, synthetic medicines, and agrochemicals. Among these heterocyclic compounds, benzimidazole and its derivatives have a significant role due to their wide variety pharmacological properties such as anticancer, antimicrobial, anti-inflammatory, anti-diabetic, anticonvulsant, antibacterial, antihypertensive, anti-cholinesterase, antimalarial, antitumor, antivirals, antiparasite, antioxidant, antiurease, CNS stimulant, and depressant (1-13). And also, literature studies have clearly demonstrated that the attachment of different groups to the benzimidazole core at different positions leads to the increase and differentiation of the activities of this class of compounds (10, 14-17). Especially, it is known that the presence of groups such as triazole, thiadiazole, oxadiazole, morpholine, piperazine, piperidine, *etc*. increases the bioactivity and biodiversity of the benzimidazole core (12-16).

In this work, we have synthesized a series of new 5,6-dichlorobenzimidazole derivatives containing the 2,4-dichlorobenzyl group at position 2 and carbothioamide structure, triazole, thiadiazole rings at position 1 of benzimidazole ring. Moreover, we have studied the urease and antioxidant properties of these compounds.

MATERIALS AND METHODS

Chemistry

The chemicals were supplied from Merck, Sigma-Alrich and Tekkim. Melting points were detected at the SMP30 Stuart melting point device and are uncorrected. The FTIR data were taken on a Perkin-Elmer 100 FTIR spectrometer as ATR (attenuated total reflectance). ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz Varian-Mercury spectrometer in the DMSO-d_6 solvent using TMS as internal standard and the chemical shifts values were given in δ ppm. The reaction times and progress were determined by TLC plates (silicagel 60, F 2.54, 0.2 mm). The elemental compositions were performed on a Carlo Erba 1106 CHN analyzer.

General procedure for the synthesis of compounds 1

2-(2,4-dichlorophenyl)-1-ethoxyethaniminium chloride (**a**) (0.011 mol) synthesized according to the reported method in the literature (18) and

4,5-dichlorobenzene-1,2-diamine (**b**) (0.010 mol) in absolute methanol (30 mL) were stirred for 2 hours at room temperature (monitored by TLC, ethyl acetate : n-hexane, 2:1). Then, plenty of water was added to the mixture to precipitate. The mixture, which was rested for a while, was filtered and washed with plenty of water and purified by recrystallization from a mixture of ethanol:water, v:v 1:1.

*5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazole (**1**).* CAS Registry Number: 1632028-81-9. Yield: 3.01 g (87 %). M.P.: 185–187 °C, FTIR (KBr): 3078 (NH), 1628 (CN), 1445, 1097, 864 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.60 (1H, s, NH), 7.77-7.40 (5H, m, Ar-H), 4.30 (2H, s, CH_2).

General procedure for the synthesis of compounds 2

Compound **1** (0.01 mol) and dry K_2CO_3 (0.03 mol) in absolute acetone (30 mL) was stirred at room temperature for 1 hour, followed by addition of ethylbromoacetate (0.01 mol) stirring at room temperature for a further 4 hours (monitored by TLC, ethyl acetate : n-hexane, 2:1). Plenty of water was added to the mixture to dissolve the excess K_2CO_3 and precipitate the expected product. The mixture, which was rested for a while, was filtered and washed with plenty of water and purified by recrystallization from a mixture of ethanol:water, v:v 1:2.

*Ethyl [5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetate (**2**).* Yield: 3.58 g (83 %). M.P.: 128–130 °C, FTIR (KBr): 1735 (C=O), 1670 (CN), 1228 (C-O) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.96 (1H, s, Ar-H), 7.82 (1H, s, Ar-H), 7.60 (1H, s, Ar-H), 7.40-7.34 (2H, m, Ar-H), 5.28 (2H, s, NCH_2), 4.31 (2H, s, CH_2), 4.10 (2H, q, *J*=8 Hz, OCH_2), 1.17 (3H, t, *J*= 8 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.4 (CH_3), 30.9 (CH_2), 45.2 (NCH_2), 61.9 (OCH_2), 112.6 (Ar-CH), 120.3 (Ar-CH), 124.7 (Ar-C), 125.2 (Ar-C), 129.1 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.6 (Ar-C), 134.9 (Ar-C), 135.7 (Ar-C), 141.9 (Ar-C), 155.7 (NCN), 168.0 (C=O). Elemental analysis: Calculated for $C_{18}H_{14}Cl_4N_2O_2$: C, 50.03; H, 3.27; N, 6.48. Found: C, 50.09; H, 3.22; N, 6.51.

General procedure for the synthesis of compound 3

A mixture of compound **2** (0.010 mol) and hydrazine monohydrate (0.050 mol) in absolute ethanol (25 mL) was stirred at room temperature for 2 hours (monitored by TLC, ethyl acetate:hexane, 3:1). The mixture was filtered and purified by recrystallization from ethanol.

*2-[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetohydrazide (**3**).* Yield: 2.84 g (68 %). M.P.: 253-254 °C, FTIR (KBr): 3301, 3153 (NH_2+NH), 1650 (CN), 1457, 1092, 868 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.51 (1H, s, NH), 7.86 (1H, s, Ar-H), 7.80 (1H, s, Ar-H), 7.62 (1H, s, Ar-H), 7.41-7.35 (2H, m, Ar-H), 5.28, 4.92 (2H, s, NCH_2), 4.56, 4.36 (2H, s, NH_2), 4.33, 4.23 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.9 (CH_2), 45.2 (NCH_2), 112.6 (Ar-CH), 120.3 (Ar-CH), 124.5 (Ar-C), 124.9 (Ar-C), 127.8 (Ar-CH), 129.1 (Ar-CH), 132.8 (Ar-C), 133.4 (Ar-CH), 133.9 (Ar-C), 134.9 (Ar-C), 135.7 (Ar-C), 142.0 (Ar-C), 155.9 (NCN), 165.9 (C=O). Elemental analysis: Calculated for $C_{16}H_{12}Cl_4N_4O$: C, 45.96; H, 2.89; N, 13.40. Found: C, 45.94; H, 2.92; N, 13.37.

General procedure for the synthesis of compound 4

A mixture of compound **3** (0.010 mol) and morpholine (0.030 mol) was heated in an oil bath at 130-140 °C reflux temperature for 16 h (monitored by TLC, ethyl acetate:hexane, v:v 2:1). The viscous residue was cooled to room temperature and acetone (10 mL) was added and allowed to cool overnight, a solid appeared. The crude product was recrystallized from a mixture of ethanol:water, 3:1 in order to obtain the desired compound.

*5,6-Dichloro-2-(2,4-dichlorobenzyl)-1-(2-morpholin-4-yl-2-oxoethyl)-1H-benzimidazole (**4**).* Yield: 2.45 g (52 %). M.P.: 193-195 °C, FTIR (KBr): 1659 (C=O), 1236 (C-O), 1094, 860, 826 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.91 (1H, s, Ar-H), 7.80 (1H, s, Ar-H), 7.61 (1H, s, Ar-H), 7.34-7.37 (2H, m, Ar-H), 5.33 (2H s, NCH_2), 4.23 (2H s, CH_2), 3.70-3.68 (2H, m, OCH_2), 3.57-3.54 (4H, m, OCH_2, morpholine-NCH_2), 3.41-3.39 (2H, m, morpholine-NCH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.9 (CH_2), 42.2 (morpholine-NCH_2), 42.4 (morpholine-NCH_2), 45.2 (NCH_2), 66.3 (OCH_2), 66.4 (OCH_2), 112.5 (Ar-CH), 120.2 (Ar-CH), 124.3 (Ar-C), 124.8 (Ar-C), 127.8 (Ar-CH), 129.1 (Ar-CH), 132.8 (Ar-C), 133.5 (Ar-CH), 133.8 (Ar-C), 134.8 (Ar-C), 136.1 (Ar-C), 142.1 (Ar-C), 156.3 (NCN), 165.0 (C=O). Elemental analysis: Calculated for $C_{20}H_{17}Cl_4N_3O_2$: C, 50.77; H, 3.62; N, 8.88. Found: C, 50.81; H, 3.59; N, 8.85.

General procedure for the synthesis of the compounds 5, 6, 7

To a solution of compound **3** (0.010 mol) in absolute ethanol (30 mL), methylisothiocyanate for compound **5**, ethylisothiocyanate for compound **6**, 4-bromophenylisothiocyanate for compound **7** was added and refluxed for 2 hours (monitored by TLC, ethyl acetate:hexane, 2:1). The mixture was cooled to room temperature and water was added. After a while, it was filtered and purified by recrystallization from a mixture of ethanol:water, v:v 3:1.

*2-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetyl}-N-methylhydrazinecarbothioamide (**5**).* Yield: 4.27 g (87 %). M.P.: 205-206 °C, FTIR (KBr): 3404, 3303, 3152 (NH), 1693 (C=O), 1616, 1554 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.25, 9.33, 8.08 (3H, s, NH), 7.88 (1H, s, Ar-H), 7.80 (1H, s, Ar-H), 7.63 (1H, s, Ar-H), 7.42-7.35

(2H, m, Ar-H), 5.09, 5.04 (2H, s, NCH_2), 4.31, 4.24 (2H, s, CH_2), 2.90 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.9 (CH_2), 31.4 (NCH_3), 45.2 (NCH_2), 112.5 (Ar-CH), 120.3 (Ar-CH), 124.6 (Ar-C), 124.9 (Ar-C), 127.9 (Ar-CH), 129.2 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.9 (Ar-C), 134.9 (Ar-C), 135.7 (Ar-C), 142.0 (Ar-C), 156.0 (NCN), 166.5 (C=O), 170.3 (CS). Elemental analysis: Calculated for $C_{18}H_{15}Cl_4N_5OS$: C, 44.01; H, 3.08; N, 14.26. Found: C, 44.05; H, 3.13; N, 14.22.

*2-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetyl}-N-ethylhydrazinecarbothioamide (**6**).* Yield: 3.84 g (76 %). M.P.: 214-216 °C, FTIR (KBr): 3382, 3282, 3153 (NH), 1694 (C=O), 1620, 1551 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.24, 9.26, 8.08 (3H, s, NH), 7.91 (1H, s, Ar-H), 7.81 (1H, s, Ar-H), 7.63 (1H, s, Ar-H), 7.41-7.34 (2H, m, Ar-H), 5.05 (2H, s, NCH_2), 4.24, 4.31 (2H, s, CH_2), 3.45 (2H, q, *J*=8 Hz, OCH_2), 1.06 (3H, t, *J*=8 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.8 (CH_3), 30.9 (CH_2), 39.3-40.6 (DMSO-d_6+CH_2), 45.1 (NCH_2), 112.5 (Ar-CH), 120.3 (Ar-CH), 124.6 (Ar-C), 124.9 (Ar-C), 127.9 (Ar-CH), 129.1 (Ar-CH), 132.8 (Ar-C), 133.4 (Ar-CH), 133.9 (Ar-C), 134.9 (Ar-C), 135.7 (Ar-C), 142.1 (Ar-C), 156.1 (NCN), 166.4 (C=O), 172.6 (CS). Elemental analysis: Calculated for $C_{19}H_{17}Cl_4N_5OS$: C, 45.17; H, 3.39; N, 13.84. Found: C, 45.18; H, 3.34; N, 13.88.

*N-(4-bromophenyl)-2-{[5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetyl}hydrazinecarbothioamide (**7**).* Yield: 4.23 g (67 %). M.P.: 184-185 °C, FTIR (KBr): 3354, 3296, 3151 (NH), 1691 (C=O), 1590, 1541 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.53, 9.89, 9.82 (3H, s, NH), 7.94 (1H, s, Ar-H), 7.82 (1H, s, Ar-H), 7.63 (1H, s, Ar-H), 7.57-7.51 (3H, m, Ar-H), 7.42-7.34 (3H, m, Ar-H), 5.19, 5.12 (2H s, NCH_2), 4.35 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.0 (CH_2), 45.3 (NCH_2), 112.6 (Ar-CH), 120.3 (Ar-CH), 124.6 (Ar-C), 124.9 (Ar-C), 127.9 (Ar-CH), 129.2 (Ar-CH), 131.5 (Ar-CH), 132.5 (Ar-C), 132.8 (Ar-C), 133.4 (Ar-CH), 133.5 (Ar-CH), 133.9 (Ar-C), 134.9 (Ar-C), 135.7 (Ar-C), 138.8 (Ar-C), 142.1 (Ar-C), 156.1 (NCN), 166.5 (C=O), 171.9 (CS). Elemental analysis: Calculated for $C_{23}H_{16}BrCl_4N_5OS$: C, 43.70; H, 2.55; N, 11.08. Found: C, 43.68; H, 2.52; N, 11.13.

General procedure for the synthesis of compounds 8, 9, 10

To the appropriate carbothioamide compound (**5**, **6**, **7**) (0.01 mol) in an ice bath, concentrated cold sulfuric acid (0.064 mol) was added dropwise and the mixture was stirred for 15 minutes. Then, the mixture was stirred at room temperature for an additional 30 minutes. Then the mixture was added to ice-water and adjusted to pH 7-8 with ammonia. The resulting product was filtered, washed with water purified by recrystallization from a mixture of ethanol:water, v:v 3:1.

*5-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-N-methyl-1,3,4-thiadiazol-2-amine (**8**).* Yield: 2.64 g (56 %). M.P.: 242-244 °C, FTIR (KBr): 3219 (NH), 1567, 1520, 1501 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.03 (1H, s, Ar-H), 7.83 (1H, s, Ar-H), 7.68-7.59 (1H, m, Ar-H), 7.32-7.38 (2H, s, Ar-H), 5.80 (2H, s, NCH_2), 4.42 (2H, s, CH_2), 2.81 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.2 (CH_2), 31.7 (CH_3), 42.3 (NCH_2), 112.5 (Ar-CH), 120.5 (Ar-CH), 124.9 (Ar-C), 125.2 (Ar-C), 127.8 (Ar-CH), 129.2 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.7 (Ar-C), 134.9 (2Ar-C), 142.1 (Ar-C), 152.2 (NCN), 155.2 (thiadiazole-C_5), 170.8 (thiadiazole-C_2). Elemental analysis: Calculated for $C_{18}H_{13}Cl_4N_5S$: C, 45.69; H, 2.77; N, 14.80. Found: C, 45.72; H, 2.73; N, 14.82.

*5-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-N-ethyl-1,3,4-thiadiazol-2-amine (**9**).* Yield: 2.28 g (47 %). M.P.: 205-207 °C, FTIR (KBr): 3203 (NH), 1581, 1563, 1516 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.04 (1H, s, Ar-H), 7.83 (1H, s, Ar-H), 7.73-7.59 (1H, m, Ar-H), 7.38-7.32 (2H, s, Ar-H), 5.79 (2H, s, NCH_2), 4.42 (2H, s, CH_2), 3.20 (2H, q, *J*=8 Hz, CH_2), 2.48 (3H, t, *J*= 8Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.6 (CH_3), 31.2 (CH_2), 39.4-40.6 (DMSO-d_6+CH_2), 42.3 (NCH_2), 112.5 (Ar-CH), 120.5 (Ar-CH), 124.9 (Ar-C), 125.2 (Ar-C), 127.8 (Ar-CH), 129.2 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.7 (Ar-C), 134.9 (Ar-C), 134. 9 (Ar-C), 142.1 (Ar-C), 152.0 (NCN), 155.4 (thiadiazole-C_5), 169.8 (thiadiazole-C_2). Elemental analysis: Calculated for $C_{19}H_{15}Cl_4N_5S$: C, 46.84; H, 3.10; N, 14.37. Found: C, 46.82; H, 3.07; N, 14.39.

*N-(4-bromophenyl)-5-{[5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-thiadiazol-2-amine (**10**).* Yield: 3.68 g (60 %). M.P.: 263-264 °C, FTIR (KBr): 3188 (NH), 1617, 1556, 1511 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.48 (1H, brs, NH), 8.09 (1H, s, Ar-H), 7.85 (1H, s, Ar-H), 7.60-7.56 (1H, m, Ar-H), 7.54-7.08 (6H, m, Ar-H), 5.92 (2H, s, NCH_2), 4.45 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 31.2 (CH_2), 42.1 (NCH_2), 112.6 (Ar-CH), 113.8 (Ar-C), 119.8 (Ar-CH), 120.5 (Ar-CH), 125.1 (Ar-C), 125.4 (Ar-C), 127.8 (Ar-CH), 129.2 (Ar-CH), 132.2 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.6 (Ar-C), 134.9 (Ar-C), 140.0 (Ar-C), 142.0 (Ar-C), 154.8 (NCN), 155.5 (thiadiazole-C_5), 165.3 (thiadiazole-C_2). Elemental analysis: Calculated for $C_{23}H_{14}BrCl_4N_5S$: C, 44.98; H, 2.30; N, 11.40. Found: C, 44.96; H, 2.32; N, 11.38.

General procedure for the synthesis of the compounds 11, 12, 13

To the appropriate carbothioamide compound (**5**, **6**, **7**) (0.01 mol) in ethanol (15 mL) 2 N 15 mL of NaOH (0.064 mol) is added and the mixture was refluxed for 2 h. Then, the mixture was cooled to room temperature and adjusted to pH 4-5 with 37% HCl. The resulting product was filtered,

washed with water and purified by recrystallization from a mixture of ethanol:water, v:v 2:1.

*5-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-4-methyl-4H-1,2,4-triazole-3-thiol (**11**).* Yield: 3.40 g (72 %). M.P.: 255-256 °C, FTIR (KBr): 2930 (SH), 1598, 1584 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 13.53 (1H, s, SH), 8.03 (1H, s, Ar-H), 7.83 (1H, s, Ar-H), 7.59 (1H, s, Ar-H), 7.40-7.33 (2H, m, Ar-H), 5.73 (2H, s, NCH_2), 4.32 (2H, s, CH_2), 3.50 (3H, s, CH_3) . ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.4 (NCH_3), 30.9 (CH_2), 45.2 (NCH_2), 112.7 (Ar-CH), 120.4 (Ar-CH), 124.8 (Ar-C), 125.2 (Ar-C), 127.8 (Ar-CH), 129.1 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.8 (Ar-C), 134.9 (Ar-C), 135.6 (Ar-C), 142.1 (Ar-C), 148.5 (NCN), 155.9 (triazole-C_5), 168.1 (triazole-C_2). Elemental analysis: Calculated for $C_{18}H_{13}Cl_4N_5S$: C, 45.69; H, 2.77; N, 14.80. Found: C, 45.71; H, 2.79; N, 14.83.

*5-{[5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-4-ethyl-4H-1,2,4-triazole-3-thiol (**12**).* Yield: 3.31 g (68 %). M.P.: 240-242 °C, FTIR (KBr):2832 (SH), 1590, 1567 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 13.58 (1H, s, SH), 8.04 (1H, s, Ar-H), 7.84 (1H, s, Ar-H), 7.59 (1H, s, Ar-H), 7.37-7.33 (2H, m, Ar-H), 5.78 (2H, s, NCH_2), 4.34 (2H, s, CH_2), 4.03 (2H, q, *J*=8 Hz, OCH_2), 1.19 (3H, t, *J*= 8 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.5 (CH_3), 30.9 (CH_2), 39.1 (triazole-NCH_2) 45.2 (NCH_2), 112.6 (Ar-CH), 120.4 (Ar-CH), 124.9 (Ar-C), 125.3 (Ar-C), 127.8 (Ar-CH), 129.1 (Ar-CH), 132.9 (Ar-C), 133.4 (Ar-CH), 133.6 (Ar-C), 134.8 (Ar-C), 135.6 (Ar-C), 142.0 (Ar-C), 147.0 (NCN), 155.9 (triazole-C_5), 167.6 (triazole-C_2). Elemental analysis: Calculated for $C_{19}H_{15}Cl_4N_5S$: C, 46.84; H, 3.10; N, 14.37. Found: C, 46.81; H, 3.14; N, 14.32.

*4-(4-Bromophenyl)-5-{[5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]methyl}-4H-1,2,4-triazole-3-thiol (**13**).* Yield: 3.93 g (64 %). M.P.: 254-255 °C, FTIR (KBr): 2893 (SH), 1587, 1578 (CN) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 13.93 (1H, s, SH), 7.96 (1H, s, Ar-H), 7.81 (1H, s, Ar-H), 7.74 (1H, s, Ar-H), 7.66-7.61 (3H, m, Ar-H), 7.40-7.29 (3H, m, Ar-H), 5.50 (2H, s, NCH_2), 4.29 (2H, s, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 30.8 (CH_2), 45.3 (NCH_2), 112.6 (Ar-CH), 120.2 (Ar-CH), 123.7 (Ar-C), 124.6 (Ar-C), 125.1 (Ar-C), 127.8 (Ar-CH), 129.1 (Ar-CH), 130.7 (Ar-CH), 132.5 (Ar-C), 132.9 (Ar-C), 133.4 (Ar-CH), 133.4 (Ar-CH), 133.7 (Ar-C), 134.8 (Ar-C), 135.7 (Ar-C), 141.9 (Ar-C), 147.5 (NCN), 155.8 (triazole-C_5), 169.5 (triazole-C_2). Elemental analysis: Calculated for $C_{23}H_{14}BrCl_4N_5S$: C, 44.98; H, 2.30; N, 11.40. Found: C, 45.01; H, 2.27; N, 11.43.

Antioxidant Activity and Radical Scavenging Assays

Antioxidant activities of the synthesized compounds were clarified using various *in vitro* antioxidant assays, including Cupric Reducing Antioxidant Capacity (CUPRAC), ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)/Persulfate and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Catechin, Trolox® and Ascorbic acid were used as positive antioxidants.

Cupric reducing antioxidant capacity (CUPRAC) assay

In order to determine the cupric ions (Cu^{2+}) reducing ability of the synthesized compounds was determined according to the literature (19, 20, 21). The standard curve was linear between 32 mM and 1.25 mM trolox (r^2=0.9989). CUPRAC values were expressed as mM Trolox® equivalent of 1 mg synthesized compound.

DPPH-Free radical scavenging assay

The DPPH radical scavenging activity of the synthesized compounds was measured using the method of Brand-Williams (20, 21, 22, 23,). Briefly, 1200 microliter of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol was added 300 µL of the synthesized compound's solution in DMSO. Then, the mixture was kept in the dark for 50 minutes, the decrease in absorbance at 517 nm was measured, using a UV-Visible spectrophotometer (1601UV-Shimadzu, Australia). All determinations were carried out in triplicate and the results are expressed as % scavenging of DPPH radical, the percentage scavenging was calculated from the Formula given below:

$$\% \text{ Scavenging} = [(\text{Odcontrol} - \text{ODtest}) / (\text{ODcontrol}) \times 100].$$

$ABTS^{\bullet+}$ Radical Cation Decolorization Assay

The ability of the synthesized compounds to scavenge $ABTS^{\bullet+}$ radical was determined according to the literature (20, 21, 24). ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was dissolved in water to a 7 mM concentration and diluted to get an absorbance of 0.700 ± 0.020 at 734 nm before use. After 5 min in the dark at room temperature, the decrease of absorbance of reaction mixture containing 200 µL of compound solution and 1800 µL of the $ABTS^{\bullet+}$ solution was measured. The percentage scavenging was calculated from the formula below:

$$\% \text{ Scavenging} = [(\text{ODcontrol} - \text{ODtest}) / (\text{ODcontrol}) \times 100].$$

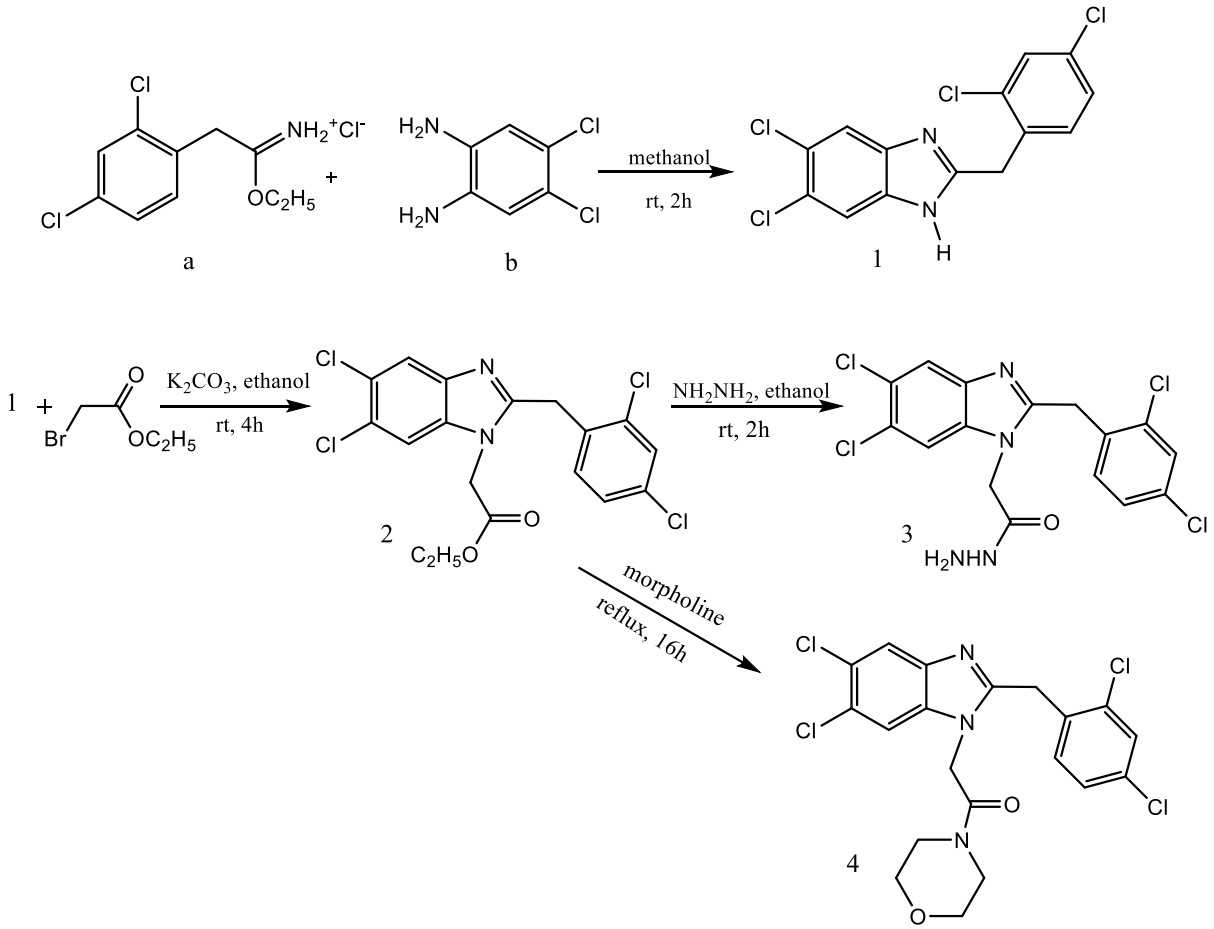
Urease inhibition assay

Urease is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The production of ammonia was measured by indophenol method and used to determine the urease inhibitory activity (25, 26, 27). The percentage remaining activity was calculated from the formula % Remaining Activity = [(ODtest)/(ODcontrol)x100]. Thiourea (S1) and

acetohydroxamic acid (S2) were used as standard inhibitors. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standards were assayed at the same reaction conditions.

RESULTS AND DISCUSSION

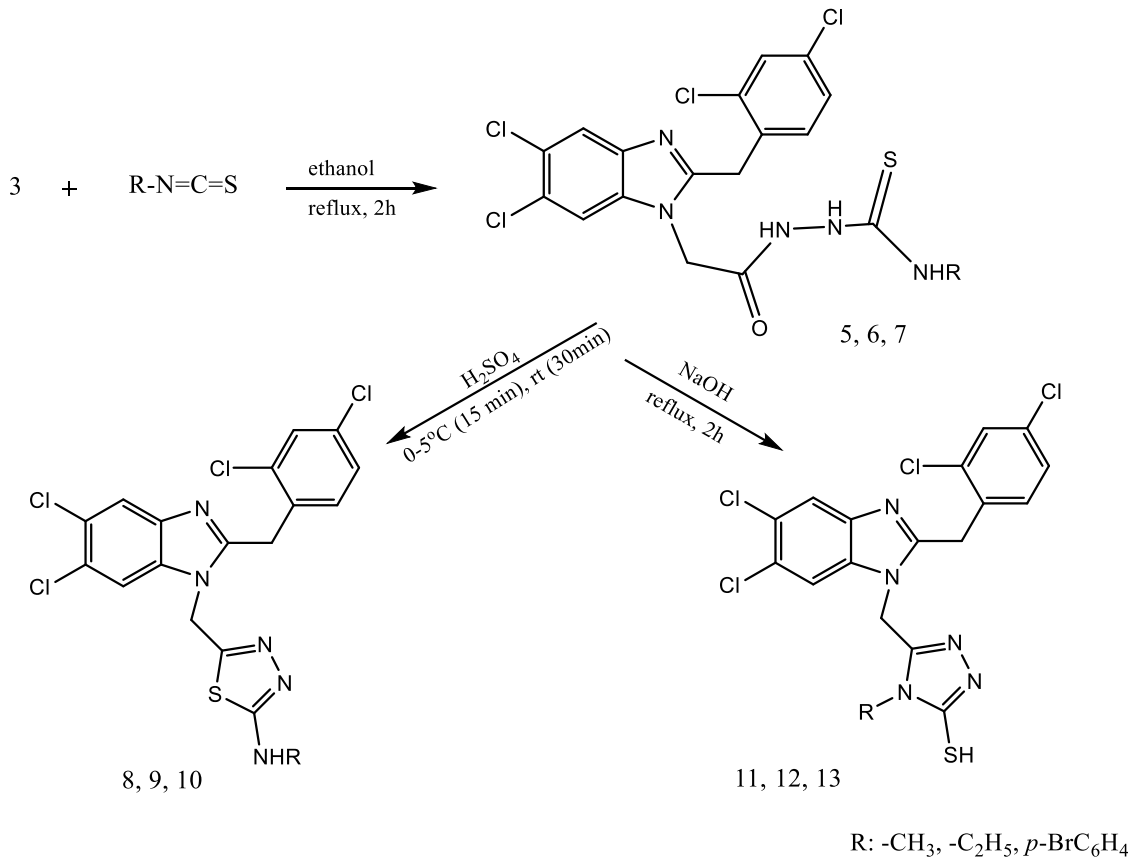
In the present study, we reported the synthesis and investigated the biological activities of antioxidant and urease inhibition of new 2-substituted benzimidazole derivatives containing triazole, thiadiazole, morpholine ring, and carbothioamide moiety. The synthetic procedures for target compounds were depicted in Schemes 1 and 2. The structures of the newly synthesized compounds were elucidated by elemental analysis, ^{1}H-^{13}C (APT) NMR, and FTIR spectral data.



Scheme 1. The synthetic routes for compounds **1**-**4**.

Imino ester hydrochloride compound (**a**) was synthesized according to the reported method in the literature (18). 5,6-Dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazole (**1**) was obtained by the nucleophilic attack of 4,5-dichlorobenzene-1,2-diamine to the imine carbon of compound **a** and subsequent removal of the ester group. The compound **1** was treated with ethylbromoacetate in the presence of potassium carbonate at room temparature to give ethyl [5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetate (**2**) followed by nucleophilic substitution with hydrazine monohydrate to obtain 2-[5,6-dichloro-2-(2,4-dichlorobenzyl)-1H-benzimidazol-1-yl]acetohydrazide (**3**).

The nucleophilic addition of morpholine nitrogen to the carbonyl group of compound **2** and followed by elimination of a mole of ethanol afforded 5,6-dichloro-2-(2,4-dichlorobenzyl)-1-(2-morpholin-4-yl-2-oxoethyl)-1H-benzimidazole (**4**). The reaction was carried out in solvent-free at 130-140 °C (reflux temperature).



Scheme 2. The synthetic routes for compounds **5**-**13**.

The synthesis of carbothioamide compounds (**5**, **6**, **7**) was accomplished by nucleophilic addition of the acetohyrazide compound (**3**) to the alkyl(aryl)isothiocyanates under reflux in ethanol. 1,3,4-Thiadiazole compounds (**8**, **9**, **10**) were obtained by the intramolecular cyclization reaction of carbothioamide compounds (**5**, **6**, **7**) in the presence of concentrated sulfuric acid. On the other hand, the synthesis of 1,2,4-triazole compounds (**11**, **12**, **13**) in the presence of 2 N NaOH was carried out by cyclization of the same compound (**5**, **6**, **7**). These compounds (**11**, **12**, **13**) may be present in the mercapto (-SH) or thioxo (C=S) tautomeric forms. The -SH protons of these compounds (**11**, **12**, **13**) resonate at 13.53, 13.58 and 13.93 ppm in the ^{1}H NMR, respectively. And also in the FT-IR spectra, the stretching bands of –SH function at 2913, 2832 and 2893 cm^{-1} and the signals of the triazole-C2 atom at 168.1, 167.6 and 169.5 ppm in ^{13}C NMR show us that these compounds are in the mercapto form. The ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds are in accordance with the structures of the synthesized compounds and the elemental data results of C, H and N atoms are within acceptable limits.

CUPRAC Antioxidant Activity Assay

With CUPRAC analysis, the exemplary compounds were examined for their capacity to reduce Cu^{+2} ions of Cu^{+}. And the absorbance of the complex Cu^{+} neocuproin was gauged at 450 nm, increasing the antioxidants with higher activity. The absorbance values for the samples were transformed to Trolox-equivalent antioxidant capacity (TEAC) values using the absorbance calibration graph [Trolox®]. And the TEAC results (mM) were shown in Figure 1. The highest antioxidant capacity was observed for the compound **11** (4.60 mM) in the CUPRAC method (Figure 1.). On the other hand, the compounds **3**, **5**, **6**, **7** and **12** showed good activity, while compounds **1**, **2**, **4**, **8** and **9** showed little activity. The others (**10** and **13**) had moderate TEAC values. Similar to the CUPRAC test results obtained, we have published benzimidazole derivative compounds containing triazole and thiophene groups with TEAC data in the range of 0.400 to 1.476 mM in one of our previous studies (20). Another study reported that a series of new benzimidazole derivative containing the triazole ring compounds with a high inhibitory activity with TEAC values at 4.16-8.67 mM in cuprac assay were synthesized (21).

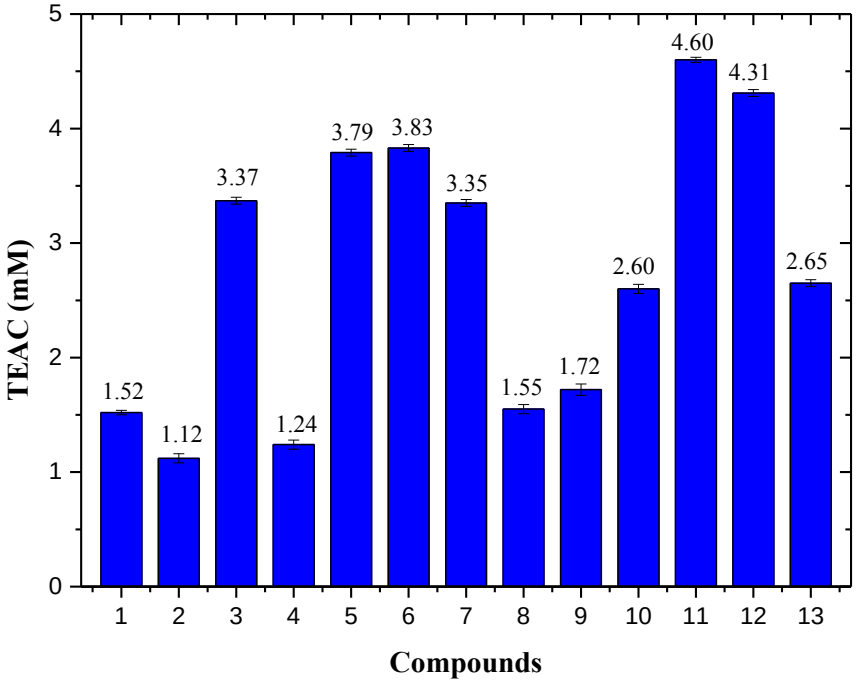


Figure 1. CUPRAC test results of all the synthesized compounds as mM TEAC (Trolox equivalent antioxidant capacity) values obtained from [Trolox]- absorbance calibration graph. TEAC values of compounds are expressed as the mean ±S.D. in triplicate.

DPPH• and ABTS•+ Radical Scavenging Activity Results

The total radical scavenging activity of the synthesized compounds was identified using DPPH and ABTS•+ radical scavenging assays and compared with catechin, ascorbic acid and Trolox® as standards. DPPH activity results of the synthesized compounds are given in Table 1. The compound **13** showed good DPPH activity at the 240 µg/mL concentration with 86.43% (Table 1). Beside this reality, it has been found that compounds **7**, **9** and **10** moderate scavenging activity at the same concentration. In the earlier published study, it has been determined that the SC_{50} results of the benzimidazole compounds containing the 1,2,4-triazole circle vary from 3.91 to 16.75 µg / mL according to the DPPH method (28). The benzimidazole compounds containing thiosemicarbazide moiety and 1,3,4-oxadiazole circle, were also effective DPPH radical scavengers, with SC_{50} results 77.36, 19.34, 13.46 and 13.27 µg/mL (23). In another study, benzimidazole derivatives containing triazole cycle were reported to be highly active with the SC50 values 7.03-31.27 µg / mL in the DPPH method (21).

Tablo 1. DPPH• radical scavenging activities of the compounds and standards at various final concentrations were expressed as the mean ±S.D. in triplicate.

Compounds and Standards	DPPH• Method				
	Radical Scavenging (%)				
	240 µg/mL	120 µg/mL	60 µg/mL	30 µg/mL	15 µg/mL
1	10.71	10.71	10.71	9.29	8.71
2	9.43	9.43	9.43	9.00	9.00
3	56.71	57.86	58.86	57.43	46.57
4	13.29	8.57	8.29	8.14	7.86
5	50.86	43.00	50.14	50.29	43.14
6	53.14	51.71	51.14	52.14	48.86
7	69.57	67.00	60.57	58.43	45.71
8	16.86	10.43	10.43	10.29	9.71
9	63.29	54.57	34.29	17.00	11.71
10	70.00	35.43	17.29	11.71	10.00
11	45.86	35.00	26.57	20.14	13.29
12	49.86	39.29	26.43	19.57	15.43
13	86.43	74.43	50.00	26.57	17.71
Catechin	90.71	90.71	90.71	90.71	85.57
Ascorbic Acid	90.71	90.71	90.71	90.71	82.29
Trolox®	90.71	90.71	90.71	85.86	80.14

ABTS•+ is produced by oxidation of ABTS with $K_2S_2O_8$ and is reduced in the presence of hydrogen-donating antioxidants. The compounds **3**, **5**, **6**, **7**, **11**, **12** and **13** showed efficient radical

scavenging activity, at all concentrations (Table 2). According to Radical Scavenging (%) activity results, the compounds can be listed **12** = **7**, **11** = **5**, **3** = **6**, **13** by decreasing degree at 24 µg/mL concentration (Table 2.).

Table 2. % $ABTS^{\bullet+}$ radical scavenging activities of the compounds and standards at various final concentrations were expressed as the mean ±S.D. in triplicate.

Compounds and Standards	ABTS•+ Method				
	Radical Scavenging (%)				
	24 µg/mL	12 µg/mL	6 µg/mL	3 µg/mL	1.5 µg/mL
1	11.43	11.43	11.29	11.29	11.29
2	8.71	8.57	8.43	8.29	8.14
3	90.43	89.86	75.57	57.29	30.57
4	15.71	12.86	12.71	11.86	11.00
5	91.00	89.57	83.00	49.71	30.43
6	90.43	90.29	76.43	48.14	31.14
7	91.14	91.14	70.14	41.71	27.14
8	13.43	13.43	13.43	12.86	12.00
9	47.00	27.43	20.00	18.00	15.00
10	33.43	23.00	20.86	19.43	18.57
11	91.00	80.14	54.71	40.00	30.71
12	91.14	82.14	54.29	38.00	31.00
13	84.29	57.14	38.57	29.14	24.29
Catechin	91.43	91.43	90.71	85.43	43.43
Ascorbic Acid	91.43	91.43	90.71	75.00	36.43
Trolox®	91.43	91.43	90.71	67.57	45.00

Urease Inhibition Results

In vitro inhibitory activity of all the synthesized compounds against Jack bean urease were investigated. Thiourea (S1) and acetohydroxamic acid (S2), clinically used for the treatment of urinary tract infections, were used as standard inhibitors. Initially, all the synthesized compounds were evaluated at the 60 µg/mL final concentration. Among these compounds, **6** exhibited the best inhibitory effect against urease with IC_{50} 2.52 µg/mL (Figure 2.). Also, the compounds **1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, **11**, **12** and **13** had higher inhibitory effect on urease and exhibited the lower IC_{50} values than acetohydroxamic acid and thiourea (Figure 2). The compound **10** demonstrated moderately inhibitory effect, according to the others. Baltas *et al.*, reported IC_{50} values of thiourea, compounds 5b and 5c as 11.91±0.33, 7.41±0.13 and 10.48±0.15 µg/mL, respectively (26). Researchers emphasized **9b** exhibited the best inhibitory effect against urease with IC_{50} value 28.89±0.11 µM. Compound **9b** inhibited urease activity by 36.07±0.41%, 68.29±0.09% and 98.43±0.28 at concentrations of 10.94, 21.87 and 43.75 µg/mL, respectively (27).

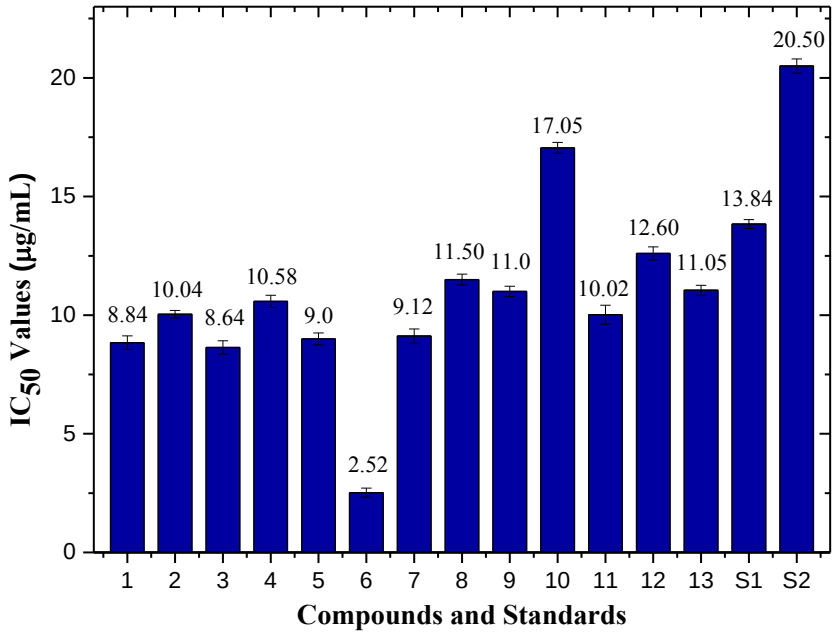


Figure 2. IC_{50} values of the synthesized compounds and standards against *Jack bean* urease. Thiourea (S1) and acetohydroxamic acid (S2)were used as standard inhibitors.

CONCLUSION

The present work led to the synthesis of novel benzimidazole derivatives containing the hybridization of the 5,6-dichlorobenzimidazole ring with different bioactive groups such as carbothioamide, morpholine, triazole ring and thiadiazole rings. Also, inhibition of antioxidant and urease enzymes of these compounds has been examined in this study. Most of the synthesized compounds showed good and moderate antioxidant activity. Especially **3**, **5**, **6**, **7**, **11**, **12**, **13** compounds showed efficient antioxidant activity in % ABTS•+ radical scavenging capacity tests. It was also found that all synthesized compounds except compound **10** had higher inhibitory effect on urease and exhibited the lower IC_{50} values (2.52-12.60 µg/mL) than acetohydroxamic acid (20.50 µg/mL) and thiourea (13.84 µg/mL), which are used as standard. The literature and this study show that the differences, location and number of substituents significantly effect the activities of these compounds.

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