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ARTICLE

Synthesis of novel tetra-substituted benzimidazole compounds containing certain heterostructures with antioxidant and anti-urease activities

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Abstract

A new series of 5,6-dimethyl-2-phenyl-1*H*-benzimidazole derivatives was synthesized. The antioxidant activities of the synthesized compounds were determined according to the cupric reducing antioxidant capacity (CUPRAC), ABTS, and DPPH assays. Many of the target compounds showed good antioxidant activity. Among these compounds, it has been determined that the carbothioamide and 1,2,4-triazole derivatives had a very good antioxidant capacity. Also, all compounds were screened for in vitro inhibitory activity against Jack bean urease. Among the synthesized molecules, the starting compound, acetate, and acetohydrazide derivatives (with IC_{50} values 12.02, 11.40, and 8.04 μg/mL, respectively) had a higher inhibitory effect on urease and exhibited a lower IC_{50} values than acetohydroxamic acid (IC_{50}: 20.50 μg/mL) and thiourea (IC_{50}: 14.04 μg/mL) as a reference inhibitors.

1 | INTRODUCTION

Urease is a nickel metalloenzyme catalyzing the hydrolysis of urea to ammonia and carbon dioxide. Urease enzyme is responsible for increasing the production of *Helicobacter pylori*, which causes gastric reflux, ulcer, and gastritis. Therefore, inhibition of urease enzyme is particularly important in the treatment of gastrointestinal and urinary tract infections. By inhibition of the enzyme, the bacteria cannot attach to the stomach and urinary system. Therefore, it is very important to develop new potential and more effective inhibitors that will be an alternative to existing urease inhibitors such as hydroximate, thiols, phosphoramide compounds, diketones, biscoumarin, some metal ions (Ag^{+1}, Hg^{+2}, Cu^{+2}, and Cd^{+2}), sulfur compounds, benzoquinone, triazoles, and some aqueous-organic solvents.[1–11] Recent studies have clearly demonstrated that benzimidazole and its derivatives can be used as potential active urease inhibitors.[12,13]

Free radicals in the body damage many biological structures such as DNA, protein, and lipid molecules. Therefore, they are associated with various types of cancer, cardiovascular, Alzheimer, Parkinson, and many other diseases.[14] Free radicals are also associated with aging, which is defined as a gradual accumulation of free radical damage.[15] Antioxidants are molecules that keep free radicals under control. Our body can produce some antioxidants on its own, but this is insufficient. In addition to natural antioxidants (such as vitamins E, A, and C, carotenoids, natural flavonoids, uric acid, bilirubin, lipoic acid, metatonin, and glutathione), many synthetic antioxidants are known (butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone, propyl gallate, and octyl gallate). Regarding their functions, there are many studies on the clinical use of natural and synthetic antioxidants.[16–19]

There is increasing interest on the benzimidazole ring because of its chemical structure reactivity and richness of biological activity. Compounds having a wide variety

of biological activities such as antimicrobial, anticancer, antiviral, antiulcer, antiprotozoal, anti-inflammatory, and analgesic efficiency activities have been reported as a result of changing groups on the benzimidazole core structure. In our previous studies, it was reported that benzimidazoles showed good levels of urease inhibitory and antioxidant activities. This study supports other studies, and how different groups and positions in the benzimidazole ring affect urease inhibition and antioxidant properties was investigated.[12,20–23]

2 | RESULT AND DISCUSSION

2.1 | Chemistry

In this study, we first synthesized the starting compound 5,6-dimethyl-2-phenyl-1*H*-benzimidazole (**1**) according to the method in the literature.[24] Then, we converted compound **1** into ester (**2**) and hydrazide (**3**) derivatives, respectively. The obtained hydrazide compound (**3**) was used as starting material for the synthesis of oxadiazole (**5**), Schiff bases (**4a**-**d**), and carbothioamide (**6a**-**b**) derivatives (Scheme 1).

Firstly, 5,6-dimethyl-2-phenyl-1*H*-benzomidazole (**1**) was synthesized from the reaction of 4,5-dimethylbenzene-1,2-diamine with ethyl benzimidate hydrochloride. Molecule **2** was obtained from the reaction of molecule **1** with ethyl bromoacetate and K_2CO_3 in dry acetone. The ester group signals of compound **2** were observed at 1.13 ppm ($OCH_2C\underline{H}_3$) and 4.10 ppm ($OC\underline{H}_2CH_3$) in the ^{1}H NMR spectrum. Also in the ^{13}C NMR spectrum, the signals of these groups were determined at 14.36 ($OCH_2\underline{C}H_3$) and 61.74 ppm ($O\underline{C}H_2CH_3$). The compound **3** was synthesized by reacting compound **2** with hydrazine monohydrate in dry ethanol. The $NHNH_2$ group signals of compound **3** appeared at 4.43 (NH_2) and 9.53 ppm (NH) in ^{1}H NMR spectrum. The molecules **4a**-**d** were obtained by reacting compound **3** with benzaldehyde for **4a**, 4-chlorobenzaldehyde for **4b**, 4-(dimethylamino)benzaldehyde for **4c**, and salicyl aldehyde for **4d**. When the ^{1}H NMR spectra of the compounds **4a**-**b** are examined, the NH proton peaks of 11.78 and 11.90 ppm (**4a**), 11.84 and 11.97 ppm (**4b**), 11.47 and 11.58 ppm (**4c**), and 11.69 and 12.08 ppm (**4d**) are seen as binary sets due to cis-trans amide conformer structure. Also, the proton signals of CH_3, NCH_2, imine (N═CH), and OH for **4d** were resonated as a double set due to cis-trans amide conformer structure. The imine carbon atoms of these compounds resonated at 144.86 (for **4a**), 143.58 (for **4b**), 145.69 (for **4c**), and 148.08 ppm (for **4d**) in the ^{13}C NMR spectrum.

The compound **5** was obtained in a basic medium by nucleophilic attack of the NH_2 group of compound **3** to CS_2 followed by intramolecular cyclization. The SH or NH (due to its tautomer) proton of the compound **5** was not observed in the ^{1}H NMR spectrum. The C_2 and C_5 carbon signals of the oxadiazole ring were observed at 159.73 and 178.33 ppm in the ^{13}C NMR spectrum, respectively. Carbothioamide derivatives (**6a**, **6b**) were obtained by nucleophilic attack of compound **3** to methylisocyanate (for **6a**) and ethylisocyanate (for **6b**). The NH signals of the compounds **6a**-**b** are in the range of 8.07 to 10.29 ppm as singlet peak in ^{1}H NMR spectra, and the C═S signals of these compounds were also determined at 170.99 (for **6a**) and 170.93 (for **6b**) ppm in ^{13}C NMR spectra.

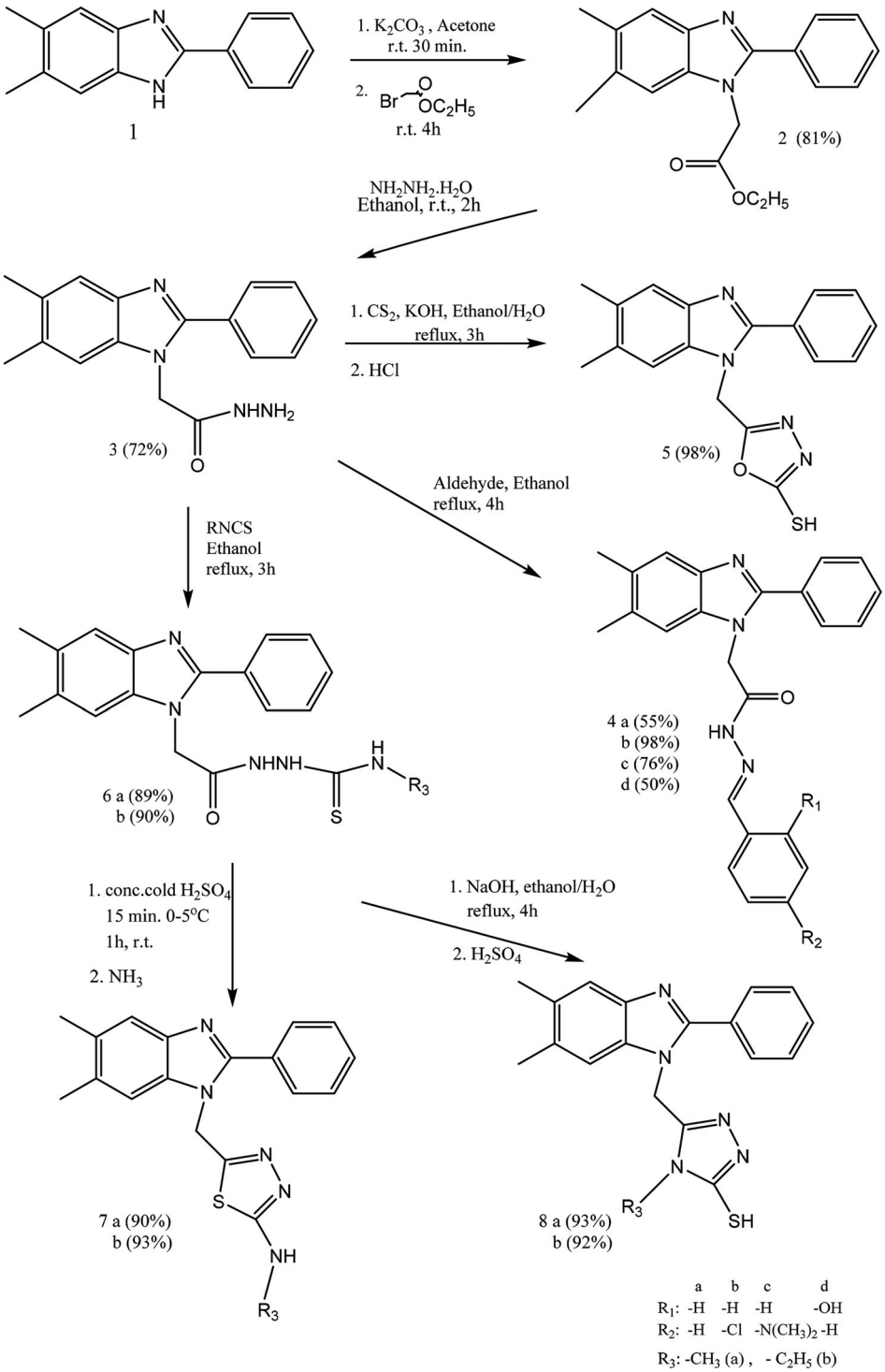
The compounds **7a** and **7b** were synthesized as a result of intramolecular cyclization of carbothioamide derivatives (**6a**, **6b**) in the presence of concentrated cold sulfuric acid. The NH signals of the compounds **6a**-**b** disappeared. NH signals attached to the thiadiazole ring at 7.62 (for **7a**) and 7.65 (for **7b**) ppm were observed in the aromatic region. In the ^{13}C NMR spectra, C_5 peaks of thiadiazole ring were observed at 153.51 (for **7a**) and 153.31 ppm (for **7b**), and C_2 peaks of thiadiazole ring were observed at 170.32 (for **7a**) and 169.36 ppm (for **7b**).

In the last step, the synthesis of benzimidazole derivatives containing triazole ring (**8a**, **8b**) was carried out by intramolecular cyclization of carbothioamide compounds in the presence of sodium hydroxide in the basic medium. In the ^{1}H NMR spectra of compounds **8a** and **8b**, SH protons are seen at 13.58 (for **8a**) and 13.65 ppm (for **8b**). Also, the triazole-C_5 and triazole-C_2 carbon peaks of compound **8a** are seen at 152.72 and 167.94 ppm, and the triazole-C_5 and triazole-C_2 carbon peaks of compound **8b** are seen at 152.62 and 167.41 ppm, respectively. The presence of NH and OH proton peaks of all synthesized compounds was determined by the exchange with D_2O. The infrared (IR) spectral data of the compounds are compatible with the structures. The results of the elemental analysis are within acceptable limits.

2.2 | CUPRAC antioxidant activity

With cupric reducing antioxidant capacity (CUPRAC) assay, the sample compounds were tested for their abilities to reduce copper (II) to copper (I) ions, and the absorbance of copper (I) neocuproine complex was measured at 450 nm, increasing with higher activity antioxidants. The absorbances measured for the samples were converted to Trolox equivalent antioxidant capacity (TEAC) values obtained from the absorbance [Trolox] calibration graph, and the mM TEAC values were given

in Figure 1 and the maximum antioxidant capacity in the CUPRAC method was observed for the compound **6b** (Figure 1). The compounds **8a**, **8b**, **6a**, and **4c** showed very good antioxidant capacity. On the other hand, the compounds **3** and **5** showed good activity, while compounds **1**, **2**, **4a**, **4b**, **4d**, **7a**, and **7b** showed little activity. As with our TEAC results, it was reported that cupric values of the triheterocyclic compounds containing thiophene and 1,2,4-triazole groups ranged from 0.400 ± 0.072 to 1.476 ± 0.025 mg TEAC/mg compounds.[25] In another study, it was expressed that benzimidazole derivatives containing a triazole nucleus were highly active in CUPRAC assay, with 4.16 to 8.67mM TEAC/mg compound values.[26]



SCHEME 1 The reaction pathway for the target compounds

2.3 | DPPH· and ABTS·+ radical scavenging activity

The total radical scavenging capacity of the compounds was determined and compared with that of Trolox, ascorbic acid, and catechin by using the 2, 2-diphenyl-2-picrylhydrazyl (DPPH·) and 2,2′-azino-bis(3--ethylbenzothiazoline-6-sulfonic acid) (ABTS·+) radical scavenging methods. DPPH radical scavenging activity results of compounds and standards are summarized in Table 1. The compounds **5** and **6b** showed fairly well DPPH radical scavenging activity, at the 120 μg/mL final concentration (Table 1). Besides this reality, it has been found that compounds **6**a and **6b** have greater scavenging activity than ascorbic acid at 3.75 μg/mL final concentration. On the other hand, the compound **8a** showed good activity at the same concentration. In an earlier published study, it was determined that SC_{50} values of compounds containing the 1,2,4-triazole ring ranged from 3.91 to 16.75 μg/mL in the DPPH method.[27] The compounds containing oxadiazole and thiosemicarbazide were also effective in DPPH radical scavengers with SC_{50} values of 19.34, 77.36, 13.46, and 13.27 μg/mL.[28] It was informed that benzimidazole derivatives containing a triazole nucleus were highly active in the DPPH method with SC_{50} values of 7.03 to 31.27 μg/mL.[29]

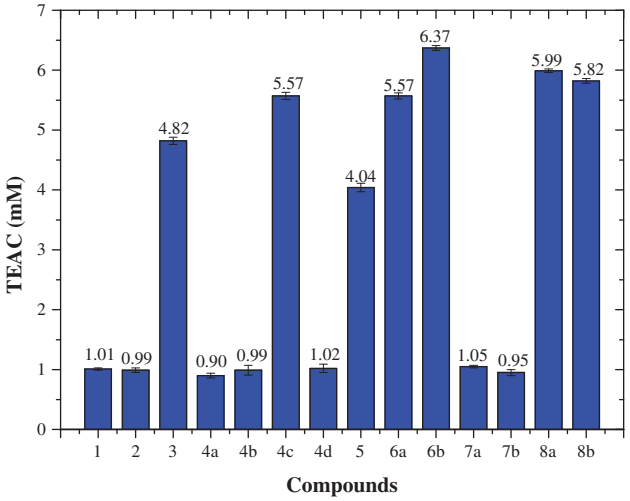


FIGURE 1 Cupric reducing antioxidant capacity (CUPRAC) test results of all the synthesized compounds as mM TEAC values obtained from [Trolox]-absorbance calibration graph. TEAC values of compounds are expressed as the mean ± SD in triplicate. TEAC, Trolox equivalent antioxidant capacity

ABTS·+ is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such as hydrogen-donating antioxidants. The compounds **3**, **5**, **6a**, **6b**, **8a**, and **8b** showed efficient radical scavenging activity at the 12.0μg/mL final concentration (Table 2). According to having effective scavenging activity by decreasing degree, compounds **8a**, **6b**, **8b**, **6a**, **5**, and **3** at the 3-μg/mL final concentration, respectively, could be

TABLE 1 % DPPH· radical scavenging values of the compounds and standards at various final concentrations

Compounds and Standards	120 μg/mL	60 μg/mL	30 μg/mL	15 μg/mL	7.5 μg/mL	3.75 μg/mL
1	7.14	6.29	6.29	6.00	5.71	5.71
2	7.43	7.29	7.14	7.14	7.00	7.00
3	59.14	53.00	46.00	32.14	27.71	19.29
4a	8.86	8.43	8.43	8.14	8.00	7.86
4b	7.86	5.86	5.71	5.71	5.57	5.57
4c	8.86	8.86	8.57	8.57	8.43	8.14
4d	11.43	10.86	10.29	9.00	8.43	7.29
5	90.71	87.40	79.86	64.14	21.57	14.71
6a	88.86	80.86	69.00	51.00	43.29	33.86
6b	91.00	82.86	71.71	55.43	44.71	40.86
7a	8.57	8.57	8.43	8.14	7.86	7.43
7b	11.14	10.71	9.86	9.43	9.00	7.86
8a	82.71	69.57	50.43	38.29	26.71	13.86
8b	45.29	35.86	29.57	23.57	17.86	14.14
Catechin	90.71	90.71	90.71	85.57	60.43	44.71
Ascorbic Acid	90.71	90.71	90.71	82.29	50.86	19.29
Trolox	90.71	90.71	85.86	80.14	65.57	25.43

Abbreviation: DPPH, 1,1-diphenyl-2-picrylhydrazyl.

TABLE 2 % $ABTS^{\cdot+}$ radical scavenging values of the compounds and standards at various final concentrations

Compounds and Standards	12.0 μg/mL	6.0 μg/mL	3.0 μg/mL	1.5 μg/mL	0.75 μg/mL	0.375 μg/mL
1	14.57	12.57	11.71	11.43	11.00	10.56
2	13.86	12.86	12.43	12.14	11.71	10.11
3	91.43	91.14	67.43	37.71	17.14	9.45
4a	13.29	12.14	11.86	11.29	11.14	10.32
4b	11.29	11.14	11.00	10.86	10.71	9.98
4c	77.86	66.86	45.71	31.57	22.57	15.39
4d	85.86	71.29	31.71	18.57	13.86	9.59
5	91.43	91.29	72.86	44.14	28.00	10.13
6a	91.29	91.29	74.00	43.57	31.00	15.43
6b	91.29	91.29	77.86	47.43	30.29	14.93
7a	11.71	10.29	10.14	10.00	9.86	8.86
7b	14.00	12.00	11.86	11.29	10.43	10.10
8a	91.43	91.29	79.14	46.43	21.71	8.78
8b	91.43	91.29	74.86	41.71	23.43	9.69
Catechin	91.43	90.71	85.43	43.43	21.86	11.55
Ascorbic Acid	91.43	90.71	75.00	36.43	18.14	9.67
Trolox	91.43	90.71	77.57	45.00	20.86	10.19

Abbreviation: ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

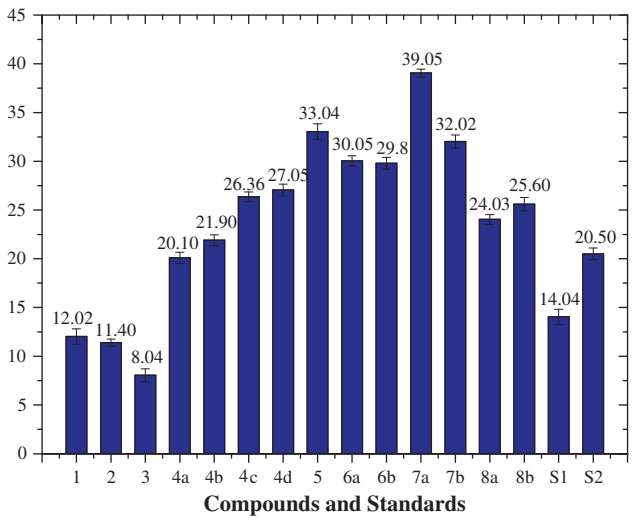


FIGURE 2 IC_{50} values of the synthesized compounds against Jack bean urease. Thiourea (S1) and acetohydroxamic acid (S2) were used as standard inhibitors

numbered (Table 2). In earlier published studies, a number of benzimidazole derivative compounds containing some different groups, such as thiophene, 1,2,4-triazole rings, salicyl, oxadiazole, thiosemicarbazide, benzimidazole derivatives containing a triazole nucleus, and 1,2,4-triazole and fluoro, have been reported to have good $ABTS^{\cdot+}$ radical scavenging activity.[26–29]

2.4 | Urease inhibition

All the compounds were screened for their in vitro inhibitory activity against *Jack bean* urease. Thiourea (S1) and acetohydroxamic acid (S2) were used as standard inhibitors. Initially, all the synthesized compounds were screened at the 60-μg/mL final concentration. Among these compounds, molecule **3** exhibited the best inhibitory effect against urease with IC_{50} 8.04 μg/mL (Figure 2). Also, compounds **1**, **2**, and **3** (with IC_{50} values 12.02, 11.40, and 8.04 μg/mL, respectively) had higher inhibitory effect on urease and exhibited lower IC_{50} values than acetohydroxamic acid and thiourea (Figure 2). Nimet et al reported that carbothioamide derivatives exhibited the best inhibitory effect against urease. IC_{50} values of carbothioamide compounds were determined as 7.41 ± 0.13 and 10.48 ± 0.15 μg/mL, respectively.[30] Researchers reported that 1,2,4-triazol-3-one compound exhibited the best inhibitory effect against urease with IC_{50} value 28.89 ± 0.11μM.[31]

3 | CONCLUSION

In this study, a new series of benzimidazole compounds with oxadiazole, triazole, thiadiazole ring, Schiff, and carbothiamide structures were synthesized as potential bioactive compounds. The antioxidant activity and urease

inhibitory effects of these synthesized compounds were investigated, and the effects of these results on groups and positions were discussed. It is seen that 1,2,4-triazole groups significantly increase antioxidant activity, and compounds **1**, **2**, and **3** show higher anti-urease results than the standards used.

4 | EXPERIMENTAL

Merck, Sigma-Aldrich, and Fluka products were used as chemical reagents. The melting points of the synthesized compounds were determined in the Q device. The IR spectra were obtained on 100 Fourier transform infrared (FTIR) Perkin-Elmer spectrophotometer as attenuated total reflection (ATR). ^{1}H and ^{13}C NMR spectra were performed in hexadeuterated dimethyl sulfoxide (DMSO-d_6) in the presence of tetramethylsilane (TMS) as an internal standard using Varian-Mercury (400 and 100 MHz, respectively). The mass spectrum data (electrospray ionization-mass spectrometry [ESI-MS], *m/z* [%]) were recorded on the Thermo Scientific Quantum Access liquid chromatography-mass spectrometry (LC-MS) spectrometer. The reaction progresses were controlled by thin-layer chromatography (TLC) plates (60 F 2.54 0.2 mm thickness silicagel-based aluminum sheets).

4.1 | General method for the synthesis of 5,6-dimethyl-2-phenyl-1*H*-benzimidazole (1)

To the solution of iminoester hydrochloride (0.011 mol) in methanol was added 4,5-dimethylbenzene-1,2-diamine (0.01 mol) and stirred at room temperature for 5 hours. The completion of the reaction was checked by TLC (ethylacetate: hexane, 1:1). The resulting product was precipitated with water and filtered and recrystallized from the ethanol-water mixture.

White solid (2.13 g), 96% yield. m.p. 250°C to 251°C (CAS Registry Number 14313-45-2). FTIR (ν_{max}/cm^{-1}): 3290-2620 (NH), 1586 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 6H, CH_3), 7.36 (s, 2H, ArH), 7.44-7.54 (m, 3H, ArH), 8.14 (d, 2H, J = 4 Hz, ArH), 12.63 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.4 ($2CH_3$), ArC [126.6, 129.3, 129.9, 130.8], 150.7 (benzimidazole-C_2). ESI-MS (*m/z*) $C_{15}H_{14}N_2$ $[M + Na]^+$: 245.99, $[M + K]^+$: 262.09.

4.2 | General method for the synthesis of ethyl 2-(5,6-dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)acetate (2)

To a 25mL absolute acetone solution containing 0.01 mol (2.22 g) of compound **1** was added about 0.033 mol (4.55 g) of K_2CO_3 and stirred for 30 minutes at room temperature. Then, 0.01 mol of ethylbromoacetate was added, and the mixture was stirred at room temperature for 4 hours. The complete reaction was controlled by TLC (ethylacetate: hexane, 2:1). The resulting product was precipitated with water and filtered. It was recrystallized from the ethanol-water mixture.

White solid (2.49 g), 81% yield. m.p. 82°C to 84°C. FTIR (ν_{max}/cm^{-1}): 1728 (C═O), 1651 (C═N), 1204 (C─O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.13 (t, 3H, CH_3, J = 8 Hz), 2.33 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.10 (q, 2H, CH_2, J = 8 Hz), 5.13 (s, 2H, NCH_2), 7.35 (s, 1H, ArH), 7.47 (s, 1H, ArH), 7.53-7.54 (m, 3H, ArH), 7.67 (d, 2H, ArH, J = 4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.3 (CH_3), 20.3 (CH_3), 20.6(CH_3), 46.4 (NCH_2), 61.7 (OCH_2), ArC [111.0, 119.6, 129.2, 130.0, 130.5, 131.0, 131.8, 135.2, 141.4], 152.7 (benzimidazole-C_2), 168.7 (C═O). ESI-MS *m/z* $C_{19}H_{20}N_2O_2$ $[M + H]^+$: 309.04.

4.3 | General method for the synthesis of 2-(5,6-dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)acetohydrazide (3)

Compound **2** (0.01 mol, 3.08 g) and 0.03 mol (2.58 mL) of hydrazine monohydrate was stirred in 20 mL of absolute ethanol at room temperature for 2 hours. The complete reaction was controlled by TLC (ethylacetate: hexane, 2:1). The resulting product was filtered and recrystallized from the ethanol-water mixture.

White solid (2.11 g), 72% yield. m.p. 214°C to 215°C. FTIR (ν_{max}/cm^{-1}): 3437, 3296, 3198 (NH + NH_2), 1651 (C═O), 1540 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 4.43 (s, 2H, NH_2), 4.77 (s, 2H, NCH_2), 7.18-7.22 (m, 1H, ArH), 7.46 (s, 1H, ArH), 7.71-7.78 (m, 3H, ArH), 7.98 (d, 2H, ArH, J = 4 Hz), 9.53 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.36 (CH_3), 20.66 (CH_3), 46.15 (NCH_2), ArC [111.0, 119.5, 129.0, 129.6, 129.9, 130.6, 130.8, 131.5, 135.3, 141.5], 153.1 (benzimidazole-C_2), 166.8 (C═O). ESI-MS *m/z* $C_{17}H_{18}N_4O$ $[M + H]^+$: 294.99.

4.4 | General method for the synthesis of Schiff-based compounds (4a-d)

A mixture of compound **3** (0.01 mol, 2.94 g) and the corresponding benzaldehyde derivatives (0.01 mol) with the catalytic amount of acetic acid in absolute ethanol (25 mL) were refluxed for 4 hours. The complete reaction was controlled by TLC (ethylacetate: hexane, 3:1). The mixture was cooled to the room temperature. The precipitated product was filtered and recrystallized from ethanol-water (2:1) to obtain pure compounds.

4.4.1 | *N′*-Benzylidene-2-(5,6-dimethyl-2-phenyl-1*H*-benzimidazol-1-yl) acetohydrazide (4a)

White solid (2.10 g), 55% yield. m.p. 194°C to 195°C. FTIR (ν_{max}/cm^{-1}): 3201 (NH), 3066 (Ar—CH), 2919 (Aliphatic-CH), 1672 (C═O), 1607, 1556 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.99, 5.44 (s, 2H, NCH_2, trans-cis conformer ratio: 14/86), 7.30-7.33 (m, 1H, ArH), 7.42-7.51 (m, 7H, ArH), 7.71-7.75 (m, 4H, ArH), 8.05, 8.25 (s, 1H, N═CH, cis-trans conformer ratio 75/25), 11.78, 11.90 (s, 1H, NH, cis-trans conformer ratio 75/25). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.5 (CH_3), 46.1 (NCH_2), ArC [111.2, 119.5, 127.4, 129.1, 129.2, 129.9, 130., 130.7, 130.7, 130.8, 131.6, 134.2, 135.7, 141.5], 144.8 (—N═CH), 153.1 (C═N), 168.8 (C═O). ESI-MS *m/z* $C_{24}H_{22}N_4O$ $[M + H]^+$: 383.10.

4.4.2 | *N′*-(4-Chlorobenzylidene)-2-(5,6-dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)acetohydrazide (4b)

White solid (4.08 g), 98% yield. m.p. 267°C to 269°C. FTIR (ν_{max}/cm^{-1}): 3194 (NH), 3061, 2957 (Ar—CH), 1678 (C═O), 1607, 1595 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.34 (s, 6H, CH_3), 4.99, 5.44 (s, 2H, NCH_2, trans-cis conformer ratio: 22/78), 7.29-7.32 (m, 1H, ArH), 7.48-7.52 (m, 6H, ArH), 7.69-7.75 (m, 4H, ArH), 8.03, 8.24 (s, 1H, N═CH, cis-trans conformer ratio 67/33), 11.84, 11.97 (s, 1H, NH, cis-trans conformer ratio: 67/33). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.5 (CH_3), 46.2 (NCH_2), ArC [111.1, 119.5, 129.1, 129.2, 128.2, 129.4, 129.9, 130.7, 130.8, 131.6, 133.2, 134.9, 135.7, 141.5], 143.5 (-N═CH), 153.1 (C═N), 168.9 (C═O). ESI-MS *m/z* $C_{24}H_{21}ClN_4O$ $[M]^+$: 417.05.

4.4.3 | 2-(5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)-*N′*-(4-(dimethylamino) benzylidene)acetohydrazide (4c)

White solid (3.23 g), 76% yield. m.p. 232°C to 233°C. FTIR (ν_{max}/cm^{-1}): 3190 (NH), 3049, 2918 (Ar—CH), 1654 (C═O), 1598 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 4.92, 5.35 (s, 2H, NCH_2,trans-cis conformer ratio: 40/60), 6.68-6.73 (m, 2H, ArH), 7.26-7.29 (m, 1H, ArH), 7.46-7.52 (m, 6H, ArH), 7.67-7.75 (m, 2H, ArH), 7.89, 8.07 (s, 1H, N═CH, cis-trans conformer ratio: 67/33), 11.47, 11.58 (s, 1H, NH, cis-trans conformer ratio: 67/33). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.5 (CH_3), 40.1 ($2CH_3$), 46.1 (NCH_2), ArC [112.1, 119.5, 121.5, 128.7, 129.1, 129.2, 129.5, 129.9, 130.7, 130.9, 131.5, 135.7, 141.5], 145.6 (-N═CH), 151.9, 153.0 (C═N), 168.2 (C═O). ESI-MS *m/z* $C_{26}H_{27}N_5O$ $[M + H]^+$: 426.15.

4.4.4 | 2-(5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)-*N′*-(2-hydroxybenzylidene)acetohydrazide (4d)

White solid (1.99 g), 50% yield. m.p. 273°C to 275°C. FTIR (ν_{max}/cm^{-1}): 3152 (NH), 2949 (Ar—CH), 2862, 2760 (aliphatic-CH), 1698 (C═O), 1612, 1553 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 6H, CH_3), 4.99, 5.41 (s, 2H, NCH_2, trans-cis conformer ratio: 40/60), 6.80-6.90 (m, 2H, ArH), 7.22-7.31 (m, 2H, ArH), 7.47-7.53 (m, 5H, ArH), 7.70-7.76 (m, 3H, ArH), 8.38, 8.47 (s, 1H, N═CH, cis-trans conformer ratio: 67/33), 10.05, 10.89 (s, 1H, OH, cis-trans conformer ratio: 67/33), 11.69, 12.08 (s, 1H, NH, cis-trans conformer ratio: 67/33). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.5 (CH_3), 46.1 (CH_2), ArC [111.2, 116.5, 119.5, 120.4, 129.2, 129.5, 129.9, 130.0, 130.7, 130.8, 131.62, 132.0, 135.7, 141.5], 148.0 (—N═CH), 153.1, 156.8 (C═N), 168.5 (C═O). ESI-MS *m/z* $C_{24}H_{22}N_4O_2$ $[M]^+$: 398.96.

4.5 | General method for the synthesis of 5-((5,6-dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (5)

To a solution of compound **3** (0.01 mol, 3.08 g) in 20 mL of absolute ethanol was added 1.00 mol (0.25 mL) of CS_2 and 1.00 mol of KOH solution (50 mL), and the mixture was refluxed for 3 hours. Water was added to the cooled mixture and acidified with HCl, and the precipitate was filtered off. The precipitated product was filtered and recrystallized from ethanol-water (2:1) to obtain pure compounds.

White solid (3.29 g), 98% yield. m.p. 232°C to 234°C. FTIR (ν_{max}/cm^{-1}): 3039 (Ar—CH), 2918 (SH), 1629, 1506 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 5.62 (s, 2H, NCH_2), 7.42-7.55 (m, 5H, ArH), 7.74 (s, 2H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3, 20.6, 39.3-40.5 (DMSO-d_6 + CH_2), ArC [111.2, 119.7, 129.3, 129.5, 129.8, 130.3, 131.7, 132.3, 134.6, 141.2], 152.5 (benzimidazole-C_2), 159.7 (oxadiazole-C_2), 178.3 (oxadiazole-C_5). ESI-MS *m/z* $C_{18}H_{16}N_4OS$ $[M]^+$: 336.98.

4.6 | General method for the synthesis of compounds 6a-b

To the solution of compound **3** (0.01 mol, 3.08 g) in 25 mL of absolute ethanol (25 mL), 0.01 mol of methyl isothiocyanate (for compound **6a**) and ethyl isothiocyanate (for compound **6b**) were added, and the mixture was refluxed for 3 hours. The complete reaction was controlled by TLC (ethylacetate: hexane, 2:1). The mixture was cooled to room temperature. The precipitated product was filtered and recrystallized from the ethanol-water (2:1) mixture.

4.6.1 | 2-(2-(5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)acetyl)-*N*-methylhydrazine-1-carbothioamide (6a)

White solid (3.26 g), 89% yield. m.p. 229°C to 230°C. FTIR (ν_{max}/cm^{-1}): 3371, 3319 (NH), 2916 (Aliphatic-CH), 2850 (SH), 1686 (C═O), 1541 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 4.88 (s, 2H, NCH_2), 7.27 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.53-7.71 (m, 5H, ArH), 8.07, 9.41, 10.26 (s, 3H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.6 (CH_3), 31.3 (CH_3), 46.2 (NCH_2), ArC [111.3, 119.5, 129.1, 129.6, 130.0, 130.4, 130.9, 131.6, 135.3, 141.4], 153.0 (benzimidazole-C_2), 167.5 (C═O), 170.9 (C═S). ESI-MS *m/z* $C_{19}H_{21}N_5OS$ $[M + H]^+$: 368.12.

4.6.2 | 2-(2-(5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)acetyl)-*N*-ethylhydrazine-1-carbothioamide (6b)

White solid (3.42 g), 90% yield. m.p. 233°C. FTIR (ν_{max}/cm^{-1}): 3358, 3208 (NH), 2972, 2923 (Aliphatic-CH), 2897 (SH), 1679 (C═O), 1538 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.11 (t, 3H, CH_3, $J = 8$ Hz), 2.34 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.50 (q, 2H, CH_2, $J = 8$ Hz), 4.92 (s, 2H, CH_2), 7.23-7.32 (m, 1H, ArH), 7.47-7.55 (m, 4H, ArH), 7.68-7.74 (m, 2H, ArH), 8.11, 9.36, 10.29 (s, 3H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.9 (CH_3), 20.3 (CH_3), 20.5 (CH_3), 38.9 (CH_2), 46.2 (NCH_2), ArC [111.3, 119.5, 129.1, 129.6, 130.0, 130.4, 130.9, 131.6, 135.3, 141.4], 153.0 (benzimidazole-C_2), 167.4 (C═O), 170.9 (C═S). ESI-MS *m/z* $C_{20}H_{23}N_5OS$ $[M + H]^+$: 382.12.

4.7 | General method for the synthesis of compounds 7a-b

A mixture of corresponding thiosemicarbazide (6a, 6b) (0.01 mol) in cold conc. sulfuric acid (20 mL) was stirred at 0–5°C for 15 min. The stirring was then continued for 1 hour at room temperature. The resulting solution was poured into ice water and alkalized to pH 8 with NH_3. The precipitate was filtered off and washed with plenty of water. The precipitated product was filtered and recrystallized from ethanol-water (2:1) to obtain pure compounds.

4.7.1 | 5-((5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)methyl)-*N*-methyl-1,3,4-thiadiazol-2-amine (7a)

White solid (3.14 g), 90% yield. m.p. 182°C to 184°C. FTIR (ν_{max}/cm^{-1}): 3278 (NH), 2967, 2919 (Ar—CH), 2858 (Aliphatic-CH), 1551 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 5.67 (s, 2H, NCH_2), 7.41-7.55 (m, 5H, ArH), 7.62 (s, 1H, NH), 7.79-7.81 (m, 2H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.7 (CH_3), 31.5 (CH_3), 43.7 (NCH_2), ArC [111.4, 119.7, 129.2, 129.5, 130.2, 130.35, 131.3, 132.0, 134.5, 141.7], 152.4 (benzimidazole-C_2), 153.5 (tiyadiazole-C_5), 170.3 (tiyadiazole-C_2). ESI-MS *m/z* $C_{19}H_{19}N_5S$ $[M + H]^+$: 350.06.

4.7.2 | 5-((5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)methyl)-*N*-ethyl-1,3,4-thiadiazol-2-amine (7b)

White solid (3.37 g), 93%, m.p. 212°C to 214°C. FTIR (ν_{max}/cm^{-1}): 3270 (NH), 2973 (Ar—CH), 1531 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.08 (t, 3H, CH_3, $J = 8$ Hz), 2.31 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.18 (q, 2H, CH_2, $J = 8$ Hz), 5.64 (s, 2H, NCH_2), 7.39 (s, 1H, ArH), 7.46 (s, 1H, ArH), 7.53-7.55 (m, 3H, ArH), 7.65 (s, 1H, NH), 7.78 (d, 2H, ArH, $J = 4$ Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.6 (CH_3), 20.3 (CH_3), 20.7 (CH_3), 39.8 (CH_2), 43.6 (NCH_2), ArC [111.4, 119.7, 129.2, 129.5, 130.2, 130.3, 131.3, 131.9, 134.5, 141.6], 152.4 (benzimidazole-C_2), 153.3 (tiyadiazole-C_5), 169.3 (tiyadizole-C_2). ESI-MS *m/z* $C_{20}H_{21}N_5S$ $[M + H]^+$: 364.13.

4.8 | General method for the synthesis of compounds 8a-b

A mixture of corresponding thiosemicarbazide (6a, 6b) (0.01 mol) in 2N NaOH solution (20 mL) was refluxed for 4h. The cooled mixture was acidified by precipitation with 37% HCl to pH 5 to 6, filtered, and washed with plenty of water. The precipitated product was filtered and recrystallized from ethanol-water (2:1) to obtain pure compounds.

4.8.1 | 5-((5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)methyl)-4-methyl-4*H*-1,2,4-triazole-3-thiol (8a)

White solid (3.24 g), 93% yield. m.p. 240°C. FTIR (ν_{max}/cm^{-1}): 3097, 3039 (Ar—CH), 2938, 2893 (Aliphatic-CH), 2749 (SH), 1575, 1500 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 6H, CH_3), 3.36 (s, 3H, CH_3), 5.61 (s, 2H, NCH_2), 7.32 (s, 1H, ArH), 7.46(s, 1H, ArH), 7.51-7.69 (m, 5H, ArH), 13.58 (s, 1H, SH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.3 (CH_3), 20.5 (CH_3), 30.3 (CH_3), 39.3-40.5 (DMSO-d_6 + NCH_2), ArC [111.3, 119.6, 129.3, 130.1, 130.2, 131.2, 131.9, 134.8, 141.4], 148.9 (benzimidazole-C_2), 152.7 (triazole-C_5), 167.9 (triazole-C_3). ESI-MS *m/z* $C_{19}H_{19}N_5S$ $[M + H]^+$: 350.20.

4.8.2 | 5-((5,6-Dimethyl-2-phenyl-1*H*-benzimidazol-1-yl)methyl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (8b)

White solid (3.33 g), 92% yield. m.p. 259°C to 260°C. IR (ν_{max}/cm^{-1}): 3089, 3038 (Ar—CH), 2910, 2845 (Aliphatic-CH), 2735 (SH), 1573, 1508 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.99 (t, 3H, CH_3, J = 8 Hz), 2.32 (s, 6H, CH_3), 3.85 (q, 2H, CH_2, J = 8 Hz), 5.67 (s, 2H, NCH_2), 7.32 (s, 1H, ArH), 7.49-7.54 (m, 4H, ArH), 7.69 (s, 2H, ArH), 13.65 (s, 1H, SH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.4 (CH_3), 20.3 (CH_3), 20.6 (CH_3), 38.8 (CH_2), 40.4 (NCH_2), ArC [111.3, 119.7, 129.2, 130.2, 130.2, 131.3, 132.0, 134.9, 141.4], 148.3 (benzimidazol-C_2), 152.6 (triazole-C_5), 167.4 (triazole-C_3). ESI-MS *m/z* $C_{20}H_{21}N_5S$ $[M + H]^+$: 364.05.

4.9 | Antioxidant activity and radical scavenging assays

Antioxidant activities of the synthesized compounds were clarified using various in vitro antioxidant assays including CUPRAC, ABTS/persulfate, and DPPH assays. Catechin, Trolox, and ascorbic acid were used as positive antioxidant.

4.10 | CUPRAC assay

In order to determine the cupric ions (Cu^{2+}), reducing ability of the synthesized compounds was determined according to the literature.[25,26] 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.

4.11 | DPPH-free radical scavenging assay

The DPPH radical scavenging activity of the synthesized compounds was measured using the method in previous studies.[26,28,29,32] Briefly, 1200 μL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol was added to 300 μL of the synthesized compound solution in DMSO. Then, in the dark for 50 minutes, decrease in absorbance at 517 nm was measured, using a UV-Visible spectrophotometer (1601UV-Shimadzu, Australia). All determinations were carried out three times. The results were expressed as % scavenging of DPPH radical. The percentage scavenging was calculated from the formula

$$\%\text{Scavenging} = [(\text{OD}_{\text{control}} - \text{OD}_{\text{test}})/(\text{OD}_{\text{control}}) \times 100].$$

4.12 | $ABTS^{\cdot+}$ radical cation decolorization assay

The ability of the synthesized compounds to scavenge $ABTS^{\cdot+}$ radical was determined according to the literature.[23,26,33] ABTS was dissolved in water to a 7mM concentration and diluted to get an absorbance of 0.700 ± 0.020 at 734 nm before usage. After 5 minutes in the dark at room temperature, the decrease in the absorbance of reaction mixture containing 200 μL of the compound solution and 1800 μL of the $ABTS^{\cdot+}$ solution was measured. The percentage scavenging was calculated from the formula

$$\%\text{Scavenging} = [(\text{OD}_{\text{control}} - \text{OD}_{\text{test}})/(\text{OD}_{\text{control}}) \times 100].$$

4.13 | 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 the indophenol method and used to determine the urease inhibitory activity.[30,31,34] The percentage remaining activity was calculated from the formula

$$\%\text{Remaining Activity} = [(\text{OD}_{\text{test}})/(\text{OD}_{\text{control}}) \times 100].$$

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.

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