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Synthesis and Antimicrobial Activity of Novel Substituted 4-[3-(1*H*-Benzimidazol-2-yl)-4-hydroxybenzyl]-2-(1*H*-benzimidazol-2-yl)phenol Derivatives[1]

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Abstract—A series of novel substituted bis-benzimidazole derivatives were synthesized by reaction of 5,5′-methylenebis(2-hydroxybenzaldehyde) with various substituted *o*-phenylenediamines in glacial acetic acid. The structure of the newly synthesized compounds was elucidated by ^{1}H and ^{13}C NMR, FT-IR, and MS spectra, and their antimicrobial activity against gram positive and gram negative bacteria and antifungal activity were evaluated. The thienyl-substituted derivative showed significant activity against *Bacillus licheniformis*. *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* (bacteria), and *Fusarium solani* (fungi). The activities of the fluoro-substituted substituted derivative against some bacterial strains and of the thienyl-substituted derivative against fungi were found to be similar to those of standard drugs.

Keywords: Bis-benzimidazoles, acetic acid, antibacterial activity, antifungal activity

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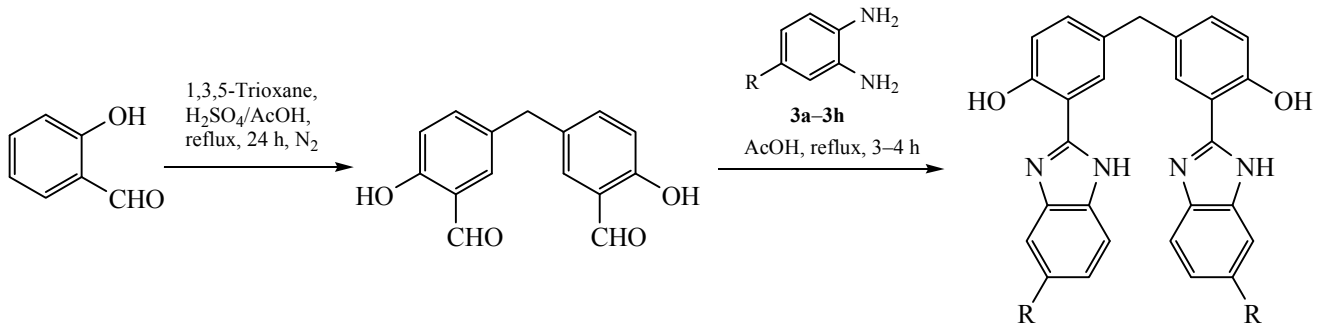
Benzimidazole is a privileged pharmacophore encountered in a number of fundamental cellular components and bioactive molecules. Indeed, a number of important drugs used in different therapeutic areas contain a benzimidazole moiety [1]. Examples are proton pump inhibitor omeprazole, anti-hypertensive drugs candesartan and telmisartan, antihelminthics albendazole and mebendazole, as well as several other kinds of investigational therapeutic agents including antitumor and anticancer [2, 3]. Literature survey revealed a number of interesting biological activities such as antitubercular, anticancer, antihelminthic, anti allergic [4], antifungal [5, 6], antihistaminic (astemizole) [7], and antioxidant [8–13]. 2-Phenylbenzimidazole was subjected to cell-based assays for cyclotoxicity and antiviral activity against a panel of RNA and DNA viruses [14]. During the past three decades several classes of anticancer drugs have been identified through both empirical screening and rational design of new compounds. These include several heterocyclic dimers such as bis-pyrrolo-benzodiazepines, bis(alkylaminophenylfurans), and bis-benzimidazoles. The basic moiety of telmisartan (reported as cytotoxicity agent in prostate cancer cell line) is also bis-benzimidazole scaffold. These heterocyclic dimers with acyclic and cyclic spacers target DNA to exhibit their anticancer activity by intercalation and alkylation mechanism, which induces DNA binding, interstrand cross-linking, and disruption of cellular processes necessary for cell maintenance and replication in cancer cells. In view of the above stated, in continuation of our previous work on the synthesis of bioactive benzimidazole derivatives [15, 16] herein we report the synthesis of new substituted 4-[3-(1*H*-benzimidazol-2-yl)-4-hydroxybenzyl]-2-(1*H*-benzimidazol-2-yl)phenol derivatives and evaluation of their anti bacterial and antifungal activities.

RESULTS AND DISCUSSION

Chemistry. 5,5′-Methylenebis(2-hydroxybenzaldehyde) (**2**) [17, 18] was prepared in good yield by electrophilic substitution reaction of salicylaldehyde (**1**) with 1,3,5-trioxane (formaldehyde trimer) in glacial acetic acid in the presence of a catalytic amount of

[1] The text was submitted by the authors in English.

Scheme 1.



1 **2** **4a–4h**

R = H (**a**), Me (**b**), HO (**c**), F (**d**), Cl (**e**), Br (**f**), cyclopentyl (**g**), thiophen-2-yl (**h**).

concentrated sulfuric acid. Various bis-benzimidazoles **4a**–**4h** were synthesized in moderate to good yields by condensation of **2** with substituted *o*-phenylenediamines **3a**–**3h** in glacial acetic acid under nitrogen (Scheme 1). *o*-Phenylenediamines **3g** and **3h** required for the synthesis of **4g** and **4h** were prepared starting from 4-bromo-2-nitroaniline which was protected with Boc_2O at 0°C and brought into Suzuki coupling reaction with cyclopentyl- or thiophen-2-ylboronic acid in the presence of Pd catalyst and Na_2CO_3 at 70°C. The resulting compound was reduced with $FeCl_3/N_2H_4 \cdot H_2O$, followed by deprotection. Compounds **3g** and **3h** were obtained in good yield, and their analytical data matched the theoretical values. 4-Cyclopentylbenzene-1,2-diamine (**3g**) has not been reported previously. The other *o*-phenylenediamines are commercially available.

The structures of all newly synthesized compounds **4a**–**4h** were confirmed by IR, ^{1}H and ^{13}C NMR, and mass spectra. In the IR spectrum of **4a**, the phenolic OH and NH stretching bands appeared at 3498 and 3144 cm^{-1}, respectively. The absorption peak at 2700 cm^{-1} was attributed to the C=N bond of benzimidazole. The ^{1}H NMR spectrum of **4a** contained two broadened singlets at δ 13.14 and 12.96 ppm due to OH and NH protons and a singlet at δ 3.98 due to bridging methylene group. In the ^{1}H NMR spectra of all compounds **4a**–**4h**, aromatic protons resonated in the region δ 6.99–7.98 ppm. The ^{13}C NMR spectra of **4a**–**4h** showed aromatic carbon signals in the region δ_C 115.3–156.9 ppm, and the CH_2 signal was observed at δ_C 42.4 ppm. The mass spectrum of **4a** displayed a strong molecular ion peak at *m*/*z* 433 $[M]^+$ ($C_{27}H_{20}N_4O_2$). Thus, the spectral data for the synthesized compounds were in agreement with their molecular structures (Scheme 1).

Biology. *Antimicrobial activity.* The antibacterial activity of newly synthesized compounds **4a**–**4h** was evaluated against three gram positive (*Bacillus licheniformis*, *Bacillus subtilis*, *Staphylococcus aureus*) and three gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*) by the agar diffusion method using ciprofloxacin as standard drug. The inhibition zone diameters and MIC values (minimum inhibitory concentration, μg/mL) are given in Table 1. Compounds **4b**–**4d** and **4h** showed excellent inhibitory activity against all bacterial strains, which may be attributed to the presence of thiopene, methyl, hydroxy, and highly electronegative fluoro substituents. Compounds **4e**, **4f**, and **4g** showed a good activity (inhibition zone ≥20 mm). It may be concluded that the presence of fluorine, chlorine, or bromine atom in the benzene ring enhances the antibacterial activity. According to the MIC values, compounds **4a**, **4c**, **4d**, and **4f** exhibit moderate to good inhibitory activity (MIC 200–25 μg/mL) against bacterial strains. Compound **4h** exhibited broad spectrum of antibacterial activity and moderate MIC values against all the tested strains. All other remaining compounds showed slightly higher MIC values.

The antifungal activity of **4a**–**4h** was evaluated against four fungal strains, *viz*. *Aspergilus niger*, *Candida albicans*, *Fusarium oxysporum*, and *Fusarium solani*, in comparison with the standard antifungal drug nystatin (Table 2). Bis-benzimidazole derivatives **4a**–**4f** turned out to be inactive against all the tested fungal strains. Compounds **4h** and **4g** showed a good activity against all fungal strains. The MIC values of **4h** and **4g** ranged from 75 to 25 μg/mL, which may be regarded as moderate to be good activity.

Table 1. Antibacterial activity[a] of compounds **4a–4h**

Comp. no.	Inhibition zone diameter, mm (minimum inhibitory concentration, μg/mL)					
	Gram-negative bacteria			Gram-positive bacteria		
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus licheniformis*	*Bacillus subtilis*	*Staphylococcus aureus*
4a	22 (50)	24 (75)	26 (50)	21(50)	23 (50)	22 (50)
4b	24 (150)	23 (17)	23 (100)	20 (200)	23 (125)	21 (125)
4c	21 (100)	21 (12)	20 (100)	25 (100)	20 (75)	22 (125)
4d	20 (25)	21 (25)	25 (25)	25 (25)	18 (25)	21 (25)
4e	26 (75)	25 (75)	24 (100)	24 (50)	21 (50)	24 (75)
4f	26 (200)	25 (17)	26 (175)	26 (200)	22 (125)	26 (125)
4g	22 (175)	23 (15)	25 (175)	20 (175)	21 (150)	23 (125)
4h	25 (200)	28 (20)	24 (175)	21 (200)	25 (175)	26 (200)
Ciprofloxacin	25 (25)	24 (25)	28 (25)	24 (25)	22 (25)	25 (25)
Control (1% DMSO)	No activity	No activity	No activity	No activity	No activity	No activity

[a] Inhibition zone diameters were measured for stock solutions with a concentration of 100 μg/mL.

EXPERIMENTAL

The melting points were determined in open capillaries and are uncorrected. The IR spectra were recorded in KBr on a Perkin Elmer Model 337 instrument. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 300 and 400 MHz instruments using DMSO-d_6 as solvent and tetramethylsilane as internal standard. Thin layer chromatography (TLC) was carried out on aluminum plates coated with silica gel 60 F_{254} (Merck; cat. no. 105 554); spots were visualized with UV light at 254 nm or alternatively by staining with aqueous basic potassium permanganate. Column chromatography was performed using silica gel (60Å, 100–200 mesh; Merck). Commercially available reagents were used as supplied, and all solvents were distilled before use. All reactions were performed in oven-dried glassware.

Table 2. Antifungal activity[a] of compounds **4g** and **4h**

Comp. no.	Inhibition zone diamter, mm (MIC, μg/mL)			
	Aspergilus niger	*Candida albicans*	*Fusarium Oxysporum*	*Fusarium Solani*
4g	19 (50)	23 (25)	21 (75)	24 (25)
4h	21 (25)	24 (50)	20 (50)	25 (75)
Nystatin	20 (25)	25 (25)	23 (25)	25 (25)
Control (1% DMSO)	No activity	No activity	No activity	No activity

[a] Inhibition zone diameters were measured for stock solutions with a concentration of 100 μg/mL.

***In vitro* antimicrobial assay.** The antimicrobial activity was evaluated by the agar well diffusion method. The activity was determined by measuring the diameter of inhibition zone (in mm). Samples of the tested compounds (50 μL, c = 1 mg/mL) were loaded into the wells on the plates. All solutions were prepared in DMSO, and pure DMSO was loaded as control. The plates were incubated at 35°C for 1–5 days and then were examined for the formation of inhibition zone. Each inhibition zone was measured three times for each bacterium culture [20, 21].

Minimal inhibitory concentration (MIC) measurement. The microorganism's susceptibility tests in nutrient and potato dextrose broths were used for the determination of MIC. Stock 1000 μg/mL solutions of the tested compounds, ciprofloxacin, and Nystatin were prepared in DMSO, followed by dilutions to concentrations of 250–25 μg/mL. Inoculated microorganism suspensions were incubated at 37°C for 1–5 days for MIC determination [22, 23].

4-Cyclopentylbenzene-1,2-diamine (3g). *tert*-Butyl (4-bromo-2-nitrophenyl)carbamate, 100 mg (0.315 mol, 1.0 equiv), was dissolved in THF/H_2O (82.5 mL), and $Pd(PPh_3)_4$ (3 mol %), cyclopentyl-

boronic acid (53 mg, 0.472 mol, 1.5 eq), and Na_2CO_3 (49 mg, 0.472 mmol, 1.5 equiv) were added at room temperature. The mixture was refluxed with stirring for 12 h. When the reaction was complete, the mixture was filtered through a celite bed, the sorbent was washed with ethyl acetate, and the organic layer was separated, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography with 20% ethyl acetate in hexane as eluent. *tert*-Butyl (4-cyclopentyl-2-nitrophenyl)carbamate was isolated as a light brick red solid. The product was reduced with $FeCl_3/N_2H_4 \cdot H_2O$ in methanol to obtain *tert*-butyl (2-amino-4-cyclopentylphenyl)carbamate which was deprotected by treatment with dilute HCl to afford 4-cyclopentylbenzene-1,2-diamine (**3g**). Yield 86%, brown solid, mp 151–163°C, R_f 0.32 (EtOAc–*n*-hexane, 1 : 2 by volume). ^{1}H NMR spectrum, δ, ppm: 6.80 s (1H, H_{arom}), 6.50–6.41 m (1H, H_{arom}, 6.14–6.01 m (1H, H_{arom}, J = 2.68 Hz), 4.73 br.s (4H, NH_2); 2.81 m (1H), 1.92–1.90 m (2H), 1.82–1.80 m (2H), 1.43–1.41 m (2H), 1.40–1.39 m (2H) (cyclopentyl). Mass spectrum: *m*/*z* 177.4 $[M]^+$.

4-(Thiophen-2-yl)benzene-1,2-diamine (3h) was synthesized in a similar way from 5-bromo-2-nitroaniline and thiophen-2-ylboronic acid. Yield 89%, white solid, mp 143–149°C, R_f 0.43 (EtOAc–*n*-hexane, 1 : 2 by volume). ^{1}H NMR spectrum, δ, ppm: 7.35 d (1H, H_{Th}, J = 2.68 Hz), 7.20 s (1H, H_{arom}), 7.00 m (1H, H_{arom}), 6.82 d (1H, H_{Th}, J = 2.38 Hz), 6.74–6.72 m (2H, H_{arom}), 6.52 d (1H, H_{Th}, J = 2.4 6 Hz), 4.53 s (4H, NH_2). Mass spectrum: *m*/*z* 191.4 $[M]^+$.

4-[3-(1*H*-Benzimidazole-2-yl)-4-hydroxybenzyl]-2-(1*H*-benzimidazole-2-yl)phenol (4a). *o*-Phenylenediamine (**3a**), 130 mg (1.171 mmol), was slowly added to a solution of 200 mg (0.781 mmol) of 5,5′-methylenebis(2-hydroxybenzaldehyde) (**2**) in glacial acetic acid, and the mixture was refluxed for 3 h, the progress of the reaction being monitored by TLC. The mixture was cooled to room temperature, diluted with water (50 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (2 × 25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using 5% ethyl acetate in pet ether as eluent. Yield 67%, white solid, mp 264–266°C, R_f 0.66 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 3498, 3144, 2700, 1630, 1547. ^{1}H NMR spectrum, δ, ppm: 13.14 s (2H, OH), 12.96 s (2H, NH), 7.98 d (2H, H_{arom}, J = 7.3 Hz), 7.70 d (2H, H_{arom}, J = 7.4 Hz), 7.58 d (2H, H_{arom}, J = 7.3 Hz), 7.29–7.23 m (6H, H_{arom}), 7.00 d (2H, H_{arom}, J = 7.4 Hz), 3.98 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 156.9 (2C), 152.8 (2C), 140.9 (4C), 134.7(2C), 130.9 (2C), 128.7 (2C), 125.7 (4C), 119.0 (2C), 116 (2C), 116.6 (2C), 115.3 (2C), 42.4 (CH_2). Mass spectrum: *m*/*z* 433.1 $[M]^+$.

Compounds **4b**–**4h** were synthesized in a similar way.

4-[4-Hydroxy-3-(5-methyl-1*H*-benzimidazol-2-yl)-benzyl]-2-(6-methyl-1*H*-benzimidazol-2-yl)phenol (4b). Reaction time 3.5 h. Yield 65%, brick red solid, mp 275–277°C, R_f 0.60 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2780, 1635, 1577. ^{1}H NMR spectrum, δ, ppm: 13.02 br.s (2H, OH), 12.03 br.s (2H, NH), 7.93 s (2H, H_{arom}), 7.56 d (2H, H_{arom}, J = 7.4 Hz), 7.41 t (2H, H_{arom}, J = 6.8 Hz), 7.23 d (2H, H_{arom}, J = 7.4 Hz), 7.07 d.d (2H, H_{arom}, J = 1.2, 4.4 Hz), 6.97 d (2H, H_{arom}, J = 7.4 Hz), 3.96 s (2H, CH_2), 2.42 s (6H, CH_3). ^{13}C NMR spectrum, δ_C, ppm: 156.3 (2C), 151.8 (2C), 138.8 (2C), 134.4 (2C), 132.7 (2C), 131.7 (2C), 131.0 (2C), 128.7 (2C), 125.1 (2C), 119.0 (2C), 116.9 (2C), 115.1 (2C), 115.0 (2C), 42.6 (CH_2), 20.9 (2C, CH_3). Mass spectrum: *m*/*z*: 461.3 $[M]^+$.

2-{2-Hydroxy-5-[4-hydroxy-3-(6-hydroxy-1*H*-benzimidazol-2-yl)benzyl]phenyl}-1*H*-benzimidazol-5-ol (4c). Reaction time 4 h. Yield 68%, white solid, mp 197–199°C, R_f 0.61 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 3422, 2725, 1624, 1574. ^{1}H NMR spectrum, δ, ppm: 12.80 br.s (2H, OH), 12.50 br.s (2H, NH), 9.35 br.s (2H, OH), 7.87 s (2H, H_{arom}), 7.44 d (2H, H_{arom}, J = 7.4 Hz), 7.20 d.d (2H, H_{arom}, J = 1.4, 7.3 Hz), 7.01–6.93 m (4H, H_{arom}), 6.74 d (2H, H_{arom}, J = 7.4 Hz), 3.95 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 156.3 (2C), 153.6 (2C), 140.9 (2C), 132.1 (2C), 131.9 (2C), 126.2 (2C), 123.1 (2C), 117.2 (2C), 115.2 (2C), 115.1 (2C), 111.5 (2C), 42.5 (CH_2.). Mass spectrum: *m*/*z*: 465.4 $[M]^+$.

4-[3-(5-Fluoro-1*H*-benzimidazol-2-yl)-4-hydroxybenzyl]-2-(6-fluoro-1*H*-benzimidazol-2-yl)phenol (4d). Reaction time 3 h. Yield 85%, white solid, mp 240–242°C, R_f 0.68 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2700, 1635, 1579, 807. ^{1}H NMR spectrum, δ, ppm: 13.18 br.s (2H, OH), 12.61 s (2H, NH), 7.97 s (2H, H_{arom}), 7.55 d (2H, H_{arom}, J = 7.4 Hz), 7.39 s (2H, H_{arom}), 7.27 d (2H, H_{arom}, J = 7.4 Hz), 7.12 s (2H, H_{arom}), 7.00 d (2H, H_{arom}, J = 7.3 Hz), 3.97 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 156.5 (2C), 152.9 (2C), 152.8 (2C), 145.5 (2C), 138.5 (2C), 134.7

(2C), 130.8 (2C), 128.7 (2C), 119.0 (2C), 116.7 (2C), 116.0 (2C), 111.9 (2C), 105.4 (2C), 42.0 (CH_2). mass spectrum: *m*/*z*: 469.3 $[M]^+$.

4-[3-(5-Chloro-1*H*-benzimidazol-2-yl)-4-hydroxybenzyl]-2-(6-chloro-1*H*-benzimidazol-2-yl)phenol (4e). Reaction time 4 h. Yield 69%, white solid, mp 270–272°C. R_f 0.66 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2730, 1560, 1598, 835. ^1H NMR spectrum, δ, ppm: 13.18 br.s (2H, OH), 12.50 br.s (2H, NH), 7.98 d (2H, H_{arom}, J = 7.4 Hz), 7.77–7.61 m (4H, H_{arom}), 7.28 d.d (4H, H_{arom}, J = 1.4, 7.4 Hz), 7.01 d (2H, H_{arom}, J = 7.4 Hz), 3.97 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 153.2 (2C), 152.8 (2C), 142.3 (2C), 137.0 (2C), 132.2 (2C), 130.1 (2C), 129.8 (2C), 129.3 (2C), 125.3 (2C), 119.0 (2C), 116.8 (2C), 116.2 (2C), 114.8 (2C), 42.0 (CH_2). Mass spectrum: *m*/*z* 501.0 $[M]^+$.

4-[3-(5-Bromo-1*H*-benzimidazol-2-yl)-4-hydroxybenzyl]-2-(6-bromo-1*H*-benzimidazol-2-yl)phenol (4f). Reaction time 3.5 h. Yield 75%, brick red solid, mp 253–255°C, R_f 0.65 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2724, 1638, 1579, 560. ^1H NMR spectrum, δ, ppm: 13.20 br.s (2H, OH), 12.50 br.s (2H, NH), 7.98 d (2H, H_{arom}, J = 7.4 Hz), 7.86 s (2H, H_{arom}), 7.59 s (2H, H_{arom}), 7.38 d.d (2H, H_{arom}, J = 1.4, 7.4 Hz), 7.28 d.d (2H, H_{arom}, J = 1.3, 7.4 Hz), 7.00 d (2H, H_{arom}, J = 7.4 Hz), 3.97 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 156.0 (2C), 152.5 (2C), 132.5 (2C), 132.0 (2C), 126.6 (2C), 125.6 (2C), 125.2 (2C), 114.1 (2C), 113.3 (2C), 112.5 (2C), 42.5 (CH_2). Mass spectrum: *m*/*z*: 591.2 $[M]^+$.

4-[3-(5-Cyclopentyl-1*H*-benzimidazol-2-yl)-4-hydroxybenzyl]-2-(6-cyclopentyl-1*H*-benzimidazol-2-yl)-phenol (4g). Reaction time 3.8 h. Yield 67%, brown solid, mp 198–200°C, R_f 0.65 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2700, 1620, 1579, 2950. ^1H NMR spectrum, δ, ppm: 13.00 s (2H, OH), 11.09 br.s (2H, NH), 7.92 s (2H, H_{arom}), 7.60–7.51 m (2H, H_{arom}), 7.49–7.39 m (2H, H_{arom}), 7.40 d (2H, H_{arom}, J = 7.4 Hz), 7.24–7.13 m (2H, H_{arom}), 6.98 d (2H, H_{arom}, J = 7.4 Hz), 4.00 s (2H, CH_2), 3.19 m (2H, cyclopentyl), 2.12 m (4H, cyclopentyl), 1.79 m (4H, cyclopentyl), 1.70–1.52 m (8H, cyclopentyl). ^{13}C NMR spectrum, δ_C, ppm: 156.2 (2C), 152.8 (2C), 140.7 (2C), 137.5 (2C), 136.0 (2C), 134.7 (2C), 131.0 (2C), 129.7(2C), 123.6 (2C), 120.0 (2C), 116.2 (2C), 114.9 (2C), 114.2 (2C), 48.7 (2C, cyclopentyl), 42.1 (CH_2), 35.2 (2C, cyclopentyl), 25.8 (2C, cyclopentyl). Mass spectrum: *m*/*z* 569.5 $[M]^+$.

4-[4-Hydroxy-3-(5-(thiophen-2-yl)-1*H*-benzimidazol-2-yl)benzyl]-2-(6-(thiophen-2-yl)-1*H*-benzimidazol-2-yl)phenol (4h). Reaction time 4 h. Yield 83%, white solid, mp 282–284°C, R_f 0.63 (EtOAc–*n*-hexane, 1 : 2, v/v). IR spectrum, ν, cm^{-1}: 2746, 1688, 1520. ^1H NMR spectrum, δ, ppm: 13.19 br.s (2H, NH), 13.14 br.s (2H, OH), 12.76 t (2H, thiophene, J = 6.8 Hz), 7.98 s (2H, H_{arom}), 7.74 t (2H, H_{arom}, J = 6.8 Hz), 7.60–7.49 m (8H, H_{arom}), 7.28 d (2H, H_{arom}, J = 7.4 Hz), 7.15 d (2H, H_{arom}, J = 7.4 Hz), 7.02 d (2H, H_{arom}, J = 7.4 Hz), 4.00 s (2H, CH_2). ^{13}C NMR spectrum, δ_C, ppm: 155.9 (2C), 152.0 (2C), 142.4 (2C), 140.9 (2C), 138.9 (2C), 138.3 (2C), 134.7 (2C), 131.0 (2C), 131.9 (2C), 128.7 (2C), 127.9 (2C), 125.6 (2C), 125.5 (2C), 120.0 (2C), 117.9 (2C), 115.1 (2C), 114.2 (2C), 42.1 (CH_2). Mass spectrum: *m*/*z* 597.3 $[M]^+$.

Supplementary data including ^1H and ^{13}C NMR and mass spectra of some compounds are available from the authors.

CONCLUSIONS

In summary, have synthesized a series of novel bis-benzimidazole derivatives and tested them for anti-bacterial and antifungal activity *in vitro*. The synthesized compounds showed a high activity against all the bacterial strains used, whereas only two compound, **4h** and **4g** were active against four fungal strains. We will focus on these two compounds in further research to improve their antimicrobial activity.

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