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An efficient synthesis of novel carbohydrate and thiosemicarbazone hybrid benzimidazole derivatives and their antimicrobial evaluation

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A library of thiosemicarbazide hybrid 2-(aldo-polyhydroxyalkyl)benzimidazole derivatives have been designed and synthesized with simple and eco-friendly methodologies. The structures of the compounds have been elucidated with the aid of elemental analysis, IR, mass and ^{1}H NMR spectral data. These novel synthesized compounds have been evaluated for their antibacterial activity against two gram-positive bacteria (*S. aureus* and *S. pyogenus*) and two gram-negative bacteria (*P. aeruginosa* and *E. coli*). The title compounds have also been studied for their antifungal activity against *C. albicans*, *A. niger* and *A. clavatus* using the broth dilution technique.

Keywords: Antibacterial activity, antifungal activity, benzimidazole, thiosemicarbazone, carbohydrate, microwave irradiation

The derivatives of carbohydrates are highly potent compounds in chemical and biological fields[1,2] and are present in natural products[3,4]. Recently, the studies on these glycomolecules[5,6], such as glycolipids[9,10], proteoglycans, glycoproteins[7,8] and antibiotics, which display the significance of carbohydrate parts (glycons) in molecular gratitude for the transmission of biological information[11,12]. The design of drug hybrids is the art of taking two molecules known to have potent biological activity and combining them such that the target molecule is more powerful than each of the individual components. In the context of saccharides, the idea of drug hybrids is of particular interest as carbohydrates provide attractive scaffolds for drug development as sugars are involved in many biological processes; selected examples include protein folding, cell-cell communication and neural development. Thus, it is now recognized that carbohydrates are at the heart of a large number of biological actions. With this inspiring biological background of carbohydrates, efficient synthesis of not only carbohydrates themselves, but also carbohydrate containing heterocycles is becoming more and more important and high-growth field in organic and medicinal chemistry[13]. Hence, scientists are pursuing the synthesis of carbohydrate-based heterocycles with renewed interest.

A variety of clinically significant species of microorganisms have become an important health problem globally[14]. One way to fight with this challenge is the appropriate usage of the available marketed antibiotics, and the other is the development of novel anti-microbial agents[15]. Hence, there will always be a vital need to discover new chemotherapeutic agents to overcome the emergence of resistance and ideally shorten the duration of therapy. Benzimidazoles express their antibacterial activity by inhibiting the bacterial nucleic acid and protein synthesis. This ability of benzimidazoles is due to their structural similarities with the purines[16,17]. As reported, 2-substituted benzimidazole derivatives are found to be pharmacologically more potent for antibacterial activity and hence the design and synthesis of 2-substituted benzimidazole is a promising area of research[18]. Previously, our group has reported benzimidazole hybrid thiosemicarbazone derivatives possessing antimicrobial, anti-cancer, anti-HIV as well as anti-malarial activities[19,20].

Despite the significant progress in antimicrobial therapy, infectious diseases caused by bacteria and fungi remain a major worldwide health problem due to rapid development of resistance towards the existing antibacterial and antifungal drugs. Thiosemicarbazone derivatives, a large group of thiourea derivatives, exhibit various biological

activities and have therefore attracted considerable pharmaceutical interest[22]. They have been evaluated over the last 50 years as antiviral[23], antibacterial[24-26], anticancer agents[27-29], anti-malarial[19,30], anti-HIV[21], *etc*., whose biological activities are a function of the parent aldehyde or ketone moiety[31,32]. Moreover, compounds with thiosemicarbazone structure are known to possess tranquilizing, analgesic, hypnotic, anti-tumor, anti-depressant, muscle relaxing, anti-fungal and anti-inflammatory properties[33,34].

Inspite of the great advances that have been made so far, further research in the development of new anti-bacterial benzimidazole derivatives are required. From the literature study, we assume that, if we substitute polyhydroxy alkyl chain at the active position of benzimidazole nucleus then it may solve one of the two or both the problems: activity and solubility. Due to this reason, we have introduced polyhydroxy alkyl chain as hydrophilic part at position C-2 of the benzimidazole. We have also synthesised thiosemicarbazone hybrid of this benzimidazole derivative, which may exhibit highly potent anti-bacterial activity as well as higher solubility. Herein, we have synthesised various thiosemicarbazone hybrid 2-substituted benzimidazole derivatives and screened them for antimicrobial activities. In addition, the importance of green chemistry in organic synthesis has encouraged our group to explore the use of microwave irradiation for organic synthesis[35,36]. Hence, a study has been undertaken using microwave irradiation for the reaction between 2-substituted polyhydroxy alkyl chain hybrid benzimidazole derivatives and phenacyl bromide in the presence of K_2CO_3 as a base using dimethylformamide (DMF) as a solvent.

Results and Discussions

Chemistry

Here, we have described an efficient protocol for the synthesis of the novel carbohydrate and thiosemicarbazone analogue benzimidazole derivatives in high yield, which does not require any chromatographic purification of the products. Accordingly, first we have carried out the reaction of Ca-gluconate.H_2O (1 equiv.) and *ortho*-phenylenediamine (OPDA) (1 equiv.) in the presence of catalytic amount of conc. HCl in ethanol-water as a green solvent system, which afforded products **P1** in 87% yield. In the second step, we performed the reaction of **P1** with two different phenacyl bromides under microwave irradiation (MWI) at 300 W for 5 to 6 min, which afforded products **P2** and **P3** in excellent yields (85-88%). Subsequently, we have synthesised various thiosemicarbazides **S1-S5**, from the reaction of various isothiocynates and hydrazine hydrate in methanol at RT. Here, we got excellent yield of the all thiosemicarbazides (92-95%). Finally, we have achieved excellent yields of the desired products **1-10** from the condensation reaction of various thiosemicarbazides **S1-S5** and compounds **P1** and **P2** in refluxing ethanol using catalytic amounts of glacial acetic acid (Scheme I).

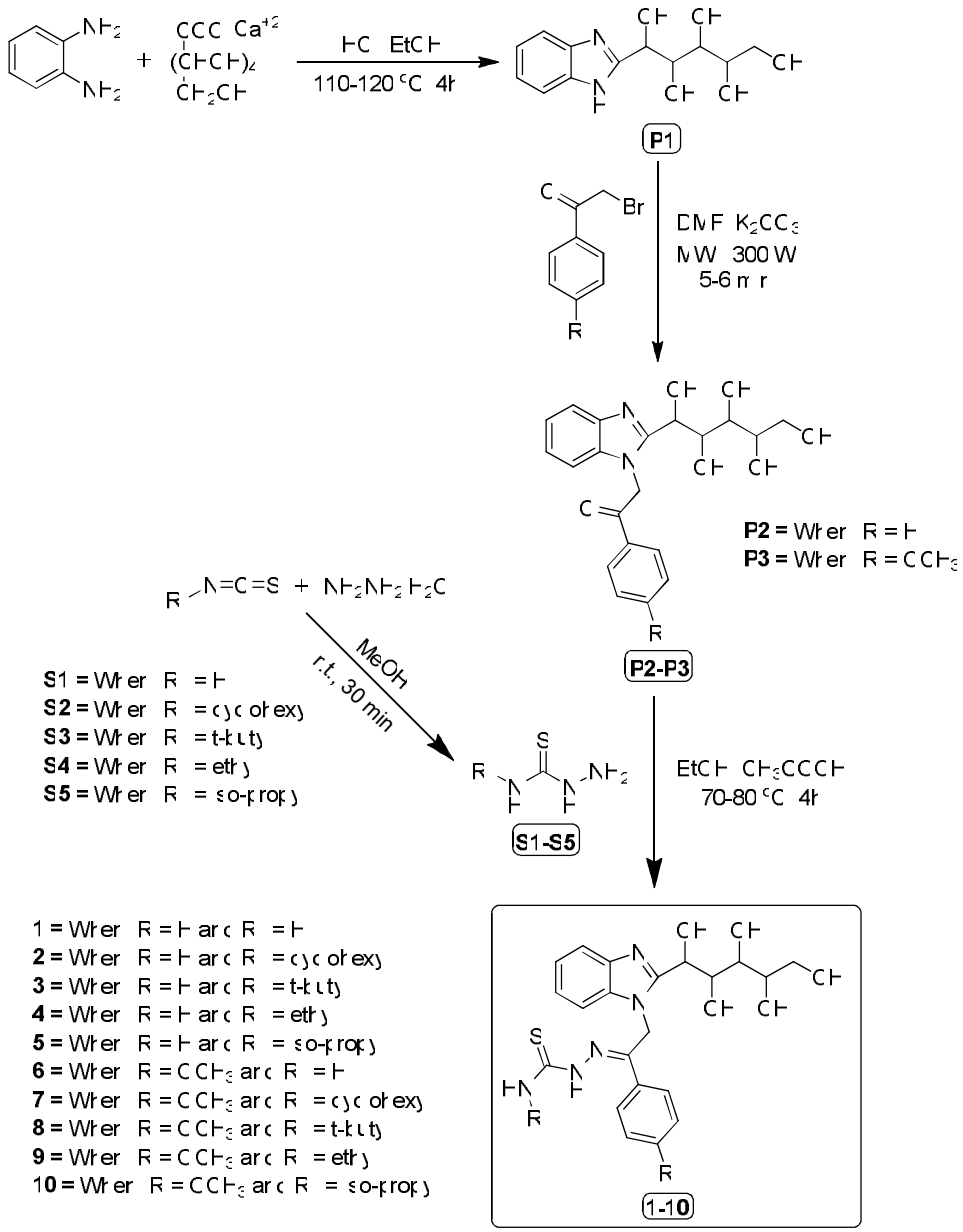
The structure of the final products **P1-P3** and **1-10** were well characterized by using spectral (IR, mass and ^{1}H NMR and elemental analysis data (ESI). In **P2** and **P3**, the IR spectra showed characteristic peaks of these derivatives at ~1700 cm^{-1} corresponding to CO. The ^{1}H NMR spectrum in DMSO-d_6 exhibited a singlet nearer to δ 11, which was attributed to the -NH group. Peaks between δ 7.2-8.2 were observed for respective aromatic protons. In compounds **1-10**, the ESI-MS spectra of the compounds show corresponding $(M)^+$ peak and $(M-1)^+$ and ^{1}H NMR shows characteristic two singlet peaks at near to δ 11 and 12 which indicates presence of two NH protons. Other characteristics protons are listed in Experimental Section.

Biological Evaluation

All newly synthesized coumarin hybrid thiosemicarbazone derivatives **P1-P3** and **1-10** were examined for antimicrobial activity against two gram-positive bacterial strains (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442, two gram-negative bacterial strains (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688) as well as three fungal strains (*Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282) using the agar dilution method[37]. Ampicillin and Chloramphenicol were used as standard control drugs for anti-bacterial activity, whereas Nystatin and Greseofulvin were used as standard control drugs for antifungal activity.

In vitro anti-bacterial activity

Reviewing of the antibacterial activities of all novel benzimidazole derivatives (Table I) indicate that all scaffolds were found to exhibit good to moderate activity against the specific microbial strain, which is described in Figure 1. Table I shows that bioassay results of the series of **P1-P3** and **1-10**



Scheme I — Synthetic strategy for the preparation of carbohydrate and thiosemicarbazone hybrid benzimidazole derivatives

compounds. Compound **P2** was found to be the most active compound that inhibits the gram positive *S. aureus* and *E. coli* bacterial growth at the lowest minimum inhibitory concentration (MIC) value of 62.5μg/mL, which indicates that compound **P2** was more potent than Ampicilin. The results revealed that most of the synthetic compounds **P1-P3** and **1-10** exhibited significant antibacterial activity against *S. aureus*. In addition, compound P2 and 5 exhibited high potent activity than Ampicilin against *S. aureus* at MIC of 62.5 μg/mL. Furthermore, compounds **P3**, **1**, **2**, **4**, **6**, **8**, **9** and **10** show higher activity than Ampicilin against *S. aureus* at MIC of 100-200 μg/mL. Also, compounds **P1** and **2** show equipotent activity against all the three strains *P. aeruginosa*, *S. aureus* and *S. pyogenus*. Moreover, compound **P3** exhibited excellent activity against *P. aeruginosa* and *S. aureus* than Ampicilin. Compound **5** and **9** displayed higher activity than Ampicilin against *S. aureus* and *S. pyogenus*.

Table I — In-*vitro* anti-bacterial activity of compounds **P1-P3** and **1-10**

Compd	MIC (µg/mL)			
	E. coli MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442
P1	125	100	250	100
P2	62.5	125	62.5	250
P3	200	100	100	125
1	250	200	125	125
2	200	62.5	100	100
3	250	200	250	250
4	125	250	100	200
5	200	250	62.5	100
6	125	200	125	125
7	200	250	250	250
8	250	200	200	200
9	125	200	125	100
10	200	250	200	200
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50

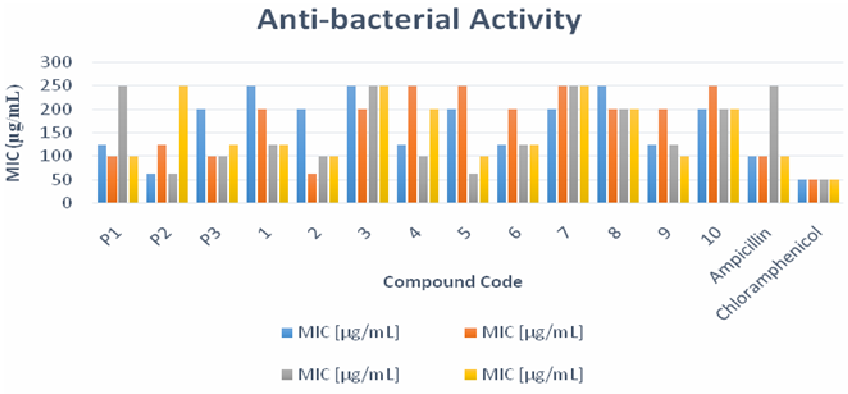


Figure 1 — Graphical representation of antibacterial activity of compounds **P1-P3** and **1-10**

In vitro antifungal activity

Antifungal activity data in Table II shows that among the **P1-P3** and **1-19** analogues, compounds **2** and **3** exhibited significant activity at 250 µg/mL and 200 µg/mL respectively against *C. albicans*. These compounds show higher activity than Greseofulvin (Standard Drug) against *C. albicans*. Furthermore, compounds **P3**, **1** and **4** displayed activity at 500 µg/mL against *C. albicans*, which was equivalent to Greseofulvin, which is described in Figure 2. The rest of the compounds show moderate activity against *C. albicans*.

Experimental Section

The reaction was performed under the modified microwave system. Melting points were determined in Optimelt MPA 100 automatic melting point apparatus and are uncorrected. TLC was run on aluminum precoated ready-made thin layer chromatography (TLC) silica gel 60 F_{254} plate (Merck, Germany) and visualization were done using iodine or UV light. IR spectra (ν_{max} in cm^{-1}) were recorded on a Perkin- Elmer FT-IR 377 spectrophotometer using KBr. ^1H NMR spectra were recorded on Bruker AV 400 MHz spectrometer using DMSO-d_6 as solvent and TMS

as the internal reference and ^{13}C NMR spectra were recorded on Bruker AV 100 MHz spectrometer using DMSO-d_6 as solvent. Mass spectra were recorded at Advion Expression CMS, USA. Acetone was used as mobile phase, electron spray ionization (ESI) is used as ion source. Elemental analysis was performed on a CHN elemental analyzer.

Synthesis of 1-(1*H*-benzimidazol-2-yl)pentane-1,2,3,4,5-pentol, P1

Ca-gluconate.H_2O (2 g, 0.009 mol of gluconic acid) and 1.1 g of *ortho*-phenylenediamine (OPDA) (0.01 mol) were taken in 100 mL flat bottom flask (FBF). About 4 mL of water, 1 mL of ethanol and 1.7 mL conc. hydrochloric acid (HCl) were added into the flask. The reaction mixture was heated on an oil bath kept at 110-120°C. The progress of the reaction was checked by TLC using ethyl acetate:toluene (2:1) as a mobile phase. After completion of the reaction, 10 mL water was added into hot solution. Then charcoaling was done. After that, 30 mL water was added into the filtrate for dilution. The solution was made alkaline, with a liquid NH_3 solution. The solid crystals were obtained upon cooling and then separated by filtration and dried. The obtained product was pure enough and did not require any chromatographic purification. The product was obtained in 87% yield. The product was confirmed by the IR, mass, ^1H NMR and elemental analysis.

Table II — In-*vitro* antifungal activity of compounds (**P1-P3** and **1-19**)

Compd	MIC (μg/mL)		
	C. albicans MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
P1	>1000	500	500
P2	1000	1000	>1000
P3	500	>1000	>1000
1	500	1000	1000
2	250	1000	1000
3	200	1000	1000
4	500	1000	1000
5	1000	>1000	>1000
6	1000	>1000	>1000
7	1000	>1000	1000
8	1500	500	500
9	1500	1500	1000
10	1000	1500	1500
Nystatin	100	100	100
Greseofulvin	500	100	100

1-(1*H*-Benzo[*d*]imidazol-2-yl)pentane-1,2,3,4,5-pentaol, P1: ^1H NMR (400 MHz, DMSO-d_6): δ 3.367-3.523 (m, 5H), 3.568-3.589 (m, 3H), 3.649 (s, 2H), 4.628 (s, 1H), 7.185-7.723 (m, 4H), 11.236 (s, 1H); ESI-MS: *m/z* 269.2 $[M+1]^+$, 270.2 $[M+2]^+$; IR (KBr): 1500 (-CN), 3398,760 (-NH-), 1259, 1031 (-OH), 1300, 1107 cm^{-1} (-OH). Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 6.01; N, 10.44. Found: C, 53.72; H, 6.00; N, 10.43%.

Synthesis of 1-aryl-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)-*N*-actaldehyde derivatives, P2 and P3

A mixture of compound **P1** (0.01 mol) and phenacyl bromide (0.01 mol) was taken in FBF. Water (20 mL) and DMF (10 mL) were added into FBF. In this reaction mixture, K_2CO_3 (0.01 mol) was added. The FBF was kept under microwave irradiation (MWI) at 300 W for 5 to 6 min. The progress of the reaction was monitored by TLC using

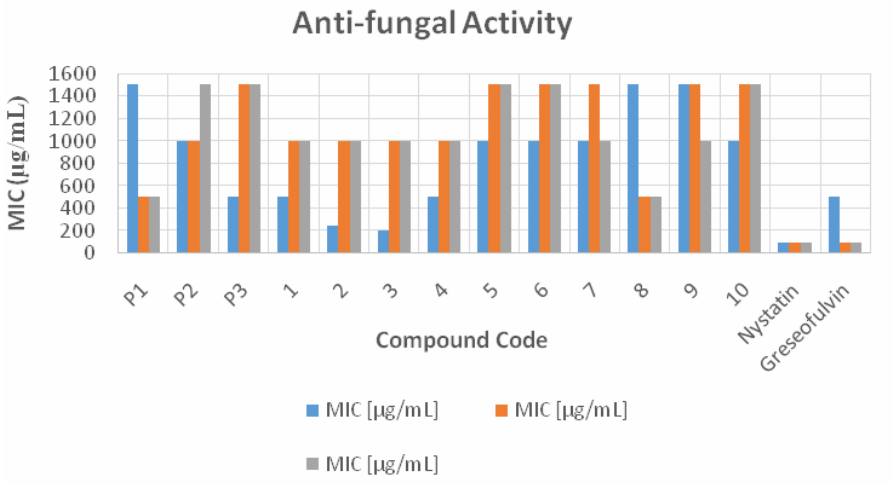


Figure 2 — Graphical representation of antifungal activity of compounds **P1-P3** and **1-19**

ethyl acetate:toluene (2:1) as a mobile phase. After completion of the reaction, the reaction mixture was poured in ice-water and the solid product thus obtained was filtered and purified by recrystallization from ethanol-water system, which afforded pure analytical grade product in excellent yield (**P1** = 88% yield and **P2** = 85% yield). All the products were confirmed from the IR, mass, ^{1}H NMR and elemental analysis.

2-(2-(1,2,3,4,5-Pentahydroxypentyl)-1*H*-benzo [*d*]imidazol-1-yl)-1-phenylethanone, P2: ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.489-3.627 (m, 3H), 3.829 (s, 2H), 3.963-4.215 (m, 5H), 4.686 (s, 1H), 5.256 (s, 1H), 7.235-7.683 (m, 4H), 7.854-8.285 (m, 5H), 11.569 (s, 1H); ESI-MS: *m/z* 387.2 $[M+1]^+$, 388.1 $[M+2]^+$; IR (KBr): 1512 (-CN), 1700 (-CO), 1230, 1039 (-OH), 1348, 1067 cm^{-1} (-OH). Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.14; H, 5.75; N, 7.26%.

1-(4-Methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydro-xypentyl)-1*H*-benzo[*d*]imidazol-1-yl)ethanone, P3: ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.215 (s, 1H), 3.367-3.716 (m, 3H), 3.931 (s, 2H), 4.025-4.297 (m, 5H), 4.628 (s, 1H), 4.998 (s, 2H), 5.698 (s, 3H), 7.103 (d, 2H), 7.286-7.697 (m, 4H), 7.892 (d, 2H), 12.258 (s, 1H); ESI-MS: *m/z* 416 $[M]^+$, 415.4 $[M-1]^+$; IR (KBr): 1510 (-CN), 1668 (-CO), 1260, 1026 (-OH), 1357, 1111 (-OH), 2912 cm^{-1} (-OCH_3). Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.61; H, 5.80; N, 6.72%.

Synthesis of N^4-monosubstituted thiosemicarbazides from isothiocynates, S1-S5

Various substituted isothiocynates (0.01 mol) and hydrazine hydrate (0.01 mol) were taken into the 100 mL FBF. 5 mL methanol was added into the FBF. The reaction mixture was stirred for 30 min at RT. The progress of the reaction was checked by TLC using ethyl acetate:toluene (2:1) as eluent. After completion of the reaction, the solid product obtained was filtered and purified by recrystallization from methanol. Pure products were afforded in good to excellent yield (92-95%). All the products were confirmed from the IR, mass, ^{1}H NMR and elemental analysis.

Synthesis of gluconate and thiosemicarbazone hybrid benzimidazole derivatives, 1-10

N^4-monosubstituted thiosemicarbazides (**S1-S5**) (0.01 mol) and compounds **P2** or **P3** (0.01 mol) were taken into the 100 mL RBF. The reaction was carried out in methanol with catalytic amount of glacial acetic acid at reflux temperature for 4 h. The progress of the reaction was checked by TLC using ethyl acetate:toluene (2:1) as eluent. After completion of the reaction, obtained solid product was filtered and purified by recrystallization from ethanol. The method afforded analytical sample in excellent yield (84-92%). All the products were identified on the basis of their elemental analysis, as well as IR, mass and ^{1}H NMR spectroscopic data.

(*E*)-2-(2-(2-(1,2,3,4,5-Pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)-1-phenylethylidene) hydrazinecarbothioamide, 1: ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.214 (s, 2H), 3.289 (s, 3H), 3.349-3.644 (m, 5H), 4.169 (s, 1H), 4.245(s, 2H), 7.268-7.453 (m, 4H), 7.523-7.854 (m, 5H), 11.836 (s, 2H), 12.031 (s, 1H); ESI-MS: *m/z* 459.15 $[M]^+$, 458.2 $[M-1]^+$; IR (KBr): 1520 (-C=N-N-), 1257, 756 (-CS), 1223, 1022 (-OH), 1324, 1111 (-OH), 1512 cm^{-1} (-C=N-). Anal. Calcd for $C_{21}H_{25}N_5O_5S$: C, 54.89; H, 5.48; N, 15.24; S, 6.98. Found: C, 54.88; H, 5.47; N, 15.23; S, 6.95%.

(*E*)-N-Cyclohexyl-2-(2-(2-(1,2,3,4,5-pentahydro xypentyl)-1*H*-benzo[*d*]imidazol-1-yl)-1-phenylethyl-idene)hydrazinecarbothioamide, 2: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.217-1.739 (m, 10H), 3.365 (s, 2H), 3.568 (s, 3H), 3.582-3.761 (m, 5H), 4.333 (s, 1H), 4.581 (s, 1H), 4.935 (s, 2H), 7.138-7.469 (m, 4H), 7.526-7.892 (m, 5H), 11.983 (s, 1H), 12.258 (s, 1H); ESI-MS: *m/z* 541 $[M]^+$, 540.6 $[M-1]^+$; IR (KBr): 1525 (-C=N-N-), 1251, 758 (-CS), 1203, 1055 (-OH), 1350, 1111 (-OH), 1525 (-C=N-), 2850 cm^{-1} (C_6H_{11}). Anal. Calcd for $C_{27}H_{35}N_5O_5S$: C, 59.87; H, 6.51; N, 12.93; S, 5.92. Found: C, 59.89; H, 6.49; N, 12.94; S, 5.91%.

(*E*)-N-(tert-Butyl)-2-(2-(2-(1,2,3,4,5-pentahydro-xypentyl)-1*H*-benzo[*d*]imidazol-1-yl)-1-phenylethyl idene)hydrazinecarbothioamide, 3: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.227-2.575 (m, 9H), 3.654 (s, 2H), 3.791 (s, 3H), 3.826-3.943 (m, 5H), 4.375 (s, 1H), 4.821 (s, 2H), 7.165-7.438 (m, 4H), 7.761-7.829 (m, 5H), 11.729 (s, 1H), 12.227 (s, 1H); ESI-MS: *m/z* 515.2 $[M]^+$, 514.1 $[M-1]^+$; IR (KBr): 1549 (-C=N-N-), 1241, 720 (-CS), 1213 (-OH), 1357 (-OH), 1530 cm^{-1} (-C=N-). Anal. Calcd for $C_{25}H_{33}N_5O_5S$: C, 58.23; H, 6.45; N, 13.58; S, 6.22. Found: C, 58.25; H, 6.43; N, 13.58; S, 6.23%.

(*E*)-N-Ethyl-2-(2-(2-(1,2,3,4,5-pentahydro-xypentyl) -1*H*-benzo[*d*]imidazol-1-yl)-1-phenylethyl idene) hydrazinecarbothioamide, 4: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.034-2.471 (m, 5H), 3.281 (s, 2H), 3.527 (s, 3H), 3.834-4.071 (m, 5H), 4.291 (s, 1H),

4.950 (s, 2H), 7.203-7.497 (m, 4H), 7.802-7.934 (m, 5H), 11.658 (s, 1H), 12.371 (s, 1H); ESI-MS: *m/z* 487.2 $[M]^+$, 486.1 $[M-1]^+$; IR (KBr): 1498 (-C=N-N-), 1201, 789 (-CS), 1200 (-OH), 1399 (-OH), 1509 cm^{-1} (-C=N-). Anal. Calcd for $C_{23}H_{29}N_5O_5S$: C, 56.66; H, 6.00; N, 14.36; S, 6.58. Found: C, 56.65; H, 5.99; N, 14.34; S, 6.59%.

(*E*)-N-Isopropyl-2-(2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)-1-phenylethylidene)hydrazinecarbothioamide, 5: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.030-1.467 (d, 6H), 2.364-2.424 (m, 1H), 3.277 (s, 2H), 3.523 (s, 3H), 3.830-4.067 (m, 5H), 4.287 (s, 1H), 4.946 (s, 2H), 7.199-7.493 (m, 4H), 7.798-7.930 (m, 5H), 11.654 (s, 1H), 12.367 (s, 1H); ESI-MS: *m/z* 501.2 $[M]^+$, 500.1 $[M-1]^+$; IR (KBr): 1496 (-C=N-N-), 1999, 787 (-CS), 1198 (-OH), 1397 (-OH), 1507 cm^{-1} (-C=N-). Anal. Calcd for $C_{24}H_{31}N_5O_5S$: C, 57.47; H, 6.23; N, 13.96; S, 6.39. Found: C, 57.45; H, 6.24; N, 13.97; S, 6.40%.

(*E*)-2-(1-(4-Methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)ethylidene)hydrazinecarbothioamide, 6: ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.271 (s, 2H), 3.523 (s, 3H), 3.829 (s, 3H), 3.835-4.062 (m, 5H), 4.282 (s, 1H), 4.941 (s, 2H), 7.194-7.488 (m, 4H), 7.793 (d, 2H), 7.907 (d, 2H), 11.649 (s, 2H), 12.362 (s, 1H); ESI-MS: *m/z* 489.2 $[M]^+$, 488.1 $[M-1]^+$; IR (KBr): 1498 (-C=N-N-), 1201, 787 (-CS), 1200 (-OH), 1399 (-OH), 1509 (-C=N-), 2914 cm^{-1} ($-OCH_3$). Anal. Calcd for $C_{22}H_{27}N_5O_6S$: C, 53.98; H, 5.56; N, 14.31; S, 6.55. Found: C, 53.96; H, 5.55; N, 14.31; S, 6.56%.

(*E*)-N-Cyclohexyl-2-(1-(4-methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)ethylidene) hydrazinecarbothioamide, 7: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.095-1.315 (m, 10H), 3.129 (s, 2H), 3.367 (m, 3H), 3.832 (s, 3H), 3.954-4.127 (m, 5H), 4.456 (s, 2H), 4.582 (s, 1H), 4.731 (s, 1H), 7.098-7.324 (m, 4H), 7.259-7.536 (m, 4H), 11.732 (s, 1H), 12.036 (s, 1H); ESI-MS: *m/z* 571 $[M]^+$, 570.6 $[M-1]^+$; IR (KBr): 1674 (-C=N-N-), 1668 (-CS), 1260, 1026 (-OH), 1357, 1111 (-OH), 1510 (-C=N-), 2928 cm^{-1} ($-OCH_3$). Anal. Calcd for $C_{28}H_{37}N_5O_6S$: C, 58.83; H, 6.52; N, 12.25; S, 5.61. Found: C, 58.85; H, 6.51; N, 12.24; S, 5.60%.

(*E*)-N-(tert-Butyl)-2-(1-(4-methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)ethylidene)hydrazinecarbothioamide, 8: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.222-2.570 (m, 9H), 3.124 (s, 2H), 3.362 (m, 3H), 3.827 (s, 3H), 3.949-4.122 (m, 5H), 4.451 (s, 2H), 4.577 (s, 1H), 7.092-7.319 (m, 4H), 7.787 (d, 2H), 7.902 (d, 2H), 11.727 (s, 1H), 12.031 (s, 1H); ESI-MS: *m/z* 545.6 $[M]^+$, 545.3 $[M-1]^+$; IR (KBr): 1669 (-C=N-N-), 1663 (-CS), 1275, 1021 (-OH), 1352, 1106 (-OH), 1505 (−C=N−), 2923 cm^{-1} ($-OCH_3$). Anal. Calcd for $C_{26}H_{35}N_5O_6S$: C, 57.23; H, 6.47; N, 12.83; S, 5.88. Found: C, 57.21; H, 6.47; N, 12.84; S, 5.87%.

(*E*)-N-Ethyl-2-(1-(4-methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*]imidazol-1-yl)ethylidene)hydrazinecarbothioamide, 9: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.222-2.370 (t, 3H), 3.012-3.217 (m, 2H), 3.124 (s, 2H), 3.362 (m, 3H), 3.827 (s, 3H), 3.949-4.122 (m, 5H), 4.451 (s, 2H), 4.577 (s, 1H), 7.092-7.319 (m, 4H), 7.787 (d, 2H), 7.902 (d, 2H), 11.727 (s, 1H), 12.031 (s, 1H); ESI-MS: *m/z* 517.2 $[M]^+$, 516.5 $[M-1]^+$; IR (KBr): 1672 (-C=N-N-), 1666 (-CS), 1278, 1024 (-OH), 1355, 1108 (-OH), 1508 (-C=N-), 2928 cm^{-1} ($-OCH_3$). Anal. Calcd for $C_{24}H_{31}N_5O_6S$: C, 55.69; H, 6.04; N, 13.53; S, 6.19. Found: C, 55.67; H, 6.05; N, 13.53; S, 6.18%.

(*E*)-N-Isopropyl-2-(1-(4-methoxyphenyl)-2-(2-(1,2,3,4,5-pentahydroxypentyl)-1*H*-benzo[*d*] imidazol-1-yl)ethylidene)hydrazinecarbothioamide, 10: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.216-2.362 (d, 6H), 3.004-3.209 (dq, 1H), 3.116 (s, 2H), 3.356 (m, 3H), 3.819 (s, 3H), 3.941-4.114 (m, 5H), 4.443 (s, 2H), 4.569 (s, 1H), 7.086-7.311 (m, 4H), 7.778 (d, 2H), 7.902 (d, 2H), 11.719 (s, 1H), 12.023 (s, 1H); ESI-MS: *m/z* 531.2 $[M]^+$, 530.3 $[M-1]^+$; IR (KBr): 1662 (-C=N-N-), 1656 (-CS), 1268, 1014 (-OH), 1345, 1098 (-OH), 1408 (-C=N-), 2918 cm^{-1} ($-OCH_3$). Anal. Calcd for $C_{25}H_{33}N_5O_6S$: C, 56.48; H, 6.26; N, 13.17; S, 6.03. Found: C, 56.50; H, 6.25; N, 13.16; S, 6.05%.

Biological assay

This work has been done in Microcare Laboratory and TRC, Surat, India.

In vitro evaluation of antimicrobial activity

The MICs of the synthesized compounds were carried out by broth micro-dilution method. DMSO was used as diluent to get the desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of

the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show: similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2000 mg/mL concentration as a stock solution. After primary screening 500, 250 and 200 mg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50 and 25 mg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

Conclusions

In this article, we have elaborated the initial efforts made toward the identification of novel, potentially active thiosemicarbazone hybrid benzimidazole derivatives, which were synthesized by simple and efficient protocols. All compounds were screened against a wide range of pathogenic bacterial and fungal strains. From the bioassay, it is clear that the introduction of thiosemicarbazone derivatives would lead to the more active anti-bacterial derivatives. Compounds P2 and P3 as well as compounds 1 to 4 exhibited excellent antimicrobial activity. Overall, from the bioassay results, it can be concluded that the findings of the present study will have a significant impact on medicinal chemists and motivate them to synthesize similar analogues, which will show enhanced bioactivity.

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