Full Paper

Synthesis and Antibacterial Activity of a Novel Series of 2,3-Diaryl-substituted-imidazo(2,1-*b*)-benzothiazole Derivatives

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Benzothiazole and imidazole compounds are extensively studied heterocyclics due to their wide spectrum of bioactivities. Among them, the imidazo(2,1-*b*)-benzothiazole derivatives are pharmacologically important because of their immunostimulant, anti-inflammatory, antifungal, antimicrobial, antitumor, and other activities. In the present research work, a novel series of 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazoles **13a–o** have been synthesized by reaction of substituted 2-aminobenzothiazoles **1–8** and an appropriately substituted α-bromo-1-(4″-substituted)-phenyl-2-(4′-substituted)-phenyl-1-ethanones **9–12** in the presence of anhydrous acetonitrile. They were characterized by physicochemical, elemental, and spectral (IR, ^{1}H-NMR, and Mass) data. All the synthesized compounds were screened for their *in-vitro* antibacterial activity against Gram-positive, Gram-negative bacteria. The investigation of antibacterial screening data revealed that most of the compounds tested have demonstrated congruent activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* as compared with the standard ampicillin. Among the series, compounds **13d**, **13h**, and **13m** exhibited excellent an antibacterial activity profile as compared with the standard. In summary, preliminary results indicate that some of the newly synthesized title compounds exhibited promising antibacterial activities and they warrant more consideration as prospective antimicrobials.

Keywords: 2-Aminobenzothiazole / Antibacterial activity / Imidazo(2,1-*b*)benzothiazole

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Introduction

Bacterial resistance to antibacterial agents or antibiotics is of grave concern in the medical community, as many species of bacteria have evolved resistance to certain antibiotics and synthetic agents. Therefore, there could be a rapidly growing global crisis in the clinical management of life-threatening infectious diseases caused by multi-drug-resistant strains of the Gram-positive pathogens like *Streptococcus*, *Enterococcus*, and *Staphylococcus*, and Gram-negative pathogens like *Escherichia*, *Salmonella*, and certain *Pseudomonas* strains. Especially the emergence of multidrug-resistant strains of Gram-positive bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is an alarming problem of ever-increasing significance [1–4]. To meet this crisis successfully, many researchers across the globe are working to unearth new compounds which can selectively attack novel targets in microorganisms. Hence, the development of novel, potent, and unique antibacterial agents is

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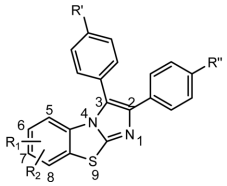
Table 1. Physicochemical data of a novel series of 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazoles **13a–o**.



Compound	R_1	R_2	R′	R″	Yield (%)	M.p. (°C)	R_f §	Mol. Formula	Mol. Weight
13a	7-Cl	8-Cl	OCH_3	OCH_3	69%	205	0.62	$C_{23}H_{16}SN_2Cl_2O_2$	455.36
13b	7-F	8-Cl	OCH_3	OCH_3	71%	186	0.54	$C_{23}H_{16}SN_2FClO_2$	438.90
13c	7-H	8-H	OCH_3	OCH_3	68%	168	0.43	$C_{23}H_{18}SN_2O_2$	386.47
13d	7-NO_2	8-H	OCH_3	OCH_3	75%	212	0.76	$C_{23}H_{17}SN_3O_4$	431.36
13e	7-Br	8-H	OCH_3	OCH_3	79%	135	0.61	$C_{23}H_{17}SN_2Br$	433.36
13f	7-F	8-H	OCH_3	OCH_3	62%	155	0.88	$C_{23}H_{17}SN_2FO_2$	404.46
13g	7-Cl	8-H	OCH_3	OCH_3	79%	190	0.81	$C_{23}H_{17}SN_2O_2Cl$	420.91
13h	6-NO_2	8-H	OCH_3	OCH_3	65%	205	0.27	$C_{23}H_{17}SN_3O_4$	431.26
13i	7-F	8-H	OCH_3	Br	56%	160	0.40	$C_{22}H_{14}SN_2OFBr$	453.33
13j	7-Cl	8-H	OCH_3	Br	71%	300	0.98	$C_{22}H_{14}SN_2OCBr$	446.34
13k	7-Cl	8-Cl	H	OCH_3	5%	300	0.84	$C_{22}H_{14}SN_2OCl_2$	425.33
13l	7-NO_2	8-H	H	OCH_3	67%	230	0.28	$C_{22}H_{15}SN_3O_3$	401.44
13m	7-NO_2	8-H	OCH_3	CH_3	60%	236	0.56	$C_{23}H_{17}SN_3O$	383.47
13n	7-Br	8-H	OCH_3	CH_3	73%	121	0.55	$C_{23}H_{17}SN_2OBr$	449.36
13o	6-NO_2	8-H	OCH_3	CH_3	68%	196	0.69	$C_{23}H_{17}SN_3O_3$	415.46

§ All synthesized compounds were purified by column chromatography using *n*-hexane and ethyl acetate (6:4) as a mobile phase and iodine vapors as visualizing agent.

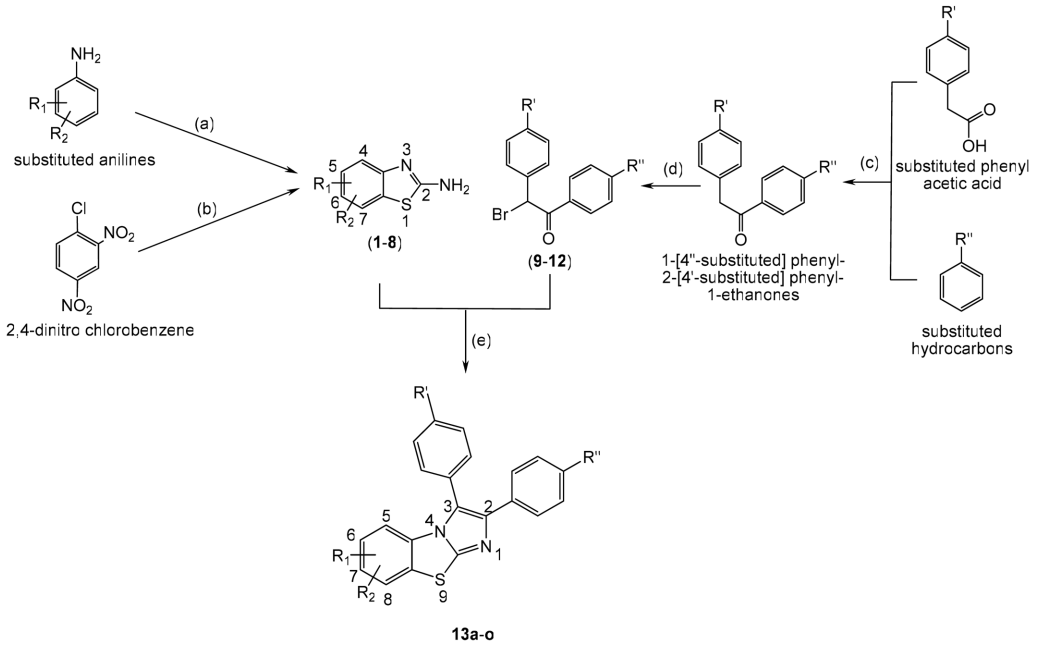
the preeminent way to overcome bacterial resistance and develop effective therapies [5].

The benzothiazole and imidazole compounds are extensively studied heterocyclics due to their wide spectrum of bioactivities. Despite numerous attempts to develop new structural prototypes in the search for more effective antimicrobials, the imidazo(2,1-*b*)-benzothiazoles still remain as one of the most versatile classes of compounds against microbes and, therefore, are an important component of molecules in drug discovery. Literature survey revealed that various compounds containing the imidazo(2,1-*b*)-benzothiazole moiety are well acknowledged to possess immunostimulant, anti-inflammatory [6, 7], antifungal, antimicrobial [8], antitumor [9], antituberculosis [10], anticonvulsant [11], and anthelmintic [12] activities. Therefore, such medicinal properties associated with these two heterocycles render them as useful structural units in drug research. These findings prompted us to synthesize imidazo(2,1-*b*)-benzothiazole derivatives as part of our ongoing research program aiming at the synthesis of a variety of heterocyclic systems for biological evaluation [13–15]. Herein, we report the synthesis of a novel series of 2,3-diaryl substituted imidazo(2,1-*b*)-benzothiazoles **13a–o** for the investigation of an antibacterial activity profile.

Results and discussion

Chemistry

The synthesis of 2,3-diaryl-substituted imidazo(2,1-*b*)benzothiazoles **13a–o** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. Reaction of substituted 2-aminobenzothizoles **1–8** with substituted α-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones **9–12** in the presence of anhydrous acetonitrile at a temperature of 65–75°C seemed to be a convenient route for the synthesis of desired molecules. Starting materials *i.e.*, various substituted 2-aminobenzothiazoles **1–8** were prepared by three different methods with respect to the position of substitution. The 2-amino-6,7-disubstituted benzothiazoles **1** and **2** were synthesized by the reaction of substituted aniline and potassium thiocyanate in the presence of glacial acetic acid at 0°C by following the literature procedure [16]. In order to produce the 2-amino-6-substituted benzothiazoles **3–7**, the literature procedure of Brewster *et al.* [17] was followed. 2-Amino-5-nitro-benzothiazole **8** was prepared by the reaction of 2,4-dinitrochlorobenzene and thiourea in the presence of pyridine under a reflux condenser for 3 h [18]. Synthesis of 1,2-(*p*-substituted)diaryl-1-ethanones was carried out by reacting appropriate phenyl acetic



13a: R_1= 7-Cl, R_2= 8-Cl, R′= OCH_3, R″= OCH_3
13b: R_1= 7-F, R_2= 8-Cl, R′= OCH_3, R″= OCH_3
13c: R_1= 7-H, R_2= 8-H, R′= OCH_3, R″= OCH_3
13d: R_1= 7-NO_2, R_2= 8-H, R′= OCH_3, R″= OCH_3
13e: R_1= 7-Br, R_2= 8-H, R′= OCH_3, R″= OCH_3
13f: R_1= 7-F, R_2= 8-H, R′= OCH_3, R″= OCH_3
13g: R_1= 7-Cl, R_2= 8-H, R′= OCH_3, R″= OCH_3
13h: R_1= 6-NO_2, R_2= 8-H, R′= OCH_3, R″= OCH_3
13i: R_1= 7- F, R_2= 8-H, R′= OCH_3, R″= Br
13j: R_1= 7-Cl, R_2= 8-H, R′= OCH_3, R″= Br
13k: R_1= 7-Cl, R_2= 8-Cl, R′= H, R″= OCH_3
13l: R_1= 7-NO_2, R_2= 8-H, R′= H, R″=OCH_3
13m: R_1= 7-NO_2, R_2= 8-H, R′= OCH_3, R″= CH_3
13n: R_1= 7-Br, R_2=8-H, R′= OCH_3, R″= CH_3
13o: R_1= 6-NO_2, R_2= 8-H, R′= OCH_3, R″= CH_3

Reagents and conditions: (a) CH_3COOH, KSCN, Br_2, NH_3, 0°C, stirring, 10 h; (b) thiourea, pyridine, reflux, stirring, 3 h; (c) H_3PO_4, $(CF_3CO)_2O$, 25°C, 1 min; (d) Br_2, $CHCl_3$, 50°C, 0.5 h; (e) anhydrous acetonitrile, methylcellosolve, P_2O_5, reflux, 4 h.

Scheme 1. Synthetic route of a novel series of 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazoles.

acid with various substituted aromatic hydrocarbons in the presence of orthophosphoric acid and trifluoroacetic anhydride [15]. Subsequently, 1,2-(*p*-substituted)diaryl-1-ethanones were subjected to bromination using liquid bromine in chloroform to obtain a-bromo-1,2-(*p*-substituted)diaryl-1-ethanones **9–12** as shown in Scheme 1. Efforts to convert compounds **1–8** into the target molecules **13a–o** under a variety of conditions [6] were not successful. Hence, an alternative method was adopted. This involved the reaction of substituted 2-aminobenzothizoles **1–8** with substituted α-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones **9–12** in the presence of anhydrous acetonitrile at a temperature of 65–75°C for 1 h with constant stirring. This way, we successfully obtained the hydrobromide salts, but the second step of the reaction was unsuccessful as per the literature procedure [19]. Therefore, we modified the second procedure by the addition of a trace quantity of phosphorus pentoxide into the reaction mixture containing methylcellosolve and continued heating under reflux for 2–6 h. This resulted in the formation of the desired compounds 2,3-diaryl-substituted imidazo(2,1-*b*)benzothiazoles **13a–o**. Structures of the synthesized compounds were established on the basis of physicochemical and ele-

Table 2. Spectral and elemental analysis data of a novel series of 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazoles **13a–o**.

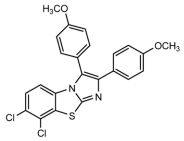
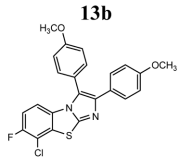
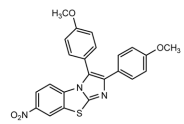
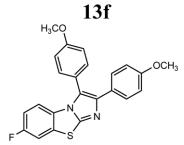
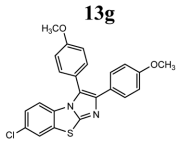
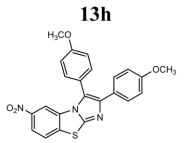
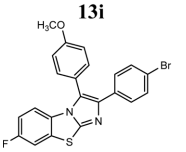
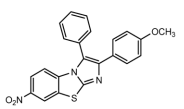
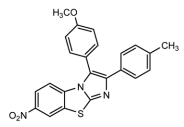
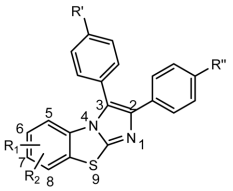
Compound	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6, δ, ppm)	Mass	Elemental (CHN) Analysis
13a 	3051.44 (aromatic C-H str), 2923.52 (methyl C-H str), 1615.78 (aromatic C=C str), 1290.83 (Ar-O-CH_3 str)	3.78 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.04–7.12 (d, 4H, Ar-H), 7.52–7.62 (m, 4H, Ar-H), 7.75 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H)	454.01 (27.3%) [M$^+$], 422.01 (21.1%), 393.02 (19.2%), 383.01 (16.8%), 321.01 (100%), 276.03 (14.2%), 239.01 (25.1%), 193.03 (35.7%), 134.01 (61.2%), 108.03 (29.4%), 90.02 (32.6%)	Anal. calcd. for $C_{23}H_{16}SN_2Cl_2O_2$: C, 60.68; H, 3.53; N, 6.14. Found: C, 60.63; H, 3.49; N, 6.08.
13b 	3087.77(aromatic C-H str), 2944.12 (methyl C-H str), 1628.99 (aromatic C=C str), 1281.64 (Ar-O-CH_3 str)	3.82 (s, 3H, OCH_3). 3.85 (s, 3H, OCH_3), 7.03–7.16 (d, 4H, Ar-H), 7.22 (s, 1H, Ar-H), 7.42–7.60 (d, 4H, Ar-H), 7.73 (s, 1H, Ar-H)		Anal. calcd. for $C_{23}H_{16}SN_2FClO_2$: C, 62.92; H, 3.69; N, 6.35. Found: C, 62.97; H, 3.69; N, 6.36.
13d 	3047.24(aromatic C-H str), 2916.90 (methyl C-H str), 1621.46 (aromatic C=C str), 1249.66 (Ar-O-CH_3 str)	3.80 (s, 6H, OCH_3), 7.15–7.23 (d, 4H, Ar-H), 7.47–7.57 (d, 4H, Ar-H), 7.88 (s,1H, Ar-H), 7.93–8.08 (d, 2H, Ar-H)	431.02 (33.4%) [M$^+$], 399.02 (18.4%), 385.03 (19.6%), 369.01 (28.2%), 324.01 (100%), 243.02 (33.5%), 189.01 (26.1%), 134.02 (67.3%), 108.02 (41.6%), 90.03 (38.6%)	Anal. calcd. for $C_{23}H_{17}SN_3O_4$: C, 64.02; H, 3.98; N, 9.76. Found: C, 63.98; H, 3.94; N, 9.72.
13f 	3062.25 (aromatic C-H str), 2934.24 (methyl C-H str), 1606.59 (aromatic C=C str), 1285.34 (Ar-O-CH_3 str)	3.74 (s, 3H, OCH_3). 3.79 (s, 3H, OCH_3), 7.11–7.19 (d, 4H, Ar-H), 7.27 (s, 1H, Ar-H), 7.51–7.67 (d, 4H, Ar-H), 7.89–7.97 (d, 2H, Ar-H)	406.02 (16.2%) [M + 1]$^+$, 404.02 (13.3%) [M$^+$], 370.02 (24.1%), 343.01 (19.4%), 322.02 (42.9%), 251.05 (28.3%), 179.02 (37.6%), 135.03 (19.2%), 134.01(100%), 107.07 (36.3%), 91.01 (31.9%)	Anal. calcd. for $C_{23}H_{17}SN_2FO_2$: C, 68.33; H, 4.25; N, 6.92. Found: C, 68.42; H, 4.24; N, 6.95.
13g 	3038.06 (aromatic C-H str), 2953.56 (methyl C-H str), 1635.13 (aromatic C=C str), 1279.75 (Ar-O-CH_3 str)	3.81 (s, 3H, OCH_3). 3.84 (s, 3H, OCH_3), 7.07–7.18 (d, 4H, Ar-H), 7.32 (s, 1H, Ar-H), 7.49–7.61 (d, 4H, Ar-H), 7.94–8.05 (d, 2H, Ar-H)		Anal. calcd. for $C_{23}H_{17}SN_2O_2Cl$: C, 65.65; H, 4.06; N, 6.64. Found: C, 65.63; H, 4.02; N, 6.68.
13h 	3072.11 (aromatic C-H str), 2941.53 (methyl C-H str), 1614.67 (aromatic C=C str), 1263.14 (Ar-O-CH_3 str)	3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 7.18–7.26 (d, 4H, Ar-H), 7.64–7.78 (d, 4H, Ar-H), 7.84 (s, 1H, Ar-H), 8.09–8.22 (m, 2H, Ar-H)	430.01 (24.1%) [M – 1], 395.02 (14.9%), 388.02 (19.6%), 372.02 (41.7%), 318.05 (100%), 273.03 (29.2), 225.02 (15.5%), 177.04 (35.2%), 134.03 (78.6%), 108.01 (32.3%), 89.02 (15.8%)	Anal. calcd. for $C_{23}H_{17}SN_3O_4$: C, 64.07; H, 3.95; N, 9.73. Found: C, 64.1; H, 3.97; N, 9.74.
13i 	3093.35 (aromatic C-H str), 2984.13 (methyl C-H str), 1650.56 (aromatic C=C str), 1302.54 (Ar-O-CH_3 str)	3.80 (s, 3H, OCH_3), 7.06–7.13 (d, 2H, Ar-H), 7.31 (s, 1H, Ar-H), 7.52–7.58 (d, 2H, Ar-H), 7. 70–7.76 (d, 2H, Ar-H), 7.83–7.89 (d,2H, Ar-H), 8.08–8.15 (d, 2H, Ar-H)	453.02 (22.5%) [M$^+$], 431.03 (26.7%), 420.01 (18.3%), 375.02 (17.4%), 327.04 (100%), 269.01 (22.4%), 227.01 (15.7%), 196.01 (17.5%), 135.02 (53.3%), 110.01 (44.9%), 88.05 (63.9%)	Anal. calcd. for $C_{22}H_{14}SN_2OFBr$: C, 58.31; H, 3.10; N, 6.20. Found: C, 58.35; H, 3.13; N, 6.16.
13l 	3042.74 (aromatic C-H str), 2941.42 (methyl C-H str), 1625.74 (aromatic C=C str), 1274.96 (Ar-O-CH_3 str)	3.88 (s, 3H, OCH_3), 7.23–7.31 (d, 4H, Ar-H), 7.61–7.82 (m, 5H, Ar-H), 7.95 (s,1H, Ar-H), 8.17–8.28 (d, 2H, Ar-H)	401.01 (24.1%) [M$^+$], 368.04 (15.6%), 351.01 (31.2%), 320.03 (72.3%), 248.02 (30.7%), 212.03 (20.5%), 182.01 (27.4%), 133.06 (100%), 110.07 (38.8%), 91.03 (33.4%)	Anal. calcd. for $C_{22}H_{15}SN_3O_3$: C, 65.83; H, 3.78; N, 10.50. Found: C, 65.79; H, 3.79; N, 10.46.
13m 	3025.45 (aromatic C-H str), 2925.43 (methyl C-H str), 1616.37 (aromatic C=C str), 1289.34 (Ar-O-CH_3 str)	1.74 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 7.13–7.21 (d, 2H, Ar-H), 7.43–7.50 (d, 2H, Ar-H), 7.64–7.71 (d, 2H, Ar-H), 7.89–7.96 (d, 2H, Ar-H), 8.12 (s, 1H, Ar-H)	417.02 (17.3%) [M + 2]$^+$, 415.01 (21.7%), 400.04 (25.1%), 382.06 (18.3%), 328.03 (53.2%), 256.03 (28.8%), 194.05 (32.4%), 134.08 (100%), 109.07 (37.1%), 89.05 (34.6%)	Anal. calcd. for $C_{23}H_{17}SN_3O$: C, 66.48; H, 4.11; N, 10.14. Found: C, 66.45; H, 4.15; N, 10.21.

Table 3. *In-vitro* antibacterial activity of 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazoles (**13a**, **b**, **d**, **f**, **g–j**, **l**, **m**, and **o**) against selected strains (MIC in μg/mL)*.



Compd.	R_1	R_2	R′	R″	Gram-positive organisms		Gram-negative organisms	
					Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
13a	7-Cl	8-Cl	OCH_3	OCH_3	64	>128	>128	>128
13b	7-F	8-Cl	OCH_3	OCH_3	32	32	>128	>128
13d	7-NO_2	8-H	OCH_3	OCH_3	0.25	0.5	0.5	0.5
13f	7-F	8-H	OCH_3	OCH_3	0.5	2	2	1
13g	7-Cl	8-H	OCH_3	OCH_3	16	32	32	>128
13h	6-NO_2	8-H	OCH_3	OCH_3	0.25	0.25	0.5	0.5
13i	7-F	8-H	OCH_3	Br	32	32	64	>128
13j	7-Cl	8-H	OCH_3	Br	64	128	>128	>128
13l	7-NO_2	8-H	H	OCH_3	2	8	2	4
13m	7-NO_2	8-H	OCH_3	CH_3	0.25	0.5	0.25	1
13o	6-NO_2	8-H	OCH_3	CH_3	4	2	1	2
Ampicillin	–	–	–	–	0.5	0.5	0.5	0.5

mental analysis and spectral data (IR, ^{1}H-NMR, and Mass), which are summarized in Tables 1 and 2, respectively.

Antibacterial activity

All the newly synthesized compounds **13a–o** were evaluated for their *in-vitro* antibacterial activity against two Gram-positive bacterial strains namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacterial strains namely, *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) using the conventional agar-dilution method [20]. Ampicillin was used as the reference standard. The results of the *in-vitro* antibacterial activity screening of the novel series of 2,3-diaryl-substituted imidazo(2,1-*b*) benzothiazoles (**13a**, **b**, **d**, **f**, **g–j**, **l**, **m**, and **o**) are summarized in Table 3. Among the series tested, three compounds (**13d**, **13h**, and **13m)** exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria while compounds **13f**, **13i**, **13l**, and **13o** showed moderate to good antibacterial activity against the tested organisms. However, all other compounds in the series were found to have less or poor activity against both Gram-positive and Gram-negative bacteria as compared to the standard. Minimum inhibitory concentration (MIC) was recorded as the lowest concentration of a compound that inhibits the growth of the tested microorganisms. In comparing the MIC values with the standard ampicillin (MIC = 0.5 μg/mL), compounds **13d**, **13h**, and **13m** exhibit the most potent *in-vitro* antibacterial activity against all evaluated organisms. Especially compounds **13d** (MIC = 0.25 to 2 μg/mL), **13h** (MIC = 0.25 to 0.5 μg/mL), and **13m** (MIC = 0.25 to 1 μg/mL) showed high antibacterial activity while compounds **13f** (MIC = 0.5 to 2 μg/mL), **13l** (MIC = 2 to 8 μg/mL), and **13o** (MIC = 1 to 4 μg/mL) showed respectable antibacterial activity. A brief investigation of the structure-activity relationship (SAR) revealed that the compounds with a nitro (-NO_2) or a halo group (especially fluoro) at position C-6 and C-7 of the imidazo(2,1-*b*)-benzothiazole nucleus contributed to a better antibacterial activity. Further, the presence of a methoxy (-OCH_3) group on the phenyl ring of either C-2 or C-3 of the imidazo(2,1-*b*)benzothiazole nucleus influenced the antibacterial activity. It is interesting to note that the introduction of a bromo group to the aromatic ring (compounds **13i** and **13j**), resulted in compounds with a poor antibacterial activity. However, the replacement of the bromo by a methyl group (-CH_3), as it is observed in compounds **13m–o**, retained the antibacterial activity. Hence, compounds **13d**, **13h**, and **13m** have exhibited excellent *in-vitro* antibacterial activity against all the test organisms and have emerged as active antibacterial agents.

Conclusion

In the present paper, we report the synthesis, spectral studies, and *in-vitro* antibacterial activity of a new series of

novel 2,3-diaryl-substituted imidazo(2,1-*b*)-benzothiazole derivatives **13a–o**. These novel heterocyclic compounds were prepared by cyclo-dehydration reaction between the various substituted 2-aminobenzothiazole derivatives **1–8** and various substituted α-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones **9–12** in the presence of anhydrous acetonitrile and methylcellosolve, under the influence of a trace quantity of phosphorus pentoxide. In general, the results of the *in-vitro* antibacterial activity are also encouraging, as out of eleven compounds tested, compounds **13d**, **13h**, and **13m** exhibited antibacterial activities, which are comparable or more potent regarding their activity than the reference drug. The MIC values of these novel compounds evidenced that the presence of a nitro group at position C-6 or C-7 of the imidazo(2,1-*b*)-benzothiazole nucleus gave rise to a better antibacterial potency. Possible improvements in the antibacterial activity can be further achieved by slight modifications in the ring substituents and/or extensive additional structural activity investigations.

Experimental

Chemistry

All research chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and were used as such for the reactions. Solvents except for those with laboratory reagent grade were dried and purified according to the literature, when necessary. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany).

Melting points of the synthesized compounds were determined in a Thermonik (Mumbai, India) melting point apparatus. IR spectra were recorded on a Thermo Nicolet IR200 FT-IR Spectrometer (Nicolet, Madison WI, USA) by using KBr pellets. The ^{1}H-NMR were recorded on Bruker AVANCE II 400 MHz (Bruker, Rheinstetten/Karlsruhe, Germany) using DMSO-d_6 as solvent. Chemical shifts are reported in δ [ppm] units with respect to TMS as internal standard. Mass spectra were recorded on Micromass Q-Tof Micro LC/MS/MS (Waters, Milford, MA, USA) under electron impact at 70 eV. The elemental analyses (C, H, N) of the compounds were performed on a CHN Elemental Analyser (Perkin Elmer 2400, Waltham, MA, USA). Results of the elemental analyses were within ± 0.3% of the theoretical values. The purity of compounds was examined by TLC on silica gel plate using *n*-hexane and ethyl acetate (6:4) as a mobile phase and iodine vapor as visualizing agent.

*General procedure for the preparation of 2-amino-6,7-disubstituted benzothiazoles **1** and **2** [16]*

Potassium thiocyanate (8 g, 0.08 mol) and of 3,4-disubstituted aniline (0.01 mol) were added to a pre-cooked (to 5°C) solution of glacial acetic acid (20 mL). The mixture was placed in a freezing mixture of ice and salt and was mechanically stirred, while 1.6 mL of bromine in 6 mL of glacial acetic acid was added from a dropping funnel at such a rate that the temperature did not rise beyond 0°C. After all the bromine had been added (105 min), the solution was stirred for an additional 2 h at 0°C and, then, at room temperature for 10 h. Then, it was allowed to stand overnight during which period an orange-colored precipitate settled at the bottom; water (6 mL) was added quickly and the slurry was heated at 85°C on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10 mL of glacial acetic acid, heated again to 85°C and was filtered hot. The combined filtrates were cooled and neutralized with concentrated ammonia solution to pH 6, when a dark yellow precipitate was collected. Recrystallized from benzene (twice) after treatment with charcoal gave the colorless plates of 2-amino-6,7-dichloro-benzothiazole **1** while pale yellow-colored plates were obtained for 2-amino-6-fluoro-7-chloro-benzothiazole **2**.

*General procedure for the preparation of 2-amino-6-substituted benzothiazole **3–7** [17]*

The appropriately substituted aniline (0.1 mol) and potassium thiocyanate (0.2 mol) were dissolved in 150 mL of glacial acetic acid, cooled in ice, and stirred mechanically while a solution of bromine (0.1 mol) in acetic acid (25 mL) was slowly added drop by drop. External cooling was applied throughout the process to keep the temperature below 10°C and the stirring was continued for thirty minutes after all of the bromine had been added. The precipitate of imino-benzothiazole hydrobromide was removed by filtration with a pump, dissolved in warm water, and the base was precipitated with alkali. The residue was recrystallized from alcohol or ligroin to yield the derivatives of 2-amino-6-substituted benzothiazole **3–7**.

*General procedure for the preparation of 2-amino-5-nitro-benzothiazole **8** [18]*

A solution of 2,4-dinitrochlorobenzene (0.05 mol) and thiourea (0.2 mol) in pyridine (50 mL) was boiled with stirring, under a reflux condenser for 3 h. After cooling, the mixture was thoroughly stirred with 500 mL of water and the solid was filtered off with suction, washed with water and dried.

General procedure for the synthesis of 1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones [15]

To a mixture of substituted phenyl acetic acid (0.0073 mol), substituted aromatic hydrocarbon (0.0088 mol), and 88–93% orthophosphoric acid (0.0088 mol) trifluoroaceic anhydride (0.029 mol) was added rapidly with vigorous stirring at 25°C. The mixture turned into a dark-colored solution with vigorous exothermic reaction. The reaction mixture was stirred for 1 min at the same temperature and poured into ice-cold water (50 mL) with stirring. Then, it was washed with cold hexane (2 × 10 mL) to obtain the title compounds which were used for the next step.

*General procedure for the synthesis of a-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones **9–12***

To a solution of 1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanone (0.2 mol) in chloroform (30 mL) kept at 50°C, bromine (0.22 mol) was added dropwise with stirring. After being stirred at 50°C for 0.5 h, the mixture was washed successively with aqueous 10% sodium thiosulphate solution and water. The solvent was removed *in vacuo* to obtain the title compounds α-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanones either as sold mass/oil crystalline compounds.

*General procedure for the synthesis of 2,3-diaryl-substituted imidazo(2,1-b) benzothiazoles **13a–o** [19]*

The various substituted 2-aminobenzothizole (**1–8**, 0.03 mol) and the appropriate α-bromo-1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanone (**9–12**, 0.029 mol) were added in 150 mL of anhydrous acetonitrile and the mixture was heated to 65–75°C for 1 h with constant stirring. After the reaction was finished, the reaction mixture was cooled, the crystals formed were recovered by filtration, washed with acetonitrile, and dried to provide the crystals of 2-imino-3-[1-[4′-substituted] phenyl-2-[4″-substituted] phenyl-1-ethanone]-2,3-dihydrobenzo-thiazole hydrobromide. Then, crystals of hydrobromide were refluxed under heating in 75 mL of methylcellosolve. After 2–4 h, phosphorus pentoxide (0.015 mol) was added and the reflux was continued for another 2–6 h. Then, the reaction mixture was cooled to about 50°C. 30 mL of 5% aqueous ammonia was added to the reaction mixture, the crystals formed were recovered by filtration and recrystallized from ethanol to provide the various 2,3-diaryl substituted imidazo(2,1-*b*)benzothiazoles **13a–o**.

Antibacterial activity

Medium

The solid media Müller–Hinton agar (MHA; beef infusion 300 g/L, casein acid hydrolysate 17.5 g/L, starch 1.5 g/L, agar 17 g/L, and distilled water 1000 mL, adjusted to pH = 7.4) was used for the antibacterial activity.

Test microorganisms

Two Gram-positive bacteria namely, *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacteria namely, *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) were used for the antibacterial activity tests.

Minimum inhibitory concentration [21]

The *in-vitro* antibacterial activity of the newly synthesized eleven compounds (**13a**, **b**, **d**, **f**, **g–j**, **l**, **m**, and **o**) was evaluated using the conventional agar-dilution method [20]. Twofold serial dilutions of the compounds and reference drug (ampicillin) were prepared in MHA. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with distilled water (9 mL). Further progressive double dilution with melted MHA was performed to obtain the required concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 μg/mL. The bacterial inoculates were prepared by suspending 24-h old bacterial colonies from MHA media in 0.85% saline. Inoculates were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL) [22]. The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Petri dishes were spot-inoculated with 1 μL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 37°C for 24 h. At the end of the incubation period, MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment in order to ensure that the solvent had no influence on bacterial growth.

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