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Iron-catalyzed cyclization of aminothiols: An easy access to benzothiazoles and evaluation of their antimicrobial and anti-biofilm activities

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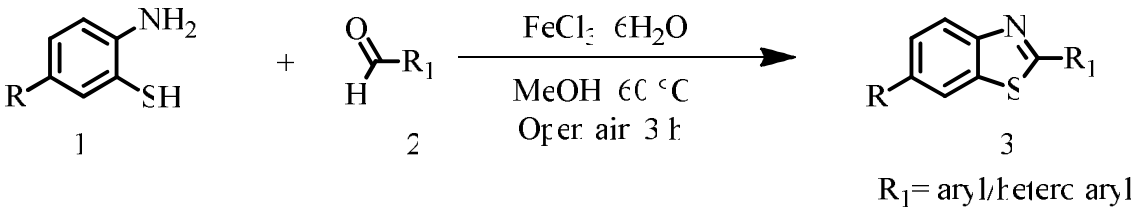
Iron-catalyzed cyclization of aminothiol with various aromatic and heteroaromatic aldehydes produces a variety of benzothiazoles in moderate to excellent yield. Compound **3o** with one carbazole functional moiety bridged between two benzothiazole scaffolds is found promising and contributes to significant antimicrobial, MBC and anti-biofilm activities.

Keywords: Cyclization, iron(III) chloride, anticancer, benzothiazoles, antimicrobial activity, *Staphylococcus aureus*

The emergence and spread of antibiotic-resistant strains of pathogenic bacteria is a major threat to public health[1,2]. *Staphylococcus aureus*, a Gram positive bacterium and a superbug of particular concern, is one of the leading causative agents of hospital-acquired infections, particularly in immuno-compromised patients, which contribute to increased rates of systemic infections and mortality[3-5]. It was earlier reported that more than 1.4 million people worldwide suffer from complications of hospital-acquired infections resulting in a mortality rate of 10-15% [6,7]. The resistance to methicillin, a first line antibiotic to *Staphylococcus aureus*, makes it even more difficult to control[8-10]. According to the recent reports of WHO, people with MRSA (methicillin-resistant *Staphylococcus aureus*) are estimated to be 64% more likely to die than people with a non-resistant form of the infection[11]. In view of the emergence of multi-drug resistance among the known pathogenic bacteria, a renewed interest has been generated towards developing newer approaches to anti-infective therapy. Some of the promising strategies for scaffold discovery include the mining of under explored microbial niches for natural products, designing screening assays that avoid rediscovering old scaffolds, and repurposing libraries of synthetic molecules for use as newer antibiotics[12]. Considering these facts, the discovery of new scaffolds which can counteract the bacterial resistance needs to be prioritized.

Benzothiazoles are a class of scaffolds which have attracted much attention in view of their important role in bioactive pharmaceutical compounds[13,14], and as new materials[15,16]. More specifically, 2-aryl benzothiazoles exhibited a diverse range of biological activities[17], such as anti-tumor[18-21], anti-viral[22], antimicrobial[23-25], and also as tracers for β-amyloid plaques in Alzheimer's disease[26]. Despite its efficacy, a plethora of strategies have emerged so far for the construction of their derivatives[27-29]. However, transition-metal-catalyzed reactions, in recent times, offer some of the most attractive routes[30-53]. These reactions mostly involve 2-aminothiophenol, thioanilides, anilides, *o*-haloanilines and Schiff bases as substrates of choice and usually proceed *via* oxidative/non-oxidative cyclization or C-S bond formation through cross-coupling or C-H functionalization. Copper[30-40] and palladium[41-47] have been the most widely used catalysts in this regard with little focus on other metals[48-53]. Moreover, most of these reactions employed additives such as bases, ligands, sometimes oxidants and are most often accompanied by longer reaction times. In this context, it has been felt that there is a pressing need to develop



Scheme I

novel catalytic routes involving direct and simpler approaches. We are engaged in iron-catalyzed reactions and recently reported the use of iron(III)chloride for the C-H functionalization of *N,N*-dialkylanilines resulting in the construction of C-C and C-O bonds[54-56]. As a part of our emphasis on iron-catalysis, we strived to develop an iron-catalytic system for the generation of various substituted benzothiazoles *via* cyclization reaction. In this paper, we describe the condensation of 2-aminothiophenol with various aldehydes using $FeCl_3.6H_2O$ as a catalyst (Scheme I) in a one pot reaction and the resulting benzothiazoles were assayed for antimicrobial and anticancer activities.

Results and Discussions

Chemistry

To begin our study, we chose aminothiol and benzaldehyde as the standard substrates to search for suitable reaction conditions (Table I). Among the various solvents examined, the yields obtained with polar, aprotic, aromatic hydrocarbons and other solvents were not convincing (Table I, entries **1-6**).

A significant upsurge in the yields was observed in case of polar protic solvents (Table I, entries **7-8**), among which, methanol produced the best (Table I, entry **7**). While optimizing the reaction conditions, we discovered that the reaction was not sensitive to moisture and air and the product yields were unaffected when the reaction was performed in open air. Having the optimized reaction conditions established, various aldehydes were employed to react with aminothiols, and representative results are compiled in Table II. The cyclization performed with various aldehydes proceeded smoothly to afford the desired benzothiazoles in moderate to excellent yields. Aromatic aldehydes tethered with electron withdrawing or electron donating substituents underwent smooth cyclization to afford the desired products in very good yields (Table I, entries **3a-i**).

It is noteworthy to mention that various potentially labile functional groups such as fluoro, bromo and alkenyl were tolerant under the reaction conditions,

Table I — Optimization of the reaction conditions[a]

Entry	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	Dimethylsulfoxide	189	3	72
2	Toluene	110	3	62
3	Acetonitrile	81	3	73
4	Chloroform	61	3	68
5	Tetrahydrofuran	65	3	67
6	Dioxane	101	3	65
7	Methanol	60	3	96
8	Acetic acid	118	3	82

[a] Reaction conditions: 1 (1 equiv), 2 (1 equiv) in solvent (7 mL) in open air.
[b] Isolated yields.

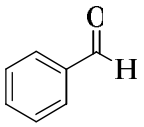
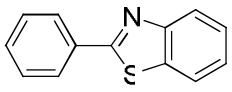
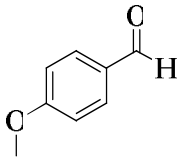
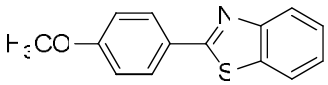
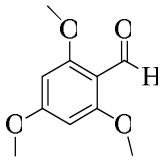
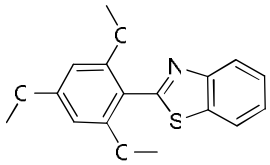
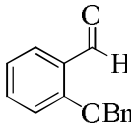
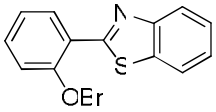
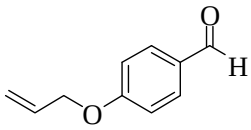
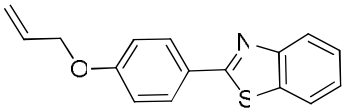
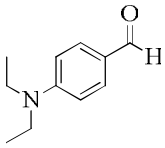
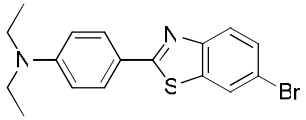
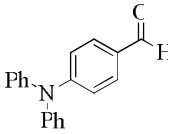
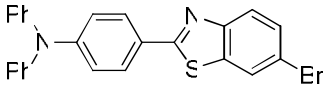
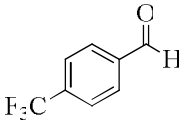
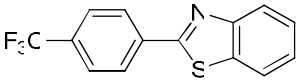
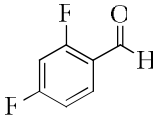
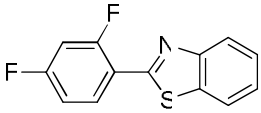
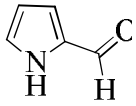
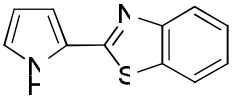
paving the way for further transformations towards various functionalities (Table I, entries **3f**, **3g** and **3i**). Thereafter, we investigated the various heteroaromatic aldehydes for cyclization. Mono, di- and tri-cyclic heteroaromatic aldehydes were also cyclized with moderate to good yields of the product (Table I, entries **3j-o**).

Biology

Antimicrobial evaluation

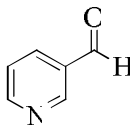
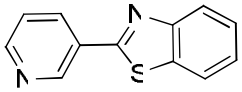
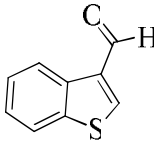
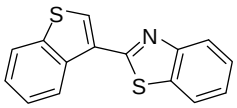
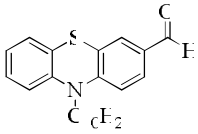
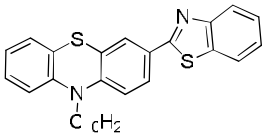
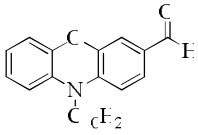
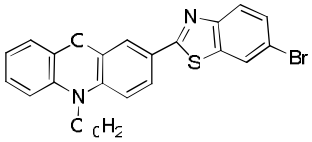
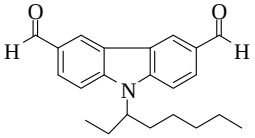
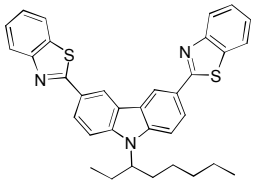
Among all the benzothiazole derivatives tested against different pathogenic reference strains, only compound **3o** showed promising antimicrobial activity against *Staphylococcus aureus* MLS-16 MTCC 2940 and *Klebsiella planticola* MTCC 530 with MIC values of 3.9 and 7.8 µg/mL, respectively. However, all the other compounds (**3a-n**) were not active against the tested pathogens even up to the highest tested concentration of >125 µg/mL. The antimicrobial activity results are shown in Table III. Based on the antimicrobial activity results, the compound **3o** was found to be quite promising and was further evaluated for minimum bactericidal concentration (MBC) and biofilm inhibition activities. The minimum bactericidal concentration (MBC) determined for compound **3o** was 7.8 µg/mL, while Ciprofloxacin (standard drug) exhibited 1.9 µg/mL

Table II — Substrate scope

Entry	R	R_1	Product	Yield (%) [b]
1	H		 **3a**	96
2	H		 **3b**	90
3	H		 **3c**	84
4	H		 **3d**	82
5	H		 **3e**	74
6	Br		 **3f**	87
7	Br		 **3g**	83
8	H		 **3h**	83
9	H		 **3i**	71
10	H		 **3j**	72

(*Contd.*)

Table II — Substrate scope (Contd.)

Entry	R	R_1	Product	Yield (%) [b]
11	H		 **3k**	77
12	H		 **3l**	79
13	H		 **3m**	72
14	Br		 **3n**[c]	70
15	H		 **3o**[c]	61

Reaction conditions: 1 (1 equiv), 2 (1 equiv), $FeCl_3.6H_2O$ (10 mol %), Methanol (7 mL), 60°C, 3 h, open air.
[b] Isolated yield based on aldehyde. [c] Chloroform used as a solvent for the formation of **3m**, **3n** and **3o**.

Table III — Antimicrobial evaluation of the synthesized compounds

	Minimum inhibitory concentration (μg/mL)			
Bacteria	Ciprofloxacin	**3a-n**	**3o**	Miconazole
Gram positive				
Micrococcus luteus MTCC 2470	0.9	>125.0	>125.0	–
Staphylococcus aureus MTCC 96	0.9	>125.0	>125.0	–
Staphylococcus aureus MLS-16 MTCC 2940	0.9	>125.0	3.9	–
Bacillus subtilis MTCC 121	0.9	>125.0	>125.0	–
Gram negative				
Escherichia coli MTCC 739	0.9	>125.0	>125.0	–
Pseudomonas aeruginosa MTCC 2453	0.9	>125.0	>125.0	–
Klebsiella planticola MTCC 530	0.9	>125.0	7.8	–
Candida albicans MTCC 3017	–	–	–	7.8

"–" No activity

against both *Staphylococcus aureus* MLS-16 MTCC 2940 and *Klebsiella planticola* MTCC 530 (Table IV).

Biofilms are structured bacterial communities comprising of single or multiple bacterial species embedded in a self-produced polymeric matrix which protects the colonized niche from competitors[57,58]. Biofilm formation is a ubiquitous defence mechanism and is a primary element in the antibiotic resistance and can cause serious chronic infections in humans *via* hospital and community environments[59]. In the clinical environments, several bacterial strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* have the ability to form biofilms[60]. These bacteria colonize the surfaces of medical implants such as stents, heart valves, vascular grafts and catheters by bacterial adhesion and biofilm formation[61]. Efforts were made in the recent past to understand the biology of biofilms and to search for novel inhibitors that can control biofilm formation and biofilm-related cellular processes[62,63]. In the present study, compound **3o**, found to be quite promising based on the antimicrobial activity results, was subjected to anti-biofilm activity using ciprofloxacin as standard control. The results suggested that compound **3o** exhibited biofilm inhibition in *Staphylococcus aureus* MLS-16 MTCC 2940 and *Klebsiella planticola* MTCC 530 with biofilm inhibitory concentration values of 2.1 and 3.6 µg/mL, respectively (Table V).

Based on the structure-activity relationship (SAR) study, it can be concluded that compound **3o** has one carbazole functional moiety bridged between two benzothiazole scaffolds which probably may be contributing to the significant antimicrobial activity as compared to other derivatives against the two bacterial strains such as *Staphylococcus aureus* and *Klebsiella planticola*. Among all the benzothiazole derivatives (Table VI) tested against a panel of four cancer cell lines, the compounds **3c**, **3f**, **3i** and **3k** were found to be quite promising. Among them,

Table IV — Minimum bactericidal concentration (MBC) for compound **3o**

Test Compd	Minimum bactericidal concentration (µg/mL)	
	Staphylococcus aureus MLS-16 MTCC 2940	*Klebsiellaplanticola* MTCC 530
3o	7.8	7.8
Ciprofloxacin (Standard)	1.9	1.9

Table V — Biofilm inhibition assay for compound 3o

Test Compd	Biofilm inhibitory concentration (µg/mL)	
	Staphylococcus aureus MLS-16 MTCC 2940	*Klebsiella planticola* MTCC 530
3o	2.1 ± 0.16	3.6 ± 0.23
Ciprofloxacin (Standard)	0.19 ± 0.12	0.44 ± 0.18

Table VI — Cytotoxicity evaluation of the synthesized compounds on different cancer cell lines (IC_{50} in µM)

S.No	Test Compd	COLO 205	A549	DU145	MCF-7
1	**3a**	16.0 ± 0.41	12.3 ± 0.26	–	–
2	**3b**	16.3 ± 0.24	–	–	13.0 ± 0.35
3	**3c**	–	–	–	6.2 ± 0.12
4	**3d**	–	–	–	72.9 ± 0.34
5	**3e**	23.1 ± 0.22	–	–	73.0 ± 0.44
6	**3f**	–	–	10.6 ±0.12	11.5 ± 0.14
7	**3g**	19.4 ± 0.16	–	–	22.6 ± 0.26
8	**3h**	14.9 ± 0.12	30.6 ± 0.15	–	15.9 ± 0.32
9	**3i**	10.1 ± 0.52	–	–	13.0 ± 0.18
10	**3j**	69.6 ± 0.48	–	18.1 ± 0.24	23.4 ± 0.24
11	**3k**	–	8.7 ± 0.11	9.2 ± 0.09	9.7 ± 0.12
12	**3l**	–	–	37.2 ± 0.16	47.9 ± 0.26
13	**3m**	–	–	–	–
14	**3n**	44.9 ± 0.32	–	–	84.1 ± 0.34
15	**3o**	75.6 ± 0.54	–	–	–
	Doxorubicin	0.8 ± 0.09	0.7 ± 0.12	0.7 ± 0.09	0.9 ± 0.08

"–" Indicates No activity COLO 205 - Human colon adenocarcinoma cell line; A549 - Human alveolar adenocarcinoma cell line; DU-145 - Human prostrate cancer cell line; MCF-7 - Human breast adenocarcinoma cell line.

3c exhibited cytotoxicity specifically against MCF-7 cell line, while compound **3f** showed cytotoxicity towards DU145 and MCF-7 cell lines. Compound **3i** showed cytotoxicity towards COLO205 and MCF-7 cell lines and compound **3k** showed cytotoxicity towards A549, DU145 and MCF-7 cell lines.

However, the compounds **3a**, **3b**, **3g**, **3h** and **3j** exhibited moderate cytotoxic activity towards all the tested cell lines. From the structure-activity relationship perspective, the compound **3c** has a trimethoxy functional moiety attached to the benzothiazole scaffold; the compound **3f** has a *N*,*N*′-diethyl functional moiety attached to the bromine substituted benzothiazole scaffold, while compounds **3i** (difluorobenzene functional moiety with two highly reactive fluorine groups) and **3k** (pyridine functional moiety) attached to the benzothiazole scaffold. These functional substituents on the benzothiazole scaffold have strong electron withdrawing or electron donating properties which probably may be contributing to the significant cytotoxicity.

Experimental Section

Materials and methods

All commercially available chemicals were used as received. Thin-layer chromatography plates were visualized by exposure to ultraviolet light (UV)/Iodine and/or by immersion in an acidic staining solution of phosphomolybdic acid followed by heating on a hot plate. ^{1}H NMR spectra were obtained on 300 MHz spectrometer and ^{13}C NMR spectra were obtained on 75 MHz spectrometer in $CDCl_3$ at 298 K with tetramethylsilane and $CDCl_3$, respectively, as the internal standard. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (δ = 7.26 for ^{1}H NMR and δ = 77.0 for ^{13}C NMR). Data for ^{1}H NMR are reported as follows: chemical shift (multiplicity, coupling constant, number of hydrogens). Multiplicity is abbreviated as follows: s (singlet), d (doublet), dd (double doublet), dt (doublet of a triplet), t (triplet), q (quartet) and m (multiplet). IR spectra were recorded on a Perkin-Elmer 1800 series FTIR spectrometer and samples were analyzed as thin films on KBr pellets. Mass spectra were carried out using Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK). High-resolution mass spectra were determined using Quadrupole time-of-flight (Q-TOF) mass spectrometer (QSTARXL, Applied Biosystems/MDS Sciex, Foster City, USA).

General experimental procedure for the synthesis of benzothiazoles

The mixture of aldehyde (1.0 mmol) and 2-aminothiophenol (1.0 mmol) in methanol (7 mL) was heated in presence of $FeCl_3.6H_2O$ (10 mol %) catalyst under open air at 60°C. The crude obtained was then concentrated under reduced pressure to remove methanol, followed by extraction with chloroform, drying over anhyd. Na_2SO_4 (s), followed by column purification to give the desired product in good yield.

Procedure for the formation of 3-(benzo[*d*]thiazol-2-yl)-10-decyl-10,10a-dihydro-4a*H*-phenothiazine, 3m: It is prepared by the general experimental procedure using the aldehyde 10-decyl-10*H*-phenothiazine-3-carbaldehyde[64].

Procedure for the formation of 3-(benzo[*d*]thiazol-2-yl)-10-decyl-10,10a-dihydro-4a*H*-phenoxazine, 3n: It is prepared by the general experimental procedure using the aldehyde 10-decyl-10*H*-phenooxazine-3-carbaldehyde[64].

2-Phenylbenzo[*d*]thiazole, 3a: Isolated by column chromatography. The title compound is a white solid (202 mg, 96% Yield). m.p.113-15°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13-8.06 (m, 3H), 7.91 (d, *J* = 7.55 Hz, 1H), 7.53-7.47 (m, 4H), 7.42-7.35 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.054, 154.091, 135.023, 133.575, 130.951, 128.999, 127.524, 126.296, 125.160, 123.199, 121.599; ESI-MS: Calcd for $C_{13}H_9NS$: *m/z* 211.28. Found: (M+1) 212.

2-(4-Methoxyphenyl)benzo[*d*]thiazole, 3b: Isolated by column chromatography. The title compound is a white solid (217 mg 90% Yield). m.p.119-22°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06-8.01 (m, 3H), 7.87 (d, *J* = 7.55 Hz, 1H), 7.51-7.43 (m, 1H), 7.39-7.32 (m, 1H), 7.03-6.98 (m, 2H), 3.88 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 167.785, 161.859, 154.175, 134.809, 130.048, 129.048, 126.379, 126.127, 124.719, 122.764, 121.430, 114.301, 113.901, 55.380; ESI-HRMS: Calcd for $C_{14}H_{12}NOS$: *m/z* 242.06341. Found: 242.06314.

(3) 2-(2,4,6-Trimethoxyphenyl)benzo[*d*]thiazole, 3c: Isolated by column chromatography. The title compound is a white solid (252 mg, 84% Yield). m.p.116-14°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25-8.20 (m, 1H), 8.05 (d, *J* = 7.55 Hz, 1H), 7.92 (d, *J* = 7.55 Hz, 1H), 7.51-7.44 (m, 1H), 7.40-7.32 (m, 1H), 6.83 (d, *J* = 9.06 Hz, 1H), 4.08 (s, 3H), 3.95 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 162.917, 155.732,

152.366, 152.191, 142.056, 135.656, 125.852, 124.457, 123.969, 122.463, 121.200, 120.048, 107.780, 60.875, 60.837, 56.069; ESI-HRMS: Calcd for $C_{16}H_{16}NO_3S$: *m/z* 302.08454. Found: 302.08415.

2-(2-(Benzyloxy)phenyl)benzo[*d*]thiazole, 3d: Isolated by column chromatography. The title compound is a yellow solid (260 mg, 82% Yield). m.p.90-94°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58-8.53 (m, 1H), 8.08 (d, *J* = 8.30 Hz, 1H), 7.87 (d, *J* = 8.30 Hz, 1H), 7.56-7.52 (m, 2H), 7.50-7.37 (m, 6H), 7.16-7.09 (m, 2H), 5.34 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 163.177, 156.278, 152.151, 149.417, 137.111, 131.729, 129.809, 128.704, 128.296, 127.889, 125.939, 124.601, 122.798, 121.428, 121.341, 116.368, 112.994, 111.467, 110.807, 71.014; ESI-HRMS: Calcd for $C_{20}H_{16}NOS$: *m/z* 318.09471. Found: 318.09369.

2-(4-(Allyloxy)phenyl)benzo[*d*]thiazole, 3e: Isolated by column chromatography. The title compound is a light yellow solid (198 mg, 74% Yield). m.p. 100-104°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-7.98 (m, 3H), 7.87 (d, *J* = 8.30 Hz, 1H), 7.55-7.42 (m, 1H), 7.35 (t, *J* = 7.55 Hz, 1H), 7.09-6.94 (m, 2H), 6.17-5.94 (m, 1H), 5.51-5.29 (m, 2H), 4.61 (d, *J* = 5.28 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 167.749, 160.847, 154.163, 134.809, 132.652, 129.025, 128.262, 126.492, 126.142, 124.733, 122.771, 121.454, 118.011, 115.036, 68.851; ESI-HRMS: Calcd for $C_{16}H_{14}NOS$: *m/z* 268.07906. Found: 268.07804.

4-(6-Bromobenzo[*d*]thiazol-2-yl)-*N*,*N*-diethylaniline, 3f: Isolated by column chromatography. The title compound is a yellow solid (313 mg, 87% Yield). m.p.128-30°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98-7.89 (m, 3H), 7.81 (d, *J* = 8.30 Hz, 1H), 7.52 (dd, *J* = 8.30, 2.26 Hz, 1H), 6.87-6.54 (m, 2H), 3.44 (q, *J* = 7.55 Hz, 4H), 1.22 (t, *J* = 7.55 Hz, 6H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.279, 153.357, 149.903, 136.141, 129.228, 129.141, 123.746, 123.119, 119.922, 117.160, 111.051, 44.455, 12.534; ESI-MS: *m/z* 361 $(M+H)^+$, 363$(M+H+2)^+$; ESI-HRMS: Calcd for $C_{17}H_{18}N_2BrS$: $(M+H)^+$ *m/z* 361.03686. Found: 361.03630.

4-(6-Bromobenzo[*d*]thiazol-2-yl)-*N*,*N*-diphenylaniline, 3g: Isolated by column chromatography. The title compound is yellow solid (379 mg, 83% Yield). m.p.158-62°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03-7.98 (m, 1H), 7.93-7.85 (m, 3H), 7.59-7.52 (m, 1H), 7.44-7.37 (m, 2H), 7.37-7.27 (m, 3H), 7.19-7.01 (m, 7H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.031, 153.132, 150.142, 146.749, 146.371, 145.939, 136.444, 132.522, 129.670, 129.512, 128.638, 128.535, 126.565, 126.508, 125.531, 125.496, 124.556, 124.174, 123.994, 123.833, 121.934, 121.445, 118.188, 116.569; ESI-HRMS: Calcd for $C_{25}H_{18}N_2SBr$: *m/z* 457.03686. Found: 457.03606.

2-(4-(Trifluoromethyl)phenyl)benzo[*d*]thiazole, 3h: Isolated by column chromatography. The title compound is light yellow solid (231 mg, 83% Yield). m.p.157-60°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (d, *J* = 8.30 Hz, 2H), 8.11 (d, *J* = 8.12 Hz, 1H), 7.94 (d, *J* = 7.74 Hz, 1H), 7.76 (d, *J* = 8.30 Hz, 2H), 7.58-7.48 (m, 1H), 7.48-7.40 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.987, 154.015, 136.733, 135.181, 132.635, 132.199, 127.720, 126.615, 125.978, 125.937, 125.747, 123.605, 121.701; ESI-HRMS: Calcd for $C_{14}H_9NF_3S$: *m/z* 280.04023. Found: 280.04028.

2-(2,4-Difluorophenyl)benzo[*d*]thiazole, 3i: Isolated by column chromatography. The title compound is yellow solid (175 mg, 71% Yield). m.p. 102-106°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50-8.37 (m, 1H), 8.10 (d, *J* = 8.12 Hz, 1H), 7.94 (d, *J* = 7.93 Hz, 1H), 7.57-7.48 (m, 1H), 7.47-7.37 (m, 1H), 7.12-6.94 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.216, 165.114, 163.185, 163.094, 161.815, 161.721, 160.105, 160.059, 159.778, 159.685, 152.354, 135.428, 135.363, 131.124, 131.093, 131.046, 131.016, 126.335, 125.295, 123.165, 121.425, 118.060, 118.033, 117.965, 117.943, 112.449, 112.427, 112.276, 112.256, 104.795, 104.584, 104.385; ESI-HRMS: Calcd for $C_{13}H_8NF_2S$: *m/z* 248.03400. Found: 248.03411.

2-(1*H*-Pyrrol-2-yl)benzo[*d*]thiazole, 3j: Isolated by column chromatography. The title compound is white solid (144 mg, 72% Yield). m.p.154-56°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, *J* = 8.12 Hz, 1H), 7.91-7.82 (m, 1H), 7.56-7.41 (m, 2H), 7.35-7.29 (m, 1H), 6.99-6.85 (m, 1H), 6.35-6.30 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 160.819, 153.885, 153.286, 133.759, 126.198, 124.449, 122.414, 121.605, 121.469, 112.756, 110.570; ESI-HRMS: Calcd for $C_{11}H_9N_2S$: *m/z* 201.04810. Found: 201.04817.

2-(Pyridin-3-yl)benzo[*d*]thiazole, 3k: Isolated by column chromatography. The title compound is purple-grey colored solid (163 mg, 77% Yield). m.p.124-26°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.32-9.27 (m, 1H), 8.75-8.69 (m, 1H), 8.42-8.35 (m, 1H), 8.10 (d, *J* = 8.30 Hz, 1H), 7.93 (d, *J* = 8.30 Hz, 1H), 7.53 (t, *J* = 8.30 Hz, 1H), 7.48-7.37 (m, 2H);

^{13}C NMR (300 MHz, $CDCl_3$): δ 164.461, 153.848, 151.516, 148.507, 134.866, 134.427, 129.580, 126.541, 125.623, 123.692, 123.399, 121.650; ESI-HRMS: Calcd for $C_{12}H_9N_2S$: *m/z* 213.04810. Found: 213.04820.

2-(Benzo[*b*]thiophen-2-yl)benzo[*d*]thiazole, 3l: Isolated by column chromatography. The title compound is yellow solid (211 mg, 79% Yield). m.p.192-94°C.^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, *J* = 8.30 Hz, 1H), 7.90-7.83 (m, 4H), 7.51 (t, *J* = 8.30 Hz, 1H), 7.43-7.38 (m, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 161.373, 153.637, 140.772, 139.474, 137.103, 134.962, 126.476, 126.068, 125.534, 125.206, 124.901, 124.499, 123.267, 122.516, 121.447; ESI-HRMS: Calcd for $C_{15}H_{10}NS_2$: *m/z* 268.02492. Found: 268.02434.

3-(Benzo[*d*]thiazol-2-yl)-10-decyl-10,10a-dihydro-4a*H*-phenothiazine, 3m: Isolated by column chromatography. The title compound is yellow solid (340 mg, 72% Yield). m.p.79-81°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.12 Hz, 1H), 7.92-7.79 (m, 3H), 7.51-7.42 (m, 1H), 7.40-7.31 (m, 1H), 7.21-7.10 (m, 2H), 6.99-6.83 (m, 3H), 3.92-3.85 (m, 2H), 1.88-1.78 (m, 2H), 1.48-1.24 (m, 14H), 0.91-0.83 (m, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 167.015, 154.126, 147.578, 144.147, 134.749, 127.715, 127.447, 127.349, 126.759, 126.185, 126.067, 125.250, 124.783, 123.916, 122.898, 122.774, 121.452, 115.542, 115.111, 47.674, 31.840, 29.491, 29.443, 29.238, 29.171, 26.824, 26.731, 22.632, 14.083; ESI-HRMS: Calcd for $C_{29}H_{33}N_2S_2$: *m/z* 473.20797. Found: 473.20713.

3-(6-Bromobenzo[*d*]thiazol-2-yl)-10-decyl-10, 10a-dihydro-4a*H*-phenoxazine, 3n: Isolated by column chromatography. The title compound is dark yellow colored solid (374 mg, 70% Yield). m.p.126-28°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, *J* = 2.26 Hz, 1H), 7.82 (d, *J* = 9.06 Hz, 1H), 7.57-7.47 (m, 2H), 7.29 (d, *J* = 2.26 Hz, 1H), 6.86-6.78 (m, 1H), 6.74-6.63 (m, 2H), 6.55-6.47 (m, 2H), 3.56-3.47 (m, 2H), 1.76-1.64 (m, 2H), 1.44-1.28 (m, 14H), 0.88 (t, *J* = 6.79 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$): δ167.588, 153.038, 144.983, 144.598, 136.252, 132.009, 129.515, 125.549, 123.872, 123.793, 123.676, 123.625, 121.688, 117.918, 115.533, 113.835, 111.647, 110.965, 44.117, 31.855, 29.579, 29.505, 29.341, 29.282, 26.845, 24.944, 22.656, 14.106; ESI-HRMS: Calcd for $C_{29}H_{32}N_2OSBr$: *m/z* 535.14132. Found: 535.14908.

2,2'-(9-(Octan-3-yl)-9*H*-carbazole-3,6-diyl)bis(benzo[*d*]thiazole), 3o: Isolated by column chromatography. The title compound is red colored solid (332 mg, 61% Yield). m.p.164-68°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.91 (s, 2H), 8.25 (d, *J* = 8.49 Hz, 2H), 8.10 (d, *J* = 8.12 Hz, 2H), 7.93 (d, *J* = 7.93 Hz, 2H), 7.53-7.46 (m, 4H), 7.42-7.35 (m, 2H), 4.21 (d, *J* = 7.36 Hz, 1H), 2.16-2.01 (m, 2H), 1.38-1.29 (m, 8H), 0.96-0.87 (m, 6H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 168.859, 154.273, 142.951, 134.856, 126.171, 125.904, 125.386, 124.668, 123.154, 122.696, 121.505, 120.253, 109.663, 47.690, 39.365, 30.899, 29.671, 28.680, 24.316, 22.949, 13.982, 10.835; ESI-HRMS: Calcd for $C_{34}H_{32}N_3S_2$: *m/z* 546.20322. Found: 546.20225.

Antimicrobial assay

The antimicrobial activity of the synthesized compounds was determined using well diffusion method against different pathogenic reference strains procured from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic bacterial and *Candida albicans* reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media Petri plates using a cork borer and the synthesized compounds at a dose range of 125 − 0.4 µg well^{-1} was added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of Ciprofloxacin and Miconazole at a dose range of 125 − 0.4 µg well^{-1} and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 30°C and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration (MIC). All experiments were carried out in duplicate and mean values are represented[65].

Minimum bactericidal concentration (MBC) assay

Bactericidal assay (NCCLS, 2000) was performed in sterile 2.0 mL microfuge tubes against two pathogenic bacterial strains, including *Staphylococcus aureus* MLS16 MTCC 2940 and *Klebsiella planticola* MTCC 530 which were cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds were prepared in Mueller Hinton broth

with different concentrations ranging from 0 to 150 µg mL^{-1}. To the test compounds, 100 µL of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5×10^8 cfu mL^{-1} (equal to 0.5 McFarland) and incubated at 37°C for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µL of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 37°C to observe the growth of test organisms. MBC is the lowest concentration of compound required to kill a particular bacterium. All the experiments were carried out in duplicate[66].

Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay[67], against two pathogenic bacterial strains such as ***Staphylococcus aureus*** MLS16 MTCC 2940 and ***Klebsiella planticola*** MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 µg/mL were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 cfu/mL. Aliquots of 100 µL were distributed in each well and then incubated at 37°C for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µL of 0.1% crystal violet solution followed by 30 min incubation at RT. Later, the crystal violet solution from the plates was discarded, thoroughly washed with distilled water 3 to 4 times and air dried at RT. The crystal violet stained biofilm was solubilized in 95% ethanol (100 µL) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose-response curves, where IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

Cytotoxicity assay

Cytotoxicity of the compounds was determined on the basis of measurement of *in vitro* growth inhibition of tumor cell lines in 96 well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using doxorubicin as a standard. The cytotoxicity was assessed against a panel of four different human tumor cell lines: COLO205 derived from human colon adenocarcinoma cells (ATCC No. CCL-222), A549 derived from human alveolar adenocarcinoma epithelial cells (ATCC No. CCL-185), DU145 derived from human prostrate cancer cells (ATCC No. HTB-81) and MCF7 derived from human breast adenocarcinoma cells (ATCC No. HTB-22) using the MTT assay[68]. The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose-response curves. IC_{50} values (in µM) were expressed as the average of two independent experiments.

Conclusion

An economical and practical method for the synthesis of benzothiazole derivatives has been reported. We believe that the simplicity of both the reaction and the product isolation will make our procedure an attractive one. The carbazole-bridged benzothiazole has been identified as a basic scaffold which has potential for further development of promising antibacterial agents.

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