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Research Article

Synthesis and Antimicrobial Evaluation of a New Series of *N*-1,3-Benzothiazol-2-ylbenzamides

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Enterococcus faecalis is a Gram-positive commensal inhabitant of the intestinal tract of humans, animals, and insects. However, it is also an opportunistic pathogen and has emerged as a leading cause of hospital-acquired extraintestinal infections. Fluoroquinolones have been frequently used to treat *E. faecalis* infections, and the emergence of fluoroquinolone-resistant *E. faecalis* strains has recently been reported in several countries. Thus, the identifications of new antibiotics specifically directed to *E. faecalis* may be envisaged. In this paper, a new series of *N*-1,3-benzothiazol-2-ylbenzamides have been designed, synthesized, and evaluated for their *in vitro* antimicrobial activities. Among the tested compounds, **3i** was active against *E. faecalis*.

1. Introduction

Drug resistance to therapeutic antibiotics poses a challenge to the identification of novel targets and drugs for the treatment of infectious diseases. Infections caused by *Enterococcus faecalis* are a major health problem. Thus, studies for the identification of novel targets and drugs for the treatment of infectious diseases are at the forefront. *E. faecalis* is a Gram-positive opportunistic pathogen which has emerged as a leading cause of hospital-acquired extraintestinal infections, including urinary tract infections (UTIs), bacteremia, wound infections, and endocarditis [1–3]. Normally, a resident of the gastrointestinal tract, extensive use of antibiotics has resulted in the rise of *E. faecalis* strains that are resistant to multiple antibiotics. We recently determined the X-ray crystallographic structure of *E. faecalis* thymidylate synthase, which should be a potential target for antibacterial therapy [4]. Fluoroquinolones have been frequently used to treat *E. faecalis* UTIs, and the emergence of fluoroquinolone-resistant *E. faecalis* (QREF) strains has recently been reported in several countries [5]. Thus, the development of new and different antimicrobial drugs, in particular acting against *E. faecalis*, is a very important goal, and most of the research program efforts in this field are directed towards the design of new agents. During the past decade, combinatorial chemistry has provided access to chemical libraries based on privileged structures [6], with heterocyclic structures receiving special attention as they belong to a class of compounds with proven utility in medicinal chemistry [7–10]. Many heterocyclic nuclei, such as 1,3,4-thiadiazole [11], benzimidazole [12], 1,3,5-triazine [13], and benzothiazole [14], have been recently reviewed as antimicrobial agents. Our attention was focused on the benzothiazole nucleus. Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor [15–18], antimicrobial [19–21], schistosomicidal [22], anti-inflammatory [23, 24], anticonvulsants [25–27], antidiabetic [28, 29], antipsychotic [30, 31], neuroprotective [32], and diuretic [33] activities. In the past, we were interested in a series of 2-mercapto-1,3-benzothiazole derivatives showing antimicrobial activity [19]. It is disclosed that the SH moiety at the C2 position of the heterocyclic nucleus led to a remarkable antibacterial activity against Gram positive and negative. Actually, in order to improve SAR studies on 1,3-benzothiazoles, we decided to investigate the isosteric relationship between 2-mercapto and 2-amino substitutions. Moreover, in this study we also aimed at widening SAR

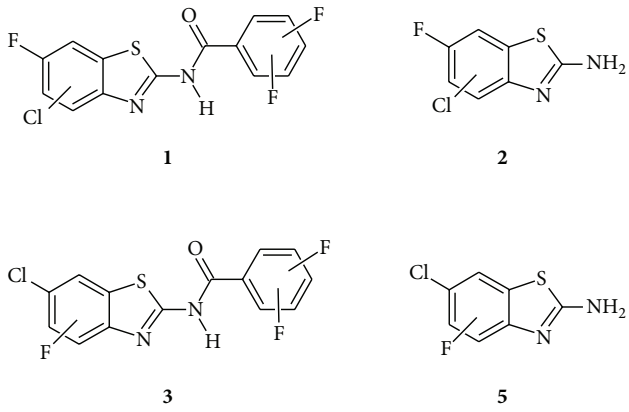
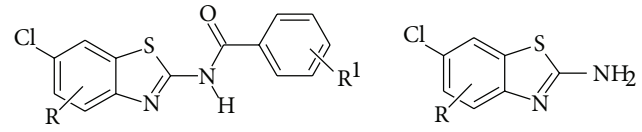


Figure 1

indications about the effects of halogen substitutions on several amides of 1,3-benzothiazole previously reported (*N*-1,3-benzothiazol-2-ylbenzamides, **1**, Figure 1). We found that these compounds exerted antifungal activity against *C. albicans* comparable to that of the reference compound sorbic acid, that is, with MIC values in the order of 250–500 μg/mL [34]. Nowadays, it is known that sorbic acid is no longer the best reference compound to be used to study antifungal activity; it is preferable to use antibiotics, such as amphotericin or fluconazole, which are much more active showing MIC values in the order of 0.5–1 μg/mL; thus, the compounds previously reported can be considered inactive against fungi. In this paper, we report a series of trifluoro-substituted-*N*-(6-chloro-1,3-benzothiazol-2-yl) benzamides (**3**, Table 1), that are position isomers of those previously reported [34]. Moreover, 2-amino-1,3-benzothiazoles (**5**, Table 1) were also synthesized and tested, and the results were compared to those obtained with their isomers previously reported (**2**, Figure 1) [35].

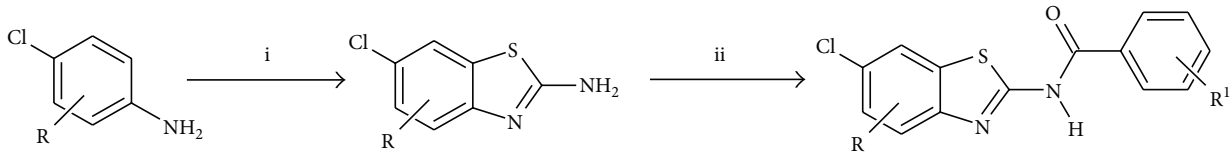
Table 1: Structures of compounds **3a-l** and **5a, b**.



Compound	R	R^1
3a	4-F	2,3-F_2
3b	4-F	2,4-F_2
3c	4-F	2,5-F_2
3d	4-F	2,6-F_2
3e	4-F	3,4-F_2
3f	4-F	3,5-F_2
3g	5-F	2,3-F_2
3h	5-F	2,4-F_2
3i	5-F	2,5-F_2
3j	5-F	2,6-F_2
3k	5-F	3,4-F_2
3l	5-F	3,5-F_2
5a	4-F	—
5b	5-F	—

2. Results and Discussion

Compounds **5a**, **b** and **3a-l** were prepared as reported in Scheme 1. The appropriate fluoro-4-chloro aniline (**4a**, **b**) was reacted with bromine and potassium thiocyanate to give the corresponding 2-amino benzothiazoles **5a**, **b**, which were reacted with the suitable difluorobenzoyl chloride to give all possible isomeric compounds **3a-l**. The antimicrobial activity of compounds **3a-l** was evaluated *in vitro* against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus 29213,* and *Enterococcus faecalis 29212, Escherichia coli 25922*) and fungi strains (*Candida albicans 10231, Candida parapsilosis 22019,* and *Candida tropicalis* 750) belonging to the ATCC collection [36, 37]. All MIC determinations were carried out according to the clinical laboratory standards institute (CLSI) guidelines. MIC values are given in μg/mL and were compared to MIC values for the standard antibacterial drugs oxacillin and norfloxacin and antifungal fluconazole. Screening results are summarized in Table 2. The combined data showed that seven compounds, out of twelve, exerted interesting inhibitory activity against *E. faecalis* with MIC values between 8 and 32 μg/mL. In particular, compound **3i** was the most active derivative giving the best antibacterial activity against *E. faecalis* with an MIC value of 8 μg/mL, while compounds **3a**, **b**, **f–h**, and **k** displayed moderate activity towards the same bacteria strain (MIC: 32 μg/mL). In order to better define structure activity relationships, it is possible to consider two subseries of compounds: the former, 6-chloro-4-fluorobenzothiazole benzamides (**3a–f**); the latter, 6-chloro-5-fluorobenzothiazole benzamides (**3g-l**). Among all the tested compounds, with the exception of the highly active compound **3i**, the best substitutions of the acyl moiety seem to be 2,3-difluoro and 2,4-difluoro in both series (**3a**, **b** and **3g**, **h**) and the worst substitution seems to be 2,6-difluoro in both series (**3d** and **3j**). Moreover, the 3,4-difluoro and 3,5-difluoro substitutions had different effects in the two series: in particular, the former decreases activity in the first series (**3e**), while it increased activity in the second (**3k**), and the opposite was true for the latter substitution (**3f** was active, and **3l** was inactive). Then, 2,5-difluorosubstitution determined a very high increase in activity in the second series giving the best compound tested (**3i**) while decreasing the activity in the first series (**3c**; MIC: 128 μg/mL). Finally, 2,6-difluoro substitution was detrimental in both series: compounds **3d** and **3j** showed no activity or low activity against *E. faecalis* (MIC > 512 μg/mL and MIC: 128 μg/mL, resp.). All the compounds did show very low or no activity against *S. aureus* and *E. coli* and all the strains of *Candida*. These results are in agreement with what had been found for a series of isomers of our compounds previously reported [34]. Moreover, intermediates **5a**, **b** were tested in order to compare results obtained with their isomers recently reported, in which the halogen atoms were inverted in their position [35]. These



4a, b **5a, b** **3a-l**

Scheme 1: Reagents and conditions: (i) Br_2, KSCN, AcOH, 30–35°C; (ii) suitable difluorobenzoyl chloride, Et_3N, dioxane, 50–60°C.

Table 2: Antimicrobial activity results of benzothiazole derivatives **3a-l** and **5a, b** (MIC, μg/mL).

	Microorganism (MIC, μg/mL)					
	Gram positive[a]		Gram negative[a]	Fungi[b]		
	S.a. 29213	*E.f.* 29212	*E.c.* 25922	*C.a.* 10231	*C.p.* 22019	*C.t.* 750
3a	64	32	R	R	R	R
3b	R	32	R	R	R	R
3c	256	256	R	R	R	R
3d	R	R	256	R	R	R
3e	R	R	R	R	R	R
3f	R	32	R	R	R	R
3g	R	32	R	R	R	R
3h	R	32	R	R	R	R
3i	128	8	R	R	R	R
3j	256	128	R	R	R	R
3k	R	32	256	R	R	R
3l	R	R	R	R	R	R
5a	256	256	R	128	64	128
5b	128	128	256	64	128	128
OXA	0.25	16	R	—	—	—
NRF	0.5	4	0.03	—	—	—
FCN	—	—	—	2	2	4

[a]Antibacterial activity was estimated by using CLSI assay [36]. Abbreviations: *S.a.*: *S. aureus*; *E.f.*: *E. faecalis*; *E.c.*: *E. coli*; OXA: oxacillin; NRF: norfloxacin; R: resistant (>512 μg/mL).

[b]Antifungal activity was estimated by using CLSI assay [37]. Abbreviations: *C.a.*: *C. albicans*; *C.p.*: *C. parapsilosis*; *C.t.*: *C. tropicalis*; *C.k.*: *C. krusei*; FCN: fluconazole; R: resistant (>512 μg/mL).

two compounds did show no antimicrobial activity, while results found as antifungals are similar to their corresponding isomers. In particular, compound **5a** was identical to its isomer against Gram-positive and -negative bacteria and against *C. parapsilosis*, while it was slightly more active than their isomers previously reported [35] on *C. albicans* and *C. tropicalis* (MIC: 128 μg/mL versus 256 μg/mL). Compound **5b** was identical to its isomer against Gram-negative and *C. tropicalis*, slightly more active against *S. aureus* (MIC: 128 μg/mL versus 512 μg/mL) and *E. faecalis* (MIC: 128 μg/mL versus 256 μg/mL) and C. *albicans* (MIC: 64 μg/mL versus 128 μg/mL), and slightly less active against *C. parapsilosis* (MIC: 128 μg/mL versus 64 μg/mL).

3. Conclusion

In conclusion, we report the synthesis and antimicrobial activity of a series of 1,3-benzothiazole derivatives (**3a-l** and **5a**, **b**). All the compounds did not show activity against *S. aureus*, *E. coli* and *C. albicans, C. parapsilosis,* and *C. tropicalis* in these tracing results previously obtained for the corresponding position isomers previously reported (**1**, **2**, Figure 1) [34, 35]. Interestingly, compounds **3a**, **b**, **f**, **g**, **h**, **i**, and **k** exerted moderate to high activity against *E. faecalis* (MIC between 8 and 32 μg/mL). In particular, compound **3i** was the most potent of the series (MIC: 8 μg/mL) and the most promising compound, while the other showed an MIC value of 32 μg/mL.

4. Experimental

4.1. General Experimental Details. Chemicals were purchased from Sigma-Aldrich or Lancaster. Yields refer to purified products and were not optimized. The structures of the compounds were confirmed by routine spectrometric and spectroscopic analyses. Only spectra for compounds not previously described are given. Purity of compounds was assessed by GC analysis. Melting points were determined on a Gallenkamp apparatus in open glass capillary tubes and are uncorrected. Infrared spectra were recorded

on a Perkin-Elmer (Norwalk, CT) Spectrum One FT spectrophotometer, and band positions are given in reciprocal centimeters cm^{-1}. ^1H NMR spectra were recorded on a Varian VX Mercury spectrometer operating at 300 MHz using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts are reported in parts per million (ppm) relative to the residual nondeuterated solvent resonance: $CDCl_3$, δ 7.26 and DMSO-d_6, δ 2.48. J values are given in Hz. GC-MS was performed on a Hewlett-Packard 6890-5973 MSD at low resolution. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 60, 0.040–0.063 mm, Merck, Darmstadt, Germany) as previously reported [38–41]. TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F_{254}, Merck). GC analyses were performed on a Varian 3800 gas chromatograph equipped with a flame ionization detector and a Jew Scientific DB-5 capillary column (30 m length × 0.25 mm ID, 0.25 μm film thickness) [42].

*4.1.1. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-2,3 difluorobenzamide (**3a**).* A mixture of **5a** (0.61 g, 3.0 mmol) and triethylamine (0.30 g, 3.0 mmol) in dry dioxane (30 mL) was stirred for 30 min at 50–60°C. A solution of 2,3-difluorobenzoyl chloride (0.53 g, 3.0 mmol) in dry dioxane (30 mL) was added dropwise. The mixture was stirred for 2 h and then poured into crushed ice. The resulting solid, so separated, was collected by filtration and washed with 1% potassium bicarbonate aqueous solution. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether 3 : 7) to give 0.32 g (31%) of **3a** as a white solid: mp 240–242°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 38), 141 (100); ^1H NMR: δ 7.18–7.25 (m, 1H, Ar *H*C-5), 7.26–7.35 (m, 1H, Ar *H*C-3′), 7.42–7.55 (m, 1H, Ar *H*C-4′), 7.63 (s, 1H, Ar *H*C-5′), 7.96 (t, J = 6.3 Hz, 1H, Ar *H*C-7), 10.08 (br s, 1H, N*H*, exch D_2O); IR (KBr): 3405 (NH), 1686 (C=O) cm^{-1}.

*4.1.2. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-2,4-difluorobenzamide (**3b**).* Prepared as reported previously for **3a** starting from **5a** and 2,4-difluorobenzoyl chloride. Yield: 33%; white solid: mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 18), 141 (100); ^1H NMR (DMSO-d_6): δ 7.12–7.55 (m, 3H, Ar *H*C-5 + Ar *H*C-3′,5′), 7.85–8.05 ppm (m, 2H, Ar *H*C-7 +Ar *H*C-6′); IR (KBr): 3418 (NH), 1673 (C=O) cm^{-1}.

*4.1.3. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-2,5-difluorobenzamide (**3c**).* Prepared as reported previously for **3a** starting from **5a** and 2,5-difluorobenzoyl chloride. Yield: 24%; white solid: mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 17), 141 (100); ^1H NMR: δ 7.00–7.25 (m, 2H, Ar *H*C-5 + *H*C-4′), 7.50–7.65 (m, 1H, Ar *H*C-6′), 7.70–7.85 (m, 1H, Ar *H*C-3′), 8.02 (d, J = 5.2 Hz, 1H, Ar *H*C-7), 10.22 ppm (br s, 1H, N*H*); IR (KBr): 3410 (NH), 1677 (C=O) cm^{-1}.

*4.1.4. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-2,6-difluorobenzamide (**3d**).* Prepared as reported previously for **3a** starting from **5a** and 2,6-difluorobenzoyl chloride. Yield: 12%; white solid: mp 245–247°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 18), 141 (100); ^1H NMR: δ 7.01 (t, J = 8.5 Hz, 2H, Ar *H*C-3′,5′), 7.14 (dd, J = 10.1, 1.8 Hz, 1H, Ar *H*C-5), 7.44–7.58 (m, 1H, Ar, *H*C-4′), 7.63 (s, 1H Ar *H*C-7), 10.37 ppm (br s, 1H, N*H*); IR (KBr): 3409 (NH), 1694 (C=O) cm^{-1}.

*4.1.5. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-3,4-difluorobenzamide (**3e**).* Prepared as reported previously for **3a** starting from **5a** and 3,4-difluorobenzoyl chloride. Yield: 14%; slightly yellowish solid: mp 226–228°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 26), 141 (100); ^1H NMR: δ 7.19 (dd, J = 9.9, 1.6 Hz, 1H, Ar *H*C-5), 7.25–7.40 (m, 1H, Ar *H*C-5′), 7.62 (s, 1H Ar *H*C-7), 7.80–7.88 (m, 1H, Ar *H*C-2′), 7.90–8.02 ppm (m, 1H, Ar *H*C-6′); IR (KBr): 3410 (NH), 1679 (C=O) cm^{-1}.

*4.1.6. N-(6-Chloro-4-fluoro-1,3-benzothiazol-2-yl)-3,5-difluorobenzamide (**3f**).* Prepared as reported previously for **3a** starting from **5a** and 3,5-difluorobenzoyl chloride. Yield: 34%; white solid: mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 36), 141 (100); ^1H NMR: δ 7.10 (tt, J = 8.2, 2.2 Hz, 1H, Ar *H*C-4′), 7.21 (dd, J = 9.9, 1.9 Hz, 1H, Ar *H*C-5), 7.47–7.57 (m, 2H, Ar *H*C-2′,6′), 7.62–7.66 ppm (m, 1H, Ar *H*C-7); IR (KBr): 3414 (NH), 1681 (C=O) cm^{-1}.

*4.1.7. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-2,3-difluorobenzamide (**3g**).* Prepared as reported previously for **3a** starting from **5b** and 2,3-difluorobenzoyl chloride. Yield: 64%; white solid: mp 241–243°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 25), 141 (100); ^1H NMR: δ 7.25–7.38 (m, 1H, Ar *H*C-4′), 7.42–7.52 (m, 1H, Ar *H*C-5′), 7.59 (d, J = 9.3 Hz, 1H, Ar *H*C-4), 7.86 (d, J = 7.1 Hz, 1H, Ar *H*C-7), 7.93–8.02 ppm (m, 1H, Ar *H*C-6′); IR (KBr): 3414 (NH), 1673 (C=O) cm^{-1}.

*4.1.8. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-2,4-difluorobenzamide (**3h**).* Prepared as reported previously for **3a** starting from **5b** and 2,4-difluorobenzoyl chloride. Yield: 64%; beige solid, mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 17), 141 (100); ^1H NMR (DMSO-d_6): δ 7.16–7.32 (m, 1H, Ar *H*C-3′), 7.40–7.56 (m, 1H, Ar *H*C-5′), 7.80–7.96 (m overlapping d at 7.84 ppm, 1H, Ar *H*C-6′), 7.84 (d overlapping m at 7.80–7.96 ppm, J = 10.2 Hz, 1H, Ar *H*C-4), 8.32 (d, J = 7.7 Hz, 1H, Ar *H*C-7), 13.04 ppm (br s, 1H, N*H*); IR (KBr): 3420 (NH), 1671 (C=O) cm^{-1}.

*4.1.9. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-2,5-difluorobenzamide (**3i**).* Prepared as reported previously for **3a** starting from **5b** and 2,5-difluorobenzoyl chloride. Yield: 64%; white solid, mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 15), 141 (100); ^1H NMR: δ 7.15–7.40 (m, 2H, Ar *H*C-3′,5′), 7.59 (d, J = 9.3 Hz, 1H, Ar *H*C-4), 7.87 (d, J = 6.9 Hz, 1H, Ar *H*C-7), 7.90–7.98 (m, 1H, Ar *H*C-6′), 10.05 ppm (br s, 1H, N*H*); IR (KBr): 3496 (NH), 1682 (C=O) cm^{-1}.

*4.1.10. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-2,6-difluorobenzamide (**3j**).* Prepared as reported previously for **3a** starting from **5b** and 2,6-difluorobenzoyl chloride. Yield: 70%; white solid, mp 247–249°C; GC-MS (70 eV, electron

impact) *m/z* (%) 342 (M^+, 18), 141 (100); ^{1}H NMR: δ 7.03 (t, J = 9.0 Hz, 1H, Ar *H*C-3′,5′), 7.34 (d, J = 9.3 Hz, 1H, Ar *H*C-4), 7.45–7.58 (m, 1H, Ar *H*C-4′), 7.85 ppm (d, J = 6.9 Hz, 1H, Ar *H*C-7); IR (KBr): 3430 (NH), 1687 (C=O) cm^{-1}.

*4.1.11. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-3,4-difluorobenzamide (**3k**).* Prepared as reported previously for **3a** starting from **5b** and 3,4-difluorobenzoyl chloride. Yield: 62%; white solid, mp > 250°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 33), 141 (100); ^{1}H NMR: δ 7.25–7.40 (m, 1H, Ar *H*C-5), 7.48 (d, J = 9.3 Hz, 1H, Ar *H*C-4), 7.76–7.85 (m, 1H, Ar *H*C-2′), 7.86 (d, J = 6.9 Hz, 1H, Ar *H*C-7), 7.87–7.98 ppm (m, 1H, Ar *H*C-6′); IR (KBr): 3408 (NH), 1682 (C=O) cm^{-1}.

*4.1.12. N-(6-Chloro-5-fluoro-1,3-benzothiazol-2-yl)-3,5-difluorobenzamide (**3l**).* Prepared as reported previously for **3a** starting from **5b** and 3,5-difluorobenzoyl chloride. Yield: 55%; white solid, mp 235–237°C; GC-MS (70 eV, electron impact) *m/z* (%) 342 (M^+, 39), 141 (100); ^{1}H NMR (DMSO-d_6): δ 7.55–7.65 (m, 1H, Ar *H*C-4′), 7.70–7.90 (m, 3H, *H*C-4,-2′,6′), 8.33 (d, J = 7.4 Hz, 1H, Ar *H*C-7), 13.2 ppm (br s, 1H, N*H*); IR (KBr): 3401 (NH), 1679 (C=O) cm^{-1}.

*4.1.13. 6-Chloro-4-fluoro-1,3-benzothiazol-2-amine (**5a**).* A mixture of **4a** (15 g, 103 mmol) and potassium thiocyanate (20 g, 206 mmol) in glacial acetic acid (250 mL) was stirred for 5 min. Bromine (24 g, 150 mmol) in glacial acetic acid (250 mL) was added dropwise to this mixture, with the temperature being kept below 30–35°C throughout the addition. Stirring was continued for an additional 1 h after addition of bromine. After cooling, the residue was removed by filtration. The filtered solution was made alkaline with 28% ammonium hydroxide, and the solid precipitate was collected and washed with water. The combined water layers were made alkaline with 28% ammonium hydroxide, and the resulting precipitate was combined with that previously collected. The combined precipitates were extracted with EtOAc. The organic layers were separated, dried (Na_2SO_4), and evaporated under vacuum. The crude residue was purified by column chromatography on silica gel (hexane/EtOAc 3 : 7) to give 14.6 g (70%) of **5a** as a slight green solid: mp 243–245°C; GC-MS (70 eV, electron impact) *m/z* (%) 202 (M^+, 100); ^{1}H NMR: δ 7.08 (dd, J = 10.2, 1.9 Hz, 1H, Ar *H*C-7), 7.30–7.40 (m, 1H, *H*C-5), 5.63 ppm (br s, 1H, NH_2); IR (KBr): 3461, 3074 (NH_2) cm^{-1}.

*4.1.14. 6-Chloro-5-fluoro-1,3-benzothiazol-2-amine (**5b**).* Prepared as reported previously for **5a** starting from **4b**. Yield: 65%; white solid: mp 234–236°C; GC-MS (70 eV, electron impact) *m/z* (%) 202 (M^+, 100); ^{1}H NMR: δ 7.30 (d, J = 9.9 Hz, 1H, Ar *H*C-4), 7.56 (d, J = 6.9 Hz, 1H, Ar *H*C-7), 5.45 ppm (br s, 1H, NH_2); IR (KBr): 3473, 3083 (NH_2) cm^{-1}.

4.2. Biology

4.2.1. Antibacterial Studies. The *in vitro* minimum inhibitory concentrations (MICs, μg/mL) were assessed by the broth microdilution method, using 96-well plates, according to CLSI guidelines [36]. Stock solutions of the tested compounds were obtained in DMSO. Stock solutions of lower concentrations were prepared for those substances which did not dissolve well. Then twofold serial dilutions in the suitable test medium between 512 and 0.5 μg/mL were plated. To be sure that the solvent had no adverse effect on bacterial growth, a control test was carried out by using DMSO at its maximum concentration along with the medium. Bacteria strains available as freeze-dried discs, belonging to the ATCC collection, were used: Gram-positive strains such as *Staphylococcus aureus* 29213 and *Enterococcus faecalis* 29212 and Gram-negative one such as *Escherichia coli* 25922. To preserve the purity of cultures and to allow the reproducibility, a series of cryovials of all microbial strains in 10% glycerol medium was set up and stored at −80°C. Precultures of each bacterial strain were prepared in cation-adjusted mueller-hinton broth (CAMHB) and incubated at 37°C until the growth ceased. The turbidity of bacterial cell suspension was calibrated to 0.5 McFarland Standard by spectrophotometric method (625 nm, range 0.08–0.10), and further the standardized suspension was diluted 1 : 100 with CAMHB to have 1-2 × 10^6 CFU/mL. All wells were seeded with 100 μL of inoculum. A number of wells containing only inoculated broth as control growth were prepared. The plates were incubated at 37°C for 24 h, and the MIC values were recorded as the last well containing no bacterial growth. The MICs were determined by using an antibacterial assayed repeated twice in triplicate. Oxacillin and norfloxacin were used as reference drugs.

4.2.2. Antifungal Studies. Antifungal studies [37] were carried out against *Candida albicans* 10231, *Candida parapsilosis* 22019, *Candida tropicalis* 750, and *Candida krusei* 6258, belonging to the ATCC collection. Preparation of stock solutions and purity of cultures preservation were obtained as previously described for antibacterial studies. Pre-cultures of each yeast strain were prepared in Sabouraud broth (SAB) 2% glucose, and incubated at 37°C until the growth ceased. The turbidity of yeast stock suspension was calibrated to 0.5 McFarland Standard by spectrophotometric method (530 nm, range 0.12–0.15), and further the standardized suspension was diluted first 1 : 50 with SAB and then 1 : 20 in the same medium to have 1–5 × 10^6 CFU/mL. All wells were seeded with 100 μL of inoculum. A number of wells containing only inoculated broth as control growth were prepared. The plates were incubated at 37°C for 24–48 h, and the MIC values were recorded as the last well containing no fungal growth. The MICs were determined by using an antifungal assay repeated twice in triplicates. Fluconazole was used as a reference drug.

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