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New 6-(4-Bromophenyl)-imidazo[2,1-*b*]thiazole Derivatives: Synthesis and Antimicrobial Activity

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Summary. New 4-alkyl/aryl-1-((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-3-thiosemicarbazides and 3-alkyl/aryl-2-((((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinones were synthesized from 6-(4-bromophenyl)-imidazo[2,1-*b*]thiazole-3-acetic acid hydrazide. Their structures were elucidated by elemental analyses and spectroscopic data. All compounds were tested for antibacterial and antifungal activities. The antimicrobial activities of the compounds were assessed by the microbroth dilution technique. The compounds were also evaluated for antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294); they exhibited varying degrees of inhibition in the *in vitro* primary screening at $6.25\,\mu g \cdot cm^{-3}$. The most active compound was 3-(4-nitrophenyl)-2-((((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone.

Keywords. Imidazo[2,1-*b*]thiazole; 4-Thiazolidinones; Antibacterial activity; Antifungal activity; Antimycobacterial activity.

Introduction

The rapidly expanding population of immunocompromised patients results in a corresponding increase of diseases caused by yeasts and other fungi. Although not life-threatening, superficial mycosis and infections of keratinized tissues such as nails, skin, and hair cause prolonged periods of distress. Dermatophytoses which are most prevalent among superficial mycosis are currently treated by the imidazole derivatives clotrimazole, miconazole, ketoconazole, econazole, and other azole antifungals which interfere with fungal ergosterol synthesis by inhibiting lanosterol 14-demethylase [1]. Derivatives of imidazo-fused heterocycles also show antifungal activity [2–4], besides antibacterial [5, 6] and antimycobacterial [7] potential. On the other hand, over the years, 4-thiazolidinones have been synthesized for a wide range of pharmaceutical and biological purposes.

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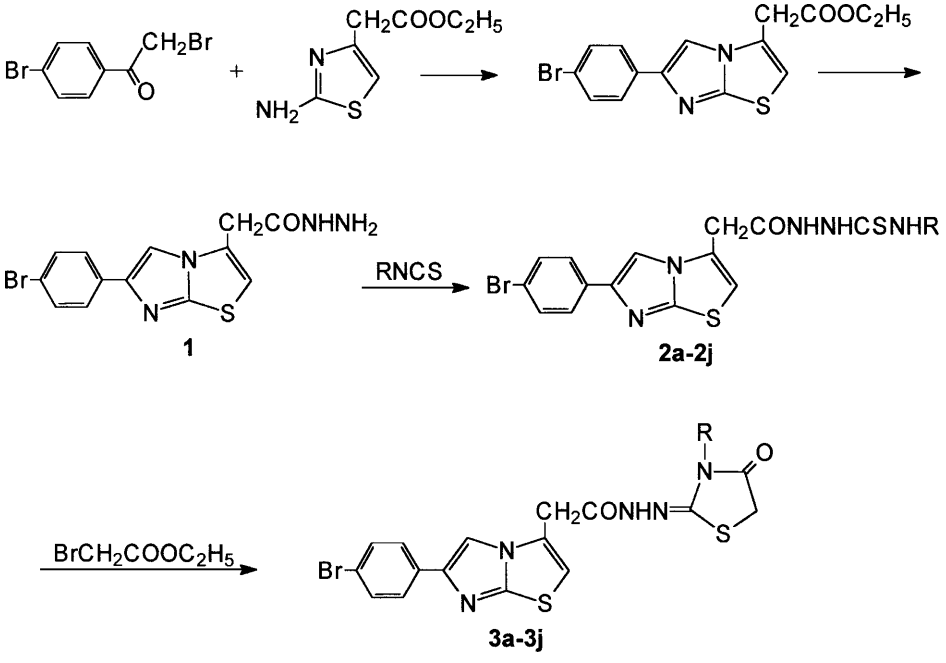
Many 4-thiazolidinone derivatives have been shown to exhibit bactericidal [8–10], fungicidal [11–13], and tuberculocidal [14–16] properties. We have previously reported on the antimicrobial activity of imidazo[2,1-*b*]thiazole derivatives [17–19]. Based on our recent findings on the antimicrobial activity of this system we prepared the new 4-substituted 1-(((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-3-thiosemicarbazides **2a–j** and 3-substituted 2-((((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinones **3a–j** with the aim to investigate their antibacterial, antifungal, and antimycobacterial activities.

Results and Discussion

The target compounds were prepared from 6-(4-bromophenyl)-imidazo[2,1-*b*]thiazole-3-acetic acid hydrazide (**1**) [20] by a two step synthesis as shown in Scheme 1. 4-Alkyl/aryl-1-(((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-3-thiosemicarbazides **2a–j** were obtained from **1** and the corresponding alkyl/arylisothiocyanates. Upon treatment with ethyl bromoacetate, **2a–j** yielded 3-alkyl/aryl-2-((((6-(4-bromophenyl)-imidazo[2,1-*b*]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinones **3a–j**. The structures of both **2a–j** and **3a–j** were assigned by elemental analysis (CHN) and spectroscopic methods (IR, ^{1}H NMR, EIMS).

The IR spectra of **2a–j** displayed bands at about 3224–3096 and 1705–1672 cm^{-1} associated with the N–H and C=O functions. The three ^{1}H NMR resonances located in the region of 10.34–8.02 ppm were assigned to the NH protons of the thiosemicarbazides and supported the structures of **2a** and **2f** [7]. New C=O bands (1754–1709 cm^{-1}) displayed by the IR spectra of 4-thiazolidinones **3a–j** provided confirmatory evidence for ring closure [4, 21].



Scheme 1

The exocyclic and ring methylene protons of **3a** and **3f** displayed two singlets at 4.04/4.17 and 3.87/4.06 ppm with inequal integrals (**3a**: 1.73:1, **3f**: 1.20:1), indicating both the presence of two isomers in inequal proportions in *DMSO*-d_6 and the coincidence of the related split signals. This may be explained on the basis of the difference in the relative stability of the (*E*) and (*Z*) isomers formed due to the rotational restriction about the exocyclic N=C bond at position 2 of the 4-thiazolidinone ring [17].

The EIMS of compounds **2a**, **2f**, **3a**, and **3f** displayed molecular ions which confirmed their molecular weights. The major fragmentation pattern involved the cleavage of the CH_2–CO, CO–NH, and NH–N bonds of the side chain [17, 18, 22, 23], yielding common fragments at $m/z = 292$ (294), 319 (321), and 335 (337). Further spectroscopic details are presented in the experimental part.

Compounds **2a**–**j** and **3a**–**j** were evaluated for *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212 as well as for antifungal activity against *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Candida parapsilosis*, *Trichophyton mentagrophytes var. erinacei* NCPF 375, *Microsporum gypseum* NCPF 580, and *Trichophyton rubrum* using the microbroth dilution method [24–28]. As can be seen in Table 1, **3h** ($R = 4\text{-}BrC_6H_4$) and **3i** ($R = 4\text{-}ClC_6H_4$) showed

Table 1. Antibacterial activity of compounds **2a**–**j** and **3a**–**j**; A: *S. aureus* ATCC 29213, B: *P. aeruginosa* ATCC 27853, C: *E. coli* ATCC 25922, D: *E. faecalis* ATCC 29212; n.t.: not tested

	A	B	C	D
	$MIC/\mu g \cdot cm^{-3}$			
2a	>32	>32	>32	>32
2b	32	>32	>32	16
2c	32	>32	>32	16
2d	32	>32	>32	16
2e	32	>32	>32	16
2f	>32	>32	>32	32
2g	>32	>32	>32	32
2h	16	>32	>32	32
2i	>32	>32	>32	32
2j	>32	>32	>32	16
3a	>32	>32	>32	>32
3b	>32	>32	>32	>32
3c	>32	>32	>32	32
3d	>32	>32	>32	16
3e	>32	>32	>32	32
3f	>32	>32	>32	>32
3g	>32	>32	>32	>32
3h	>32	>32	>32	8
3i	>32	>32	>32	8
3j	>32	>32	>32	>32
Cefotaxime	2	8	0.06	n.t.
Tetracycline	n.t.	n.t.	n.t.	16

Table 2. Antifungal activity of compounds **2a–j** and **3a–j**; A: *C. albicans* ATCC 10231, B: *C. parapsilosis* ATCC 22019, C: *C. krusei* ATCC 6258, D: *C. parapsilosis*, E: *T. mentagrophytes var. erinacei* NCPF 375, F: *M. gypseum* NCPF 580, G: *T. rubrum*; itraconazole: quality control strain *C. parapsilosis* ATCC 22019, $MIC = 0.25\,\mu g \cdot cm^{-3}$; n.t.: not tested

	A	B	C	D	E	F	G
	$MIC/\mu g \cdot cm^{-3}$						
2a	32	32	>32	32	4	>32	>32
2b	>32	>32	>32	>32	8	>32	>32
2c	>32	>32	>32	>32	16	>32	>32
2d	>32	>32	>32	>32	16	>32	>32
2e	>32	>32	>32	>32	16	>32	>32
2f	32	>32	>32	>32	32	>32	>32
2g	>32	>32	>32	>32	16	>32	>32
2h	>32	>32	>32	>32	16	>32	>32
2i	>32	>32	>32	>32	16	>32	>32
2j	32	>32	>32	>32	32	>32	>32
3a	32	32	32	>32	32	>32	>32
3b	>32	>32	>32	>32	>32	>32	>32
3c	>32	>32	>32	>32	8	>32	>32
3d	>32	>32	>32	>32	16	>32	>32
3e	>32	>32	32	>32	32	>32	>32
3f	>32	>32	>32	>32	16	>32	>32
3g	>32	>32	>32	>32	8	>32	>32
3h	>32	>32	>32	>32	>32	>32	>32
3i	>32	>32	>32	>32	32	>32	>32
3j	32	32	>32	>32	>32	>32	>32
Ketoconazole	n.t.	0.25	0.5	n.t.	n.t.	n.t.	n.t.
Itraconazole	n.t.	0.25	0.5	n.t.	0.5	0.5	1

the highest activity against *E. faecalis* ATCC 29212 ($MIC = 8\,\mu g \cdot cm^{-3}$). Derivative **2a** ($R = CH_3$) was most active against *Trichophyton mentagrophytes var. erinacei* NCPF 375 ($MIC = 4\,\mu g \cdot cm^{-3}$, Table 2). Compounds **2a–j**, **3a–f** and **3h–j** were also evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay (Microplate Alamar Blue Assay (MABA) [29]). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [29]. As can be seen from Table 3, thiosemicarbazide derivatives **2a–j** were generally more active than the thiazolidinone derivatives **3a–f** and **3h–j**. However, the most active compound was compound **3j** with a 4-nitrophenyl group at position 3 of the thiazolidinone ring.

Experimental

Melting points were determined with a Büchi 530 melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded as KBr discs using a Perkin-Elmer 1600 FTIR spectrophotometer. ^1H NMR spectra (*DMSO*-d_6/*TMS*) were run on a Bruker AC 200 spectrometer at 200 MHz.

Table 3. Antimycobacterial activity of **2a–j**, **3a–f**, and **3h–j**; n.a.: not active

	R	Assay	$MIC/\mu g \cdot cm^{-3}$	Inhibition/%
2a	CH_3	Alamar	>6.25	29
2b	C_2H_5	Alamar	>6.25	16
2c	C_3H_7	Alamar	>6.25	n.a.
2d	C_4H_9	Alamar	>6.25	n.a.
2e	$CH_2=CH–CH_2$	Alamar	>6.25	2
2f	C_6H_5	Alamar	>6.25	n.a.
2g	4-$C_6H_4CH_3$	Alamar	>6.25	1
2h	4-C_6H_4Br	Alamar	>6.25	29
2i	4-C_6H_4Cl	Alamar	>6.25	14
2j	4-$C_6H_4NO_2$	Alamar	>6.25	n.a.
3a	CH_3	Alamar	>6.25	7
3b	C_2H_5	Alamar	>6.25	n.a.
3c	C_3H_7	Alamar	>6.25	18
3d	C_4H_9	Alamar	>6.25	n.a.
3e	$CH_2=CH–CH_2$	Alamar	>6.25	n.a.
3f	C_6H_5	Alamar	>6.25	2
3h	4-C_6H_4Br	Alamar	>6.25	n.a.
3i	4-C_6H_4Cl	Alamar	>6.25	n.a.
3j	4-$C_6H_4NO_2$	Alamar	>6.25	37

EI mass spectra were determined on a VG Zab Spec (70 eV) instrument. Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer; the results were in good agreement with the calculated values. The starting materials were either commercially available or synthesized according to the references cited.

4-Alkyl/aryl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazides **2a–j***; general procedure*

To a solution of 0.005 mol **1** in 30 cm^3 EtOH, 0.005 mol of the appropriate isothiocyanate were added. The resulting mixture was heated under reflux for 3 h. After cooling, the precipitate was separated and purified by washing with hot EtOH.

4-Methyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2a**; $C_{15}H_{14}BrN_5OS_2$)

Yield: 77%; m.p.: 240–241°C; IR (KBr): $\nu = 3224$ (N–H), 1673 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6, δ, 200 MHz): 10.09 (s, 1H, NH), 9.24 (s, 1H, NH), 8.20 (s, 1H, H-5), 8.02 (s, 1H, NH), 7.77 (d, $J = 8.5$ Hz, 2H, ar), 7.58 (d, $J = 8.5$ Hz, 2H, ar), 7.08 (s, 1H, H-2), 3.81 (s, 2H, CH_2), 2.91 (d, $J = 4.3$ Hz, 3H, NCH_3) ppm; EIMS: m/z (%) = 425, 423 ($(M+2)^+$, M^+; 0.31, 0.79), 352, 350 (94, 94), 337, 335 (13, 19), 321, 319 (37, 53), 294, 292 (88, 89), 211 (100), 89 (7), 73 (70).

4-Ethyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2b**; $C_{16}H_{16}BrN_5OS_2$)

Yield: 91%; m.p.: 244–245°C; IR (KBr): $\nu = 3204$ (N–H), 1672 (C=O) cm^{-1}.

4-Propyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2c**; $C_{17}H_{18}BrN_5OS_2$)

Yield: 75%; m.p.: 234–235°C; IR (KBr): $\nu = 3208$ (N–H), 1673 (C=O) cm^{-1}.

4-Butyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2d**; $C_{18}H_{20}BrN_5OS_2$)

Yield: 80%; m.p.: 246–247°C; IR (KBr): $\nu = 3221$ (N–H), 1673 (C=O) cm^{-1}.

4-Allyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2e**; $C_{17}H_{16}BrN_5OS_2$)

Yield: 90%; m.p.: 233–234°C; IR (KBr): $\nu = 3200$ (N–H), 1672 (C=O) cm^{-1}.

4-Phenyl-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2f**; $C_{20}H_{16}BrN_5OS_2$)

Yield: 79%; m.p.: 213–214°C; IR (KBr): $\nu = 3180$ (N–H), 1672 (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 10.34 (s, 1H, NH), 9.74 (s, 1H, NH), 9.62 (s, 1H, NH), 8.25 (s, 1H, H-5), 7.74 (d, $J = 8.5$ Hz, 2H, ar), 7.56 (d, $J = 8.5$ Hz, 2H, ar), 7.48–7.17 (m, 5H, ar), 7.11 (s, 1H, H-2), 3.88 (s, 2H, CH_2) ppm; EIMS: m/z (%) = 487, 485 ($(M+2)^+$, M^+; 0.06, 0.15), 352, 350 (57, 56), 337, 335 (10, 13), 321, 319 (21, 35), 294, 292 (54, 54), 211 (46), 151 (0.79), 135 (100), 77 (67).

4-(4-Methylphenyl)-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2g**; $C_{21}H_{18}BrN_5OS_2$)

Yield: 83%; m.p.: 221–222°C; IR (KBr): $\nu = 3131$ (N–H), 1674 (C=O) cm^{-1}.

4-(4-Bromophenyl)-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2h**; $C_{20}H_{15}Br_2N_5OS_2$)

Yield: 77%; m.p.: 226–227°C; IR (KBr): $\nu = 3096$ (N–H), 1672 (C=O) cm^{-1}.

4-(4-Chlorophenyl)-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2i**; $C_{20}H_{15}BrClN_5OS_2 \cdot H_2O$)

Yield: 70%; m.p.: 223°C; IR (KBr): $\nu = 3460$, 3131 (O–H, N–H), 1676 (C=O) cm^{-1}.

4-(4-Nitrophenyl)-1-((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-3-thiosemicarbazide (**2j**; $C_{20}H_{15}BrN_6O_3S_2$)

Yield: 82%; m.p.: 220–221°C; IR (KBr): $\nu = 3205$ (N–H), 1705 (C=O) cm^{-1}.

3-Alkyl/aryl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinones **3a**–**j***; general procedure*

A mixture of 0.005 mol **2**, 0.005 mol of ethyl bromoacetate, and 0.02 mol of fused sodium acetate in 25 cm^3 of anhydrous ethanol was heated under reflux for 3 h. The reaction mixture was cooled, diluted with H_2O, and allowed to stand overnight. The precipitate was filtered, dried, and purified either by recrystallization from EtOH (**3a**–**e**) or by washing with hot EtOH (**3f**–**j**).

3-Methyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3a**; $C_{17}H_{14}BrN_5O_2S_2$)

Yield: 92%; m.p.: 251–252°C; IR (KBr): $\nu = 3147$ (N–H), 1717 (C=O ring), 1663 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6, δ, 200 MHz): 10.61 (s, 1H, NH), 8.22 (s, 1H, H-5), 7.76 (d, $J = 8.4$ Hz, 2H, ar), 7.57 (d, $J = 8.2$ Hz, 2H, ar), 7.05 (s, 1H, H-2), 4.04, 3.87 (2s, 4H, CH_2 and SCH_2), 3.08 (s, 3H, NCH_3) ppm; EIMS: m/z (%) = 465, 463 ($(M + 2)^+$, M^+; 91, 86), 407, 405 (79, 75), 352, 350 (2, 2), 337, 335 (4, 7), 321, 319 (66, 71), 294, 292 (100, 99), 211 (73), 57 (5).

3-Ethyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3b**; $C_{18}H_{16}BrN_5O_2S_2$)

Yield: 98%; m.p.: 271°C; IR (KBr): $\nu = 3180$ (N–H), 1709 (C=O ring), 1689 (C=O) cm^{-1}.

3-Propyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3c**; $C_{19}H_{18}BrN_5O_2S_2$)

Yield: 84%; m.p.: 226–227°C; IR (KBr): $\nu = 3192$ (N–H), 1717 (C=O ring), 1673 (C=O) cm^{-1}.

3-Butyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3d**; $C_{20}H_{20}BrN_5O_2S_2$)

Yield: 91%; m.p.: 235–236°C; IR (KBr): $\nu = 3198$ (N–H), 1718 (C=O ring), 1671 (C=O) cm^{-1}.

3-Allyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3e**; $C_{19}H_{16}BrN_5O_2S_2$)

Yield: 82%; m.p.: 224–225°C; IR (KBr): $\nu = 3186$ (N–H), 1719 (C=O ring), 1671 (C=O) cm^{-1}.

3-Phenyl-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3f**; $C_{22}H_{16}BrN_5O_2S_2$)

Yield: 95%; m.p.: 278–279°C; IR (KBr): $\nu = 3241$ (N–H), 1739 (C=O ring), 1677 (C=O) cm^{-1}; ^1H NMR (*DMSO*-d_6, δ, 200 MHz): 11.25 (s, 1H, NH), 8.14 (s, 1H, H-5), 7.58 (d, $J = 8.2$ Hz, 2H, ar), 7.41 (d, $J = 8.5$ Hz, 2H, ar), 7.35–7.11 (m, 5H, ar), 6.90 (s, 1H, H-2), 4.17, 4.06 (2s, 4H, CH_2 and SCH_2) ppm; EIMS: m/z (%) = 527, 525 ($(M + 2)^+$, M^+; 97, 90), 407, 405 (0.63, 0.31), 352, 350 (0.63, 0.79), 337, 335 (25, 66), 321, 319 (74, 77), 320, 318 (100, 93), 294, 292 (92, 92), 211 (89), 119 (38), 77 (45).

3-(4-Methylphenyl)-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3g**; $C_{23}H_{18}BrN_5O_2S_2$)

Yield: 99%; m.p.: 281–282°C; IR (KBr): $\nu = 3228$ (N–H), 1733 (C=O ring), 1647 (C=O) cm^{-1}.

3-(4-Bromophenyl)-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3h**; $C_{22}H_{15}Br_2N_5O_2S_2$)

Yield: 99%; m.p.: 266–267°C; IR (KBr): $\nu = 3114$ (N–H), 1749 (C=O ring), 1704 (C=O) cm^{-1}.

3-(4-Chlorophenyl)-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3i**; $C_{22}H_{15}BrClN_5O_2S_2$)

Yield: 92%; m.p.: 276–277°C; IR (KBr): $\nu = 3119$ (N–H), 1748 (C=O ring), 1705 (C=O) cm^{-1}.

3-(4-Nitrophenyl)-2-(((6-(4-bromophenyl)-imidazo[2,1-b]thiazol-3-yl)-acetyl)-hydrazono)-4-thiazolidinone (**3j**; $C_{22}H_{15}BrN_6O_4S_2$)

Yield: 99%; m.p.: 265–266°C; IR (KBr): $\nu = 3107$ (N–H), 1754 (C=O ring), 1707 (C=O) cm^{-1}.

Microbiology

All compounds to be tested were dissolved in *DMSO* at a stock concentration of 3200 $\mu g \cdot cm^{-3}$. The final desired concentrations were prepared with RPMI 1640 medium for *Candida* species and dermatophytes and with *Mueller-Hinton* broth for bacteria. The final *DMSO* concentration was adjusted to 1%.

Antibacterial activity

*MIC*s were determined by the microbroth dilution method using the National Committee for Clinical Laboratory Standards (NCCLS) recommendations [24, 25]. *Mueller-Hinton* broth (Oxoid, Hemakim, Turkey) was used as the test medium. An inoculum of approximately 5×10^5 $CFU \cdot cm^{-3}$ was delivered per well. Serial twofold dilutions of the test compounds (32–0.25 $\mu g \cdot cm^{-3}$) and extra dilutions (0.12–0.015 $\mu g \cdot cm^{-3}$) for antibiotic standards were prepared. Plates were incubated for 16–20 h at 35°C in an ambient air incubator. The lowest concentration of the test compounds inhibiting visible growth was taken as the *MIC* value.

Antifungal activity

Antifungal activity for *Candida* species

*MIC*s were determined by the microbroth dilution method using the NCCLS recommendations [26]. RPMI broth was prepared from RPMI 1640 medium (Sigma, St. Louis, Mo, USA) supplemented with 0.3 g of glutamine per dm^3, buffered with 3-(*N*-morpholino)-propanesulfonic acid (*MOPS*), and adjusted to *pH* 7.0. A working suspension of the inoculum was prepared by a 1:100 dilution of the 0.5 *McFarland* standard yeast suspension in 0.85% saline followed by a 1:20 dilution in RPMI broth. Twofold dilutions of test compounds from 32 to 0.25 $\mu g \cdot cm^{-3}$ were prepared with the working suspension of the inoculum. Extra dilutions (0.12–0.015 $\mu g \cdot cm^{-3}$) were added for itraconazole and ketoconazole. The plates were incubated at 35°C for 48 h in ambient air. The *MIC* is the lowest concentration of a compound that substantially inhibits growth of the organism as detected visually.

Antifungal activity for dermatophytes

*MIC*s were determined by the microbroth dilution method according to a modification of previously described procedures [27, 28]. RPMI 1640 with glutamin and buffered at *pH* 7.0 with *MOPS* was used for broth microdilution susceptibility testing. Test inocula of approximately 0.4×10^3 to 5×10^3 $CFU \cdot cm^{-3}$ were evaluated. Dermatophytes were grown on potato dextrose agar slants. (Acumedia, Baltimore, MD, USA) at 30°C for 7 days. Sterile normal saline 0.85% was added to the slant, and the culture was gently swabbed with a cotton tip applicator to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile tube. Heavy particles were allowed to settle for 5 min. The upper homogeneous suspensions were collected and vortexed. The densities of the conidial suspensions were read and adjusted to 70 to 82% transmittance at 530 nm. These suspensions were diluted

1:500 in RPMI 1640 yielding 0.4×10^3 to 5×10^3 CFU $\cdot$ cm^{-3} per well. Twofold dilutions of test compounds (32–0.25 μg $\cdot$ cm^{-3}) and additional dilutions for itraconazole (0.12–0.015 μg $\cdot$ cm^{-3}) were prepared. For quality control assessment, *C. parapsilosis* ATCC 22019 was used. The microdilution plates were incubated at 35°C for 4 days. The minimum concentration at which no growth was observed was taken as the *MIC* value.

Antimycobacterial activity

A primary screening was conducted at 6.25 μg $\cdot$ cm^{-3} (or a molar equivalent of the highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay (Microplate Alamar Blue Assay (MABA) [29]). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [29]. Compounds effecting less than 90% inhibition in the primary screen (*MIC* >6.25 μg $\cdot$ cm^{-3}) were not generally evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against *M. tuberculosis* H_{37}Rv to determine the actual minimum inhibitory concentration using MABA. The *MIC* was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

Radiometric susceptibility test

A total of 0.1 cm^3 of BACTEC 12B passaged inoculum was delivered without prior dilution into 4 cm^3 of test medium. Subsequent determination of bacterial titers yielded average titers (three experiments) of 1×10^5, 2.5×10^5, and 3.25×10^4 CFU $\cdot$ cm^{-3} of BACTEC 12B medium for *M. tuberculosis* H_{37}Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium; then, 0.1 cm^3 were delivered to the test medium yielding 5.0×10^5 and 1.25×10^5 CFU per BACTEC vial for H_{37}Rv. Twofold drug dilutions were prepared in either *DMSO* (Sigma) or distilled deionized H_2O and delivered *via* a 0.5 cm^3 insulin syringe in a 50 mm^3 volume. Drug-free control vials consisted of solvent with bacterial inoculum and solvent with a 1:100 dilution of bacterial inoculum (1:100 controls). Vials were incubated at 37°C, and the *Gl* was determined in a BACTEC 460 instrument (Becton Dickinson) until the *Gl* of the 1:100 controls reached at least 30. All vials were read the following day, and the *Gl* and daily change in *Gl* (ΔGl) were recorded for each drug dilution. The *MIC* was defined as the lowest concentration for which the ΔGl was less than the ΔGl of the 1:100 control. If the *Gl* of the test sample was greater than 100, the sample was scored as resistant even if the ΔGl was less than the ΔGl of the 1:100 control.

Alamar blue susceptibility test (MABA)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, CT, USA) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile H_2O to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either *DMSO* or distilled deionized H_2O, and subsequent twofold dilutions were performed in 0.1 cm^3 of 7H9GC (no Tween 80) in the microplates. BACTEC 12B passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 cm^3 were added to the wells. Subsequent determination of bacterial titers yielded 1×10^6, 2.5×10^6, and 3.25×10^5 CFU $\cdot$ cm^{-3} in plate wells for *M. tuberculosis* H_{37}Rv. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 0.1 cm^3 to the wells resulted in final bacterial titers of 2.0×10^5 and 5×10^4 CFU $\cdot$ cm^{-3} for H_{37}Rv. Wells containing drug only were used to detect autofluorescence. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 mm^3 of 10X alamar Blue solution (Alamar Biosciences/Accumed, Westlake, OH, USA) and 12.5 mm^3 of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37°C.

Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥ 50000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, MA, USA) in the bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or less than 50000 FU were measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37°C, and results were recorded 24 h after reagent addition. Visual *MIC*s were defined as the lowest concentration of drug that prevented a color change. For fluorometric *MIC*s, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as 1 – (test well FU/mean FU of triplicate B wells) × 100. The lowest drug concentration effecting an inhibition of above 90% was considered as the *MIC*.

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