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5-Nitroimidazole derivatives as possible antibacterial and antifungal agents

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Abstract

Some novel 1-[2-[[5-(2-furanyl)-4-substituted 4*H*-1,2,4-triazol-3-yl]thio]ethyl]-2-methyl-5-nitro-1*H*-imidazoles (**3**), 1-[3-[[5-(2-furanyl/2-thienyl)-4-substituted 4*H*-1,2,4-triazol-3-yl]-thio]-2-hydroxypropyl]-2-methyl-5-nitro-1*H*-imidazoles (**5**) and 1-[3-[(*N*,*N*-disubstituted thiocarbamoyl)-thio]-2-hydroxypropyl]-2-methyl-5-nitro-1*H*-imidazoles (**7**) were synthesized and evaluated for in vitro antibacterial and antifungal activity. Some of **5** were found to be effective against bacteria and fungi (minimum inhibitory concentration (MIC) 7.3–125 μg/ml), whereas **7** were found to be effective against fungi (MIC 3–25 μg/ml).

Keywords: 5-Nitroimidazoles; 1,2,4-Triazoles; Dithiocarbamates; Antibacterial activity; Antifungal activity

1. Introduction

Metronidazole and related N-1 substituted 5-nitroimidazoles like ornidazole, secnidazole and tinidazole are widely used in the treatment of diseases caused by protozoa and anaerobic bacteria [1–3]. Furthermore, some 5-nitroimidazoles have been shown to sensitize hypoxic tumor cells to the effects of ionizing radiation [4]. It has been speculated that a reactive intermediate formed in the microbial reduction of the 5-nitro group of nitroimidazoles covalently binds to the DNA of the microorganism, triggering the lethal effect. Potential reactive intermediates include the nitroxide, nitroso, hydroxylamine and amine. The ability of nitroimidazoles, especially metronidazole, to act as radiosensitizing agents is also related to their reductive potentials. 1,2,4-Triazoles and dithiocarbamic acid esters are also reported to show antifungal and antibacterial activity [5–9]. In view of the wide continued interest in the activity spectrum and profile of the nitroimidazoles [10,11] and in continuation of our work on the synthesis and antimicrobial evaluation of 1,2,4-triazoles and dithiocarbamic acid esters [12–14], some new nitroimidazole derivatives bearing a 1,2,4-triazolylthioethyl, 1,2,4-triazolylthiopropyl or *N*,*N*-disubstituted dithiocarbamoyl–propyl residue at N-1 were synthesized and evaluated for in vitro antibacterial and antifungal activity.

2. Chemistry

1-(2-Chloroethyl)-2-methyl-5-nitro-1*H*-imidazole (**1**) and 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitro-1*H*-imidazole (ornidazole, **4**) was reacted with the anion generated from 5-(2-furanyl/2-thienyl)-4-substituted 2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**2**) [12,15,16] in the presence of K_2CO_3, to afford 1-[2-[[5-(2-furanyl)-4-substituted 4*H*-1,2,4-triazol-3-yl]thio]ethyl]-2-methyl-5-nitro-1*H*-imidazoles (**3**) and 1-[3-[[5-(2-furanyl/2-thienyl)4-substituted 4*H*-1,2,4-triazol-3-yl]thio]-2-hydroxypropyl]-2-methyl-5-nitro-1*H*-imidazoles (**5**), respectively. Treatment of **4** with potassium *N*,*N*-disubstituted dithiocarbamates **6** [14] furnished 1-[3-[(*N*,*N*-disubstituted thiocarbamoyl)thio]-2-hydroxypropyl]-2-

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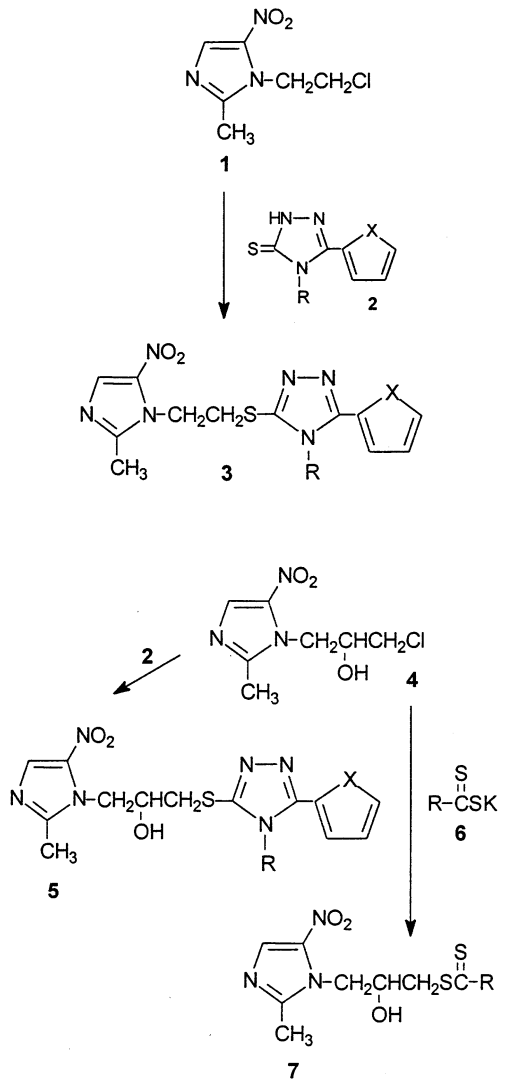
methyl-5-nitro-1*H*-imidazoles (**7**) (Scheme 1, Table 1). Analytical (CHN) and spectral data (IR, ^{1}H NMR, EIMS) supported the designed structures.

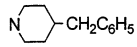
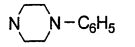
The O–H (3446–3188 cm^{-1}), =C–H (3176–3096 cm^{-1}), C=C/C=N (1610–1421 cm^{-1}) and NO_2 (1570–1515 cm^{-1}, 1366–1358cm^{-1}) stretching vibrations observed in the IR spectra provided substantial proof for the formation of the desired products **3**, **5** and **7**.

The ^{1}H NMR spectra of **3** displayed the N–CH_2 resonances at about δ 4.67–4.70 ppm as triplets. The S–CH_2 group in **3a** was observed as a multiplet together with the N–CH_3 singlet at about δ 3.56–3.68 ppm while the S–CH_2 protons of **3d** and **3e** resonated as triplets at about δ 3.60–3.62 ppm. Compounds **5** and **7** are chiral molecules, thus the geminal hydrogens on the methylene groups adjacent to the chiral center experience different magnetic environments. In rigid systems where averaging is not possible, the geminal hydrogens absorb at different δ values on the ^{1}H NMR scale and show large couplings in the range of 15 Hz, which are characteristic of geminal interactions [17]. Thus, the N–CH_2 protons at N-1 of the imidazole moiety, both in **5** and **7** absorbed as double doublets



Scheme 1. Synthetic routes to compounds **3**, **5** and **7**.

Table 1
Physical constants of compounds **3**, **5** and **7**

Comp.	R	X	Yield(%)	M.p.(°C)	Formula (mol.wt.)
3a	CH_3	O	22	128-130	$C_{13}H_{14}N_6O_3S$ (334.35)
3b	C_2H_5	O	26	128-132	$C_{14}H_{16}N_6O_3S$ (348.38)
3c	C_4H_9	O	12	124-126	$C_{16}H_{20}N_6O_3S$ (376.43)
3d	C_6H_5	O	48	188-191	$C_{18}H_{16}N_6O_3S.1/2H_2O$ (405.43)
3e	$C_6H_4Cl(4)$	O	33	149	$C_{18}H_{15}ClN_6O_3S$ (430.87)
3f	$C_6H_4CH_3(4)$	O	49	105-110	$C_{19}H_{18}N_6O_3S.H_2O$ (428.47)
5a	CH_3	O	57	168-171	$C_{14}H_{16}N_6O_4S$ (364.38)
5b	C_2H_5	O	79	190-191	$C_{15}H_{18}N_6O_4S$ (378.40)
5c	CH_2-CH=CH_2	O	70	79-83	$C_{16}H_{18}N_6O_4S.H_2O$ (408.43)
5d	C_4H_9	O	70	79-83	$C_{17}H_{22}N_6O_4S$ (406.46)
5e	C_6H_{11}	O	80	74-80	$C_{19}H_{24}N_6O_4S.H_2O$ (450.51)
5f	C_6H_5	O	78	202-203	$C_{19}H_{18}N_6O_4S$ (426.44)
5g	$C_6H_4Br(4)$	O	75	178-183	$C_{19}H_{17}BrN_6O_4S$ (505.35)
5h	$C_6H_4Cl(4)$	O	76	169-172	$C_{19}H_{17}ClN_6O_4S$ (460.89)
5i	$C_6H_4F(4)$	O	81	105-108	$C_{19}H_{17}FN_6O_4S.1/2H_2O$ (453.45)
5j	$C_6H_4CH_3(4)$	O	45	188	$C_{20}H_{20}N_6O_4S$ (440.48)
5k	$C_6H_4NO_2(4)$	O	34	182-184	$C_{19}H_{17}N_7O_6S.H_2O$ (489.48)
5l	C_6H_5	S	81	210-211	$C_{19}H_{18}N_6O_3S_2$ (442.51)
5m	$C_6H_4Cl(4)$	S	71	179-180	$C_{19}H_{17}ClN_6O_3S_2$ (476.96)
7a		-	81	105-108	$C_{20}H_{26}N_4O_3S_2$ (434.58)
7b		-	26	101-105	$C_{18}H_{23}N_5O_3S_2.H_2O$ (439.56)

centered at about δ 4.23–4.56 ppm with coupling constants in the ranges 14.1–13.7 and 9.4–2.5 Hz. The S–CH_2 protons also adjacent to the chiral center absorbed as double doublets centered at about δ 3.38–3.39 ppm due to magnetic unequivalence, but showed smaller coupling constants in **5** (5.8–5.6 and 2.1–1.7 Hz) where the rotation about the S–CH_2 bond was not as much impeded as it was about the N–CH_2 bond, which is in direct relation with the rigid imidazole system. The large coupling observed for the S–CH_2 protons in **7** (δ 3.59 ppm; 13.6 and 4.8 Hz) implicated restriction of rotation about the S–C bond of the dithiocarbamate moiety. The variation in the vicinal coupling constants observed for these protons was attributed to the variation in the dihedral angle between the N–CH_2 and S–CH_2 protons and the C*H*OH proton. The absorption positions and splitting patterns of the other protons were in accordance with the literature [12,17,18].

The molecular ions at m/z 444, 421 and 334 observed in the EIMS of representative examples, (**3a**, **5i** and **7b**), provided further evidence for the formation of the expected structures. The fragmentation routes primarily involved losses of OH (m/z 17), NO (m/z 30), NO_2 (m/z 46) and HNO_2 (m/z 47) from the molecular ion, which are characteristic of compounds carrying OH and NO_2 functions. Diagnostic fragments derived from the imidazole and 1,2,4-triazole or *N*,*N*-disubstituted dithiocarbamate systems were in accordance with the structures reported in the literature [14,19–21].

3. Results and discussion

Compounds **3**, **5** (except **5j** and **5k**) and **7** were evaluated for antibacterial and/or antifungal activity against representative bacteria — *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* UC 57, *Shigella flexneri*, *Pseudomonas aeruginosa* ATCC 1539, *Proteus mirabilis* and *Salmonella typhi*; and fungi — *Trichophyton tonsurans* NCPF 245, *T. mentagrophytes* var. *erinacei* ATCC 375, *T. mentagrophytes*, *Microsporum gypseum* NCPF 580, *M. canis* and *M. audouinii* [22,23]. As can be seen in Table 2, although not as active as the standard ampicillin, **5a**–**i** were found to be active against *S. aureus* ATCC 6538 and/or *S. epidermidis* ATCC 12228 where ornidazole was devoid of activity. These results indicate that incorporation of the 3-[[5-(2-furanyl)-4-substituted 4*H*-1,2,4-triazol-3-yl]thio]-2-hydroxypropyl moiety imparts activity against aerobic bacteria since **3**, which differs from **5a**–**i** in bearing an ethyl instead of the 2-hydroxypropyl residue between the imidazole and 1,2,4-triazole systems; **5l** and **5m**, which bear the 2-thienyl instead of the 2-furanyl moiety at the 5-position of the 1,2,4- triazole ring; and **7**, which does not bear the 1,2,4-triazole nucleus were found to be devoid of activity. Table 2 also contains results of antifungal activity tests of **5** and **7** where miconazole was used as the standard. The most active compound was **7a** (*T. tonsurans* NCPF 245; minimum inhibitory concentration (MIC) 3 μg/ml) exerting about one half the activity of onidazole. Although the rest of the compounds

Table 2
Antimicrobial activity of **5** and **7** (MIC μg/ml)

Comp.	Microorganism [a](MICμg/ml)							
	A	B	C	D	E	F	G	H
5a	58.6	n.a.	12.5	12.5	25	25	>25	25
5b	58.6	n.a.	25	25	25	25	>25	25
5c	58.6	n.a.	25	25	25	25	>25	25
5d	29.2	62.5	25	>25	25	25	25	25
5e	7.3	125	25	25	25	25	>25	25
5f	29.2	31.2	25	25	25	25	>25	25
5g	14.6	62.5	25	>25	25	25	>25	25
5h	29.2	3.66	25	25	25	25	>25	25
5I	14.6	62.5	25	25	25	25	>25	25
7a	n.a.	n.a.	3	25	25	25	25	25
7b	n.a.	n.a.	25	>25	25	25	25	25
Ornidazole	n.a.	n.a.	1.6	6.2	6.2	6.2	0.8	6.2
Miconazole	n.t.	n.t.	0.2	0.2	0.2	0.2	0.2	0.2
Ampicillin	0.06	1.95	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.

[a] A = *S. aureus* ATCC 6538, B = *S. epidermidis* ATCC 12228, C = *T. tonsurans* NCPF 245, D = *T. mentagrophytes* var. *erinacei* ATCC 375, E = *T. menta-grophytes*, F = *M. gypseum* NCPF 580, G = *M. canis*, H = *M. audounii*. n.a. = not active, n.t. = not tested.

showed varying degrees of inhibition (MIC 12.5–25 μg/ml), none were as effective as miconazole (MIC 0.2 μg/ml) or ornidazole (MIC 0.8–6.2 μg/ml).

4. Experimental

4.1. Chemistry

Melting points were determined with a Büchi (Tottoli) melting point apparatus in open capillaries and are uncorrected. IR, ^{1}H NMR and EIMS were recorded on Shimadzu 2100S, Perkin–Elmer 1600 FTIR, Bruker AC 200 (200 MHz) and VG Zab Spec (EI, 70 eV) instruments, respectively. The reactions were monitored by TLC (Silica Gel 60 F_{254} Merck Art. 5735).

4.1.1. Synthesis of 1-(2-chloroethyl)-2-methyl-5-nitro-1H-imidazole (1)

To a suspension of 1-(2-hydroxyethyl)-2-methyl-5-nitro-1*H*-imidazole (0.03 mol) in benzene (20 ml), $SOCl_2$ (4.5 ml) was added and the reaction mixture was heated under reflux for 2 h. After cooling the solvent was decanted and the remaining crystalline precipitate was washed with petroleum ether, neutralized by the addition of saturated $NaHCO_3$ solution, washed with H_2O and filtered to afford **1**, which was used without further purification.

4.1.2. Synthesis of 1-[2-[[5-(2-furanyl)-4-substituted 4H-1,2,4-triazol-3-yl]thio]ethyl]-2-methyl-5-nitro-1H-imidazoles (3a–f)

To a solution/suspension of **2** (0.005 mol) in CH_3COCH_3 (30 ml), **1** (0.005 mol) and K_2CO_3 (0.02 mol) were added. The reaction mixture was refluxed for 21 h, cooled and poured into ice water. The precipitate was collected by filtration. Compounds **3a**, **3b** and **3d** were purified by washing with H_2O and **3c**, **3e** and **3f** were recrystallized from C_2H_5OH.

Spectral data for **3a**. IR [ν cm^{-1}, KBr]: 3107 (=C–H), 1610 (C=N/C=C), 1522 (NO_2), 1481, 1460, 1430 (C=N/C=C), 1364 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.47 (s, 3H, CH_3), 3.56–3.68 (m, 5H, N–CH_3 and S–CH_2), 4.67 (t, 3H, N–CH_2), 6.47 (dd, $J = 3.0$, 1.6 Hz, 1H, furan C_4–H), 7.10 (d, $J = 3.4$ Hz,1H, furan C_3–H), 7.94–7.99 (m, 2H, furan C_5–H and imidazole C_4–H). EIMS [m/z (%)]: 334 (M^+, 2), 319 (5), 288 (100), 220 (7), 208 (27), 181(57), 154 (12), 139 (8), 121 (7), 108 (88), 94 (30), 80 (22), 67 (16).

Spectral data for **3d**. IR [ν cm^{-1}, KBr]: 3124 (=C–H), 1595 (C=N/C=C), 1524 (NO_2), 1498, 1477, 1467 (C=N/C=C), 1358 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.44 (s, 3H, CH_3), 3.62 (t, $J = 6.3$ Hz, 2H, S–CH_2), 4.70 (t, $J = 6.3$ Hz, 2H, N–CH_2), 6.15 (d, $J = 3.2$ Hz, 1H, furan C_3–H), 6.52 (dd, $J = 3.2$, 1.6 Hz, 1H, furan C_4–H), 7.43–7.50 (m, 2H, ar.), 7.59–7.71 (m, 3H, ar.), 7.81 (s, 1H, furan C_5–H), 8.02 (s, 1H, imidazole C_4–H).

Spectral data for **3e**. IR [ν cm^{-1}, KBr]: 3105 (=C–H), 1527 (NO_2), 1513, 1493, 1464, 1421 (C=N/C=C), 1364 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.41 (s, 3H, CH_3), 3.60 (t, $J = 6.4$ Hz, 2H, S–CH_2), 4.69 (t, $J = 6.4$ Hz, 2H, N–CH_2), 6.16 (d, $J = 3.4$ Hz, 1H, furan C_3–H), 6.51 (dd, $J = 3.4$, 1.6 Hz, 1H, furan C_4–H), 7.29 (d, $J = 8.3$ Hz, 2H, ar.), 7.41 (d, $J = 8.3$ Hz, 2H, ar.), 7.75 (s, 1H, furan C_5–H), 7.96 (s, 1H, imidazole C_4–H).

4.1.3. Synthesis of 1-[3-[[5-(2-furanyl/2-thienyl)-4-substituted 4H-1,2,4-triazol-3-yl]thio]-2-hydroxypropyl]-2-methyl-5-nitro-1H-imidazoles (5a–m)

To a solution/suspension of **2** (0.005 mol) in CH_3COCH_3 (30 ml), **4** (0.005 mol) and K_2CO_3 (0.02 mol) were added. The reaction mixture was refluxed for 21 h, cooled and poured into H_2O. The precipitate was collected by filtration.

Compounds **5a**, **5c**, **5f**, **5h**, **5l** and **5m** were purified by washing with H_2O; **5b**, **5e**, **5i**, **5j** and **5k** by recrystallization from C_2H_5OH; **5d** from C_2H_5OH:H_2O and **5g** by washing with ether.

Spectral data for **5i**. IR [ν, cm^{-1}, KBr]: 3188 (OH), 3096 (=CH), 1603 (C=N/C=C), 1515 (NO_2), 1463, 1430 (C=N/C=C), 1362 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.34 (s, 3H, CH_3), 3.39 (dd, $J = 5.8$, 2.1 Hz, 2H, S–CH_2), 4.04–4.12 (m, 1H, CH), 4.23 (dd, $J =$ 13.7, 9.2 Hz, 1H, N–CH_2), 4.55 (dd, $J = 13.7$, 2.5 Hz, 1H, N–CH_2); 5.58 (d, $J = 5.3$ Hz, 1H, OH); 6.27 (d, $J = 3.3$ Hz, 1H, furan C_3–H), 6.53 (dd, $J = 3.4$, 2.0 Hz, 1H, furan C_4–H), 7.47 (d, $J = 8.5$ Hz, 2H, ar.), 7.56 (dd, $J = 9.0$, 4.8 Hz, 2H, ar.), 7.74 (d, $J = 1.1$ Hz, 1H furan, C_5–H), 7.99 (s, 1H, imidazole C_4–H). EIMS [m/z (%)]: 444 (M^+, 53), 427 (77), 414 (52), 398 (40), 397 (83), 384 (45), 366 (74), 354 (58), 334 (60), 316 (78), 305 (45), 288 (49), 286 (75), 274 (88), 244 (66), 230 (84), 229 (55), 202 (85), 169 (90), 147 (70), 134 (82), 132 (80) 120 (84), 104 (88), 98 (100), 93 (20), 78 (86), 65 (90).

Spectral data for **5j**. IR [ν, cm^{-1}, KBr]: 3230 (O–H), 3121 (=CH), 1529 (NO_2), 1514, 1467, 1427 (C=N/C=C), 1366 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.43 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.39 (dd, $J = 5.8$, 2.1 Hz, 2H, S–CH_2), 4.04–4.12 (m, 1H, CH), 4.23 (dd, $J = 13.7$, 9.2 Hz, 1H, N–CH_2), 4.56 (dd, $J = 13.7$, 2.5 Hz, 1H, N–CH_2), 5.00 (d, $J = 5.4$ Hz, 1H, OH), 6.15 (d, $J = 3.4$ Hz, 1H, furan C_3–H), 6.51 (dd, $J = 8.4$, 1.8 Hz, 1H, furan C_4–H), 7.29 (d, $J = 8.4$ Hz, 2H, ar.), 7.34 (d $J = 8.4$ Hz, 2H, ar.), 7.82 (d, $J = 1.2$ Hz, 1H, furan C_5–H), 8.01 (s, 1H, imidazole C_4–H).

Spectral data for **5l**. IR [ν, cm^{-1}, KBr]: 3247 (O–H), 3110 (=CH), 1570 (NO_2), 1524, 1498, 1464, 1444 (C=N/C=C), 1365 (NO_2). ^{1}H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.44 (s, 3H, CH_3), 3.38 (dd, $J = 5.6$, 1.7 Hz, 2H, S–CH_2), 4.04–4.12 (m, 1H, CH), 4.23 (dd, $J =$ 14.1, 9.4 Hz, 1H, N–CH_2), 4.55 (dd, $J = 14.1$, 2.7 Hz,

1H, N–CH_2), 5.65 (s, 1H, OH), 6.72 (d, $J = 3.4$ Hz, 1H, thiophene C_3–H), 7.00 (dd, $J = 3.9$, 3.8 Hz, 1H, thiophene C_4–H), 7.52–7.66 (m, 5H, ar.), 8.01 (s, 1H, imidazole C_4–H), 8.31 (s, 1H, thiophene, C_5–H).

4.1.4. Synthesis of 1-[3-[(N,N-disubstituted thiocarbamoyl)thio]-2-hydroxypropyl]-2-methyl-5-nitro-1H-imidazoles (**7a–b**)

To a solution of **6** (0.0055 mol) in CH_3COCH_3 (30 ml), (0.0055 mol) **4** was added and the mixture was refluxed for 4 h. The solid was separated by filtration and the filtrate was allowed to cool to room temperature. The precipitate formed after cooling was collected by filtration and recrystallized from C_2H_5OH.

Spectral data for **7b**. IR [ν, cm^{-1}, KBr]: 3446 (OH), 3176 (=CH), 1600 (C=N/C=C), 1533 (NO_2), 1471, 1431 (C=N/C=C), 1361 (NO_2). ^1H NMR [200 MHz, δ ppm, DMSO-d_6]: 2.46 (s, 3H, CH_3), 3.28–3.46 (m, CH_2 piperazine and solvent H_2O), 3.59 (dd, $J = 13.6$, 4.8 Hz, 2H, S–CH_2), 3.94–4.09 (m, 1H, CH), 4.25 (dd, $J =$ 13.9, 8.9 Hz, 5H, piperazine N–CH_2 and CH_2), 4.54 (dd, $J = 13.9$, 3.5 Hz, 1H, N–CH_2), 5.56 (d, $J = 5.8$ Hz, 1H, OH), 6.81 (t, $J = 7.2$ Hz, 1H, ar.), 6.95 (d, $J = 8.1$ Hz, 2H, ar.), 7.25 (d, $J = 7.3$ Hz, 2H, ar.), 8.00 (s, 1H, imidazole C_4–H). EIMS [m/z (%)]: 421 (M^+, 10), 404 (32), 375 (7), 352 (4), 293 (5), 263 (4), 238 (25), 205 (41), 189 (10), 184 (5), 171 (7), 162 (50), 133 (20), 132 (98), 120 (100), 104 (52), 91 (52), 91 (24), 85 (14), 78 (34), 77 (47), 63 (32).

4.2. Microbiology

4.2.1. Antibacterial activity [22]

The disk diffusion method was used for the preliminary antibacterial evaluation of **3**, **5** and **7**. The MICs of **5**, which showed inhibition in the preliminary tests, were determined by the microbroth dilution technique using Mueller–Hinton broth (Difco Laboratories, Detroit, MI). Serial two-fold dilutions of the test compounds (235–0.6 μg/ml and 250–0.4 μg/ml) in DMSO were prepared. The inoculum was prepared in broth, which had been kept at 37°C overnight, and was diluted with broth to give a final concentration of 10^5 cfu/ml in the test tray. The trays were covered to prevent drying. After incubation at 37°C for 18–20 h the trays were examined for growth. The lowest concentration of the test compounds inhibiting visible growth was taken as the MIC value.

4.2.2. Antifungal activity [23]

All the compounds to be tested were dissolved in DMSO at a concentration of 4000 μg/ml and the final concentration was reduced to 200 μg/ml with sterile distilled water. No effect of DMSO (5%) was observed upon growth of dermatophytes.

The dermatophyte strains which were grown on slant medium of Sabouraud (Difco) were transferred to 3.5 ml nutrient broth (NB, Diagnostic Pasteur) and incubated for 3–5 days at 25°C. At the end of the incubation period these strains were transferred into screwcapped bottles containing sterilized beads and shaken for 4–5 min in a vortex (IKA-VF, Germany). The suspensions of the cultures were adjusted to have an absorbance degree of 0.6 at 450 nm in the spectrophotometer. Eight different dilutions of the test compounds between 25 and 0.2 μg/ml were prepared in microplates by serial dilutions from top to bottom. Then all the wells except the 12th wells (positive control) were filled with 10 μl of the standardized strains. These plates were incubated at 25°C for 5 or 6 days. The minimum concentration at which no growth was observed was taken as the MIC value.

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