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ORIGINAL ARTICLE

Antimicrobial evaluation of diaminothiazoloylbenzothiazoles

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Abstract A series of 2-(4-amino-2-aryl/alkylaminothiazol-5-oyl) benzothiazole derivatives were synthesized from amidinothioureas and 2-(2-bromoacetyl)benzothiazole with triethylamine. Their structures were established on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses. All the synthesized compounds were screened for their antibacterial, antifungal and antimycobacterial potential. All the compounds showed significant activity against the microorganisms tested.

1. Introduction

It is a well-known fact that infectious microorganisms, i.e. bacteria and fungi, cause serious diseases and are responsible for nearly one-half of the deaths in India. Benzothiazole derivatives are fascinating chemical products used in the field of medicine as they have been found to possess a wide spectrum of biodynamic properties. Many of them have been reported to have antitumor (Aiello et al., 2008), antimicrobial (Sareen et al., 2006), antileishmanial (Delmas et al., 2004), anticonvulsant (Ugale et al., 2012), antidiabetic (Pattan et al., 2005), and anti-inflammatory (Venkatesh and Pandeya, 2009) activities. For this study we have prepared novel derivatives of diaminothiazoloylbenzothiozoles **3a–i** (see Table 4). All the synthesized compounds were screened for their antibacterial, antifungal and antituberculosis activities.

2. Experimental

2.1. Materials and methods

The reagents and solvents used were of AR grade. All chemicals were purchased from Merck Specialities Pvt. Ltd. and HiMedia Laboratories Pvt. Ltd.

The spectra were recorded on JEOL DRX 300 or DPX 300 NMR spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C NMR spectra), JEOL SX 102/DA-6000 mass spectrometer (using argon/xenon, 6 kV, 10 mA as the FAB gas and *m*-nitrobenzyl alcohol as the matrix) for FAB mass spectra and Nicolet 400D FTIR spectrometer. Melting points were uncorrected. Elemental analysis was done at the Central Drug Research Institute, India.

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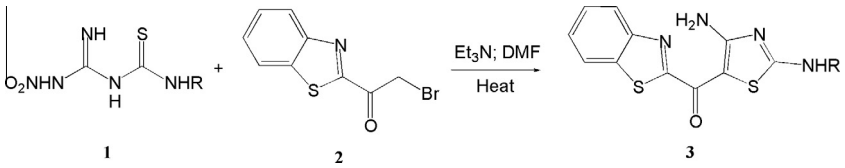

Scheme 1 Synthetic route of molecule **3**.

*2.2. General procedure for the synthesis of 2-(4-amino-2-aryl/alkyl aminothiazol-5-oyl)benzothiazoles **3a–i***

The reaction sequences employed for the synthesis of title compounds are shown in Scheme 1. 2-(4-Amino-2-aryl/alkyl aminothiazol-5-oyl)benzothiazoles **3a–i** were prepared according to the following method (Abbs Fen Reji et al., 2009).

A solution of 1-aryl/alkyl-3-(*N*-nitroamidino)thiourea **1a–i** (1 mmol) in DMF (2 mL) was added to a solution of 2-(2-bromoacetyl)benzothiazole **2** (0.254 g, 1 mmol), which was prepared from 2-(1-hydroxyethyl)benzothiazole (Sawhney and Singh, 1970; Gupta et al., 1980; Joshua and Rajasekharan, 1974; Hunter, 1925a,b, 1926) in DMF (2 mL). The reaction mixture was stirred well and triethylamine (0.15 mL, 1 mmol) was added. The reaction mixture was warmed at 35–40 °C for 10 min. It was then cooled and poured into ice-cold water with constant stirring. A yellowish orange precipitate thus obtained was filtered, washed with water and dried. The crude product was crystallized from methanol:water (2:1) and then from benzene:petroleum ether (1:1) to give a yellowish orange crystalline solid.

*2.3. 2-(4-Amino-2-phenylaminothiazol-5-oyl)benzothiazole **3a***

Yield 60%, m.p. 293–95 °C Analysis: Found: C, 57.75: H, 3.50: N, 15.69%; Calc. for $C_{17}H_{12}N_4OS_2$ (352.43): C, 57.93: H, 3.43: N, 15.90%; IR (KBr) cm^{-1}: 3454, 3285, 3137, 3103, 3050, 1625, 1599, 1566, 1526, 1499, 1445, 1356, 1237, 1188, 1034, 891, 749, 690; ^{1}H NMR: (300 MHz, DMSO-d_6) δ: 7.12 (t, J = 7.35 Hz, 1H, 1ArH), 7.40(t, J = 7.8 Hz, 2H, 2ArH), 7.49–7.65 (m, 2H, H-5, H-6), 7.72 (d, J = 8.1 Hz, 2H, 2ArH), 8.09 (d, J = 8.1 Hz, 1H, H-4), 8.20(d, J = 7.8 Hz, 1H, H-7), 8.64 (br, 1H, NH), 8.76 (br, 1H, NH), 11.08 (s, 1H, NH);^{13}C NMR: (75 MHz, DMSO-d_6) δ: 91.1, 119.4, 122.9, 123.7, 123.9, 126.6, 126.9, 129.1, 135.9, 139.3, 152.9, 168.7, 169.6, 170.6, 171.3; FABMS: 353 (MH^+).

*2.4. 2-[4-Amino-2-(4-chlorophenylamino)thiazol-5-oyl]benzothiazole **3b***

Yield 59%, m.p. 335–38 °C Analysis: Found: C, 52.57: H, 2.79: N, 14.65%; Calc. for $C_{17}H_{11}ClN_4OS_2$ (386.88): C, 52.77: H, 2.87: N, 14.48%; IR (KBr) cm^{-1}: 3461, 3272, 3210, 3136, 3083, 1634, 1605, 1526, 1492, 1465, 1357, 1256, 1189, 1093, 892, 825, 757, 667; ^{1}H NMR: (300 MHz, DMSO-d_6) δ: 7.44 (d,J = 9 Hz, 2H, 2ArH), 7.51–7.67 (m, 2H, H-5, H-6), 7.76 (d,J = 8.7 Hz, 2H, 2ArH), 8.10 (d,J = 7.8 Hz, 1H, H-4), 8.21 (d, J = 7.8 Hz, 1H, H-7), 8.69 (br, 1H, NH), 8.72 (br, 1H, NH), 11.18 (s, 1H, NH); FABMS: 387 (MH^+).

*2.5. 2-[4-Amino-2-(4-methoxyphenylamino)thiazol-5-oyl]benzothiazole **3c***

Yield 63%, m.p. 254–55 °C Analysis: Found: C, 56.30: H, 3.75: N, 14.80%; Calc. for $C_{18}H_{14}N_4O_2S_2$ (382.46): C, 56.52: H, 3.69: N, 14.65%; IR (KBr) cm^{-1}: 3455, 3293, 3187, 3067, 2931, 2842, 1617, 1537, 1468, 1324, 1261, 1186, 1102, 1026, 897, 828, 755, 728, 690;^{1}H NMR: (300 MHz, DMSO-d_6)δ: 3.76 (s, 3H, OCH_3), 6.98 (d, J = 9 Hz, 2H, 2ArH), 7.47–7.66 (m, 4H, H-5, H-6, 2ArH), 8.08 (d, J = 7.8 Hz, 1H, H-4), 8.19 (d, J = 7.5 Hz, 1H, H-7), 8.57 (br, 1H, NH), 8.78 (br, 1H, NH), 10.92 (s, 1H, NH); FABMS: 383 (MH^+).

*2.6. 2-[4-Amino-2-(4-ethoxyphenylamino)thiazol-5-oyl]benzothiazole **3d***

Yield 65%, m.p. 258–60 °C Analysis: Found: C, 57.80: H, 4.18: N, 14.36%; Calc. for $C_{19}H_{16}N_4O_2S_2$ (396.48): C, 57.55: H, 4.07: N, 14.13%; IR (KBr) cm^{-1}: 3461, 3299, 2982, 2935, 1620, 1529, 1479, 1445, 1324, 1255, 1175, 1104, 1054, 899, 831, 763, 729;^{1}H NMR: (300 MHz, DMSO-d_6)δ: 1.33 (t, J = 6.9 Hz, 3H, CH_3), 4.03 (quartet,J = 6.9 Hz, 2H, CH_2), 6.96 (d, J = 9 Hz, 2H, 2ArH), 7.50–7.68 (m, 4H, H-5, H-6, 2ArH), 8.08 (d, J = 6 Hz, 1H, H-4), 8.20 (d, J = 6 Hz, 1H, H-7), 8.56 (br, 1H, NH), 8.76 (br, 1H, NH), 10.89 (s, 1H, NH); FABMS: 397 (MH^+).

*2.7. 2-[4-Amino-2-(4-methylphenylamino)thiazol-5-oyl]benzothiazole **3e***

Yield 60%, m.p. 282–85 °C Analysis: Found: C, 58.74: H, 3.73: N, 15.43%; Calc. for $C_{18}H_{14}N_4OS_2$ (366.46): C, 58.99: H, 3.85: N, 15.29%; IR (KBr) cm^{-1}: 3457, 3279, 3130, 3082, 2927, 2861, 1625, 1605, 1526, 1465, 1357, 1256, 1189, 1020, 892, 814, 761, 752, 621;^{1}H NMR: (300 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 7.20 (d, J = 8.1 Hz, 2H, 2ArH), 7.50–7.68(m, 4H, H5, H-6, 2ArH), 8.08 (d,J = 7.8 Hz, 1H, H-4), 8.20 (d,J = 7.8 Hz, 1H, H-7), 8.60 (br, 1H, NH), 8.75 (br, 1H, NH), 10.98 (s, 1H, NH); FABMS: 367 (MH^+).

*2.8. 2-(4-Amino-2-ethylaminothiazol-5-oyl)benzothiazole **3f***

Yield:65%,m.p. 255–56 °C Analysis: Found: C, 51.41: H, 3.90: N,18.55%; Calc. for $C_{13}H_{12}N_4OS_2$ (304.39): C, 51.29: H, 3.97: N, 18.41%; IR (KBr) cm^{-1}: 3467, 3285, 3233, 3175, 3067, 2972, 2928, 2850, 1623, 1592, 1558, 1450, 1351, 1093, 882, 818, 757, 722;^{1}H NMR: (300 MHz, DMSO-d_6) δ: 1.18 (t, J = 7.0 Hz, 3H, CH_3), 3.35 (br, 2H, CH_2), 7.45–7.62 (m, 2H, H-5, H-6),8.07 (d, J = 7.8 Hz, 1H, H-4), 8.16 (d, J = 7.8 Hz,

1H, H-7), 8.39 (br, 1H, NH), 8.78 (br, 1H, NH), 8.94 (br, 1H, NH); FABMS: 305 (MH^+).

2.9. 2-(4-Amino-2-n-propylaminothiazol-5-oyl)benzothiazole **3g**

Yield: 63%, m.p. 211–13 °C; Analysis: Found: C, 52.95: H, 4.58: N, 17.45%; Calc. for $C_{14}H_{14}N_4OS_2$ (318.42): C, 52.80: H, 4.43: N, 17.60%; IR (KBr) cm^{-1}: 3360, 3218, 3134, 3067, 2967, 2933, 2867, 1639, 1592, 1552, 1506, 1472, 1357, 1155,1093, 891, 823, 778, 683, 622; ^1H NMR: (300 MHz, DMSO-d_6) δ: 0.91 (t, $J = 7.4$ Hz, 3H, CH_3), 1.58 (sextet, $J = 6.7$ Hz, 2H, CH_2), 3.38 (br, 2H, CH_2),7.45–7.63 (m, 2H, H-5, H-6), 8.06 (d, $J = 6.9$ Hz, 1H, H-4), 8.16 (d, $J = 7.5$ Hz, 1H, H-7), 8.40(br, 1H, NH), 8.79 (br, 1H, NH), 8.95 (br, 1H, NH); ^{13}C NMR: (75 MHz, DMSO-d_6) δ: 11.3, 21.9, 39.2, 91.1, 122.8, 123.8, 126.4, 126.8, 135.8, 139.3, 153.1, 169.5, 170.6, 171.3; FABMS: 319 (MH^+).

2.10. 2-(4-Amino-2-n-butylaminothiazol-5-oyl)benzothiazole **3h**

Yield: 65%, m.p. 182–185 °C; Analysis: Found: C, 54.33: H, 4.93: N, 16.59; Calc. for $C_{15}H_{16}N_4OS_2$ (332.44): C, 54.19: H, 4.85: N, 16.85%; IR (KBr) cm^{-1}: 3352, 3279, 3198, 3162, 3050, 2962, 2917, 2858, 1634, 1600, 1539, 1465, 1357, 1309, 1152, 1081, 891, 818, 771, 737, 612; ^1H NMR: (300 MHz, DMSO-d_6) δ: 0.90 (t, $J = 7.4$ Hz, 3H, CH_3), 1.35 (sextet, $J = 7.3$ Hz, 2H, CH_2), 1.51 (quintet, $J = 7.1$ Hz, 2H, CH_2), 3.33 (br, 2H, CH_2), 7.45–7.64 (m, 2H, H-5, H-6), 8.06 (d, $J = 7.8$ Hz, 1H, H-4), 8.17(d, $J = 7.5$ Hz, 1H, H-7), 8.42 (br, 1H, NH), 8.92 (br, 1H, NH), 9.00 (br, 1H, NH); FABMS: 333 (MH^+).

2.11. 2-(2-Allylamino-4-aminothiazol-5-oyl)benzothiazole **3i**

Yield: 63%, m.p. 254–55 °C; Analysis: Found: C, 53.29: H, 3.91: N, 17.57%; Calc. for $C_{14}H_{12}N_4OS_2$ (316.40): C, 53.14: H, 3.82: N, 17.71%; IR (KBr) cm^{-1}:3486, 3299, 3238, 3083, 3050, 2967, 2933, 2894, 2842, 1626, 1599, 1565, 1506, 1458, 1322, 1094, 1013, 958, 891, 825, 764, 729; ^1H NMR (300 MHz, DMSO-d_6) δ: 4.02 (m, 2H, CH_2), 5.11–5.32(m, 2H, CH_2), 5.82–6.00 (m, 1H, CH), 7.45–7.64 (m, 2H, H-5, H-6), 8.07 (d, $J = 7.8$ Hz, 1H, H-4), 8.17 (d, $J = 7.5$ Hz, 1H, H-7), 8.43 (br, 1H, NH), 8.77 (br, 1H, NH), 9.09 (br, 1H, NH); FABMS: 317 (MH^+).

3. Biological evaluation

3.1. Antimicrobial activity

The disk diffusion test was performed using standard procedures. The inoculum suspension of each bacterial strain was swabbed on the entire surface of Mueller–Hinton agar plates (MHA, pH 7.3 ± 0.1, HiMedia). Sterile 6-mm filter paper disks, which were previously impregnated with the compounds (**3a–i**) dissolved in the solvent ethyl acetate, were aseptically placed on MHA surfaces. Sterile paper disks impregnated with 10% DMSO were used as the negative controls, whereas a disk containing penicillin was placed in the plate as a positive control. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37 °C for 24 h. Diameters of inhibition zones were measured.

In vitro antimicrobial activity was evaluated against eight pathogenic microorganisms: *Pseudomonas* sp. MTCC-*6538*, *Escherichia coli MTCC-1671*, *Klebsiella* sp. MTCC-7407, *Bacillus* sp. *MTCC*-1134, *Streptococcus* sp. MTCC-1936 and fungal strains of *Penicillium* sp. IC-201211 and *Aspergillus niger* IC-281011. For *Mycobacterium tuberculosis* MB- H37Rv, 1% cetrimide agar was used as the substrate and sputum swab containing microbial population was made in the plate. Then the disks impregnated with compounds were placed in the plates and the zone of inhibition was measured.

Table 2 Zones of inhibition of compounds **3a**–i, standard (Flucanazole) with different fungal strains.

Compound	Zone of inhibition (mm)	
	Penicillium	*Aspergillus niger*
3a	9	10
3b	11	9
3c	10	8
3d	9	9
3e	9	11
3f	11	10
3g	10	8
3h	8	8
3i	9	9
Flucanazole	12	12

NA Not active.

Table 1 Zones of inhibition of compounds **3a–i**, standard (penicillin) with different bacterial strains.

Compound	Zone of inhibition(mm)					
	E. coli	*Klebsilla*	*Bacillus*	*Streptococcus*	*Staphyllococcus*	*Pseudomonas*
3a	13	12	8	7	11	10
3b	13	14	10	11	14	13
3c	8	NA	NA	NA	NA	NA
3d	9	NA	NA	NA	NA	NA
3e	8	NA	NA	NA	NA	NA
3f	NA	NA	NA	NA	NA	NA
3g	8	NA	NA	NA	NA	NA
3h	9	NA	NA	NA	NA	NA
3i	NA	NA	NA	NA	NA	NA
Control	15	12	12	12	10	10

NA Not active.

Table 3 Zones of inhibition of compounds **3a–i**, with mycobacterium tuberculosis.

Compound	Zone of inhibition (mm)				
	0.5 mg	1 mg	1.5 mg	2 mg	Control
3a	NA	NA	2	3	3
3b	NA	1	2	4	3
3c	NA	NA	NA	2	3
3d	3	5	6	8	3
3e	NA	3	4	4	2
3f	3	6	7	8	3
3g	NA	NA	NA	NA	NA
3h	NA	NA	2	2	4
3i	2	3	3	5	2

NA Not Active.

Table 4 2-(4-Amino-2-aryl/alkylaminothiazol-5-oyl)benzothiazoles **3a–i**.

Compound	*R*
3a	C_6H_5–
3b	4-ClC_6H_4–
3c	4-$MeOC_6H_4$–
3d	4-$EtOC_6H_4$–
3e	4-MeC_6H_4–
3f	C_2H_5–
3g	N-C_3H_7–
3h	N-C_4H_9–
3i	Allyl–

4. Results and discussion

The structures of all the compounds were established on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and tested for in vitro antimicrobial activity. The antibacterial and antifungal screening results of these compounds are shown in Table 1 and Table 2 'respectively'. The drug susceptibility test against *Mycobacterium tuberculosis* is shown in Table 3.

From the above-mentioned results, it may be concluded that the derivatives of benzothiazoles possess moderate to potent antimicrobial activity. Compounds **3a** and **3b** were found to be more effective against all bacterial strains and most of the compounds were active against *E. coli*. All the compounds were found to have moderate antifungal activity. When tested against *M. tuberculosis*, compounds **3d**, **3f**, and **3i** showed the maximum activity when compared with control. Compounds **3b**, **3e**, and **3h** showed moderate activity. Thus the study ascertains the value of benzothiazole drugs which could be of considerable interest for the development of new drugs.

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