Asian Journal of Chemistry Vol. 20, No. 2 (2008), 992-998

Synthesis and Antimicrobial Activity of 1-(Benzothiazol-2'-yl)-5-phenyl-tetrazole

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In the present study, a new series of 1-(benzothiazol-2'-yl)-5-phenyl-tetrazole were synthesized by the reaction of Schiff base (2-aminobenzothiazole and substituted benzaldehyde) with phosphorous pentachloride and sodium azide. The chemical structures of the synthesized compounds were confirmed by means of IR, ^{1}H NMR, mass spectral and elemental analysis. The synthesized compounds were screened for antibacterial (*Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*), antifungal (*Aspergillus niger* and *Candida albicans*). The synthesized compounds exhibited good antibacterial and significant antifungal activity at the dose of 250 µg/mL by Paper disc diffusion method.

Key Words: Tetrazole, Benzothiazole, Analgesic, Antibacterial, Antifungal.

INTRODUCTION

Tetrazole derivatives were reported to possess antibacterial[1], antifungal1[2], virucidal[3], antipyretic[4], analgesic[5], antiinflammatory[5], anticonvulsant[6], antiallergic[7], bronchodilator[7], anorectic agent[8] and MAO inhibitory activity[9]. Benzothiazole derivatives were reported to possess antibacterial[10], antifungal[10], analgesic[11], antiinflammatory[12,13], anticonvulsant[14], antihistaminic[15], anthelmintic[16], antitumor[17] and localanaesthetic[18] activities. Therefore, it was envisaged that compounds containing both the chemical moieties would result in compounds of interesting biological activities. In this present study 2-aminobenzothiazole were treated with different substituted aromatic aldehydes to produce Schiff's base[19]. The Schiff bases were chlorinated with phosphorous pentachloride and cycloaddition reactions with sodium azide to produce tetrazole derivatives[20]. The chemical structures of the synthesized compounds were confirmed by means of IR,

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^{1}H NMR, mass spectral and elemental analysis. The synthesized compounds were screened for antibacterial (*Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*) and antifungal (*Aspergillus niger* and *Candida albicans*).

EXPERIMENTAL

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB104 with KBr pellets. ^{1}H NMR spectra were recorded on 400 MHz-Joel GSX 400 using DMSO-d_6 as solvent. The chemical shifts are reported as ppm downfield from tetra methyl silane. Mass spectra were recorded on Shimadzu GC-MS QP 5050A. Microanalyses for C, H, N were performed in Heraeus CHN Rapid Analyzer and analyses indicated by the symbols of the elements are within ± 0.4 % of the theoretical values. ^{1}H NMR and IR spectra were consistent with the assigned structures. The purity of the compounds was checked by TLC on pre-coated aluminum sheets (Silica gel 60 F_{254}) using (4:1) CCl_4: petroleum ether (40-60 °C) as mobile phase and visualized by iodine vapours.

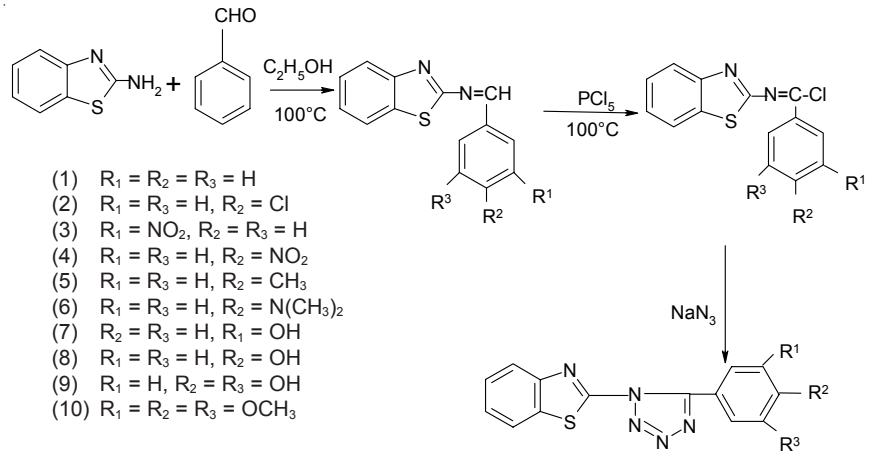
General method for the synthesis of Schiff base: A mixture of 2-aminobenzothiazole (0.01 mol), substituted benzaldehyde (0.01 mol) and a drop of acetic acid was dissolved in ethanol (25 mL) and heated on a steam bath for 45-60 min. The reaction mixture was allowed to stand at room temperature for 24 h. The product separated out was filtered, dried under vacuum and recrystallized by using warm ethanol.

General procedure for the preparation of tetrazole: Schiff's base (0.004 mol) and PCl_5 (0.004 mol) was heated at 100 °C for 1 h. When the evolution of fumes of HCl ceased, excess of PCl_3 was removed under reduced pressure and the residual imidoyl chloride was treated with an ice-cold solution of sodium azide (0.0075 mol) in water (25 mL), sodium acetate (0.004 mol) and acetone (30 mL) with stirring. Stirring was continued for overnight, there after acetone was removed under reduced pressure. The remaining aqueous portion was extracted with chloroform and dried (**Scheme-I**).

1-(Benzothiazol-2'-yl)-5-phenyl-tetrazole (1): Yield = 71 %, m.p. 98-100 ºC, R_f =0.65. ^{1}H NMR (DMSO-d_6) δ: 7.94 (m, 2H; 4',7'-H), 7.65 (m, 2H; 5',6'-H), 6.89-7.68 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3384, 2102, 1529, 1157 (tetrazole), 741, 719. EI-MS m/z: 279.30 (Calcd. for $C_{14}H_9N_5S$: 279.32) Anal. Calcd. for $C_{14}H_9N_5S$: C, 60.19; H, 3.25; N, 25.08. Found: C, 60.29; H, 3.28; N, 25.13.

1-(Benzothiazol-2'-yl)-5-(4-chlorophenyl)tetrazole (2): Yield = 70 %, m.p. 108-110 ºC, R_f = 0.66. ^{1}H NMR (DMSO-d_6) δ: 8.15 (m, 2H; 4', 7'-H), 7.90 (m, 2H; 5',6'-H), 6.97-7.78 (m, 5H; 2",3",4",5",6"-H). IR (KBr,

cm^{-1}): 3384, 2104, 1528, 1158 (tetrazole), 741, 719, 541. EI-MS m/z: 313.80 (m.w. 313.76). Anal. (%) calcd. for $C_{14}H_8N_5SCl$: C, 53.58; H, 2.57; N, 22.33. Found: C, 53.74; H, 2.61; N, 22.48.



Scheme-I. Synthetic scheme of 1-(benzothiazol-2'-yl)-5-phenyl-tetrazole

1-(Benzothiazol-2'-yl)-5-(3-nitrophenyl)tetrazole (3): Yield = 30 %, m.p. 178-180 ºC, R_f = 0.63. ^{1}H NMR (DMSO-d_6) δ: 7.87(m, 2H; 4', 7'-H), 7.64 (m, 2H; 5',6'-H), 7.01-7.46 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3384, 2102, 1535,1347 (NO_2), 1158 (tetrazole), 751, 726. EI-MS m/z: 324.30 (m.w. 324.32). Anal. (%) calcd. for $C_{14}H_8N_6O_2S$: C, 51.84; H, 2.48; N, 25.92. Found: C, 51.80; H, 2.42; N, 25.84.

1-(Benzothiazol-2'-yl)-5-(4-nitrophenyl)tetrazole (4): Yield = 66 %, m.p. 238-240 ºC, R_f = 0.57. ^{1}H NMR (DMSO-d_6) δ: 8.41(m, 2H; 4', 7'-H), 8.00 (m, 2H; 5',6'-H), 6.91-7.96 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3384, 2110, 1523, 1347 (NO_2), 1158 (tetrazole), 764, 750. EI-MS m/z: 324.28 (m.w. 324.32). Anal. (%) calcd. for $C_{14}H_8N_6O_2S$: C, 51.84; H, 2.48; N, 25.92. Found: C, 51.76; H, 2.54; N, 25.98.

1-(Benzothiazol-2'-yl)-5-*p*-tolyl-tetrazole (5): Yield = 80 %, m.p. 120-122 ºC, R_f = 0.81. ^{1}H NMR (DMSO-d_6) δ: 7.99 (m, 2H; 4', 7'-H), 7.62 (m, 2H; 5',6'-H), 6.82-7.46 (m, 5H; 2",3",4",5",6"-H), 2.48 (s, 3H; CH_3). IR (KBr, cm^{-1}): 3384, 2106, 1527, 1158 (tetrazole), 741, 720. EI-MS m/z: 293.38 (m.w. 293.35). Anal. (%) calcd. for $C_{15}H_{11}N_5S$: C, 61.41; H, 3.77; N, 23.88. Found: C, 61.52; H, 3.82; N, 23.95.

1-(Benzothiazol-2'-yl)-5-(4-aminodimethylphenyl)tetrazole (6): Yield = 62 %, m.p. 174-176 ºC, R_f = 0.79. ^{1}H NMR (DMSO-d_6) δ: 8.25 (m, 2H; 4', 7'-H), 8.01 (m, 2H; 5',6'-H), 7.25-7.79 (m, 5H; 2",3",4",5",6"-H), 3.57 (ss, 6H; 2-CH_3). IR (KBr, cm^{-1}): 3384, 2110, 1532, 1157 (tetrazole), 763, 754. EI-MS m/z: 322.40 (m.w. 322.39) Anal. (%) calcd. for $C_{16}H_{14}N_6S$: C, 59.60; H, 4.37; N, 26.07. Found: C, 59.67; H, 4.28; N, 26.16.

1-(Benzothiazol-2'-yl)-5-(3-hydroxyphenyl)tetrazole (7): Yield = 57 %, m.p. 118-120 ºC, R_f = 0.58. ^{1}H NMR (DMSO-d_6) δ: 10.59 (s, 1H; OH), 7.63 (m, 2H; 4', 7'-H), 7.48 (m, 2H; 5',6'-H), 6.97-7.33 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3607 (OH), 3384, 2105, 1527, 1156 (tetrazole), 741, 719. EI-MS m/z: 295.30 (m.w. 295.32). Anal. (%) calcd. for $C_{14}H_9N_5OS$: C, 56.93; H, 3.07; N, 23.72. Found: C, 56.89; H, 3.12; N, 23.78.

1-(Benzothiazol-2'-yl)-5-(4-hydroxyphenyl)tetrazole (8): Yield = 67 %, m.p. 210-212 ºC, R_f = 0.59. ^{1}H NMR (DMSO-d_6) δ: 10.45 (s, 1H; OH), 8.45 (m, 2H; 4', 7'-H), 8.19 (m, 2H; 5',6'-H), 7.49-7.88 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3648 (OH), 3384, 2102, 1519, 1156 (tetrazole), 751, 725. EI-MS m/z: 295.30 (m.w. 295.32). Anal. (%) calcd. for $C_{14}H_9N_5OS$: C, 56.93; H, 3.07; N, 23.72. Found: C, 56.88; H, 3.10; N, 23.82.

1-(Benzothiazol-2'-yl)-5-(3,4-dihydroxyphenyl)tetrazole (9): Yield = 73 %, m.p. 128-130 ºC, R_f = 0.50. ^{1}H NMR (DMSO-d_6) δ: 10.75 (s, 1H; OH), 9.78 (s, 1H; OH), 7.92(m, 2H; 4', 7'-H), 7.66 (m, 2H; 5',6'-H), 6.93-7.42 (m, 5H; 2",3",4",5",6"-H). IR (KBr, cm^{-1}): 3647 (OH), 3583 (OH), 2104, 1527, 1156 (tetrazole), 741, 719. EI-MS m/z: 311.30 (m.w. 311.32). Anal. (%) calcd. for $C_{14}H_9N_5O_2S$: C, 54.00; H, 2.91; N, 22.50. Found: C, 54.12; H, 2.96; N, 22.56.

1-(Benzothiazol-2'-yl)-5-(3,4,5-trimethoxyphenyl)tetrazole (10): Yield = 48 %, m.p. 120-122 ºC, R_f = 0.53. ^{1}H NMR (DMSO-d_6) δ: 8.25 (m, 2H; 4', 7'-H), 7.90 (m, 2H; 5',6'-H), 6.95-7.68 (m, 5H; 2",3",4",5",6"-H), 3.05 (s, 3H; 3-OCH_3), 2.95 (s, 3H; 4-OCH_3), 2.85 (s, 3H; 5-OCH_3). IR (KBr) cm^{-1}: 3506, 3384, 3048, 2102, 1537, 1158 (tetrazole), 751, 723. EI-MS m/z: 369.40 (m.w. 369.39). Anal. (%) calcd. for $C_{17}H_{15}N_5O_3S$: C, 52.27; H, 4.09; N, 18.96. Found: C, 52.32; H, 4.14; N, 19.08.

Antimicrobial activity: The antibacterial activity[21] of the synthesized compounds was tested against gram(+) bacteria (*Staphylococcus aureus* NCCS 2079 *Bacillus cereus* NCCS 2106) and gram(-) bacteria (*Escherichia coli* NCCS2065 and *Pseudomonas aeruginosa* NCCS2200) using nutrient agar medium and fungi (*Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 3471) using sabourand dextrose agar medium.

Paper disc diffusion method: The sterilized (autoclaved at 120 °C for 0.5 h), liquified medium (40-50 °C) was inoculated (1 mL/100 mL of medium) with the suspension of the microorganism (matched to McFarland barium sulphate standard) and poured into the petri dish to give a depth of 3-4 mm. The paper discs impregnated with the test compounds (250 µg/mL for antibacterial & 250 µg/mL for antifungal activity using dimethyl sulphoxide as solvent) were placed on the solidified medium. The plates were refrigerated (pre-incubated) for 2 h at 4 °C and then incubated at 37 °C for 24 and 48 h for antibacterial and antifungal activity, respectively at the end of which the zone of inhibition was observed (Tables 1 and 2).

Amoxycillin (10 µg/disc), cefaclor (30 µg/disc) and fluconazole (100 µg/disc) were used as standards. The data are presented in Table-1.

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE COMPOUNDS

Compd.	Antibacterial activity†				Antifungal activity‡	
	SA	BC	EC	PA	AN	CA
1	17 (70)	22 (75)	22 (70)	22 (90)	21	23
2	21 (50)	24 (60)	25 (50)	23 (60)	16	17
3	20 (60)	23 (70)	26 (50)	25 (50)	13	16
4	18 (85)	24 (50)	25 (60)	24 (55)	18	21
5	20 (70)	23 (60)	24 (65)	23 (60)	20	23
6	16 (90)	21 (100)	21 (85)	21 (100)	19	20
7	18 (80)	22 (90)	22 (80)	21 (90)	14	16
8	20 (80)	24 (60)	22 (75)	23 (70)	16	18
9	18 (75)	22 (80)	22 (90)	22 (90)	16	18
10	17 (100)	21 (90)	19 (100)	21 (90)	21	23
Cefaclor	19	22	19	20	-	-
Amoxycillin	21	27	24	22	-	-
Fluconazole	-	-	-	-	22	25
Control	-	-	-	-	-	-

SA = *S. aureus*; BC = *B. cereus*; EC = *E. coli*; PA = *P. aeruginosa*;
AN = *A. niger*; CA = *C. albicans*
Zone of Inhibition in ‡mm and †MIC in µg/mL

Minimum inhibitory concentration: The minimum inhibitory concentration[22] (MIC) against the bacterial strains was determined by the test tube dilution technique using Mueller-Hinton nutrient broth. A series of glass tubes containing different concentrations of the synthesized compounds (in dimethyl sulphoxide) with the medium was inoculated with the required amount of inoculum to obtain a suspension of microorganism, which contains 105 CFU/mL. One growth control tube was prepared without the addition of microorganism. The tubes were incubated at 37 °C for

24 h. The minimum inhibitory concentration (MIC-μg/mL) was considered to be the lowest concentration that exhibited the same turbidity as the blank tube. The data are presented in Table-1.

RESULTS AND DISCUSSION

The structure of the synthesized compounds was characterized by IR, ^{1}H NMR, mass spectral and elemental analysis. All the synthesized compounds exhibited good antibacterial activity against *S. aureus, B. cereus, E. coli* and *P. aeruginosa* and significant antifungal activity by paper disc diffusion method (*A. niger* and *C. albicans*). The MIC of the compounds **1** to **10** for *S. aureus* (50-100 μg/mL), *B. cereus* (50-100 μg/mL), *E. coli* (50-100 μg/mL) and *P. aeruginosa* (50-100 μg/mL). Compounds **2**, **3**, **4** and **8** exhibited highest activity against *S. aureus, B. cereus, E. coli* and *P. aeruginosa*, respectively. 1-(Benzothiazol-2'-yl)-5-(4-chlorophenyl)-tetrazole exhibited highly significant antibacterial activity against all tested organism. 1-(Benzothiazol-2'-yl)-5-phenyl-tetrazole (**1**), 1-(benzothiazol-2'-yl)-5-*p*-tolyl-tetrazole (**5**) and 1-(benzothiazol-2'-yl)-5-(3,4,5-trimethoxy phenyl)-tetrazole (**10**) exhibited highly significant antifungal activity against *A. niger* and *C. albicans* at the concentration 250 μg/mL by paper disc diffusion method.

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(*Received*: 1 January 2007; *Accepted*: 29 September 2007) AJC-5929