Chem Sci Trans., 2013, **2(2),** 473-478
DOI:10.7598/cst2013.414

Chemical Science Transactions
ISSN/E-ISSN: 2278-3458/2278-3318

RESEARCH ARTICLE

Synthesis, Structural Study and Antimicrobial Screening of Substituted Bis-benzothiazole Derivatives

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Received 20 October 2012 / Accepted 12 November 2012

Abstract: A series of {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-6-substituted-benzothiazol-2-yl-amines have been synthesized by oxidative cyclization of 1-{4-[4-(3-aryl thiocarbamido)-3-carboxy-benzyl]-2-carboxy-phenyl}-3-aryl thiocarbamides using the solution of bromine in chloroform followed by the basification with dilute ammonium hydroxide solution. Initially 1-{4-[4-(3-aryl thiocarbamido)-3-carboxy-benzyl]-2-carboxy-phenyl}-3-aryl thio-carbamides were prepared by the interaction of different aryl isothiocyanates with 4,4'-methylene-bis-(anthranilic acid). The latter was obtained by treating the mixture of anthranilic acid and concentrated hydrochloric acid with 3% aqueous formaldehyde followed by neutralization with sodium hydroxide. The acetylation of bis-benzothiazoles afforded di-acetyl derivatives. The structures of synthesized compounds have been established on the basis of chemical transformation, elemental analysis, equivalent weight determination and IR, ^{1}H NMR spectral studies. The title compounds have been assayed for their biological activity against gram-positive as well as gram-negative microorganisms.

Keywords: Synthesis, Structural study, Antimicrobial screening, Bis-benzothiazoles

Introduction

During this decade there has been considerable interest in the synthesis of substituted or fused benzothiazoles, because benzothiazole motif is an important skeleton in naturally occurring biologically active compounds. In addition to this, benzothiazole is having potent biological properties such as antitumor[1,2], antimicrobial[3,4] and LTD[4] receptor antagonist like orexin[5]. Benzothiazole derivatives exhibit antifungal[6,7], anti-inflammatory[8,9], antitubercular[10] and muscle relaxant[11] activities. Numbers of benzothiazole are known for their manifold medicinal property particularly as antibacterial agents[12,13]. Benzothiazole derivatives are also used as vasodilator[14] and schistosomicidal agents[15]. Benzothiazoles are the most active heterocyclic compounds having wide range of pharmacological activities. The literature survey reveals the various substituted benzothiazoles possessing wide spectrum of therapeutic activities[16,17]. Aryl benzothiazoles bearing a substituent at C_2 are of great interest as these structural frame works have proved to be important class of bicylic privileged sub-

structures owing to their utility as imaging agents for *β*-amyloid, chemiluminescent agents, anti-tumour agents, calcium channel antagonists, antituberculosis, antiparasitics and also as photosensitizers[5,18]. In view of these observations about the utility of fused heterocyclic compounds in different fields and as a part of wider programme to provide alternative routes for the synthesis of heterocyclic compounds, we report herein the synthesis of substituted bis-benzothiazole systems using reagents *N*-aryl isothiocyanates followed by the oxidative cyclization using bromine in chloroform.

Experimental

The melting points of all synthesized compounds were recorded using hot paraffin-bath and are uncorrected. Chemicals used were of AR grade. ^{1}H-NMR spectra were recorded with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1} in nujol mull and as KBr pellete. Purity of the compounds was checked on silica gel-G plates by TLC.

*Synthesis of 4,4'-methylene-bis-(anthranilic acid) (**3**)*

The parent compound 4,4'-methylene-bis-(anthranilic acid) **3** was prepared by dissolving anthranilic acid **1** (0.01 mole) in distilled water (15 mL) and 36.5% hydrochloric acid (2.5 mL) at 50 ^{0}C. The mixture was then treated with 3% aqueous formaldehyde **2** (3.5 mL) at 20 ^{0}C with stirring for 1 h and neutralized with 10% sodium hydroxide solution, light yellow precipitate was obtained. It was washed with hot water and crystallized from acetic acid, **3** (82%), m.p. 232 ^{0}C. (Found: C, 61.99; H, 4.83; N, 9.73. Calcd. for $C_{15}H_{14}N_2O4$: C, 62.93; H, 4.89; N, 9.79%).

*Synthesis of 1-{2-carboxy-4-[3-carboxy-4-(3-phenyl thiocarbamido)-benzyl]-phenyl} -3-phenyl thiocarbamide (**4a**)*

A mixture of 4,4'-methylene-bis-(anthranilic acid) **3** (0.01 mole) and phenyl isothiocyanate (0.02 mole) in benzene (15 mL) was refluxed for 1.5 h. Then benzene was distilled off, a solid mass was obtained. It was washed with petroleum ether (60-80 ^{0}C) and crystallized from ethanol to yield 1-{2-carboxy-4-[3-carboxy-4-(3-phenyl thiocarbamido)-benzyl]-phenyl}-3-phenyl thiocarbamide **4a**, (78%), m.p. 210 ^{0}C. (Found: N, 9.62; S, 11.41. Calcd. for $C_{29}H_{24}N_4O_4S_2$: N, 10.07; S, 11.51%); ν_{max} 3482, 3412 (NH), 3151 (OH), 1689 (C=O), 1321 (C-N), 1227 cm^{-1} (C=S); δ ($CDCl_3$+DMSO-d_6) 8.07 (2H, s, OH), 6.45-8.11 (16H, m, Ar-H), 4.31 (2H, s, NH), 3.91 (2H, s, NH), 2.42 (2H, s, CH_2)[23,24]. This reaction was extended to synthesize other compounds **4b-g**: **4b** (70%), m.p. 240 ^{0}C (Found: N, 9.42; S, 10.88. Calcd. for $C_{31}H_{28}N_4O_4S_2$: N, 9.58; S, 10.95); **4c** (68%), m.p. 222 ^{0}C (Found: N, 9.52; S, 10.85. Calcd. for $C_{31}H_{28}N_4O_4S_2$: N, 9.58; S, 10.95); **4d** (75%), m.p. 205 ^{0}C (Found: N, 9.37; S, 10.61. Calcd. for $C_{31}H_{28}N_4O_4S_2$: N, 9.58; S, 10.95); **4e** (72%), m.p. 234 ^{0}C (Found: N, 8.87; S, 10.05. Calcd. for $C_{29}H_{22}N_4O_4S_2Cl_2$: N, 8.96; S, 10.24%); **4f** (70%), m.p. 178 ^{0}C (Found: N, 8.66; S, 10.21. Calcd. for $C_{29}H_{22}N_4O_4S_2Cl_2$: N, 8.96; S, 10.24%); **4g** (76%), m.p. 195^{0}C (Found: N, 8.76; S, 10.10. Calcd. for $C_{29}H_{22}N_4O_4S_2Cl_2$: N, 8.96; S, 10.24%).

*Synthesis of {4-[4-(benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-benzothiazol-2-yl-amine (**5a**)*

A paste of 1-{2-carboxy-4-[3-carboxy-4-(3-phenyl thiocarbamido)-benzyl]-phenyl}-3-phenyl thiocarbamide **4a** (1.5 g) was prepared in benzene. To this a solution of bromine in chloroform was added drop by drop with constant stirring. The colour of bromine was initially disappeared. The addition was continued till brown colour of bromine persisted.

The reaction mixtute was left overnight at room temperature. The separated solid was crystallized by ethanol. It was acidic to litmus and on determination of equivalent weight found to be {4-[4-(benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-benzothiazol-2-yl-amine hydrobromide, m.p. 178 ^{0}C. It on basification with dilute ammonium hydroxide solution afforded a free base. It was crystallized from ethanol and identified as {4-[4-(benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-benzothiazol-2-yl-amine **5a** (80%), m.p. 226 ^{0}C. (Found: C, 62.55; H, 3.57; N, 9.78; S, 11.49. Calcd. for $C_{29}H_{20}N_4O_4S_2$: C, 63.04; H, 3.62; N, 10.14; S, 11.59%); ν_{max} 3473, 3464 (NH), 3135 (OH), 1667 (C=O), 1542 (C=N), 1400 (C-N), 703 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 8.11 (2H, s, OH), 6.58-7.93 (14H, m, Ar-H), 3.67 (2H, s, NH), 2.56 (2H, s, CH_2). This reaction was extended to synthesize other compounds **5b-g**: **5b** (70%), m.p.218 ^{0}C (Found: C, 63.77; H, 4.10; N, 9.54; S, 10.86. Calcd. for $C_{31}H_{24}N_4O_4S_2$: C, 64.13; H, 4.13; N, 9.65; S, 11.03%); ν_{max} 3465, 3442 (NH), 3123 (OH), 1693 (C=O), 1531 (C=N), 1378 (C-N), 698 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 8.13 (2H, s, OH), 6.76-7.71 (12H, m, Ar-H), 4.16 (2H, s, NH), 2.56 (2H, s, CH_2), 2.29 (6H, s, Ar-CH_3); **5c** (74%), m.p. 189^{0}C (Found: C, 64.12; H, 4.01; N, 9.67; S, 10.85. Calcd. for $C_{31}H_{24}N_4O_4S_2$: C, 64.13; H, 4.13; N, 9.65; S, 11.03%); **5d** (80%), m.p. 160 ^{0}C (Found: C, 63.83; H, 4.05; N, 9.71; S, 10.97. Calcd. for $C_{31}H_{24}N_4O_4S_2$: C, 64.13; H, 4.13; N, 9.65; S, 11.03%); **5e** (65%), m.p. 172 ^{0}C (Found: C, 55.56; H, 2.79; N, 8.88; S, 10.28. Calcd. for $C_{29}H_{18}N_4O_4S_2Cl_2$: C, 56.03; H, 2.89; N, 9.01; S, 10.30%); **5f** (70%), m.p. 153 ^{0}C (Found: C, 55.42; H, 2.75; N, 8.75; S, 10.21. Calcd. for $C_{29}H_{18}N_4O_4S_2Cl_2$: C, 56.03; H, 2.89; N, 9.01; S, 10.30%); **5g** (78%), m.p. 170 ^{0}C (Found: C, 55.94; H, 2.82; N, 9.07; S, 10.33. Calcd. for $C_{29}H_{18}N_4O_4S_2Cl_2$: C, 56.03; H, 2.89; N, 9.01; S, 10.30%).

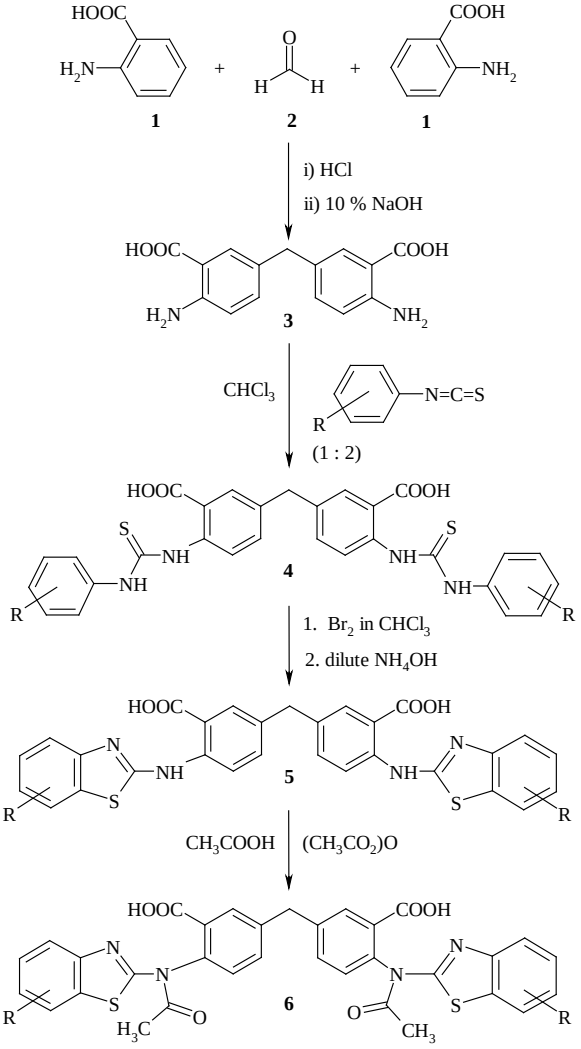
*Synthesis of {4-[4-(benzothiazol-2-yl-acetamido)-3-carboxy-benzyl]-2-carboxy-phenyl} -benzothiazol-2-yl-acetamide (**6a**)*

A mixture of {4-[4-(benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-benzothiazol-2-yl-amine **5a** (0.01 mole) and acetic anhydride (0.02 mole) in glacial acetic acid (15 mL) was refluxed for 2 h. The reaction mixture was cooled and poured in a little crushed ice with water, a faint yellow coloured solid precipitated was crystallised from aqueous ethanol to give **6a** (76%), m.p.166 ^{0}C (Found: C, 62.22; H, 3.70; N, 8.71; S, 9.98. Calcd. for $C_{33}H_{24}N_4O_6S_2$: C, 62.46; H, 3.78; N, 8.83; S, 10.09%); ν_{max} 3151 (OH), 1708 (C=O), 1610 (C=N), 1368 (C-N), 691 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 8.52 (2H, s, OH), 6.21-8.11 (14H, m, Ar-H), 2.91 (2H, s, CH_2), 2.29 (6H, s, CO-CH_3). This reaction was extended to synthesize other acetyl derivatives **6b-g** from **5b-g** respectively: **6b** (72%), m.p. 177 ^{0}C; **6c** (70%), m.p. 125 ^{0}C; **6d** (68%), m.p. 201 ^{0}C; **6e** (75%), m.p. 167 ^{0}C; **6f** (70%), m.p. 155 ^{0}C; **6g** (75%), m.p. 190 ^{0}C.

Results and Discussion

The parent compound 4,4'-methylene-bis-(anthranilic acid) **3** was prepared by dissolving anthranilic acid **1** (0.01 mole) in distilled water (15 mL) and 36.5% hydrochloric acid (2.5 mL) at 50 ^{0}C. The mixture was then treated with 3% aqueous formaldehyde **2** (3.5 mL) at 20 ^{0}C with stirring for 1 h and neutralized with 10% sodium hydroxide. It was transformed into 1-{4-[4-(3-aryl thiocarbamido)-3-carboxy-benzyl]-2-carboxy-phenyl}-3-aryl thiocarbamides **4a-g** by condensing it with different aryl isothiocyanates (0.02 mole) in refluxing benzene medium for 1.5 h. The compounds **4a-g** were then transformed into {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-6-substituted-benzothiazol-2-yl-amines **5a-g** by their oxidative cyclization using the solution of bromine in chloroform. The reaction mixtures were left over night at room temperature and separated solids were

crystallized by ethanol. These were acidic to litmus and on determination of equivalent weight found to be the {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-carboxy-benzyl]-2-carboxy-phenyl}-6-substituted-benzothiazol-2-yl-amine hydrobromides. These on basification with dilute ammonium hydroxide solution afforded the free bases **5a-g**. Compounds **5a-g** on acylation with mixture acetic anhydride and glacial acetic acid afforded di-acetyl derivatives **6a-g** (Scheme 1).



Where, 4a-g : H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 3-Cl, 4-Cl

5,6a-g : H, 4-CH_3, 5-CH_3, 6-CH_3, 4-Cl, 5-Cl, 6-Cl

Scheme 1

Antimicrobial screening

The synthesized compounds **5a-g** were screened for their antibacterial activity using cup plate diffusion method[19,20]. The bacterial organisms used included both gram-positive as well

as gram-negative strains like *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and *A. aerogenes*. Sensitivity plates were seeded with a bacterial innoculum of $1x10^6$ CIU mL^{-1} and each well (diameter 10 mm) was loaded with 0.1 mL of test compound solution (1000 µg mL^{-1}) in DMF, so that concentration of each test compound was 100 µg mL^{-1}. The zones of inhibition were recorded after incubation for 24 h at 37 ^{0}C, using Vernier caliper. Inhibition zone record of the compounds clearly indicated that **5b**, **5c** and **5d** were highly active against *E. coli*, *S. aureus*, *S. typhi* and moderately active against *A. aerogenes*. Majority of the compounds were found inactive against *B. subtilis* (Table 1).

To determine minimum inhibitory concentration (MIC), the serial dilution technique[21] was followed using nutrient broth medium. The MIC values of compounds **5b**, **5c** and **5d** were determined against *E. coli*, *S. aureus* and *S. typhi*, which were found to be 70, 76 and 80 µg mL^{-1} respectively.

Screening of these compounds **5a-g** having the concentration 1%, for antifungal activity using paper disc method[22] showed that **5b**, **5c** and **5e** were highly active against *A. niger*, whereas other compounds showed low to moderate activity. The zones of inhibition were recorded after incubation for 48 h at 37 ^{0}C (Table 1).

Table 1. Antibacterial and antifungal activity of compounds **5a-g**

Compounds	Antibacterial activity					Antifungal activity
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	*A. aerogenes*	*A. niger* (Conc. 1%)
5a	++	+	–	+	+	+
5b	+++	+++	+++	–	++	+++
5c	+++	+++	+++	+	++	+++
5d	+++	+++	+++	++	++	+
5e	++	+	–	+	+	+++
5f	–	+	++	–	+	++
5g	+	++	+	+	++	–

(–) : Inactive (12 mm and less) (+) : Weakly active (13-16 mm) (++) : Moderately active (17-20 mm) (+++) : Highly active (21 mm and above)

Acknowledgement

Thanks are due to Director, R.S.I.C., Central Drug Research Institute, Chandigarh for providing analytical and spectral data. Author is thankful to Ms. Poonam P. Yadav and Ms. Vaishali R. Patil for their generous help during the work and also to Dr. V. D. Nanoty, Principal, Shri Radhakisan Laxminarayan Toshniwal College of Science, Akola for providing necessary facilities.

References

1 Hatchinson B I, Jenning S A, Vishnuvajala B R, Westwell A W and Stevens M F G, *J Med Chem.*, 2002, **45**, 744-748.

2 Hutchinson B I, Chua M S, Browne H L, Trapani V, Bradshaw T D, Westwell A D and Stevens M F G, *J Med Chem.*, 2001, **14**, 1446.

3 Guru N and Srivastava S D, *J Sci Indian Res.*, 2001, **60**, 601.

4 Desai K G and Desai K R, *Indian J Chem.*, 2005, **44B**, 2093.

5 Itoh T and Mase T, *Org Lett.*, 2007, **9**, 3687-3689.

6 Chavan A A and Pai N R, *Molecules*, 2004, **59**, 297.
7 Krause G, Frank R and Vasilev G N, *Biochem Physiol Pflanz.*, 1979, **174**, 128.
8 Bahekar S S and Shinde D B, *J Korean Chem Soc.*, 2003, **47**, 237.
9 Srivastava S K, Yadav R and Srivastava S D, *Indian J Chem.*, 2004, **43B**, 399.
10 Caleta I, Grdisa M, Mrvos D S, Cetina M, Tralic K V, Pavelic K and Karminski G, *IL Farmaco.*, 2004, **59**, 297-305.
11 Rajeeva N, Shrinivasulu and Shantakumar S M, *Eur J Chem.*, 2009, **6**, 775.
12 Mistry K and Desai K R, *Indian J Chem.*, 2006, **45B**, 1762.
13 Sayeed A, Rafique H, Hameed A and Rasheed S, *Pharm Chem J.*, 2008, **42**, 191.
14 Gurupadayya B M, Manohara Y N and Gopal M, *Indian J Heterocycl Chem.*, 2006, **15**, 113-116.
15 Kohichiro Y, Katsumi G, Kazuya Y, Tominori M and Goro T, *J Med Chem.*, 1990, **33**, 2192.
16 Shah V H and Mehta D S, *Indian J Heterocycl Chem.*, 2001, **11**, 139-144.
17 Korpe G V, Deshmukh S P and Fokmare A K, *Indian J Heterocycl Chem.*, 2001, **10**, 287.
18 Henriksen G, Houser A I, Westwell A D, Yousefi B H, Schwaiger M, Drzezga A and Wester H J, *J Med Chem.*, 2007, **50(6)**, 1087-1089.
19 Barry A L, The Antimicrobial Suspectibility Test; Principle and Practices, Illus Lea & Fibiger (Philadephia, Pa, USA), 1976, 180.
20 Cavanagh F, Analytical Microbiology (Academic Press, New York), 1963, 126.
21 Nishina C, Enoki N, Tawata S, Mori A, Kobayashi K and Fukushima, *Agric Bio Chem.*, 1987, **51**, 139.
22 Ananthanarayan R and Jagram Pancka G K, Text Book of Microbiology, 4th Edn., (Orient Longmans), 1990.
23 Colthup N B, Dally L H and Wiberly S E, Introduction to Infrared and Raman Spectroscopy (Academic Press, New York), 1964.
24 Silverstein R M, Bassler G C and Morrill T C, Spectrometric Identification of Organic Compounds, 4th Edn., (John wiley & Sons, New York), 1981.