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Synthesis, structural study and biological evaluation of pharmacologically important substituted bis-benzothiazole systems

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ABSTRACT

A series of {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-nitro-benzyl]-2-nitro-phenyl}-6-substituted-benzothiazol-2-yl-amines have been synthesized by oxidative cyclization of 1-{4-[4-(3-aryl thiocarbamido)-3-nitro-benzyl]-2-nitro-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-nitro-benzyl]-2-nitro-phenyl}-3-aryl thiocarbamides were prepared by the interaction of different aryl isothiocyanates with 4,4'-methylene-bis-(2-nitro aniline). The latter was obtained by treating the mixture of 2-nitro aniline 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, biological evaluation, bis-benzothiazoles.

INTRODUCTION

There has been considerable interest during this decade in the synthesis of substituted or fused benzothiazoles, because benzothiazole motif is an important skeleton in naturally occurring biologically active compounds. Added 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. Various benzothiazole are known for their manifold medicinal activity particularly as antibacterial agents [12,13]. Besides benzothiazole derivatives are used as vasodilator [14] and schistosomicidal agents [15].

Benzothiazoles represent the most active class of heterocyclic compounds possessing wide spectrum of pharmacological activities. The literature survey reveals the various substituted benzothiazoles possessing wide range 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 findings about the utility of fused heterocyclic compounds in various fields and as a part of wider programme to provide alternative routes for the

synthesis of heterocyclic compounds, we report herein the synthesis of pharmacologically important substituted bis-benzothiazole systems using reagents N-aryl isothiocyanates followed by the oxidative cyclization using bromine in chloroform.

MATERIALS AND METHODS

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-(2-nitro aniline) (3).
The parent compound 4,4'-methylene-bis-(2-nitro aniline) (**3**) was prepared by dissolving 2-nitro aniline (**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 hr. and neutralized with 10% sodium hydroxide solution, yellow precipitate was obtained. It was washed with hot water and crystallized from acetic acid, (**3**) (80%), m.p. 208^{0}C. (Found: C, 53.67; H, 4.02; N, 19.11. Calcd. for $C_{13}H_{12}N_4O4$: C, 54.16; H, 4.16; N, 19.44%).

Synthesis of 1-{2-nitro-4-[3-nitro-4-(3-phenyl thiocarbamido)-benzyl]-phenyl}-3-phenyl thiocarbamide (4a).
A mixture of 4,4'-methylene-bis-(2-nitro aniline) (**3**) (0.01 mole) and phenyl isothiocyanate (0.02 mole) in chloroform (15 mL) was refluxed for 1.5 hr. Then chloroform 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-nitro-4-[3-nitro-4-(3-phenyl thiocarbamido)-benzyl]-phenyl}-3-phenyl thiocarbamide (**4a**), (75%), m.p. 56^{0}C. (Found: N, 14.63; S, 11.23. Calcd. for $C_{27}H_{22}N_6O_4S_2$: N, 15.05; S, 11.46%); ν_{max} 3448, 3378 (NH), 1618 (C=N), 1521 (N=O),1310 (C-N), 1234 cm^{-1} (C=S); δ ($CDCl_3$+DMSO-d_6) 6.58-7.24 (16H, m, Ar-H), 4.52 (4H, s, NH), 2.69 (2H, s, CH_2) [19,20]. This reaction was extended to synthesize other compounds (**4b-g**): (**4b**) (75%), m.p. 69^{0}C (Found: N, 14.12; S, 10.83. Calcd. for $C_{29}H_{26}N_6O_4S_2$: N, 14.33; S, 10.92); (**4c**) (70%), m.p. 84^{0}C (Found: N, 14.29; S, 10.88. Calcd. for $C_{29}H_{26}N_6O_4S_2$: N, 14.33; S, 10.92); (**4d**) (78%), m.p. 60^{0}C (Found: N, 14.37; S, 10.96. Calcd. for $C_{29}H_{26}N_6O_4S_2$: N, 14.33; S, 10.92); (**4e**) (72%), m.p. 91^{0}C (Found: N, 13.32; S, 10.09. Calcd. for $C_{27}H_{20}N_6O_4S_2Cl_2$: N, 13.39; S, 10.20%); (**4f**) (68%), m.p. 64^{0}C (Found: N, 13.24; S, 10.17. Calcd. for $C_{27}H_{20}N_6O_4S_2Cl_2$: N, 13.39; S, 10.20%); (**4g**) (70%), m.p. 78^{0}C (Found: N, 13.40; S, 10.13. Calcd. for $C_{27}H_{20}N_6O_4S_2Cl_2$: N, 13.39; S, 10.20%).

Synthesis of {4-[4-(benzothiazol-2-yl-amino)-3-nitro-benzyl]-2-nitro-phenyl}-benzothiazol-2-yl-amine (5a).
A paste of 1-{2-nitro-4-[3-nitro-4-(3-phenyl thiocarbamido)-benzyl]-phenyl}-3-phenyl thiocarbamide (**4a**) (1.5 gm) was prepared in chloroform. 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-nitro-benzyl]-2-nitro-phenyl}-benzothiazol-2-yl-amine hydro-bromide, m.p. 86^{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-nitro-benzyl]-2-nitro-phenyl}-benzothiazol-2-yl-amine (**5a**) (78%), m.p. 95^{0}C. (Found: C, 57.80; H, 3.23; N, 15.05; S, 11.49. Calcd. for $C_{27}H_{18}N_6O_4S_2$: C, 58.48; H, 3.24; N, 15.16; S, 11.55%); ν_{max} 3468, 3354 (NH), 1622 (C=N), 1500 (N=O), 1339 (C-N), 691 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 7.00-8.82 (14H, m, Ar-H), 3.38 (2H, s, NH), 2.55 (2H, s, CH_2). This reaction was extended to synthesize other compounds (**5b-g**): (**5b**) (78%), m.p.118^{0}C (Found: C, 59.49; H, 3.75; N, 14.47; S, 10.91. Calcd. for $C_{29}H_{22}N_6O_4S_2$: C, 59.79; H, 3.78; N, 14.43; S, 10.99%); ν_{max} 3428, 3332 (NH), 1642 (C=N), 1525 (N=O), 1312 (C-N), 708 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 7.10-7.98 (12H, m, Ar-H), 3.43 (2H, s, NH), 2.58 (2H, s, CH_2), 2.34 (6H, s, Ar-CH_3); (**5c**) (75%), m.p. 136^{0}C (Found: C, 59.61; H, 3.63; N, 14.31; S, 10.92. Calcd. for $C_{29}H_{22}N_6O_4S_2$: C, 59.79; H, 3.78; N, 14.43; S, 10.99%); (**5d**) (82%), m.p. 104^{0}C (Found: C, 59.33; H, 3.65; N, 14.34; S, 10.88. Calcd. for $C_{29}H_{22}N_6O_4S_2$: C, 59.79; H, 3.78; N, 14.43; S, 10.99%); (**5e**) (70%), m.p. 102^{0}C (Found: C, 51.66; H, 2.47; N, 13.52; S, 10.23. Calcd. for $C_{27}H_{16}N_6O_4S_2Cl_2$: C, 52.00; H, 2.56; N, 13.48; S, 10.27%); (**5f**) (75%), m.p. 133^{0}C (Found: C, 51.86; H, 2.39; N, 13.26; S, 10.29. Calcd. for $C_{27}H_{16}N_6O_4S_2Cl_2$: C, 52.00; H, 2.56; N, 13.48; S, 10.27%); (**5g**) (76%), m.p. 98^{0}C (Found: C, 51.53; H, 2.53; N, 13.40; S, 10.14. Calcd. for $C_{27}H_{16}N_6O_4S_2Cl_2$: C, 52.00; H, 2.56; N, 13.48; S, 10.27%).

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

A mixture of {4-[4-(benzothiazol-2-yl-amino)-3-nitro-benzyl]-2-nitro-phenyl}-benzothiazol-2-yl-amine (**5a**) (0.01 mole) and acetic anhydride (0.02 mole) in glacial acetic acid (15 mL) was refluxed for 2.5 hr. The reaction mixture was cooled and poured in a little crushed ice with water, a cream coloured solid precipitated was crystallised from aqueous ethanol to give (**6a**) (70%), m.p.155^{0}C (Found: C, 58.02; H, 3.30; N, 13.05; S, 9.91. Calcd. for $C_{31}H_{22}N_6O_6S_2$: C, 58.30; H, 3.44; N, 13.16; S, 10.03%); ν_{max} 1701 (C=O), 1628 (C=N), 1545 (N=O), 1313 (C-N), 697 cm^{-1} (C-S); δ ($CDCl_3$+DMSO-d_6) 6.78-7.66 (14H, m, Ar-H), 2.83 (2H, s, CH_2), 2.16 (6H, s, CO-CH_3). This reaction was extended to synthesize other acetyl derivatives (**6b-g**) from (**5b-g**) respectively: (**6b**) (65%), m.p. 166^{0}C; (**6c**) (72%), m.p. 109^{0}C; (**6d**) (72%), m.p. 116^{0}C; (**6e**) (68%), m.p. 147^{0}C; (**6f**) (75%), m.p. 138^{0}C; (**76g**) (68%), m.p. 172^{0}C.

RESULTS AND DISCUSSION

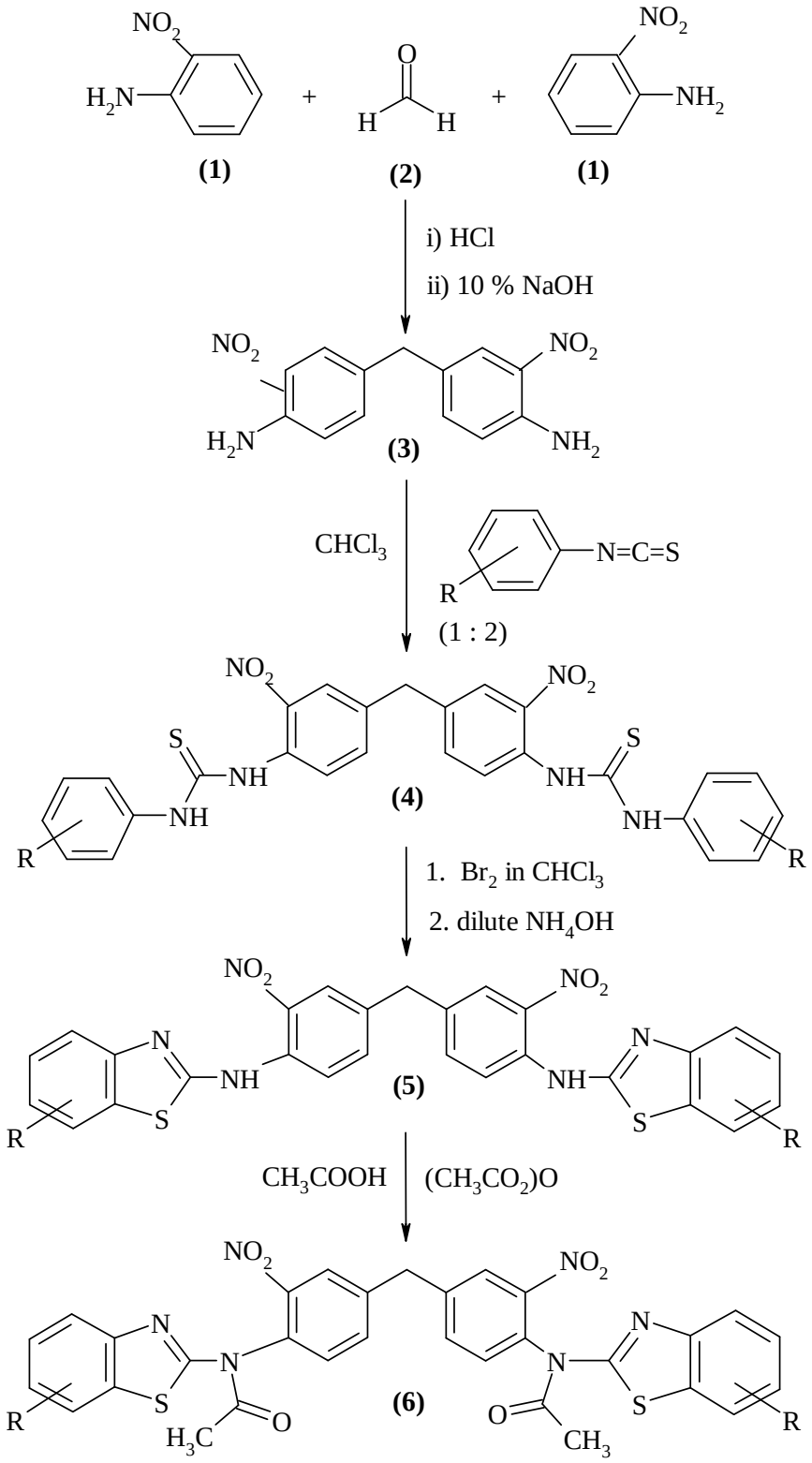
The parent compound 4,4'-methylene-bis-(2-nitro aniline) (**3**) was prepared by dissolving 2-nitro aniline (**1**) (0.01mole) in distilled water (15 mL) and 36.5% hydrochloric acid (2.5 mL) at 50^{0}C. The mixture was then treated with 3% aqueous formaldehyde (**2**) (3.5 mL) at 20^{0}C with stirring for 1 hr. and neutralized with 10% sodium hydroxide. It was transformed into 1-{4-[4-(3-aryl thiocarbamido)-3-nitro-benzyl]-2-nitro- phenyl}-3-aryl thiocarbamides (**4a-g**) by condensing it with different aryl isothiocyanates (0.02 mole) in refluxing chloroform medium for 1.5 hr. The compounds (**4a-g**) were then transformed into {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-nitro-benzyl]-2-nitro-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-nitro-benzyl]-2-nitro-phenyl}-6-substituted-benzothiazol-2-yl-amine hydrobromides. These on basification with dilute ammonium hydroxide solution afforded 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**).

Biological evaluation

The synthesized compounds (**5a-g**) were screened for their antibacterial activity using cup plate diffusion method [21,22]. 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 hr. at 37^{0}C, using Vernier caliper. Inhibition zone record of the compounds clearly indicated that (**5b**), (**5d**) and (**5e**) 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 [23] was followed using nutrient broth medium. The MIC values of compounds (**5b**) (**5d**) and (**5e**), were determined against *E. coli*, *S. aureus* and *S. typhi*, which were found to be 79, 88 and 76 µg ml^{-1} respectively.

Screening of these compounds (**5a-g**) having the concentration 1%, for antifungal activity using paper disc method [24] showed that (**5d**), (**5e**) and (**5f**) were highly active against *A. niger*, whereas other compounds showed low to moderate activity. The zones of inhibition were recorded after incubation for 48 hr. at 37^{0}C.



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)

CONCLUSION

In present work, synthesis of {4-[4-(6-substituted-benzothiazol-2-yl-amino)-3-nitro-benzyl]-2-nitro-phenyl}-6-substituted-benzothiazol-2-yl-amines (**5a-g**) and its acetyl derivatives (**6a-g**) have been reported. The method applied for the synthesis is quite efficient. Biological evaluation of these compounds revealed that, most of the compounds have better antibacterial and antifungal activities.

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