Novel Synthesis and Antimicrobial Activity of 3-Substituted 5-bromo-7-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazoles

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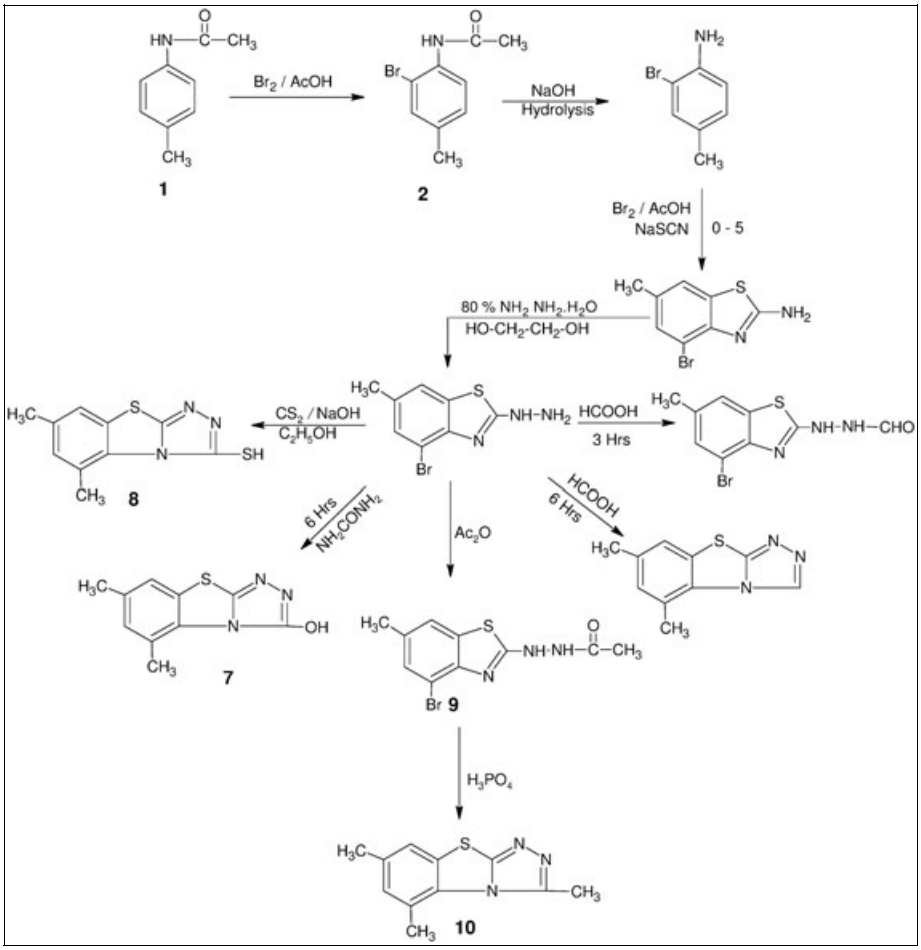


4-Methyl acetanilide (**1**) on treatment with bromine in acetic acid, followed by hydrolysis with dilute HCl/NaOH solution, yielded 2-bromo-4-methyl aniline (**2**), which on treatment with sodium thiocyanate in acetic acid afforded 2-amino-4-bromo-6-methyl benzothiazole (**3**). Compound **3** in ethylene glycol was heated at 150°C with 80% hydrazine hydrate to get 4-bromo-2-hydrazino-6-methyl benzothiazole (**4**). This hydrazino compound **4** on heating with formic acid for 3 h yielded 4-bromo-2-hydrazinoformyl-6-methyl benzothiazole (**5**). Same compound **4** when heated independently with formic acid for 6 h/urea for 3 h/carbon disulfide in alkali afforded 5-bromo-7-methyl (**6**)/5-bromo-3-hydroxy-7-methyl (**7**)/5-bromo-3-mercapto-7-methyl (**8**)-1,2,4-triazolo-[3,4-*b*]-benzothiazoles, respectively. Compound **4** on heating with acetic acid/acetic anhydride gave acetyl benzothiazolyl derivative **9**, which on cyclization with orthophosphoric acid yielded 5-bromo-3,7-dimethyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (**10**). All these newly synthesized compounds were screened for antimicrobial activity against *Escherichia coli* (Gram −ve), *Bacillus subtilis* (Gram +ve), *Erwinia carotovora*, and *Xanthomonas citri* using ampicillin, streptomycin, and penicillin as a standard for comparison.

INTRODUCTION

1,2,4-Triazole and their derivatives are important class of organic compounds with diverse agriculture, industrial and biological activities [1–3], including antimicrobial [4,5] anticonvulsant [6,7], and anti-inflammatory [8]. Similarly, benzothiazoles are known to possess different activities such as anticancer [9], anthelmintic activity [10], and antitubercular activity [11].

A survey of literature reveals such fused substituted tricyclic triazoles are prepared by different methods [12,13] but little work is carried out on bromo derivative of such fused tricyclic triazoles. Hence, it was thought worthwhile to synthesize 5-bromo-7-methyl as a substituent on benzene moiety in the 1,2,4-triazolo-[3,4-*b*]-benzothiazole system by following series of reactions and study the chemistry and biological activity of these compounds.

As the first step, 2-bromo-4-methyl aniline (**2**) was prepared by treating 4-methyl acetanilide (**1**) with bromine in acetic acid, followed by hydrolysis with dilute HCl/NaOH solution.

To the solution of sodium thiocyanate in glacial acetic acid, 2-bromo-4-methyl aniline (**2**) was added. The mixture was stirred well and bromine in glacial acetic acid was added dropwise maintaining the temperature below 5°C. The residue is filtered, dissolved in hot water, and neutralized by alkali. The obtained product 2-amino-4-bromo-6-methyl benzothiazole (**3**) was recrystallized by ethanol.

On the basis of elemental analysis and spectral data, the resulting product **3** has assigned the structure 2-amino-4-bromo-6-methyl benzothiazole. The IR spectrum showed absorption bands at 3440 cm^{-1} and 3340 cm^{-1} due to asymmetric and symmetric stretching of —NH_2 group, respectively. The PMR spectrum exhibited singlet at δ 2.4 due to Ar-CH_3, broad peak of δ 6.0 due to —NH_2 protons, and two singlets in the region δ 7.0–7.5 due to two Ar-H protons. The mass spectrum reveals molecular ion peaks at 244 (M + 2, 98%) and 242 (M^+, 100%). It also confirmed the presence of one bromine atom.

2-Amino-4-bromo-6-methyl benzothiazole (**3**) in ethylene glycol, as solvent, was heated with 80% hydrazine hydrate hydrochloride over an oil bath for 3 h at 150°C to get the product, 4-bromo-2-hydrazino-6-methyl benzothiazole (**4**). The IR spectrum of **4** showed absorption bands at 3452 cm^{-1} and 3353 cm^{-1} due to —NH_2 asymmetric and symmetric stretching, respectively. The mass spectrum exhibits molecular ion peaks of equal intensity at 259 (M + 2) and 257 (M^+) confirming the formation of compound **4** with one bromine atom.

Compound **4** was heated with formic acid for 3 h to get expected tricyclic triazolo benzothiazole (**6**), but it resulted in the formation of 4-bromo-2-hydrazinoformyl-6-methyl benzothiazole (**5**). On the basis of elemental analysis and spectral data, the structure of the resulting product has been assigned. The IR spectrum of the compound **5** in KBr showed absorption bands at 3246 cm^{-1} and 3184 cm^{-1} due to N—H stretching and absorption band of medium intensity at 2856 cm^{-1} accompanied by a strong carbonyl (—C=O) stretching absorption band at 1683 cm^{-1} indicating the presence of an aldehyde group. The ^{1}H NMR spectrum in $CDCl_3$ showed peak at δ 2.5 due to Ar-CH_3, δ 2.7 due to —NH proton, δ 7.4–7.7 due to Ar-H, and δ 9.5 due to aldehydic proton.

The mass spectrum of the compound exhibits peaks of equal intensity at 287 (M + 2, 48%) and 285 (M^+, 50%) which corresponds to the molecular weight and also confirms the presence of one bromine atom.

When same reaction mixture was refluxed for 6 h, the expected tricyclic triazolo benzothiazole, 5-bromo-7-methyl-1,2,4-trizolo-[3,4-*b*]-benzothiazole (**6**) was obtained. The IR spectrum showed the absence of absorption band at 3246 cm^{-1}, 3184 cm^{-1}, 2856 cm^{-1}, and 1683 cm^{-1} indicating the absence of —NH and —CHO groups, respectively. It supports the formation of cyclized product. ^{1}H NMR spectrum exhibits singlets at δ 2.5 due to Ar-CH_3 peaks, δ 7.4–7.7 due to Ar-H and singlet at δ 8.3 due to aryl —CH proton. Moreover, the mass spectrum exhibits molecular ion peak of equal intensity at 269 (M + 2, 88%) and 267 (M^+, 90%) which corresponds to its molecular weight and confirms the presence of one bromine atom.

Compound **4** on heating with urea at 200°C for 3 h afforded the product to which, on the basis of elemental analysis and spectral data, was assigned the structure 4-bromo-3-hydroxy-6-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (**7**).

The IR spectrum showed characteristics absorption bands at 3493 cm^{-1} and C—O stretching band at 1250 cm^{-1} which indicates the presence of —O—H group. Moreover, it also exhibits strong absorption band at 1717 cm^{-1} due to C=O group. It indicates that compound **7** exists in its tautomeric form.

^{1}H NMR spectrum of compound **7** in dimethyl sulfoxide (DMSO) showed two singlets at δ 7.4 and 7.6 due to two Ar-H protons. The singlets at δ 2.3 and 2.6 can be assigned to Ar-CH_3 and O—H proton, respectively. The mass spectrum shows molecular ion peaks of equal intensity at 286 (M^+ + 2) and 284 (M^+).

Another 3-substituted tricyclic triazolo benzothiazole, 3-mercapto-5-bromo-7-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (**8**) was obtained by refluxing 4-bromo-2-hydrazino-6-methyl benzothiazole (**4**) with carbon disulfide in the presence of alkali for 3 h, followed by neutralization with hydrochloric acid solution.

The compound dissolves in sodium hydroxide solution and gets reprecipitated with hydrochloric acid solution indicating the presence of mercapto (—SH) group.

The IR spectrum shows the absorption band at 2750 cm^{-1} due to mercapto group. ^{1}H NMR spectrum of

compound **8** in DMSO showed singlets at δ 2.5, 3.6, 7.6, and 7.9 due to Ar-CH_3, —S—H, and two Ar-H protons, respectively. The mass spectrum exhibits molecular ion peaks of equal intensity at 301 (M + 2) and 299 (M^+) which corresponds to its molecular weight.

The preparation of trisubstituted triazolo benzothiazole was undertaken with 3-methyl substituent on triazolo ring and 5-bromo-7-methyl substituent on benzene ring. 4-Bromo-2-hydrazino-6-methyl benzothiazole (**4**) on heating with acetic acid/acetic anhydride gave acetyl thiazolyl derivative **9**. The acetyl derivative shows the IR absorption bands at 3274 cm^{-1}, 3188 cm^{-1}, and 1710 cm^{-1} due to NH and C=O stretching, respectively. ^{1}H NMR spectrum exhibits peaks at δ 2.3, 2.6, 7.3–7.5, 10.1, and 10.5 due to Ar-CH_3, —$COCH_3$, Ar-H, and N—H protons, respectively. The mass spectrum exhibits molecular ion peaks of equal intensity at 301 (M + 2, 98%) and 299 (M^+, 100%) which corresponds to its molecular weight.

The acetyl thiazolyl derivative **9** on cyclization with orthophosphoric acid afforded the product to which, on the basis of elemental analysis and spectral data, was assigned the structure, 5-bromo-3,7-dimethyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (**10**).

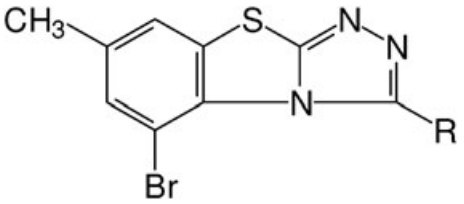
The IR spectrum of **10** showed the absence of strong absorption band in the region 1650–1750 cm^{-1} indicating the absence of —$COCH_3$ group. The ^{1}H NMR spectrum exhibited two singlets at δ 2.3 and 2.5 due to Ar-CH_3 and —CH_3 attached to triazole ring, respectively, and two singlets at δ 7.4 and 7.6 due to Ar-H protons.

ANTIMICROBIAL SCREENING: RESULTS AND DISCUSSION

The compounds **6–8** and **10** were tested for their antimicrobial activity by cup plate agar diffusion method against *Escherichia coli* (Gram -ve), *Bacillus subtilis* (Gram +ve), *Erwinia carotovora*, and *Xanthomonas citri* using ampicillin, streptomycin, and penicillin as a standard for comparison. The antibacterial screening data of the compounds are presented in Table 1. DMSO was used as a control solvent. Compounds **6** and **10** are more active than compounds **7** and **8** against *B. subtilis*, whereas compound **8** is active against *E. coli*. Compared with other compounds, compound **7** is active against *X. citri* and *E. carotovora*, and inactive against *E. coli*.

Table 1

Evaluation of antimicrobial activity of 3-substituted 5-bromo-7-methyl-1,2,4-triazolo-[3,4,-*b*]-benzothiazole.



Sr. no.	Comp.	R	Antimicrobial activity (zone of inhibition in mm) *E. coli*	*E. carotovora*	*B. subtilis*	*X. citri*
1	**6**	–H	1.5	03	03	01
2	**7**	–OH	00	04	02	03
3	**8**	–SH	03	03	01	02
4	**10**	–CH_3	02	03	03	02
5	Ampicillin		16	21	17	18
6	Streptomycin		20	22	22	18
7	Penicillin		15	24	18	20
8	Control		00	00	00	00

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded in Nujol/potassium bromide pellets on Bomen MB 104FT infrared spectrophotometer. ^{1}H NMR spectra were obtained on a Gemini 200 MHz spectrometer with trimehylsilane (TMS) as an internal standard and mass spectra on FT VG-7070H mass spectrometer using the GI technique at 70 eV. Elemental analysis was carried out on Heraeus CHN-O Rapid analyzer. Purity of the compound was checked by thin layer chromatography (TLC).

Synthesis of 2-amino-4-bromo-6-methyl benzothiazole (3). 2-Bromo-4-methyl aniline (18.6 g, 0.2*M*) and sodium thiocyanate (16 g, 0.2*M*) were dissolved in glacial acetic acid (150 mL). The solution was cooled in freezing mixture. Bromine (32 g, 10 mL, 0.2*M*) in glacial acetic acid (25 mL) was added with stirring and maintaining temperature below 5°C. The mixture was allowed to stand for 1 h at room temperature. The resulting hydrobromide was dissolved in hot water and neutralized with 10% NaOH to obtain base. The amine thus obtained was filtered, washed with water, and recrystallized in aqueous ethanol (50 mL ethanol + 50 mL water) to get the product 11 g (45%), mp 208–210°C. IR (KBr): 3440 cm^{-1} (asymmetric stretching of —NH_2), 3340 cm^{-1} (N—H symmetrical stretching of —NH_2), 2922 cm^{-1} (Ar-C—H stretching), 3052 cm^{-1} (Ar-H stretching), 1630 cm^{-1} (—C=N stretching), 650 cm^{-1} (C—S stretching); ^{1}H NMR ($CDCl_3$): δ 2.4 (s, 3H, Ar-CH_3), 6.0 (br, 2H, —NH_2), 7.0–7.5 (two singlets, 2H, Ar-H); MS: *m/z* 244 (M + 2, 98%), 242 (M^+, 100%), 163, 136; Anal. Calcd for $C_8H_7BrN_2S$ (243): C, 39.5; H, 2.88; N, 11.52; Br, 32.92; S, 13.16. Found: C, 39.2; H, 2.62; N, 11.38; Br, 32.8; S, 13.0.

4-Bromo-2-hydrazino-6-methyl benzothiazole (4). Hydrazine hydrate (80%, 17 mL) was taken in a flask cooled to 5°C and concentrated HCl (11 mL) was added with stirring. The flask was kept at room temperature for few minutes and then 2-amino-4-bromo-6-methyl benzothiazole (11 g) was added in portions. Ethylene glycol (44 mL) was added into the flask. The contents of the flask were heated at 150°C on an oil bath for 3 h. On cooling, the product 4-bromo-2-hydrazino-6-methyl benzothiazole crystallized out. It was filtered at pump, washed with cold water, and recrystallized from ethyl alcohol to give 10.5 g (90%), mp 246–248°C. IR (KBr): 3452 cm^{-1} (asymmetric N—H stretching in

—NH_2), 3353 cm^{-1} (symmetric N—H stretching in —NH_2); MS: *m/z* 259 (M + 2), 257 (M^+); Anal. Calcd for $C_8H_8BrN_3S$ (258): C, 37.2; H, 3.1; N, 16.2; Br, 31.0; S, 12.40. Found: C, 36.9; H, 2.9; N, 16.0; Br, 30.8; S, 12.2.

4-Bromo-2-hydrazinoformyl-6-methyl benzothiazole (5). A mixture of 4-bromo-2-hydrazino-6-methyl benzothiazole (0.516 g, 0.002*M*) and formic acid (5 mL) in 50-mL round-bottom flask was refluxed on an oil bath at 150°C for 3 h. The contents of the flask were cooled and poured on crushed ice with stirring. The white precipitate obtained was recrystallized from ethanol to give 0.37 g (64.68%), mp 238–240°C. IR (KBr): 3246 cm^{-1}, 3184 cm^{-1} (N—H stretching), 2856 cm^{-1} (C—H stretching of CHO group), 1683 cm^{-1} (C=O stretching of CHO); ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 3H, Ar-CH_3), 2.7 (s, 2H, —NH_2), 7.4–7.7 (2s, 2H, Ar-H), 9.5 (s, 1H, CHO); MS: *m/z* 287 (M + 2, 48%), 285 (M^+, 50%); Anal. Calcd for $C_9H_8BrN_3OS$ (286): C, 37.76; H, 2.79; N, 14.68; Br, 27.97; S, 11.18. Found: C, 37.68; H, 2.74; N, 14.53; Br, 27.72; S, 11.02.

5-Bromo-7-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (6). Reaction mixture of 4-bromo-2-hydrazino-6-methyl benzothiazole (0.516 g, 0.002*M*) and formic acid (5 mL) was refluxed on an oil bath at 150°C for 6 h. The contents of the flask were cooled and poured on crushed ice with stirring. The white precipitate obtained was filtered at pump, washed with cold water, and recrystallized from 1,4-dioxane to get 0.32 g (62%), mp 276–278°C. IR (KBr): absence of absorption band in the region 3400–3100 cm^{-1} due to NH and at 2856 cm^{-1} and 1683 cm^{-1} due to —CHO; ^{1}H NMR: δ 2.5 (s, 3H, Ar-CH_3), 7.4–7.7 (2s, 2H, Ar-H), 8.3 (s, 1H, CH); MS: *m/z* 269 (M + 2, 98%), 267 (M^+, 100%); Anal. Calcd for $C_9H_6BrN_3S$ (268): C, 40.29; H, 2.23; N, 15.67; Br, 29.85; S, 11.94. Found: C, 40.14; H, 2.12; N, 15.48; Br, 29.62; S, 11.8.

5-Bromo-3-hydroxy-7-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (7). A mixture of 4-bromo-2-hydrazino-6-methyl benzothiazole (1.29 g, 0.005*M*) and urea (3 g, 0.05*M*) was crushed together in a mortar. Then the powdered mixture transferred into 50-mL round-bottom flask and heated on an oil bath at 190°C for 3 h. The contents of the flask were cooled. The solid obtained was then dissolved in 10% NaOH and filtered. The clear filtrate cooled and acidified with HCl to obtain white precipitate. It was filtered, washed with cold water, and recrystallized from 1,4-dioxane to obtained 0.66 g (46.4%), mp 278–280°C. IR (KBr): 3493 cm^{-1} (O—H), 1717 cm^{-1} (C=O stretching), 1203 cm^{-1} (C—O in —C—OH); ^{1}H NMR: δ 2.3 (s, 3H, Ar-CH_3), 2.6 (s, 1H, O—H), 7.8–7.5 (2s, 2H, Ar-H); Anal. Calcd for $C_9H_6BrN_3OS$ (284): C, 38.02; H, 2.11; N, 14.78; Br, 28.16; S, 11.26. Found: C, 37.98; H, 2.02; N, 14.66; Br, 28.0; S, 11.12.

5-Bromo-3-mertcapto-7-methyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (8). 4-Bromo-2-hydrazino-6-methyl benzothiazole (1.29 g, 0.005*M*) in 40 mL of ethanol was mixed with sodium hydroxide (0.4 g in 5 mL water) and then carbon disulfide (0.5 mL) was added. The mixture was refluxed on water bath for 3 h at 60°C. The solid left behind dissolved in 10% NaOH, filtered, and the clear filtrate acidified in cold condition with concentrated hydrochloric acid afforded product. It was washed with water and recrystallized from hot nitrobenzene to give 0.68 g (45.7%), mp 263–265°C. IR (KBr): 2750 cm^{-1} (S—H stretching); ^{1}H NMR: δ 2.5 (s, 3H, Ar-CH_3), 3.6 (s, 1H, S—H), 7.6–7.9 (2s, 2H, Ar-H); MS: *m/z* 301 (M + 2), 299 (M^+); Anal. Calcd for $C_9H_6BrN_3S_2$ (300): C, 36; H, 2.00; N, 14.00; Br, 26.66; S, 21.33. Found: C, 35.90; H, 1.96; N, 13.94; Br, 26.42; S, 21.14.

2-Acetyl-4-bromo-6-methyl benzothiazole (9). 4-Bromo-2-hyrdazino-6-methyl benzothiazole (1.29 g, 0.005*M*) was refluxed with acetic anhydride (2 mL) and glacial acetic acid (4 mL) on an oil bath at 150°C for 1.5 h. The reaction mixture was cooled and poured with stirring over crushed ice. The acetyl derivative thus obtained was filtered, washed with cold water, and recrystallized from hot ethanol to give 1.2 g (80%), mp 208–210°C. IR (KBr): 3274 cm^{-1}, 3188 cm^{-1} (N—H stretching), 1710 cm^{-1} (C—O stretching in C=O); ^{1}H NMR: δ 2.3 (s, 3H, Ar-CH_3), 2.6 (s, 3H, $COCH_3$), 7.3–7.5 (s, 2H, Ar-H), 10.5 (s, 1H, N—H); MS: *m/z* 301 (M + 2, 98%), 299 (M^+, 100%); Anal. Calcd for $C_{10}H_{10}BrN_3OS$ (300): C, 40.0; H, 3.33; N, 14.0; Br, 26.66; S, 10.66. Found: C, 39.92; H, 3.12; N, 13.94; Br, 26.52; S, 10.44.

5-Bromo-3,7-dimethyl-1,2,4-triazolo-[3,4-*b*]-benzothiazole (10). The acetyl derivative (1 g, 0.0035*M*) was refluxed on an oil bath with acetic anhydride (5 mL) and syrupy phosphoric acid (0.75 mL) for 1.5 h at 150°C. The reaction mixture was cooled, poured in 150 mL ice cold water with stirring, then rendered alkaline with ammonia solution. The solid product was filtered, washed with water, and recrystallized from hot ethanol to obtain 0.38 g (40.4%), mp 260–262°C; ^{1}H NMR: δ 2.3 (s, 3H, Ar-CH_3), 2.5 (s, 3H, $COCH_3$), 7.3–7.6 (s, 2H, Ar-H); Anal. Calcd for $C_{10}H_8BrN_3S$ (282): C, 42.55; H, 2.32; N, 14.89; Br, 28.36; S, 11.32. Found: C, 42.42; H, 2.12; N, 14.76; Br, 28.22; S, 11.18.

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