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Synthesis And Antimicrobial Activity Of 3-Aryl Substituted 5-Bromo -7-Ethoxy -1, 2, 4-Triazolo-[3, 4-*B*] Benzothiazoles By Oxidative Cyclisation

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Abstract

4-ethoxy acetanilide 1 on treatment with bromine in acetic acid, followed by hydrolysis with dil. HCl/NaOH solution, yielded 2-bromo-4-ethoxy aniline 2 which on treatment with sodium thiocyanate in acetic acid afforded 2-amino-4-bromo-6-ethoxy benzothiazole 3. Compound 3 in ethylene glycol was heated at 150 °C with 80% hydrazine hydrate to get 4-bromo-6-ethoxy-2-hydrazino benzothiazole 4. This hydrazino compound 4 on heating with 2-hydroxy-3-methoxy-benzaldehyde / 4-hydroxy-3-methoxy benzaldehyde / 2-hydroxybenzaldehyde / naphthaldehyde/cinnamylaldehyde / 4-dimethyl aminobenzaldehyde to obtain corresponding hydrazones (5a-5f). [4-bromo 2(substituted phenyl/naphthyl)-6-ethoxy benzothiazolyl hydrazone].

These hydrazones (5a- 5f) in benzene independently were refluxed on water bath for three hours with Attenburrow's MnO₂ to obtain 3-(2'-hydroxy-3'-methoxy phenyl (6a)/4'-hydroxy-3'-methoxy phenyl (6b)/2'-hydroxy phenyl(6c)/1'-naphthyl(6d)/cinnamyl (6e)/4'-dimethyl amino phenyl(6f))-5-bromo-7-ethoxy-1,2,4-triazolo-[3,4-*b*]-benzothiazoles respectively.

All these newly synthesized compounds were screened for antimicrobial activity against *E.Coli* (Gram -ve), *B. subtilis* (Gram +ve), *E. Carotovara* 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 activities²⁻⁴, including antimicrobial⁵⁻⁶ anti-convulsant⁷⁻⁸ and anti-inflammatory⁹. Similarly benzothiazoles are known to possess different activities such as anticancer¹⁰, anthelmintic activity¹¹, antitubercular activity¹².

A survey of literature reveals such fused substituted tricyclic triazoles are prepared by different methods¹³⁻¹⁴ but little work is carried out on bromo derivative of such fused tricyclic triazoles. Hence it was thought worthwhile to synthesize 5-bromo-7-ethoxy 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-ethoxy aniline (2) was prepared by treating 4-ethoxy acetanilide (1) with bromine in acetic acid, followed by hydrolysis with dil. HCl / NaOH solution.

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



On the basis of elemental analysis and spectral data the resulting product (3) has assigned the structure 2-amino-4-bromo-6-ethoxy benzothiazole. The I.R. spectrum showed absorption bands at 3440 cm^{-1} and 3340 cm^{-1} due to asymmetric and symmetric stretching of $-\text{NH}_2$ group respectively. The PMR spectrum exhibited broad peak of δ 6.0 due to $-\text{NH}_2$ protons and two singlets in the region δ 7.0 - 7.5 due to two Ar-H protons.

2-amino-4-bromo-6-ethoxy benzothiazole shows triplet at δ 1.4 and quatrate at δ 4.2 due to $-\text{OCH}_2\text{CH}_3$, while The mass spectrum reveals molecular ion peaks at 274 ($M+2$, 98%) and 272 (M^+ , 100%). It also confirmed the presence of one bromine atom.

2-Amino-4-bromo-6-ethoxy benzothiazole (3) in ethylene glycol as solvent was heated with 80% hydrazine hydratehydrochloride over an oil bath for three hours keeping temp. at 150°C to get the product, 4-bromo-6-ethoxy-2-hydrazino benzothiazole (4). The I.R. spectrum of (4) showed absorption bands at 3320 cm^{-1} and 3203 cm^{-1} due to $-\text{NH}_2$ asymmetric and symmetric stretching respectively. The mass spectrum exhibits molecular ion peaks of equal intensity at 289 ($M+2$) and 287(M^+) which also confirming the formation of compound (4) with one bromine atom.

This 4-bromo-6-ethoxy-2-hydrazino benzothiazole (4) in ethanol was refluxed on water bath for three hours independently with 2-hydroxy-3-methoxy-benzaldehyde/4-hydroxy-3-methoxy benzaldehyde/2-hydroxybenzaldehyde/naphthaldehyde/cinna-mladehyde/4-dimethyl aminobenzaldehyde to obtain corresponding hydrazones (5a-6f). [4-bromo 2(substituted phenyl/naphthyl)-6-ethoxy benzothiazolyl hydrazone]. The I.R. spectra of hydrazones showed stretching absorption bands in the region $3450\text{--}3100\text{ cm}^{-1}$ due to $-\text{N-H}$ stretching. The presence of broad singlet in their PMR spectra in the region δ 2.5 to δ 4.5 confirmed the presence of $-\text{NH}$ proton. The mass spectrum of the compound (5a) shows molecular peak at 391(M^+) which corresponds to molecular weight of the compound.

These hydrazones (5a- 5f) in benzene independently were refluxed on water bath for three hours with Attenburrow's MnO_2^{23} to obtain 3-(2'-hydroxy-3'-methoxy phenyl (6a)/4'-hydroxy-3'-methoxy phenyl (6b)/2'-hydroxy phenyl(6c)/1'-naphthyl(6d)/cinnamyl (6e) /4'-dimethyl amino phenyl(6f)-5-bromo-7-ethoxy-1,2,4-triazolo[3,4-*b*]-benzo- thiazoles respectively. The I. R. spectra of these triazolo benzothiazoles observed the absence of strong bands in the region 3450cm^{-1} - 3100 cm^{-1} due to $-\text{NH}$ stretching, however the absence of broad singlet in PMR spectra of these triazolo benzothiazole in the region δ 2.5 - δ 4.5 confirms the formation of cyclised products.

The mass spectrum of compound (6f) exhibits molecular peak at 417 which corresponds to its molecular weight. It confirms the formation of 5-bromo-3-(4'- dimethyl amino phenyl)-7-ethoxy 1,2,4-triazolo-[3,4-*b*]-benzothiazole products

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. ^1H NMR spectra were obtained on a Gemini 200 Mz spectrometer with 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 a Heraeus CHN-O Rapid analyser. Purity of the compound was checked by TLC and elemental analysis.

Synthesis of 2-Amino-4-bromo-6-ethoxy benzothiazole (3)



2-Bromo-4-methyl aniline (21.6 gm, 0.2 M) and sodium thiocyanate (16 gm, 0.2 M) were dissolved in glacial acetic acid (150 ml). The solution was cooled in freezing mixture. Bromine (32 gm, 10 ml, 0.2 M) in glacial acetic acid (25 ml) was added with stirring and maintaining temperature below 25°C. The mixture was allowed to stand for one hour at room temp. 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 aq. alcohol to get the product 13 gm. (60 %), M.P 210 °C., IR (KBr): 3440 cm^{-1} (Asymmetric stretching of $-\text{NH}_2$), 3340 cm^{-1} (N-H Symmetrical stretching of $-\text{NH}_2$), 3052 cm^{-1} (Ar-H stretching), 1630 cm^{-1} ($-\text{C}=\text{N}$ stretching), 1325 cm^{-1} (Ar-C-O stretching). $^1\text{H NMR}$ (CDCl_3): δ 1.4 (triplet, 3H, CH_3) and δ 4.2 (quartate, 2H, CH_2) due to $-\text{OCH}_2\text{CH}_3$, δ 6.0 (broad, 2H, NH_2), δ 7.0-7.5 (two singlet, 2H, Ar-H), m/z 244 (M^+ , 98 %), 242 (M^+ , 100%), 163, 136

4-Bromo-6-ethoxy-2-hydrazino benzothiazole (4)

Hydrazine hydrate (80%, 9 ml) was taken in a flask, cooled to 5°C and concentrated HCl (6 ml) was added to it with stirring. The flask was kept at room temperature for few minutes and then 2-amino-4-bromo-6-ethoxy benzothiazole (6 gm) was added in portions. Ethylene glycol (24 ml) was added into the flask. The contents of the flask were heated at 140 °C on an oil bath for three hours and then cooled. The separated product, 4-bromo-6-ethoxy-2-hydrazino benzothiazole was filtered, washed with cold water and crystallized from ethyl alcohol to give 3.8 gm (61%), M. P. 280°C, IR (KBr) : 3320 cm^{-1} (asymmetric N-H stretching of $-\text{NH}_2$), 3203 cm^{-1} (symmetric N-H stretching of $-\text{NH}_2$) m/z : 289 (M^+), 287 (M^+)

General procedure

4-bromo- 2-substituted-6-ethoxy benzothiazolyl hydrazone. (5a-5f)

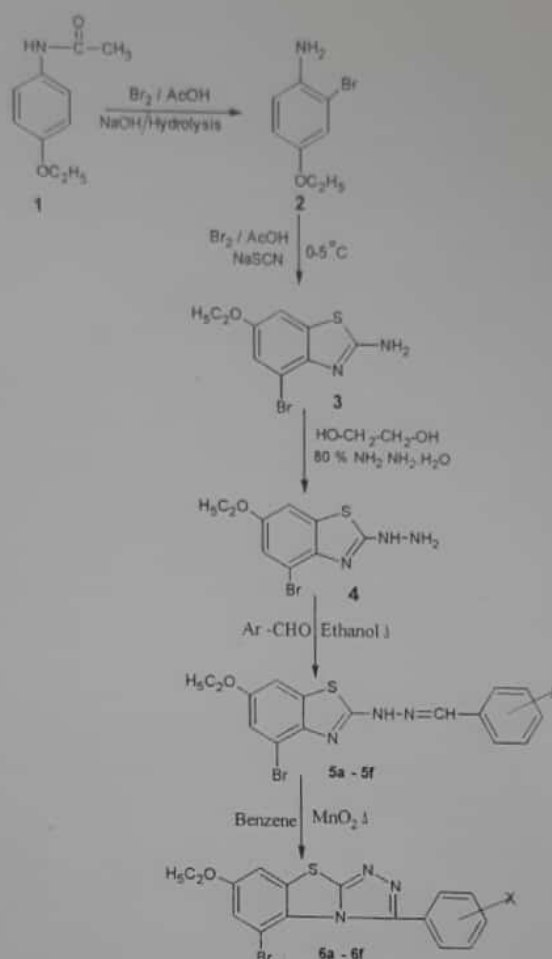
The mixture of 4-bromo 6-ethoxy 2-hydrazino benzothiazole (2.88 gm, 0.01M) was dissolved in ethanol (50 ml) and aromatic aldehydes (2-hydroxy-3-methoxy-benzaldehyde / 4-hydroxy-3-methoxy benzaldehyde/2-hydroxybenzaldehyde / naphthaldehyde/cinnamylaldehyde / 4-dimethyl aminobenzaldehyde) (0.01 M) in ethanol (25 ml) was refluxed on water bath for two hours. The reaction mixture was cooled, the solid product obtained, filtered at pump, washed with ethanol and recrystallized from hot benzene

4-bromo- 2-(2'-hydroxy-3'-methoxy phenyl)-6-ethoxy benzothiazolyl hydrazone. (5a)

Cream coloured powder, Yield : 71% , M. P. 245 °C IR (KBr) : 3423 cm^{-1} (O-H stretching) , 3209 cm^{-1} (N-H stretching) , Anal. Calculated for $\text{C}_{17}\text{H}_{16}\text{BrN}_3\text{O}_5$, C : 48.34%; H : 3.79%; N : 9.9 . Found : C : 48.12%; H : 3.62%; N : 9.58



Scheme : 1



4-bromo-2-(4'-hydroxy-3'-methoxy-phenyl)-6-ethoxy benzothiazolyl hydrazone. (5b)

Yellow powder, Yield 75%, M. P. 214 °C, IR (KBr) : 3448 cm^{-1} (O-H stretching), 3200 cm^{-1} (N-H stretching), Anal. Calculated for $\text{C}_{17}\text{H}_{16}\text{BrN}_3\text{O}_2\text{S}$, C : 48.34%; H : 3.79%; N : 9.95, Found : C : 48.00; H : 3.50; N : 9.62

4-bromo-2-(2'-hydroxy phenyl)-6-ethoxy benzothiazolyl hydrazone. (5c)

cream coloured powder, Yield : 74.7%, M. P. 215 °C. IR (KBr) : 3180 cm^{-1} (-OH Stretch) 3174 cm^{-1} (N-N Stretch), Anal. Calculated for $\text{C}_{16}\text{H}_{14}\text{BrN}_3\text{O}_2\text{S}$, C : 48.99%; H : 3.60%; Br : 20.37%; N : 10.71%; S : 8.17%;, Found : C : 48.14%; H : 3.42%; Br : 20.12%; N : 10.43%; S : 8.03%;

4-bromo-2-(1'-naphthyl)-6-ethoxy benzothiazolyl hydrazone. (5d)

Yellow powder, Yield: 68%, M. P. 233 °C, I.R. (KBr): 3389 (N-H stretching), 3053 (-C-H stretch in aromatic ring), 1541 (C=N stretch), 1290 (C-N stretch), Anal. Calculated for



$C_{20}H_{16}BrN_3OS$, C : 56.34%; H : 3.79%; N : 9.86%, Br : 18.74%; S : 7.52%; Found C : 56.12%; H : 3.63%; N : 9.54%, Br : 18.44%; S : 7.42%;

4-bromo-2-(Cinnamyl)-6-ethoxy benzothiazolyl hydrazone (5e).

Yellow powder., Yield: 70.9% M. P. 231 °C. IR (KBr) 3423 cm^{-1} (N-H stretching), Anal. Calculated for $C_{18}H_{16}BrN_3OS$, C : 53.74%; H : 4.01%; N : 10.44%, Br : 19.86%; S : 7.97%; Found : C : 53.62%; H : 3.94%; N : 10.23%, Br : 19.72%; S : 7.847%;

4-bromo-2-(4'-dimethyl amino phenyl)-6-ethoxy benzothiazolyl hydrazone (5f)

Yellow powder, Yield :62% M. P. 138 °C. IR (KBr) : 3302 (N-H stretching), Anal. Calculated for $C_{18}H_{19}BrN_4OS$, C : 51.55%; H : 4.57%; N : 13.36, Br : 19.05%; S : 7.65%; , Found : C : 51.32%; H : 4.42%; N : 13.24%, Br : 18.96%; S : 7.58%;

General procedure

3-Substituted-5-bromo-7-ethoxy 1,2,4 triazolo [3,4-*b*]-benzothiazole. (6a-6f)

4-Bromo-2-substituted -6-methyl benzothiazolyl hydrazone (0.002 M) was taken in dry benzene (50 ml). To this was added Attenbarrow's active manganese dioxide¹⁴ (2.0 gm, 0.016 M) and the mixture was refluxed on water bath for three hours. Contents were poured on hot condition in petridish, benzene solvent was removed by distillation. Obtained solid product was recrystallized from hot ethanol,

3-(2'-hydroxy-3'-methoxy phenyl)-5-bromo-7-ethoxy 1,2,4 triazolo[3,4-*b*]benzothiazole. (6a)

Cream powder, Yield : 37%, M. P. 290°C IR (KBr):3200 cm^{-1} (O-H stretching) Band due to N-H stretching absent, Anal. Calculated for $C_{17}H_{14}BrN_3O_3S$, C : 48.57; H : 3.3%; N : 10.0, Br : 19.01%; S : 7.63%; , Found : : C : 48.20%; H : 3.12%; N : 9.86%, Br : 19.01%; S : 7.63%;

3-(4'-hydroxy-3'-methoxy phenyl)-5-bromo-7-ethoxy-1,2,4-triazolo-[3,4-*b*] benzothi-azole. (6b)

Cream powder, Yield :64% M. P. 165°C I.R. (KBr) 3279 cm^{-1} broad O-H stretching band due to N-H stretching absent. Anal. Calculated for $C_{17}H_{14}BrN_3O_3S$, C : 48.57%; H : 3.3%; N : 10.0, Br : 19.01%; S : 7.63%; Found : : C : 47.92%; H : 3.08%; N : 9.72%, Br : 18.84%; S : 7.42%;

3-(2'-hydroxy phenyl)-5-bromo-7-ethoxy 1,2,4 triazolo-[3,4-*b*] benzothiazole. (6c)

Cream powder, Yield 76 %, M. P. 148 °C, I.R. (KBr) 3275 cm^{-1} (O-H stretching), Band due to N-H stretching absent Anal. Calculated for $C_{16}H_{12}BrN_3O_2S$, C : 49.24; H : 3.10; N : 10.77, Br : 20.471%; S : 8.22%; Found : : C : 49.10; H : 3.08; N : 10.62%; Br : 20.471%; S : 8.22%

3-(1'-Naphthyl)-5-bromo-7-ethoxy 1,2,4 triazolo-[3,4-*b*] benzothiazole. (6d).

Cream powder, Yield 74%, M. P. 134°C I.R. (KBr) Band due to N-H stretching absent. Anal. Calculated for $C_{20}H_{14}BrN_3OS$, C : 56.61%; H : 3.08%; N : 9.90%, Br : 18.83%; S : 7.56%; Found : : C : 56.42%; H : 2.94%; N : 9.72%, Br : 18.54%; S : 7.38%

3-(2'-Cinnamyl)-5-bromo-7-ethoxy 1,2,4 triazolo [3,4-*b*] benzothiazole. (6e)

Cream powder, Yield 62%, M.P. 190 °C, I.R. (KBr) Band absorption due to N-H stretching absent. Anal. Calculated for $C_{18}H_{14}BrN_3OS$, C : 54.01%; H : 3.53%; N : 10.50%, Br : 19.96%; S : 8.01%; Found : : C : 53.94%; H : 3.42%; N : 10.44%, Br : 19.82%; S : 7.2%

3-(4'-dimethyl amino phenyl)-5-bromo-7-ethoxy 1,2,4-triazolo [3,4-*b*] benzothiazole. (6f)



Cream powder, Yield 67 % M. P. 120 °C, I.R. (KBr) Band due to N-H stretching absent, Mass: 416 (M⁺) and 418 (M+2); Anal. Calculated for C₁₈H₁₇BrN₄OS, C: 51.79%; H: 4.11%; N: 13.42%, Br: 19.15%; S: 7.68%; Found: C: 51.62%; H: 4.02%; N: 13.28%, Br: 19.01%; S: 7.53%

Result and Discussion

The structures of these tricyclic triazolo benzothiazoles (6a- 6f) were assigned on the basis of their elemental analysis and spectral data

The I. R. spectra of these triazolo benzothiazoles, observed the absence of strong bands in the region 3450cm⁻¹ - 3100 cm⁻¹ due to -NH stretching, however the absence of broad singlet in PMR spectra of these triazolo benzothiazole in the region δ 2.5 - δ 4.5 confirms the formation of cyclised products.

The mass spectrum of compound (6f) exhibits molecular peak at 417 which corresponds to its molecular weight. It confirms the formation of 5-bromo-3-(4'-dimethylamino phenyl)-7-ethoxy 1,2,4-triazolo-[3,4-*b*]-benzothiazole products.

Antibacterial Activity :

The compound 6a to 6f If were tested for their antimicrobial activity by cup plate agar diffusion method against *E.coli* (Gram -ve) *B.subtilis* (Gram +ve), *E. carotovara* and *Xanthomonas citri* using ampicillin, streptomycin. and penicillin as a standard for comparison. The antibacterial screening data of the compounds is presented in table No.1. Dimethyl sulphoxide was used as a control (solvent). Compound 1a and 1b is more active than compound 1c and 1d against *B.subtilis* while compound 1d is active against *E. coli*. As compare to other compounds. Compounds 1c is active against *Xanthomonas citri* and *E. carotovara* while it is inactive against *E. Coli*.

Result and discussion

The compounds 6a to 6f were tested for their antimicrobial activity by the cup plate agar diffusion method against *E. Coli*, *Erwinia carotovara*, *Bacillus subtilis*, and *Xanthomonas citri*. The antibacterial screening data of the compound are presented in table-1

Table : 1

Sr. No.	Comp.	Antimicrobial activity (zone of inhibition in mm)			
		<i>E.coli</i>	<i>Erwinia</i>	<i>Bacillus</i>	<i>Xanthom-Onas citri</i>
1	6a	08	00	00	00
2	6b	16	14	12	13
3	6c	12	04	06	08
4	6d	00	4	00	04
5	6e	14	13	10	12
6	6f	10	10	08	07
	Ampicillin	16	18	17	15
	Streptomycin	20	18	22	18
	Penicillin	15	20	18	17
	Control	00	00	00	00



Strong inhibition zone were found with compound 6b (16 mm) against *E. coli*. While moderate inhibition zone were reported with compound 6c (12 mm), 6e (14 mm), 6f (10 mm), and low inhibition zone with compound 6a (8 mm). The compound 6d does not show inhibition zone against *E. coli*, Species.

The moderate inhibition zone reported against *Erwinia carotovora* with compound 6b (14 mm), 6e (13 mm), , 6f (10 mm) and less inhibition zone with compound 6c (4 mm), 6d (4 mm) while no inhibition zone with compound Va.

The compound 6b (12 mm), 6e (10 mm), reported moderate zone of inhibition against *Bacillus subtilis* species while compound 6c (6 mm), and 6f (8 mm) reported less zone of inhibition. The compound Va and 6d does not show any zone of inhibition.

Similarly moderate zone of inhibition reported with compound 6b (13 mm), 6e (12 mm) against *Xanthomonas citri* and less inhibition zone with compound 6c (8 mm) 6f (7 mm) 6d (4 mm) while compound Va does not show any one of inhibition against *Xanthomonas citri* species.

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