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Synthesis and structural studies of novel benzothiazole derivative and evaluation of their antimicrobial activity

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Abstract

Benzothiazole are important group of compounds reported to have various biological activities and hence the present study was undertaken in order to synthesize same new compounds built upon this nucleus with the hope to enhance the biological properties of newly designed compounds. In the present work 2-substituted benzothiazole (a) was prepared from p-tolnidine via cyclization reaction then N-2-benzothiazolyl thiourea was synthesized by reacting (a) with ammonium thiocyanate. Then its semicarbazide derivative was formed reacting with hydrazine hydrate in ethylene glycol which was reacted with various acetophenons to form respective derivatives. Chemical structure of product and their purity was ascertained by TLC, MP and various spectral methods as FTIR and NMR. The inhibition of microorganism under standard condition was determined to demonstrate antimicrobial activity of derivatives using gram positive and gram negative bacteria such as *Staphylococcus aureus, Pseudomonas. aeruginosa,, Escherichia coli* with ampicillin as standard compounds. IR and NMR spectral data were supporting the presence of synthesized compounds. Derivatives showed significant zone of inhibition against the both gram positive and gram negative bacteria. The metanitroacetophenone derivative showed excellent activity against *P. aeruginosa.*

INTRODUCTION

Benzothiazole and its derivatives are bicyclic ring system and have identified showing a multiple applications. From the ancient time a number of 2- substituted benzatiazole derivatives were investigated and identified for their various pharmacological activities. Benzothiazole derivatives have been intensively studied for their antitumour [1-3], neurotransmission blocker [4-6], calmodulin (CaM) antagonists [7], neuroprotective agent [8, 9] and other biological activities [10-14].

In view of these observations, some novel N-2-benzothiazolylthiourea derivatives have been synthesized in order to examine their in vitro antimicrobial activities against various gram positive and gram negative microorganisms in comparison with drug ampicillin in accordance with the method described in literature.

MATERIALS AND METHODS

Experimental

Chemicals

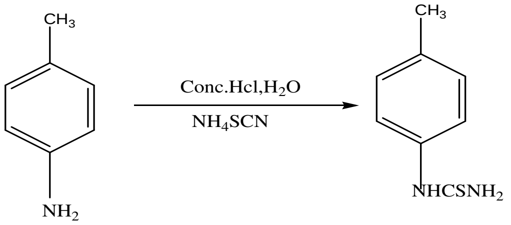
The reagents and solvents were commercially available (CDH, Rankem, Merck) and of synthetic grade. Solvents and reagents were dried prior to use over anhydrous sulfate or fused calcium chloride.

Synthetic scheme

The synthesis part of the present work was divided in to following

1. *Synthesis of p-tolyl thiourea*

Para-toludine (5.35gm) was dissolved in a mixture of conc. HCL (4.3ml) and water (11.6ml) by heating on a water bath. Cooled the contents added solid ammonium thiocyanate (3.8gm) and heated the mixture on the water bath for 22hrs. Precipitated product was cooled and filtered .Then washed with water and dried. Recrystallized with aqueous methanol gave colored crystals. Yield 4.12gm (77%), (Fig 1)



Para-toludine Para-tolyl thiourea

Fig 1: Synthesis of p-tolyl thiourea [S-1]

2. *Synthesis of 2-Amino-6-methyl benzothiazole*

A solution of bromine (6.8ml) 0.17 mol, in $CHCl_3$ (50ml) was poured in to a mixture of the dry powdered Para tolyl thiourea (20 gm) 0.13mol and $CHCl_3$ (60ml) which was heated to boil contained in a three neck flask. Heat was evolved and the mixture rapidly gives a clear solution which was kept at below 30 °C by external heating. When the initial reaction subsided the flask was fitted with a stirrer and a condenser carrying a calcium chloride tube and the mixture refluxed until the evolution of Hydrogen bromide ceased. The hydrogen bromide derivative of 2-amino-6-methyl benzothiazole kept for overnight and crystallizes out (m.p.168° C). The cold mixture was filtered and the insoluble residue suspended in water and treated with sodium sulphite until traces of free bromine get evolved, The mixture was again filtered and the precipitate was collected and dried. Yield 3.25 gm (65%) (Fig 2)

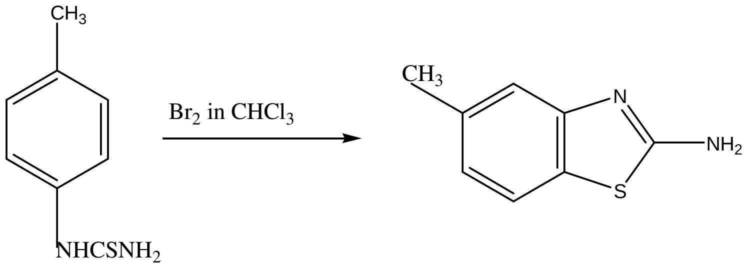


Fig 2: Synthesis of 2-Amino-6-methyl benzothiazole [S-2]

3. *Synthesis of N-2-benzothiazolylthiourea*

Benzoyl chloride (14gm) 0.1mol was added drop wise to NH_4SCN (7.6gm) 0.1mol in dry acetone 50ml with stirring .After the initial reaction subsided mixture was heated for 5 mins. Then hot solution of 2-amino benzothiazole (15gm) 0.1mol in dry acetone (50ml) was added with constant stirring. After refluxing for 1hr. the mixture was poured into the water a crystalline solid precipitated out slowly. After filtration the solid was heated with 10%NaOH solution (300ml) and filtered again. The filtrate was acidified with concentrated hydrochloric acid then made alkaline by addition of little ammonia. The solid obtained was filtered washed with water, dried and recrystallized with ethanol .yield (2.68gm) m. pt. 207 ° C (Fig 3)

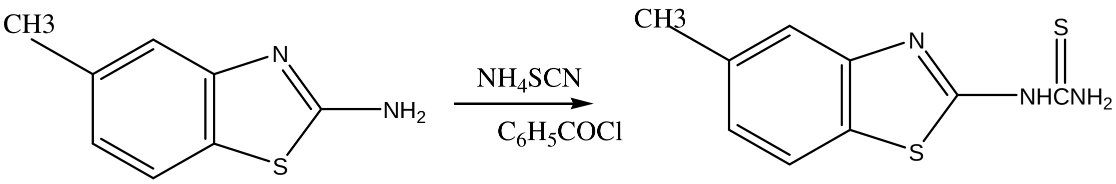


Fig 3: preparation of N-2-benzothiazolylthiourea [S-3]

4. *Synthesis of thiosemicarbazide derivative of the N-2benzothiazolylthiourea*

N-2-benzothiazolylthiazolyl (20gm) 0.82mol and hydrazine hydrate 80% (0.11 mol) in 50ml of ethylene glycol refluxed while stirring for 4hrs. The color of the reaction changes to green and the homogenous solution appears. A white solid was precipitated at the end of the reflux period. The mixture was cooled and the product was filtered and washed with water, air dried and recrystallized from ethanol (m.p.178° C). Yield 2.55gm (51 %), (Fig 4)

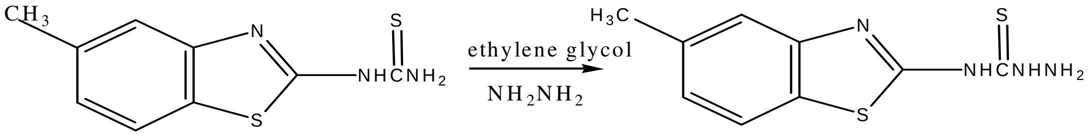


Fig 4: Synthesis of thiosemicarbazide derivative of the N-2-benzothiazolylthiourea **[S4]**

5. *Synthesis of Derivative S-5*

Thiosemicarbazide derivative (1.5 mmol) ,m-nitro acetophenone (2.2mmol) and glacial acetic acid 2-3drops were taken in absolute ethanol (20ml) and refluxed on a water bath for 10hrs till a different spot on TLC may appear. On cooling solid get separated which was filtered washed with water and recrystallized with ethanol. Yield 250mg (50%), m.p. 215° C. (Fig 5)

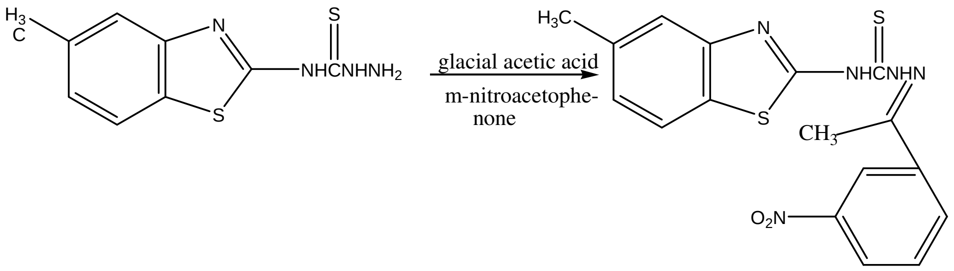


Fig 5: Synthesis of Derivative [S5]

6. *Synthesis of Derivative S-6*

Thiosemicarbazide derivative (1.5mmol) ,p-bromoacetophenone (2.2mmol) and glacial acetic acid 2-3drops were taken in absolute ethanol (20ml) and refluxed on a water bath for 10hrs till a different spot on TLC may appear. On cooling solid get separated which was filtered washed with water and recrystallized with ethanol. Yield 200mg (40%) m.p. 175℃ (Fig 6)

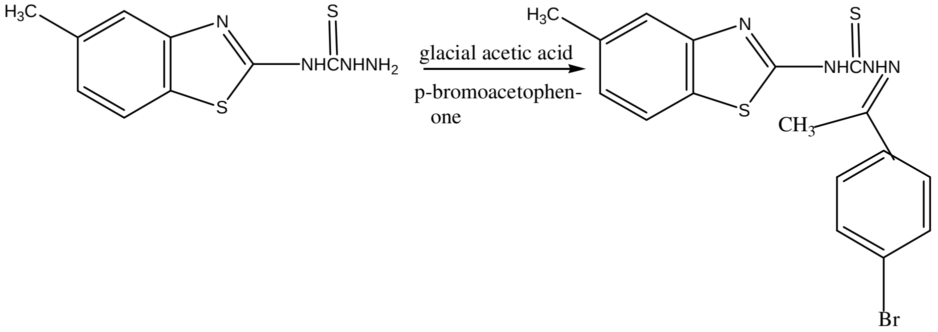


Fig 6: Synthesis of Derivative S-6 [S6]

7. *Synthesis of Derivative S-7*

Thiosemicarbazide derivative (1.5mmol) ,p-chloroacetophenone (2.2mmol) and glacial acetic acid 2-3drops were taken in absolute ethanol (20ml) and refluxed on a water bath for 10hrs till a different spot on TLC may appear. On cooling solid get separated which was filtered washed with water and recrystallized with ethanol .Yield 220mg (40%) m.p. 165 ℃ (Fig 7)

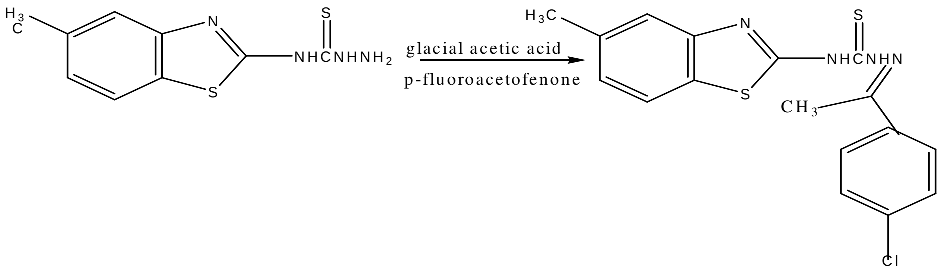


FIG 7: Synthesis of Derivative S-7 [S7]

8. Structural characterization

Authentication of chemical structure and compounds purities of all synthesized compounds and derivatives were evaluated by using several analytical methods as thin layer chromatography, Fourier Transform Infra Red Spectroscopy and Nuclear magnetic resonance. Thin layer chromatography and Infra Red Spectroscopy was done in I.T.S. (Paramedical) College, Muradnagar, Ghaziabad. Samples were sanded laboratory of CDRI, Lucknow for Nuclear magnetic resonance analysis.

9. Biological Evaluation

The microbial activity of the compound was assayed by agar disc diffusion method. The antimicrobial activity was tested for bacteria, gram negative and gram positive. Gram positive organisms were *Staphylococcus aureus*, and *Staphylococcus epidermidis*, and gram negative microorganism were *Escherichia coli*, and *Pseudomonas aeruginosa* All compounds were used by keeping 1mg/ml disc concentration, Ampicillin (17-22 mm, zone of inhibition) was taken as standard.

RESULTS

Due to the prominent activities exhibited by the benzothiazole, different compounds based on benzothiazole as a basic moiety have been synthesized with the hope to enhance the biological properties of newly designed compounds. Re crystallization of synthesized compounds was carried out by an appropriate solvent. Mainly single solvents were used wherever possible but the solvent mixtures were also employed as an alternative.

1. Structural characterization: Chemical structure of synthesized compound was determined various methods which shows the confirmation of synthesized compounds. Data of Fourier Transform Infra Red Spectroscopy and Nuclear magnetic resonance are given in the table 1.

2. Biological Evaluation: The inhibition of microorganisms under standardized conditions was utilized to demonstrate microbial action of the compounds.For present work efficacy of three compounds was detected against *S.aureus*, *P.aeruginosa*, and *E.coli*. The concentration of test compound used was 1mg/ml and ampicillin was taken as the standard drug. Zone of inhibition of ampicillin was measured 22, 21 and 21 mm for S.aureus, *P.aeruginosa*, and *E.coli*. respectively. There was a measurable difference of zone of inhibition for all synthesized compounds against each microorganism. Data of each measurement are given in the table 2 and figure 1, 2 and 3. From the results of antibacterial activity conducted on compound S-5 shows excellent activity against *P.aeruginosa* while compound S-6 shows excellent activity against *E.coli*. While compound S-7 shows moderate activity against all three bacterias.

Table 1.

Compounds	**IR SPECTRAL DATA (cm $^{-1}$)**	**NMR SPECTRAL DATA(δ)**
S-1	3851.15w (N-H *str*),3010.34m(Aliphatic C-H *str*),1624.73w(N-H *ben*), 1314.25w(Aromatic C-H *ben*)	2.33 (2H, s) NH$_2$, 3.62 (1H) NH , 7.19-7.10 (4H, d) Ar-H,2.24 (3H, s) CH$_3$
S-2	3402.2s (N-H *str*$_{(assym)}$)3276.8m (N-H *str*$_{(sym)}$) 1417.58w (Ar-C and Ar -N Ring *str*) 1242.07,1230.5w (C-S *str*)	7.60-7.23 (3H) Ar-H, 2.88 (2H, s) NH$_2$, 2.32 (3H,S) CH$_3$
S-3	3172.68m (Aromatic C- H *str*), 2912.3w (Aliphatic C-H *str),* 1577.66 (N-H *ben*)	7.55-7.22 (3H) Ar-H, 2.32 (3H,s) CH$_3$, 3.6 (1H s) NH, 3.0 (2H, s) NH$_2$
S-4	3417.6 (N-H *str*), 3238.26s, 3134.1(NHNH *str*)., 3134.11m (Aromatic C-H *str*	8.11-7.35 (3H s) Ar-H, 3.8 NH, 2.4 (3H) NHNH$_2$, 3.07 (3H s) CH$_3$
S-5	3437.1S (N-H *str*), 3167.2m(Aromatic C-H *str*), 1630.2s(C=N *str*) 1550.8w(NH *ben*) 2316m(Ar C-NO$_2$*str*), 1259, 1235w(C-S *str*)	7.14-7.60 (7H) Ar-H,3.01(3H,s) CH3 7.82 (1H s) NH, 3.8 (1H s) NH 2.32 (3 H s) CH$_3$
S-6	3436.6s(N-H *str*), 3166.1m(Ar C-H *str*) 1612s(C=N *str*) 1582.1w(N-H *ben*) 1261, 1238w(CS *str*), 960.2(C-Br *str*)	7.26-7.11 (7H) Ar-H, 2.6 (1H).s) NH, 2.36 (3H s) CH3, 1.60 (3H, s) CH3 7.56 (CH,S) NH
S-7	3439 w (N-H *str*), 3165.3m (Ar0 C-H *str*) 1677.9s (C=N *str*) 1585.5s (N-H *ben*) 801.7, 734.8w(Ar C-Cl *str*), 1260s (C=S *str*)	7.90-7.44 (7H) Ar-H, 3.4 (2H, s) NH, 3.03 (3H,s) CH$_3$, 2.58 (3H, s) CH$_3$ 8.2 (1 H, s) NH

Table 2: Zone of inhibition of ampicilline synthesized derivatives.

Compounds	S.aureus	P.aeruginosa	E. coli
Ampicillin(std)	22 mm	21 mm	21 mm
1E	11 mm (50%)	18 mm (85%)	12 mm (57%)
1F	12 mm (54%)	14 mm (67%)	17 mm (81%)
1G	10 mm (45%)	13 mm (62%)	11 mm (52%)

Fig 1: Percentage Activity v/s Derivatives synthesized in Case of *S. Aureus*

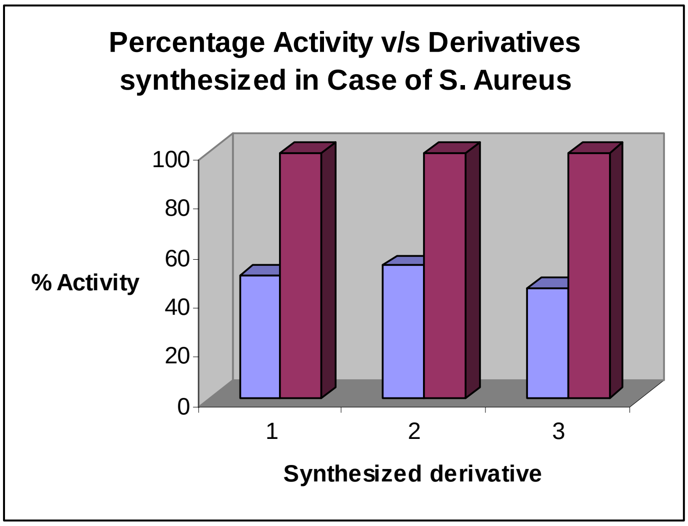


Fig 2: Percentage Activity v/s Derivatives synthesized in Case of *P.aeruginosa*

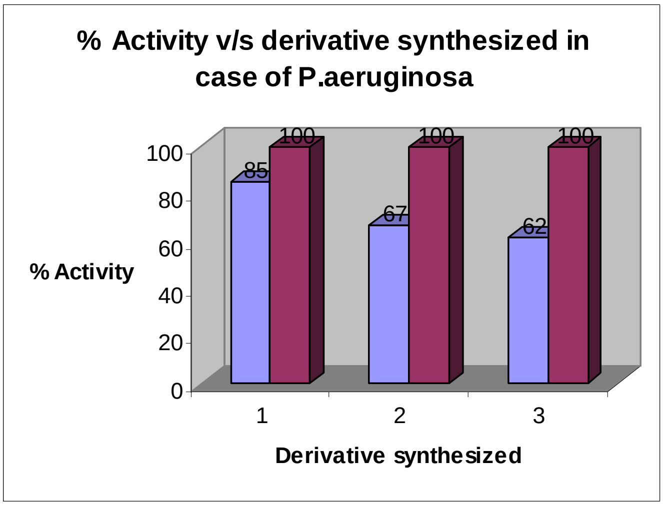
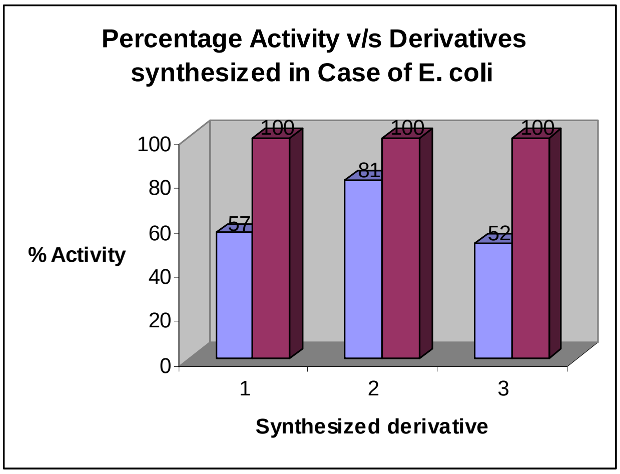


Fig 3: Percentage Activity v/s Derivatives synthesized in Case of E. coli



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