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# Synthesis of novel thiobiuret derivatives of 2-aminobenzothiazole and their antimicrobial studies.

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**ABSTRACT**

Novel thiobiuret derivatives of 2-aminobenzothiazole were synthesized and their structures confirmed by physical and spectroscopic studies such as $^1$HNMR, IR and elemental analysis. The antimicrobial activities of synthesized compounds were evaluated against some of the gram +ve and gram –ve bacterial strains and with fungal strains. Among the synthesized compounds, some of the compounds showed good activity and rest of the compounds showed moderate antibacterial and antifungal activity compared to standard dugs. This work provided a series of derivatives based on conjugation of thiourea with 2-aminobenzothiazole with moderate to good potent antimicrobial activity.

**Keywords:** 2-aminobenzothiazole; antimicrobial activity; Gram-negative bacteria; Gram-positive bacteria; Thiobiuert.

## 1. INTRODUCTION

Antimicrobial resistance is an increasingly pressing public health issue, which currently imposes a high burden on health care systems globally and is projected to have a significant and worsening impact in the coming decades. In the absence of effective treatments against an increasing number of microbial infections, infectious diseases will become more difficult to treat, thereby increasing their morbidity and mortality.

Consequently, efforts to prevent, mitigate, and overcome antimicrobial resistance are of global importance. Antimicrobial resistance development and spread is a natural response to the selective pressures placed upon microorganisms as a result of antimicrobial consumption and reduced infection prevention & control, particularly in healthcare settings. Therefore, strategies to combat resistance need careful evaluation, planning and implementation.

Benzothiazoles are bicyclic ring system with multiple applications. Number of 2-amino benzothiazoles were intensively studied for the various pharmalogical property such as antibacterial activity [1-2], Antifungal activity [3], Anticonvulsant activity [4], Anthelmintic activity [5-6], Anti cancer activity [7], Anti imflamatory activity [8], α2A Receptor Antagonist activity [9], diuretic potential [10], Muscle relaxant property [11], (Antifeedant activity, Acaricidal activity, Contact toxicity, Stomach toxicity) activities [12], antitubercular activity [13].

Replacement of oxygen atom in urea by sulphur atom produces thiourea which has been successfully used in many infectious diseases. Thiourea can be readily synthesized by different synthetic routes among which condensation of primary and secondary amine with isothiocyanate, thiophosgene or its derivatives constitutes the most widely accepted general methods [14-16]. The thiourea derivatives are studied for Antimalarial activity [17], antimicrobial activity [18].

In this connection, by keeping these rational points in our mind and as a part of the on-going programme in developing novel bioactive molecules, thiobiuret derivatives of 2-aminobenzothiazole were designed and synthesized to evaluate their antimicrobial efficacy.

## 2. MATERIALS AND METHODS

All chemicals phenylchloroformate, pyridine, KSCN, glacial acetic acid, chloroform Hexane, ethylacetete, acetone, DMSO, monomethylamine, benzyl amine, ethylamine, propylamine, butylamine, cyclohexylamine, TEA, DCM and other chemicals were purchased from s, d-fine chemicals, Merck, India. Methyl, ethyl, propyl urea and thiourea were procured from sigma Aldrich. All the solvents used for the synthesis and analysis were of analytical

grade. TLC was carried out on precoated silica gel plates prepared in laboratory using silica gel. $^1$H NMR spectra were obtained on a 400 MHz Bruker FT-NMR spectrometer instrument using DMSO as solvent and TMS as an internal standard. Elemental analysis was obtained by using VARIO EL III CHNS Elementar.

**2.1 General procedure for the preparation of 2-aminobenzothiazole.**

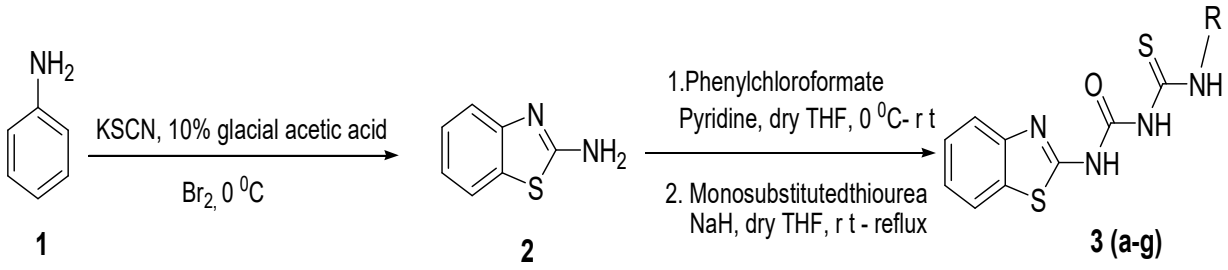
To the cooled and stirred solution of aniline (0.95g, 0.01 *M*) and potassium thiocyanate (0.97g, 0.01 *M*) in glacial acetic acid (20 mL), bromine (4.95g, 0.01 *M*) was added from dropping funnel at such a rate that the temperature does not rise beyond 0$^o$C. After the complete addition of bromine, the solution was stirred for an additional 2hr at 0$^o$C. Then it was allowed to stand for overnight during this period an orange precipitate settled at the bottom, water (6 mL) was added quickly slurry was heated at 85$^o$C on steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10 mL of glacial acetic acid, heated again to 85$^o$C and filtered in hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to p$^H$ 6, when dark yellow precipitate was appeared and recrystallized from benzene to obtain the 2-aminobenzothiazole.[19] Spectroscopic parameters of 2 aminobenzothiazole (2).

Yield-82%; M. P. 127-128$^o$C; IR (KBr): $\gamma_{max}$ cm$^{-1}$ 3410, 3252.5, 3040.5, 1645.7, 1535, 1462, 1319.5, 1102; $^1$HNMR (60 MHz, $CDCl_3$, δ ppm): δ7.65-7.55 (m, 4H, Ar-H), 5.55-5.71 (S, 2H, -C-$NH_2$).

**2.1.1 General procedure for synthesis of thiobiuret derivatives of 2-aminobenzothiazole compounds.** [20]

Phenyl chloroformate (0.015mmol) was added drop wise at such a rate to keep the temperature below 10$^0$C to the solution of 2-aminobenzothiazole (0.01mmol) and pyridine (2.47mmol) in dry THF (10 mL) and stirred at 0$^0$ C in an ice bath. The mixture was stirred for 0.5 h. Then reaction was continued by stirring at room temperature for 5-6hr and filtered. The white to light yellow solid was collected and washed with DCM to obtain crude benzothiazol-2-yl-carbamate (75-83%).

A mixture of monoN-substitutedThiourea (0.013mmol) and sodium hydride (5mmol) stirred for 30 minutes and then a solution of crude benzothiazol-2-yl-carbamate (0.01mmol) in dry THF was added. Then the reaction was carried at room temperature (rt) to reflux for 10-12hr. The reaction mixture cooled to rt followed by concentration to about 1/3 of the initial volume on rotavapor. Hexane was added to the residue and the obtained precipitate was collected by filtration under reduced pressure to yield the crude product. When necessary, the isolated material was purified by chromatography on silica gel with $CHCl_3$–EtOAc as the eluent.




1 2 3 (a-g)

R = $CH_3$, $CH_3$-$CH_2$,$CH_3(CH_2)_2$,$CH_3(CH_2)_3$, $(CH_3)_3C$, Ph, cyclohexane.

**Scheme - 1: Synthesis of thiobiuret derivatives of 2-aminobenzothiazole compounds.**

**Table - 1: Physical characterization data of Thiobiuret derivatives of 2-aminobenzothiazole compounds.**

| Entry | R | Yield (%) | Molecular formula | Elemental analysis (%) Calculated(found) | | | | $^1$HNMR (DMSO, δ ppm) |
|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S | |
| 3a | $CH_3$- | 85 | $C_{10}H_{10}N_4OS_2$ | 44.76 (45.05) | 3.70 (3.75) | 20.88 (21.15) | 23.90 (24.10) | 7.39-8.35(m, 4H, ArH-Bz), 6.4(2H, NHCO), 6.6(1H, NH, imide), 2.3(d, 2H, $CH_2$, NHCSNH), 2.41(t, 3H, $CH_3$). IR (KBr, $cm^{-1}$): 3412(N-H), 3155(C-H), 1751(N=C-N), 1577(C=C), 1110(C=S), 1199(C-N), 1730 (C=O). |
| 3b | $CH_3CH_2$- | 86 | $C_{11}H_{12}N_4OS_2$ | 46.99 (47.05) | 5.00 (5.35) | 19.84 (20.15) | 22.71 (23.12) | 7.45-8.45(m, 4H, ArH-Bz), 6.51(2H, NHCO), 6.67(1H, NH, imide), 2.5(d, 2H, α$CH_2$, NHCSNH), 3.6(q, 2H, α$CH_2$), 1.1(t, 3H, β$CH_3$). IR (KBr, $cm^{-1}$): 3415(N-H), 3160(C-H), 1760(N=C-N),1580(C=C),1205(C-N), 1120(C=S), 1738 (C=O). |
| 3c | $CH_3(CH_2)_2$- | 90 | $C_{12}H_{14}N_4OS_2$ | 48.62 (49.15) | 5.44 (5.69) | 18.90 (19.15) | 21.64 (22.10) | 7.4-8.4(m, 4H, ArH-Bz), 6.57(2H, NHCO), 6.75(IH, NH, imide), 2.55(2H, α$CH_2$, NHCSNH), 3.6(q, 2H, β $CH_2$, 1.7(t, 2H, β$CH_2$), 1.15(t, 3H, β$CH_3$).IR (KBr, $cm^{-1}$): 3420(N-H), 3162(C-H),1757(N=C-N), 1555(C=C), 1202(C-N), m1115(C=S), 1720(C=O). |
| 3d | $CH_3(CH_2)_3$- | 88 | $C_{13}H_{16}N_4OS_2$ | 50.30 (50.37) | 5.84 (5.95) | 18.05 (18.20) | 20.66 (20.71) | 7.4-8.35(m, 4H, ArH-Bz), 6.5(2H, NHCO), 6.65(s, 1H, NH, imide), 2.45 (2H, α$CH_2$, NHCSNH), 3.55(m, 2H, α$CH_2$), 1.65(m, 2H, β$CH_2$), 1.4 (m, 2H, γ$CH_2$), 1.05(t, 3H, δ$CH_3$).IR (KBr, $cm^{-1}$): 3430(N-H), 3165(C-H), 1761(N=C-N),1566(C=C),1210(C-N), 1105(C=S), 1720(C=O). |
| 3e | $(CH_3)_3C$- | 91 | $C_{13}H_{16}N_4OS_2$ | 50.30 (50.45) | 5.61 (5.65) | 18.05 (18.20) | 20.66 (21.15) | 7.45-8.45(m, 4H, ArH-Bz), 6.55(2H, NHCO), 6.71(s, 1H, NH, imide), 2.4 (2H, α$CH_2$, NHCSNH), 1.15(s, 9H, $CH_3$).IR (KBr, $cm^{-1}$): 3425(N-H), 3160(C-H),1750(N=C-N), 1560(C=C), 1210(C-N), 1127(C=S), 1719 (C=O). |
| 3f |  | 82 | $C_{15}H_{18}N_4OS_2$ | 53.54 (53.65) | 5.99 (6.15) | 16.65 (16.79) | 19.06 (19.75) | 7.41-8.30(m, 4H, ArH-Bz), 6.35(2H, NHCO), 6.42(s, 1H, NH imide),2.7 (2H,α$CH_2$,NHCSNH),3.52(m,1H,CH,Cyclohexane),1.45-1.82(m,10H,$CH_2$ Cyclohexane).IR (KBr, $cm^{-1}$): 3432(N-H),3155(C-H),1751(N=C-N), 1555(C=C), 1995(C-N), 1190(C-C), 1490(C=C), 1116(C=S), 1741C=O). |
| 3g |  | 85 | $C_{15}H_{12}N_4OS_2$ | 54.52 (54.72) | 4.27 (4.31) | 16.96 (17.21) | 19.41 (19.85) | 7.25-8.35(m, 4H, ArH-Bz), 5.1(s, 1H, NHCO), 6.5(s, 1H, NHCONH, imide),4.3(s,1H,NHCSNH),6.7(m,2H,αCHNCS,ArH),6.65-7.2(m,5H, ArH). IR (KBr,$cm^{-1}$):3432(N-H),3155(C-H),1751(N=C-N), 1555(C=C), 1995(C-N). |

**2.1.2. Biological activity**

**2.1.3. Antibacterial assay**

The synthesized compounds were screened for antibacterial efficacy with gram +ve and gram -ve bacteria according to the procedure of Kato K *et al.,* [21] with slight modifications.

**2.1.4. General Method for antibacterial assay:**

*In vitro* antibacterial assays of synthesized compounds were performed against *Staphylococcus aureus, Escherichia coli*, *Klebesiella pnemoniae and Pseudomonas auregenosa* by following agar well diffusion method. The bacterial strains were cultivated in Muller-Hinton broth and the inocculum concentration was adjusted by the method of mid-logarithmic phase (OD 600=0.5). The molten media was prepared by adding Muller-Hinton agar in sterile distilled water and autoclaved for 1 hr. The autoclaved molten media was poured into pre-sterilized 90 mm petriplate and allowed to solidify. Then, the media was scooped out at the center by using 8 mm sterilized cup-borer and inocculum were spread over the media and 50 µL of stock solution of compounds (10 µg/well) was added to the well made in the petriplate and kept for 3-4 days at 37 $^0$C. All the synthesized compounds were tested in triplicate; Streptomycin was used as positive control and DMSO as negative control. The zone of inhibition was measured in mm and presented in **Table-2.0.**

**2.1.5. Antifungal activity**

The synthesized compounds were tested for their antifungal efficiency by following the procedure of Kato *et al.,* with slight modifications.

**Table - 2: Antibacterial activity of thiobiuret derivatives of 2-amiobenzothiazole compounds**

| Compounds[a] | Inhibitory Zone (diameter) mm[b] | | | |
|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Pseudomonas auregenosa*** | ***Klebesiella pneumoniae*** |
| 3a | 06 | 05 | 06 | 06 |
| 3b | 06 | 06 | 05 | 06 |
| 3c | 07 | 06 | 06 | 07 |
| 3d | 08 | 07 | 07 | 08 |
| 3e | 06 | 06 | 07 | 07 |
| 3f | 07 | 07 | 06 | 07 |
| 3g | 07 | 06 | 07 | 06 |
| Streptomycin | 13 | 11 | 10 | 11 |

[a] Concentration of compounds and reference drug: 10 µg/well.

[b] Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

**Table - 3: Antifungal activity of thiobiuret derivative s of 2-amiobenzothiazole compounds**

| Compounds[a] | Inhibitory Zone (diameter) mm[b] | | |
|---|---|---|---|
| | ***Fusarium monoliforme*** | ***Aspergillus niger*** | ***Aspergillus flavus*** |
| 3a | 06 | 06 | 06 |
| 3b | 07 | 05 | 05 |
| 3c | 07 | 06 | 07 |
| 3d | 07 | 07 | 07 |
| 3e | 06 | 06 | 06 |
| 3f | 06 | 06 | 05 |
| 3g | 07 | 07 | 07 |
| Nystatin | 11 | 09 | 11 |

[a] Concentration of compounds and reference drug: 10 µg/mL

[b] Values are mean of three determinations, the ranges of which are less than 5% of the mean in all cases.

### 2.1.6 General method of antifungal assay:

*In vitro* antifungal assay of synthesized compounds were performed against *Aspergillus niger, Aspergillus flavus* and *Fusarium monoliforme* by using agar well diffusion method. [22] The fungal cultures were raised by growing on PDA media of pH 7.4 for six days at 25 $^0$C. The spores were harvested in sterilized normal saline (0.9 % NaCl in distilled water) and its concentration was adjusted to 1 x $10^6$/ml with a Haemocytometer. The autoclaved molten media (20mL) was poured in to each 90 mm sterilized petriplate and allowed to solidify. To study the growth response of fungi species, 0.4 mL of the synthesized compounds (10 µg/mL) was poured in to each plate and spreaded uniformly over the agar media. A volume of 10 µl spore suspension was poured in to the small depression made at the center of the plate and kept for 6 days at 25 $^0$C. After six days of incubation, the plates were observed and compared with their respective controls. The control plates contained only DMSO for which fungal growth is taken as 100% growth (no inhibition). The fungicidal activity of the synthesized compounds was assessed by comparing the zone of fungal growth in treated plates with that of control plates in mm and the results are presented in **Table-3.0**.

## 3. RESULTS AND DISCUSSION

Novel thiobiuret derivatives of 2-aminobenzthiazole compounds were synthesized and their structures were confirmed by TLC, elemental analysis and $^1$H NMR. The synthesized compounds were tested for both antimicrobial activity.

### 3.1. Antibacterial activity

### 3.1.1. Structural activity relationship of synthesized compounds

All the compounds synthesized were screened with strains of gram +ve and gram -ve bacteria such as *Staphylococcus aureus, Klebesiella pneumoniae, Pseudomonas auregenosa* and *Escherichia coli.* Streptomycin was used as positive control and DMSO as a negative control. The concentration used for both test compounds and that of standard remains the same. Among synthesized compounds, the antibacterial activity increases with the increase of carbon chain length of the alkyl group of mono-N-substituted thiourea conjugated with 2-aminobenzothiazole and also due to the presence of suphar atom in thiourea. The presence of hydrophobic alkyl chain in the molecule may helps the compounds to interact/penetrate more with cell membrane of the microorganisms their by inactivating them.

### 3.1.2. Antifungal activity

The synthesized compounds were tested against fungal strains such as *Aspergillus niger, Aspergillus flavus and Fusarium monoliforme.* Nysatin was used as positive control and DMSO as a negative control. Among all the compounds synthesized, compounds with long alkyl chain showed more activity than the rest of the compounds synthesized, from the literature it is evident that thiourea derivative are good antifungal agents. The conjugation of thiourea with 2-amino benzothiazole may enhance the antifungal activity of the synthesized compounds.

## 4. CONCLUSION

In summary, in an effort to discover novel antimicrobials agents, the thiobiuret derivatives of 2-aminobenzothiazole were synthesized and evaluated for their antimicrobial efficacy. From the results it is evident that some of the compounds showed promising antibacterial and antifungal activity. The present work demonstrate that the conjugation of thiourea derivatives with 2-aminobenzothiazole might be results in an enhanced antimicrobial activity.

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