JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# SYNTHESIS AND DEVELOPMENT OF $N_1$-ALKYL/ARYL-$N_5$-(6-NITRO-1, 3-BENZOTHIAZOL-2-YL)-1, 5-THIOBIURETS AND THEIR ASSESSMENT FOR ANTIMICROBIAL EFFICACY

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**Abstract :** In the pursuit of more biologically potent compounds, we envisioned to synthesize a series of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1,3-benzothiazol-2-yl)-1,5-thiobiurets by reacting 6-nitro-2-aminobenzothiazole with phenylchloroformate using anhydrous pyridine in dry THF at room temperature (RT) initially to produce corresponding isocyanates. The resulting isocyanates is then refluxed for 10-12 hours with monoalkyl/aryl urea derivatives in the presence of sodium hydride (NaH) in dry THF. The synthesized thiobiuret compounds were identified using $^1$H NMR and $R_f$ values, and subsequently tested for antibacterial activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, as well as antifungal activity against Aspergillus niger, Aspergillus flavus, and Fusarium moniliforme. Some of the thiobiurets displayed significant activity, while others showed moderate activity.

**Index Terms** - Antimicrobial resistance, thiobiuret, 6-nitro-2-aminobenzothiazole, zone of inhibition, isocyanates and Tetrahydrofuran (THF).

## 1.0 Introduction

The management of microbial infectious diseases continues to pose a significant and complex challenge for researchers across the globe. Despite the introduction of numerous new antimicrobial agents, their clinical efficacy is constrained in addressing a growing range of severe systemic infections due to their elevated potential for toxicity, emergence of drug resistance through genetic alterations, pharmacokinetic variations, and/or inadequacies in their antimicrobial potency [1].

Over the last few decades, there has been a growing interest in heterocyclic compounds and their derivatives due to their significant medicinal importance. The versatile and readily available 2-aminobenzothiazole scaffolds have garnered attention for their multiple applications in synthetic organic chemistry and the biological field, owing to their potent pharmacological activities [2]. In the realm of fused heterocycles, 2-aminobenzothiazole plays a crucial role in the development of various biologically active drugs [3-4], including those with antihelmintic [5], antitumor [6-7], anti-inflammatory [8], anti-tubercular [9-10], antimicrobial [11-12], analgesic [13-14], antileishmanial [15], and anticonvulsant activities [16-17].

Thiobiuret derivatives have demonstrated potential as glycoenzyme inhibitors [18], anticonvulsant, hypnotic [19], analgesic [20], and antimicrobial agents [21].

In light of these findings, it was deemed necessary to investigate the synthesis of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1,3-benzothiazol-2-yl)-1, 5-thiobiurets **4(a-l)** through the reaction of 6-nitro-2-aminobenzothiazole with phenylchloroformate using anhydrous pyridine in dry THF medium at room temperature (RT) in the sequential steps as illustrated in **(Scheme-1).**

## 2.0 Experimental work:

### Materials and Methods:

All chemicals such as ammonia, monomethylamine, benzyl amine, ethylamine, propylamine, butylamine, cyclohexylamine, TEA, DCM, and other chemicals were acquired from s, d-fine chemicals, Merck, India. Methyl, ethyl, propyl urea, and thiourea were purchased from Sigma Aldrich. The solvents utilized for synthesis and analysis were all of analytical grade. TLC was conducted on silica gel plates that were pre-coated in the laboratory. 1H NMR spectra were recorded using a 400 MHz Bruker FT-NMR spectrometer with DMSO as the solvent and TMS as an internal standard. Elemental analysis was performed using VARIO EL III CHNS Elementar.

**General procedure for the preparation of 6-nitro-1, 3-benzothiazol-2-amine.[22]**

P-nitroaniline (0.98g, 0.01 M) and potassium thiocyanate (0.97g, 0.01 M) were taken in glacial acetic acid (20 mL) and then cooled and stirred. Bromine (4.95g, 0.01 M) was slowly added from a dropping funnel to the solution to maintain a temperature of $0^oC$. After the addition of all the bromine, the solution was stirred for an additional 2 hours at $0^oC$. The mixture was left overnight, resulting in the formation of an orange precipitate at the bottom. Water (6 mL) was added to quickly form slurry, which was then heated to $85^oC$ on a steam bath and filtered while hot. The orange residue was transferred to a reaction flask, treated with 10 mL of glacial acetic acid, heated to $85^oC$, and filtered while hot. The combined filtrate was cooled, neutralized with concentrated ammonia solution to attain a pH of 6, leading to the appearance of a dark yellow precipitate. This precipitate was recrystallized from benzene to yield the 6-nitro-1, 3-benzothiazol-2-amine.

**6-Nitro-2-aminobenzothiazole (2):**

Yield -72%; M. P. 160-163$^oC$; IR (KBR): $\gamma_{max}$ $cm^{-1}$ 3435, 3040, 1535; $^1H$ NMR ($CDCl_3$) δ 8.62 (s, 1H, Ar), 9.23(s, 1H, Ar), 5.23(s, 2H, $NH_2$).

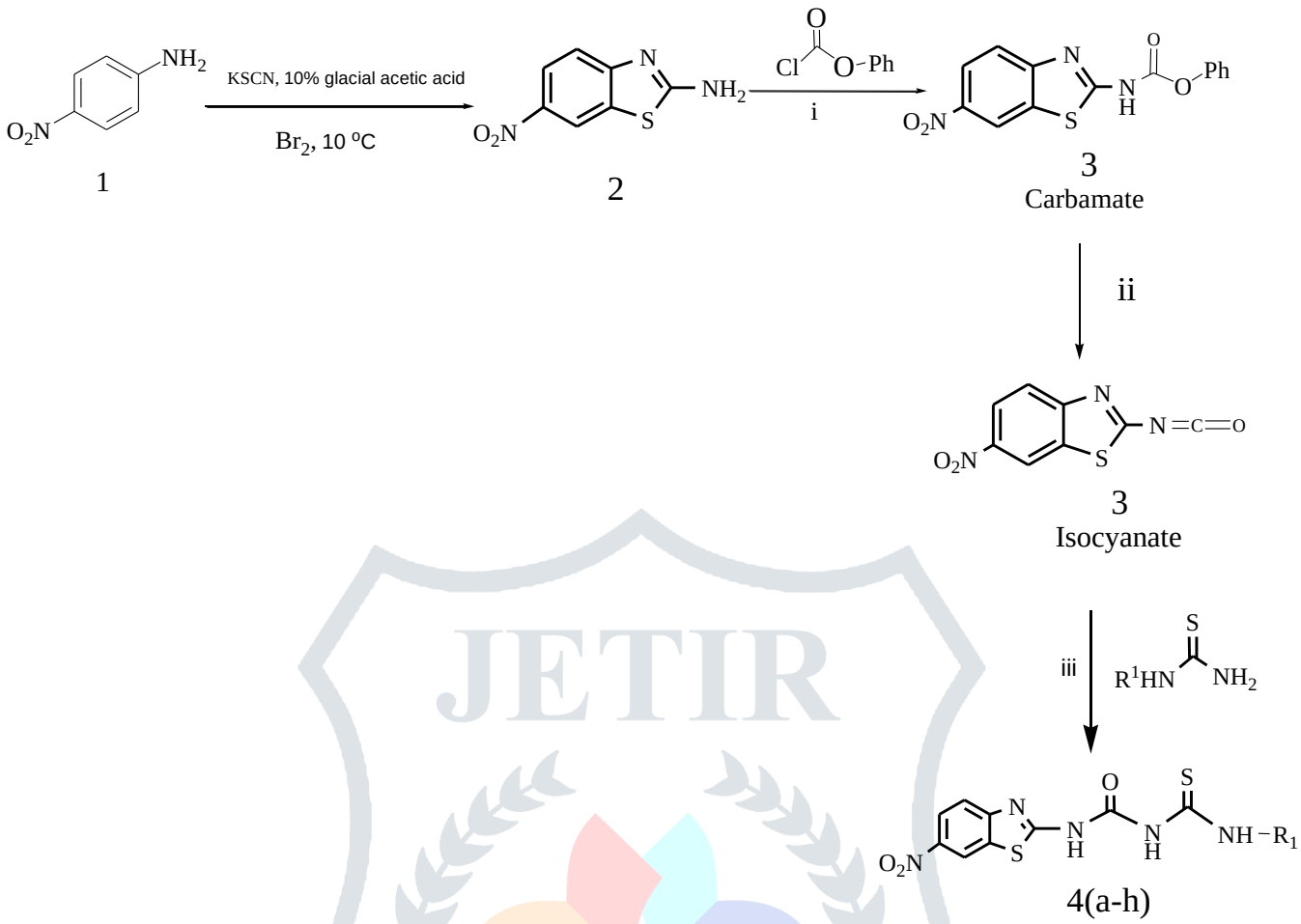
**General procedure for synthesis of the mono substituted N-Alkyl/aryl thiourea derivatives.[23]**

Ammonium thiocyanate (20 mmol, 1.52 g) was dissolved in dry acetonitrile (50 mL) and then mixed with Silica gel (18.48 g). The resulting mixture was stirred at room temperature for 30 min. After removing the acetonitrile using rotovapor, the reagent was dried in vacuo (15 mmHg) at room temperature for 3hr.

The slurry of $NH_4SCN/SiO_2$ (20mmol, 20g) in 1, 2-dichloroethane (100 mL) was mixed with benzyl chloride (1.7g, 10mmol) and stirred at $25^0C$ for 2 hr. Then, an amine (20 mmol) was added and stirred for an additional 1hr. The supported reagent was filtered out, and the filtrate was washed with 5% HCl, brine, and dried over anhydrous $Na_2SO_4$. The crude product was obtained after evaporating the solvent under reduced pressure, and then purified by recrystallization (methanol). Finally, hydrazine hydrate (10 mmol) was added and the resulting mixture was stirred for 1hr, resulting in the extraction of N-benzylthiourea (89%) by flash column chromatography. N-benzylthiourea was obtained in 90% yield by this method.

**General procedure for synthesis of $N_1$-alkyl/aryl-$N_5$-6-(nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets.[24]**

The 6-nitro-2-aminobenzothiazole (0.01mmol) and pyridine (2.47mmol) were dissolved in 10 mL of dry THF and stirred at 0 $^0C$ in an ice bath. The mixture was stirred for 0.5 hr. Then, phenyl chloroformate (0.015mmol) was added drop wise at such a rate to keep the temperature below 10 $^0C$. The reaction was stirred at room temperature for 5-6 hrs and filtered. The white to light yellow solid was collected and washed with DCM to obtain crude benzothiazol-2-yl-carbamates (80-90%) which is then converted into isocyanates on reaction of carbamates with anhy.$AlCl_3$ in dry THF. A mixture of mono N-substituted thioureas (0.013mmol) and sodium hydride (5mmol) stirred for 30 mins and then a solution of crude benzothiazol-2-yl-isocyanates (0.01mmol) in dry THF was added. The mixture was refluxed for 10-12 hrs before cooling to r.t. and 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 chromatography on silica gel with $CHCl_3$–EtOAc as the eluent.

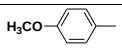



(i) anhy. Py, 0-RT, Dry THF, 5-6hrs (ii) anhy $Alcl_3$, anhy THF (iii) anhy.THF, NaH, Reflux, 10-12hrs

**Scheme-1: Synthesis of $N_1$-alkyl/aryl-$N_5$-6-(nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets**

**Table-1: Physical constants of $N_1$-alkyl/aryl-$N_5$-6-(nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets.**

| Entry | R | Yield (% | Molecular formula | Elemental analysis (%) Calculated (found) | | | | $^1$HNMR(DMSO, δ ppm) |
|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S | |
| 4a | $CH_3$- | 90 | $C_{10}H_{11}N_5O_3S_2$ | 38.33 (38.45) | 3.54 (3.75) | 22.35 (22.55) | 20.47 (20.50) | 9.35(d, 1H, ArH-Bz), 8.6 (d, 1H, ArH-Bz), 8.3(dd, 1H, ArH-Bz), 6.5(s, 1H, NHCO), 6.3(s, 1H, NHCONH, imide), 2.2(s, 1H, NHCSNH), 2.6 (s, 3H, $CH_3$). IR (KBr, $cm^{-1}$): 3490 (N-H), 3255 (C-H), 1790 (N=C-N), 1590 (C=C), 1105(C=S), 1726 (C=O), 1260 (C-N). |
| 4b | $CH_3CH_2$- | 88 | $C_{11}H_{15}N_5O_2S_2$ | 42.16 (42.35) | 4.82 (4.91) | 22.35 (22.51) | 20.46 (20.51) | 9.2(d, 1H, ArH-Bz), 8.3 (d, 1H, ArH-Bz), 8.2 (dd, 1H, ArH-Bz), 6.55(s, 1H, NHCO), 6.34(s, 1H, NHCONH, imide), 2.1(s, 1H, NHCSNH), 3.45(q, 3H, $CH_2$), 1.15(t, 3H, $CH_3$). IR (KBr, $cm^{-1}$): 3482 (N-H), 3210 (C-H), 1775 (N=C-N), 1579 (C=C), 1117(C=S), 1749(C=O), 1251 (C-N). |
| 4c | $CH_3(CH_2)_2$- | 89 | $C_{12}H_{17}N_5O_2S_2$ | 44.02 (44.25) | 45.23 (45.37) | 21. 39 (21.41) | 19.59 (20.05) | 9.25(d, 1H, ArH-Bz), 8.25 (d, 1H, ArH-Bz), 8.33(dd, 1H, ArH-Bz), 6.35(s, 1H, NHCO), 6.12(s, 1H, NHCONH, imide), 2.0(s, 1H, NHCSNH), 3.55(t,3H, α$CH_2$), 1.63(m, 3H, β$CH_2$), 1.1 (t, 3H, γ$CH_2$).IR (KBr, $cm^{-1}$): 3470 (N-H), 3245 (C-H), 1770(N=C-N), 1569 (C=C), 1120(C=S), 1738(C=O), 1255 (C-N). |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4d | $CH_3(CH_2)_3$- | 92 | $C_{13}H_{17}N_5O_3S_2$ | 43.93 (44.05) | 4.82 (5.15) | 19.7 (20.1) | 18.04 (18.55) | 9.2(d, 1H, ArH-Bz), 8.5 (d, 1H, ArH-Bz), 8.3(dd, 1H, ArH-Bz),6.5(s, 1H, NHCO), 6.2(s, 1H, NHCONH, imide), 2.3(s, 1H, NHCSNH), 3.5(t, 2H, $\alpha CH_2$), 1.6 (m, 2H, $\beta CH_2$), 1.35 (m, 2H, $\gamma CH_2$), 1.12 (t, 3H, $\delta CH_3$).IR (KBr, $cm^{-1}$): 3475 (N-H), 3234 (C-H), 1757(N=C-N), 1576 (C=C), 1126(C=S), 1719(C=O), 1249 (C-N). |
| 4e | $(CH_3)_3C$- | 90 | $C_{13}H_{17}N_5O_3S_2$ | 43.93 (44.05) | 4.82 (4.95) | 19.7 (19.9) | 18.04 (18.17) | 9.1(d, 1H, ArH-Bz), 8.35 (d, 1H, ArH-Bz), 8.39(dd, 1H, ArH-Bz), 6.7(s, 1H, NHCO), 6.45(s, 1H, NHCONH,imide),2.5(s,1H, NHCSNH),1.2 (s, 9H, $\delta CH_3$).IR (KBr, $cm^{-1}$): 3455 (N-H), 3230 (C-H),1745(N=C-N),1557(C=C),1129(C=S),1726(C=O),1235(C-N). |
| 4f |  | 95 | $C_{15}H_{19}N_5O_5S_2$ | 47.23 (47.35) | 5.02 (5.15) | 18.36 (18.55) | 16.81 (16.95) | 9.5(d, 1H, ArH-Bz), 8.7 (d, 1H, ArH-Bz), 8.5(dd, 1H, ArH-Bz), 6.5(s, 1H, NHCO), 6.75(s, 1H, NHCONH, imide), 2.2(s,1H,NHCSNH), 1,46-1.75(m, 10H,$CH_2$),3.5(m,1H,CH,Cyclohexane). IR (KBr, $cm^{-1}$): 3465(N-H), 3235 (C-H), 1753 (N=C-N), 1625 (C=C), 1130(C=S), 1742(C=O) 1215(C-N),1160(C-C), 1460(C=C). |
| 4g |  | 90 | $C_{15}H_{13}N_5O_3S_2$ | 47.99 (48.15) | 3.49 (3.69) | 18.65 (19.15) | 17.08 (16.97) | 9.2(d, 1H, ArH-Bz), 8.5 (d, 1H, ArH-Bz), 8.3(dd, 1H, ArH-Bz), 5.5(s, 1H, NHCO) 8.7(s, IH, NHCONH, imide), 5.2(s, 1H, NHCSNH), 6.55-7.3 (m, 5H, ArH).IR (KBr, $cm^{-1}$): 3452 (N-H), 3229 (C-H), 1780(N=C-N), 1618 (C=C), 1135(C=S), 1715(C=O), 1205(C-N), 1170 (C-C), 1475 (C=C). |
| 4h |  | 92 | $C_{16}H_{15}N_5O_4S_2$ | 47.40 (47.55) | 3.73 (3.75) | 17.27 (17.29) | 15.82 (16.19) | 9.1(d, 1H, ArH-Bz), 8.4 (d, 1H, ArH-Bz), 8.1(dd, 1H, ArH-Bz), 5.5(s, 1H, NHCO), 8.5(s, 1H, NHCONH, imide),5.1(s, 1H, NHCONH), 6.5-6.8 (m, 4H, ArH), 3.78 (s, 3H, $OCH_3$). IR (KBr, $cm^{-1}$): 3444 (N-H), 3224(C-H), 1765 (N=C-N), 1637 (C=C), 1442 (N-C=S), 1190(C-N), 1144(C-C), 1120(C=S),1738(C=O),1480(C=C) |

**Antibacterial assay**

The antibacterial assay was carried out against gram +ve and gram -ve bacteria by following the procedure of Kato K *et al.,*[25] with slight modifications.

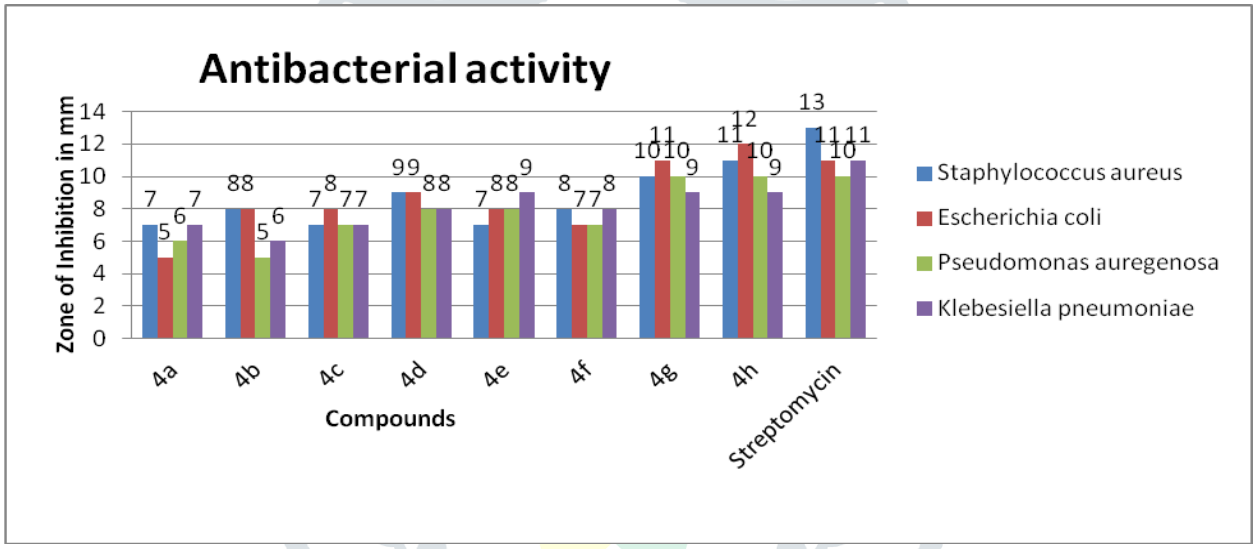
**General method for antibacterial assay:**

*In vitro* antibacterial assays were performed against *Staphylococcus aureus*, *Escherichia coli*, *Klebesiella pnemoniae and Pseudomonas auregenosa* by using agar well diffusion method.[25] 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/mL) 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 water as negative control. The zone of inhibition was measured in mm and presented in **table-2 and graph-1** respectively.

**Table-2: Antibacterial activity of $N_1$-alkyl/aryl-$N_5$- (6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets.**

| Compounds[a] | Zone of Inhibition (diameter) mm[b] | | | |
|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Pseudomonas auregenosa*** | ***Klebesiella pneumoniae*** |
| 4a | 07 | 05 | 06 | 07 |
| 4b | 08 | 08 | 05 | 06 |
| 4c | 07 | 08 | 07 | 07 |
| 4d | 09 | 09 | 08 | 08 |
| 4e | 07 | 08 | 08 | 09 |
| 4f | 08 | 07 | 07 | 08 |
| 4g | 10 | 11 | 10 | 09 |
| 4h | 11 | 12 | 10 | 09 |
| Streptomycin | 13 | 11 | 10 | 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.




**Graph-1: Antibacterial activity of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets.**

**Antifungal activity:**

The antifungal activities of the synthesized compounds were evaluated by following the procedure of Kato *et al.*,[26] with slight modifications.

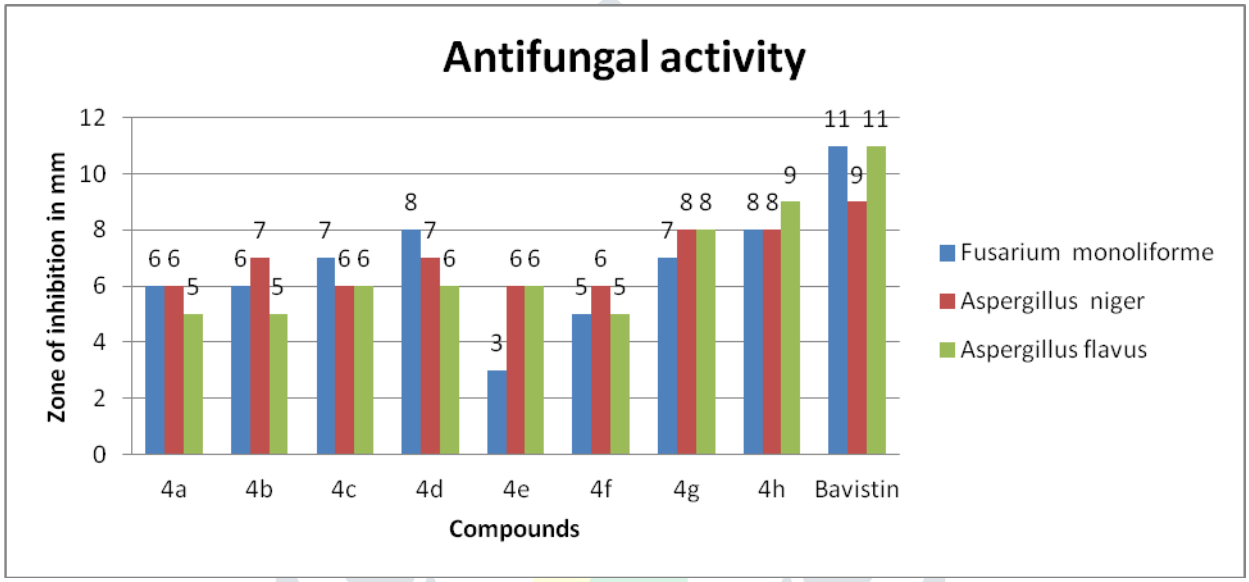
**General method of antifungal assay:**

*In vitro* antifungal assays were performed against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium monoliforme* by using agar well diffusion method. 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 distilled water for which fungal growth is taken. The zone of inhibition was measured in mm and presented in **table-3 and graph-2** respectively

**Table-3: Antifungal activity of activity of $N_1$-alkyl/aryl-$N_5$- (6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets:**

| Compounds[a] | Zone of Inhibition (diameter) mm[b] | | |
|---|---|---|---|
| | *Fusarium monoliforme* | *Aspergillus niger* | *Aspergillus flavus* |
| 4a | 06 | 06 | 05 |
| 4b | 06 | 07 | 05 |
| 4c | 07 | 06 | 06 |
| 4d | 08 | 07 | 06 |
| 4e | 03 | 06 | 06 |
| 4f | 05 | 06 | 05 |
| 4g | 07 | 08 | 08 |
| 4h | 08 | 08 | 09 |
| Bavistin | 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.




**Graph-2: Antifungal activity of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets.**

## 3.0 Results and Discussion:

We have synthesized a new class of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets. The product obtained was characterized by TLC, elemental analysis and $^1$H NMR. The synthesized compounds were used for antimicrobial activity.

**Structural activity relationship of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets:**

**Antibacterial activity**

All Compounds synthesized were tested against 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 all the synthesized compounds **4c, 4d, 4g, 4f** and **4h** with electron releasing group and electron withdrawing groups in benzothiazole moiety with substituted thiourea showed better activity over the other compounds. The following factors may be held responsible for the enhancement of antibacterial activity, viz., the presence of electron releasing groups like $OCH_3$, $CH_3$ and electron withdrawing group $NO_2$ enhance the antibacterial activity as well as the antifungal activity. The presence of these helps the molecule to interact/penetrate more with cell membrane of the microorganisms thereby inactivating them.

**Antifungal activity:**

All 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 synthesized compounds, compounds with electron releasing group and electron withdrawing groups **4c, 4d, 4g** and **4h** showed better activity over the other compounds, other compounds showed mild to moderate antifungal activity. Here also the factors explained under antibacterial activity equally holds well.

**Acknowledgement**
Authors are thankful to the department of Chemistry, Maharani's Science College for Women, Bangalore-560001, India for providing the necessary laboratory.

**Conflict of Interest:**
The authors confirm there is no conflict of interest.

**4.0 Conclusion:**
In the present work efforts have been made to synthesize newer derivatives of $N_1$-alkyl/aryl-$N_5$-(6-nitro-1, 3-benzothiazol-2-yl)-1, 5-thiobiurets via novel route of synthesis. Among the synthesized compounds **4c, 4d, 4g** and **4h** showed better antibacterial activity against gram +ve and gram -ve bacteria such as *Staphylococcus aureus, Klebesiella pneumoniae, Pseudomonas auregenosa* and *Escherichia coli* and antifungal activity against *Aspergillus niger, Aspergillus flavus and Fusarium monoliforme*. The rest of the compounds showed mild to moderate antibacterial and antifungal activity.

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