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Design, synthesis, and evaluation of antimicrobial activity of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

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ABSTRACT

Novel alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole were synthesized through a three-step process. Initially, 6-nitro-2-aminobenzothiazole was treated with phenylchloroformate in anhydrous pyridine and dry THF at room temperature (RT) to obtain the corresponding carbamates which on further reaction with anhydrous aluminium chloride and anhy. pyridine in dry THF gives corresponding isocyanates. These isocyanates were then refluxed for 10-12 hours with monoalkyl/aryl urea derivatives in the presence of sodium hydride (NaH) in dry THF. The resulting alkyl/aryl urea derivatives of 6-methoxy-2-aminobenzothiazole were characterized using 1H NMR and Rf values. Subsequently, their antibacterial activity against Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae, and Pseudomonas aeruginosa, as well as antifungal activity against Aspergillus Niger, Aspergillus flavus, and Fusarium monoliforme, was evaluated. While some of the synthesized compounds demonstrated significant activity and the remaining compounds exhibited moderate activity.

Keywords: Antimicrobial resistance, Derivatives, 6-nitro-2-aminobenzothiazole, Zone of inhibition and Tetrahydrofuran (THF).

1. INTRODUCTION

Benzothiazole derivatives have attracted considerable attention due to their antibacterial [1-2], antidiabetic [3], Receptor Antagonist 2A [4] and anticonvulsant properties [5]. Conversely, urea and its derivatives are acknowledged as significant organic compounds with diverse applications such as tranquillizing drugs, antioxidants, dyes, corrosion inhibitors, agrochemicals, and antidiabetic agents [6-8].

Moreover, urea derivatives have been associated with pharmaceutical uses like anti-melanoma [9], antidiuretics [10], antagonists of human vanilloid VR1 receptors [11], anti-tuberculosis [12], anti-hyperglycemic agents [13], and inhibitors of Murine receptor A and Murine receptor B [14].

Given the wide range of biological functions of benzothiazole and urea derivatives mentioned above, we introduce a simple method for synthesis of new benzothiazole derivatives containing 6-NO2-2-aminobenzothiazole and monosubstituted alkyl/aryl urea derivatives through carbamate and isocyanate chemistry.

2. EXPERIMENTAL WORK

2.1. 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 and propyl urea, 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.

2.2. General procedure for the preparation of 6-nitro-1, 3-benzothiazol-2-amine [15]

A solution of p-nitroaniline (0.92g, 0.01 M) and potassium thiocyanate (0.97g, 0.01 M) in glacial acetic acid (20 mL) was mixed and stirred after cooling. Bromine (4.95g, 0.01 M) was then slowly added from a dropping funnel to the solution to prevent the temperature from exceeding 0°C. Once

all the bromine was added, the solution was stirred for an additional 2 hours at 0°C. After standing overnight, an orange precipitate formed at the bottom. Water (6 mL) was added quickly to create a slurry, which was then heated to 85oC 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 again to 85oC, and filtered while hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to reach a pH of 6. A dark yellow precipitate appeared, which was recrystallized from benzene to yield the 6-substituted-1,3-benzothiazol-2-amine.

6-Nitro-2-aminobenzothiazole (2):

Yield-72%;; M. P. 160-163oC; IR (KBR): γ max cm-1 3435, 3040, 1535; 1H NMR (CDCl3) δ 8.62 (s, 1H, Ar), 9.23(s, 1H, Ar), 5.23(s, 2H, NH2).

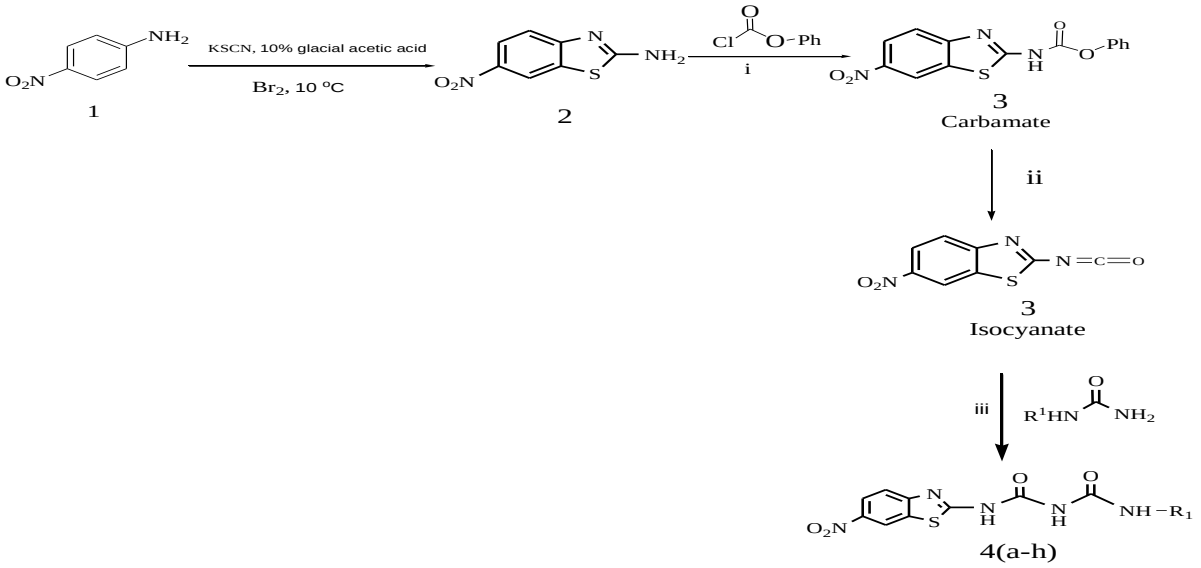
2.3. General procedure for synthesis of the mono substituted Alkyl/aryl urea derivatives [16]:

To the stirred solution of silicon tetraisocyanate (19.6 g., 0.1 mole) in 150 ml. of anhydrous benzene in a three necked round-bottomed flask a solution of amine (39.7g, 0.4 mole) in 100 ml. of anhydrous benzene is added slowly. The mixture is heated at the reflux temperature for 30 minutes; the benzene is then removed using a rotary evaporator. Dilute isopropyl alcohol (200 ml) is added to the residue and the resulting mixture is heated at the reflux temperature for 30 minutes. The hot mixture is filtered through a 0.5-in. layer of celite contained in a coarse-grade sintered-glass funnel. The gelatinous silica is washed with two 75-ml. portions of acetone and is then pressed and drained. The combined filtrates are evaporated to dryness on a steam bath. The crude cyclohexyl urea (m.p.183-189°, 50.0 g., 90% yield) is recrystallized from 200 ml. of isopropyl alcohol to give 35g. (67%) of product, m. p. 188-190°. Concentration of the mother liquor affords additional product which is less pure.

2.4. General procedure for synthesis of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole [17]:

Solution of 6-nitro-2-aminobenzothiazole (0.01mmol) and pyridine (2.47mmol) in dry THF (10 mL) stirred at 0 0C in an ice bath. The mixture was stirred for 0.5 h. phenyl chloroformate (0.015mmol) was added drop wise at such a rate to keep the temperature below 100C. 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-carbamate (80-90%), which on further treatment with anhy. AlCl3 in dry THF yields corresponding isocynates.

A mixture of mono N-substituted ureas (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 CHCl3–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 alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

Antibacterial assay:

The antibacterial assay was carried out against gram +ve and gram -ve bacteria by following the procedure of Kato K et al., with slight modifications.[18]

General method for antibacterial assay:

In vitro antibacterial tests were conducted on Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa using the agar well diffusion method. The bacterial strains were grown in Muller-Hinton broth, and the inoculum concentration was adjusted during the mid-logarithmic phase (OD 600=0.5). The molten media was prepared by mixing Muller-Hinton agar with sterile distilled water and autoclaved for 1 hour. After autoclaving, the molten media was poured into pre-sterilized 90 mm petri plates and allowed to solidify. Subsequently, the media was removed from the center using an 8 mm sterilized cup-borer, and the inoculum was spread over it. A 50 µL stock solution of compounds (10 µg/mL) was added to the wells created in the petriplate, which was then kept at 37°C for 3-4 days. All synthesized compounds were tested in triplicate, with Streptomycin serving as the positive control and water as the negative control. The zone of inhibition was measured in millimeters and presented in table-2 and graph-1, respectively.

Table-1: Physical characterization data of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

Entry	R	Yield (%)	Molecular formula	Elemental analysis (%) Calculated(found)				^{1}HNMR(DMSO, δ ppm)
				C	H	N	S	
4a	CH_3-	88	$C_{10}H_{11}N_5O_4S$	40.40 (40.75)	3.73 (3.75)	23.56 (23.65)	10.79 (10.81)	9.15(d, 1H, ArH-Bz), 8.6 (d, 1H, ArH-Bz), 8.3(dd, 1H, ArH-Bz), 5.52(s, 1H, NHCO, urea), 9.64(s, 1H, NHCONH, imide), 5.2(s, 1H, NHCONH), 3.1(s, 3H, CH_3). IR (KBr, cm^{-1}): γ-3435 (N-H); 3055 (Ar-C-H); 1719 (C=O); 1432 (C=N);1249 (C-N); 1605(C=C).
4b	CH_3CH_2-	90	$C_{11}H_{13}N_5O_4S$	42.44 (42.55)	4.21 (4.25)	22.50 (22.52)	10.30 (10.35)	9.20(d, 1H, ArH-Bz), 8.5 (d, 1H, ArH-Bz), 8.35(dd, 1H, ArH-Bz), 5.5(s, 1H, NHCO, urea), 9.7(s, 1H, NHCONH, imide), 5.25 (s, 1H, NHCONH), 3.1(q, 2H, αCH_2), 1.25(t, 3H, βCH_3). IR (KBr, cm^{-1}):γ-3440 (N-H); 3050 (Ar-C-H); 1721 (C=O); 1435 (C=N); 1255 (C-N); 1610 (C=C).
4c	$CH_3(CH_2)_2$-	92	$C_{12}H_{15}N_5O_4S$	44.30 (44.45)	4.65 (4.77)	21.53 (21.55)	9.86 (9.95)	9.12(d, 1H, ArH-Bz), 8.52 (d, 1H, ArH-Bz), 8.4(dd, 1H, ArH-Bz), 5.53(s, 1H, NHCO, urea), 9.6(s, 1H, NHCONH, imide), 5.3(s, 1H, ,NHCONH), 3.1(t, 2H, αCH_2), 1.65(m, 2H, βCH_2), 1.05 (t, 3H, γCH_3). IR (KBr, cm^{-1}): γ-3442 (N-H); 3060 (Ar-C-H);

								1725 (C=O); 1436 (C=N); 1257 (C-N); 1610(C=C).
4d	$CH_3(CH_2)_3$-	88	$C_{13}H_{17}N_5O_4S$	46.01 (46.03)	5.05 (5.15)	20.64 (21.75)	9.45 (9.55)	9.25(d, 1H, ArH-Bz), 8.6(d, 1H, ArH-Bz), 8.3(dd, 1H, ArH-Bz), 5.51(s, 1H, NHCO, urea), 9.6 (s, 1H, NHCONH, imide), 5.5(s, 1H, ,NHCONH), 3.1(t, 2H, αCH_2), 1.65(m, 2H, βCH_2), 1.4 (m, 2H, γCH_2), 1.1 (t, 3H, δCH_3). IR (KBr, cm^{-1}):γ-3455 (N-H); 3058 (Ar-C-H); 1721 (C=O); 1439(C=N); 1257 (C-N); 1630 (C=C).
4e	$(CH_3)_3C$-	90	$C_{13}H_{17}N_5O_4S$	46.01 (46.15)	5.05 (5.15)	20.64 (20.94)	9.45 (9.55)	9.17(d, 1H, ArH, Bz), 8.5 (d, 1H, ArH, Bz), 8.31(dd, 1H, ArH, Bz), 5.5(s, 1H, NHCO, urea), 9.7(s, 1H, NHCONH, imide), 5.27(s, 1H, NHCONH), 1.45 (s, 9H, δCH_3). IR (KBr, cm^{-1}):γ-3450 (N-H); 3055 (Ar-C-H); 1730 (C=O); 1440 (C=N); 1250 (C-N); 1615 (C=C).
4f		85	$C_{15}H_{19}N_5O_4S$	49.30 (49.45)	5.24 (6.01)	19.70 (19.85)	8.78 (9.35)	9.1(d, 1H, ArH-Bz), 8.45(d, 1H, ArH-Bz), 8.25(dd, 1H, ArH-Bz), 5.45(s, 1H, NHCO, urea), 9.65(s, 1H, NHCONH, imide), 5.9(s, 1H, NHCONH),1.4-1.7 (m, 10H, CH_2), 3.45 (m, 4H, αCH_2), IR (KBr, cm^{-1}): γ-3462 (N-H); 3055 (Ar-C-H); 1715 (C=O); 1436 (C=N); 1247 (C-N); 1612 (C=C), 1100 (C-C).
4g		70	$C_{15}H_{13}N_5O_4S$	50.13 (50.99)	3.65 (3.75)	19.49 (19.51)	8.92 (9.95)	8.9 (d, 2H, ArH-Bz), 8.5 (d, 2H, ArH-Bz), 8.0(dd, 1H, ArH-Bz), 5.1(s, 1H, NHCO, urea), 9.63(s, 1H, NHCONH, imide), 5.2(s, 1H ,NHCONH), 6.9 -7.55 (m, 5H, ArH),IR (KBr, cm^{-1}): γ-3450 (N-H); 3053(Ar-C-H); 1705(C=O);1426 (C=N); 1237(C-N); 1617(C=C).

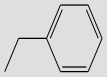
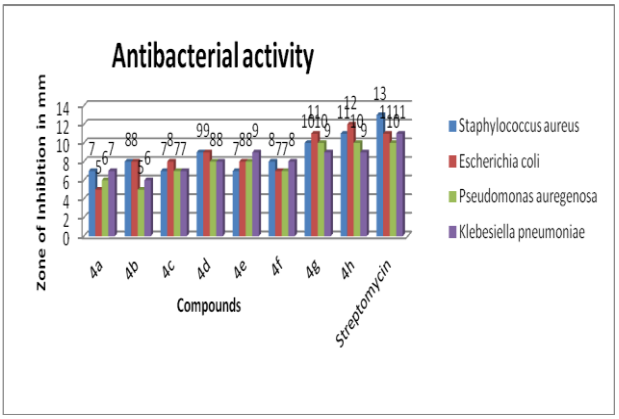
4h		75	$C_{16}H_{15}N_5O_4S$	51.47 (51.55)	4.05 (4.32)	18.76 (18.05)	8.59 (8.17)	9.0(d, 1H, ArH-Bz), 8.52 (d, 1H, ArH-Bz), 8.25(dd, 1H, ArH-Bz), 5.15(s, 1H, NHCO, urea), 9.37(s, 1H, NHCONH, imide), 5.1(s, 1H, ,NHCONH), 4.1(s, 2H, CH_2Ar), 6.7-7.40 (m, 2H, ArH), IR (KBr, cm^{-1}): γ-3460(N-H); 3054(Ar-C-H); 1720(C=O); 1440(C=N); 1260(C-N); 1612(C=C).

Table – 2: Antibacterial Activity

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 alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

Antifungal activity:

The synthesized compounds were assessed for their antifungal properties using the method outlined by Kato et al., with minor adjustments. [19]

General method of antifungal assay:

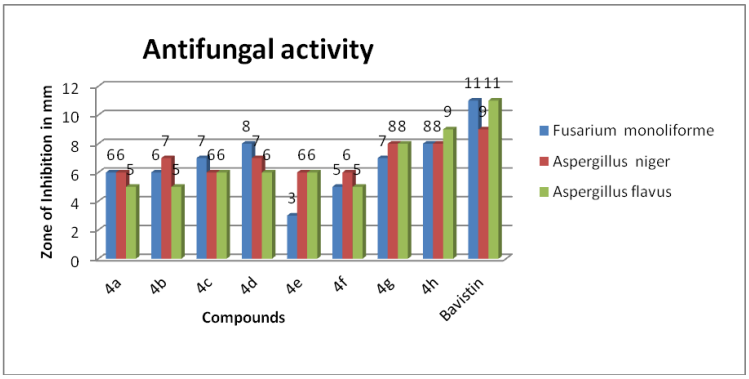
In vitro antifungal assays were performed against Aspergillus niger, Aspergillus flavus and Fusarium monoliforme by using agar well diffusion method. The fungal cultures were raised by growing on PDA media of pH 7.4 for six days at 25^{0}C. The spores were harvested in sterilized normal saline (0.9 % NaCl in distilled water) and its concentration was adjusted to 1 x 106/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

Table - 3: Antifungal activity of activity of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole

Compounds[a]	Zone of inhibiton (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 alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

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 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 and graph-2 respectively.

3. RESULTS AND DISCUSSION

A novel category of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole has been successfully created. The products were identified using TLC, elemental analysis, and 1H NMR. These synthesized compounds were then tested for their antimicrobial properties.

Structural activity relationship of alkyl/aryl urea derivatives of 6-nitro-2-aminobenzothiazole.

Antibacterial studies:

All synthesized compounds were tested against gram-positive and gram-negative bacteria strains including Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli. Streptomycin served as the positive control, while DMSO was used as the negative control. The concentration remained consistent for both the test compounds and the standard. Among the synthesized compounds, compounds 4d, 4g and 4h with electron-releasing or electron-withdrawing groups in the benzothiazole moiety and ureas exhibited superior activity compared to other compounds. Factors such as the presence of electron-releasing groups like CH3, and electron-withdrawing groups like NO2 were found to enhance antibacterial and antifungal activities. These groups facilitate better interaction and penetration of the molecule with the cell membrane of microorganisms, leading to their inactivation.

Antifungal studies:

The synthesized compounds underwent testing against fungal strains including Aspergillus niger, Aspergillus flavus and Fusarium monoliforme.. Nysatin served as the positive control, while DMSO served as the negative control. Compounds 4C, 4d, 4g and 4h with electron releasing and electron withdrawing groups exhibited superior activity compared to other compounds in the series, while the remaining compounds demonstrated mild to moderate antifungal activity. These findings also apply to the factors influencing antibacterial activity.

4. CONCLUSION

We present new alkyl/aryl urea derivatives of 6-methoxy-2-aminobenzothiazole compounds in this communication. Compounds 4b, 4d, 4g, and 4h exhibited enhanced activity against bacterial strains Staphylococcus aureus, Klebesiella pneumoniae, Pseudomonas auregenosa, and Escherichia coli, as well as antifungal activity against Aspergillus niger, Aspergillus flavus and Fusarium monoliforme., respectively. Conversely, the remaining compounds in the series displayed mild to moderate antibacterial and antifungal activity.

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Conflict of Interest

The authors confirm there is no conflict of interest.

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