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Design, Synthesis and Antimicrobial Activity of N_1-Alkyl/Aryl-N_5-(1, 3-Benzothiazol-2-Yl)-1, 5-Biurets.

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ABSTRACT: *A series of novel N_1-alkyl/aryl-N_5-(1, 3-benzothiazol-2-yl)-1, 5-biurets were synthesized, characterized by ^{1}HNMR, IR and elemental analysis. Synthesized molecules were screened for antimicrobial activity against some of the gram +ve and gram –ve bacterias and with t some of the fungal strains. Among the synthesized compounds 7d, 7g and 7h showed good activity and rest of the compounds showed moderate antibacterial and antifungal activity compared to standard dugs.*

KEYWORDS: *2-aminobenzothiazole, antimicrobial activity, Gram-negative bacteria, Gram-positive bacteria, Biuerts.*

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I. INTRODUCTION

Emerging and re-emerging infectious diseases have left us facing a counter charge from infections. Infections with drug resistant organisms remain an important problem in clinical practice that is difficult to solve. Thus there is a abruptly growing global crisis in the clinical management of severe infectious disease, which demands for the discovery and development of novel antimicrobial leads with high efficacy.[1-2]

Benzothiazoles found to be most interesting biophore in research because it is used as a synthon for the synthesis of bioactive structures.[3] It is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities such as anticancer activity,[4] antioxidant activity,[5] **a**ntiviral activity,[6] antimicrobial activity.[7] Benzothiazoles are an extremely important part of compounds that occur widely as biologically active natural products, as well as in the form of marketed drugs or drug candidates. The feasible positions for substitution group at C-2 position exhibit various pharmacological activities such as antimicrobial activity, [8-9] antitubercular activity, [10-11] Raf-1 inhibitor.[12]

In this scenario, 2-aminobenzothiazole represent an important benzene fused thiazole bicyclic ring scaffolds which have been reported with a wide range of pharmaceutical and agrochemical applications. A number of 2-aminobenzothiazoles have shown wide range of biological activity such as antibacterial activity, [13-16] antidiabetic activity, [17] antifungal activity, [18] antihelmenetic activity, [19] antiimflamatory activity, [20] antimicrobial activitity. [21-26]

On the other hand, Several urea derivatives are associated with diverse pharmaceutical applications such as diuretics, [27] antagonists of human vanilloid VR1 receptors, [28] anti-tuberculosis agents, [29] anti-melanoma agents, [30] potent inhibitors of influenza virus neuraminidase, [31] HIV-1 protease inhibitors, [32] anti-hyperglycemic agents, [33] and as inhibitors of Murine receptor A and Murine receptor B. [34]

In continuation of our research work of heterocyclic conjugation, we have synthesized novel biurets by the conjugation of heterocyclic 2-amino benzothiazole with monosubstituted urea by considering the above mentioned biological importance of 2-aminobenzothiazole and substituted urea.

II. MATERIALS AND METHODS:

All chemicals ammonia, 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 urea etc. 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 6-Substituted-1, 3-benzothiazol-2-amine. [35]

A mixture of aniline (0.95g, 0.01 M) and potassium thiocyanate (0.97g, 0.01 M) in glacial acetic acid (20 mL) was cooled and stirred. To this solution bromine (4.95g, 0.01 M) was added from dropping funnel at such a rate that the temperature does not rise beyond 0^oC. After all the bromine has been added, the solution was stirred for an additional 2hr at 0^oC. It was allowed to stand for overnight during which period an orange precipitate settled at the bottom, water (6 mL) was added quickly slurry was heated at 85^oC 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^oC 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 6-substituted-1, 3-benzothiazol-2- amine.

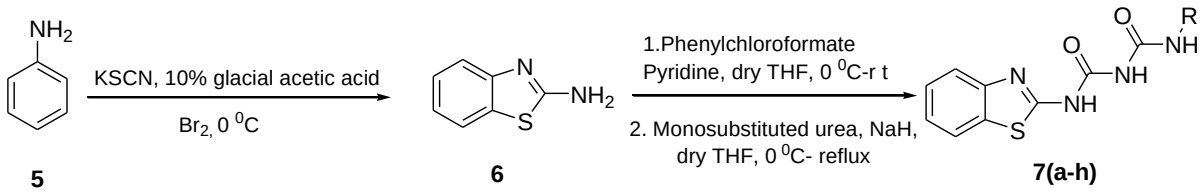
2-amino-1, 3-benzothiazole

Yield - 82%; M. P. 127-128^OC; IR (KBr): γ_{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).

2.2 General procedure for synthesis of N_1-alkyl/aryl-N_5-6-(substituted-1, 3-benzothiazol-2-yl)-1, 5-biurets. [36]

Solution of 2-aminobenzothiazole (0.01mmol) and pyridine (2.47mmol) in dry THF (10 mL) stirred at 0^0 C 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 10^0C. The reaction was stirred 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 (80-90%).

A mixture of mono N-substituted urea (0.013mmol) and sodium hydride (5mmol) stirred for 30 mins and then a solution of crude benzothiazol-2-yl-carbamate (0.01mmol) in dry THF was added. The mixture was refluxed for 10-12hr 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.



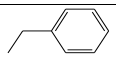
5 **6** **7(a-h)**

R = CH_3, CH_3 CH_2,$CH_3(CH_2)_2$,$CH_3(CH_2)_3$, $(CH_3)_3C$, Ph, CH_2Ph.

Scheme: Synthesis of N_1-alkyl/aryl-N_5-6-(substituted-1, 3-benzothiazol-2-yl)-1, 5-biurets.

Table-1.0: Physical characterization data of N_1-alkyl/aryl-N_5-(1, 3-benzothiazol-2-yl)-1, 5-biurets.

Entry	R	Yield (%)	Molecular formula	Elemental analysis (%) Calculated (found)				^{1}HNMR (DMSO, δ ppm)
				C	H	N	S	
7a	CH_3-	85	$C_{10}H_{10}N_4O_2S$	47.90 (47.73)	4.25 (4.93)	22.45 (22.55)	12.71 (13.01)	7.44-8.4(m, 4H, ArH-Bz), 5.4(2H, NH, urea), 9.5(s, 1H, NH, imide), 3.1(d, 2H, αCH_2, NHCONH), 2.9(t, 3H, CH_3). IR (KBr, cm^{-1}): 3414 (N-H), 2972 (C-H), 1616 C=C), 1149 (C-N), 1755 (C=O).
7b	CH_3CH_2-	90	$C_{11}H_{12}N_4O_2S$	51.68 (53.10)	5.80 (5.85)	20.90 (19.93)	11.82 (11.25)	7.40-8.35(m, 4H, ArH-Bz), 5.6(s, 2H, NH, urea), 9.2 (s, 1H, NH, imide), 3.1(s, 2H, αCH_2, NHCONH), 2.9(q, 2H, βCH_2), 1.1(t, 3H, βCH_3).IR (KBr, cm^{-1}): 3445 (N-H), 2990 (C-H), 1630 (C=C), 1119 (C-N), 1710 (C=O).
7c	$CH_3(CH_2)_2$-	89	$C_{12}H_{14}N_4O_2S$	51.45 (51.60)	5.75 (6.05)	19.98 (20.50)	11.44 (11.50)	7.41-8.30(m,4H, ArH-Bz), 5.1(s, 2H, NH, urea), 9.3(s, 1H, NH, imide), 3.3 (s, 2H, αCH_2, NHCONH),3.4(q,2H,αCH_2),1.7(q,2H,β CH_2), 1.1(t, 3H, γCH_3). IR (KBr, cm^{-1}): 3420 (N-H),2980(C-H),1620 (C=C),

								1155 (C-N), 1735 (C=O).
7d	$CH_3(CH_2)_3$-	90	$C_{13}H_{16}N_4O_2S$	53.04 (53.45)	6.16 (7.05)	19.03 (19.35)	10.89 (11.12)	7.33-8.39 (m, 4H, ArH-Bz), 5.55 (2H, NH, urea), 9.0 (IH, NH, imide), 3.0(t, 2H, αCH_2, NHCONH), 3.35(q, 2H, αCH_2), 1.45(m, 2H, βCH_2), 1.3 (m, 2H, γCH_2), 1.05(t, 3H, δCH_3). IR(KBr, cm^{-1}): 3410 (N-H),2960(C-H), 1610(C=C), 1140(C-N), 1720 (C=O)..
7e	$(CH_3)_3C$-	90	$C_{13}H_{16}N_4O_2S$	53.04 (53.75)	6.16 (6.30)	19.03 (19.15)	10.89 (11.25)	7.40-8.42(m, 4H, ArH-Bz), 5.6(2H, NH, urea) 9.1(IH, NH, imide), 3.2(t, 2H, αCH_2, NHCONH), 1.42(s, 9H, CH_3). IR (KBr, cm^{-1}): 3415(N-H), 2970(C-H), 1605(C=C), 1155 (C-N), 1755 (C=O).
7f		85	$C_{15}H_{18}N_4O_2S$	56.23 (57.25)	6.29 (6.50)	17.49 (18.25)	10.35 (10.51)	7.35-8.47(m,4H,ArH-Bz), 5.4(2H,NH,urea)9.2(IH, NH,imide),3.3(t,2H,αCH_2,NHCONH), 1.25.45(m,10H,CH_2),3.45(m, 1H,αCH). IR (KBr, cm^{-1}): 3420(N-H), 2960 (C-H), 1590 (C=C),1120(C-N), 1150(C-C), 1470 (C=C), 1740 (C=O).
7g		88	$C_{15}H_{12}N_4O_2S$	57.31 (57.35)	4.49 (4.60)	17.82 (16.99)	10.30 (10.50)	7.45-8.5(m, 5H, ArH-Bz), 5.4(s, 1H, NHCO, urea), 9.5(s, 1H, NHCONH,imide),5.6(s,1H,NHCONH) , 7.1-7.55(m, 5H, ArH). IR (KBr, cm^{-1}): 3450(N-H),2950(C-H),1580(C=C), 1160(C-N), 1729(C=O).
7h		83	$C_{16}H_{14}N_4O_2S$	58.52 (57.95)	4.91 (5.15)	17.06 (17.25)	9.76 (9.85)	7.45-8.45(m,5H,ArH-Bz),5.45(s, 1H, NHCO, urea), 9.7(s, 1H,NHCONH, imide), 5.62(s,1H,NHCONH),4.55(s,2H,αCH_2, Benzyl), 7.12-7.35(m, 5H, CH_2-Ar), IR (KBr, cm^{-1}): 3455(N-H), 2960 (C-H), 1590(C=C), 1195(C-N), 1749 (C=O).

2.3 Antibacterial assay

General method for antibacterial assay:

The antibacterial assay was carried out against gram +ve and gram -ve bacteria by following the procedure of Perez. C et al., [37] with slight modifications.

In vitro antibacterial assays were performed against Staphylococcus aureus, Escherichia coli, Klebesiella pnemoniae and Pseudomonas auregenosa by using 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. 4 Antifungal activity:

General method of antifungal assay:

The antifungal activities of the synthesized compounds were evaluated by following the procedure of Singh. I et al [38] with slight modifications.

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 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**.

Table-2.0: Antibacterial activity of synthesized biurets:

Compounds[a]	Inhibitory Zone (diameter) mm[b]			
	Staphylococcus aureus	Escherichia coli	Pseudomonas auregenosa	Klebesiella pneumoniae
7a	06	06	07	07
7b	07	08	07	06
7c	07	08	08	07
7d	09	08	07	08
7e	07	06	07	06
7f	07	06	07	06
7g	09	08	09	09
7h	10	07	08	07
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-3.0: Antifungal activity synthesized biurets:

Compounds[a]	Inhibitory Zone (diameter) mm[b]		
	Fusarium monoliforme	Aspergillus niger	Fusarium monoliforme
7a	05	05	05
7b	06	05	06
7c	063	06	06
7d	07	06	07
7e	06	05	04
7f	06	05	05
7g	07	06	07
7h	08	07	08
Nystatin	11	09	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.

III. RESULTS AND DISCUSSION:

We have synthesized a novel N_1-substituted-(6-substituted-1.3-benzothizol-2-yl)-N_5-(N_1-substitutedurea) biurets. The product obtained was characterized by TLC, elemental analysis and ^{1}H NMR. The synthesized compounds were screened for antimicrobial activitity.

3.1 Structural activity relationship.

3.1.1 Antibacterial activity:

All the synthesized Compounds 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, compounds with long carbon chain in substituted urea and phenyl ring showed better activity over the other compounds. The presence of these helps the molecule to interact/penetrate more with cell membrane of the microorganisms thereby inactivating them.

3.1.2 Antifungal activity:

All synthesized Compounds were tested against fungal strains such as Aspergillus niger, Aspergillus flavus and Fusarium monoliforme. Nystatin was used as positive control and DMSO as a negative control. Among all the synthesized compounds, compounds with long carbon chain in substituted urea and phenyl ring showed better activity over the other compounds, the other compounds in the series showed mild to moderate antifungal activity. Here also the factors explained under antibacterial activity equally holds good.

IV. CONCLUSION

In an effort to discover a novel antimicrobials agents, among the synthesized biuret derivatives, some of the compounds with long carbon chain and phenyl ring in urea moiety in biuret deivatives showed equipotent antimicrobial and antifungal activity with the conventional antimicrobial drugs and others showed moderate to mild activity and these can be considered as novel antimicrobial agents.

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