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Development and creation of a Novel conjugates of alkyl/aryl ureas and 6 methoxy-2-aminobenzothiazole as antimicrobial agents.

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ABSTRACT

In quest for biologically more potent compounds, we envisioned synthesizing novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole by the treatment of 6-methoxy-2-aminobenzothiazole with phenylchloroformate using anhydrous pyridine in dry THF at room temperature (RT) initially to obatain corresponding isocyanates. The resultant isocyanate is further refluxed for 10-12 hrs with monoalkyl/aryl ureas in presence of sodium hydride (NaH) in dry THF. The synthesized conjugates were characterized by 1H NMR and Rf values, and subsequently evaluated for 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. Among the synthesized compounds, some of the biurets demonstrated significant activity, while rest of the compounds exhibited moderate activity.

Keywords: Antimicrobial resistance, Conjugates, 6-methoxy-2-aminobenzothiazole, zone of inhibition, isocyanates and Tetrahydrofuran (THF).

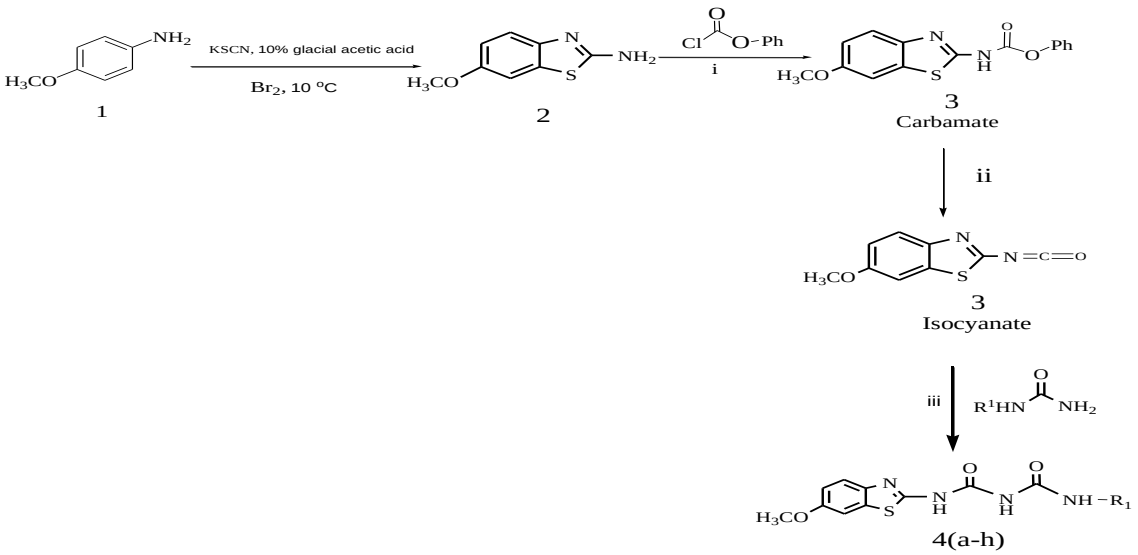
1. INTRODUCTION

Recently fused heterocyclic compounds pursue great attention in the field of medicinal chemistry due to their significant contribution in the biological profile of drug. Benzothizole ring found to be possessing many pharmacological activities such as anthelmintic activities [1], Ant-inflammatory activities [2-3], Anti-tubercular activity [4], antimicrobial activity [5-6], analgesic activity [7], anticonvulsant activity [8-9], Antitumor activity [10-11]. 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. For example, Frentizole is a combination of urea derivative with benzothiazole which is used as a nontoxic antiviral and immune suppressive agent1, it must be emphasized that combination of 2-aminobenzothiazoles with other heterocyclic is a well-known approach to design new drug like molecules, which allows achieving new pharmacological profile, action, toxicity lowering [12-13].

Alkyl/aryl ureas are a family of well-known compounds. They are important intermediates [14]. Urea and its derivatives are versatile organic compounds present in a number of naturally occurring compounds and are associated with a wide range of therapeutic and pharmaceutical applications as peptidomimetics [15], protease inhibitors [16], cytokinin analogous [17], HIV-1 Raf kinase inhibitors [18], glycoenzyme inhibitor [19], glycogen phosphorylase [20] and antagonists [21].
In continuation of our efforts to synthesize novel antimicrobial agents, in the literature there are ample avenues for the versatile biological activity of 2-aminobenzothiazole and 2-aminobenzothiazole derivatives.

On the other hand, it is evident from the literature; substituted urea and biuret are the class of compounds which exhibits immunerable number of pharmacological properties which has led to its distinctive therapeutics.

In this connection, the above mentioned biological activities of substituted 2-aminobenzothiazole and substituted urea and biuret compounds inspired us to design and synthesize novel compounds



(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 conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole.

incorporating 6-methoxy-2-aminobenzothiazole with substituted urea compounds for antimicrobial activity.

2. 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 and propyl ureas 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. 1H 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-methoxy-1, 3-benzothiazol-2-amine [22]

A mixture of p-anisidine (0.92g, 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°C. After all the bromine has been added, the solution was stirred for an additional 2 hrs at 0°C. 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°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°C and filtered in hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH-6, when dark yellow precipitate was appeared and recrystallized from benzene to obtain the 6-methoxy-1, 3-benzothiazol-2- amine.

6-Methoxy-2-aminobenzothiazole (2):

Yield-78% M. P. 260-262oC; IR (KBR): γ max cm-1 3357, 3325, 1620; 1H NMR (CDCl3) δ 3.79 (s, 3H, Ar), 7.35 (s, 1H, Ar), 5.45(s, 2H, NH2).

General procedure for synthesis of the mono substituted Alkyl/arylurea derivatives [23]

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.

2.2. General procedure for synthesis of synthesis of novel conjugates of alkyl/aryl urea and 6-methoxy-2-aminobenzothiazole [24]

Solution of 6-methoxy-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 10^{0}C. 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%) and then transformed into corresponding isocyanates by treating the carbamates with anhy. AlCl3 in dry THF.

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 10-12hr 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 CHCl3–EtOAc as the eluent.

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.

2.3. 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. [27] 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-1: Physical characterization data of novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole.

Entry	R	Yield (%)	Molecular formula	Elemental analysis (%) Calculated(found)				^{1}HNMR (DMSO, δ ppm)
				C	H	N	S	
4a	CH_3-	88	$C_{10}H_{14}N_4O_2S$	47.61 (47.65)	4.79 (4.81)	22.21 22.25)	12.71 (12.55)	7.75(d, 1H, ArH-Bz), 7.25(d, 1H, ArH-Bz), 8.2 (dd, 1H, ArH-Bz), 3.75(s, 3H, OCH_3), 5.5(s, 1H, NHCO, urea), 9.7(s, 1H, NHCONH, imide), 5.2(s, 1H, ,NHCONH), 3.1(s, 3H, CH_3),. IR (KBr, cm^{-1}): γ-3425 (N-H); 3050 (Ar-C-H); 1715 (C=O); 1430 (C=N); 1251 (C-N); 1125 (C-O), 1595 (C=C).
4b	CH_3CH_2-	90	$C_{12}H_{16}N_4O_3S$	48.6 (48.69)	5.44 (5.45)	18.91 (18.95)	10.82 (10.85)	7.81(d, 1H, ArH, Bz), 7.17 (d, 1H, ArH, Bz), 8.15(dd, 1H, ArH, Bz), 3.7(s, 3H, OCH_3), 5.3(s, 1H, NHCO, urea), 9.72(s, IH, NHCONH, imide), 5.15(s, 1H, ,NHCONH), 3.0(t, 2H, αCH_2), 1.25(t, 3H, βCH_3),. IR (KBr, cm^{-1}): γ-3430 (N-H); 3040 (Ar-C-H); 1717 (C=O); 1450 (C=N); 1245 (C-N); 1119 (C-O), 1605 (C=C).
4c	$CH_3(CH_2)_2$-	88	$C_{13}H_{18}N_4O_3S$	50.31 (50.37)	5.85 (5.89)	18.05 (18.07)	10.33 (10.46)	7.7 (d, 1H, ArH-Bz), 7.05 (d, 1H, ArH-Bz), 8.25(dd,1H, ArH-Bz), 3.9(s, 3H, OCH_3),5.5(s, 1H, NHCO, urea), 9.80(s, 1H, NHCONH, imide), 5.25(s, 1H, ,NHCONH), 3.0(t, 2H, αCH_2), 1.6(m, 2H, βCH_2), 1.1(t, 3H, γCH_3). IR (KBr, cm^{-1}): γ-3429 (N-H); 3037 (Ar-C-H); 1709 (C=O); 1422 (C=N); 1245 (C-N); 1135 (C-O), 1605 (C=C).
4d	$CH_3(CH_2)_3$-	92	$C_{13}H_{18}N_4O_2S$	53.04 (53.55)	6.16 (6.07)	19.03 (19.05)	10.89 (10.91)	7.65(d, 1H, ArH-Bz), 7.1(d, 1H, ArH-Bz), 8.21(dd, 1H, ArH-Bz), 3.8(s, 3H, OCH_3), 5.5(s,1H,NHCO, urea), 9.75(s, 1H, NHCONH, imide), 5.2(s,1H, ,NHCONH), 3.1(t,2H, αCH_2), 1.6(m, 2H, βCH_2), 1.3(m, 3H, γCH_2), 1.1(t, 3H, δCH_3). IR (KBr, cm^{-1}): γ-3427 (N-H); 3046 (Ar-C-H); 1720 (C=O); 1440 (C=N); 1255 (C-N); 1135 (C-O), 1585 C=C stretching.
4e	$(CH_3)_3C$-	90	$C_{14}H_{20}N_4O_3S$	51.83	6.21	17.27	9.88	7.69(d, 1H, ArH-Bz), 7.15 (d, 1H, ArH-Bz), 8.2(dd, 1H, ArH-Bz), 3.7(s, 3H, OCH_3), 5.8(s, 1H, NHCO, urea), 9.8 (s, 1H, NHCONH,

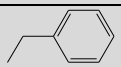
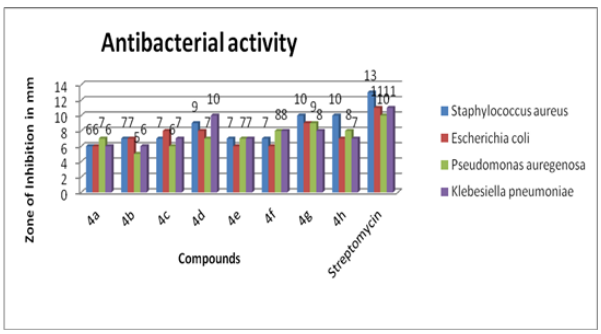
								imide), 5.35(s, 1H, ,NHCONH), 1.15(s, 9H, CH_3). IR (KBr, cm^{-1}): γ-3433 (N-H); 3043 (Ar-C-H); 1716(C=O); 1430 (C=N); 1265 (C-N); 1145 (C-O), 1615 (C=C).
4f		90	$C_{16}H_{24}N_4O_3S$	54.84 (55.01)	6.33 (6.35)	15.99 (16.29)	9.15 (9.17)	7.75(d, 1H, ArH-Bz), 7.2(d, 1H, ArH-Bz), 8.3 (dd, 1H, ArH-Bz), 3.85(s, 3H, OCH_3), 5.5(s, 1H, NHCO, urea), 9.72(s, 1H, NHCONH, imide), 3.3(m, 2H, 5.15(s, 1H, ,NHCONH), 1.25-1.6(m, 10H, CH_2). 1609 C=C stretching, 1150 C-C, 1470 C=N stretching.
4g		85	$C_{16}H_{16}N_4O_3S$	55.80 (55.95)	4.68 (4.71)	16.27 (16.31)	9.31 (9.35)	7.8 (d, 1H, ArH-Bz), 7.24 (d, 1H, ArH-Bz), 8.15(dd, 1H, ArH-Bz),3.7(s, 3H, OCH_3), 5.51(s,1H,NHCO,urea),9.79(s, 1H,NHCONH, imide),5.15(s,1H,,NHCONH),7.15-7.9(m,5H, ArH). IR (KBr,cm^{-1}): γ-3405 (N-H); 3017 (Ar-C-H); 1719(C=O); 1425(C=N); 1260 (C-N); 1137 (C-O), 1628 (C=C).
4h		88	$C_{17}H_{18}N_4O_3S$	56.97 (56.55)	5.06 (5.17)	15.63 (15.75)	8.95 (8.73)	7.85(d, 1H, ArH-Bz), 7.19 (d, 1H, ArH-Bz), 8.35(dd, 1H, ArH-Bz), 3.7(s, 3H, OCH_3), 5.4(s,1H,NHCO,urea),9.72(s, 1H,NHCONH, imide),5.15(s,1H,,NHCONH),4.15(s,2H,CH_2-Ar). 6.95-7.35(m, 5H, ArH)). IR (KBr, cm^{-1}): γ- 3423(N-H);3023(Ar-C-H); 1729(C=O); 1445 (C=N); 1250 (C-N); 1165 (C-O), 1630 (C=C).

Table -2: Antibacterial activity of novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole:

Compounds[a]	Zone of inhibition (diameter) mm[b]			
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas auregenosa***	***Klebesiella pneumoniae***
4a	06	06	07	06
4b	07	07	05	06
4c	07	08	06	07
4d	09	08	07	10
4e	07	06	07	07
4f	07	06	08	08
4g	10	09	09	08
4h	10	07	08	07
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 novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole.

Antifungal activity:

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

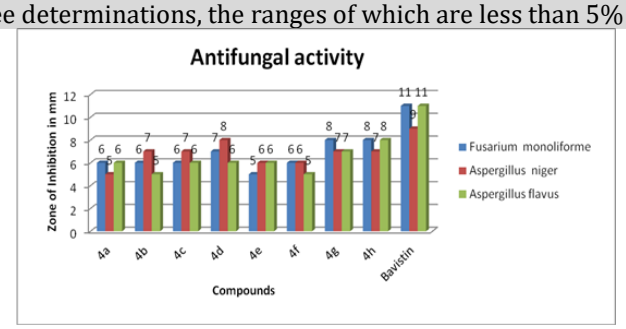
General method of antifungal assay:

In vitro antifungal assays were performed against Aspergillus niger, Aspergillus flavus and Fusarium monoliforme by using agar well diffusion method.[26] 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

Table -3: Antifungal activity of activity of novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole.

Compounds[a]	Zone of inhibition (diameter) mm[b]		
	Fusarium monoliforme	*Aspergillus niger*	*Aspergillus flavus*
4a	06	05	06
4b	06	07	05
4c	06	07	06
4d	07	08	06
4e	05	06	06
4f	06	06	05
4g	08	07	07
4h	08	07	08
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 novel conjugates of alkyl/aryl urea and 6-methoxy-2-aminobenzothiazole.

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 center of the plate and kept for 6 days at 25°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

We have synthesized a new class of novel conjugates of alkyl/aryl urea and 6-methoxy-2-aminobenzothiazole. The compounds obtained were characterized by TLC, elemental analysis and ^{1}H NMR. The synthesized compounds were used for both antimicrobial and antioxidant activities.

3.1. Structural activity relationship of novel conjugates of alkyl/aryl ureas and 6-methoxy-2-aminobenzothiazole.

Antibacterial activity:

All 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 4d, 4g and 4h with electron releasing group and electron withdrawing groups in benzothiazole moiety or in substituted urea or 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 enhances 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.

3.2. 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 with electron releasing group 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 well.

4. CONCLUSION

All the newly synthesized conjugates of alkyl/aryl ureas and 6-mehoxy-2-aminobenzothiazole were analyzed with different spectral techniques and screened *in vitro* for their antibacterial activity against both Gram-positive and Gram-negative reveals all compounds exhibited good activity against all strains and as antifungal activity as well.

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Conflict of Interest

The authors confirm there is no conflict of interest.

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