STUDIES ON ANTIBACTERIAL EFFICACY OF SUBSTITUTED THIOUREAS

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Abstract

The aim of this study was to evaluate the possible antibacterial potential of certain disubstituted thioureas against bacterial pathogens. For this purpose, clinical isolates of two gram positive (*Staphylococcus aureus and Enterococcus faecalis*) and two grams negative (*Escherichia coli* and *Pseudomonas aeroginosa)* were tested. Antibacterial potency of the compounds was evaluated by standard growth inhibitory assay methods. All the tested compounds showed varying degrees of strain specific antibacterial potential against the tested strains, of which C4 showed superior activity against *E.faecalis* a*nd P.aeruginosa*. C1 exhibited the least antibacterial activity against *S. aureus*. These promising findings suggest the antibacterial efficacy of the bioactive compounds against multi-drug resistant bacterial pathogens and serving them as an alternative antimicrobial agent against diseases caused by these organisms.

Keywords: disubstituted thiourea, *staphylococcus aureus, enterococcus faecalis, escherichia coli, pseudomonas aeroginosa,* antibacterial agent

1. Introduction

In recent years, multiple drug resistance has been developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases (Westh et al., 2004). This resistance problem needs a re-newed effort, resulting in researching effective antibacterial agents against pathogenic microorganisms resistant to current antibiotics (Soulsby, 2005). Besides, though conventional antibiotics are strong medicine and save lives, they cause more harmful effects than good ones when they are not used in right way (Neu, 1992).

Several thiourea derivatives have been reported to have marked antibacterial, antifungal, antitubercular, and antitumour properties. Also, these compounds find wide applications in pharmacology. The most widely used method for the synthesis of disubstituted thioureas is the condensation of a compound having an amino function with isothiocyanate. Aromatic and aliphatic amines can be added to aryl/alkyl isothiocyanates to give these derivatives. This is an excellent N-alkyl-N'-(4-alkylphenyl) thiourea method for the synthesis of 1, 3- disubstituted thioureas. The present work aims at the synthesis and antimicrobial studies of certain 1,3-disubstituted thioureas.

2. Materials and Methods

2.1. Materials

Ammonia, carbon disulphide, aniline, lead nitrate, ethyl amine, methyl amine and all the solvents were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Muller Hinton Agar, Agar Agar, and sterile discs for antibacterial studies were obtained from Nice Chemicals Pvt. Ltd., India.

2.2. Preparation of phenyl isothiocynate

Ammonia (100 ml) and carbon disulphide (50 ml) were mixed together in a long-necked round bottom flask. To this mixture, aniline (60 ml) was added slowly with constant shaking. The reaction is exothermic so it was cooled in ice bath before each addition. After complete addition of aniline, boiling solution of lead nitrate (200g in 400 ml water) was added

slowly with vigorous shaking. The addition was completed by taking half an hour, then the round bottom flask was kept aside for 10 minutes, when all the reactions are completed, and excess of carbon disulphide was removed. From the reaction mixture isothiocyanate was separated by steam distillation. It was then separated by using a separating funnel and dried over anhydrous calcium chloride.

2.3. Synthesis of N-alkyl-N'-(4-alkylphenyl) thioureas

An equimolar amount of aliphatic amines and phenyl isothiocyanate was mixed together and stirred for few minutes. The mixture was stirred well till a solid product separates out. It was then washed with ether to remove isothiocyanate and with dilute HCl to remove aniline if they were found excess, which can be identified by their smell. The product was then crystallised from ethanol when colourless crystals of 1,3-disubstituted thioureas were obtained. The synthesized products are; N-Methyl-N'-(4-methylphenyl) thiourea (C1), N -Ethyl-N'-(4-methylphenyl) thiourea (C2), N-Methyl-N'-(4-phenyl) thiourea (C3) and N-Ethyl-N'-(4-phenyl) thiourea (C4).

2.4. Antimicrobial Analysis

2.4.1. Bacterial Strains

For antimicrobial analysis, two grams positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial stains were isolated from clinical specimens. All the tested strains were maintained in nutrient agar slants at 4°C.

2.4.2. Susceptibility tests

Susceptibility tests were performed by a modified agar well diffusion method (Okunji et al., 1990; Okeke et al., 2001). The inoculum size of the test strains were standardized according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 1993). The bacterial strains were inoculated in Mueller Hinton Broth (Hi-media, Mumbai, India) and incubated at 37°C in a shaker water bath for 3 - 6 h until the culture attained a turbidity of 0.5 McFarland unit. The final inoculum size was adjusted to 5 x 105 cfu/ml. Antibacterial potential of substituted thioureas were tested against *S. aureus. E. faecalis, E. coli and P.aeruginosa* in modified agar well diffusion method.

2.4.3. Determination of inhibitory zone diameter (IZD)

One ml of standard suspension of each bacterial strain was spread evenly on Mueller-Hinton Agar (Hi-media, Mumbai, India) plates using a sterile glass rod spreader and the plates were allowed to dry at room temperature. Subsequently six mm diameter wells were bored in the agar and 100 µl volumes of 100 mg/ml of each reconstituted compounds was pipetted into wells. After holding the plates at room temperature for 2 h to allow diffusion of extract into the agar and incubated at 37°C for 24 h. Inhibition Zone Diameter (IZD) was measured to the nearest millimeter (mm). 5% DMSO was used as negative control. The tests were performed in triplicate for each microorganism used and the final results were expressed as the arithmetic average of triplicate experiments.

2.4.4. Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentrations (MICs) of the compounds against all the test strains were determined by broth dilution assay method (NCCLS, 1993). Two-fold serial dilutions of all the extracts (0.25 to 2 mg/ml) were prepared in tubes with Mueller Hinton Broth (Hi-media, Mumbai, India) as diluent. Each dilution was seeded with 20 µl of test micro-organisms to the standard concentration (5 x 10^5 cfu/ml). The tubes were incubated at 37°C for 24 h. The least concentration of the compound or standard drug showing no visible growth was taken as the MIC.

2.4.5. Determination of minimal bactericidal concentration (MBC)

Minimal bactericidal concentration (MBC) determination was determined by aspirating

0.01 ml of the culture medium from each tube (in the macro broth MIC assay) showing no apparent growth when sub-culturing it on fresh MHA. The later was incubated at 37°C for 24 h. The MBC was read as the least concentration of the extract showing no visible growth on MHA subculture. Using the values of MIC and MBC, the MIC index (MBC/MIC) of each extract was computed against each test strain.

3. Results and Discussion

Table 1 shows the results of antibacterial potential of the synthesized compounds. Compounds are labelled as C1 for N-Methyl-N'-(4-methylphenyl) thiourea, C2 for N -Ethyl-N'-(4-methylphenyl) thiourea, C3 for N-Methyl-N'-(4-phenyl) thiourea and C4 for N-Ethyl-N'-(4-phenyl) thiourea. All compounds showed varying degrees of strain specific inhibitory action (IZD ranged from 14– 28 mm). Compound C4 was found to be more potent against all the strain tested. DMSO (negative control) showed no inhibitory action against any of the test strains. The photographs demonstrating the antibacterial activity of the said systems against the test species are shown in Fig. 1. Table 2 shows the results of MIC, MBC and MIC index values of the compounds (C1-C4) against the test strains. The MIC values for C1, C2, C3 & C4 against the test strains were ranged from 0.50 to1.00 mg/ml, MBC values were ranging from 0.50-1.25 mg/ml and MIC index values against the test strains were ranged from 1-1.33.

Table 1: Results of antibacterial potential of the compounds

Microorganisms	Inhibitory Zone Diameter (IZD) (mm)				
	C1	C2	C3	C4	DMSO (control)
S. aureus	14	17	18	20	0
E.faecalis	21	19	20	28	0
E. coli	18	18	18	20	0
P.aeruginosa	16	16	15	28	0

Note: Values are the average of triplicate experiments.

Table 2: Results of MIC, MBC and MIC index values of the compounds

Compound	Microrganisms	MIC (mg/ml)	MBC (mg/ml)	MIC index
C1	*S. aureus* *E.faecalis* *E. coli* *P.aeruginosa*	0.75 1.00 0.50 1.00	0.75 1.00 0.50 1.00	1 1 1 1
C2	*S. aureus* *E.faecalis* *E. coli* *P.aeuoginosa*	0.75 0.75 1.00 1.00	0.75 1.00 1.00 1.25	1 1.33 1 1.25

C3	*S. aureus* *E.faecalis* *E. coli* *P.aeruginosa*	0.75 0.75 0.75 1.00	1.00 1.00 1.00 1.25	1.33 1.33 1.33 1.25
C4	*S. aureus* *E.faecalis* *E. coli* *P.aeruginosa*	0.50 0.75 0.75 0.50	0.50 0.75 0.75 0.50	1 1 1 1

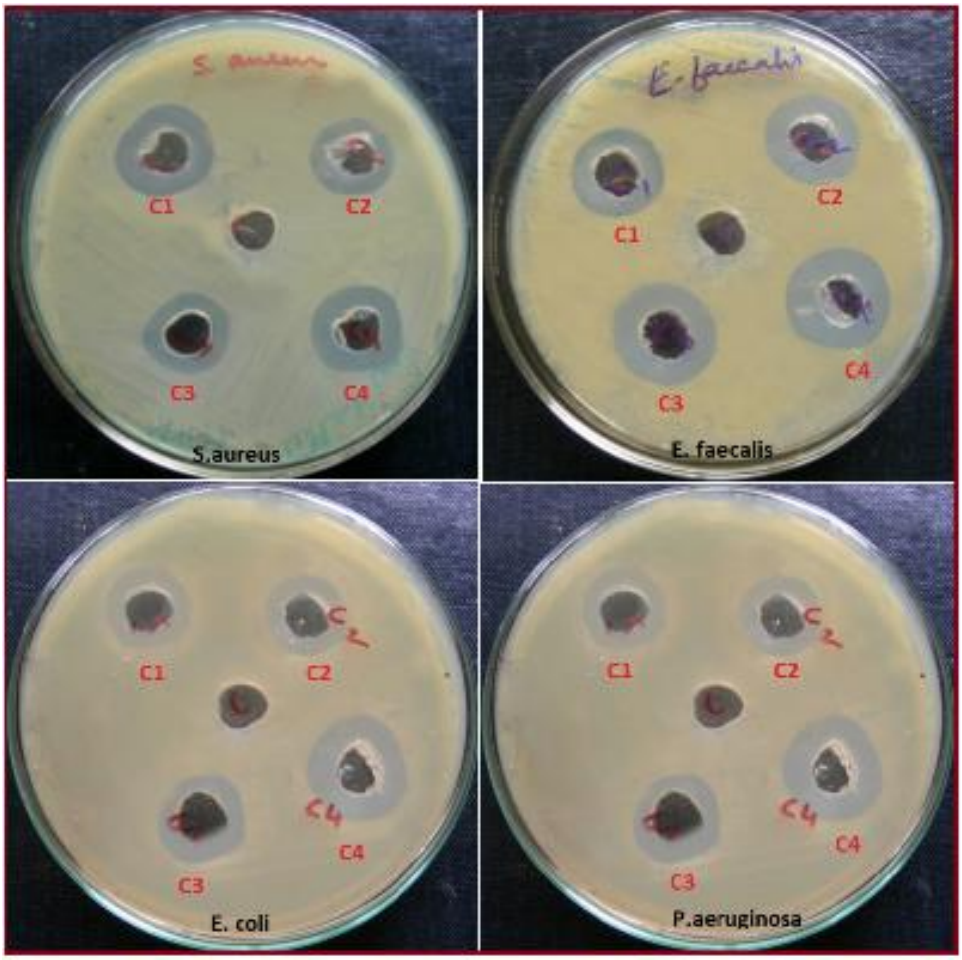

Figure 1: Antibacterial activity of the compounds against the test species

The results revealed that all of the tested compounds showed antibacterial activity againt *E. faecalis, S.aureus, E.coli* and *P. aeruginosa.* Among them highest activity was shown by C4 against *E.faecalis* and *P. aeruginosa* (IZD-28 mm) in agar well diffusion method and least zone of inhibition by C1against *S.aureus* (IZD-16 mm). All other compounds exhibited a zone of inhibition between the above two ie, between 18-28 mm. This is very significant and comparable to some antibiotics.

Results of our foregoing findings revealed that all the tested compounds exhibited growth inhibitory activity against the bacterial strains evaluated. The Minimum inhibitory

concentration (MIC) and Minimum bactericidal concentration (MBC) of the compounds ranges from 0.50mg/ml-1.25mg/ml. MIC index of C1 was 1, indicating MIC and MBC were same for this compound, but others showed slight variation in their bacteriostatic and bactericidal concentration. MIC was 0.50mg/ml for C1 against E. coli and C4 against *S.aureus* and *P.aeruginosa;* for them MBC was also same. MIC and MBC of C1 against *S. aureus* & C2 against *S. aureus* C4 against *E.faecalis & E. coli* were same ie, 0.75 mg/ml. For C3, MIC and MBC were not same against all tested strain indicating that higher concentration was required to kill them than inhibiting the growth (table 2).

The mechanism by which these materials are able to inactivate bacteria is not understood completely, but studies suggest that when bacteria were treated with antimicrobial materials, changes took place in its membrane morphology which was evident from the morphological and structural changes in the bacterial cells. They have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like changes in the permeability of the cell membrane leaving the bacterial cells incapable of properly regulating transport through the plasma membrane and gradually resulting into cell death.

4.Conclusion

The work now presented is based on the synthesis and antimicrobial studies of 1,3-disubstituted thioureas by making use phenyl isothiocynate and amines. It has been reported that substituted thioureas are formed by the condensation of a compound having an amine function with isothiocyanate. The synthesized products have excellent antimicrobial action against the tested bacterial species. These antimicrobial properties can be of great significance in therapeutic treatments and pharmacological applications. All the four compounds can be used against diseases caused by these microorganisms and further studies on their chemical constituents and side effect are needed to pinpoint the findings. This report may serve as a footstep on this aspect.

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