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Potent antibacterial activity of 1,3-bis(N-substituted thioamido) guanidines

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ABSTRACT

Bis-(N-p-chlorophenyl thioamido), bis-(N-phenyl thioamido) and bis-(N-tolyl thioamido) derivatives were tested for antibacterial activity against *Staphylococcus aureus & Escherechia coli* bacterial species. Antibacterial activity was measured by paper disc diffusion method. Inhibition zones indicated that 25 & 50 µg/ml of p-chloro derivative showed strong antibacterial activity against *S. aureus* and *E. coli* respectively. Synthesized compounds were also tested for antifungal activity against Candida albicans & Aspergillus niger fungal species. N-tolyl & N-phenyl derivatives showed good antifungal activity.

Key words: Antimicrobial activity, 1,3-bis(N-substituted thioamido)guanidines.

INTRODUCTION

The basic mechanism of antibiotic action against bacterial cells are: (i) Inhibition of cell wall synthesis (e.g. penicillin) (ii) Inhibition of protein synthesis (e.g. tetracyclines) (iii) Alternation of cell membrane (e.g. polymixins) (iv) Inhibition of nucleic acid synthesis (e.g. quinolones) and (v) Antimetabolite action (e.g. sulphonamides)[1]. Inhibition of cell wall synthesis is most common mechanism of antibiotic action. Synthetic compounds may effect the integrity of cell membrane leading to cell death [2,3].

Synthetic antimicrobial agents form ingredients in commercial productst[4-6] like soaps, paints etc. but require multiphase clinical trial to accept as commercial drugw . Although penicillin, methicillin and vancomycin have been used successfully to cure these infections, the resistance strains to these antibiotics have also evolved[8-9]. It is seen when thiourea linkage present in certain compounds, produces antimicrobial activity[1]p. Synthesized compounds possess thiourea & guanidine moieties. Biological activity of thiouriedo compounds have been studied by Pandeya & Co-workers[11-12]. Compounds with thiourea moiety have been reported to show antithyroidal[13], hypoglycemic[13], anticonvulsant[14] , anaesthetic[15] and antibacterial[12-16] activities. Similarly compounds incorporating a guanidine moiety are reported to have various bioactivities[17-19]. Keeping these facts in mind it was planned to synthesize novel compounds possessing thiourea & guanidine moieties for better bioactivities.

MATERIAL AND METHODS

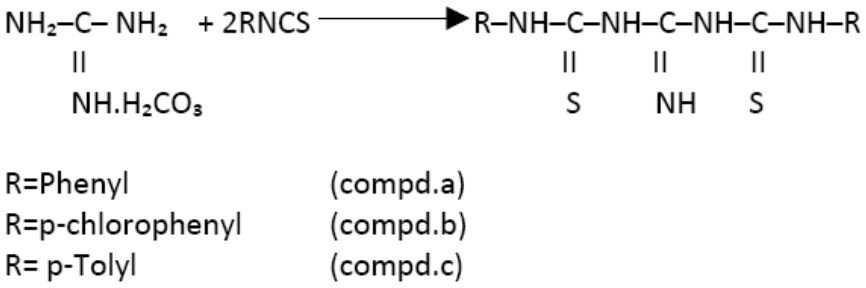
All chemicals used were of analar grade. Substituted isothiocyanates were prepared according to literature method[20]. A mixture of guanidine carbonate (0.05m) with respective isothiocyanates (0.1m) in acetone (25 ml) & ethanol (25ml) was refluxed for 12 hrs on a water bath to

get crystals of respective thioamido derivatives. (scheme-1)

Synthesized compounds were characterized by PMR , FT–IR , UV SPECTRA & MP and elemental analysis . Mueller Hinton Agar (MHA) were from Merck , amplicillin & nystatin from Himedia (Mumbai) While other chemicals were purchased from Merk of highest purity available .

Antomicrobial Activity

Four representative strains *S.aureus* (ATCC 23564), *E.coli* (ATCC 25922), *C. albicans* (ATCC 2091) and *A.niger* were used . Apure culture of *A. niger* was procured from Botany Department BHU. Stock solutions of the testing compounds were prepared in dimethylformamide. The antibacterial study was carried by disc diffusion techniques [21,22]. Filter paper discs of uniform size impregnated with concentration of test compounds (100µg/disc) are placed over inoculated agar surface containing microbial strain. After keeping for ~ 30minutes at room temperature , they were incubated at 37°c for 24 hrs.The zone of inhibition were measured in terms of diameter in mm and the values of antibacterial and antifungal activity were compared against standard references ampicillin (10µg) and nystatin (100 µg) , (Table 1). The presented values of inhibition zones are average of three separate experiments.



Scheme 1

Minimum inhibitory concentrations (MICs) were determined by agar double ditution method. Graded amount of synthesized compounds were incorporated Into measured amount agar media. The media were subsequently inoculated and incorporated . Stock solutions were serially double diluted to get 200,100,50,25 12.5,6.25,3.12 etc. µg/ml concentration of test compounds and used for study , 1ml of solution was added into each sterilized

Table 1: Antimicrobial activity data of compounds showing zones of Inhibition (mm)

Compound (µg/disc)	Antibacte rial activity		Antifungal activity	
	S.aureus	***E. coli***	***C.ablicans***	***A.niger***
a(100µg)	15	NZ	14	20
b(100µg)	24	16	13.5	16
c(100µg)	18	14	22	NZ
Ampicillin(10µg)	25	12	-	-
Nystatin(100µg)	-	-	20	17

NZ: No Zone observed

petri-dish containing graded amount of MH agar media (previously cooled at 60°c). Under aseptic conditions dilute bacterial suspension was inoculated on agar surface using sterilized swabs . After inoculation , the dishes were incubated at 37°C for 24 hrs. The MICs ,the lowest concentration of drug that inhibited the growth of bacteria was noted (Table 2).

Table 2: Antibacterial activity data of synthesized compounds showing MICs (µg/ml)

Compound	*S.aureus*	*E.coli*
a	100	> 200
b	25	50
c	50	100

RESULTS AND DISCUSSION

In disc diffusion method bis-(N-p-chlorophenyl thioamido) derivative (100µg) showed strong antibacterial activity against S. aureus & E. coli producing zones of inhibition 24 mm & 16 mm respectively and were comparable to ampicillin (10 µg) which produced inhibition zones 25 mm and 12 mm respectively . In the same experiment bis –(N–p – tolyl) derivative produced inhibition zone 14 mm against *E. coli.* MIC of p - chlorophenyl derivative was found 25 µg & 50µg against S.aureus & E. coli for complete inhibition of bacterial growth. In antifungal activity bis–(N- phenyl) derivative (100µg) & bis-(N-p-tolyl) derivative (100µ g) produced inhibition zones 20 mm and 22 mm against A. niger and C. albicans respectively and were comparable to Nystatin (100 units) which produced zones of inhibition 17 mm & 20 mm in the same experiment. The results indicate that compound b is potent antibacterial agent against *S. aureus & E. coli* while compound a & compound c showed good antifungal activity against *A. niger* and *C. albicans.* Therefore compounds deserve further studies for utilizing as antiseptic agent.

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