ANTIMICROBIAL

ACTIVITY

ANTIMICROBIAL SCREENING OF THE COMPOUNDS

INTRODUCTION :

A large number of heterocyclic compounds show antibacterial and antifungal activities. Some of them are synthesised by microorganism and are called as antibiotics. Some other compounds which are not synthesised by microbes but show good antibacterial and antifungal activities used for controlling microbes and in the treatment of infections and diseases. Many dyes, sulphonamides, quinolines, pyrazoles, thiadiazoles, indoles show antibacterial activities and are of therapeutic use.

The antibacterial activities of newly synthesised compounds are observed by incarporating these compounds in the nutritional media used for the cultivation of various test microbes. The microbes used usually pathogens. The microbial activity is examined by studying the growth inhibition pattern of the microbes on media containing these compounds. The methodology employed for testing consists of small paper discs previously impregnated with specific compounds with known concentration. The sensitivity of pathogens to different synthetic compounds is determined by measuring the diameter of growth inhibition zones.

Thus, the compounds in the present study were tested for their antibacterial activity using paper disc method.

By using paper disc method the antibacterial activity was observed against Staphylococus aureus (gram +ve) and Escherichia coli (gram -ve) bacteria. These bacterial species are pathogenic. S. aureus causes septic in wounds, burns etc. and are acute pathogenic lesions in man. It also causes tonsillistis, pharyngitis, sinusitis, and pneumonia. E. coli causes diarrhoea or gastroenteritis, perticularly in infants, children and adults. It also causes urinary track infections, pyrogenic infections and septicaemia etc.

EXPERIMENTAL PROCEDURE :

The drug reported in the present study were screened for their antibacterial activity by paper disc method. The compound is allowed to diffuse through a solid medium, so that a gradient is established, the concentration being decreasing with distance. The test bacterium is seeded in the medium and its sensitivity to the synthesised compound was determined by measuring the zones of growth inhibitions.

PREPARATION OF MEDIUM AND MATERIALS:

All the glass wares and other materials were sterilised. All media were adjusted to a correct hydrogen ion concentration (pH) between 7.1 and 7.5.

NUTRIENT MEDIUM COMPOSITION :

1)	Peptone	: 5 gm.
2)	Agar Agar powder	: 10 gm.
3)	Meat extract	: 5 gm.
4)	Sodium chloride	:2.5 gm.
5)	Distilled water	:500 ml.

MATERIAL :

i) Nutrient medium (20 ml. for each petri dish)
ii) Sterile petridishes.
iii) Sterile pipettes.
iv) Old grown culture (24 hrs.)in test tube.
v) Solution of the compound of known concentration.

Nutrient medium is sterilised by autoclaving at 121^{o}C and at 15 lb/sq.inch pressure for 20-25 min. It was then poured into sterilised glass plat (20 ml. per plate) and cooled at room temp. A suitable dilution of growth culture of the test bacteria was spread over media and plate dried at 37^{o}C for 0.5 hr. A filter

paper discs (6 mm diameter, commercially used) charged with the compound of 10 mg/ml. concentration in acetone and applied with sterile forceps after 24 hrs of incubation. The degree of sensitivity was determined by measuring growth inhibition zones around the disc*.

Similarly one plate with tetracycline (std. compound) and other with acetone were charged and incubated for 24 hrs. for comparison and control of the solvent respectively.

Zones of inhibitions of growth were measured in mm. and compared with zone of standard antibiotics i.e. tetracycline. On this basis the percentage inhibition is calculated.

The results of antimicrobial screening have been reported in the tables - A, B, C, D, and E.

Zone of tetracycline is considered as 100% inhibition for gram (+ve) and gram (-ve) bacteria.

* Text Book of Microbiology by R. Anantnarayan and Jayram Panikar, Orient Longman, 2nd Edn.

TABLE - A

Compound No.	Name of the Compound	Antimicrobial Activity % Inhibition			
		S. aureus		E. coli	
		10	100	10	100
6a	3-(6-Chloro-4-methyl-2-oxoquinolino-1-amidyl)-2-(2-nitrophenyl)-1,3-thiazolidin-4(1H)-one	50	53	-	-
6b	3-(6-Chloro-4-methyl-2-oxoquinolino-1-amidyl)-2-(4-nitrophenyl)-1,3-thiazolidin-4(1H)-one	59	46	-	-
6c	3-(6-Chloro-4-methyl-2-oxoquinolino-1-amidyl)-2-(2-chlorophenyl)-1,3-thiazolidin-4(1H)-one	41	41	61	62
6d	3-(6-Chloro-4-methyl-2-oxoquinolino-1-amidyl)-2-(3,4-dichlorophenyl)-1,3-thiazolidin-4(1H)-one	-	25	72	63
Standard	Tetracycline	100	100	100	100

PART - I

ANTIMICROBIAL ACTIVITY

The compound 6a-d of this series were tested for their antimicrobial activity by paper disc method against S. aureau and E. coli at 10 and 100 ppm concentration using tetracycline as standard compound for comparison (Table-1). Ethanol was used as control of solvent in both the cases. The compound 6c exhibited moderate growth inhibitor activity against both the type of bacterial strains. Compound 6a and 6b showed no activity against E. coli and S. aureus. Compound No.6d showed moderate activity against E. coli and less activity against S. aureus at higher concentration.

The presence of the halogen atom in 2-substituted phenyl ring of thiozolidine enhances the antibacterial activity.

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TABLE - B

Compound No.	Name of the Compound	Antimicrobial Activity % Inhibition			
		S. aureus		E. coli	
		10	100	10	100
6a	3-(8-Chloro-4-methyl-2-oxoquinolino-1-acetamidyl)-2-Phenyl-1,3-thiazolidin-4(1H)-One.	59	47	44	54
6b	3-(8-Chloro-4-methyl-2-oxoquinolino-1-acetamidyl)-2-(4-nitrophenyl)-1,3-thiazolidin-4(1H)-One.	59	50	50	58
6c	3-(8-Chloro-4-methyl-2-oxoquinolino-1-acetamidyl)-2-(2-chlorophenyl)-1,3-thiazolidin-4(1H)-One.	-	38	56	54
6d	3-(8-Chloro-4-methyl-2-oxoquinolino-1-acetamidyl)-2-(3,4-dichlorophenyl)-1,3-thiazolidin-4(1H)-One.	41	44	55	54
Standard	Tetracycline	100	100	100	100

PART - II

All the N^1-substituted thiazolidinones were tested for their antibacterial activity by method described in Part-I of the dissertation against Staphylococus aureus and Escherichia coli bacteria. The result of antibacterial screening indicated that the moderate activity was exhibited 6a, 6b, 6d against S. aureus as well as against E. coli. The compound 6c was not so spectacular at lower concentrations against S. aureus.

One generalisation can be made from their result that the compounds 6a-c with substitution pattern R=Chlorophenyl nitrophenyl and R_3 = Cl appear to be more active than other substituted compounds. These compounds may have considerable medicinal value as a drug.

TABLE - C

Compound No.	Name of the Compound	Antimicrobial Activity % Inhibition			
		S. aureus		E. coli	
		10	100	10	100
6a	3-(4,6-Dimethyl-2-oxoquinolino -1-amidyl)-2-(2-nitrophenyl) -1,3-thiazolidine-4(1H)-one	45	41	67	63
6c	3-(4,6-Dimethyl-2-oxoquinolino -1-amidyl)-2-(2-chlorophenyl) -1,3-thiazolidine-4(1H)-one	64	47	72	67
6b	3-(4,6-Dimethyl-2-oxoquinolino -1-amidyl)-2-(4-nitrophenyl) -1,3-thiazolidine-4(1H)-one	50	56	44	54
6d	3-(4,6-Dimethyl-2-oxoquinolino -1-amidyl)-2-(3,4-dichlorophenyl) -1,3-thiazolidine-4(1H)-one	45	41	61	62
Standard	Tetracycline	100	100	100	100

PART - III

The compound 6a-d were tested in vitro for their antibacterial activity against Staphylocous aureus (gram +ve) and Escherichia coli (gram -ve) bacteria at 10 and 100 ppm concentration. The control of experiments was run in a similar manner. The zones of inhibitation were measured in mm. The results showed that the compounds possess moderate antibacterial activity (55-60%) as compared to standard compounds. Most of the tested compounds were effective at higher concentrations and not so spectacular at lower concentrations. The compounds with substituent pattern R = o-chlorophenyl/3,4-dichloro phenyl in 2-substituted phenyl ring were found to exhibit high antibacterial activity as compared to the compounds with substituent pattern R = o-nitrophenyl/p-nitrophenyl.

TABLE - D

Compound No.	Name of the Compound	Antimicrobial Activity % Inhibition			
		S. aureus		E. coli	
		10	100	10	100
6a	3-(6-Chloro-2-methyl-4-oxoquinolino-1-acetamidyl)-2-(phenyl)-1,3-thiazolidin-4(1H)-one	45	34	50	41
6b	3-(6-Chloro-2-methyl-4-oxoquinolino-1-acetamidyl)-2-(2-nitrophenyl)-1,3-thiazolidin-4(1H)-one	55	41	-	46
6c	3-(6-Chloro-2-methyl-4-oxoquinolino-1-acetamidyl)-2-(4-nitrophenyl)-1,3-thiazolidin-4(1H)-one	59	41	56	50
6d	3-(6-Chloro-2-methyl-4-oxoquinolino-1-acetamidyl)-2-(3,4-dichlorophenyl)-1,3-thiazolidin-4(1H)-one	68	53	89	63
Standard	Tetracycline	100	100	100	100

PART - IV

All thiazolidinones (6a-d) of this series were tested for their antibacterial activity against Staphylococus aureus (gram +ve) and Escherichia coli (gram -ve) bacteria. The compound 6d was found to be relatively more potent against S. aureus and E. coli, where as the compound 6c exhibited moderate active against same bacterial strains under study. Here, the presence of the substitution pattern R = 3,4-dichlorophenyl/o-nitrophenyl and 2-substituted phenyl ring and together with halogen atom (R=Cl) in the quinolone ring enhances the anti- bacterial activity.

TABLE - E

Compound No.	Name of the Compound	Antimicrobial Activity % Inhibition			
		S. aureus		E. coli	
		10	100	10	100
6a	3-(8-Chloro-2-methyl-4-oxoquinolino-1-amidyl)-2-(phenyl)-1,3-thiazolidin-4(1H)-one	41	31	61	42
6b	3-(8-Chloro-2-methyl-4-oxoquinolino-1-amidyl)-2-(4-nitrophenyl)-1,3-thiazolidin-4(1H)-one	68	53	94	79
6c	3-(8-Chloro-2-methyl-4-oxoquinolino-1-amidyl)-2-(2-nitrophenyl)-1,3-thiazolidin-4(1H)-one	64	50	94	75
6d	3-(8-Chloro-2-methyl-4-oxoquinolino-1-amidyl)-2-(2-chlorophenyl)-1,3-thiazolidin-4(1H)-one	77	59	89	92
Standard	Tetracycline	100	100	100	100

PART - V

All the compounds of this class were tested for their anti- bacterial activity by paper disc method against Staphylocous aureus (gram +ve) and Escherichia coli (gram -ve) at 10 and 100 ppm concen- trations using tetracycline as a standard. The compound 6d showed strong moderate to good antibacterial activity where as the compound no.6a and 6b were found to be moderately active. The compound 6a showed less activity. The thiazolidinones with substituents R = o-chlorophenyl/p-nitro- phenyl enhances the antibacterial activity and of are of are of considerable importance as a drug.