CHAPTER-III

ANTIMICROBIAL SCREENING

ANTIMICROBIAL SCREENING OF THE COMPOUNDS

INTRODUCTION :

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

Thus, the compounds in the present study were tested for their antibacterial activity using Kirby-Baur* diffusion method against various gram (+ve) and gram (-ve) bacteria. The gram (+ve) bacteria selected were Staphylococus aureus; Staphylococcus citreus and gram (-ve) included Pseudomonas aerugenosa; Klebsiella pneumoniae; Escherichia coli. These bacterial species are pathogenic. S. aureus and S. citreus cause septic in wounds and burns. They cause acute pyogenic lesions in man. S. aureus causes tonsillitis, pharyngitis, sinusitis and pneumonia.

E. coli causes diarrhoea or gastroenteritis particularly in infants, children and adults. It also causes urinary tract infections, pyogenic infections and septicaemia whereas P. aerugenosa causes chronic diseases

which are in the form of localised or generalised infections. Localised infections are common in wounds, eye and urinary tract infections. K. pneumoniae causes urinary infections, abscesses, meningitis and septicaemia.

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

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A) ANTIBACTERIAL ACTIVITY :

The compounds reported in the present study were tested for their bacteriostatic activity by Agar plate diffusion method and the zones of inhibition were measured to know the activity. The following bacterial species were used for the evaluation of antimicrobial activity.

a) Types of bacteria :

i) Pseudomonas aerugenosa (Gram negative),

ii) Escherichia coli (Gram negative),

iii) Klebsiella pneumoniae (Gram negative),

iv) Staphylococus aureus (Gram positive),

v) Staphylococus citreus (Gram positive).

b) Materials :

i) Nutrient agar (12-15 ml),

ii) Sterile petri dishes,

iii) Old grown culture (24 hours) in test tube,

iv) Sterile pipettes,

v) Test tubes containing solution of the compounds to be tested with known concentration in acetone.

c) Preparation of sub-culture :

A uniform suspension of test organisms of 24 hours old culture was prepared in test tube containing sterile saline solution. To this suspension (1 ml) was poured in each of the sterile petridishes. A sterile nutrient agar was then added in each petri dish. The dishes were rotated to ensure the uniform mixing of micro-organism in the agar medium which

was then allowed to solidify. The agar cups was prepared with sterile cork borer with suitable dimension. The solution of each compound to be tasted for antibacterial activity was added by sterile pipette aseptically into each cup. The control of the solvent used was acetone. The plates were incubated at 37°C for 24 hours. The concentration of test compound in acetone was 5 mg/ml. After incubation the inhibitory zones around the agar cups were observed. The dimeter of inhibition zone was meausred in terms of mm. The principle lying in this testing is the sensitivity of micro-organism to organic compound by diffusion through agar medium.

d) Zones of inhibition

i) Strong growth inhibitor : +++
(zone size 15-20 mm)

ii) Moderate growth inhibitor : ++
(Zone size 9-14 mm)

iii) Less growth inhibitor : +
(zone size 6-8 mm)

iv) No growth inhibitor : -

The antibacterial screening data of the tested compounds have been tabulated in table XVIa.

ANTIBACTERIAL SCREENING RESULTS (Part-I)

Table - XVIa

Antibacterial screening data of the compunds (IV-VIII)

Compd. No.	Name of the compound	Gram +ve		Gram -ve		
		SA	SC	Psed.	Kleb.	E.coli
IVa	N^1-Hydrazido-4-methyl quinolin-2-(1H)-one.	-	-	++	-	+++
IVb	N^1-Hydrazido-4,8-dimethyl quinolin-2 (1H) one.	++	+	+++	+++	+++
IVc	N^1-Hydrazido-4-methyl-7-chloro-quinolin-2(1H) one.	+		+	+	+
Va	4-Phenyl-1-(4'-Methyl, quinolin-2'-one-1'-yl)-thiosemicarbazide.	++	++	+++	-	-
Vb	4-Phenyl-1-(4',8'-dimethyl) quinolin-2'-one-1'-yl) thiosemicarbazide.	++	-	-	+++	-
Vc	4-Phenyl-1-(4'-Methyl,7'-chloro-quinolin-2'-one-1'-yl) thiosemi-carbazide.	++	-	+	++	-
VIa	1-Phenyl,2-(4'-Methyl quinolin-2'-one-1'-yl)-5-mercapto-1,3,4-triazole.	+	-	-	-	++
VIb	1-Phenyl,2-(4',8'-dimethyl, quinolin-2'-one-1'-yl)-5-mercapto-1,3,4-triazole.	+	-	-	-	++
VIc	1-Phenyl, 2-(4'-methyl,7'-chloro-quinolin-2'-one-1'-yl)-5-mercapto-1,3,4-triazole.	+	-	-	-	++

Compd. No.	Name of the compound	Gram +ve		Gram -ve		
		SA	SC	Psed.	Kleb.	E.coli
VIIa	5-Anilino-2-(4'-Methyl, quinolin-2'-one-1'-yl)-1,3,4-oxadiazole.	++	-	-	-	+++
VIIb	5-Anilino-2-(4',8'-dimethyl, quinolin-2'-one-1'-yl)-1,3,4- oxadiazole.	++	-	-	-	+++
VIIc	5-Anilino-2-(4'-Methyl,7'-chloro-quinolin-2'-one-1'-yl)-1,3,4-oxadiazole.	++	-	-	+	++
VIIIa	5-Anilino-2-(4'-Methyl, quinolin-2'-one-1'-yl)-1',3,4-thiadiazole.	++	-	+	+	++
VIIIb	5-Anilino-2-(4',8'-dimethyl quinolin-2'-one-1'-yl) -1,3,4-thiadiazole.	++	-	+	+	+++
VIIIc	5-Anilino-2-(4'-Methyl,7'-chloro-, quinolin-2'-one-1'-yl)-1,3,4-thiadiazole.	++	-	++	+	++

Table - XVIIa

Antibacterial screening data of the compounds (III'-VII')

Compd. No.	Name of the compound	Gram +ve		Gram -ve		
		SA	SC	Psed.	Kleb.	E.coli
III'a	N^1-Methyl hydrazido, 4-methyl quinolin-2(1H) one.	-	-	-	-	++
III'b	N^1-Methyl hydrazido-4,8-dimethyl quinolin-2(1H)one.	-	-	-	-	+++
III'c	N^1-Methyl hydrazido,4-methyl, 7-chloro-quinolin-2(1H)-one.	-	-	-	-	-
IV'a	4-Phenyl-1(4'-methyl, quinoline-2'-one) methyl-oxo-thiosemi-carbazide.	+	+	++	+	+
IV'b	4-Phenyl-1-(4'-8'-dimethyl quinoline-2-one) methyl-oxo-thiosemicarbazide.	+	++	++	++	++
IV'c	4-phenyl-1-(4'-methyl,7'-chloro-quinolin-2'-one)methyl-oxo-thiosemicarbazide.	++	-	++	+	++
V'a	1-Phenyl, 2-(4'-methyl quinolin-2-one-meth-1'-yl) -5-mercapto-1,3,4-triazole.	++	-	+	++	++
V'b	1-phenyl, 2-(4',7'-dimethyl quinolin -2'-one-meth-1'-yl)-5-mercapto-1,3,4-triazole.	++	-	++	++	+++

Compd. No.	Name of the compound	Gram +ve		Gram -ve		
		SA	SC	Psed.	Kleb.	E.coli
V'c	1-phenyl-2-(4'-methyl, 7'-chloro-quinolin-2'-one-meth-1'-yl)-5-mercapto,1,3,4-triazole.	+	-	+	++	++
VI'a	5-Anilino-2-(4'-methyl, quinolin-2'-one, meth-1'-yl) -1,3,4-oxadiazole.	+	+	+	-	++
VI'b	5-Anilino-2- (4',8'-dimethyl, quinolin -2'-one, meth-1'-yl)- 1,3,4-oxadiazole.	+	+	++	+	++
VI'c	5-Anilino-2-(4'-methyl, 7'-chloro-quinolin-2'-one, meth-1'-yl)-1,3,4-oxadiazole.	+	-	++	++	++
VII'a	5-Anilino-2-[4'-methyl, quinolin-2' -one, meth-1'-yl)-1,3,4-thiadiazole.	++	++	++	-	++
VII'b	5-Anilino-2-(4',8'-dimethyl, quinolin-2'-one, meth-1'-yl) 1,3,4-thiadiazole.	++	+++	++	+	++
VII'c	5-Anilino-2-(4'-Methyl,7'-chloro-quinolin-2'-one,meth-1'-yl) 1,3,4-thiadiazole.	++	++	++	+	++

Table - XVIIIa

Antimicrobial Screening data of hydrazones (I'a-c)

Compd. No.	Name of the compound	Gram +ve		Gram -ve		
		SA	SC	Psed.	Kleb.	E.coli
I"a	N^1-Citralidene hydrazido-4-methyl quinolin-2(1H)-one.	-	-	++	+	-
I"b	N^1-Citralidene hydrazido-methyl,4,8-dimethyl quinolin-2(1H) one.	+	-	++	++	-
I"c	N^1-Citralidene methyl hydrazido-4-methyl,7-chloro-quinolin-2(1H) one.	+	-	++	+	-

RESULTS AND DISCUSSION

Part - I

The synthesized compounds were tested against Staphylococcus aureus and Staphylococcus citreus (Gram +ve) and Pseudomonas aerugenosa, Klebsiella pneumoniae, Escherichia coli Gram (-ve) bacteria by Agar plate diffusion technique. Most of compound included in the Part - I of the chapter-II were found to be more active against Gram (-ve) than Gram (+ve) bacteria.

Out of three compounds IVa-c, the hydrazide IVb was found to be more active than rest of the phenyl substituted quinolino-hydrazides against gram -ve bacteria. This may be attributed to the presence of electron donating - methyl substitutent attached to C_8-position of the quinoline ring system. The same compound (IVb) is moderately active against Staphylococus aureus but inactive against Staphylococus citreus. Compound IVc is least active against gram +ve and gram -ve bacteria among three.

Among the semicarbazides Va-c the unsubstituted thiosemicarbazide Va was found to be more active as compared with the rest of two thiosemicarbazides Vb and Vc. The compound Vb is found moderately active against Staphylococus aureus (gram +ve) and most active against K. pheumoniae. The compound Vc also found to be less active.

2-Quinolinone containing three membered triazole ring at N^1-position i.e. VIa-c exhibited moderate activity against E. coli bacteria while less activity against S. aureus and no activity against the rest of the microbial

species under testing.

2-Quinolinone containing oxadiazole nucleus at N^1-position (VIIa-c) were observed to be moderately active against S aureus and more active against E. coli except compound VIIc. Compounds VIIa-c showed no activity against rest of the bacterial species.

2-Quinolinone containing thiadiazole were found to be moderately active against gram -ve bacteria as compared to gram +ve bacteria. Promising activity has been observed against E. coli than rest of the bacterial species. Here, also the presence of electron donating methyl group in phenyl ring in quinoline nucleus increases the activity. These compounds observed to be moderately active against S. aureus bacteria while no activity against A. citreus.

In general, it has been observed that the substitution of the thiadiazole nucleus at N^1-position of the 4-methyl 2-quinolinone enhances the antibacterial activity as compared to triazole and oxadiazole nucleus.

Among all the compounds included in Part-I hydrazide IVb is of considerably important as an antibacterial drug.

ANTIMICROBIAL SCREENING OF THE COMPOUNDS

Part - II

Among the compound of this series III'a-c, the compound III'b was found to be most active against E. coli while no activity was noticed against rest of the bacterial species under testing.

N^1-substituted quinolinoyl thiosemicarbazides IV'a-c were found to be moderately active against P. aerugenosa, K. pneumonie, E. coli and less active against gram +ve bacteria except IVb and IVc which exihibited moderate activity against S. citreus and S. aureus respectively.

4-Methyl-2-quinolinone substituted with triazole nucleus at N^1-position have exhibited moderate to good activity against gram -ve bacteria. The compound V'a and V'c showed moderate activity against S. aureus bacteria and no activity against S. citreus.

4-Methyl quinolinone substituted at N^1-position with oxadiazole nucleus VI'a-c have shown moderate activity against gram -ve bacteria and less activity against gram -ve bacteria.

4-Methyl,2-quinolinone substituted with thiadiazole nucleus at N^1-position VII'a-c exhibited moderate to good activity against S. aureus and S. citreus bacteria. Same compounds were observed to be moderately active against gram -ve bacteria.

In general, it has been observed that substitution of the triazole, oxadiazole and thiadiazole nucleus at N^1-position of the 4-methyl 2-quinolone increases the activity as compared to their corresponding hydrazides and thiosemicarbazides.

Among all the compounds included in the present study,

methyl triazole (V'b) and methyl oxadiazole (VII'b) substituted at N^1-position of the 4-methyl 2-quinolinone nucleus are of considerable medicinal importance as drugs.

N^1-Citralidene hydrazido derivative of 4-Methyl quinoline-2(1H)-one (I"a-c) were found to be less active than gram -ve bacteria and are not of considerable medicinal importance.