CHAPTER-III

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ANTIMICROBIAL SCREENING OF THE COMPOUNDS

1. Introduction, experimental, antimicrobial screening data of the compounds

2. Results and discussion

3. Conclusion

ANTIMICROBIAL SCREENING OF THE COMPOUNDS

INTRODUCTION :

Most of the heterocyclic compounds exhibit antifungal and antibacterial activities. Some them are synthesised by microorganism and are called as antibiotics. Some heterocyclic compounds which are not synthesised by microbes, show antifungal and antibacterial activities to control microbes. Different dyes, sulphonamides, pyrazoles, thiadiazoles, indoles, quinolines found to show antibacterial activities and are of therapeutic use.

Antibacterial activities of newly synthesised compounds were observed by incarporating these compounds in the nutritional media used for cultivation of various test microbes. The microbes used are usually pathogens. The microbial activity of the compounds is examined by the study of growth inhibition pattern of the microbes on media containing these compounds.

Diffusion assays can be carried out on a solid medium, usually an agar medium, suitable for the growth of the test organisms. There are two types of diffusion assay as cylinder method and paper disc method.

In the cylinder method, a portion of the antibiotic solution diffuses from a reservoir or cylinder into the surrounding agar. In Paper-disc method, only defined aamount of the solution is applied to the disc. In this method, plates of seeded agar

medium are prepared and inoculated. The solutions to be assayed or solutions of reference compound are added at a volume of 0.1 ml to discs of sterile filter paper having 12.8 mm in diameter, laid on the surface of the seeded agar. The solutions are applied to discs lying on a glass plate and the solvents are allowed to evaporate before placing the discs on the seeded agar medium for assay.

The methodology employed for testing the synthesised compounds involves small paper discs previously impregnated with specific compounds with known concentration. The sensitivity of pathogens to different synthesised compounds is determined by measuring the diameter of growth inhibition zones in mm. Hence, the compounds in the present study were tested for their antibacterial activity using Paper disc method against some gram +ve bacteria like S.citreus and S.albus and grame -ve bacteria like E.coli and K.pneumoniae. These bacterial species are pathogenic causing diseases.

EXPERIMENTAL PROCEDURE :

The compounds 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 glasswares and other materials were sterilized. 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

MATERIALS :

a) Nutrient medium (20 ml for each petri dish)

b) Sterile petridishes

c) Sterile pipettes

d) Old grown culture (24 hrs.) in test tube

e) Solution of the compound of known concentration

Nutrient medium is sterilised by autoclaving at 121^{o}C and at 15 Ib/Sq, inch pressure for 20-25 min. It was then poured into serilised glass plate (20 ml per plate) and cooled at room temperature. 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 disc (6 mm diameter, commercially used) charged with the compound of 0.1 mg/ml concentration in DMF 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 Streptomycin (std. compound) and other with DMF were charged and incubated for 24 hrs. for comparison and control of the solvent respectively.

Zones of inhibition of growth were measured in mm. and compared with zone of standard antibiotic i.e. Streptomycin, Zones of inhibition for streptomycin by S.albus, S.citreus (gram +ve), E.coli and K.pneumoniae (gram -ve) were found to be 20, 23, 19, 21 respectively. By comparing the zones of inhibition of std. with sample the activity of the compounds is determined.

The results of antimicrobial screening have been reported in the Table A, B, C, D, E, F and G for the compounds included in the Schemes, I,II, III A, III B, IV A, IV B, and V respectively.

ZONES OF INHIBITION :

		Zone of inhibition in mm
i)	Strong growth inhibitor	15 - 20
ii)	Moderate growth inhibitor	9 - 14
iii)	Less growth inhibitor	6 - 8
iv)	No growth inhibitor	-

TABLE - A

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE AND OTHER DERIVATIVES OF N^1-ACETYLHYDRAZIDO-7-METHOXY-4-METHYL-QUINOLIN-2(1H) ONE (5a-e, 7,8,10).

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial Activity S.albus	S.citreus	E.coli	K.pneumoniane
5a	N^1-(2'-Nitrobenzylidene acetylhydrazido)-7-methoxy-4-methyl quinolin-2(1H)one	15	17	14	12
5b	N^1-(2',4'-Dichloro benzylidene acetylhydrazido)-7-methoxy-4-methyl quinolin-2(1H) one	12	11	14	15
5c	N^1-(4'-Hydroxybenzylidene acetylhydrazido)-4-methoxy-4-methyl quinolin-2(1H) one	11	13	15	12
5d	N^1-(4'-chloro benzylidene acetylhydrazido)-7-methoxy-4-methyl quinolin-2(1H) one	17	15	12	15
5e	N^1-(4'-Dimethyamino benzylideneacetylhydrazido)-7-methoxy-4-methyl-quinolin-2(1H) one	16	15	10	14
7	5-Phenyl-2(N^1-7'-methoxy 4'-methyl quinolin-2'-one-1'-yl)-5-mercapto-1,3,4-triazole	13	10	11	9
8	5-Anilino-2(7'-methoxy-4'-methyl quinolin-2'-one meth-1'-yl)-1,3,4 thiadiazole	10	12	8	10

Compd. No.	Name of the Compound	Antimicrobial Activity			
10	3,6-Dimethyl-1(7'-methoxy-4-methyl quinolin-2-one-1'-yl methyl oxo)-4-oxo-pyrano [4,3-c] pyrazole	15	16	10	13
Std. Compd.	Streptomycin	20	23	19	21

PART I : ANTIMICROBIAL SCREENING : RESULTS AND DISCUSSION

The compounds 5a-e, 7,8, and 10 of this series were tested for their antimicrobial activity by 'Paper disc' method against S.albus, S.citreus, E.coli, K.pneumoniae using Streptomycin as a standard compound for comparison. The antibacterial screening data of the compounds have been incorporated in Table A. Ethanol was used as a control of the solvent in both the cases. Compounds 5a, 5d, 5e, 10 showed good activity against both types of bacteria than other compounds.

Here, N^1-(N-Arylidene acetyl hydrazido)-7-methoxy-2-quinolones in which R= 4-chlorophenyl, 4-dimethyl amino, 2-nitrophenyl enhance the antibacterial activity. N^1-methyl substituted triazolo and thiazolo derivatives showed low activity. However, N^1-methyl substituted pyranopyrazole derivatives moderate to good antibacterial activity.

(SCHEME - I)

TABLE - B

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDROZONE DERIVATIVES OF N^1-ACETYL HYDRAZIDO-7-CHLORO-4-METHYL QUINOLIN-2(1H)ONE (5a-f)

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial activity S.albus	S.citreus	E.coli	K.pneumoniane
5a	N^1-(2'-Nitrobenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H)one	12	14	10	11
5b	N^1-(2',4'-Dichlorobenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H) one	17	16	14	12
5c	N^1-(2'-Hydroxybenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H) one	15	16	15	17
5d	N^1-(4'-chlorobenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H) one	13	13	14	10
5e	N^1-(4'-Methoxybenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H) one	14	15	13	17
5f	N^1-(2',4'-Dinitrobenzylidene acetylhydrazido)-7-chloro-4-methyl quinolin-2(1H) one	12	10	13	10
Std. Compd.	Streptomycin	20	23	19	21

PART II : ANTIMICROBIAL SCREENING : RESULTS AND DISCUSSION

All the synthesised new quinolone derivatives 5a-f given in Table-B of this series were tested for their antibacterial activity against S.albus, S.citreus, E.coli and K.pneumoniae. Compounds 5c and 5e were found to be relatively more potent against K.pneumoniae. Compounds 5b, 5c, 5e showed good activity against gram +ve and gram -ve bacteria. From the antibacterial screening data, it is clear that N^1-(N-aryliden acetylhydrazido) 7-chloro-4-methyl quinolin-2(1H)one with the substitution pattern, R=2,4-dichlorophenyl, 4-methoxyphenyl along with 7-chloro substituent in 4-methyl quinolin-2(1H)one nucleus (Scheme-II) enhances the antibacterial activity. Hence these compounds are of considerable medicinal value.

TABLE - C

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE AND OXADIAZOLE DERIVATIVES OF N^1-HYDRAZIDO-4,8-DIMETHYL QUINOLIN-2(1H)ONE (5a-e AND 6)

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial activity S.albus	S.citreus	E.coli	K.pneumonae
5a	N^1-(4'-Chlorobenzylidene hydrazido)-4,8-dimethyl quinolin-2(1H)one	13	8	11	9
5b	N^1-(4'-Hydroxy-3'-methoxy benzylidenehydrazido)-4,8-dimethyl quinolin-2(1H)one	10	-	10	13
5c	N^1-(4'-Hydroxybenzylidene hydrazido-4,8-dimethyl quinolin-2(1H)one	8	14	7	12
5d	N^1-(2'-Chlorobenzylidene hydrazido)-4,8-dimethyl quinolin-2(1H)one	11	11	9	13
5e	N^1-(2'4'-Dinitrobenzylidene hydrazido)-4,8-dimethyl quinolin-2(1H)one	12	15	12	13
6	5-(4',8'-Dimethyl quinolin-2'(1H)one-1'-yl)-2-chloro-1,3,4-oxadiazole	8	7	10	9
Std. Compd.	Streptomycin	20	23	19	21

PART - III A : ANTIMICROBIAL ACTIVITY : RESULTS AND DISCUSSION :

The compounds 5a-e and 6 of the series were tested for their antimicrobial activity by paper disc method against S.albus, S.citreus (gram +ve), E.coli and K.pneumoniae (gram -ve) using streptomycin as a standard compound for comparison (Table-C). The compound 5e exhibited comparatively good activity against all four types of bacterial strains where as 5d showed moderate activity against S.albus and S.citreus. Compound 5a exhibited moderate activity against S.albus where as compound 5b showed good activity against K.pneumoniae. Compound (6) exhibited less activity against all four types of bacterial strains.

The presence of halogen atom (Cl) in the compound enhances the antibacterial activity. (SCHEME - III A)

TABLE - D

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE DERIVATIVES OF N^1-ACETYLHYDRAZIDO-4,8-DIMETHYL QUINOLIN-2(1H)ONE (5a-f)

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial activity S.albus	S.citreus	E.coli	K.pneumoniae
5a	N^1-(2'-Hydroxybenzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	8	11	10	8
5b	N^1-(4'-Hydroxybenzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	-	10	9	7
5c	N^1-(3'-Hydroxybenzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	11	9	8	7
5d	N^1-(4'-Dimethyl amino benzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	11	13	9	11
5e	N^1-(2'4'-Dichlorobenzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	9	9	14	16
5f	N^1-(4'-Methoxybenzylidene acetylhydrazido)-4,8-dimethyl quinolin-2(1H)one	15	14	10	14
Std. Compd.	Streptomycin	20	23	19	21

PART - III B : ANTIMICROBIAL ACTIVITY : RESULTS AND DISCUSSION

All the compounds of the series (5a-f) given in Table-D were tested for their antibacterial activity by 'Paper disc' method against S.albus, S.citreus (gram +ve), E.coli and K.pneumoniae (gram -ve) bacteria using Streptomycin as a standard compound for comparision. From the data given in the Table-D, it is observed that compound 5e exhibited moderate activity against E.coli and K.pneumoniae. The compound 5f showed better antibacterial activity towards all the bacterial strains.

Thus, N -arylidene acetylhdrazido-4,8-dimethyl-quinolin--2(1H)ones containing R=2,4-dichlorophenyl and 4-methoxy phenyl substituents enhance the antibacterial activity. (SCHEME - III B)

TABLE - E

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE AND OXADIAZOLE DERIVATIVES OF N^1-HYDRAZIDO-4,6-DIMETHYL QUINOLIN-2(1H)-ONE

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial activity S.albus	S.citreus	E.coli	K.pneumoniae
5a	N^1-(4'-chlorobenzylidene hydrazido)-4,6-dimethyl quinolin-2(1H)one	6	8	10	9
5b	N^1-(2'4'-Dichlorobenzylidene hydrazido)-4,6-dimethyl quinolin-2(1H)one	10	8	6	7
5c	N^1-(4'-Hydroxybenzylidene hydrazido)-4,6-dimethyl quinolin-2(1H)one	9	7	8	9
5d	N^1-(4'-HYdroxy-3'-methoxy benzylidene hydrazido)-4,6-dimethyl quinolin-2(1H)one	10	8	10	6
7	5-(4',6'-Dimethyl quinolin-2'(1'H)one-1'-yl)-2-chloro-1,3,4-oxadiazole	-	10	-	-
Std. Compd.	Streptomycin	20	23	19	21

PART - IV A : ANTIMICROBIAL ACTIVITY : RESULTS AND DISCUSSION :

All the newly synthesized quinoline derivatives 5a-d and 7 were tested for their antibacterial activity against S.albus, S.citreus, E.coli and K.pneumoniae bacteria. The results of antibacterial screening have been included in the Table-E. Most of the compounds exhibited less activity (about 50%) against bacterial strains used in the present study as compared with the standard compound. (SCHEME - IV A)

TABLE - F

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE DERIVATIVES OF N^1-ACETYLHYDRAZIDO-4,6-DIMETHYL QUINOLIN-2(1H)ONE (5a-e) :

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antibacterial activity S.albus	S.citreus	E.coli	K.pneumoniae
5a	N^1-(2'4'-Dichlorobenzylidene acetyl hydrazido)-4,6-dimethyl quinolin-2(1H)one	14	10	13	12
5b	N^1-(2'-Hydroxybenzylidene acetylhydrazido)-4,6-dimethyl quinolin-2(1H)one	11	9	7	10
5c	N^1-(4'-Hydroxybenzylidene acetylhydrazido)-4,6-dimethyl quinolin-2(1H)one	8	11	9	11
5d	N^1-(3'-Hydroxybenzylidene acetylhydrazido)-4,6-dimethyl quinolin-2(1H)one	8	10	9	10
5e	N^1-(2'-Nitrobenzylidene acetylhydraziido)-4,6-dimethyl quinolin-2(1H)one	10	15	14	11
Std. Compd.	Streptomycin	20	23	19	21

PART - IV B :

All the newly synthesized quinolones 5a-e from this series were tested for their antibacterial activity against S.albus, S.citreus, E.coli and K.pneumoniae using the Streptomycin as a standard compound for comparison. The results of antimicrobial screening study have been reported in the Table-F. From the antibacterial screening data, it was found that compound 5e is highly potent against E.coli as well as S.citreus. Compound 5e exhibited moderate activity against S.albus as well as K.pneumoniae.

Hence, the presence of the substitution pattern R=2,4-dichlorophenyl and 2-nitrophenyl in N^1-arylidene acetylhydrazido-4,6-dimethyl-quinolin-2(1H)one enhances the antibacterial activity.

(SCHEME - IV B)

TABLE - G

ANTIMICROBIAL SCREENING DATA OF SOME NEW HYDRAZONE DERIVATIVES OF N^1-ACETYLHYDRAZIDO-8-CHLORO-4-METHYL QUINOLIN-2(1H)ONE (5a-f)

(Zone of inhibition in mm)

Compd. No.	Name of the Compound	Antimicrobial activity S.Albus	S.citreus	E.coli	K.pneumoniae
5a	N^1-(4'-Chlorobenzylidene acetylhydrazido)-8-chloro-4-methyl quinolin-2(1H)one	7	10	9	11
5b	N^1-(2'4'-Dichlorobenzylidene acetylhydrazido)-8-chloro-4-quinolin-2(1H)one	10	8	11	7
5c	N^1-(4'-Hydroxybenzylidene acetylhydrazideo)-8-chloro-4-methyl-quinolin-2(1H)one	-	11	8	8
5d	N^1-(4'-Methoxybenzylidene acetylhydrazido)-8-chloro-4-methyl quinolin-2(1H)one	11	9	8	7
5e	N^1-(2'-Nitrobenzylidene acetylhydrazido)-8-chloro-4-methyl quinolin-2(1H)one	8	10	9	11
5f	N^1-(3',4',5'-Trimethoxy benzylidene acetyl hydrazido) -8-chloro-4-methyl quinolin-2(1H)one	10	9	9	10
Std. Compd.	Streptomycin	20	23	19	21

PART - V : ANTIMICROBIAL ACTIVITY : RESULTS AND DISCUSSION :

The compounds 5a-f were tested invitro for their antibacterial activity against S.ablus, S.citreus, E.coli, K.pneumoniae using streptomycin as standard commpoud. The control of the solvent experiment was done in the similar manner and the zones of inhibition were measured in mm. The results incorporated in Table-G, showed that compounds 5a-f showed moderate to less activity against different bacterial stains used in the present study. (SCHEME - V)

CONCLUSION :

Among the different series of N^1-substituted hydrazido, N^1-substituted acetylhydrazido quinolin-2(1H)ones included in the present dissertation the N^1-acetylhydrazido-7-chloro-4-methyl quinolin-2(1H)one and N^1-acetylhydrazido-7-methoxy-4-methyl quinolin-2(1H)one hydrazone derivatives are found to exhibit the promising antibacterial activity and hence they have considerable value as the drugs.