Chapter - V
Testing of antibacterial activity of compounds synthesised in chapter II - IV

TESTING OF ANTIBACTERIAL ACTIVITY OF COMPOUNDS SYTHESISED IN CHAPTER II TO VI

INTRODUCTION[1-4]

The therapeutics known before the time of Ehrlich were cinchona for malaria, ipeac for amoebic dysentery and mercury for treating syphilis. The diseases of protozoal and spirochaetal origin have been made to respond to synthetic chemotherapeutic agents during the first two decades of 19th century. The microbiologist and clinical personnel overlooked the possibility that the bacteriostatic compounds would inhibit rapid reproduction of pathogenic bacteria and enable the leucocytes and other defence mechanism of the host to cope with few static invaders.

Paul Ehrlich, the father of chemotherapy used the term chemotherapy to describe the cure of an infectious disease without injury to the host known as chmotherapeutic agents and classified according to diseases and the infections, such as antibacterial, antiprotozoal, antiviral, antineoplastic, antitubercular and antifungal agents.

This part describes methods used for 'invitro' assessment of antibacterial agents. Antibacterial substances and preparations are classified as disinfectants, antiseptics and chemotherapeutic agents. The term disinfectant is used to eliminate or destroy infection and should be capable of killing a wide range of bacteria. An antiseptic is used to control or eliminate bacterial infection. A chemotherapeutic agent is an antibacterial substance administered systematically for the treatment of infection, may be either bacteriostic or bacteriocidal in its action, its main function is to prevent the multiplication of infective organism.

Antibacterial Agents

They are one type of chemotherapeutic agents used against the bacterial diseases and divided into two types according to their action onbacteria namely bacteriostatic and bactericidal agents. An agent is considered "bacteriostatic" when it inhibits further growth or multiplication of bacteria and classed as "bactericidal" when it kills the bacteria. Antimicrobial agents are the chemosterapeutic substances that destroy or inhibit the growth of micro organisms in the living tissue Antibiotics are substances produced by living organisms and are sufficiently non-toxic to be used as antimicrobial agents.

CLASSIFICATION OF ANTIBACTERIAL AGENTS

1. Alcohols and related compounds

Various alcohols and alcohol derivatives have been used as antiseptics, e.g. ethanol and propanol. The antibacterial values of straight chain alcohols increase with an increase in the molecular weight and beyond C_8 the activity begins to fall off. The isomeric alcohols show a drop in activity from primary to secondary to tertiary.

2. Acids and their Derivatives

Salicyclic acid has strong antiseptic and germicidal properties being a carboxylated phenol. The presence of the carboxy group appears to have -ve effect. Benzoic acid is used externally as an antiseptic and employed in lotions and ointments.

3 Iodine containing Compounds

Not many iodine containing compounds are widely used as antiseptics in the medicine today. But iodine as a tincture or in aqueous solution with an iodine is still widely used as an antiseptic, virucide, fungicide and amebicide.

4 Chlorine containing Compounds

The bactericidal properties of hypochiorite was first studied by Robert Koch in 1881. N-Chloro-compounds are represented by amides, imides and amidines in which one or more of the hydrogen atoms attached to nitrogen have been replaced by chlorine, like chloroamine-T, halozone, chlorozidin, etc.

5 Oxidizing Agents

Oxidizing agents are of value as antiseptics depending on the liberation of oxygen, like H_2O_2, other metal peroxides, urea peroxide etc.

6 Bacteriostatic Dyes

Prior to the advent of the sulfonamides and the antibiotics, the organic dyes have been used extensively as antibacterial agents. Their medical significance was first recognized by Churchman[5] who reported in 1912 on the inhibitory effect of crystal violet on Gram-positive organism. The yellow acridine dyes have been first introduced by Ehrlich for control of trypanosomal infections. Browing[6] in 1913 discovered their antibacterial properties which led to their wide clinical use. The acridines exert a bactericidal and bacteriostatic action against both Gram-positive and Gram-negative organisms.

7. Antibacterial Antibiotics

In the twenty years since the discovery of erythromycine, more than fifty antibacterial antibiotics with a common chemical feature - a macrolytic lactone has been described. These macrolides are of great interest because of their antibacterial activity, primarily against Gram positive bacterial and mycoplasma species like methymycin, erythromycin and carbomycin. The streptomycins and enomycine comprise the aminoglycoside antibacterial antibiotics.

8. 8-Hydroxyquinoline

8-Hydroxyquinoline or oxime is unique among the isomeric hydroxy quinolines. It alone exhibits antimicrobial activity attributed by its ability to chelate metals.[7]

9. Antibacterial Metal Ions

Metals and their salts other than mercury and silver are less important as practical antibacterial agents. Both organic and inorganic copper salts, used mainly as industrial fungicides and preservatives, are strongly bacteriostatic but lack significant bactericidal properties. The zinc pyrithione is both highly antibacterial and antifungal. Sprowls and Poc[8] studied the antibacterial activity of a number of metallic salts in solution and found them more effective.

DETOXICATION OF ANTIBACTERIALS

P-Amino benzoic acid is a growth factor for certain microorganisms and competitively inhibits the bacteriostatic action of sulfonamides. The metabolites identified in man are p-aminobenzoylglucuronide : p-amino hippuric acid : p-acetylaminobenzoylglucuronide : p-acetylaminohippuric acid and p-acetylaminobenzoic acid. The aromatic nitriles appear to undergo primarily hydroxylation and to a lesser extent, hydrolysis with or without oxidation.

BACTERIA

In 1928, a German Scientist C.E.Ehrenberg used the term 'bacterium' The bacteria are small microscopic organism with a relatively simple and primitive form of the cellular organisation known as "Procaryotic". The staining reactions of bacteria are of greatest importance in their differentiation and identification. In 1884, danish physician Gram discovered the stain known as Gram-stain. Staining reaction has widest application which divided all bacteria into two catagories, namely "Gram-(+)ve"

and "Gram-(-)ve". The Gram(+)ve bacteria resist decolourisation and remain stained as dark purple colour while Gram(-)ve bacteria are decolourised.

Bacteria can be classified according to their morphological characteristics as lower and higher. The lower bacteria are of generally unicellular structures, never in the form of a mycelium or sheathed filaments, e.g. cocci, bacilli, vibrios, spirille and spirochaetes. The micro-organisms capable of producing disease in animal or human being are known as "Pathogenic". Most of the micro-organisms present on the skin and mucous membranes are non-pathogenic.

CLASSIFICATION OF IMPORTANT ORGANISMS

CLASS : SCHIZOMYCETES

Order 1	Family 2	Genus 3	Species 4
A Eubacterials	Micrococeaceae	Staphylococcus Micrococcus Sarcina	Staph. Aureus M.tetragenus S.lutes
	Lactobaci- llaceas	Streptococcus Peptostreptococcus Diplococcus Lactobacillus	Str.pyogenes Pep.putridis D.pheumonae L.acidophilus
	Neisseriaceae	Neisseria	N.gonorrhoeae N.meningitidis N.catarrhalis
	Corynebac- teriaceae	Corynebacterium Listeria Erysipelothrix	C.diphtheriae E.rhusiopathiae L.monocytogenes
	Achromobac- teriaceae	Alceligenes	Alc.faecalis
	Enterobac- teriaceae	Escherichia Klebsiella Citrobacter Cloaca Hafnia	Esch.coli K.pneumoniae K.aerogenes Cit.Freundii Cl.Cloacae Haf.alvei

CONTD...

Order 1	Family 2	Genus 3	Species 4
		Serretia	Ser.marcescens
		Salmonella	Salm.typhosa
		Shigella	Sh.dysenteriae
		Proteus	Pr.vulgaris
	Brucellaceas	Pasteurella	P.pestis
			P.psuedotuberculosis
		Fancisella	F.tularensis
		Brucella	Br.melitensis
			Br.abortus
			Br.Suis
		Haemophilus	H.influanzaa
			H.duoreyi
		Bordetella	Bord.pertussis
		Moraxella	M.lacunata
		Actinobacillus	A.mallei
			A.lignieresii
	Bacteriodaceae	Bacteroids	Bact.fragilis
		Fusobacterium	F.fusiforms
		Streptobacillus	St.moniliformis
		Sphaerophorus	Sph.necrophorous
	Bacillaceae	Bacillus	B.anthracis
			B.subtilis
		Clostridium	Cl.tetani.
			Cl.welchii
B.Pseudo-minadales	Pseudomin-adaceae	Pseudomonas	Ps.aeruginosa
	Spirillaceae	Vibrio	V.cholerae
		Spirillum	Sp.minus
C.Mycopla-smatales	Mycoplas-mataceae	Mycoplasma M.mycoides	M.pneumoniae
D.Actinomy-cetatea	Mycobact-riaceae	Mycobacterium	Myco.tuberculosis
			Myco.laprae
	Actinomy-cetaceae	Actinomyces	A.israeli
			A.bovis
		Nocardia	N.madurae
	Strepto-mycetaceae	Streptomyces	Strepto.griseus

CONTD...

Order 1	Family 2	Genus 3	Species 4
E.Spirocha-etales	Spirocha-etaceae	Spirochaeta	Non.pathogenic
		Saprospirs	
	Trepone-mataceae	Borrelia	Bor.duttoni
			Bor.recuraentis
			Bor.vincenti
		Treponema	Tr.pallidum
		Tr.partenue	
		Leptospira	L.icterohaemorrhagies

We have employed three bacterial species namely E.coli., S.aureus and B.Subtilis for testing the antibacterial activity of these compounds.

EVALUATION OF ANTIBACTERIAL ACTIVITY

Varieties of 'invivo' screening methods has been used to evaluate the antibacterial activity. Testing in mice has become standard, the sensitivity of bacteria to antimicrobial agents is tested by the same methods as in other form of microbiological assey.

Invitro Methods

'Invitro testing is useful for antibacterial spectrum determination of a compound and comparing it with other agent.

Several types of procedure are in use for assaying the potency of antibiotic preparation for therapeutic purpose. These methods have been modified and used for sensitivity test of unknown organisms.

1. Serial dilution in Broth[9]

Serial dilutions of the drug being assayed are made in uniform amounts of stadnard broth in culture tubes. These are inoculated with a uniform number of cells to test organism. After incubation, turbidity (or its absence) is measured by turbidimeter and turbidities (amounts of growth) are compared with a dilution series made in the same way but with antibiotic reference standard of measured potency.

2. Streak Assay in Agar (loc.cit.)[9]

Graded dilutions of the substance to the tested are placed in a series of petri dishes in which is poured about 10 ml. Of melted and cooled agar, contents mixed with drug dilution. After agar has hardened, the plates marked into several sectors, each of which is streaked with different test organism.

3. Diffusion tests

Diffusion tests on solid media have been adopted by most of the laboratories wherein the antimicrobial agent is held in a reservoir from which it diffuses through agar medium to form a diffusion gradient to which the microorganisms, growing in or on the agar, are exposed. The size of inhibition zone depends upon the factor that influence the diffusion of the antimicrobial agent as well as the rate of growth of the organism.

a) Agar Strip diffusion test for sensitivity

This is a simple technique which has originally been used by Fleming. A strip of agar is cut from the centre at a place of suitable culture medium. Appropriate amount of antimicrobial agent is added to molten agar and pipetted into the gutter in the medium and the surface of the agar is inoculated by stroking cultures to be tested

b) Replica plate method to show bacteriostatic and bactericidal action[10]

A zone of inhibition of growth around a dish may indicate that the antimicrobial agent is either bacteriocidal or bacteriostatic The presence or absence of living organisms within the zones of apparent complete inhibition of growth on diffusion plates have been shown by replica plate method.

c) Diffusion tests with filter paper-disks for DETERMINING SENSITIVITY[11]

This constitute a simple and reliable technique, impregnating small disk of standard filter paper with given amount of antibiotic placing them on plates of culture medium inoculated with the organism to be tested. After incubation the degree of sensitivity by measuring the easily visible areas of inhibition of growth which has been produced by the diffusion of antibiotic from the disk into the surrounding medium is determined.

DISCUSSION

Certain characteristic features of representative species i.e. one gram +ve and one gram -ve bacterial strains have been briefly described here.

1. Staphylococcus Aureus Family : Micrococcaceae

In 1878, Koch observed micrococcus like organisms in pus; Pasteur (1880) cultivated these cocci in liquid media. Ogaston (1881) found it present in pus of acute chronic abscesses and found it pathogenic for mice and guinea pigs.

They are Gram-(+) cocci, avoid or spheroidel, non-motile, arranged in group of clusters; grow on nutrient agar and produce colonies, which are golden yellow, white or lemon yellow in colour; serobes or faculative anaerobes; biochemical activities and haemolytic power are variable; pathogenic strains produce coagulase, ferment glucose, lactose, mannitol with production of acid, liquefy gelatin and produce pus in the lesion.

Genus : Staphylococcus

Staphylococcus is differentiated from micrococcus, another genus of the same family, by the ability to utilise glucose, amnnitol and pyruvate anaerobically. Staphylocci are found on the skin or mucous membranes of the animal body, especially of the nose and mouth, where they often occur in large numbers even under normal conditions.

Species : Staphylococcus aureus

The individual cells are 0.8 to 0.9μ in diameter. They are avoid or spherical, non-motile, noncapsulated, non-sporing, stain with ordinary aniline dyes and Gram-(+)ve, typically arranged in groups. These are aerbbes or facultative anaerobes and grow easilyon nutrient agar. The optimum temperature for the growth is 37°C but the range of temperature varies from 10° to 40°C, optimum pH is 7.4 to 7.6.

2 Escharichia coli - Family : Enterobacteriaceae

They are Gram(-)ve rods, motile with peritrichate flagella, or non-motile. They do not form spores, and are primarily environmental saprophytes and scavengers, found in the intestinal tract of man or lower animals, hence the family name.

Genus : Escherichia

This genus comprises Escherichia coli and several varients, and is of particular interest since they occur commonly in the normal intestinal tract of man and animals. Escherichia coli is most distinctively fecal species.

Species : Escherichia Coli

Escherichia in 1885 discovered Escherichia coli from the faeces of the new born who showed the organisms in the intestine within 3 days after birth. These are Gram (-)ve rods 2 to 4μ. Commonly seen in coccobacillery from and rarely in filamentous forms. E.coli are generally non-pathogenic and incriminated as pathogens, because sometimes strains have been found to produce septicemia, inflammations of liver gall bladder, appendix, meningitis, pneuomonia and other infections.

Invitro testing

Bacteriostatic activity can be determined on solid or liquid, media, each depends on assessing the extent of inhibition of growth. We have adopted the disk or Cupplate method" for the sensitivity testing.

The Disk Method

After the report of the "International Collaborative Study"[12] involved with investigating 'the disk test', the method recommended has been adopted in Sweden. In U S.A. the modified Kirby-Bauer[13] technique has been adopted as an official method by 'Food and Drugs Administration'.

The main stimulus for standardization in U.K. has come from recommendation of use of the controlled single disk method [14] In this method, nutrient agar of appropriate composition is heavily inoculated with the desired organism all over the surface of the solidified agar or mixed with agar, while still fluid, before pouring the plate. If an antibiotic solution of unknown potency is being assayed, the organism used is stock strain of known sensitivity to standard doses of antibiotic. Measured strengths of the antibiotic solution are applied to the inoculated agar in disks of uniform thickness, or sterile filter paper, are placed on the surface of the agar plate before incubating. The width of the zone indicates, the sensitivity of the organism being tested though the presecne or absence of a zone is of greater significance.

Factors influencing inhibition Zone sizes

1 Ingredients of Culture Media

Many substances are present in culture media which may affect the zone of inhibition, common ingredients such as peptone, tryptone, yeast extract and agar may vary in their mineral content and many of them may influence the activity of some antibiotics. It is well known that Ca,Mg and Fe affect sensitivity zones produced by tetracyclines and gentamycin.

2. Choice of Medium

Consistent and reproducible results are obtained in media prepared especially for sensitivity testing the plates must be poured flat with an even depth, very thin plates are unsatisfactory.

3. Effect of pH

The activity of amino-glycosides is enhanced in alkaline media and reduced in acidic media, the reverse isshown by tetracyclin.

4. Size of Inoculum

Although many antibiotics are not markedly affected by large number of organisms, all inhibition zones are diminished by heavy inocula. Overnight broth cultures of organisms and suitable suspensions from solid media can be diluted accurately to give optimum inocula for sensitivity testing. In practice, satisfactory results can be achieved by taking a loopful of a well grown culture, or a suitably made suspension of organisms and spreading it with dry sterile swab.[15]

The performance of Diffusion Technique :

1 Composition of Nutrient Agar

Peptone - 2 g; NaCl - 2 g.

Meat extract - 3.2 g; Agar-agar Powder - 8 g.

pH - 7.4 ; Distilled Water - 1000 ml.

2 Strength of Antibiotics

Until very recently, there has been little or no agreement regarding the strength of antibiotic disks for use 'invitro' sensitivity tests.

3. Storage of disks

Disks should always be kept cool and dry and when applied to the medium should be pressed firmly to ensure proper contact and even diffusion disks may be applied to culture media very convenient with fine pointed forceps, dissecting needles or hypodermic needles.

4. Incubation Time

It should ideally be the minimum required for the normal growth of the organism. Prolonged incubation of a culture may result in inactivation of the antibiotic and result in the subsequent growth of organism.

5. Controls

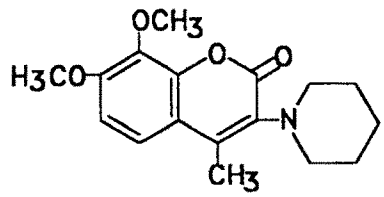
For the correct interpretation of results and recognition of any source of error in disk diffusion sensitivity tests, the correct use of control organism isessential. If multiple disks, necessitating the use of whole plates are used. Control plates should be set up for every drug and medium. For routine daily use, the organisms are most conveniently kept in a refrigerator at 4°C on sterile throat swabs, a jar full of such swabs can be impregnated at one time as they keep well at least a week.

The compounds described in Chapter II to IV have been screened for antibacterial activity and tabulated in table - 1 to 7 under the following heads

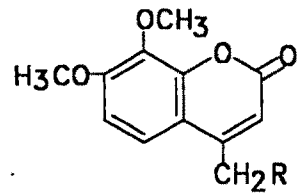
1. Mannich bases from Amines via bromomethylation.
2. Mannich bases from Aminoacids.
3. Schiff bases
4. Oxadiazoles and Hydrazides
5. Amides from Amino acids
6. Anilides and amides from 2° amines
7. Sulfonamides

N.B. : Tables 1 to 7

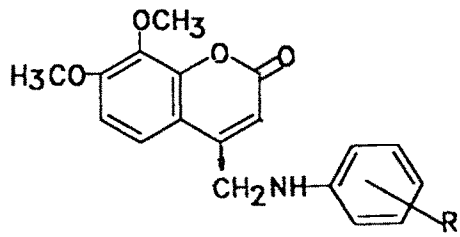
1. (*) indicate zone diameter of growth inhibition in mm.
2. (+) Zone diameter less than 15 mm.
3. (++) Zone diameter more than 15 mm.
4. (-) indicates no inhibitory zone around the disk.
5. Solvent used : Dimethylformamide.
6. All the results were compared with standard drug Ampicillin.



I-A



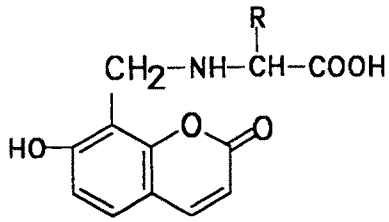
I-B(1-S)



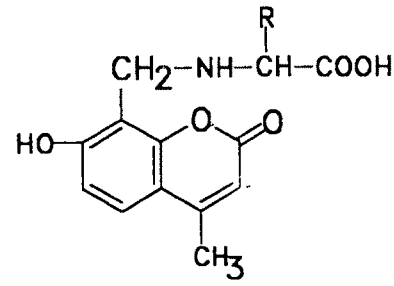
I-C(1-11)

Table - 1 : Antibacterial Activity of Mannich Bases From Amines Via Bromomethylation

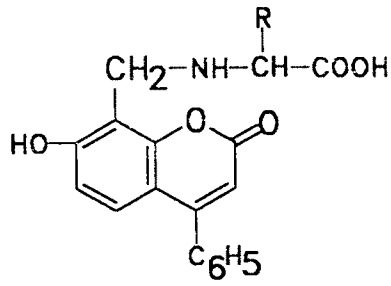
Structure No. I	R	Interpretation zone of inhibition at 100 and 500 ppm conc.			
		E. coli		S. aureus	
		100	500	100	500
A - 1	-	+	++	-	-
B - 2	Piperidino	+	++	-	-
3	Morpholino	-	-	-	-
4	N-phenylpiperazino	-	++	-	-
5	4-Methylpiperidino	-	-	-	-
C - 1	2-CH_3	-	-	-	+
2	3-CH3	-	-	-	+
3	4-CH_3	-	-	-	+
4	4-COOC2H5	-	-	-	-
5	3-$COCH_3$	-	-	-	-
6	4-$COCH_3$	-	+	+	+
7	4-Br	-	-	-	-
8	H	-	-	-	+
9	1-Naphthyl	+	+	+	+
10	4-NO2	+	+	-	+
11	3,4-Cl2	-	-	-	+



II–A(1–6)



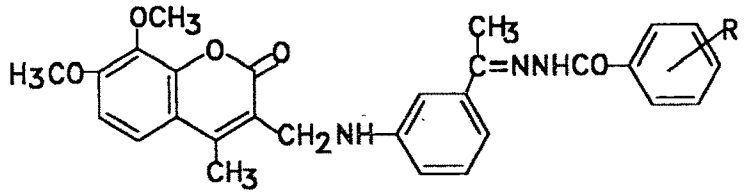
II–B(1–8)



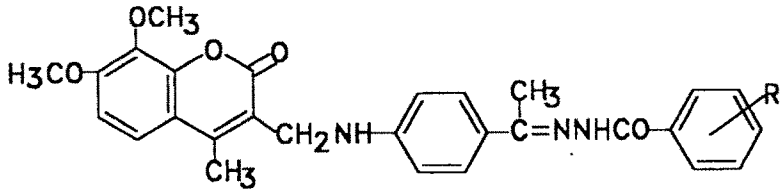
II–C(1–4)

Table - 2 : Antibacterial Activity of Mannich Bases From Aminoacids

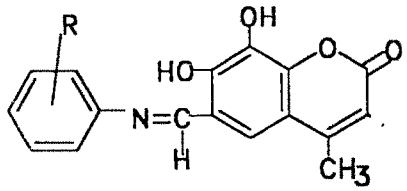
Structure No. II		R	Interpretation zone of inhibition at 100 and 500 ppm conc.			
			E. coli		S. bacillus	
			100	500	100	500
A -	1	CH_3	-	+	-	+
	2	$CH_2C_6H_5$	-	-	-	+
	3	$CH_2CH_2SCH_3$	-	-	-	+
	4	CH_2OH	-	-	-	+
	5	$CH(CH_3)_2$	-	-	-	+
	6	$CH_2CH(CH_3)_2$	-	-	-	+
B -	1	CH_3	-	-	-	+
	2	$CH_2C_6H_5$	-	-	-	+
	3	$CH_2CH_2SCH_3$	-	-	-	+
	4	CH_2OH	-	-	-	-
	5	$CH(OH)CH_3$	-	-	-	-
	6	$CH(CH_3)_2$	-	+	+	+
	7	$CH_2CH(CH_3)_2$	-	-	-	-
	8	Sarcocin	-	-	-	+
C -	1	CH_3	-	-	-	+
	2	$CH_2C_6H_5$	-	-	-	+
	3	$CH_2CH_2SCH_3$	+	+	+	+
	4	$CH(CH_3)_2$	+	+	-	+



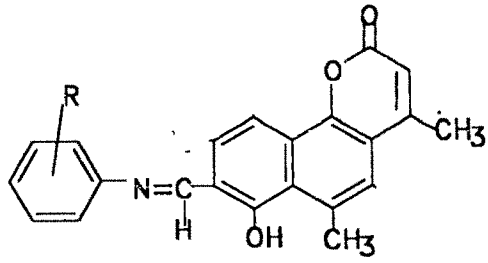
III A (1–7)



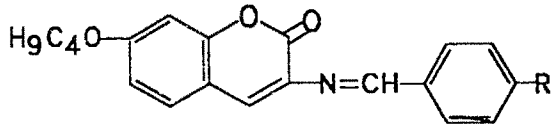
III B (1–6)



III C (1–4)



III D (1–10)



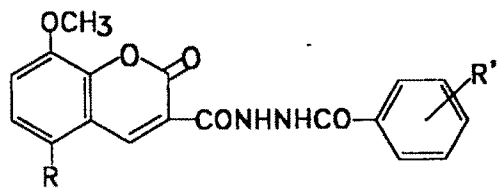
III–C(1–10)

Table - 3 : Antibacterial Activity of Schiff Bases

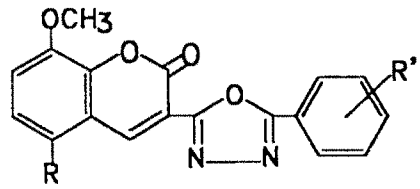
Structure No. III		R	Interpretation zone of inhibition at 100 and 500 ppm conc.			
			E. coli		S. aureus	
			100	500	100	500
A -	1	3-CH_3	-	-	-	-
	2	2-NO_2	-	++	-	++
	3	3-Br	-	+	-	+
	4	2-Cl	-	++	-	++
	5	4-Cl	-	++	-	++
	6	3-Cl	-	-	-	-
	7	4-OH	-	-	-	-
B -	1	3-CH_3	-	-	-	-
	2	2-NO_2	-	+	-	+
	3	3-Br	-	+	-	+
	4	3-OCH_3	-	-	-	-
	5	2-Cl	-	++	-	++
	6	4-Cl	-	-	-	-
C -	1	4-CH_3	-	++	-	++
	2	4-$COOC_2H_5$	-	++	-	++
	3	3,4-Cl_2	-	+	-	+
	4	2,4-Cl_2	-	++	-	++

contd... Table - 3 : Antibacterial Activity of Schiff Bases

Structure No III		R	Interpretation zone of inhibition at 100 and 500 ppm conc.			
			E. coli		S. aureus	
			100	500	100	500
D -	1	H	-	-	-	-
	2	4-Br	-	-	-	-
	3	4-NO_2	-	-	-	-
	4	2,4-Cl_2	-	-	-	-
	5	3,4-Cl_2	-	+	-	+
	6	4-CH_3	-	-	-	-
	7	4-$COOC_2H_5$	-	+	-	+
	8	1-Naphthyl	-	-	-	-
	9	2-Naphthyl	-	-	-	-
	10	3-NO_2	-	-	-	-
E -	1	4-NO_2	-	++	-	-
	2	3-NO_2	-	-	-	-
	3	2-NO_2	-	-	-	-
	4	2,4-Cl_2	-	++	-	-
	5	3,4-Cl_2	-	++	-	-
	6	4-Cl	-	++	-	-
	7	3-Cl	-	++	-	-
	8	2-Cl	-	++	-	-
	9	4-CH_3	-	-	-	-
	10	3-OCH_3	-	-	-	-



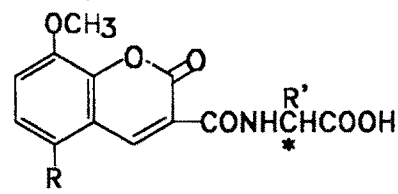
IV (1–9)



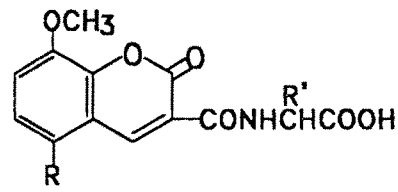
V (1–6)

Table - 4 : Antibacterial Activity of Hydrazides and Oxadiazoles

Structure		R	R'	Interpretation zone of inhibition at 100 and 500 ppm conc.			
				E. coli		S. aureus	
				100	500	100	500
IV	1	Br	2-NO_2	-	+	-	-
	2	Br	3-NO_2	-	-	-	-
	3	Br	4-NO_2	-	+	-	-
	4	Br	2-Cl	-	+	-	-
	5	Br	3-OCH_3	-	-	-	-
	6	Br	4-CH_3	-	++	-	-
	7	Br	4-OCH_3	-	+	-	-
	8	Br	H	-	++	-	-
	9	H	H	-	-	-	-
V	1	Br	2-NO_2	-	-	-	-
	2	Br	3-OCH_3	-	++	-	-
	3	Br	2-Cl	-	++	-	-
	4	Br	4-CH_3	-	+	-	-
	5	Br	3-NO_2	-	-	-	-
	6	H	H	-	-	-	-



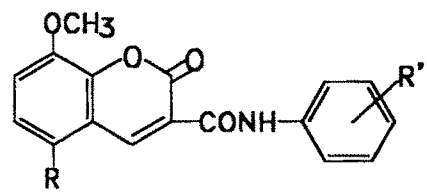
VI-A (1-8)



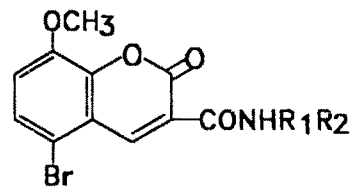
VI-B (1-7)

Table - 5 : Antibacterial Activity of Carboxamides from aminoacids

Structure VI		R	R'	Interpretation zone of inhibition at 100 and 500 ppm conc.			
				E. coli		S. aureus	
				100	500	100	500
A	1	H	CH_3	-	+	-	+
	2	H	$CH(CH_3)_2$	-	+	-	+
	3	H	$CH_2CH_2SCH_3$	-	++	-	+
	4	H	CH_2OH	-	+	-	++
	5	H	$CH(OH)CH_3$	-	++	-	+
	6	Br	CH_3	-	+	-	+
	7	Br	$CH(CH_3)_2$	-	+	-	+
	8	Br	$CH(OH)CH_3$	-	-	-	-
B	1	H	H	-	++	-	+
	2	H	β-alanine	-	-	-	-
	3	H	CH_3	-	++	-	++
	4	H	$CH(CH_3)_2$	-	++	-	++
	5	H	$CH_2CH_2SCH_3$	-	++	-	++
	6	H	$CH_2C_6H_5$	-	+	-	++
	7	Br	β-alanine	-	+	-	+



VII.–A (1–15)

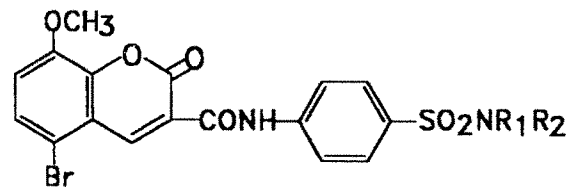


VII.–B (1–7)

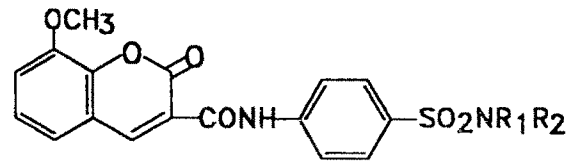
Table - 6 : Antibacterial Activity of Carboxanilides and Amides from amines

Structure VII A	R	R'	Interpretation zone of inhibition at 100 and 500 ppm conc.			
			E. coli		S. aureus	
			100	500	100	500
1	Br	H	-	++	-	+
2	Br	4-$COOC_2H_5$	-	++	-	++
3	Br	4-CH_3	-	+	-	-
4	Br	2-CH_3	-	-	-	-
5	Br	4-NO_2	-	+	-	+
6	Br	3-NO_2	-	-	-	-
7	Br	2,4-Cl_2	-	++	-	++
8	Br	3,4-Cl_2	-	+	-	++
9	Br	3-$COCH_3$	-	-	-	-
10	Br	4-$COCH_3$	-	-	-	-
11	Br	4-OCH_3	-	-	-	-
12	Br	4-Br	-	-	-	-
13	Br	2-Naphthyl	-	-	-	-
14	H	2-NO_2	-	-	-	-
15	H	2-CH_3	-	-	-	-

VII B	R_1	R_2	Interpretation zone of inhibition at 100 and 500 ppm conc.			
			E. coli		S. aureus	
			100	500	100	500
1	CH_3	C_6H_5	-	++	-	++
2	C_2H_5	C_6H_5	-	++	-	++
3	C_6H_5	C_6H_5	-	-	-	-
4	Morpholino		-	++	-	++
5	N-Phenylpiperazino		-	++	-	-
6	4-Methyl piperidino		-	++	-	++
7	3-Methyl piperidino		-	-	-	-



VIII-A(1-7)



VIII-B(1-8)

Table - 7 : Antibacterial Activity of Sulfonamides

Structure VIII		R_1	R_2	Interpretation zone of inhibition at 100 and 500 ppm conc.			
				E. coli		S. aureus	
				100	500	100	500
A	1	CH_3	C_6H_5	-	++	-	-
	2	C_2H_5	C_6H_5	-	++	-	-
	3	Piperidino		-	-	-	-
	4	4-Methyl piperidino		-	-	-	-
	5	3-Methyl piperidino		-	++	-	-
	6	Morpholino		-	-	-	-
	7	N-Phenyl piperazino		-	-	-	-
B	1	CH_3	C_6H_5	-	++	-	-
	2	C_2H_5	C_6H_5	-	-	-	-
	3	Piperidino		-	-	-	-
	4	4-Methyl piperidino		-	++	-	-
	5	3-Methyl piperidino		-	++	-	-
	6	2-Methyl piperidino		-	-	-	-
	7	Morpholino		-	++	-	-
	8	N-Phenyl piperazino		-	-	-	-

REFERENCES

1 Salle, S.J.McGraw Hall, Fundamental Principles of Bacteriology, New Delhi, (1967).

2. Pelczar and Reid; Microbiology, McGraw Hill, New Delhi (1965).

3 Martin Frobisher; Fundamental Microbiology, Saurders and Co., Japan (1968).

4. Frobisher, M; "Fundamental Microbiology, (8th ed.) W.B.Saunder Company, Philadelphia.

5 J.W.Churchman; J.Exptl.Med.**16**, 221 (1912).

6. C.H. Browing and W.Gilmor; J.Pathol,Bacteriol., **18**, 144 (1913).

7. A.Albert et al. Brit.J.Expt.pathol., **34**, 119 (1953).

8. J.B.Sprowla and C.F.Poc., J.Am.Pharm.Assoc., **32**, 4 (1943).

9 M.Frobisher; Fundamentals of Microbiology, 8th ed., P.295, W.B.Saunder Co., Philadelphia.

10. S.D. Elerk and G.R.F. Hilson; J.Clin, Path, **1**, 37 (1954).

11. J.C.Gould and J.H. Bowia; Edinb. J., **59**, 178 (1952).

12 H.M. Ericsson; Acta.Path.Microbiol., Scant.Sect. B.supply-217 (1971).

13. W.M.M.Kirby and A.W.Bauer et al., Amer J. Clin.Path., **45**, 493 (1966).

14. E.Strokes; Clinical Bacteriology, 3rd ed. Edward Arnold London (1968).

15. D.Fleminhan et al., Med.Lab.Technol., **29**, 198 (1972).