C H A P T E R - I I I

STUDY OF ANTIBACTERIAL ACTIVITY

CHAPTER - III

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3.1 INTRODUCTION :

The compounds the synthesis of which is reported were tested for their antibacterial activity against Bacillus-megaterium (Gram Positive) and E. Coli (Gram Negative) micro-organism. These cultures were obtained from Haffkins institute, Bombay and were subcultured on nutrient agar medium once a month.

Cup-plates method was followed for the evaluation of the antibacterial activity of compounds. The principle lying in this testing is the sensitivity of micro-organism to organic compounds by diffusion through agar medium. The compounds were dissolved in redistilled acetone with concentration 5 mg. per ml.

3.2 CUP-PLATE METHOD :

The materials which used in this experiment are as follows :

i) Nutrient agar

ii) 18-24 hours old growth culture in nutrient broth

iii) Sterile pipettes

iv) Sterile test tube containing solution of test compounds (5 mg/ml.)

Nutrient agar was made as :

Peptone (1 gm.), Sodium chloride (0.5 gm), meat extract (0.3 gm), Water (distilled) (100 ml.), pH (7.2).

3.3 EXPERIMENTAL PROCEDURE OF CUP-PLATE METHOD :

A uniform suspension of test organism of 24 hours old culture is prepared in test tube containing sterile saline water. One ml. of this suspension is added in each sterile petridishes aseptically. A sterile nutrient agar medium was melted under pressure and moderately cooled agar is then poured in each petridish. The dishes are roteted to ensure the uniform mixing of micro-organism in the agar medium which is then allowed to solidify.

The agar cups were prepared with sterile cork borer with suitable dimension. The solution of each compound to be tested for antibacterial activity is added by sterile pipette aseptically in to each cup. The controll solvent used was acetone. The plates were incubated at 37^{o}C for 24 hours. After incubation the inhibitory zones around the agar cups were observed the diameter of inhibition in mm. Thus the antibacterial activity observed and results are tabulated in Table No.1.

Table 1

3.4 ANTIBACTERIAL SCREENING RESULTS OF VARIOUS COMPOUNDS

Sr. No.	Name of the Compounds	Bacillus megaterium (Gram positive)	E. Coli (Gram negative)
IIIa	2-Lepidyl-p-aminobenzene Sulfonamide	+ + +	+ + +
IIIb	2-Lepidyl-p-amino-o-toludo Sulfonamide	+ +	+ +
IIIc	2-Lepidyl-p-amino-m-toludo Sulfonamide	+	+
IVa	2-Lepidyl-p-acetamido-benzene Sulfonamide	+ + +	+ + +
IVb	2-Lepidyl-p-acetamido-o-toludo Sulfonamide	+ + +	+ +
IVc	2-Lepidyl-p-acetamido-m-toludo Sulfonamide	+ +	+
IVd	2-Lepidyl-p-toluene Sulfonamide	+	+ +
Va	2-Lepidyl-benzene-p-Sulfonamide	+	+ +
Vb	2-Lepidyl-O-toludo-p-Sulfonamide	+	+
Vc	2-Lepidyl-m-toludo-p-Sulfonamide	+	+

Zone of inhibition

+++ Strong growth inhibitor (zone size 32 - 35 mm)

++ Moderate growth inhibitor (zone size 29 - 31 mm)

+ Less growth inhibitor (zone size 25 - 28 mm)

3.5 DISCUSSION :

All the compounds the synthesis of which is reported earlier tested for their antibacterial activity against bacillus-megaterium (Gram positive) and E.Coli (Gram negative) bacteria. It is observed from the result (Table No.1) that all these lepidine sulfonamides show moderate to strong antibacterial activity against gram positive and gram negative bacteria without exception.

The strong activity has been observed for compounds 2-Lepidyl-p-amino-benzone sulfonamide IIIa and 2-Lepidyl-p-acetamidobenzene sulfonamide IVa, against both gram positive and gram negative bacteria. Where as 2-Lepidyl-p-anino-o-toludo Sulfonamide IIIb, and 2-Lepidyl-p-acetamido-m-toludo Sulfonamide IVc show moderate antibacterial activity against gram positive bacteria.

The compound 2-Lepidyl-p-acetamido-o-toludide Sulfonamide (IVb) shows very strong antibacterial activity against gram positive bacteria but moderate activity against gram negative bacteria.

Compounds 2-Lepidyl-p-toluene Sulfonamide IVd and 2-Lepidyl benzene-p-Sulfonamide Va show moderate antibacterial activity against gram negative bacteria.

The 2-Lepidyl-Sulfonamide compounds such as 2-Lepidyl-p-amino-m-toludo-sulfonamide (IIIc), and 2-Lepidyl-o-toludo-p-Sulfonamide (Vb), 2-Lepidyl-m-toludo-p-Sulfonamide (Vc) show less than moderate activity against both gram positive (Bacillus megaterium) and gram negative (E.Coli) bacteria. The compound IVc show less antibacterial activity against gram negative bacteria, which have been tested in laboratory.

3.6 CONCLUSION :

The results show that when sulfonamide group ($-SO_2-NH-$) is involved in condensation the antibacterial activity is very strong towards gram positive (Bacillus megaterium) and gram negative (E.Coli) bacteria. But when ($-NH-$) group is involved in the linking it results in to less active products towards both gram positive and gram negative bacteria. Also it is observed from Table No. 1 that any substituent in the benzene ring close to Sulfonamide group decreases the antibacterial activity of the original compound i.e. 2-Lepidyl-p-aminobenzene sulfonamide (IIIa) is a strong growth inhibitor than the 2-Lepidyl-p-amino-o-toludo sulfonamide (IIIb) in which $-CH_3$ group is at meta to ($-SO_2-NH_2-$) which in turn is a more growth inhibitor than that of 2-Lepidyl-p-amino-m-toludo-Sulfonamide IIIc.

Also it has been observed that when -NH- involved in condensation between 2-Lepidyl and Sulfanilamide moiety the antibacterial activity of the products is found minimum

towards both gram negative (E.Coli) and gram positive (Bacillus megaterium) bacteria. Thus all these 2-Lepidyl Sulfonamides show antibacterial activity against gram positive and gram negative ranging from very strong to moderate and therefore, very useful in medicine as drugs.

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