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Review Article

Antimicrobial Activity of Chloro, Nitro, Methyl Substituted Schiff's Bases

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ABSTRACT

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. The compounds were screened for their antimicrobial activity against bacteria like S. aureus, E. coli, S. typhi, S. paratyphi and P. vulgaris by using agar disc diffusion method. The Minimum Inhibitory Concentration (MIC) values of the synthesized compounds were also calculated by serial dilution method.

INTRODUCTION

An 'antimicrobial' is a substance that kills or inhibits the growth of microorganisms[1] such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substance used on non-living objects or outside the body. Antimicrobial chemotherapy has been an important medicinal treatment since the first investigations of antibacterial dyes by Ehrlich in the beginning of the twentieth century. However, by the late 1940s bacteria resistant to antimicrobials were soon recognized as a serious problem in clinical environments, such as hospitals and care facility. (Martin 1998). The discovery of antibacterials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials. However, with the development of antibacterials, microorganisms have adapted and become resistant to previous

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antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change and become resistant to the poisons and/or heavy metals. Antimicrobials are used worldwide in human medicine, food, agriculture, livestock and household products. In many cases the use of antibiotics is unnecessary or questionable. Consumption of antibiotic is linked to bacterial resistance. In hospitals, most common resistant bacteria include methicillin-resistant enterococci and gram negative rods, including the *Enterobacteriaceal* and *Pseudomonas aeruginosa*. (Beovice 2006). Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low.

There are mainly two classes of antimicrobial drugs.

1. Those obtained from natural sources

Beta-Lactam antibiotic (such as Penicillin, Cephalosphorins)

Protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides)

2. Synthetic Agents

Sulphonamides, Cotrimoxazole, Quinolones

Anti-virals

Anti-fungals

Anti-cancer drugs

Anti-malarials

Anti-tuberculosis drugs

Anti-leprotics

Anti-protozoals

Literature survey reveals that the heterocycles like pyrazoles, pyrimidines, thiazoles, thiazolines, chalcones, coumarones etc. show good antimicrobial activity.[2-5] Cleiton M. da Silva et al [6] studied the short review compiles examples of the most promising antimalarial, antibacterial, antifungal and antiviral Schiff bases. Aliasghare Jorrahpour et al[7] studied the synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-schiff bases of isatin and their derivatives. Bagihalli et al [8] synthesized some substituted coumarins and their complexes with Co(II), Ni(II) and Cu(II) and studied the antimicrobial activity against *E. coli, S. aureus, S. pyogenes* and *P. aeruginosa* by MIC method. Antimicrobial activity of oleanolic acid from salvia officinalis and related compounds on Vancomycin-Resistant Enterococci (VRE) [9].

Present Work:

The review of literature survey clearly mentioned that the chloro, nitro, methyl substituted benzaldehyde have medicinal, biological, pharmacological, industrial and agricultural values but very less work has been carried out on the derivatives of substituted benzaldehyde groups. Hence, it was thought interesting to study antimicrobial activity of chloro, nitro, methyl substituted Schiff's bases against pathogenic microorganisms and help to find better alternative against drug resistant pathogenic microorganism. The work presented in this chapter deals with the study of antimicrobial activity of chloro, nitro, methyl substituted Schiff's bases synthesized by us in Part-I against different test organisms namely, *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Proteus vulgaris, Staphylococcus aureus* to cover antibacterial and antifungal class.

1] Escherichia coli :- A gram negative bacillus. E. coli are parasites occurs in the lower portion of the intestine of human and animals, causative agent of gastroenteritis, urinary tract infections.

2] Salmonella typhi :- A gram negative causative agent of typhoid.

3] Salmonella paratyphi:- A gram negative causative agent of paratyphoid.

4] Proteus vulgaris:- A gram negative commensal in intestine and present on skin.

5] Staphylococcus Aureus :- A gram positive coccus, causing skin infections like pimples and wound infections.

The following compounds were tested.

1. 4-Nitrophenylidiene-4'-aniline

2. 4-Nitrophenylidiene-4'-nitroaniline

3. 4-Chlorophenylidiene-4'-aniline

4. 4-Chlorophenylidiene-4'-nitroaniline

5. 4-Methoxyphenylidiene-4'-aniline

6. 4-Methoxyphenylidiene-4'-nitroaniline

7. 4-Dimethylphenylidiene-4'-aniline

8. 4-Dimethylphenylidiene-4'-nitroaniline

EXPERIMENTAL

The antibacterial activities of all the eight ligands synthesized were tested to evaluate their efficiencies against animal pathogenic organisms. All the chemicals and media were purchased from M/s. Hi-Media Pvt. Ltd., Mumbai, India. The Department of Biotechnology, Brijlal Biyani Science College, Amravati was kind enough to carry out the antimicrobial activity studies. The organisms used were *E. coli, S. typhi, S. paratyphi, P. vulgaris* and *S. aureus*.

For the evaluation of in-vitro antimicrobial activity, the following three conditions must be fulfilled.

i) First the substance to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated.

ii) Secondly, favourable conditions (nutritional, environmental etc.) must provided to offer a maximum opportunity for optimum growth of the organisms in absence of antimicrobial agent, and

iii) Thirdly, there should be a method for measuring antibacterial response obtained by antimicrobial agent.[10]

Various methods have been proposed and adopted for the measurement of antibacterial activity[11], these are,

1] Agar streak dilution method

2] Agar diffusion (cup, paper disc, cylinder) method

3] Turbidometric method

4] Serial dilution method

5] Specific method (specific for measuring the action of specific sbstance)

In the present research work, agar disc diffusion method was used to find out the activity of all synthesized compounds against the microbes. The Minimum Inhibitory Concentrations (MIC) were measured by serial dilution method.

A] Media Used:

1. Nutrient Agar Medium:- The agar medium consist of -

Beef extract -- 1.5 gms

Yeast extract -- 1.5 gms

Peptone -- 5.0 gms

NaCl -- 5.0 gms

Agar powder -- 20.0 gms

Distilled water-- 1000 ml

pH -- 7.4 ± 0.2 (at 25°C)

2. Nutrient Broth Medium :- The broth medium consist of -

Yeast extract -- 1.5 gms

Beef extract -- 1.5 gms

Peptone -- 5.0 gms

NaCl -- 5.0 gms

Distilled water-- 1000 ml

Ph -- 7.4 ± 0.2 (at 25°C)

Both the above cited media used were of bacteriostatic grade. Above media were found to be suitable for the growth of all four organisms used in the present work.

B] Slant Preparation:

Nutrient agar medium was dissolved in distilled water and was sterlized by autoclaving. About 5 ml of molten media was transferred aseptically in previously sterilized test tubes. The test tubes were then plugged tightly and placed in a slanting position to cool and solidify.

C] Stock Culture:

Culture was grown on nutrient agar slants by incubating them for 24 hrs at 37°C.

D] Culture Dilution:

One loopful of stock culture was added to 5 ml of nutrient broth medium for inoculation. The inoculated broth was incubated for 24 hrs at 37°C. For all experimental purposes 24 hrs fresh diluted culture of the organisms were used.

E] Preparation of Sample Solution:

To study the antimicrobial activity the dilutions of the compounds used in the present study were prepared by using 1,4-dioxane solvent. To check the potency of compounds, the solutions were prepared with 500 mg/ml concentration. 1 ml of this solution was added to 5 ml of nutrient broth solution containing organism to be tested. Test tubes with organism and medium with 1,4-dioxane, were used as controls. These test tubes were kept for incubation at 37°C for 24 hrs. The solutions of standard drugs were prepared in distilled water.

F] Disk Diffusion Method:-

The disk diffusion method is also known as Kirby-Bauer disk diffusion method. In this method, every time fresh sterile nutrient agar medium was prepared. The proceedings were carried out aseptically. All the glassware and apparatus required were sterilized. In each sterile petridish, 15-20 ml of molten medium was added. It was allowed to solidify at room temperature. A sterile cotton swab was dipped into 24 hrs fresh diluted culture of organism under study and the inoculum was spread evenly over the entire surface of petriplate by swabbing in three directions. Then 6 mm discs of sterlized Whatmann filter paper No. 42 was moistened throughly with the same

concentration of each of the compound and with the standard drug solution also. The moist discs were placed on the surface of inoculated plate. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24 hrs. The diameter of inhibition zone was observed and measured with the help of ruler.[38]

G] Serial Dilution Method [12,13]:

The following procedure was followed in serial dilution method to determine the MIC of various compounds. Nutrient broth was prepared by dissolving 13 gms of dehydrated medium in 1 litre of distilled water. The pH of the medium was adjusted to 7.4. The 5 ml of the medium was distributed in each test tube. All the test tubes were sterilized at 121°C for 20 min. The 0.01 M solution of the test compounds were prepared in 1,4-dioxane solvent. Various amounts of the above stock solution was aseptically added to the various nutrient broth test tubes (viz. 0.5, 1.0, 1.2, 1.4, 1.6, 1.8, ... 5.8, 6.0 ml). Fresh culture of the test bacterium was inoculated in each test tube (0.2 ml culture). All the test tubes were incubated at 37°C for 24 hrs. Uninoculated test tube was kept as a control in which nutrient broth and 5 ml of the solvent was taken. After 24 hrs of incubation, all the test tubes were observed for MIC against test bacterium.

RESULTS AND DISCUSSION

The five microorganisms studied are disease causing microbials. The synthesized compounds showed the remarkable antibacterial activities. For testing the antimicrobial activity the compounds were assayed against *E. coli, S. typhi, S. paratyphi, P. vulgaris* and *S. aureus*. For determining the MIC value, all these compounds were dissolved in ethanol, the MIC value between 200-1000 □g/ml. such compounds are active in inhibiting the growth of organism tested. Generally, less is the concentration more is the active compound. The Minimum Inhibitory Concentration values (MIC values) were determined by serial dilution method.[14] The comparative study of MIC values of the compound is given in Table-1.

The following Schiff's bases were prepared :-

1. 4-Nitrophenylidiene-4'-aniline (A_1)

2. 4-Nitrophenylidiene-4'-nitroaniline (A_2)

3. 4-Chlorophenylidiene-4'-aniline (B_1)

4. 4-Chlorophenylidiene-4'-nitroaniline (B_2)

MIC values of Schiff's bases in µg/ml

Table – 1

Sr. No.	Compd. No.	*E. coli*	*S. typhi*	*S. paratyphi*	*P. vulgaris*	*S. aureus*
1.	A_1	1400	700	800	840	860
2.	A_2	800	600	650	650	700
3.	B_1	1650	1000	1080	1100	1040
4.	B_2	1000	980	980	1040	1000
5.	C_1	2200	2900	1850	1800	1800
6.	C_2	1550	1400	1460	1480	1450
7.	D_1	1000	1000	1060	1100	1140
8.	D_2	1200	1280	1400	1450	1500

From the Table-1, it is clear that (A_1) ranges from 700-1400 □g/ml, the MIC values of (A_2) ranges from 600-800 □g/ml. The Schiff base is active because of nitro group. The MIC value of (B_1)

ranges from 1000-1650 □g/ml, the MIC value of (B_2) ranges from 980-1040 □g/ml. The Schiff base (B_1) and (B_2) are good inhibitory active due to the presence of one chloro and one nitro group in their structure respectively. In short nitro group and chloro group are electron withdrawing groups. The nitro group is more electron withdrawing than chloro group, so that the compounds having such groups in their structure have more inhibiting active. The MIC value of (C_1) ranges from 1800-2900 □g/ml, the MIC value of (C_2) ranges from 1400-1550 □g/ml, (C_2) is more active as compared to (C_1). (C_1) is less reactive because of the presence of methyl substituent in its structure, □CH_3 group is electron donating group. The MIC value of (D_1) ranges from 1000-1140 □g/ml, the MIC value of (D_2) ranges from 1200-1500 □g/ml. From the result it has been observed that the presence of nitro group increases the activity and increase in activity is also related to the number of nitro groups. However, if chloro group is introduced in the structure, the increase in activity is more.

The order was found to be NO_2, NO_2 > NO_2Cl > NO_2.

Also the presence of methyl group decrease the activity. As number of methyl group increases the decrease in activity is more. This may be due to the electron withdrawing nature of □NO_2 group and electron donating nature of □CH_3 group. Thus, the relationship between the structure and activity of the compounds are established.

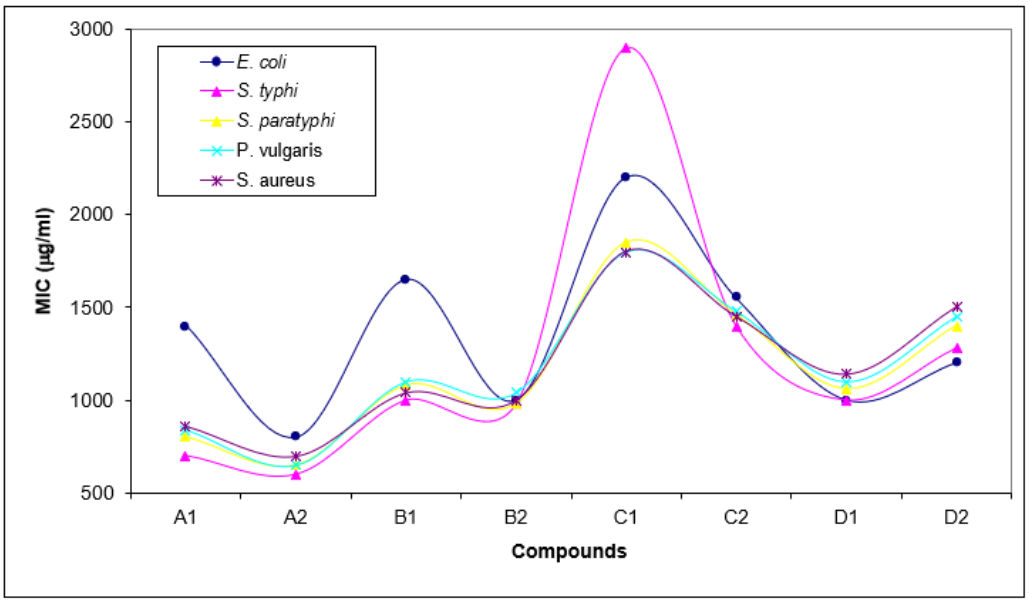

Figure - 1
MIC Values of Schiff bases in □g/ml

REFERENCES

1. 'Antimicrobial' - Definition from the Merriam Webster Online Dictionary (2009).
2. Prasanna D.S., Kavitha C.V., Raghava B., Vinaya K. and Rangappa K.S.: Investigational New Drugs, 28(4), 454 (2010).
3. Hussie M.A., Kafafy A.H.N. and Moty A.S.G.: Acta Pharm., 59, 365 (2009).
4. Solankee A., Kapadia K., Ciric A. and Geronikaki A.: European J. Medicinal Chemistry, 45(2), 510 (2010).
5. Aswale S.S. : Ph.D. Thesis, submitted to S.G.B. Amravati University, Amravati (2006).

6. Cleiton M. da Silva, Daniel L. da Silva, Luzia V. Modolo, Rosemeire B. Alves, Maria A. de Resende, Cleide V.B. Martins, Angelo de Fatima: J. Advanced Research, 2(1), 1-8 (Jan. 2011).
7. Jarrahpour Aliasghar, Khalili Dariush, Salmi Erik, Brunel Jean Michel : Molecules, 12(8), 1720-1730 (2007).
8. Bagihalli G.B., Badami P.S. and Patil S.A.: J. Enzyme Inhibition and Medicinal Chemistry, 24(2), 381 (2009).
9. Peach and Tracy: Berlin, III, 639 (1955).
10. Ananthanarayan R. and Panikar C.K.J. : "Textbook of Microbiology", 6th Ed., Orient Longmann, Hyderabad (2003).
11. Cappuceino J.C. and Sherman N.: "Microbiology : A Laboratory Manual", Weslex Publishing Co. (1983).
12. Aneja K.R.: "Experiments in Microbiology, Plant Pathology and Biotechnology", New Age Publishers, 69 (2005).
13. Booth C.: "Extremophiles, Methods in Microbiology", Academic Press, 543 (2006).
14. Donald C.D. and William A.R. : "An Essay Method of Antibiotics: A Laboratory Manual", Medical Encyclopedia Inc. (1995).

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