INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article……!!!

Received; accepted

ANTIBACTERIAL STUDIES OF SCHIFF BASE COMPLEXES AND POLYMER SUPPORTED SCHIFF BASE COMPLEXES

Raja Sidambaram R[1]*, Jisha J[2] and Mary NL[2]

1. Department of Medical Laboratory Technology, Stella Maris College (Autonomous), Chennai, Tamil Nadu, India
2. Department of Chemistry, Stella Maris College (Autonomous), Chennai, Tamil Nadu, India

Keywords:
Antibacterial activity, disc diffusion, MIC, Schiff bases

For Correspondence:

Raja Sidambaram R.

Department of Medical Laboratory Technology, Stella Maris College (Autonomous), Chennai, Tamil Nadu, India

E-mail:
rajasidambaram@gmail.com

ABSTRACT

With increased microbial resistance to antibiotics from natural sources, we are bound to look for synthetic chemicals with antibacterial activity. Many such chemotherapeutic agents such as norfloxacin, ciprofloxacin and others are widely used. Ten Schiff base metal ion complexes derived from salicylidene thiosemicarbazone and 5-bromosalicylidene thiosemicarbazone (SALTSC and 5-BrSALTSC) were synthesized, characterized and screened for their *in vitro* antibacterial activity against common pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*. The sensitivity of the compounds tested both by disc diffusion and tube dilution techniques. Among these compounds 5-BrCuSALTSC was found to be highly active against *S.aureus* at a concentration of 100µg/disc and against *E.coli* at a concentration of 150µg/disc. 5-BrNiSALTSC was found to be highly active against *E.coli* at a concentration of 150µg/disc. These compounds, if found to be non-toxic to human and animal tissues, can be used for treating human infections. They may also be used at higher concentrations in veterinary medicine.

INTRODUCTION

Many synthetic Schiff bases have been reported possessing antibacterial [1-7], antifungal [8-10], antimicrobial [11-13] and antiviral [14, 15] activities. Also certain polymeric Schiff bases have been found to possess antitumor activity. The Schiff bases have the highest degree of hydrolysis at pH 5 and the solubility in water is also high at this pH. Antitumor activity of the Schiff bases towards acidic tumours increases considerably with the slight increase in water solubility [16].

Schiff bases are important intermediates for the synthesis of some bioactive compounds such as beta-lactams. The base catalysed condensation of acetyl chlorides with N-arylaldimines occurs by initial acylation at the nitrogen atom and leads to β-lactams of interest in penicillin chemistry [17].

It has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases [18]. The compounds having antimicrobial activity may act either by killing the microbe or by inhibiting multiplication of the microbe by blocking their active sites [19]. Schiff bases derived from salicylaldehydes are well known as polydentate ligands, coordinating as deprotonated or neutral forms [20]. Keeping in view the promising use of potentially metal-based antibacterial therapy that has provoked wide interest into this diversified area, we, therefore, wish to report here some metal-based [Cu(II), Ni(II), Zn(II), Co(II) and Cd(II)] complexes incorporated with salicylidene thiosemicarbazone and 5-bromosalicylidene thiosemicarbazone and their *in vitro* antibacterial activity. To compare the antibacterial activity we have synthesized polymer supported Schiff base complexes derived from styrene acrylonitrile (SAN) copolymer and 5-bromosalicylidene thiosemicarbazone.

MATERIALS AND METHODS

Preparation of Schiff base complexes [21, 22]

A mixture of 0.735ml of salicylaldehyde (0.01M) and 0.0919g of thiosemicarbazide (0.01M) dissolved in ethanol refluxed for two hours on a water bath till a clear solution was obtained. It was allowed to cool and stirred using a magnetic stirrer. The Schiff base separated out was filtered and recrystallised from hot ethanolic solution. The Melting Point of synthesized Schiff base was 203^0C. 5-bromosalicylaldene thiosemicarbazone was prepared by combining brominated salicylaldehyde (0.01M) and thiosemicarbazide (0.01M).

Salicylaldene thiosemicarbazone/ 5-bromosalicylaldene thiosemicarbazone (0.01 M) was mixed with corresponding metal ion (0.01M) in ethanol. The resulting solution was refluxed for two hours. Sodium acetate was also added to control the pH. The refluxed solution was cooled and the complex filtered. The filtered complex washed several times with ethanol and dried over anhydrous calcium chloride in a desiccator. The polymeric supported Schiff base complexes were synthesized by using styrene acrylonitrile (SAN) copolymer with 5-bromosalicylidene thiosemicarbazone according to the literature method [23, 24].

Antibacterial studies

The synthesized Schiff base ligand, metal ion complex and polymer supported complexes were screened *in vitro* for their antibacterial activity against one Gram-positive, *Staphylococcus aureus and* two Gram-negative, *Escherichia coli and Klebsiella pneumoniae bacterial* strains by Kirby-Bauer disc diffusion method [25] at lower concentration of 10µg/disc, 20µg/disc and 30µg/disc. Among the ten complexes five of them which were moderately or slightly active were tested again at a concentration of 50µg/disc, 100µg/disc and 150µg/disc against *S.aureus* and *E.coli.* The minimum inhibitory concentration (MIC) of these five complexes was also determined by tube dilution method.

Disc diffusion method: When an antibiotic-impregnated disc is placed on a solid growth medium previously inoculated with the test bacterium, the disc picks up moisture and the antibiotic diffuses radially outward through the agar, producing an antibiotic concentration gradient. The antibiotic is present at high concentrations near the disc and affects even minimally susceptible microorganisms while resistant organisms will grow up to the disc. As the distance from the disc increases, the antibiotic concentration drops and only more susceptible pathogens are inhibited. A clear zone or ring is present around an antibiotic disc after incubation if the agent inhibits bacterial growth.

With a sterile inoculation loop four or five discrete colonies of the pathogen grown on nutrient agar medium, was picked and suspended in a tube of Tryptone Soya broth, to have a bacterial concentration equivalent to 0.5 McFarland standard. A sterile cotton swab was dipped in the standardized bacterial test suspension and used to evenly inoculate the entire surface of a plate of Mueller-Hinton agar. After the agar surface has dried for about 5 minutes, the appropriate antibiotic test discs were placed on it with sterilized forceps or a needle. The plate was incubated aerobically in an incubator at 37^0C. After 24 h of incubation, the diameter of each zone of inhibition measured to the nearest mm.

Tube dilution method: In broth dilution test, a series of Tryptone Soya broth tubes containing chemical concentrations in the range of 10 to 100µg/tube was prepared and inoculated with standard volume of test bacterial suspension in sterile saline. The lowest concentration of the antibiotic resulting in no growth after 16 to 20 h of incubation is the MIC of that compound.

RESULTS

The antibacterial activity of ten Schiff base complexes was screened *in vitro* against two Gram-negative *(E.coli* and *K.pneumoniae)* and one Gram-positive *(S.aureus)* bacterial strains by using the solvents Dimethyl formamide (DMF) and Dimethyl sulphoxide (DMSO) as controls. The complexes either exhibited slight or no inhibitory effect on the growth of different tested strains. The effect of compounds which were taken in a concentration of 10µg/disc, 20µg/disc and 30µg/disc on the growth of tested bacteria is given in Table 1. From the data it was found out that the polymeric supported samples were inactive against *S. aureus* and *E. coli* and were slightly active against K. *Pneumoniae.*

Table 1- Effect of Schiff bases on the growth of tested bacteria at lower concentrations.

Complexes	**Concentration-10µg/disc**			**Concentration-20µg/disc**			**Concentration-30µg/disc**		
	S.A	*K.P*	*E.C*	*S.A*	*K.P*	*E.C*	*S.A*	*K.P*	*E.C*
SALTSC	+	+	+	+	+	+	+	+	+
CuSALTSC	++	+	+	++	+	+	+	++	++
5BrCuSALTSC	+	+	+	+	+	+	++	++	+
NiSALTSC	+	+	+	+	+	+	+	++	+
5-BrNiSALTSC	+	+	+	+	+	+	+	+	+
ZnSALTSC	+	+	+	+	+	+	+	-	+
5BrCuSALTSC-SAN	-	+	-	-	+	-	+	-	+
5BrNiSALTSC-SAN	-	+	-	-	+	-	-	++	-
5BrCoSALTSC-SAN	-	+	-	-	+	-	-	+	-
5-BrCdSALTSC-SAN	-	-	-	-	+	-	-	+	-

Key to symbols:

Moderately active = ++ (inhibition zone 9-12mm)

Slightly active = + (inhibition zone 6-9mm)

Inactive = - (inhibition zone < 6 mm)

Abbreviations:

S.A= *Staphylococcus aureus*

K.P= *Klebsiella pneumoniae*

E.C= *Escherichia coli*

Table 2- Effect of Schiff bases on the growth of tested bacteria at higher concentrations.

Complex	Concentration of complex-50µg/disc		Concentration of complex-100µg/disc		Concentration of complex-150µg/disc	
	S.aureus	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*
SALTSC	+	++	+	-	+	-
CuSALTSC	+	++	++	++	+	++
5BrCuSALTSC	++	+	+++	++	+++	+++
NiSALTSC	+	-	+	-	+	+
5BrNiSALTSC	+	+	++	++	+++	-

Key to symbols:

Highly active = +++ (inhibition zone >12mm)

Moderately active = ++ (inhibition zone 9-12mm)

Slightly active = + (inhibition zone 6-9mm)

Insignificant = - (inhibition zone < 6 mm)

Table 3- The minimum inhibitory concentration of Schiff base complexes on the growth of tested bacteria.

Schiff base complex	MIC for *S.aureus*	MIC for *E.coli*	MIC for *K.pneumoniae*
SALTSC	>100µg	>100µg	60µg
CuSALTSC	>100µg	>100µg	60µg
5-BrCuSALTSC	>100µg	>100µg	60µg
NiSALTSC	>100µg	>100µg	60µg
5-BrNiSALTSC	>100µg	>100µg	>100µg

In the next stage we selected five complexes and tested their activity against *S.aureus* and *E.coli* at a concentration of 50µg/disc, 100µg/disc and 150µg/disc. The test results are given in Table 2. The compounds taken in 50µg/disc showed growth inhibition of S. aureus, but only with 5-BrCUSALTSC the growth inhibition was significant. *E.coli* showed significant growth inhibition with compounds SALTSC and CuSALTSC. The compounds taken in 100µg/disc showed growth inhibition of *S.aureus.* But compounds CuSALTSC, 5-BrCuSALTSC and 5-BrNiSALTSC showed significant growth inhibition. Both CuSALTSC and 5-BrCuSALTSC exhibited growth inhibition of *E.Coli.* (=12mm).

The compounds 5-BrCuSALTSC and 5-BrNiSALTSC (150µg/disc) showed better inhibition of 13mm and 14mm respectively. CuSALTSC, 5-BrCuSALTSC and NiSALTSC showed growth inhibition of *E.coli.*

The broth tube dilution method was used to determine the minimum inhibitory concentration (MIC). The culture that showed no growth in the presence of the lowest concentration of the complex represents the MIC of the complex against the test bacteria. The MIC of the compounds is given in Table 3.

DISCUSSION

It is evident from the above data that the antibacterial activity significantly increased on coordination. This enhancement in the activity may be rationalised on the basis of their structures mainly possessing an additional azomethine bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system. This process, in turn, increases the lipophilic nature of the central metal atom. This favours its permeation more efficiently through the lipid layer of the microorganism thus making the chelate compounds bactericidal.

The scope for future research in this field is to subject the synthetic compounds to *in vitro* cytotoxic studies on normal and cancer cell lines. If found toxic only for cancer cells, the compounds can be subjected to *in vivo* animal studies to establish their chemotherapeutic efficacy. With positive animal test results, the compounds can be used in Human clinical trial studies and such an effort may pave way for new drug developments for antimicrobial and chemotherapeutic use.

REFERENCES

1. Sari N, Arslan S, Logoglis E. & Sakiyan I., Antibacterial activities of some new amino acid Schiff bases, *G.U. J. Science,* 2003; 16: (2) 283-288.

2. Karia F D, Parsania P H., Synthesis, biological and thermal properties of Schiff bases of bisphenol, C. *Asian J. Chem,*1999. 11: (3) 991-995.

3. El-Masry A H, Fahmy H H, Wahed SHA., Synthesis and antimicrobial activity of some new benzimidazole derivatives, *Molecules,* 2000; 5 :(12) 1429-1438.

4. Baseer M A, Jadhav V D, Phule R M, Archana Y V, Vibhute Y B.,Synthesis and antibacterial activity of some new Schiff bases,*Orient. J. Chem.*,2000;16:(3) 553-556.
5. Amir M, Hasan S M, Wadood A., Synthesis and antibacterial activity of 1-isonicotinyl-3-methyl-4-(substituted phenyl hydrazono)-2-pyrazolin-5-ones, *Orient.J.Chem,* 2002; *18*: 351-353.

6. Sridhar S K, Saravanan M, Ramesh A., Eur. *J.Med and Chem., 2001; 3: 615.*

7. Rathod AS., *Orient J. Chem,* 2000; 16: 549.

8. Singh W M, Dash BC., Synthesis of some new Schiff bases containing thiazole and oxazole nuclei and their fungicidal activity, *Pesticides,* 1988; 22: (11) 33-37.

9. Rajendran S P, Karvembu R., Synthesis and antifungal activities of Schiff bases derived from 3-amino-2H-pyrano[2,3]-quinolin-2 ones., *Ind. J. Chem.,* 2002; *41B,* 222-224.

10. Calis U, Yarim M, Kokasal M, Ozalp M., Synthesis and antimicrobial activity evaluation of some new adamantane derivatives**,** *Arzneimittel-Forschiney,* 2002; 52: (10) 778-81.

11. Pandeya S N, Sriram D, Nath G., Synthesis and antimicrobial activity of Schiff and Mannich bases of isatin and its derivatives with pyrimidine, *Farmaco,*1999; 54: 624-628.

12. Shaikh K A, Baseer M A, Mote N A., Asian J. Chem., 2001; 13: 496.

13. Karia F D, Parsania PH, Synthesis, biological and thermal properties of Schiff bases of bisphenol, *C. Asian J. Chem.,* 1999; 11: (3) 991-995.

14. Sridhar SK, Pandaya S N, De Cleroq E., Bollettino Chimico farmaceutico, 2001; 140: 302.

15. Sridhar S K, Pandaya S N, De Cleroq E., *Chem. Abstr, 2002*; 16: 129-*136.*

16. Schmid GH. Organic Chemistry, 1996; 3rd Ed. Mosby, New York.

17. Isaacs N S., Chem. *Soc. Rev,* 1976; 5:181.

18. Phatak P, Jolly V S, Sharma K P., Synthesis and biological activities of some new substituted arylazo Schiff bases, *Orient. J. Chem.,* 2000; 16 :(3) 493-494.

19. Mishra A P., *J.Indian Chem,* 1999; Soc. 46: 3537.

20. Boyd DB., Electronic structures of cephalosporins and penicillins. Inductive effect of the 3-position side chain in cephalosporins, J.Med. *Chem,* 1984; 27: (1), 63–66.

21. Mary N L, Geetha Parameswaran., Kinetics and Mechanism of Thermal Decomposition of Schiff Base Complexes of Lanthanum and Cerium by TG Studies, *Synth. React. Inorg. Met-Org. Chem.,* 1993; 23(7), 1209-1227.

22. Ayesha D, Shivaji J, Megha R, Mazahar F., Synthesis and spectral studies of some co-ordination compounds, *Rasayan J. Chem.,* 2010; 3: 133-136.

23. Wang et al., *J. Appl. Polym. Sci,* 2000; 75: 1138-1143.

24. Dhathathreyan A, Mary N L, Radhakrishnan G, Collins S J., Langmuir and Langmuir-Blodgett, Films of Schiff Base Modified Styrene-Maleic Anhydride Copolymers, *Macromolecules*, 1996; 29: 1827-1829.

25. Lalitha M K., 2011; Manual on Antimicrobial Susceptibility Testing, Publication of Indian Association of Medical MicrobiologistsSari N, Arslan S, Logoglis E. & Sakiyan I., Antibacterial activities of some new amino acid Schiff bases, *G.U. J. Science,* 2003; 16: (2) 283-288.