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Antibacterial Activities of Some Transition Metal Schiff Base Complexes

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ABSTRACT

A new series of four transition metal complexes of a Schiff base derived from salicylaldehyde and glycine, viz. [N-salicylidene glycinato diaqua cobalt (II) dimer] $(SGCo)_2$, [N-salicylideneglycinato-di-aqua-nickel(ll)dimer] $(SGN)_2$, [N-salicylideneglycinato-aqua-copper(II)] (SGC) and [N-salicylidene glycinato diaqua zinc(II) dimer] $(SGZ)_2$ have been synthesized and characterized through a rapid, simple, and efficient methodology in excellent yield. These compounds were screened for *in vitro* antibacterial activities against six pathogenic bacteria, such as *Shigella sonnei, Escherichia coli, Bacilus subtilis, Sarcina lutea, Staphylococcus aureus and Pseudomonas arioginosa*. The antibacterial activity was determined by the disc diffusion method using DMSO as solvent. The results indicate that (SGC) compound exhibit a significant antibacterial activity, depending on the bacterial strain and $(SGCo)_2$, $(SGN)_2$ and $(SGZ)_2$ compounds show a moderate sensitivity even with higher doses. All these compounds were found to possess cytotoxic effect.

***Keywords*:** Antibacterial activity; Schiff base transition metal complexes; minimum inhibitory concentration; Brine shrimp lethality

1. INTRODUCTION

During the last few decades, increased incidence of bacterial resistance to existing drugs has become a major concern throughout the world and necessitates continuing research into new classes of antibiotics[1]. Extensive use of antibacterial drugs and their resistance against bacterial infections has led to severe health problems. Of particular concern are severe infections caused by multidrug-resistant Gram-positive pathogens, such as *Staphylococcus* species[2, 3], which has become a serious problem in hospitals and in the community. Resistance

of wide spectrum antibacterial agents has prompted discovery and modification toward new chemical agents or antibiotics with a potent, wide therapeutic window, broad spectrum activity, and new mode of action.

The extensive investigations with a new antibacterial agents or metal complexes have been reported[4-6]. The advantages of such compounds are their diverse structural aspects viz. Metal, ligands, chelating agents having various functional groups and donor atoms capable of exerting selective toxicity. Consequently special attention has been given by researchers to schiff base complexes. Chohan *et al*[7] studied the antimicrobial and toxicological activity of some mixed ligand transition metal complexes of Schiff bases. Ali *et al*[8, 9] showed that transition metal schiff base complexes are biologically active and possess pronounced anticancer properties. In the present paper, the antibacterial activities of four Schiff base complexes namely [N-salicylidene glycinato diaqua cobalt (II) dimer] $(SGCo)_2$, [N-salicylideneglycinato- di-aqua-nickel (ll) dimer] $(SGN)_2$, [N-salicylideneglycinato-aqua-copper(II)] (SGC) and [N-salicylidene glycinato diaqua zinc(II) dimer] $(SGZ)_2$ have been studied. In addition the cytotoxic effect and minimum inhibitory concentration (MIC) have also been evaluated.

2. EXPERIMENTAL

2. 1. General

All the chemicals used throughout the research work were purchased from BDH (England) and used without further purification. Solvents were distilled prior to use.

All the reagents used were of commercial grade.

2. 2. General Procedure for the Preparation of the Schiff Base complexes

The procedure was similar to that described elsewhere[10-13]. Alcoholic solutions of salicylaldehyde and glycine were mixed with saturated aqueous solution of metal acetates in 1:1:1 molar ratio. The whole mixture was refluxed for 2–3 hours when crystalline product was obtained. The crystals were separated out from the mother liquor, recrystallized several times from alcoholic solution, dried in an oven at 50 °C and finally stored in a desiccator.

2. 3. Characterization of the complexes

The synthesized compounds were characterized by IR spectra (as KBr disc by a Shimadzu FTIR, Japan) and the data obtained from elemental analysis for C, H and N by using Perkin 240C analyzer and metals by atomic absorption spectrophotometer (Shimadzu, Japan). The weight loss for the coordinated water was determined by using DTA-TG analyzer (Mettler Instrument Corp. Highstown, NJ)

2. 4. Antibacterial Screening

Antibacterial activities of the compounds were measured by observing the growth response of various microorganisms. The susceptibilities of such growth rate of microorganisms were measured *in vitro* by disc diffusion method[14].

A loop full of the given test strain was inoculated in 30 ml of nutrients broth and incubated for 24 hours in an incubator at 30 °C in order to activate the bacterial strain activity. 20 ml of the nutrients agar media was added in to 120 mm diameter petridishes.

0.1 ml of the activated strain was inoculated into the media when it reached the temperature of 37 °C.

The media was allowed to solidify. After solidification of the media, a sterilized (BBL, Cocksrvile, U.S.A) filter paper disc (3 mm diameter) for sample and standard drug *kanamycin* (30 µg/disc) disc were taken in the petridishes. The test samples were applied on the disc with the help of a micropipette in an aseptic condition, controls were run (for each bacterial strain and each solvent), where pure solvent was applied on the disc in the petridishes. The petridishes were incubated for 24 hours at 37 °C.

The inhibition zone formed by the two compounds against the particular test bacterial strain determined the antibacterial activity of the synthetic complexes. Therefore, the diameter of zones showing complete inhibition (mm) was measured and the growth inhibition was calculated with reference to positive control.

2. 4. 1. Preparation of stock solution of Test samples for antibacterial screening

Exactly 3 mg, 10 mg and 20 mg of SGC were dissolved separately in 1ml of DMSO to get concentration of 30, 100 and 200 µg/disc respectively. Similarly 20 mg, 40mg and 60 mg of $(SGN)_2$ were dissolved separately in 1ml of DMSO to get concentration of 200, 400 and 600 µg/disc respectively. Exactly 40 mg, 60 mg and 80 mg of $(SGZ)_2$ and $(SGCo)_2$ compounds were similarly dissolved in 1 ml of DMSO to get concentration of 400, 600 and 800 µg/disc respectively.

2. 5. Minimum Inhibitory Concentration (MIC)

The MIC of the test compounds were determined by serial tube dilution technique[15] against the same bacteria as used for antibacterial screening. Nutrient agar media was used for this purpose. Decreasing concentrations of test compounds were prepared in serial two fold dilution using the stock solution.

Bacterial suspension (10 µl) containing 10^7 cells/ml was inoculated into all tubes. After incubation for 24 hours at 37 °C, the test tube with no visible growth of the microorganism was taken to represent the MIC value of sample in µg/ml.

2. 6. Brine shrimp lethality bioassay

The cytotoxic effect of the test compounds were studied by method as described by Attaur Rahman *et al*[16]. Brine shrimp (Artemia salina) eggs were hatched in artificial sea water (prepared by dissolving 38 g NaCl in one liter distilled water) at room temperature under constant aeration for 48 hours.

Stock solutions of the complexes (10 mg/ml) in DMSO) were added to each vial, so that final concentration of the compounds became 0, 20, 40, 60, 80 and 100 µg/ml after diluting them to 5 ml with sea water.

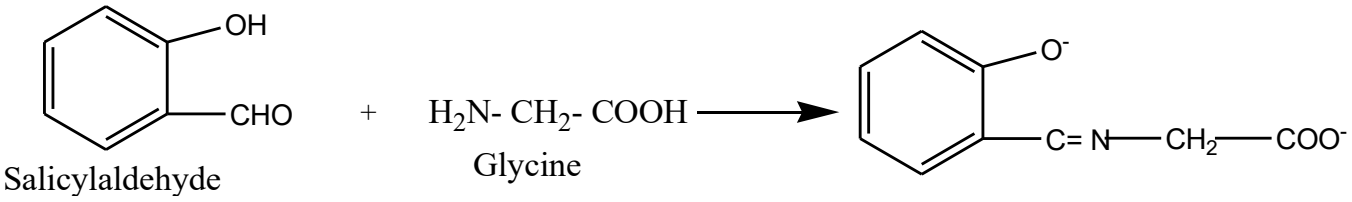
To each vials, 10 living shrimps were added and allowed to stay there for 24 hours. The survived nauplii in each vial were counted and the results were noted.

3. RESULTS AND DISCUSSION

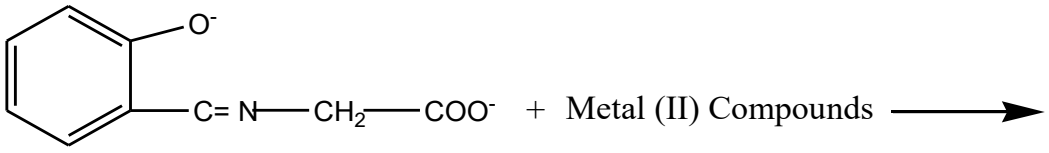
3. 1. Synthesis

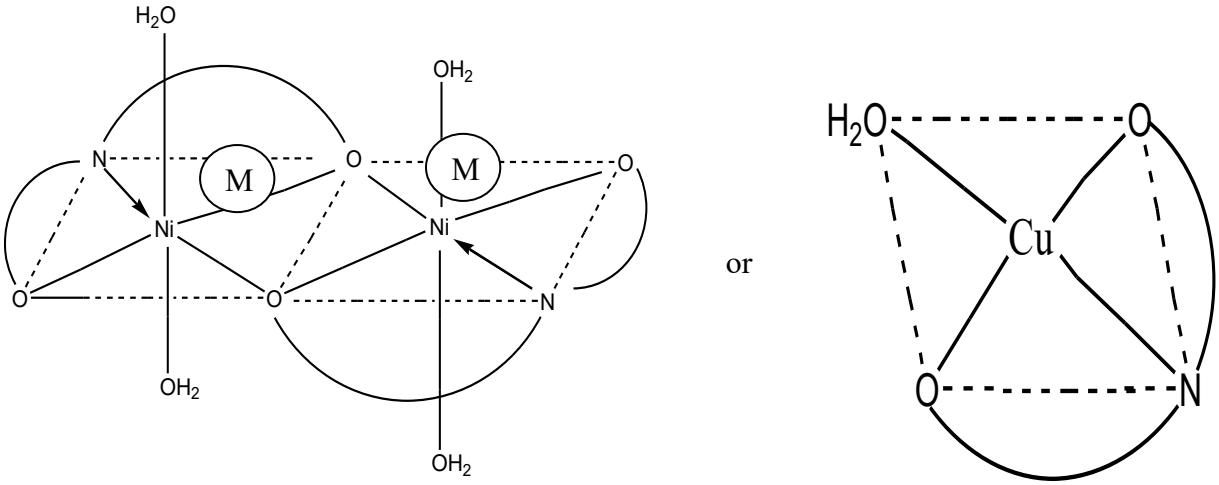
The synthetic routes of metal complexes are outlined in the following Scheme I and Scheme II. Metal complexes were prepared by condensation of Salicylaldehyde and Glycine in ethanol in good yield.

SCHEME: I



SCHEME: II

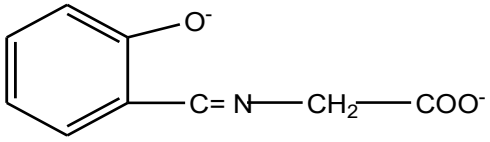




(a) (b)

Figure 1. Structure of Schiff base complexes
(a) M= Ni, Zn or Co, dimeric octahedral structure for Ni, Zn and Co complexes
(b) Square planar structure for Cu complex

Here, the skeleton **-O-N-O-** represents the following base ligand:



The purity of the final products have been verified with the help of IR spectral, elemental analytical data for the metals, carbon, hydrogen, and nitrogen content, which are in accordance with the structures shown in figure 1. The melting points of these compounds could not be measured owing to their thermal instability above 200 ºC probably due to the loss of coordinated water and also the burning of organic part of the molecule. From the TG-DTA thermogram (not shown here) the percentages of water content have been calculated corresponding to the weight loss owing to the endothermic effects between 200-220 ºC. For $(SGCo)_2$ $(SGN)_2$, (SGC) and $(SGZ)_2$ these values were found to be 12.8 %, 12.55 %, 6.6 % and 12.4 % by weight respectively. These values were very much similar to those of the theoretical values for the coordinated water contents. All the data presented in table 1 is in good agreement with the molecular structure proposed earlier[10-13].The complexes with nickel, zinc and cobalt were found to be dimeric and that with copper was monomeric.

Table 1. Physical and chemical analysis data.

Test compound	**Yield %**	**Physical form**	**Solubility**	**Elemental analytical data, %**	**IR spectra, cm^{-1}**
$(SGCo)_2$	50	Brown crystalline	Ethanol Methanol DMSO and Acetone	C=39.22(39.70) O=3.98(4.04) H=5.32(5.15) Co=21.57(21.69) H_2O=12.78(13.23)	1287s (C-N) 1691-1598w (C=N) 3516s, 3442s, 3296s (phenolic -OH) 563s (Co-N) 500 s(Co-O)
$(SGN)_2$	50	Greenish yellow crystal	Ethanol Methanol DMSO and Acetone	C=37.17(39.75) O=4.01(4.04) H=5.26(5.10) Ni=20.96(21.60) H_2O=12.55(13.25)	3600w,3400w (H_2O) 1640s(C=N) 1307sh(C-N) 560s(Ni-N) 487s(Ni-O)

(SGC)	60	Black crystal	Ethanol Methanol DMSO and Acetone	C=41.55(41.93) O=3.06(3.11) H=5.24(5.44) Cu=24.33(24.67) H_2O=6.55(6.98)	1323s,1303s (C-N) 1629s,1602s (C=N) 3566sh,3550w (H_2O) 585-560w(Cu-N) 478s (Cu-O)
$(SGZ)_2$	50	White crystalline	Ethanol Methanol DMSO and Acetone	C=38.81(38.94) O=3.66(3.60) H=4.98(5.05) Zn=23.38(23.57) H_2O=12.31(12.97)	1287s (C-N) 1612s, 1575s, 1541s (C=N) 3313s (phenolic -OH)

3. 2. Biological Activities

The antibacterial activities of these complexes were measured in terms of zone of inhibition are shown in Table 2. The test compounds showed a good sensitivity against a number of pathogenic bacteria. The results were compared with standard drug disc of *kanamycin* (30 μg/disc).

Table 2. Results of antibacterial activities of the four compounds

Name of the bacterial strains		Diameter of zone inhibition (mm)													
		$(SGZ)_2$, μg/disc			$(SGCo)_2$, μg/disc			(SGC), μg/disc			$(SGN)_2$, μg/disc			Solvent DMSO	Standard Drug (*Kanamycin*) 30 μg/disc
		400	600	800	400	600	800	30	100	200	200	400	600		
Gram negative bacteria	*S. sonnei*	15	18	21	13	16	19	07	07	11	11	13	15	0	31
	E. coli	9	11	15	7	10	14	10	13	15	13	18	22	0	30

	P. arioginosa	6	7	22	5	9	16	R	10	14	R	R	R	0	30
Gram positive bacteria	***B. subtilis***	8	9	11	7	9	12	-	-	-	-	-	-	0	26
	S. lutea	8	9	10	6	8	9	-	-	-	-	-	-	0	25
	St. aureus	7	8	10	6	8	11	16	19	22	R	R	R	0	27

R = Resistant and (-) = Not done.

Potency of (SGC) against all test organisms was quite comparable with that of standard drug *kanamycin* at dose 30µg/disc. Somewhat better results were obtained when tested with higher doses (100 µg/disc and 200 µg/disc) of the complexes. Other three compounds [$(SGN)_2$, $(SGCo)_2$ and $(SGZ)_2$] showed moderate activity even at high doses. The solvent DMSO showed no activity against any bacterial strain. MIC values of the test compounds were determined as µg/ml and are shown in Table 3.

Table 3. Results of minimum inhibitory concentration of the four compounds

Test organisms	**SGC**	**$(SGN)_2$**	**$(SGZ)_2$**	**$(SGCo)_2$**
	MIC µg/ml	MIC µg/ml	MIC µg/ml	MIC µg/ml
St. aureus	32	256	128	32
E. coli	64	128	128	16
S. soneei	32	128	64	32
P. aeruginosa	128	512	128	128

The brine shrimp lethality bioassay has been chosen to assess the *in vitro* cytotoxic effect of the test compounds. Median lethal concentration (LC_{50}) of brine shrimp lethality was measured from the plots of percentage of mortality versus concentration of the samples (Fig. 2). LC_{50} of $(SGN)_2$, (SGC), $(SGZ)_2$, and $(SGCo)_2$ were found to be 55.22, 40, 42 and 32.5 µg/ml respectively.

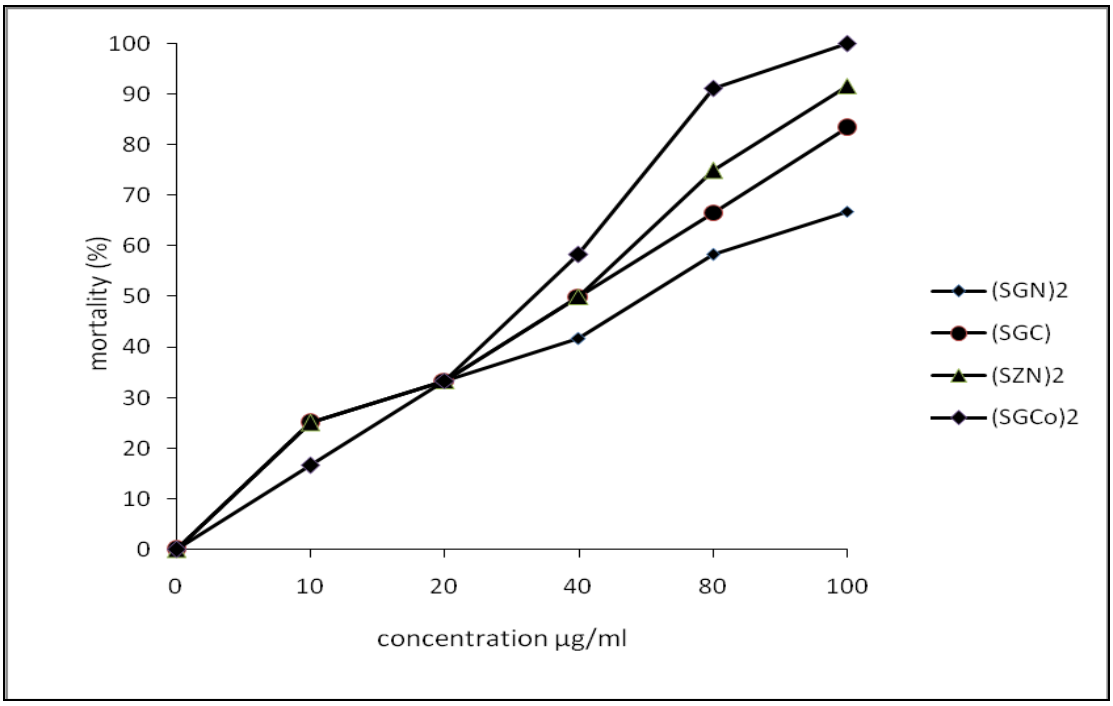


Figure 2. Brine shrimp lethality bioassay of the test compounds

From the results discussed above it is clear that the synthesized complexes are biologically active. Among the complexes studied SGC is the most efficient.

4. CONCLUSIONS

In conclusion, we have described simple, rapid and efficient protocol for the synthesis of a new series of four transition metal complexes of a schiff base derived from salicylaldehyde and glycine, viz. [N-salicylidene glycinato diaqua cobalt (II) dimer] $(SGCo)_2$, [N- salicylideneglycinato- di-aqua-nickel (ll) dimer] $(SGN)_2$, [N-salicylideneglycinato-aqua-copper(II)] (SGC) and [N-salicylidene glycinato diaqua zinc(II) dimer] $(SGZ)_2$ with excellent yields. All the synthesized compounds have been investigated for their antibacterial activities. With our newly synthesized compounds, it is evident that (SGC) compound exhibit a significant antibacterial activity and $(SGCo)_2$, $(SGN)_2$ and $(SGZ)_2$ compounds show a moderate sensitivity even with higher doses. All these compounds were found to possess cytotoxic effect. Therefore, these compounds may be used as new antibacterial drugs after performing further research works with advanced technology.

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