



## Green Synthesis, Characterisation and Biological Evaluation of Schiff base of Drug and its Silver Complex

### Chemistry

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### ABSTRACT

The work presented in this paper concerns the synthesis, characterization and biological evaluation of silver complex of Schiff base synthesized from Sulfamethoxazole & m-nitro benzaldehyde by microwave irradiation followed by complexation with silver metal. The synthesized Schiff base & its silver complex have been characterized by elemental analysis, FTIR & NMR spectroscopy. Also they have been screened in vitro for their antibacterial and antifungal activities against two bacteria namely Escherichia coli & Staphylococcus aureus and two fungus Candida albicans & Aspergillus niger. The new synthesized silver complex has exhibited significant inhibition for both bacterial and fungal pathogens. Also it has been observed that incorporation of silver metal in Schiff base has reduced its MIC value without reducing the diameter of inhibition zone.

### KEYWORDS:

Schiff base, Microwave irradiation, MIC, Silver, Complexes

#### Introduction:

Sulfamethoxazole (SMZ or SMX), is an antibiotic. It was used for bacterial infections such as urinary tract infections, bronchitis and prostatitis and is effective against both gram negative and positive bacteria such as *Listeria monocytogenes* and *E. coli*.

It is a sulfonamide and bacteriostatic. It resembles a component of folic acid. It prevents folic acid synthesis in the bacteria that must synthesize their own folic acid. Mammalian cells and some bacteria do not synthesize but require preformed folic acid (vitamin B9), they are therefore insensitive to sulfamethoxazole<sup>[2]</sup>. The trade name for SMZ is Gantanol. Other names include: sulfamethalazole, sulfisomezole and sulfamethazole.

Schiff bases are characterized by azomethine group (-N=CH-). Several studies showed that the presence of a lone pair of electrons in SP<sup>2</sup> hybridized orbital of N atom of the azomethine group is of biological importance moreover Schiff bases are excellent chelating agents, especially when a functional group like -OH or -SH is present close to azomethine group so as to form a five or six membered ring with the metal ion<sup>[6]</sup>. Sulphur is one of elements that strongly coordinates silver in geometries from linear to octahedral<sup>[7]</sup>.

Fungal infection usually are not only limited to the contamination of surface tissues. Recently there was a considerable increase in the incidence of systemic fungal infection, which are potentially life-threatening<sup>[8]</sup>. To overcome this problem attempt has been made to prepare complex of silver with Schiff base obtained from SMZ and selected substituted benzaldehyde.

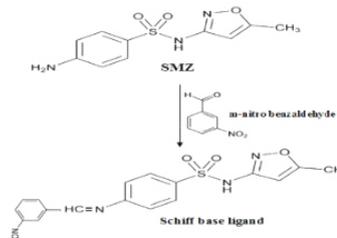
#### Materials and Methods:

All the chemicals and solvents used were of A. R. grade. All were purchased from a commercial shop and were used without further purification. Melting points were determined on a Digital Automatic Melting Point Apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on silica gel plates (Fluka-Kiesel gel, 0.2 mm thickness) and the plates were scanned under 254 nm ultraviolet light. All the compounds were analysed satisfactorily for C, H, N and S using micro analytical technique on ELEMENTAL analyser at SAIF, COCHIN. The synthesis of Ligand and its complex were carried out in glass vessel connected to water condenser in a

Scientific Microwave Synthesiser Model: CATA-2R of capacity 32 litre with a maximum power output of 850 W and microwave frequency 2450 MHz. A thermocouple used to monitor the temperature inside the vessel of the microwave. The microwave reactions were performed using on/off cycling to control the temperature. Completion of reaction was monitored by performing TLC and melting point. Infrared spectra of ligand and its silver complex (in a KBr matrix) were recorded in the 4000-350 cm<sup>-1</sup> region on Perkin Elmer FT-IR spectrophotometer. The NMR spectra were recorded on Bruker Avance II 400 FT NMR Spectrometer in DMSO using TMS as the internal standard. Antibacterial and Antifungal activities of synthesized Ligand & complex were recorded as diameter of Inhibition Zones in mm in Bio-Genics, Research and Training Centre in Biotechnology, Hubli, Karnataka.

#### Synthesis of Schiff base Ligand

The ligand was synthesized by reported method<sup>[9,10]</sup> using methanol as a solvent. The equimolar solution of SMZ and m-nitro benzaldehyde (1:1 ratio) in methanol were mixed thoroughly and small amount of glacial acetic acid was added. The mixture was subjected to microwave irradiation at an interval of P-5, Watt: 425 (Power 50%) for 5min. The progress of the reaction and purity of the products were monitored by TLC. After the completion of the reaction, the product was poured into ice cold distilled water and stirred well. The solid separated was filtered, re-crystallized from ethanol and dried under reduced pressure.



**Fig-1: Scheme of preparation of schiff's base from sulfame thoxazole (SMZ)**

#### Synthesis of silver complex:

The synthesis of silver complex was conducted in the absence of light

and the product is also stored in the dark at all times. The equimolar solutions of ligand and AgNO<sub>3</sub> in methanol were mixed thoroughly in 1:1 ratio & the reaction mixture was irradiated in the microwave synthesizer at an interval of 2 min at P-5, Watt: 425 for about 5-10 min. The progress of the reaction and purity of the product was monitored by TLC using silica gel. After the completion of the reaction, the obtained product was poured into cold distilled water and stirred well. The obtained product was filtered off, re-crystallized with methanol and finally washed with petroleum ether. The final product was dried under reduced pressure over anhydrous calcium chloride in a desiccator.

**In-Vitro Antibacterial Screening**<sup>[11,12]</sup>: The antibacterial activities of salfamethoxazole, the synthesized ligand & its silver complex against test bacteria *Escherichia coli* & *Staphylococcus aureus* at different concentration were checked by Agar diffusion method. In the method the composition of nutrient medium used was Peptone-10gm, NaCl-10gm & Yeast extract 5gm, Agar 20gm in 1000ml of distilled water. The stock cultures of selected bacteria were revived by inoculating in broth media & grown at 37°C for 18 hrs. 20ml sterilized nutrient agar medium was poured in each Petri-dish. After 18hrs of solidification, old cultures of bacteria spread over the medium evenly using a spreader. The test compounds measured quantities were dissolved in DMSO to get the concentrations 25, 50, 100, 250, 500 & 1000 µg ml<sup>-1</sup> using sterile cork borer (6mm in diameter) holes (wells) were made in each dish & then test compounds dissolved in DMSO were poured in to these holes. Finally the dishes were incubated at 37°C for 24 hrs. The clear inhibition zones were detected around each well. The diameters of inhibition zones were noted and are tabulated in **Table-4**.

**In-Vitro Antifungal Screening**<sup>[11,12]</sup>: *Aspergillus niger* & *Candida albicans* fungi were selected as test microorganism for which the growth inhibition capacity of the synthesized Schiff base ligands their silver complexes were screened & also sulfamethoxazole drug was screened against selected fungi for comparisons. Same technique, Agar diffusion method, was used for antifungal analysis but the composition of nutrient media used was Sucrose -30g/L, Sodium nitrate 02g/L, K<sub>2</sub>HPO<sub>4</sub> -01g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5g/L, FeSO<sub>4</sub> 0.01g/L & Agar-20g/L. The stock cultures of selected fungi were revived by inoculating in broth media & grown at 27°C for 48 hrs.

The plates of above mentioned agar nutrient media were prepared and wells were made in the plate using cork borer. Each plate was inoculated with 48hrs. Old culture of the test fungi was spread evenly on the media in the plate using spreader. The test compounds were dissolved in DMSO solvent to get concentration such as 25, 50, 100, 250, 500 and 1000 µg ml<sup>-1</sup>

The wells were filled with compounds of different concentrations and all plates were incubated at 27°C for 96hrs. The diameter of inhibition zone were recorded in mm and are tabulated in **Table No.5**. Amphotericin B was used as a standard drug under same conditions for each organism.

## Results and Discussion:

**Elemental Analysis:** Micro analytical data of Ligand and complex with proposed molecular formula are given in the following Table-1;

**Table-1: Physicochemical data and elemental analysis**

Ligand Compound	Colour	Molecular Formula	MP °C	CHNS Elemental analysis (calculated) observed			
				C%	H%	N%	S%
Ligand	Yellow	C <sub>17</sub> H <sub>14</sub> O <sub>3</sub> N <sub>4</sub> S	160	53.05 (52.85)	36.81 (36.63)	14.62 (14.51)	8.42 (8.29)
Complex	White	C <sub>34</sub> H <sub>28</sub> O <sub>10</sub> N <sub>8</sub> S <sub>2</sub> Ag <sub>2</sub>	290	35.80 (37.36)	2.12 (2.2)	10.82 (10.26)	5.53 (5.86)

The results obtained from elemental analytical measurements are in good agreement with calculated results. From the proposed molecular formula of Ligand and complex it confirms that the ratio of Ligand and metal in the complex is 2:1.

## FTIR Analysis:

The IR spectrum of complex was compared with that of Schiff base ligand in order to determine the mode of chelation. The observed characteristic peaks (in cm<sup>-1</sup>) in the IR spectra of Schiff base Ligand & its silver complex are mentioned in the following table. The band corresponding to azomethine group of Ligand (1616 cm<sup>-1</sup>) is shifted to lower wave number (1602cm<sup>-1</sup>) in the IR spectrum of complex which indicates the involvement of azomethine nitrogen in co-ordination with silver ion<sup>[13,14]</sup>. The absence of Sulfonamide -NH band and shifts in the asymmetric & symmetric stretching frequencies of sulfonyl group(SO<sub>2</sub> group) in the spectrum of silver complex indicating the involvement of this group in chelation with silver ion by nitrogen & oxygen donor atoms. The bands related to isoxazole ring stretching vibrations in Ligand at 1464 & 1401 suffered a shift at 1480 & 1427 in the spectra of complex indicating that isoxazole moiety is participated in co-ordination<sup>[15]</sup>. There is no appreciable change in frequency of nitro group which indicates that this group is not involved in chelation. The bonding of Ligand through nitrogen & oxygen donor atoms to silver ion is further supported by the appearance of new absorption bands at 549&580 cm<sup>-1</sup>

in the spectrum of complex which are assigned to Ag-O & Ag-N respectively.

**Table – 2: The important characteristic peaks in the IR spectra of Ligand and its silver complex**

Com pound	N-H (SO <sub>2</sub> NH)	CH= N	S=O (asy.)	S=O (symm.)	Isoxazole ring	N=O	Ag-N	Ag-O
Ligand	3483	1616	1344	1157	1464,1401	1529	-	-
Complex	-	1602	1349	1136	1480,1427	1530	580	549

## <sup>1</sup>HNMR Analysis:

The involvement of nitrogen atoms of groups SO<sub>2</sub>NH and HC=N in co-ordination via deprotonation is further supported by the disappearance of the singlet corresponding to SO<sub>2</sub>NH and HC=N groups at 10.2 & 10.9 ppm respectively in the NMR spectrum of the complex<sup>12</sup>.

**Table 3: NMR Spectral data of Ligand and its silver complex**

Compound	SO <sub>2</sub> NH	HC=N	Aromatic protons
Ligand	10.9	10.2	6.5-8.5
Complex	Disappeared	Disappeared	6.5-8.5

## In - Vitro Antimicrobial Screening:

The biological activities of synthesized complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods respectively. The biological activities were done at 0.025, 0.050, 0.250, 0.500 and 1 mg/ ml in DMSO solvent by using bacteria-*Staphylococcus aureus* & *Escherichia coli* and fungi *Aspergillus Niger* & *Candida albicans* as follows:

## Antibacterial and antifungal analysis<sup>13,14</sup>:

Composition of media used for antibacterial analysis is peptone-10g, sodium chloride 10g, yeast extract 5g, Agar 20g in 1000 ml of distilled water. Media used for antifungal analysis is sucrose 30g, sodium nitrate 2g, K<sub>2</sub>HPO<sub>4</sub> 1g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5g, KCl 0.5g, FeSO<sub>4</sub> 0.01g, Agar 20g. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of above media were prepared and wells were made by using sterile cork borer of 6mm diameter in the plate. Each plate was inoculated with 18 hrs old cultures and spread evenly on the plate. After 20 min, the wells were filled with compound and antibiotic at different concentrations. All the plates were incubated at 37°C for 24 hrs and the diameter of inhibition zone were noted. For antifungal activity the plates were incubated at 27°C for 96 hrs.

The antibacterial and antifungal activities are recorded as diameter of inhibition zone in the tables 4 and 5 and as minimum inhibition concentration (MIC) in table 6.

**Table 4: Diameter of inhibition zone in mm exhibited by complexes on selected bacteria**

Pathogen→ Conc. (µg/ml)→ Sample	Antibacterial activity											
	S. aureus						E. coli					
	25	50	100	250	500	1000	25	50	100	250	500	1000
SMZ	0	0	0	0	0	0	0	0	0	0	0	10
Ligand	0	0	0	0	6	15	0	0	0	0	6	10
Complex	0	0	5	6	8	10	2	4	5	8	9	11
Ciprofloxacin	25	28	31	34	36	*	26	29	32	34	38	*

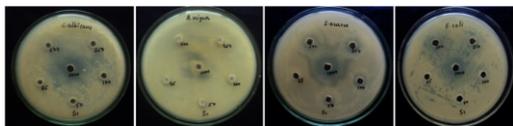
**Table 5: Diameter of inhibition zone in mm inhibited by complexes on selected fungi**

Pathogen n→ Conc.→ (µg/ml) Sample	Antifungal activity											
	A. niger						C. albicans					
	25	50	100	250	500	1000	25	50	100	250	500	1000
SMZ	0	0	0	0	0	0	0	0	0	0	0	0
Ligand	0	0	0	10	15	25	0	0	0	12	15	30
Complex	0	0	0	0	5	19	0	0	0	5	10	31
Amphotericin	0	0	2	3	5	7	0	2	7	9	13	15

**Table 6: Minimum inhibition concentration (MIC)**

Compound	Microbial species	MIC (µg/ml)
Ligand	S.aureus	500
Complex	S.aureus	100
Ligand	E.coli	500
Complex	E.coli	25
Ligand	A.niger	250
Complex	A.niger	500
Ligand	C.albicans	500
Complex	C.albicans	250

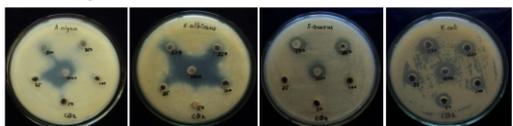
The above mentioned results reveal that the parent drug SMZ is inactive against selected pathogens at screened concentrations. But synthesized Ligand & complex are showing both antibacterial and potent antifungal activity. Incorporation of silver metal in Ligand has reduced its MIC value except in case of A. niger and improved diameter of inhibition zone in case of E. coli and S. aureus



**SMZ DRUG ACTIVITY**

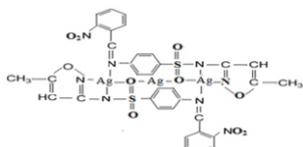


**Schiff base Ligand Activity**



**Complex Activity**

On the basis of Elemental, IR & NMR spectral data analysis, the following structure has been proposed for the synthesized complex



**Conclusion:** Microwave irradiation synthetic method is user-friendly and eco-friendly. The synthesized Ligand links with silver metal in Tetra dentate manner. When the synthesized Schiff base Ligand & its silver complex were screened for their antimicrobial activity against selected bacterial and fungal strains, it was observed that both Ligand and complex showed potent antifungal activity than the standard drug and incorporation of silver atom in Schiff base has reduced its MIC value appreciably except in case of A. Niger.

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