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Microwave Aided Synthesis and Biological Screening of Drug Based Schiff Base Complexes of Silver

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ABSTRACT: This paper describes the potential use of silver complexes of Schiff bases obtained from Sulfamethoxazole/Trimethoprim and Isatin as new antimicrobial drugs. An introduction to fungal and bacterial infections concentrating on *Candida albicans, Aspergillus niger, Escherichia coli* and Staphylococcus *aureus* is provided. The detailed synthesis of Schiff bases and their silver complexes in alcoholic medium using catalyst under microwave irradiation are provided. The synthesized complexes were characterized by IR and NMR spectroscopy, and microanalysis. Complexes were screened, in vitro, for their antifungal activity against *Candida albicans* and *Aspergillus niger* and antibacterial activity against Escherichia coli and *Staphylococcus aureus*. The new silver complexes were found to be more active than the parent drugs.

Keywords: Microwave irradiation, MIC, IR, Silver and Drug

I. INTRODUCTION

***Candida albicans**:* Candida infections of virtually every tissue of the human body have been reported, with the most common manifestations being superficial lesions of the mouth or vagina. It is capable of causing a wide range of infections and can be particularly problematic for immuno-compromised individuals such as AIDS patients, transplant recipients, cancer patients, burn patients and premature infants. The improper use of broad spectrum antibiotics can also lead to a Candida infection due to the lack of friendly bacteria to keep it under control. It can also cause more serious and potentially life-threatening systemic infections of organs, including the kidney, liver and brain. [1]

***Aspergillus niger*:** It is a mold (a type of fungus) .It causes a disease called black mold on certain fruits and vegetables and is a common contaminant of food .It has been associated with otomycosis, cutaneous infections and pulmonary disease. There are few reports of A. niger causing pneumonia.

***Escherichia coli*:** Escherichia coli is a Gram-negative bacterium that is commonly found in the lower intestine of humans and warm-blooded animals. The bacterium produces toxins, known as Shiga toxins, which damage the lining of the intestines and other target organs such as the kidneys. They can cause diarrhoea, urinary tract infections, meningitis, wound infections and pneumonia. [2]

***Staphylococcus aureus*:** It is a Gram-positive spherical bacterium that is commonly found in the nasopharynx or on our skin. It can cause infections ranging from minor skin abscesses to serious diseases such as meningitis, endocarditis and septicaemia.

Silver in Health Care: The antimicrobial properties of silver have been known for centuries. It was used in ancient Greece and Rome as a disinfectant, while the Macedonians used it to encourage the healing of wounds. With the advent of modern antibiotics in the 20th century, silver became less favoured. However, in more recent times silver has been incorporated into many medicinal and commercial products due to its high antimicrobial potency. Such products include wound plasters, dressings, mobile phones, washing machines, antiperspirant sprays, silverlined catheters, Ag/AgCl electrode, disposable ECG electrodes, clean-room paints, and even toothbrushes, hair dryers, bedding and clothing.[3, 4].

The purpose of this work was to synthesize new silver (I) complexes that would have novel structural features and would be effective antimicrobial and anticancer agents

II. 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 analyzed satisfactorily for C, H, N and S using micro analytical technique on ELEMENTAL analyzer at SAIF, COCHIN. The synthesis of Ligands and their complexes were carried out in open glass vessel on aScientific Microwave SynthesizerModel: CATA-2Rof capacity 32 litre with a 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 their silver complexes (in a KBr matrix) were recorded in the 4000-400 cm^{-1} region on Perkin Elmer FT-IR spectrophotometer. The NMR spectra were recorded on BruckerAvance II 400 FT NMR SPECTROMETER in DMSO using TMS as the internal standard. Antibacterial and Antifungal activities of synthesized complexes were recorded as diameter of Inhibition Zones in mm in Bio-Genics, Research and Training Centre in Biotechnology, Hubli, Karnataka.

Synthesis of Schiff's base ligands (SBL_1):

The ligands SBL1 and SBL2 were synthesized by reported method[7,8] using methanol as a solvent. Initially the equimolar ratio of methanolic solutions of 6.3g Sulphamethoxazole/7.3g Trimethoprim and methanolic solution of isatin(3.7g) were mixed thoroughly and few drops of glacial acetic acid was added. The mixture was subjected to microwave irradiation at an interval of 2 min at 550 W for about 10-15 min. The progress of the reaction and purity of the products were monitored by TLC using silica gel. After the completion of the reaction, the obtained product was poured into ice cold distilled water and stirred well. Solid separated was filtered and recrystallized from suitable solvent. The crystalline products were dried under vacuum or reduce pressure under anhydrous $CaCl_2$ and kept in a desiccator till further use (yield: 75%).

Sulfamethoxazole -Isatin Schiff Base Ligand (SBL_1) - Melting point, 141 °C. FT-IR (KBr, ν cm^{-1}); 3375 (N-H stretching of SO_2NH), 1320 & 1148 (asymmetric and symmetric stretching frequencies of SO_2 group),3203(N-H of Isatin), 1735(>C=O)1616 (>C=N),1499.8(>C=N of Isoxazole ring). NMR (DMSO, ppm) 2.2ppm (CH_3 proton); 6.1ppm (Isoxazole ring proton); 6.6-7.6ppm (aromatic ring protons); 11ppm (SO_2NH proton).

Trimethoprim-Isatin Schiff Base Ligand (SBL_2)- Melting point, 160 °C. FT-IR (KBr, ν cm^{-1}); 3465 &3316(symmetric and asymmetric stretching frequencies of NH_2 group); 3115(N-H of Isatin); 1728 (>C=O); 1595(>C=N); 1500(pyrimidine >C=N-). NMR (DMSO, ppm) 2.5(>CH_2 proton); 3-4ppm (OCH_3 proton); 6.2 ppm (NH_2 protons); 6.6-7.6ppm (aromatic ring protons) [5].

Synthesis of silver complexes: The syntheses of all silver complexes were conducted in the absence of light and the products were also stored in the dark at all times.

The equimolar methanolic solutions of ligand and the metal salt were mixed thoroughly in 1:1 ratio and 0.1% methanolic KOH was added to adjust the pH of the reaction mixture within 7-8 and was then irradiated in the microwave synthesizer at an interval of 1 min at 500 W for about 15-20 min. The progress of the reaction and purity of the products were 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 desiccators. **Ag – Sulfamethoxazole-Isatin Complex (C_1)**-Melting point, 244 °C. FT-IR (KBr, ν cm^{-1}); 3435 (intermolecular H-bonding):3366(N-H of SO_2NH), 1276 & 1135 (asymmetric and symmetric stretching frequencies of SO_2 group),3226(N-H of Isatin), 1732(>C=O);1607 (>C=N),1472(>C=N of Isoxazole ring);580(Ag-N). NMR (DMSO, ppm) 2.2ppm (CH_3 proton); 6.1ppm (Isoxazole ring proton); 6.6-7.6ppm (aromatic ring protons); 11ppm (SO_2NH proton).**Ag–Trimethoprim-Isatin Complex (C_2)**-Melting point, 193 °C 3464 &3342(symmetric and asymmetric stretching frequencies of NH_2 group); 3202(N-H of Isatin);1728(>C=O);1591(>C=N); 467(Ag-N). NMR (DMSO, ppm) 2.5(>CH_2 proton);3-4ppm (OCH_3 proton); 6.5 ppm (NH_2 protons); 6.6-7.6ppm (aromatic ring protons).

RESULT AND DISCUSSION

Elemental analysis data studies: Micro analytical data of the complexes with proposed molecular formula are given in the following table:

Table1: Physicochemical data and elemental analysis.

Ligand Or Complex	Molecular Formula	CHNS Elemental analysis (calculated) observed			
		C%	H%	N%	S%
SBL1	$C_{18}H_{14}O_4N_4S$	55.74 (56.54)	4.01 (3.66)	14.80 (14.65)	8.50 (8.37)
SBL2	$C_{22}H_{21}O_4N_5$	61.80 (63.00)	4.78 (5.01)	15.75 (16.70)	--
C1	$C_{36}H_{28}O_8N_8S_2Ag_2$	43.66 (44.08)	2.86 (2.85)	11.56 (11.43)	6.64 (6.53)
C2	$C_{44}H_{42}O_8N_{10}Ag_2$	46.20 (50.00)	3.29 (3.98)	12.00 (13.28)	------ ------

The results obtained from elemental analytical measurements are in good agreement with calculated results from the empirical formula of each compound and confirms that the composition of the metal complexes corresponds to 2:2 (metal - ligand) stoichiometry.

INFRARED SPECTRA

The IR spectra of the complexes were compared with those of the free ligands carefully in order to confirm formation of silver complex. The N-H band of Isatin in ligands is shifted to higher wave number while azomethine band in ligands is shifted to lower wave number in the IR spectra of complexes which indicates the coordination of isatin nitrogen as well as azomethine nitrogen to silver ion. This is supported by the formation the formation of new band in complexes (580 & 467 cm^{-1}) which is assigned to Ag-N [6].

In the IR spectrum of SBL1, N-H stretching of the sulfonamide group appear at 3462 &3375 and C=N band of isoxazole ring appear at1499.But in IR spectrum of its silver complex, N-H stretching of the sulfonamide group appear at lower wave number(3435 & 3366) and C=N band of isoxazole ring is disappeared. From these observations, the involvement of SO_2NH group and also isoxazole ring nitrogen in co-ordination is confirmed. In the IR spectrum of SBL2, the NH_2 stretchings of amino group appear at 3465 & 3316 and pyrimidine C=N band at 1500.

In IR spectrum of its silver complex, the NH_2 stretching frequency of amino group appeared at 3464 & 3342 and pyrimidine C=N band is disappeared which indicates the involvement of these groups in co-ordination with silver ion.

ANTIMICROBIAL ACTIVITY:

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 Antibacterial and Antifungal activities analysis 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 [7]:

Antibacterial analysis:

Composition of media used for Antibacterial analysis is peptone-10g, NaCl 10g, Yeast extract 5g, Agar 20g in 1000 ml of distilled water.

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 ^{0}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^{0}C for 24 hrs and the diameter of inhibition zone were noted.

Antifungal analysis:

Composition of media used for Antifungal analysis is Sucrose 30g, sodium nitrate 2g, K_2HPO_4 1g, $MgSO_4.7H_2O$ 0.5g, KCl 0.5g, $FeSO_4$ 0.01g, Agar 20g

Initially, the stock cultures of fungi were revived by inoculating in broth media and grown at 27 ^{0}C for 48 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 27^{0}C for 96 hrs and the diameter of inhibition zone were noted.

All the results are tabulated in the following tables;

Table 2: Diameter of inhibition zone in mm exhibited by complexes on selected bacteria

Pathogen→ Conc.(mg/ml)→ Sample ↓	Antibacterial activity									
	S. aureus					E. coli				
	0.025 mg	0.050 mg	0.250 mg	0.500 mg	1mg	0.025 mg	0.050 mg	0.250 mg	0.500 mg	1mg
C_1	0	4	6	9	12	0	5	7	12	16
C_2	0	0	5	8	10	4	6	8	9	12

Table 3: Diameter of inhibition zone in mm inhibited by complexes on selected fungi.

Sample	Antifungal activity									
	A. niger					*C. albicans*				
	0.025 mg	0.050 mg	0.250 mg	0.500 mg	1mg	0.025 mg	0.050 mg	0.250 mg	0.500 mg	1mg
C_1	0	12	18	24	*	17	20	30	35	*
C_2	0	0	12	16	19	0	8	12	14	21

***Inhibition zones were too big to measure.**

Table 4: The results of antibacterial and antifungal activity are presented in the following table as minimum inhibition concentration(MIC) in mg/ml.

Sample	Antibacterial activities		Antifungal activities	
	S. aureus	*E. coli*	*A. niger*	*C. albicans*
C1	0.05	0.05	0.050	0.025
C2	0.25	0.025	0.250	0.050

Table 5: Comparative statement of Inhibition zone of compounds in mm.

Compound	Pathogens	
	S. aureus	*E. coli*
DMSO	NA	NA
$AgNO_3$	18 (at conc.**5** mg/ml)	15 (at conc.**5**mg/ml)
Sulfamethoxazole	11.8 (at conc.**1** mg/ml)	10.2 (at conc.**1**mg/ml)
C1	12 (at conc.**1** mg/ml)	16 (at conc.**1**mg/ml)
Trimethoprim	20 (at conc.**5** mg/ml)	NA
C2	10 (at conc.**1**mg/ml)	12 (at conc.**1**mg/ml)

NA= not active

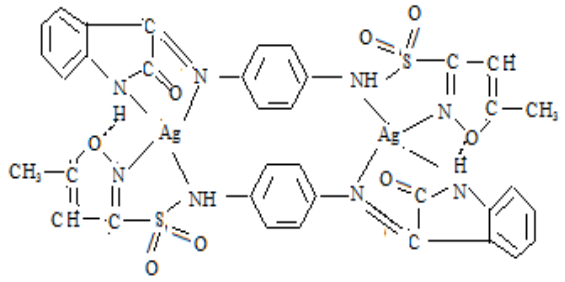
Table 6: Comparative statement of MIC values of compounds in mg/ml.

Compound	Pathogen	
	S. aureus	*E. coli*
$AgNO_3$	1.25	>2.50
Sulfamethoxazole	0.065	0.1
C1	0.050	0.050
Trimethoprim	2.5	NA even at conc.5mg/ml
C2	0.25	0.025

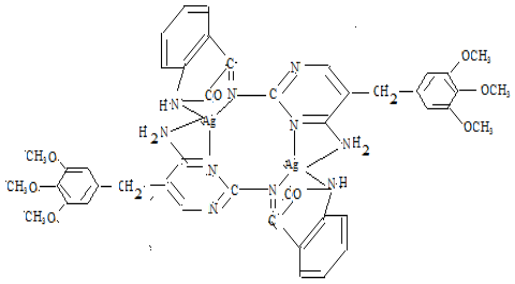
The above mentioned results reveal that both the synthesized complexes C_1 and C_2 show more antibacterial activity than their corresponding parent drug. However C_1-complex shows potent antifungal activities against *C. albicans* as well as *Aspergillus niger* with appreciable zone of inhibition diameter. But C_2 is inactive towards fungus *C. albicans*.

On the basis of elemental analysis, IR and NMR spectral data the following structures have been proposed for the C_1 and C_2 complexes.

Proposed Structure:



Complex – C_1



Complex – C_2

CONCLUSION

Microwave assisted synthesis has reduced reaction time from hours together to few minute with better yield compared to classical synthesis methods. From IR spectral data, formation of silver complexes are confirmed. From comparative statement, it is evident that Trimethoprim drug is inactive against *E. coli* but its synthesized Schiff base silver complex is exhibiting better activity against the same bacteria that too at very low concentration (12mm at1mg/ml) and also it is exhibiting enhanced activity against *S. aureus* than parent drug and $AgNO_3$. Schiff base silver complex of sulfamethoxazole is found to have potent antifungal activity against selected pathogen compared to antifungal agents like Miconazole and Fluconazole.

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