

**SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF
BENZENE SULFONAMIDE, 4-AMINO-N- (5-METHYL-3-ISOXAZOLYL)
MONOSILVER SALT AND RELATED COMPOUNDS.**

R.A.Herole*¹, V.S. Velingkar ²

¹Smt. S.S.Patil College of Pharmacy, Dept. of Pharmaceutical Chemistry, At, Post and Tal.

Chopada, Dist.Jalgaon, Maharashtra, 425107, India.

²Prin.K.M. Kundnani College of Pharmacy, Dept. of Pharmaceutical Chemistry, Mumbai-05, India.

Corresponding author*: ramesherole@yahoo.co.in

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ABSTRACT:

The synthesis of sulfa drug-metal complexes has received much attention due to their effective application as chemotherapeutic agents to be employed for the prevention and cure of bacterial infection in human being. Further more, sulfa drugs and their metal complexes, posses many applications as diuretic, antiglaucoma, antiepileptic etc¹⁻². The present work report the synthesis, characterization and antimicrobial screening of synthesized silver- sulfa drug complexes.

Keyword: sulfonamide, silver; metal complexes; antibacterial activity.

1. INTRODUCTION

The sulfonamides were the first effective chemotherapeutic agents to be employed systematically for the prevention and cure of bacterial infection in human being. The sulfa drugs exert their antibacterial action by the competitive inhibition of the enzyme dihydropterae synthetase towards the substrate P-aminobenzoate³⁻⁴. Several authors have reported the antimicrobial activity of sulfa drugs and their metal complexes¹⁻⁴. Studies on their metal chelates could have much physiological and pharmacological relevance, because the metal chelates of sulfa drugs have been found to be more bacteriostatic than the drugs themselves⁵. Besides, silver has been used in medicine for many years⁶. In antibacterial therapy, microorganisms

show resistance to the existing antibacterial agents. To overcome this problem attempt has been made to prepare complexes of silver with selected sulfonamides so as to attain broad-spectrum antibacterial activity with minimum toxicity and resistance. The title compounds were synthesized by preparing sodium salt of sulfonamides as an intermediate, which were further complexed with silver nitrate. These compounds were then characterized by physical, spectral and elemental analysis. Investigation of antimicrobial activity of these compounds was carried out by using Broth Dilution method.

2. MATERIALS AND METHODS

The sulfonamides sulfamethoxazole, sulfacetamide sodium, sulfadimidine sodium and sulfaquinoxaline were obtained as gift samples from Nicholas Piramal India Ltd. Mumbai. Sulfadoxine was obtained from Ipca Laboratories Ltd. Mumbai. The bacterial strain *Staphylococcus aureus* (NCTC-3750), *Pseudomonas auriginosa* ('S'Fisher's immunotype-4) were procured from the Haffkine Institute, Lower Paral, Mumbai. The completion of reaction was monitored by using stationary phase silica gel GF₂₅₄ and mobile phase chloroform: methanol: 25% ammonia, ethyl acetate: methanol, ethanol: 25% ammonia etc. Melting points were recorded by using open capillary method and further checked by Visual Melting Point Apparatus (model: MR - VIS, Lab India). The values were uncorrected. The UV spectra of compounds were recorded on UV-Visible spectrophotometer (UV-1601, Shimadzu). The infrared (IR) spectra were recorded using KBr pellet technique (model: FTIR - 4200, Shimadzu), ¹H-NMR, (δ in ppm) spectra of compounds were recorded in DMSO D₆ using 300 MHz Varian Mercury Plus Spectrometer. Mass spectra and elemental analysis were performed on Q-T of Micro YA-105 mass spectrometer and by using kjeldahl's method respectively.

2.1 Experimental Work: The sodium salt of sulfonamide was prepared by using sodium methoxide and selected substituted sulfonamide. The various silver sulfonamide complexes were prepared by reacting silver nitrate with sodium salts of sulfonamides⁶⁻⁹.

Step 1: Sulfonamides were added to a solution of sodium methoxide, which was prepared by dissolving sodium in absolute methanol. On shaking for sometime the entire reaction mixture

crystallized to a solid cake. It was warmed for a few minutes on water bath to complete the reaction. The precipitate obtained was then filtered and the yield was reported.

Step 2: Silver nitrate 5.1 gm (0.03 mole) in 100 ml of distilled water was added with vigorous stirring to a solution of selected sodium salt of sulfonamide (0.03mole) in 100 ml of distilled water. Immediately a voluminous white precipitate formed which was filtered off with suction (using a fine filter paper) and washed with cold water until the washings were entirely free of silver ions. The washed precipitate was then spreaded upon a porous plate and allowed to dry in the dark for 24 hrs. It was then removed from the plate and dried in the dark in vacuum desiccators over potassium hydroxide sticks for 48 hrs. And finally in the oven at 100°C for 4 hrs.

2.2 Microbiology: The technique used for the study of antimicrobial screening was Broth Dilution method.

Broth Dilution Method¹⁰⁻¹²: Prior sterilized Mueller Hinton Broth was distributed into tubes so as to get the concentration from 200 μ g/ml to 10 μ g/ml. after addition of synthesized compounds. Stock solution of synthesized compounds was prepared at 1000 μ g/ml in DMSO (Dimethyl sulphoxide). This was added into the respective Mueller Hinton Broth in order to give the concentration of the drug into the respective broth tube as mentioned above. (200 μ g/ml to 10 μ g/ml). Inoculum of the bacterium to be tested (10^7 microorganism / ml) was inoculated into the respective tubes containing broth and title compound, this was incubated for 24 hrs. and results were noted for the bacterial growth. The

minimum concentration, at which there was no visible growth, was taken as minimum inhibitory concentration (MIC) value of those particular compounds.

3. RESULTS AND DISCUSSION

Sodium salt of selected sulfonamide was prepared by using the alcoholic sodium metal. The silver sulfonamide complexes were prepared by using silver nitrate and selected sodium salt of sulfonamides. All the complexes were obtained in very good yields (85% - 95%). These complexes were further characterized by physical and spectral analysis. The characterization of complexes by physical method was performed by recording their melting points in open capillary tube method as well as by Visual melting range apparatus. Which are found to be uncorrected. The observed nitrogen contents were found to be within 1% range of calculated nitrogen contents. Spectral analysis of UV, IR, ^1H NMR, and Mass spectral data are tabulated in Table no.1. ^1H NMR spectra of various complexes showed expected delta (δ) values of protons. Mass spectral data of acetamide- N- [(4-amino phenyl) sulfonyl] monosilver salt verifies all the fragments. Most of title compounds showed better activity against *Staphylococcus aureus* and *Pseudomonas auriginosa*. All compounds were selected for the determination of minimum inhibitory concentration (MIC). They were compared against parent sulfonamide and were found to possess better antimicrobial activity. Complex A \approx 1 (Benzene sulfonamide, 4-amino-N- (5-methyl-3-isoxazolyl) monosilver salt) was found to be most active against both *Staphylococcus aureus* and *Pseudomonas auriginosa*, which showed

MIC at 100 $\mu\text{g/ml}$. Complex \approx A2 (Benzene sulfonamide, 4-amino-N- (5,6 dimethoxy-4-pyrimidinyl) monosilver salt) was found to be effective against *Pseudomonas auriginosa*, Which showed MIC at 50 $\mu\text{g/ml}$. Complex \approx A3 (Acetamide, N- [(4- amino phenyl) sulfonyl] monosilver salt) showed the activity against *Pseudomonas auriginosa* and *Staphylococcus aureus*, which showed MIC at 40 $\mu\text{g/ml}$. and 50 $\mu\text{g/ml}$. respectively. Complex \approx A4 (N 1 - (4, 6-dimethylpyrimidin-2-yl) sulfanilamide monosilver salt exhibit better antimicrobial activity against *Staphylococcus aureus*, which showed MIC at 50 $\mu\text{g/ml}$. Complex \approx A5 (N 1 – Quinoxalinylyl sulfanilamide) was found to be most active against both *Staphylococcus aureus* and *Pseudomonas auriginosa*, which showed MIC at 100 $\mu\text{g/ml}$. control DMSO did not show any antibacterial activity. The results are tabulated in Table no.2.

CONCLUSION:

From the experimental findings, the results are found to be encouraging. It can thus be concluded that such title compounds may be utilized as novel antibacterial agents. Some more compounds can also be prepared for further antibacterial as well as antifungal studies.

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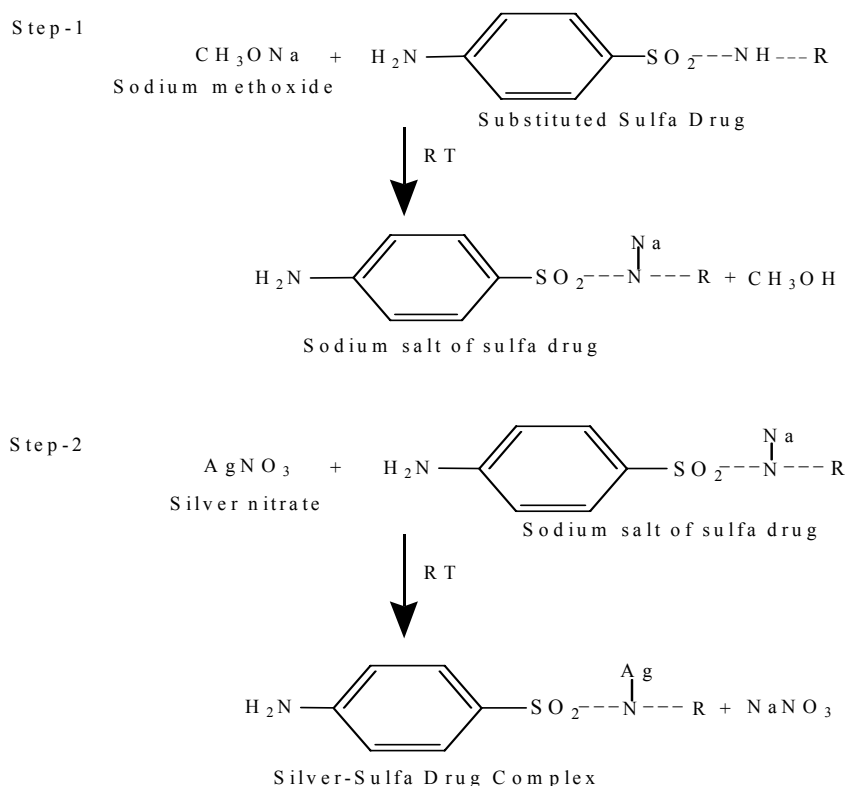
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Scheme I: synthesis of metal-sulfa drug complexes



RT = room temperature

R = -COCH₃, isoxazole, dimethyl pyrimidine, dimethoxy pyrimidine, quinoxalinyll etc.

Table No.1: spectral analysis of metal-sulfa drug complexes.

S. N.	Compound	% Yield	Physical Constant (M.P. ^o c)	UV λ (nm)	FTIR ν (cm ⁻¹) Kbr	¹ HNMR δ ppm	Mass spectrum M/e	Elemental Analysis					
								calculated			observed		
								C	H	N	C	H	N
1	A 1	95	273	259	3388,3322,1614, 1597,1500, 1301,1165	2.3,6.1, 5.8,6.59, 7.57	-----	33.40	2.7	8.7	33.3	2.6	8.12
2	A 2	87	320	259	3469,3365,1619, 1570,1501,1236, 1318,1183	-----	-----	34.58	3.12	13.45	34.4	3.2	13.4
3	A 3	93	241	263	3454,3358,1638, 1598,1366,1183	1.9,5.8, 6.53,7.5	321,279, 156,108	29.97	2.8	8.7	29.8	2.5	8.5
4	A 4	91	275	275	3424,3349,1638, 1555,1501,1344, 1182	-----	-----	37.46	3.38	14.57	37.3	3.3	14.6
5	A 5	95	271	253	3467,3366,1628, 1504,1349,1183	-----	-----	35.44	2.95	13.78	35.4	2.8	13.6

The compounds A \approx 1, A \approx 2, A \approx 3, A \approx 4 and A \approx 5 refer to the silver sulfamethoxazole silver sulfadoxine, silver sulfacetamide, silver sulfadimidine and silver sulfaquinoxalline respectively.

Table No. 2: Minimum Inhibitory Concentrations of metal- sulfa drug complexes against Gram positive and Gram negative bacteria.

Sr.No.	Title compounds	<i>Staphylococcus aureus</i> $\mu\text{g/ml}$		<i>Pseudomonas auriginosa</i> $\mu\text{g/ml}$	
		Drug	Complex	Drug	Complex
1.	A 1	67	100	88	100
2.	A 2	58	100	62	50
3.	A 3	48	50	52	40
4.	A 4	34	50	73	100
5.	A 5	74	100	80	100
6.	DMSO (control)	Nil	Nil	Nil	Nil