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Ministry of higher eduction
And scientific Research
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A Project
Submitted to the Department of pharmaceutical chemistry in the College of Pharmacy/University of Basrah in partial Fulfillment of the Requirements for the BSc degree in Pharmacy

Synthesis ,characterization and biological studies of sulfa-drug based transition metal complex

By
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ABSTRACT

The sulfa drug based ligand and transition metal complex have been synthesized and the constitution of the products have been settled on the basis of spectroscopic data. The products have been evaluated for antimicrobial activity. Some compounds are found to have antimicrobial activity comparable to that of standard drugs, viz.sulfamethoxazole, sulfadiazine. The products have also been characterized by elemental analysis, conductivity measurement, molecular weight determination and magnetic susceptibility measurements and P^H metric method.

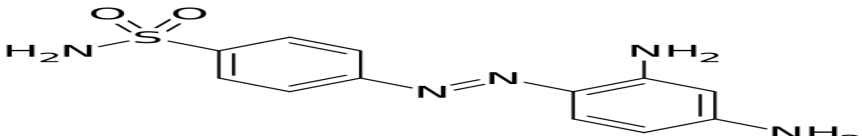
INTRODUCTION TO THE CHEMISTRY OF SULFONAMIDES

General:

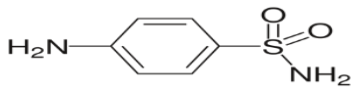
A sulfonamide grouping is derived from a sulfonic acid group by replacing its hydroxyl group with an amino group. Sulfonamides, also known as sulfa drugs, have a history that dates back to almost 70-80 years. A sulfonyl group plays a very important role as a key constituent of number of biologically active molecules. Sulfonamides occupy a unique position in the drug industry and exhibit a wide spectrum of biological activities].

The first clinically used sulfonamide was named prontosil I that showed protective action against streptococci in mice .Prontosil was active in vivo, but ineffective in vitro, which led to the conclusion that prontosil itself was not the active drug. When

metabolized in the body, prontosil produces sulfanilamide II, which is the real active agent .It acts by interfering with p-aminobenzoic acid utilization by the infecting bacteria.



I.

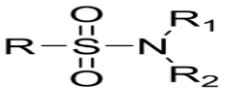


II

This discovery started great efforts in the investigation and production of new sulfonamides. Several drugs containing sulfonamide functionality are in clinical use which include antibacterial and antifungal drugs .carbonic anhydrase inhibitors], anti-inflammatory agents anticonvulsant agent anti-migraine agents hypoglycemic, protease inhibitors and agents acting against diabetic mellitus They are also found to have extensive applications in cancer chemotherapy Viagra, a sulfa drug is one of the recent block bluster molecules used for erectile dysfunction Some sulfonamides have proved to be useful as herbicides and fungicides Aryl sulfonyl substituted derivatives have been used as protecting groups for oxygen and nitrogen functionality. Sulfonamide derivatives of azo dyes have been reported to improve stability and lubrication (11)

Structure:

Sulfonamides are compounds, which have a general structure represented by III. In this structure, R may be alkyl, aryl or hetero aryl etc. R1, R2 may be hydrogen, alkyl, aryl or hetero aryl groups.



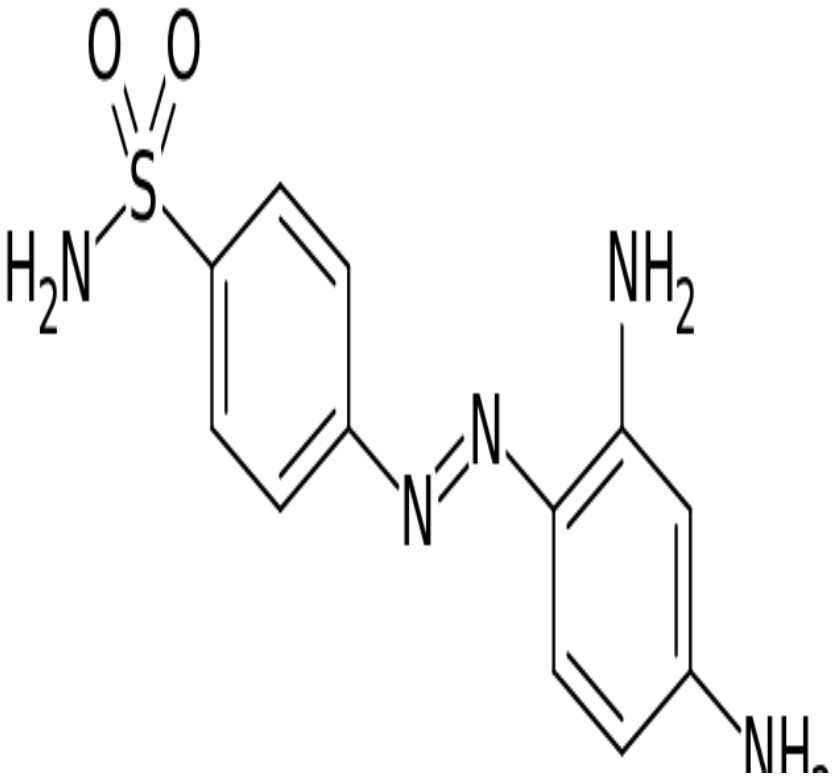
(III)

Allergies to sulfonamide are common. According to data the overall incidence of adverse drug reactions to sulfa antibiotics is approximately 3%, close to (11)penicillin;[[. hence medications containing sulfonamides are prescribed carefully. It is important to make a distinction between sulfa drugs and other sulfur-containing drugs and additives, such as sulfates and sulfites, which are chemically unrelated to the sulfonamide group, and do not cause the same hypersensitivity reactions seen in the sulfonamide

History of Sulfonamides:

In 1932, the German dye manufacturing company prepared a red azo dye, named prontosil for its dye properties]. Remarkably, it was discovered that prontosil showed antibacterial action when it was used to dye wool. In 1935, Gerhard Domagk published the results of his research work indicating that prontosil was capable of curing staphylococcal infections in mice and rabbits . In 1939, Domagk earned nobel prize in medicine for this important discovery but anorder from Hitler prevented Domagk from accepting the honour [23]. After sulfanilamide discovery, thousands of chemical variations were studied and the best therapeutic results were obtained from the compounds in which one hydrogen of the SO2NH2 group was replaced by heterocyclic ring .(11) To date more than twenty thousand sulfanilamide derivatives, analogs and related compounds have been synthesized. These synthesis have resulted in the discovery of new compounds with varying pharmacological properties

Prontosil is an antibacterial drug discovered in 1932[1] by a research team at the Bayer Laboratories of the IG Farbenconglomerate in Germany. It has a relatively broad effect against Gram-positive cocci but not against enterobacteria. The discovery and development of this first sulfonamide drug opened a new era in medicine.[2]


O
O
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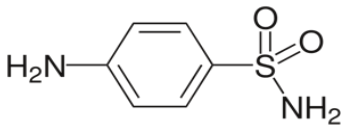
H2N
NH2
N
N
NH2

Prontosil structure

Prontosil ——————————sulfanilamide

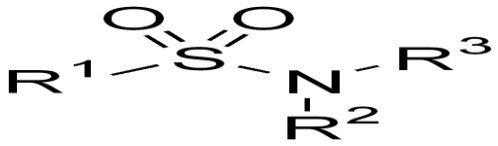
All sulfonamides are derivatives of sulfanilamide (p-amino benzene sulfonamide). The sulfanilamide [7]compound is more active in the protonated form. The drug has very low solubility and sometimes can crystallize in the kidneys, due to its first pK_a of around 10. This is a very painful experience, so patients are told to take the medication with copious amounts of water. Newer analogous compounds prevent this complication because they have a lower pK_a, around 5–6. making them more likely to remain in a soluble form.



Sulfanilamide structure

Sulfonamide (also called **sulphonamide**, **sulfa drugs** or **sulpha drugs**) is the basis of several groups of drugs. The original antibacterial sulfonamides are synthetic antimicrobial agents that contain the sulfonamidegroup. Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame.

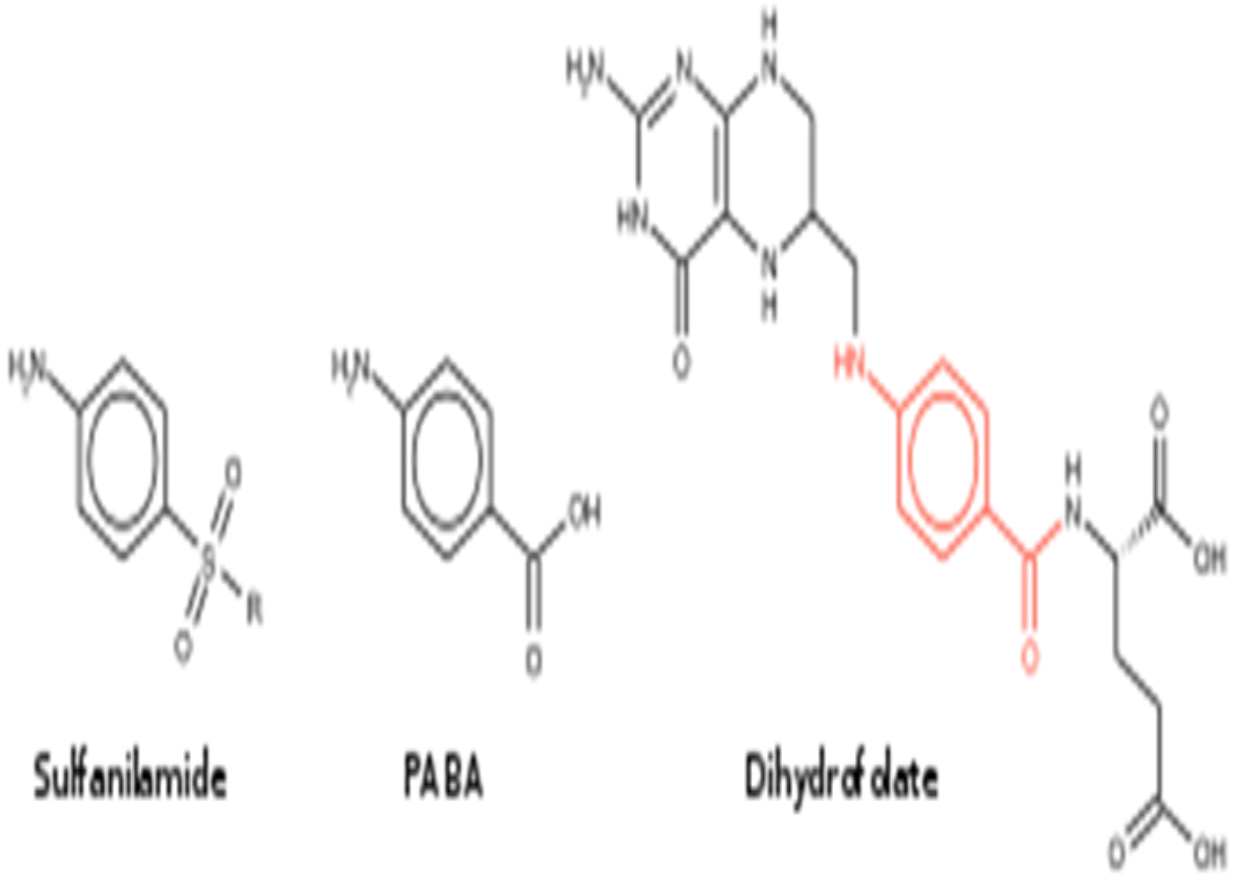
The sulfonylureas and thiazide diuretics are newer drug groups based upon the antibacterial sulfonamides[3,4]



Antimicrobial

Main article: Dihydropteroate synthase inhibitor

In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase (DHPS), an enzyme involved in folate synthesis. Sulfonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Humans, in contrast to bacteria, acquire folate (vitamin B_9) through the diet.[6]


Sulfanilamide
PABA
Dihydrofolate

Structural similarity between sulfonamide (left) and PABA (center) is the basis for the inhibitory activity of sulfa drugs on tetrahydrofolate(right) biosynthesis

Other uses

Sulfonamides are used to treat allergies and cough, as well as antifungal and antimalarial functions. The moiety is also present in other medications that are not antimicrobials,
including thiazidediuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide,
and torsemide), acetazolamide, sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib).
Sulfasalazine, in addition to its use as an antibiotic, is also used in the treatment of inflammatory bowel disease.

Mechanism of action

- Structural analogs of para-aminobenzoic acid (PABA)
- Inhibit dihydropteroate synthase - needed for folic acid synthesis
- Prevent normal bacterial utilization of PABA for the synthesis of folic acid[9]

Preparation

Sulfonamides are prepared by the reaction of a sulfonyl chloride with ammonia or an amine. Certain sulfonamides (sulfadiazine or sulfamethoxazole) are sometimes mixed with the drug trimethoprim, which acts against dihydrofolate reductase

dihydrofolate reductase. As of 2013, the Republic of Ireland is the largest exporter worldwide of sulfonamides, accounting for approximately 32% of total exports. [8]

Pharmacokinetic[9]
(1) Absorbed from the stomach and small intestine
(2) Distributed widely to tissues and body fluids (CSF), placenta,
and fetus. Plasma protein bound 20-95%
Sulfadiazine and sulfisoxazolev may be effective in meningeal
infections .
(3) Metabolized in the liver by acetylation
(4) Eliminated mainly in the urine as the unchanged drug and metabolic product
• In acid urine, the eliminated are insoluble and may precipitate, thus induce renal disturbance.

Classification[9]
(1) Oral absorbable agents
• Short-acting agents (4-9hr): sulfisoxazole
• Medium-acting agents (10-17hr): sulfamethoxazole (SMZ) • Long-acting agents (7 days):sulfadoxine
(2) Oral non-absorbable agents sulfasalazine
(3) Topical agents
Sulfacetamide,mafenideacetateandsilver sulfadiazine

Clinical uses[9]

(1) Systemic infections

• Cerebral meningitis

• Tympanitis

• Uncomplicated urinary tract infections

• Combined with TMP in treating complicated urinary tract infections, respiratory infections, GI infections

(2) Intestinalinfections

• Ulcerative colitis, enteritis, other inflammatory bowel disease

- sulfasalazine
(3) Infections of burn and wound • Sulfadiazine sliver

Combination agents[9]
• Sulfadoxine+pyrimethamine(Fansidar)- malaria second-line
• Sulfadiazine+pyrimethamine — acute toxoplasmosis
• Co-trimoxazole Sulfamethoxazole+trimethoprim — a wide variety of infections.
Individually they both are bacteriostatic but the combination is bacteroicidal

Adverse reactions

1. Hypersensitivity
• Skin rash and fever is common
• Stevens-Johnson syndrome is rare, but is a serious and potentially fatal type of skin and mucous membrane eruption[9]
2. Gastrointestinal effects
• Nausea, vomiting, and diarrhea is common
• Mild hepatic dysfunction, hepatitis is uncommon
3. Urinary tract disturbances • May precipitate in acid urine — Crystalluria and hematuria
4. Hematopoietic disturbance
Acute hemolysis in G-6PD, Kernicterus in newborn, Granulocytopenia, thrombocytopenia, and aplastic anemia

Example on sulfadrug:-

Trimethoprim (TMP)[9]

- TMPinhibitsbacterialdihydrofolicacidreductase
- Preventstheformationofactivetetrahydro form of folic acid
- 50,000timeslessefficientininhibitionof mammalian dihydrofolic acid reductase
- TMPgiventogetherwithsulfonamides,produces sequential blocking of folic acid synthesis, resulting in marked enhancement (synergism) of the bacteriostatic activity.

Trimethoprim-Sulfamethoxazole (TMP-SMZ)

- Sequential interference with folic acid synthesis results in bacterial synergism often with bactericidal activity
- Sulfonamides are structural analogues of para-amino benzoic acid (PABA), competitively inhibiting synthesis of dihydrofolic acid
- Trimethoprim is an analogue of the pteridine portion of dihydrofolic acid inhibiting synthesis of tetrahydrofolic acid

(TMP-SMZ)
• Resistance is reduced because of the sequential interference with steps involved in folic acid synthesis
• Sulfas: decreased permeability (plasmid mediated), increased production of PABA
• TMP: synthesis of DHFR with decreased affinity for TMP (plasmid-mediated), overproduction of DHFR
• Resistance to both TMP and SMZ has been increasing • Combination antibiotic with 1:5 ratio of TMP to SMZ achieves a serum ratio of 1:20
• Available both orally or parenterally
• Both agents are well distributed achieving good levels in the lungs, kidneys, biliary tree and the central nervous system
• Both are partially metabolized in the liver and are excreted in the urine.
• The serum half-life is 9-11h, however it is prolonged in subjects with renal insufficiency[9]

Spectrum of activity
Trimethoprim-Sulfamethoxazole
(TMP-SMZ)

Excellent broad spectrum activity against a diversity of microorganisms
• Gram negatives: E. coli, klebsiella, proteus, salmonella, shigella, vibrio, B. cepacia, H.influenzae, Neisseria spp. • Gram positives: staphylococci, streptococci, listeria, not enterococci
• Miscellaneous: pneumocystis, nocardia, chlamydia[9]

Clinical uses
• Urinary tract infections
• Prostatitis
• Treatment of moderately severe to severe pneumocystis pneumonia
• Upper and lower respiratory infections caused by susceptible organisms
• Diarrheal illnesses due to salmonella, shigella and enterotoxigenic E.coli[9]

Adverse effects
□Hypersensitive reactions: rash, fever
□GI effects: nausea, vomiting diarrhea □Toxicity from TMP-SMZ including fever,
rashes, Stevens Johnson syndrome, is dramatically increased in subjects with AIDS. The reason for this is unclear.[9]

Experimental section

The drug,chmical and solvents used in this study were of analytical grade and used as obtained from Aldrich without further purification.

IR spectra of all compound were recorded on FT-IR (UV-1100 uv vis spectroscopy spectrophotometer)

Uv-vis spectrophotometry (.1100 uv)with mached quartz cuvettes was used for all absorbance measurement.

Melting points were determined by open tube capillary method by (melting point testing machine with microscopy

Materials and Methods

All the chemicals used were of AR grade, procured from Alfa/ Aesar and Aldrich. Solvents used were of analytical grade. IR spectra (KBr pellets) were recorded in the region 4000–400 cm^{-1} on a FT-IR spectrum BX-II spectrophotometer. 1H NMR spectrum was recorded with a model Bruker Advance DPX-300 spectrometer operating at 300 MHz using DMSO-d6 as a solvent and TMS as an internal standard. EPR spectra of Cu(II) complex was recorded as polycrystalline sample and in the DMSO solution, at room temperature (RT) on E4-EPR spectrometer using the DPPH as the g-marker. Electronic spectra were recorded in DMSO solution on a Shimadzu UV mini-1240 spectrophotometer. Magnetic moment measurements (Gouy balance) were made at room temperature using $CuSO_4.5H_2O$ as a calibrant. The thermal analyses (TGA and DTA) were carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 10°C min^{-1}using Shimadzu TG–50H and DTA–50H thermal analyzers.

Preparation of ligand

The sulfanilamide azo-dye was prepared by the gradual addition of an aqueous solution of 0.01 mol of $NaNO_2$ to a concentrated HCl 1:1 solution of 0.01 mole of sulfanilamide with stirring and kept for about 20 min in an ice bath at –10°C. A solution of salicylaldehyde (0.01 mol) and sodium hydroxide (5 g) and doubly distilled deionized water (100 mL) was added drop wise to the resulting solution with stirring and the resulting mixture was reacted for 4 h at 0°C. The reaction mixture was acidified with hydrochloric acid. The crude material was recrystallized from ethanol and then dried. Then 0.01 mol p-toluidine was slowly added to a solution of 0.01 mol of the azo compound in 30 mL ethanol. After refluxing the reaction mixture for 2 h, the precipitate was cooled and collected by filtration. The formed solid product was separated by filtration, purified by crystallization from ethanol, washed several times with diethyl ether and dried in vacuum over anhydrous calcium chloride to give orange crystals

Preparation of metal complexes

Preparation of metal complexes

Metal salt Al(No3)3.H2O and CUSO4 was dissolved to get solution(0.01 mol).Ethanol solution of sulfonamide was prepared (0.01mol)

Both of above solution were mixed at 80c and stirred at 800rpm for 4hr

Cool the reaction mixture till change in coloration occure indicating ppt of metal complexes

Complexes formed were recoverd by vacum filteration from reaction mixture.

Washed and recrystallized with appropriate solvent

FT-IR Spectra

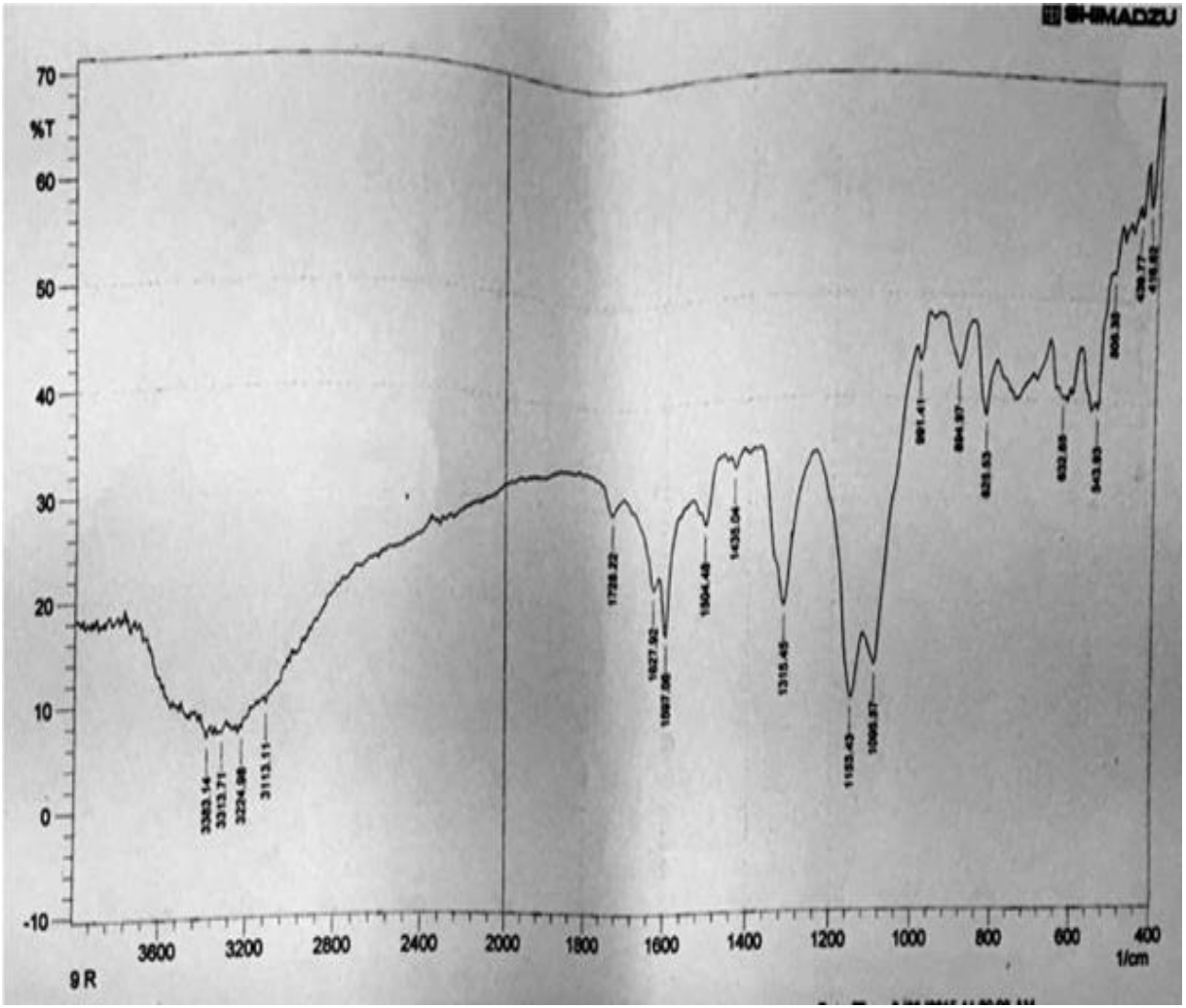
Infrared spectroscopy is used for identifying functional groups in pure organic and inorganic Compounds. (10)The absorption of infrared light brings about the vibration of the molecules].An infrared spectrum originates from the different modes of vibration and rotation of a molecule. The infrared spectrum of a compound tells about the functional groups that are present in compounds/Complexes. When the ligand forms a complex with a metal ion, there is a shift in the frequency of the region or disappearance of the region indicated that the ligand is involved in the complexationThe infrared spectroscopy has been used to study the mode of coordination of Drugs/Ligands and their metal complexes.

The IR spectra of the free ligand and its metal complexes were measured in the region of 4000-400/ cm and.proposed assignments for the spectral bands are shown in Table (1)Tentative band assignments (cm)of some.characteristic bands of sulfadiazine and their related systems were reported.The IR spectra of all complexes show abroad band at around 3440 cm-1 and a strong band between1610-1655/ cm .These may be assigned to asymmetric O-H stretching, which indicates the presence of water molecule in the complexes(10)

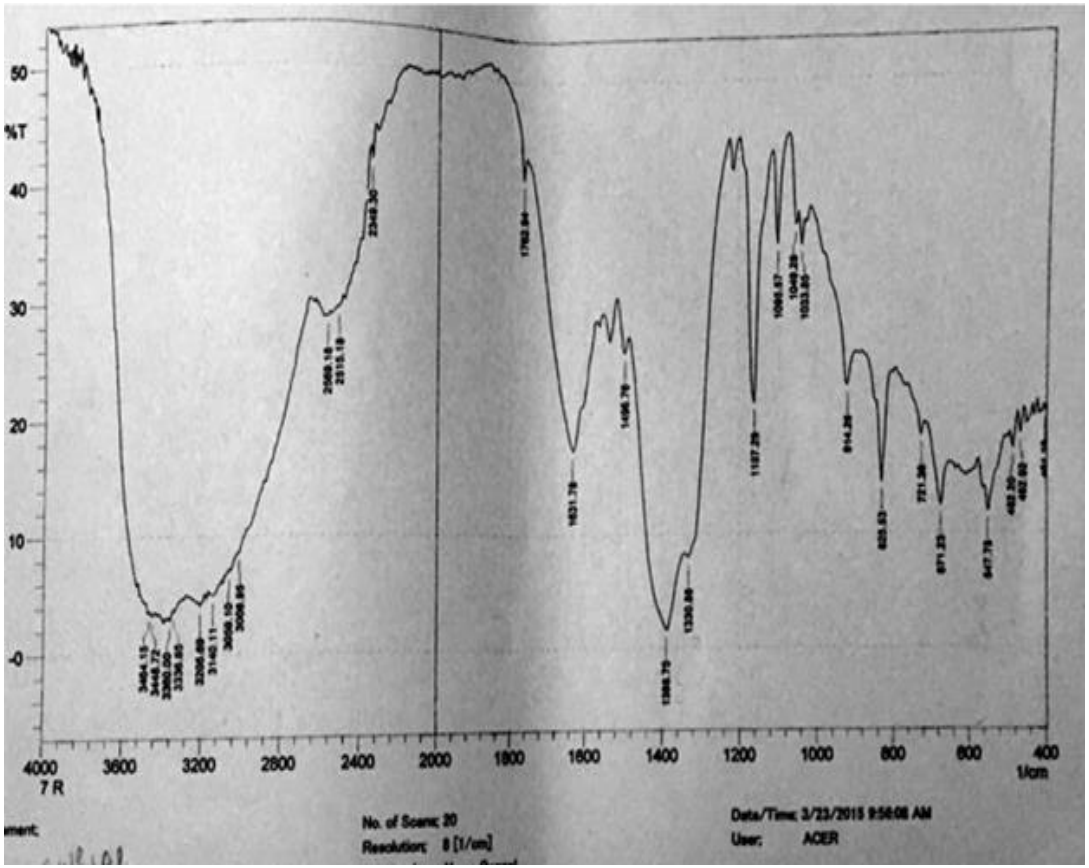
Sulfa-cu complex	S-o- broud band at1095.57	S-o-NH broud band at1315.43	NH- broud band at 3484.72
Sulfa-Al complex	1057.20	1380.75	3485.72

Spectral characteristic

The absorption spectra of the reaction product of sulfonamide with metals show maximum absorption(hmax) at310 and 460nm for sulfa-cu and sulfa -Al complex respectively with 1*10^-3M concentration for the complexes.and the absorption spectra of sulfa drug did not have any band in this region as shown in figure 1,2,3) the for more than 24hr at temperature range (20-30)



Figure(1). Ft-IR spectra of sulfa-cu complex.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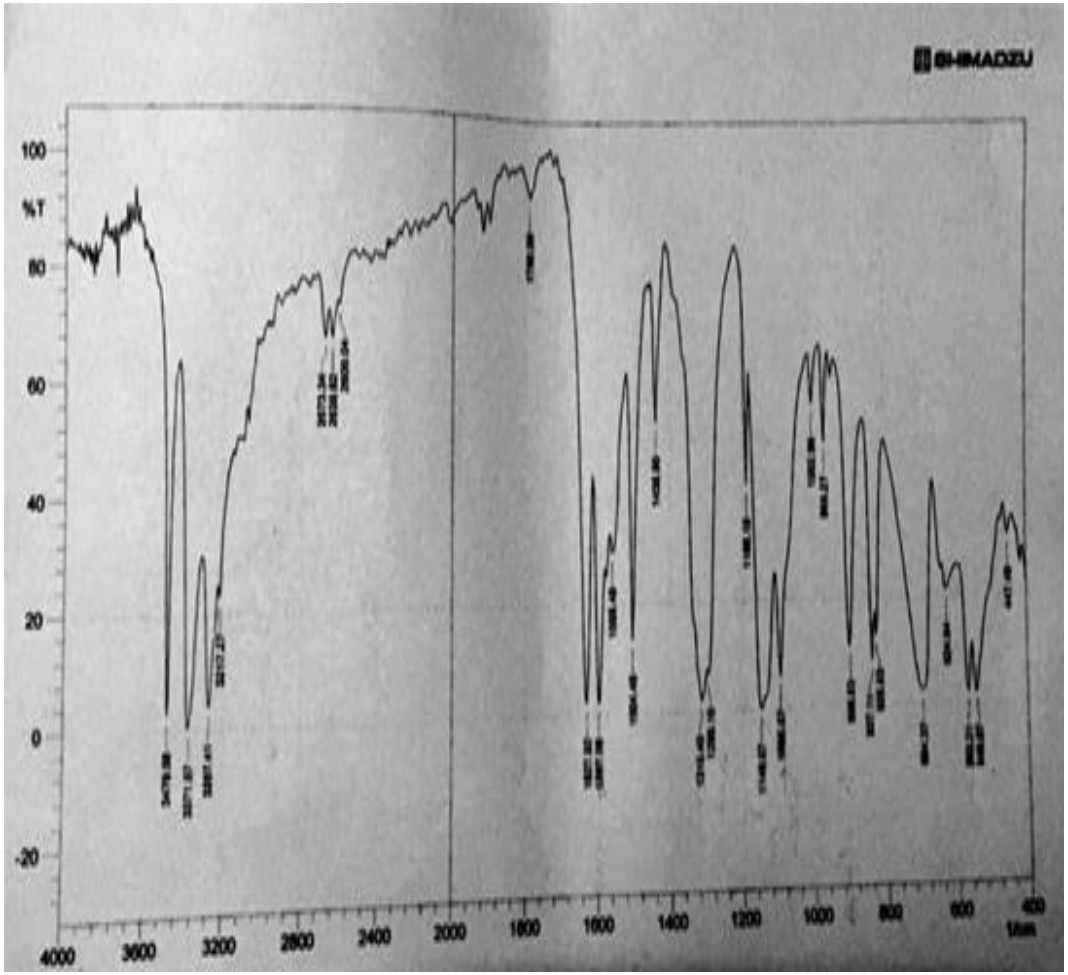
Figure(2) **Ft_**



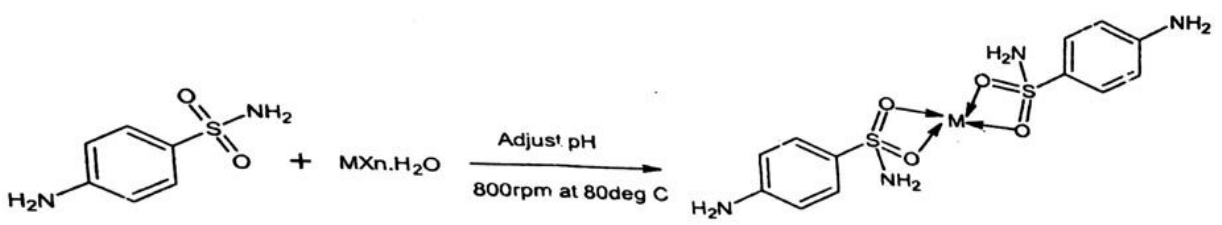
Figure(3)sulfamide

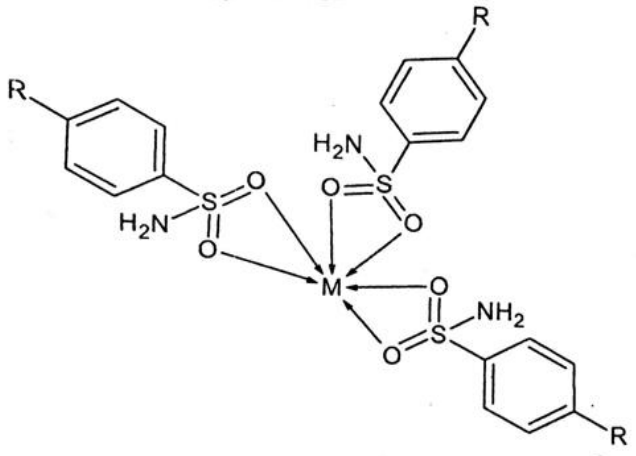
The IR spectra of the complex shows a sharp band at 1612 cm^{-1}, attributed to v(–C=N–), which is shifted to lower frequency on going from the free ligand (at 1625 cm^{-1}) to the complex. This is indicative of the coordination of the imine nitrogen to the metal. Deprotonation of all phenolic functions is confirmed by the lack of –OH stretching bands in the IR at 3400 cm^{-1} for copper complex The band at 1212 $cm^{-1}$for [HL] is ascribed to the phenolic C–O stretching vibrations. This band is shifted to lower frequencies due to O–metal coordination. The v(NH) mode of the sulfonamide group/amino group in the uncoordinated Schiff base remains unchanged in the spectrum of this complex. This suggests that the sulfonamide nitrogen or amino group is not taking part in coordination. The bands, in this ligand, due to vasym (–SO_2) and vsym (–SO_2) appear at 855 and 640 cm^{-1}, respectively. These remain almost unchanged in the spectrum of complex, indicating that sulfonamide oxygens are not participating in coordination. In

the free ligand, the sharp band observed at 1496 cm^{-1} is due to v(–N=N–) stretching frequency of azo–dye. In copper(II) complex, this band remains quite unchanged confirming the noninvolvement of the azo-dye nitrogen in complex formation [17]. The IR spectrum of copper(II) complex exhibits two bands at 1590 and 1435 cm^{-1} which are characteristic for vasym (–COO–) and vsym (–COO–) of the symmetrical group COO– present in bridging complexes [18]. For copper(II) complex two new bands appear in their IR spectrum at 434 cm^{-1} and 454 cm^{-1}respectively which are absent from the IR spectrum of the free ligand, these can be assigned to vM–N and vM–O band.

Scheme synthesis

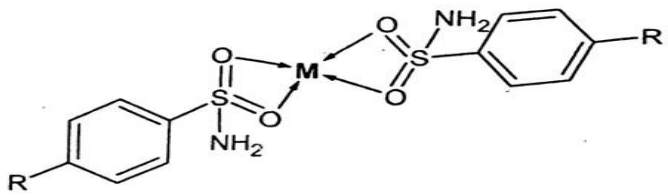
The reaction between the sulonamide and metals can be reprasnted in fig(4)ur





AL:sulfonamide ,1:3ratio

ulfonamid complex ratio 1:2complex



Results and Discussion

Copper complex was prepared by reaction of copper salt with ligand. The molar conductivity at room temperature of 10^{-3} M solutions of complex in DMSO was at 16 $\Omega^{-1}cm^{2}$ mol^{-1} corresponding to nonelectrolyte. The copper complex was sparingly soluble in water, ethanol, acetone and most of the organic solvents but completely soluble in DMSO and DMF. Higher melting point of the copper complex than the free ligand indicated the stability of the complex. The analytical data revealed that copper complex possessed 2:2 copper to ligand stoichiometry based on elemental analysis, the complex was assigned the composition

Conclusion

Our proposed structure of azo-dye Schiff base ligand on the basis of the IR, 1H NMR, mass, UV–vis. spectra has potential binding sites towards the copper ion and act as bidentate chelate by coordinating through hydroxyl oxygen and azomethine nitrogen. Spectral characterizations of the new complex showed that Cu(II) form four coordinate square-planar complex with 2:2 (copper:ligand) stoichiometry. The proposed structure of complex was geometrically optimized and their structural parameters were calculated on the basis of using DMOL3 program were performed in Materials Studio package program and the calculated data were correlated with the current experimental data. The complex can effectively cleave plasmid DNA in the presence of H_2O_2 as an oxidant. The mycological studies reveal that the compounds act as anti pathogenic agents and anti pathogenic behavior provokes on coordination.

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Conflict of interest:

there no conflict of interest.

Funding of research:

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