CRYSTAL GROWTH AND NON-LINEAR APPLICATION OF SULPHA DRUG-AN INVITRO ANTIBACTERIAL EFFECT AGAINST ESBL (EXTRENDED SPECTRUM OF BETA LACTAMSE) E.COLI

P.Dhatchana moorthy[1], V.S.Sangeetha[2], S.Suguna[3], V.Bhakyajothi[4]

Assistant professor,

[1]Department of chemistry ,Dhanalakshmi Srinivasan College of Arts & Science for Women(Autonomous)

Perambalur

ABSTRACT

A probe into the literature survey clearly reveals no work has been carried out for the synthesis of 4-(Phenyl sulfonyl) morpholine and 4-(Phenyl sulfony) morpholine potassium hydrogen phosphate using TLC 4-(Phenyl sulfonyl)morpholine and 4-(Phenyl sulfony) morpholine potassium hydrogen phosphate. Aim and scope of the work is highly effective medicinal hydrazide derivatives have been synthesized from Schiff base route. The structure of the ligand THC and its complexes were carried various spectral studies. From these studies, I know about the knowledge of organic chemistry and spectroscopy. In my present work, we have to synthesis of and 4-(Phenyl sulfonyl) morpholine and 4-(Phenyl sulfony) morpholine potassium hydrogen phosphate establish the structure through the analytical (elemental and TLC) and spectral (IR, H^1 NMR) methods of characterization. The objective of the proposed work are

KEY WORDS: sulpha drug ,TLC, FTIR AND H^1 NMR

INTRODUCTRION

Synthesis of sulpha drugs and its importance

Sulfa drugs containing sulfonamide functional group which have extensive biological activities revolutionalised the field of medical sciences [**1**]. Folic acid, an important chemical for synthesis of bacterial DNA and RNA, is inhibited by sulfonamides; production of new DNA and RNA is decreased by the deficiency of

tetra hydro folate which ultimately decayed the bacteria. Microorganism's normal growth is inhibited due to mistaken attempt by bacteria to convert sulfonamide instead of p-amino benzoic acid for synthesis of folic acid. It also shows relatively high thermal stability. Furthermore, it can be grown to large crystals by the solvent evaporation method, which is much easier for large crystal growth than the commonly used high temperature solid-state method. All these make the crystal a new potential NLO material in the IR region.

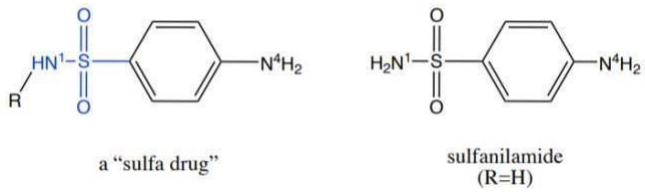
Various biological aspects of the metal complexes (exclusively depend on the ease of cleaving the bond between the metal ion and the ligand. It is therefore, important to understand coordination behaviour and relationship of the metals and the ligands in biological systems. In view of the versatile chemistry of sulfonamides as ligand we have started a program [**3**] in synthesizing and designing various metal-based sulfonamides and to investigate their structural and biological behaviour.Nonlinear optics (NLO) includes the interactions of different frequencies (including frequency doubling and tripling) of light with a material. NLO materials have profound applications in optical and electro-optical devices such as optical data storage, optical signal processing, optical communication, optical data processing devices [**4**], for all-optical data conversion, format switching and wavelength/sign conversion. The study of NLO response (hyper polarizabilities) of organic compounds is an interesting topic and has attained the attention of researchers since last one decade. The interest in organic compounds having high

NLO response, have attracted considerable attention because of their ease of synthesis, stability and usefulness as advance NLO materials. Firstly, the NLO response of organic compounds was reported by Davydov and colleagues in 1970s. Afterward, large number of organic compounds have been explored for their linear and nonlinear optical properties, both experimentally as well as theoretically [**5**].

Structure and Crystallization of sulpha drug

Sulfa drugs were some of the very first antibiotic agents ever discovered and remain a cornerstone of medical science. Also called sulfonamides, they are

characterized by this eponymous group, which consists of a sulfonyl singly bonded to an amine. The sulfonamide is positioned adjacent to a phenyl ring, as seen in sulfanilamide, one of the simplest sulfa drugs.



a "sulfa drug" sulfanilamide (R=H)

Sulfa drugs inhibit the folate pathway by binding to dihydropteroate synthase (DHPS) in the place of its natural substrate pABA. Mutations in DHPS that result in resistance to sulfa drugs occur near this protrusion, "relegating sulfonamide-based therapies to second- or third- line options. The sulfa drug is toxic to all cells which undergo the rapid cancer cell division. To improve the medicinal effect and reducing toxicity of the drug, it is used only their derivatives as a potential drug. The chemical modification of sulfanilamide drug gives the broader antibacterial activity and different pharmacological actions [**6**].

Today, sulfonamides are mainly used in a fix dose combination with trimethoprim (TMP), a di hydro folatereductase (DHFR) inhibitor. Co-tri moxazole, a combination of sulfa meth oxazole (SMX), and TMP, is the most commonly prescribed. This cheap and orally bioavailable combination is used as a second-line therapy to treat a wide variety of bacterial infections including urinary tract infections (UTIs), bronchitis, traveler's diarrhea, and methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Nonlinear optical semi-organic materials play a wide role in fast-developing fields such as upto electronics, optical modulation, optical switching frequency shifting, signal processing, sensor, and optical data storage for the development of technologies in telecommunication and information process (**7**).

The activity of the sulfa drugs has been extensively studied and can be explained in the following manner. Sulfonamides are typically administered in doses that are bacteriostatic, meaning they prevent or limit bacterial multiplication.

Sulfonamides can be classified into three major groups: oral absorbable agents, oral non absorbable agents and topical. Oral absorbable agents may be further classified as short, medium, or long acting sulfonamides. Sulfonamides are absorbed from the stomach and small intestine and widely distributed to tissues. Sulfonamides and inactivated metabolites are excreted by the kidney mainly through glomerular filtration [**9**].

X-ray powder diffraction (XRPD) is the most convenient way to determine crystal forms, since different crystal forms produce different diffraction patterns. Although a relatively large amount of API is usually required for detailed investigation and the sample is also reusable. Theoretically, a slight difference in diffraction pattern should indicate different crystal forms; however, slight differences are frequently observed for identical crystal forms of actual candidates due to many reasons including difference in crystallinity, difference in particle size and habit, and contamination of the small amount of the different crystal form. Other spectroscopic methods, including infrared, Raman, and near-infrared spectroscopy, may also differentiate crystal forms [**10**].

EXPIRIMENTAL METHODS

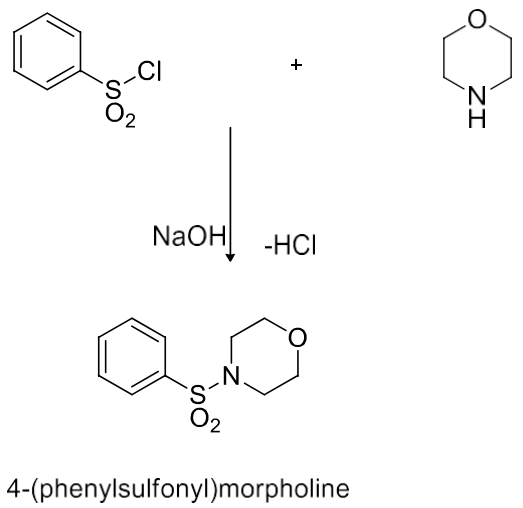
4.2.1. SYNTHESIS OF 4-(PHENYLSULPHONYL)MORPHOLINE (MBS)

CHEMICALS REQUIRED:

Benzenesulphonyl chloride : 2.5523 ml Morpholine : 1.725 ml Ethanol

3.5 g of the benzene sulphonyl chloride (2.5523mole) taken in 1:1 molar ratio. Add one sodium pellet dissolved in 10 ml of water. 1.7 ml of morpholine (1.725 moles) was taken in a round bottom flask and 10 mL of ethanol was added. To this solution, 10 mL ethanolic solution of 3g of the benzene sulphonyl chloride(2.5523 moles) was added and stirred well for one hour by keeping the reaction mixture on a magnetic stirrer. After one hour a crude solid was obtained (**Scheme 3**). This crude solid was washed with water two to three times and dried then finally washed with ethanol and kept in over a vacuum for two days. The crude sample

was re crystallised from ethanol. The purity of the compound was checked by Thin Layer Chromatography (TLC).



4-(phenylsulfonyl)morpholine

Scheme 1. Synthesis of 4-(Phenylsulphonyl)morpholine.

Co-crystaliztion of 4-(phenylsulphonyl)morpholine with potassiumdihydro orthophosphate (mbspdo) chemicals

MATERIALS REQUIRED:

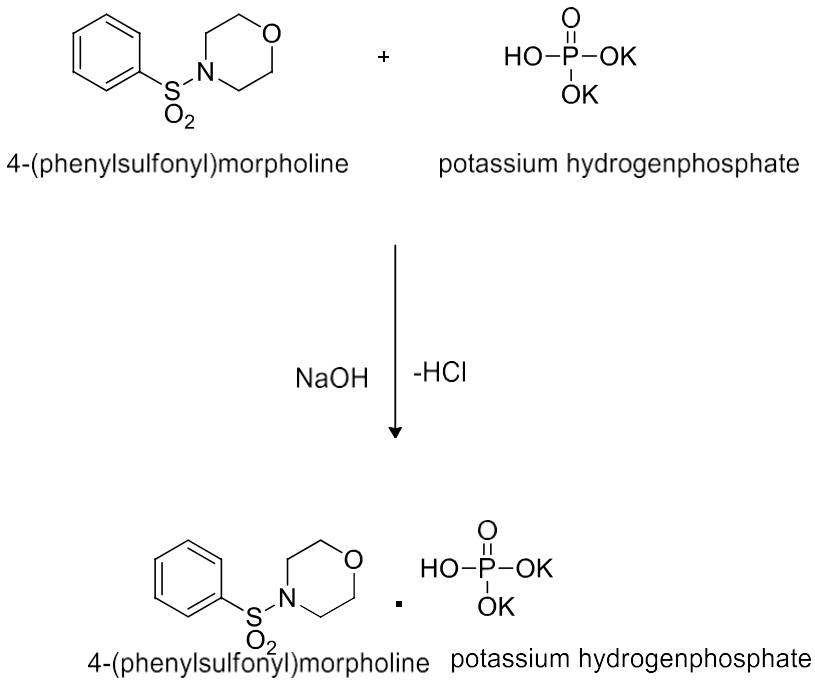
Benzene sulphonamide : 0.0022026 g

Potassium dihydro orthophosphate : 0.29975 g

Ethanol

0.5 g of the benzene sulphonamide (0.022026 moles) taken in 1:1 molar ratio. 0.2 g of potassium di hydro orthophosphate was taken in a round bottom flask and 10 mL of water was added. To this solution, 10 mL ethanolic solution of 0.5 g of the benzene sulphonamide (0.0022026 moles) was added and stirred well for one hour by keeping the reaction mixture on a magnetic stirrer. After one hour a crude solid was obtained (**Scheme 4**). This crude solid was washed with water two to three times and dried then finally washed with ethanol and kept in over a vacuum for two days. The crude sample was re crystallised from ethanol. The purity of the

compound was checked by Thin Layer Chromatography (TLC)



Scheme 2. Synthesis of 4-(Phenylsulphonyl)morpholine with potassium hydrogenphosphate.

Analytical techniques:

Elemental Analysis:

Our objective is to detect the presence of nitrogen, sulphur, chlorine, bromine and iodine in organic compounds by **Lassaigne's** test. A small piece of dry sodium was melted in a fusion tube. Then 0.1 g of solid substance was added to the molten sodium. It was heated gently at first, then to red hotness. Quickly plunged red hot end of tube into 10mL distilled water in a china dish. It is stirred well with broken end of tube, boiled and filtered.

Test for nitrogen:

Few crystals of ferrous sulphate was added with 1ml of fusion extract. It was boiled, cooled and then added two-ml of diluted sulfuric acid. Sodium

cyanide is converted to sodium ferrocyanide on treating with ferrous sulphate. The green colour solution developed, it indicates the presence of nitrogen.

$$Na + \underset{\text{(From organic compound)}}{C + N} \xrightarrow{\text{Fuse}} NaCN$$

$$FeSO_4 + 2NaOH \rightarrow \underset{\text{(Green ppt.)}}{Fe(OH)_2} + Na_2SO_4$$

$$Fe(OH)_2 + \underset{\text{(From Sodium extract)}}{2NaCN} \rightarrow Fe(CN)_2 + 2NaOH$$

$$Fe(CN)_2 + 4NaCN \rightarrow \underset{\text{Sod. ferrocyanide}}{Na_4[Fe(CN)_6]}$$

$$3Na_4[Fe(CN)_6] + 4FeCl_3 \rightarrow \underset{Ferric\ ferrocyanide}{Fe_4[Fe(CN)_6]_3} + 12NaCl$$

Test for halogen

One-ml of dilute nitric acid is mixed with one-ml of fusion extract. It is boiled, cooled and then added 1ml of silver nitrate solution. The halide ions chloride, bromide and iodide ions are giving only curdy white, pale yellow and yellow precipitate respectively but the compound MBS and MBSPDO does form curdy precipitate which is soluble in ammonium hydroxide. Hence we have conclude the sample MBS and MBSPDO has presence of only chlorine is confirmed.

Test for sulphur

1. Lead acetate test

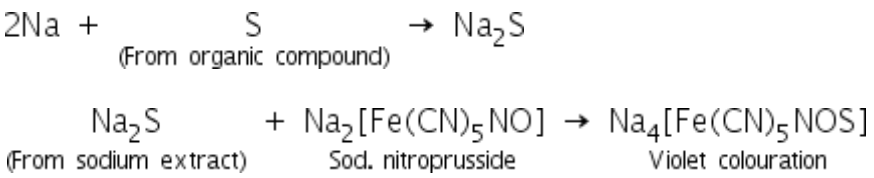
Sodium sulphide formed during the preparation of Lassaigne's extract reacts with lead acetate to yield lead sulphide as black precipitate.

$$Na_2S + (CH_3COO)_2Pb \rightarrow \underset{\text{Black precipitate}}{PbS} + 2CH_3COONa$$

2. Sodium nitro prusside test

During the preparation of Lassaigne's extract, sulphur from the organic compound reacts with sodium to form sodium sulphide. It gives a purple colour with sodium

nitroprusside due to the formation of sodium thionitroprusside.



From this test conclude the synthesized compound has both nitrogen and sulphur is present in it.

Antibacterial activity

Disc diffusion method was used for antibacterial activity. The methicillin Resistant *Staphylococcus aureus* (MRSA) pure isolates used in this study. A stock solution of extract was prepared by dissolving 10 mg/Ml of ethanol to produce a final concentration of 1 mg/mL. The stock solution was then loaaded to sterile disc at concentrations of 25, 50, and 100 µg. 30 *µg amoxicillin clavanulate used as positive control and ethanol serve as negative control.* Each of the disc were allowed to air dry and place over the agar plate previously swabed with test pathogen and incubated at 37° C and zone of inhibition was measured.

RESULTS AND DISCUSSION

Spectral Characterization:

FT-IR Spectral studies:

In order to study of functional group of the synthesized co-crystal, the IR spectrum was compared with the general functional ranges. The IR spectrum of co-crystal showed characteristic broad band at 3463 cm^{-1} can be attributed to ν(O-H) and aromatic ν(ArC-H) stretching vibrations also overlapped with this broad peak. It is indicated, the co-crystal was connected through ionic interaction as well as weak O…H hydrogen bonding. The weak interaction was depends on the concentration of the solution. Another distinctive vibration expected for S=O observed at 1105 cm^{-1}. In the carbonyl group stretching vibrations also appeared at 1653 cm^{-1} is due to some solvent impurity present in the compound. The phosphate counter anion also appeared at 1047 and 598 cm^{-1} and all other peaks are good agreement with the proposed structure. The FT-IR spectral data are given in table 4 and figure 1.

Table 4 Important IR bands of Schiff base with their assignments.

Vibrations	ν(O-H)	ν(S=O)	ν(P=O)	ν(P-O)
Peak (cm^{-1})	3463	1105	1047	598

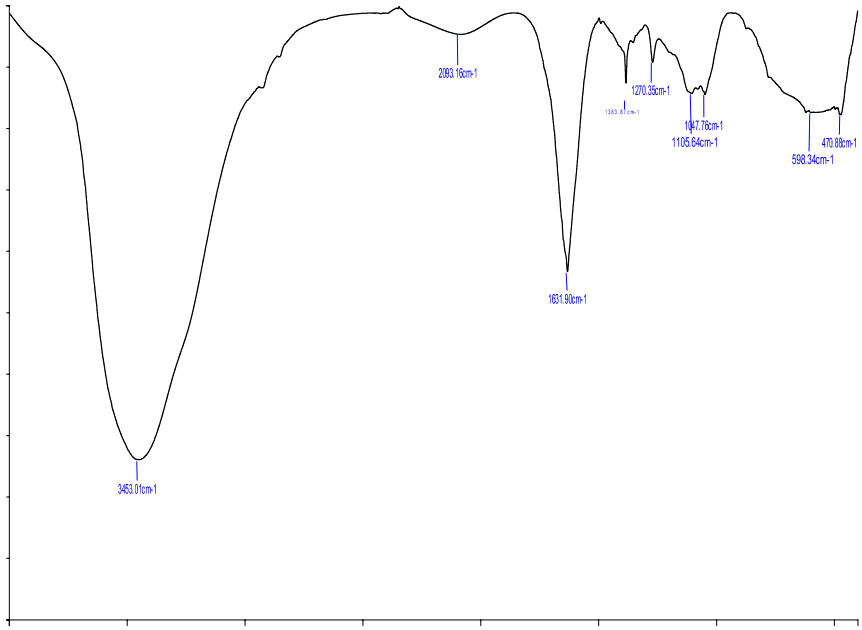


Figure 1. FT-IR spectrum of 4-(phenylsulfonyl)morpholin-4-ium dihydrogenphosphate

NMR spectra analysis

In ^{1}H NMR spectrum, the co-crystal showed only five signals for cation and one signal for counter anion. This is due to center of symmetry present in the molecule. The morpholine protons are highly distinctive in nature. It appeared as two unique triplets in the aliphatic region. Signal at δ = 3.5 and 4.11 ppm clearly indicates, molecule contains four aliphatic protons. Broad peak at δ = 7.0 ppm represent the morpholine nitrogen atom having positive charge. On the other hand, the phenyl ring shows only three signals as discussed early due to C2 symmetry. On the phenyl ring, C2 & C6 as well as C3 & C5 carbon on the same environment and gives peak at δ = 7.86 and 7.62 ppm as a doublets. The hydrogen attached to C4 carbon appeared as multiplet at δ =

7.71 ppm. The characteristic phosphate anion was appeared as broad singlet at δ = 2.0 ppm. The detailed assignments of protons were given in table 5 and figure 2.

Table 5 ^{1}H NMR Spectroscopic Data (δ) of 4-(phenylsulfonyl)morpholin-4-ium dihydrogenphosphate

S. No	Position Assignment	^{1}H (δ, ppm)
1	1	--
2	2, 6	7.86, d
3	3, 5	7.62, m
4	4	7.71, m
5	1’	7.0, s
12	2’, 6’	3.50, t
13	3’, 5’	4.11, t
14	anion	2.0, s

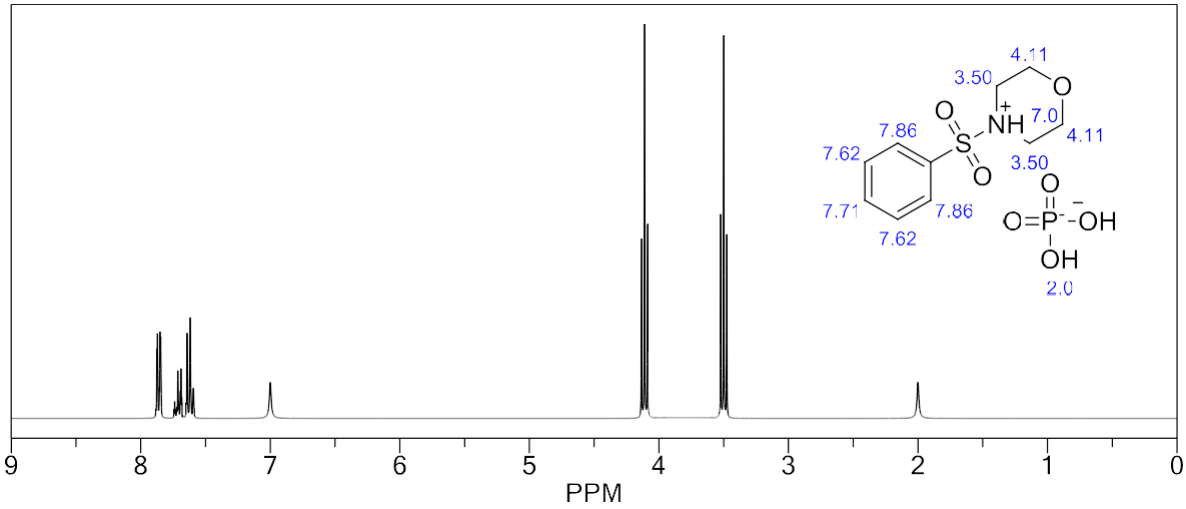


Figure 2. ^{1}H NMR spectrum of 4-(phenylsulfonyl)morpholin-4-ium dihydrogenphosphate

ANTIBACTERIAL STUDY:

According to the results of disc diffusion assay (plate 2), this compound has active compounds that are effective for the prevention of infections caused by MRSA. The maximum zone produced by the compound against the MRSA was 16 mm at 100 µg. The lowest zone of growth inhibition was 10 mm at 25-50 µg and no activity was recorded at negative control.while the positive control (AMC) showed inhibition diameters 20 mm (table 5). The percentage of relative zone of inhibition of synthesized compound was calculated as 80%. It was reported that, sulfonamide showed the highest inhibitory effect on gram positive bacteria i.e. *S. aureus, N. asteroides, N. farcinia and B. subtilis (*Bekdemir et al., 2004).

Antimicrobial activities of the sulfonamides depend on substituent and their position in the benzene ring [**52**].

Plate 2 Antibacterial activity of synthesized compound against *S.aureus*

Table 6. Zone of inhibition of synthesized compound against *S.aureus*

Organism	Zone and inhibition mm in dm					
	NC	Amc	25 µg	50 µg	100 µg	RIZD
S.aureus	2	20	12	12	18	80%

CONCLUSION

The chapter I of the thesis present about introduction and summary ofthe previous work done on the Schiff base and the application of the bases and relevance for carrying out this work. The chapter II, consist of for parts, deals with the Aim and Scope of the present work. The chapter III of the thesis discuss about probe into the literature survey clearly reveals no work has been carried out for the synthesis of 4-(Phenylsulphonyl)morpholine and potassiumhydro orthophosphate using4-(Phenylsulphonyl)morpholine and potassiumhydro orthophosphate in detail study of Schiff base.

In chapter IV of thesis explain the materials and methods of Schiff base. The chapter IV, consist of four parts, deals with synthesis and characterization of 4-

(Phenyl sulphonyl)morpholine and potassiumhydrogenphosphate.

Chapter V covers experiental data and Results. The elemental analysis shows the presence of nitrogen is confirmed by using sodium fusion extract. The FT-IR spectral study information. The ^{1}H-NMR spectral studies of the ligand. The morpholine protons are highly distinctive in nature shows C_2 symmetry and S=O. The number of singal snappeared in the ^{1}H –NMR, confirm the expected position of H and C atoms as per the molecular formula and structure of the ligand. The signals appeared in both the spectra gives the exact position of each proton and carbon respectively as expected. The bioassays indicated that the synthesized compounds showed potential antibacterial agents.

Future work:

In future, an attempt has been for the biological studies such as Anti-caners activities and Anti-oxidant activities for synthesized derivative.

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