SYNTHESIS, CHARACTERISATION AND ANTI BACTERIAL STUDIES OF [E]-2-(3,4,5- TRIMETHOXY BENZYLIDENE) HYDRAZINE CARBOTHIOAMIDE

V S Sangeetha[1] ,D Deepa[2] , M Kanmani[3], R Shanmuga Priya[4]

[1]Department of chemistry, DhanalakshmiSrinivasan College of Arts and Science for Women(Autonomous)Perambalur,621212,Tamilnadu, India

ABSTRACT

The chemistry of macromolecule like shiff bases get major attensionbecouse of its immobilization activities and hence they are alternatives for biopolymer biosensors in lot of various industries. The polymer based macromolecule coordinate with metal and the resulting material act as sensors .In this work, we synthesised (E)-2-(3,4,5trimethoxy benzylidene)hydrazinecarbothioamide and establish its structure through the analytical (elemental and TLC) and spectral (IR, H1 NMR and C13 NMR) methods of characterization .The structure of the ligand THC and its complexes were carried out. The changes in the structural features of the ligands are observed in the fingerprint region (4000-400 cm-1). The bands due to the metal ligand bonds are mainly observed in the far IR region (600-100 cm-1).In the present study, IR spectra of the compounds were recorded using Perkin Elmer spectrum RXI using KBr pellets at frequency range 4000-400 cm-1.1H NMR of the ligands were recorded using Bruker 300 MHz Avance –II FT-NMR Spectrometer with DMSO-d6 as the solvent and TMS as internal standard.[13]C NMR of the synthesized compounds were recorded on 75 MHz Bruker Spectrometer at 298.6 K using DMSO-d6 as solvent.

Key words: Ligand, complex Schiff bases,[1]HNMR,[13]CNMR, *DMSO,antibacterial,* FT-IR *etc.*

INTRODUCTION

Organic base is an organic compound which are usually, but not always, proton acceptors. Nitrogen containing organic bases can be easily be protonated. All the amines and heterocyclic compounds containing nitrogen as hetetro atoms such as Imidazole, benzimidazole, histidine, Pyridine, alkanamines, guanidine, ,methylamine, phosphazene bases are examples of organic bases.

Schiff bases are playing an important role in decarboxylation, transamination and C-C bond cleavage reactions taking places in living organisms .Transamination and Decarboxylation are important steps in the biosynthesis of neurotransmitters , hormones, and pigments. The chemistry of macromolecules like Schiff bases has been receiving good attention in various industry areas because they are one of the apt immobilization alternatives for biopolymer biosensors. The coordinating ability of the polymer-based macromolecules like shiffbases with metal permits these materials to act as sensors.

It is interested to synthesize the various ligands and complexes because these ligands and complexes that having sulphur and nitrogen have wide applications for the preparation of of drugs. And because of its medical application , there is considerable interest in the preparation and structural stusies of these compounds. There having problem of using Drug resistances for medical purpose as antibacterial agents. The preparation of metal complexes by the a process of chelation with the coordination of transition metal ions can used to overcome these problems. N atoms are the hetero atom in Schiff bases and they are the basic elements. The donor atoms in Schiff base derivatives can act as good chelating agents for the transition of metal ions. Research studies shows that, Schiff bases and their metal complexes have been widely studied due to their import fungicidal-bactericidal , anticancer , antiparasitic, , properties.

Schiff Bases

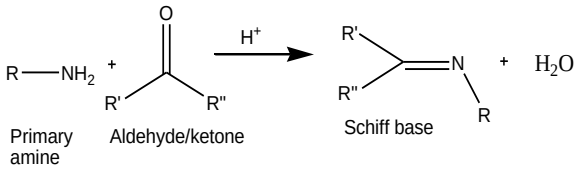
Schiff bases are condensation products of primary amines and carbonyl compound .The project has been aimed at the synthesis of some novel heterocyclic compounds like Schiff bases and their cyclisation to produce (Azetidinones) Betalactam derivatives of biological significance.

A Schiff base (-C=N-R) is a nitrogen analog of an aldehydes or ketones in which the C=O group is replaced by amine group. It is usually formed by condensation of an aldehydes or ketones with a primary amine and they are explained with schemes. According to the following scheme-I,
Where R may be an alkyl or aryl groups Schiff base that contain aryl substituents are substantially more readily synthesized, while those which contain alkyl substituent are relatively unstable. Schiff base of aldehydes are relatively unstable and readily polymerizablewhile those of aromatic aldehydes having effective conjugation are more stable.

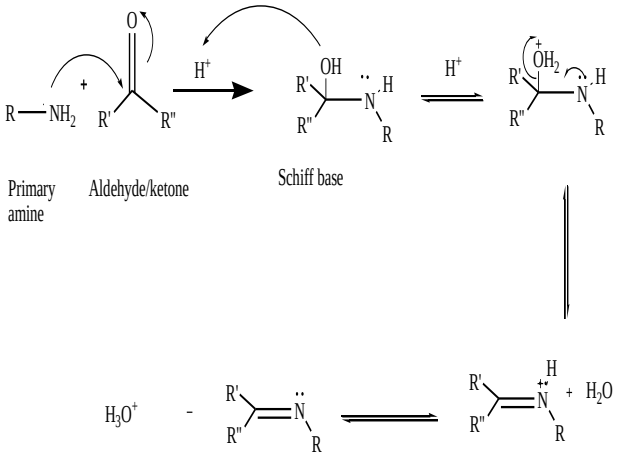
Mechanism of formation of Schiff base

The mechanism of Schiff base formation is another variation on the theme of nucleophilic addition to the carbonyl group. In this case the nucleophilic is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or carbinolamine loses water by either acid or base acid catalyzed pathways. Since the carbinolamine is an alcohol it undergoes acid catalyzed dehydration.

Typically the dehydration of the carbinolamine is the rate–determining step of Schiff base formation and that is why the reaction is catalyzed by acid. Yet the acid concentration cannot be too high because amines are basic compounds. If the amine is prorogated and become non-nucleophilic equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore many Schiff base syntheses are best carried out at mildly acidic (Scheme 2).



Scheme 1. General synthesis of Schiff base



Scheme 2: General mechanism of formation of Schiff base

Schiff bases metal complexes

Transition metal are known to form Schiff bases complexes and Schiff bases have often been used as chelating legends in the field of coordination chemistry

Their metal complexes have been of great interest for many years, It is well known that N,O and S atom play a key role in the coordination of metals at the active . sites of numerous metallic biomolecules[27]. Schiff base metal complexes have been widely studied because they have industrial, Antifungal, Antibacterial, Anticancer, Antiviral and herbicidal applications[28,29].

They serve as models for biologically important species and find applicationin biomimetic catalytic reactions. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities .

There is certain metalloid – elements without which the normal functioning of living organism is inconceivable. Among these metallo – elements so called, metal of life four member form an island. These are Na, Mg, K and Ca the transition elements are V, Cr, Mn, Fe, Co, Ni, Cu and Zn.

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EXPERIMENTAL METHOD

Materials and methods

synthesis of (*e*)-2-(3,4,5-trimethoxybenzylidene) hydrazinecarbothioamide:

synthesis of THC:

chemicals required**:**

3,4,5-trimethoxybenzaldehyde (0.0076 mole)	=1.5g
thiosemicarbazide(0.0076 mole)	=0.7g
ethanol	=10mL
diethyl ether	=10mL

3,4,5-trimethoxybenzaldehyde and thiosemicarbazidewere taken in 1:1 molar ratio. 0.7 g of thiosemicarbazide(0.0076 mole) was taken in a round bottom flask and 40 mL of ethanol was added. To this solution, 10 mL ethanolic solution of 1.5gof 3,4,5-trimethoxybenzaldehyde (0.0076 mole) 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 . This crude solid was washed with water two to three times and dried then finally washed with diethyl ether and kept in over a vacuum for two days. The crude sample was recrystallised from ethanol. The Thin Layer Chromatography(TLC) is used to check purity of the compound .

Scheme 3 :Synthesis of (*E*)-2 (3,4,5 trimethoxybenzylidene)hydrazinecarbothioamide

Scheme 4: Thioamide and thioltautomerism of
(*E*)-2-(3,4,5trimethoxybenzylidene) hydrazinecarbothioamide

Analytical techniques - Elemental Analysis:

Our objective is to detect the presence of nitrogen, sulphur, chlorine, bromine and iodine in organic compounds by Lassaigne'stest.A small piece of dry sodium was melted in a fusion tube. Then 0.1g 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 sulphatewas added with 1ml of fusion extract. It was boiled, cooled and then added two-ml of diluted sulfuric acid. Sodium cyanide on treating with ferrous sulphate converted to sodium ferrocyanide. The green colour solution developed, it indicates the presence of nitrogen

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 white, pale yellow and yellow precipitate respectively but the compound THCdoes not formany precipitate which is sparingly soluble in ammonium hydroxide pale. Hence we have conclude the sample THChasabsence of halogen is confirm

Test for sulphur -Lead acetate test

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

Sodium nitroprusside test

Sulphur from the organic compound reacts with sodium to form sodium sulphide during the preparation of Lassaigne's extract . It gives a purple colour with sodium nitro prusside due to the formation of sodium thionitroprusside

TLC

Thin Layer Chromatography has been used as an analytical tool, especially in organic chemistry because of its high speed of separation and its applicability in a large number of chemical compounds. The high sensitivity of TLC is used to check the purity of the samples. With the help of TLC, it is possible to know whether a reaction is complete and had followed the expected course.

Thin Layer Chromotography was made by dipping a glass plate in slurry of silica gel G, prepared by shaking silica gel G with chloroform-methanol (2:1) mixture at room temperature. The homogeneity of the compounds was monitored by this TLC plates and visualized by iodine vapour.

Spectral methods

Infrared spectroscopy

The best methods for the characterization for metal complexes are IR spectroscopy. The basic theory involved in IR is that upon complexation the stretching vibration modes of the ligands changes due to strengthening or weakening of the bonds involved in the bond formation and hence cause in subsequent change in the position of the bands appearing in the IR Spectrum. The changes in the structural features of the ligands are observed as changes in bands observed, mainly in the fingerprint region (4000-400 cm^{-1}). The bands due to the metal ligand bonds are mainly observed in the far IR region (600-100 cm^{-1}).

In the present study, IR spectra of the compounds were recorded using Perkin Elmer spectrum RXI using KBr pellets at frequency range 4000-400 cm^{-1} and Shimadzu FT IR 400 Spectrophotometer, frequency range 4000-400 cm^{-1} using KBr

Nuclear Magnetic Resonance spectroscopy

^{1}H NMR:

Differences in the magnetic properties of various magnetic nuclei present in the molecule can be Studying by NMR spectroscopy and also it enables us to deduce the positions of this nucleus within the molecule. One can find out how many different kinds of environments there are in the molecule and also which atoms are present in neighboring groups. Usually, the number of atoms present in each of these environments is measured. Therefore, the diagnostic features of the NMR Spectra are the number of signals, position of signals, splitting pattern of signals and area of signals. ^{1}H NMR of the ligands were recorded using Bruker 300 MHz Avance –II FT-NMR Spectrometer with DMSO-d_6 as the solvent and TMS as internal standard

^{13}CNMR:

^{13}C has a spin quantum number equal to ½ and its nuclear magnetic resonance can be observed in a magnetic field of 23,500 gauss at 25.2 mega cycles per second. ^{1}H spectrum is normally

obtained by sweeping either the excitation frequency or the held through the region of precession frequencies. The inefficiency of this method is clear from the fact that only one line can be observed at a given point in time. The problem arises when ^{13}C with intrinsically narrow lines covering a wide absorption range are studied. It is, therefore, advantageous to excite the whole band of frequencies simultaneously. It is done by strong pulse of radio-frequency covering a large band of frequencies which is capable of exciting all resonance of interest at once. At the end of the pulse period, the nuclei will process freely with their characteristic frequencies reflecting with the chemical environment (Ele. Org. spec-231 &) and exhibit chemical shifts. ^{13}C NMR of the synthesized compounds were recorded on 75 MHz Bruker Spectrometer at 298.6 K using DMSO-d_6 as solvent .

RESULT AND DISCUSSION

Thin layer chromatography(TLC)

Analytical TLC was performed on pre-coated aluminum sheets of silica (60F254) and visualized by short-wave UV light at λ 254 nm. Flash column chromatography wascarried out on silica gel (230-400 mm) and semi-automated purification was carried out crystallization by slow evaporation method. Solvent systems are reported by column volume (CV) with the solvent flow rate as stated. A single spot on TLC silica gel glass plate with ethanol confirmed the purity of the synthesized sample. TLC plate of the compound(*E*)-2-(3,4,trimethoxybenzylidene) hydrazinecarbothioamide is shown in Fig 1.

Rf= distance travelled by given sample

Distance travelled by solvent

$R_f = \frac{3.2}{3.4} = 0.94\ cm^{-1}$

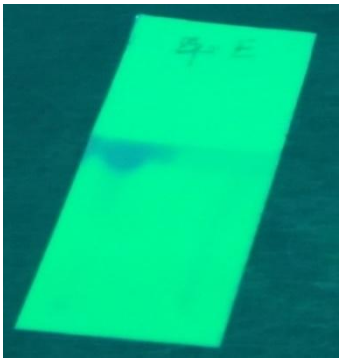

Fig. 1 TLC plate for (*E*)-2-(3,4,5-rimethoxybenzylidene)hydrazinecarbothioamide in ethanol as a eluent**.**

Spectral Characterization:

FT-IR Spectral studies:

In order to study of functional group of the THC Schiff base, the IR spectrum ofTHC was compared with the general functional ranges. The IR spectrum of THCshowed characteristic band at 3393 and 3157 cm^{-1} can be attributed to ν(NH2) and ν(NH) respectively. Aromatic ν(ArC-H) A sharp band appeared at 3026 cm^{-1}. Generally carbonyl group appears at 1680-1700cm^{-1} but the compound THC has appears at 1603 cm^{-1}, this is due to amide group present in the compound which decreases the carbonyl functional group. νC=N and νCNC stretching frequency appears at 1533 and 1106 cm^{-1} respectively. The FT-IR spectral data are given in table 1 and figure 2

Table .1 Important IR bands of THCwith their assignments.

Compounds	ν(NH2)	ν(N-H)	ν(ArC-H)	ν(C=O)	ν(C=N)	ν(CNC)
THC	3393	3157	3026	1603	1533	1106

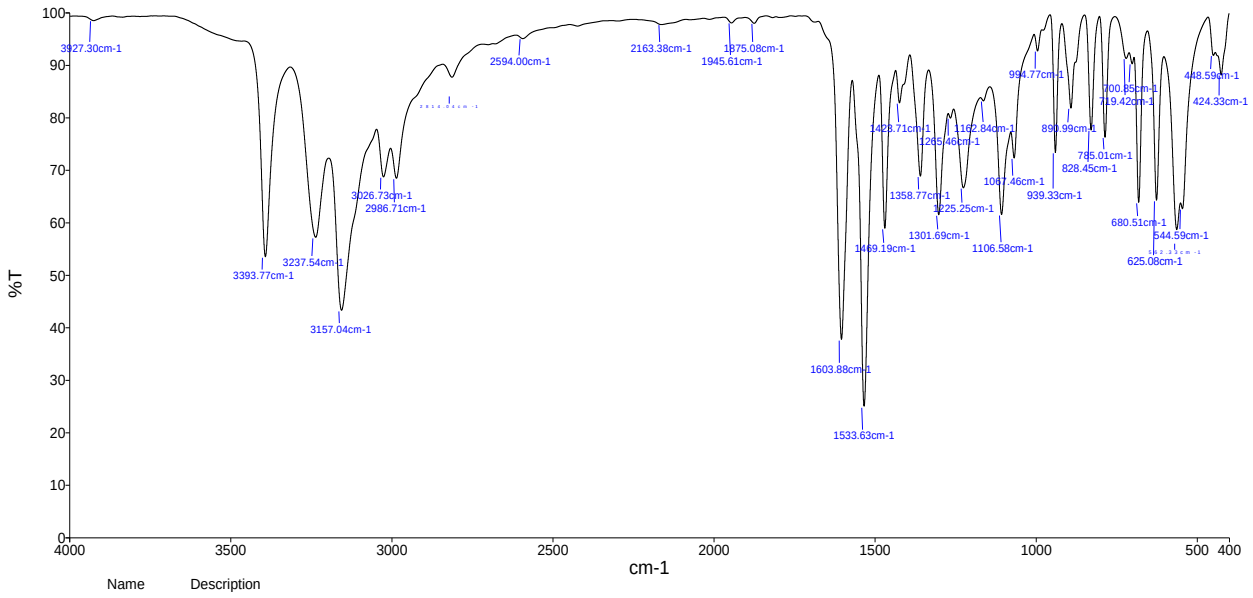

Fig 2.FT-IR Spectrum of (*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazinecarbothioamide

^{1}H NMR and ^{13}C NMR spectrum:

^{1}H NMR spectrum of the compound THC was recorded in DMSO-d_6 (300 MHz) and the ^{1}H-NMR spectrum of THC is shown in Fig. 3 and 3a.A sharp singlet appears at δ 7.1 ppm which is due to phenyl ring of two ortho position proton.A chemical shift appears at 11.4 ppm this is due to amide proton which involeamido-thioltautomerism. The azomethine proton shows singet higher chemical shift δ 8.5 ppm this is due to phenyl ring present in the adjacent position. The NH2 group shows two singlet one is at δ 8.2ppm andanother one is at δ 8.1 ppm due to different chemical environment. A singlet with very intensity appears at 3.8 ppm this is due totwomethoxy group present at meta positions and a singlet appears at 3.6 ppm which is due to para substituted methoxyproton. ^{13}C NMR spectrum of the compound THC was recorded in DMSO-d_6 (75 MHz) and the ^{13}C-NMR spectrum of THC is shown in Fig. 4. A peak appears at 178ppm which is due to carbon of thiocarbonylgroup.Four different environment Aromatic carbons appears at 153,139,130 and 105 and the azomethine carbon appears at 142 ppm. A very intensity peak appears at 56 ppm this is due to carbon of two methoxy group present at meta positions and a low intensity peak appears at 60 ppm which is due to para substituted methoxycarbon.

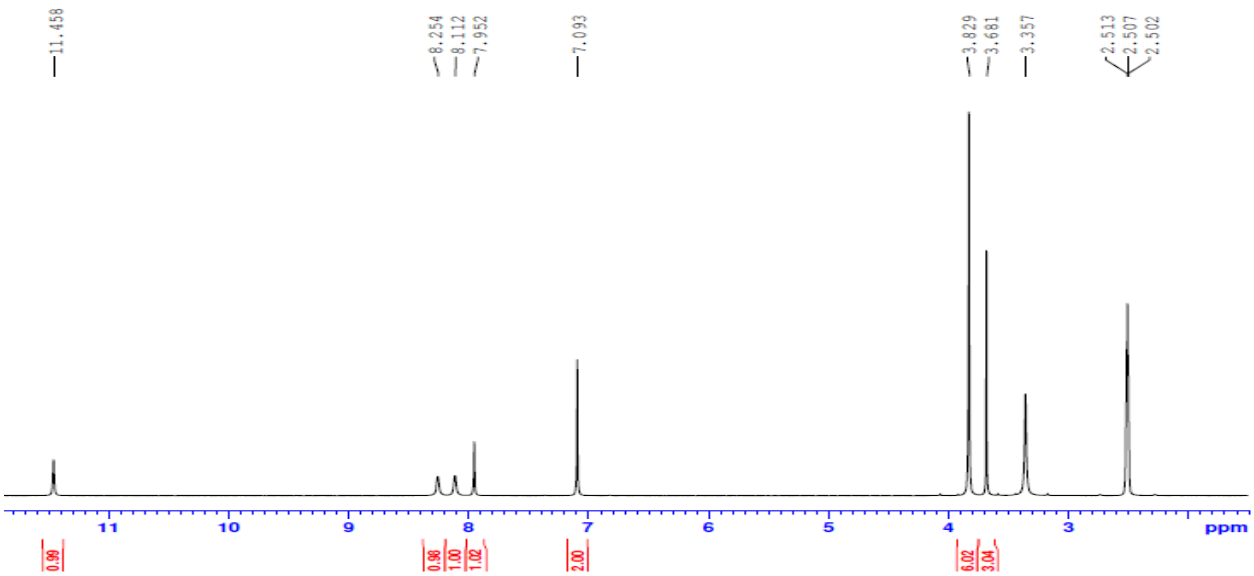

Fig. 3.[1]H-NMR spectrum of (*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazinecarbothioamide

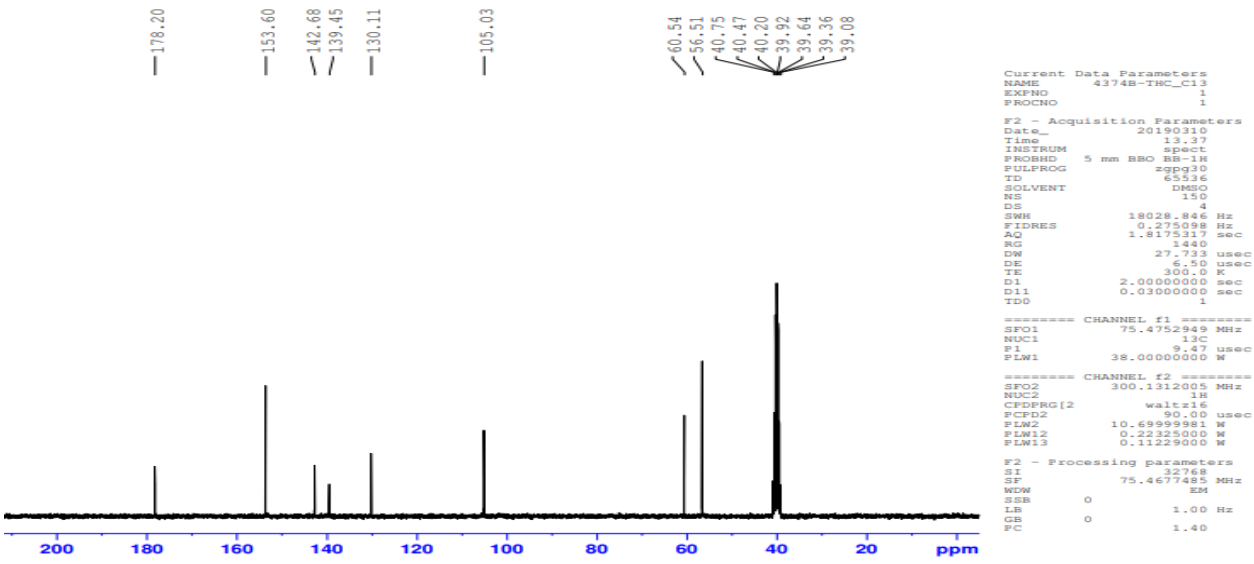

Fig .3a.Expanded[1]H-NMR spectrum of (*E*)-2-(3,4,5-trimethoxybenzylidene) hydrazinecarbothioamide

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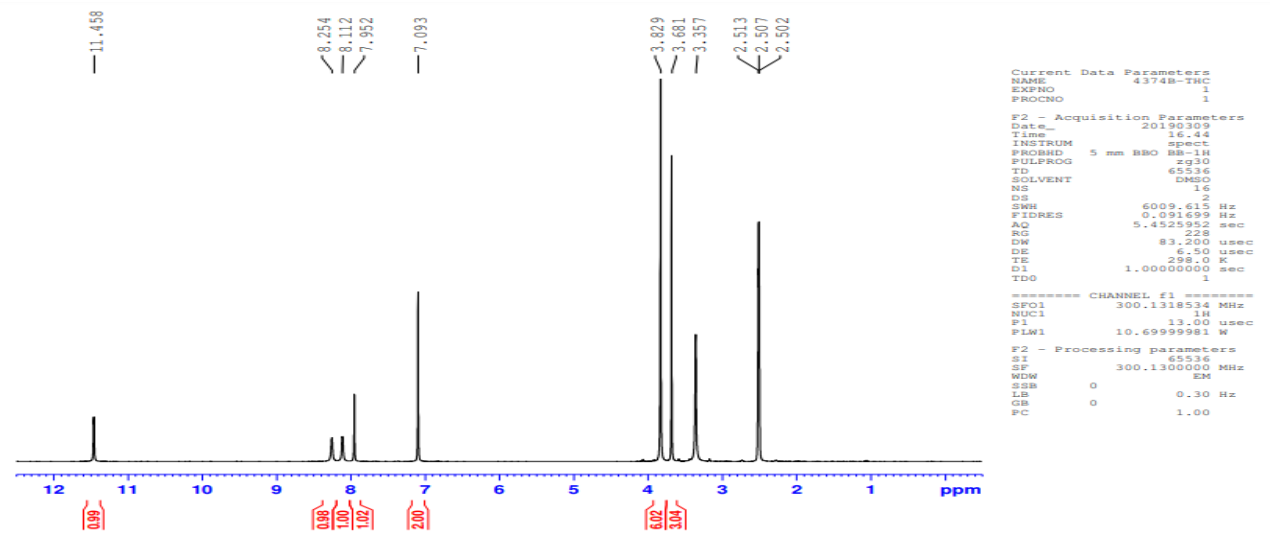

Fig 4.^{13}C-NMR spectrum of (*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazinecarbothioamide

CONCLUSION

The present study deals with synthesis and characterization of (*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazinecarbothioamide. The elemental analysis shows the presence of nitrogen and is confirmed by using sodium fusion extract.From the FT-IR spectral study information ,that is frequencies around ν 3393 and 1603 cm^{-1} ,confirm the presence of amide group and thiocarbonyl group .

In the ^{1}H and ^{13}C NMR spectral studies of the ligand THC the number of signals appeared in the ^{1}H NMR and ^{13}C NMR spectra 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 give the exact position of each proton and carbon respectively as expected. In future it is possible to do the biological studies such as antimicrobial activities, anti-cancer activities and anti-oxidant activities of synthesized THC.

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