SYNTHESIS CHARACTERISATIONAND BIOFILM STUDIES OF [E]-[2,6 DICHLOROBENZYLIDENE]HYDRAZINE CARBOTHIAMIDE

V S Sangeetha[1] ,D Deepa[2] , P Devi[3] ,V Bhakyajothi[4]
[1]Dhanalakshmi College Of Arts And Science For Women(Autonomous),
Perambalur,Tamilnadu, India

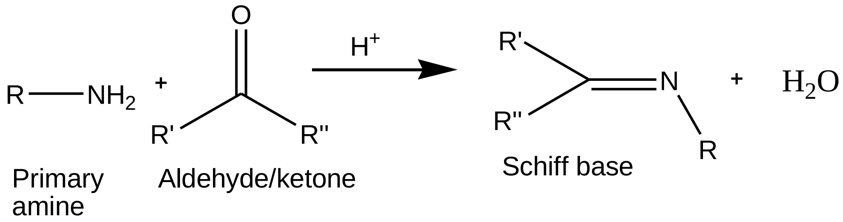
Abstract

A probe into the literature survey clearly reveals no work has been carried out for the synthesis of (E)-2-(2, 6-dichlorobenzylidene)hydrazinecarbothioamide (DHC)using 2,6-dichlorobenzaldehyde and thiosemicarbazide.Aim and scope of the work is highly effective medicinal hydrazide derivative has been synthesized from Schiff base route and also synthesized its some transition metal complexes.The structure of the ligand DHC 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 (E)-2-(2, 6-dichlorobenzylidene) hydrazinecarbothioamide and establish the structure through the analytical (elemental and TLC) and spectral (IR, H^1 NMR and C^{13} NMR) methods of characterization.

INTRUODUCTION:

A Schiff base may be a compound with the final structure R2C=NR’ (R’≠H).They can be thought of a sub-class of imines, being either secondary ketimines or secondary aldimines counting on their structure. The term is usually synonymous with azomethine that refers specifically to secondary aldimines (i.e.R-CH=NR’where R’≠ H).The term Schiff base is normally applied to these compounds when they are being used as ligands to form coordination complexes with metal ions. Schiff bases are condensation products of primary amines and carbonyl compound and they were discord by a German Chemistry Nobel Prize winner Hugo Schiff in 1864[1].The project has been aimed toward the synthesis of some novel heterocyclic compounds like Schiff bases and their cyclisation to supply (Azetidinones) Betalactam derivatives of biological significance.

A Schiff base is formed by the reaction of nucleophilic addition to the carbonyl group. In this reaction amine is the nucleophile. In the first step, the amine reacts with aldehyde or ketone to give an unstable addition compound called carbinolamine. It loses water by either acid or base catalyzed pathways.Since the carbinolamine is associate alcohol, it undergoes acid catalyzed dehydration.Typically the dehydration of the carbinolamine is that the rate determinative step of Schiff base formation which is why the reaction is catalyzed by acids.The acid concentration should not be too high because amines are basic compounds and may be protonated and then becomes non-nucleophile. In that condition, the equilibrium is pulled to the left and carbinolamine formation cannot occur. 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 according to the following scheme-1.



Scheme 1.

General synthesis of Schiff base

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 polymerizable while those of aromatic aldehydes having effective conjugation are more stable.Schiff bases area unit a number of the foremost wide used organic compounds.They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers.Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties.Imine or azomethine teams area unit gift in varied natural, natural – derived and non-natural compounds.The imine cluster gift in such compounds has been shown to be important to their biological activities.

EXPERIMENTAL METHOD

4.1 Characterization techniques used:

Some physical methods were used to elucidate the bonding and structure of the synthesized ligands and complexes and to confirm the expected properties. While the ligands were characterized by usual methods such as analytical technique such as TLC, molar conductance, magnetic susceptibility and spectral techniques such as IR, UV-Visible, NMR and mass spectral techniques, it differs for complexes depending on the nature of the ligands and the metal ions involved. The presence of paired or unpaired electrons of the metal ions imparts the magnetic behavior of the complexes.

Chemicals used as,

All the chemicals used were of Merck and Sigma Aldrich products, available commercially in AR grade. The purchased chemicals were used without any further purification.

The physicochemical techniques employed for the present study is discussed below.

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.

SYNTHESIS, OF (*E*)-2-(2, 6-DICHLOROBENZYLIDENE)HYDRAZINECARBOTHIOAMIDE

SYNTHESIS OF DHC:

CHEMICALS REQUIRED:

2,6-dichlorbenzaldehyde (0.0057mole) = 1g
Thiosemicarbazide(0.0057mole) =0.523g
Ethanol =10mL
Diethyl ether =10mL

2,6-dichlorbenzaldehyde (0.0057 mole)and thiosemicarbazide were taken in 1:1 molar ratio. 0.523g of thiosemicarbazide(0.0057mole) was taken in a round bottom flask and 10 mL of ethanol was added. To this solution, 10 mL ethanolic solution of 1g of 2,6-dichlorbenzaldehyde(0.0057 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 recrystallized from ethanol. The purity of the compound checked by Thin Layer Chromatography(TLC).

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 1 mL of fusion extract. It was boiled, cooled and then added 2 mL of diluted sulfuric acid. Sodium cyanide is converted to sodium ferrocyanide on treating with ferrous sulphate($FeSO_4$). The green colour solution developed, it indicates the presence of nitrogen.

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

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

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

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

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

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 white, pale yellow and yellow precipitate respectively but the compound DHC does formwhite precipitate which is soluble in ammonium hydroxide pale. Hence we have conclude the sample DHC has chlorine is confirmed.

$$\mathrm{Na} + \underset{\text{(From organic compound)}}{\mathrm{Cl}} \rightarrow \mathrm{NaCl}$$

$$NaCl + AgNO_3 \rightarrow \underset{\substack{\text{Silver chloride}\\ \text{(White ppt.)}}}{AgCl} + NaNO_3$$

$$AgCl + 2NH_4OH \rightarrow \underset{\text{(Soluble)}}{[Ag(NH_3)_2]Cl} + 2H_2O$$

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.

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

Sodium nitroprusside test:

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

$$2Na + \underset{\text{(From organic compound)}}{S} \rightarrow Na_2S$$

$$\underset{\text{(From sodium extract)}}{Na_2S} + \underset{\text{Sod. nitroprusside}}{Na_2[Fe(CN)_5NO]} \rightarrow \underset{\text{Violet colouration}}{Na_4[Fe(CN)_5NOS]}$$

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

RESULT AND DISCUSSION

Analytical and spectral studies(FT-IR, ^{1}H-NMR and ^{13}C-NMR of (*E*)-2-(2, 6-dichlorobenzylidene)hydrazinecarbothioamide

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-(2,6-dichlorobenzylidene)hydrazinecarbothioamide is shown in Fig 5.1.

$$R_f= \frac{Distance\ \ travelled\ \ by\ Sample}{Distance\ \ travelled\ \ by\ solvent}$$

$$R_f= \frac{3.2}{3..6}$$

$= 0.88\ cm^{-1}$

Spectral Characterization:

FT-IR Spectral studies:

In order to study of functional group of the DHC Schiff base, the IR spectrum of DHC was compared with the general functional ranges. The IR spectrum of DHC showed characteristic band at 3410 and 3148 cm^{-1} can be attributed to ν(NH2) and ν(NH) respectively Aromatic ν(ArC-H) A sharp band appeared at 3010 cm^{-1}. Generally carbonyl group appears at 1680-1700cm^{-1} but the compound DHC has appears at 1592 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 1525 and 1102 cm^{-1} respectively. The FT-IR spectral data are given in table 5.2.1and figure 5.2.1a

Table 5.2. 1. Important IR bands of DHC ligand and its complexes with their assignments

Compounds	ν(NH2)	ν(N-H)	ν(ArC-H)	ν(C=O)	ν(C=N)	ν(CNC)
DHC	3410	3148	3010	1592	1525	1102

.

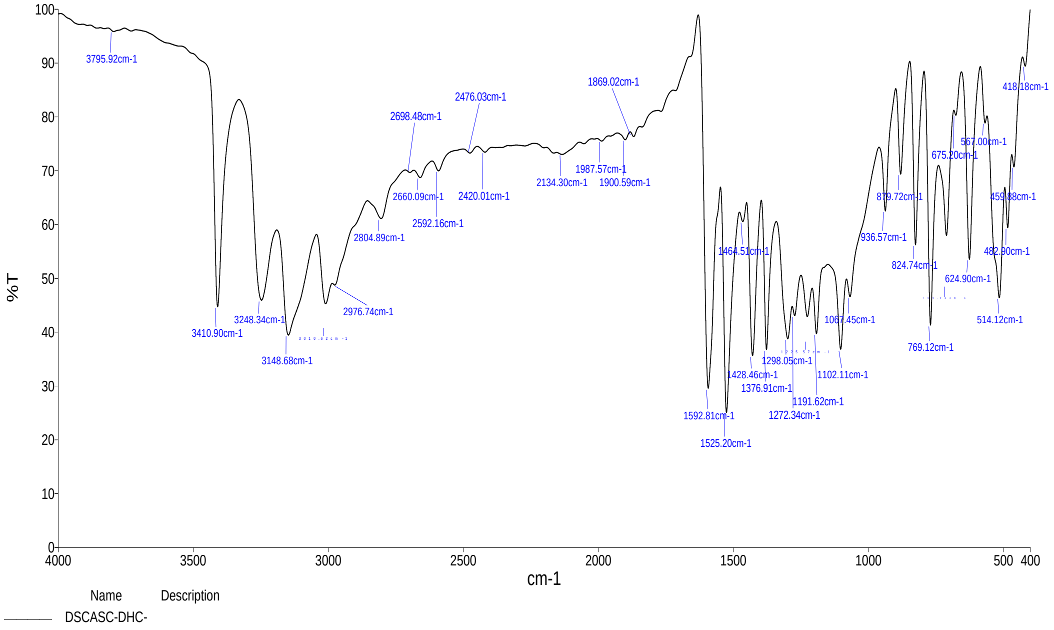
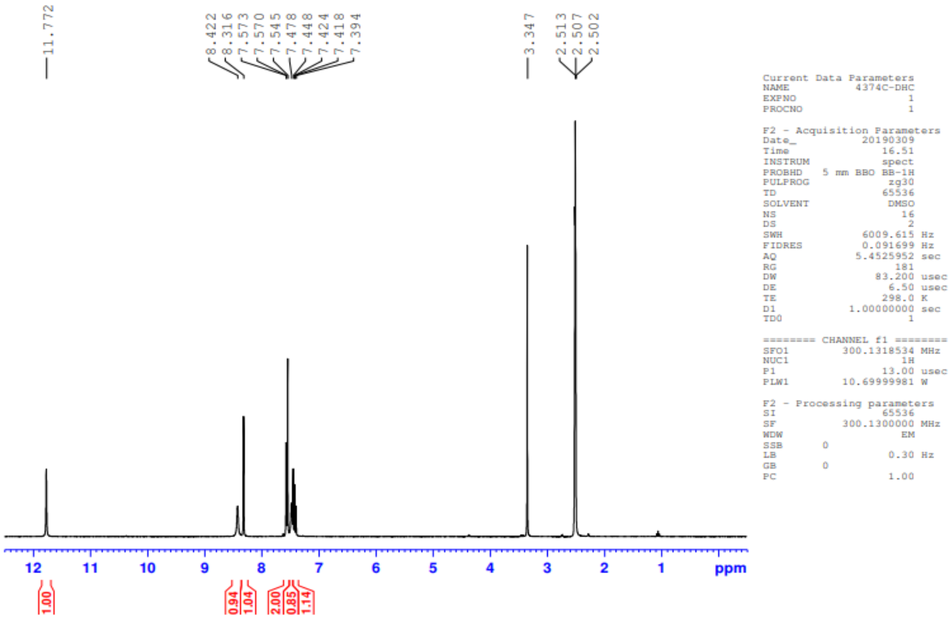


Fig5.2.1a FT-IR Spectrum of (*E*)-2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide

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

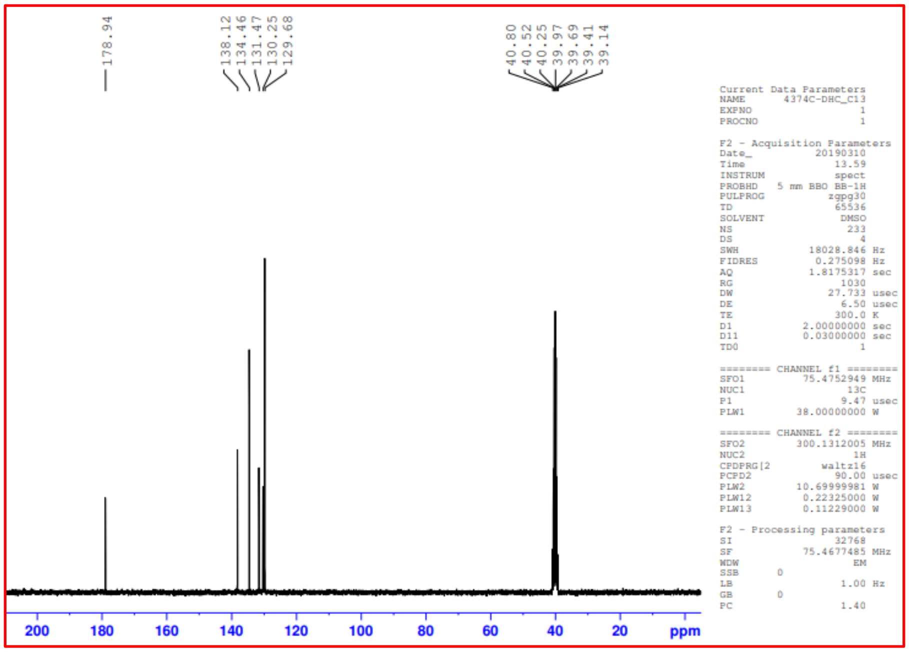
^{1}H NMR down of the compound DHC was recorded in DMSO-d_6 (300 MHz) (Fig. 5.2.2, 5.2.2a). The ligand DHC shows a singlet at δ 11.7 ppm is assigned to amido-thiol SH proton. A doublet appeared at 7.5 ppm which is corresponding to phenyl ring protons of meta position. Two singlets appeared in the range one is at 8.4 ppm and another one is at 8.3 ppm this is due to NH_2 protons of thiosemicarbazide part. A triplet appeared at δ 7.4 ppm this is due to para position of aromatic ring proton. A sharp signal at δ 7.3 ppm is assigned to azomethine (-CH=N-) proton. ^{13}C NMR spectrum of the ligand DHC was recorded in DMSO-d_6 (75MHz) (Fig 5.2.2b, 5.2.2c). Amide group thiocarbonyl carbon is appeared at δ 178 ppm. Azomethine carbon shows at 138 ppm and phenyl ring carbons appeared at 134, 131, 130 and 129ppm.

Fig 5.2.2.^{1}H-NMRspectrum of (*E*)-2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide



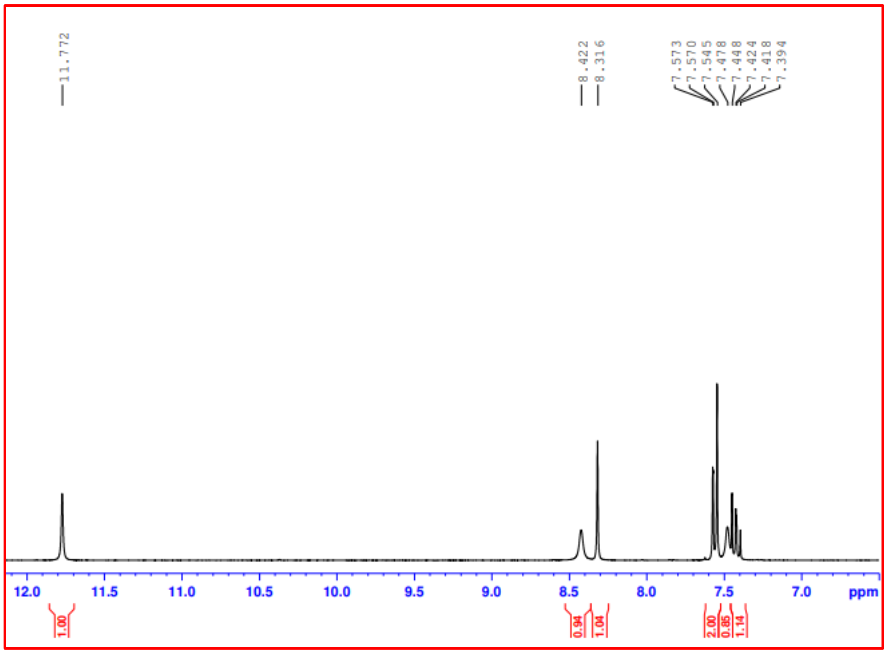
```
Current Data Parameters
NAME             4374C-DHC
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20190309
Time                 16.51
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      16
DS                       2
SWH               6009.615 Hz
FIDRES            0.091699 Hz
AQ               5.4525952 sec
RG                     181
DW                  83.200 usec
DE                    6.50 usec
TE                   298.0 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           300.1318534 MHz
NUC1                    1H
P1                   13.00 usec
PLW1           10.69999981 W

F2 - Processing parameters
SI                   65536
SF             300.1300000 MHz
WDW                     EM
SSB       0
LB                    0.30 Hz
GB        0
PC                    1.00
```

Fig 5.2.2a. Expanded^{1}H-NMR spectrum of (*E*)-2-(2, 6 -dichlorobenzylidene)hydrazinecarbothioamide



```
Current Data Parameters
NAME       4374C-DHC_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190310
Time               13.59
INSTRUM            spect
PROBHD   5 mm BBO BB-1H
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                   233
DS                     4
SWH            18028.846 Hz
FIDRES          0.275098 Hz
AQ             1.8175317 sec
RG                  1030
DW                27.733 usec
DE                  6.50 usec
TE                 300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          75.4752949 MHz
NUC1                 13C
P1                  9.47 usec
PLW1         38.00000000 W

======== CHANNEL f2 ========
SFO2         300.1312005 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         10.69999981 W
PLW12         0.22325000 W
PLW13         0.11229000 W

F2 - Processing parameters
SI                 32768
SF            75.4677485 MHz
WDW                   EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```

Fig 5.2.2.b^{13}C-NMR spectrum of (*E*)-2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide



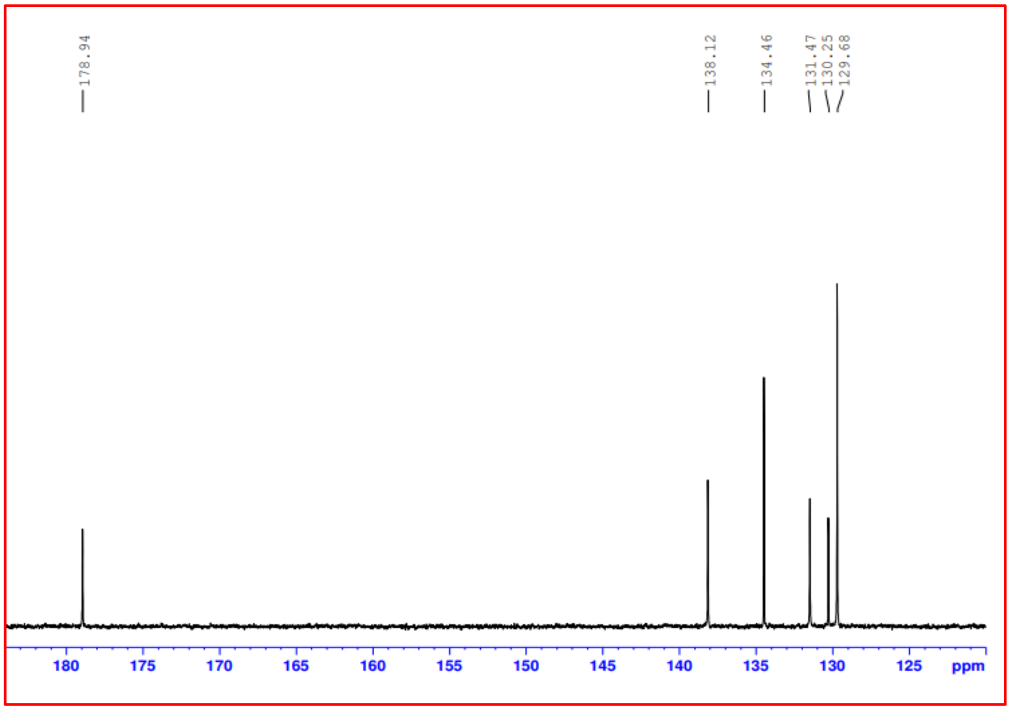


Fig 5.2.2c.expanded^{13}C-NMR spectrum of(*E*)-2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide

EFFECT ON BIO FILM FORMATION:

A modified crystal violet assay was employed to test the effect of plant extract on biofilm formations. Two-fold serial dilutions of plant extracts were made in sterile 96-well tissue culture plates containing 50 µl of Mueller–Hinton broth per well. The tested concentration range from 20 mg/ml to 0.156 mg/ml. A 50 µl of fresh bacterial suspension (1.0 McFarland) was added to each well. Growth control (cells + broth), media control (only broth) and blank control (broth + extract) were included. After incubation at 37 °C for 48 h, the biofilm biomass was assayed using the crystal violet staining assay as described above. The percentage of biofilm inhibition was calculated using the following formula:

Concentration (0.01mg per 1ml)	Percentage
10µl	40.55
25µl	34.10
50µl	39.17
100µl	53.91

The results of in vitro anti-biofilm (strong producer) activity of 53.91 are tested and results were presented in Table.The bacteria used in this part of investigation have been selected from the bacteria used for antibacterial activity depending on their biofilm formation potential.Anti-biofilm activity is presented in three types of results. The influence on biofilm formation varied among the tested strains. P. Aeruginosa formed the thickest biofilm (moderate producer) at 39.17 and two other bacteria were classified as insignificantly biofilm producer at concentration 34.10.

CONCLUSION

The metal complexes of Schiff base and the important application of the bases and relevance for carrying out this work.The detail study of Literature review of Schiff base, the synthetic route and analytical studies of Schiff base.The deals with synthesis and characterization of (*E*)-2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide. The elemental analysis shows the presence of nitrogen is confirmed by using sodium fusion extract.The FT-IR spectral study information. The frequencies around ν 3410 cm^{-1} confirm the presence of amide group. The stretching frequency of ν(C=N), (CNC) and ν(C=O) ofDHC are shows expected ranges.The ^{1}H and ^{13}C NMR spectral studies of the ligand DHC. The signals appeared in both the spectra give the exact position of each proton and carbon respectively as expected. In mass spectral study of the ligand DHC is discussed.Finally biofilm studies also carried out.

REFERENCE:

1. Ashraf M. A., Mahmood K., Wajid A.: Synthesis, Characterization and Biological Activity of Schiff Bases. *IPCBEE,*(2011), 10, 1–7
2. Kalaivani S., Priya N. P., Arunachalam S.: Schiff bases: facile synthesis, spectral characterization and biocidal studies. *IJABPT*, (2012), 3, 219–223
3. Souza P., Garcia-Vazquez J. A., Masaguer J. R.: *Transition Met. Chem.,* (1985), 10, 410.
4. Prashanthi Y., Kiranmai K., Subhashini N. J. P., Shivaraj: Synthesis, potentiometric and antimicrobial studies on metal complexes of isoxazole Schiff bases. *Spectrochim. Acta Part A,* (2008), 70, 30–35.
5. Ashraf M., Wajid A., Mahmood K., Maah M., Yusoff I.: Spectral Investigation of the Activities of Amino Substituted Bases. *Orient. J. Chem.,*(2011), 27, 2, 363–372.
6. Golcu A., Tumer M., Demirelli H., Wheatley R.: Cd(II) and Cu(II) complexes of polydentate Schiff base ligands: synthesis, characterization, properties and biological activity. *Inorg. Chim. Acta*, (2005), 358, 1785–1797.
7. Rice L.B.: Unmet medical needs in antibacterial therapy. *BiochemPharmacol*., (2006), 71, 7, 991–995.
8. Yang X., Wang Q., Huang Y., Fu P., Zhang J., Zeng R.: Synthesis, DNA interaction and antimicrobial activities of copper (II) complexes with Schiff base ligands derived from kaempferol and polyamines. *Inorg. Chem. Com*., (2012), 25, 55–59.
9. Sundriyal S., Sharma R. K., Jain R.: Current advances in antifungal targets and drug development. *Curr. Med. Chem*., (2006), 13, 11, 1321–1335.
10. Rehman W., Baloch M. K., Muhammad B., Badshah A., Khan K. M.: Characteristic spectral studies and in vitro antifungal activity of some Schiff bases and their organotin (IV) complexes. *Chin. Sci. Bull.,*(2004), 49, 2, 119–122.
11. Pandeya S. N., Sriram D., Nath G., De Clercq E.: Synthesis, antibacterial, antifungal and anti-HIV activity of Schiff and Mannich bases of isatin with N-[6-

chlorobenzothiazol-2-yl]thiosemicarbazide. *Indian J. Pharm. Sci*.,(1999), 61, 358–361

12. Sridhar S. K., Pandeya S. N., Stables J. P., Ramesh A.: Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives. *Eur. J. Pharm. Sci.*,(2002), 16, 129–132.
13. S. D.H. Brown and W.E. Smith, Enzyme Chemistry-Impact and Applications, *Chapmann and Hall, London,* (1990).
14. K. Singh, M.S. Barwa and P. Tyagi, *Eur. J. Med. Chem*.,(2007),42,394.
15. P.G. Cozzi, *Chem. Soc. Rev*., (2004),33, 410.
16. S. Chandra and J. Sangeetika, *J. Ind. Chem. Soc.*,(2004),81, 203.
17. H. Schiff, *Ann. Chem. Pharm*.,(1869), 150,193.
18. C. K. Jørgensen, *Acta Chem. Scand*., (1957), 11, 73.
19. F. Tuna, G.T. Pascu, J.P. Sutter, M. Andruh, S. Golhen, J. Guillevic and H. Pritzkow, Inorg. Chim. Acta, (2003), 342,131.
20. R. Srinivasan, I. Sougandi, K. Velavan, R. Venkatesan, BabuVerghese and P. SambasivaRao, Polyhedron,(2004), 23, 1115.
21. Shu – Fei Huang, Yi – Chian Chou, P. Mishra, Chin – Jhan Lee, S. Mohanta and Ho – Hsiang Wei, Inorg. Chim. Acta, (2004), 357, 1627
22. H. Schiff, *Ann. Chem. Pharm*., (1869), 150,193.
23. H. Schiff, *Ann. Chem. Pharm*.,(1869), 151,186.
24. M. Delepine, *Bull. Soc. Chim*., (1899), 21, 943.
25. P. Pfeiffer, T. Hesse, H. Pfitzinger, W. Scholl and H. Thielert, *J. Prakt. Chem*.,(1937), 149, 217.
26. P. Pfeiffer, E. Buchholz and O. Baver, *J. Prakt. Chem*., (1931), 129, 163.
27. V. Cassel1ata, P. Vigato, D.E. Fenton and M. Vidali, *Chem. Soc. Rev.*,(1979), 79, 199.
28. J. Sessler and J. Sibcr, *Tetrahedron,* (1993), 49, 8727.
29. M. Yildiz, Z. Kilic and T. Hökelek, *J. Mol. Struct*.,(1998),1, 441.