SYNTHESIS AND CHARACTERISATION OFCOPPER II COMPLEX OF PYRIDINE DERIVATIVE AND ITS ANTI CONVULSANT PROPERTY

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ABSTRACT

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Pyridine is structurally similar tobenzene,The pyridine ring possesing six π electrons that are delocalized over the ring. In Pyridine fivemethine (=CH−) group and one nitrogen atom constitute six a membered ring . It is a weakly basic colorless liquid with unpleasant fish smell .The pyridine rings occurs in importance compounds including pharamaceuticals ,and vitamins .Basicity of pyridine is governed by availability of lone pair of electron and is behave as tertiary amine. A probe into the literature survey clearly reveals that no work has been carried out for Cu(II) complex of(2,3 –dichlorobenzylidenepicolinohydrazide) 2,3 diclorobenzaldhyde and 2- pyridine carboxylic acid hydrayide.work deals with highly effective medicinal isoniazide derivatives, have been synthesized from Schiff base route and also its Cu(II) metal complex were synthesized.The structure of the ligand dichlorobenzylidenepicolinohydrazide and its Cu(II) complex were undergone various spectral studies. In present work an attempt has been made for the synthesis of (2,3dichlorobenzylidene nicotinohydrazide) its metal complex of Cu(II) and to establish the structure through the Analytical Techniques such as Elemental test and TLC and Spectral Studies such as IR ,1H NMR and 13C1 NMR methods used for their characterizations..

INTRODUCTION

Pyridine and fused heterocyclic pyrimidine derivative have received significance due to their biologically activity .For instance , triazolopyrimidine has a strong structural correlation with the nonselective adenosine receptor antagonist nucleus thus it represented an attractive key intermediate for obtaining adenosine receptor antagonists .Fused trizolopyrimidines and tetrazolopyrimidines have been reported as antibacterial and antifungal agent.Other examples are pyrazolepridimidine and their analogs are well known in the fields of medicinal chemistry and chemotherapy owing to their antimetabolite, anticancer ,antiviral,antitumor activities And thiazolo (3,2-a) pyrimidine derivatives shown anti-inflammatory and anticancer activities. Pyridine compound used in antibiofouling agent

A neurotoxic tetra-pyridine compound named Nemertelline found in the marine hoplonemertine worm Amphiporusangulatus. Variety of toxins produced by these worms are used both in hunting their prey and in defending themselves from predators. Potential application of this compound is as an antifouling agent for boats and other marine installations . Pyridine derivatives having many biological and medicinal applications.

Pyridines oxime derivatives and naphthiridine have high activity against HIV ant also show antibacterial activity. Oximes-Pyridine derivatives are used as antidotes against organophosphorus compound poisoning .Some thiopyridine derivatives are showing cytotoxic (DPPH) and antioxidant (SOD) activities. Structure of the compounds related to the DPPH and SOD activities . The QSAR studies show that dipole moment and elcetrophilic index were the most important descriptors for correlating the molecular structure of compounds with their respective SOD activities. Results indicated that molecules with high moment and elcetrophilic index values also had high SOD activity. Lowest atomic polarizability (MATS4p) compounds will have highest DPPH activity.

The pyridine derivatives containing thiazolidinones exhibit antidiabetic activities. The results reveal that a number of these compounds show very effective anti-diabetic activities, few among these compounds showed appreciable anti-diabetic activity and these findings could lead on to the event of novel class of anti-diabetic drugs in coming time. Some ligands which springs from acetyl pyridine have anti-amoebic activity, however, when this ligands is including ruthenium(II) to afford a posh , the anti-amoebic activity drastically increase.

EXPERIMENTAL METHOD

Thin layer chromatography(TLC)

Analytical TLC was performed on precoated aluminum sheets of silica (60F254) and isualized by UV light at λ 254 nm. Solvent systems are reported by column volume (CV) with the solvent flow as stated. A single spot on TLC silica gel glass plate with ethanol confirmed the purity of the synthesized sample.

Rf=$Distancetravelledby Sample/ Distancetravelledbysolvent$

Infrared spectroscopy:

Spectral studies give sufficient information about the structure of the compound. The Infrared spectrum is one of the spectra. The IR spectroscopy is widely used as a characterization technique for metal complexes. The basic theory involved is that the stretching

modes of the ligands changes upon complexation thanks to weakening or strengthening of the bonds involved within the bond formation leading to subsequent change within 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

Nuclear Magnetic Resonance spectroscopy:

1H NMR :

NMR may be a study of transitions between the magnetically inducted spin states. It is concerned with the magnetic properties of atomic nuclei with an integral value I. This technique consists of exposing the protons in an organic molecule to a powerful field. The protons will process at different frequencies. Studying a molecule by NMR spectroscopy enables us to record differences within the magnetic properties of varied magnetic nuclei present and to deduce the positions of this nucleus within the molecule. One can deduce 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. 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 .

13CNMR :

There are many differences between 1H NMR and 13C NMR spectra both in the mode of recording as well as appearance. The spin quantum number, I for 12C is equal to zero since 12C isotope has an even number of protons and even number of neutrons and hence no magnetic spin. It is, therefore, non-magnetic and does not give any NMR signal. The natural abundance of 13C is only about 1.1% and has an odd number of neutrons. So, 13C 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. 1H 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 13C with intrinsically narrow lines covering a wide absorption range are studied. 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 endof the pulse period, the nuclei will process freely with their characteristic frequencies reflecting with the chemical environment (Ele. Org. spec-231 &) and exhibitchemical shifts. 13CNMRofthesynthesizedcompoundswererecordedon75MHzBrukerSpectrometerat298.6 K using DMSO-d6 as solvent

Synthesis of(2,3 – dichlorobenzylidene) Picolinohydrazide and its cu(ii) complex

0.875g of 2,3 – dichlorobenzaldehydeis taken in a clean conical flask .Then it is dissolved in 10ml of methanol. 0.685g of 2- pyridine carboxylic acid hydrayideis dissolved in 20ml of dislilledwater.Then these two mixture are mixed using magnetic stirrer.After 10 minutes in stirring the white precipitate crude is yielded.The crude sample was recrystallised from ethanol.The purity of the compound was checked by thinlayerchromatography(TLC)

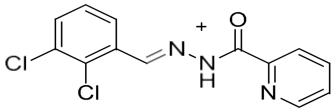


Figure 2 a -Structure of(2,3-dichlorobenzylidene)picolinohydrazide

Synthesis of metal complexes (1)

0.5g of dichlorobenzylidenepiclinohydrazideis dissolved in 40 ml of ethanol and$CuSO_4.5H_2O$ of o.4244g is dissolved in 20 ml of distilled water The reaction mixture was takenin a round bottom flask and kept over a magnetic stirrer with hot plate and stirred under 100°C. After 2h, the product separated as a solid was washed, filtered, washed with petroleum ether and dried invaccum

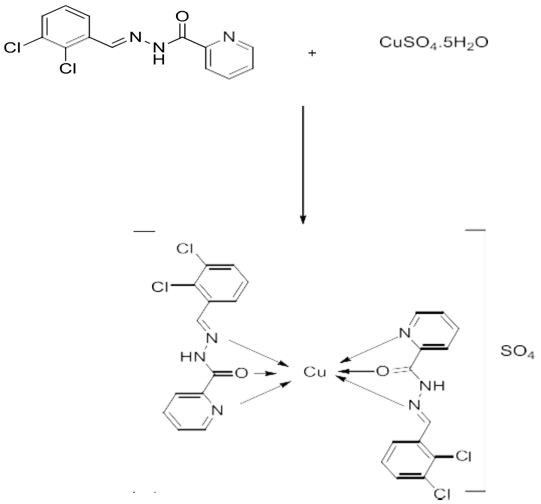


Figure 2 b –Synthesis of metal complex(1)

Procedure 3 : Synthesis of metal complexes (2)

0.5g of Dichlorobenzylidenenicotinohydrazidedissolvedin 40 ml of ethanol

and$CuSO_4.5H_2O$ of o.4244g is dissolved in 20 ml of distilled water The reaction mixture was takenin a round bottom flask and kept over a magnetic stirrer with hot plate and stirred under 100°C. After 2h, the product separated as a solid was washed, filtered, washed with petroleum ether and dried invaccum

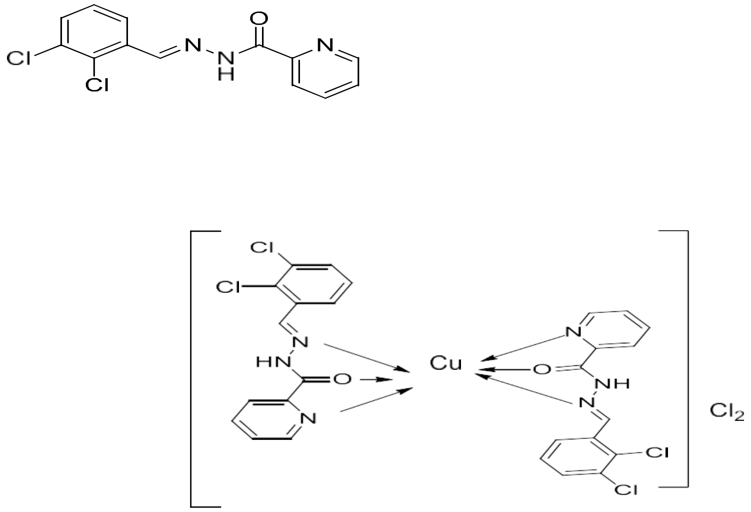


Figure 2 c –Synthesis of metal complex(2)

Solubility test

Solubility of compound was tested using water, methanol, ethanol, hexane, dichloromethane, benzene, ethylacetate, chloroform and DMSO. 1 mg of compound was added to 10 ml of solvent and solubility was tested under three different conditions such as cold condition and hot condition corresponding to the boiling point of solvent.

Crystallization

After synthesis the compound is dissolved 0.1g in 10ml ethanol and kept in a conicl flask at RT for several days until the formation of crystals.

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 assistance of TLC, it's possible to understand 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 UV light.

Characterization

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 and NMR techniques, it differs for complexes counting on the character of the ligands and therefore the metal ions involved. The presence of paired or unpaired electrons of the metal ions imparts the magnetic behavior of the complexes.

FTIR

Infrared spectra were obtained on a Bomem FT IR MB-102 spectrometer in KBr pellets.

NMR

1H NMR (200 MHz) and 13C NMR (50 MHz) spectra were recorded on BrukerAvance DRX200 spectrometer

Biological activity-anti convulsant property

Zebrafish were maintained during a light- and temperature controlled aquaculture facility under a typical 14:10 h light/ dark photoperiod. Adult zebrafish were housed in 1.5 l tanks at a density of 10–15 fish per tank and fed twice per day (dry flake and/or flake supplemented with live brine shrimp). Water quality was continuously monitored: temperature, 28–30C; pH 7.4 were maintained.

Drugs used

Pentylenetetrazole (PTZ) was used to induce zebrafish epileptic seizure. phenytoin (PHT) as negative control were selected for the development and validation of zebrafish epileptic seizure model. 100 mg/ml Stock solutions were prepared in 100% dimethyl sulfoxide (DMSO) and stored at -20 °C,

Treatment group

Fish of three months old were selected with a weight range of 0.5–0.6 g. Animal were divided into following groups, Group I: Vehicle control (10% DMSO); Group II: Pentylenetetrazole (PTZ-Negative control group); Group III: Phenytoin 80 mg/kg (PHY) + PTZ (170 mg/kg); Group IV: compound 80 mg/kg + PTZ (170 mg/kg). In the experiment 5 fisher group were used

Drug Treatment

Fish was captured individually by fish holding net, then transfer into ice water to give anesthesia. Fish was taken out once anaesthetized and weighed to calculate the dose and therefore the injection volume. A soft sponge of roughly 20 mm tall was saturated with water and set into 60 mm Petri dish . On the sponge made a cut of 10–15 mm deep to restrain and hold the fish for injection. Intraperitoneal injection was made employing a dissecting microscope by inserting the needle into the midline between the pelvic fins. Precautions include using a small injection volume of 10 µl per gram body weight. After injection, fish was immediately transferred to the tank and subjected for behavior recording

T-maze Test

The T-maze consists of 1 long (18′) and two short (12′) arms. One of the short arms is connected to a deeper square chamber (9 × 9′) which function a positive environment for the fish . Favorable environment is that the chamber which is deeper and wider compare to other arms in T-maze and once fish finds it, they spend the majority of their time in it. The T-maze behavior test was performed within the behavior room of constant temperature of 25°C – 26°C

Table 1: scoring standard for induced T-maze SeizureTable

SCORE	Behavior phenotype
0	Short swim mainly in the bottom of the tank.
1	Increased swimming activity and high frequency of opercular movement.
2	Burst swimming, left and right movements, and erratic movements.
3	Circular movements.
4	Clonic seizure-like behavior (abnormal whole-body rhythmic muscular contraction).
5	Fall to the bottom of the tank, tonic seizure-like behavior (sinking to the bottom of the tank, loss of body posture, and principally by rigid extension of the body).
6	Death.

RESULTS AND DISCUSSION

Synthesis of (*E*)-*N'*-(2,3-dichlorobenzylidene) picolinohydrazide :

The compounds are crystallized (plate 1) and tested for solubility among different solvent (plate 2).The data given in table 2 reveals that the synthesized compound was insoluble among hexane and water under RT and cold condition. Analytical TLC was performed on pre coated aluminum sheets of silica (60F254) and visualized by short wave UV light at λ 254 nmSolubilityis higher in methanol, ethanol, ethylacetate, benzene, chloroform, DMSO and dichloromethane TLC (plate 3) reveals that the compound is pure and having higher Rf value ranges from higher value of ethyl acetate (0.95) and lower value of DCM (0.44). The TLC plate for (E)-N’-(2,4-dichlorobenzylidene)nicotinohydrazidein above solvent actas eluent. The elemental analysis showed positive on nitrogenonly.

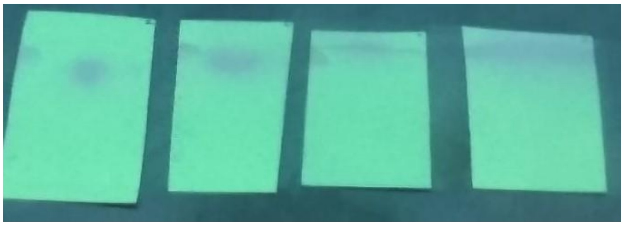
Table 2.solubility and R_f value of compound vs solvent

Solvent	Solubility		Rf Value
	RT	Hot	
Water	Insoluble	Insoluble	_
Methanol	Soluble	Soluble	0.69cm-1
Ethanol	Soluble	Soluble	0.70cm-1
Hexane	Insoluble	Insoluble	_
Benzene	Soluble	Soluble	0.77cm-1
Ethyl acetate	Soluble	Soluble	0.80cm-1
Chloroform	Soluble	Soluble	0.84cm-1
Dimethylsulphoxide	Soluble	Soluble	0.90cm-1
Dichloromethane	Soluble	soluble	0.94cm-1

Photoplates

Plate1:Synthesizedcompound Plate 2:SolibilityTest

Plate 3:TLC Analysis

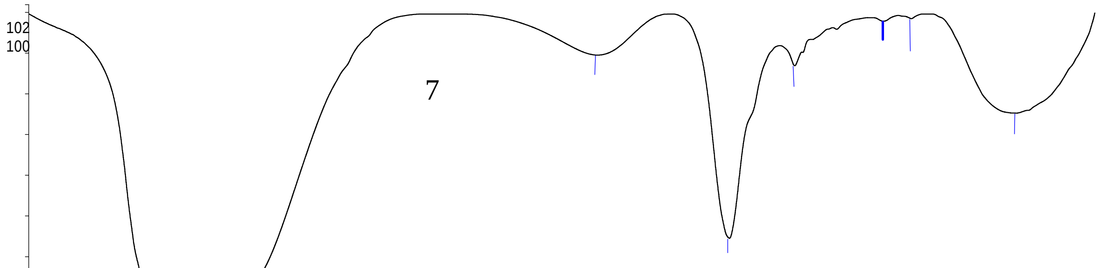


FTIR analysis

In order to study of functional group of the synthesized Schiff base, the IR spectrum was compared with the general functional ranges. The IR spectrum of Schiff base showed characteristic broad band at 3439 can be attributed to ν(N-H) and aromatic ν(ArC-H) band also overlapped with the N-H stretching vibrations. It is indicated, the Schiff base also having intermolecular O…H hydrogen bonding. The weak interaction was depends on the concentration of the solution. Generally carbonyl group stretching vibrations appears at 1680-1700cm^{-1} but in this case appeared at 1603 cm^{-1}, this is due to amide group present in the compound which decreases the carbonyl functional group. In finger print region, all peaks are good agreement with the proposed structure. The FT-IR spectral data are given in table 3 and figure1.

Table 3 Important IR bands of Schiff base with their assignments.

Vibrations	ν(N-H)	ν(N-N)	ν(C=O)	ν(C=N)
Peak (cm^{-1})	3439	2080	1634	1413



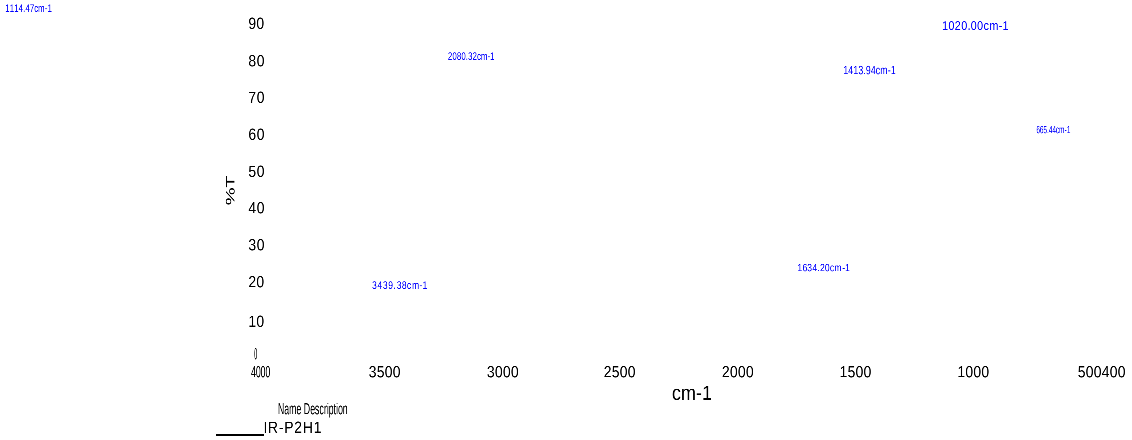


Figure 1. FT-IR spectrum of (*E*)-*N'*-(2,3-dichlorobenzylidene)picolinohydrazide

In ^{1}H NMR spectrum, the proton attached to C7 carbon showed as a singlet at δ= 8.36 ppm. It was the unique proton appeared as a sharp singlet without multiplicity and used to calibrate other protons. The characteristic amine N-H was appeared as broad singlet at δ=8.0ppm. The three protons attached on the phenyl ring were appeared as a two doublets at δ= 7.46 and 7.65 ppm and one multipletaround δ=7.34ppm. On the other hand, the four protons associated with pyridine ring were identified as two doublets at δ= 8.37 and 8.71 ppm and two multipletsat δ= 7.83 and 8.02 ppm. The detailed assignments of protons were given in table 4 and figure 2.

Table 4 NMR Spectroscopic Data (δ) of (*E*)-*N'*-(2,3-dichlorobenzylidene)picolinohydrazide

S. No	Position Assignment	^{1}H (δ, ppm)	^{13}C(δ, ppm)
1	1	7.46, d	132.5
2	2	--	142.7
3	3	--	130.8
4	4	--	134.8
5	5	7.65, d	125.3
6	6	7.34, m	128.3
7	7	8.36, s	138.7
8	8	--	--
9	9	8.0, br	--
10	10	--	157.6
11	1’	--	--
12	2’	--	151.3
13	3’	8.37, d	122.1
14	4’	8.02, m	137.5
15	5’	7.83, m	126.7
16	6’	8.71, d	147.6

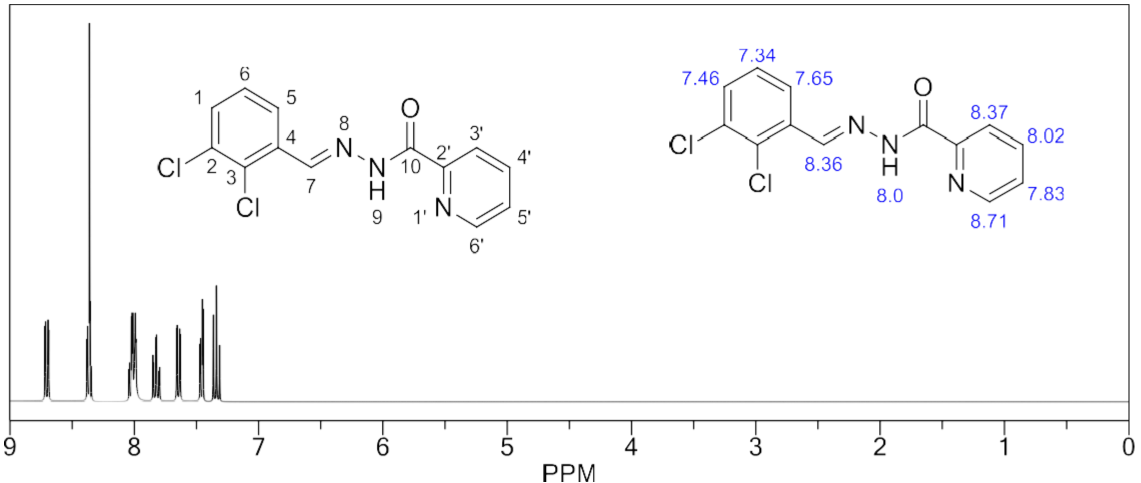


Figure 2.^{1}H NMR spectrum of (*E*)-*N*'-(2,3-dichlorobenzylidene)picolinohydrazide

In ^{13}C NMR spectrum, the distinguishable amide carbonyl appeared at δ= 157.6 ppm and it clearly indicates that molecule having amide group on its skeleton. Next, the carbon attached to the adjacent of nitrogen atom on pyridine ring was appeared at δ= 151.3 and 147.6 ppm. The newly formed imine quaternary carbon peak appeared around at δ= 138.7 ppm. The chlorine attached quaternary carbon appeared at δ= 130.8 and 142.7 ppm. The remaining six C-H carbons are showed six signals in the range of δ= 122.1 to 137.5 ppm. Assigning of this carbon was showed in table 4 and figure3.

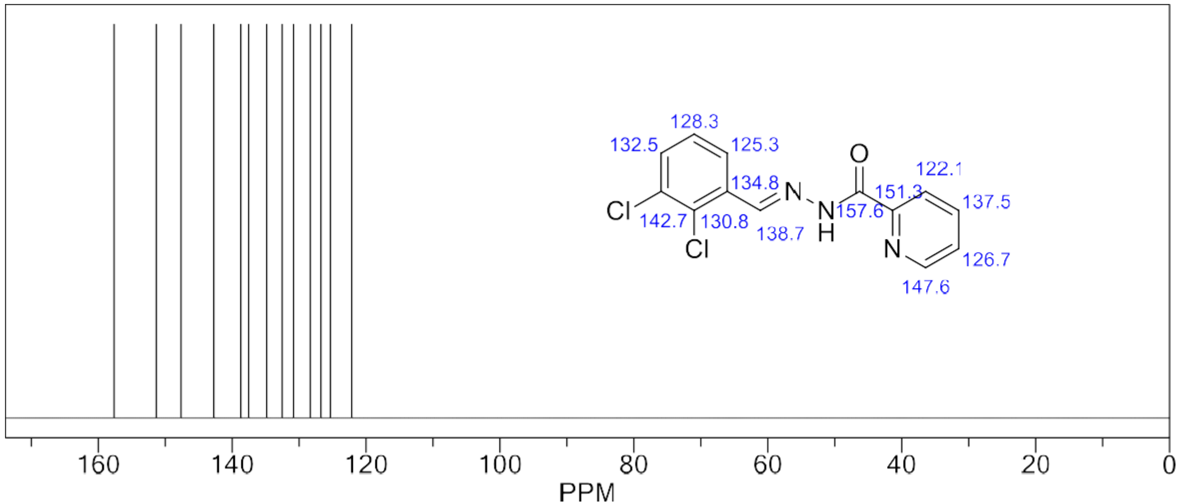


Figure 3.^{13}C NMR spectrum of (*E*)-*N*'-(2,3-dichlorobenzylidene)picolinohydrazide

Anti Convulsant Property

The behavioral analysis shownin table 6 reveals that movement of adult zebrafishin T maze towards unfavourable and favourable. In control 80% are favourablemoved to favourable and PTZ alne80% were unfavourable. The induced seizure is reverted byPhenytoinwithin 2h and only 40% moved unfavourable. The tested compound showed within 2 h 80% are maintained favourable condition. On the other hand, animals immersed into PTZ solution presented behavioral epileptic seizures, classified in different scores as shown in Table 6.

Pentylenetetrazole (PTZ) induced seizure in adult zebrafish clonus-like behavior and have had maximum unfavourable score. The PTZ concentrations induced seizures with score 4 in two h and 5 in 3h. Where asincontrol group the score ranges 0-1 an ideal experimental condition exhibited spontaneous usual swimming movements consisted by repeated constant short swims. Group 4 PTZ seizure is inverted by Phenytoin with in3 h and the score is 3≥2≥1 between 0-3 h. Similarly the synthesized tested compound has score 1 with in2 h and completely reduced the Seizure score to 0 from3.

Table 5.T-maze behavior analysis among control and treatment

Groups	Number of Zebra fishes	Favourable Environment	Unfavourable Environment
Group I–Control (untreated)	5	4	1
Group II – PTZ (Negative control)	5	1	4
Group III – Phenytoin + PTZ	5	3	2
Group IV – compound + PTZ	5	4	1

Table 6. T-maze Seizure score analysis among control and treatment

Group	Event of Behaviour	Scores		
		1h	2h	3h
\Group I – Control (untreated)	Short bottom swimming Increased swimming	0	1	1
Group II - PTZ (Negative control)	Circular erratic, clonic movements Fall to the bottom of the tank	3	4	5
Group III - Phenytoin + PTZ	Circular movements, Burst swimming, increaesed swimming and opercula activity	3	2	1
Group IV - MU compound + PTZ	Circular movements, Increased swimming and Short swim	3	1	0

The elemental analysis shows the presence of nitrogen is confirmed by using sodium fusionextract.The FT-IR spectral study information. The frequencies around v 3393 and 1603 cm^{-1} On firm the presence of carbonyl group is confirmed. The ^{1}H-NMR and ^{13}C- NMR spectral studies of the compound. The number of singalsnappearedin the ^{1}H –NMR reveals presence of pyridine ring. The signals appeared in both the spectra gives the exact position of each proton and carbon respectively asexpected.This is first time reported that the compound (*E*)-*N*'-(2,3-dichlorobenzylidene) picolinohydrazide have had anti convalescent property.In future, an attempt has been for the biological studies such as Anti-oxidant and Alzheimeractivities.

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