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Synthesis and Spectral Characterization of (E)-2-Amino- N'-[1-(2, 4-dihydroxy phenyl) Ethylidene] Benzohydrazide: Its Cytotoxicity

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Abstract: *Anthraniloyl hydrazones contains one free -NH_2 group attached to the aromatic ring and the other is the hydrazinic –Nitrogen. The ^{1}H-NMR and D_2O exchange studies inferred that the amide N-C=O and phenolic –OH are involved in hydrogen bonding. The mass spectrum of the ligand shows dimer peak. The anthranil hydrazides possesses antioxidant activity and also some of the hydrazone ligands are used in the treatment of iron overload diseases. As these Schiff base ligands are known for their wide range of applications in pharmaceutical and industrial fields for their antitumor properties the cytotoxicity of the ligand is studied. The ligand ADHPEBH is characterized by different analytical methods like mass, ir, ^{1}H-nmr and D_2O exchange. This paper provides an overview of the synthesis, analytical characterization, types of hydrogen bonding and cytotoxicity of the title compound.*

KeyWords: *(E)-2-amino-N'-[1-(2,4-dihydroxyphenyl) ethylidene] benzohydrazide (ADHPEBH), characterization, cytotoxicity, hydrogen bonding, synthesis.*

I. Introduction

Schiff base ligands occupy a central role in the development of coordination chemistry. These ligands are well known for their wide range of applications in pharmaceutical and industrial fields (1-3). The hydrazones derived from condensation of O-hydroxy, methoxy-aldehydes and ketones with hydrazides are potential polynucleating ligands possessing azomethine, amide and phenol or methoxy functions which offer varying bonding possibilities in metal complexes. Moreover the hydrazone group plays an important role for the antimicrobial activity and possesses interesting antibacterial, antifungal(4-6), antitubercular activities(7-11).

In the present work, an attempt is made to synthesize amino dihydroxy phenyl ethylidene benzohydrazide (ADHPEBH). But the title compound obtained as a dimer with intermolecular hydrogen bonding. The present study involves the synthesis, characterization, hydrogen bonding and cytotoxicity of the title compound.

II. Experimental

IR spectra were recorded on Perkin-Elmer 435 Spectrophotometer. ^{1}H-NMR were recorded in $CDCl_3$/DMSO-d_6 on a bruker WH (270 MHZ) spectrometer, mass spectra were recorded on Micro Mass V.G70-70H spectrometer operating at 70 ev using direct inlet system.

2.1 Materials

All the chemicals and solvents were of Analar grade. Solvents such as water,ethanol were purified before use by following standard procedures[12].

2.2 Synthesis of the ligand

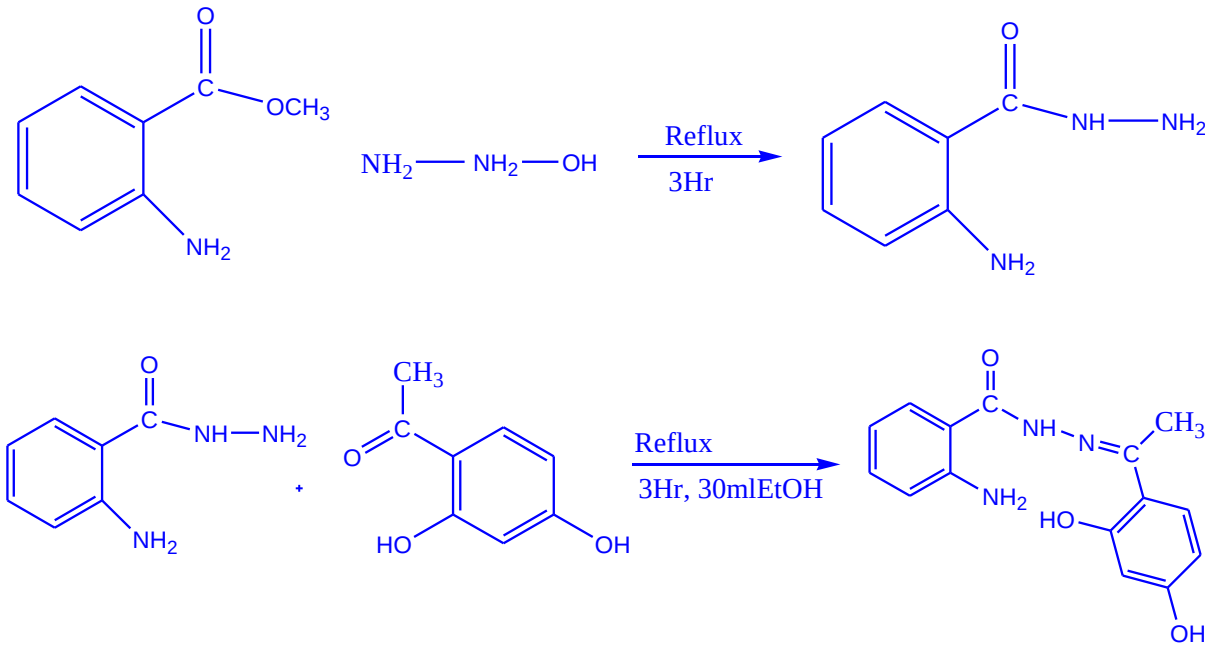
The ligand ADHPEBH was synthesized in two steps. The first step involves synthesis of anthranil hydrazide and the second step involves condensation of anthranil hydrazide with resorcinol acetophenone.

Synthesis of anthranil hydrazide

An equimolar mixture of methyl anthranilate and hydrazine hydrate are refluxed for 3 hr on water bath. On slow evaporation brown coloured solid compound anthranil hydrazide separated out. The purity of the compound was checked by TLC.

Synthesis of amino dihydroxy phenyl ethylidene benzohydrazide

(0.003 mole) of anthraniloyl hydrazide and (0.003 moles) of resorcinol acetophenone was dissolved in 30 ml of ethanol by adding a drop of sulphuric acid and refluxed for 3 hr on water bath. On slow evaporation yellow coloured solid separated. The compound was recrystallised from 1:1 ratio of acetone-water.The purity of the compound was checked by TLC. The melting point of the compound was found to be 280˚c to 283˚c. The ligand was prepared for the first time. It is obtained as dimer with intermolecular hydrogen bondings. The ligand was confirmed by NMR, D_2O exchange, IR and Mass spectral analysis.



III. Results and Discussion

3.1 Mass Spectra

The mass spectrum of the ligand exhibits molecular ion dimerization peak at m/z 650 (2M+55)which is in good agreement with molecular mass of the ligand. The m/z value indicates that the ligand exists as dimer (fig.1).

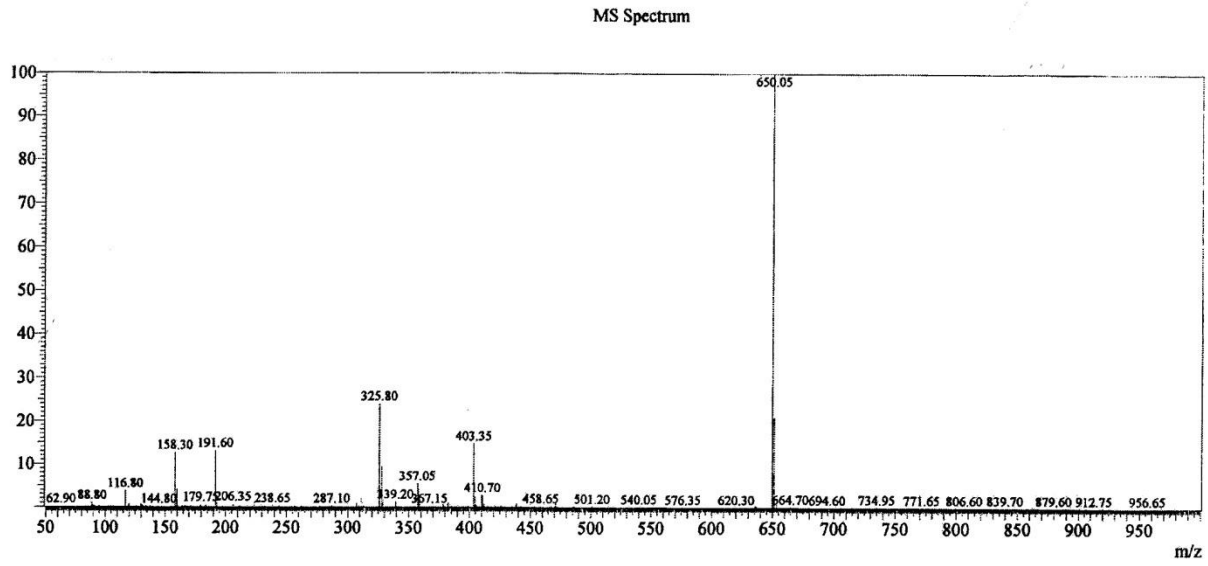


Figure. 1 Mass spectrum of ADHPEBH

3.2 Infrared Spectra

The IR spectra of the ligad(ADHPEBH) exhibits strong absorptions at ν3380cm^{-1}, ν3276 cm^{-1}, ν3000 cm^{-1}, ν1616 cm^{-1} and ν700-800 cm^{-1} assignable to $\nu_{OH,}$ $\nu_{(N-H),}$ $\nu_{(C-H)}$ aromatic, $\nu_{(C=O)}$ and the out of plane deformation of the aromatic ring respectively. The observation of the phenolic (OH) and amide (C=O) bands ascertains the formation of intermolecular hydrogen bonding. The appearance of the sharp peak of phenolic (OH) and the important amide C=O stretching shifted to lower wave number gives evidence for the formationof intramolecular hydrogen bonding of the type amide N-C=O….H[13]and intermolecular hydrogen bonding between phenolic –OH….N (hydrazinic nitrogen of the second ligand). In addition there are no peaks in the

1540-1560 cm^{-1} region indicates absenceof C=N stretch and existence of the ligand in keto (N-C=O) form in solid state.There is a C-N stretch appearing at 1386cm^{-1}.

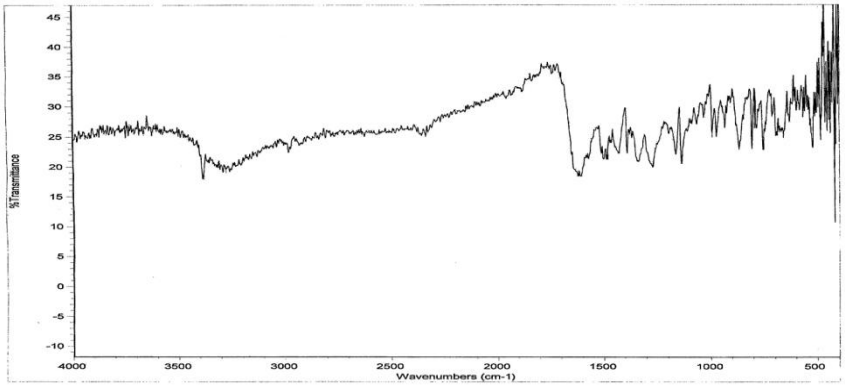


Figure.2 IR spectrum of ADHPEBH

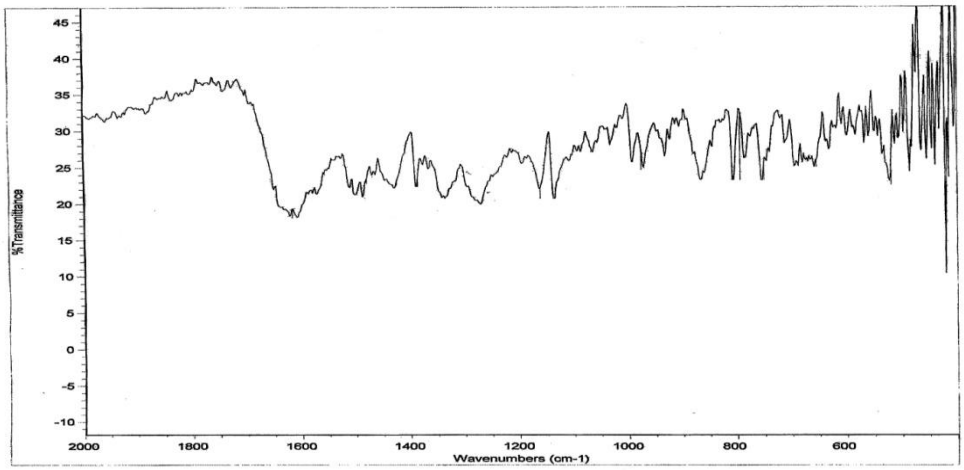


Figure.3 IR spectrum of ADHPEBH

3.2 ^{1}H-NMR

The ^{1}H-NMR spectrum of the ligand (ADHPEBH) shows signals assigned to the protons of (phenolic OH,aromatic NH, aromatic CH, -CH_3) at (13.2, 5.4, 7-8, 4) ppm down field to TMS. The down field of the OH signal compared with that reported in literature is most probably due to the intermolecular hydrogen bonding established between the hydrogen atom of the phenolic hydroxyl group and the hydrazinic nitrogen[14,15] as previously provided by the IR data.

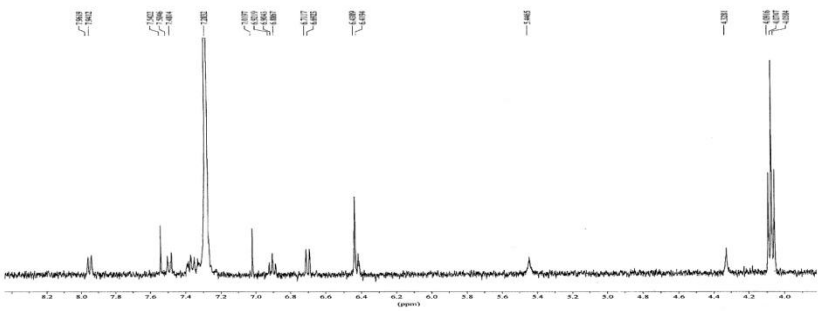


Figure.4 ^{1}H-NMR spectrum of ADHPEBH

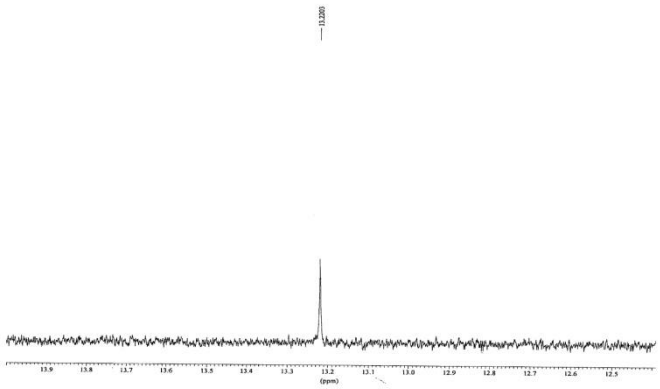


Figure.5 ^{1}H-NMR spectrum of ADHPEBH

3.3 D_2O exchange

The D_2O exchange of the ligand retains the OH peak and suppression of NH peak shows that the aromatic NH_2 has not involved in hydrogen bonding. From the D_2O exchange it is inferred that the two ligands are bonded through two types of hydrogen bonding[16] . The first hydrogen bonding is between amide C=O of one ligand and amide proton of same ligand (intramolecular hydrogen bonding). The second hydrogen bonding is between phenolic OH and hydrazinic nitrogen of the second ligand. This further confirms the existence of the ligand as dimer form through intermolecular hydrogen bonding.

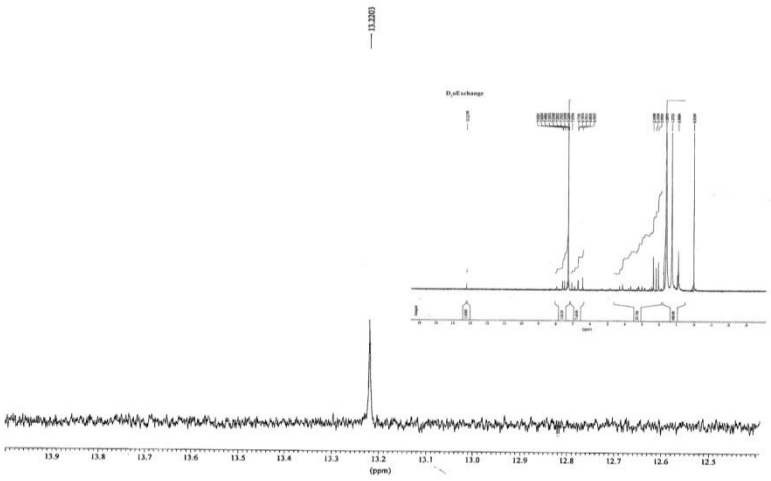


Figure.6 D_2O exchange of ADHPEBH

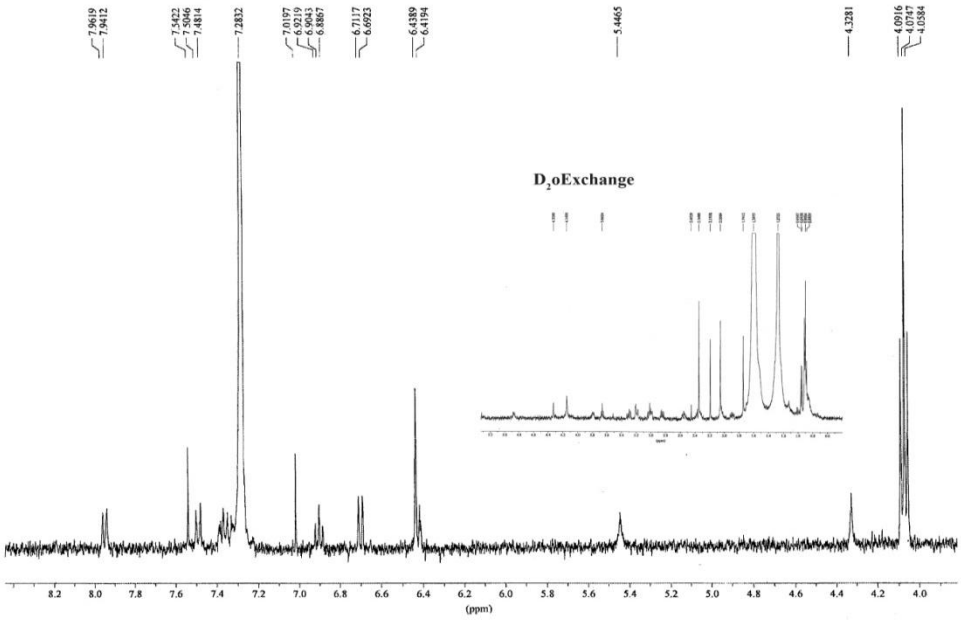


Figure. 7 D_2O exchange of ADHPEBH

IV. Cytotoxicity

4.1 Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

1.8 Cell lines and Culture medium A549 (Human lung carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India)

4.2 Preparation of Test Solutions

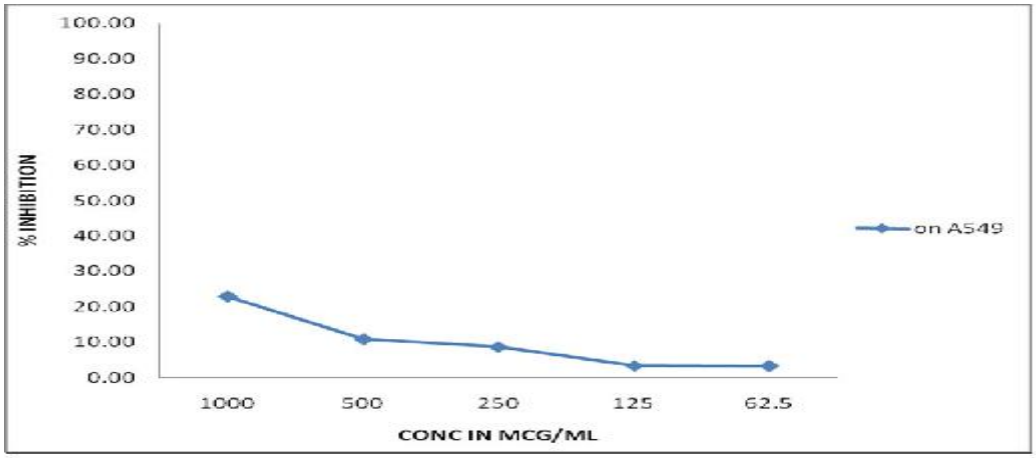
For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

4.3 Determination of cell viability by MTT Assay

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using MEM containing 10% FBS. To each well of the 96 well micrometre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 ml of different test concentrations of test drugs were added on to the partial monolayer in micrometre plates. The plates were then incubated at 37o C for 3 days in 5% CO2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37o C in 5% CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and

concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line. The cytotoxic data of the ligand is given in the table below.

$$\% \text{ Growth Inhibition} = 100 - \left[\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \text{ X } 100 \right]$$

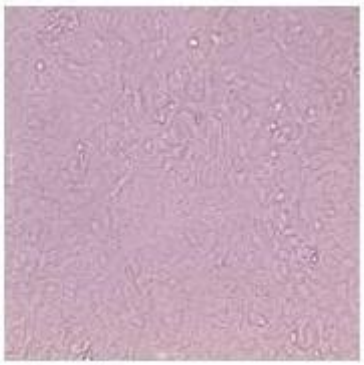


Cytotoxic effect of ADHPEBH on A549 cell line

Table. 1 Cytotoxic properties of ADHPEBH against A549 cell line (Human lung carcinoma)

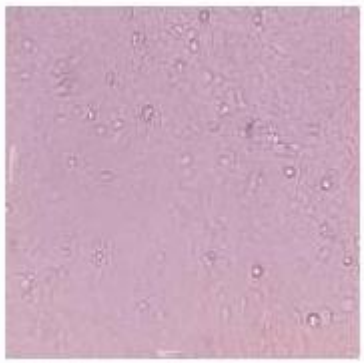
Sl.No	Name of Test drug	Test conc. (µg/ml)	% cytotoxicity	CTC_{50} (µg/ml)
1	ADHPEBH	1000 500 250 125 62.5	22.79±2.4 10.79±1.7 8.68±1.6 3.35±0.6 3.28±1.2	>1000

Photos



Control

1000 µg/ml



500 µg/ml

V. Conclusions

The IR spectra gave information regarding inter and intra molecular hydrogen bondings. The appearance of sharp peak of phenolic OH, shifting of C=O peak to lower wave numbers, absence of C=N stretch and appearance of C-N stretch provides evidence for hydrogen bonding. Mass spectrum showed dimerization peak. Appearance of OH signal in D_2O exchange and at downfield in ^{1}NMR indicates its involvement in hydrogen bonding. Since the ligand has good donors and good acceptors for hydrogen bonding it is planned to synthesize metal complexes and study their inter and intra molecular hydrogen bonding. The ligand ADHPEBH is having cytotoxic activity which is studied against A549 cell line. The studies show that the ligand concentration is directly proporational to cytotoxic activity.

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