SYNTHESIS CHARACTERISATION AND ANTIBACTERIAL ACTIVITY STUDIES OF PICOLIOHYDRAZIDE(2-PYRIDINE CARBOXYLIC ACID HYDRAZIDE)

J.Senthilkumaran[1], G.Annamalai[2], R.Shanmugapriya[3], M.Deepa[4]

Assistant professor,

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

Perambalur

ABSTRACT

Hydrazides derivatives are the type of organic compounds containing a nitrogen-nitrogen covalent bond with one of the substituents being an acyl group. Hydrazides and their derivatives have gained prominence because of their antibacterial,anti-inflammatory, anticancer, antiplatelet, antimalarial, analgesic and antioxidant activity. In addition to this they are also employed as potential inhibitors for controlling corrosion of many metals including mild steel [1].Hydrazide–hydrazones constitute a class of organic compounds, which attracts the attention of medicinal chemists due to the fact that they contain azomethine group (–NH–N=CH–) connected with carbonyl group, which is responsible for their different pharmaceutical applications and makes possible the synthesis of different heterocyclic scaffolds.

Keywords:Hydrazides ,anti-inflammatory ,hydrazones, antiplatelet, antimalarial, analgesicetc

INDRODUCTION

Hydrazides

Hydrazides behave similarly to hydrazines, because an alkyl or an acyl radical bound to the NH end of NH-NH2 does not influence

significantly the reactivity of the NH2 group. Carbonyl compounds are frequently used as derivatization reagents. Some of the hydrazides and analogous hydrazones are psychopharmacological agent such as monoamine oxidase (MAO) inhibitor and serotonin antagonists.Recently, sulfonylhydrazides have been widely used as an environmentally friendly sulfur source since they are stable, readily accessible and odor-free. The main route to synthesize hydrazide–hydrazone derivatives is the heating of appropriate hydrazides of carboxylic or heterocarboxylic acids with different aldehydes or ketones in various organic solvents like ethanol, methanol or butanol .A lot of biologically important hydrazide–hydrazone derivatives with a number of functional groups have been synthesized from many different carbonyl compounds.

A variety of methods have been used to prepare hydrazides, some of them involve the reaction of acids or their derivatives with hydrazine hydrate, anhydrides and acid chlorides, although the more popular method for the preparation of hydrazides achieved by the hydrazinolysis. Hydrazone compounds as it was mentioned above due to their azo methane group activities have drawn the attention of many researchers in synthesizing numerous compounds of hydrazones by the well-known hydrazinolysismethod .

Hydrazides are known to include an essential category of biologically active organic compounds(Tabanca et, al., 2013) These hydrazides and their condensation products were stated to possess a wide range of biological activities as well as antibacterial activity ,tuberculostatic properties , HIV inhibitors , pesticidal, antifungal . Some of the hydrazides and analogous hydrazones are psychopharmacological agent such as monoamine oxidase (MAO) inhibitor and serotonin antagonists .Hydrazide-hydrazones compounds are not only intermediates but they are likewise very effective organic compounds in their own right. When they are used as intermediates, new derivatives can be synthesized by using the active hydrogen component of –CONHN=CH- azometinegroup.

REVIEW OF LITERATURE

Studies shows series of 4-(morpholin-4-yl)-N'-(arylidene)benzohydrazides were synthesized using appropriate synthetic route. Schiff bases mediated different 4-(morpholin-4-yl)-N'-(arylidene) benzohydrazides synthesized and Antimycobacterial activity of the was

carried out by luciferase reporter phages (LRP) assay. Percentage reduction in relative light units (RLU) for isoniazid was also calculated. The test compounds showed significant antitubercular activity against Mycobacterium tuberculosis H37Rv.Amongst the compounds tested 4-(morpholin-4-yl)-N'-(arylidene)benzohydrazidesfound to be the most potent, activity against M. tuberculosis H37Rv and to have a greater activity against clinical isolates[14]

Newhydrazide derivatives of imidazo[1,2-a]pyridine were synthesized and evaluated for antituberculosis activity. The reaction of 2-[(2-carboxyimidazo[1,2-a] pyridine-3-yl)sulfanyl] acetic acid hydrazide with various benzaldehydes gave N-(arylidene)-2-[(2-carboxyimidazo[1,2-a]pyridine-3-yl)sulfanyl]acetic acid hydrazide derivatives. The chemical structures of the compounds were elucidated by IR,(1)H-NMR, FAB-MS spectral data and elemental analysis. Antituberculosis activities of the synthesized compounds were determined by broth microdilution assay, the MicroplateAlamar Blue Assay in BACTEC 12B medium. The results of BACTEC 460 Radiometric System against *Mycobacterium tuberculosis* H37Rv was 6.25 microg/mL; the tested compounds showed significant inhibition .

New compounds containing 4-hydroxy-N'-(1,3-thiazoldin-2-yldene)benzohydrazide moiety is basically for superior antimycobacterial activity studied by QSAR and further, the biological activity of the compound can be predicted before synthesis. The 3D-QSAR studies for the set of 4-hydroxy-N'-(1,3-thiazoldin-2-yldene)benzohydrazide and their derivatives were carried out by using V-life MDS (3.50). The various statistical methods such as Multiple Linear Regression (MLR), Partial Least Square Regression (PLSR), Principle Component Regression(PCR) and K nearest neighbour (kNN) were used. The kNN showed good results having cross validated r^2 0.9319, r^2 for external test set 0.8561 and standard error of estimate 0.2195. The docking studies were carried out by using Schrodinger GLIDE module which resulted in good docking score in comparison with the standard isoniazid. The designed compounds were further subjected for synthesis and biological evaluation. Antitubercular evaluation of these compounds showed that (4.a), (4.d) and (4.g) found as potent inhibitor of H37RV .

novel series of 4-pyrrol-1-yl benzoic acid hydrazide analogs, some derived 5-substituted-2-thiol-1,3,4-oxadiazoles, 5-substituted-4-amino-1,2,4-triazolin-3-thione and 2,5-dimethyl pyrroles have been synthesized in good yields and characterized by IR, NMR, mass spectral and elemental analyses. Compounds were evaluated for their preliminary in vitro antibacterial activity against some Gram-positive and Gram-negative bacteria and compounds were screened for antitubercular activity against Mycobacterium tuberculosis H37Rv strain by broth dilution assay method. Some compounds showed very good antibacterial and antitubercular activities.

Ali Akbar Khandar et al. *(2017)*Have been synthesized dibromido{N-[(pyridin-2-yljN)methylidene]picolinohydrazide-j2N00,O}-cadmium methanol monosolvate and diiodido-{N-[(pyridin-2-yl-jN)methylidene] picolinohydrazide-}cadmium . Complex (I) crystallizes as the methanol monosolvate. In both compounds, the Cd2+ cation is ligated by oneO atom and two N atoms of the tridentate ligand, and by two bromide anions forming a Br2N2O pentacoordination sphere for (I), and by two iodide anions forming an I2N2O pentacoordination sphere for (II), both with a distorted square-pyramidal geometry. In the crystal of complex (I), molecules are linked by pairs of N—H_ _ _O and O—H_ _ _Br hydrogen bonds, involving the solvent molecule, forming dimeric units, which are linked by C—H_ _ _Br hydrogen bonds forming layers parallel to (101). In the crystal of complex (II), molecules are linked by N—H hydrogen bonds, forming chains propagating along [010]. In complex (II), measured at room temperature, the two iodide anions are each disordered over two sites; the refined occupancy ratio is 0.75 (2):0.25 (2).

HadiAdibia et al *(2012)*Have been synthesized and Characterized the Hydrazide-Hydrazone Derivatives 3-Pyridine Carboxylic Acid as Antimycobacterial Tuberculosis Agents. Benzaldehyde derivatives react with nicotinicacidhydrazide to form nicotinoylhydrazones. The synthesized compounds were screened against *M. tuberculosis* H37Rv, clinical isolates of *M. tuberculosis* and MDR clinical isolates of *M. tuberculosis*using the proportion test. The minimum inhibitory concentration (MIC) of *N'*-(4-methylphenyl) nicotinicacidhydrazone

and *N'*-(4-(*N*,*N*-dimethyl)phenyl)nicotinicacidhydrazone exhibited activity between 40 and 200μg/mL and could be a good start point to find new lead compounds against *M. tuberculosis*.

Fatehia et al *(2010)*Have been synthesized compound with antimicrobial activity on some novel phthalazinones derivatives. A simple and efficient synthesis of [4-(3, 4-dimethylphenyl)-5, 6, 7, 8-tetrabromo-1-oxo-1Hphthalazin-2-yl]-acetic acid hydrazide**IV** has been carried out. The obtained hydrazide**IV** has been used in synthesis of some interesting heterocycles such as pyrazolone, thiazolidinone, pyrimidine benzoxazinelactam , rhodanine, quinazoline, benzoxazinone**.**. Some of the prepared compounds evaluated for their antibacterial activitiesagainst gram positive and gram-negative strain of bacteria for in vitro antibacterial activities [20]

GadadaNaganagowdaet al.*(2014)*Have beensynthesizedSynthesis and Antimicrobial Activity of New Schiff Base Compounds Containing 2-Hydroxy-4-pentadecylbenzaldehyde Moiety. Various novel Schiff base compounds have been synthesized by reaction of 2-hydroxy-4-pentadecylbenzaldehyde with substitutedbenzothiophene-2-carboxylic acid hydrazide and different substituted aromatic or heterocyclic amines in the presence of aceticacid in ethanol.The structures of all these compounds were confirmed by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data and have been screened for antibacterial and antifungal activity.[21]

Heng-Yu Qian et al., ***(2012)***Have been synthesized, Characterization, X-Ray Crystal Structures and Antibacterial Activities of Oxidovanadium(V) Complexes with Hydrazone and HydroxamateLigandsTwo new oxidovanadium(V) complexes, [VOL1L] (**1**) and [VOL2L]·CH3OH (**2**·CH3OH), where L1 and L2 are the dianionicform of *N'*-(3-bromo-2-hydroxybenzylidene)picolinohydrazide (H2L1) and 2-chloro-*N'*-(2-hydroxy-3-methoxybenzylidene) benzohydrazide (H2L2), respectively, and L is the monoanionic form of 2-hydroxybenzohydroxamic acid(HL), were prepared and characterized by elemental analysis, infrared and electronic spectroscopy. Structures of thecomplexes were further confirmed by single crystal X-ray determination. The V atoms in the complexes are in

octahedral coordination. The hydrazone ligands coordinate to the V atoms through the phenolate O, imino N, and enolate O atoms. The hydroxamate ligand coordinates to the V atom through the carbonyl and hydroxy O atoms. The complexes show effective antibacterial activity against *B. subtilis*, *S. aureus*and *E. coli*. The presence of Cl substitute group in the complex may enhance the antibacterial activity.[22]

SevimRollas et al., ***(2007)*** Have been synthesized Biologically active Hydrazone Derivatives. There has been considerable interest in the development of novel compounds with anticonvulsant, antidepressant, analgesic, anti inflammatory, antiplatelet, antimalarial, antimicrobial, antimycobacterial, antitumoral, vasodilator, antiviral and anti schistosomiasis activities. Hydrazones possessing an azometine -NHN=CH- proton constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated theirbiological activities. These observations have been guiding for the development of newhydrazones that possess varied biological activities.[23]

VitaSurzhkoa et al.,***(2017)***Have been Synthesisedpicolinohydrazides and their evaluation as ligands in the zinc-catalyzed hydrosilylation of ketones. A set of picolinohydrazides was prepared by reaction between hydrazines and either 2-picolinic acid or ethyl pyridine-2-carboxylate, and characterized. These molecules were evaluated as ligands in the zinc-catalyzed hydrosilylation of ketones. Thus, several aromatic and aliphatic ketones were successfully reduced by diethoxymethylsilane as the hydride source in the presence of a catalytic system made of diethylzinc combined *in situ* to the picolinohydrazides described herein.

SunandaDey et al., ***(2019)***Have beenSynthesised Aggregation-Induced Emission-Active Hydrazide-Based Probe: Selective Sensing of Al3+, HF2 −, and Nitro Explosives The AIE activity of H-PNAP is selectively quenched by 2,4,6- trinitrophenol (TNP) and 2,4-dinitrophenol (DNP) out of different nitroaromatic compounds with a limit of detection (LOD) of 7.79 × 10−7 and 9.08 × 10−7 M, respectively. The probe is nonemissive in aqueous medium (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HEPES buffer, pH 7.2); however, it shows a strong emission to Al3+ (λem, 490 nm) in the presence of 17 other biological metal ions, and the LOD is 2.09 nM which is far below the WHO recommended value (7.41 mM). The emission of the [Al(PNAP)- (NO3)2] complex is quenched by

HF2 − (F− and PO4 3− are weak quencher), and the LOD is as low as 15 nM. The probable mechanism of the sensing feature of the probe has been authenticated by 1H nuclear magnetic resonance titration, mass spectrometry, Fourier transform infrared spectroscopy, Benesi− Hildebrand plot, and Job's plot in each case. The probe has some practical applications such as recovery of Al3+ from the drinking water sample, construction of the INHIBIT logic gate. .[28]

Edward A. kesicki et al., ***(2016)*** havebeen analysed 2-Aminothiazoles as Anti-Tubercular Agents. The 2-aminothiazole series has anti-bacterial activity against the important global pathogen *Mycobacterium tuberculosis*.We explored the nature of the activity by designing and synthesizing a large number of analogs and testing these for activity against M. tuberculosis, as well as eukaryotic cells. We determined that the C-2 position of the thiazole can accommodate a range of lipophilic substitutions, while both the C-4 position and the thiazole core are sensitive to change. The series has good activity against *M. tuberculosis* growth with sub-micromolar minimum inhibitory concentrations being achieved. A representative analog was selective for mycobacterial species over other bacteria and was rapidly bactericidal against replicating *M. tuberculosis*. The mode of action does not appear to involve iron chelation. We conclude that this series has potential for further development as novel antitubercular agents.[29]

Rosita Dian et al., ***(2019)***Have beenSynthesisedAmphiphilicPyridinoyl-hydrazone Probe forColorimetric and Fluorescence pH SensingA new pH sensor based on a substituted aroylhydrazide with a flexible side chain anda terminal trimethyl ammonium group (PHA+) was designed and synthesized. The terminalquaternary ammonium guarantees excellent solubility in water. At the same time, the probe is verysoluble in hydrophobic environments. The pyridinoyl-hydrazone moiety acts as the pH-sensitivefluorophore/chromophore probe. Extensive physicochemical characterization has been performed onthe bromide salt PHABr. DFT calculations, based on single-crystal X-ray data, permitted to rationalizethe optical behavior. Molecular dynamics simulations permitted to clarify the mode of interactionwith lipid membrane. The ability of the probe to change color and fluorescence in response to differentpH and media of di_erent polarity has been investigated. PHABr shows a remarkable pH-dependentbehavior in both absorption and fluorescence spectra with high sensitivity and strong on-off switcheffect at neutral pH, perceptible even to the naked eye.[30]

Sraa Abu- Melha et al.,**(2018)**Have been SynthesisedPyridylthiosemicarbazide of synthesis, crystal structure, DFT/B3LYP, molecular docking studies and its biological investigations Furthermore, its geometry optimization, calculated vibrational frequencies, non-linear optical properties, electrostatic potential and average local ionization energy properties of molecular surface were being evaluated using Jaguar program in the Schrödinger's set on the basis of the density functional concept to pretend themolecular geometry and predict properties of molecule performed by the hybrid density functional routine B3LYP. Furthermore, the docking study of *N*-(pyridin-2-yl)hydrazine carbothioamide were applied against negative *Escherichia coli* bacterial and gram positive *Staphylococcus aureus bacterial* strains by Schrödinger suite program using XP glide protocol.[31]

Agnieszka et al., ***(2012)***Have been Synthesized and studied Tuberculostatic activity of Novel N-Methyl-4-(pyrrolidin-1-yl) picolinohydrazide and N-Methylpyrimidine-2-carbohydrazide Derivatives These compounds were used as starting materials to obtain methyl N- methyl hydrazine carbodithioates which, on reaction with either triethylamine or hydrazine, gave corresponding 1,3,4-oxadiazioles or 1,2,4-triazoles with the free NH2 group at the N-4 position, respectively. Compounds particularly containing cyclic amines at N-4 of the 1,2,4-triazole ring, were also obtained. Synthesized compounds were tested in vitro for their activityagainst Mycobacterium tuberculosis. The structure–activity relationship analysis for obtained compounds was done.[32]

Shilah et al., ***(2016)*** Have been Synthesised the general secretion (Sec) pathway is a conserved essential pathway in bacteria and is the primary route of protein export across the cytoplasmic membrane. We developed a targetbasedwhole cell assay to screen for potential inhibitors of LepB, the sole signal peptidase in Mycobacterium tuberculosis,using a strain engineered to underexpressLepB (LepB-UE).We screened 72,000 compounds against both the Lep-UE and wild-type (wt) strains. We identified the phenylhydrazone(PHY) series as having higher activity against the LepB-UE strain. We conducted a limited structure−activity relationship determination around a representative PHY compound with differential activity (MICs of 3.0 μM against the LepB-UE strain and 18 μM against the wt); several analogues were less potent against the LepB overexpressing strain. A number of chemical modifications around the hydrazone moiety resulted in improved potency. Inhibition of LepB activity was observed for a number of compounds in a biochemical assay using cell membrane fraction derived from M. tuberculosis. Compounds did not increase cell

permeability, dissipate membrane potential, or inhibit an unrelated mycobacterial enzyme, suggesting a specific mode of action related to the LepB secretory mechanism.[33]

EXPERIMENTALMETHODS

Infrared spectroscopy:

Most of the spectra give sufficient information about the structure of the compound. The Infra Red spectrum is one of the spectra. Unlike UV spectrum which comprises of relatively few peaks, IR technique provides a spectrum containing a large number of absorption bands from which a wealth of information can be derived about the structure of an organic compound. The absorption of Infra-Red radiations causes the various bands in a molecule to stretch and bend with respect to one another.

The IR spectroscopy is generally utilized as a portrayal method for metal edifices. The fundamental hypothesis included is that the extending methods of the ligands changes upon complexation because of debilitating or fortifying of the securities engaged with the security development bringing about ensuing change in the situation of the groups showing up in the IR Spectrum. The adjustments in the primary highlights of the ligands are seen as changes in groups noticed, fundamentally in the unique mark area (4000-400 cm-1). The groups because of the metal ligand bonds are for the most part seen in the far IR district (600-100 cm-1).

In the current examination, IR spectra of the mixes were recorded utilizing Perkin Elmer range RXI utilizing KBr pellets at recurrence range 4000-400 cm-1at ACIC, St. Joseph′s College (Autonomous), Trichirapalli and Shimadzu FT IR 400 Spectrophotometer, recurrence range 4000-400 cm-1 utilizing KBr circle at St Joseph's College, Trichy. **Nuclear Magnetic Resonance spectroscopy:**

^{1}H NMR:

NMR is 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. Now, these processing protons are irradiating with steady changing frequencies and

observe the frequencies at which absorptions occur. The signals obtained corresponding to the absorption is known as NMR Spectrum.Studying a molecule by NMR spectroscopy enables us to record differences in the magnetic properties of various 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. ^{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 at SASTRA University, Tanjore.

^{13}CNMR:

There are many differences between ^{1}H NMR and ^{13}C NMR spectra both in the mode of recording as well as appearance. The spin quantum number, I for ^{12}C is equal to zero since ^{12}C 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 ^{13}C is only about 1.1% and has an odd number of neutrons. So, ^{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. 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 at SASTRA University Tanjore.

Synthesis ofpicoliohydrazide(2-pyridine carboxylic acid hydrazide)(34)

REQUIREMENTS:

2,4-dichlorobenzaldehyde -0.875 g

2-pyridine carboxylic acid hydrazide -0.685 g

Methanol	-10 ml
Distilled water	-20 ml

PROCEDURE: 0.875g of 2, 3-dichlorobenzaldehyde is taken in a conical flask. Then it is dissolved in 10ml of methanol. 0.685g of 2-pyridie carboxylic acid hydrazide is dissolved in 20ml water. Then these two mixtures are mixed using magnetic stirrer. After 10 minutes in stirring the white precipitate crude is yielded. The crude sample was re crystallized from ethanol. The purity of the compound was checked by Thin Layer Chromatography (TLC).

Fig3: Structure of (E)-N'-(2,4-dichloroezylidene)picolinohydrazide.

SOLUBILITY TEST:

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

Thin Layer Chromatography:

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 Chromatography 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 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 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.

UV analysis:

The synthesized compound is subjected to UV-visible analysis. DMSO used as blank. The OD is recorded between 200-700nm.

FTIR:

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

NMR:

1H NMR (200 MHz) and 13C NMR (50 MHz) spectra were recored on BrukerAvance DRX200 spectrometer at the SASTRA university, thanjavur.

Analytical techniques

Elemental Analysis:

Our objective is to detect the presence of nitrogen, sulphur, chlorine, bromine and iodine in synthesized ligands by Lassaigne's test. 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 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 white, pale yellow and yellow precipitate respectively but the compound does not form pale yellow precipitate . Hence we have conclude the ligand has bromine is absent.

$$Na + \underset{\text{(From organic compound)}}{Br} \rightarrow NaBr$$

$$NaBr + AgNO_3 \rightarrow \underset{\substack{\text{Silver bromide}\\ \text{(Pale yellow ppt.)}}}{AgBr} + NaNO_3$$

.

Test for sulphur:

1. Lead acetate test:

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

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

BIOLOGICAL ACTIVITY:

Antitubercle activity

Disc diffusion method was used for antibacterial activity. The *M.smegmatis* MTCC300 strain is used in this study. Dubose oleic medium with bovine albumin used for assay. A stock solution of extract was prepared by dissolving 1 mg/Ml of ethanol. The stock solution was then loaded to sterile disc at concentrations of 25, 50, and 100µg. 30 μgrifampicin used as positive control and ethanol serve as negative control. each of the disc were allowed to air dry and place over the test pathogen swabbed on agar platesand incubated at 37° C under anaerobic condition for 3 days. The zone of inhibition was measured

RESULTS AND DISCUSSION

Efforts are being made here to investigate the coordination properties of 2, 3-dichlorobenzaldehyde and picolinic acid synthesizes as given in figure 4. The compounds are crystallized(plate 1) and tested for solubility among different solvent (plate 2). The data given in table 1 reveals that the synthesized compound was insoluble among hexane and water under RT and cold condition. Solubility is higher in methanol, ethanol, ethylacetate, DMSO and dichloromethane and partial in benzene and chloroform. TLC(plate 3) reveals that the compound is pure and having higher R_f value 0.9 among polar solvent,moderate in benzene (0.6) and no mobility in hexane solvent.

Figure 4. Synthesis of (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide :

Table 1. Solubility test for MBSPDO

S.no.	Solvents	Room temperature	Hot condition	Rf value
1	Water	Insoluble	Insoluble	-
2	Methanol	soluble	soluble	0.95 cm-1
3	Ethanol	soluble	soluble	0.97 cm-1
4	Hexane	Insoluble	Insoluble	-
5	Benzene	soluble	Soluble	0.66 cm-1
6	Ethyl acetate	soluble	Soluble	0.90 cm-1
7	Chloroform	soluble	soluble	0.94cm-1
8	Dimethylsulphoxide	soluble	soluble	0.97cm-1
9	Dichloromethane	soluble	soluble	0.90 cm-1

Spectral Characterization:

FT-IR Spectral studies:

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 3429 cm^{-1} can be attributed to ν(N-H) and aromatic ν(ArC-H) stretching vibrations appeared at 3079 cm^{-1}. It is indicated, the Schiff base also having intermolecular O…H hydrogen bonding. The weak interaction was depends on the concentration of the solution. In this spectrum was recorded with very dilute sample. Another distinctive vibration expected for N-N observed at 1910 cm^{-1}. Generally carbonyl group stretching vibrations appears at 1680-1700 cm^{-1} but in this case appeared at 1605 cm^{-1}; this is due to amide group present in the compound which decreases the carbonyl functional group. The newly generated C=N stretching vibration appeared at 1451 cm^{-1} along with other finger print region signal and all other peaks are good agreement with the proposed structure. The FT-IR spectral data are given in table 2 and figure 5.

Table 2 Important IR bands of Schiff base with their assignments.

Vibrations	ν(N-H)	ν(ArC-H)	ν(N-N)	ν(C=O)	ν(C=N)
Peak (cm^{-1})	3429	3079	1910	1605	1451

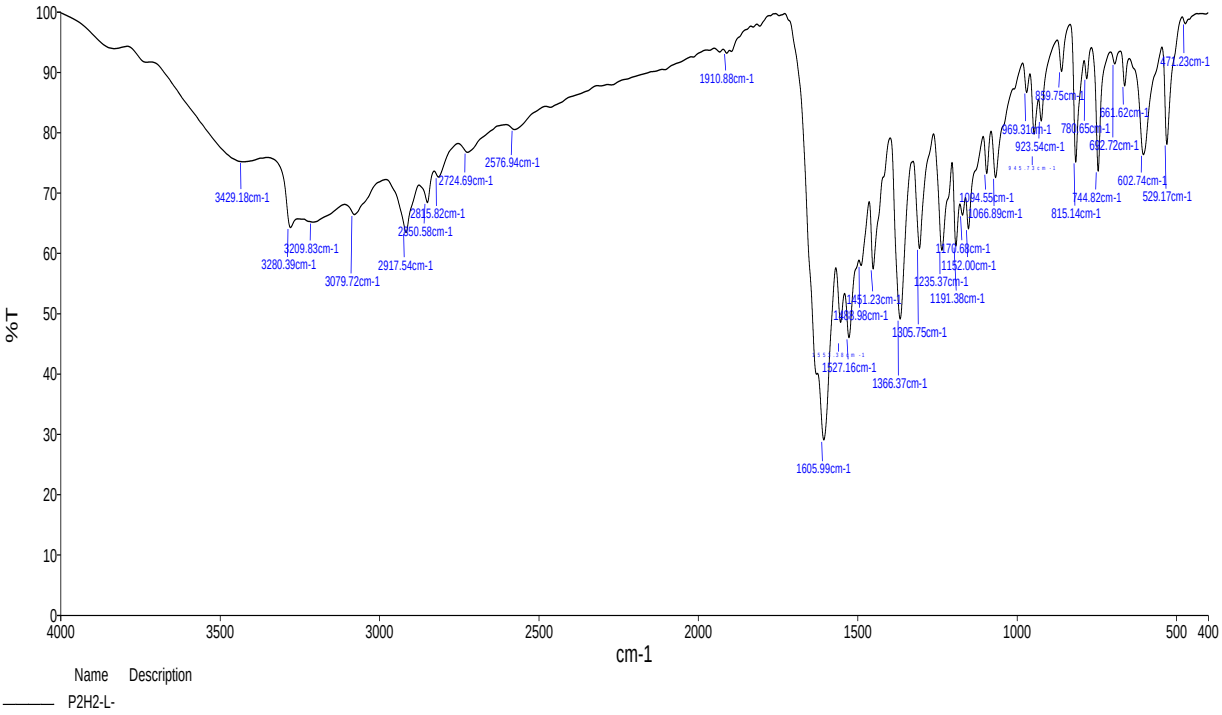


Figure 5. FTIR spectrum of synthesized compound

NMR spectra analysis

In ^{1}H NMR spectrum, the proton attached to C5 & C7 carbon showed as a singlet . It was the unique proton appeared as a sharp singlet without multiplicity and used to calibrate other peaks. The characteristic amine N-H was appeared as broad singlet . The three protons attached on the phenyl ring were appeared as a two doublets at □ □ □ 7.40 and 7.98 ppm and one sharp singlet discussed earlier. On the other hand, the four protons associated with pyridine ring were identified as two doublets at □ □ □ □ 8.35 and 8.68 ppm and two multiplets at □ □ □ □ 7.80 and 8.05 ppm. The detailed assignments of protons were given in table 3 and figure 6.

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

S. No	Position Assignment	^{1}H (δ, ppm)	^{13}C(δ, ppm)
1	1	--	132.8

2	2	--	131.0
3	3	7.70, s	129.1
4	4	--	128.3
5	5	7.40, d	127.0
6	6	7.98, d	129.0
7	7	8.36, s	138.7
8	8	--	--
9	9	8.0, br	--
10	10	--	157.0
11	1’	--	--
12	2’	--	151.8
13	3’	8.35, d	122.4
14	4’	8.05, m	137.5
15	5’	7.80, m	127.3
16	6’	8.68, d	148.0

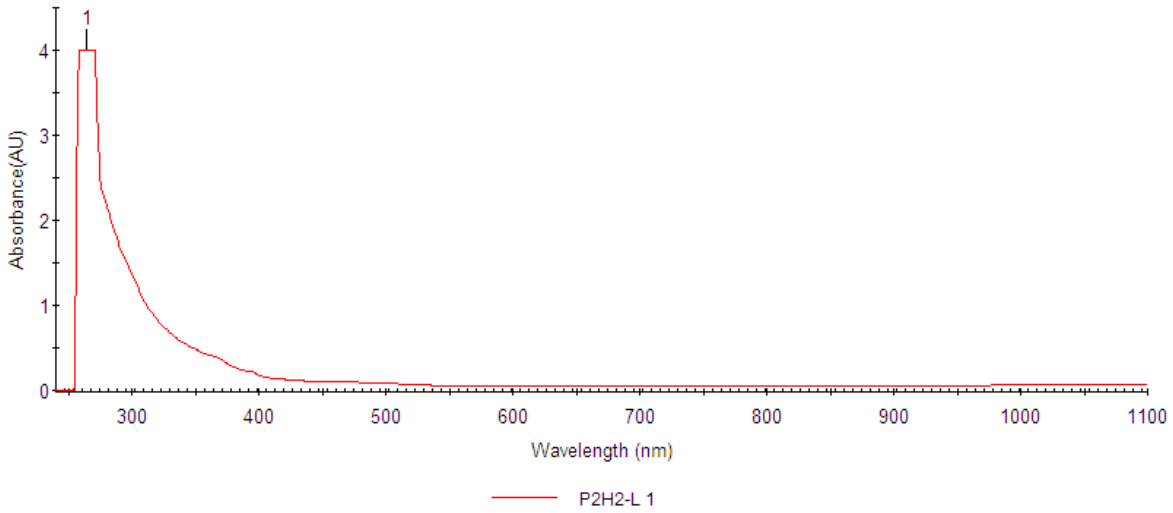
Figure 6.^{1}H NMR spectrum of (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide

In ^{13}C NMR spectrum, the discernible amide carbonyl appeared at □ □ □ 157.0 ppm and it clearly indicates that molecule having amide group on its skeleton. Next, the carbon attached to the adjacent to the nitrogen atom on pyridine ring was appeared at □ □ □ □ 151.8 and 148.0 ppm. The newly formed imine carbon peak appeared around at □ □ □ □138.7 ppm. The chlorine attached quaternary carbon appeared at □ □ □ □ 131.0 and 128.3 ppm. The remaining six CH carbons are showed six signals in the range of □ □ □ □ 122.4 to 137.5 ppm. Peak assigning of other carbons was showed in table 3 and figure 7.

Figure 7.^{13}C NMR spectrum of (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide

UV-Visible absorption spectra analysis

Figure 8 shows UV-visible absorption spectra of (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide in solvent which exhibit absorption bands at 264 nm. Normally, pyridine absorbs at visible region and its linked with other azo unit reduces its absorption. The longer wavelength bands can be attributed to the π-π* transitions of the (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide.



Name	No.	Peak(nm)	Peak(AU)	No.	Valley(nm)	Valley(AU)
P2H2-L	1	264.35	4.0000			

Fiure 8. UV spectrum of (*E*)-*N*'-(2,4-dichlorobenzylidene)picolinohydrazide

*IN VITRO*ANTIBACTERIAL STUDY(35)

According to the results of disc diffusion assay (plate 4), this compound has active compounds that are effective for the prevention of infections caused Mycobacterium. The maximum zone produced by the compound 17 mm at 100 µg . The lowest zone of growth inhibition was 10 mm at 25-50µg and no activity was recorded at negative control. while the positive control (AMC) showed inhibition diameters 20 mm (table 4). The percentage of relative zone of inhibition of synthesized compound was calculated as 80%..

Table 4.antibacterial activity of compound

Organism	Zone and inhibition mm in dm				
	NC	Rif pc	25 µg	50 µg	100 µg
M.smegmatis	_	20	12	12	18

IN SILICO DOCKING WITH MYCOLIC ACID SYNTHESE

These compounds showed great hydrophobic contributions and for each established system 100 ns of molecular dynamics simulations were performed and the binding free energy was calculated.The analysis of the intermolecular interactions is useful for identification and optimization of contacts between ligands and target given in figure 9. The results for the ligand molecules selected are shown in table 5. Ligand performs hydrophobic interactions of its dihydroxybenzene ring with the TRP 60, GLU84 and GLY 59 with 2.40≥2.05≥2.28 A hydrogen bond distance. The free energy is -7.2 and – 7.5. Hydrogen interactions with the same residues have already been verified in other studies

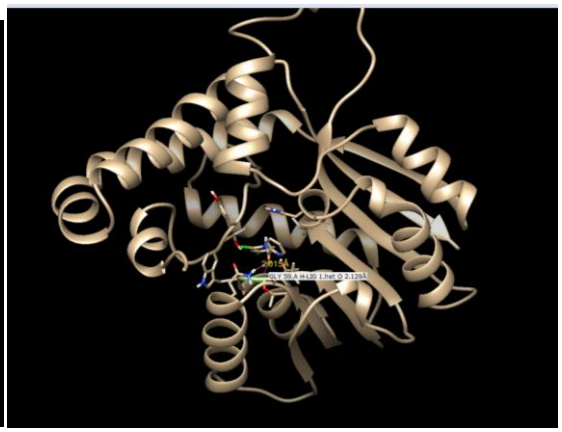


Figure 9. In silico docking of picolinohydrazide ligand with Mycloic acid synthase

Table 5.scoring of intermolecular interaction between receptor and ligand

Pose	Score	Residues	Distance of hydrogen
Pose 1	**-7.5**	TRP 60 LIG1N	2.40
Pose 2	**-7.2**	GLU84 –LIG1O GLY 59-LIG 1O	2.053 2.286

CONCLUSION

In the present study, substituted picolinohydrazidewas synthesized and evaluated as antibacterial compared with standard drug rifampicin and results interpret that having nitrogen as heteroatom in the heterocyclic nucleus found to be more potent than the standard drug rifampicin.In silico study revals that the synthesized compound capable to inhibit mycolic acid synthase and might be a novel antimycobacterial agent.

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