SYNTHESIS CHARECTERIZATION OF PYRAZINEAMIDE AND ITS ACETYLCOLINESTERASE INIHIBITION ASSAY

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Abstract

A probe into the literature survey clearly reveals no work has been administered for the synthesis of pyrazinamide(TLC) using Teraphthaldehyde . therefore this study aimed to figure and produce highly effective medicinal hydrazide derivatives are synthesized from Schiff base route. The structure of the ligand THC and its complexes were carried various spectral studies. From these studies, i do know about the knowledge of chemistry and spectroscopy. The main objective of this study is to synthesis of pyrazineamide and teraphthaledehyde to determine the structure through the analytical (elemental and TLC) and spectral (IR, H1 NMR and C13 NMR) methods of characterization

INTRODUCTION

Pyrazinamide may be a synthetic pyrazinoic (Fig 1) acid amide derivative with bactericidal property. Pyrazine belongs to the six membered heterocyclic diazines with two nitrogen within the same ring at 1, 4 positions, the opposite members being the pyridazine and pyrimidine with the 2 nitrogens at 1, 2 and 1, 3 positions respectively. Another pyrazine containing heterocycle is that the quinoxaline or benzopyrazine. Both pyrazine and quinoxaline derivatives are quite important thanks to their crucial roles in natural and artificial compounds (1).

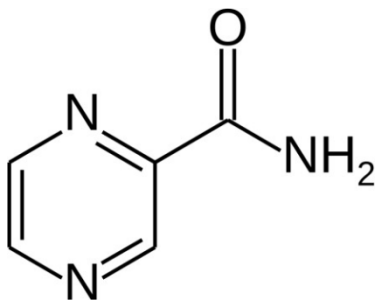


Figure 1. structure of Pyrazinamide

Schiff's bases are a crucial class of organic compounds . They were first reported by Hugo Schiff in 1864. Schiff's bases are condensation products of primary amines with carbonyl compounds. The common structural feature of those compounds is that the azomethine group with the overall formula RHC = N-R1, where R and R1 are alkyl, aryl, cycloalkyl, or heterocyclic groups . Structurally, a Schiff's base (also referred to as imine or azomethine) may be a nitrogen analogue of an aldehyde or ketone during which the group (>C = O) is replaced by an imine or azomethine group (2)

The pyrazi -namide has good antibacterial effect, but there are side effects, and therefore the main side effect is to cause liver damage. additionally , the water/fat-solubility of pyrazinamide isn't good. Pyrazinamide is especially active against slowly multiplying intracellular bacilli (unaffected by other drugs) by an unknown mechanism of action. Its bactericidal action depends upon the presence of bacterial pyrazinamidase, which removes the amide group to supply active pyrazinoic acid. The structure of pyrazinamide was optimised by structure modification with alkyl chains/six membered rings and bioisosterism . aside from their other bioactivities like antidiabetic anti- inflammatory ,antimicrobial and diuretic, pyrazine derivatives, like pyrazinamide, have an important role in controlling tuberculosis a life threatening disease (3)

Pyrimidine derivatives comprise a diverse and interesting group of drugs is extremely important for their biological activities. Dihydropyrimidine and their derivatives have attracted increasing interest owing to their therapeutic and pharmaceutical properties, such as antiviral, antitubercular, antimicrobial agent antagonists of the human adenosine A2A receptor, cyclooxygenase-2 inhibitory activity tyrosine kinase inhibitors, antiamoebic activity, cytotoxicity and acetyl cholinesterase inhibitor activity.

Pyrazinamide (PZA) is a first-line drug that exhibits unique sterilizing activity towards both drug-susceptible and MDR-TB. It is responsible for the killing of the persistent tubercle bacilli during the initial intensive phase of chemotherapy, allowing treatment to be shortened from 9 months to 6 months for drug susceptible cases (4). PZA

therapy has been linked to improved outcomes for both non-MDR and MDR-TB, and is being considered as a part of the longer term regimens in combinations with bedaquiline, delamanid, PA-824 and moxifloxacin, which are currently in phase three trials Tuberculosis (TB) claimed more livels than the other communicable disease . this is often primarily because TB is rampant precisely within the areas where it's most challenging to treat thanks to the poverty of these areas and therefore the lengthy treatment course. Adding to the problem in effective treatment is widespread drug resistance. Of the four front-line anti- TB drugs (isoniazid, rifampin, pyrazinamide (PZA), and ethambutol), PZA has been an irreplaceable component in clinical TB regimens thanks to its unique activity against persisters in chronic Mtb infections and skill to shorten treatment times (5) it's for these reasons that PZA is suggested by the WHO as an integral component within the treatment of multidrug resistant TB.

Pyrazinamide, the pyrazine analogue of nicotinamide, is an anti-tuberculous agent. it's a white crystalline powder, stable at temperature , and sparingly soluble in water. Pyrazinamide (PZA), which is an analogue of nicotinamide, is a crucial first- line drug utilized in the short-course treatment of tuberculosis. This antibiotic plays a key role in shortening the duration of antituberculous treatment due to its activity against the persisting tubercle bacilli at an acidic pH . PZA, which may be a prodrug barren of significant antibacterial activity, is metabolized into its active form, pyrazinoic acid (POA),by the amidase activity of theMycobacterium tuberculosis nicotinamidase/pyrazinamidase (6)

Tuberculosis (TB) may be a common and fatal chronic communicable disease , and it's easy to occur in children . The disease is mainly caused by Mycobacterium tuberculosis infection, also from M. bovis, M. africanum, M. canetti, M. microti, and other species of M. tuberculosis infection, but the mycobacteria don't usually infect healthy(4/6) (7) . Pyrazinamide (PZA)—is successfully wont to shorten the time needed for treatment up to 2 thirds. it's characterized by its unique ability to kill dormant sorts of mycobacteria PZA is endowed with multiple mechanisms of action supported its parent form or its metabolite pyrazinoic acid (POA). one among the primary theories related to the mechanism of action of PZA is predicated on transport of PZA into the mycobacterial cell and its subsequent activation via nicotinamidase(8). Hybrid

compounds supported a mixture of the first-line antitubercular pyrazinamide (PZA) and a formerly identified antimycobacterial scaffold of 4-arylthiazol-2-

amine were designed. Eighteen compounds were prepared, characterized and tested for in vitrogrowth inhibition activity against M. tuberculosis H37Rv.(9)

Alzheimer's disease (AD) is that the leading explanation for dementia among older people. it's a chronic and progressive neurodegenerative disease that's neuropathologically characterized by the extracellular deposition of β-amyloid aggregates and intraneuronal neurofibrillary tangles. Neuronal loss at the affected regions causes a deficit within the production of the neurotransmitter acetylcholine, resulting in cortical cholinergic dysfunction. A series of novel and biologically interesting pyrazinamide condensed azetidinones were synthesized and structurally analyzed. The azetidinones were prepared using triethylamine in 1,4-dioxane as an efficient catalyst. The importance of substitutions at the azetidinone fourth positions was studied by examining acetylcholinesterase and butyl cholinesterase inhibitory activities. novel pyrazinamide condensed azetidinones was prepared with pyrazinamide Schiff's bases and chloroacetylchloride within the presence of catalytic amounts of 1,4-dioxane and triethylamine reported as AchE inhibitor.

EXPERIMENTAL METHOD

4.1 Characterization techniques used:

Some physical methods were wont to elucidate the bonding and structure of the synthesized ligands and complexes and to verify the expected properties. While the ligands were characterized by usual methods like analytical technique like TLC, molar conductance, magnetic susceptibility and spectral techniques like IR, UV- Visible, NMR and mass spectral 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.

Chemicals used as

All the chemicals used were of Merck and Sigma Aldrich products, available commercially in AR grade. The purchased chemicals were used with none further purification.The physicochemical techniques employed for this study is discussed below.

4.1.1 TLC:

Thin Layer Chromatography has been used as an analytical tool, especially in chemistry due to its high speed of separation and its applicability during a sizable amount of chemical compounds. The high sensitivity of TLC is employed to see 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 colloid G, prepared by shaking colloid G with chloroform-methanol (2:1) mixture at temperature . The homogeneity of the compounds was monitored by this TLC plates and visualized by iodine vapour.

4.2 Spectral methods:

4.2.1 Infrared spectroscopy:

Most of the spectra give sufficient information about the structure of the compound. The Infra Red spectrum is one among the spectra. Unlike UV spectrum which comprises of relatively few peaks, IR technique provides a spectrum containing an outsized number of absorption bands from which a wealth of data are often derived about the structure of an compound . The absorption of Infra-Red radiations causes the varied bands during a molecule to stretch and bend with reference to each other .

The IR spectroscopy is widely used as a characterization technique for metal complexes. the essential 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 within the IR Spectrum. The changes within the structural features of the ligands are observed as changes in bands observed, mainly within the fingerprint region (4000-400 cm-1). The bands thanks to the metal ligand bonds are mainly observed within the far IR region (600-100 cm-1).

In the present study, IR spectra of the compounds were recorded using Perkin Elmer spectrum RXI using KBr pellets at frequency range 4000-400 cm-1at ACIC, St. Joseph′s College (Autonomous), Trichirapalli and Shimadzu FT IR 400 Spectrophotometer, frequency range 4000-400 cm-1 using KBr disc at St Joseph’s College, Trichy

4.2.2 Nuclear Magnetic Resonance spectroscopy:

4.2.3 ^{13}C NMR:

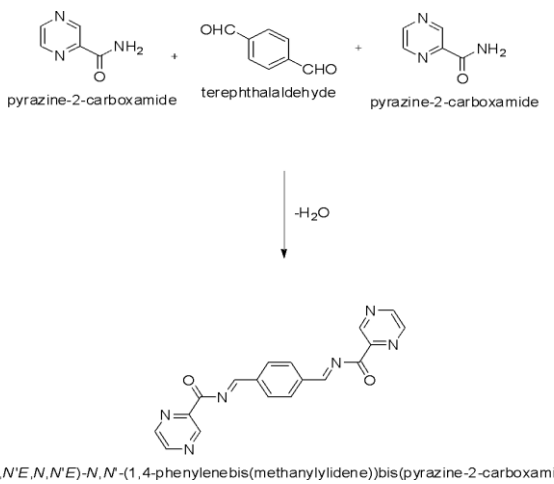
There are many differences between 1H NMR and 13C NMR spectra both within the mode of recording also as appearance. The spin quantum number, I for 12C is adequate to zero since 12C isotope has a good number of protons and even number of neutrons and hence no magnetic spin. It is, therefore, non-magnetic and doesn't give any NMR signal. The natural abundance of 13C is merely about 1.1% and has an odd number of neutrons. So, 13C features a spin quantum number adequate to ½ and its nuclear resonance are often observed during a magnetic flux of 23,500 gauss at 25.2 mega cycles per second. 1H spectrum is generally obtained by sweeping either the excitation frequency or the held through the region of precession frequencies. The inefficiency of this method is obvious from the very fact that just one line are often observed at a given point in time. the matter arises when 13C with intrinsically narrow lines covering a good absorption range are studied. It is, therefore, advantageous to excite the entire band of frequencies simultaneously. it's done by strong pulse of radio-frequency covering an outsized band of frequencies which is capable of exciting all resonance of interest directly . At the top of the heart beat period, the nuclei will process freely with their characteristic frequencies reflecting with the chemical environment (Ele. Org. spec-231 &) and exhibit chemical shifts. 13C NMR of the synthesized compounds were recorded on 75 MHz Bruker Spectrometer at 298.6 K using DMSO-d6 as solvent at SASTRA University Tanjore.

4.3 SYNTHESIS OF PYRAZINEAMIDE DERIVATIVES

4.3.1 SYNTHE IS OF THC CHEMICALS REQUIRED:

Pyrazineamide	0.5g
Teraphthaldehyde	0.2722g
Ethanol	40mL
HCL	1mL

Pyrazineamide and teraphthaldehyde were taken in 0.5 g of pyrazineamide (0.00406 mole) was



taken during a round bottom flask and 40 mL of ethanol was added 0.2722gof teraphthaldehyde(0.00406 mole) and After half-hour added 1ml HCL pour to the present solution keeping the Schiff base reaction . After eight hours a heating , finally yellow coloured solution formed (Scheme 3). This solution was filter to small conical flask and kept during a fourteen days.Finally formed to the crude sample was washed with acetone and dry to recrystallised from ethanol. The purity of the compound was checked by Thin Layer Chromatography(TLC).

Figure 2. schematic representation of compound synthesis

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 hot condition like the boiling point of solvent. Solubility also tested under cold condition and RT.

TLC analysis.

Pre-coated colloid 60 F254, 20 × 20 cm TLC plate was purchased from E. Merck (India) Limited, (Mumbai, India). The plates were developed in horizontal twin trough glass chamber with . St

UV analysis

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

FTIR analysis

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

NMR analysis

1H NMR (200 MHz) and 13C NMR (50 MHz) spectra were recorded on Bruker Avance DRX200 spectrometer at the SASTRA University, Thanjavur

***In vitro* AChE assay**

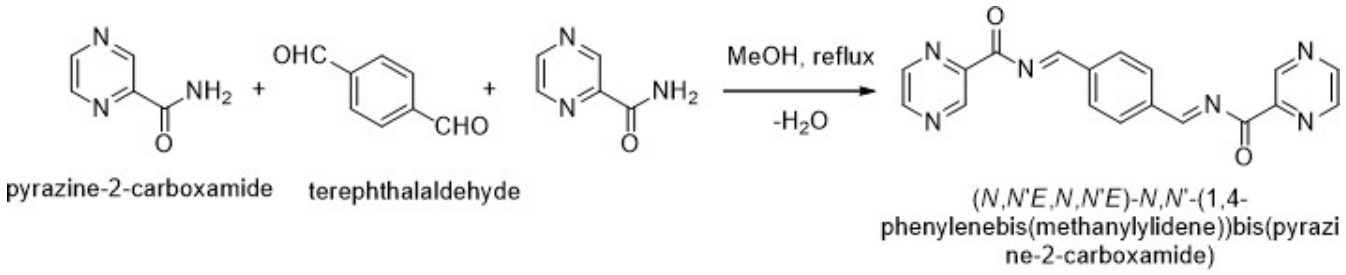
All of the assays were administered in 0.1 M KH_2PO_4/K_2HPO_4 buffers at pH 8.0 employing a Shimadzu UV-2450 spectrophotometer. Enzyme solutions were prepared at a degree of two .0 units/mL in 2-mL aliquots. The assay media (1 mL) consisted of phosphate buffer (pH 8.0), 50 μL of 0.01 M DTNB, 10 μL of enzyme, and 50 μL of 0.01 M substrate (ACh chloride solution). Test compounds were added to the assay solution and pre-incubated at 37 °C with the enzyme for 15 min followed by the addition of the substrate. The activity decided by measuring the rise in absorbance at 412 nm at 1-min intervals at 37 °C. Calculations were performed consistent with Ellman's method. Each concentration was assayed in triplicate. The in vitro assay wont to test BuChE was almost like the tactic used for AChE.

Docking simulations

The initial ChEs coordinates were obtained from the PDB (PDB IDs: 1EVE and 1P0P). The co-crystallized ligands and water molecules of the crystal structure were removed, and therefore the hydrogen atoms were added using the Chimera 1.8 software. For docking studies, we utilized several protein conformations previously obtained through the MD simulation procedures mentioned above. All solvent molecules and therefore the co-crystallized inhibitor were faraway from the structures to supply sterically unimpeded cavities for ligand docking. Docking was performed by using Molegro Virtual Docker (MVD) software package because this algorithm maintains a rigid macromolecule while allowing ligand flexibility. The identification of ligand binding modes is completed by iteratively evaluating variety of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule.

RESULT AND DISCUSSION

5.1 Synthesis of *N*,*N*'-(1,4-phenylenebis(methanylylidene))bis(pyrazine-2-carboxamide)



The compounds were synthesized as outlined in above Scheme by a reaction of schiffbase was given in plate 1. Plate 2 reveals solubility test. The synthesized compound was found to be highly soluble in methanol, ethanol, Benzene, ester and Chloroform and insoluble in water, hexane, acetone and dichloromethane at temperature also as hot condition (table 1). Further the TLC was performed on pre-coated aluminum sheets of silica (60F254) and visualized by short-wave UV light at λ 254 nm. Solvent systems are reported by column volume (CV) with the solvent flow as stated. one spot on TLC colloid glass plate with ethanol confirmed the purity of the synthesized sample and best eluted with chloroform and dichloromethane. TLC plate of the compound pyrazineamide and teraphthaldehyde and merchandise is shown in Fig 2. The optimum mobile phase was used the Rf value of pyrazinamide were ranges between 0.5 to 0.8. least retention factor was 0.2 and 0.3 for chloroform and hexane. the utmost was Rf value 0.8 in acetone. solvent like chloroform and hexane the character spot of pyrazinamide is retained on stationary phase and in nonpolar moved with solvent front. Similar TLC separations were

performed by Mishra et al (48) and reported the range of Rf, 0.74 to 0.90. chemsketch analysis shows the formula is C18H12N6O2 and weight is 344.32688.

Table 1 solubility and *Rf* value of compound Vs solvent

Solvent	Solubility		Rf value	
	RT	Hot	pyr	Product
Water	Absent	Absent	0.775	0.75
Methanol	Present	Present	0.755	0.68
Ethanol	Present	Present	0.48	0.648
Hexane	Absent	Absent	0.28	0.307
Benzene	Present	Present	0.275	0.625
Ethyl acetate	Present	Present	0.358	0.584
Chloroform	Present	Present	0.072	0.2
Acetone	Absent	Absent	0.38	0.888

Dichloromethane	Absent	Absent	0.15	0.525

5.2 Spectral Characterization:

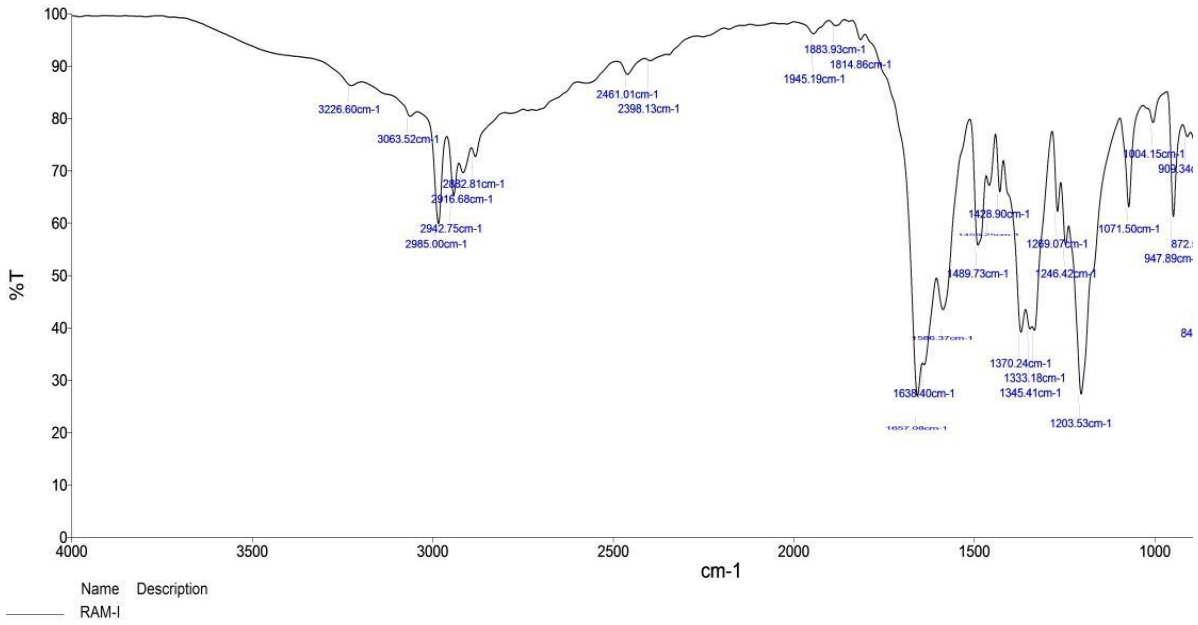
FT-IR Spectral studies:

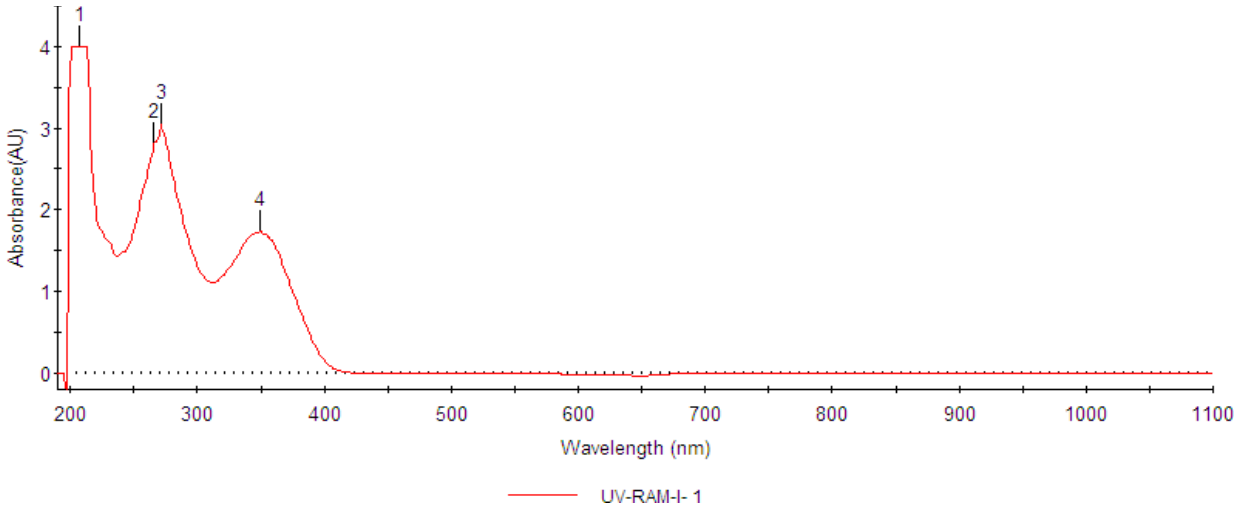
In order to review of functional group of the synthesized Schiff base, the IR spectrum was compared with the overall functional ranges. Generally group stretching vibrations appears at 1680-1700 cm-1 but during this case appeared at 1638 cm-1; this is often thanks to amide group present within the compound which decreases the carbonyl functional group. The IR spectrum of Schiff base showed aromatic ν(ArC-H) stretching vibrations appeared at 3063 cm-1 and also showed olefinic ν(C-H) stretching vibrations appeared at 2985 cm-1. The newly generated C=N bond stretching vibration appeared at 1489 cm-1 along side other finger print region signal and every one other peaks are good agreement with the proposed structure. The FT-IR spectral data are given in table 2 and figure 1.

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

Vibrations	ν(ArC-H)	ν(C-H)	ν(C=O)	ν(C=N)
Peak (cm^{-1})	3063	2985	1638	1489

Figure 3. Important IR bands of THC with their assignments.





Name	No.	Peak(nm)	Peak(AU)	No.	Valley(nm)	Valley(AU)
UV-RAM-I-1	**1**	**206.95**	**4.0000**			
	2	**266.25**	**2.8378**			
	3	**271.20**	**3.0762**			
	4	**349.20**	**1.7369**			

Figure 4. UV spectrum of compound

UV-Visible absorption spectra analysis

Figure 4 shows UV-visible absorption spectra of N,N'-(1,4-phenylenebis(methanylylidene))bis(pyrazine-2-carboxamide) in DMSO which exhibit absorption bands at 206, 264, 271 and 349 nm. The longer wavelength bands are often attributed to the π-π* transitions and intramolecular charge transfer (ICT) of the N,N'-(1,4- phenylenebis(methanylylidene))bis(pyrazine-2-carboxamide)

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

NMR spectra analysis

In 1H NMR spectrum, the proton attached to C7 & C7' carbon showed as a singlet at δ = 8.17 and 9.48 ppm. it had been the unique proton appeared as a pointy singlet without

multiplicity and wont to calibrate other peaks. The four protons attached on the phenyl ring were appeared as a pointy singlet at δ = 8.10 ppm. On the opposite hand, the three protons related to pyrazine

ring were identified as two singlets at δ = 8.76 and 9.34 ppm. thanks to symmetric nature, number of signals less compared to number of proton present within the molecule. The detailed assignments of protons got in table 3 and figure 5.

Table 3 NMR Spectroscopic Data (δ) of *N*,*N*'-(1,4-phenylenebis(methanylylidene))bis(pyrazine- 2-carboxamide)

S. No	Position Assignment	^{1}H (δ, ppm)	^{13}C(δ, ppm)
1	1	--	136.0
2	2	8.10, s	129.3
3	3		
4	4	--	136.0
5	5	8.10, s	129.3
6	6		
7	7/7’	8.17, s/9.48, s	163.7
8	8/8’	--	--
9	9/9’	--	173.6
10	10/10’	--	148.4
11	11/11’	--	--
12	12/12’	8.76, s	148.1
13	13/13’		145.9
14	14/14’	--	--
15	15/15’	9.34, s	143.3

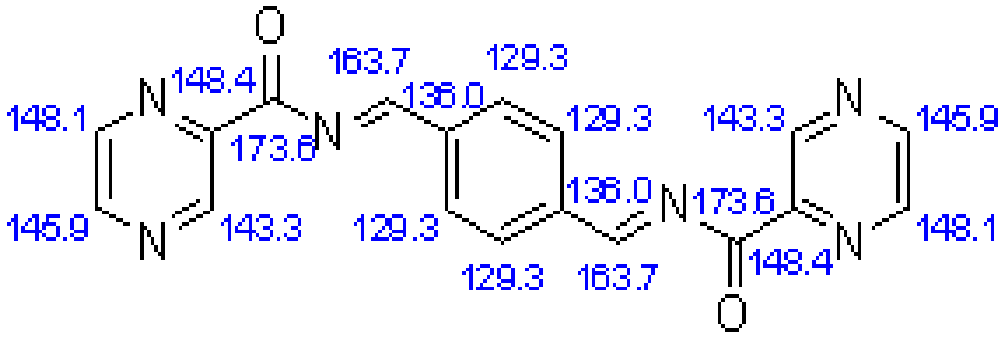
Nuclear Magnetic Resonance spectroscopy:

NMR spectra analysis:

In ^{1}H NMR spectrum, the proton attached to C2 & C7 carbon showed as a singlet at δ = 8.17 and 9.34 ppm. It was the unique proton appeared as a sharp singlet without multiplicity and used to calibrate other peaks. The three protons attached on the phenyl ring were appeared as two doublets at δ = 8.76, 8.17,8.48 and 8.10 ppm and one singlet as discussed early. On the other hand, the four protons associated with pyrazine ring were identified as two doublets at δ = 8.36 & 8.68 ppm . The detailed assignments of protons were given in table 2 and figure 2. 1H NMR spectrums showed signals in the range 8.3 ppm, and these signals were the evidence of the secondary amide bonding to the ligand

Figure 5. ^{1}H NMR spectrum of *N*,*N*'-(1,4-phenylenebis(methanylylidene))bis(pyrazine-2-carboxamide)

In 13C NMR spectrum, the discernible amide carbonyl appeared at δ = 173.6 ppm and it clearly indicates that molecule having amide group on its skeleton. thanks to symmetric in nature, thee phenyl ring shows just one signal for four C-H carbons at δ = 129.3 ppm. Next, the four carbon of pyrazine ring was appeared at δ = 145.9, 148.1, 145.9 and 148.1 ppm. The newly formed imine carbon peak appeared around at δ = 163.7 ppm. Peak assigning of other carbons was showed in table 3 and figure 6.



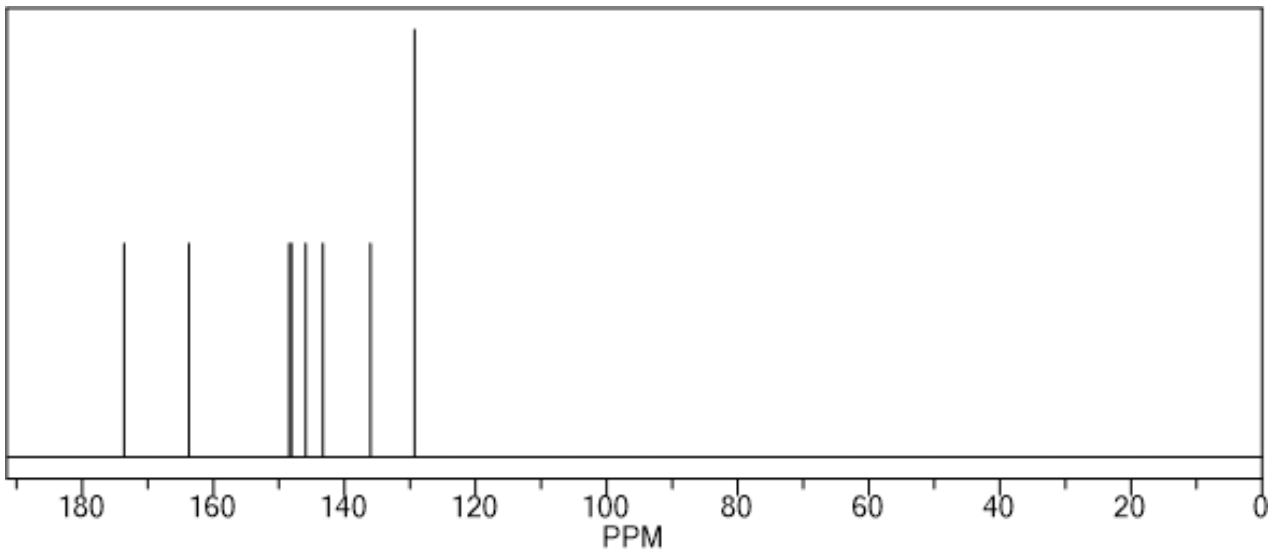


Figure 6. ^{13}C NMR spectrum of *N*,*N*'-(1,4-phenylenebis(methanylylidene))bis(pyrazine-2-carboxamide)

5.3. AchE activity

To determine the potential interest of the synthesized compound, AChE inhibitory potency was evaluated consistent with a modified Ellman method with commercially obtainable Eserine because the reference standard (49). Initially, all the obtained compounds were tested at 10-100µg and 10,25,50 µg concentration for normal and therefore the ChE inhibitory results are outlined in Table 4. With reference to the activity results, it had been determined that activity is concentration dependent and showed better inhibitory activity against AChE maximum 66% for compound and 77% for normal at 100 µg. no activity was found in 10 µg of compound but standard showed 53% inhibition. The IC50 shows synthesized compound has better activity than standard.

Table 4.AChE inhibition assay of compound and standard

Sample	OD	% of inhibition AchE	Ic50
Control	0.09		
100 µg	0.06	66	50 µg
50 µg	0.04	44	
25 µg	0.02	22	
10 µg	-	-	
Eserine 100 µg	0.07	77	
50 µg	0.06	75	69 µg
25 µg	0.055	62	
10 µg	0.046	53	

5.4.Docking reports

Docking of ligand of selected compound during this study was administered within the site of ChEs. three top poses for every ligand were returned within the simulation(figure 7), out of which one best pose for every ligand was selected on the idea of their MolDock score (-9.8, -9.2, -7.2). pose and three were single hydrogen bonded with AchE with GLY(114) and TYR(118). The results obtained using MVD, shown in terms of MolDockScore; Interaction residues, H-bonding distance respectively are given in table 5. Docking clearly shows that the majority of the designed compounds exhibited good to moderate inhibitory activities toward the AChE

Table 5. Docking score of compound with AchE

pose	**Score**	**Amino acid**	**H bond Distance**
1	**-9.8**	**GLY 114- LIG N**	**2.465**

2	**-9.2**	**---**	**Pseudo hydrogen bond**
3	**-7.2**	**TYR118- HHLIGN**	**2.471**

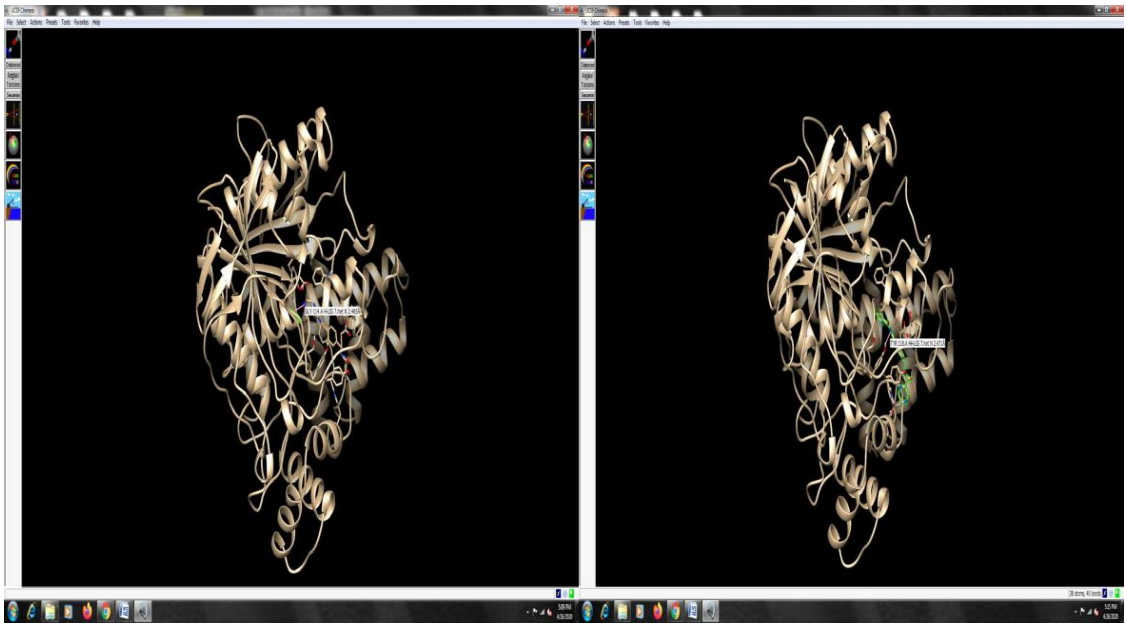


Plate 4. Docking interaction of ligand and receptor using autodoc

SUMMARY AND CONCLUSION

It deals with synthesis and characterization of Pyrazineamide and teraphthaldehyde. the basic analysis shows the presence of nitrogen is confirmed by using sodium fusion extract. The FT-IR spectral study information. The frequencies around ν 3393 and 1638 cm cm-1 confirm the presence of amide group and thiocarbonyl group. indicates amide group. vThe 1H and 13C NMR spectral studies of the ligand THC. The signals appeared in both the spectra give the precise position of every proton and carbon respectively needless to say . Confirmation of the chemical structure of the synthesized compounds was substantiated by TLC, different spectral data IR, 1 H NMR, mass spectra and elemental analysis. The docking studies as described above provide estimation on inhibitory activities of the docked ligand with high score -9.8. Ligand can bind strongly to the enzyme active region due to its dual binding sites. The results showed that the series of novel pyrazinamide derivatives fits well within the site of both cholinesterase enzymes acetylcholinesterase (AChE) and half the maximal inhibitory concentreation was 50µg. to hold out the in vivo AcHE biological studies and anti-cancer activities

Proton NMR

Nuclear Magnetic Resonance spectroscopy:

NMR spectra analysis:

In ^{1}H NMR spectrum, the proton attached to C2 & C7 carbon showed as a singlet at $\delta = 8.26$ and 8.31 ppm. 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 at $\delta = 8.37$ ppm. The three protons attached on the phenyl ring were appeared as two doublets at $\delta = 9.96$ & 8.89 ppm and one singlet as discussed early. On the other hand, the four protons associated with pyridine ring were identified as two doublets at $\delta = 8.36$ & 8.68 ppm figure 2. 1H NMR spectrums showed signals in the range 8.3 ppm, and these signals were the evidence of the secondary amide bonding to the ligand [42].

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