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Synthesis, characterisation and chemotherapeutical studies of 2- Pyrazinoyl hydrazide and 2-pyrazinoyl hydrazones

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Abstract
Salicyclic acid has been suggested to be the simplest model for substances in natural waters[1] and a convenient ligand substrate for biologically relevant tyrosine phenoxide and glutamic /aspartic carboxylate functions[2]. It can adopt several bonding modes[3] and act as an effective agent for the synthesis of photoactive carboxylato polynuclear manganese complexes[4]. Salicylates of different metal ions[5] have wide spread applications both as antiseptic and medicinal agents[6].The mononegative hydrazone ligands coordinate to the metal atoms through azomethine nitrogen, carbonyl oxygen and sulphur of thiophene ring (HL1)/ nitrogen of pyridine ring(HL2) and found to have square pyramidal geometry around each vanadium ion. Ligands and complexes were screened for in vitro antibacterial activity and antifungal activity at different concentrations against bacteria viz. Gram positive Bacillus subtilis, Micrococcus Luteus and Gram negative Pseudomonas aeruginosa, Pseudomonas mendocina and fungi Verticillum, Cladosporium, Tinospora. A distinct enrichment in biocidal active

Keywords: Pyrazinoyl hydrazide and 2-pyrazinoyl hydrazones

Introduction
Salicyclic acid has been suggested to be the simplest model for substances in natural waters [1] and a convenient ligand substrate for biologically relevant tyrosine phenoxide and glutamic /aspartic carboxylate functions [2]. It can adopt several bonding modes [3] and act as an effective agent for the synthesis of photoactive carboxylato polynuclear manganese complexes [4]. Salicylates of different metal ions [5] have wide spread applications both as antiseptic and medicinal agents [6]. Incorporation of α-dimine as second ligand in meta l-salicylato frame increases the cytotoxicity and the neutral molecules get easily transported across the cell membranes[7]. Here we have undertaken the preparation of 2-pyrazinoyl hydrazide and its various derivatives with various aromatic and heterocyclic aldehyes.

Materials and Methods:
Pyrazine-2-carboxylic acid, 2-pyrazinoyl hydrazone, was procured from Williams Lab., U.K. while substituted benzaldehyde were obtained from Johnson chemical company. p-Aminoacetophenone was procured from Aldrich chemical company. Metal salts were of BDH grade. All other chemicals used were of AR grade. Solvents were purified and dried use by usual procedures. Pyrazine-2-carboxylic acid hydrazide prepared beforehand according to published procedure. [Merck F.E. Chem Abstr. 30 (1936) 6994]. The elemental analysis were carried out at the RSIC, CDRI, Lucknow. The electronic spectra were recorded on a schimadzu spectrophotometer and spectra (KBr/nujol) on a Perkin Elmer spectrophotometer. Molar luctance was measured using an Elico conductivity bridge. The magnetic susceptibility was determined by the Guoy method. Molecular weight was rmined by Rast's method also.The Lanthanide contents of the complexes were determined by the oxalate oxide method [(I.M.Kolthoff and P.S. Elving Treatise on analytical chemistry Vol. 8 Part 2 (Interscience New York (1963) p-34 & 51)] and the nitrate contents by the nitron method.

Results and disscussion
Infrared Spectral Studies: The infrared spectral data of pyrazine-2-carboxylic acid hydrazide and its various derivatives with benzal-dehyde, anisaldehyde, p-(N, N-diethylamino) benzaldehyde, cinnamaldehyde, vanillin (4-hydroxy-3-methoxy benzaldehyde), salicylaldehyde, furfuraliyde are discussed in following sub sections.

(a) Frequencies due to (NH) and intra molecular hydrogen bonding

All the compounds synthesized show medium sharp and broad bands with a double structure in the range 3400-3000 cm^{-1} assigned to v(NH) vibrations, another medium broad band observed in region 3100-2840 cm^{-1} indicates stong intramolecular hydrogen bonding in the molecule, resulting in the lowering of v(OH) vibrations[8] From I.R. spectra of these compounds, (NH) rocking modes are also observed as medium sharp bands in the region -10-90-1020 cm^{-1}, while (NH) out of the plane deformation are observed as medium sharp band in the region 720-580 cm-[1(19).]

(b) Frequencies due to amide band I, II & III

The sharp absorption from the compounds observed in the region 1680-1640 cm^{-1}, 1620-1580 cm^{-1} 1390-1300 cm-1 and 1190-1105 cm, are assigned10 to amide band I: v(C=O), amide band II: v(CHN) of immine-N and NH bending modes, amide band III: [v(C=O) + v(C=N)+ γ(CO) + γ(CN)] and amide band IV: [γ(NCO)] + γ(C-O)] respectively. These bands indicate that the ligands primarily exist in the keto from in the solid state. Sharp absorption due to amide band IV [γ (N-CO) +γ (C-O)] observed in the region 1190-1105 cm^{-1} also confirms the existence of pyrazine-2-carboxylic acid hydrazide and its various derivatives in the keto from in the solid state[11].

(c) Frequencies due to pyrazine ring of 12yrazine-2-carboxylic acid drazide

The sharp absorption band due to symmetric and antisymmetric v(C=C) and v(C=N) of pyrazine observed as sharp absorption band in the region 1580-1505 cm^{-1} are also found to be interfered by the sharp absorptions due to amide-II. The double bond character of both these vibrations is considered to be increased due to the donation of electrons by the nitrogen atom of the pyrazine ring [12,13]. The pyrazine ring breathings modes are observed in all the ligands in the region 1090-1020 cm^{-1}, out of plane, pyrazine ring deformations are observed as a medium sharp absorption in the region 985- 920 cm^{-1}, while in-plane pyrazine ring deformation are observed in the region 720-500 cm^{-1} as sharp absorption bands.

(d) Frequencies due to aldehydic adduct

The sharp absorption due to v (O-CH_3)) are observed in the region 2650-2620 cm^{-1} as well as 1090-1020 Cm^{-1} in the IR spectra of pyrazine carboxylic acid hydrazide. (CH) bending modes and (CH) in-plane deformations due to benzene ring as well as pyrazine ring observed in the region 1495-1410 cm^{-1} and 1290-1200 cm^{-1} respectively. In the IR spectra of 2-furfuralidene-2'--pyrazinol hydrazone sharp band observed at 620 cm^{-1} and 590 cm^{-1}, are tentatively assigned to furan ring deformations in the infrared spectra of free ligands. Very sharp and broad absorptions observed at 3500 cm^{-1} and 3450 cm^{-1} respectively [14-17] are assigned to v (OH).

2.5 Nuclear Magnetic Resonance (^{1}H NMR) Studies

Preliminary studies of the ^{1}H NMR spectra of the ligands under investigation, showed that the froms of spectra depend upon a great deal on the concentration of the solutions. Solutions of the vacuum[18] dried compounds, having concentration 5 to10%, using CH_3OD as solvent, were taken, all signals of NMR of the organic compounds appeared to the left of TMS signals i.e., δ=0.000 p.p.m. in zero down field (data summarized in table 2.5). In the NMR spectra a sharp singlet observed in the range δ = 0.4 – δ 1.76 p.p.m., may be attributed to the proton of the CH attached to the aldehydic group, while sharp doublet observed at δ 4.36 p.p.m., of free ligand (PAH), are tentatively assigned to two protons of -NH2 group. This range of absorption is not observed in all the derivatives of PAH prepared by aldehydic condensation at primary amino group, it indirectly confirms the said condensation. The sharp signals observed in the region δ 2.28 - δ 2.72 p.p.m. are tentatively assigned to -CH=N proton. This azomethine proton is also observed due to resonance in the absorption region δ 8.60[19] δ 8.73 p.p.m. however these are interfered due to pyrazine ring protons, in nearby the e region. The signals due to NH proton in the ligands are observed in the region δ 4.90 - δ 5.06 p.p.m. the signals due to pyrazine ring protons, occur as multiplets at δ 8.52 p.p.m. (PAH), while absorption due to pyrazine ring protons in the NMR spectra of PAH derivatives are observed in[20] the region δ 8.62 - δ 8.97 p.p.m., however only in some cases, these resonances are coupled with absorption due to azomethine (CH=N) proton. The sharp multiplets observed in NMR spectra of the PAH derivatives, observed at δ 6.90 – δ 7.10 p.p.m. have been assigned to various aromatic protons interacting in aromatic ring. The induced field of circulating electrons (ring current) reinforces the applied field at the aromatic protons, which are termed as deshielded. As a result NMR absorption due to such deshielded aromatic protons is observed with higher δ values in p.p.m. because of large down field, the same reasoning can also be proposed for pyrazine ring protons[21,22]. The observation of sharp doublet as well as sharp singlet observed at δ 1.88 - δ 2.20 p.p.m. may possibly assigned to the proton exhibiting keto-enol tautomerism[23,24].

2.6. Chemotherapeutical Studies

2.6.1. Evaluation of antitubercular properties

The chemotherapy of infections by mycobacterium tuberculosis, the causative organisms of tuberculosis, presents peculiar difficulties which are not encountered in the chemotherapy of other bacterial infections. They are due to the unusual chemical make-up of the organism, its slow growth and complexities of the lesions it produces. The tubercle bacillus contains proteins, polysaccharides & lipds, Koch's old tuberculin is a water-soluble protein fraction of M. tuberculosis, which when injected into an animal, which is already infected with tuberculosis, produces at the site of injection a characteristic slowly developing inflammatory reaction called the tuberculin reaction. This reaction cannot be obtained in normal animals and is due to the presence of antibodies developed against the tuberculous infection.

Five types of tubercle bacillus are known, but only the human and me are found in clinical infection in men. Infection by the bovine type is almost exclusively by the alimentary tract whilst infection by the human type is mainly through the respiratory tract.

(a) Mycobacteria

The mycobacteria are slender, straight or curved rods ranging from 0.3 to 0.6 by 0.5 to 4.0 µm. They usually occur singly or in clusters but may occasionally exhibit branching and filamentous forms. They are difficult to stain with the usual microbiological dyes, but they stain readily by ziehl-

neelsen technique (initial staining with basic fuschin washed with I and alcohol) probably because of the high fat content of the organisms, they are not decolourized by the acid-alcohol and therefore have been acidfast organisms. The treatment of infections in man caused by acidfast bacteria [25,26] is still one of the most challenging problems today in the field of chemotherapy. The factors that make the management of mycobacterial diseases difficult in many instances, and even impossible in some are,

1. Inadequacy of defense mechanisms in infected human hosts,
2. The metabolic characteristic of myco bacteria,
3. The development of drug resistance in the organisms,
4. The lack of bactericidal activity of most of the available drugs and
5. Therapeutic agents to produce untoward effects that may preclude their administration or necessitate their use in suboptimal sources.

(i) Assessment of tuberculostatic activity

(a) In vitro tests: The first test for tuberculostatic activity of a drug usually consists in determining the minimum concentration at which it inhibits the growth of cultures of the micro organisms. This information, however, is not sufficient, to produce with any certainity the purity of drug in the living animal for many compounds which are inactive when tested in vivo either because they are inactivated by the body fluids or antagonized by certain cellular constituents or because they fail to reach the tuberculous lesion in a sufficiently high concentration.

(b) In vivo tests

A variety of experimentally infected animals have been used for the evaluation of antituberculosis drug including guinea-pigs, mice, rabbits and monkeys. The character of the infection varies in different species. In the guinea-pig even a mild infection tends to be progressive and ultimately fatal; in this species the tubercle bacilli occur mainly extracellularly and are found in great quantities in tectoc tissue. The mouse is much more resistant to tuberculosis than the guinea-pig and tubercle bacilli frequently occur intracellularly. Man has a great resistance to tuberculosis than the guinea pig and the majority of such infections are controlled by the natural deficiencies of the body, tubercle bacilli occur both extracellularly and inytracellularly and large quantities are found in casious lesions species, differences in the character of the disease may make it difficult to predict from animal experiments the clinical effectiveness of new antituberculosis drugs. The drugs used to treat infections caused by acid fast bacteria comprise a heterogenous group that ranges from relatively simple synthetic organic substances to antibiotics. Microbiological techniques, similar to those employed with other organisms have been widely used to determine the in-vitro sensitivity of tubercle bacilli, typical mycobacteria for various drugs. However, the media[27,28] employed must be selected as the basis of their ability to support optimal growth of mycobacteria and the culture must be incubated for at least 6 to 8 weeks before the results become significant. Progress in the study of drugs of potential value in the treatment of tuberculosis has depended on the development of methods for infecting experimental animals with the human strain of M.tuberculosis. Immunological and cytological responses to a tuburculous infection, vary greatly from one species to another, this often makes extrapolation of the results of treatment of the experimental disease to naturally occurring human infection, very difficult [29].

There are number of antitubercular drugs like ethambutol, ethananamide, rifampicin, cycloserine, viomycin, isoniazid, pyrazine carboxylic acid hydrazide, aminosalicylic acid and streptomycin etc. and from them isoniazid is one of the potent antitubercular drug. It is both tuberculostatic and tuberculocidal in-vitro. The minimal. tuberculostatic concentration is 0.025 to 0.05 mcg/ml. The drug does not produce immediate inhibition of rowth of M.tuberculosis, regardless of the concentration to which the organisms are exposed [30]. Isoniazid has an important, though restricted, applications in the therapy of disease. It is an example of competitive inhibition affecting a restricted group of microorganism, the mycobacteria. It has proved to be useful in the control of tuberculosis, in humans and is most effective given when alternately with streptomycin. Because it is a structural analog of pyridoxin or vitamin B6, isoniazid can block pyridoxin catalyzed reactions in micro organism. This accounts for its antimicrobial activity. Pyrazine-2-carboxylic acid hydrazide and its hydrazones with various aldehydes listed in table were screened for tuberculostatic activity to study SAR (structural activity relationship). The antimycobacterial activity of the compounds were studied in-vitro against mycobacterium tuberculosis H_{37} R_v at Central Drug Research Institute, Lucknow.

(ii) Preparation of lowenstein-jensen egg medium

1. Mineral salt solution

potassium dihydrogen phosphate (KH_2PO_4)	=	2.40 g.
Magnesium sulphate ($MgSO_4$)	=	0.24 g.
Magnesium citrate	=	0.60 g.
Aspargin	=	3.60 g
GlyceroI	=	12.00ml.
Water	=	600.00 ml.

All the ingredients were dissolved in water and sterilized by heating in autoclave at 121°C for twenty minutes.

2. Malachite green solution

A 2% w/v solution of malachite green in water was prepared and kept the dye solution in the incubator for 1-2 hour. The complete medium was prepared as-follows:

Mineral salt solution	-	600 ml.
Malachite green solution	-	20m1
Fresh egg yolk (fluid)	-	1200 ml

iii) Testing of Uberculostatic activity

All the ligands were studied in-vitro in serial dilutions upto a maximum dose of 100 mcg per ml. A 2 mg/ml suspension of the samples under study were prepared in tween-80-saline solution (10% tween-80 in 0.9% NaCl sol'n) and 1 mg/ml of stock solution of standard 2-pyrazinoyl hydrazide was prepared in tween-80-saline (0.05% tween-80 in 0.9% NaCl sol'n) and it was serially diluted with the same soIvent to a final dilution of 10 mcg/ml Slant was prepared by heating the L.J. Medium at 800-82^0 C for 60 minutes for three days and this slant was inoculated with Mycobacterium tuberculosis H37R and incubated at 37°C, one loopfull of $H_{37}R_v$, grown on L.J. medium slant was removed and added to 0.5 mI of 0.05% tween-80-saline contained in ¼ oz screw capped bottles having glassbeads; the bottles were shaken for some time, one loopfull is used to inoculate each tube containing

L.J.medium with or without test compound and one such tube is used for two sets of compounds only. 2.0 ml of L.J. egg medium was added, aseptically, with the help of a graduated pipette, in 26 sets of eight sterile media bottles fitted with screw cap (capacity 20 ml). Bottle no. 1 of each set contained 3.8 ml of the sterilized broth and the bottles were numbered (1-8). In bottle no. 1, of all the sets, was added (0.2 ml) sample. The contents of the bottle no. 1 was thoroughly mixed, two ml from bottle no. 1 was withdrawn into bottle no. 2, the contents were mixed, two ml from bottle no. 2 was withdrawn into bottle no. 3 and this process is repeated upto bottle 6, two ml from bottle no. 7 was withdrawn and discarded, bottle no. 8 containing 2.0 ml of medium was taken as reference, the above same process was repeated for other samples and standard, each of the bottle was inoculated with one loopfull of inoculam of $H_{37}R_v$ prepared above. AII the bottles were incubated at 37°C for six weeks. After six weeks bottles were pbserved in comparison to the control (bottle no. 8) for any growth.

Table: 2.4 Ultra violet and infra red frequencies (cm^{-1}) and their tentative assignment of pyrazine-2 carboxylic acid hydrazide (PAH) and its aldehydic derivatives

PAH ($C_5H_6N_4O$)	**PAH-BENZ ($C_{12}H_{10}N_4O$)**	**PAH-ANSL ($C_{13}H_{12}N_4O_2$)**	**PAH-VANI ($C_{13}H_{12}N_4O_3$)**	**Tentative assignment**
1	2	3	4	
1475s 1420s	1480ms 1450ms 1420vs	1490sh 1460sh 1420vs	1470vs 1450s 1420vs	C-H bending modes of benzene ring and hetero ring.
1340vs	1360vs 1340vs	1355ms 1340sh 1310vs	1380ms 1300ms	Amide band III v (C=O) + v (CN) + γ(CO) + γ(CN)
1205vs	1210s	1290sh 1250vs 1200b	1290ms 1250s 1220sh	C-H in-plane deformation
1105ms	1180s 1160ms 1135ms	1175vs 1160vs 1120s	1190ms 1160ms 1120vs	Amide band IV γ (NCO) + γ (CO)
1030vs	1090b 1070ms 1030vs	1070vs 1040vs	1080b 1060ms 1020vs	Pyrazine ring breathing modes and NH rocking modes.
----	985s 960vs 920vs	960ms 920ms	950sh 940vs 930s	Pyrazine ring deformation
880vs	880s	885vs	900vs	δ(N-N)
825vs	835s 760vs 740ms	830vs 810vs 775s	860vs 820vs 790b 740s	
700vs 680b 620s	700vs	720ms 700ms 640b	700vs 650b 620s 580b	In- plane pyrazine ring deformation and (NH) out of plane deformation.

s-sharp. Ms- medium sharp, vs-very sharp, sh-shoulder, b-broad, vb-very broad

Table: 2.4 Ultra violet and infra red frequencies (cm^{-1}) and their tentative assignment of pyrazine-2 carboxylic acid hydrazide (PAH) and its aldehydic derivatives

PAH ($C_5H_6N_4O$)	**PAH-BENZ ($C_{12}H_{10}N_4O$)**	**PAH-ANSL ($C_{13}H_{12}N_4O_2$)**	**PAH-VANI ($C_{13}H_{12}N_4O_3$)**	**Tentative assignment**
5	6	7	8	
1360vs	1360vs 1300vs	1360vs 1330sh 1300vs	1390s 1330s	Amide band III v(C=0)+V(CN)+ γ (CO) + γ (CN)
1290ms 1220s	1195s	1275s 1200ms	1280b 1240s 1220s	C-H in-plane deformation
1180ms 1140s 1120s	1180s 1140s 1120s	1150s 1120b	1190s 1150vs 1110s	Amide band IV γ (NCO) + γ (CO)
1050s 1020s	1060ms 1025vs	1070s 1020s	1080sh 1060vs 1020ms	Pyraine ring breathing modes and NH rocking modes.
980s 940s	970vs	980ms 920ms	940vs	Pyrazine ring deformation.
910s	900vs	880vs	900ms	(N-N)
810vs	830sh 820s	820s 760vs	880ms 820vs	

	740vs		740vs	
700vb	720s 700s 620s	720ms	700vs 620s 590vs	In-plane pyrazine ring deformation and (NH) out of lane deformation

s-sharp, ms-medium sharp, vs-very sharp, sh-shoulder, b-broad, vb-very broad

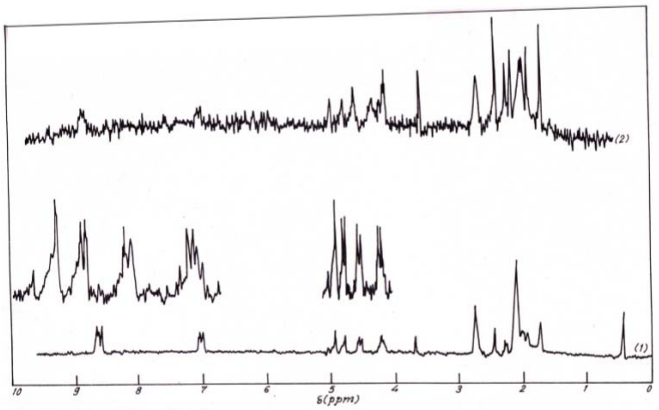

Fig.(2.3): ^1H NMR Spectra of:
(1): Pyrazine-2-carboxylic acid hydrazide (PAH) ($C_5H_6N_4O$).
(2): PAH-BENZ (Benzylidene-2-pyrazinoyl hydrazone) ($C_{12}H_{10}N_4O$).

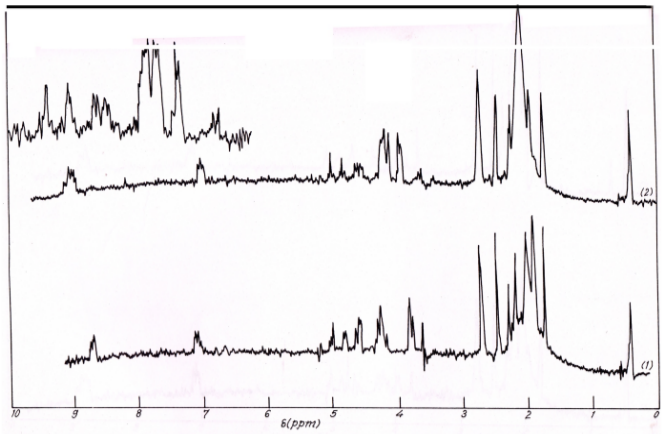

Fig.(2.5): ^1H NMR Spectra of:
(1): PAH-PDEAB (p-(N,N'-diethylamino) benzylidene-2-pyrazinoyl hydrazone) ($C_{16}H_{19}N_5O$).
(2): PAH-CAH (Cinnamalidene-2-pyrazinoyl hydrazone) ($C_{14}H_{12}N_4O$).

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