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Structural Chemistry and In Vitro Antitubercular Activity of Acetylpyridine Benzoyl Hydrazone and Its Copper Complex against *Mycobacterium smegmatis*

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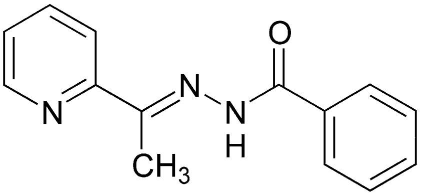
Abstract—Acetylpyridine benzoyl hydrazone (APBH) **1** and its copper complex $[\{(APBH)CuCl\}_2]\cdot(EtOH)$ **2** were structurally characterized by elemental analysis, magnetic measurements, spectroscopy, electrochemistry and single crystal X-ray diffraction studies. The ligand assumes Z-isomeric form and planar geometry in solid state, coordinating through pyridyl nitrogen, azomethine nitrogen and the carbonyl oxygen of the benzoyl group. The copper complex is dimeric and has a distorted octahedral geometry in which the two copper atoms are bridged by two chloride atoms. Antimycobacterial screening of ligand and its copper compound against *Mycobacterium smegmatis* shows clear enhancement in the antitubercular activity upon copper complexation.

The heterocyclic hydrazones constitute an important class of biologically active drug molecules which have attracted attention of medicinal chemists due to their wide ranging pharmacological properties including iron scavenging and antitubercular activities.[1–3] Although biological activities of many of these compounds were shown to be related to their metal chelating abilities in the past,[4–8] evidence confirming the formation of a lipid-soluble copper chelate which facilitates cell internalization has been provided only recently in case of isonicotinoyl hydrazide (INH) which is the most effective first-line antitubercular compound used in the DOT program advocated by WHO.[9,10] We were thus motivated to undertake a systematic study of preparation and characterization of acetylpyridine benzoyl hydrazone (APBH) and its copper complex including their single crystal x-ray structures along with the in vitro antimycobacterial activities against *Mycobacterium smegmatis* which clearly indicates synergistic enhancement in the activity upon metal complexation.



(1)

Acetylpyridine benzoyl hydrazone (APBH)

Experimental

All chemicals and solvents were of analytical grade and were used as supplied. Methyl benzoate and hydrazine hydrate were purchased from Merck (Mumbai, India) while copper chloride was the product of Qualigens (Mumbai, India).

Elemental analyses were carried out in the Microanalytical Lab of University of Pune, India. Magnetic susceptibility of the copper compound was measured at 303 K on a Faraday balance having field strength of 7000 KG. IR spectra were recorded in Nujol in the range 4000–450 cm^{-1} on a Perkin–Elmer 283B FTIR

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instrument. The ^1H NMR was recorded in DMSO-d_6. Electronic spectra were recorded in DMSO on a Genesys-2 UV–vis-NIR spectrophotometer in the range 200–1100 nm. Cyclic Voltammetric measurements were made in DMSO solvent on a Bioanalytical System BAS CV-27 with an XY recorder using Pt as working electrode against SCE as standard and Pt wire as auxiliary electrode. Tetraethyl ammonium perchlorate (TEAP) was used as a supporting electrolyte.

Synthesis

2-Acetylpyridine benzoylhydrazone (**1**, APBH) was prepared by stirring a mixture of methanolic solution of 2-acetyl pyridine (0.445g, 1.0 mmol) and benzoyl hydrazide (0.50g, 1.0 mmol) in methanol for 2 h after which the solvent was stripped off on a rotary evaporator and the white solid obtained was dried in vacuum. Benzoyl hydrazide was prepared according to a literature method.[11] Single crystals suitable for X-ray diffraction studies were obtained from benzene: ethyl acetate (7:3 V/V) solvent mixture of the crude product (mp 149 °C). Anal. calcd for $C_{14}H_{13}N_3O$: C,70.21; H,5.43; N,17.55. Found: C, 70.10; H, 5.47; N, 17.42%, IR (nujol): ν (C=O) 1654 cm^{-1}, ν (C=N) 1616, 1579 cm^{-1}, ^1H NMR (DMSO-d_6), δ (–NH) 10.87, δ (–CH3) 2.46 ppm.

The copper complex *[{(APBH)CuCl}$_2$·(EtOH)]* (**2**) was prepared by refluxing the ethanolic mixture of APBH and copper chloride dihydrate in the molar ratio (1:1) for 1 h. The precipitate obtained was filtered and single crystals suitable for X-ray diffraction studies were grown from the mother liquor by slow evaporation (mp > 350 °C). Anal. calcd For $C_{29}H_{28}Cl_2Cu_2N_6O_3$: C, 49.25; H, 3.96; N, 11.88; Cu, 17.98. Found: C,49.14; H,3.90; N, 11.78; Cu,17.86%. IR (nujol): ν (C=N) 1599, 1564 cm^{-1}, ^1H NMR (DMSO-d_6), δ (–CH_3) 2.25 ppm.

Table 1. Crystal data and refinement summary for **1** and **2**

	1	**2**
Empirical formula	$C_{14}H_{13}N_3O$	$C_{30}H_{30}Cl_2Cu_2N_6O_3$
Formula weight	239.27	720.58
Temperature (K)	200	200
Wavelength	0.71073	0.71073
Crystal system	Orthorhombic	Monoclinic
Space group	*Pbcn*	*P*2$_1$/*c*
a (Å)	18.888(3)	8.5137(8)
b (Å)	7.9514(7)	10.9719(7)
c (Å)	16.0749(15)	16.3181(14)
α (°)	90	90
β (°)	90	92.396(11)
γ (°)	90	90
U (Å^3)	2414.3(5)	1523.0(2)
Z	8	2
ρ(calc) (Mg m^{-3})	1.317	1.541
μ(Mo-K_α) (mm^{-1})	0.086	1.613
F(000)	1008	736
Crystal size (mm)	0.40×0.25×0.15	0.35×0.25×0.10
2θ range (°)	5.56–51.10	6.22–52.32
Reflections measured	4041	11806
Independent reflections	1877	2800
R_{int}	0.0430	0.0288
Parameters	215	256
wR_2 (all data)	0.0973	0.0945
S (all data)	0.894	1.000
R_1 [I > 2σ(I)]	0.0370	0.0350
Max diff. peak/hole (eÅ^{-3})	0.143/−0.113	0.560/−0.361

X-ray crystallography of 1 and 2. Crystal data and numerical parameters for the data collection and refinement for compounds **1** and **2** are summarized in Table 1. Data were measured using a Stoe IPDS area detector diffractometer with graphite-monochromatised Mo-K_α radiation; wherein the crystals were cooled to 200 K with an Oxford Cryostream low-temperature attachment. The structures were solved by direct method and refined against F^2 (all data, anisotropic thermal parameters for all non-H atoms, all H atoms located and fully refined with isotropic thermal parameters), using the SHELXTL software suite.[12]

Crystallographic data (excluding structural factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 173601 and 173602. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

Antimycobacterial assay against *M. smegmatis*

2YT agar medium was prepared using 1% yeast extract, 2% trypton, 1.5% agar, 1% NaCl in 250 mL distilled water by maintaining the pH of the medium at 7 using 10% NaOH solution. This medium was then sterilized by autoclaving at 120 °C for 15 min. After cooling to 50 °C the medium was poured into 85 mm diameter Petri dishes (approx. 25 mL each) and setting aside at 37 °C. After a few hours, Petri dishes were stored in the cold room at 4 °C. Freshly prepared 100 μL of inoculum of *M. smegmatis* was spread in each dish and 20 μL (100 μg) solution of the test compound was poured in each well. 20 μL DMSO was used as negative control. The plates were kept at 37 °C for 24 h after which the diameter of the inhibition zones was measured.[13] Ciprofloxacin (100 μM) was used as a standard reference drug for comparison.

Structural data

The molecular structures of **1** and **2** are shown in Figures 1 and 2. The selected bond distances and bond angles for the ligand and its copper complex are compared in Table 2.

The crystal structure of **1** shows the central C_4N_2O unit of the molecule to be planar, and in the Z-isomeric form due to the placement of oxygen atom O(1) *cis* to the azomethine nitrogen N(2), an arrangement which remains unaltered in its copper complex. The phenyl ring is rotated out of the molecular plane (mean torsion angle about C(8)–C(9) = 47.2°) to avoid an unfavorable steric interaction between the hydrogens bonded to N(3) and C(10). This interaction is absent in **2** due to deprotonation of N(3) and the phenyl ring is almost coplanar with the rest of the ligand. In case of ligand **1**, the pyr-

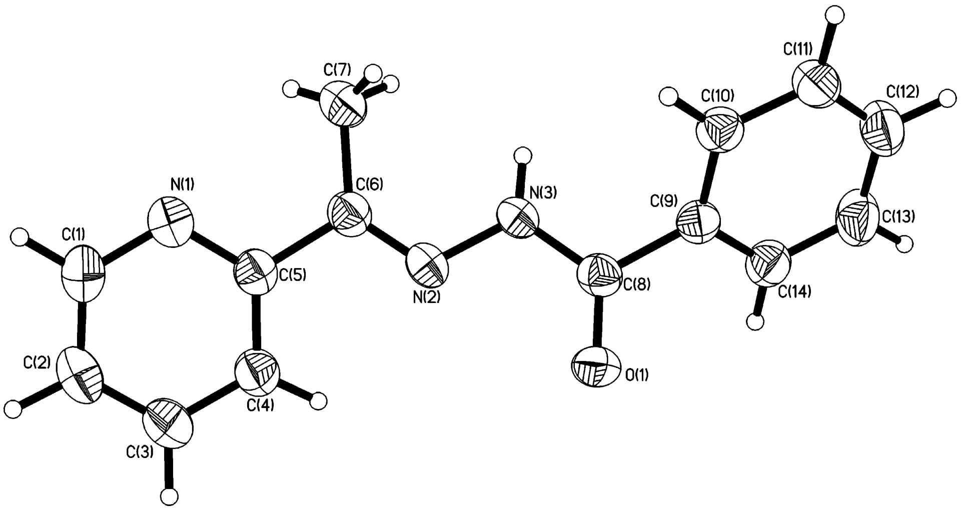

Figure 1. Molecular structure of APBH (1) (50% thermal ellipsoids).

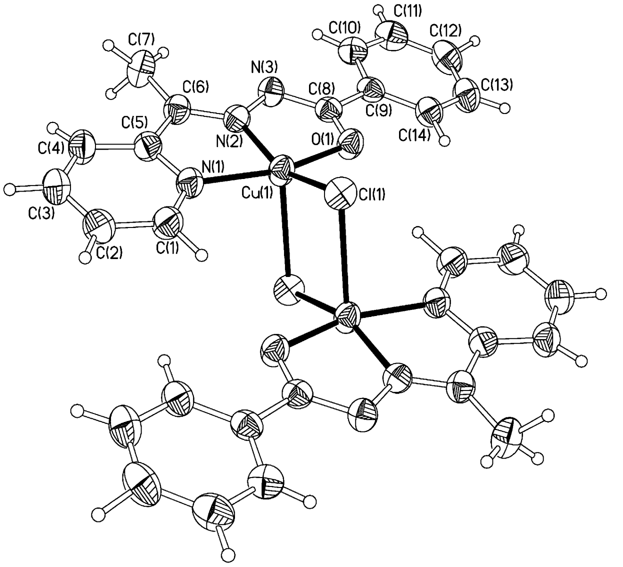

Figure 2. Molecular structure of [{(APBH)CuCl}$_2$]·(EtOH) (2) (50% thermal ellipsoids).

idyl ring is rotated out of the plane. In case of ligand **1**, the pyridyl ring is rotated out of the plane, to a small extent (24.9°). The pyridyl and azomethine nitrogens are placed at trans positions in 1 which on copper coordination attain cis placement. The C=O bond length in the hydrazide side chain is 1.219(2) Å which is typical of the ketonic linkage (1.23 Å).[14] It is lengthened [1.279(2)] Å on copper coordination, with concomitant shortening of N(3)–C(8) bond from 1.347(3) to 1.326(3) Å, due to a contribution from the enolic tautomer of **2**. The coordination environment around the central copper atom in **2** is best described as five-coordinated wherein the atoms N(1), N(2), O(1) and Cl(1) constitute the distorted square with slight displacement of the central copper atom towards the apical halogen atom. The apical metal–halogen bond is found to be longer [2.6434(7) Å] than the axial metal–halogen bond [2.2603(7) Å]. Complex **2** is thus a dimer, with a nearly rectangular $Cu_2(\mu\text{-}Cl)_2$ core similar to that found in the analogous copper compound of dimethylenediamine, $[Cu_2Cl_2(dmen)_2]$,[15] although the Cu–Cl bond lengths in the latter are slightly longer. The octahedral coordination around Cu(1) in the present dimer is completed by the weakly interacting deprotonated N(3) atom of the adjacent dimer at $\{x-1,\ y,\ z\}$.with the Cl(1′)–Cu(1)··N(3″) bond angle being 165.9°. These interactions, operating pairwise, nonetheless organize the dimers into chains running parallel to the crystal *a*-axis (Fig. 3).

Table 2. Selected bond distances (Å) and bond angles (°) for **1** and **2**

Bond distances	1	2
N(1)–C(5)	1.325(3)	1.359(3)
C(5)–C(6)	1.487(2)	1.464(4)
C(6)–N(2)	1.264(3)	1.287(3)
N(2)–N(3)	1.375(2)	1.370(3)
N(3)–C(8)	1.347(3)	1.326(3)
C(8)–O(1)	1.219(2)	1.279(3)
Cu(1)–N(1)		2.013(2)
Cu(1)–N(2)		1.928(2)
Cu(1)–O(1)		1.964(2)
Cu(1)–Cl(1)		2.2603(7)
Cu(1)–Cl(1′)		2.6434(7)
Bond angles		
C(5)–C(6)–N(2)	114.47(15)	112.8(2)
C(5)–C(6)–C(7)	118.2(2)	123.0(2)
N(2)–C(6)–C(7)	127.12(16)	124.2(3)
C(6)–N(2)–N(3)	119.02(14)	123.1(2)
N(2)–N(3)–C(8)	117.02(13)	107.3(2)
N(3)–C(8)–C(9)	115.99(13)	116.8(2)
N(3)–C(8)–O(1)	123.32(15)	125.4(2)
O(1)–C(8)–C(9)	120.68(18)	117.7(2)
N(1)–Cu(1)–N(2)		80.30(9)
N(1)–Cu(1)–O(1)		159.67(9)
N(2)–Cu(1)–O(1)		79.54(8)
N(1)–Cu(1)–Cl(1)		100.16(7)
N(2)–Cu(1)–Cl(1)		156.43(6)
O(1)–Cu(1)–Cl(1)		99.37(6)
N(1)–Cu(1)–Cl(1)#1		93.06(6)
N(2)–Cu(1)–Cl(1)#1		110.35(6)
O(1)–Cu(1)–Cl(1)#1		91.47(6)
Cl(1)–Cu(1)–Cl(1)#1		93.20(2)
Cu(1)–Cl(1)–Cu(1)#1		86.80(2)

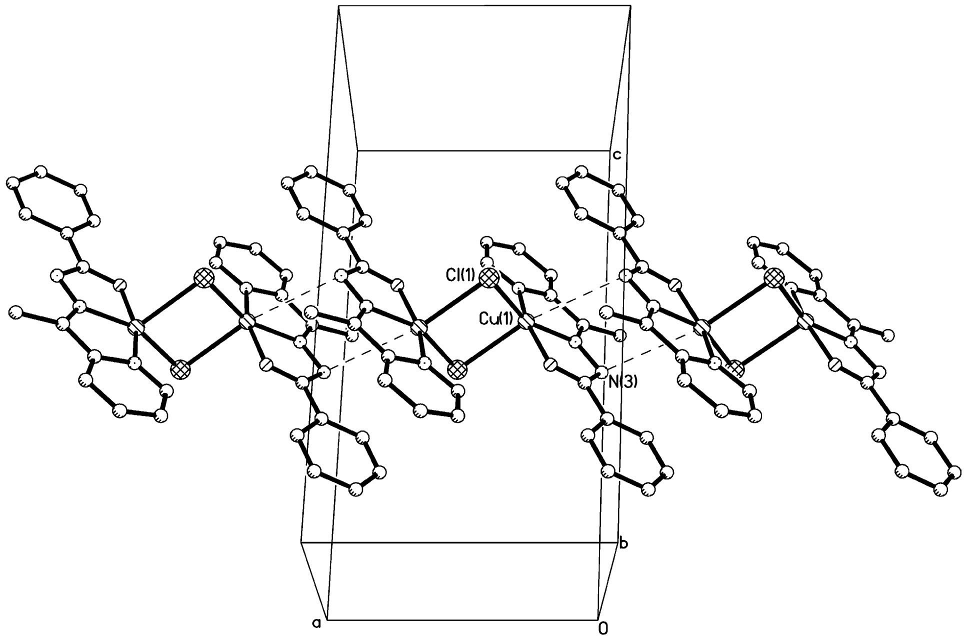

Figure 3. Chain of dimers running parallel to the crystal *a*-axis in **2**, showing the weak Cu(1)–N(3″) interaction which completes the octahedral coordination around Cu(1).

Spectroscopy and magnetism

The imine frequency ($\nu_{(C=N)}$) in the hydrazone ligand **1** is observed at 1616 cm^{-1}, which is shifted to 1599 cm^{-1} upon copper complexation indicating the involvement of the azomethine nitrogen in metal coordination. This is further supported by the shift of the peak due to –CH$_3$ group at $\delta = 2.25$–2.46 ppm in the copper complex. Similarly the involvement of the carbonyl function in the side chain of the enolic tautomer of the ligand in metal complexation is indicated by the disappearance of the band at 1654 cm^{-1}. It is supported by the loss of the peak due to imino group at $\delta = 10.57$ ppm in the NMR complex of the complex. The binding of the pyridyl nitrogen to the metal is indicated by the displacement of $\nu_{(CN)}$ frequency from 1579 to 1564 cm^{-1}.

The copper compound shows a much-lowered magnetic moment of 1.30 B.M similar to many analogous chloride-bridged copper complexes[16–18] indicating strong antiferromagnetic interactions between the two copper centers. The electronic spectrum of the ligand in DMSO shows the ligand-based transitions at 35,700 ($\varepsilon = 3819\,M^{-1}$ cm^{-1}) and 25,700 ($\varepsilon = 3774\,M^{-1}$ cm^{-1}) cm^{-1} respectively. However, in the visible region of the spectrum instead of three transitions usually observed for the octahedral copper compounds[19–21] only one broad absorption is seen for the present compound around 13,600 cm^{-1} ($\varepsilon = 28\,M^{-1}$ cm^{-1}) and is ascribed to $E_{2g} \rightarrow T_{2g}$[22] transition.

Cyclic voltammetry

The cyclic voltammograms of the ligand and its copper complex in DMSO are shown in Figure 4 which show ligand-based reductions at −0.65 V (quasireversible reduction of the aroyl hydrazone moiety[23]) and −1.38 V [azomethine (C=N) reduction], respectively. The Cu(II)/Cu(I) redox couple in the metal complex is observed at +0.25 volts indicating a facile reduction of the copper center which perhaps contributes to its enhanced antimycobacterial activity.

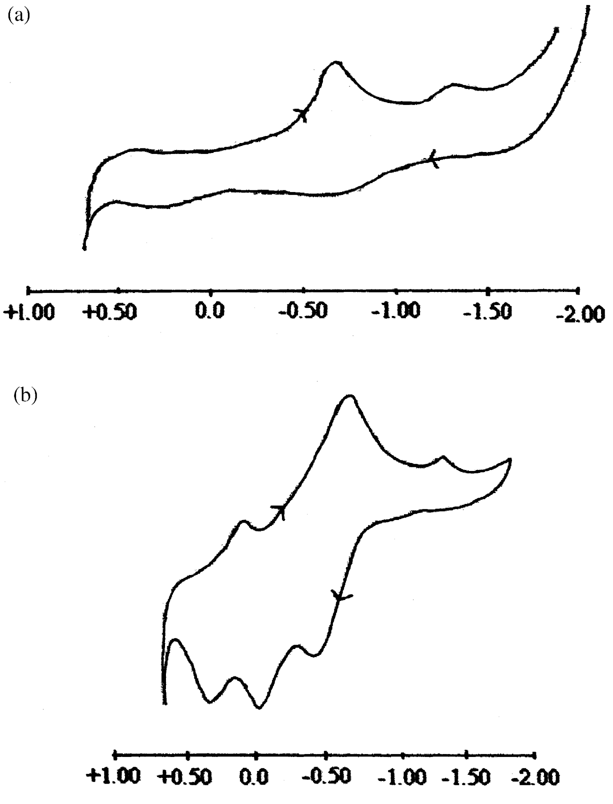

Figure 4. Cyclic voltammograms of ligand **1** and its copper complex **2**.

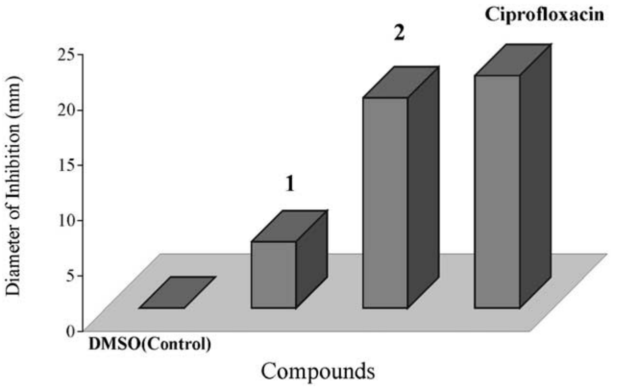

Figure 5. Antimycobacterial activity of **1** and **2** against *M. smegmatis*.

Antimycobacterial activity

The results of antimycobacterial assay for the ligand and its copper complex against *M. smegmatis* are shown in Fig. 5 which indicate that copper conjugation synergistically enhances the antitubercular activity of the parent ligand by three fold making it comparable with that of ciprofloxacin.

This enhancement may in part be due to increase in cell permeability as a result of increase in lipophilicity of the metal conjugate also observed in case of the metal conjugates of antitubercular compounds such as isoniazid, thiacetazone and ethambutol.[24,25] It has been suggested that the intracellular reduction of Cu(II) to Cu(I) species may also lead to the activation of oxygen which could be lethal for bacteria.

In conclusion, the present study has shown that copper conjugation may be advantageous in designing highly effective drugs in antitubercular therapy.

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