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Nickel(II) 2,6-Diacetylpyridine Bis(isonicotinoylhydrazonate) and Bis(benzoylhydrazonate) Complexes: Structure and Antimycobacterial Evaluation. Part XI

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Abstract—The reaction of 2,6-diacetylpyridine (dap) and isonicotinoyl- or benzoylhydrazide leads to bishydrazones H_2dapin (**1a**) and H_2dapb (**1b**), respectively. The condensation can either take place as a bimolecular kinetic process between the two reactants or as a monomolecular metal-templated synthesis in the presence of nickel(II) ions. In the latter case the reaction products are charged 2,6-diacetylpyridine bis(hydrazone) nickel(II) complexes, which can be easily deprotonated to neutral hydrazonates. Diffractometric analysis of one of these $[Ni(dapb)]_2$ (**8b**) has shown a binuclear structure with two octahedral nickel(II) ions bridged by two helicoidal dap (bishydrazonates) in a spheroidal structure of C_{2V} symmetry. The synthesized complexes **8** are promising as antimycobacterial agents against *M. tuberculosis H37Rv*. In particular, **8b** displays significant activity (MIC = 0.025 μg/mL) 10-fold higher than rifampin and equal to isoniazid, while its ligand is ineffective. Compound **8b** is also capable of reducing HIV-induced cytopathogenic effect in human T_4 lymphocytes.

Introduction

In the last few years a renewed interest in metal-based therapy has been raised: in fact, on coordination, bioactive ligands might improve their bioactivity profiles, while inactive ligands may acquire pharmacological properties.[1–6] In addition, metal-coordination is one of the most efficient strategies in the design of repository, slow-release or long-acting drugs.[7] Furthermore, metal complexes have gained importance as enzyme inhibitors: in fact, they can either strongly attach to enzymes (through covalent and ionic bonds) preventing substrate interaction or perturbing the active site, or else disturb metals that are essential for enzymatic action.[8]

The metal coordination capability of isonicotinoylhydrazide (isoniazid, INH), primary antituberculous drug, and its hydrazones (ISNEs) has been widely exploited for many biochemical and pharmacological applications.[9,10] In many cases coordination has been used to confer antibacterial and antiproliferative properties to different INH derivatives.[11–16] In particular it was reported that Cu^{2+} improved the uptake of ISNEs into both susceptible and resistant mycobacteria,[17] whose emergence is greatly involved in the current recrudescence of TB world-wide, TB in turn being greatly involved in AIDS pandemic.

Selectivity and potency of INH against *M. tuberculosis* has so far remained unattainable. However, its efficacy results greatly reduced against resistant mycobacteria.[18,19]

In the last years we have tried to extend the spectrum of action of the drug by functinalizations of INH, aimed to increase its lipophilic character, achieving some interesting results.[20–22]

More recently we have addressed our interest to metal coordination as a strategy to ameliorate lipophilicity. In fact, coordination, locking the polar electronegative atoms in the inner core around the metal and confining the apolar residues in an external lipophilic envelope,

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should favour diffusion through biomembranes.[23,24] In this perspective a number of copper(II), nickel(II) and cobalt(II) complexes of active ISNEs of stoichiometry $[ML_2(H_2O)_2]$, endowed with antimycobacterial activity comparable to rifampin (RMP), have been prepared;[25,26] according to our suggestion they might act as inside cell repositories for the active ligands.

In pursuing this search, in the present paper we describe the preparation and the antimycobacterial activity of the 2,6-diacetylpyridine bis(isonicotinoylhydrazone) H_2dapin (**1a**) and its isostere bis(benzoylhydrazone) H_2dapb (**1b**): moreover, we prepared nickel(II) complexes of these well-known versatile ligands,[27,28] with the objective to extend the lipophilic external envelope of complexes (Fig. 1).

The antimycobacterial in vitro activity against *M. tuberculosis H37Rv* of the two ligands and their Ni^{II} chelates has been evaluated in comparison with established anti-TB drugs.

In addition, taking into account the aim of our continuing search on new INH-analogues, complex **8b** was assayed for anti-HIV-1 activity, which is also reported in this note.

Results and Discussion

Chemistry

The reaction of 2,6-diacetylpyridine (dap) with INH or benzoylhydrazide (BH) in 1:2 molar ratio in methanol at room temperature quantitatively leads to the formation of bishydrazones H_2dapin (**1a**) and H_2dapb (**1b**).

When the condensation between dap and hydrazides is carried out in the presence of Ni^{2+}, the reaction takes place within the coordination sphere by a metal-templated first-order kinetic process (see Kinetics) and neutral or charged Ni^{II} complexes of **1a** and **1b** are formed (Scheme 1).

2,6-Diacetylpyridine has often been used to synthesize hydrazonic ligands.[27] The reaction of these ligands with chlorides of the first row transition metals usually gives pentagonal bipyramidal complexes, in which the five equatorial coordination sites are occupied by hydrazones in a planar arrangement of four adjacent pentadentate rings. Two molecules of solvents or two chloride ions usually occupy the two residual apical sites of the bipyramid.[28–30]

Even though the pentacoordination has often been observed in these complexes, it must be reminded that the two oxygen atoms of the carbonyl groups are rather weak donors whose coordination is favoured by the planar arrangement of the hydrazone structure and by the chelate effect. Thus, in solution carbonyl displacement equilibria driven by the metal and/or by the nature of hydrazone acyl group, must be expected. Alternatively these structures can be stabilised by deprotonation to hydrazonates where the negatively charged oxygens are firmly bonded to the metal.[27–30]

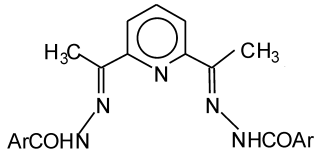
The reaction of H_2dapin and H_2dapb with $NiCl_2$ in methanol (1:1 molar ratio), as well as the $NiCl_2$ templated reaction of dap with INH or BH (1:2 molar ratio), leads to soluble charged species (Scheme 1). Evaporation of the solvent allows the recovering of mixtures whose composition shows small but significant differences for each preparation.

In fact, the crude products obtained in different experiments, if redissolved in methanol, show equivalent conductances ranging from 145 to 160 ohm^{-1} mol^{-1} cm^2, values consistent with the existence of both mono- and di-positively charged species in equilibrium (Scheme 1). Accordingly, the IR spectra of all the samples show medium NH stretchings centred at 3320–3300 cm^{-1} and two very strong stretching vibrations at 1680 and 1660 cm^{-1}, respectively, attributed to the uncoordinated and nickel(II) coordinated carbonyl groups, their relative intensity being different in each sample.

These data are consistent with the existence of competition for Ni^{II} coordination sites between the bishydrazone carbonyl oxygens, chloride ions and eventually solvent molecules (Scheme 1). During each preparation, equilibria can be driven towards mono- (**3**, **5**, **6**) or di-positively (**2**) charged species by subtle unwanted changes of the experimental conditions, occurring probably during the evaporation of the solvent.

When the Ni^{2+} templated synthesis is carried out with an excess of hydrazide (Ni^{2+}/dap/hydrazide 1:1:4), only neutral enolate complexes (**7**, **8**) are obtained, since the coordinated hydrazones are quantitatively deprotonated by the unreacted hydrazide. It is known, in fact, that coordination to transition metals may increase the acid dissociation constant of some hydrazones by 10^5 to 10^8 factor.[31,32]

With deprotonated ligands, besides pentagonal bipyramidal, other coordination geometries have been observed where the hydrazonates lack the planar array to give rise to dinuclear or sometimes tetranuclear highly symmetric structures, by helicoidal wrapping. A delicate balance of electronic and steric effects can drive the mononuclear pentacoordinate towards polynuclear species or viceversa.[27,28,33,34]



1a, Ar=Py (H_2dapin)
1b, Ar=Ph (H_2dapb)

Figure 1.

On the other hand, the reaction of **1a** and **1b** with $Ni(CH_3COO)_2$ in methanol (Ni^{2+}/dap/hydrazide 1:1:2), leads to quantitative formation of enolates **7** and **8**, since the acetate ion acts as a base strong enough to quantitatively deprotonate the bishydrazones.

The dinuclear structure of **8b** was unambiguously shown by the X-ray diffractometric analysis; **8a** could not be obtained as crystals suitable for the diffractometric analysis. The poor solubility of the latter complex prevented its spectroscopic characterization; however, the lack of NH and C=O stretchings in the IR spectrum proved that **8a**, similar to **8b**, is an enolate.

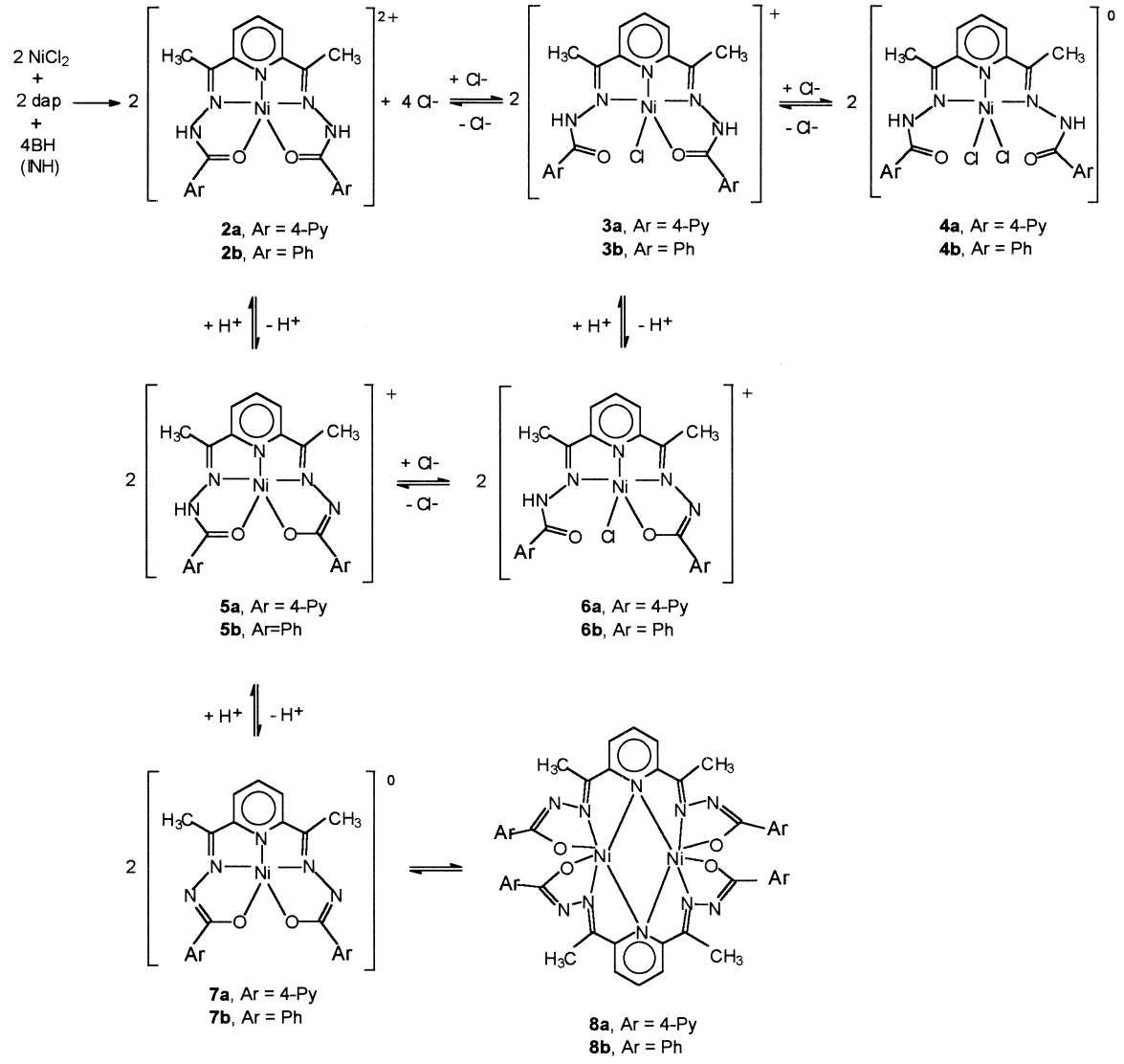
Kinetics

The reaction between dap and INH or BH takes place in methanol as a second-order single kinetic stage. The second-order rate constants, k_2 (mol^{-1} s^{-1}), determined by the plots of k_{obs} versus hydrazide (Fig. 2) are reported in Table 1.

The reaction of dap/hydrazide/nickel(II) in 1:1:1 ratio takes place in two different kinetic stages. We attribute the first one, too fast to be measured, to the formation of a ternary complex in accordance with the equation:

$$Ni^{2+} + dap + hydrazide \rightarrow [Ni(dap)(hydrazide)]^{2+}$$

The second stage, which we have measured and reported in Tables 1 and 2 as k_1, is a rather slow first-order kinetic process which can be conceivably attributed to an intramolecular condensation. According to this interpretation, the reactants, held in close proximity by the metal, are forced to react within the coordination sphere of nickel(II) in a single first-order kinetic stage by a metal-templated synthesis. The 'matchmaking' role



Scheme 1. For simplicity the coordinated molecules of solvent have not been reported in the Scheme.

played by the metal in these reactions recalls that of a rudimentary enzyme, but, of course, the strong metal–ligand interaction does not allow any catalytic cycle.[35]

If the same condensations are performed by adding an excess of hydrazide to the 1:1 molar ratio solution of nickel(II)/ dap, a two terms kinetic law is observed:

$$k_{obs} = k_1 + k_2 \text{ [hydrazide]}$$

The first-order term k_1 (s^{-1}) accounts for the metal-templated synthesis, the second-order term k_2 (s^{-1} mol^{-1}) for the bimolecular nucleophilic attack of the 'free' hydrazide onto co-ordinated dap. The two different contributions are clearly evidenced by the intercept and by the slope of the plots of k_{obs} versus hydrazide, respectively (Tables 1 and 2, Figure 2).

The k_1 values calculated by means of the intercept of the plots agree, within the range of experimental error, with those calculated from the pure first-order reaction of the intermediate ternary complexes $[Ni(dap)(hydrazide)]^{2+}$ above described.

Crystal structure of $[Ni(dapb)]_2$ (8b)

The crystal structure of compound **8b** consists of discrete binuclear molecules of $[Ni(dapb)]_2$ in an I-non-standard monoclinic packing. The binuclear complex possesses a C2 axis of symmetry which coincides with a binary crystallographic axis; consequently the two ligands are coordinated to nickel atoms in the same way. A view of the molecular structure is shown in Figure 3a and b together with the atomic numbering scheme. Each ligand, that in principle could act as pentadentate by means of η2-coordination of pyridine nitrogen atom (N1) is able to exhibit an esa coordination mode.

The two metal centres show a distorted octahedral geometry; the distortions are evidenced by the N2–Ni–O1 and N1–Ni–N2 angles which are 78.62(7)° and 76.22(7)°

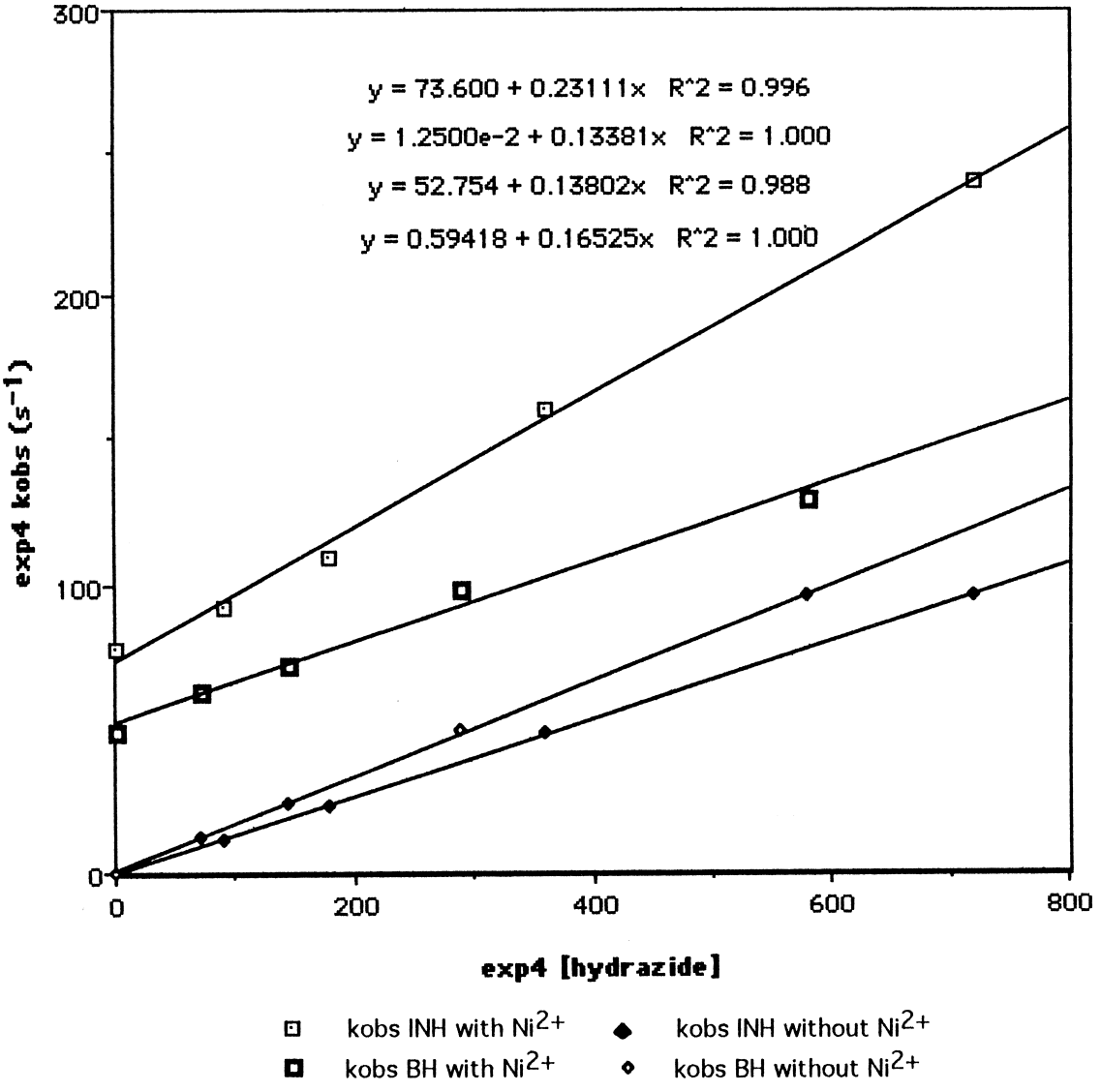


Figure 2. Plots of k_{obs} (s^{-1}) versus hydrazide concentration for the condensation of dap and hydrazide in the presence and in the absence of Ni^{2+}.

significantly different from 90° and by the deviations of the O2–Ni–N1 [153.42(6)] and O1–Ni–N1 154.77(7)° from linearity.

The Ni...Ni separation of 3.0933(8) Å is comparable with those found in other related binuclear complexes reported in the April 2000, 5.19 release of the CSD;[36] it is also comparable with the value reported for the $[Ni(dapz)]_2$ complex[37] where the two 2,6-diacetylpyridine-bis(1′-phthalazinylhydrazone) (H_2dapz) ligands show the same coordination type toward metal centres. On the contrary, the very similar 2,6-diacetylpyridine-bis{[DL-hydroxy(phenyl)acetic] hydrazone} and the 2,6-diacetylpyridine-bis(octanoylhydrazone) ligands chelate a single metal centre.[29,38]

The molecular packing is essentially due to van der Waals interactions. There are also several inter and intramolecular contacts involving heteroatoms *N* and *O* less than the sums of the van der Waals radii. Some of these can be properly described as weak hydrogen bonds.

Microbiology

The antimycobacterial in vitro activity of nickel(II) chelates **8a** and **8b**, in comparison with their ligands H_2dapin and H_2dapb, has been tested against *M. tuberculosis H37Rv* (ATCC 27294, susceptible both to RMP and INH) according to the Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF) anti-tuberculosis drug discovery program.[39–41] The results are collected in Table 3.

As it was to be expected because of the presence of the isoniazid moiety, **1a** and **8a** reach 99% inhibition of *M. tuberculosis H37Rv* growth with MIC values 0.39 and 0.78 μg/mL, respectively.

On the contrary, H_2dapb (**1b**) is ineffective (MIC > 12.5 μg/mL), in line with our previous finding about hydrazone analogues in which isonicotinoyl moiety was replaced by isostere acyl groups.[25]

Surprisingly its nickel(II) binuclear complex **8b** displays very significant activity. In fact, its MIC = 0.025 μg/mL is 10-fold lower than that of RMP and reaches INH, so far the sole established anti-TB drug which possesses such a MIC value against *M. tuberculosis*. Instead, charged mononuclear complexes from the 2,6-diacetylpyridine bisbenzoylhydrazone in different mixtures showed MICs > 12.5 μg/mL.

It is worth noting that, for both binuclear complexes **8a** and **8b**, no breakdown was evidenced in conditions mimicking those employed for antimycobacterial assays (phosphate buffer: pH 6.5/6.8; 37 °C; 15 days).

A precise estimation of the stability constants of the complexes in water is precluded by their poor solubility. However, spectrophotometric titrations have allowed us to establish that the concentration of Ni^{2+} ion in the condition of the assays is less than 10^{-10} M. It is known that nickel is generally an essential nutrient for bacteria, required as trace elements at nanomolar concentrations, but at micro- or millimolar concentrations it is toxic.[28,41] However, taken together, our experimental data rule out the possibility that the observed antimycobacterial activity rises from a free metal ion toxic effect.

In addition $[Ni(dapb)]_2$ **8b** has been submitted to the anti-HIV-1 in vitro screening in the context of AIDS Antiviral Screening Program of the US-NCI.[42] In the preliminary cytotoxicity assay on uninfected human T_4 lymphocytes it displayed low cytotoxicity (IC_{50} > 100

Table 1. Pseudo-first-order and first-order rate constants (k_{obs}, s^{-1}) dependence on the concentration of isonicotinoylhydrazide (methanol, 25 °C, μ = 0.1)

10^4 [dap]	10^4 [inh]	10^4 [$NiCl_2$]	10^4 k_{obs} (s^{-1})	k_1 (s^{-1})	k_2 (mol^{-1} s^{-1})
2.50	90	—	12.0	—	1.3
2.50	180	—	23.5		
2.50	360	—	49.2		
2.50	720	—	104		
2.50	2.50	2.6	78.0[a]		
2.50	90	2.6	92.0	76.0	2.2
2.50	180	2.6	110		
2.50	360	2.6	160		
2.50	720	2.6	240		

[a]First-order rate constant.

Table 2. Pseudo-first-order and first-order rate constants (k_{obs}, s^{-1}) dependence on the concentration of benzoylhydrazide (methanol, 25 °C, μ = 0.1)

10^4 [dap]	10^4 [bh]	10^4 [$NiCl_2$]	10^4 k_{obs} (s^{-1})	k_1 (s^{-1})	k_2 (mol^{-1} s^{-1})
2.50	72	—	13.0	—	1.0
2.50	145	—	24.1		
2.50	290	—	49.5		
2.50	580	—	106		
2.50	2.50	2.6	50.0[a]		
2.50	72	2.6	63.0	52	1.6
2.50	145	2.6	72.2		
2.50	290	2.6	98.6		
2.50	580	2.6	130.0		

[a]First-order rate constant.

Table 3. Antimycobacterial in vitro activity expressed as MIC (μg/mL)[a] against *M. tuberculosis H37Rv*

Compd[b]	% Inhibition	MIC (μg/mL)
1a	99	0.39
1b	65	> 12.5
8a	99	0.78
8b	99	0.025
RMP		0.25
INH[c]		0.025–0.05

[a]MIC is defined as the lowest concentration of compound inhibiting 90% of the inoculum relative to controls.
[b]Charged mononuclear complexes from 2,6-diacetylpytidine bis(benzoylhydrazone) in different mixtures showed MIC > 12.5 μg/mL.
[c]Ref 39.

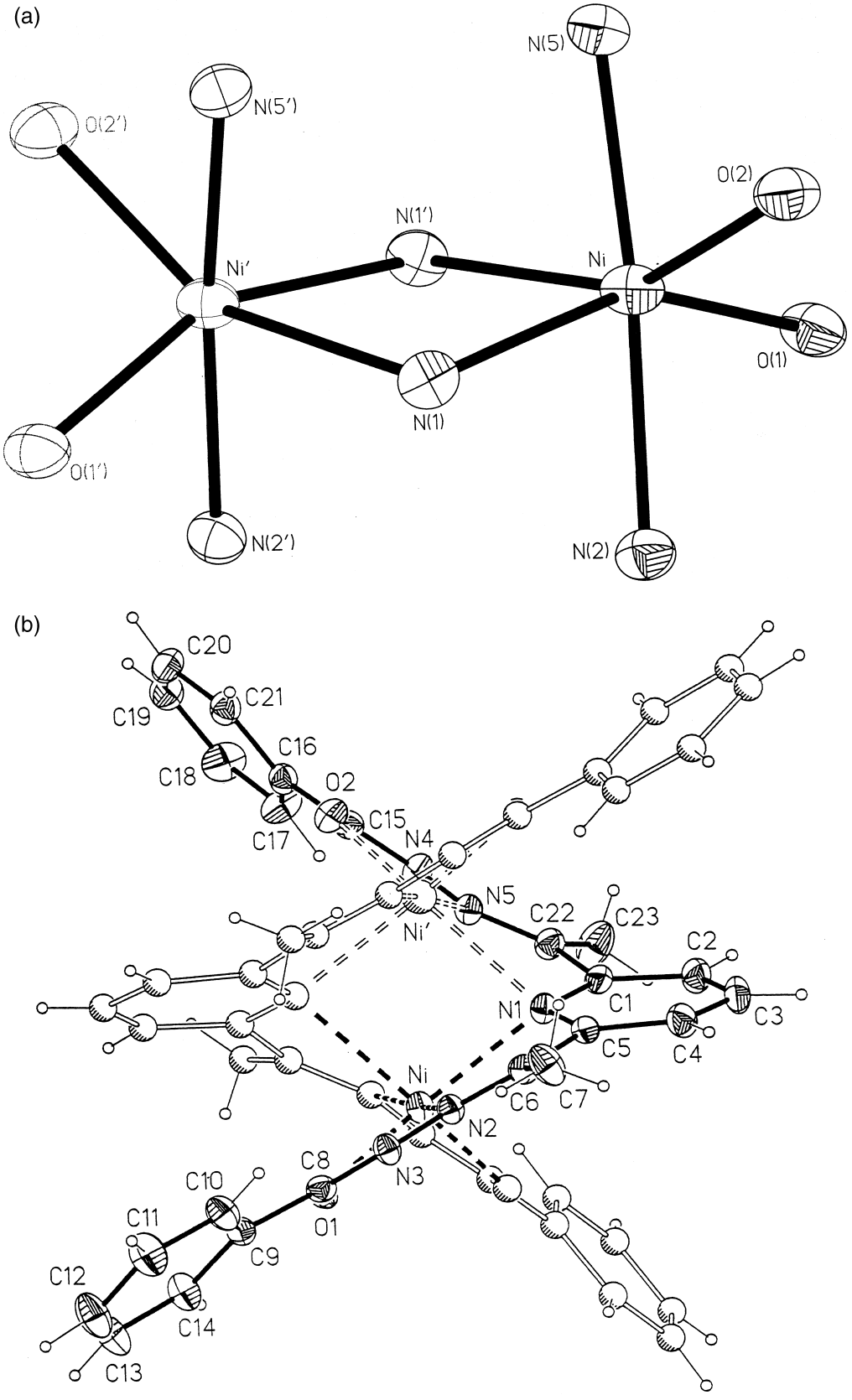


Figure 3. (a) View of the coordination polyhedron around the two equivalent nickel atoms related by the symmetry operation 1/2-x, y,-z. Thermal ellipsoids are drawn at 30% level. (b) View of the dimer along the crystallographic two-fold axis showing the numbering Scheme of the asymmetric unit. The bridging N-bonds are represented by shaded lines. Thermal ellipsoids are drawn at 30% level while hydrogen and equivalent atom size is arbitrary.

μM). Its capability of protecting infected lymphocytes from HIV-induced cytopathogenicity reaches 39.07% at 1 μM dose (the activity criterion for this assay is 50% protection).

Conclusions

2,6-diacetylpyridine bishydrazones act with respect to nickel(II) as pentadentate ligands which can be easily deprotonated leading to enolate complexes. X-ray diffractometric analysis shows that nickel(II) 2,6-diacetylpyridine bis(benzoylhydrazonate) **8b** is a dimer endowed with an external spheroidal lipophilic structure which could account for the observed bioactivity of both complexes **8a** and **8b**.

In fact, **8a** has MIC inferior to 1 μg/mL; its isostere **8b**, obtained from the pharmacologically inactive ligand **1b**, proves to be very effective against *M. tuberculosis H37Rv* reaching the MIC level reported for INH.

Compound **8b** is also capable of reducing HIV-induced cytopathogenic effects in human T_4 lymphocytes, with a favourable therapeutical index. It is known that HIV-sieropositivity is the strongest risk factor for TB and other mycobacterial diseases; thus, although the protection percentage is moderate, this result steps up the interest in this metalchelate. It is worth designing new structural modifications to enhance its anti-HIV potency.

In conclusion, **8b** could be considered as a novel antimycobacterial lead compound, *not related to INH*. In fact, according to TAACF criteria, a novel compound showing MIC $\leq$ 1 μg/mL is considered an excellent lead. This result is noteworthy, taking into account that today dramatic health problems, such as AIDS pandemic, multi-drug resistance, etc., have made the search for new anti-TB drugs even more urgent. It is hopeful that positive results will be found in the further assays against single-drug resistant *M. tuberculosis* and *M. avium* complex that are ongoing.

Microbiology

Antimycobacterial activity. The antimycobacterial in vitro activity was assayed according the Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF) antituberculosis drug discovery program, coordinated by the Southern Research Institute (Birmingham, Alabama, USA) under the direction of the National Institute of Allergy and Infectious Diseases (NIAID).

All compounds were initially screened against *Mycobacterium tuberculosis H37Rv* (ATCC 27294) at the single concentration 12.5 μg/mL in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[39] Compounds exhibiting fluorescence are then tested in the BACTEC 460 radiometric system too.[40] Compounds demonstrating at least 90% inhibition are retested at lower concentrations against *M. tuberculosis H37Rv* to determine the actual Minimum Inhibitory Concentration (MIC), using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampin (RMP) was used as reference drug. Generally MIC $\leq$1 μg/mL in a novel compound class is considered an excellent lead.

Anti-HIV-1 activity

The in vitro anti-HIV drug testing system was performed in the National Cancer Institute Laboratories of Bethesda (USA) according to reported procedures.[42] The assay involves the killing of T_4 lymphocytes by HIV. T_4 lymphocytes (CEM cell lines) are exposed to HIV at a virus to cell ratio (MOI) of approximately 0.05. Each candidate agent, dissolved in DMSO, was added at varying concentrations ranging from 10^{-8} to 10^{-4} M. Uninfected cells treated with the test compound serve as toxicity control, and infected and uninfected cells without the compound as basic controls. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 6 days; the tetrazolium salt, XTT, was added to all wells, and cultures were again incubated to allow formazan colour development by viable cells. Individual wells were analysed spectrophotometrically to quantify formazan production and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity. All tests were compared with positive AZT and 2′,3′-dideoxycytidine (ddC)- treated controls carried out at the same time under identical conditions.

In such a test system, activity is expressed as the 50% effective concentration (EC_{50}) which represents the concentration of test compound resulting in 50% reduction of the viral cytopathic effect, whereas the cytotoxic dose (IC_{50}) represents the concentration of agent resulting in 50% inhibition of growth of normal uninfected cells.

Experimental

Chemistry

Elemental analyses (C, H, N) were performed at Redox Labs, Cologno Monzese, Milano (Italy).

Physical measurements. IR spectra were recorded as Nujol mulls in the range 4000–300 cm^{-1} using CsI disks on a Perkin-Elmer FT-IR model 1730 spectrophotometer.

UV–visible spectra were scanned in the thermostated cell compartment of a Cary 219 or a Perkin-Elmer Lambda 3 spectrophotometer.

A radiometer 4 conductivity bridge provided with a thermostatated cell was used for the measures of molar conductivity.

Kinetic experiments

1.5 mL of a methanol solution of dap (5×10^{-4} M) was thermostated at 25 °C. 1.5 mL of the hydrazide

dissolved in methanol at the desired concentration were added to the dap solution. A similar procedure was followed for the template reactions; in the latter case anhydrous $NiCl_2$ (5.5×10^{-4} M) was added to the dap solution. No significative rate change was observed if $NiCl_2$ was added to the hydrazide rather than to the dap solutions. When required pseudo-first-order conditions were ensured by an at least 20 times excess of hydrazide with respect to dap. The kinetic calculations were performed using optical density changes at 380 nm by a home-made best fitting procedure. The ionic strength was maintained constant at the value of 0.1 M by addition of anhydrous $LiClO_4$ to the methanol solution. No significative change in the reaction rate was observed by addition of 5% of water.

2,6-diacetylpyridine bis(isonicotinoylhydrazone) and 2,6-diacetylpyridine bis(benzoylhydrazone) were prepared according to reported procedures.[27,28]

Preparation of nickel(II) 2,6-diacetylpyridine bis(isonicotinoylhydrazonate) 8a and nickel(II) 2,6-diacetylpyridine bis(benzoylhydrazonate) 8b. A mixture of $Ni(CH_3COO)_2$ (1 mmol; 0.176 g), 2,6-diacetylpyridine (1mmol; 0.163 g) and isoniazid (2 mmol; 0.274 g) or benzoylhydrazine (2 mmol, 0.272 g), each dissolved in 10 mL of CH_3OH, was refluxed under stirring for 10 h. After cooling the reaction mixture, the product was filtered off and obtained as pure solid.

Nickel(II) 2,6-diacetylpyridine bis(isonicotinoylhydrazonate) 8a. Yield: 95%. IR (nujol), cm^{-1}: 1600 (C=N), 1145 (C–O). Anal: $C_{42}H_{34}N_{14}O_4Ni_2$. Found: C, 55.12; H, 3.14; N, 21.31.

Nickel(II) 2,6-diacetylpyridine bis(benzoylhydrazonate) 8b. Yield: 98%. IR (nujol), cm^{-1}: 1590 (C=N), 1156 (C–O). Anal: $C_{46}H_{38}N_{10}O_4Ni_2$. Found: C, 60.19; H, 4.37; N, 15.09.

Crystal structure analysis. Crystal data. $C_{46}H_{38}N_{10}O_4Ni_2$. $M_r = 3649.14$, monoclinic, I2/a, a = 15.496(3), b = 16.220(3), c = 16.416(3)Å, ß = 90.21(2)°, V = 4126.05(2) Å^3, Z = 8, Dc = 1.4686 g cm^{-3}, λ (MoK_α) = 0.71073 Å, μ = 0.797 cm^{-1}, F(000) = 1888, T = 296 K, R = 0.0311 for 3663 independent reflections.

Crystals suitable for X-ray analysis were obtained by recrystallization from methanol solutions. A crystal of dimensions $0.20\times0.20\times0.30$ mm was used for intensity-data collection at 296 K with a Siemens R3m/V four-circle diffractometer using graphite-monochromated MoK_α(λ = 0.71073 Å) radiation. Accurate unit-cell dimensions and crystal orientation matrices were obtained from least-squares refinement of 2θ, ω, χ and jϕ values of 30 strong reflections in the range $12 < 2\theta < 30°$. Crystal and electronic stability was confirmed by the constancy of three check reflections measured every 100 min of X-ray exposure. Of 7541 reflections measured by the ω/2θ scan technique, in the 2θ range 3.64–5θ.12; 3663 ($R_{int} = 0.037$) having net intensity $F > 4.0\ \sigma(F)$ were used in the solution and refinement. The diffraction data were processed with the learnt-profile procedure[43] and then corrected for Lorentz-polarization effects. Absorption correction was applied by fitting a pseudo-ellipsoid to the azimutal scan data of 15 suitable reflections with high χ angles.[44]

Structure determination. The structure was solved by standard direct methods and subsequently completed by a combination of least squares technique and Fourier Synthesis. All non-hydrogen atoms were refined anisotropically.

Hydrogen atoms were added at calculated positions and included in the structure factor calculations with a common thermal parameter (U = 0.06 Å^2), and during the refinement they were allowed to ride on their respective parent carbons. The structure model was refined by full-matrix least squares technique, minimising the function $w(Fo^2-Fc^2)^2$, converging to $R = \Sigma|Fo - Fc|/\Sigma Fo = 0.0311$ and $R' = [\Sigma w(Fo^2 - Fc^2)^2/\Sigma w^-(Fo^2)^2]^{1/2} = 0.061$ with the final weighting Scheme $= 1/[\sigma^2(Fo^2) + (0.0309^*P)^2 + 0.00^*P]$ where $P = (Max(Fo^2) + 2^*Fc^2)/3$.

The last difference map showed the largest electron density residuals (maximum and minumum range = ±0.26 eÅ^{-3}). Neutral-atom scattering factors and anomalous dispersion corrections were taken into account.[45]

Data reduction and structure solutions and drawings were performed with the SHELXTL-PLUS package,[46] while structure refinement and final geometrical calculations were carried out with SHELXL-97[47] and PARST program[48] respectively, on DEC MicroVax/3400 computer.

Acknowledgements

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