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Isoniazid-Related Copper(II) and Nickel(II) Complexes with Antimycobacterial In Vitro Activity. Part 9†

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Abstract—Isonicotinoylhydrazones **1**, obtained by the primary antituberculous agent Isoniazid, have been used as monoanionic ligands (L) to prepare copper(II) **2** and nickel(II) **3** octahedral complexes of stoichiometry $[ML_2(H_2O)_2]$. Their antimycobacterial in vitro activity was evaluated against *Mycobacterium tuberculosis H37Rv* in comparison with the ligands. Complexes **2a**, **2b**, **2f**, **3b**, **3d** and **3g** displayed MIC values $\leqslant 0.2$ μg/mL.

The recrudescence of tuberculosis (TB) coincident with the spread of AIDS is nowadays a challenging health problem all over the world. The search for new antimycobacterial agents is justified by many important reasons, mainly: (a) the emergence of multidrug resistant (MDR) strains of *Mycobacterium tuberculosis*; (b) the spreading of opportunistic infections due to non-tubercular mycobacteria (NTM), *M. avium* complex (MAC) in particular; (c) the poor effectiveness of the present treatment on immunodepressed patients; (d) the complexity of the current therapeutic regimens.[1–3]

Pursuing our efforts to discover novel agents against TB and other AIDS-associated pathologies, we have systematically modified the structure, and consequently the chemical-physical features, of Isoniazid (INH); it is still a primary anti-TB drug, the utilisation of which however is limited by the development of INH-resistant *M. tuberculosis* strains.[1] In particular the enhancement of lipophilicity could extend its activity spectrum to NTM strains[4] and, possibly, to other AIDS-associated pathogens as well as to some neoplasias. We were able to verify this rationale in several INH-analogues so far explored. The most satisfactory results always dealt with substances bearing fluorine or trifluoromethyl groups.[5–8]

On this basis, we designed new transition series metals complexes of isonicotinoylhydrazones (ISNE) **1** (Scheme 1), in which coordination to the metal ion should produce structures with a polar core (the metal and the electronegative atoms involved in coordination) surrounded by a lipophilic envelope formed by aromatic rings and aliphatic substituents. Such an arrangement should guarantee some advantages, such as easier diffusion through biomembranes. In particular the increased lipophilicity might help to extend the parent drug activity spectrum to *M. avium* complex (MAC), not susceptible to INH.[1,2] In fact, MAC possesses a complex lipid wall architecture that acts as a barrier excluding the majority of the established anti-TB drugs.[9] It is worthy of note that MAC infections are the most frequent cause of lethality in terminal AIDS patients.[2]

INH and its hydrazones have been reported as forming stable metal complexes behaving as mono-, bi- and sometimes poly-dentate ligands.[10,11] A number of these complexes possesses antitubercular activity.[12,13] In particular some reports demonstrate that the capture of INH and ISNEs by both INH-susceptible and INH-resistant *M. tuberculosis* strains is enhanced by copper(II) ions.[14]

The binary neutral copper(II) and nickel(II) complexes **2** and **3** represented in Scheme 1 have been obtained in methanol solution by reaction of ISNEs **1** with the metal acetate or chloride, in molar ratio 2:1, at 50 °C. Concentrated solutions, left at room temperature for

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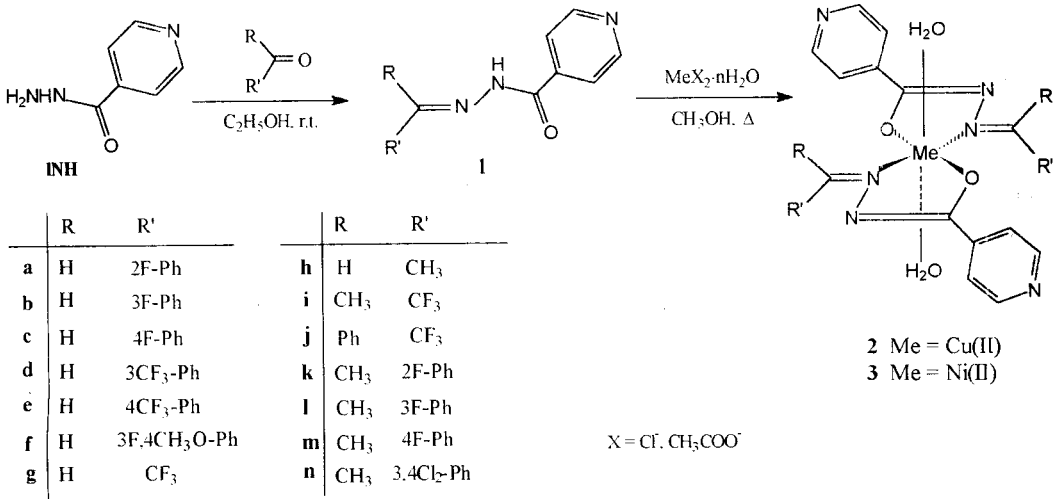

	R	R'
a	H	2F-Ph
b	H	3F-Ph
c	H	4F-Ph
d	H	$3CF_3$-Ph
e	H	$4CF_3$-Ph
f	H	3F,$4CH_3O$-Ph
g	H	CF_3

	R	R'
h	H	CH_3
i	CH_3	CF_3
j	Ph	CF_3
k	CH_3	2F-Ph
l	CH_3	3F-Ph
m	CH_3	4F-Ph
n	CH_3	3,$4Cl_2$-Ph

Scheme 1.

Table 1. Antimycobacterial in vitro activity, expressed as MIC (μg/mL),[a] against *M. tuberculosis H37Rv*[b] of isonicotinoylhydrazones **1** and metalchelates **2–3**

Compd	% Inhibition	MIC (μg/mL)	Compd	% Inhibition	MIC (μg/mL)	Compd	% Inhibition	MIC (μg/mL)
1a	100	0.05	**1l**[27]	100	< 0.05	**3a**	99	0.39
1b	99	< 0.2	**1m**[27]	99	< 0.2	**3b**	99	0.1
1d	99	0.1	**1n**[27]	100	< 0.2	**3c**	98	1.56
1e	100	< 0.2	**2a**	99	0.1	**3d**	99	0.1
1f	99	< 0.2	**2b**	99	0.1	**3e**	99	6.25
1g[27]	100	0.05	**2e**	100	< 12.5	**3f**	99	0.39
1h[27]	99	0.025	**2f**	99	0.1	**3g**	99	0.2
1i[27]	99	0.05	**2i**	99	0.39	**3i**	99	3.13
1j	100	6.25	**2j**	99	12.5	**RMP**[c,24]		0.06–0.25
1k[27]	100	0.2	**2m**	99	0.39	**INH**[d,24]		0.025–0.05

[a]MIC is defined as the lowest concentration inhibiting 90% of the inoculum relative to controls.
[b]RMP and INH susceptible ATCC 27294 strain.
[c]RMP, Rifampin.
[d]INH, Isoniazid.

24 h, generally provide powders which were further purified by repeated washing with hot methanol.

The formation of complexes **2–3** is clearly evidenced by monitoring the electronic spectra of the reagents according to Job's method of continuous variation.[15,16] The experimental data indicate the formation of 1:2 complexes when a methanol solution of a ligand **1** is added to a solution of the metal acetate in the same solvent. The observed marked increase of pH after coordination strongly suggests the formation of enolates.[17] The resulting complexes are not charged and show poor solubility both in polar and apolar solvents. The optical density of 50% MeOH:H_2O solutions of complexes **2** and **3** has been measured in the pH range 3.5–12.0. All the solutions at 300 nm showed maximum absorption at pH between 6.5 and 7.0. In acid, a marked variation of absorption at pH 4.5–5.0 suggests the breakdown of the complexes probably connected with enolate protonation. On the alkaline side, the complexes showed much greater stability; we did not detect any breakdown up to pH 12.0.

In the IR spectra of ISNEs **1** the ν C=O of the amido groups are observed in the range 1705–1660 cm^{-1} as strong bands.[18] In the spectra of the metal chelates these absorptions are substituted by new bands of lower intensity between 1624 and 1600 cm^{-1} which are consistent with nitrogen co-ordinated C=N stretching vibrations. In the complexes, moreover, a broad absorption centred at about 3350 cm^{-1} substitutes the sharper band around 3250 cm^{-1} in accordance with water coordination and ligand NH deprotonation upon coordination. The enolate coordination is also confirmed by a new band at 1060 cm^{-1} attributable to single C–O stretching. The strong skeleton absorption of the isonicotinic ring at about 1545 cm^{-1} is not affected by the complexation, indicating the non-participation of the pyridine nitrogen in coordination.[19]

The magnetic moments, μ_{eff}, per metal ion grammo-atom,[20] range for copper(II) complexes between 2.0 and 2.1 BM. These values are consistent with d^9 electronic configurations in octahedral environment presenting no metal–metal interaction.[21] Nickel(II) complexes show

μ_{eff} comprised between 3.7 and 3.8 BM, values consistent with d^8 electronic configurations in octahedral geometry.[22]

On the basis of these findings and of the analytical results we suggest for complexes **2** and **3** an octahedral coordination geometry with four equatorial co-ordination sites occupied by two molecules of ISNE enolate and axial sites occupied by two water molecules.

The antimycobacterial activity of metalcomplexes **2** and **3** together with that of free ligands has been evaluated according to the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program, co-ordinated by Southern Research Institute (Birmingham, AL, USA); it consists of several subsequent in vitro and in vivo assays, the latter of which are still being carried out.

In particular the initial screening is conducted at 12.5 μg/mL against *M. tuberculosis H37Rv* (ATCC 27294, susceptible both to rifampin, RMP and isoniazid, INH), using the Microplate Alamar Blue Assay (MABA).[23] Compounds exhibiting fluorescence are then tested in the BACTEC 460 radiometric system.[24] Compounds demonstrating at least 90% inhibition are re-tested at lower concentrations in the MABA and/or in the BACTEC 460 systems to determine the actual Minimum Inhibitory Concentration (MIC), defined as the lowest concentration inhibiting 90% of the inoculum relative to controls. Concurrently, MICs on *M. avium* complex (ATCC 25291, a strain susceptible to clarithromycin) are determined in the MABA.

All tested hydrazones **1** and metal complexes **2** and **3** produced 99 to 100% growth inhibition (sole exception **3c**, 98%) at 12.5 μg/mL.

Complexes **2a**, **2b**, **2f**, **3b**, **3d** and **3g** are the most active, with MICs $\leq$0.2 μg/mL, while other compounds **2** and **3** having MICs ranging from 0.39 and 12.5 μg/mL. No clear-cut correlation between the kind of coordinating ion and the activity levels, has appeared (e.g. compare **2b** and **3b**), although even if some copper complexes (**2a**, **2f** and **2i**) showed at least 3-fold more activity than the corresponding nickel ones.

The MIC values of ligands **1**, ranging from 0.025 to 0.2 (except for **1j**), reveal very significant activity, equal or higher than that of rifampin used as reference drug; the potency of **1a**, **1g**, **1h**, **1i** and **1l** is similar to that of the parent-drug INH (Table 1).

Hydrazone isosteres of ISNEs, i.e. nicotinoyl-, benzoyl- and pyrazinoyl hydrazones, similarly obtained and tested, failed to inhibit the growth of *M. tuberculosis H37Rv* (% inhibition between 53 and 0),[25] confirming that the isonicotinic acyl moiety is necessary for activity.[26]

Moreover, except **1b** and **1f**, which show similar inhibition degrees, hydrazones **1** are more potent than their copper(II) and nickel(II) complexes, suggesting that the antitubercular activity of the metal complexes could be mainly due to slow release of the ligands, inside the mycobacterial cell. In fact bioactivity was never revealed in complexes of inactive ligands such as hydrazones isosteres of ISNEs **1**.[25] Thus the active principle of these compounds should be ultimately the same ligand, while the metal should play a role mainly connected with their enhanced capacity to cross the mycobacterial cell wall so providing the inhibitory agent for a long period. However a deeper investigation is underway on the action mechanism of these active compounds, taking into account the recent discoveries on the molecular action mechanism of INH.[26,28]

Compounds **1**–**3** have been tested against *M. avium* complex without reaching the required cut-off of 90% inhibition at 12.5 μg/mL. However, **1b** and **1e**, possessing 82%, and **2d** and **3e**, producing 40 and 49% inhibition levels respectively appear to be moderately active. Such findings are of some interest in that INH is not considered active against *M. avium* complex.[1,3] In fact, in the same experimental conditions, INH displays MIC >32 μg/mL.[24]

Preliminary data about the cytotoxicity evaluation, performed in vitro on VERO cells, revealed that compounds **1**–**3** are scarcely cytotoxic with safety indexes ($SI = IC_{50}/MIC$) ranging from 25.6 to 625.[29]

Other TAACF screening levels, i.e. evaluation against single DR *M. tuberculosis* strains and on infected macrophages culture, are underway.

Furthermore, when assayed against a panel of 60 tumor cell lines in the context of US-NCI antitumor screening program,[30] **3i** and **2f** were found to possess cytotoxic properties, as MG-MID values[31] reveal ($-\log GI_{50} =$ 8.50 and 4.62 M, respectively). In addition, **2a**, at 10 μM dose, produced 41.14% protection from HIV-induced cytopathic effect on human lymphocytes T_4.[32]

Taken together, the results so far collected hopefully will allow us in the near future to identify efficient antimycobacterial agents endowed with some advantages with respect to the parent drug INH.

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Compd	IC_{50}	S.I.	Compd	IC_{50}	S.I.
1a	8.4	168	**2f**	>10	>100
1b	>6.25	>31.25	**2i**	>10	>25.6
1d	>10	>100	**INH**	>1000	
1k	>125	>625	**RMP**	110–147	
1m	>10	>50			

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