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# Antimycobacterial In Vitro Activity of Cobalt(II) Isonicotinoylhydrazone Complexes. Part 10

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**Abstract**—Octahedral cobalt(II) complexes of isonicotinoylhydrazones, which were obtained from the primary antituberculous agent isoniazid, have been synthesised and characterised. Their antimycobacterial in vitro activity has been evaluated against *Mycobacterium tuberculosis H37Rv*: they exhibit MIC values ranging from $<0.1$ to 0.39 μg/mL, showing them to be generally more active than previously reported analogous Cu(II) and Ni(II) complexes. 

Tuberculosis (TB) is presently considered as the most dangerous infective disease world-wide and one of the major AIDS-associated infections. According to alarming data furnished by the World Health Organization (WHO), more than a third of the entire world population is afflicted with TB and it is believed it could affect 300 million people within the next 10 years. The simultaneous presence of HIV infection, the spreading of drug-resistant strains of *Mycobacterium tuberculosis* and the scarce compliance with the lengthy complex therapies often complicate the treatment of TB.[1,2] Therefore, the search for new antituberculous agents is required, in spite of the availability of effective drugs such as isoniazid (INH) and rifampin (RMP).

Recently we have reported copper(II) and nickel(II) chelates ($MeL_2(H_2O)_2$) of isonicotinoylhydrazones (ISNE = L), which were obtained from INH and fluorinated carbonyl compounds. Most of them display significant antimycobacterial in vitro activity and low cytotoxicity.[3] The ligands are effective against *M. tuberculosis H37Rv* and also, to some extent, against *M. avium* complex, the latter being unsusceptible to parent-drug INH.[3] We have suggested that the antituberculous activity of these complexes should be ultimately due to the slow release of the active ligands, while the metal ion should play a role mainly connected with the enhanced capability to cross the mycobacterial cell wall. In fact, the polar donor atoms of ISNE, as enolate, co-ordinate the metal ion while the hydrophobic moieties form an outer lipophilic envelope that should facilitate the diffusion through biomembranes. Consequently, these chelates could be considered as repositories for the active ligands and potentially long-acting antituberculous agents.

In fact, a renewed interest in metal-based chemotherapies has stemmed from the possibility of designing slow release drugs.[4] In addition, co-ordination to a metal ion has often been reported to enhance the antiinfective and antiproliferative properties of several ligands.[5–10]

All these considerations prompted us to extend our research to analogous cobalt(II) complexes **2a**–**g** (Scheme 1), obtained by the reaction of ISNEs **1** with Co(II) nitrate, in molar ratio 2:1, at 50 °C in methanol solution. The brown microcrystalline powders were separated from the stirred solutions by dripping 0.1 N $CH_3ONa$. After 2 h they were filtered at room temperature, washed with methanol and dried in vacuo to constant weight. Complexes **2** are scarcely soluble in methanol, ethanol and water and moderately soluble in DMSO and DMF. Their structures were characterised by means of elemental analyses, IR and UV spectroscopies and magnetic measurements.

The formation of neutral 1:2 complexes was monitored by means of the electronic spectra of the reagents, according to Job's method of continuous variation.[11,12]

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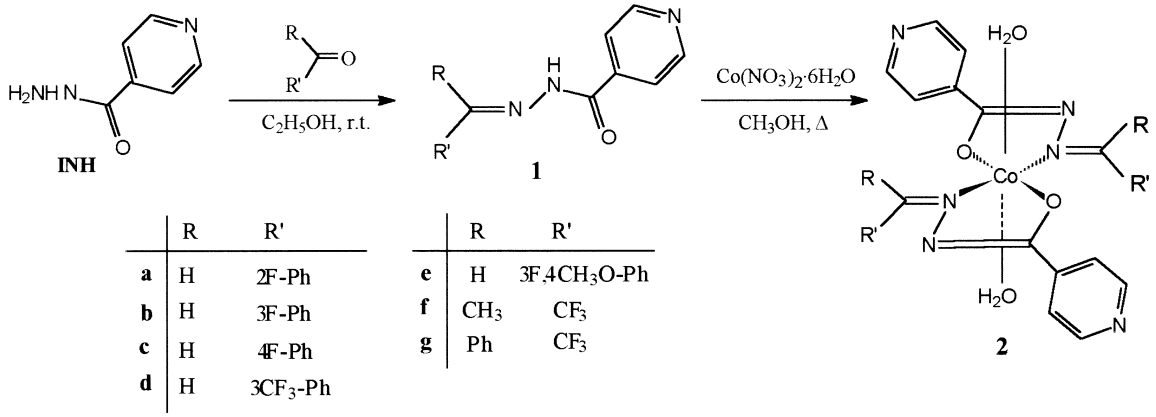


| | R | R' |
|---|---|---|
| **a** | H | 2F-Ph |
| **b** | H | 3F-Ph |
| **c** | H | 4F-Ph |
| **d** | H | 3$CF_3$-Ph |

| | R | R' |
|---|---|---|
| **e** | H | 3F,4$CH_3O$-Ph |
| **f** | $CH_3$ | $CF_3$ |
| **g** | Ph | $CF_3$ |

**Scheme 1.**

The IR spectra of **2** lack the strong carbonyl absorption at 1705–1660 $cm^{-1}$ typical of the free ligands. The enolate structure is also supported by (a) a medium band between 1630 and 1600 $cm^{-1}$ due to the nitrogen co-ordinated C=N stretching, (b) a band at 1050 $cm^{-1}$ due to the enolic C–O stretching, and (c) a broad absorption centred at about 3350 $cm^{-1}$ attributable to the presence of water in the co-ordination sphere.[13]

The magnetic moments, $\mu_{eff}$, per metal ion grammoatom, range between 3.9 and 4.0 BM.[14] These values are consistent with $d^7$ low spin electronic configuration in octahedral environment without any metal–metal interaction.[15]

Therefore, we propose for Co(II) complexes **2** the same octahedral co-ordination geometry evidenced for the analogous Cu(II) and Ni(II) chelates, with four equatorial co-ordination sites occupied by two ISNE enolates and the axial sites occupied by two molecules of water (Scheme 1).

The behaviour of complexes **2** in MeOH/$H_2O$ 1:1 solution, in the pH range 3.5–12.0, is very similar to that of the Cu(II) and Ni(II) analogues.[3] Maximum absorption is detected at 300 nm at pH between 6.0 and 7.0 and no breakdown is evidenced up to pH 12.0. The stability has also been verified in conditions mimicking those employed for antimycobacterial assays (phosphate buffer: pH 6.5/6.8; 37 °C; 10 days).[16]

In contrast, the complexes undergo breakdown at pH 4.0–5.0.

The antimycobacterial in vitro activity of Co(II) complexes **2** has been evaluated according to the protocols of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program.[17–19]

All tested complexes **2** produce 98–99% inhibition of *M. tuberculosis H37Rv* growth with MICs ranging between $<0.1$ and 0.39 μg/mL (with the exception of **2g**) (Table 1).

The in vitro cytotoxicity in VERO cells, expressed as $IC_{50}$ (μg/mL), is generally 10 μg/mL and the selectivity indexes ($SI = IC_{50}/MIC$) are 74 for **2b** and >50 for **2d** and **2e**.

In comparison with their respective ligands, complexes **2** exhibit similar (**2a**, **2b**, **2d**, **2g**) or lower (**2e**, **2f**) potency.[3] Compound **2g**, obtained from α,α,α-trifluoroacetophenone ISNE, is the least effective among the tested Co(II) chelates, similar to the Cu(II) one; the ligand itself is less active than other ISNEs.

The comparison with previously reported complexes[3] shows that compounds **2** are always more potent than their Ni(II) analogues and often than the Cu(II) ones. In particular, **2c**/**2f** and **2a** are 8- and 4-fold more active than the corresponding Ni(II) complexes, respectively. **2a** is moderately more active and **2d** is 4-fold more effective than the respective Cu(II) analogues.

Therefore, the replacement of Cu(II) or Ni(II) with Co(II) as co-ordinating metal ion represents an improvement in the antimycobacterial activity of these isonicotinoylhydrazone metal complexes, on the whole. The pharmacological data so far collected and the comparison with the free ligands seem to support the hypothesis that they should act like a repository of the active ISNEs, similarly to the reported Cu(II) and Ni(II) analogues.[3]

**Table 1.** Antimycobacterial in vitro activity of **2a**–**g**, expressed as MIC (μg/mL), against *M. tuberculosis H37Rv*[17]

| Compound | % Inhibition | MIC (μg/mL) | Compound | % Inhibition | MIC (μg/mL) |
|---|---|---|---|---|---|
| **2a** | 99 | $<0.1$ | **2f** | 99 | 0.39 |
| **2b** | 99 | $\leq 0.1$ | **2g** | 99 | 6.25-12.5 |
| **2c** | 99 | 0.2 | **RMP**[19] | | 0.06-0.25 |
| **2d** | 98 | $\leq 0.2$ | **INH**[19] | | 0.025-0.05 |
| **2e** | 99 | 0.2 | | | |

RMP = Rifampin; INH = Isoniazid

However, further pharmacological evaluation against INH-resistant strains of *M. tuberculosis* and in TB-infected macrophages as well as against *M. avium* is ongoing.[18,19] A comprehensive profile of these transition metal complexes as antituberculous agents showing some advantages, if any, compared to INH, is expected to be acquired in the near future.

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## References and Notes

1. Sensi, P.; Gialdroni Grassi, G. Antimycobacterial agents. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M. E. Ed., John Wiley & Sons: New York, 1996; Vol. 2, pp 575–635.
2. Mandell, G. L.; Petri, W. A., Jr. Antimicrobial Agents. Drugs used in the Chemotherapy of Tuberculosis, *Mycobacterium avium* Complex Disease, and Leprosy. In *Goodman & Gilman's the Pharmacological Basis of Therapeutics*, 9th ed.; McGraw-Hill: New York, 1996; pp 1155–1174.
3. Bottari, B.; Maccari, R.; Monforte, F.; Ottanà, R.; Rotondo, E.; Vigorita, M. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 657.
4. Bharti, N.; Maurya, M. R.; Naqvi, F.; Bhattacharya, A.; Bhattacharya, S.; Azam, A. *Eur. J. Med. Chem.* **2000**, *35*, 481.
5. Lowe, G.; Droz, A. S.; Vilaivan, T.; Weaver, G. W.; Park, J. J.; Pratt, J. M.; Tweedale, L.; Kelland, L. R. *J. Med. Chem.* **1999**, *42*, 3167.
6. Von Nussbaum, F.; Miller, B.; Wild, S.; Hilger, C. S.; Schumann, S.; Zorbas, H.; Beck, W.; Steglich, W. *J. Med. Chem.* **1999**, *42*, 3478.
7. Navarro, M.; Pérez, H.; Sánchez-Delgado, R. A. *J. Med. Chem.* **1997**, *40*, 1937.
8. Sánchez-Delgado, R. A.; Navarro, M.; Pérez, H.; Urbina, J. A. *J. Med. Chem.* **1996**, *39*, 1095.
9. Ramadan, A. M. *J. Inorg. Biochem.* **1997**, *65*, 183.
10. Malhotra, R.; Singh, J. P.; Dudeja, M.; Dhindsa, K. S. *J. Inorg. Biochem.* **1992**, *46*, 119.
11. Foley, R. T.; Anderson, R. C. *J. Am. Chem. Soc.* **1948**, *70*, 1195.
12. Job, P. *Ann. Chim.* **1928**, *9*, 113; **1936**, *11*, 97.
13. Infrared 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.
14. Magnetic measurements were performed according to the procedure described in ref 3.
15. Cotton, F. A.; Wilkinson, G. In *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1966.
16. pH measurements were performed on a Metrohm 632 pH-Meter calibrated on $CH_3OH/H_2O$ 1:1 solutions of buffer phosphate. UV spectra were recorded in the thermostated cell compartment of a Cary 219 or a Perkin–Elmer Lambda 3 spectrophotometer.
17. Compounds are initially screened against *M. tuberculosis H37Rv* (ATCC 27294, susceptible both to rifampin, RMP and isoniazid, INH), at 6.25 μg/mL, in BACTEC 12B medium, using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition are re-tested 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. Concurrent with the determination of MICs, compounds are tested for cytotoxicity ($IC_{50}$) in VERO cells at concentrations $\leq$62.5 μg/mL or 10× the MIC for *M. tuberculosis H37Rv*. After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. Compounds for which the selectivity index ($SI = IC_{50}$/MIC ratio) >10 have in vitro activity confirmed in the BACTEC 460 at 6.25 μg/mL.
18. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Freguson, R. M.; Gilman, R. H. *J. Clin. Microb.* **1998**, *36*, 362.
19. Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.