European Journal of Pharmaceutical Sciences 70 (2015) 45–54

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Antitubercular activity of Ru (II) isoniazid complexes

Inara de Aguiar [a], Aline Tavares [a], Antonio C. Roveda Jr. [a], Augusto C.H. da Silva [a], Leonardo B. Marino [b], Érica O. Lopes [b], Fernando R. Pavan [b], Luiz G.F. Lopes [c], Douglas W. Franco [a,*]

[a] Instituto de Química, Universidade de São Paulo, São Carlos, SP 13566-590, Brazil
[b] Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Julio de Mesquita Filho", Araraquara, SP 14801-902, Brazil
[c] Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza, CE 60455-970, Brazil

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form 15 January 2015
Accepted 16 January 2015
Available online 28 January 2015

Keywords:
Tuberculosis
Resistant strains
Metal complexes
Metallodrugs
Tetraammine- and pentaammine ruthenium complexes
Isoniazid

ABSTRACT

Despite the resistance developed by the *Mycobacterium tuberculosis* (MTb) strains, isoniazid (INH) has been recognized as one of the best drug for treatment of Tuberculosis (Tb). The coordination of INH to ruthenium metal centers was investigated as a strategy to enhance the activity of this drug against the sensitive and resistant strains of MTb. The complexes *trans*-$[Ru(NH_3)_4(L)(INH)]^{2+}$ (L = SO_2 or NH_3) were isolated and their chemical and antituberculosis properties studied. The minimal inhibitory concentration (MIC) data show that $[Ru(NH_3)_5(INH)]^{2+}$ was active in both resistant and sensitive strains, whereas free INH (non-coordinated) showed to be active only against the sensitive strain. The coordination of INH to the metal center in both $[Ru(NH_3)_5(INH)]^{2+}$ and *trans*-$[Ru(NH_3)_4(SO_2)(INH)]^{2+}$ complexes led to a shift in the INH oxidation potential to less positive values compared to free INH. Despite, the ease of oxidation of INH did not lead to an increase in the *in vitro* INH activity against MTb, it might have provided sensitivity toward resistant strains. Furthermore, ruthenium complexes with chemical structures analogous to those described above were synthesized using the oxidation products of INH as ligands (namely, isonicotinic acid and isonicotinamide). These last compounds were not active against any strains of MTb. Moreover, according to DFT calculations the formation of the acyl radical, a proposed intermediate in the INH oxidation, is favored in the $[Ru(NH_3)_5(INH)]^{2+}$ complex by 50.7 kcal mol^{-1} with respect to the free INH. This result suggests that the stabilization of the acyl radical promoted by the metal center would be a more important feature than the oxidation potential of the INH for the antituberculosis activity against resistant strains.

1. Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis* (MTb), is the second leading cause of death from an infectious disease and it is surpassed by the human immunodeficiency virus (HIV) (WHO, 2012). The chemotherapy of tuberculosis is in general based on the administration of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol (WHO, 2009). Among these drugs, the prodrug isoniazid (isonicotinic acid hydrazide, INH), first synthesized in 1912 (Meyer and Mally, 1912), is the most prescribed (Riccardi et al., 2009; Rozwarski et al., 1998). INH action involves the inhibition of the mycolic acid biosynthesis, which is one of the most important cell wall components of MTb (Winder and Collins, 1970). In fact, the most accepted action mechanism of this prodrug requires a conversion of the INH into an acyl radical promoted by the KatG enzyme (Lei et al., 2000). This radical is able to link with NAD^+ and form a covalent adduct (Vilcheze et al., 2006) potentially capable of inhibiting the FASII enoyl-ACP reductase InhA (Lei et al., 2000; Nguyen et al., 2002; Rawat et al., 2003; Vilcheze et al., 2006). Despite this, the mechanism of action of INH has been related to occurs by other pathways, as the inhibition of nucleic acids (Gangadharam et al., 1963), phospholipids (Brennan et al., 1970) synthesis and NAD^+ metabolism (Bekierkunst, 1966; Zatman et al., 1954).

Unfortunately, MTb have become resistant to INH due to their high ability to change DNA under selective pressure (Barry et al., 1998). The most common alterations related to INH resistance occur in the genes: *katG*, *inhA*, *ahpC*, *kasA* and *ndh* (Schroeder et al., 2002). The *katG* gene encodes the KatG protein (catalase-peroxidase-peroxynitritase T), responsible for the INH activation and also for mutations in the gene. This is the most accepted mechanism for INH resistance (Kapetanaki et al., 2005). Alterations in the *inhA* gene can occur in two different ways: through mutations in the gene promoter region and through mutations in the InhA domain responsible for the binding with the NAD-INH adduct. In

* Corresponding author. Tel.: +55 16 3373 9970.
E-mail address: douglas@iqsc.usp.br (D.W. Franco).

http://dx.doi.org/10.1016/j.ejps.2015.01.008
0928-0987/

general, resistant strains which have a mutated KatG show a loss of catalase-peroxidase activity, which is a protective mechanism (Pym et al., 2002). In an attempt to compensate this loss and adapt the protective fitness, these bacteria overexpress the AhpC protein, an alkyl hydroperoxide reductase, through mutations in the *ahpC* promoter region. The mutation in the -46 position is one of the most found mutation (Hazbón et al., 2006; Hoshide et al., 2013; Jnawali et al., 2013).

Mutations in *katG* and *inhA* do not cover the totality of resistant clinical isolates (Mdluli et al., 1998). Mdluli and co-authors (Mdluli et al., 1998) conducted a study aiming at a new target to INH. They found that a treatment with a dose of 1 μg mL^{-1} of INH could generate a saturated hexacosanoic acid accumulation in a 12 kDa protein known as AcpM. The same study (Mdluli et al., 1998) showed an upregulation of an 80 kDa-protein of the same amino terminal of AcpM under a treatment with INH. Actually, such an 80 kDa was a complex covalently formed by AcpM, INH and β-ketoacyl ACP synthase, called KasA.

The discovery of new anti-TB drugs is essential for the overcoming the major problems related to tuberculosis: (1) nonadherence of the patients due to the duration and complexity of the treatment; (2) treatment side effects; (3) emergence of MDR (Multidrug Resistant) and XDR (Extensive Drug Resistant) strains; (4) drug interactions between antiretroviral and available anti-TB drugs and (5) need for a drug capable of acting in latent bacteria (van den Boogaard et al., 2009). The treatment duration is one of the main problems of the disease, since it results in nonadherence by the patient. The attempts to shorten the therapy include studies with Sutezolid (phase II of clinical development) (Williams et al., 2009), rifamycins (Rosenthal et al., 2007), nitroimidazoles (Lin et al., 2012) and fluoroquinolones (present in Oflotub, ReMox and Rifaquin trials) (Zumla et al., 2013).

One of the strategies to overcome the problems related to INH therapy is the coordination of this prodrug to metal complexes (Basso et al., 2010; Oliveira et al., 2006, 2004; Rodrigues et al., 2012; Sousa et al., 2012; Vasconcelos et al., 2008), as previously performed with $[Fe(CN)_5(INH)]^{3-}$ and $[Ru(CN)_5(INH)]^{3-}$. These compounds have been tested *in vitro* and *in vivo* against tuberculosis. $[Fe(CN)_5(INH)]^{3-}$ exhibited good activity (0.2 μg mL^{-1}) as opposed to $[Ru(CN)_5(INH)]^{3-}$, which was not active (Sousa et al., 2012). Indeed, there has been growing concern about the medicinal applications of coordination compounds of iron and ruthenium against cancer, parasitosis and tuberculosis (Pavan et al., 2011, 2010; Santos et al., 2013). The association of bioactive molecules with metal complexes may result in a potentialization of drug activity and, in some cases, improvement on other physical chemical properties, like an increase in water solubility of these molecules regarding the free drug. For example, the coordination of benznidazole (Bz), the mostly used drug in therapy of Chagas' disease (Silva et al., 2008), to the tetraammineruthenium complex *trans*-$[Ru(Bz)(NH_3)_4(SO_2)](CF_3SO_3)_2$ resulted in a ninefold increase in water solubility and a thousand-fold smaller dose (*in vivo* assays) regarding the optimal dose of Bz (Silva et al., 2008). After the coordination of Bz to the ruthenium(II) complex, the reduction potential of the nitro group (in Bz) was shifted to more positive values ($\Delta E = 0.10$ V). The highest activity observed for *trans*-$[Ru(Bz)(NH_3)_4(SO_2)](CF_3SO_3)_2$ regarding the non-coordinated Bz was attributed to this feature.

A similar strategy was used in the present study, i. e. $[Ru(NH_3)_5(INH)](BF_4)_2$ and *trans*-$[Ru(NH_3)_4(SO_2)(INH)](BF_4)_2$ complexes (Fig. 1) were synthesized and their antituberculosis activity investigated. Analogous ruthenium(II) complexes with isn (isonicotinamide) and ina (isonicotinic acid) ligands, known as two of the isoniazid oxidation products (Johnsson et al., 1995), were also synthesized (Fig. 1). The antituberculosis activity of these compounds was also investigated. DFT calculations were carried out so as to verify the relative stability of the possible intermediates generated during the INH oxidation.

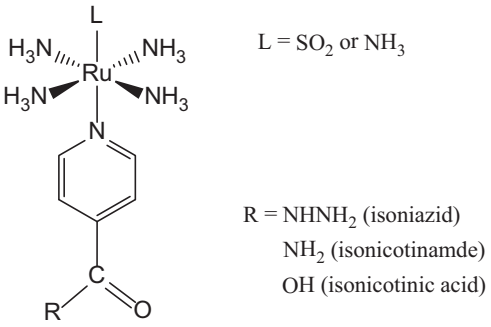

Fig. 1. Chemical structure of the ruthenium(II) complexes.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were of analytical grade (Aldrich, Merck or Panreac) and used without further purification.

2.2. Synthesis of the complexes

The precursors *trans*-$[Ru(NH_3)_4(SO_2)Cl](BF_4)_2$ (Vogt et al., 1965) and $[Ru(NH_3)_5Cl]Cl_2$ (Kuehn and Taube, 1976), and the complexes $K_3[Ru(CN)_5(INH)]$ (Johnson and Shepherd, 1983), $Na_3[Fe(CN)_5(INH)]$ (Sousa et al., 2012), $[Ru(NH_3)_5(isn)](BF_4)_2$, $[Ru(NH_3)_5(INH)](BF_4)_2$ (Gaunder and Taube, 1970), *trans*-$[Ru(NH_3)_4(SO_2)(isn)](BF_4)_2$ (Isied and Taube, 1974), $[Ru(NH_3)_5(ina)](BF_4)_2$ (Chou et al., 1992) were synthesized as previously reported. The well known complexes *trans*-$[Ru^{II}(NH_3)_4(NO^+)(L)]^{3+}$, where *L* is isonicotinamide (isn), pyridine (py), 4-picoline (pic), nicotinamide (nic), isonicotinic acid (ina) or imidazol (imN) were synthesized by following earlier described procedures (Roveda et al., 2014; Tfouni et al., 2003).

2.2.1. trans-$[Ru(NH_3)_4(SO_2)(ina)](BF_4)_2$

0.40 g of isonicotinic acid were added to 10 mL of water and solid NaOH was added until the total dissolution of the ligand. The final pH was adjusted to 9.0 (using trifluoroacetic acid) and the solution was deaerated by argon flux for 30 min. *trans*-$[Ru(NH_3)_4(Cl)(SO_2)]Cl$ (0.15 g, 0.5 mmol) was dissolved in 9.0 mL of previously deaerated water (under argon) and sodium carbonate was added until reach pH 9.0. The isonicotinic acidic solution was added to this pale green-yellow solution through Teflon tubing. The color changed to orange and a suspension was formed. The reaction was kept under stirring for 15 min and then 3.0 mL of 6.0 M HBF_4 solution were added. The product was precipitated with an excess of acetone, filtered and dried under vacuum. Anal. calcd.: C, 13.60; H, 3.23; N, 13.21. Found: C, 12.93; H, 3.15; N, 13.22. ^{1}H NMR (400 MHz): δ 8.99 ppm (d, 2H), δ 8.56 ppm (d, 2H).

2.2.2. trans-$[Ru(NH_3)_4(SO_2)(INH)](BF_4)_2$

trans-$[Ru(NH_3)_4(SO_2)Cl](Cl)_2$ (0.10 g, 0.49 mmol) was dissolved in 9.0 mL of deaerated water and sodium carbonate was added until reach pH 9.0. Isoniazid was added to this solution (0.68 g, 4.98 mmol) and the mixture was left to react for 30 min at room temperature. 3.0 mL of HBF_4 6.0 M were then added. The orange precipitate was collected by filtration, washed with cold 6.0 M HBF_4 and dried under vacuum. The solid was suspended in HBF_4 and filtrated. Yield: 75%. Anal. Calc. C, 13.25; H, 3.52; N, 18.02.