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Impact of Metal Nature on Bioactivity of Metal Chelates of Monensin Antibiotic: Synthesis and Anti-tubercular Activity of Metal-Monensin Complexes

MOSTAFA MIMOUNI [1*], BOUCHRA FILALI BABA[4], ISMAIL WARAD[2], VIJAY MASAND[3], ABDELALI KERBAL[4] AND TAIBI BEN HADDA[1*]

[1]Laboratoire de Chimie des Matériaux, Faculté des Sciences, Université Mohammed Premier, 60000 Oujda, Morocco.

[2]Department of Chemistry, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.

[3]Department of Chemistry, VidyaBharati Mahavidyalaya, Camp, Amravati, Maharashtra, India-444602.

[4]Laboratoire de Chimie Organique, Faculté des Sciences Dhar Mehraz, 30000 Fès, Morocco.

Abstract- Metal-carboxylic ionophore complexes, where Metal = Ag, Tl, Li, Na, K, Rb, Mg, Mn, Cs, Sr, Ba, Cu and Ionophore = monensin (MON-H), have been synthesized. The molecular structures of three complexes have been solved by X-ray diffraction methods. The metal chelates were tested against human tuberculosis line for potential anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB). The metal chelates overall showed enhanced activity and amongst the ionophore. This increased activity could possibly be due to the increased hydrophobic character that the alkyl and alkyloxy groups confer on the molecule after coordination to metals. Moreover, hydrophobic molecules with rigid and globular structure have been shown to have the ability to induce localized permeability across the membrane.

Introduction

The World Health Organization's reports on tuberculosis (TB) are highly alarming. Almost nine million new cases occurred in 2004 and nearly 2 million people die every year. It is estimated that one-third of the world's population is infected by *Mycobacterium tuberculosis*, the bacillus responsible for TB. Moreover, TB incidences are growing globally 1% every year. Also, the worldwide emergence of multi-drug resistant TB is considered to aggravate the current pandemic. [1] New clinical methodologies are therefore, sturdily required addressing this issue.

A large number of antibiotics are recognized for their ability to act as complexing agent towards various transition metals. Many studies have indicated a strong relationship of metals and biological activity. [2] Non-biologically active compounds become active and less biologically active become more upon coordination/chelation with the metal ions. With the same intentions many transition metal chelates have been prepared and studied for their properties e.g., antitumoral activity or cancer-kinase inhibition by ruthenium-staurosporin derivatives,[2] HIV-integrase inhibition by bimetallic complexes (Mg_2-DKA) where DKA = diketo acid derivatives,[3] antibacterial properties by coordination of palladium(II) with three antibiotics of tetracycline family (tetracycline, doxycycline and chlortetracycline),[4] and antibiotics (Amoxicillin, dicluxacillin) complexing ability to form metal chelates and their bactericidal and fungicidal properties.[5] In continuation of our work on anti-tubercular activity of ruthenium(II)-polypyridyl complexes.

Keywords: Monensin; Metal Chelates; *Mycobacterium tuberculosis*; X-ray structure.

*Corresponding author: taibi.ben.hadda@gmail.com (T. Ben Hadda) and mimouni_8@yahoo.ca (M. Mimouni)

[6] In continuation of our work on ionophores as like Lasalocid acid (LASA-H) [7-8] and Calcimycin (CALC-H) [11], we report herein the results of our studies on the metal chelates of Monensin acid (MON-H).[9, 10] Tentative structures have been proposed on the basis of analytical, potentiometric, spectral, and obtained crystallographic data.

In order to establish the biological role of metals, this carboxylic ionophore and its metal chelates [9, 10] have been screened for anti-tubular activity against *Mycobacterium Tuberculosis* $H_{37}Rv$ and the results are reported.

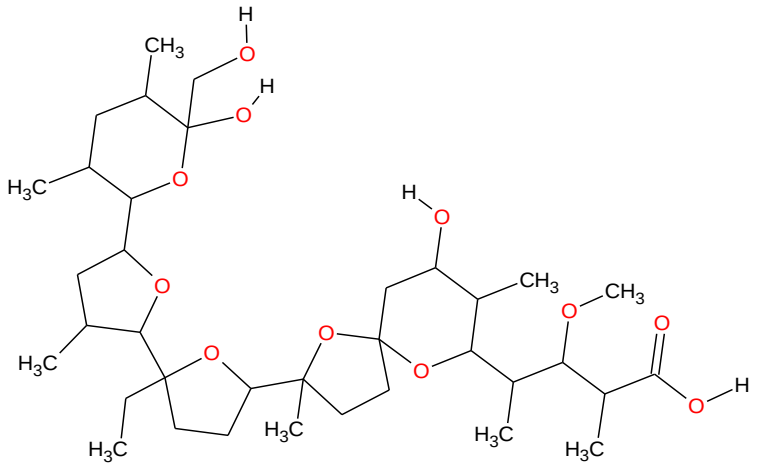

Figure 1. Chemical structure of free monensin acid (MON-H).

Experimental

Monensin ionophore (90–95%) is purchased from commercial source. All manipulations with the substances were performed in normal atmosphere. The complexes are characterized by ^{1}H and ^{13}C NMR by using Bruker AC 400 MHz spectrometer and their stereochemistry is confirmed by crystallographic structures.

Synthesis of the metal-monensin complexes: 1-10

The metal-monsensin salts (Metal = Na^{+}, K^{+}, Rb^{+}, Cs+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}) have been previously prepared in our laboratory at Oujda,[9-10] by using the procedure described in literature.[12-14]. Monensin acid is well known to exhibit significant preference to form complexes with monovalent cations such as: Li^{+}, Na^{+}, K^{+}, Rb^{+}, Ag^{+} and Tl^{+}.[16-19]

Antitubercular evaluation

Primary screening was conducted at 6.25 □g mL^{-1} against M. tuberculosis H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[15] Compounds exhibiting The most effective inhibitors are **5-9** and which produce 99-100% inhibition of the *Mycobacterium tuberculosis* with (0.2 to 0.7 µg/mL). Molecules **1-4** and **10** are effective too but produce appreciable growth inhibition (90-94 % inhib.) with (0.74 to 5.8 µg/mL), in comparable conditions. The increase in activity could be due to the increase of the hydrophobic character that the alkyl, alkyloxy and fluorescence were tested in the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against M. tuberculosis H37Rv to determine the MIC using MABA. MIC is defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls.[15]

Results and Discussion

Evaluation of anti-tuberculosis activity (in vitro)

Twenty two compounds containing antibiotics and their metal complexes (ionophore = calcimycin, lasalocid, monensin and M = Ag^{+}, Li^{+}, K^{+}, Cs^{+}, Rb^{+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}) have been evaluated as anti-tuberculosis agents through the TAACF tuberculosis screening program, surprisingly the metal-ionophore complexes have been shown to inhibit significantly the growth of *Mycobacterium tuberculosis* $H_{37}Rv$ using both Alamar and Maba assay at the first level adopted for *in vitro* screening. Not surprisingly, some of these ionophore derivatives are currently being modified in the goal to be examined at the *in vivo* stage of the tuberculosis screening program. The antituberculosis data are summarized in Tables 1-3. alkylamino groups confer on the molecule after coordination to metals. Hydrophobic molecules with rigid, globular structure have been shown to have the ability to across the membranes and induce localized permeability changes leading to leakage out of the membrane. The combination of lipophilic character and cationic character of metallic cations while also hydrophobic and very easily

inserted into the membrane, are much less likely to cause disruption of the lipid packing order. That can be explained by possible cell membrane disturbances. The enhanced antitubercular inhibition observed in the presence of most active complexes is then more likely due to their interaction with some intracellular target. The presence of a strong or poor electron-withdrawing group must alter the nature of the compound in such a way as to promote binding to the target(s). All the Metal-Ionophore complexes individually exhibited varying degrees of inhibitory effect on the growth of the tested *Mycobacterium Tuberculosis*.

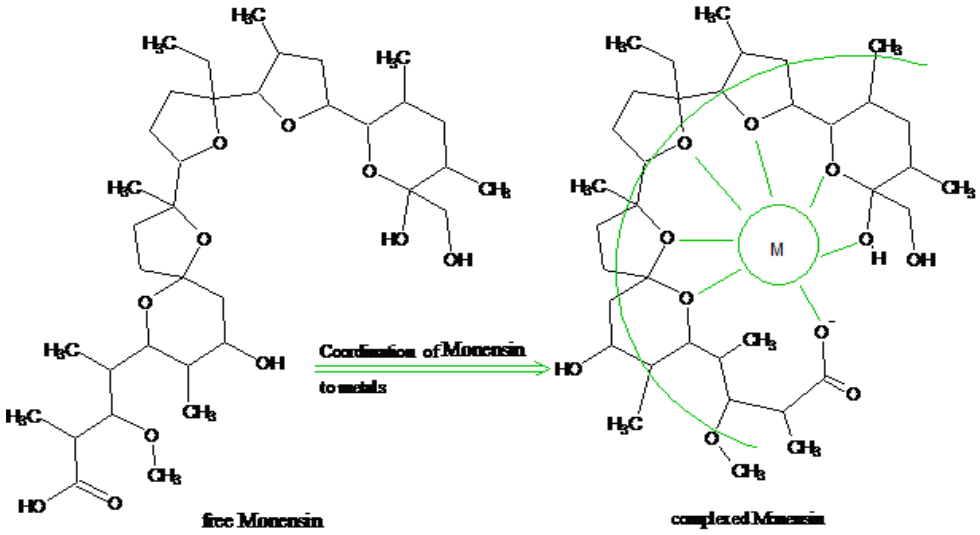

Table 1 shows that the activity of the carboxylic ionophore complexes became more pronounced when coordinated with the metal ions. The biological activity of the ionophores follows the order: $Ba^{2+} > Mg^{2+} > Ca^{2+} > Li^{+}$. Furthermore, the data in Table 1 show that *Mycobacterium Tuberculosis* was inhibited to a greater degree by the Mg(II), Ba(II) for monensin complexes. The importance of this lies in the fact, that these complexes could reasonably be used for the treatment of some common diseases caused by other *Mycobacterium Tuberculosis* multi-drug resistant.

Table 3. Anti-tubercular activity data of Metal-monensin complexes.

Corp ID (TAACF code)	**Chemical structure**	**Antitubercular Activity**			
		IC90[b] (µg/mL)	**IC50**[b] (µg/mL)	**%Inhib.**[a] at 6.25 µg/mL	**Activity**[b]
1 (407147)	[Li (MON)]	1.034	0.731	99	Active
2 (407148)	[Na (MON)]	10.5	5.393	90	Active
3 (407149)	[K (MON)]	2.498	1.337	99	Active
4 (407150)	[Rb(MON)]	3.489	1.748	100	Active
5 (407151)	[Cs(MON)]	1.322	0.694	100	Active
6 (407152)	$[Mg(MON)_2]$	0.728	0.332	100	Active
7 (407153)	$[Ca(MON)_2]$	0.864	0.42	100	Active
8 (407154)	$[Sr(MON)_2^-]$	1.216	0.601	100	Active
9 (407155)	$[Ba(MON)_2]$	0.558	<0.2	100	Active
10 (407156)	$[Cu(MON)_2]$	25.744	5.856	100	Active

[a]Essai: ALAMAR. [b]Essai: MABA

Conclusion

In this paper we reported the preparation, isolation, and characterization of a new polydentate antibiotic ionophore monensin, and its complexes with K(I), Na(I), Ag(I), Tl(I), Ba(II), Ca(II), Mn(II) and Mg(II). It is tentatively proposed that the ionophore ligand coordinate through the oxygen's of the heterocyclic rings and the oxygen of the carboxylate group, forming stable chelate ring structures. The synthesized metal complexes, in comparison to the uncomplexed antibiotic ligand, were screened for their antibacterial activity against pathogenic bacterial specie (*Mycobacterium Tuberculosis*). The activity of the Metal-Ionophor complexes became more pronounced when coordinated with the metal ions.

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