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In vitro effect of free and complexed indium(III) against *Mycobacterium tuberculosis*

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Abstract

In mycobacteria, the study of inhibition by metal ions has been limited by the absence of suitable molecular vectors. Recently, we reported on the inhibitory activity of a family of chelators, macrocyclic compounds (MCC), against *Mycobacterium tuberculosis*. In this study equimolar concentrations of the free cations vanadium(IV), arsenic(III), iron(III), indium(III) and bismuth(III), and as 1:1 complexes with the MCC 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid (TETA) were tested in vitro against *M. tuberculosis* using the Bactec™ 460 TB radiometric technology (Becton-Dickinson, MD, USA). Radiometric inhibition above 80% was obtained with free indium(III) and bismuth(III), and ranged from 80% to 99%, with the complexes of TETA with vanadium(IV), bismuth(III) and indium(III), in the order of increasing activity. The highest radiometric inhibition levels were obtained with the $[In(TETA)]^-$ complex, which caused drops of up to 4 log units in cellular viability. The minimal inhibitory concentration of this compound was evaluated at 3 μM.

Keywords: *Mycobacterium tuberculosis*; Macrocyclic compounds; Metal complexes

1. Introduction

Metal ions, free or complexed, are known for their disinfectant properties and in some cases as effective therapeutic agents against bacterial diseases. As early as 1908 research in this area resulted in the awarding of the Nobel Prize to Paul Ehrlich for his development of chemotherapeutic arsenical drugs [1]. Before the age of antibiotic therapy, gold compounds were introduced in the treatment of tuberculosis, although their effectiveness against the disease was not scientifically confirmed but initially based on the observation of Robert Koch, who had reported in vitro activity of gold cyanide against *Mycobacterium tuberculosis* [2].

In mycobacteria, the study of inhibition by metal ions has been limited by the absence of suitable molecular vectors. Although in non-biological systems mycobacterial siderophores of the mycobactin family are known to form stable complexes with a number of metal ions [3–6], attempts at using these siderophores to vectorize cations with potential antimycobacterial activity failed due to their high ferri-specificity [7]. More recently non-siderophore chelators have been developed and tested against mycobacterial species showing enhanced activity

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when in the form of complexes [8–15]. However, it seems clear, that in regards to mycobacterial pathogens the chemotherapeutic potential of metal ions has not been fully explored.

Recently, we reported on the inhibitory activity against *M. tuberculosis* of chelators belonging to the family of macrocyclic compounds (MCC) [16]. In this study, we explore the in vitro activity against *M. tuberculosis* of free cations: vanadium(IV), arsenic(III), iron(III), indium(III) and bismuth(III); and their complexes with the MCC 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid (TETA).

2. Materials and methods

2.1. Organisms

The *M. tuberculosis* H37Rv strain was selected for this study. Prior to the experiments, the strain was precultured in 12B vials for use with the Bactec™ 460 TB radiometric instrument (Becton-Dickinson, Microbiology Systems, Cockeysville, MD, USA). Otherwise, it was stored as frozen suspensions at −70 °C.

2.2. Drugs

The compound 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid (TETA), was synthesized from the MCC cyclam (1,4,8,11-tetraazacyclotetradecane) by condensation with potassium chloroacetate in aqueous basic solution, keeping the pH below 10 and the temperature between 40 and 60 °C during the reaction. The mixture was then acidified to pH 2 with hydrochloric acid. The precipitate obtained was recrystallized and identified as pure TETA (Fig. 1) [17]. The final product was characterized by melting point, elemental analysis, ^{1}H and ^{13}C NMR. Cyclam was obtained commercially from Strem Chemicals (Bischheim, France).

The standard solution of arsenic(III) was prepared by dissolving a known amount of As_2O_3 (p.a.) in ionized water, and the solution of oxovanadium(IV) was prepared from $V_2O_5 \cdot 5H_2O$ by dissolving in a solution of H_2SO_4 and standardized with $KMnO_4$. The other metal ions were prepared from nitrate salts, of analytical grade with demineralized water (obtained by a Millipore/Milli-Q system) and were standardized by titration with K_2H_2EDTA solutions as described [18].

The metal complexes of TETA and vanadium(IV), arsenic(III), iron(III), indium(III) and bismuth(III) were prepared by the addition of aqueous solutions of $Fe(NO_3)_3$, arsenic(III), vanadium(IV), $Bi(NO_3)_3$ and $In(NO_3)_3$, to the MCC in a 1:1 ratio, followed by the increase of the pH of the mixture with a solution of KOH till the complete formation of the complex (between pH 4.5 and 6) [19].

To prepare the TETA complexes and to study the biological effect of the free cations $Fe(NO_3)_3$, As_2O_3, V_2O_5, $Bi(NO_3)_3$ and $In(NO_3)_3$were purchased from Merck (VWR International, Lisbon, Portugal).

Equimolar concentrations, of 116 μM, of the cations, TETA and the TETA complexes were used in the in vitro studies.

2.3. Drug sensitivity studies

For examining the antimycobacterial activity of different MCC-metal complexes, stock solutions of 5 mg ml^{-1} were prepared in sterile distilled water, sterilized by filtration using 0.2 μm Filtropur filters (Sarstedt, Nümbrecht, Germany), and frozen at −20 °C until use. Volumes of 0.1 ml of the appropriate dilutions were injected into Bactec™ 12B vials (Becton Dickinson, Sparks, MD, USA), to obtain concentrations ranging from 10 to 300 mg L^{-1}.

In vitro activities were evaluated using methodologies applied to the Bactec™ 460 TB instrument (Becton Dickinson, Sparks, MD, USA), as previously described [16,20]. This instrument uses the radiometric detection of bacterial growth through the capture and measure of released ^{14}C-labelled CO_2 resulting from the metabolism of ^{14}C-labelled palmitic acid by the organisms in the Bactec™ 12 B growth medium. The ^{14}C-labelled CO_2 captured by the detector is expressed as a numerical value called the Bactec™ growth index (GI) which ranges from 1 to 999. As the GI during exponential growth is a measure of respiratory activity that is proportional to the number of viable bacteria in the vials [21], it was used in this simple relation to express inhibition. Thus, relative growth was calculated in relation to a vial

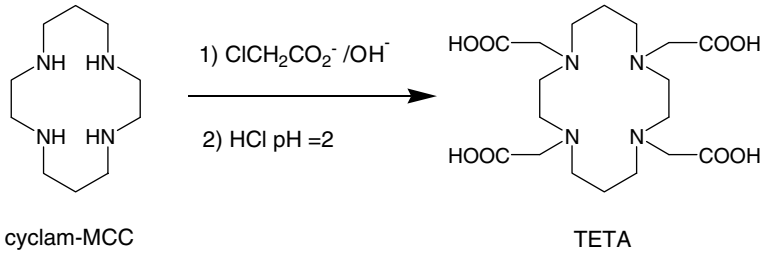

Fig. 1. Macrocyclic compound TETA and a scheme of its synthesis.

having received the same initial inoculum but no added compound as control, representing 100% of radiometric growth, or 0% of radiometric inhibition. Results were expressed as the average value obtained from the readings of four consecutive days during exponential growth. Since relative growth was calculated on the basis of GI readings, and is therefore in reference to a measure of respiratory activity, it was also compared to bacterial counts carried out at the end of the experiment by plating serial 10-fold dilutions of the bacterial suspensions from the individual Bactec™ 12B vials onto Middlebrook 7H11 agar plates, results were expressed in CFU ml^{-1}. Viability was determined from the comparison of CFU ml^{-1} from individual Bactec™ vials at the beginning and at the end of the experiments.

2.4. Effect of free and complexed indium

To further evaluate the effect of free and complexed indium(III) on the in vitro growth of *M. tuberculosis*, equimolar concentrations of 116 μM of $In(NO_3)_3$, TETA and $[In(TETA)]^-$ were added during exponential growth to individual Bactec™ 12 B vials, having received the same initial inoculum, but no added compound. Thus, compounds were added six days after inoculation and left to incubate at 37 °C with the experiment lasting a total of 14 days. Results expressed as the Bactec™ growth index (GI) and as bacterial counts in CFU ml^{-1}, represented the average of three independent experiments.

Minimal inhibitory concentrations (MIC) were determined by testing a wide range of concentrations using procedures recommended for the Bactec™ 460 radiometric system [16,20]. Briefly, a control was prepared containing a 10^2 dilution of the initial inoculum used in the drug containing vials, termed 1:100 control. When the growth index (GI) of the 1:100 control vial reached 30, the GI was read one additional day. The difference in the GI values (ΔGI) was calculated for these two days. The MIC, corresponding to the drug concentration resulting in more than 99% of inhibition of the bacterial population, was defined as the lowest concentration amongst those tested for which the ΔGI of the drug containing vial was less than the ΔGI of the 1:100 control, obtained from the reading after the GI had reached 30.

3. Results

Preliminary observations of the effect of equimolar concentrations of 116 μM of the free cations: vanadium(IV), arsenic(III), iron(III), indium(III) and bismuth(III); and their TETA complexes are shown in Table 1. Calculated on the basis of GI readings, radiometric inhibition levels above 80% were obtained with free indium(III) (88%) and with bismuth(III) (93%), whereas there was no apparent inhibitory activity with arsenic(III), iron(III) and vanadium(IV). For all the cations, radiometric inhibition was enhanced when they were used in the form of complexes. Radiometric inhibition of and above 80% was obtained with the TETA complexes of vanadium(IV) (80%), bismuth(III) (96%) and indium(III) (99%). Important differences in the radiometric inhibition levels between the free and complexed forms were observed in the case of arsenic(III) and vanadium(IV) and their TETA complexes as well as iron(III) and $[Fe(TETA)]^-$, as compared to closer levels observed with bismuth(III) and $[Bi(TETA)]^-$, and indium(III) and $[In(TETA)]^-$. However, the observed differences in radiometric inhibition levels, calculated on the basis of GI readings, were not reflected by the bacterial counts, as there were no marked differences in viability values between the free and complexed forms except in the case of indium(III).

The negative viability values obtained with the $[In(TETA)]^-$ complex, led us to further evaluate the effect of free and complexed indium(III) on the in vitro growth of *M. tuberculosis*. Results of this evaluation are summarized in Fig. 2 and represent the average of three independent experiments, using equimolar concentrations of 116 μM of the compounds. Drops averaging

Table 1
Drug sensitivity studies of 116 μM concentrations of free cations and their TETA complexes on the growth of *Mycobacterium tuberculosis*

Cations	Free cations		TETA complexes	
	Radiometric inhibition (%)[a]	Bacterial viability[b]	Radiometric inhibition (%)[a]	Bacterial viability[b]
None	0 ± 0	+2.53	49 ± 12	+1.64
Arsenic(III)	0 ± 0	+1.96	72 ± 1	+1.65
Iron(III)	3 ± 5	+2.29	62 ± 3	+1.67
Vanadium(IV)	0 ± 0	+2.15	80 ± 1	+1.42
Bismuth(III)	93 ± 2	+0.32	96 ± 1	+0.28
Indium(III)	88 ± 3	+1.37	99 ± 0	−2.75

[a] Relative growth was calculated in relation to a control vial having received the same initial inoculum but no added compound, representing 100% of radiometric growth, or 0% of radiometric inhibition. Results were expressed as the average value obtained from the Bactec™ 460 readings of four consecutive days during exponential growth.

[b] Bacterial viability was expressed as the difference in the logarithms of the number of colony forming units per ml at the end of the experiment (t_f) and that of the day of inoculation (t_0) according to the expression: $\log[(\text{CFU ml}^{-1})_{t_f}] - \log[(\text{CFU ml}^{-1})_{t_0}]$.

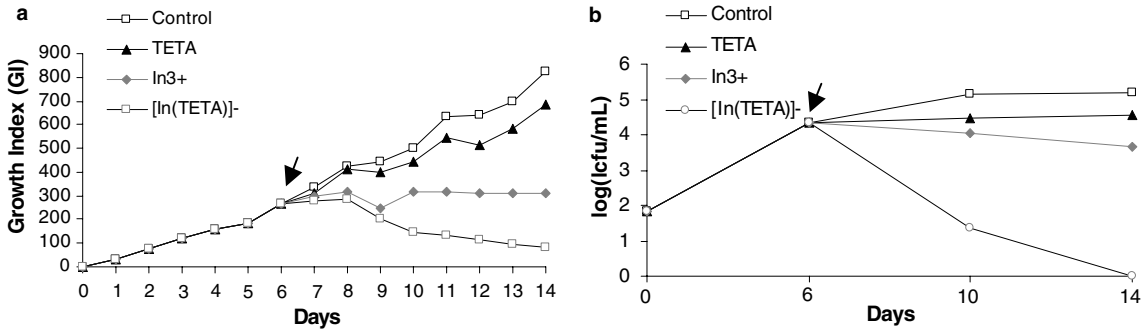

Fig. 2. Effect of free and complexed indium on *Mycobacterium tuberculosis* H37Rv growth monitored as a function of time and expressed as: (a) the radiometric growth index (GI) using the Bactec™ 460 TB instrument (Becton-Dickinson, MD, USA), or (b) the logarithms of viable counts in CFU ml^{-1}. Arrows indicate when $In(NO_3)_3$, TETA or $[In(TETA)]^-$ were added to the drug free vials, six days after inoculation with the *M. tuberculosis* strain. Results represent the averages of three independent experiments.

up to 4 log units in cellular viability, observed with the $[In(TETA)]^-$ complex, confirm the in vitro bactericidal effect of this compound on *M. tuberculosis*. Minimal Inhibitory Concentrations were obtained at 3.6 μM for $[In(TETA)]^-$, 116 μM for $In(NO_3)_3$ and 116 μM for TETA.

4. Discussion

In this study, radiometric Bactec™ methodologies were used to study the in vitro activity against *M. tuberculosis* of the free cations: vanadium(IV), arsenic(III), iron(III), indium(III) and bismuth(III); and their complexes with the MCC 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid (TETA).

For all the cations, radiometric inhibition based on Bactec™ GI readings, was enhanced when they were used in the complexed form, the highest levels being observed with the $[In(TETA)]^-$ complex. The large range in the variation of the radiometric inhibition levels between the free and complexed forms of arsenic(III), iron(III) and vanadium(III), may reflect regulated uptake or possible extrusion mechanisms as regards the free cation. Mycobacteria, especially environmental species, are known to be resistant to heavy metals [22–24], although the mechanisms by which this may take place have only recently begun to be explored. The possible role of the *M. tuberculosis* Mramp, a divalent cation transporter of the Nramp family, in the extrusion of a variety of metal ions, has recently been suggested [25]. Also recent has been the discovery of the SmtB/ArsR family of metallo-regulatory transcriptional regulatory proteins in *M. tuberculosis* [26–28]. These regulators repress the expression of operons encoding proteins involved in di-and multivalent heavy metal sequestration, transport, export and reduction by binding to the operator promoter regions of genes repressing transcription. However, when their effector binding sites become occupied by metal ions in response to metal toxicity, a conformational change takes place, allowing transcription to proceed [29]. In *M. tuberculosis* NmtR has been shown to form complexes with Zn(II), Co(II) and Ni(II) [27,28] and CmtR with Cd(II) and Pb(II) [26].

Our previous investigations provided evidence that the use of an appropriate molecular vector as the delivery agent may allow bypassing a highly regulated mechanism [30]. Using *Mycobacterium aurum* as a model, it was suggested that the toxicity of iron(III), observed at concentrations above 5 μM, depended on whether the uptake of the delivery agent was subject to the cells regulatory mechanisms, as in the case of homologous mycobactin, or not, as with heterologous mycobactins or synthetic chelators. Thus, whereas iron starvation is bacteriostatic [31], iron uptake, when not regulated, is bactericidal, at concentrations as low as 5 μM, as reported.

Another aspect involves the choice of the cations. The cations vanadium(IV), arsenic(III), indium(III) and bismuth(III), are known to affect diverse biological processes in biological systems in some cases competing with iron(III) in vital functions [32–35].

Contrary to variation of the radiometric inhibition, there were no marked differences in bacterial viability between the free and complexed forms except in the case of indium(III). The Bactec™ radiometric GI is a measure of respiratory activity. Thus, although radiometric inhibition levels were calculated on the basis of GI readings, and were therefore in reference to a measure of respiratory activity, these were also compared to cellular viability determined from the comparison bacterial counts in CFU ml^{-1} from individual Bactec™ vials at the beginning and at the end of the experiments. In previous investigations using an iron(III) complex of a MCC, it had been concluded, from the comparison of bacterial viability counts to reductions in the GI, that radiometric inhibition was not determined by direct inhibition of the respiratory activity [16]. However, in this study, where a larger number of cations and their TETA complexes were tested, the results showing a

degree of radiometric inhibition seemingly high as compared to drops in bacterial viability counts, suggest that the reductions in the GI may in fact be determined by direct inhibition of the respiratory activity, indicating that the arsenic(III) and vanadium(IV) TETA complexes, $[Fe(TETA)]^-$, and possibly $[Bi(TETA)]^-$ are likely to be acting as respiratory poisons without direct killing effect. In contrast $[In(TETA)]^-$ appears to be bactericidal.

Drops averaging up to 4 log units in cellular viability, observed with the $[In(TETA)]^-$ complex, confirmed the in vitro bactericidal effect of this compound on *M. tuberculosis*. The MIC values for this complex was low, 3 μM, considering no attempts of derivatization of TETA, for improving uptake and in vitro activity against *M. tuberculosis* were attempted. Macrocyclic compounds (MCC) seem a promising tool in drug development based on the fact that their properties can be changed considerably by modifying the backbone or by introducing *N*-substituents [17,36,37]. In addition, they already have medical applications as contrast-enhancing agents for magnetic resonance imaging (MRI), in nuclear medicine for radioimmunoscintigraphy or radioimmunotherapy and in chelation therapy [38–42].

The results presented in this investigation provide evidence that cations may be useful against mycobacterial infections when appropriate molecular vectors are provided and, that the macrocyclic family of compounds seem promising chelators to use in their molecular vectorization. Further studies should also include the evaluation and improvement of intracellular uptake of the MCC chelators, testing the complexes for intramacrophagic clearance of mycobacterial infections and the elucidation of their mechanisms of action in mycobacterial species.

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References

[1] Mukhopadhyay, R., Li, J., Bhattacharjee, H. and Rosen, B.P. (1998) Metalloid resistance mechanisms In: Resolving the Antibiotic Paradox (Rosen, B.P. and Mobashery, S., Eds.), pp. 159–181. Kluwer Academic/Plenum Publishers, New York.

[2] Benedek, T.G. (2004) The history of gold therapy for tuberculosis. J. Hist. Med. All. Sci. 59, 50–89.

[3] Andres, Y., MacCordick, H.J. and Hubert, J.C. (1991) Complexes of mycobactin from *Mycobacterium smegmatis* with scandium, yttrium and lanthanum. Biol. Met. 4, 207–210.

[4] MacCordick, H.J. (1985) Restrictive spatial modeling in structure and stability concepts of metallo-mycobactins. Nouv. J. Chim. 9, 535–538.

[5] Snow, G.A. (1969) Metal complexes of mycobactin P and of desferrisideramines. Biochem. J. 115, 199–205.

[6] Snow, G.A. (1970) Mycobactins: iron-chelating growth factors from mycobacteria. Bacteriol. Rev. 34, 99–125.

[7] Barclay, R. and Ratledge, C. (1986) Metal analogues of mycobactin and exochelin fail to act as effective antimycobacterial agents. Zbl. Bakt. Hyg. A 262, 203–207.

[8] Bottari, B., Maccari, R., Monforte, F., Ottanà, R., Rotondo, E. and Vigorita, M.G. (2001) Antimycobacterial in vitro activity of cobalt(II) isonicotinoylhydrazone complexes. Bioorg. Med. Chem. Lett. 11, 301–303.

[9] Bottari, B., Maccari, R., Monforte, F., Ottanà, R., Vigorita, M.G., Bruno, G., Nicolò, F., Rotondo, A. and Rotondo, E. (2001) Nickel(II) 2,6-diacetylpyridine bis(isonicotinoylhydrazonate) and bis(benzoylhydrazonate) complexes: structure and antimycobacterial evaluation. Part XI. Bioorg. Med. Chem. 9, 2203–2211.

[10] Maccari, R., Ottana, R., Bottari, B., Potondo, E. and Vigorita, M.G. (2004) In vitro advanced antimycobacterial screening of cobalt(II) and copper(II) complexes of fluorinated isonicotinoylhydrazones. Bioorg. Med. Chem. Lett. 14, 5731–5733.

[11] Maiti, A. and Ghosh, S. (1989) Synthesis and reactivity of the oxovanadium(IV) complexes of two N–O donors and potentiation of the antituberculosis activity of one of them on chelation to metal ions: part IV. J. Inorg. Biochem. 36, 131–139.

[12] Para, A., Klisiewicz-Panszczyk, T. and Jurek, I. (2001) Synthesis and in vitro tuberculostatic activity of Co(II), Cu(II) and Ni(II) complexes of dialdehyde starch dithiosemicarbazone. Acta Pol. Pharm. 58, 405–408.

[13] Pin, Y. and Zhang, X.P. (1989) Synthesis and characterization of new chromium(III), vanadium(IV), and titanium(III) complexes with biologically active isonicotinic acid hydrazide. J. Inorg. Biochem. 37, 61–68.

[14] Sandbhor, U., Padhye, S., Billington, D., Rathbone, D., Franzblau, S., Anson, C.E. and Powell, A.K. (2002) Metal complexes of carboxamidrazone analogs as antitubercular agents. 1. Synthesis, X-ray crystal-structures, spectroscopic properties and antimycobacterial activity against *Mycobacterium tuberculosis* H(37)Rv. J. Inorg. Biochem. 90, 127–136.

[15] Stojiljkovic, I., Kumar, V. and Srivivasan, N. (1999) Non-iron metalloporphyrins: potent antibacterial compounds that exploit haem/Hb uptake systems of pathogenic bacteria. Mol. Microbiol. 31, 429–442.

[16] David, S., Ordway, D., Arroz, M.-J., Costa, J. and Delgado, R. (2001) Activity against *Mycobacterium tuberculosis* with concomitant induction of cellular immune responses by tetraaza macrocycle with acetate pendant arms. Res. Microbiol. 152, 569–576.

[17] Delgado, R. and Fraústo da Silva, J.J.R. (1982) Metal complexes of cyclic tetraazatetraacetic acids. Talanta 29, 815–822.

[18] Schwarzenbach, G. and Flaschka, H. (1969) Complexometric Titrations. Methuen & Co., London.

[19] Clarke, E.T. and Martell, A.E. (1991) Stabilities of trivalent metal ion complexes of the tetraacetate derivatives of 12-, 13- and 14-membered tetraazamacrocycles. Inorg. Chim. Acta 190, 37–46.

[20] Rastogi, N., Goh, K.S., Bryskier, A. and Devallois, A. (1996) In vitro activities of levofloxacin used alone and in combination with first- and second-line antituberculosis drugs against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. 40, 1610–1616.

[21] Rastogi, N., Goh, K.S. and David, H.L. (1989) Drug susceptibility testing in tuberculosis: a comparison of the proportion methods using Lowenstein-Jensen, Middlebrook 7H10 and 7H11 agar media and a radiometric method. Res. Microbiol. 140, 405–417.

[22] Chapman, J.S. and Speight, M. (1971) Tolerance of atypical mycobacteria: the effect of metal ions in various concentrations. Am. Rev. Respir. Dis. 103, 372–376.

[23] Falkinham III, J.O., George, K.L., Parker, B.C. and Gruft, H. (1984) In vitro susceptibility of human and environmental isolates of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum* to heavy-metal salts and oxyanions. Antimicrob. Agents Chemother. 25, 137–139.

[24] Steingrube, V.A., Murphy, D., McMahon, S., Chapman, J.S. and Nash, D.R. (1975) The effect of metal ions on the atypical mycobacteria: growth and colony coloration. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 230, 223–236.

[25] Wagner, D., Maser, J., Moric, I., Boechat, N., Vogt, S., Gicquel, B., Lai, B., Reyrat, J.-M. and Bermudez, L. (2005) Changes in the phagosomal elemental concentrations by *Mycobacterium tuberculosis* Mramp. Microbiol. 151, 323–332.

[26] Cavet, J.S., Graham, A.I., Meng, W. and Robinson, N.J. (2003) A cadmium–lead-sensing ArsR-SmtB repressor with novel sensory sites. J. Biol. Chem. 278, 44560–44566.

[27] Cavet, J.S., Meng, W., Pennella, M.A., Appelhoff, R.J., Giedroc, D.P. and Robinson, N.J. (2002) A nickel–cobalt sensing ArsR-SmtB family repressor: contributions of cytosol and effector binding sites to metal selectivity. J. Biol. Chem. 277, 38441–38448.

[28] Pennella, M.A., Shokes, J.E., Cosper, N.J., Scott, R.A. and Giedroc, D.P. (2003) Structural elements of metal selectivity in metal sensor proteins. Proc. Natl. Acad. Sci. USA 100, 3713–3718.

[29] Busenlehner, L.S., Pennella, M.A. and Giedroc, D.P. (2003) The SmtB/ArsR family of metalloregulatory transcriptional repressors: structural insights into prokaryotic metal resistance. FEMS Microbiol. Rev. 27, 131–143.

[30] Bosne-David, S., Bricard, L., Ramiandrasoa, F., Déroussent, A., Kunesch, G. and Andremont, A. (1997) Evaluation of growth promotion and inhibition from mycobactins and nonmycobacterial siderophores (desferrioxamine and FR160) in *Mycobacterium aurum*. Antimicrob. Agents Chemother. 41, 1837–1839.

[31] Bosne, S., Papa, F., Clavel-Sérès, S. and Rastogi, N. (1993) A simple and reliable EDDA method for mycobactin production in mycobacteria: optimal conditions and use in mycobacterial speciation. Curr. Microbiol. 26, 353–358.

[32] Domenico, P., Reich, J., Madonia, W. and Cunha, B.A. (1996) Resistance to bismuth among gram-negative bacteria is dependent upon iron and its uptake. J. Antimicrob. Chemother. 38, 1031–1040.

[33] Baysse, C., De Vos, D., Naudet, Y., Vandermonde, A., Ochsner, U., Meyer, J.-M., Budzikiewicz, H., Schafer, M., Fuchs, R. and Cornelis, P. (2000) Vanadium interferes with siderophore-mediated iron uptake in *Pseudomonas aeruginosa*. Microbiology 146, 2425–2434.

[34] Rogers, H.J., Synge, C. and Woods, V.E. (1980) Antibacterial effect of scandium and indium complexes of enterochelin on *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 18, 63–68.

[35] Rogers, H.J., Woods, V.E. and Synge, C. (1982) Antibacterial effect of the scandium and indium complexes of enterochelin on *Escherichia coli*. J. Gen. Microbiol. 128, 2389–2394.

[36] Costa, J., Delgado, R., Drew, M.G.B. and Felix, V. (1998) Design of selective macrocyclic ligands for the divalent first-row transition metal ions. J. Chem. Soc., Dalton Trans., 1063–1071.

[37] Costa, J., Delgado, R., Drew, M.G.B. and Felix, V. (1999) Methyl pyridine derivatives of 14-membered tetraaza macrocycles. A new host with high selectivity for cadmium. J. Chem. Soc., Dalton Trans., 4331–4339.

[38] Andersen, O. (1999) Principles and recent developments in chelation treatment of metal intoxication. Chem. Rev. 99, 2683–2710.

[39] Anderson, C.J. and Welch, M.J. (1999) Radiometal-labeled agents (non-technetium) for diagnostic imaging. Chem. Rev. 99, 2219–2234.

[40] Eisenwiener, K.-P., Prata, M.I.M., Buschmann, I., Zhang, H.-W., Santos, A.C., Wenger, S., Reubi, J.-C. and Mäcke, H.R. (2002) NODAGATOC, a new chelator-coupled somatostatin analogue labeled with $^{67/68}$Ga and ^{111}In for SPECT, PET, and targeted therapeutic applications of sSomatostatin receptor (hsst2) expressing tumors. Bioconjugate Chem. 13, 530–541.

[41] Meares, C.F. (1986) Chelating agents for the binding of metal ions to antibodies. Nucl. Med. Biol. 13, 311–318.

[42] Parker, D. (1990) Tumour targeting with radiolabelled macrocycle-antibody conjugates. Chem. Soc. Rev. 19, 271–291.