3-Acylated tetratic and tetronic acids as natural metal binders: myth or reality?

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3-Acylated tetramic and tetronic acids as natural metal binders: myth or reality?

Mehdi Zaghouani and Bastien Nay*

3-Acylated tetramic and tetronic acids are characterized by a low pKa and are likely to be deprotonated under physiological conditions. In addition, their structure makes them excellent chelators of metallic cations. We will discuss the significance of these chemical properties with regard to the biological properties and mechanisms of action of these compounds, highlighting the importance of considering them as salts or chelates for biological purposes, rather than acids.

However, the metal-binding properties of ATas and ATos are still poorly considered when studying their biological properties, with a few exceptions compiled herein. Following general comments and a survey of ATa and ATo metal adducts found in the literature, emphasis will be put on those with proved or well studied biological relevance, in order to answer the title question.

2 Physicochemical properties of 3-acylated tetramic and tetronic acids in the biological context

Owing to their low pKa (typically <4), ATas and ATos are expected to be deprotonated in vivo. They can form four tautomers, with ratios depending on the substitution pattern and solvents, as studied by NMR, and usually favoring the endo

Scheme 1 Structure and biosynthetic origin of 3-acylated tetramic (a) and tetronic (b) acids.

Scheme 2 Tautomerism of ATas and ATos (X = NH or O).
enol forms (Scheme 2).3 Each tautomer can behave as a powerful bidentate chelator for various metals, leading to stable six-membered ring complexes, thus reminiscent of the acetylacetonate ligand (acac) which is widely used in organometallic chemistry.4 Depending on the cation valence, the stoichiometry of the ATa or ATo complex varies with a ligand-to-metal ratio from 1 (monovalent cations like Na+) to 3 (trivalent cations like Fe3+). The following discussion will include affinity and binding constants when available (these data are rarely found in the literature).

Early on, natural product chemists have been exploiting this chelation ability to reveal 3-acyltetramic acids, giving deep orange-red to brown “ferric colors” in the presence of FeCl3.6 Iron complexes of tenuazonic acid (1, Figure 1), generated by FeCl3 addition to acidified culture filtrates, were quantified by measuring the absorbance of ethyl acetate or butanol extract solutions at 450 nm (λmax of the iron tetramate adduct).1 The green fluorescence under UV light (254, 310 or 360 nm), obtained by spraying a Tdc1 solution on TLC, was also used to enhance the detection sensitivity.7 Chelation (e.g. of Na+, Ca2+, Cu2+) has often been used for crystallization purposes and stable copper chelates can be readily formed for storing.6 Mention should be done that some protonated forms of ATas or ATos are not stable, unlike their salt forms (e.g. geodin A,8 2, or pachydermin,9 3, which is a 3-oxalyltetramic acid, Figure 2). Finally, metal chelates exhibit greater lipophilicity than their pending acid form, which can improve cell penetration through lipid bilayers.4,5 Among 3-acyltetramic acids, tenuazonic acid (1, Figure 1a) and its salts have been extremely studied since its discovery in 1957.6 Isolated at pH 7 from Alternaria tenuis (A. tenuis),11,12 A. longipes,10 Aspergillus sp. F1404,13 Pyricularia oryzone3,4,15 or from Phoma sorghina (as a 10:5:2:1:5 mixture of Mg2+/Ca2+/Na+ salts, with trace amounts of Zn2+ and K+, showing the relative affinities of 1 for these metals).14 In particular, the stability of ATa complexes with metals of group IIA (especially Mg2+ and Ca2+) has been correlated with the strength of these cations as Lewis acids, the favorable size of the chelate ring and the cationic radius. Considering Mg2+, the rate of complexation by electron-donating ligands has been known to be fast (105 s–1) with the rate determining step being controlled by the loss of coordinated water.16 The mycotoxin demonstrated a strong affinity for Na+ in aqueous solutions, as shown by the spontaneous conversion of the Fe3+ complex, obtained from FeCl3-complemented culture filtrates, into the sodium adduct (see also Section 5).11 However, the formation of a copper complex could facilitate the extraction by chloroform.10 Tenuazonic acid complexes with Cu(II), Fe(III), Ni(II) and Mg(II) metals were deeply studied by Lebrun et al. using microanalysis, mass spectrometry, infrared spectroscopy and voltametry, establishing the following stoichiometry: Cu(1–H)2, Fe(1–H)2, Ni(1–H)2, Mg(1–H)2.15a In crystalline Cu(1–H)2, the metal is bound by the amide and acetyl carbonyls, as shown by X-ray crystallography (Figure 1b),17 but it can be different in the solution or amorphous states. The regioselectivity of metal complexation by ATas and ATos may thus be matter of debate with two possible coordination sites: (i) between the external carbonyl (exo-enol) and the amide, as observed in Cu(1–H)2; (ii) between the external carbonyl and the internal carbonyl (endo-enol), as observed in the sodium complex of tetronasin 35 (Figure 3, Section 4.2) or in the manganese complex of cyclopiazonic acid 10 (Figure 6c, Section 6.2). Discrimination of the various tautomers can be achieved by IR spectroscopy, analyzing the 1700-1500 cm–1 region, which, by comparison with metal acetylacetonates, showed characteristic absorption bands at 1600-1570 cm–1 for the metal bound C=O and at 1550-1520 cm–1 for the C=C bond.15a The free carbonyl should absorb at 1700-1650 cm–1. This last band signs the presence of a free amide in the range of 1675-1669 cm–1, or a free intracyclic ketone in the range of 1710-1700 cm–1. However, these values also strongly depend on the metal bound, with a difference of 30 cm–1 between the two extremes (i.e. those of Ni3+ and Fe3+ complexes).

Metal associations with 1 were biologically active as antiviral,13 cytotoxic against tumor cell lines (with marked differences depending on the enantiomer or diastereomer)18 or phytotoxic,10,11,14,15 while the mechanism of action of 1 involved the inhibition of amino acid incorporation in the ribosome during protein biosynthesis.19 Copper complexes of synthetic ATas proved sometimes better candidates for antimicrobial purposes compared to their parent acids (on Bacillus subtilis, Staphylococcus aureus), while complexation had no such effect with ATos.20 Finally, mention should be made of synthetic derivatives of 1 not only synthesized for medicinal purposes,21,22 but also for their chelating properties targeting metal pollutants (Cd2+, Pb2+)23 and structural or physicochemical studies.14,24

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**Figure 1** Structure of 1-tenuazonic acid 1 (a) and copper bis(tenuazonate) chelate crystallographic structure (b, retrieved from the CCDC database).17

**Figure 2** Structures of geodin A (2) and of pachydermin (3), known to be unstable as acids.
3 Occurrence of metal 3-acylated tetramatides and tetrotanates (1954-2015)

Several ATas and ATos have been described as chemical associations with metals, mainly alkali or alkaline earth metals Na⁺, K⁺, Mg²⁺ or Ca²⁺. However, transition metal complexes have occasionally been reported as naturally occurring (Zn²⁺ or Fe³⁺) or as synthetic (Cu²⁺, Ni²⁺, Co²⁺, Cd²⁺, Hg²⁺ or Pt²⁺). These compounds are gathered in Tables 1 and 2 (see also Figures S1 and S2 for structures).

In the next sections, biologically relevant studies – and to the best of our knowledge, the only ones being correlated to metal binding properties for ATAs and ATOs – will be highlighted to stress the importance of this phenomenon. The biological properties can be specific to chemical structures and mechanisms of action (e.g. cyclosporine A 10 inhibiting SERCA, Section 6.2) or generalizable to compound series, especially those sharing structural features like a long-chain acyl group on the tetramate or tetratomic core, giving amphiphilic molecules (e.g. ionophores, Section 4, or phosphate mimics, Section 6.1).

Table 1 List of reported metal chelates of 3-acytletic acids (see Figure S1 in ESI for the structures of 4, 6-9, 11-15, 19-21).

<table>
<thead>
<tr>
<th>AT or ATO</th>
<th>Sources</th>
<th>Metals (references)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancorinoside A (4)</td>
<td>Ancorina sp.</td>
<td>Mg⁺⁻ (25)</td>
</tr>
<tr>
<td>C₂₃₇₋₄₅ (5)</td>
<td>Pseudomonas aeruginosa</td>
<td>Fe₃⁺ (26a,27,28)</td>
</tr>
<tr>
<td>Chaunolines A-C (6-8) and F-14329 (9)</td>
<td>Cha unopsis sp.</td>
<td>Mg²⁺ (29)</td>
</tr>
<tr>
<td>Cyclosporine A (10)</td>
<td>Aspergillus fumus, Penicillium cyclosporum</td>
<td>Ca²⁺ (30,31,32,33)</td>
</tr>
<tr>
<td>Cylindramide (11)</td>
<td>Halichondria cilindracea</td>
<td>Mg²⁺ (34)</td>
</tr>
<tr>
<td>Erythroskynins (12)</td>
<td>Penicillium islandicum</td>
<td>Mg²⁺ (35,36)</td>
</tr>
<tr>
<td>Fuligurin A (13)</td>
<td>Fuligo septica</td>
<td>Mg²⁺ (37)</td>
</tr>
<tr>
<td>Geodin A (2)</td>
<td>Geodia sp.</td>
<td>Mg²⁺ (38)</td>
</tr>
<tr>
<td>Harzianic acid (14)</td>
<td>Trichoderma harzianum, fungal strain F-1531</td>
<td>Mg²⁺ (39)</td>
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<tr>
<td>Ikaragamycin (15)</td>
<td>Streptomyces sp. 8063</td>
<td>Mg²⁺ (40)</td>
</tr>
<tr>
<td>Magnesidin (16)</td>
<td>Pseudomonas magnesiorubra</td>
<td>Mg²⁺ (41)</td>
</tr>
<tr>
<td>Magnesidin A (16)</td>
<td>Vibrio gazogens</td>
<td>Mg²⁺ (42)</td>
</tr>
<tr>
<td>Melophins A-C (17-19)</td>
<td>Melophilus sarasinorum</td>
<td>Mg²⁺ (43)</td>
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<tr>
<td>Olefinic (20)</td>
<td>Streptomyces parvalus</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (45,46)</td>
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<tr>
<td>Pachydermin (3)</td>
<td>Chemonia pachydermis</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (47)</td>
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<tr>
<td>Physarorubicin</td>
<td>Physarium polycephalum</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (48)</td>
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<tr>
<td>Speradine A (22)</td>
<td>Aspergillus tamarii</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (49)</td>
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<tr>
<td>Streptolydigin (23)</td>
<td>Streptomyces lydicus</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (50)</td>
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<tr>
<td>Tenuazonic acid (1)</td>
<td>Alternia alternata (= A. tenuis), A. longipes, Phoma sordida, Pyricularia oryzae</td>
<td>Ca²⁺⁻, Mg²⁺⁻, Na⁺⁻ (51)</td>
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Synthetic derivatives -

Table 2 List of reported metal chelates of 3-acytletic acids (see Figure S2 in ESI for the structures of 24-32, 34).

<table>
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<th>AT or ATO</th>
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<td>Agglomerins A-D (24-27)</td>
<td>Enterobacter agglomerens</td>
<td>Na⁺⁻ (50)</td>
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<tr>
<td>Aminosyringos A and B (28,29)</td>
<td>Micromonospora sp. TP-A0316i</td>
<td>Na⁺⁻⁻ (51)</td>
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<tr>
<td>Chlorothricin (30)</td>
<td>Streptomyces antibioticus</td>
<td>Na⁺⁻⁻ (52)</td>
</tr>
<tr>
<td>Kijanimicin (31)</td>
<td>Actinomadura kijani a</td>
<td>Na⁺⁻⁻ (53)</td>
</tr>
<tr>
<td>Quartzomycin (e.g. 32)</td>
<td>Arcyria tospalis orientalis</td>
<td>Na⁺⁻⁻ (55)</td>
</tr>
<tr>
<td>RK-682 (33)</td>
<td>Actinomyces strain DSM 7357</td>
<td>Na⁺⁻⁻⁻, Mg²⁺⁻⁻ (56,57)</td>
</tr>
<tr>
<td>Tetrocarcin A</td>
<td>Micromonospora callosa</td>
<td>Na⁺⁻⁻ (58)</td>
</tr>
<tr>
<td>(antlermicin A) (34)</td>
<td>Streptomyces longipaliforme</td>
<td>Ca²⁺⁻, Na⁺⁻⁻ (59,60)</td>
</tr>
<tr>
<td>Tetronasin (= M-139603) (35)</td>
<td>Streptomyces sp.</td>
<td>Mg²⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻˓</td>
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concentration of 100 µg/mL when cultivated on non-fermentable substrates (like glycerol requiring functional mitochondria), while its aglycon part was found more active at 1.25–5 µg/mL. Moreover, 20 was shown to deplete the Mg^{2+} and Ca^{2+} contents of mitochondria, two essential elements for cell processes. It inhibits yeast respiration and converts growing and resting cells into respiratory-deficient mutants, as a result of the fragmentation of yeast mitochondrial DNA possibly induced by Mg^{2+} depletion. The ATa 20 increases the inner membrane ion permeability of rat liver mitochondria, but not that of the plasma membrane, inducing swelling of non-respiring mitochondria in isosomotic solutions. In addition, it disturbs the respiration states and ATPase activities. Small concentrations of Mg^{2+} prevented these effects, suggesting that 20 could displace Mg^{2+} cations from magnesium phosphates which are ligands of ATPases. In fact, Mg^{2+} depletion in mitochondria could indirectly induce non selective permeability of the inner membrane to ions. A detergent-like action could yet be excluded by two facts: (1) 20 was effective at low concentrations; (2) 20 did not induce membrane leakiness as shown by the remaining sensitivity of the ATPase activity to carboxyatractyloside, a specific inhibitor of the ADP/ATP membrane translocator (the adenine nucleotides only crossed the inner membrane through this translocator, even in the presence of 20). The effect of 20 was similar to that of other ionophore antibiotics and divalent cation binders like calimycin (= A23187) and primycin, increasing the permeability of the mitochondrial inner membrane to Mg^{2+}.

4.2 Tetronasin and tetronycin

Both the structurally related ATos tetronasin (= M-139603, 35, pKa 1.8) and tetronycin (36, pKa 2.52) were isolated in the early 1980’s (Figure 3), the first as a sodium adduct from Streptomyces longisporoflavus and the second as a mixture of sodium and calcium adducts from Streptomyces sp. NRRL 11266. The sodium adduct of 35 was soluble in organic solvents but not in water while all metal adducts of 36 were soluble in dichloromethane. When used with the polyether ionophore lasalocid, they had a synergistic effect (3-12 folds) for the transport of Pr^{3+} across lipid bilayers (egg yolk phospholipid vesicles). The binding constants (K) of 35 were evaluated in a methanol-water (7:3) mixture, showing marked affinities for potassium (K = 340 M^{-1}), sodium (K = 500 M^{-1}), magnesium (K = 1300 M^{-1}), and above all for calcium (K > 10^4 M^{-1}), to be compared with rubidium (K = 54 M^{-1}) or lithium (not detected) in membrane-mimicking organic solvents, both compounds selectively bind Na^+ and Ca^{2+} (having similar ionic radii, 0.95 and 1.0 Å, respectively), and could have a preformed geometry for sodium binding, yet extremely reminiscent of the solid state structure (Figure 3). Compound 35 is active against a variety of ruminal bacteria and 36 against Gram-positive bacteria. 35 facilitates Ca^{2+}/H^+ exchanges across the membrane (Ca^{2+} efflux), leading to depolarization of the ruminal bacteria membrane, internal pH decrease and strong ATP depletion due to the energy expenditure used to maintain the ionic gradients. This effect is potentiated by high concentrations of metal ions like Ca^{2+} and Na^+. By such mechanisms, tetronasin is used as a feedlot ionophore used to enhance cattle productivity, interfering with the nitrogen metabolism of ruminal microorganisms, changing the fermentation stoichiometry and lowering methane emissions.

Therefore, in both chemical series, the observed biological effects are strongly correlated to the ability of the ATa and ATo structures to bind metallic cations and disturb ion exchanges across the membranes. In particular, the enhanced lipophilicity of divalent cation complexes can be regarded as a key feature of ATAs and ATos to function as ionophores in vivo.

5 3-Acyltetramic acids as siderophores

Some 3-acyltetramic acids, such as C_{12}-TA (5) and harzianic acid (14), have been reported to be potent siderophores (Figure 4). To the best of our knowledge, there has been no report concerning such a function for 3-acyltetronic acids in the literature, although their ability to bind iron cannot be excluded. The case of C_{12}-TA (5), or 3-(1-hydroxydecyldiene)-5-(2-hydroxy-ethyl)pyrrolidine-2,4-dione, has been somewhat controversial. This compound is formed as a degradation product of the autoinducer 3-oxo-1-acylhomoserine lactone (Scheme 3a) involved in the quorum sensing of bacterial populations of Pseudomonas aeruginosa, an opportunistic pathogen in humans. Not only 5 displayed antibacterial properties against Gram-positive bacterial competitors of P. aeruginosa (it was inactive on Gram-negative bacteria), but it was also found to be a potent siderophore. It could contribute to iron sequestration (as a Fe(5-H)_3 complex, Scheme 3b) by P. aeruginosa in the quest for this element. Indeed iron is...
essential to bacterial life and pathogenesis. The relative affinity constant ($K_d$) of the bidentate chelator 5 for iron(III) was indeed found to be $1.6 \times 10^{-29}$ M$^3$. Applying a standardization method to compare this affinity with known hexadentate chelators showed that 5 was a stronger chelator than EDTA, but weaker than pyoverdin which is also produced by *P. aeruginosa*. However, in 2012, it was reported that 5 is not sufficient to provide a siderophore mutant of *P. aeruginosa* with the iron required for its growth.$^{27}$ The same study indicated that metal chelation is not responsible for the observed bacteriostatic effect. Compound 5 would rather behave as an ionophore (see Section 4) dissipating the membrane potential and the pH gradient of Gram-positive bacteria, similarly to reutericyclin, a structurally related 11-acylated ATA (38, Figure 4),$^{28}$ as reported previously.$^{28}$ Additional coordination chemistry studies partially explained these observations. It was demonstrated that, at physiological pH, the binding ability of 5 (pKa = 5.0)$^{69}$ for iron is limited (as demonstrated by spectrometric methods) due to the hydrolysis of the Fe(5–H)$_2$ complex into the hydrated Fe(5–H)$_3$ complex, finally leading to insoluble Fe(OH)$_n$. The chelating property of 5 is indeed only sufficient at low pH, which raises questions on its role as a siderophore during bacterial growth. Furthermore, to satisfy the octahedral six coordinate structure of Fe(III), three bidentate ATA ligands are necessary, which dramatically decreases their efficiency as iron chelators, unless it is present at relatively high concentrations.

In this section, mention should be done of harzianic acid (14, Figure 4), an ATA first isolated in 1994 from *Trichoderma harzianum* and sharing some structural features with 5 (a long acyl chain and a 2-hydroxyethyl substituent at C-5).$^{70}$ Compound 14 was found to inhibit serine/threonine phosphatase type 2A when it was associated to Zn$^{2+}$ (as isolated from the fungus strain F-1531), but not as the acid form.$^{71}$ It was also antifungal and displayed plant-growth promoting activities.$^{72}$ The siderophore capability of 14 was demonstrated by a colorimetric method on agar plates in the presence of chrome azurol S competing for iron chelation, which is blue under normal conditions but turning orange in the presence of a siderophore.$^{73}$ Unlike C$_{12}$-TA, characterization of the complex by mass spectrometry revealed a 1:1 stoichiometry for the iron complex, in favour of (14–H)FeCl$_2$. The relative constant affinity was determined to be 1.79 x $10^{-25}$ M. Thanks to its iron-chelating properties, 14 could be able to regulate the availability of iron in the plant rhizosphere by participating to iron solubilisation.

6 Cation-dependent interaction of 3-acylated tetramic and tetronic acids with proteins

6.1 Cation-dependency of protein tyrosine phosphatase inhibition by the tetronic acid RK-682

Concerning the 3-acytetronic acid series, the cation-dependency of the inhibition of protein tyrosine phosphatase (PTP) by RK-682 (33, Figure 5a) has recently been reported.$^{74}$ RK-682 (3-hexadecanoyl-5-hydroxymethyl-tetronic acid) was first isolated from the actinomycete strain DSM7357, presumably as a Na$^+$ salt (M = Na$^+$), along with analogous 3-alkanoyl-5-hydroxymethyltetronic acids with variable alkanoyl chain lengths and substitutions.$^{75}$ The compounds were originally described as inhibitors of HIV-1 protease with IC$_{50}$ values between 84 and 135 µM. Compound 33 more specifically inhibited PTP and cell growth at phase G1 (thus in a different way than potassium orthovanadate).$^{75}$ This activity was observed on CD45 at IC$_{50}$ = 54 µM, and on vaccinia H1-related protein at 2 µM. ATO 33 was later found to have a particular affinity for Ca$^{2+}$ since a calcium adduct was formed during silica gel chromatography as revealed by ICP-AE spectroscopy,$^{76}$ while a silica complex of 33 had also previously been reported.$^{77}$ During this work, the FAB mass
spectra could considerably be simplified by exchanging the cation Ca\(^{2+}\) with Li\(^+\).\(^{76b}\)

Sodeoka and co-workers suggested that the ATo anion could be used as a mimic of the dephosphorylation transition state (Figure 5b) to inhibit PTP and dual-specificity protein phosphatases (DSP). Based on this assumption and with the aim to find selective inhibitors of DSPs, they constructed a library focused on the core tetronate structure of 33.\(^{21,77}\) The membrane permeability and selectivity of these analogues were improved by the conversion of the acidic exo-enol into a neutral enamine. Kinetic analysis at pH 6 (calc. pKa of \(33 = 3.1\)) showed that two molecules of 33 are necessary to inhibit the phosphatase.\(^{77b}\) In addition, Pilli and co-workers recently showed that the presence of divalent cations like Mg\(^{2+}\) or Ca\(^{2+}\) depletes the inhibitory activity of 33, presumably by sequestering the tetronate anion.\(^{74}\) Indeed, the PTP active site is positively charged at the surface of the enzyme (Figure 5c) and could only interact with free tetronate anions. The high affinity of the tetronate chelator for specific cations like Mg\(^{2+}\) or Ca\(^{2+}\) could thus be crucial in the control of the PTP-tetronate interaction which depends on the anionic nature of the ATo core.

6.2 The SERCA protein-cyclopiazonic “acid” complex

Cyclopiazonic acid (10, Figure 6a) furnishes an interesting case of controversial studies, which has only been solved in 2009. It was first identified in 1968 as the main mycotoxin of Penicillium cyclopium, giving an intense orange-red colour upon treatment with FeCl\(_3\) on TLC, and easily forming a library focused on the core tetronate structure of 33.\(^{21,77}\) The membrane permeability and selectivity of these analogues were improved by the conversion of the acidic exo-enol into a neutral enamine. Kinetic analysis at pH 6 (calc. pKa of \(33 = 3.1\)) showed that two molecules of 33 are necessary to inhibit the phosphatase.\(^{77b}\) In addition, Pilli and co-workers recently showed that the presence of divalent cations like Mg\(^{2+}\) or Ca\(^{2+}\) depletes the inhibitory activity of 33, presumably by sequestering the tetronate anion.\(^{74}\) Indeed, the PTP active site is positively charged at the surface of the enzyme (Figure 5c) and could only interact with free tetronate anions. The high affinity of the tetronate chelator for specific cations like Mg\(^{2+}\) or Ca\(^{2+}\) could thus be crucial in the control of the PTP-tetronate interaction which depends on the anionic nature of the ATo core.

7 Conclusion

During this discussion, we showed that ATas and ATos can be potent binders of metals. These compounds are marked by a low pKa and would thus be deprotonated under common physiological conditions. Their chelating properties could lead to relatively stable metal complexes, with enhanced lipophilicity allowing them to interact with cell or organelle membranes. Metal chelates can also be good ligands for specific proteins like SERCA. In addition, the structural analogy of tetronate anions with the phosphate anions (with a negative charge delocalized on three oxygens) suits them to interact with protein phosphatases, while the anion character allows possible interactions with positively charged protein surfaces. To conclude, considering the poor availability of some metal species in the environment, it is not excluded that ATas and ATos serve specifically as metallophores for the benefit of their producers. The intrinsic metal-chelating property of ATas and ATos is thus a reality that should be systematically taken into account when studying their biological effects from molecular to cellular levels, which could also depend on the nature of the metal partner and on the stability of the resulting complex.

Acknowledgements
We are grateful to Dr. M.-H. Lebrun who brought to our attention the use of a fluorimetric method to detect ATAs,[7] and the recent findings on the biosynthesis of tenuazonic acid.† This work was supported by the Agence nationale de la Recherche (ANR grant number ANR-12-BS07-0028-01, SYNBIORG), by the Centre national de la Recherche (CNRS interdisciplinary call Physico-Chemistry-Biology 2011) and by the Muséum national d’histoire naturelle.

Notes and references

‡ During the edition process, a biosynthetic study of the mycotoxin tenuazonic acid (1) in Magnaporthe oryzae was reported, showing that 1 is formed from isoleucine and acetocetyl-coenzyme A by TeA synthetase 1 which is a unique "NRPS-PKS" hybrid enzyme starting with a NRPS module: C.-S. Yun, T. Motoyama, H. Osada, Nat. Comm., 2015, 6, 8758.


69 The pKa of 3 (= 5.0) was found significantly higher than that of a shorter-chain ATa (pKa = 2.5 for the corresponding 3-acetyltetrameric acid), as a result of electronic effects, decreased solvation and an aggregation phenomenon (see ref. 27).