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

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HIGHLIGHT ARTICLE

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

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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.

1 Introduction

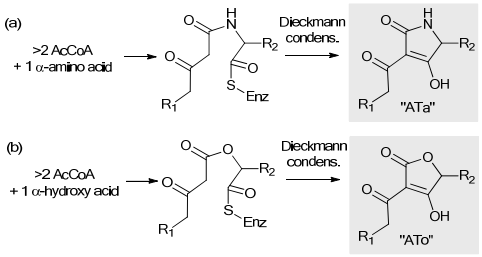
3-Acylated tetramic (ATa)[1] and tetronic (ATo)[2] acids are complex polyketides either originated from the hybrid PKS-NRPS‡ or from the glycerate-incorporating PKS pathways, respectively, mainly produced by micro-organisms. The ATa and ATo heterocyclic cores arise from a Dieckmann condensation of the corresponding *N*-(β-ketoacyl)amino acid or the 2-*O*-(β-ketoacyl)glyceric acid thioesters (Scheme 1). They have been correlated to many biological activities, especially as antibiotics. The tricarbonylmethine feature (enolizable into four possible enol tautomers, Scheme 2) makes them particularly acidic with low pKa values (2.5–4.0, depending on the substitution pattern).[3] Deprotonation is thus expected to take place under physiological pH conditions (pH ~7.4), resulting in tetramate or tetronate salts. Indeed many have been isolated as salts (most commonly bound to Na^+, Ca^{2+} or Mg^{2+} cations) even though the acid forms have been prevalently reported.

Given the putative occurrence of ATa and ATo salts in vivo, the question arises whether they exert their biological effects as metal adducts or as acids. In addition, the enol tautomers (Scheme 2) provide powerful chelators for the previously mentioned alkali or for other biologically relevant metals, including transition metals such as iron, copper, zinc or manganese, or for toxic and non-biologically relevant elements like cadmium, ruthenium or platinum.1[d] ATas and ATos can thus be regarded as metal binders (or metallophores) with potential biological significance. Furthermore, some of them are only stable under their salt or chelate form, and many have been stored as copper chelates (see Section 2). Metal chelation provides ATas and ATos with different physicochemical properties, like increased lipophilicity,[4] which is of particular importance in the physiological context.

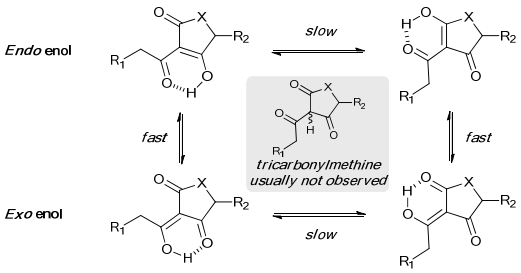
However, the metal-binding properties of ATas and ATos are still poorly considered when studying their biological properties, with a few exceptions compiled herein. Following general comments and a survey of ATa and ATo metal adducts found in the literature, emphasis will be put on those with proved 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),[3] 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).

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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.[5] 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 Fe^{3+}). 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 $FeCl_3$.[6] Iron complexes of tenuazonic acid (**1**, Figure 1), generated by $FeCl_3$ addition to acidified culture filtrates, were quantified by measuring the absorbance of ethyl acetate or butanol extract solutions at 450 nm (λ_{max} of the iron tetramate adduct).[7] The green fluorescence under UV light (254, 310 or 360 nm), obtained by spraying a $TbCl_3$ solution on TLC, was also used to enhance the detection sensitivity.7

Chelation (*e.g.* of Na^+, Cs^+, Cu^{2+}) 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* as a Mg^{2+} salt, **1** was easily converted into a stable, chloroform-soluble, green Cu^{2+} chelate for storing. Its acid form was indeed prompt to slow epimerization into isotenuazonic acid over 2.5 years. Pure salts or mixtures (Na^+, K^+, Ca^{2+}, Mg^{2+} or Fe^{3+}) of tenuazonic acid (pKa 3.5)[10] were also isolated from *A. alternata* (= *A. tenuis*),[11,12] *A. longipes*,[10] *Aspergillus sp.* F1404,[13] *Pyricularia oryzae*[14,15] or from *Phoma sorghina* (as a 10.5:2:1.5 mixture of Mg^{2+}/Ca^{2+}/Na^+ salts, with trace amounts of Zn^{2+} and K^+, showing the relative affinities of **1** for these metals).[16] In particular, the stability of ATa complexes with metals of group IIA (especially Mg^{2+} and Ca^{2+}) has been correlated with the strength of these cations as Lewis acids, the favorable size of the chelate ring and the cationic radius. Considering Mg^{2+}, the rate of complexation by electron-donating ligands has been known to be fast (10^5 sec^{-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 Fe^{3+} complex, obtained from $FeCl_3$-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)$_3$, 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 Ni^{2+} and Fe^{3+} 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 (Cd^{2+}, Cs^+, Pb^{2+})[23] and structural or physicochemical studies.1d,[24]

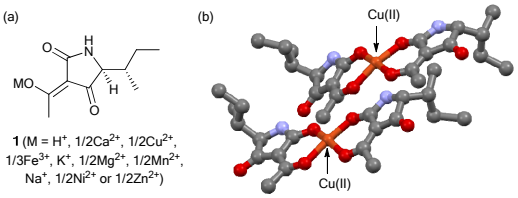


Figure 1 Structure of L-tenuazonic acid **1** (a) and copper bis(tenuazonate) chelate crystallographic structure (b, retrieved from the CCDC database).[17]

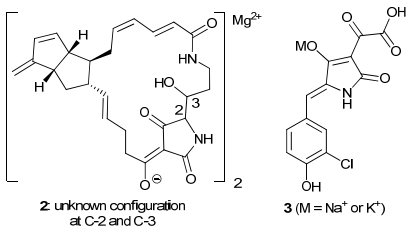


Figure 2 Structures of geodin A (**2**) and of pachydermin (**3**), known to be unstable as acids.

3 Occurrence of metal 3-acylated tetramates and tetronates (1954-2015)

Several ATas and ATos have been described as chemical associations with metals, mainly alkali or alkaline earth metals Na^+, K^+, Mg^{2+} or Ca^{2+}. However, transition metal complexes have occasionally been reported as naturally occurring (Zn^{2+} or Fe^{3+}) or as synthetic (Cu^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+} or Pt^{2+}). 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.* cyclopiazonic acid **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 tetramic core, giving amphiphilic molecules (*e.g.* ionophores, Section 4, or phosphate mimics, Section 6.1).

Table 1 List of reported metal chelates of 3-acyltetramic acids (see Figure S1 in ESI for the structures of **4**, **6**-**9**, **11**-**13**, **15**-**19**, **21**-**23**).

Names	Sources	Metals (references)
Ancorinoside A (**4**)	*Ancorina sp.*	$Mg^{2+(a)}$ (25)
C_{12}-TA (**5**)[b]	*Pseudomonas aeruginosa*	$Fe^{3+(c,d)}$, $Ga^{3+(d)}$ (26a,27,28)
Chaunolidines A-C (**6**-**8**) and F-14329 (**9**)	*Chaunopycnis sp.*	$Al^{3+(d)}$, $Cu^{2+(d)}$, $Fe^{3+(d)}$, $Mg^{2+(d)}$, $Zn^{2+(d)}$ (29)
Cyclopiazonic acid (**10**)	*Aspergillus flavus, Penicillium cyclopium*	$Ca^{2+(c)}$, $Cu^{2+(d)}$, $Fe^{3+(d)}$, $Mg^{2+(c)}$, $Mn^{2+(d)}$, $Na^{+(d)}$ (30,31,32,33)
Cylindramide (**11**)	*Halichondria cylindrata*	$Ca^{2+(c)}$, $Cu^{2+(d)}$, $Mg^{2+(c)}$ (34)
Erythroskyrin (**12**)	*Penicillium islandicum*	$Mg^{2+(d)}$, $Na^{+(d)}$, $Fe^{3+(d)}$, $Cu^{2+(d)}$ (35,36)
Fuligorubin A (**13**)	*Fuligo septica*	$Ca^{2+(c)}$ (37)
Geodin A (**2**)	*Geodia sp.*	$Mg^{2+(a)}$ (8)
Harzianic acid (**14**)	*Trichoderma harzianum,* fungal strain F-1531	$Fe^{3+(c)}$, $Zn^{2+(a)}$ (38,39)
Ikarugamycin (**15**)	*Streptomyces sp.* 8603	$Cu^{2+(d)}$, $Fe^{3+(d)}$, $Na^{+(d)}$ (40,41)
Magnesidin[(e)]	*Pseudomonas magnesiorubra*	$Mg^{2+(a,d)}$ (42)
Magnesidin A (**16**)[(e)]	*Vibrio gazogenes*	$Mg^{2+(a)}$ (43)
Melophlins A-C (**17**-**19**)	*Melophlus sarasinorum*	$Ga^{3+(d)}$, $La^{3+(d)}$, $Mg^{2+(d)}$, $Ru^{3+(d)}$, $Zn^{2+(d)}$ (44)
Oleficin (**20**)	*Streptomyces parvulus*	$Ca^{2+(c)}$, $Mg^{2+(c)}$, $Na^{+(d)}$ (45,46)
Pachydermin (**3**)	*Chamonixia pachydermis*	$K^{+(a)}$, $Na^{+(a)}$ (9)
Physarorubinic acid (**21**)	*Physarum polycephalum*	$Ca^{2+(a)}$ (47)
Speradine A (**22**)	*Aspergillus tamarii*	$Ca^{2+(c)}$ (48)
Streptolydigin (**23**)	*Streptomyces lydicus*	$Mg^{2+(c)}$ (49)
Tenuazonic acid (**1**)	*Alternia alterna (= A. tenuis), A. longipes, Phoma sorghina, Pyricularia oryza*	$Ca^{2+(a,d)}$, $Cu^{2+(d)}$, $Fe^{3+(a,d)}$, $K^{+(a)}$, $Mg^{2+(a,d)}$, $Mn^{2+(d)}$, $Na^{+(a)}$, $Ni^{2+(d)}$, $Tb^{3+(d)}$, $Zn^{2+(d)}$ (6,10-15)
Synthetic derivatives	-	Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pt^{2+}, Ba^{2+}, Mg^{2+}, Rh^{+}, Zn^{2+} (24)

[(a)] as isolated; [(b)] 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione; [(c)] suspected interaction *in vivo*; [(d)] *in vitro* interaction, staining reagent or synthetic material; [(e)] Magnesidin is a 1:1 mixture of 3-hexanoyl- and 3-octanoyltetramates while magnesidin A is the octanoyl derivative of this mixture.

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

Names	Sources	Metals (references)
Agglomerins A-D (**24**-**27**)	*Enterobacter agglomerans*	$Na^{+(a)}$ (50)
Arisostatins A and B (**28**, **29**)	*Micromonospora sp.* TP-A0316i	$Na^{+(a,e)}$ (51)
Chlorothricin (**30**)	*Streptomyces antibioticus*	$Na^{+(c)}$, $Cs^{+(c)}$ (52)
Kijanimicin (**31**)	*Actinomadura kijaniata*	$Na^{+(b,c)}$, $K^{+(c)}$, $Rb^{+(c)}$, $Cu^{2+(c)}$, $Zn^{2+(c)}$ (53,54)
Quartromicins (*e.g.* **32**)[(d)]	*Amycolatopsis orientalis*	$Na^{+(a)}$, $K^{+(a)}$, $Ca^{2+(a)}$, $Mg^{2+(a)}$ (55)
RK-682 (**33**)	Actinomycete strain DSM 7357	$Na^{+(a,c)}$, $Mg^{2+(c)}$ (56,57)
Tetrocarcin A (antlermicin A) (**34**)[(f)]	*Micromonospora chalcea*	$Na^{+(c)}$ (58)
Tetronasin (= M-139603) (**35**)	*Streptomyces longisporoflavus*	$Ca^{2+(a)}$, $Na^{+(a)}$ (59,60)
Tetronomycin (**36**)	*Streptomyces sp.* NRRL 11266	$Ag^{+(c)}$, $Ca^{2+(a,c)}$, $K^{+(c)}$, $Mg^{2+(a,c)}$, $Na^{+(a,c)}$, $Pr^{3+\,(c)}$, $Rb^{+(c)}$ (61,62)
Synthetic derivatives	-	$Cu^{2+(c)}$, $Cd^{2+(c)}$, $Cs^{+(c)}$, $Pb^{2+(c)}$ (23,20)

[(a)] as isolated; [(b)] suspected interaction *in vivo*; [(c)] *in vitro* interaction, staining reagent or synthetic material; [(d)] The metal composition was determined as follows: Na, 70%; K, 19%; Ca, 10%; Mg, 1%;[55a] Quartromicins are difficult to be freed from the metals by conventional methods;[55b] [(e)] The sodium salt can be isolated at pH 7 while the acid form, though unstable, can be isolated at pH 3.5;[51] [(f)] pKa = 3.9.

4 3-Acylated tetramic and tetronic acids as ionophores and their interactions with membranes

The studies of ATas and ATos as ionophores are probably the oldest and most documented ones. Ionophores are defined as lipid-soluble small molecules that bind specific ions, masking their charge and passively shuttling them across the lipid bilayer down electrochemical ion gradients.[63] ATa and ATo antibiotics could be such binders, disturbing cellular ion homeostasis. The studies of the tetramate oleficin (**20**) and the tetronates tetronasin (**35**) and tetronomycin (**36**) will be exposed.

4.1 Oleficin, an ionophore inhibiting respiration

Oleficin (**20**, Figure 3) is a polyenoyltetramic acid isolated from *Streptomyces parvulus* as a dark-red solid.[45] Its sodium salt is moderately soluble in water. At pH 7.4, **20** is capable of selectively transferring the divalent cations Mg^{2+} and Ca^{2+} from water to an organic phase (butanol/toluene), presumably through the formation of the neutral complexes as M(**20**–H)$_2$, ($M \neq K^+$, Na^+).[46] Active against Gram-positive bacteria or the Yoshida subcutaneous sarcoma in mice with a toxicity at LD_{50} = 40 mg/kg,[46a] **20** also inhibited the growth of yeasts at the

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.[64]

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.[64] 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 isoosmotic solutions.[46,64] 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:[47] (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)[65] and primycin,[66] increasing the permeability of the mitochondrial inner membrane to Mg^{2+}.[47]

4.2 Tetronasin and tetronomycin

Both the structurally related ATos tetronasin (= M-139603, **35**, pKa 1.8) and tetronomycin (**36**, pKa 2.52) were isolated in the early 1980's (Figure 3), the first as a sodium adduct from *Streptomyces longisporoflavus*[59] and the second as a mixture of sodium and calcium adducts from *Streptomyces* sp. NRRL 11266.[61,62a] 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 phospholipidic vesicles).[62b] 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).[62a] In membrane-mimicking organic solvents, both compounds selectively bind Na^+ and Ca^{2+} (having similar ionic radii, 0.95 and 1.0 Å, respectively),[62a] and could have a preformed geometry for sodium binding, yet extremely reminiscent of the solid state structure (Figure 3).[59]

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.[60] 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.[60,67]

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-hydroxydecylidene)-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-*N*-acylhomoserine lactone **37** (Scheme 3a) involved in the quorum sensing of bacterial populations of *Pseudomonas aeruginosa*, an opportunistic pathogen in humans.[26] 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

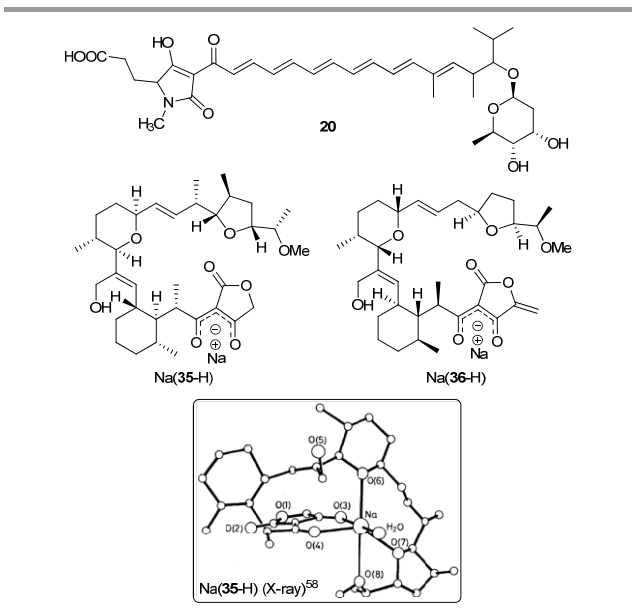


Figure 3 Structure of ionophores: oleficin (**20**), tetronasin (**35**) and tetronomycin (**36**) (box: crystal structure of **35**, reproduced from Ref. 59 with permission from The Royal Society of Chemistry).

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 x 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 *N*-acylated ATa (**38**, Figure 4),[68] 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)$_3$ complex into the hydrated Fe(**5**–H)$_2^+$ complex, finally leading to insoluble Fe(OH)$_3$. The chelating property of **5** is indeed only efficient 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]

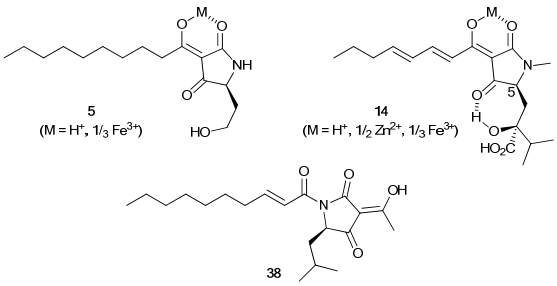
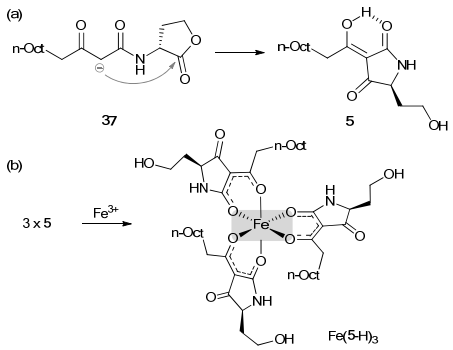


Figure 4 Structure of putative ATa siderophores, C_{12}-TA (**5**) and harzianic acid (**14**), and of the structurally related reutericyclin (**38**).



Scheme 3(a) Conversion of 3-oxo-*N*-acylhomoserine lactone **37** into C_{12}-TA (**5**); (b) Proposed iron chelate with **5**.[26]

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-acyltetronic 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.[56] 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 G_1 (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,[76a] while a silica complex of **33** had also previously been reported.[57] During this work, the FAB mass

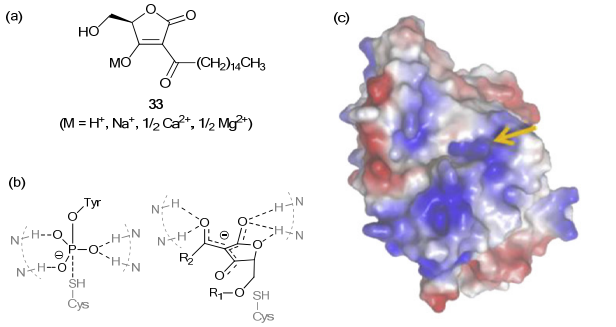


Figure 5 (a) Structure of RK-682 (**33**); (b) Model of the dephosphorylation transition state in the PTP active site (left) and of the tetronate mimic (adapted from ref. 77b); (c) Charge distribution on PTP-1B (red: negative charges; blue positive charges) and binding pocket of phosphorylated tyrosine (arrow). Reproduced from ref. 74. Copyright © 2015 published by Elsevier Masson SAS. All rights reserved.

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.[77a] 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 greenish precipitating copper chelate on treatment of an aqueous solution of the Na^{+} salt with $Cu(OAc)_2$.[30] It was later reisolated as an undefined metal complex (presumably the natural form) from *Aspergillus flavus* and shown to be toxic on cockerels at an oral LD_{50} of 19 mg/kg.[31] Compound **10** has been extensively studied due to its ability to specifically inhibit the Ca^{2+}-ATPase of the sarcoplasmic and endoplasmic reticuli (SERCA), but not other ATPases.[78] This inhibition was competitive with ATP and Ca^{2+}, and it was shown that **10** interferes with the ATP-induced $E_1 \rightarrow E_2$ conformational changes related to Ca^{2+}-transport (Figure 6b).[79] **10** tightly binds E_2 and stabilizes the enzyme in an inactive conformational state (E_2-**10**) with decreased affinity for Ca^{2+}.[80] The first crystal structures of cyclopiazonic acid-SERCA complexes were reported in 2007, independently by two groups,[81,82] whose data showed discrepancies on the position of **10** in its binding pocket within the calcium access channel. In both cases, no metal chelate was observed in the binding pocket, and the protonated ATa **10** was shown to interact with water and the protein through hydrogen bonds. An additional study the same year showed that the binding of **10** also depends on Mg^{2+} concentration and that **10** hardly interacts with SERCA in the absence of Mg^{2+}, yet without structural evidence.[32] In 2009, reconsideration of the crystallographic data finally shed light on this interaction (Figure 6c), providing additional crystallographic matter in favour of a complex of SERCA, MgF_4^{2-}, an ATP analogue and (**10**–H)-Mn^{2+} (Mn^{2+} replacing Mg^{2+} for X-ray anomalous scattering).[33] From these data, it was clear that the space supposedly filled by a molecule of water in the first crystallographic analysis of the **10**-SERCA complex[81] was in fact filled by a Mn^{2+} or Mg^{2+} cation chelated by **10**. Not only this study showed that a divalent metal chelate of **10** is the active species for SERCA inhibition, but it also provided bases for fragment-based drug design. Such synthetic works to access cyclopiazonic acid analogues as drugs and structure-activity relationships were reported in 2011.[83]

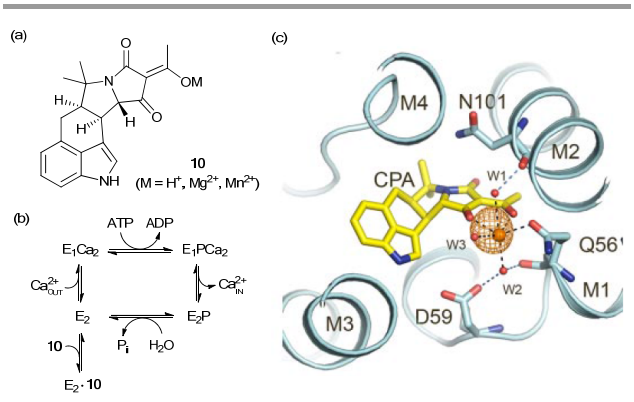


Figure 6 (a) Structure of cyclopiazonic acid (**10**); (b) Enzyme cycle model showing **10** inhibiting the enzyme at state E_2;[79] (c) Transmembrane domain of SERCA with bound **10**, showing amino acid residues interacting with **10** (= CPA) and the Mn^{2+} cation (orange sphere) coordinated at the SERCA- cyclopiazonic acid binding interface. Reprinted from ref. 33 with permission © 2008 The American Society for Biochemistry and Molecular Biology. All rights reserved.

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.

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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 acetoacetyl-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, *Nature Comm.*, 2015, **6**, 8758.

1 For leading reviews on natural tetramic acids: (a) B.J.L. Royles, *Chem. Rev.*, 1995, **95**, 1981; (b) E.L. Ghisalberti, in *Studies in Natural Products Chemistry*, Atta-ur-Rahman (Ed.), Elsevier Science, 2003, pp. 109–154; (c) R. Schobert and A. Schlenk, *Bioorg. Med. Chem.,* 2008, **16**, 4203; (d) G. Athanasellis, O. Igglessi-Markopoulou and J. Markopoulos, *Bioinorg. Chem. Appl.,* 2010, 315056; (e) X. Mo, Q. Li and J. Ju, *RSC Adv.,* 2014, **4**, 50566.

2 For leading reviews on natural tetronic acids: (a) see also ref. 1c and 1d; (b) A.L. Zografos, D. Georgiadis, *Synthesis,* 2006, **19**, 3157; (c) D. Georgiadis, in *Natural Lactones and Lactams*: T. Janecki (Ed.), Wiley-VCH, 2013, pp. 1–49; (d) W. Tao, M. Zhu, Z. Deng and Y. Sun, *Sci. China Chem.,* 2013, **56**, 1364; (e) L. Vieweg, S. Reichau, R. Schobert, P.F. Leadlay and R.D. Süssmuth, *Nat. Prod. Rep.,* 2014, **31**, 1554; (f) M.H. Lacoske and E.A. Theodorakis, *J. Nat. Prod.,* 2015, **78**, 562.

3 T. Yamaguchi, K. Saito, T. Tsujimoto and H. Yuki, *J. Heterocyclic Chem.,* 1976, **13**, 533.

4 W.O. Foye, *J. Pharm. Sci.,* 1961, **50**, 93.

5 H. Lamprey, *Ann. New York Acad. Sci.,* 1960, **88**, 519.

6 T. Rosett, R.H. Sankhala, C.E. Stickings, M.E.U. Taylor and R. Thomas, *J. Biochem.,* 1957, **67**, 390.

7 M.-H. Lebrun, F. Dutfoy, F. Gaudemer, G. Kunesch, A. Gaudemer, *Phytochemistry*, 1990, **29**, 3777

8 R.J. Capon, C. Skene, E. Lacey, G.H. Gill, D. Wadsworth and T. Friedel, *J. Nat. Prod.,* 1999, **62**, 1256.

9 G. Lang, A.L.J. Cole, J.W. Blunt and M.H.G. Munro, *J. Nat. Prod.,* 2006, **69**, 151.

10 Y. Mikami, Y. Nishijima, H. Iimura, A. Suzuki and S. Tamura, *Agric. Biol. Chem.,* 1971, **35**, 611.

11 D.J. Robeson and M.A.F. Jalal, *J. Inorg. Biochem.,* 1991, **44**, 109.

12 R.A. Meronuck, J.A. Steele, C.J. Mirocha and C.M. Christensen, *Appl. Microbiol.,* 1972, **23**, 613.

13 F.A. Miller, W.A. Rightsel, B.J. Sloan, J. Ehrlich, J.C. French and Q.R. Bartz, *Nature,* 1963, **200**, 1339.

14 N. Umetsu, J. Kaji and K. Tamari, *Agric. Biol. Chem.,* 1972, **36**, 859.

15 (a) M.-H. Lebrun, P. Duvert, F. Gaudemer, A. Gaudemer, C. Deballon and P. Boucly, *J. Inorg. Chem.,* 1985, **24**, 167; (b) M.-H. Lebrun, L. Nicolas, M. Boutar, F. Gaudemer, S. Ranomenjanahary, A. Gaudemer, *Phytochemistry,* 1988, **27**, 77.

16 P.S. Steyn and C.J. Rabie, *Phytochemistry,* 1976, **15**, 1977.

17 A. Dippenaar, C.W. Holzapfed and J.C.A. Boeyens, *J. Cryst. Mol. Struct.,* 1977, **7**, 189.

18 C.O. Gitterman, *J. Med. Chem.,* 1965, **8**, 483.

19 L. Carrasco and D. Vazquez, *Biochim. Biophys. Acta,* 1973, **319**, 209.

20 (a) K. Tanaka, K. Matsuo, Y. Nakaizumi, Y. Morioka, Y. Takashita, Y. Tachibana, Y. Sawamura and S. Kohda, *Chem. Pharm. Bull.,* 1979, **27**, 1901; (b) K. Matsuo, I. Kitagushi, Y. Takata and K. Tanaka, *Chem. Pharm. Bull.,* 1980, **28**, 2494.

21 M. Sodeoka, R. Sampe, S. Kojima, Y. Baba, T. Usui, K. Ueda and H. Osada, *J. Med. Chem.,* 2001, **44**, 3216.

22 Y.-C. Jeong, Z. Bikadi, E. Hazai and M.G. Moloney, *ChemMedChem,* 2014, **9**, 1826.

23 J. Rouleau, A. Korovitch, C. Lion, M. Hémadi, N.-T. Ha-Duong, J.-M. El Hage Chahine and T. Le Gall, *Tetrahedron,* 2013, **69**, 10842.

24 (a) O. Markopoulou, J. Markopoulos and D. Nicholls, *J. Inorg. Biochem.* 1990, **39**, 307; (b) B.T. Heaton, C. Jacob, J. Markopoulos, O. Markopoulou, J. Nähring, C.-K. Skylaris and A.K. Smith, *J. Chem. Soc. Dalton Trans.,* 1996, **8**, 1701; (c) M. Petroliagi, O. Igglessi-Markopoulou and J. Markopoulos, *Heterocyclic Comm.,* 2000, **6**, 157; (d) E. Gavrielatos, C. Mitsos, G. Athanasellis, B.T. Heaton, A. Steiner, J.F. Bickley, O. Igglessi-Markopoulou and J. Markopoulos, *J. Chem. Soc., Dalton Trans.,* 2001, 639; (e) E. Gavrielatos, G. Athanasellis, O. Igglessi-Markopoulou and J. Markopoulos, *Inorg. Chim. Acta,* 2003, **344**, 128; (f) D. Matiadis, O. Igglessi-Markopoulou, V McKee, J. Markopoulos, *Tetrahedron,* 2014, **70**, 2439.

25 E. Ohta, S. Ohta and S. Ikegami, *Tetrahedron,* 2001, **22**, 4699.

26 G.F. Kaufmann, R. Sartorio, S.-H. Lee, C.J. Rogers, M.M. Meijler, J.A. Moss, B. Clapham, A.P. Brogan, T.J. Dickerson and K.D. Janda, *Proc. Natl. Am. Soc. USA,* 2005, **102**, 309.

27 A.A. Romano, T. Hahn, N. Davis, C.A. Lowery, A.K. Struss, K.D. Janda, L.H. Böttger, B.F. Matzanke and C.J. Carrano, *J. Inorg. Biochem.,* 2012, **107**, 96.

28 C.A. Lowery, J. Park, C. Gloeckner, M.M. Meijler, R.S. Mueller, H.I. Boshoff, R.L. Ulrich, C.E. Barry, III, D.H. Bartlett, V.V. Kravchenko, G.F. Kaufmann and Kim D. Janda, *J. Am. Chem. Soc.,* 2009, **131**, 14473.

29 Z. Shang, L. Li, B.P. Esposito, A.A. Salim, Z.G. Khalil, M. Quezada, P.V. Bernhardt and R.J. Capon, *Org. Biomol. Chem.,* 2015, **28**, 7795.

30 C.W. Holzapfel, *Tetrahedron,* 1968, **24**, 2101.

31 (a) J.W. Kirksey and R.J. Cole, *Appl. Microbiol.,* 1973, **26**, 827; R.T. Gallager, J.L. Richard, H.M. Stahr and R.J. Cole, *Mycopathol.,* 1978, **66**, 31.

32 C. Montigny, M. Picard, G. Lenoir, C. Gauron, C. Toyoshima and P. Champeil, *Biochemistry,* 2007, **46**, 15162.

33 M. Laursen, M. Bublitz, K. Moncoq, C. Olesen, J.V. Møller, H.S. Young, P. Nissen and J.P. Morth, *J. Biol. Chem.,* 2009, **284**, 13513.

34 N. Cramer, S. Helbig, A. Baro, S. Laschat, R. Diestel, F. Sasse, D. Mathieu, C. Richter, G. Kummerlöwe, B. Luy and H. Schwalbe, *ChemBioChem,* 2008, **9**, 2474.

35 B.H. Howard and H. Raistrick, *Biochem. J.,* 1954, **57**, 212.

36 S. Junzo, S. Shibata, U. Sankawa, H. Taguchi and Y. Shibanuma, *Chem. Pharm. Bull.,* 1965, **10**, 1240.

37 I. Casser, B. Steffan and W. Steglich, *Angew. Chem. Int. Ed.,* 1987, **26**, 586.

38 M.N. Preobrazhenskaya, E.N. Olsufyeva, O.V. Miroshnikova, J.J. Plattner, D. Chu, and S.S. Printsevskaya, *J. Antibiot.,* 2007, **4**, 235.

39 F. Vinale, M. Nigro, K. Sivasithamparam, G. Flematti, E.L. Ghisalberti, M. Ruocco, R. Varlese, M. Marra, S. Lanzuise and A. Eid, *FEMS Microbiol. Lett.,* 2013, **2**, 123.

40 (a) S. Ito and Y. Hirata, *Tetrahedron Lett.,* 1972, **12**, 1181; (b) S. Ito and Y. Hirata, *Bull. Chem. Soc. Jap.,* 1977, **7**, 1813.

41 L.A. Paquette, D. Macdonald and L.G. Anderson, *J. Am. Chem. Soc.,* 1990, **25**, 9292.

42 (a) N. M. Gandhi, J. Nazareth, P.V. Divekar, H. Kohl and N.J. De Souza, *J. Antibiot.,* 1973, **12**, 797; (b) H. Kohl, S.V. Bhat, J.R. Patell, N.M. Gandhi, J. Nazareth, P.V. Divekar, N.J. De Souza, H.G. Berscheid and H.W. Fehlhaber, *Tetrahedron Lett.,* 1974, **12**, 983.
43 N. Imamura, K. Adachi, H. Sano, *J. Antibiot.,* 1994, **2**, 257.
44 B. Biersack, R. Diestel, C. Jagusch, F. Sasse and R. Schobert, *J. Inorg. Biochem.,* 2009, **1**, 72.
45 (a) J. Gyimesi, I. Ott, I. Horváth, I. Koczka and K. Magyar, *J. Antibiot.,* 1971, **24**, 277; (b) I. Horváth, J. Gyimesi and Z. Méhesfalvi-Vajna, *Tetrahedron Lett.,* 1973, **36**, 3643; (c) J. Gyimesi and Z. Méhesfalvi-Vajna, I. Horváth, *J. Antibiot.,* 1978, **31**, 626.
46 L. Mészáros, L. Hoffmann, T. König and I. Horváth, *J. Antibiot.,* 1980, **33**, 494.
47 A. Nowak and B. Steffan, *Liebigs Ann.,* 1997, **9**, 1817.
48 M. Tsuda, T. Mugishima, K. Komatsu, T. Sone, M. Tanaka, Y. Mikami, M. Shiro, M. Hirai and Y. Ohizumi and J. Kobayashi, *Tetrahedron,* 2003, **18**, 3227.
49 S. Zorov, Y. Yuzenkova, V. Nikiforov, K. Severinov and N. Zenkin, *Antimicrob. Agents Chemother.,* 2014, **3**, 1420.
50 J. Shoji, R. Sakazaki, T. Hattori, K. Matsumoto, N. Uotani and T. Yoshida, *J. Antibiot.,* 1989, **12**, 1729.
51 Y. Igarashi, K. Takagi, Y. Kan, K. Fujii, K.-i. Harada, T. Furumai and T. Oki, *J. Antibiot.,* 2000, **53**, 233.
52 M. Brufani, S. Cerrini, W. Fedeli, F. Mama and R. Muntwyler, *Helv. Chim. Acta*, 1972, **55**, 2094.
53 J.A. Waitz, A.C. Horan, M. Kalyanpur, B.K. Lee, D. Loebenberg, J.A. Marquez, G. Miller, M.G. Patel, *J. Antibiot.,* 1981, **34**, 1101.
54 A.K. Mallams, M.S. Puar, R.R. Rossman, A.T. McPhail, R.D. Macfarlane, R.L. Stephens, *J. Chem. Soc. Perkin Trans. 1,* 1983, 1497.
55 (a) T. Kusumi, A. Ichikawa, H. Kakisawa, M. Tsunakawa, M. Konishi and T. Oki, *J. Am. Chem. Soc.*, 1991, **113**, 8947; (b) M. Tsunakawa, O. Tenmyo, K. Tomita, N. Naruse, C. Kotake, T. Miyaki, M. Konishi and T. Oki, *J. Antibiot.,* 1992, **45**, 180.
56 (a) B.E. Roggo, F. Petersen, R. Delmendo, H.-B. Jenny, H.H. Peter and J. Roesel, *J. Antibiot.,* 1994, **47**, 136; (b) B.E. Roggo, P. Hug, S. Moss, R. Raschdorf and H.H. Peter, *J. Antibiot.,* 1994, ***47***, 143.
57 M. Sodeoka, R. Sarape, T. Kagamizono and H. Osada, *Tetrahedron Lett.*, 1996, **37**, 8775.
58 K. Kobinata, M. Uramoto, T. Mizuno, K. Isono, *J. Antibiot.,* 1980, **33**, 244.
59 D.H. Davies, E.W. Snape and P.J. Suter, *J. Chem. Soc. Chem. Comm.,* 1981, 1073.
60 C.J. Newbold, R.J. Wallace and N.D. Walker-Bax, *FEMS Microbiol. Lett.,* 2013, **338**, 161.
61 C. Keller-Juslén, H.D. King, M. Kuhn, H.-R. Loosli, W. Pache, T.J. Petcher, H.P. Weber and A. von Wartburg, *J. Antibiot.,* 1981, **35**, 142.
62 (a) J. Grandjean and P. Laszlo, *Tetrahedron Lett.,* 1983, **24**, 3319; (b) J. Grandjean and P. Laszlo, *J. Am. Chem. Soc.,* 1984, **106**, 1472.
63 D.L. Nelson and M.M. Cox, *Lehninger Principles of Biochemistry* (2005), 4th Ed., W.H. Freeman and Company: New York, Chapter 11, pp. 369–420.
64 L. Kovác, V. Poliachová and I. Horváth, *Biochim. Biophys. Acta,* 1982, **721**, 349.
65 P.W. Reed and H.A. Lardy, *J. Biol. Chem.,* 1972, **246**, 6970.
66 L. Mészáros, L. Hoffmann, M. Paróczai, T. König, I. Horváth, *J. Antibiot.,* 1980, **33**, 523.
67 C.J. Newbold, R.J. Wallace and N. McKain, *J. Anim. Sci.,* 1990, **68**, 1103.
68 A. Höltzel, M.G. Gänzle, G.J. Nicholson, W.P. Hammes, G. Jung, *Angew. Chem. Int. Ed.*, 2000, **39**, 2766.
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-acetyltetramic acid), as a result of electronic effects, decreased solvation and an aggregation phenomenon (see ref. 27).
70 R. Sawa, Y. Mori, H. Iinuma, H. Naganawa, M. Hamada, S. Yoshida, H. Furutani, Y. Kajimura, T. Fuwa and T. Takeuchi, *J. Antibiot.,* 1994, **47**, 731.
71 M. Kawada, Y. Yoshimoto, H. Kumagai, T. Someno, I. Momose, N. Kawamura, K. Isshiki and D. Ikeda, *J. Antibiot.,* 2004, **57**, 231.
72 F. Vinale, G. Flematti, K. Sivasithamparam, M. Lorito, R. Marra, B.W. Skelton and E.L. Ghisalberti, *J. Nat. Prod.,* 2009, **72**, 2032.
73 F. Vinale, M. Nigro, K. Sivasithamparam, G. Flematti, E.L. Ghisalberti, M. Ruocco, R. Varlese, R. Marra, S. Lanzuise, A. Eid, S.L. Woo and M. Lorito, *FEMS Microbiol. Lett.,* 2013, **347**, 123.
74 V.M.T. Carneiro, D.B.B. Trivella, V. Scorsato, V.L. Beraldo, M.P. Dias, T.J.P. Sobreira, R. Aparicio and R.A. Pilli, *Eur. J. Med. Chem.,* 2015, **97**, 42.
75 T. Hamaguchi, T. Sudo and H. Osada, *FEBS Lett.,* 1995, **372**, 54.
76 (a) M. Sodeoka, R. Sampe, S. Kojima, Y. Baba, N. Morisaki and Y. Hashimoto, *Chem. Pharm. Bull.,* 2001, **49**, 206; (b) N. Morisaki H. Kobayashi, K. Nagasawa, Y. Baba, M. Sodeoka and Y. Hashimoto, *Chem. Pharm. Bull.,* 2003, **51**, 1341.
77 (a) T. Usui, S. Kojima, S.-i. Kidokoro, K. Ueda, H. Osada and M. Sodeoka, *Chem. Biol.,* 2001, **8**, 1209; (b) G. Hirai and M. Sodeoka, *Acc. Chem. Res.,* 2015, **48**, 1464.
78 N.W. Seidler, I. Jona, M. Vegh and A. Martonosi, *J. Biol. Chem.,* 1989, **264**, 17816.
79 F. Soler, F. Plenge-Tellechea, I. Fortea and F. Fernandez-Belda, *Biochemistry,* 1998, **37**, 4266.
80 F. Martínez-Azorín, *FEBS Lett.,* 2004, **576**, 73.
81 K. Moncoq, C.A. Trieber and H.S. Young, *J. Biol. Chem.,* 2007, **282**, 9748.
82 M. Takahashi, Y. Kondou and C. Toyoshima, *Proc. Natl. Am. Soc. USA,* 2007, **104**, 5800.
83 (a) S. Yao, D. Gallenkamp, K. Wölfel, B. Lüke, M. Schindler and J. Scherkenbeck, *Bioorg. Med. Chem.,* 2011, **19**, 4669; (b) W.R.C. Beyer, K. Woithe, B. Lüke, M. Schindler, H. Antonicek and J. Scherkenbeck, *Tetrahedron,* 2011, **67**, 3062.