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# New insights into molecular recognition of 1,1-bisphosphonic acids by farnesyl diphosphate synthase



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#### ABSTRACT

As part of our project pointed at the search of new antiparasitic agents against American trypanosomiasis (Chagas disease) and toxoplasmosis a series of 2-alkylaminoethyl-1-hydroxy-1,1-bisphosphonic acids has been designed, synthesized and biologically evaluated against the etiologic agents of these parasitic diseases, *Trypanosoma cruzi* and *Toxoplasma gondii*, respectively, and also towards their target enzymes, *T. cruzi* and *T. gondii* farnesyl pyrophosphate synthase (FPPS), respectively. Surprisingly, while most pharmacologically active bisphosphonates have a hydroxyl group at the C-1 position, the additional presence of an amino group at C-3 resulted in decreased activity towards either *T. cruzi* cells or *Tc*FPPS. Density functional theory calculations justify this unexpected behavior. Although these compounds were devoid of activity against *T. cruzi* cells and *Tc*FPPS, they were efficient growth inhibitors of tachyzoites of *T. gondii*. This activity was associated with a potent inhibition of the enzymatic activity of *Tg*FPPS. Compound **28** arises as a main example of this family of compounds exhibiting an ED<sub>50</sub> value of 4.7  $\mu$ M against tachyzoites of *T. gondii* and an IC<sub>50</sub> of 0.051  $\mu$ M against *Tg*FPPS.

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# 1. Introduction

Farnesyl pyrophosphate synthase (FPPS) can be considered as a valid target not only for bone related disorders, but also for different parasitic diseases.<sup>1</sup> FPPS catalyzes the consecutive condensation of IPP with DMAPP and with geranyl diphosphate (GPP) to produce farnesyl diphosphate (FPP). FPP is the substrate for enzymes catalyzing the first committed step for the biosynthesis of sterols, ubiquinones, dolichols, heme a, and prenylated proteins. FPP could be condensed with an additional molecule of IPP by the geranylgeranyl pyrophosphate synthase (GGPPS) to form the 20-carbon isoprenoid GGPP. In trypanosomatids, isoprenoid biosynthesis occurs via the classical mevalonate pathway (Scheme 1). The FPPS gene has been cloned from *Trypanosoma cruzi* and *Trypanosoma brucei.*<sup>2,3</sup> Both of these genes are single copy. RNA interference showed that the *T. brucei* FPPS gene is essential.<sup>3</sup>

In Apicomplexan parasites such as *Toxoplasma gondii* isoprenoids are biosynthesized through the DOXP/MEP pathway as illustrated in Scheme 2.<sup>4</sup> In addition, *T. gondii* possesses a bifunctional FPPS/GGPPS (*Tg*FPPS) that is able to catalyze the formation of both FPP and GGPP.<sup>5,6</sup> The FPPS gene appears to be essential in all organisms.<sup>7,8</sup> Comparison of the amino acid sequence of FPPSs from different organisms (bacteria to higher eukaryotes) shows the presence of seven conserved regions including two aspartate-rich domains that are very important for the catalytic action and most likely act as the binding sites for IPP and the allylic substrates. All the FPPSs that have been characterized are homodimeric enzymes, and require divalent cations such as Mg<sup>2+</sup> or Mn<sup>2+</sup> for activity.<sup>9</sup>

On the other hand, bisphosphonates (**2**) are pyrophosphate (**1**) analogues in which a methylene group replaces the oxygen atom bridge between the two phosphorus atoms of the pyrophosphate moiety. The substitution of carbon with different side chains has generated a large family of compounds. Several bisphosphonates are potent inhibitors of bone resorption and are in clinical use for the treatment of different bone disorders.<sup>10</sup> Acidocalcisomes are equivalent in composition to the bone mineral; the accumulation of bisphosphonates in these organelles, as they do in bone mineral, facilitates their antiparasitic action.<sup>11</sup> Aminobisphosphonates such as pamidronate (**3**), alendronate (**4**), and risedronate (**5**), were first found to be effective in the inhibition of *T. cruzi* in vitro and in vivo without toxicity to the host cells (Fig. 1).<sup>12</sup> In addition, some bisphosphonates were growth inhibitors of *T. gondii, T. brucei* 

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Scheme 1. Isoprenoid biosynthesis in trypanosomatids.



Scheme 2. Isoprenoid biosynthesis in Apicomplexan parasites.

2*rhodesiense*, *Leishmania donovani* and *Plasmodium falciparum*.<sup>13–16</sup> In vivo testing in mice has shown that risedronate can significantly increase the survival of mice infected by *T. cruzi*.<sup>17,18</sup> All these results indicate that bisphosphonates are promising candidate drugs to treat infections by *T. cruzi* and other pathogenic parasites. In fact, they have already been developed to treat other diseases and consequently have low toxicity; their structures are simple and easy to synthesize; these compounds have shown effective inhibitory activity against *T. cruzi* in vitro<sup>12</sup> and in vivo.<sup>17,18</sup> Of particular interest are linear bisphosphonates, specifically, 2-2alkyl(amino)ethyl derivatives, which can be considered as promising antiparasitic agents.<sup>19,20</sup> These bisphosphonate derivatives exhibit potent cellular activity against intracellular *T. cruzi*, which is one of the clinically relevant forms of this parasite, having  $IC_{50}$  values at the low nanomolar level against the target enzyme (*Tc*FPPS).<sup>19,20</sup> In addition, at the present time, linear 1-hydroxy-, 1-alkyl-, and 1-amino-1,1-bisphosphonates such as **6–9** can be considered as useful structures to establish rigorous SAR studies



Figure 1. Chemical structures of representative FDA-approved bisphosphonates clinically employed for different bone disorders.



Figure 2. Chemical structures of representative members of bisphosphonic acids derived from fatty acids.

as antiparasitic agent targeting *Tc*FPPS.<sup>5,21–24</sup> In fact, these compounds show a broad range of antiparasitic activity against trypanosomatids and Apicompexan. For example, 6 is a potent growth inhibitor of T. cruzi (amastigotes)<sup>21</sup> and also against T. gondii (tachyzoites),<sup>5,24</sup> whereas **7** is effective against *P*. falciparum.<sup>5</sup> Besides,  $\alpha$ -fluoro-1,1-bisphosphonates of formula **10** and **11** are devoid of cellular activity against T. cruzi or TcFPPS, but they are extremely potent inhibitors of the enzymatic activity of T. gondii FPPS exhibiting IC<sub>50</sub> values of 35 nM and 60 nM, respectively.<sup>25</sup> The high selectivity observed by these fluorine-containing bisphosphonates against TgFPPS versus TcFPPS can be rationalized by the evidence that the amino acid sequences of these enzymes have less than 50% identity.<sup>16</sup> (Fig. 2). As mentioned before, **12–14** are promising anti-T. cruzi agents. For example, **12** exhibit an ED<sub>50</sub> value of 0.84 µM against T. cruzi (amastigotes),<sup>19</sup> which is fifteen times more potent than the well-known antiparasitic agent WC-9 under the same assays conditions.<sup>26</sup> In addition, **13** is an extremely potent inhibitor of the enzymatic activity of TcFPPS ( $IC_{50} = 0.058$  - $\mu$ M) and of *Tg*FPPS (IC<sub>50</sub> = 0.095  $\mu$ M),<sup>20</sup> whereas the long chain length derivative 14 is an effective growth inhibitors of intracellular T. cruzi proliferation (ED<sub>50</sub> =  $0.67 \mu$ M) compared to benznidazole  $(ED_{50} = 2.77 \,\mu\text{M})$ <sup>21</sup> Sulfur-containing bisphosphonates also presents good prospective as putative lead drugs. For example, 15 and 16 are potent anti-Toxoplasma agents and, to a lesser extent, efficient anti-T. cruzi agents.<sup>27</sup> For example, **15** has a potent cellular activity against tachyzoites of *T. gondii* ( $ED_{50} = 1.8 \mu M$ ), which was associated with a potent inhibition of the enzymatic activity of *Tg*FPPS (IC<sub>50</sub> = 0.021  $\mu$ M).<sup>27</sup> Compound **15** is also effective towards *Tc*FPPS (IC<sub>50</sub> = 0.097  $\mu$ M).<sup>27</sup> Moreover, **16** exhibits an extremely potent inhibitory action against TgFPPS with an IC<sub>50</sub> value as low as 0.009  $\mu$ M<sup>27</sup> (Fig. 2). It is worth mentioning that similar activity have also been reported for some lipophilic bisphosphonates, including 2-alkylaminoethyl compounds lacking the hydroxyl group at C-1, against another Apicomplexan parasite, P. falciparum.<sup>28,29</sup>

# 2. Rationale

The present study was motivated to get further insight into the molecular recognition processes of 2-alkyl(amino)ethyl-1,1-bis-phosphonates taking compounds **12–14** as reference structures. We have recently demonstrated that *Tc*FPPS inhibitors **12** and **13** bind to the allylic site of the enzyme with the phosphates group

of the bisphosphonate unit coordinating three Mg<sup>2+</sup> atoms,<sup>30</sup> which bridge the compounds to the enzyme in a similar way to that observed for the physiological substrates.<sup>31,32</sup> Binding of either **12** or **13** is enthalpically unfavorable. The favorable entropy, which dominates the favorable free-energy, results from a delicate balance between two opposing effects: the unfavorable loss of conformational entropy, due to freezing of single bond rotations of the inhibitor (and binding site side chains), and the favorable increase of entropy associated with burial of the hydrophobic alkyl chains.<sup>30</sup> The nitrogen atom at the C-3 position is very important to maintain a potent inhibition of the enzymatic activity of *Tc*FPPS, but does not coordinate any Mg<sup>2+</sup> atom at the active site<sup>30</sup> as we had been initially considered.<sup>19,20</sup> It has a crucial role to drive the spatial alignment of the alkyl chains for better fitting.

We have envisioned that the introduction of a hydroxyl group at the C-1 position in compounds **12** and **13** is a relevant structural variation for a number of reasons: (a) it is known that the presence of an electron withdrawing group at C-1 would enhance the ability to coordinate Ca<sup>2+</sup> or Mg<sup>2+</sup> in a tridentate manner;<sup>32–38</sup> (b) the presence of an electron withdrawing group at C-1 of 1,1-bisphosphonic acids would increase acidity in at least one order of magnitude compared to those where these groups are absent mimicking the pK<sub>a</sub> value of pyrophosphoric acid;<sup>39–43</sup> (c) most of the bisphosphonates clinically in use for the treatment of bone disorders have a hydroxyl group bonded at C-1; <sup>44–46</sup> (d) compound **6** impairs its efficiency as inhibitor of the enzymatic activity agaisnt *Tc*FPPS when the hydroxyl group is absent as occurs with compound **8**.<sup>21,22</sup>

Bearing in mind the above statements, the role of the hydroxyl group at C-1 on biological activity in a variety of bisphosphonates is still uncertain. Experimental evidence indicates that that the hydroxyl group at C-1 does not interact with  $Mg^{2+}$  at the active site of FPPS suggesting that the function of this group is circumvented to influence the ability of the adjacent bisphosphonic unit to coordinate  $Mg^{2+}$  as well as to increase the  $pK_a$  of the *gem*-phopshonate functionality.<sup>47–49</sup>

### 3. Results and discussion

As previously discussed, we selected 2-alkylaminoethyl-1-hydroxy-1,1-bisphosphonic acid derivatives 27-31 as the title compounds for the present study. These compounds were straightforwardly prepared starting from benzyl bromoacetate and the corresponding linear amine. Nucleophilic displacement reaction between each amine and benzyl bromoacetate in acetonitrile as a solvent, according to slightly modified published procedures,<sup>50,51</sup> afforded the respective benzyl *n*-alkylaminoacetates **17–21** in yields ranging 29-84%. Benzyl groups were cleaved by catalytic hydrogenation employing palladium on charcoal as catalyst to give the respective free acids 22-26 in 46-98% yields. These 2-(n-alkylamino)acetic acids were the substrates to prepare the title compounds 27-31. Then, in independent experiments, on treatment with phosphorous acid and phosphorous trichloride employing benzenesulfonic acid as a solvent at 65 °C followed by hydrolysis, 22-26 were converted into 27-31 according to the widely employed method for the preparation of 1-hydroxy-1,1-bisphosphonic acids from carboxylic acids (Scheme 3).<sup>52</sup>



Scheme 3. Synthetic approach for the preparation of modified alkylaminoethyl bisphosphonates.

Biological evaluation of 2-alkylaminoethyl-1-hydroxy-1,1-bisphosphonates has lead to surprising results. Contrary to it was expected, all of these compounds were almost devoid of biological activity as inhibitors of T. cruzi proliferation and also as inhibitor of the enzymatic activity of TcFPPS confirming our previous finding in compound 29.27 However, some of these compounds showed an extremely potent inhibition of the enzymatic activity of TgFPPS. For example 28 and 30 are potent inhibitors of TgFPPS exhibiting  $IC_{50}$  values of 0.051  $\mu$ M and 0.039  $\mu$ M, respectively. This enzymatic activity was associated with an efficient cellular activity showing ED<sub>50</sub> values of 4.7 µM and 2.0 µM, respectively. Risedronate was used as positive control ( $ED_{50} = 2.4 \mu M$ ). This selectively observed towards the target enzymes (TcFPPS vs TgFPPS) has previously been observed and it can be justified by the fact that sequences of these enzymes have less than 50% identity.<sup>16</sup> Moreover, these results are more comprehensible taking into account that TgFPPS is a bifunctional enzyme, hence as TgFPPS also catalyzes formation of both FPP (C-15) and GGPP (C-20).<sup>5,6</sup> it is reasonable to assume that its enzymatic activity could be inhibited by compounds of long chain length, which are structurally rather similar to GGPP in contrast to TcFPPS, the enzyme that catalyses formation of FPP as final product exclusively. The biological evaluation is presented in Table 1.

The lack of biological activity of this family of compounds against T. cruzi is quite unexpected and cannot be attributable simply to the presence of a hydroxyl group at C-1. In fact, either compound 6 or risedronate (5), both bearing a hydroxyl group at C-1, are effective inhibitors of TcFPPS.<sup>21,22,53</sup> Why our title compounds are devoid of antiparasitic activity against T. cruzi?. In risedronate the nitrogen atom is bonded one position further than is bonded in our compounds. Evidently, the position of this nitrogen atom has a strong influence in the observed biological activity. Removal of the hydroxyl group keeping the nitrogen atom at C-3 results in extremely potent 2-alkylaminoethyl bisphosphonates, such as 12-14. Therefore, these two groups did not produce the expected synergistic effect. On the contrary, the combination of these two groups reduced their activity. We have attempted to rationalize this behavior using density functional theory (DFT) calculations. Analogs of 12-14 (12a) and 27-31 (27a) carrying, for the sake of simplicity, N-methylamino groups instead of longer alkylamino moieties were submitted to energy minimizations at the B3LYP/6-311+G(d,p) level, simulating the presence of water



Figure 3. Simplified models of 12–14 (12a) and 27–31 (27a) to carry out molecular modeling studies.

as solvent with the polarizable continuum method (PCM). In order to reproduce better the possible conformation of these compounds in the biological environment, the molecules **12a** and **27a** were considered to have the acidic hydrogen atoms expected to appear at physiological pH ( $\approx$ 6.5), that is two hydrogen atoms for **12a**, and only one for the more acidic **27a**.<sup>39,40</sup> One magnesium atom was added to complete each molecule (Fig. 3).

Results show that the minimization always leads to the formation of a six-membered ring containing C-1, both P atoms, two O atoms and the Mg atom, being the distance between each O and the Mg of 1.91–1.96 Å. However, the 'exocyclic' atoms generate different hydrogen bond patterns (even in the simulated water environment) with distinct geometries and energies. It is known that the strength of the hydrogen bonds depends, under the geometric criteria, on acceptor-hydrogen distances as short as possible (for very strong bonds, the distance can be even shorter than 2 Å), and donor-hydrogen-acceptor angles as close to 180° as possible.<sup>54,55</sup> Table 2 shows the results for the main conformers of each analog. For 12a, the most stable geometry shows a strong '1,3-diaxial' hydrogen bond between an axial O-H and an axial O bonded to different P atoms ( $d_{H-O} = 1.82$  Å), and a very strong hydrogen bond between the other acidic hydrogen (equatorial) and the nearby nitrogen atom ( $d_{H-N} = 1.68$  Å), which fixes the conformation of the side chain (Fig. 4). Another conformer, with similar energy, has the same hydrogen bond features but a different conformation of the carbon chain. On the other hand, compound **27a** does not have two acidic hydrogens. Thus, the only acidic hydrogen should be involved in either the 1,3-diaxial H-bond interaction or the interaction with the N. The most stable geometry (Fig. 4, Table 2) shows the strong diaxial interaction  $(d_{H-O} = 1.62 \text{ Å}, \theta = 161^{\circ})$ , and two weaker interactions (Table 2),

Table 1

Biological activity of 2-alkylaminoethyl-1-hydroxy-1,1-bisphosphonic acids against TcFPPS, TgFPPS, T. cruzi (amastigotes), and tachyzoites of T. gondii

Compound	TcFPPS IC50 (µM)	ED <sub>50</sub> T. cruzi amastigotes (µM)	TgFPPS IC50 (µM)	ED <sub>50</sub> T. gondii tachyzoites (µM)	
27	>10	>20	0.067 + 0.064	>10	
28	>10	>20	$0.051 \pm 0.006$	4.68 ± 1.19	
29 30	>10 >10	>20 46% at 10 µM cytotoxic at 10 µM	>1.0 0.039 ± 0.033	≥ 10 μM 2.00 ± 0.95	
31	>10	42% at 10 µM	$0.125 \pm 0.023$	14.9% at 10 µM	
Benznidazole Risedronate	$0.027 \pm 0.01^{53}$	$\begin{array}{c} 1.44 \pm 0.97 \\ 55.0 \pm 5.0^{53} \end{array}$	$0.074 \pm 0.017^5$	$2.4 \pm 0.7^{24}$	



**Figure 4.** Most stable geometries of simple models **12a** (a) and **27a** (b). Hydrogen bonds were labelled as strong (s) when  $d_{Acceptor-H} < 2$  Å and  $\theta_{donor-H-Acceptor} > 145^{\circ}$ , or as weak (w) if they do not meet both of these criteria.

as deduced from directional factors ( $\theta = 124-134^{\circ}$ ). Other conformations, with higher energies show other bonding patterns: conformer 4 shows a similar pattern, whereas conformers 2 and 3 show a strong hydrogen bond between the acidic hydrogen and the nitrogen atom, as occurred with **12a** (Table 2). However, these conformers have energies surpassing in about 2 kcal/mol that of the most stable conformer. The strength of the bond in conformer 3 of **27a** is equivalent to that observed for the main conformer of **12a**, but the hydrogen bond in conformer 2 is slightly weaker, as deduced from the distance and angles (Table 2).

These results might be a clue that in compounds like **12–14**, having two acidic hydrogen atoms, the flexibility of the carbon chain is strongly reduced, to the point of giving an almost 'fixed' conformation carrying the nitrogen atom in a favorable arrangement for biological action. On the other hand, compounds like **27–31**, which carry both a hydroxyl group at C-1 and a N atom on C-2 display a higher flexibility of the carbon chain, thus generating a manifold of conformations, for which only some (less stable) display the arrangement needed for an optimal molecular recognition.

NMR analyses support the above statement. Compound **32** is an interesting example of an isosteric analog of compounds **12–14**.<sup>56</sup> It has been demonstrated that **32** forms a rather stable six-mem-

bered ring via a hydrogen bond based on the chemical shift of the phosphorus atom at the C-3 position as illustrated in Figure 5.<sup>56</sup> In addition, proton NMR data of **28** and the *n*-propyl derivative of **12–14** (compound **33**), performed in anhydrous deuterated DMSO, indicated the occurrence of this conformational restriction on these compounds. Two signals of protons bonded to heteroatoms were observed in the <sup>1</sup>H NMR spectrum of **33** at 7.70 ppm and 8.95 ppm, respectively. The first signal moved upfield (5.40 ppm) when 1 equiv of Mg<sup>2+</sup> was added. A similar behavior was observed from the proton NMR spectrum of **28**. Once again, two signals that appeared at 6.69 ppm and 9.11 ppm. The first one moved upfield (4.41 ppm) when one equivalent of Mg<sup>2+</sup> was added.

In summary, it can be concluded that the activity against *T. cruzi* of compounds of type **12–14** can be attributed due to these compounds adapt a restricted conformation having no tension of torsion that would be benefit for molecular recognition, whereas the 1-hydroxy derivatives **27–31** present a manifold of conformations that would not lead to a successful interaction with the target enzyme. Nevertheless, these bisphophonate derivatives exhibited a selective and potent inhibitory action towards *Tg*FPPS. Efforts in optimizing lead structures **12–14** are currently being pursued in our laboratory.

# 4. Experimental section

# 4.1. General

The glassware used in air- and/or moisture-sensitive reactions was flame-dried and reactions were carried out under an argon atmosphere. Unless otherwise noted, chemicals were commercially available and used without further purification. Solvents were distilled before use. Acetonitrile was distilled from phosphorus pentoxide.

Nuclear magnetic resonance spectra were recorded using a AM-500 MHz spectrometer. Chemical shifts are reported in parts per million ( $\delta$ ) relative to tetramethylsilane. Coupling constants are reported in Hertz. <sup>13</sup>C NMR spectra were fully decoupled. <sup>31</sup>P NMR spectra are referenced with respect to the peak of 85% H<sub>3</sub>PO<sub>4</sub> as external reference. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet.

Table 2

Main conformers<sup>a</sup> of **12a** and **27a**, calculated by B3LYP/6-311+G(d,p) with PCM in water: energies and geometrical features of the hydrogen bonds

Conformer	Energy (kcal/mol)	Carbon chain <sup>b</sup>	Hydrogen bonds atoms involved	$d_{\text{H-Acc}}$ (Å)	$\theta_{\text{Don-H-Acc}}$ (°)
12a					
1	0.00	Equat.	$H(PO_{ax})-O_{ax}(P')$	1.82	154
			H(PO <sub>eq</sub> )-N	1.68	157
2	0.59	Axial	$H(PO_{ax})-O_{ax}(P')$	1.89	154
			H(PO <sub>eq</sub> )-N	1.73	155
3	6.50	Equat.	$H(PO_{ax})-O_{ax}(P')$	1.86	154
			$H(N)-O_{eq}(P)$	2.25	139
4	8.49	Equat.	$H(N)-O_{eq}(P)$	2.19	139
27a					
1	0.00	Equat.	$H(PO_{ax})-O_{ax}(P')$	1.62	161
			H(O <sub>ax</sub> )-N	2.01	124
			$H(N)-O_{eq}(P)$	2.24	134
2	1.77	Equat.	$H(O_{ax})-O_{eq}(P)$	1.91	135
			H(PO <sub>eq</sub> )-N	1.78	153
3	2.17	Axial	$H(O_{eq})-O_{ax}(P)$	1.95	133
			H(PO <sub>eq</sub> )-N	1.67	157
4	3.75	Equat.	$H(PO_{ax})-O_{ax}(P')$	1.62	162
			$H(O_{ax})-O_{eq}(P)$	2.82	108
			$H(N)-O_{eq}(P)$	2.14	142
5	4.86	Axial	$H(PO_{ax})-O_{ax}(P')$	1.59	160
			$H(O_{eq})-O_{eq}(P)$	2.25	127

<sup>a</sup> For each of the nine H-bond/carbon chain arrangements, the conformer with the orientation of the *N*-methyl group with lower energy is shown.

<sup>b</sup> The designation of substituents as axial or equatorial was made considering the presence of a six-membered cycle formed by the atoms C-1-P-1-O-Mg-O-P-2.



Figure 5. Chemical structures of compound 32, exhibiting a typical six-membered hydrogen bond (see Ref. 56), and its isosteric analogue 33.

High-resolution mass spectra were obtained using a hybrid quadrupole time of flight mass spectrometer with MS/MS capability.

Melting points are uncorrected. Analytical TLC was performed on commercial 0.2 mm aluminum-coated silica gel plates ( $F_{254}$ ) and visualized by 254 nm UV or immersion in an aqueous solution of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (0.04 M), Ce(SO<sub>4</sub>)<sub>2</sub> (0.003 M) in concentrated H<sub>2</sub>SO<sub>4</sub> (10%). Elemental analyses were conducted by UMYM-FOR (CONICET-FCEyN). The results were within ±0.4% of the theoretical values.

#### 4.2. Synthesis of 2-(n-Alkylamino)acetic acids

### 4.2.1. General procedure

To a solution of the corresponding *n*-alkylamine (1.00 g, 10 mmol) in anhydrous acetonitrile (15 mL) cooled at 0 °C was added dropwise benzyl bromoacetate (2.29 g, 10 mmol). Then, triethylamine (2.7 mL, 19.4 mmol) was added and the reaction mixture was stirred overnight. The solvent was evaporated and the residue was purified by column chromatography (sílica gel) eluting with a mixture of hexane–EtOAc (19:1) to afford the corresponding benzyl esters **17–21** as colorless oils. Then, a solution of respective benzyl ester (8.0 mmol) in ethyl acetate (50 mL) in the presence of palladium on charcoal (50 mg) was treated with hydrogen at 3 atm in a Parr apparatus. The reaction mixture was shaken for 6 h and the mixture was filtered through a fritted glass funnel. The solvent was evaporated to yield the corresponding free 2-(*n*-alkylamino) acetic acids that were used in the next step without further purification.

# **4.3.** 1-[(*n*-Alkylamino)ethyl]-1-hydroxy-1,1-bisphosphonic acids

#### 4.3.1. General procedure

To a flame dried 100 mL three neck flask having an addition funnel and a reflux condenser through which water at 0 °C was circulated the corresponding 2-(*n*-alkylamino) acetic acid (2.9 mmol), H<sub>3</sub>PO<sub>3</sub> acid (273 mg, 2.9 mmol), and anhydrous benzenesulfonic acid (1.0 g, 6.3 mmol) under argon atmosphere. The reaction mixture was heated to 65 °C, then PCl<sub>3</sub> (500  $\mu$ L, 5.8 mmol) was added dropwise with vigorous stirring. The reaction was stirred at 65 °C for 16 h, and then allowed to cool to room temperature. Then, cold water (60 mL) was added and the reaction was stirred at 100 °C for an additional 5 h. The reaction was cooled to room temperature and the pH was adjust to 4.3 with a 50% aqueous NaOH solution. Acetone (20 mL) was added, and the resulting mixture was cooled to 0 °C for 24 h. The product was filtrated and crystallized from water–ethanol.

## 4.3.2. 2-(*n*-Pentylamino)acetic acid (22)

White solid; 46% yield; mp 185–190 °C (desc.); <sup>1</sup>H NMR (500.13 MHz, CD<sub>3</sub>OD)  $\delta$  0.94 (t, *J* = 6.8 Hz, 3H), 1.37 (m, 4H), 1.68 (p, *J* = 7.5 Hz, 2H), 2.97 (dist t, *J* = 7.9 Hz, 2H), 3.47 (s, 2H); <sup>13</sup>C NMR (125.77 MHz, CD<sub>3</sub>OD)  $\delta$  14.1, 23.2, 26.9, 29.7, 48.7, 50.6,

170.9. HRMS (ESI) calcd for  $C_7H_{16}O_2NNa [M+Na]^+$  168.1000; found: 168.1010.

## 4.3.3. 1-[(*n*-Pentylamino)ethyl]-1-hydroxy-1,1-bisphosphonic Acid (27)

White solid; 31% yield; mp 186–190 °C; <sup>1</sup>H NMR (500.13 MHz, D<sub>2</sub>O)  $\delta$  0.79 (t, *J* = 7.1 Hz, 3H), 1.26 (m, 4H), 1.62 (p, *J* = 7.4 Hz, 2H), 3.02 (t, *J* = 7.5 Hz, 2H), 3.40 (t, *J* = 11.7 Hz, 2H); <sup>13</sup>C NMR (125.77 MHz, D<sub>2</sub>O)  $\delta$  13.0, 21.4, 25.0, 27.7, 48.2, 49.9, 70.3 (t, *J* = 137.5 Hz); <sup>31</sup>P NMR (202.46 MHz, D<sub>2</sub>O)  $\delta$  14.82. HRMS (ESI) calcd for C<sub>7</sub>H<sub>19</sub>O<sub>7</sub>NP<sub>2</sub>Na [M+Na]<sup>+</sup> 314.0534; found: 314.0527.

### 4.3.4. 2-(Hexylamino)acetic acid (23)

White solid; 91% yield; mp 196–199 °C (desc.); <sup>1</sup>H NMR (500.13 MHz, CD<sub>3</sub>OD)  $\delta$  0.92 (t, *J* = 6.9 Hz, 3H), 1.35 (m, 4H), 1.39 (p, *J* = 7.8 Hz, 2H), 1.67 (p, *J* = 7.6 Hz, 2H), 2.97 (dist t, *J* = 7.9 Hz, 2H), 3.46 (s, 2H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta$  14.3, 23.5, 27.2, 27.3, 32.4, 48.7, 50.6, 170.9. HRMS (ESI) calcd for C<sub>8</sub>H<sub>18</sub>O<sub>2</sub>N [M+H]<sup>+</sup> 160.1338; found: 160.1343.

# 4.3.5. 1-[(*n*-Hexylamino)ethyl]-1-hydroxy-1,1-bisphosphonic Acid (28)

White solid; 38% yield; mp 189–190 °C (desc.); <sup>1</sup>H NMR (500.13 MHz, D<sub>2</sub>O)  $\delta$  0.77 (t, *J* = 7.2 Hz, 3H), 1.25 (m, 4H), 1.29 (p, *J* = 6.6 Hz, 2H), 1.61 (p, *J* = 7.5 Hz, 2H), 3.02 (t, *J* = 7.5 Hz, 2H), 3.39 (t, *J* = 11.6 Hz, 2H); <sup>13</sup>C NMR (125.77 MHz, D<sub>2</sub>O)  $\delta$  13.2, 21.6, 25.2, 25.3, 30.4, 48.2, 50.0, 70.4 (t, *J* = 138.4 Hz); <sup>31</sup>P NMR (202.46 MHz, D<sub>2</sub>O)  $\delta$  14.69. HRMS (ESI) calcd for C<sub>8</sub>H<sub>21</sub>O<sub>7</sub>NP<sub>2</sub>Na [M+Na]<sup>+</sup> 328.0691; found: 328.0684.

# 4.3.6. 2-(n-Heptylamino)acetic acid (24)

White solid; 67% yield; mp = 187–191 °C; <sup>1</sup>H NMR (500.13 MHz, CD<sub>3</sub>OD)  $\delta$  0.91 (t, *J* = 7.0 Hz, 3H), 1.32 (m, 4H), 1.37 (m, 4H), 1.67 (p, *J* = 7.5 Hz, 2H), 2.97 (m, 2H); 3.46 (s, 2H); <sup>13</sup>C NMR (125.77 MHz, D<sub>2</sub>O)  $\delta$  13.6, 22.2, 28.5, 28.7, 29.4, 31.3, 47.4, 49.2, 169.5. HRMS (ESI) calcd for C<sub>9</sub>H<sub>20</sub>O<sub>2</sub>N [M+H]<sup>+</sup> 174.1494; found: 174.1510. Anal. Calcd for (C<sub>9</sub>H<sub>19</sub>O<sub>2</sub>N): C, 62.39; H, 11.05; N, 8.08. Found C, 62.05; H, 10.62; N, 7.74.

# 4.3.7. 1-[(*n*-Heptylamino)ethyl]-1-hydroxy-1,1-bisphosphonic acid (29)

White solid; 10% yield; mp 155–159 °C; <sup>1</sup>H NMR (500.13 MHz, CDCl<sub>3</sub>)  $\delta$  0.90 (t, *J* = 7.0 Hz, 3H), 1.18 (m, 6H), 1.24 (m, 2H), 1.60 (p, *J* = 7.4 Hz, 2H), 3.01 (t, *J* = 7.6 Hz, 2H), 3.39 (t, *J* = 11.7 Hz, 2H); <sup>13</sup>C NMR (125.77 MHz, D<sub>2</sub>O)  $\delta$  13.3, 21.8, 25.3,25.5, 27.8, 30.7, 48.3, 49.9, 70.3 (t, *J* = 137.7 Hz); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  15.31. HRMS (ESI) calcd for C<sub>9</sub>H<sub>24</sub>O<sub>7</sub>NP<sub>20</sub> [M+H]<sup>+</sup> 320.1030; found: 320.1037. Anal. Calcd for (C<sub>9</sub>H<sub>23</sub>O<sub>7</sub>NP<sub>2</sub>.1.50H<sub>2</sub>O): C, 31.22; H, 7.57; N, 4.05. Found C, 31.53; H, 7.75; N, 4.36.

### 4.3.8. 2-(Octylamino)acetic acid (25)

White solid; 96% yield; <sup>1</sup>H NMR (500.13 MHz, CD<sub>3</sub>OD)  $\delta$  0.90 (t, *J* = 6.8 Hz, 3H), 1.33 (m, 10H), 1.68 (p, *J* = 7.5 Hz, 2H), 2.97 (dist t, *J* = 7.9 Hz, 2H), 3.46 (s, 2H); <sup>13</sup>C NMR (125.77 MHz, CD<sub>3</sub>OD)  $\delta$  14.4, 23.7, 27.2, 27.6, 30.17, 30.19, 32.9, 48.7, 50.6, 170.9. HRMS (ESI) calcd for  $C_{10}H_{22}O_2N$  [M+H]<sup>+</sup> 188.1651; found: 188.1669.

# **4.3.9.** 1-[(*n*-Octylamino)ethyl]-1-hydroxy-1,1-bisphosphonic acid (30)

White solid; 43% yield; <sup>1</sup>H NMR (500.13 MHz, DMSO- $d_6$ )  $\delta$  0.85 (t, *J* = 6.9 Hz, 3H), 1.25 (m, 10H), 1.54 (p, *J* = 7.1 Hz, 2H), 2.90 (dist. t, *J* = 7.1 Hz, 2H), 3.22 (t, *J* = 10.7 Hz, 2H); <sup>13</sup>C NMR (125.77 MHz, DMSO- $d_6$ )  $\delta$  13.9, 22.1, 25.6, 25.9, 28.46, 28.48, 31.1, 47.4, 49.8, 69.4 (t, *J* = 132.3 Hz); <sup>31</sup>P NMR (DMSO- $d_6$ )  $\delta$  14.85. HRMS (ESI) calcd for C<sub>10</sub>H<sub>25</sub>O<sub>7</sub>NP<sub>2</sub>Na [M+Na]<sup>+</sup> 356.1004; found: 356.0994.

# 4.3.10. 2-(Decylamino)acetic acid (26)

White solid; mp 184–187 °C; <sup>1</sup>H NMR (500.13 MHz, CD<sub>3</sub>OD)  $\delta$  0.89 (t, *J* = 6.9 Hz, 3H), 1.24 (m, 14H), 1.67 (p, *J* = 7.5 Hz, 2H), 2.97 (dist t, *J* = 8.9 Hz, 2H), 3.46 (s, 2H); <sup>13</sup>C NMR (125.77 MHz, CD<sub>3</sub>OD)  $\delta$  14.4, 23.7, 27.2, 27.6, 30.2, 30.4, 30.5, 30.6, 33.0, 48.7, 50.6, 170.9. HRMS (ESI) calcd for C<sub>12</sub>H<sub>26</sub>O<sub>2</sub>N [M+H]<sup>+</sup> 216.1964; found: 216.1995.

# 4.3.11. 1-[(*n*-Decylamino)ethyl]-1-hydroxy-1,1-bisphosphonic acid (31)

White solid; mp 188–190 °C; <sup>1</sup>H NMR (500.13 MHz, DMSO- $d_6$ )  $\delta$  0.84 (t, J = 6.7 Hz, 3H), 1.25 (m, 14H), 1.54 (m, 2H), 2.90 (dist. t, J = 7.0 Hz, 2H), 3.22 (t, J = 11.1 Hz, 2H); <sup>13</sup>C NMR (125.77 MHz, DMSO- $d_6$ )  $\delta$  13.9, 22.1, 25.6, 25.9, 28.5, 28.7, 28.8, 28.9, 31.3, 47.3, 50.0, 69.2 (t, J = 130.0 Hz); <sup>31</sup>P NMR (DMSO- $d_6$ )  $\delta$  15.19. HRMS (ESI) calcd for C<sub>12</sub>H<sub>30</sub>O<sub>7</sub>NP<sub>2</sub> [M+H]<sup>+</sup> 362.1498; found: 362.1493.

# 5. Drug screening

### 5.1. T. cruzi amastigote assays

Gamma-irradiated (2000 Rads) Vero cells ( $3.4 \times 10^4$  cells/well) were seeded in 96 well plates (black, clear bottom plates from Greiner Bio-One) in 100 µL RPMI media (Sigma) with 10% FBS. Plates were incubated overnight at 35 °C and 7% CO<sub>2</sub>. After overnight incubation, Vero cells were challenged with  $3.4 \times 10^5$  trypomastigotes/well (CL strain overexpressing a tdTomato red fluorescent protein) in 50 µL volume and incubated for 5 h at 35 °C and 7% CO<sub>2</sub>. After infection, cells were washed once with Hanks solution (150 µL/well) to eliminate any extracellular parasites and compounds were added in serial dilutions in RPMI media in 150 µL volumes. Each dilution was tested in quadruplicate. Each plate also contained controls with host cells and no parasites (for background check), and controls with parasites and no drugs (positive control). Drugs were tested on T. cruzi at 1.56, 3.125, 6.25, 12.5, 25 µM. For each set of experiments, benznidazole was also used as a positive control 0.39, 0.78, 1.56, 3.125, and 6.25 µM. After drug addition, plates were incubated at 35 °C and 7% CO<sub>2.</sub> At day 3 post-infection, plates were assayed for fluorescence.<sup>57</sup> IC<sub>50</sub> values were determined by non-linear regression analysis using SigmaPlot. There was no evident cytotoxicity on the host cells (visual assay) with any of the drugs tested at concentrations as high as 25 µM.

### 5.2. T. gondii tachyzoites assays

Experiments on *T. gondii* tachyzoites were carried out as described previously<sup>58</sup> using *T.gondii* tachyzoites expressing red fluorescent protein.<sup>59</sup> Cells were routinely maintained in hTerT cells grown in High Glucose Dulbecco's modified Eagle's medium (DMEM-HG) supplemented with 1% fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, at 37 °C in a humid 5% CO<sub>2</sub> atmosphere. Confluent monolayers grown in 96-well black plates with optical bottoms (black, clear bottom plates from Greiner Bio-One) were

used and drugs dissolved in the same medium and serially diluted in the plates. Freshly isolated tachyzoites were filtered through a 3 µm filter and passed through a 22 gauge needle, before use. The cultures were inoculated with 10<sup>4</sup> tachyzoites/well in the same media. The plates were incubated at 37 °C and read daily in a molecular devices fluorescence plate reader. To preserve sterility the plates were read with covered lids, and both excitation (510 nm) and emission (540 nm) were read from the bottom.<sup>59</sup> For the calculation of the EC<sub>50</sub>, the percent of growth inhibition was plotted as a function of drug concentration by fitting the values to the function:  $I = I_{max} C/(EC_{50} + C)$ , where *I* is the percent inhibition,  $I_{max} = 100\%$  inhibition, *C* is the concentration of the inhibitor, and EC<sub>50</sub> is the concentration for 50% growth inhibition. There was no evident cytotoxicity on the host cells with any of the drugs tested (visual assay).

## 6. Computational methods

All the DFT calculations were performed using the GAUSSIAN09 program,<sup>60</sup> the B3LYP<sup>61</sup> functional and the 6-311+G(d,p) basis set. Calculations were carried out with full geometry optimization, using standard termination conditions, and including in all cases the effect of the solvent (water) through the Tomasi's polarized continuum model (PCM)<sup>62</sup> as implemented in GAUSSIAN09. Several different input geometries were used in order to find the most important conformers; the Mg atom always appeared coordinated to two oxygen atoms, and the hydrogen bonding usually drove the energy minimization processes.

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### Supplementary data

Supplementary data associated (copies of the <sup>1</sup>H NMR, <sup>13</sup>C NMR and <sup>31</sup>P NMR spectra as well as Cartesian coordinates of compounds **12a** and **27a**) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.11.010.

#### **References and notes**

- 1. Sun, S.; McKenna, C. E. Expert Opin. Ther. Patents 2011, 21, 1433.
- 2. Montalvetti, A.; Bailey, B. N.; Martin, M. B.; Severin, G. W.; Oldfield, E.;
- Docampo, R. J. Biol. Chem. 2001, 276, 33930.
  Montalvetti, A.; Fernandez, A.; Sanders, J. M.; Ghosh, S.; Van Brussel, E.; Oldfield, E.; Docampo, R. J. Biol. Chem. 2003, 278, 17075.
- 4. Moreno, S. N. J.; Li, Z.-H. Expert Opin. Ther. Targets **2008**, 12, 253.
- Ling, Y.; Li, Z.-H.; Miranda, K.; Oldfield, E.; Moreno, S. N. J. Biol. Chem. 2007, 282,
- 30804. 6. Li. Z.-H.: Cintrón, R.: Koon, N. A.: Moreno, S. N. I. *Biochemistry* 2
- Li, Z.-H.; Cintrón, R.; Koon, N. A.; Moreno, S. N. J. Biochemistry 2012, 51, 7533.
   Blanchard, L.; Karst, F. Gene 1993, 125, 185.
- Bianchard, E., Karst, F. Oche 1999, 120, 100.
   Song, L.; Poulter, C. D. Proc. Natl. Acad. Sci. U.S.A. 1994, 91, 3044.
- 9. Ogura, K.; Koyama, T. *Chem. Rev.* **1998**, 98, 1263.
- 10. Rodan, G. A. Annu. Rev. Pharmacol. Toxicol. 1998, 38, 375.
- 11. Docampo, R.; Moreno, S. N. J. Cell Calcium 2011, 50, 113.
- Urbina, J. A.; Moreno, B.; Vierkotter, S.; Oldfield, E.; Payares, G.; Sanoja, C.; Bailey, B. N.; Yan, W.; Scott, D. A.; Moreno, S. N. J.; Docampo, R. J. Biol. Chem. 1999, 274, 33609.
- Martin, M. B.; Grimley, J. S.; Lewis, J. C.; Heath, H. T., III; Bailey, B. N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J. A.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. J. Med. Chem. 2001, 44, 909.
- Yardley, V.; Khan, A. A.; Martin, M. B.; Slifer, T. R.; Araujo, F. G.; Moreno, S. N. J.; Docampo, R.; Croft, S. L.; Oldfield, E. Antimicrob. Agents Chemother. 2002, 46, 929.

- Martin, M. B.; Sanders, J. M.; Kendrick, H.; de Luca-Fradley, K.; Lewis, J. C.; Grimley, J. S.; Van Brussel, E. M.; Olsen, J. R.; Meints, G. A.; Burzynska, A.; Kafarski, P.; Croft, S. L.; Oldfield, E. J. Med. Chem. 2002, 45, 2904.
- 16. Rodriguez, J. B.; Szajnman, S. H. Expert Opin. Ther. Patents 2012, 22, 311.
- Garzoni, L. R.; Waghabi, M. C.; Baptista, M. M.; De Castro, S. L.; Meirelles Mde, N.; Britto, C. C.; Docampo, R.; Oldfield, E.; Urbina, J. A. Int. J. Antimicrob. Agents 2004, 23, 286.
- Bouzahzah, B.; Jelicks, L. A.; Morris, S. A.; Weiss, L. M.; Tanowitz, H. B. *Parasitol. Res.* 2005, 96, 184.
- Szajnman, S. H.; García Liñares, G. E.; Li, Z.-H.; Galizzi, M.; Jiang, C.; Bontempi, E.; Ferella, M.; Moreno, S. N. J.; Docampo, R.; Rodriguez, J. B. *Bioorg. Med. Chem.* 2008, 16, 3283.
- Rosso, V. S.; Szajnman, S. H.; Malayil, L.; Galizzi, M.; Moreno, S. N. J.; Docampo, R.; Rodriguez, J. B. *Bioorg. Med. Chem.* **2011**, *19*, 2211.
- Szajnman, S. H.; Bailey, B. N.; Docampo, R.; Rodriguez, J. B. Bioorg. Med. Chem. Lett. 2001, 11, 789.
- 22. Szajnman, S. H.; Montalvetti, A.; Wang, Y.; Docampo, R.; Rodriguez, J. B. Bioorg. Med. Chem. Lett. 2003, 13, 3231.
- 23. Szajnman, S. H.; Ravaschino, E. L.; Docampo, R.; Rodriguez, J. B. Bioorg. Med. Chem. Lett. 2005, 15, 4685.
- 24. Ling, Y.; Sahota, G.; Odeh, S.; Chan, J. M. W.; Araujo, F. G.; Moreno, S. N. J.; Oldfield, E. J. Med. Chem. 2005, 48, 3130.
- 25. Szajnman, S. H.; Rosso, V. S.; Malayil, L.; Smith, A.; Moreno, S. N.; Docampo, R.; Rodriguez, J. B. Org. Biomol. Chem. 2012, 10, 1424.
- Cinque, G. M.; Szajnman, S. H.; Zhong, L.; Docampo, R.; Schvartzapel, A. J.; Rodriguez, J. B.; Gros, E. G. J. Med. Chem. 1998, 41, 1540.
- Recher, M.; Barboza, A. P.; Li, Z.-H.; Galizzi, M.; Ferrer-Casal, M.; Szajnman, S. H.; Docampo, R.; Moreno, S. N. J.; Rodriguez, J. B. *Eur. J. Med. Chem.* **2013**, *60*, 431.
- Singh, A. P.; Zhang, Y.; No, J.-H.; Docampo, R.; Nussenzweig, V.; Oldfield, E. Antimicrob. Agents Chemother. 2010, 54, 2987.
- No, J. H.; de Macedo Dossin, F.; Zhang, Y.; Liu, Y.-L.; Wei Zhu, W.; Feng, X.; Yoo, J. A.; Lee, E.; Wang, K.; Hui, R.; Freitas-Junior, L. H.; Oldfield, E. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 4058.
- Aripirala, S.; Szajnman, S. H.; Jakoncic, J.; Rodriguez, J. B.; Docampo, R.; Gabelli, S. B.; Amzel, L. M. J. Med. Chem. 2012, 55, 6445.
- 31. Laskovics, F. M.; Poulter, C. D. Biochemistry 1981, 20, 1893.
- 32. Poulter, C. D.; Argyle, J. C.; Mash, E. A. J. Biol. Chem. 1978, 253, 7227.
- Van Beek, E.; Löwik, C.; Que, I.; Papapoulos, S. J. Bone Miner. Res. 1996, 11, 1492.
   Ebetino, F. H.; Francis, M. D.; Rogers, M. J.; Russell, R. G. G. Rev. Contemp. Pharmacother. 1998, 9, 233.
- 35. Jung, A.; Bisaz, S.; Fleisch, H. Calcif. Tissue Res. 1973, 11, 269.
- Matczak-Jon, E.; Kurzak, B.; Kamecka, A.; Kafarski, P. *Polyhedron* 2002, *21*, 321.
   Gumienna-Kontecka, E.; Jezierska, J.; Lecouvey, M.; Leroux, Y.; Kozlowski, H. J.
- Inorg. Biochem. 2002, 89, 13.
  Gumienna-Kontecka, E.; Silvagni, R.; Lipinski, R.; Lecouvey, M.; Marincola, F. C.; Crisponi, G.; Nurchi, V. M.; Leroux, Y.; Kozlowski, H. Inorg. Chim. Acta 2002, 339, 111
- 39. Marma, M. S.; Xia, Z.; Stewart, C.; Coxon, F.; Dunford, J. E.; Baron, R.; Kashemirov, B. A.; Ebetino, F. H.; Triffit, J. T.; Russell, R. G. G.; McKenna, C. E. J. Med. Chem. 2007, 50, 5967.

- 40. McKenna, C. E.; Shen, P.-D. J. Org. Chem. 1981, 46, 4573.
- 41. Berkowitz, D. B.; Bose, M. J. Fluorine Chem. 2001, 112, 13.
- 42. Blackburn, G. M.; England, D. A.; Kolkmann, F. Chem. Commun. 1981, 930.
- 43. Romanenko, V. D.; Kukhar, V. P. Chem. Rev. 2006, 106, 3868.
- Roelofs, A. J.; Thompson, K.; Ebetino, F. H.; Rogers, M. J.; Coxon, F. P. Curr. Pharm. Des. 2010, 16, 2950.
- 45. Reszka, A. A.; Rodan, G. A. Mini.-Rev. Med. Chem. 2004, 4, 711.
- 46. Russell, R. G. G.; Rogers, M. J. Bone 1999, 25, 97.
- 47. Huang, C.-H.; Gabelli, S. B.; Oldfield, E.; Amzel, L. M. Proteins 2010, 78, 888.
- Cao, R.; Chen, C. K.-M.; Guo, R.-T.; Wang, A. H.-J.; Oldfield, E. *Proteins* 2008, *73*, 431.
   Gabelli, S. B.; McLellan, J. S.; Montalvetti, A.; Oldfield, E.; Docampo, R.; Amzel, L.
- Gabelli, S. B.; McLellan, J. S.; Montalvetti, A.; Oldneid, E.; Docampo, R.; Amzel, L. M. Proteins 2006, 62, 80.
- Zuliani, V.; Carmi, C.; Rivara, M.; Fantini, M.; Lodola, A.; Vacondio, F.; Bordi, F.; Plazzi, P. V.; Cavazzoni, A.; Galetti, M.; Alfieri, R. R.; Petronini, P. G.; Mor, M. Eur. J. Med. Chem. 2009, 44, 3471.
- 51. Kruijtzer, J. A. W.; Liskamp, R. M. J. Tetrahedron Lett. 1995, 6969.
- Kieczykowski, G. R.; Jobson, R. B.; Melillo, D. G.; Reinhold, D. F.; Grenda, V. J.; Shinkai, I. J. Org. Chem. 1995, 60, 8310.
- Demoro, B.; Caruso, F.; Rossi, M.; Benítez, D.; Gonzalez, M.; Cerecetto, H.; Parajón-Costa, B.; Castiglioni, J.; Galizzi, M.; Docampo, R.; Otero, L.; Gambino, D. J. Inorg. Biochem. **2010**, 104, 1252.
- 54. Pan, Z.; Chen, J.; Lu, G.; Geng, Y.-Z.; Zheng, H.; Ji, Q. J. Chem. Phys. 2012, 136, 164313.
- 55. Swiatla-Wojcik, D. Chem. Phys. 2007, 342, 260.
- Delain-Bioton, L.; Turner, A.; Lejeune, N.; Villemin, D.; Hix, G. B.; Jaffrès, P.-A. Tetrahedron 2005, 61, 6602.
- Canavaci, A. M.; Bustamante, J. M.; Padilla, A. M.; Pereza Brandan, C. M.; Simpson, L. J.; Xu, D.; Boehlke, C. L.; Tarleton, R. L. *PLoS Negl. Trop. Dis.* 2010, 4, e740.
- 58. Gubbels, M. J.; Li, C.; Striepen, B. Antimicrob. Agents Chemother. 2003, 43, 309.
- Agrawal, S.; van Dooren, G. G.; Beatty, W. L.; Striepen, B. J. Biol. Chem. 2009, 284, 33683.
- GAUSSIAN 09, Revision C.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Comperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. GAUSSIAN Inc., Wallingford CT, 2009.
- (a) Lee, C.; Yang, W.; Parr, R. G. Phys. Rev. B. 1988, 37, 785; (b) Becke, A. D. J. Chem. Phys. 1993, 98, 5648.
- 62. Tomasi, J.; Mennucci, B.; Cammi, R. Chem. Rev. 2005, 105, 2999.