European Journal of Medicinal Chemistry 60 (2013) 431–440

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and biological evaluation of sulfur-containing 1,1-bisphosphonic acids as antiparasitic agents

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ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form
6 December 2012
Accepted 10 December 2012
Available online 20 December 2012

Keywords:
Farnesyl diphosphate synthase
Trypanosoma cruzi
Toxoplasma gondii
Chagas disease
Toxoplasmosis
Antiparasitic agents

ABSTRACT

As part of our efforts aimed at searching for new antiparasitic agents, 2-alkylmercaptoethyl-1,1-bisphosphonate derivatives were synthesized and evaluated against *Trypanosoma cruzi*, the etiologic agent of Chagas disease, and *Toxoplasma gondii*, the responsible agent for toxoplasmosis. Many of these sulfur-containing bisphosphonates were potent inhibitors against the intracellular form of *T. cruzi*, the clinically more relevant replicative form of this parasite, and tachyzoites of *T. gondii* targeting *T. cruzi* or *T. gondii* farnesyl diphosphate synthases (FPPSs), which constitute valid targets for the chemotherapy of these parasitic diseases. Interestingly, long chain length sulfur-containing bisphosphonates emerged as relevant antiparasitic agents. Taking compounds **37**, **38**, and **39** as representative members of this class of drugs, they exhibited ED_{50} values of 15.8 μM, 12.8 μM, and 22.4 μM, respectively, against amastigotes of *T. cruzi*. These cellular activities matched the inhibition of the enzymatic activity of the target enzyme (*Tc*FPPS) having IC_{50} values of 6.4 μM, 1.7 μM, and 0.097 μM, respectively. In addition, these compounds were potent anti-*Toxoplasma* agents. They had ED_{50} values of 2.6 μM, 1.2 μM, and 1.8 μM, respectively, against *T. gondii* tachyzoites, while they exhibited a very potent inhibitory action against the target enzyme (*Tg*FPPS) showing IC_{50} values of 0.024 μM, 0.025 μM, and 0.021 μM, respectively. Bisphosphonates bearing a sulfoxide unit at C-3 were also potent anti-*Toxoplasma* agents, particularly those bearing long aliphatic chains such as **43**–**45**, which were also potent antiproliferative drugs against tachyzoites of *T. gondii*. These compounds inhibited the enzymatic activity of the target enzyme (*Tg*FPPS) at the very low nanomolar range. These bisphosphonic acids have very good prospective not only as lead drugs but also as potential chemotherapeutic agents.

1. Introduction

The isosteric replacement of the oxygen atom bridge of inorganic pyrophosphate (**1**) with substituted methylene groups gives rise to a class of drugs known as bisphosphonates (**2**) [1], which became compounds of pharmacological importance since calcification studies carried out many decades ago [2–4]. Several bisphosphonates such as, pamidronate (**3**), alendronate (**4**) and risedronate (**5**) are in clinical use for the treatment and prevention of osteoclast-mediated bone resorption associated with various bone disorders (Fig. 1) [5–8].

Besides their use in long-term treatment of different bone disorders, bisphosphonates exhibit a wide range of biological activities, such as antibacterial agents [9], anticancer agents [10–13], as selective inhibitors of acid sphingomyelinase [14], in stimulation of γδ T cells [15], and, particularly, as antiparasitic agents [16–20]. Some years ago, selected bisphosphonates, comprising the FDA-approved pamidronate (**3**) and alendronate (**4**), were found to be potent inhibitors of *Trypanosoma cruzi* proliferation in *in vitro* and *in vivo* assays without toxicity to the host cells [21]. Based on the previous findings, other bisphosphonates were found to be potent antiproliferative agents against other trypanosomatids such as *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, and *L. mexicana*, and Apicomplexans such as *Toxoplasma gondii* and *Plasmodium falciparum* [17–20].

Bone mineral has a similar mineral composition than acidocalcisomes, which are acidic organelles of high-density with a high concentration of phosphorus present as pyrophosphate and polyphosphate, which is associated to calcium and other cations. Then, it is reasonable to anticipate that accumulation of bisphosphonates in

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http://dx.doi.org/10.1016/j.ejmech.2012.12.015

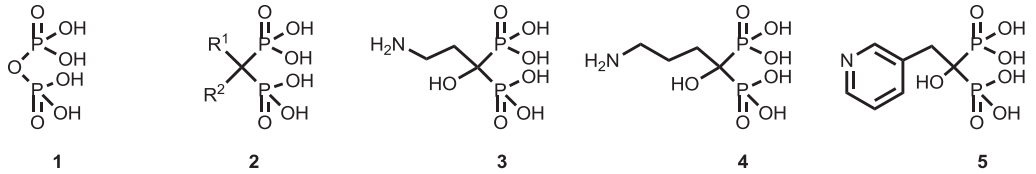

Fig. 1. . General formula and chemical structure of representative FDA-approved bisphosphonates clinically employed for the treatment of bone disorders.

these organelles facilitates their antiparasitic action [22,23]. FPPS catalyzes the two committed biosynthetic steps to form farnesyl diphosphate from dimethylallyl and isopentenyl diphosphates [20,21].

Bisphosphonates derived from fatty acids are promising antiparasitic agents, in particular, 2-alkyl(amino)ethyl derivatives. These compounds exhibit 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 [24,25]. In addition, 1-hydroxy-, 1-alkyl-, and 1-amino-bisphosphonates such as **6**–**9** have been mainly useful in SAR studies as antiparasitic agents [26–29]. For example, bisphosphonate **6** is a potent growth inhibitor against *T. cruzi* (amastigotes) [26] and also against *T. gondii* (tachyzoites) [29,30], while **7** is effective against *P. falciparum* [30]. Compounds **10** and **12** have cellular activity against *T. gondii*, the latter one being unusually effective against the target enzyme (IC_{50} = 93 nM) [30,31]. In addition, in contrast to what would be expected, α-fluoro-1,1-bisphosphonates are devoid of activity against *T. cruzi* cells and *Tc*FPPS regardless of the chain length [31]. However, these compounds behave as extremely potent inhibitors of the enzymatic activity of *T. gondii* FPPS [31]. Actually, **13** and **14** possess IC_{50} values of 35 nM and 60 nM, respectively, toward *Tg*FPPS, that is, they are even more effective than risedronate (IC_{50} = 74 nM) used as positive control (Fig. 2) [31]. The high selectivity observed by these drugs toward *Tg*FPPS versus *Tc*FPPS is not surprising bearing in mind that the amino acid sequences of these enzymes have less than 50% identity [20].

Trypanosoma cruzi and *Toxoplasma gondii* are the etiologic agents of American trypanosomiasis (Chagas disease) and toxoplasmosis, respectively, two major parasitic diseases according to the World Health Organization [20,21]. Chemotherapy for this two parasitic diseases, based on empirically discovered drugs, is still a challenge [23,32–34]. *T. cruzi* has a complex life cycle involving blood-sucking Reduviid insects and mammals [35]. This parasite has four main morphological forms and the amastigote form is the more relevant replicative form of the parasite [35]. This blood-sucking activity is the main way of dissemination of Chagas disease, while infection *via* the placenta or by blood transfusion is the mechanism responsible where this

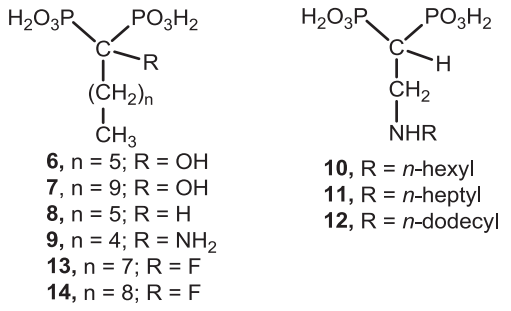

Fig. 2. Chemical structure of representative members of bisphosphonic acids derived from fatty acids.

disease is not endemic [36]. The opportunistic parasite *T. gondii* is able to infect humans (basically all warm-blooded mammals) by contact with feces of infected cats, by eating undercooked meat or *via* the placenta from pregnant women [37,38]. Two asexual forms are able to affect humans: the tachyzoite form can invade cells and multiplies leading to host cell death, while the bradyzoite form proliferates slowly and forms cysts in muscle [39]. The main goal in toxoplasmosis is to develop a drug that is able to eliminate the cyst stage of the parasite to avoid recrudescence of the disease [20].

2. Rationale

In the last years, many efforts have been made to understand how bisphosphonic acids inhibit FPPS at the molecular level [40–42]. Recently, we were able to determine that *Tc*FPPS inhibitors **10** and **11** bind to the allylic site of the enzyme [43] with the phosphate groups of the bisphosphonate moiety coordinating three Mg^{2+} atoms that bridge the compound to the enzyme in a similar way that was observed for the physiological substrates [44,45]. The nitrogen atom at the C-3 position is very important to maintain a high degree of biological activity.

Analyses of the 2-alkylaminoethyl-1,1-bisphosphonates–*Tc*FPPS complexes have indicated that methyl substitution at the *N*-linked carbon of the alkyl chain would be favorable for binding [43]. Then, **18** was envisioned for this purpose (Scheme 1). In addition, in order to study a potential synergistic effect, it was considered to add a hydroxyl group at C-1, present in many pharmacological important bisphosphonic acids, in the reference structure **11** to afford the 2-alkylaminoethyl-1-hydroxy-1,1-bisphosphonic acid **21**.

To assess the necessity of the amine group for inhibitory activity against *T. cruzi* or *T. gondii*, as well as their corresponding target enzymes *Tc*FPPS and *Tg*FPPS, we decided to replace it for a sulfide, sulfoxide, sulfone and methylalkylsulfonium group.

3. Results and discussion

Preparation of the methyl analog of the lead structure **10** (compound **18**) was conducted according to previously published procedures [24,25]. Briefly, the versatile Michael acceptor **16** [46–48], which was straightforwardly obtained from commercially available tetraethyl methylenebis(phosphonate) (**15**), was reacted with 2-heptylamine in methylene chloride to afford the Michael adduct **17**. This compound was hydrolyzed by treatment with bromotrimethylsilane in methylene chloride followed by digestion with methanol [49] to afford the free bisphosphonic acid **18**. Additionally, the 1-[(*n*-alkylamino)ethyl]-1-hydroxy-1,1-bisphosphonic acid derivative **21** was readily prepared from *n*-heptylamine. Coupling reaction between this compound and benzyl bromoacetate in acetonitrile [50] afforded the expected benzyl *n*-alkylaminoacetate **19** in 84% yield, which was hydrogenated employing palladium on charcoal as catalyst to yield the free acid **20** in 67% yield, which was the substrate to form the title compound **21**. Then, on treatment with phosphorous acid and phosphorous trichloride employing benzenesulfonic acid as a solvent at 65 °C followed by hydrolysis, **20** was