Bioorganic & Medicinal Chemistry Letters 24 (2014) 49–53

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of α-hydroxyalkylphosphonates as new antimicrobial agents

Ana Maria Faísca Phillips [a,*], Maria Teresa Barros [a,*], Marta Pacheco [b], Ricardo Dias [b]

[a] Department of Chemistry, CQFB-REQUIMTE, Faculty of Sciences and Technology, Universidade Nova de Lisboa, Campus de Caparica, Quinta da Torre, 2829-516 Caparica, Portugal
[b] Microbiology and Biotechnology Laboratory, Center for Biodiversity, Functional and Integrative Genomics, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 4 November 2013
Revised 29 November 2013
Accepted 1 December 2013
Available online 8 December 2013

Keywords:
Phosphonates
Antimicrobial activity
Clinical isolates
Gram-positive
Gram-negative

ABSTRACT

A set of α-quaternary 3-chloro-1-hydroxyalkylphosphonates, analogues of fosfomycin and fosfonochlorin, some of which are new compounds, was synthesized. The compounds were screened for bioactivity against several clinical and standard microbial isolates. Some were found to have moderate activity. The activity was higher with phenyl protection of the phosphoryl ester groups and α-phenyl substitution. Compound **11** was as effective or more potent than fosfomycin or chloramphenicol against several Gram-negative bacteria as well as against some Gram-positive ones.

Antibiotics are in great demand. Although many successes have been achieved in the fight against infectious diseases in the last century, the continuous development of resistance by bacteria to the current antibiotics, and even the development of multidrug resistant strains, has left us with a 'rather bare antibiotic cupboard to meet the challenges of new outbreaks'.[1] Bacterial infections are still a major cause of death in the developing world. Another problem is resistant bacteria from hospitals, which cause 'community-acquired' infections, difficult to treat, particularly in immuno-compromised patients. These resistant bacteria have also acquired toxins that make them more virulent. Examples are Gram-positive bacteria, like *Staphylococcus aureus* and *Enterococcus* spp., which frequently display multidrug resistance,[2] and Gram-negative *Stenotrophomonas maltophilia*. Drug resistance develops as a result of gene mutations, rearrangements and genetic transfer between different bacteria.[3] These are serious problems to populations and to public health systems due to the morbidity and mortality they cause, and the costs related to the implementation of effective control measures, and led the World Health Organization to select 'antimicrobial resistance' as the theme for World Health Day 2011.

The development of effective antimicrobial control measures involves both a search for novel compounds and finding new uses for older drugs. One revival is fosfomycin, a phosphonic acid derivative with a broad spectrum of activity, used mainly for the treatment of uncomplicated urinary tract and gastrointestinal infections.[4] Recent studies showed its potential for the treatment of mutidrug-resistant, including extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae infections[5] like those caused by *Escherichia coli* and *Klebsiella pneumoniae*, and other Gram-negative species such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[6,7] In fact fosfomycin is one of the very few antibiotics presently available to treat Gram-negative infections,[8] besides colistin and the new tigecycline and doripenem.[4] Fosfomycin acts by inactivating the enzyme UDP-*N*-acetylglucosamine enolpyruvyl transferase (Mur A), thereby irreversibly blocking condensation of uridine diphosphate-*N*-acetylglucosamine with phosphoenolpyruvate, one of the first steps in the peptidoglycan bacterial cell wall synthesis. Normally it is used together with another antibiotic to prevent the development of resistance, and it is compatible to many.

We have been interested in the chemistry of phosphonic acid derivatives for the past few years.[9] Many compounds of this class are biologically active, due to their structural similarity to phosphates and carboxylates, competing with them for enzyme binding sites.[10] Examples of other potent phosphonate antibiotics are fosmidomycin, which is also a potent antimalarial, plumbemycin A and fosfonochlorin, an antibiotic with spheroplast-forming activity isolated from cultures of *Fusarium* sp., moderately active against some species of Gram-negative bacteria (Fig. 1).[11]

Inspired by the structure of fosfomycin, we decided to synthesize analogues that could have antimicrobial activity. It is known

* Corresponding authors. Tel./fax: +351 212948300.
E-mail addresses: amfp@fct.unl.pt, ana.faisca@fct.unl.pt (A.M. Faísca Phillips), mtb@fct.unl.pt (M.T. Barros).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.12.002

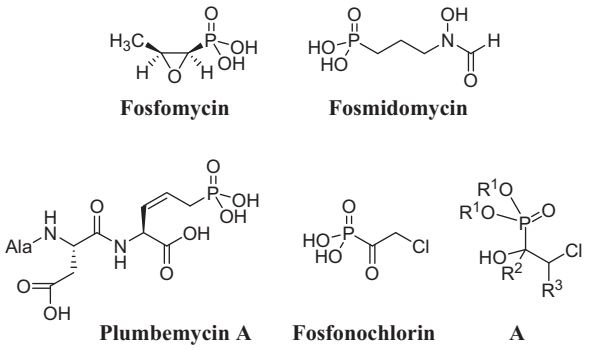


Figure 1. Phosphonate antibiotics.

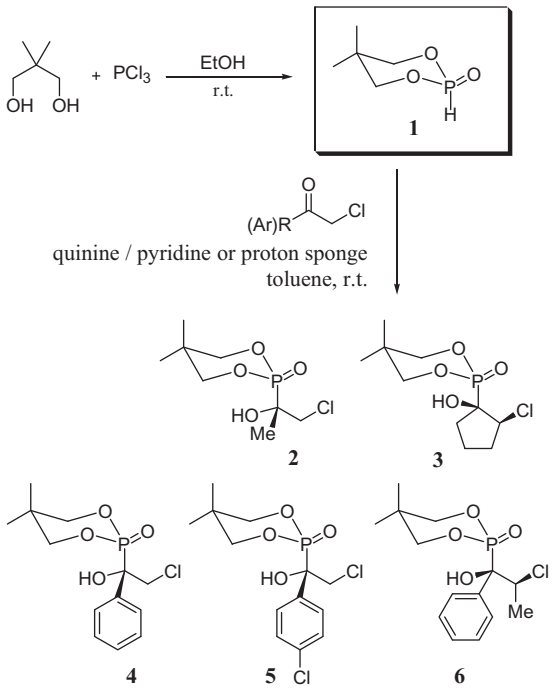
that the enzymes that cause resistance to fosfomycin, namely, Fos A, Fos B, and Fos X, function by nucleophilic attack at the carbon atom α to phosphorus.[12] If this centre were to be a quaternary carbon atom, reaction at this position would become more difficult, and interesting biological activity may result. We recently developed an organocatalyzed method for the synthesis of β-chloro-α-quaternary hydroxyphosphonates **A**, which are also structurally related to fosfonochlorin.[13] There are existing examples of biologically active α-hydroxyphosphonic acids which are enzymes inhibitors, but not of biologically active α-quaternary β-chloro-α-hydroxyphosphonates.[13] We produced a set of these compounds to screen for potential bioactivity and for structure-activity relationship studies. In this paper we report the synthesis and characterization of some new phosphonates, not previously described in the literature, and our results on the evaluation of the full set for potential antimicrobial activity.

The synthesis of the required α-hydroxyphosphonates was based on a method recently developed by us,[13] which involves an organocatalyzed regioselective modified Pudovik reaction between a dialkylphosphite and a α-haloketone.[14,15] This reaction may be carried out under mild conditions using catalytic amounts of quinine in the presence of a stoichiometric base such as proton-sponge or pyridine.[13] When these conditions are used, β-chloro-α-hydroxyphosphonates are obtained. The reaction is compatible with aliphatic, cyclic or aromatic α-haloketones. Since phosphonic acids are negatively charged at nearly all physiological pH values,[16] compounds bearing these groups are very polar. High polarity can be a set back in a drug, preventing efficient delivery to target organs, since highly ionized species do not pass readily across mucosal and cellular membranes. The volume of distribution and bioavailability will be relatively low. In drugs, phosphonates and phosphates are usually derivatized as neutral esters that can break down in the body to release the parent drug, since this modification alters membrane permeability and improves oral (GI permeability), brain, tumour and cellular delivery. A few studies have shown the influence of the nature of the phosphonate ester groups on drug delivery and bioavailability. For example, the acyclic nucleotide analogue 9-[2-(phosphonomethoxy)ethoxy]adenine, a potent and selective inhibitor of the human immunodeficiency virus-1 (HIV-1) and immunodeficiency virus-2 (HIV-2), has very low bioavailability.[17] Derivatization as bis[pivaloyloxy) methyl]ester or diphenyl ester provide good oral bioavailability.[17] A similar effect of the phenyl group was shown in Adefovir, 9-[2-(phosphonomethoxy)ethyl]adenine, a nucleotide analogue used clinically to treat chronic hepatitis B virus infection. It also enhanced the in vitro activity against HSV-2 (G-strain) when compared with the unprotected drug (IC_{50} = 77 μM vs 119 μM in the unprotected drug).[18] With the endopeptidase inhibitor CGS 25462, diphenyl ester derivatization provided a 200-fold increase of the active drug in the plasma above its IC_{50} value.[19]

We chose two different types of ester protecting groups: cyclic phosphonate esters, which are usually more stable than the analogous alkyl esters, and phenyl phosphonate esters which are much more labile.[17,20] The two series of hydroxyphosphonates to be screened were prepared as shown in Schemes 1 and 2.

For the phosphonates shown in Scheme 1, the intermediate cyclic phosphite ester **1** needed was obtained via the reaction of 2,2-dimethyl-1,3-propanediol with phosphorus trichloride in the presence of ethanol, according to a previously published procedure.[21] Hydroxyphosphonates **2**–**6** were obtained after the modified Pudovik reaction (Scheme 1). Diphenyl phosphite is commercially available, and with it hydroxyphosphonates **8**–**12** could be synthesized (Scheme 2). 2-Chloroindanone, needed for the synthesis of **12**, was obtained via another organocatalyzed reaction, a thiourea-catalyzed α-chlorination, using *N*-chlorosuccinimide as the halogen source.[22] To the best of our knowledge, phosphonates **9**, **11** and **12** have not been previously described in the literature.[23]

In the synthesis of compounds **9**, **11** and **12**, the desired product was obtained as the major product, together with small amounts of enolphosphate, as determined by ^{31}P NMR spectroscopy. In each case there was also usually, in addition, an extra signal at δ = 128.5 ppm, due to unreacted starting material in the phosphite form (<5%).[24] Phosphonates **9** and **12** were obtained as mixtures of diastereoisomers in approximately a 90:10 ratio. The major diastereoisomers were isolated by chromatography on silica gel and subsequently recrystallized. Their structures were established by one- and two-dimensional NMR spectroscopic techniques and by infrared spectroscopy. Their composition was confirmed by elemental analysis. In the case of cyclopentanol derivatives **9** and **12**, NOESY experiments provided some information (included in the Supplementary data section). C-1 is quaternary and the hydroxyl proton did not appear as an independent signal in the spectrum showing coupling to the C-2 proton. It appears as a very broad singlet in the spectra of most of the compounds in these series. The nearest protons which could show cross peaks with H-2 and hence help to establish the stereochemistry at C-2 are the aromatic protons on the phosphorus ester substituents. They also showed no cross peaks with H-2, but this fact cannot be taken unambiguosly



Scheme 1. Synthesis of cyclic alkylphosphonate esters.