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Original article

The effect of novel [3-fluoro-(2-phosphonoethoxy)propyl]purines on the inhibition of *Plasmodium falciparum*, *Plasmodium vivax* and human hypoxanthine–guanine–(xanthine) phosphoribosyltransferases

Ondřej Baszczyňski [a], Dana Hocková [a], Zlatko Janeba [a,*], Antonín Holý [a], Petr Jansa [a], Martin Dračínský [a], Dianne T. Keough [b], Luke W. Guddat [b]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, CZ-166 10 Prague 6, Czech Republic
[b] The School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072 QLD, Australia

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ABSTRACT

Protozoan parasites from the *Plasmodiidae* family are the causative agents of malaria. Inhibition of hypoxanthine–guanine–(xanthine) phosphoribosyltransferase (HG(X)PRT) has been suggested as a target for development of new anti-malarial therapeutics. Acyclic nucleoside phosphonates (ANPs) are potent and selective inhibitors of plasmodial HG(X)PRTs. A new series of ANPs, based on the chemical structure and inhibitory activity of three ANPs, 2-(phosphonoethoxy)ethyl with either guanine or hypoxanthine as the base (PEEG and PEEHx) and 3-hydroxy-2-(phosphonomethoxy)propyl with guanine as the base (HPMPG), were prepared. These compounds are stereoisomers of 3-fluoro-(2-phosphonoethoxy)propyl (FPEPs) and 3-fluoro-(2-phosphonomethoxy)propyl (FPMPs) analogues. Both the (*R*)- and (*S*)-isomers of these fluorinated derivatives have higher K_i values (by 10- to 1000-fold) for human HGPRT and *Plasmodium falciparum* HGXPRT than the non-fluorinated ANPs. Possible explanations for these changes in affinity are proposed based on docking studies using the known crystal structures of human HGPRT in complex with PEEG.

1. Introduction

Malaria continues to be a serious life-threatening disease leading to immense human suffering and mortality. The WHO global burden report (2010) suggests that there are 225 million new malaria cases each year with an estimated one million fatalities. The causative agent for malaria is the protozoan parasite family *Plasmodiidae* with the *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) species responsible for the majority of infections [1–3]. Traditional drug therapeutics for the treatment of malaria include chloroquine [4,5], atovaquone/proguanil (Malarone) and sulfadoxine/pyrimethamine (Fansidar). Current medications are based on artemisinin combination therapies (ACTs) [4,6] where artemisinin or its analogues are used together with other therapeutic drugs. These compounds do however have disadvantages including side effects [7], the emergence of drug-resistant strains of *Pf* and *Pv* [8,9], and the cost of manufacturing these compounds so that they can be used in countries where such expense cannot be met. There is, thus, a need for the development of new anti-malarial drugs to eradicate this disease.

Hypoxanthine–guanine–(xanthine) phosphoribosyltransferase (HG(X)PRT) [10] is an enzyme of the purine salvage pathway [11]. This enzyme catalyzes the formation of an *N*-glycosidic bond between the N^9 atom of purine base and the C^1 atom of α-D-5-phosphoribosyl-1-pyrophosphate (Fig. 1) [12].

The rationale for HG(X)PRT being an anti-malarial drug target is that the human host cell possesses two metabolic pathways to synthesize the nucleoside monophosphates essential for DNA and RNA production, but the malarial parasite possesses only one of these. For humans the two pathways are *de novo* synthesis (starting from small molecules) and salvage of preformed purine bases. The *Pf* and *Pv* parasites are unable to synthesize the purine ring and rely on the transport of these bases from the host cell to synthesize their nucleoside monophosphates. They are, therefore, dependent on this less energy utilizing salvage pathway. Thus, inhibition of the critical enzyme in this pathway (HG(X)PRT) should result in cessation of parasitic replication.

Acyclic nucleoside phosphonates (ANPs) are nucleotide analogues [13] containing a stable carbon–phosphorous bond instead

* Corresponding author. Tel.: +420 220 183 143; fax: +420 220 183 560.
E-mail addresses: janeba@uochb.cas.cz, zjaneba@yahoo.com (Z. Janeba).

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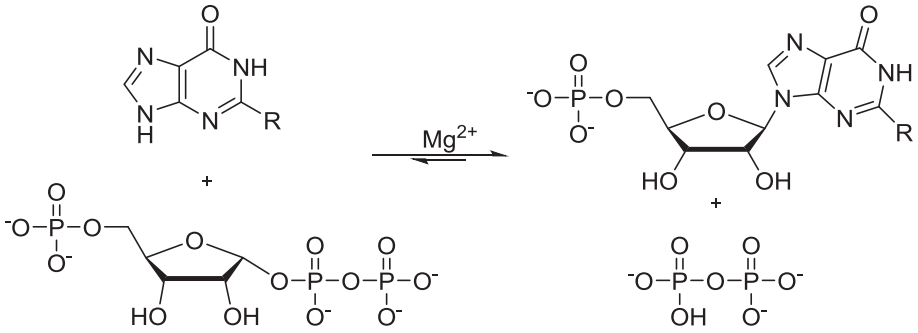

Fig. 1. The reactions catalyzed by the 6-oxopurine PRTases. The naturally occurring bases are: (a) guanine R = NH_2; (b) hypoxanthine R = H; (c) xanthine R = OH.

of a phosphate ester which can be hydrolyzed by esterases within the cell. Three ANPs (cidofovir, adefovir, tenofovir) are currently in use as antiviral agents [14] though their mode of action is different from that proposed for the ANPs described here. This different mechanism is due to two reasons: (i) the fact, that these antiviral agents do not contain a 6-oxopurine base attached to the phosphonate moiety; and (ii) such compounds inhibit either RNA reverse transcriptases or DNA polymerases. Several ANPs containing hypoxanthine and guanine bases connected to a phosphonate group with variable chemical linkers have been demonstrated to be good inhibitors of *Pf*HGXPRT and *Pv*HGPRT (Fig. 2) [15]. These compounds were the first HG(X)PRT inhibitors that selectively discriminate between human and the parasite enzymes. A broad spectrum of ANPs as inhibitors of human, *Pf* and *Pv* HG(X)PRTs have subsequently been investigated [16–20]. The chemical structures of the ANPs which form the basis of the new fluorinated inhibitors reported in this paper are given in Fig. 2 (K_i values in Table 1) [15]. The first such fluorinated analogue, racemic 9-[3-fluoro-2-(phosphonomethoxy)propyl]guanine (FPMPG) [21] was already reported [15] to have activity towards *Pf*HGXPRT in preliminary tests.

As the ANPs in Fig. 2, Table 1 are good inhibitors of *Pf*HGXPRT, fluorinated derivatives where $-CH_2F$ is attached to the phosphonate moiety were synthesized to test how this chemical change affected the affinity for these enzymes. Both the (*R*)- and (*S*)-isomers of these compounds were prepared. Here, we report the synthesis and inhibitory activity of the enantiomeric ANPs containing FPMP (**3**,**4**) and FPEP (**10**,**11**) phosphonate tails with two different 6-oxopurine bases, hypoxanthine or guanine. These compounds were subsequently docked into the known crystal structures of human HGPRT in complex with PEEG, PEEHx or HPMPG in an attempt to explain the differences in the K_i values.

2. Results and discussion

2.1. Chemistry

The starting compounds (**1**,**2**, **a**,**b**) for the synthesis of the target FPMPHx and FPMPG (**3**,**4**, **a**,**b**) were prepared according to a previously established procedure (Scheme 1) [22]. Two different methods were used to obtain the desired products (**3**,**4**, **a**,**b**). The first method, sequential hydrolysis of the starting compounds consisted of two separate steps without isolation of the intermediates. 6-Chloropurine derivatives (**1**,**2**, **a**,**b**) were treated with aqueous trifluoroacetic acid, followed by phosphonate ester cleavage using bromotrimethylsilane in acetonitrile [21]. The second method, a more convenient and direct approach to the synthesis of the corresponding 6-oxopurine phosphonic acids (**3**,**4**, **a**,**b**), was performed using microwave-assisted hydrolysis (Scheme 1) [23].

Novel compounds with elongated phosphonate tails FPEPHx and FPEPG (**10**,**11**, **a**–**c**) were prepared *via* an efficient stepwise synthesis (Scheme 2). Initially, the key intermediates (**7**, **a**–**c**) were synthesized from the fluorohydrines (**5**, **a**–**c**) which were prepared according to a previously published method [24]. Our previous experience [25] with the "oxa"-Michael additions of secondary alcohols to diethyl vinylphosphonate (DEVP) led us to apply this reaction for introducing the ethylphosphonate moiety to the fluorohydrines (**5**, **a**–**c**). O-Alkylation of **5**, **a**–**c** with the DEVP, under catalysis with KOH [26] or Cs_2CO_3, afforded compounds **6**, **a**–**c**. Trityl groups were removed by treatment with the ion exchange resin, Dowex D50 X 8 in H^+ cycle, to give the desired hydroxy derivatives (**7**, **a**–**c**).

Mitsunobu reaction of **7**, **a**–**c** with 6-chloropurine and 2-amino-6-chloropurine gave esters **8**,**9**, **a**–**c** (Scheme 3) in acceptable yields [27]. Additionally, in the case where 2-amino-6-chloropurine is the base, it was necessary to decompose the unwanted triphenylphosphine adducts at the end of the reaction. This reaction was performed by heating with the addition of the small amount of water for several hours. Diesters **8**,**9**, **a**–**c** were subsequently transformed to the corresponding 6-oxopurine phosphonic acids (**10**, **a**–**c**, Scheme 3), using the same procedures as described above for compounds **3**,**4**, **a**–**c** (Scheme 1).

In search for novel inhibitors of *Bordetella pertussis* adenylyl cyclase toxin (CyaA) and in order to study antiviral, antineoplastic, and immunomodulatory activities within this new class of ANP analogues we also prepared adenine, 6-cyclopropyl-2-aminopurine

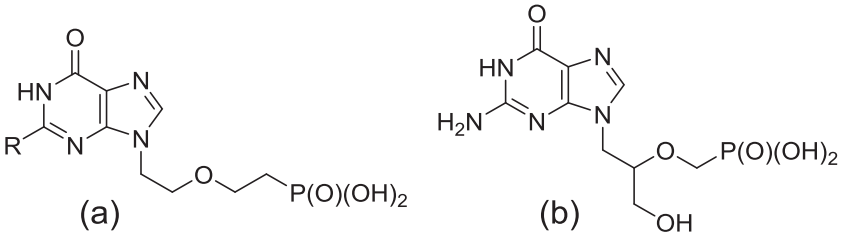

Fig. 2. Three ANP inhibitors of human HGPRT and *Pf*HGXPRT: (a) PEEHx where R = H; PEEG where R = NH_2; (b) (*RS*)-HPMPG.