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# Novel nucleotide analogues bearing (1*H*-1,2,3-triazol-4-yl)phosphonic acid moiety as inhibitors of *Plasmodium* and human 6-oxopurine phosphoribosyltransferases

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ABSTRACT

A novel family of acyclic nucleoside phosphonates (ANPs) bearing a (1*H*-1,2,3-triazol-4-yl)phosphonic acid group in the acyclic side chain have been prepared in order to study the influence of the hetaryl rigidizing element on the biological properties of such compounds. The key synthetic step consisted of a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) between diethyl ethynylphosphonate and the corresponding azidoalkyl precursor. Two ANPs in this family, bearing a guanine base, exhibited the highest potency for the human 6-oxopurine phosphoribosyltransferase irrespective of the stereochemistry on the C-2′ atom. Four compounds inhibited *Plasmodium falciparum* 6-oxopurine phosphoribosyltransferase with little differences in their $K_i$ values irrespective of whether the base was guanine, hypoxanthine or xanthine but only two, with guanine as base, inhibited *Pv*HGPRT.



## 1. Introduction

Acyclic nucleoside phosphonates (ANPs)[1] represent an important class of antimetabolites that mimic the naturally occurring nucleoside monophosphates. Extensive structure-activity relationship (SAR) studies have been carried out and several distinct classes of ANPs with diverse biological activities have been identified. 2-(Phosphonomethoxy)ethyl or PME (*e.g.* PMEA, Fig. 1), 2-(phosphonomethoxy)propyl or PMP, and 3-hydroxy-2-(phosphonomethoxy)propyl or HPMP (*e.g.* (*S*)-HPMPC, Fig. 1) analogues have antiviral properties,[2–4] and 2-(phosphonoethoxy)ethyl or PEE derivatives (such as PEEHx and PEEG, Fig. 1), bisphosphonates (Fig. 1) and aza-ANPs (Fig. 1) have antimalarial[5–9] and/or antimycobacterial[10,11] activity. Several different chemical types of ANPs, including modified PMEA analogues, have also been studied as potent inhibitors of bacterial adenylate cyclases, namely adenylate cyclase toxin from *Bordetella pertussis* and edema factor from *Bacillus anthracis*.[12–14] Such analogues may have potential for treatment or prevention of toxaemia caused by the invasion of these bacteria into the human host.

Inhibition of plasmodial hypoxanthine-guanine-(xanthine) phosphoribosyltransferases (HG(X)PRTs) by the ANPs is well-correlated with their antimalarial activity.[5–9] These enzymes catalyze the formation of the 6-oxopurine mononucleotides from the 6-oxopurine nucleobases and 5-phospho-α-D-ribosyl-1-pyrophosphate (Fig. 2).[15] HG(X)PRTs are key enzymes of the purine salvage pathway and since malarial parasites lack the *de novo* pathway for purine nucleotide synthesis, this enzyme is a validated target for the development of new antimalarials.[5–9] Importantly, the mode of action of the ANPs is different from the currently used drugs, so represents a new approach to developing antimalarial therapeutics.

Novel types of ANPs bearing (1*H*-1,2,3-triazol-4-yl)phosphonic acid group attached to the acyclic side chain (general structure **A**, Fig. 1) have been synthesized as a continuation of the extended SAR studies carried out by our group. Compounds of type **A** (Fig. 1) bearing 6-oxopurine bases were designed as potential inhibitors of plasmodial HG(X)PRTs since it has been reported[16] that the optimal length of the aliphatic linker between the nucleobase and the phosphonate group is 5 or 6 atoms (in contrast to antiviral ANPs

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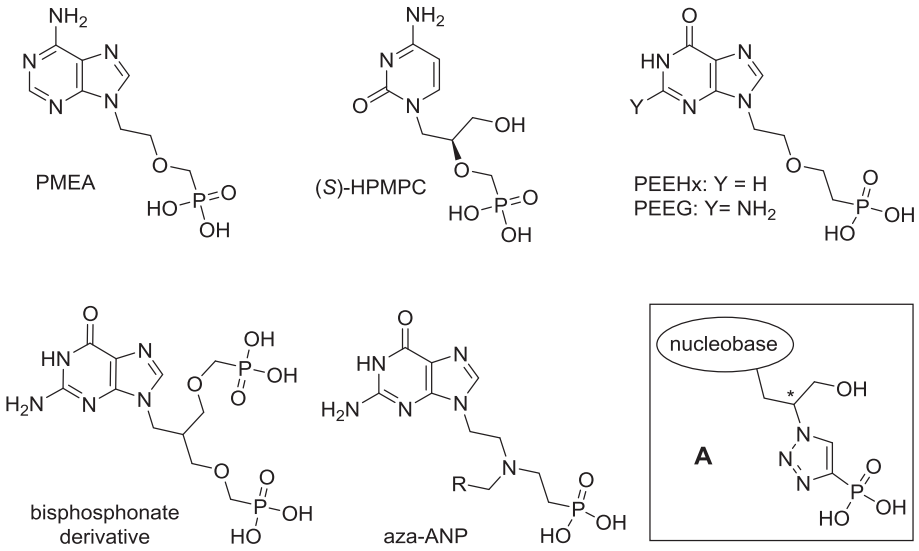


**Fig. 1.** Examples of chemical structures of biologically active acyclic nucleoside phosphonates (ANPs). Top row: The antiviral compounds PMEA and (*S*)-HPMPC, and antimalarial compounds PEEHx and PEEG. Bottom row: an antiplasmodial bisphosphonate, the general structure of the aza-ANPs, and the general scaffold of the newly designed (1*H*-1,2,3-triazol-4-yl)phosphonates **A**.

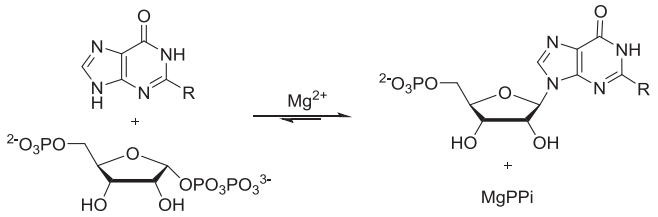


**Fig. 2.** Reaction catalyzed by the HG(X)PRTs. The naturally occurring bases are hypoxanthine (R = H), guanine (R = $NH_2$) and xanthine (R = OH).

with 4-atom-linkers). In comparison to the flexible PEE or modified PEE analogues which are potent HG(X)PRTs inhibitors, derivatives **A** (Fig. 1) have the 1*H*-1,2,3-triazol-4-yl moiety integrated into the acyclic chain to rigidify the linker, thus, possibly leading to increased affinity.

An efficient synthetic methodology to access the desired 6-oxopurine ANPs with the (1*H*-1,2,3-triazol-4-yl)phosphonic acid moiety has been developed and optimized. To show the full scope of this synthetic approach, the whole series of ANPs with various purine or pyrimidine bases attached were prepared in good overall yields.

## 2. Results and discussion

### 2.1. Chemistry

Since the designed compounds **A** (Fig. 1) contain a stereogenic centre at the C-2′ atom, both (*R*)- and (*S*)-enantiomers were synthesized side by side for their subsequent biological evaluations. The key synthetic step involved the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) between diethyl ethynylphosphonate[17] and suitable intermediate bearing an azido group. In general, two strategies could be utilized for the introduction of the 1,2,3-triazole ring into target ANPs: a) using the “click” CuAAC chemistry between diethyl ethynylphosphonate and acyclic nucleoside analogue having the azido group in the side aliphatic chain or b) the preparation of suitable intermediate bearing diethyl (1*H*-1,2,3-triazol-4-yl)phosphonate moiety for its subsequent attachment to purine or pyrimidine nucleobases.

The first approach was applied for the synthesis of adenine ANPs by analogy to previously reported procedures.[18,19] The synthesis started with alkylation of adenine with commercially available enantiomerically pure tritylated (*R*)-(+)- and (*S*)-(−)-glycidols, (*R*)-**2** and (*S*)-**2** (Scheme 1), to give compounds (*R*)-**3** and (*S*)-**3**, respectively.[18] Intermediates (*R*)-**3** and (*S*)-**3** were then benzoylated at the exocyclic amino group to form derivatives (*R*)-**4** and (*S*)-**4**,[18] which were further converted into their mesylate derivatives and, subsequently, to azido derivatives (*R*)-**5** and (*S*)-**5**, respectively, using $NaN_3$ (Scheme 1).[19]

The CuAAC between azido compounds (*R*)-**5** or (*S*)-**5** and diethyl ethynylphosphonate,[17] using CuI and DiPEA in DMF,[20] provided the desired 1,4-substituted triazole derivatives (*R*)-**6** or (*S*)-**6** in good yields (Scheme 1). The removal of benzoyl and trityl groups using methylamine in toluene[21] and 80% aq. acetic acid, respectively, followed by removal of phosphonate ethyl ester moieties with $Me_3SiBr$/MeCN with ensuing hydrolysis,[22] afforded final products (*R*)-**8** or (*S*)-**8** (Scheme 1).

The second synthetic strategy seems to be more efficient and more broadly applicable since the preformed aliphatic precursor bearing (1*H*-1,2,3-triazol-4-yl)phosphonate group can be directly attached to suitably modified purines or pyrimidines. At first, the starting tritylated (*R*)-(+)- and (*S*)-(−)-glycidols, compounds (*R*)-**2** and (*S*)-**2** (Scheme 2), were successively treated with freshly prepared sodium benzyloxide in DMF (without isolation of the products),[23] with MsCl in pyridine (to give (*R*)-**9** and (*S*)-**9**, respectively), and finally with $NaN_3$ in a DMF/HMPA mixture to afford azido derivatives (*R*)-**10** and (*S*)-**10**, respectively, in overall yields higher than 60% (Scheme 2).

To prepare the desired 1,2,3-triazole intermediates (*R*)-**12** and (*S*)-**12** (Scheme 2), azides (*R*)-**10** and (*S*)-**10** can be either first debenzylated and then cyclized under the CuAAC conditions or first cyclized and then debenzylated. Both approaches were tentatively tested and the former approach was selected as it gave higher yields. Thus, removal of the benzyl group from compounds (*R*)-**10** and (*S*)-**10**, using a $NaBrO_3/Na_2S_2O_4$ reagent under two-phase conditions (a method compatible with the present azido group),[24] afforded hydroxyl derivatives (*R*)-**11** and (*S*)-**11**, respectively, which were then converted by the above described CuAAC