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Acyclic nucleoside phosphonates containing 9-deazahypoxanthine and a 5-membered heterocycle as selective inhibitors of plasmodial 6-oxopurine phosphoribosyltransferases

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Keywords: malaria; acyclic nucleoside phosphonates; 9-deazahypoxanthine; 6-oxopurine phosphoribosyltransferase; purine salvage

Abstract

Acyclic nucleoside phosphonates (ANPs) are an important class of therapeutic drugs that act as antiviral agents by inhibiting viral DNA polymerases and reverse transcriptases. ANPs containing a 6-oxopurine instead of 6-aminopurine or pyrimidine bases are inhibitors of the purine salvage enzyme, hypoxanthine-guanine-[xanthine]-phosphoribosyltransferase (HG[X]PRT). Such compounds, and their prodrugs, are able to arrest the growth of *Plasmodium falciparum* (*Pf*) in cell culture. A new series of ANPs has been synthesized and tested as inhibitors of human HGPRT, *Pf*HGXPRT and *Plasmodium vivax* (*Pv*) HGPRT. The novelty of these compounds is that they contain a 5-membered heterocycle (imidazoline, imidazole or triazole) inserted between the acyclic linker(s) and the nucleobase, namely 9-deazahypoxanthine. Five of the compounds were found to be micromolar inhibitors of *Pf*HGXPRT and *Pv*HGPRT but no inhibition of human HGPRT was observed under the same assay conditions. This demonstrates selectivity of this type of compounds for the two parasitic enzymes compared to the human counterpart and confirms the importance of the chemical nature of the acyclic moiety in conferring affinity/selectivity for these three enzymes.

Introduction

Malaria remains one of the most important of all human protozoan borne infections. This disease is caused by one of five plasmodial species of which *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) are the most deadly and widespread.[1,2] Limitations of current vaccines[3] and increasing development of resistance to antimalarial drugs underline the need to develop new antimalarial therapeutics against previously unused molecular targets.[4,5]

Parasites of the genus *Plasmodium* lack the enzymes for *de novo* synthesis of purine bases and rely on the transport of these nucleobases from the host. Hypoxanthine-guanine-[xanthine] phosphoribosyltransferase (HG[X]PRT) is the key enzyme for the production of the essential metabolites (GMP, IMP and AMP) which, in malarial parasites, can only be produced from IMP.[6] Thus, these parasites depend on the activity of HG[X]PRT for their survival and reproduction.

Acyclic nucleoside phosphonates (ANPs)[7] constitute a distinguished class of antivirals.[8] A number of structurally modified ANPs were also shown to be potent and/or selective inhibitors of *Pf*HGXPRT and *Pv*HGPRT.[9] These include acyclic nucleoside bisphosphonates (ANbPs)[10] and aza-acyclic nucleoside phosphonates (aza-ANPs).[11] Crystal structures in complex with human HGPRT confirmed that bisphosphonate analogues are able to fit into all three key binding pockets of the enzyme, namely the binding site of the purine base, the 5'-phosphate group, and pyrophosphate.[10,11b] Furthermore, several bisphosphonates also exhibited potent inhibitory properties against bacterial (*Escherichia coli* and *Mycobacterium tuberculosis*) 6-oxopurine PRTs.[12]

Transition state analogues known as immucillins (e.g. immucillinHP, **1**, Fig. 1)[13] and chemically more stable acyclic immucillin phosphonates (e.g. compounds **2** and **3**, Fig. 1),[14], are potent inhibitors of 6-oxopurine PRTs. Compounds **2** and **3** have K_i values of 10.6 nM and 0.65 nM for *Pf*HGXPRT and K_i values for human HGPRT of 4.9 μM and 385 nM, a decrease of 466- and 592-fold, respectively.[14a] However, the structure of compound **2** in complex with *Pf*HGXPRT does not provide an explanation for the wide disparity in K_i values between the *Pf* and human enzymes. When the immucillin phosphates, immucillin phosphonates or their acyclic analogues bind in the active site, the side chain of D148 (*Pf*) or D137 (human) moves close to the purine ring and forms a hydrogen bond with N^7. This aspartic acid residue is located at the top of the 5′-phosphate binding pocket and points away from the nucleobase when GMP[15] or ANPs with either guanine of hypoxanthine bind.[9a]

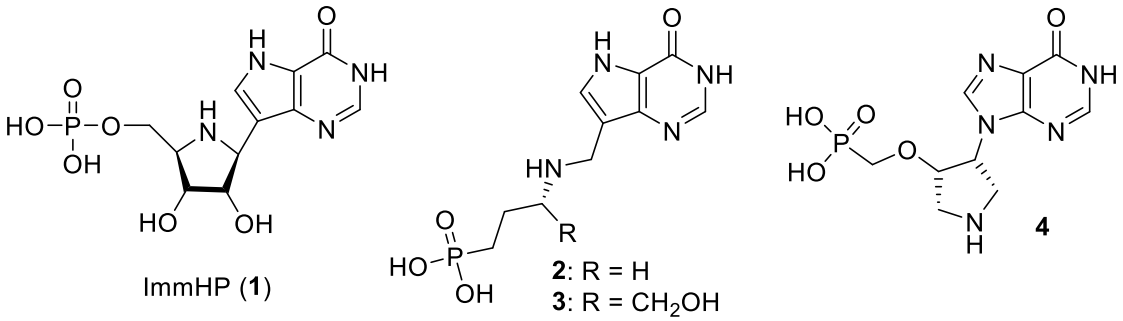


Figure 1. Chemical structure of a) immucillinHP (ImmHP, **1**),[13] b) acyclic immucillin phosphonates **2** and **3**,[14] and c) pyrrolidine based nucleotide analogue **4**.[16]

For the study reported herein, 9-deazahypoxanthine was chosen as the base since the acyclic immucilin phosphonates that contain this nucleobase are selective and potent inhibitors of the malarial enzymes.[14] It has been previously reported[16] that ANPs with a locked conformation *via* a pyrrolidine ring (e.g. compound **4**, Fig. 1) are excellent inhibitors of the 6-oxopurine PRTs. We have speculated that the increased flexibility of the acyclic moiety of ANPs allows them to bind optimally into the active sites of these enzymes potentially leading to the decrease of selectivity. On the other hand, an introduction of some rigidity to the acyclic chain of ANPs could help to fine-tune the selectivity among the parasitic and human enzymes. Thus, novel series of ANPs (**A**) and ANbPs (**B**) were designed (Fig. 2), where different 5-membered heterocyclic moieties, either partially saturated (imidazoline) or aromatic (imidazole or triazole), were inserted between 9-deazahypoxanthine and an aliphatic linker(s) bearing the phosphonate moiety to examine the effect of such inclusion on selectivity. In total, 18 compounds were synthesized and evaluated as potential inhibitors of *Pf* HGXPRT, *Pv*HGPRT and human HGPRT.

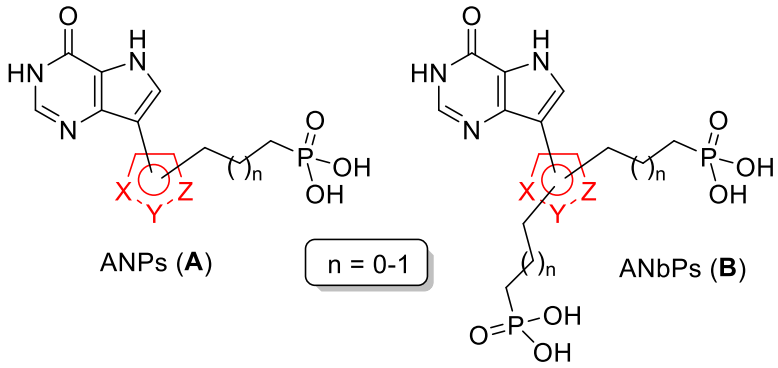
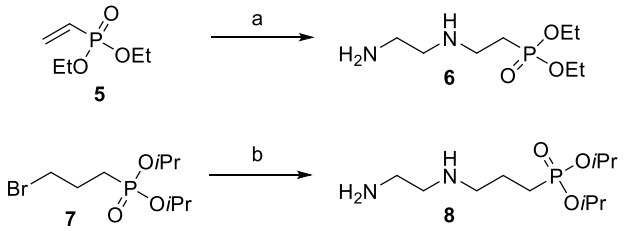


Figure 2. General structures of target acyclic nucleoside phosphonates (ANPs, **A**) and acyclic nucleoside bisphosphonates (ANbPs, **B**) with the 5-membered heterocycle (in red) attached to the C-9 position of 9-deazahypoxanthine.

Results and discussion

Chemistry

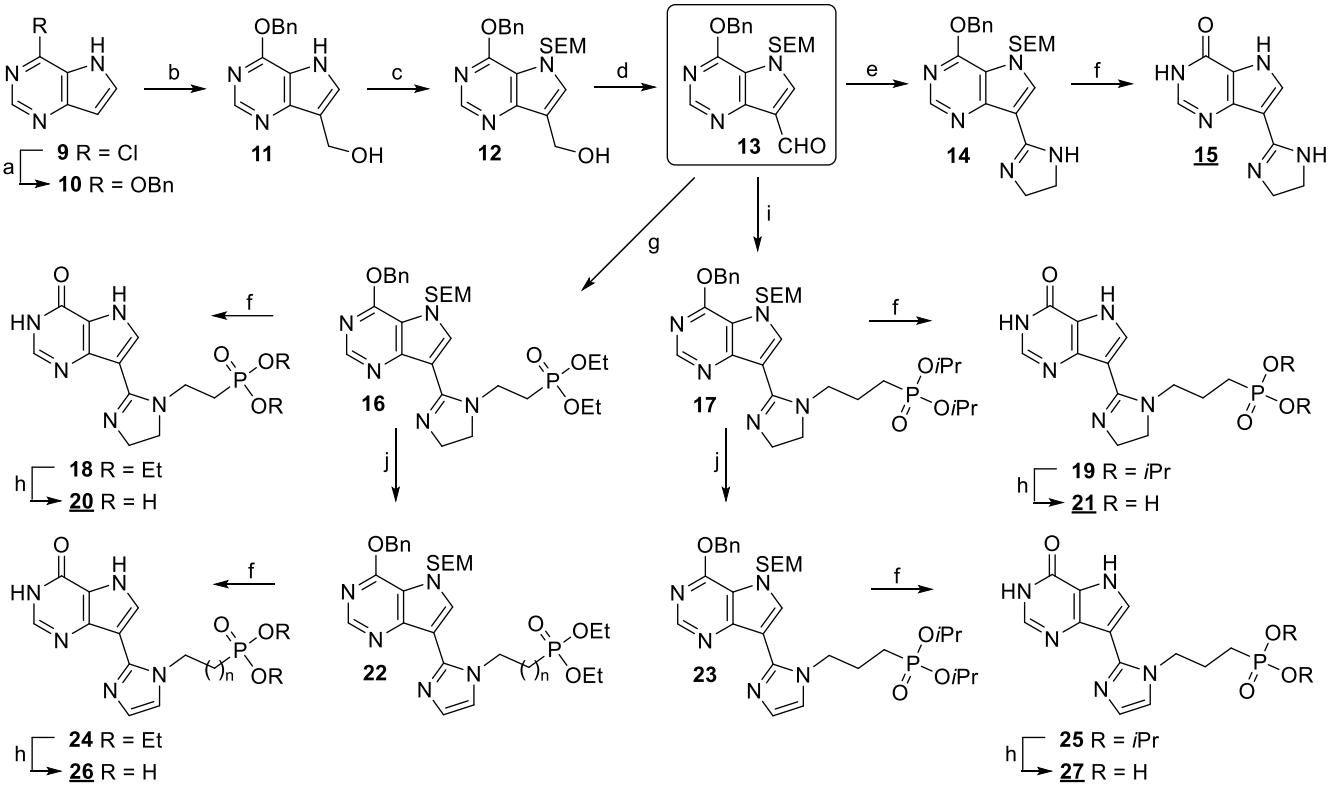
Compounds **6** and **8** (Scheme 1) are the ideal synthetic precursors for the synthesis of *N*-(phosphonoalkyl)imidazoline derivatives. Compound **6** was prepared using aza-Michael addition of diethyl vinylphosphonate (DEVP, **5**) to ethylendiamine (EDA) according to a recently published methodology using only water without additional catalyst or organic solvent.[17] Reaction of diisopropyl (3-bromopropyl)phosphonate (**7**) with EDA, serving both as a reactant and solvent, was used for the synthesis of compound **8**.



Scheme 1. Synthesis of *N*-(phosphonoalkyl)ethylendiamines **6** and **8**. *Reagents and conditions:* a) EDA, H_2O, RT, overnight, 95%; b) EDA, RT, 2 h, 96%.

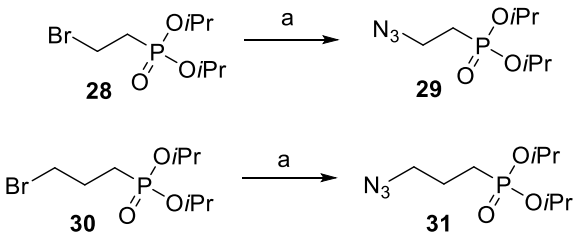
Compounds **10** and **11** (Scheme 2) were prepared from 6-chloro-9-deazapurine **9** on a large scale according to the previously reported procedure.[18] Compound **11** was selectively protected at position *N*-7 using SEMCl under basic conditions (NaH in THF) providing alcohol **12** which was subsequently oxidized with Dess-Martin reagent in acetic acid to form aldehyde **13** in a high yield. Compound **13** can be subsequently transformed to 2-imidazoline derivatives. A number of different synthetic procedures for the formation of imidazoline ring from an aldehyde have been reported.[19] However, in this case, these approaches failed because of the low reactivity of aldehyde **13** and, apparently, harsher reaction conditions were required. We decided to modify the previously described cyclisation,[19b] and MW-assisted heating (90 or 120 °C) of **13** for 1 h with either EDA, compound **6**, or compound **8** gave imidazoline derivatives **14**, **16**, and **17** (Scheme 2), respectively, in good yields. Aromatization of compounds **16** and **17** with $KMnO_4$ in dioxane[20] afforded corresponding imidazole analogues **22** and **23**. All protected 9-deazapurine derivatives **14**, **16**, **17**, **22**, and **23** were converted into 9-deazahypoxanthine analogues **15**, **18**, **19**, **24**, and **25**, respectively, using 75% aqueous trifluoroacetic acid. The unreacted *N*-7-hydroxymethyl side-products can be alternatively eliminated by treatment of the reaction mixture with

7 M ammonia in methanol followed by evaporation to dryness. Removal of ester groups from phosphonates **18**, **19**, **24**, and **25** under standard conditions (Me_3SiBr/MeCN),[21] followed by hydrolysis gave free phosphonic acids **20**, **21**, **26**, and **27** (Scheme 2). 2,6-Lutidine was added to neutralize HBr present in commercial Me_3SiBr and/or formed during the reaction progress.



Scheme 2. Synthesis of the imidazoline and imidazole derivatives. *Reagents and conditions:* a) BnOH, NaH, 90 °C, overnight, 69%; b) CH_2=O, K_2CO_3, H_2O/dioxane (1/1), 90 °C, 18 h, 85%; c) 1. BSA, Me_3SiBr, MeCN, RT, 5 min.; 2. NaH, SEMCl, THF, 0 °C – RT, 2 h, 81%; d) Dess-Martin reagent, AcOH, RT, 1 h, 89%; e) EDA, I_2, K_2CO_3, *t*-BuOH, MW, 120 °C, 1 h, 61%; f) 75% CF_3COOH, RT, overnight, 71–91%; g) **6**, I_2, K_2CO_3, *t*-BuOH, MW, 90 °C, 1 h, 76%; h) Me_3SiBr, 2,6-lutidine, MeCN, RT, overnight, 38–63%; i) **8**, I_2, K_2CO_3, *t*-BuOH, MW, 90 °C, 1 h, 72%; j) $KMnO_4$, dioxane, 80 °C, overnight 61–63%.

Next, phosphonoalkylazides **29** and **31** (Scheme 3), precursors for "click-chemistry", were prepared by treatment of corresponding bromoalkyphosphonates **28** and **30** with NaN_3 in toluene at 90 °C and in the presence of tetrabutylammonium bromide (Bu_4NBr) as a phase-transfer catalyst.



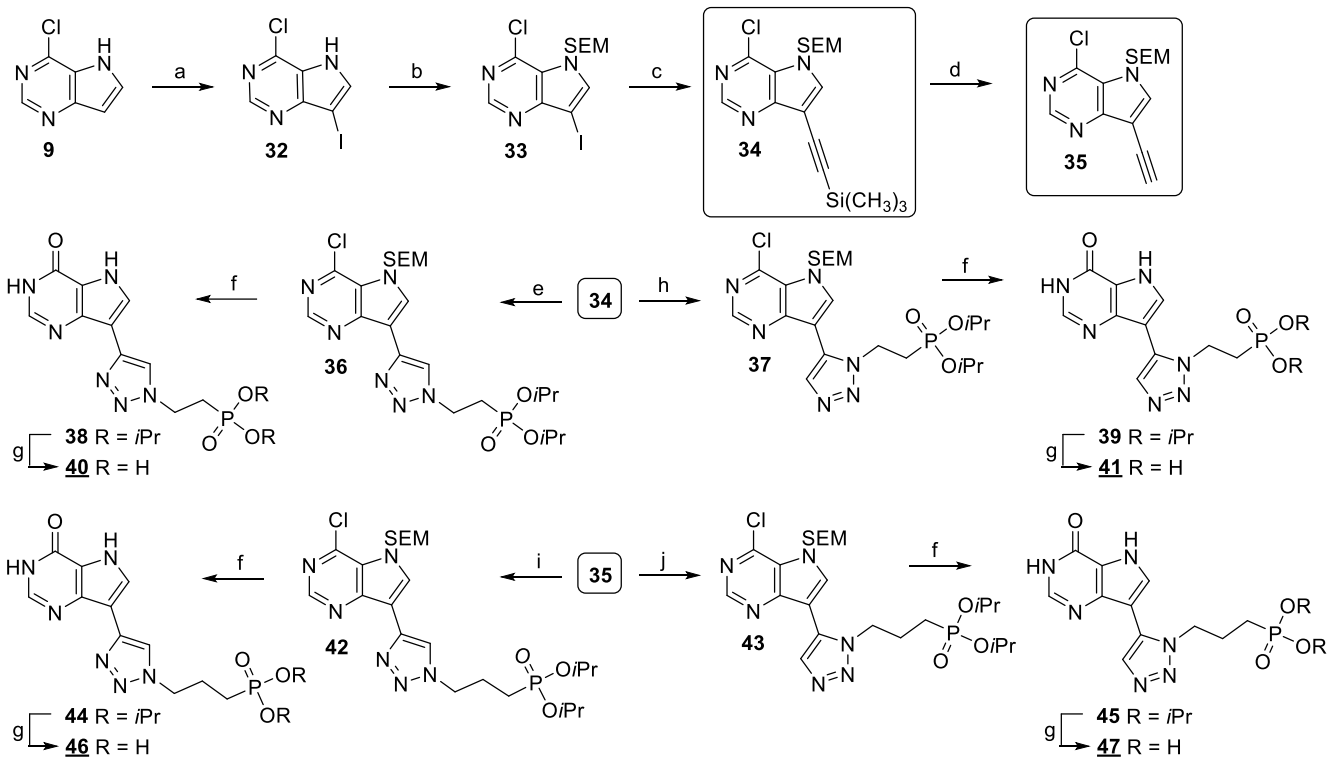
Scheme 3. Synthesis of azidoalkylphosphonates **29** and **31**. *Reagents and conditions:* a) NaN_3, Bu_4NBr, toluene, 90 °C, overnight, 78% (**29**) and 92% (**31**).

For the synthesis of 1,4- and 1,5-disubstituted 1,2,3-triazole analogues, compound **9** (Scheme 4) was first converted into 9-iodo-9-deazapurine derivative **32** with NIS in THF and then into SEM-derivative **33** with SEMCl and NaH in DMF. Treatment of compound **33** with TMS-acetylene under standard Sonogashira reaction conditions[22] afforded TMS-derivative **34** in excellent yield. The SEM group of compound **34** was removed with tetrabutylammonium fluoride (TBAF) in THF to give the unprotected acetylene derivative **35**.

Two methods were used for the formation of the 1,2,3-triazole ring: a) Cu(I)-catalyzed[22] azide alkyne Huisgen[24] "click" cycloaddition (CuAAC) for the regioselective assembly of 1,4-disubstituted 1,2,3-triazoles, and b) pentamethylcyclopentadienyl ruthenium chloride [Cp*RuCl] complexes[25] catalyzed azide alkyne cycloaddition (RuAAC) leading to the formation of 1,5-disubstituted 1,2,3-triazoles with high regioselectivity.

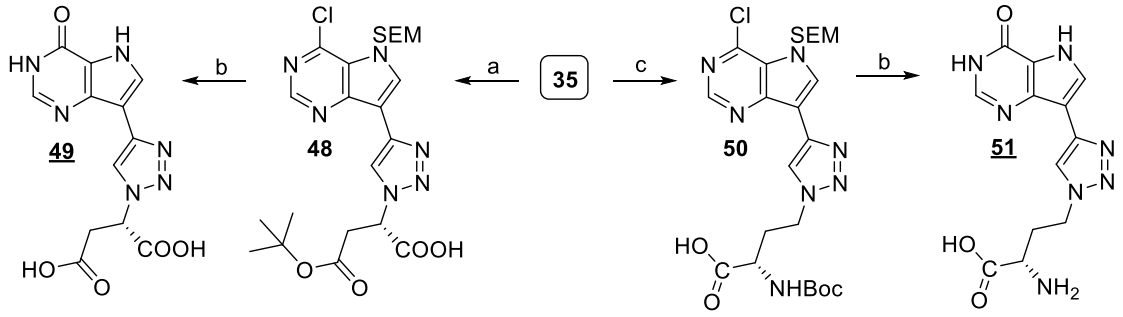
Thus, acetylene derivative **35** (or TMS-acetylene derivative **34** after in-situ TMS removal) was reacted with azides **29** or **31** in DMF under presence of CuI at room temperature to obtain 1,4-disubstituted triazoles **36** (28%) and **42** (67% of a crude product), respectively (Scheme 4).

For the second method, a variety of [Cp*RuCl] complexes can be employed. In our case, Cp*Ru(COD)Cl exhibiting excellent activities at room temperature,[26] was chosen as a selective catalyst for preparation of 1,5-disubstituted 1,2,3-triazoles. Reaction of intermediate **35** (or **34** after in-situ TMS removal) with azides **29** and **31** was carried out in dry toluene, as a convenient aprotic solvent for [Cp*RuCl]-catalyzed cycloadditions,[26] and gave the desired products **37** (67%) and **43** (88%), respectively, in excellent yields. Formed triazoles **36**, **37**, **42** and **43** were treated with 75% aqueous trifluoroacetic acid at 60 °C to form compounds **38**, **39** and **44**, **45** (Scheme 4), respectively. Finally, the desired free phosphonic acids **40**, **41**, **46**, and **47** were obtained in good yields after standard removal of the phosphonate ester moieties.



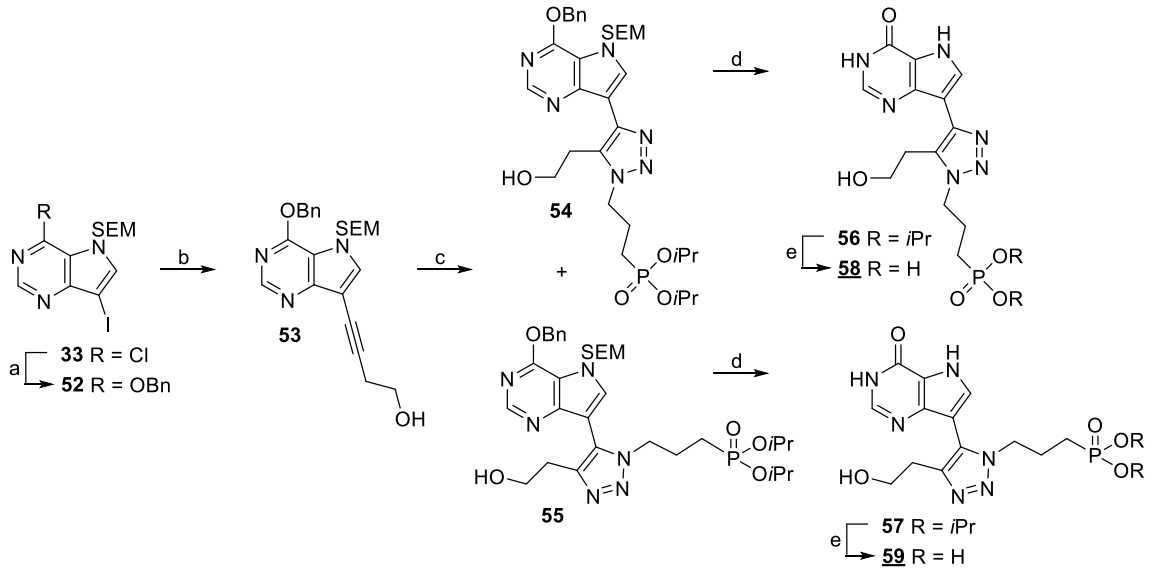
Scheme 4. Synthesis of monosubstituted triazole derivatives. *Reagents and conditions:* a) NIS, THF, r.t., 1 h, 84%; b) NaH, SEMCl, DMF, RT, overnight, 92%; c)TMS-acetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 0 °C – RT, 3 h, 94%; d) TBAF, THF, RT, 1 h, 59%; e) 1. TBAF, THF, RT, 30 min; 2. **29**, CuI, Et_3N, DMF, RT, 4 h, 28%; f) 75% CF_3COOH, 60 °C, 5-16 h, 64–93%; g) Me_3SiBr, 2,6-lutidine, MeCN, RT, overnight, 49–93%; h) 1. TBAF, THF, RT, 30 min; 2. **29**, Cp*Ru(COD)Cl, toluene, RT, 16 h, 67%; i) **31**, CuI, Et_3N, DMF, RT, 16 h, 67%; j) **31**, Cp*Ru(COD)Cl, toluene, RT, 16 h, 88%.

The CuAAC methodology was also used for the synthesis of carboxylic acids **49** and **51** (Scheme 5), bearing a carboxylic acid moiety in the side chain. Cu(I)-catalyzed reaction of acetylene derivative **35** with *(S)*-2-azido-4-(*tert*-butoxy)-4-oxobutanoic acid or 4-azido-2-((*tert*-butoxycarbonyl)amino)butanoic acid in DMF gave in both cases complex reaction mixtures which, after laborious isolation and purification, afforded intermediates **48** (13%) and **50** (12%). Their subsequent treatment with aqueous 75% trifluoroacetic acid at 60 °C gave the desired products **49** and **51** in good yields (Scheme 5).



Scheme 5. Synthesis of monosubstituted triazole derivatives **49** and **51**. *Reagents and conditions:* a) *(S)*-2-azido-4-(*tert*-butoxy)-4-oxobutanoic acid, CuI, Et_3N, DMF, RT, 16 h, 13%; b) 75% CF_3COOH, 60 °C, 5 h, 61% (**49**) and 87% (**51**); c) 4-azido-2-((*tert*-butoxycarbonyl)amino)butanoic acid, CuI, Et_3N, DMF, RT, 16 h, 12%.

SEM-protected 6-(benzyloxy)-9-iodo-9-deazapurine **52** (Scheme 6) was selected as another suitable starting material for cross-coupling reactions. Compound **52** was obtained by the treatment of chloro derivative **33** with BnOH and NaH (Scheme 6). The benzyloxy group was chosen for its stability under alkaline conditions during cross-coupling reactions. The reaction of compound **52** with 3-butyn-1-ol under the standard Sonogashira conditions ($PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, RT) proceeded only with approximately 40% conversion (UPLC-MS) and 9-deiodo derivative was the major product in the reaction mixture. Thus, we decided to modify the procedure using $Pd(PPh_3)_4$, CuI, and Cs_2CO_3 in DMF and the desired alcohol **53** was obtained in a 91% yield.



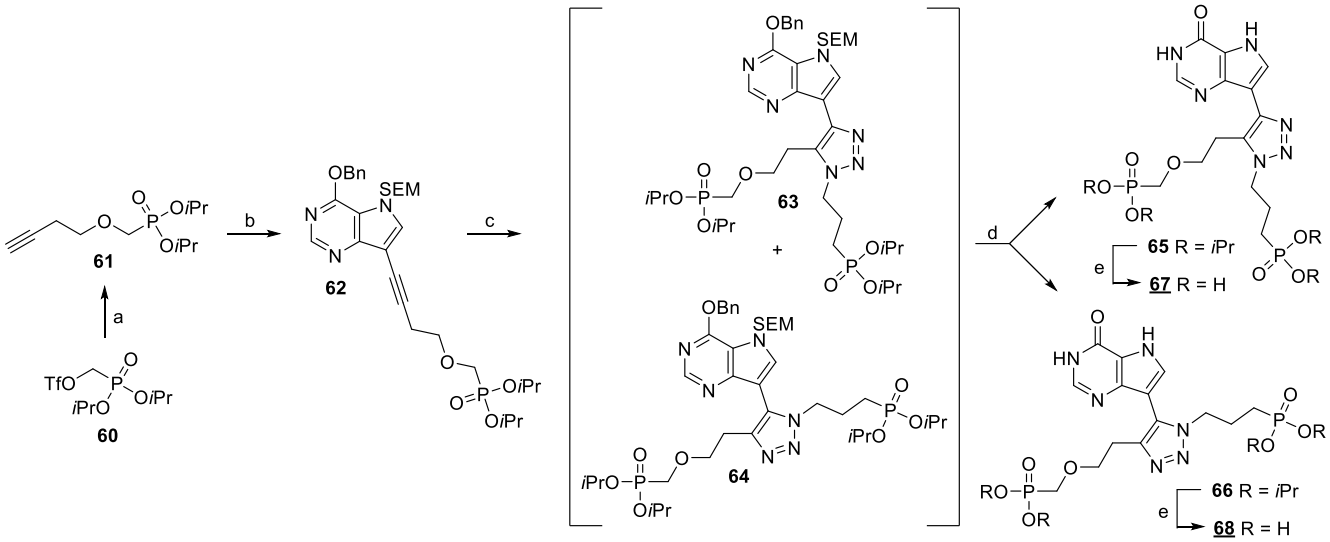
Scheme 6. Synthesis of triazole derivatives bearing two aliphatic linkers. *Reagents and conditions:* a) BnOH, NaH, 90 °C, overnight, 91%; b) 3-butyn-1-ol, $Pd(PPh_3)_4$, CuI, Cs_2CO_3, DMF, RT, 3 h, 91%; c)

31, Cp*Ru(PPh_3)$_2$Cl, toluene, 100 °C, 2 days, 22% (**54**) and 41% (**55**); d) 75% CF_3COOH, RT, overnight, 44–49%; e) Me_3SiBr, 2,6-lutidine, MeCN, RT, overnight, 75–77%.

As already mentioned above, cycloaddition of alkyl azides with terminal alkynes catalyzed by copper(I) ion leads exclusively to 4-substituted 1,2,3-triazols in high yields. However, this type of reaction cannot be applied for the cycloadditions with internal alkynes. This fact was confirmed by mechanistic studies showing that such reactions involve terminal copper acetylide and proceed through a stepwise non-concerted process.[27] In contrast to CuAAC, the Ru-complexes were reported to catalyze the cycloaddition reactions of internal alkynes. We decided to utilize the [Cp*RuCl] catalytic system for the cycloaddition of internal alkynes with organic azides to form 1,4,5-trisubstituted 1,2,3-triazoles.[26] Initial experiments were conducted with alkyne derivative **53**, azide **31**, and Cp*Ru(COD)Cl as a catalyst in toluene at room temperature. But the reaction afforded only traces of desired product **55**, even when the reaction time was prolonged to 3 days or when the reaction mixture was heated to 100 °C. The problem with the elevated temperature may lie in the fact that the cyclooctadiene (COD) ligand in Cp*Ru(COD)Cl is labile at higher temperatures and heating of the reaction results in rapid deactivation of the catalyst.[26] Compared to Ru-catalyst with COD ligand, Cp*Ru(PPh_3)$_2$Cl with more stable phosphine ligands in the bis(triphenylphosphine) complex enables to perform cycloadditions at temperatures up to 110 °C.[26] But the cycloaddition of internal alkyne **53** with azide **31** and Cp*Ru(PPh_3)$_2$Cl as catalyst in toluene at 100 °C for 2 days afforded only a mixture of products **54** and **55** (Scheme 6), however, with higher amounts of 1,4,5-trisubstituted 1,2,3-triazole **55**. Compounds **54** and **55** were successfully separated using silica gel column chromatography and subsequently treated with aqueous 75% trifluoroacetic acid to afford 6-oxo-9-deazapurine products **56** and **57**. To prepare the final free phosphonic acids **58** and **59** (Scheme 6), the phosphonate ester groups of phosphonates **56** and **57** were cleaved under the standard conditions using Me_3SiBr/MeCN followed by hydrolysis.

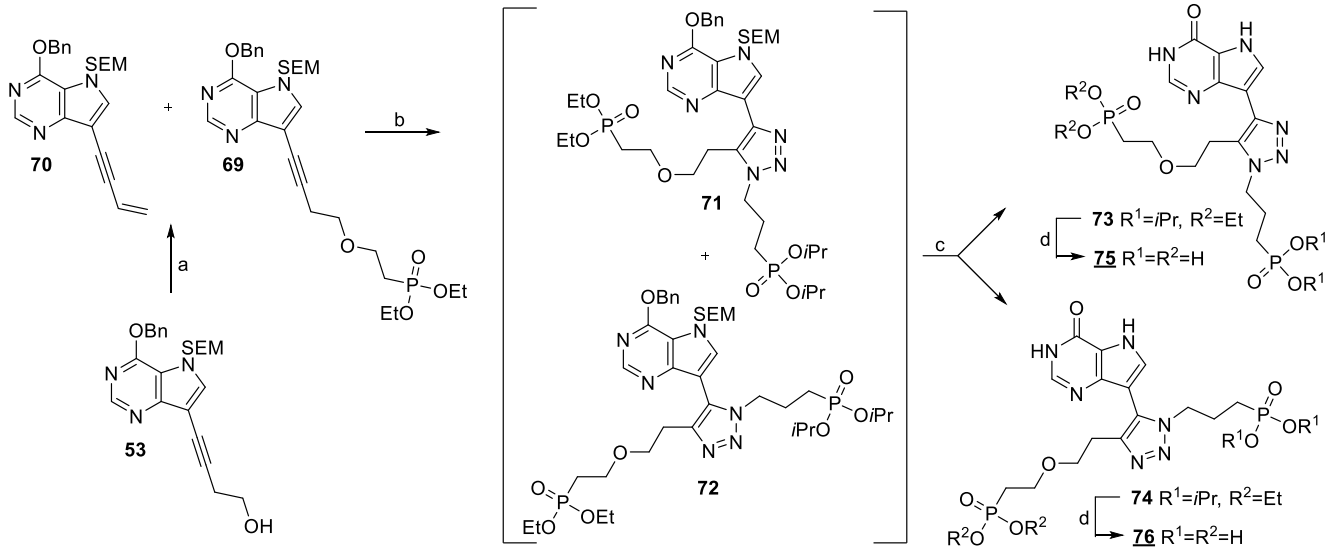
For the synthesis of other triazole bisphosphonate derivatives, key internal alkyne derivative **62** (Scheme 7) was prepared. Alkylation of 3-butyn-1-ol with triflate **60** in THF using BuLi as a base afforded terminal alkyne **61** in high yield. The treatment of compound **61** with intermediate **52** under the above described Sonogashira conditions gave the protected phosphonate **62** in a high yield (Scheme 7). Compound **62** was also prepared by the treatment of alcohol **53** with triflate **60** and NaH in THF at room temperature, but in a lower yield (52%) and this approach was less suitable for the scale-up. The cycloaddition of compound **62** with azide **31** using catalyst Cp*Ru(PPh_3)$_2$Cl was not selective again and only an

inseparable mixture of 3,4,5-trisubtituted triazol **63** and expected 1,4,5-trisubstituted triazol **64**. After treatment of the mixture of **63** and **64** with aqueous 75% trifluoroacetic acid, bisphosphonates **65** and **66** (Scheme 7) were successfully separated from each other using C18 silica gel flash chromatography. The standard use Me_3SiBr/MeCN for removal of phosphonate ester moieties followed by hydrolysis led to the formation of bisphosphonic acids **67** and **68** (Scheme 7).



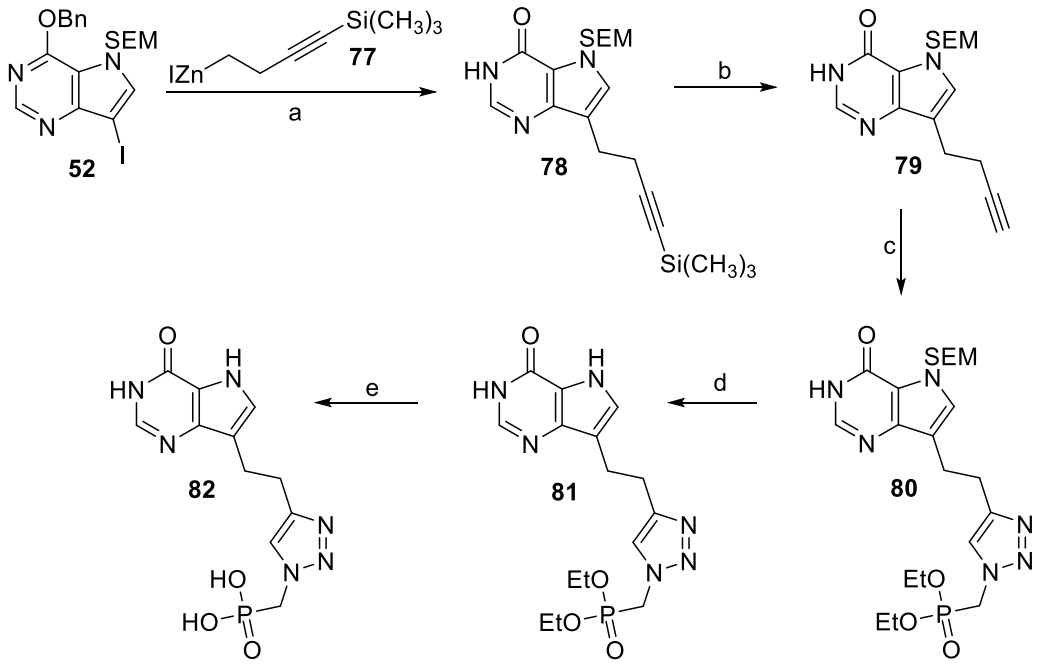
Scheme 7. Synthesis of bisphosphonates **67** and **68**. *Reagents and conditions:* a) 3-butyn-1-ol, BuLi, THF, -78 °C – RT, overnight, 87%; b) **52**, $Pd(PPh_3)_4$, CuI, Cs_2CO_3, DMF, RT, 3 h, 79%; c) **31**, $Cp^*Ru(PPh_3)_2Cl$, toluene, 100 °C, 2 days, 45% (**63** + **64**); d) 75% CF_3COOH, RT, overnight, 36% (**65**) and 29% (**66**); e) Me_3SiBr, 2,6-lutidine, MeCN, RT, overnight, 80–81%.

A different approach was employed for the preparation of prolonged bisphosphonates, where the PME linker is replaced with PEE linker. The first reaction step involved modified oxa-Michael addition on diethyl vinylphosphonate (DEVP, **5**) to introduce the ethyl phosphonate moiety.[28] Treatment of above prepared alcohol **53** with **5** and a catalytic amount of KOH in dioxane afforded desired phosphonate **69** together with vinylacetylen derivative **70** as a by-product (Scheme 8). Compound **69** was further treated with azide **31** and $Cp^*Ru(PPh_3)_2Cl$ in toluene at 100 °C to give an inseparable mixture of protected phosphonates **71** and **72**. Treatment of the mixture with aqueous 75% trifluoroacetic acid led to the formation of phosphonates **73** and **74** which were subsequently separated on the C18 silica gel column. The phosphonate ester groups of **73** and **74** were cleaved under the standard conditions using Me_3SiBr/MeCN followed by hydrolysis to form final bisphosphonic acids **75** and **76** (Scheme 8).



Scheme 8. Synthesis of bisphosphonates **75** and **76**. *Reagents and conditions:* a) DEVP (**5**), KOH, dioxane, RT, overnight, 59% (**69**) and 12% (**70**); b) **31**, Cp*Ru(PPh$_3$)$_2$Cl, toluene, 100 °C, 2 days, 56% (**71** + **72**); c) 75% CF$_3$COOH, RT, overnight, 35% (**73**) and 25% (**74**); d) Me$_3$SiBr, 2,6-lutidine, MeCN, RT, overnight, 82–85%.

Finally, we intended to compare the above rigid analogues, containing triazole ring attached directly to the C-9 position of 9-deazahypoxanthine, with more flexible compounds where the triazole moiety is attached to 9-deazahypoxanthine via a two-carbon linker. For that reason, model phosphonic acid **82** (Scheme 9) has been designed.



Scheme 9. Synthesis of compound **82**. *Reagents and conditions:* a) $PdCl_2$(dppf), CuI, DMA, 80 °C, overnight, 41%; b) TBAF, THF, RT, 3 h, 93%; c) $N_3CH_2P(O)(OEt)_2$, CuI, Et_3N, DMF, RT, overnight, 75%; d) 75% CF_3COOH, RT, overnight, 47%; e) Me_3SiBr, 2,6-lutidine, MeCN, RT, overnight, 78%.

Preliminary attempts to introduce (trimethylsilyl)butynyl moiety to compound **52** (Scheme 9) by its treatment with (4-(trimethylsilyl)but-3-yn-1-yl)lithium or (4-(trimethylsilyl)but-3-yn-1-yl)copper(I) failed. Subsequently, Negishi coupling under the reaction conditions reported by Storch et al.[29] was successfully applied. Zinc reagent **77**,[29] prepared using highly reactive Rieke zinc,[30] was heated with compound **52** in the presence of $PdCl_2$(dppf) and CuI in dry DMA to give desired compound **78** in a 41% yield. The benzyl group was cleaved during the reaction but this partial deprotection enabled us to separate product **78** from the deiodo by-product, 6-*O*-benzyl-7-((2-trimethylsilyl)ethoxy)methyl-9-dezapurine, which were formed in about 1:1 ratio. Removal of the trimethylsilyl group with TBAF gave terminal alkyne **79** in high yield. Subsequent cycloaddition of compound **79** with diethyl (azidomethyl)phosphonate[31] was performed using CuI in DMF to afford 1,4-disubstituted triazole derivative **80** in a 75% yield. An attempt to prepare the corresponding 1,5-disubstituted triazole, regioisomer of compound **80**, failed as the treatment of alkyne **79** with $Cp^*Ru(PPh_3)_2Cl$ in toluene provided only a complex reaction mixture with very low conversion (~5% of the desired product according to UPLC-MS analysis). Treatment of triazole **80** with aqueous 75% trifluoroacetic acid led to the formation of phosphonate **81** (47%) which was subsequently converted to final free phosphonic acid **82** (78%) with Me_3SiBr/MeCN followed by hydrolysis (Scheme 9).

Inhibition studies of novel 9-deazahypoxanthine acyclic nucleoside phosphonates

Five of the six triazole analogues bearing a single acyclic linker, namely compounds **40**, **41** and **46** (Scheme 4), **49** and **51** (Scheme 5), do not inhibit any of the three HG[X]PRTs at concentrations between 60 - 100 μM. Similarly, triazole bisphosphates **67** and **68** (Scheme 7) and **75** and **76** (Scheme 8), as well as analogues where the 5-membered ring in the linker is imidazole, as in compounds **26** and **27** (Scheme 2), do not inhibit any of the three enzymes. In these cases, the rigid heterocycles do not appear to allow either the purine base or the acyclic linker with polar groups to position themselves properly in the active sites.

Table 1 shows the five new derivatives with build in heterocycle that proved to be selective micromolar inhibitors of the *Pf* and/or *Pv* enzymes. None of these ANPs inhibit the human enzyme. The compounds

containing 2-imidazoline, **20** and **21** (Scheme 2), or 1,2,3-triazole, **47** (Scheme 4) and **59** (Scheme 6), attached directly to the C-9 position of the 9-deazahypoxanthine base and with the phosphonopropyl moiety on the nitrogen next to the C–C bond between the nucleobase and the 5-membered hetrocycle are low micromolar inhibitors of *Pf*HGXPRT and *Pv*HGPRT. Phosphonate **59** which contains additional hydroxyethyl moiety has a K_i value of 2-3 μM for the two malarial enzymes. Interestingly, compound **58** (Scheme 6), a regioisomer of inhibitor **59**, differing only in the position of an attachment of the phosphonopropyl linker to the triazole ring, does not inhibit any of the HG[X]PRTs tested. Again, this can be attributed to the inability of derivative **58** to accommodate itself properly in the critical binding areas in the active site of either enzyme.

Table 1. K_i values of 9-deazahypoxanthine ANPs for human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT.

Compound	5-Membered heterocycle	K_i (μM)			SI (*Pf*)*	SI (*Pv*)*
		human	*Pf*	*Pv*		
20	2-imidazoline	NI[a]	6.6	29	>14	>3
21	2-imidazoline	NI[b]	4.7	6	>20	>17
47	1,2,3-triazole	NI[c]	2	1	>50	>100
59	1,2,3-triazole	NI[d]	3	2	>17	>25
82	1,2,3-triazole	NI[e]	NI	2	NI	>50

[a][inhibitor] = 73 μM; [b][inhibitor] = 74 μM; [c][inhibitor] = 71 μM; [d][inhibitor] = 11.7 μM; [e][inhibitor] = 61 μM.
*Selectivity index. Lowest value likely, given none of the compounds inhibited human HGPRT at the concentrations listed above, which represents their maximum solubility for **20**, **21**, **47** and **82**. The absorbance of **59** absorbed very strongly at the wavelength of the experiment. Thus, this was its maximum value that could be measured.

Compound **15** (Scheme 2) did not inhibit either the human or plasmodial enzymes at concentrations of 108 μM in contrast to **20** and **21** (Scheme 2) which contain a linker with the phosphonate group. This is as expected for an ordered sequential reaction mechanism as the nucleobase cannot enter the active site prior to *P*Rib-*PP*. The attachment of a phosphonate group to the five-membered ring in **20** and **21**, however, led to the inhibition of the two parasitic enzymes only. Therefore, it can be concluded that this can simply occur when the 5′-phosphate binding site is occupied. Though compounds **20** and **21** containing a non-aromatic imidazoline ring are low μM inhibitors of *Pf*HGXPRT and *Pv*HGPRT, their "oxidized" aromatic forms, compounds **26** and **27** (Scheme 2), respectively, did not inhibit the enzymes at similar concentrations. This indicates that some, although limited, flexibility of the acyclic linker of these novel ANPs is beneficial to exert biological activity.

Compound **82** with a two-carbon linker between the aromatic five-membered ring (triazole) and 9-deazahypoxanthine did not inhibit either the human or *Pf* enzymes but does inhibit *Pv*HGPRT which shows that important differences exist between the active sites of the two plasmodial enzymes.

Conclusions

This study nicely demonstrates how the introduction of a rigid element into the acyclic linker of ANPs can affect the potency and selectivity of the HG[X]PRT inhibitors. In general, such insertion seems to lead to the decreased potency of these inhibitors compared to parent ANPs with the fully aliphatic linkers, but at the same time, to more favorable selectivity and, thus, possibly to less off-target effects of potential antimalarial treatments. The insertion of different 5-membered heterocyclic rings between the phosphonoalkyl linker(s) and a purine base influences the K_i values of these compounds for all three enzymes. Inserting imidazole moiety completely abolished binding whereas the insertion of imidazoline and suitably substituted triazole (preferably phosphonopropyl attached to the nitrogen next to the C–C bond between the nucleobase and heterocycle) did result in inhibition of the two plasmodial enzymes with K_i values between 1-29 μM, while no inhibition of human HGPRT, even at concentrations up to 100 μM, was observed. The reason for this could be due to the flexibility of the mobile loops that surround the active site of 6-oxopurine PRTs. However, it is clear that the nature of the linker connecting different groups of the inhibitor can be a crucial factor for conferring both affinity and selectivity between the human and plasmodial enzymes. The important SAR observations reported herein may be exploited in future design and development of potent and completely selective inhibitors of plasmodial 6-oxopurine PRTs.

Experimental section

General

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. TLC was performed on TLC aluminium sheets – silica gel 60 F_{254} (Merck), chromatographic systems are described in the text. Column chromatography was performed on silica gel 230-400 mesh, 60 Å (Merck). Reverse phase HPLC separation were performed on a Waters Delta 600 instrument with a Waters 486 Tunable Absorbance Detector using column Phenomenex Gemini C-18 (10 μm, 250 x 21.2 mm, flow 10 ml/min preparative column). ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance II 600 (^{1}H at 600 MHz and ^{13}C at 151 MHz) and/or Bruker Avance II 500 (^{1}H at 500 MHz and ^{13}C at

126 MHz) spectrometers in $CDCl_3$, DMSO or D_2O (NaOD additive) and referenced to TMS, ^{13}C chloroform signal (δ 77.0) or dioxane used as internal standard (δ 3.75 and δ 67.19). Mass spectra were measured on UPLC–MS (Waters SQD-2). HRMS data were taken on a LTQ Orbitrap XL spectrometer. Microwave experiments were carried out in 10 mL vial in CEM Discover (Explorer) microwave apparatus operated at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300W. Chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic). 6-Chloro-9-deazapurine was purchased from Santiago (Czech Republic), DEVP from Epsilon Chemie (France). Tetrahydrofuran (THF) was distilled from lithium aluminium hydride under argon. Other anhydrous solvents are commercially available. Solvents were dried by standard procedures and stored over molecular sieves (4 Å). Chromatographic system Eluent 1: cyclohexane : choroform : methanol = 6 : 4 : 1.

Detailed synthesis and full compound characterization can be found in the Electronic Supporting Information.

Determination of K_i values. Recombinant human HGPRT, *Pf*HGXPRT and *Pv*HGPRT were purified as described previously.[10] Human HGPRT and *Pv*HGPRT were stored in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4, 200 μM *P*Rib-*PP*, 1 mM dithiothreitol (DTT), −80°C. *Pf*HGXPRT was stored in 0.01 M phosphate, pH 7.4, 200 μM *P*Rib-*PP*, 60 μM hypoxanthine, 1 mM dithiothreitol (DTT), −80°C.[10] Enzyme assays were conducted in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4, 25°C. The K_i values were calculated at a fixed concentration of guanine (60 μM) and at variable concentrations of *P*Rib-*PP* (100 −1000 μM), depending on the $K_{m(app)}$, in the presence of the inhibitor and using the equations, $v_o = V_{max}.[S]_o/K_{m(app)} + [S]_o$ and $K_{m(app)} = K_m[1+ [I]/K_i]$.

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