Journal Pre-proof

Sulfide, sulfoxide and sulfone bridged acyclic nucleoside phosphonates as inhibitors of the *Plasmodium falciparum* and human 6-oxopurine phosphoribosyltransferases: Synthesis and evaluation

Tomáš Klejch, Dianne T. Keough, Marina Chavchich, Jye Travis, Jan Skácel, Radek Pohl, Zlatko Janeba, Michael D. Edstein, Vicky M. Avery, Luke W. Guddat, Dana Hocková

PII: S0223-5234(19)30811-6

DOI: https://doi.org/10.1016/j.ejmech.2019.111667

Reference: EJMECH 111667

To appear in: *European Journal of Medicinal Chemistry*

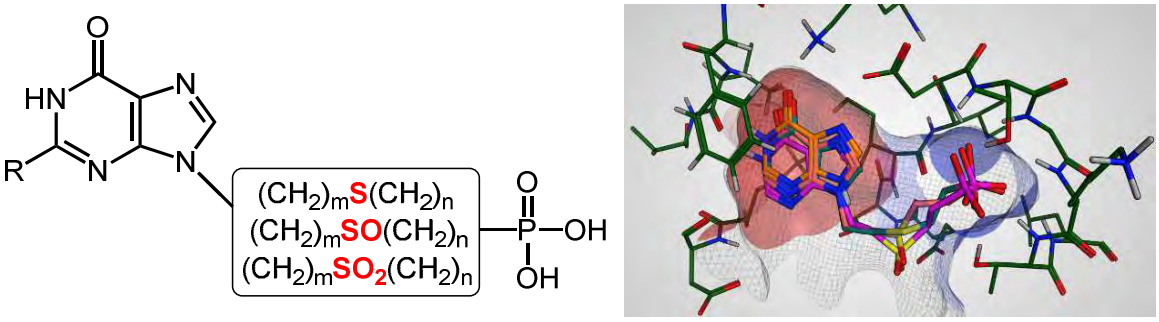
Received Date: 23 July 2019

Revised Date: 29 August 2019

Accepted Date: 30 August 2019

Please cite this article as: Tomáš. Klejch, D.T. Keough, M. Chavchich, J. Travis, J. Skácel, R. Pohl, Z. Janeba, M.D. Edstein, V.M. Avery, L.W. Guddat, D. Hocková, Sulfide, sulfoxide and sulfone bridged acyclic nucleoside phosphonates as inhibitors of the *Plasmodium falciparum* and human 6-oxopurine phosphoribosyltransferases: Synthesis and evaluation, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.111667.

Graphical abstract



Sulfide, Sulfoxide and Sulfone Bridged Acyclic Nucleoside Phosphonates as Inhibitors of the *Plasmodium falciparum* and Human 6-Oxopurine Phosphoribosyltransferases: Synthesis and Evaluation

Tomáš Klejch,[a] Dianne T. Keough,[b] Marina Chavchich,[c] Jye Travis,[b, c] Jan Skácel,[a] Radek Pohl,[a] Zlatko Janeba,[a] Michael D. Edstein,[c] Vicky M. Avery,[d] Luke W. Guddat,[b]* Dana Hocková[a]*

[a]*The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, CZ-16610 Prague 6, Czech Republic*

[b]*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane 4072, Australia*

[c]*Department of Drug Evaluation, Australian Defence Force Malaria and Infectious Disease Institute, Enoggera, 4051, Australia*

[d]*Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, 4111, Australia*

[*]address for corespondence

hockova@uochb.cas.cz

luke.guddat@uq.edu.au

Keywords:

Hypoxanthine-guanine-xanthine phosphoribosyltransferase, sulfur-containing drugs, sulfide oxidation, *Plasmodium falciparum*, acyclic nucleoside phosphonates

Abstract

Hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT) is a recognized target for antimalarial chemotherapeutics. It synthesises all of the 6-oxopurine nucleoside monophosphates, IMP, GMP and XMP needed by the malarial parasite, *Plasmodium falciparum* (*Pf*). *Pf*HGXPRT is also indirectly responsible for the synthesis of the 6-amino nucleoside monophosphate, AMP. The acyclic nucleoside phosphonates (ANPs) are a class of *Pf*HGXPRT inhibitors. Prodrugs of these compounds are able to arrest the growth of *Pf* in cell culture. In the search for new inhibitors of *Pf*HGXPRT, a series of sulfur containing ANPs (thia-ANPs) has been designed and synthesized. These compounds are based on the structure of 2-(phosphonoethoxy)ethylguanine (PEEG) and PEEHx which consist of a purine base (*i.e.* guanine or hypoxanthine) linked to a phosphonate group by five atoms *i.e.* four carbons and one oxygen. Here, PEEG and PEEHx were modified by substituting a sulfide, sulfoxide or a sulfone bridge for the oxygen atom in the linker. The effect of these substitutions on the K_i values for human HGPRT and *Pf*HGXPRT was investigated and showed that most of the thia-ANPs distinctively favour *Pf*HGXPRT. For example, the thia-analogue of PEEHx has a K_i value of 0.2 μM for *Pf*HGXPRT, a value 25-fold lower than for the human counterpart. Prodrugs of these compounds have IC_{50} values in the 4-6 μM range in antimalarial cell-based assays, making them attractive compounds for further development as antimalarial drug leads.

1. Introduction

Plasmodium falciparum hypoxanthine-guanine-xanthine phosphoribosyltransferase (*Pf*HGXPRT) catalyses the formation of the 6-oxopurine nucleoside monophosphates, IMP, GMP and XMP, from 5-phospho-α-D-ribosyl-1-pyrophosphate and hypoxanthine, guanine or xanthine (**Fig. 1**). For this reaction to proceed, the presence of a divalent metal ion is required. This is the only route that these malaria parasites possess to make nucleotides essential for DNA/RNA production as they are unable to synthesise the purine ring *de novo*.[1] The preformed purine bases can be obtained directly from the host cell. A second source of hypoxanthine is also available within the parasite. This is via the action of purine nucleoside phosphorylase which converts inosine, transported from the host cell, to hypoxanthine and ribose-1-phosphate.[1]

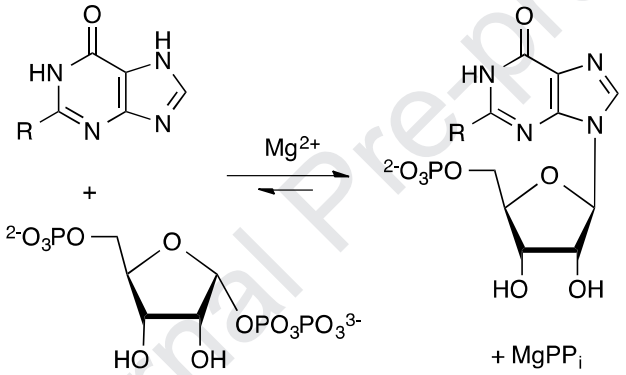


Figure 1. The reaction catalysed by *Pf*HGXPRT. R = -H (hypoxanthine); -NH_2 (guanine); R = -OH (xanthine).

In addition to its role in the production of the 6-oxopurine nucleoside monophosphates, *Pf*HGXPRT is also indirectly responsible for the synthesis of adenosine monophosphate (AMP) as *Pf* does not possess adenine phosphoribosyltransferase or adenylate kinase activity.[2] Thus, AMP can be produced from IMP which, in turn, is only synthesized by HGXPRT. AMP is also synthesized in the erythrocytes and can then be transported into the *Pf* cells, providing another source of this metabolite to the parasite.[3] The genome of *Pf* is AT rich and, therefore, AMP is required in large amounts in rapidly dividing cells.[4] Hence, as *Pf*HGXPRT is responsible for the production of all of its purine nucleoside monophosphates, its activity is crucial for cell proliferation and survival. Because of this central role in the parasites' metabolism, *Pf*HGXPRT is a recognized target for the development of antimalarial therapeutics and inhibitors of *Pf*HGXPRT are highly sought after

as antimalarial drug candidates.[5] Development of inhibitors of *Pf*HGXPRT is timely as *P. falciparum* resistance exists to all currently available antimalarial drugs, including the artemisinin derivatives, necessitating an urgent need to develop new antimalarials to clear parasites from the blood and prevent recrudescence.[6],[7]

Acyclic nucleoside phosphonates (ANPs) are one class of developed inhibitors of the 6-oxopurine phosphoribosyltransferases.[8],[9],[10],[11],[12],[13] These flexible compounds contain a 6-oxopurine base connected to a phosphonate group by a series of atoms, referred to as the "linker". These compounds were designed based on the chemical structure of GMP, IMP or XMP. Thus, they occupy two areas in the active site of the 6-oxopurine PRTases, that where the purine base and the 5′-phosphate group bind. The absence of a glycosidic bond and the presence of the C-P bond renders these ANPs resistant to enzymatic hydrolysis *in vivo*.[14],[15]

Two simple ANPs that mimic the 6-oxopurine nucleoside monophosphates contain five atoms in the linker, four carbons and one oxygen.[16],[17] These compounds are 2-(phosphonoethoxy)ethylguanine (PEEG, **Fig. 2A**) and 2-(phosphonoethoxy)ethylhypoxanthine (PEEHx). Both are good inhibitors of *Pf*HGXPRT with K_i values of 0.1 and 0.3 μM, respectively. In comparison, the K_i values of PEEG and PEEHx for human HGPRT are 10-fold higher, being 1 and 3.6 μM, respectively. Thus, these ANPs are better inhibitors of *Pf*HGXPRT than human HGPRT. An additional substituent attached to the PEE-linker can modify the activity[18],[19] or completely reverse the selectivity[20]. The K_i values of GMP and IMP for *Pf*HGXPRT are 10 μM and 3.6 μM, respectively, which are 100-fold and 12-fold higher than for PEEG or PEEHx.[16] Thus, these ANPs bind considerably more strongly to *Pf*HGXPRT than the products of the reaction. This makes them excellent starting points for the development of new inhibitors of *Pf*HGXPRT.

The crystal structures of human HGPRT in complex with PEEG (PDB code: 3GGJ)[16] or GMP (PDB code; 1HMP)[21] are compared in **Figs. 2B** and **2C**. These images demonstrate that, when the different ligands bind, conformational changes in the active site structure of the enzyme occur. As a result, there are differences in the non-covalent bonding networks that form when these two ligands bind in the active site (**Figs. 3A** and **3B**). This variability in the structure of the enzyme makes it difficult to predict how different ligands will bind.

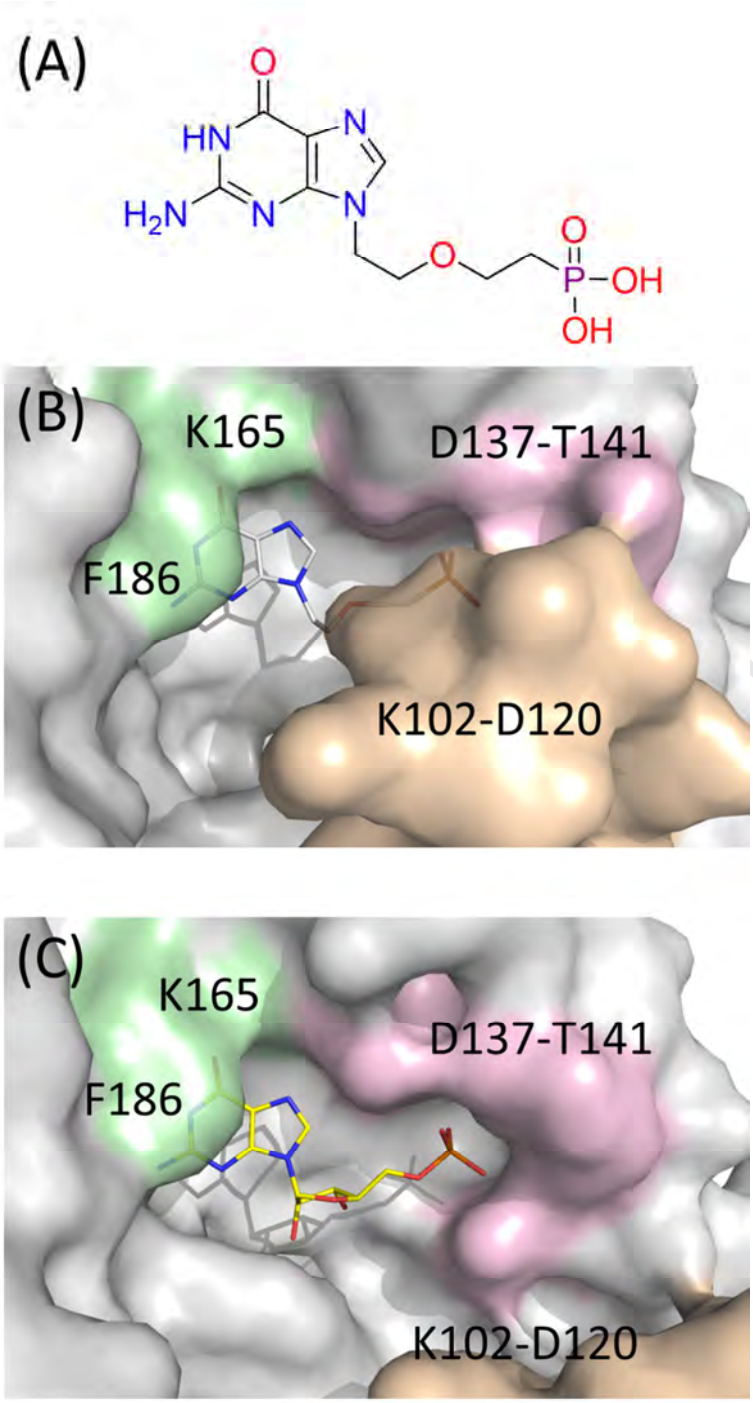


Fig. 2. Overlay of the crystal structures of human HGPRT in complex with either PEEG or GMP. (**A**) Chemical structure of PEEG. (**B**) The Connolly surface map of the human HGPRT.PEEG complex (PDB code: 3GGJ, 2.5 Å resolution).[16] Green is the location where residues F186 and K165 of the purine binding site are located; pink is the location of the 5′-phosphate binding pocket (residues D137-T14); the large mobile loop (residues K102-D120) is coloured in beige and partially covers the active site. **(C)** The Connolly surface map of the human HGPRT.GMP complex (PDB code: 1HMP, 2.6 Å resolution).[21]

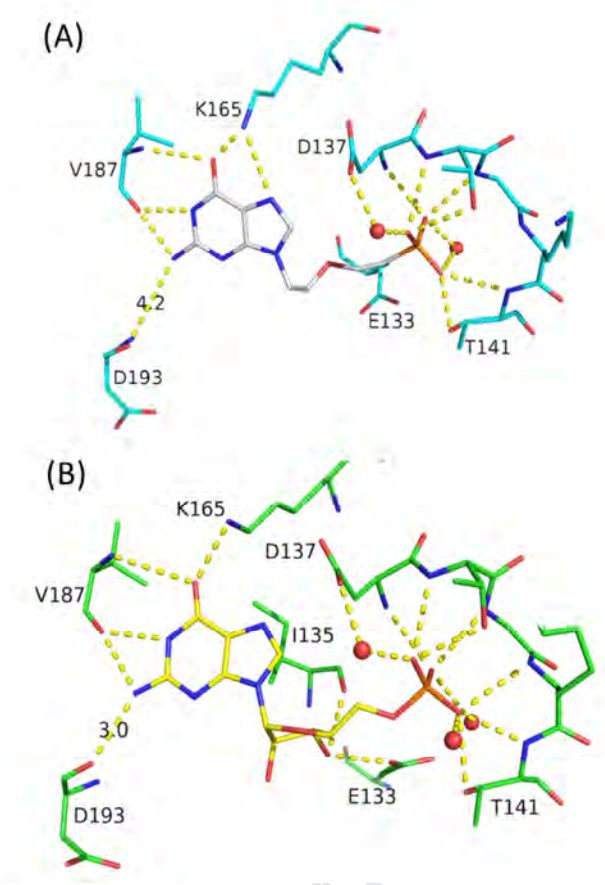


Fig. 3. Hydrogen bonding network between PEEG (A) or GMP (B) with amino acid residues when these two ligands are bound in the active site of human HGPRT. (**A**) The human HGPRT.PEEG complex.[16] (**B**) The human HGPRT.GMP complex.[21]

The strategy adopted here for the design of new inhibitors of *Pf*HGXPRT is to make modifications to the structure of PEEG (**Fig. 2A**) or PEEHx. These include replacing the oxygen in the PEE-linker by a sulfide, a sulfoxide or a sulfone (**Fig. 4**, m = 1, n = 1). A second variation is to move the sulfur moiety further along the "linker" so that it is one atom distal from the purine base (**Fig. 4**, m = 2, n = 0). Previously, only a few sulfur-containing ANPs had been reported as antiviral compounds[22] or purine nucleoside phosphorylase inhibitors.[23] A sulfur analogue of PMEA (Adefovir) was prepared in various oxidation states via oxidation with sodium periodate.[24] Here, the synthesis of ten ANPs containing

sulfur in diverse oxidation states is reported together with the inhibition constants of these thia-ANPs for human HGPRT and *Pf*HGXPRT.

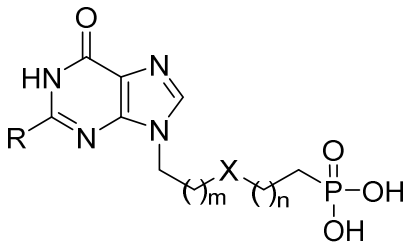


Fig. 4. The structure of the new sulfur-containing compounds, the thia-ANPs. R = -H or -NH_2; X = S, SO or SO_2; m = 1 or 2; n = 1 or 0, n + m = 2.

The prodrugs of PEEG inhibit the growth of *Pf* in cell culture[25] (**Fig. 5**). The presence of the phosphonate group in the ANPs is responsible for their highly polar character and negative charge at physiological pH. This is why the prodrug-approach is needed to improve the cell penetration.[26] **Fig. 5** demonstrates that there are differences in the IC_{50} values for the various prodrugs even when the parent compound is the same.[25] This is attributed to the influence of the attachment on the ability of the prodrug to cross membranes and/or the hydrolysis of the prodrug group by inherent enzymes to release the active inhibitor.[27] Here, prodrugs of three of the best thia-ANP inhibitors were synthesized and tested for antimalarial activity in *Pf in vitro* assays.

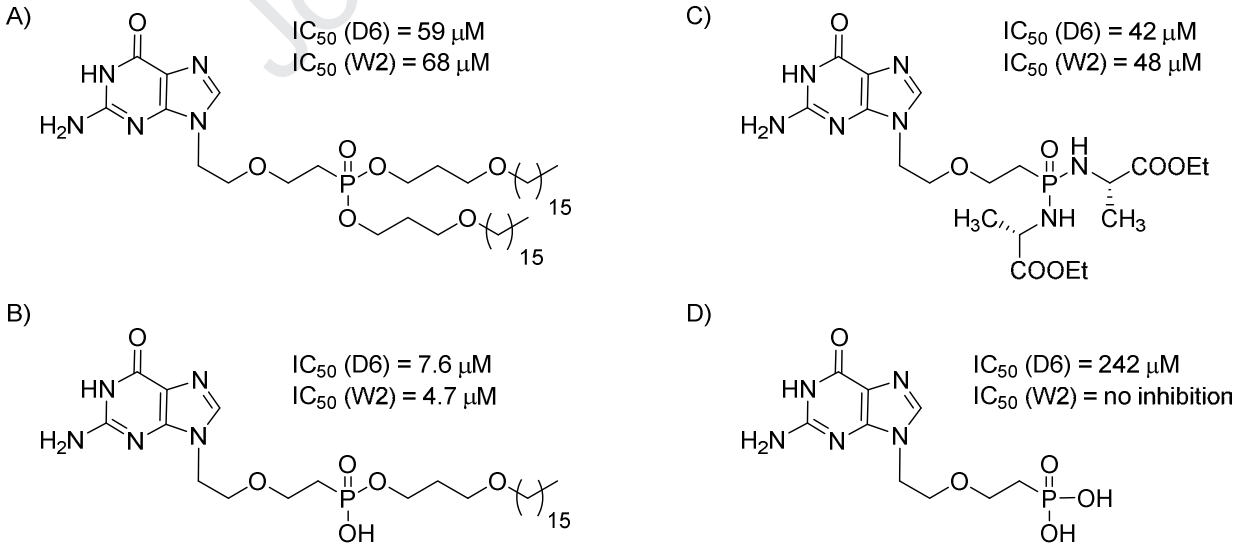


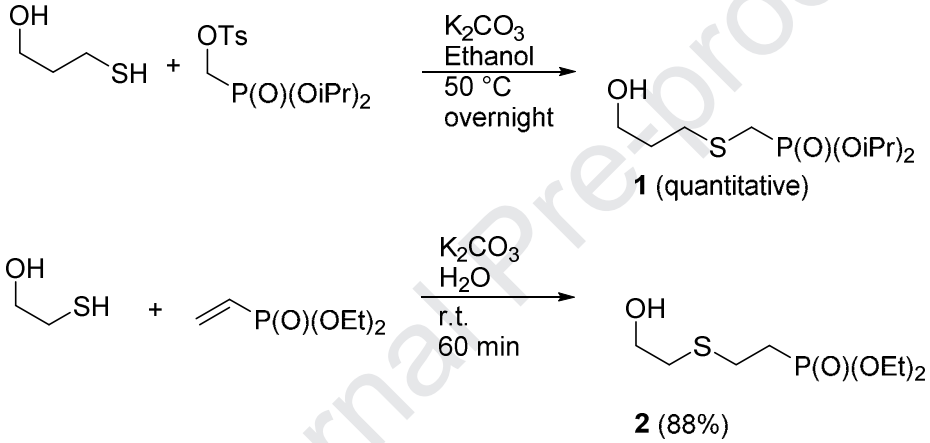
Fig. **5.** Three prodrugs of PEEG (**A-C**) and their IC_{50} values against *Pf* grown in erythrocyte cell culture compared to the parent compound itself, PEEG (**D**).[25] D6 strain is sensitive to

most drugs and W2 strain is resistant to chloroquine. Compounds **A**, **B** and **C** exhibited cytotoxicity values in human cells of > 300 μM.

2. Results and discussion

2.1 Chemistry

The first step in preparation of thia-ANPs is the synthesis of the phosphonate bearing acyclic moieties **1** and **2** (**Scheme 1**), either via alkylation or by Michael addition. Due to the high nucleophilicity of the thiol group, no protection of the hydroxy group was required and the desired compounds were prepared chemoselectively under mild conditions in very high yields.



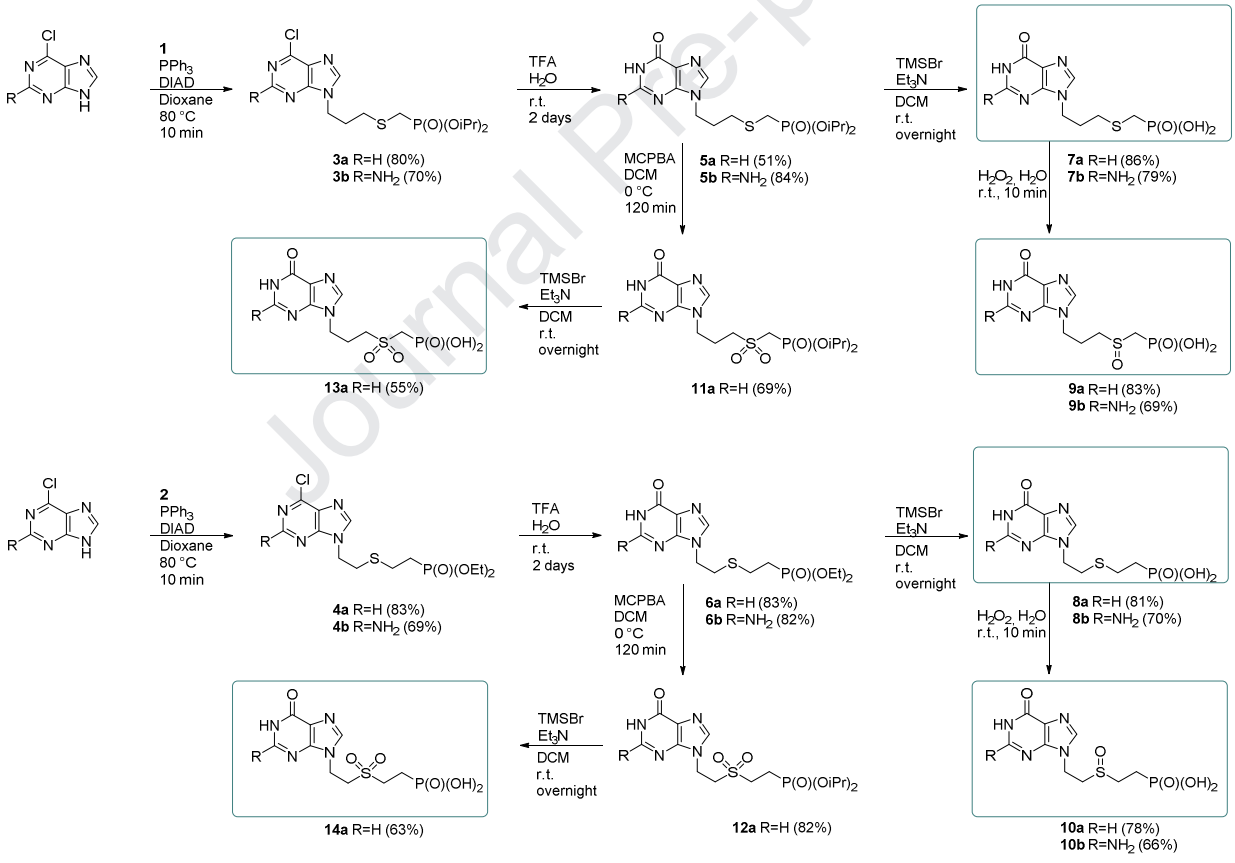
Scheme 1: Preparation of phosphonate-containing thia linkers

Derivatives **3a**,**b** and **4a**,**b** were obtained by Mitsunobu reaction with alcohols **1** and **2** and 6-chloropurine or 2-amino-6-chloropurine, respectively (**Scheme 2**). We used a protocol with an increased reaction temperature, which significantly decreased the reaction time.[28] With these conditions, no decomposition of the starting material was observed. Hydrolysis with 75% trifluoroacetic acid (TFA) afforded hypoxanthine derivatives **5a** and **6a** and guanine derivatives **5b** and **6b**. Standard treatment with bromotrimethylsilane (TMSBr) followed by hydrolysis yielded free phosphonates **7a**,**b** and **8a**,**b** (**Scheme 2**).[18]

In the next stage, sulfur oxidation was carried out. Oxidation methods had to be selected carefully due to possible nucleobase oxidation. Treatment of hypoxanthine or guanine derivatives **5-6** with $Br_2(CHCl_3)/KHCO_3(H_2O)$[29] lead selectively to the synthesis of the sulfoxides while allowing convenient colorimetric monitoring of the reaction. However, a deprotection step was required to prepare free phosphonic acids, which proved to be

problematic. Direct oxidation of free phosphonic acids proved to be a more convenient process (**Scheme 2**). Treatment of free phosphonic acids **7-8** with an equimolar amount of hydrogen peroxide in water[30] afforded the target sulfoxides with high purity and no undesired byproducts. Also, no additional transformations were required.

Oxidation to sulfone was more challenging. No further reaction was observed when sulfoxides **9-10** were treated with excess hydrogen peroxide. Heating with sodium periodate in water[31] resulted in a nonselective nucleobase oxidation. A similar result was achieved when the Oxone in methanol method[32] was employed. Finally, the hypoxanthine derivatives were successfully oxidized in a two-step procedure. First, compounds **5a** and **6a** were treated with excess of MCPBA in dichloromethane[33] and then the resulting sulfones **11a** and **12a** were subjected to ester cleavage to form target compounds **13a** and **14a** (**Scheme 2**).

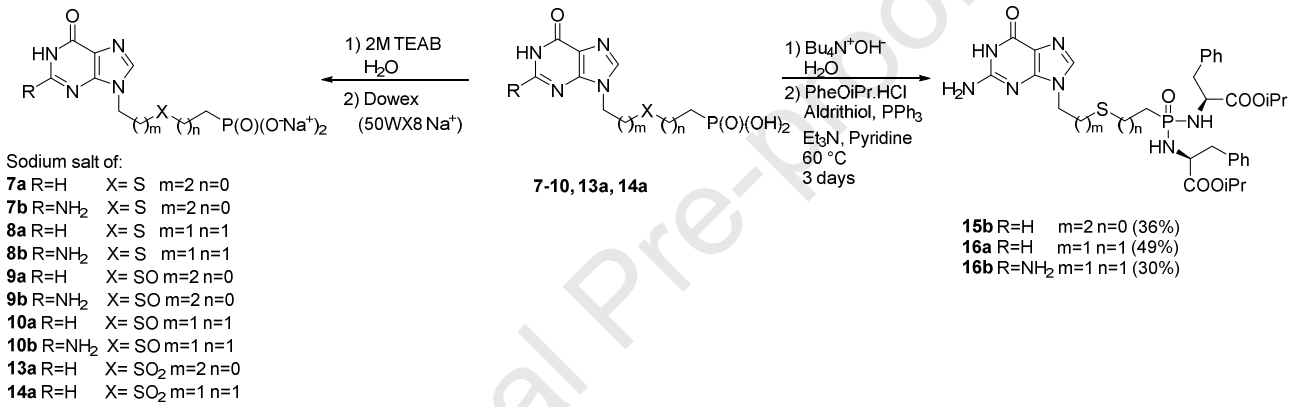


Scheme 2: Synthesis of target thia-ANPs

To ensure suitable solubility of samples for enzymatic assays, the free phosphonic acids were converted to their sodium salts (**Scheme 3**) by treatment with triethylammonium

bicarbonate, followed by purification by reverse phase flash chromatography and then passage through Dowex 50 in Na^+ cycle.

Phosphoramidate prodrugs, for the antimalarial assays, were synthesized from the most promising thia-ANP inhibitors. The goal was to improve membrane penetration of these highly polar phosphonic acids. First, phosphonic acids **7b** and **8a**,**b** were converted to tetrabutylammonium salts for better solubility. These were treated by a standard procedure[34] to obtain the desired bisamidate prodrugs **15b** and **16a**,**b** (**Scheme 3**). When derivatives **9** and **10** were subjected to the same procedure, sulfoxide moieties were reduced to sulfides during the reaction, so only sulfide bridged prodrugs were prepared.



Scheme 3: Conversion of phosphonic acids to sodium salts and phosphoramidate prodrugs

2.2 Analysis of the synthesized structures and docking into human HGPRT

Compounds **7a**, **7b**, **8a** and **8b** are isosteres of PEEG or PEEHx. Thus, the binding modes of these compounds are likely to be similar to that of PEEG or PEEHx and there is an expectation that their location and conformation should be well predicted by docking calculations. The docking with human HGPRT shows that, when only the sulfide is present (at position 3 in the linker), a change in the conformation of the linker could occur, but the location of both the base or the phosphonate in the active site can remain conserved (**Fig. 6**). Likewise, when the sulfide is moved by one atom, docking into the active site of *Pf*HGXPRT is readily achievable and the binding mode also appears well conserved.

To study the influence of the oxidation state on the bonding angle and the whole geometry of the linker chain, derivatives with sulfur in different oxidation states, sulfide **8a**, sulfoxide **10a** and sulfone **14a** were docked into the *Plasmodium falciparum* enzyme (PDB

code 3OZF)[35] (**Fig. 7**). The introduction of a sulfone or sulfoxide into the linker does have the potential to create steric clashes or new contacts resulting in an alternative binding mode for these compounds relative to PEEG or PEEHx. Nevertheless, the docking of these compounds does show that they can be accommodated into the active site of *Pf*HGXPRT without significantly altering the binding mode (**Fig. 7**).

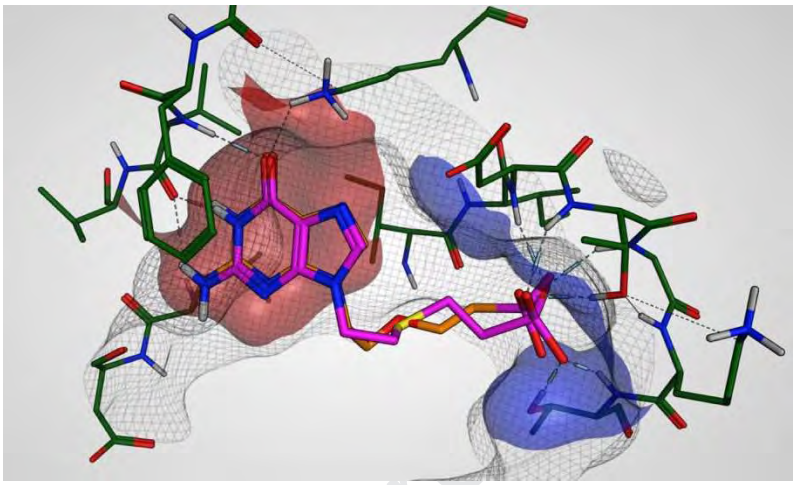


Figure 6. Docking of 8b into the active site of human HGPRT. The docked **8b** is depicted with magenta carbon atoms. Overlaid is PEEG as it appears in the crystal structure of the complex with HGPRT (PDB code 3GGJ).[16]

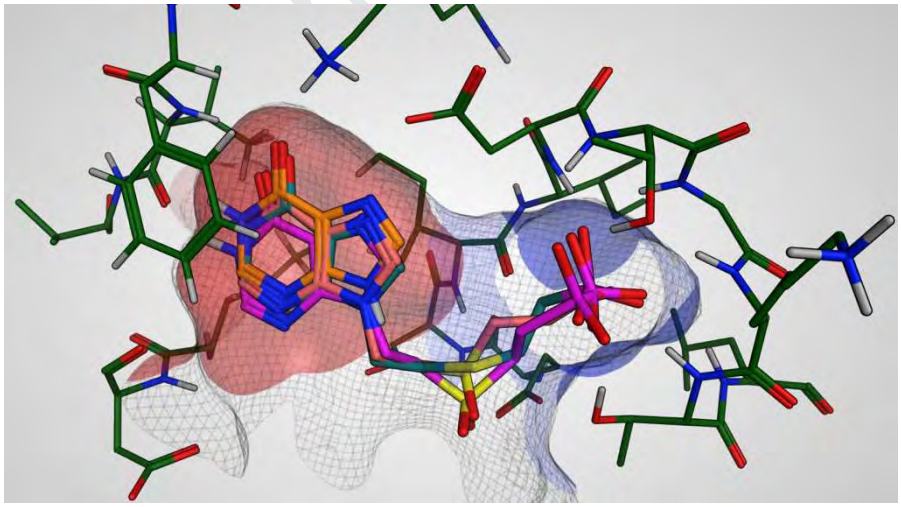


Figure. 7. Docking of three thia-ANPs into the active site of *Pf*HGXPRT. 8a (sulfide) has green carbon atoms, **10a** (sulfoxide) orange carbon atoms, and **14a** (sulfone) magenta carbon atoms docked into *Pf*HGXPRT (PDB code 3OZF).[35]

2.3 Comparison of the inhibition of human HGPRT and* Pf*HGXPRT by the thia-ANPs

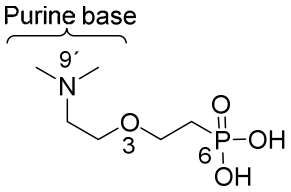
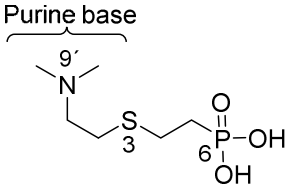
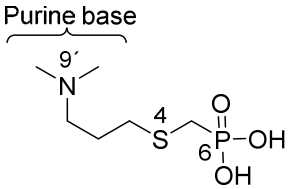
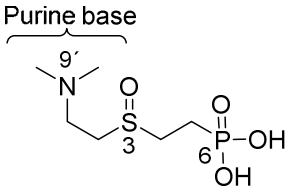
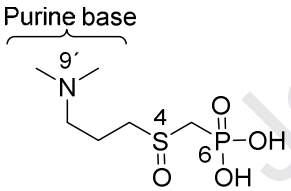
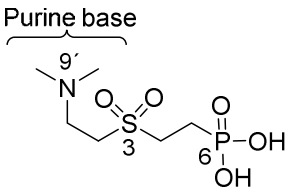
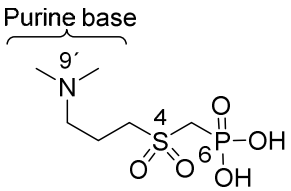
Table 1 shows the inhibition constants for the ten newly synthesized thia-ANPs. Compounds **8a** and **8b** have similar K_i values to those of PEEG and PEEHx (**Table 1**), an indication that they should bind to human HGPRT and *Pf*HGXPRT in a similar mode to PEEG and PEEHx (**Fig. 6**). However, moving the sulfide group so that it is one atom further away from the N^9 atom of the base (**7a** and **7b**) results in dramatic increases in K_i values. Comparing **7a** with **8a** (hypoxanthine derivatives) and **7b** with **8b** (guanine derivatives), 16-fold and 4-fold increases (human HGPRT) and 16 and 11-fold increases (*Pf*HGXPRT) are observed. These increases show that a simple relocation of the sulfur atom reduces binding. This may be due to a change in the conformation of the linker (as predicted by the docking) or reduced complementarity of these compounds with the active site of the enzyme.

In **14a** and **13a**, a sulfone replaces the sulfide (*cf* with **8a** and **7a**). Comparing **8a** and **14a**, this change results in ~20-fold increases in K_i for both the human and *Pf* enzymes suggesting that these attachments are also sterically unfavourable. In contrast, however, there is little change in the K_i values for human HGPRT and *Pf*HGXPRT for **7a** and **13a**, so the addition of the oxygen atoms one position distal to the base has little effect.

For the sulfoxides, there is not a significant change in the K_i values compared with the sulfides (*cf.* **8a** and **8b** with **10a** and **10b; 7a** and **7b** with **9a** and **9b)**. There is one exception, this is **7a** which has a much lower K_i value than **9a** for human HGPRT. Thus, the introduction of a sulfoxide moiety does not have any effect on the binding of the thia-ANPs for *Pf*HGXPRT.

Interestingly, most of the thia-ANPs show a strong preference for binding to *Pf*HGXPRT rather than human HGPRT (**Table 1**, see selectivity). For example, **7a**, **8a** and **14a** have more than 20-fold lower K_i values for *Pf*HGXPRT compared to human HGPRT. Thus a promising group of inhibitors resulted from the introduction of sulfur containing motif into the ANP-scaffold.

Table 1. The K_i values for the thia-ANPs with human HGPRT and *Pf*HGXPRT

Structure	Code (**Scheme 2**)	**Nucleobase**[a]	**Human** K_i (μM)	***Pf*** K_i (μM)	**Selectivity** Human/*Pf*
	PEEHx[b]	Hx	3.6±0.2	0.3±0.04	12
	PEEG [b]	G	1±0.5	0.1±0.02	10
	**8a**	Hx	5±2	0.2±0.06	25
	8b	G	0.4±0.1	0.1±0.05	4
	**7a**	Hx	>80	3±0.5	>26
	7b	G	1.4±0.5	1.1±0.3	1.3
	**10a**	Hx	4±1.5	1.0±0.2	4
	10b	G	0.2±0.1	0.3±0.1	0.7
	**9a**	Hx	11±3	4±2	2.8
	9b	G	3±1	0.2±0.1	15
	**14a**	Hx	>90	4±1	>22
	14b	G	---[c]	---[c]	---[c]
	**13a**	Hx	46±15	4.5±0.5	10
	13b	G	---[c]	---[c]	---[c]

[a]Hx = hypoxanthine, G = guanine. [b]data from [16]. [c]Not synthesized.

2.4 The IC_{50} values for the phosphoramidate prodrugs of the thia-ANP inhibitors

Phosphoramidate prodrugs (**15b**, **16a** and **16b; Scheme 3**) of the thia-ANP inhibitors **7b**, **8a** and **8b** (**Table 1**) were synthesized and tested against the asexual stages of two *Pf* strains (chloroquine-sensitive D6 and chloroquine-resistant W2) in erythrocyte cell culture. The IC_{50} values (**Table 2**) are in the 4-6 μM range, which is similar to that for one of the prodrugs of PEEG (**Fig. 4B)**. This also corresponds well with the fact that the K_i values for *Pf*HGXPRT for the four parent compounds, **7a**, **8a**, **8b** and PEEG[16] are similar, 0.1, 0.2, 1 and 0.1 μM (**Table 1**). The prodrugs exhibited no cytotoxicity in tested human cell lines (**Table 2**).

Table 2. The IC_{50} values for three phosphoramidate prodrugs in *Pf in vitro* assays and cytotoxicity of the prodrugs in human cells.

Prodrug (Scheme 1)	Parent compound (Scheme 2)	IC_{50} (μM) D6[a]	IC_{50} (μM) W2[b]	Viability [%][c] HepG2[d]	Viability [%][c] CCRF-CEM[e]
15b	**7b**	4.0 ± 0.6	5.2 ± 1.0	95	98
16a	**8a**	4.4 ±1 .6	5.4 ± 0.1	90	100
16b	**8b**	5.4 ± 0.5	4.4 ± 0.1	83	106

[a]D6 is the strain of *Plasmodium falciparum* that is sensitive to most drugs. [b]W2 is resistant to chloroquine. [c]Percent cell viability at a fixed prodrug concentration (10 μM) versus untreated control. [d]Human hepatocellular carcinoma cell line. [e]Human T-cell leukemia cell line.

3. Conclusion

Chemoselective sulfur alkylation and oxidation to sulfoxide or sulfone at various stages of synthesis were used to synthesize ten thia-ANPs whose chemical structures were based on that of PEEG/PEEHx. The insertion of sulfur in three of its five oxidation states resulted in a range of K_i values for *Pf*HGXPRT (0.1-4.5 μM). For human HGPRT, this range increased to 0.2-90 μM. This implies that thia-ANPs distinctively favour *Pf*HGXPRT over its human counterpart. Therefore, subtle variations in the chemical structure of the linker connecting the purine base to the phosphonate group can strongly influence how the ligand binds in the

active site of human HGPRT and *Pf*HGXPRT. Prodrugs of the most potent inhibitors of *Pf*HGXPRT have antimalarial activity in cell culture with IC_{50} values between 4 and 6 μM and are not toxic in human cell lines. Thus, further elaboration and modification of this chemical scaffold should result in new compounds that are relatively cost effective to synthesize, are potent and selective inhibitors of *Pf*HGXPRT and are active in biological assays.

4. Experimental section

4.1 General remarks

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and prepared compounds were dried at 30 °C at 2 kPa. Starting compounds and reagents were purchased from commercial suppliers (Sigma-Aldrich, Fluorochem, Acros Organics, Carbosynth, TCI) and used without further purification or were prepared according to the published procedures.

Dioxane was dried by activated neutral alumina (drysphere®). Dry dichloromethane and pyridine was purchased from Acros Organics.

Analytical TLCs were performed on silica gel pre-coated aluminium plates with fluorescent indicator (Merck 60 F254). Flash column chromatographies were carried out by Teledyne ISCO CombiFlash Rf200 with dual absorbance detector. Various types of columns were used: a) Teledyne ISCO columns RediSepRf HP Silica GOLD in sizes 12 g, 40 g, 80 g and 120 g; b) Teledyne ISCO columns RediSepRf HP C18 Aq GOLD in sizes 50 g and 100 g; c) column Chromabond Flash DL 40, DL 80, DL 120 and DL 200, filled with FLUKA silica gel 60; d) Interchim puriFlash C18 Aq in sizes F0040 and F0080. Eluents used were cyclohexane-ethyl acetate 6:4 mixture (A), ethyl acetate modified with 10 % of methanol (B), chloroform (C), methanol (D) and water (E).

Preparative HPLC purifications were performed on Waters Delta 600 chromatography system with columns packed with C18 reversed phase resin (Phenomenex Gemini 10 μm 21 x 250 mm, Phenomenex Gemini 5 μm 21 x 250 mm, Phenomenex Luna 10 μm 21 x 250 mm) using gradient H_2O/MeOH as eluent.

Mass spectra, UV absorbancy and purity of compounds were measured on Waters UPLC-MS system consisted of Waters UPLC H-Class Core System (column Waters Acquity UPLC BEH C18 1.7 mm, 2.1 x 100 mm), Waters Acquity UPLC PDA detector and Mass

spectrometer Waters SQD2. The universal LC method was used (eluent H_2O/ CH_3CN, gradient 0 – 100 %, run length 7 min) and MS method (ESI+ and/or ESI-, cone voltage = 30 V, mass detector range 100 – 1000 Da). Purity of the final compounds was >95%.

High-resolution mass spectra were measured on a LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific).

NMR spectra were recorded on Bruker Avance 400 or 500 spectrometers referenced to the residual solvent signal or a specified additive.

Dowex® 50W resin was turned to Na^+ cycle by treatment of Dowex® 50W resin in H^+ cycle with 1M NaOH aq. solution, followed by water wash to neutral pH.

4.2 General methods

4.2.1 Method A: General procedure for Mitsunobu reaction with 6-chloropurine

In a 60 mL vial, triphenylphosphine (2.00 g, 7.5 mmol, 1.5 eq.), 6-chloropurine (0.77 g, 5.0 mmol, 1 eq.) and the corresponding alcohol (6.0 mmol, 1.2 eq.) was suspended in 40 mL of dry dioxane under an argon atmosphere. The suspension was heated up to 80 °C and then DIAD (1.5 mL, 7.5 mmol, 1.5 eq.) was added dropwise via syringe. The mixture turned homogeneous and started to change to a green colour in a few minutes. After 10 minutes, the reaction was completed and the solvent was evaporated in vacuo. The residue was loaded onto silicagel and purified by flash chromatography using solvent gradient A to B to yield the title compound as a colourless oil.

4.2.2 Method B: General procedure for Mitsunobu reaction with 2-amino-6-chloropurine

In a 60 mL vial, triphenylphosphine (2.00 g, 7.5 mmol, 1.5 eq.), 2-amino-6-chloropurine (0.85 g, 5.0 mmol, 1 eq.) and the corresponding alcohol (6.0 mmol, 1.2 eq.) was suspended in 40 mL of dry dioxane under an argon atmosphere. The suspension was heated up to 80 °C and then DIAD (1.5 mL, 7.5 mmol, 1.5 eq.) was added dropwise via syringe. The mixture turned homogeneous and started to change to a green colour in a few minutes. After 15 minutes, the reaction was completed. Water (5 mL) and MeOH (5 mL) were added and the mixture was stirred for a further 60 minutes at 80 °C. After that, the solvent was evaporated in vacuo. The residue was loaded onto silicagel and purified by flash chromatography using solvent gradient A to B to yield the title compound as a colourless oil.

4.2.3 Method C: General procedure for hydrolysis of 6-chloropurines and 2-amino-6-chloropurines

In a 100 mL round-bottom flask, the 6-chloropurine or 2-amino-6-chloropurine derivative (3 mmol) was dissolved in 20 mL of 75% trifluoroacetic acid and the reaction mixture was stirred at room temperature for 2 days. After the reaction went to completion the solvent was evaporated and the residue was codistilled with water (3 times) and ethanol. The residue was purified by flash chromatography using solvent gradient C to D. The product was obtained as a sticky white solid.

4.2.4 Method D: General procedure for deprotection of phosphonate compounds

In a 50 mL round-bottom flask the phosphonate compound (1 mmol) was dissolved/suspended in 10 mL of dry dichloromethane under an argon atmosphere. Trimethylsilyl bromide (1 mL, 7.5 mmol, 7.5 eq.) was added dropwise and the reaction mixture was stirred overnight. If a suspension was present, it was dissolved during the reaction. After completion, methanol (5 mL) was added and the reaction mixture was evaporated. The residue was dissolved in 10 mL of H_2O/MeOH (1:1) mixture, stirred for 30 minutes and solvent evaporated. The crude product was used for subsequent reactions without further purification. For the preparation of sodium salt, 2 M TEAB (0.5 mL) was added, the mixture was evaporated and then purified by reverse phase flash chromatography using solvent gradient E to D. The fractions containing product were combined, evaporated, dissolved in a minimal amount of water, passed through a short column of DOWEX 50 in Na^+ cycle and lyophilised to yield the final products as white foams.

4.2.5 Method E: General procedure for oxidation of sulfides to sulfoxides

In a 4 mL vial, the phosphonic acid (0.5 mmol) was dissolved in water (2 mL). Hydrogen peroxide (100 μL, 30%, 1 eq.) was added while stirring. After the starting compound was consumed, 2M TEAB (0.5 mL) was added and the reaction mixture was purified by reverse phase flash chromatography using solvent gradient E to D. The fractions containing product were combined, evaporated, dissolved in a minimal amount of water, passed through a short column of DOWEX 50 in Na^+ cycle and lyophilised to yield the final products.

4.2.6 Method F: General procedure for oxidation of sulfides to sulfones

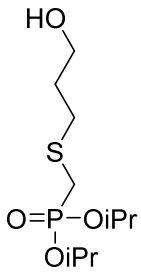
In a 30 mL vial the hypoxanthine derivative (1 mmol) was dissolved in 5 mL of DCM and the solution was cooled to 0 °C. A solution of MCPBA (3 mmol, 3 eq.) in DCM (5 mL) was added and the reaction mixture was stirred for 2 hours. After the reaction was completed the solvent was evaporated and the residue was purified by reverse phase flash chromatography using solvent gradient C to D to yield the sulfone as a white solid.

4.2.7 Method G: General procedure bisamidate prodrug synthesis

To the starting phosphonic acid, tetrabutylammonium hydroxide (1.0 M in water, 2 eq.) was added and the solvent was evaporated. The residue was purified by reverse flash column chromatography using solvent gradient E to D and lyophilised. To the dry lyophilisate (0.40 g, 0.5 mmol) in a 30 mL vial isopropyl-phenylalanine hydrochloride (0.43 g, 1.77 mmol, 3.5 eq.) was added. The vial was evacuated and filled with argon three times. Dry pyridine (20 mL) and dry triethylamine (0.8 mL) were added and the solution was heated to 60 °C for 15 minutes. In a second vial triphenylphosphine (0.70 g, 2.62 mmol, 5.24 eq.) and 2,2´-dipyridyldisulfide (0.81 g, 3.67 mmol, 7.34 eq.) were dissolved in dry pyridine (10 mL) under argon and the solution was added into the first vial. The resulting solution was heated at 60 °C for 3 days and then the solvent was evaporated. The residue was purified by flash column chromatography using solvent gradient C to D. The product containing fractions were evaporated and purified by preparative HPLC. The product was obtained as white solid after lyophilisation.

4.3 Compounds synthesis

*4.3.1 Diisopropyl 5-hydroxy-2-thiapentanephosphonate (**1**)*



In a 250 mL round-bottom flask potassium carbonate (8.6 g, 62 mmol) was suspended in 120 mL of ethanol. To this suspension 3-mercaptopropanol (5 mL, 59 mmol) and diisopropyl tosyloxymethylphosphonate (17.5 g, 50 mmol) was added and the reaction mixture was stirred at reflux overnight. The reaction mixture was evaporated and the residue was dissolved in 100 mL of water and extracted with chloroform (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and evaporated to give **1b** (13.7 g, quantitative) as a yellowish oil.

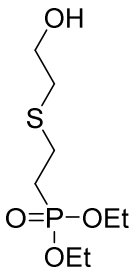
^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.77 (dq, *J* = 12.4, 6.2 Hz, 2H, POC**H**), 3.79 (t, *J* = 5.8 Hz, 2H, C**H$_2$**OH), 2.92 (t, *J* = 6.8 Hz, 2H, C**H$_2$**CH$_2$CH$_2$OH), 2.69 (d, *J* = 12.7 Hz, 2H, PC**H$_2$**), 1.84 (p, *J* = 6.4 Hz, 2H, C**H$_2$**CH$_2$OH), 1.36 (t, *J* = 6.0 Hz, 12H, POCHC**H$_3$**).

^{31}P NMR (162 MHz, Chloroform-*d*) δ 25.13.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 71.4 (d, *J* = 7.2 Hz, PO**C**H), 60.2 (**C**H$_2$OH), 31.5 (**C**H$_2$CH$_2$OH), 30.0 (**C**H$_2$CH$_2$CH$_2$OH), 26.4 (d, *J* = 152.0 Hz, P**C**H$_2$), 24.1 (d, *J* = 17.9 Hz, POCH**C**H$_3$).

MS (ESI+) m/z = 271 $[M + H]^+$.

*4.3.2 Diethyl 5-hydroxy-3-thiapentanephosphonate (**2**)*



In a 100mL round-bottom flask potassium carbonate (1.38 g, 10 mmol) and 2-mercaptoethanol (2.1 mL, 30 mmol) was dissolved in 50 mL of distilled water. To this solution diethyl vinylphosphonate (3.0 mL, 20 mmol) was added and the reaction mixture was stirred for 1 hour at room temperature. Then it was extracted with chloroform (3x25 mL). The combined organic layers were washed with saturated $KHCO_3$ (25 mL) and brine (25 mL), dried over $MgSO_4$ and evaporated to give **1a** (4.3 g, 88% yield) as a colourless oil.

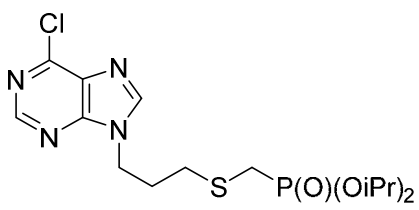
^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.18 – 4.00 (m, 4H, POC**H$_2$**), 3.73 (t, *J* = 6.1 Hz, 2H, C**H$_2$**OH), 3.47 (s, 1H, O**H**), 2.80 – 2.74 (m, 2H, PCH$_2$C**H$_2$**), 2.72 (t, *J* = 6.1 Hz, 2H, C**H$_2$**CH$_2$OH), 2.12 – 1.96 (m, 2H, PC**H$_2$**), 1.31 (t, *J* = 7.1 Hz, 6H, POCH$_2$C**H$_3$**).

^{31}P NMR (162 MHz, Chloroform-*d*) δ 31.17.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 61.9 (d, *J* = 6.5 Hz, PO**C**H$_2$), 60.7 (**C**H$_2$OH), 35.1 (**C**H$_2$CH$_2$OH), 26.8 (d, *J* = 137.3 Hz, P**C**H$_2$), 24.8 (d, *J* = 3.9 Hz, PCH$_2$**C**H$_2$), 16.4 (d, *J* = 6.0 Hz, POCH$_2$**C**H$_3$).

MS (ESI+) m/z = 243 $[M + H]^+$.

*4.3.3 Diisopropyl 5-(6-chloropurin-9-yl)-2-thiapentanephosphonate (**3a**)*



Prepared by Method A from **1b**. Yield 1.61 g (80%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.60 (s, 1H, H-2), 8.21 (s, 1H, H-8), 4.72 – 4.48 (m, 2H, POC**H**),

4.37 (t, *J* = 7.0 Hz, 2H, ArC**H₂**), 2.65 (t, *J* = 6.7 Hz, 2H, $ArCH_2CH_2C\mathbf{H_2}$), 2.56 (d, *J* = 13.1 Hz, 2H, PC**H₂**), 2.17 (p, *J* = 6.9 Hz, 2H, $ArCH_2C\mathbf{H_2}$), 1.21 (d, *J* = 6.2 Hz, 12H, POCHC**H₃**).

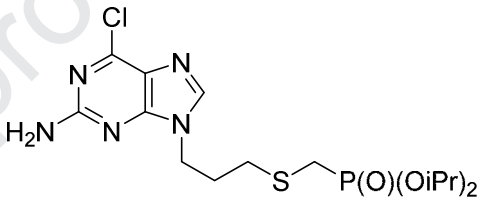
^{31}P NMR (162 MHz, Chloroform-*d*) δ 24.20.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 151.8 (C-2), 151.7 (C-4), 150.7 (C-6), 145.7 (C-8), 131.9 (C-5), 71.3 (d, *J* = 7.0 Hz, PO**C**H), 42.9 (Ar**C**H_2), 30.0 (d, *J* = 2.8 Hz, $ArCH_2CH_2\mathbf{C}H_2$), 28.4 ($ArCH_2\mathbf{C}H_2$), 25.9 (d, *J* = 151.9 Hz, P**C**H_2), 23.9 (dd, *J* = 9.6, 4.4 Hz POCH**C**H_3).

MS (ESI+) m/z = 407 $[M + H]^+$.

*4.3.4 Diisopropyl 5-(2-amino-6-chloropurin-9-yl)-2-thiapentanephosphonate (**3b**)*

Prepared by Method B from **1b**. Yield 1.47 g (70%).



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.83 (s, 1H, H-8), 5.36 (s, 2H, N**H₂**), 4.76 (dq, *J* = 12.4, 6.2 Hz, 2H, POC**H**), 4.24 (t, *J* = 6.9 Hz, 2H, ArC**H₂**), 2.75 (t, *J* = 6.6 Hz, 2H $ArCH_2CH_2C\mathbf{H_2}$), 2.75 (d, *J* = 13.5 Hz, 2H, PC**H₂**), 2.32 – 2.05 (m, 2H, $ArCH_2C\mathbf{H_2}$), 1.34 (d, *J* = 6.1 Hz, 12H, POCHC**H₃**).

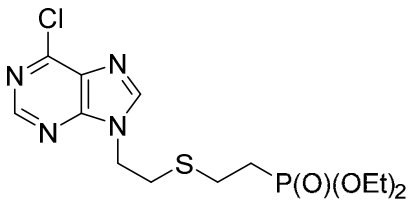
^{31}P NMR (162 MHz, Chloroform-*d*) δ 24.52.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.1 (C-2), 153.8 (C-4), 151.4 (C-6), 142.5 (C-8), 125.4 (C-5), 71.4 (d, *J* = 7.1 Hz, PO**C**H), 42.2 (Ar**C**H_2), 30.1 (d, *J* = 3.5 Hz, $ArCH_2CH_2\mathbf{C}H_2$), 28.3 ($ArCH_2\mathbf{C}H_2$), 26.0 (d, *J* = 151.2 Hz, P**C**H_2), 24.1 (dd, *J* = 10.2, 4.4 Hz, POCH**C**H_3).

MS (ESI+) m/z = 422 $[M + H]^+$.

*4.3.5 Diethyl 5-(6-chloropurin-9-yl)-3-thiapentanephosphonate (**4a**)*

Prepared by Method A from **1a**. Yield 1.56 g (83%).



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.77 (s, 1H, H-2), 8.37 (s, 1H, H-8), 4.54 (t, *J* = 6.5 Hz, 2H, ArC**H₂**), 4.21 – 3.96 (m, 4H, POC**H₂**), 3.09 (t, *J* = 6.5 Hz, 2H, $ArCH_2C\mathbf{H_2}$), 2.76 (dd, J = 16.4, 10.2 Hz, 2H, $PCH_2C\mathbf{H_2}$), 2.01 (dt, J = 18.0, 8.3 Hz, 2H, PC**H₂**), 1.33 (t, *J* = 7.1 Hz, 6H, $POCH_2C\mathbf{H_3}$).

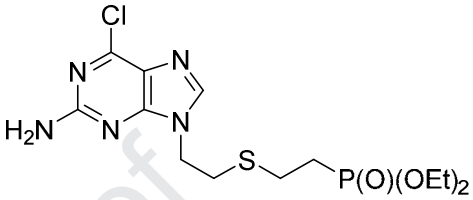
^{31}P NMR (162 MHz, Chloroform-*d*) δ 30.37.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 152.1 (C-2), 151.6 (C-4), 151.1 (C-6), 145.6 (C-8), 131.2 (C-5), 62.0 (d, *J* = 6.6 Hz, PO**C**H$_2$), 44.0 (Ar**C**H$_2$), 31.6 (ArCH$_2$**C**H$_2$), 26.6 (d, *J* = 137.9 Hz, P**C**H$_2$), 25.2 (d, *J* = 3.6 Hz, PCH$_2$**C**H$_2$), 16.5 (d, *J* = 5.9 Hz, POCH$_2$**C**H$_3$).

MS (ESI+) m/z = 379 $[M + H]^+$.

*4.3.6 Diethyl 5-(2-amino-6-chloropurin-9-yl)-3-thiapentanephosphonate (**4b**)*

Prepared by Method B from **1a**. Yield 1.35 g (69%).



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.78 (s, 1H, C-8), 5.66 (s, 2H, -N**H$_2$**), 4.26 (t, *J* = 7.0 Hz, 2H, ArC**H$_2$**), 4.10 (dtt, *J* = 14.1, 7.0, 3.4 Hz, 4H, POC**H$_2$**), 2.95 (t, *J* = 7.1 Hz, 2H, ArCH$_2$C**H$_2$**), 2.89 – 2.80 (m, 2H, PCH$_2$C**H$_2$**), 2.13 (dd, *J* = 18.5, 16.9 Hz, 2H, PC**H$_2$**), 1.32 (t, *J* = 7.1 Hz, 6H, POCH$_2$C**H$_3$**).

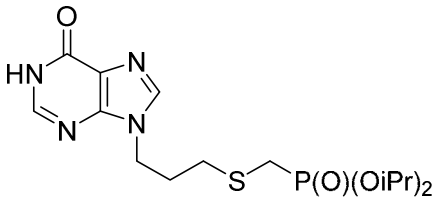
^{31}P NMR (162 MHz, Chloroform-*d*) δ 30.89.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.4 (C-2), 153.8(C-4), 151.3 (C-8), 142.0 (C-6), 124.9 (C-5), 62.0 (d, *J* = 6.6 Hz, PO**C**H$_2$), 43.2 (Ar**C**H$_2$), 30.7 (ArCH$_2$**C**H$_2$), 26.5 (d, *J* = 136.3 Hz, P**C**H$_2$), 24.6 (d, *J* = 3.3 Hz, PCH$_2$**C**H$_2$), 16.5 (d, *J* = 5.9 Hz, POCH$_2$**C**H$_3$).

MS (ESI+) m/z = 394 $[M + H]^+$.

*4.3.7 Diisopropyl 5-(hypoxanthin-9-yl)-2-thiapentanephosphonate (**5a**)*

Prepared by Method C from **2b**. Yield 0,59 g (51%).



^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H, H-1), 8.11 (s, 1H, H-8), 8.04 (s, 1H, H-2), 4.57 (dhept, *J* = 7.8, 6.2 Hz, 2H, POC**H**), 4.22 (t, *J* = 7.0 Hz, 2H, ArC**H$_2$**), 2.82 (d, *J* = 13.3 Hz, 2H, PC**H$_2$**), 2.64 (t, *J* = 7.1 Hz, 2H ArCH$_2$CH$_2$C**H$_2$**,), 2.09 (p, *J* = 7.4 Hz, 2H, ArCH$_2$C**H$_2$**), 1.23 (d, *J* = 6.2 Hz, 12H, POCHC**H$_3$**).

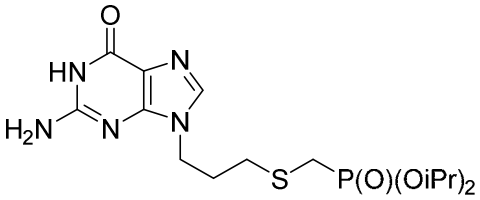
^{31}P NMR (162 MHz, DMSO-d_6) δ 25.30.

^{13}C NMR (101 MHz, DMSO-d_6) δ 157.1 (C-6), 148.8 (C-2), 145.9 (C-4), 140.7 (C-8), 124.4 (C-5), 70.7 (PO**C**H), 42.8 (Ar**C**H$_2$), 29.9 (d, *J* = 4.0 Hz, ArCH$_2$CH$_2$**C**H$_2$), 29.4 (ArCH$_2$**C**H$_2$), 25.2 (d, *J* = 147.6 Hz, P**C**H$_2$), 24.2 (dd, *J* = 12.7, 4.2 Hz, POCH**C**H$_3$).

MS (ESI+) m/z = 389 $[M + H]^+$.

*4.3.8 Diisopropyl 5-(guanin-9-yl)-2-thiapentanephosphonate (**5b**)*

Prepared by Method C from **3b**. Yield 1.02 g (84%).



^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H, H-1), 7.69 (s, 1H, H-8), 6.42 (s, 2H, N**H$_2$**), 4.59 (ddt, *J* = 12.3, 7.8, 6.1 Hz, 2H, POC**H**), 4.00 (t, *J* = 7.1 Hz, 2H, ArC**H$_2$**), 2.84 (d, *J* = 13.3 Hz, 2H, PC**H$_2$**), 2.63 (t, *J* = 7.1 Hz, 2H ArCH$_2$CH$_2$C**H$_2$**), 2.02 (p, *J* = 7.2 Hz, 2H, ArCH$_2$C**H$_2$**), 1.24 (d, *J* = 6.2 Hz, 12H, POCHC**H$_3$**).

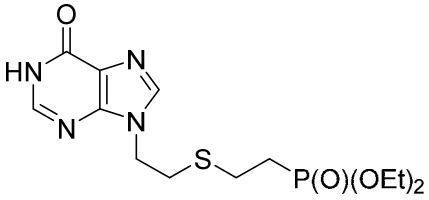
^{31}P NMR (162 MHz, DMSO-d_6) δ 25.33.

^{13}C NMR (101 MHz, DMSO-d_6) δ 157.2 (C-6), 153.9 (C-2), 151.6 (C-4), 137.8 (C-8), 117.1 (C-5), 70.64 (d, *J* = 6.7 Hz, PO**C**H), 42.1 (Ar**C**H$_2$), 30.0 (d, *J* = 4.1 Hz, ArCH$_2$**C**H$_2$), 29.2 (ArCH$_2$**C**H$_2$), 25.3 (d, *J* = 147.4 Hz, P**C**H$_2$), 24.2 (dd, *J* = 12.8, 4.3 Hz, POCH**C**H$_3$).

MS (ESI+) m/z = 404 $[M + H]^+$.

*4.3.9 Diethyl 5-(hypoxanthin-9-yl)-3-thiapentanephosphonate (**6a**)*

Prepared by Method C from **2a**. Yield 0.90 g (83%).



^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.99 (s, 1H, NH-1), 8.26 (s, 1H, H-8), 8.06 (s, 1H, H-2), 4.41 (t, *J* = 6.8 Hz, 2H, ArC**H$_2$**), 4.18 – 3.99 (m, 4H, POC**H$_2$**), 3.03 (t, *J* = 6.8 Hz, 2H, ArCH$_2$C**H$_2$**), 2.75 (dd, *J* = 17.2, 9.1 Hz, 2H, PCH$_2$C**H$_2$**), 2.03 (dt, *J* = 18.1, 8.4 Hz, 2H, PC**H$_2$**), 1.30 (t, *J* = 7.1 Hz, 6H, POCH$_2$C**H$_3$**).

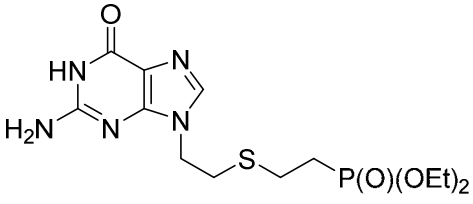
^{31}P NMR (162 MHz, Chloroform-*d*) δ 30.73.

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.4 (C-6), 148.9 (C-4), 145.7 (C-2), 140.4 (C-8), 124.1 (C-5), 62.0 (d, *J* = 6.6 Hz, PO**C**H$_2$), 43.8 (Ar**C**H$_2$), 31.8 (ArCH$_2$**C**H$_2$), 26.5 (d, *J* = 137.1 Hz, P**C**H$_2$), 25.0 (d, *J* = 3.6 Hz, PCH$_2$**C**H$_2$), 16.4 (d, *J* = 6.0 Hz, POCH$_2$**C**H$_3$).

MS (ESI+) m/z = 361 $[M + H]^+$.

*4.3.10 Diethyl 5-(guanin-9-yl)-3-thiapentanephosphonate (**6b**)*

Prepared by Method C from **3a**. Yield 0.92 g (82%)



^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H, H-1), 8.27 (s, 1H, H-8), 6.72 (s, 2H, N**H$_2$**), 4.19 (t, *J* = 6.7 Hz, 2H, ArC**H$_2$**), 4.06 – 3.92 (m, 4H, POC**H$_2$**), 2.98 (t, *J* = 6.7 Hz, 2H, ArCH$_2$C**H$_2$**), 2.72 – 2.59 (m, 2H, PCH$_2$C**H$_2$**), 2.09 – 1.97 (m, 2H, PC**H$_2$**), 1.23 (t, *J* = 7.0 Hz, 6H, POCH$_2$C**H$_3$**).

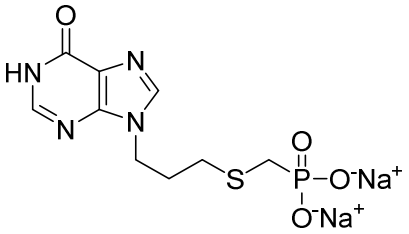
^{31}P NMR (162 MHz, DMSO-d_6) δ 30.91.

^{13}C NMR (101 MHz, DMSO-d_6) δ 156.7 (C-6), 154.4 (C-2), 151.3 (C-4), 138.0 (C-8), 61.7 (d, *J* = 6.3 Hz, PO**C**H$_2$), 43.1 (Ar**C**H$_2$), 30.7 (ArCH$_2$**C**H$_2$), 26.1 (d, *J* = 133.9 Hz, P**C**H$_2$), 24.5, PCH$_2$**C**H$_2$, 16.7 (d, *J* = 5.8 Hz, POCH$_2$**C**H$_3$).

MS (ESI+) m/z = 376 [M + H]$^{+}$.

*4.3.11 Sodium 5-(hypoxanthin-9-yl)-2-thiapentanephosphonate (**7a**)*

Prepared by Method D from **5a**. Yield 0.30 g (86 %).



^{1}H NMR (400 MHz, D$_2$O) δ 8.22 (s, 1H, H-8), 8.20 (s, 1H, H-2), 4.34 (t, *J* = 6.9 Hz, 2H, ArC**H$_2$**), 2.70 (d, *J* = 14.1 Hz, 2H, PC**H$_2$**), 2.66 (d, *J* = 7.1 Hz, 2H ArCH$_2$CH$_2$C**H$_2$**), 2.18 (p, *J* = 7.0 Hz, 2H, ArCH$_2$C**H$_2$**).

^{31}P NMR (162 MHz, D$_2$O) δ 18.93.

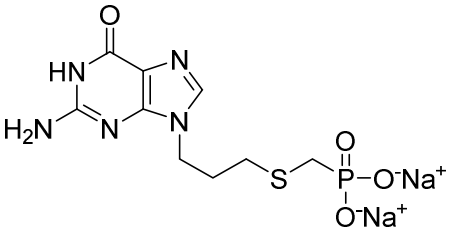
^{13}C NMR (101 MHz, D$_2$O) δ 158.4 (C-6), 148.6 (C-4), 145.8 (C-2), 142.4 (C-8), 123.3 (C-5), 43.4 (Ar**C**H$_2$), 30.3 (d, *J* = 7.8 Hz, ArCH$_2$CH$_2$**C**H$_2$), 28.9 (d, *J* = 131.5 Hz, P**C**H$_2$), 28.9 (ArCH$_2$**C**H$_2$).

MS (ESI+) m/z = 305 [M -2 Na +3 H]$^{+}$.

HR-MS (ESI^{-}) m/z: calcd for $C_9H_{12}O_4N_4PS$ = 303.0322 [M − 2Na + H]$^{-}$, found 303.0321 [M − 2Na + H]$^{-}$.

*4.3.12 Sodium 5-(guanin-9-yl)-2-thiapentanephosphonate (**7b**)*

Prepared by Method D from **5b**. Yield 0.28 g (79%).



^{1}H NMR (400 MHz, D$_2$O) δ 8.86 (s, 1H, H-8), 4.29 (t, *J* = 6.9 Hz, 2H, ArC**H$_2$**), 2.74 (d, *J* = 13.7 Hz, 2H, PC**H$_2$**), 2.66 (t, *J* = 7.0 Hz, 2H ArCH$_2$CH$_2$C**H$_2$**), 2.15 (p, *J* = 7.0 Hz, 2H, ArCH$_2$C**H$_2$**).

^{31}P NMR (162 MHz, D$_2$O) δ 25.44.

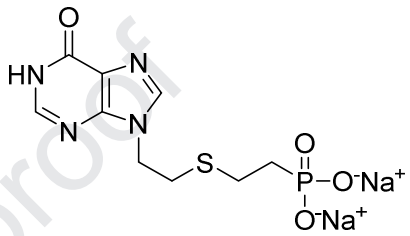
^{13}C NMR (101 MHz, Deuterium Oxide) δ 158.86 (C-6), 153.56 (C-2), 151.42 (C-4), 140.18 (C-8), 115.96 (C-5), 42.45 (Ar**C**H_2), 29.98 (d, *J* = 7.1 Hz, $ArCH_2CH_2$**C**H_2), 28.36 ($ArCH_2CH_2$), 28.09 (d, *J* = 134.1 Hz, P**C**H_2).

MS (ESI+) m/z = 320 $[M -2 Na +3 H]^+$.

HR-MS (ESI^-) m/z: calcd for $C_9H_{13}O_4N_5PS$ = 318.0431 $[M - 2Na + H]^-$, found 318.0431 $[M - 2Na + H]^-$.

*4.3.13 Sodium 5-(hypoxanthin-9-yl)-3-thiapentanephosphonate (**8a**)*

Prepared by Method D from **6a**. Yield 0.28 g (81%).



^{1}H NMR (400 MHz, D_2O) δ 8.07 (s, 1H, H-8), 8.01 (s, 1H, H-2), 4.33 (t, *J* = 6.5 Hz, 2H, ArC**H$_2$**), 3.01 (t, *J* = 6.5 Hz, 2H, $ArCH_2$C**H$_2$**), 2.69 (dt, *J* = 10.9, 6.2 Hz, 2H, PCH_2C**H$_2$**), 1.72 – 1.51 (m, 2H, PC**H$_2$**).

^{31}P NMR (162 MHz, D_2O) δ 21.22.

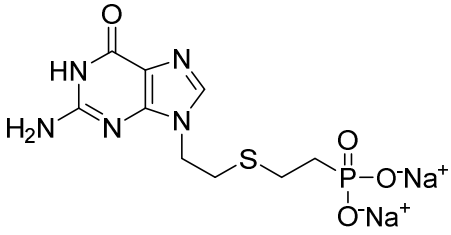
^{13}C NMR (101 MHz, D_2O) δ 165.6 (C-6), 151.7 (C-4), 149.4 (C-2), 141.2 (C-8), 123.1 (C-5), 43.1 (Ar**C**H_2), 31.0 ($ArCH_2$**C**H_2), 30.1 (d, *J* = 123.8 Hz, P**C**H_2), 26.8 (PCH_2**C**H_2)

MS (ESI+) m/z = 305 $[M -2 Na +3 H]^+$.

HR-MS (ESI^-) m/z: calcd for $C_9H_{12}O_4N_4PS$ = 303.0322 $[M - 2Na + H]^-$, found 303.0321 $[M - 2Na + H]^-$.

*4.3.14 Sodium 5-(guanin-9-yl)-3-thiapentanephosphonate (**8b**)*

Prepared by Method D from **6b**. Yield 0.25 g (70%).



^{1}H NMR (400 MHz, D_2O) δ 7.84 (s, 1H, H-8), 4.27 (t, *J* = 6.4 Hz, 2H, ArC**H$_2$**), 3.07 (t, *J* = 6.5 Hz, 2H, $ArCH_2$C**H$_2$**), 2.78 (dd, *J* = 17.4, 6.5 Hz, 2H, $ArCH_2$C**H$_2$**), 1.81 – 1.65 (m, 2H, PC**H$_2$**).

^{31}P NMR (162 MHz, D_2O) δ 21.75.

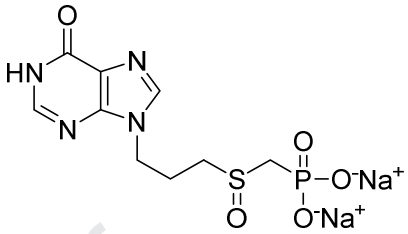
^{13}C NMR (101 MHz, D_2O) δ 168.3 (C-6), 161.0 (C-2), 151.4 (C-4), 139.0 (C-8), 117.7 (C-5), 42.9 (Ar**C**H_2), 31.2 ($ArCH_2$**C**H_2), 30.3 (d, *J* = 123.8 Hz, P**C**H_2), 27.1 (PCH_2**C**H_2).

MS (ESI+) m/z = 320 $[M -2 Na +3 H]^+$.

HR-MS (ESI$^-$) m/z: calcd for $C_9H_{13}O_4N_5PS$ = 318.0431 [M − 2Na + H]$^-$, found 318.0431 [M − 2Na + H]$^-$.

*4.3.15 Sodium 5-(hypoxanthin-9-yl)-2-oxo-2-thiapentanephosphonate (**9a**)*

Prepared by Method E from **7a**. Yield 0.15 g (83%).



^{1}H NMR (400 MHz, D_2O) δ 8.10 (s, 1H, H-8), 8.00 (s, 1H, H-2), 4.27 (t, *J* = 6.9 Hz, 2H, ArC**H$_2$**), 3.19 (dd, *J* = 14.9, 3.7 Hz, 2H, PC**H$_2$**), 3.04 (dt, J = 13.9, 7.2 Hz, 1H, $ArCH_2CH_2C\mathbf{H}_2$), 2.87 (dt, J = 13.9, 7.2 Hz, 1H, $ArCH_2CH_2C\mathbf{H}_2$), 2.25 (p, *J* = 7.2 Hz, 2H, $ArCH_2C\mathbf{H_2}$).

^{31}P NMR (162 MHz, D_2O) δ 10.55.

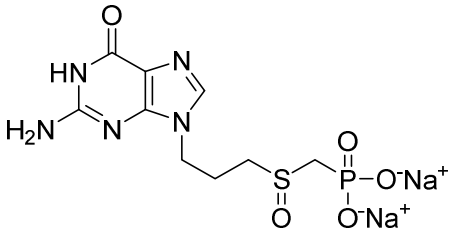
^{13}C NMR (101 MHz, D_2O) δ 158.7 (C-6), 149.3 (C-4), 146.8 (C-2), 142.6 (C-8), 123.4 (C-5), 51.0 (d, *J* = 125.3 Hz, P**C**H_2), 50.2 (d, *J* = 5.1 Hz, $ArCH_2CH_2\mathbf{C}H_2$), 44.0 (Ar**C**H_2), 23.5 ($ArCH_2\mathbf{C}H_2$).

MS (ESI+) m/z = 321 [M -2 Na +3 H]$^+$.

HR-MS (ESI$^-$) m/z: calcd for $C_9H_{12}O_5N_4PS$ = 319.0272 [M − 2Na + H]$^-$, found 319.0271 [M − 2Na + H]$^-$.

*4.3.16 Sodium 5-(guanin-9-yl)-2-oxo-2-thiapentanephosphonate (**9b**)*

Prepared by Method E from **7b**. Yield 0.13 g (69%).



^{1}H NMR (400 MHz, D_2O) δ 7.65 (s, 1H, H-8), 4.02 (t, *J* = 7.2 Hz, 2H, ArC**H$_2$**), 3.16 (dt, *J* = 13.7, 7.9 Hz, 1H $ArCH_2CH_2C\mathbf{H}_2$), 3.08 (dd, *J* = 13.8, 8.9 Hz, 2H, PC**H$_2$**), 2.79 (dt, *J* = 13.8, 7.2 Hz, 1H $ArCH_2CH_2C\mathbf{H}_2$), 2.16 (p, *J* = 7.5 Hz, 2H, $ArCH_2C\mathbf{H_2}$).

^{31}P NMR (162 MHz, D_2O) δ 8.10.

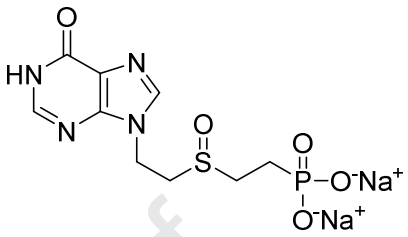
^{13}C NMR (101 MHz, D_2O) δ 157.4 (C-6), 154.0 (C-2), 150.9 (C-4), 138.9 (C-8), 113.3 (C-5), 50.1 (d, *J* = 125.5 Hz, P**C**H_2), 49.4 (d, *J* = 5.4 Hz, $ArCH_2CH_2\mathbf{C}H_2$), 42.8 (Ar**C**H_2), 22.4 ($ArCH_2\mathbf{C}H_2$).

MS (ESI+) m/z = 336 [M -2 Na +3 H]$^+$.

HR-MS (ESI$^-$) m/z: calcd for $C_9H_{13}O_5N_5PS$ = 334.0381 [M − 2Na + H]$^-$, found 334.0380 [M − 2Na + H]$^-$.

*4.3.17 Sodium 5-(hypoxanthin-9-yl)-3-oxo-3-thiapentanephosphonate (**10a**)*

Prepared by Method E from **8a**. Yield 0.14 g (78%).



^{1}H NMR (400 MHz, D_2O) δ 8.07 (s, 1H, H-8), 8.07 (s, 1H, H-2), 3.48 – 3.38 (m, 2H, ArC**H$_2$**), 3.31 (dt, *J* = 13.9, 5.8 Hz, 2H, ArCH$_2$C**H$_2$**), 3.13 – 2.89 (m, 2H, PCH$_2$C**H$_2$**), 1.85 (dddd, *J* = 19.9, 18.1, 14.6, 9.0 Hz, 2H, PC**H$_2$**).

^{31}P NMR (162 MHz, D_2O) δ 22.48.

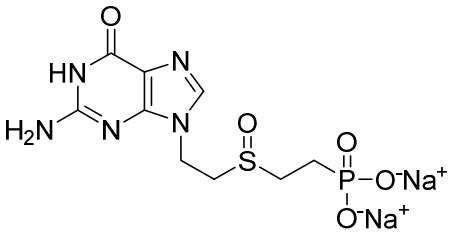
^{13}C NMR (101 MHz, D_2O) δ 158.3 (C-6), 148.7 (C-4), 145.8 (C-2), 141.9 (C-8), 123.3 (C-5), 50.1 (Ar**C**H$_2$), 46.1 (d, *J* = 2.3 Hz, PCH$_2$**C**H$_2$), 38.5 (ArCH$_2$**C**H$_2$), 20.8 (d, *J* = 131.9 Hz, P**C**H$_2$).

MS (ESI+) m/z = 321 [M -2 Na +3 H]$^+$.

HR-MS (ESI$^-$) m/z: calcd for $C_9H_{12}O_5N_4PS$ = 319.0272 [M − 2Na + H]$^-$, found 319.0271 [M − 2Na + H]$^-$.

*4.3.18 Sodium 5-(guanin-9-yl)-3-oxo-3-thiapentanephosphonate (**10b**)*

Prepared by Method E from **8b**. Yield 0.13 mg (66%).



^{1}H NMR (400 MHz, D_2O) δ 7.64 (s, 1H, H-8), 4.35 (t, *J* = 6.5 Hz, 2H, ArC**H$_2$**), 3.25 (ddt, *J* = 42.6, 13.7, 6.5 Hz, 2H, ArCH$_2$C**H$_2$**), 3.11 – 2.86 (m, 2H, PCH$_2$C**H$_2$**), 1.74 (dddd, *J* = 23.9, 20.9, 10.7, 7.0 Hz, 2H, PC**H$_2$**).

^{31}P NMR (162 MHz, D_2O) δ 20.98.

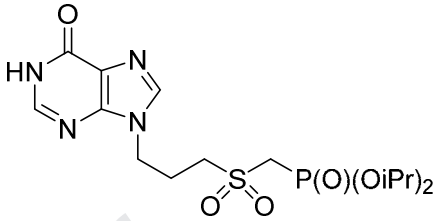
^{13}C NMR (101 MHz, D_2O) δ 157.5 (C-6), 153.9 (C-2), 151.0 (C-4), 138.9 (C-8), 113.8 (C-5), 49.6 (Ar**C**H$_2$), 45.9 (d, *J* = 2.5 Hz, PCH$_2$**C**H$_2$), 38.1 (ArCH$_2$**C**H$_2$), 20.8 (d, *J* = 132.3 Hz, P**C**H$_2$).

MS (ESI+) m/z = 336 [M -2 Na +3 H]$^+$.

HR-MS (ESI$^-$) m/z: calcd for $C_9H_{13}O_5N_5PS$ = 334.0381 $[M - 2Na + H]^-$, found 334.0379 $[M - 2Na + H]^-$.

*4.3.19 Diisopropyl 5-(hypoxanthin-9-yl)-2,2-dioxo-2-thiapentanephosphonate (**11a**)*

Prepared by method F from **5a**. Yield 0.30 g (69%).



^{1}H NMR (400 MHz, D_2O) δ 8.14 (s, 1H, H-8), 8.12 (s, 1H, H-2), 4.39 (t, *J* = 6.6 Hz, 2H, ArC**H$_2$**), 4.07 (d, *J* = 17.4 Hz, 2H, PC**H$_2$**), 3.36 – 3.26 (m, 2H, POC**H**), 2.40 (dt, *J* = 14.4, 6.8 Hz, 2H, $ArCH_2C$**H$_2$**), 1.23 (t, *J* = 5.9 Hz, 12H, $POCHC$**H$_3$**).

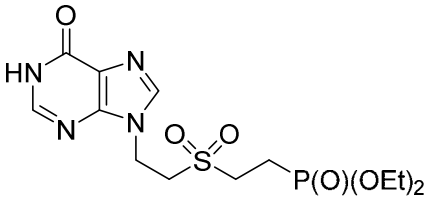
^{31}P NMR (162 MHz, D_2O) δ 13.79.

^{13}C NMR (101 MHz, D_2O) δ 158.4 (C-6), 148.8 (C-4), 145.9 (C-2), 142.1 (C-8), 123.3 (C-5), 75.0 (d, *J* = 6.8 Hz, PO**C**H), 51.5 ($ArCH_2CH_2$**C**H_2), 49.6 (d, *J* = 139.5 Hz, P**C**H_2), 42.5(Ar**C**H_2), , 23.0 (dd, *J* = 17.2, 4.4 Hz, $POCH$**C**H_3), 21.7 ($ArCH_2$**C**H_2).

MS (ESI+) m/z = 421 $[M + H]^+$.

*4.3.20 Diethyl (2-((2-(hypoxanthine-9-yl)ethyl)sulfonyl)ethyl)phosphonate (**12a**)*

Prepared by Method F from **6a**. Yield 0.32 g (82%).



^{1}H NMR (400 MHz, D_2O) δ 8.13 (s, 1H, H-8), 8.09 (s, 1H, H-2), 4.83 – 4.63 (m, 2H, ArC**H$_2$**), 4.12 – 3.97 (m, 4H, POC**H$_2$**), 3.84 (t, *J* = 6.2 Hz, 2H, $ArCH_2C$**H$_2$**), 3.31 – 3.17 (m, 2H, PCH_2C**H$_2$**), 2.30 – 2.13 (m, 2H, PC**H$_2$**), 1.22 (t, *J* = 7.1 Hz, 6H, $POCH_2C$**H$_3$**).

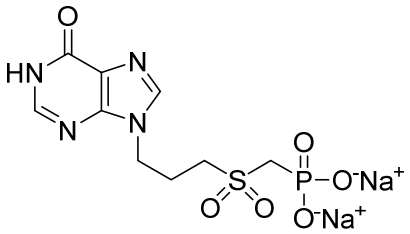
^{31}P NMR (162 MHz, D_2O) δ 31.38.

^{13}C NMR (101 MHz, Deuterium Oxide) δ 158.4 (C-6), 148.8 (C-4), 146.1 (C-2), 142.1 (C-8), 123.4 (C-5), 64.0 (d, *J* = 6.7 Hz, PO**C**H_2), 50.7 (Ar**C**H_2), 47.0 (d, *J* = 3.0 Hz, PCH_2**C**H_2), 37.5 ($ArCH_2$**C**H_2), 16.9 (d, *J* = 143.6 Hz, P**C**H_2), 15.5 (d, *J* = 5.7 Hz, $POCH_2$**C**H_3).

MS (ESI+) m/z = 393 $[M + H]^+$.

4.3.21 *Sodium 5-(hypoxanthin-9-yl)-2,2-dioxo-2-thiapentanephosphonate (**13a**)*

Prepared by Method D from **11a**. Yield 0.11 mg (69%).



^{1}H NMR (400 MHz, D_2O) δ 8.17 (s, 1H, H-8), 8.14 (s, 1H, H-2), 4.40 (t, *J* = 7.0 Hz, 2H, ArC**H$_2$**), 3.46 (d, *J* = 14.6 Hz, 2H, PC**H$_2$**), 3.37 (t, *J* = 7.6 Hz, 2H $ArCH_2CH_2C$**H$_2$**), 2.39 (p, *J* = 7.1 Hz, 2H, $ArCH_2C$**H$_2$**).

^{31}P NMR (162 MHz, D_2O) δ 5.44.

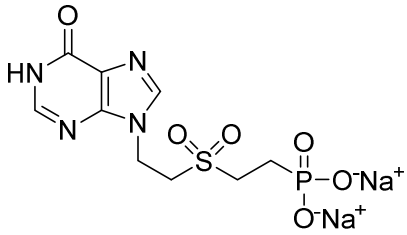
^{13}C NMR (101 MHz, D_2O) δ 158.6 (C-6), 148.9 (C-4), 145.8 (C-2), 142.2 (C-8), 123.5 (C-5), 52.1 (d, *J* = 112.6 Hz, P**C**H_2), 50.7($ArCH_2CH_2$**C**H_2), , 42.7 (Ar**C**H_2), 22.2 ($ArCH_2$**C**H_2).

MS (ESI+) m/z = 337 $[M -2 Na +3 H]^+$.

HR-MS (ESI^-) m/z: calcd for $C_9H_{12}O_6N_4PS$ = 335.0221 $[M - 2Na + H]^-$, found 335.0220 $[M - 2Na + H]^-$.

4.3.22 *Sodium 5-(hypoxanthin-9-yl)-3,3-dioxo-3-thiapentanephosphonate (**14a**)*

Prepared by Method D from **12a**. Yield 0.12 g (63%).



^{1}H NMR (400 MHz, D_2O) δ 8.13 (s, 1H, H-8), 8.12 (s, 1H, H-2), 4.76 (t, *J* = 6.4 Hz, 2H, ArC**H$_2$**), 3.84 (t, *J* = 6.3 Hz, 2H, $ArCH_2C$**H$_2$**), 3.30 (dd, *J* = 17.1, 7.4 Hz, 2H, PCH_2C**H$_2$**), 1.93 – 1.77 (m, 2H, PC**H$_2$**).

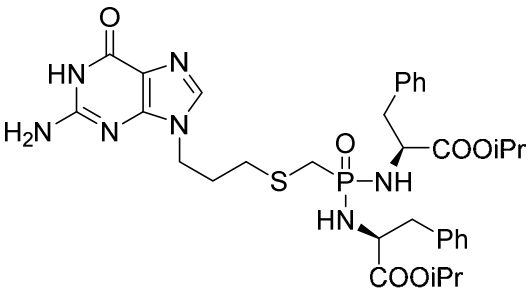
^{31}P NMR (162 MHz, D_2O) δ 20.31.

^{13}C NMR (101 MHz, D_2O) δ 158.5 (C-6), 148.8 (C-4), 146.0 (C-2), 142.2 (C-8), 123.3 (C-5), 50.1 (Ar**C**H_2), 49.4 ($ArCH_2$**C**H_2), 37.1, (PCH_2**C**H_2), 20.1 (d, *J* = 130.2 Hz, P**C**H_2).

MS (ESI+) m/z = 337 $[M -2 Na +3 H]^+$.

HR-MS (ESI^-) m/z: calcd for $C_9H_{12}O_6N_4PS$ = 335.0221 $[M - 2Na + H]^-$, found 335.0220 $[M - 2Na + H]^-$.

4.3.23 *9-(5-(bis((S-1-isopropoxycarbonyl-2-phenylethyl)amino)phosphoryl)-4-thiapentyl)guanine (**15b**)*



Prepared by Method G from **7b**. Yield 125 mg (36%).

^{1}H NMR (500.0 MHz, DMSO-d_6): 10.55 (bs, 1H, NH), 7.69 (s, 1H, H-8), 7.10 – 7.29 (m, 10H, H-*o,m,p*-Ph), 6.46 (bs, 2H, NH_2), 4.77, 4.80 (2 × sep, 2 × 1H, J_{vic} = 6.2 Hz, $(CH_3)_2C\mathbf{H}$); 4.50 (t, 1H, $J_{H,P} = J_{NH,2}$ = 11.3 Hz, NH-Phe), 4.27 (dd, 1H, $J_{H,P}$ = 12.6 Hz, $J_{NH,2}$ = 10.6 Hz, NH-Phe), 3.93 – 4.00 (m, 3H, H-2-Phe, $NC\mathbf{H_2}CH_2CH_2S$), 3.90 (m, 1H, H-2-Phe), 2.78 – 2.90 (m, 4H, H-3-Phe), 2.50 – 2.55 (m, 2H, $NCH_2CH_2C\mathbf{H_2}S$), 2.32, 2.38 (2 × dd, 2 × 1H, J_{gem} = 14.6 Hz, $J_{H,P}$ = 12.4 Hz, CH_2P), 1.87 – 1.94 (m, 2H, $NCH_2C\mathbf{H_2}CH_2S$), 1.00, 1.05, 1.11, 1.16 (4 × d, 4 × 3H, J_{vic} = 6.2 Hz, $(C\mathbf{H_3})_2CH$).

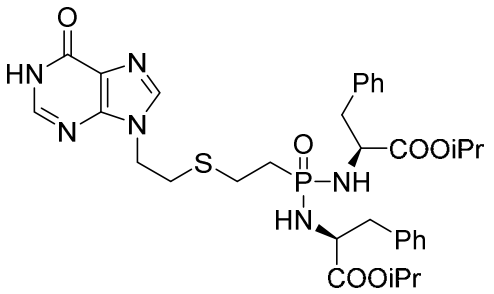
^{13}C NMR (125.7 MHz, DMSO-d_6): 172.6 (d, $J_{C,P}$ = 2.9 Hz, C-1-Phe), 172.4 (d, $J_{C,P}$ = 5.5 Hz, C-1-Phe), 157.1 (C-6), 153.7 (C-2), 151.4 (C-4), 137.7 (CH-8), 137.3, 137.2 (C-*i*-Ph), 129.7, 129.8 (CH-*o*-Ph), 128.3, 128.3 (CH-*m*-Ph), 126.7, 126.7 (CH-*p*-Ph), 116.8 (C-5), 68.0, 68.2 ($(CH_3)_2\mathbf{C}H$), 54.5, 54.4 (CH-2-Phe), 41.8 ($N\mathbf{C}H_2CH_2CH_2S$), 40.4, 40.3 (CH_2-3-Phe), 29.7 (d, $J_{C,P}$ = 3.8 Hz, $NCH_2CH_2\mathbf{C}H_2S$), 29.0 ($NCH_2\mathbf{C}H_2CH_2S$), 28.2 (d, $J_{C,P}$ = 116.6 Hz, CH_2P), 21.7, 21.7, 21.6, 21.5 ($(\mathbf{C}H_3)_2CH$).

^{31}P{^{1}H} NMR (202.4 MHz, DMSO-d_6): 23.64.

MS (ESI+) m/z = 698 $[M + H]^+$.

HR-MS (ESI^+) m/z: calcd for $C_{33}H_{44}O_6N_7NaPS$ = 720.2704 $[M + Na]^+$, found 720.2704 $[M + Na]^+$.

*4.3.24 9-(5-(bis((S-1-isopropoxycarbonyl-2-phenylethyl)amino)phosphoryl)-3-thiapentyl)hypoxanthine (**16a**)*



Prepared by Method G from **8a**. Yield 115 mg (49%).

^{1}H NMR (500.0 MHz, DMSO-d_6): 12.33 (bs, 1H, NH), 8.09 (s, 1H, H-8), 8.03 (s, 1H, H-2), 7.10 – 7.30 (m, 10H, H-*o,m,p*-Ph), 4.83, 4.78 (2 × sep, 2 × 1H, J_{vic} = 6.2 Hz, $(CH_3)_2C\mathbf{H}$), 4.61 (t, 1H, $J_{H,P} = J_{NH,2}$ = 10.9 Hz, NH-Phe), 4.29, 4.24 (2 × dt, 2 × 1H, J_{gem} = 14.3 Hz, J_{vic} = 7.0 Hz, $SCH_2C\mathbf{H_2}N$), 4.16 (dd, 1H, $J_{H,P}$ = 12.6 Hz, $J_{NH,2}$ = 10.7 Hz, NH-Phe), 3.95 (ddt, 1H, $J_{2,NH}$ =

10.9 Hz, $J_{H,P}$ = 9.3 Hz, $J_{2,3}$ = 7.3 Hz, H-2-Phe), 3.83 (dddd, 1H, $J_{2,NH}$ = 10.7 Hz, $J_{H,P}$ = 8.9 Hz, $J_{2,3}$ = 8.1 Hz, 6.4 Hz, H-2-Phe), 2.81 – 2.92 (m, 3H, H-3a-Phe1, H-3-Phe2, SC**H$_2$**CH$_2$N), 2.71 (dd, 1H, J_{gem} = 13.4 Hz, $J_{3b,2}$ = 7.9 Hz, H-3b-Phe1), 2.26 – 2.45 (m, 2H, PCH$_2$C**H$_2$**S), 1.40 – 1.65 (m, 2H, PC**H$_2$**CH$_2$S), 1.18, 1.12, 1.07, 1.01 (4 × d, 4 × 3H, J_{vic} = 6.2, (C**H$_3$**)$_2$CH).

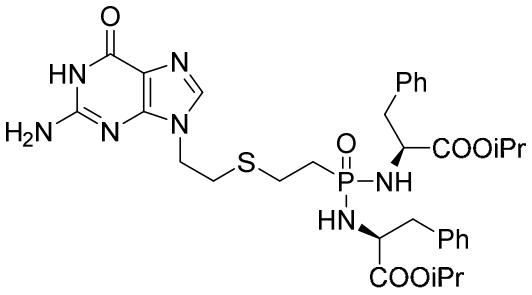
^{13}C NMR (125.7 MHz, DMSO-d_6): 172.9 (d, $J_{C,P}$ = 2.1, C-1-Phe), 172.8 (d, $J_{C,P}$ = 4.4, C-1-Phe), 156.9 (C-6), 148.6 (C-4), 145.8 (CH-2), 140.6 (CH-8), 137.5 (C-*i*-Ph), 129.7, 129.6 (CH-*o*-Ph), 128.3, 128.3 (CH-*m*-Ph), 126.7, 126.7 (CH-*p*-Ph), 124.1 (C-5), 68.1, 67.9 ((CH$_3$)$_2$**C**H), 54.6, 54.1 (CH-2-Phe), 42.8 (SCH$_2$**C**H$_2$N), 40.2, 40.2 (CH$_2$-3-Phe), 30.6 (S**C**H$_2$CH$_2$N), 30.0 (d, $J_{C,P}$ = 109.1, SCH$_2$**C**H$_2$P), 24.5 (S**C**H$_2$CH$_2$P), 21.7, 21.7, 21.6, 21.5 ((**C**H$_3$)$_2$CH).

^{31}P{^{1}H} NMR (202.4 MHz, DMSO-d_6): 26.26.

MS (ESI+) m/z = 683 $[M + H]^+$.

HR-MS (ESI^+) m/z: calcd for $C_{33}H_{43}O_6N_6NaPS$ = 705.2595 $[M + Na]^+$, found 705.2592 $[M + Na]^+$.

*4.3.25 9-(5-(bis((S-1-isopropoxycarbonyl-2-phenylethyl)amino)phosphoryl)-3-thiapentyl)guanine (**16b**)*



Prepared by Method G from **8b**. Yield 103 mg (30%).

^{1}H NMR (500.0 MHz, DMSO-d_6): 10.56 (bs, 1H, NH), 7.66 (s, 1H, H-8), 7.31 – 7.08 (m, 10H, H-*o*,*m*,*p*-Ph), 6.54 (bs, 2H, NH$_2$), 4.83, 4.75 (2 × sep, 2 × 1H, J_{vic} = 6.2 Hz, (CH$_3$)$_2$C**H**), 4.67 (t, 1H, $J_{H,P}$ = $J_{NH,2}$ = 12.1 Hz, NH-Phe), 4.20 (dd, 1H, $J_{H,P}$ = 12.8 Hz, $J_{NH,2}$ = 10.9 Hz, NH-Phe), 4.05, 4.01 (2 × dt, 2 × 1H, J_{gem} = 14.1 Hz, J_{vic} = 7.2 Hz, SCH$_2$C**H$_2$**N), 3.95, 3.82 (2 × m, 2 × 1H, H-2-Phe), 2.93 – 2.80 (m, 3H, H-3a-Phe1, H-3-Phe2), 2.75 (t, 2H, J_{vic} = 7.2 Hz, SC**H$_2$**CH$_2$N), 2.70 (dd, 1H, J_{gem} = 13.4 Hz, $J_{3b,2}$ = 7.9 Hz, H-3b-Phe1), 2.47 – 2.33 (m, 2H, PCH$_2$C**H$_2$**S), 1.75 – 1.53 (m, 2H, PC**H$_2$**CH$_2$S), 1.18, 1.09, 1.06, 1.00 (4 × d, 4 × 3H, J_{vic} = 6.2 Hz, (C**H$_3$**)$_2$CH).

^{13}C NMR (125.7 MHz, DMSO-d_6): 173.0 (d, $J_{C,P}$ = 2.1, C-1-Phe), 172.8 (d, $J_{C,P}$ = 4.3, C-1-Phe), 157.1 (C-6), 153.8 (C-2), 151.4 (C-4), 137.6 (CH-8), 137.4, 137.5 (C-*i*-Ph), 129.6, 129.7 (CH-*o*-Ph), 128.3, 128.4 (CH-*m*-Ph), 126.7, 126.8 (CH-*p*-Ph), 116.7 (C-5), 68.1, 67.9 ((CH$_3$)$_2$**C**H), 54.6 (CH-2-Phe), 54.1, 42.3 (SCH$_2$**C**H$_2$N), 40.3 (CH$_2$-3-Phe), 40.2, 30.0 (S**C**H$_2$CH$_2$N), 29.8 (d, $J_{C,P}$ = 108.6, SCH$_2$**C**H$_2$P), 24.3 (S**C**H$_2$CH$_2$P), 21.7 ((**C**H$_3$)$_2$CH), 21.6, 21.6, 21.5.

$^{31}P\{^{1}H\}$ NMR (202.4 MHz, DMSO-d_6): 26.85.

MS (ESI+) m/z = 698 $[M + H]^+$.

HR-MS (ESI^+) m/z: calcd for $C_{33}H_{44}O_6N_7NaPS$ = 720.2704 $[M + Na]^+$, found 720.2703 $[M + Na]^+$.

4.4 Docking

The docking study was performed using Molecular Operating Enviroment software (MOE).[36]] Crystal structure of *Plasmodium falciparum* hypoxanthine-guanine-xanthine phosphoribosyltransferase (PDB ID: 3OZF, resolution 1.9Å) was prepared with MOE QuickPrep tool with default setup and the structure was not minimized. Structures of all final compounds above were properly protonated, deprotonated and minimized to RMS gradient of 0.001. For docking studies, rigid dock protocol was chosen with structure waters excluded and ligands rotate bonds was enabled. Default placement and refinement method was used with 50 retained structures after the first refinement and 20 retained structures after the second refinement. As positions of nucleobases in PRTs are mostly highly conserved,7,11 pharmacophore restrains were applied for nucleobase (features volume in brackets): both aromatic rings (1.3). For all calculations, Amber12:EHT mixed force-field was used with R-Field solvent model.

4.5 Determination of K_i values

The K_i values for human HGPRT were determined as previously described.[12] The K_i values for *Pf*HGXPRT were determined under the same conditions but with xanthine as substrate. The reaction was followed at 255 nm and the $\Delta\varepsilon$ for the reaction was 4670 M^{-1} cm^{-1}. The K_m for xanthine is 189±18 μM and 45±5 μM for *P*Rib-*PP* with xanthine as substrate.

4.6 Evaluation of *in vitro* antimalarial activity

The IC_{50} values were determined as previously described.[37] *P. falciparum* D6 (Sierra-Leone) laboratory line, sensitive to most antimalarial drugs and W2 (Indochina) line, resistant to chloroquine and pyrimethamine, were maintained as previously described[38] in medium consisted of 10.4 g/L RPMI-1640-LPLF powder (Gibco BRL), 5.97 g/L HEPES buffer (MP Biomedicals, USA), 2.0 g/L D-glucose (BDH chemicals, Australia), 0.05 g/L hypoxanthine (Sigma, USA) and 40 mg/L gentamycin (Pfizer, Australia), supplemented with 0.21%

$NaHCO_3$ and 10% human plasma prior to use. Red blood cells O (Rh+) type were obtained from the Australian Red Cross Blood Service and added to 4% haematocrit. Cultures were routinely synchronised using D-sorbitol.[39] To evaluate the antimalarial activity of the ANPs, the ^{3}H-hypoxanthine growth inhibition assay was utilized, where the uptake of ^{3}H-hypoxanthine by malaria parasites is used as a surrogate marker for parasite growth. For these assays, stock solutions of the tested compounds were made to concentrations of 20-40 mM in DMSO or water and subsequently diluted in hypoxanthine-free complete media prior to assay. The assays (in 96-well plate format) were initiated when the majority of parasites (>90%) were at early trophozoite (ring) stage. Parasite cultures (100 μL per well) at 0.5% initial parasitemia and 2% hematocrit in hypoxanthine-free RPMI1640-LPLF medium were exposed to ten 2-fold serial dilutions of the compounds and chloroquine (CQ) (reference drug) for 96 hours, with ^{3}H-hypoxanthine (0.2 μCi/well) added ~48 hours after beginning of the experiment.

The ^{3}H-hypoxanthine incorporation data were analyzed and sigmoidal growth inhibition curves were produced by non-linear regression analysis of the ^{3}H-hypoxanthine incorporation data versus log-transformed concentrations of the compounds using Graphpad Prism V5.0 software (GraphPad Software Inc. USA). The inhibitory concentration (IC_{50}) that results in 50% inhibition of parasite growth was determined. The IC_{50} values were based on three independent experiments with mean ± SD calculated.

Acknowledgments

This work was supported by the Czech Science Foundation (Grant No. 16-06049S), by funds from the National Health and Medical Research Council, Australia (Grant No. 1147368), by the subvention for development of research organization (Institute of Organic Chemistry and Biochemistry, RVO 61388963) and by Gilead Sciences (Foster City, CA, USA). We wish to thank the technical excellence of Kerryn Rowcliffe for *in vitro* antimalarial drug testing and the Australian Red Cross Blood Service for the provision of human blood and plasma for *in vitro* cultivation of *P. falciparum* lines. The views expressed in this article are those of the authors and do not necessarily reflect those of the Australian Defence Force or any extant policy.

References

[1] M. B. Cassera, Y. Zhang, K. Z. Hazleton, V. L. Schramm, Purine and Pyrimidine Pathways as Targets in Plasmodium falciparum, Curr. Top. Med. Chem. 11 (2011) 2103−2115.

[2] S. Mehrotra, M. P. Bopanna, V. Bulusu, H. Balaram, Adenine metabolism in Plasmodium falciparum, Experimental Parasitol. 125 (2010) 147-151.

[3] R. G. Ducati, H. A. Namanja-Magliano, V. L. Schramm, Transition-state inhibitors of purine salvage and other prospective enzyme targets in malaria, Future Med. Chem. 5 (11) (2013) 1341-1360.

[4] A. F.Cowman, B. S. Crabb, The Plasmodium falciparum genome - a blueprint for erythrocyte invasion. Science 298 (2002) 126-128.

[5] M. Berg, P. Van der Veken, A. Goeminne, A. Haemers, K. Augustyns, Inhibitors of the Purine Salvage Pathway: A Valuable Approach for Antiprotozoal Chemotherapy, Curr. Med. Chem. 17 (2010) 2456−2481.

[6] E. A. Ashley et al., Spread of Artemisinin Resistance in Plasmodium falciparum Malaria N. Engl. J. Med. 371 (2017) 411-423.

[7] N. V. Thanh et al., Rapid decline in the susceptibility of Plasmodium falciparum to dihydroartemisinin–piperaquine in the south of Vietnam, Malaria Journal 16 (2017) 27.

[8] W. S. Eng, D. Hocková, P. Špaček, Z. Janeba, N. P. West, K. Woods, L. M. J. Naesens, D. T. Keough, L. W. Guddat, First Crystal Structures of Mycobacterium tuberculosis 6-Oxopurine Phosphoribosyltransferase: Complexes with GMP and Pyrophosphate and with Acyclic Nucleoside Phosphonates Whose Prodrugs Have Antituberculosis Activity. J. Med. Chem. 58 (11) (2015) 4822–4838.

[9] E. Doleželová, D. Terán, O. Gahura, Z. Kotrbová, M. Procházková, D. Keough, P. Špaček, D. Hocková, L. Guddat, A. Zíková, 2018. Evaluation of the Trypanosoma brucei 6-oxopurine salvage pathway as a potential target for drug discovery. PLoS Neglected Tropical Diseases. 12, e0006301.

[10] D. Hocková, D. T. Keough, Z. Janeba, T. H. Wang, J. de Jersey, L. W. Guddat, Synthesis of Novel N-Branched Acyclic Nucleoside Phosphonates As Potent and Selective Inhibitors of Human, Plasmodium falciparum and Plasmodium

vivax 6-Oxopurine Phosphoribosyltransferases. J. Med. Chem. 55 (2012) 6209−6223.

[11] P. Špaček, D. T. Keough, M. Chavchich, M. Dračínský, Z. Janeba, L. Naesens, M. D. Edstein, L. W. Guddat, D. Hocková, Synthesis and Evaluation of Asymmetric Acyclic Nucleoside Bisphosphonates as Inhibitors of Plasmodium falciparum and Human Hypoxanthine-Guanine-(Xanthine) Phosphoribosyltransferase J. Med. Chem. 60 (2017) 7539–7554.

[12] D. T. Keough, D. Hocková, Z. Janeba, T. H. Wang, L. Naesens, M. D. Edstein, M. Chavchich, L. W. Guddat, Aza-acyclic Nucleoside Phosphonates Containing a Second Phosphonate Group As Inhibitors of the Human, Plasmodium falciparum and vivax 6□Oxopurine Phosphoribosyltransferases and Their Prodrugs As Antimalarial Agents. J. Med. Chem. 58 (2015) 827-846.

[13] K. Clinch, D. R. Crump, G. B. Evans, K. Z. Hazleton, J. M. Mason, V. L. Schramm, P. C. Tyler, Acyclic phosph(on)ate inhibitors of Plasmodium falciparum hypoxanthine-guanine-xanthine phosphoribosyltransferase. Bioorg. Med. Chem. 21 (2013) 5629–5646.

[14] E. De Clercq, A. Holý, Acyclic nucleoside phosphonates: a key class of antiviral drugs Nature Reviews Drug Discovery 4 (2005) 928–940.

[15] J. Balzarini, D. Schols, K. Van Laethem, E. De Clerq, D. Hocková, M. Masojidkova, A. Holý, Pronounced in vitro and in vivo antiretroviral activity of 5-substituted 2,4-diamino-6-[2-(phosphonomethoxy)ethoxy] pyrimidines. J. Antimicrob. Chemother. 59 (1) (2007) 80–86.

[16] D. T. Keough, D. Hocková, A. Holý, L. M. J. Naesens, T. S. Skinner-Adams, J. de Jersey, L. W. Guddat, Inhibition of Hypoxanthine-Guanine Phosphoribosyltransferase by Acyclic Nucleoside Phosphonates: A New Class of Antimalarial Therapeutics J. Med. Chem. 52 (2009) 4391–4399.

[17] M. Česnek, D. Hocková, A. Holý, M. Dračínský, O. Baszczyňski, J. de Jersey, D. T. Keough, L. W. Guddat, Synthesis of 9-phosphonoalkyl and 9-phosphonoalkoxyalkyl purines: Evaluation of their ability to act as inhibitors of Plasmodium falciparum, Plasmodium vivax and human hypoxanthine–guanine–(xanthine) phosphoribosyltransferases. Bioorg. Med. Chem. 20 (2012) 1076–1089.

[18] Špaček, P.; Keough, D. T.; Chavchich, M.; Dračínský, M.; Janeba, Z.; L. Naesens, M. D. Edstein, L. W. Guddat, D. Hocková, Synthesis and evaluation

of symmetric acyclic nucleoside bisphosphonates as inhibitors of the Plasmodium falciparum, Plasmodium vivax and human 6-oxopurine phosphoribosyltransferases and the antimalarial activity of their prodrugs J. Bioorg. Med. Chem. 25 (2017) 4008–4030.

[19] O. Baszczyňsky, D. Hocková, Z. Janeba, A. Holý, P. Jansa, M. Dračínský, D. T. Keough, L. W. Guddat, The effect of novel [3-fluoro-(2-phosphonoethoxy)propyl]purines on the inhibition of Plasmodium falciparum, Plasmodium vivax and human hypoxanthine–guanine–(xanthine) phosphoribosyltransferases. Eur. J. Med. Chem. 67 (2013) 81-89.

[20] D. Hocková, A. Holý, M. Masojídková, D. T. Keough, J. de Jersey, L. W. Guddat, Synthesis of branched 9-[2-(2- phosphonoethoxy)ethyl]purines as a new class of acyclic nucleoside phosphonates which inhibit Plasmodium falciparum hypoxanthine−guanine−xanthine phosphoribosyltransferase. Bioorg. Med. Chem. 17 (2009) 6218−6232.

[21] J. C. Eads, G. Scapin, Y. Xu, C. Grubmeyer, J. C. Sacchettini, The crystal structure of human hypoxanthine-guanine phosphoribosyltransferase with bound GMP. Cell 78 (1994) 325-334.

[22] Ch. U. Kim, B. Y. Luh, P. F. Misco, J. J. Bronson, M. J. M. Hitchcock, I. Gazzouli, J. C. Martin, Acyclic Purine Phosphonate Analogues as Antiviral Agents. Synthesis and Structure-Activity Relationships. J. Med. Chem. 33 (1990) 1207–1213.

[23] J. L. Kelley, J. A. Linn, E. W. McLean, J. V. Tuttle, 9-[(Phosphonoalkyl)benzyl]guanines. Multisubstrate Analogue Inhibitors of Human Erythrocyte Purine Nucleoside Phosphorylase. J. Med. Chem. 36 (1993) 3455–3463.

[24] D. Villemin, F. Thibault-Starzyk, Synthesis of a New Sulfur Analog of PMEA. Synth. Commun. 23 (8) (1993) 1053–1059.

[25] D. T. Keough, P. Špaček, D. Hocková, T. Tichý, S. Vrbková, L. Slavětínská, Z. Janeba, L. Naesens, M. D. Edstein, M. Chavchich, T. H. Wang, J. de Jersey, L. W. Guddat, Acyclic Nucleoside Phosphonates Containing a Second Phosphonate Group Are Potent Inhibitors of 6□Oxopurine Phosphoribosyltransferases and Have Antimalarial Activity J. Med. Chem. 56 (2013) 2513−2526.

[26] U. Pradere, E. C. Garnier-Amblard, S. J. Coats, F. Amblard, R. F. Schinazi, Synthesis of Nucleoside Phosphate and Phosphonate Prodrugs. Chem. Rev. 114 (2014) 9154.

[27] D. Hocková, Z. Janeba, L. Naesens, M. D. Edstein, M. Chavchich, D. T. Keough, L. W. Guddat, Antimalarial activity of prodrugs of N-branched acyclic nucleoside phosphonate inhibitors of 6-oxopurine phosphoribosyltransferases. Bioorg. Med. Chem. 23 (17) (2015) 5502–5510.

[28] T. Klejch, R. Pohl, Z. Janeba, M. Sun, D. T. Keough, L. W. Guddat, D. Hocková, Acyclic nucleoside phosphonates with unnatural nucleobases, favipiravir and allopurinol, designed as potential inhibitors of the human and Plasmodium falciparum 6-oxopurine phosphoribosyltransferases Tetrahedron 74 (2018) 5886-5897.

[29] J. Drabowitz, W. Midura, M. Mikolajczyk, A Convenient Procedure for Oxidation of Sulphides to Sulphoxides by Bromine/Aqueous Potassium Hydrogen Carbonate Reagent in a Two Phase System. Synthesis of ^{18}O-Sulphoxides Synthesis (1979) 39-40.

[30] M. Recher, A. P. Barboza, Z. Li, M. Galizzi, M. Ferrer-Casal, S. H. Szajnam, R. Docampo, S. N. J. Moreno, J. B. Rodriguez, Design, synthesis and biological evaluation of sulfur-containing 1, 1-bisphosphonic acids as antiparasitic agents. Eur. J. Med. Chem. 60 (2013) 431–440.

[31] S. Ozaki, T. Nagase, H. Tamai, H. Mori, A. Hoshi, M. Ilgo, Synthesis and Antitumor Activity of α-Alkylthiomethyl-, α-Alkylsulfinylmethyl-,α-Alkylsulfonylmethyl-, and α-Acylthiomethyl-5－fluorouracils. Chem. Pharm. Bull. 35 (1987) 3894-3897.

[32] B. M. Trost,D. P. Curran, Chemoselective oxidation of sulfides to sulfones with potassium hydrogen persulfate. Tetrahedron Lett. 22 (1981) 1287-1290.

[33] S. C. Söderman, A. L. Schwan, 1,2-Dibromotetrachloroethane: An Ozone-Friendly Reagent for the in Situ Ramberg−Bäcklund Rearrangement and Its Use in the Formal Synthesis of E ‑Resveratrol J. Org. Chem. 77 (2012) 10978-10984

[34] R. L. Mackman, A. S. Ray, H. C. Hui, L. Zhang, G. Birkus, C. G. Boojamra, M. C. Desai, J. L. Douglas, Y. Gao, D. Grant, G. Laflamme, K.-Y. Lin, D. Y. Markevitch, R. Mishra, M. McDermott, R. Pakdaman, O. V. Petrakovsky, J. E.

Vela, T. Cihlar, Discovery of GS-9131: Design, Synthesis and Optimization of Amidate Prodrugs of the Novel Nucleoside Phosphonate HIV Reverse Transcriptase (RT) Inhibitor GS-9148. Bioorg. Med. Chem. 18 (2010) 3606–3617.

[35] K. Z. Hazleton, M. Ho, M. B. Cassera, K. Clinch, D. R. Crump, I. Rosario, E. F. Merino, S. C. Almo, P. C. Tyler, V. L. Schramm, Acyclic Immucillin Phosphonates: Second-Generation Inhibitors of Plasmodium falciparum Hypoxanthine- Guanine-Xanthine Phosphoribosyltransferase. Chem. Biol. 19 (2012) 721-730.

[36] "Molecular Operating Environment (MOE)," in Chemical Computing Group Inc, Montreal, QC, Canada, 2016

[37] M. M. Kaiser, D. Hocková, T. H. Wang, M. Dračínský, L. Poštová-Slavětínská, E. Procházková, M. D. Edstein, M. Chavchich, D. T. Keough, L. W. Guddat, Z. Janeba, Synthesis and Evaluation of Novel Acyclic Nucleoside Phosphonates as Inhibitors of Plasmodium falciparum and Human 6-Oxopurine ChemMedChem 10 (2015) 1707-1723.

[38] W. Trager, J. B. Jensen, Human malaria parasites in continuous culture Science 193 (1976) 673-675.

[39] C. Lambros, J. P. Vanderberg, Synchronization of Plasmodium falciparum erythrocytic stages in culture. J Parasitol. **1979** Jun;65(3):418-20. PubMed PMID: 383936.

Thia-ANPs (sulfur bridged acyclic nucleoside analogues) were designed and synthesized

Thia-ANPs are (sub)micromolar inhibitors of human and *Pf*HGPRT

Thia-ANPs distinctively favour *Pf*HGXPRT over its human counterpart

Thia-ANP prodrugs have antimalarial activity IC_{50} values between 4 and 6 μM