DOI: 10.1002/cmdc.200800231

Synthesis of Bicyclic *N*-Arylmethyl-Substituted Iminoribitol Derivatives as Selective Nucleoside Hydrolase Inhibitors

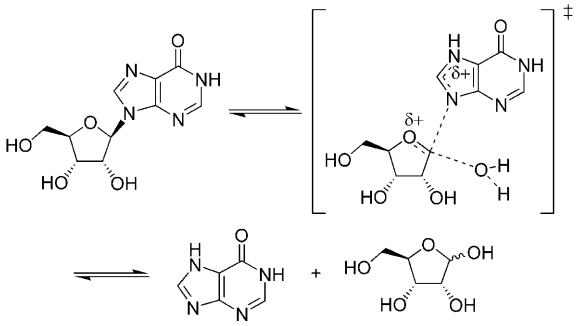
Maya Berg,[a] Gunther Bal,[a] Annelies Goeminne,[a] Pieter Van der Veken,[a] Wim Versées,[b] Jan Steyaert,[b] Achiel Haemers,[a] and Koen Augustyns*[a]

The purine metabolism of Trypanosoma *and* Leishmania *spp. provides a good target in the search for new selective drugs. Bicyclic* N*-arylmethyl-substituted iminoribitols were developed as inhibitors of* T. vivax *nucleoside hydrolase, a key enzyme of the purine salvage pathway. The obtained results and structure–activity data confirmed our model for inhibitor binding with a hydrogen bond between a nitrogen atom of the nucleobase mimetic and the protonated Asp40 from the enzyme. This interaction depends on an optimal pK_a value, which can be influenced by the electronic properties of the substituents. These compounds are potent, selective inhibitors of nucleoside hydrolase and are inactive toward human nucleoside phosphorylase.*

Introduction

African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas' disease), and Leishmaniasis are caused by parasites of the genus *Trypanosoma*: *Trypanosoma b. brucei*, *Trypanosoma cruzi*, and various *Leishmania* spp., respectively. Together these three infections cause more than one hundred thousand deaths annually and therefore remain a major health problem in developing countries. The currently used drugs are toxic, and resistance of the parasite is a growing problem.[1] It has been proposed that the purine metabolism of these parasites could be a good target in the search for new selective drugs. Unlike mammals, parasites are not able to synthesise purines de novo and are dependent on the purine salvage pathway for their survival.[2]

In these parasites, purine bases are obtained by cleavage of the N-glycosidic bond of nucleosides by nucleoside hydrolases (NH).[3] NHs provide the essential purines for the biosynthesis of purine nucleotides by phosphoribosyltransferases.[4] At present, four types of NHs have been identified on the basis of their substrate specificity.[5] In our research we focus on the purine-specific IAG-NH (inosine-adenosine-guanosine-preferring NH) that was isolated from *Trypanosoma vivax*, a parasite that causes Souma disease, a sleeping-sickness-like disease in cattle.[3] The reaction mechanism of this enzyme is based on the formation of an oxocarbenium-ion-like transition state in which the ribose oxygen carries a partial positive charge and the N7 atom is protonated (Scheme 1).[6] In contrast, mammals use purine nucleoside phosphorylase (PNP) to cleave their nucleosides. PNP catalyses a similar reaction, but uses a phosphate nucleophile to effect phosphorolysis of the N-ribosidic bond, whereas NH hydrolyses this bond with a water nucleophile (Scheme 1). The catalytic mechanism, however, is different from IAG-NH: due to the absence of aromatic residues in the hPNP active site, aromatic stacking interactions, which are important for binding with *Tv*NH are absent. Moreover, PNP has a preference for 6-oxopurine substrates. These two features allow selectivity with IAG-NH inhibitors.[7] NHs are absent in mammals, and therefore they are good targets for the development of new anti-trypanosomal compounds.[2]



Scheme 1. Nucleoside-hydrolase-catalysed hydrolysis of a ribonucleoside (exemplified by inosine). This proceeds via an S_N1-like mechanism with an oxocarbenium-ion-like transition state.[8]

The strongest NH inhibitors that are known to date, the immucillins (Figure 2, **A** and **B**), are transition-state-analogue inhibitors that mimic the oxocarbenium-ion-like transition state.[9,10] They are characterised by an iminoribitol (1,4-dideoxy-1,4-imino-D-ribitol) moiety with a deazapurine analogue that is attached to C1′.[11] The iminoribitol nitrogen atom is partially protonated at physiological pH and mimics the partial positive charge that develops on O4′ in the oxocarbenium-ion-like transition state.[12] The nucleobase mimetic is a purine-like

[a] *M. Berg, Dr. G. Bal, Dr. A. Goeminne, Prof. Dr. P. Van der Veken, Prof. Dr. A. Haemers, Prof. Dr. K. Augustyns*
Department of Medicinal Chemistry, University of Antwerp
Universiteitsplein 1, 2610 Antwerp (Belgium)
Fax: (+32) 3820 2739
E-mail: koen.augustyns@ua.ac.be

[b] *Prof. Dr. W. Versées, Prof. Dr. J. Steyaert*
Group of Structural Biology Brussels
Department of Molecular and Cellular Interactions, VIB
Vrije Universiteit Brussel, Pleinlaan 2, 1050, Brussels (Belgium)

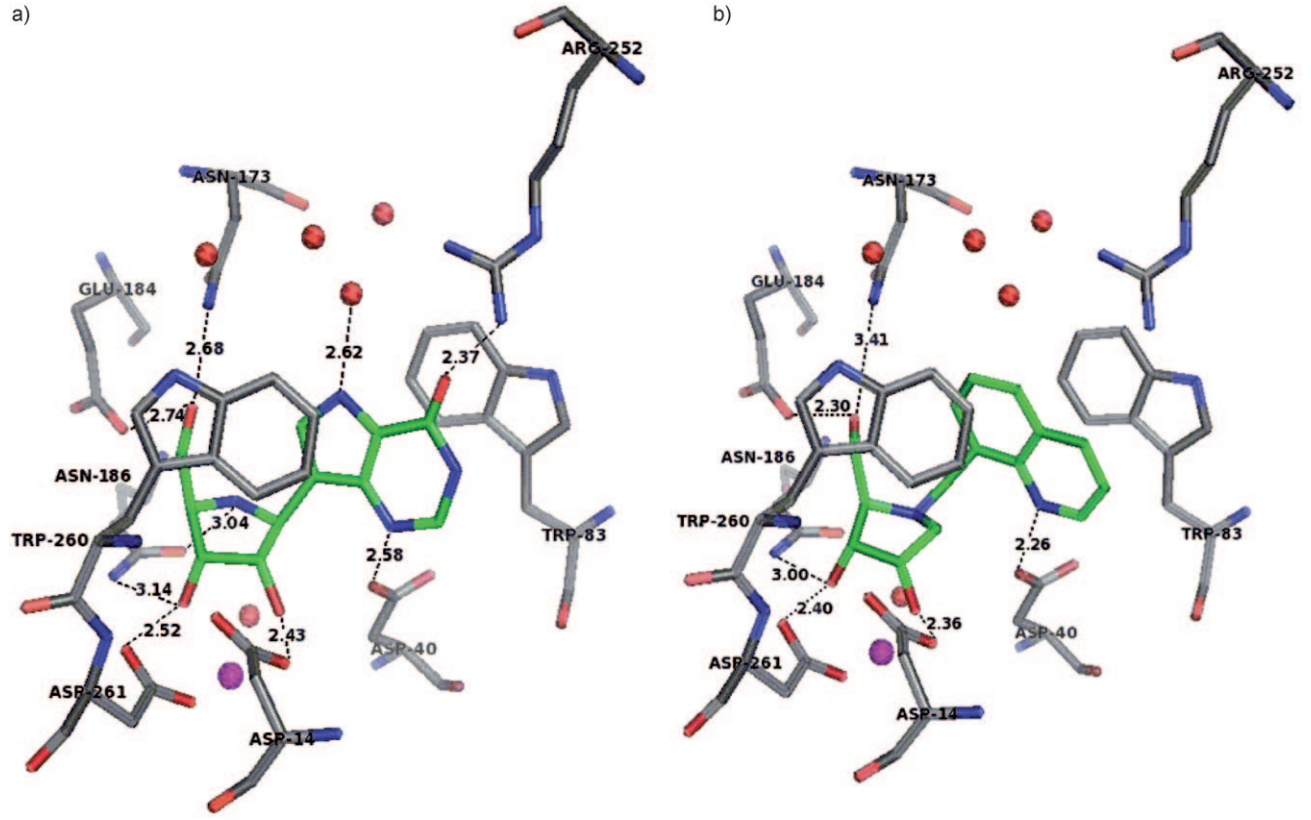


Figure 1. Inhibitors a) **A** and b) **E** in the active site of the target enzyme. Interaction distances are given in Å. The active site residues are depicted in grey (carbon atoms), blue (nitrogen atoms) and red (oxygen atoms). The carbon atoms of the inhibitors are depicted in green, the colour codes for nitrogen and oxygen atoms are the same as for the active site residues. Water molecules are depicted as red spheres, and the Ca^{2+} ion is depicted as a magenta sphere. The colours of the enzyme surface are according to the elements. The images were made with PyMOL (PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA, USA).

moiety to retain the purine specificity of IAG-NH that is imposed by parallel aromatic stacking interactions between the purine ring of the substrate and two Trp residues (Trp83 and Trp260) in the active site (Figure 1).[13] Based on the immucillins, our research group developed inhibitors with substituents such as alkylguanidine groups or tetrazole moieties on C1′.[14,15] Molecular modelling studies (Figure 1) suggest that attachment of the base moiety to the iminoribitol nitrogen atom can result in a correct orientation to fit into the active site of the target enzyme. Results from our group confirmed that the activity is conserved or even improved with N-substitution of iminoribitol with a methylene linker.[14] We previously synthesised a small library of novel N-arylmethyl-substituted iminoribitols, which were screened for inhibitory activity against IAG-NH from *T. vivax* (*Tv*NH).[16] Three very potent inhibitors were identified (Figure 2): compound **C** (immucillin H analogue, K_i = 4.4 nM), compound **D** (immucillin A analogue, K_i = 4.1 nM), and compound **E** (K_i = 10.8 nM). These compounds are among the most active inhibitors of IAG-NH that have been reported. Docking experiments on a series of inhibitors revealed an interaction between a nitrogen atom in the nucleobase mimic and the Asp40 residue in the active site of the target enzyme (Figure 1).[16] We proposed that this interaction plays an important role in the binding of these inhibitors in the enzyme. Inhibitor **E**, one of the most active inhibitors that was developed

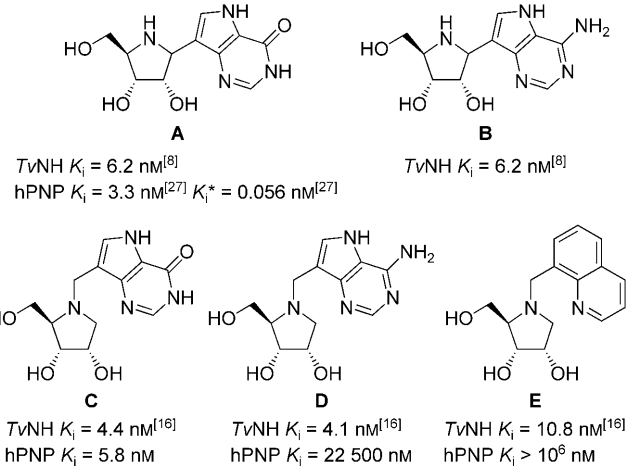
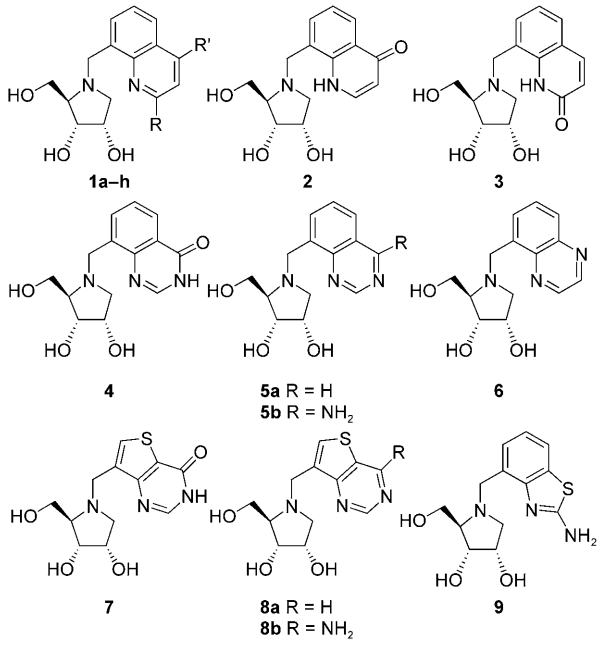


Figure 2. Inhibition constants (K_i) for immucillin H (**A**) and immucillin A (**B**) (K_i* is the equilibrium dissociation constant obtained after slow-onset inhibition), and derivatives (**C**, **D** and **E**); hPNP values for compounds **C**, **D**, and **E** are reported herein for the first time.

in this series (K_i = 10.8 nM), provided us with a good lead structure for the further development of potent NH inhibitors. In contrast to the immucillins, compound **E** has no purine characteristics and is easier to synthesise. Furthermore, we report

herein for the first time the high selectivity of compound **E** for NH over human PNP (hPNP).

The objective of this current study was to further investigate the SAR by designing a set of new inhibitors (Figure 3) by starting from structure **E** as a lead compound. We report the synthesis and inhibitory activity towards *Tv*NH and hPNP.



1a R = H R' = Cl
1b R = H R' = OMe
1c R = Me R' = Cl
1d R = Cl R' = H
1e R = OMe R' = H
1f R = H R' = NH_2
1g R = Me R' = NH_2
1h R = NH_2 R' = H

Figure 3. Overview of the target compounds.

Results and Discussion

Synthesis

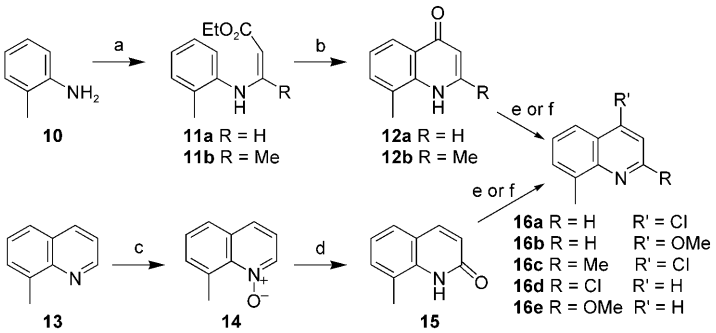
Target compounds were prepared by coupling 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-protected iminoribitol with the corresponding heteroarylmethyl bromides. Protected iminoribitol was synthesised from D-gulonolactone as described in literature.[17,18]

8-Methylquinolines **16a–e** were obtained by following two pathways (Scheme 2). In one pathway *o*-toluidine **10** and ethyl propiolate or ethyl acetoacetate were condensed, followed by ring closure at high temperature to 4-quinolones **12a–b**.[19,20] The second pathway started from 8-methylquinoline **13** to form *N*-oxide **14**, which was then converted into 2-quinolone **15**. Various substituents were introduced by starting from quinolones **12** and **15**. To add a chloro substituent (in **16a,c,d**), quinolone was heated at reflux in $POCl_3$,[19] and by using methyl iodide at 80 °C the methoxy substituent (in **16b,e**) was obtained.[19]

Quinolines **16a–e** were converted into their 8-bromomethyl analogues **17a–e** (Scheme 3) with *N*-bromosuccinimide (NBS)/dibenzoyl peroxide[21] and then coupled to protected iminoribitol. Subsequent deprotection of **18a–e** afforded target compounds **1a–e**. Aminoquinolines **1f–h** were obtained by amination of **1a,c,d** with ammonia. Compounds **2** and **3** were isolated as side products of this amination procedure, from **1a** and **1d** respectively.[22]

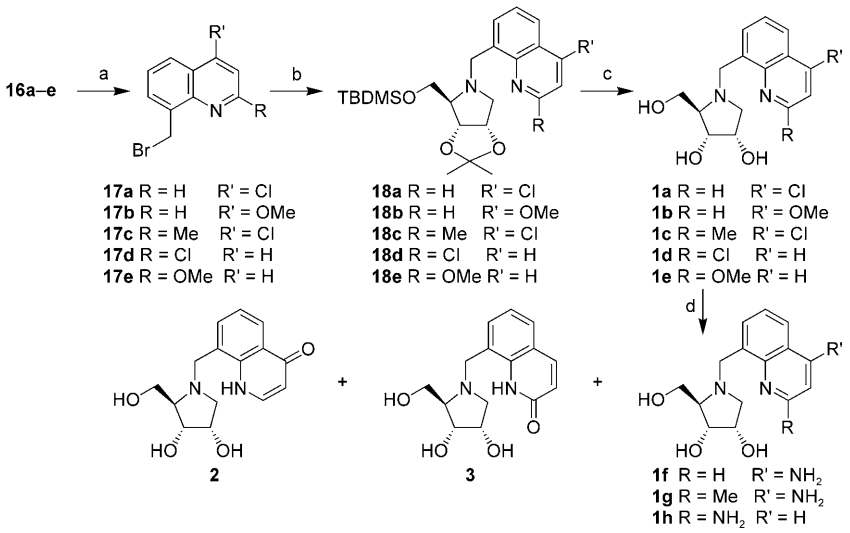
Quinazolines **4**, **5a** and **5b** (Scheme 4) were synthesised from 2-amino-3-methylbenzoic acid **19** and formamidine acetate.[23] Intermediate 4-quinazolinone **20** was chlorinated with $POCl_3$ to afford 4-chloro compound **21** and transformed in 8-methylquinazoline **22** by using *p*-toluenesulfonylhydrazide followed by basic hydrolysis in ethanol.[24] Both **21** and **22** were brominated to compounds **25** and **23**. Subsequently, compounds **25** and **23** were coupled to protected iminoribitol to afford **26** and **24** respectively. Unsubstituted quinazoline **5a** and oxoquinazoline **4** were obtained by trifluoroacetic acid (TFA) deprotection of compounds **24** and **26**, respectively. Compound **26** was aminated with ammonia to afford **27**, which was transformed into **5b** by deprotection.

Quinoxaline **6** (Scheme 5) was prepared from commercially available 5-methylquinoxaline **28**, which was brominated to afford **29**, then coupled to afford **30** and deprotected as described for the quinazolines.

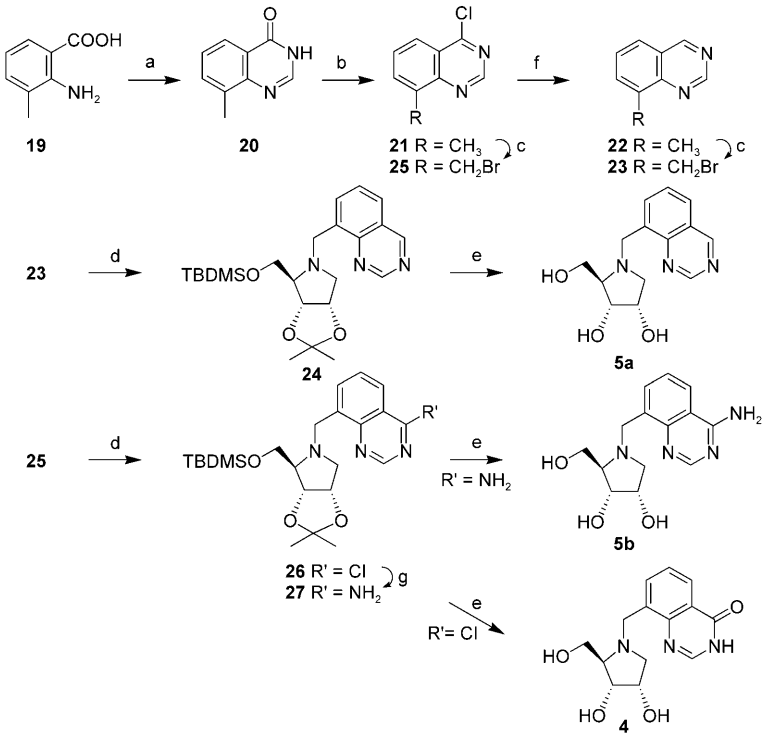


Scheme 2. Reagents and conditions: a) ethyl propiolate for R = H, ethyl acetoacetate for R = Me, MeOH, reflux, 80 °C for 4 h, 40 °C for 48 h; b) PhOPh, reflux, 15 min; c) *m*-CPBA, $CHCl_3$, RT, overnight; d) benzoyl chloride, NaOH (aq), CH_2Cl_2, 0 °C, 1 h; e) $POCl_3$, reflux, 5 h; f) MeI, DMF, K_2CO_3, 80 °C, 3–5 h.

Thienopyrimidines **7–8** were synthesised by starting from methyl-3-amino-4-methyl-thiophene-2-carboxylate **31** (Scheme 6) to form 4-thienopyrimidinone **32**.[23] The general procedure for bromination (to form **33**), coupling (to form **34**) and deprotection resulted in target compound **7**. To obtain **8a**, 4-thienopyrimidone **32** was chlorinated with $POCl_3$ to give **35** followed by dehalogenation to give **39**. A one-pot reaction combining the bromination and coupling step (compounds



Scheme 3. Reagents and conditions: a) NBS, dibenzoyl peroxide, CCl_4, reflux, 6 h; b) protected iminoribitol, DMF, K_2CO_3, 40 °C, 2 h; c) TFA/H_2O (1:1), RT, overnight; d) NH_4OH, dioxane, 150 °C, overnight.



Scheme 4. Reagents and conditions: a) formamidine acetate, formamide, reflux, 3 h; b) $POCl_3$, ACN, reflux, 5 h; c) NBS, dibenzoyl peroxide, CCl_4, reflux, 6 h; d) protected iminoribitol, DMF, K_2CO_3, 40 °C, 2 h; e) TFA/H_2O (1:1), RT, overnight; f) 1. *p*-toluenesulfonylhydrazide, dioxane, reflux, overnight, 2. NaOH (1 N), EtOH, reflux, 4 h; g) NH_4OH, dioxane, 30 °C, 5 days.

40–**41**) yielded target compound **8a**. Compound **8b** was prepared by amination of **37**, followed by deprotection of **38**.

Aminobenzothiazole **9** (Scheme 7) was obtained by starting from commercially available 2-amino-4-methyl-benzothiazole **42**, which was first protected to give **43**. The standard procedure was then used (bromination to give **44**, then coupling to give **45** and deprotection) to obtain target compound **9**.

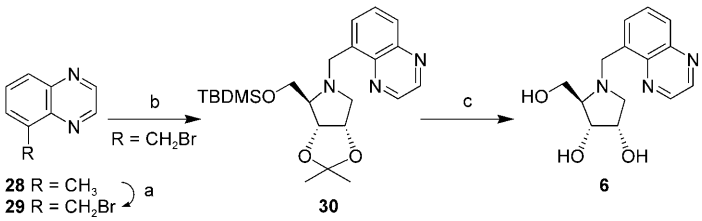
Biochemical results

All target compounds were tested in vitro as competitive inhibitors of *Tv*NH by using *para*-nitrophenol-β-D-ribofuranoside as a substrate. Lead compound **E** has a K_i value of 10.8 nM.[16] By introducing a chlorine atom in the 4-position (in **1a**), which acts as a weak electron acceptor, the activity remains the same. When electron donors such as an amino group (in **1f**) or a methoxy group (in **1b**) are substituted at the 4-position, the pK_a of the quinoline nitrogen atom rises, and the activity drops. Quinolones (**2** and **3**) are more than 1000-fold less active. This confirms our model with a hydrogen bond between the protonated Asp40 and the nucleobase N atom.[15] Substitution at position 2 (**1c**, **1d**, **1e**, **1g** and **1h**) also decreases the potency considerably (up to 170-fold) when compared with their 4-substituted analogues. Miles and co-workers already suggested that increasing the bulk of the 2-substituent destroys the affinity for base-specific IAG-NH. This confirms the stringent leaving-group requirements of IAG-NH.[9]

Introducing a second nitrogen atom in the ring (e.g. **5a**) lowers the pK_a and results in a drop of activity.[25] The isomer quinoxaline **6** is even less active. When an amino group is introduced on the quinazoline **5b**, the effect of this mesomeric donor causes a rise in the pK_a (closer to the pK_a of lead compound **E**) and consequently a rise in inhibitory activity. Quinazolone **4** loses activity relative to unsubstituted quinazoline **5a**, but in a less drastic way than observed with the quinolines (twofold).

When the phenyl moiety in quinazolines **4** and **5a**–**b** is replaced by its isostere thiophene (compounds **7**, **8a**–**b**), activities remain about the same. A sulfur bioisostere of **1g**, aminobenzothiazole **9**, shows however a much higher inhibitory activity.

Selectivity is an important matter in inhibitor development. All active target compounds, including previously synthesised compounds **C**, **D** and **E**, were tested in vitro as inhibitors of hPNP (Table 1). hPNP is especially important in T-cells, and its inhibition could lead to T-cell-mediated immunosuppression.[7] Therefore the cross reactivity of NH inhibitors that are targeted against parasites could lead to unwanted host toxicity. PNP does not have aromatic residues in its active site, hence aromatic stacking interactions, which are important for binding with *Tv*NH, are not involved in binding to hPNP.[26] Moreover, hPNP has a preference for 6-oxopurines (inosine, guanosine) as sub-



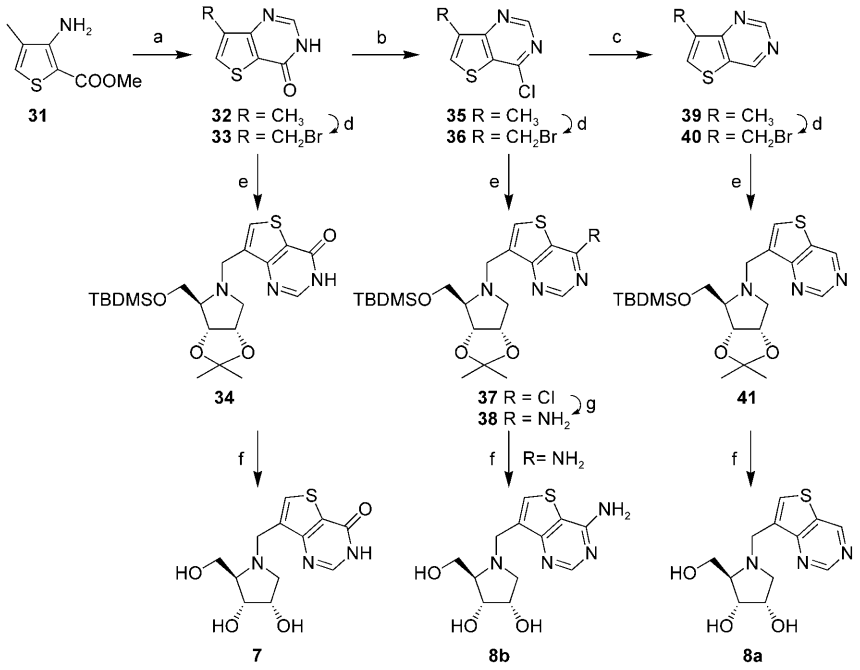
Scheme 5. Reagents and conditions: a) NBS, dibenzoyl peroxide, CCl_4, reflux, 6 h; b) protected iminoribitol, DMF, K_2CO_3, 40 °C, 2 h; c) TFA/H_2O (1:1), RT, overnight.

strates.[7] This can be explained by the interaction pattern in the active site of the PNP–immucillin H complex (Figure 4). The most important interactions between the enzyme and the transition state analogue are: 1) a hydrogen bond between NH7 of bound immucillin H and the carbonyl group of PNP Asn243, 2) a hydrogen bond between O6 of immucillin H and the amide NH_2 group of PNP Asn243, while the same O6 forms a hydrogen bond with a water molecule, which is in close contact with the bulk solvent and Glu201, and 3) a hydrogen bond between the NH1 from the purine ring with the carboxyl oxygen atoms of PNP Glu201. The enzymatic interactions at N7 and O6 seem to be essential for tight substrate–inhibitor binding. Compound **C** shows that the presence of both features is crucial for high activities ($K_i = 5.8$ nM) towards hPNP. Therefore inhibitors with structural features of adenosine and quinolines discriminate between hPNP and *Tv*NH. Active target compounds such as **1a**, **b**, **f**, **5a**–**b**, **7**, **8a**–**b**, and **9** are inactive towards hPNP because they lack a purine NH7 and mostly also a carbonyl group.

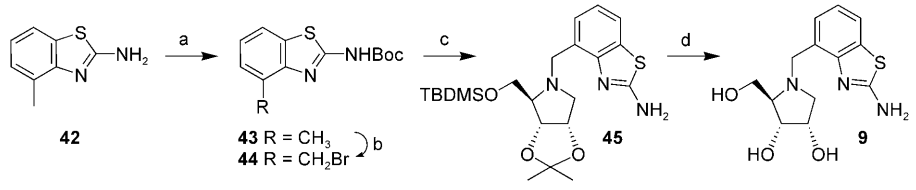
Conclusions

We synthesised a series of bicyclic heterocyclic analogues of lead compound quinoline **E.** These compounds are potent and selective inhibitors of IAG-NH of *T. vivax*, and have no activity on human purine nucleoside phosphorylase. Although we could not improve the inhibitory activity of **E** against *Tv*NH, the results and the obtained structure–activity data confirm the importance of the quinoline nitrogen atom of target compound **E**. This nitrogen atom optimally has a calculated pK_a around 5, meaning that it is mostly unprotonated at physiological pH, so that it can form a hydrogen bond with the protonated Asp40. Compounds with a higher pK_a at this nitrogen atom, such as **1f**, are at least partially protonated and hence show decreased potency. Compounds with a lower pK_a such as **5a** also show decreased potency, because of the lower availability of the free electron pair of the nitrogen atom for the formation of a hydrogen bond with the protonated Asp40.

We can conclude that this N–Asp40 interaction depends on an optimal pK_a, which can be influenced by the electron donating/accepting properties of substituents. These data can be very useful in future inhibitor design. A full report on the antiparasitic activity of these compounds will be published in the near future.



Scheme 6. Reagents and conditions: a) formamidine acetate, formamide, reflux, 3 h; b) $POCl_3$, ACN, reflux, 5 h; c) 1. *p*-toluenesulfonylhydrazide, dioxane, reflux, overnight, 2. NaOH (1 N), EtOH, reflux, 4 h; d) NBS, dibenzoyl peroxide, CCl_4, reflux, 3 h; e) protected iminoribitol, $KHCO_3$, DMF, overnight, RT; f) TFA/H_2O (1:1), RT, overnight; g) NH_4OH, dioxane, 30 °C, 5 days.



Scheme 7. Reagents and conditions: a) 1. Boc_2O, Et_3N, RT, overnight, 2. Boc_2O, DMAP, 40 °C, overnight; b) NBS, dibenzoyl peroxide, CCl_4, reflux, 4 h; c) protected iminoribitol, K_2CO_3, DMF, 40 °C, 4 h; d) TFA/H_2O (1:1), RT, 4 h.

Experimental Section

All starting materials and dry solvents were obtained from Acros Organics or Aldrich. Column chromatography was performed on a

Table 1. Inhibition of *Tv*NH and hPNP.

Compd	K_i [nM]		SI[a]
	*Tv*NH	hPNP	
A	6.2 ± 0.7[8]	3.3 ± 0.2 (K_i) 0.056 ± 0.015 (K_i*)[27][b]	0.53
B	6.2 ± 0.3[8]	ND[c]	ND
C	4.4 ± 0.5[16]	5.8 ± 1.5	1.32
D	4.1 ± 0.7[16]	22500 ± 5500	> 5000
E	10.8 ± 1.2[16]	> 10^6	> 92500
1a	14 ± 2	> 10^6	> 70000
1b	29 ± 6	> 10^6	> 34000
1c	369 ± 47	ND	ND
1d	314 ± 49	ND	ND
1e	1258 ± 157	ND	ND
1f	50 ± 7	> 5.10^5	> 10000
1g	71296 ± 14681	ND	ND
1h	8362 ± 1136	ND	ND
2	13104 ± 1926	> 10^6	76
3	187000 ± 24069	ND	ND
4	109 ± 15	> 10^6	> 9000
5a	58 ± 10	> 10^6	> 17000
5b	15 ± 4	> 10^6	> 66000
6	139 ± 26	> 10^6	> 7100
7	20 ± 3	77605 ± 18843	> 3800
8a	42 ± 9	> 10^6	> 23800
8b	19 ± 4	> 10^6	> 52600
9	40 ± 6	> 10^6	25000

[a] Selectivity index: K_i(hPNP)/K_i(*Tv*NH). [b] K_i* for slow-onset inhibition. [c] ND = not determined.

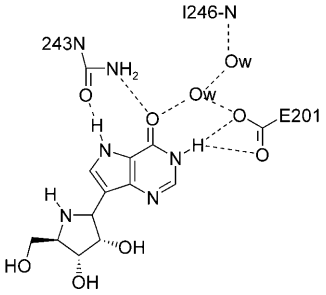


Figure 4. Noncovalent interactions in the PNP–immucillin H complex; interactions with the iminoribitol moiety were left out.[7]

Flashmaster II (Jones Chromatography, Mid Glamorgan, UK) with Isolute columns pre-packed with silica gel (30–90 μM) for normal phase and C_{18} (30–90 μM) for reversed-phase chromatography. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer (400 MHz), coupling constants are reported in Hz. Electrospray ionisation (ESI) mass spectra were acquired on an ion trap mass spectrometer (Bruker Daltonics esquire 3000^{plus}). LC–MS spectra were recorded on an Agilent 1100 Series HPLC system that was equipped a HILIC silica column (2.1 × 100 mm, 5 μm, Atlantis HILIC, Waters), coupled with a Bruker Daltonics esquire 3000^{plus} mass spectrometer (solvent A: H_2O with 0.1% formic acid, solvent B: ACN with 0.1% formic acid; gradient: 90% B→40% B, 12 min, 0.2 mL min^{-1}). HPLC was performed on a Gilson instrument equipped with a C_{18} column (4.6 × 25 cm, 5 μm, Ultrasphere ODS) for system I, and a HILIC Silica column (2.1 × 100 mm, 5 μm, Atlantis HILIC, Waters) for system II (system I: solvent A: H_2O with 0.1% TFA, solvent B: ACN with 1% TFA; gradient I: 5% B→50% B, 36 min, 1 mL min^{-1}; system II: solvent A: H_2O with 0.1% formic acid, solvent B: ACN with 0.1% formic acid; gradient II: 90% B→ 50% B, 20 min, 0.4 mL min^{-1}).

Chemistry

General procedures

Procedure A (chlorination):[19,20] $POCl_3$ (16 equiv) was added to 8-methylheteroarylone (1 equiv) under N_2 flow. The mixture was heated under stirring at 80 °C for 3 h, poured onto ice and neutralised with NH_4OH. The H_2O layer was extracted with $CHCl_3$ (3×). The organic layers were collected and dried over Na_2SO_4 followed by evaporation under reduced pressure to yield the crude product.

Procedure B (methoxylation):[19] A mixture of 8-methylheteroarylone (1 equiv), MeI (1 equiv), DMF (15 mL) and anhydrous K_2CO_3 (1.5 equiv) was heated under stirring at 80 °C for 5 h. H_2O (100 mL) was added and the mixture was extracted with $CHCl_3$ (3×). The organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The pure product was isolated after recrystallisation with MeOH.

Procedure C (bromination):[21] The 8-methylheteroaryl compound (1 equiv) was dissolved in CCl_4 (30 mL) and *N*-bromosuccinimide (1.05 equiv) and dibenzoyl peroxide (0.003 equiv) were added. The mixture was heated at reflux under stirring for 2–4 h. The hot solution was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL) and washed with saturated $NaHCO_3$ (2 × 100 mL) and H_2O (100 mL). After drying over Na_2SO_4, the organic layers were evaporated under reduced pressure. The pure product was obtained after recrystallisation with EtOH.

Procedure D (iminoribitol coupling): The protected iminoribitol[17,18] (1 equiv) was dissolved in DMF (2 mL). K_2CO_3 (2 equiv) and the 8-bromomethylquinoline (1 equiv) were added. The mixture was stirred at 40 °C for 2 h, diluted with H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The organic layers were collected and dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography.

Procedure E (deprotection): The crude product from procedure D was dissolved in TFA/H_20 (1:1, 10 mL per mmol product) and the solution was stirred at room temperature overnight. TFA was evaporated under reduced pressure, and the residue was dissolved in H_2O. During the synthesis of the quinoline derivatives, an excess of Amberlyst was added for neutralisation. The Amberlyst was filtered off and washed extensively with MeOH and H_2O. These layers were collected and evaporated under reduced pressure. The crude product was purified by column chromatography.

Procedure F (one-pot reaction): The methyl heteroaryl compound (1 equiv) was dissolved in CCl_4 (30 mL). *N*-Bromosuccinimide (1.05 equiv) and dibenzoyl peroxide (0.003 equiv) were added. The mixture was heated at reflux under stirring for 2–4 h then allowed to cool to 40 °C. The protected iminoribitol[18,19] (1 equiv) was dissolved in DMF (2 mL), and K_2CO_3 (2 equiv) was added. This mixture was stirred at 40 °C for 2 h, then filtered and washed with CCl_4. After evaporation, the residue was diluted with H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography.

Procedure G (amination):[22] 1,4-Dideoxy-1,4-imino-*N*-(chloro-8-aryl)-methyl-D-ribitols (1 equiv) was dissolved in NH_4OH (10 mL) in a pressure-resistant barrel and heated overnight at 170 °C. The NH_4OH was evaporated under reduced pressure to yield the crude product. This residue was purified by column chromatography (gradient $CHCl_3$/MeOH 4:1, 1% Et_3N), to give the pure product. As a side-product of this reaction, the corresponding heteroarylone was formed.

Procedure H (alternative amination): 5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-(chloro-8-aryl)-methyl-D-ribitols (1 equiv) was dissolved in dioxane (3 mL). NH_4OH (30 mL) was added, and the mixture was stirred for 4–7 days at room temperature. The NH_4OH was evaporated under reduced pressure, and the crude product was purified by column chromatography.

Compounds

(*Z*)-Ethyl-3-(*o*-toluidino)acrylate (11a): *o*-Toluidine (**10**, 1.00 mL, 9.35 mmol) was added to MeOH (10 mL) followed by ethyl propiolate (1 equiv).[19] The solution was heated at reflux under stirring at 80 °C for 4 h and then heated at 40 °C for 48 h. The MeOH was evaporated under reduced pressure to give the crude product. This residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 4:1) to give the pure product; yield: 1.15 g (60%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.29$ (t, $J = J' = 7.1$ Hz, 3H), 2.29 (s, 3H), 4.17 (q, $J = J' = J'' = 7.1$ Hz, 2H), 4.85 (d, $J = 8.2$ Hz, 1H), 6.87–6.93 (m, 1H), 7.14–7.18 (m, 3H), 7.52 ppm (d, $J = 8.2$ Hz, 1H); ES^+-MS: *m/z*: 228 $[M+Na]^+$.

8-Methylquinolin-4(1*H*)-one (12a): Diphenyl ether (75 mL) was heated at 120 °C in a round-bottomed flask,[19,20] then **11a** (1.95 g, 9.51 mmol) was added, and the temperature was quickly raised to, and kept at 250 °C for 15 min under N_2 flow. After cooling to 70 °C, hexane (150 mL) was added. The obtained precipitate was filtered off and washed with hexane. This was then recrystallised from EtOH; yield: 0.80 g (53%). ES^+-MS: *m/z*: 181.5 $[M+Na]^+$.

(*Z*)-Ethyl-3-(*o*-toluidino)-but-2-enoate (11b): *o*-Toluidine (**10**, 6.00 g, 56 mmol), ethyl acetoacetate (7.28 g, 56 mmol), $CaSO_4$ (9.70 g, 56 mmol), abs MeOH (30 mL) and a few drops of acetic acid were added together and heated at reflux under stirring at 80 °C for 2 h.[19] The $CaSO_4$ was then filtered off, and the solvents were evaporated under reduced pressure; yield: 10.68 g (87%); ES^+-MS: *m/z*: 241.7 $[M+Na]^+$.

2,8-Dimethylquinolin-4(1*H*)-one (12b): In a round-bottomed flask diphenyl ether (75 mL) was heated at 120 °C.[19,20] Then **11b** (2.02 g, 9.21 mmol) was added and the temperature was quickly raised to, and kept at 250 °C for 15 min under N_2 flow. After cooling to 70 °C, hexane (150 mL) was added. The precipitate that was obtained was filtered off and washed with hexane. It was then recrystallised from EtOH; yield: 0.81 g (51%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 2.52$ (s, 3H), 2.58 (s, 3H), 6.20 (s, 1H), 7.26 (t, $J = J' = 7.9$ Hz, 1H), 7.51 (d, $J = 7.1$, 1H), 8.13 ppm (d, $J = 8.9$ Hz, 1H); ES^+-MS: *m/z*: 195.6 $[M+Na]^+$.

8-Methylquinoline-*N*-oxide (14): *m*CPBA (1.22 g, 7.10 mmol) was added in portions to a stirred solution of 8-methylquinoline (**13**, 1.00 g, 6.98 mmol) in $CHCl_3$ (20 mL). This was stirred at room temperature overnight. Then an aqueous solution of saturated $NaHCO_3$ was added until CO_2 evolvement ceased. NaOH (1.8 mL, 2.34 mmol) was added to achieve a basic environment. The mixture was extracted with $CHCl_3$ (3×50 mL) and then washed with a saturated NaCl solution (1×100 mL). The organic layers were dried over Na_2SO_4 and evaporation under reduced pressure to yield the crude product, which was purified by flash column chromatography (SiO_2, EtOAc to EtOAc/MeOH, 9:1); yield: 0.66 g (58%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.19$ (s, 3H), 7.17 (dd, $J = 8.4$ Hz, $J' = 6.1$ Hz, 1H), 7.39–7.44 (m, 2H), 7.62–7.65 (m, 2H), 8.40 ppm (dd, $J = 6.0$ Hz, $J' = 0.9$ Hz, 1H); ES^+-MS: *m/z*: 160.0 $[M+H]^+$, 182.0 $[M+Na]^+$.

8-Methylquinolin-2(1*H*)-one (15): Benzoyl chloride (0.88 g, 6.3 mmol) was added slowly to a vigorously stirred mixture of **14** (0.84 g, 5.25 mmol) and NaOH (0.49 g, 12.07 mmol) in H_2O (10 mL) and CH_2Cl_2 (5 mL). When the addition was nearly complete, a short reflux was observed. The flask was therefore cooled in an ice bath, and the addition was resumed. After 1 h stirring, the precipitate was filtered off, rinsed well with H_2O and CH_2Cl_2 and dried in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane/EtOAc 1:1, EtOAc/MeOH 9:1); yield: 0.51 g, 61%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.48$ (s, 3H), 6.65 (d, $J = 9.5$ Hz, 1H), 7.12 (t, $J = J' = 7.6$ Hz, 1H), 7.35 (d, $J = 7.3$ Hz, 1H), 7.42 (d, $J = 7.8$ Hz, 1H), 7.76 (d, $J = 9.5$ Hz, 1H), 9.29 ppm (s, 1H); ES^+-MS: *m/z*: 160.0 $[M+H]^+$, 182.0 $[M+Na]^+$.

4-Chloro-8-methylquinoline (16a): Compound **12a** (1.00 g, 6.26 mmol) was used in procedure A to give the crude product; yield: 0.85 mg (77%). ^{1}H NMR (400 MHz, $CDCl_3$), $\delta = 2.78$ (s, 3H), 7.39–7.55 (m, 3H), 8.01 (d, $J = 8.4$ Hz, 1H), 8.73 ppm (d, $J = 4.6$ Hz, 1H); ES^+-MS: *m/z*: 177.6 $[M+H]^+$.

4-Methoxy-8-methylquinoline (16b): Compound **12a** (0.88 g, 5.50 mmol) was used in procedure B to give the crude product; yield: 0.45 mg (47%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.81$ (s, 3H), 4.05 (s, 3H), 6.76 (d, $J = 5.2$ Hz, 1H), 7.40 (t, $J = J' = 7.4$ Hz, 1H), 7.56 (d, $J = 6.9$ Hz, 1H), 8.08 (d, $J = 8.3$ Hz, 1H), 8.81 ppm (d, $J = 5.2$ Hz, 1H); ES^+-MS: *m/z*: 174 $[M+H]^+$.

4-Chloro-2,8-dimethylquinoline (16c): Compound **12b** (0.81 g, 4.66 mmol) was used in procedure A to give the crude product; yield: 0.95 g (99%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.58$ (s, 3H), 2.72 (s, 3H), 7.15 (s, 1H), 7.28 (t, $J = J' = 7.7$ Hz, 1H), 7.41 (d, $J = 6.8$ Hz, 1H), 7.87 ppm (d, $J = 8.3$ Hz, 1H); ES^+-MS: *m/z*: 191.6 $[M+H]^+$.

2-Chloro-8-methylquinoline (16d): Compound **15** (0.15 g, 0.95 mmol) was used in procedure A to give the crude product; yield: 0.16 g (97%). ES^+-MS: *m/z*: 178.1 $[M+H]^+$.

2-Methoxy-8-methylquinoline (16e): Compound **15** (0.20 g, 1.26 mmol) was used in procedure B to give the crude product; yield: 0.08 g (37%); ES^+-MS: *m/z*: 196.0 $[M+Na]^+$.

8-Bromomethyl-4-chloroquinoline (17a): Compound **16a** (0.50 g, 2.82 mmol) was used in procedure C to give the crude product; yield: 0.72 g (99%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.22$ (s, 2H), 7.53 (d, $J = 4.7$ Hz, 1H), 7.61 (t, $J = J' = 7.2$ Hz, 1H), 7.89 (d, $J = 6.2$ Hz, 1H), 8.23 (d, $J = 8.6$ Hz, 1H), 8.86 ppm (d, $J = 4.7$ Hz, 1H); ES^+-MS: *m/z*: 257.8 $[M+H]^+$, 279.8 $[M+Na]^+$.

8-Bromomethyl-4-methoxyquinoline (17b): Compound **16b** (0.50 g, 2.82 mmol) was used in procedure C to give the crude product; yield: 0.44 g (67%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.05$ (s, 3H), 5.21 (s, 2H), 6.78 (d, $J = 5.2$ Hz, 1H), 7.46 (t, $J = J' = 8.1$ Hz, 1H), 7.81 (d, $J = 7.1$ Hz, 1H), 8.2 (d, $J = 7.3$ Hz, 1H), 8.84 ppm (d, $J = 5.2$ Hz, 1H); ES^+-MS: *m/z*: 274.0 $[M+Na]^+$.

8-Bromomethyl-4-chloro-2-methylquinoline (17c): Compound **16c** (0.50 g, 2.60 mmol) was used in procedure C to give the crude product; yield: 0.61 g (86%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.74$ (s, 3H), 5.24 (s, 2H), 7.41 (s, 1H), 7.52 (t, $J = J' = 8.2$ Hz, 1H), 7.85 (d, $J = 7.1$ Hz, 1H), 8.15 ppm (dd, $J = 8.5$ Hz, $J' = 1.2$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 25.5$, 29.6, 122.3, 124.7, 124.9, 126.3, 131.5, 136.0, 142.6, 146.2, 158.6 ppm.

8-Bromomethyl-2-chloroquinoline (17d): Compound **16d** (0.16 g, 0.92 mmol) was used in procedure C to give the crude product; yield: 0.23 g (98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.10 (s, 2H), 7.33–7.37 (m, 1H), 7.43–7.48 (m, 1H), 7.72 (dd, J = 8.2 Hz, J' = 1.3 Hz, 1H), 7.80 (dd, J = 7.2 Hz, J' = 1.2 Hz, 1H), 8.00–8.03 ppm (m, 1H).

8-Bromomethyl-2-methoxyquinoline (17e): Compound **16e** (0.08 g, 0.46 mmol) was used in procedure C to give the crude product; yield: 0.10 g (91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.04 (s, 3H), 5.06 (s, 2H), 6.83–6.87 (m, 1H), 7.42 (t, J = J' = 6.5 Hz, 1H), 7.55–7.61 (m, 1H), 7.64 (dd, J = 7.2 Hz, J' = 1.3 Hz, 1H), 7.99 ppm (dd, J = 8.0 Hz, J' = 0.8 Hz, 1H).

1,4-Dideoxy-1,4-imino-*N*-(4-chloroquinolin-8-yl)methyl-D-ribitol (1a): Compound **17a** (0.14 g, 0.30 mmol) was used in procedure D to yield the intermediate product (**18a**), which was used in general procedure E without further purification. The crude product (0.06 g) was purified by flash column chromatography (RPC_{18}, H_2O/MeCN, 2:8), to give a yellow syrup; yield: 0.040 g (77%). HPLC (II, λ = 214 nm): t_R = 4.96 min (100%); ^{1}H NMR (400 MHz, CD_3OD): δ = 3.13 (s, 1H), 3.46 (dd, J = 12.3 Hz, J' = 4.1 Hz, 1H), 3.66 (s, 2H), 3.87 (d, J = 3.4 Hz, 2H), 4.19–4.25 (m, 2H), 5.27 (d, J = 13 Hz, 1H), 7.77–7.81 (m, 2H), 7.98 (d, J = 7.0 Hz, 1H), 8.41 (d, J = 8.5 Hz, 1H), 8.87 ppm (d, J = 4.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 58.2, 58.7, 68.1, 70.9, 72.6, 72.9, 123.7, 127.2, 128.0, 129.0, 133.7, 134.5, 145.0, 148.3, 151.5 ppm; ES$^+$-MS: m/z: 309.0 $[M+H]^+$; LC–MS: t_R = 5.1 min; m/z: 309.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-methoxyquinolin-8-yl)methyl-D-ribitol (1b): Compound **17b** (0.07 g, 0.28 mmol) was used in procedure D to yield the intermediate product (**18b**), which was then used in general procedure E without further purification. The crude product was purified by column chromatography (RPC_{18}, H_2O to H_2O/MeCN, 2:8), to give a yellow syrup; yield: 0.12 g (99%). HPLC (II, λ = 214 nm): t_R = 5.12 min (100%);^{1}H NMR (400 MHz, CF_3CO_2D): δ = 3.76 (d, J = 13.2 Hz, 1H), 3.96 (d, J = 10.4 Hz, 1H), 4.27 (s, 2H), 4.43 (s, 3H), 4.64 (s, 1H), 4.71–4.86 (m, 2H), 5.32–5.50 (m, 2H), 7.47 (d, J = 6.7 Hz, 1H), 7.97 (t, J = J' = 8.2 Hz, 1H), 8.41 (d, J = 7.1 Hz, 1H), 8.74 (d, J = 8.5 Hz, 1H), 8.98 (d, J = 6.9 Hz, 1H); ^{13}C NMR (100 MHz, CF_3CO_2D): δ = 65.9, 71.9, 72.2, 72.5, 74.6, 75.1, 104.4, 121.6, 124.6, 129.9, 131.4, 139.8, 143.0, 143.6, 149.0, 174.3 ppm; ES$^+$-MS: m/z: 305.0 $[M+H]^+$, 327.0 $[M+Na]^+$; LC–MS: t_R = 5.3 min, m/z 305.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-chloro-2-methylquinolin-8-yl)methyl-D-ribitol (1c): Compound **17c** (0.12 g, 0.45 mmol) was used in procedure D to yield the intermediate product (**18c**), which was then used in general procedure E without further purification. The crude product was purified by column chromatography (RPC_{18}, H_2O/MeCN, 2:8), to yield a gum; yield: 0.06 g (66%). HPLC (II, λ = 214 nm): t_R = 4.59 min (98.3%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.69 (s, 3H), 2.89 (dd, J = 11.1 Hz, J' = 3.9 Hz, 1H), 3.07 (d, J = 10.8 Hz, 1H), 3.18 (dd, J = 11.2 Hz, J' = 4.7 Hz, 1H), 3.27–3.30 (m, 1H), 3.78 (dd, J = 11.8 Hz, J' = 3.7 Hz, 1H), 3.94 (dd, J = 11.9 Hz, J' = 3.3 Hz, 1H), 4.00–4.03 (m, 1H), 4.06–4.08 (m, 1H), 4.20 (d, J = 12.8 Hz, 1H), 7.57 (s, 1H), 7.56–7.61 (m, 1H), 7.77 (d, J = 6.9 Hz, 1H), 8.18 ppm (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 24.8, 58.5, 58.8, 60.0, 70.9, 72.0, 73.3, 123.8, 125.9, 126.1, 127.7, 132.9, 133.6, 144.5, 147.9, 161.0 ppm; ES$^+$-MS: m/z: 323.0 $[M+H]^+$, 346.0 $[M+Na]^+$; LC–MS: t_R = 5.3 min, m/z 323.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(2-chloroquinolin-8-yl)methyl-D-ribitol (1d): Compound **17d** (0.74 g, 0.29 mmol) was used in procedure D to yield the intermediate product (**18d**) without further purification, which was then used in general procedure E. The crude product was purified by column chromatography (RPC_{18}, H_2O/MeCN, 2:8) to give a gum; yield: 0.016 g (59%). HPLC (II, λ = 214 nm): t_R = 4.73 min (100%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.55 (dd, J = 9.7 Hz, J' = 6.3 Hz, 1H), 2.82–2.86 (m, 1H), 2.99–3.02 (m, 1H), 3.72 (dd, J = 11.6 Hz, J' = 4.1 Hz, 1H), 3.88 (m, 2H), 3.91–3.99 (m, 2H), 4.74 (d, J = 12.8 Hz, 1H), 7.48 (d, J = 3.5 Hz, 1H), 7.58 (t, J = J' = 8.1 Hz, 1H), 7.79 (d, J = 7.0 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 8.30 ppm (d, J = 8.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 56.0, 59.3, 62.1, 70.9, 71.7, 73.7, 123.5, 127.9, 128.8, 129.0, 133.0, 136.5, 141.4, 147.7, 151.6 ppm; ES$^+$-MS: m/z: 309.0 $[M+H]^+$; LC–MS: t_R = 5.4 min, m/z 309.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(2-methoxyquinolin-8-yl)methyl-D-ribitol (1e): Compound **17e** (0.08 g, 0.28 mmol) was used in procedure D to yield the intermediate product (**18e**) without further purification, which was then used in general procedure E. The crude product was purified by column chromatography (RPC_{18}, H_2O/MeCN, 2:8) to give a gum; yield: 0.035 g (42%). HPLC (II, λ = 214 nm): t_R = 4.57 min (100%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.63 (dd, J = 9.6 Hz, J' = 7.1 Hz, 1H), 2.89 (q, J = J' = J'' = 4.4 Hz, 1H), 3.08–3.12 (m, 1H), 3.69 (d, J = 4.3 Hz, 2H), 3.92 (t, J = J' = 4.8 Hz, 1H), 3.97–4.02 (m, 1H), 4.07 (s, 3H), 4.11 (d, J = 12.9 Hz, 1H), 4.64 (d, J = 12.9 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 7.35 (t, J = J' = 7.7 Hz, 1H), 7.68 (dd, J = 7.9 Hz, J' = 6.3 Hz, 2H), 8.06 ppm (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 53.7, 54.8, 59.1, 62.3, 70.9, 72.5, 74.3, 113.5, 124.4, 126.4, 128.1, 131.9, 135.2, 140.3, 146.1, 163.1 ppm; ES$^+$-MS: m/z: 305.0 $[M+H]^+$; LC–MS: t_R = 5.4 min, m/z 305.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-aminoquinolin-8-yl)methyl-D-ribitol (1f): Compound **1a** (0.15 g, 0.48 mmol) was used in procedure G to give a syrup; yield: 0.054 g (40%). HPLC (I, λ = 254 nm): t_R = 5.94 min (100%), (I, λ = 214 nm): 5.90 min (100%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.52 (dd, J = 10.0 Hz, J' = 5.6 Hz, 1H), 2.83–2.88 (m, 1H), 2.99 (dd, J = 10.0 Hz, J' = 5.7 Hz, 1H), 3.63–3.68 (m, 2H), 3.78 (t, J = J' = 5.6 Hz, 1H), 3.92–3.96 (m, 2H), 4.73 (d, J = 13.1 Hz, 1H), 6.66 (d, J = 13.0 Hz, 1H), 7.40 (t, J = J' = 8.1 Hz, 1H), 7.61 (d, J = 6.9 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 8.21 ppm (d, J = 5.9 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 58.0, 59.5, 62.7, 70.9, 71.1, 73.6, 103.5, 119.4, 123.1, 125.5, 132.6, 134.5, 145.0, 147.1, 156.5 ppm; ES$^+$-MS: m/z: 290.0 $[M+H]^+$; LC–MS: t_R = 5.2 min, m/z 290.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4(1*H*)-quinolon-8-yl)methyl-D-ribitol (2): Compound **1a** (0.15 g, 0.48 mmol) was used in procedure G to give a syrup; yield: 0.024 g (17%). HPLC (I, λ = 254 nm): t_R = 6.75 min (100%), (I, λ = 214 nm): 6.71 min (98%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.51–2.56 (m, 1H), 2.85–2.90 (m, 1H), 2.93–2.98 (m, 1H), 3.75 (dd, J = 11.3 Hz, J' = 4.4 Hz, 1H), 3.82 (d, J = 13.6 Hz, 1H), 3.93–3.99 (m, 2H), 4.03 (m, 1H) 4.55 (d, J = 13.6 Hz, 1H), 6.35 (d, J = 7.2 Hz, 1H), 7.34 (t, J = J' = 7.3 Hz, 1H), 7.60 (d, J = 7.0 Hz, 1H), 7.93 (d, J = 7.2 Hz, 1H), 8.20 ppm (d, J = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 58.2, 58.8, 61.1, 70.5, 71.1, 73.6, 109.7, 124.8, 125.7, 127.0, 128.7, 133.4, 140.9, 141.3, 180.4 ppm; ES$^+$-MS: m/z: 291.0 $[M+H]^+$; LC–MS: t_R = 4.6 min, m/z 291.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-amino-2-methylquinolin-8-yl)methyl-D-ribitol (1g): Compound **1c** (0.036 g, 0.11 mmol) was used in procedure G to give a powder; yield: 0.011 g (34%). HPLC (I, λ = 254 nm): t_R = 8.15 min (100%), (I, λ = 214 nm): 8.06 min (90%); ^{1}H NMR (400 MHz, CD_3OD): δ = 2.42–2.45 (m, 4H), 2.75–2.77 (m, 1H), 2.91 (d, J = 4.3 Hz, 1H), 3.69 (d, J = 13.5 Hz, 1H), 3.76 (dd, J = 11.3 Hz, J' = 4.1 Hz, 1H), 3.93 (d, J = 5.6 Hz, 1H), 4.00 (dd, J = 10.9 Hz, J' = 3.6 Hz, 2H), 4.56 (d, J = 13.6 Hz, 1H), 6.19 (s, 1H), 7.29

(t, $J=J'=7.2$ Hz, 1H), 7.54 (d, $J=7.0$ Hz, 1H), 8.14 ppm (d, $J=7.9$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=20.1$, 58.0, 58.8, 61.0, 70.4, 70.9, 73.5, 109.7, 124.4, 125.4, 125.9, 128.2, 133.2, 140.8, 153.0, 177.3 ppm; ES$^+$-MS: *m/z*: 304.0 $[M+H]^+$; LC–MS: $t_R=8.1$ min, *m/z* 304.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(2-aminoquinolin-8-yl)methyl-D-ribitol (1h): Compound **1d** (0.083 g, 0.27 mmol) was used in procedure G to give a syrup; yield: 0.016 g (21.0%). HPLC (II, $\lambda=214$ nm): $t_R=1.96$ min (100%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=3.05$ (d, $J=2.9$ Hz, 1H), 3.43–3.45 (m, 1H), 3.48 (d, $J=7.3$ Hz, 1H), 3.94 (dd, $J=12.3$ Hz, $J'=2.5$ Hz, 1H), 4.05–4.09 (m, 1H), 4.20–4.22 (m, 2H), 4.34 (d, $J=13.1$ Hz, 1H), 4.97 (d, $J=13.1$ Hz, 1H), 6.89 (d, $J=9.0$ Hz, 1H), 7.25 (t, $J=J'=7.6$ Hz, 1H), 7.55 (d, $J=7.1$, 1H), 7.72 (d, $J=7.9$ Hz, 1H), 8.01 ppm (d, $J=9.0$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=$ 57.7, 58.2, 60.2, 71.3, 71.8, 73.0, 114.0, 123.2, 124.2, 130.0, 130.2, 132.3, 140.4, 145.0, 159.0 ppm; ES$^+$-MS: *m/z*: 290.0 $[M+H]^+$; LC–MS: $t_R=9.5$ min, *m/z* 290.0 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(2(1*H*)-quinolon-8-yl)methyl-D-ribitol (3): Compound **1d** (0.083 g, 0.27 mmol) was used in procedure G to give a powder, 0.014 g (18%). HPLC (I, $\lambda=254$ nm): $t_R=9.58$ min (100%), (I, $\lambda=214$ nm): 9.53 min (95.65%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=2.46$ (dd, $J=10.0$ Hz, $J'=6.1$ Hz, 1H), 2.78–2.82 (m, 1H), 2.92–2.96 (m, 1H), 3.71–3.78 (m, 2H), 3.89–3.94 (m, 2H), 4.04–4.08 (m, 1H), 4.55 (d, $J=13.5$ Hz, 1H), 6.63 (d, $J=9.5$ Hz, 1H), 7.21 (t, $J=J'=7.6$ Hz, 1H), 7.45 (d, $J=7.3$ Hz, 1H), 7.61 (d, $J=7.8$ Hz, 1H), 7.99 ppm (d, $J=9.5$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=$ 58.7, 59.2, 63.1, 71.2, 71.6, 74.1, 121.6, 121.7, 123.7, 125.7, 128.7, 132.3, 138.8, 143.2, 160.5 ppm; ES$^+$-MS: *m/z*: 291.0 $[M+H]^+$; LC–MS: $t_R=6.6$ min, *m/z* 291.0 $[M+H]^+$.

8-Methylquinazolin-4(3*H*)-one (20): 2-Amino-3-methylbenzoic acid (**19**, 2.0 g, 13.20 mmol) was dissolved in formamide (0.52 mL, 13.20 mmol), then formamidine acetate (4.12 g, 39.60 mmol) was added.[23] The solution was stirred at 160 °C for 6 h, before it was cooled to room temperature and dissolved in hot 2 N NaOH. The solution was heated for 10 min with charcoal and filtered through celite under reduced pressure. The celite was washed several times with hot 2 N NaOH. The filtrate was neutralised with 2 N HCl (100 mL). The solution was left overnight to precipitate. The precipitated crystals were filtered, washed with cold H_2O and dried; yield: 2.46 g (95%).^{1}H NMR (400 MHz, [D_6] DMSO): $\delta=2.51$ (s, 3H), 7.40 (t, $J=7.6$ Hz, 1H), 7.67 (d, $J=7.1$ Hz, 1H), 7.97 (d, $J=7.8$ Hz, 1H), 8.13 (s, 1H), 12.26 ppm (s, 1H); ES$^+$-MS: *m/z*: 161.1 $[M+H]^+$, 183.1 $[M+Na]^+$.

4-Chloro-8-methylquinazoline (21): Compound **20** (0.85 g, 5.3 mmol) was suspended in MeCN (120 mL), then $POCl_3$ (16 equiv) was added under N_2 flow.[19] The solution was heated at reflux under stirring for 4 h. The mixture was evaporated on ice, and then ice was added to the residue. This was neutralised by adding 10 N NH_4OH. The mixture was left to precipitate at 4 °C. The precipitate was isolated, dried and purified by flash column chromatography (SiO_2, hexane/EtOAc, 7:3); yield: 0.78 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=2.75$ (s, 3H), 7.50 (t, $J=J'=7.7$ Hz, 1H), 7.75 (m, 1H), 8.04 (d, $J=8.4$ Hz, 1H), 9.02 ppm (s, 1H); ES$^+$-MS: *m/z*: 179.5 $[M+H]^+$.

8-Methylquinazoline (22): Compound **21** (1.80 g, 10.11 mmol) and *p*-toluenesulfonylhydrazide (1.88 g, 10.11 mmol) were dissolved in CH_2Cl_2 (30 mL).[24] The solution was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was dissolved in EtOH (30 mL). A solution of 1 N NaOH (30 mL) was added, and the mixture was heated at reflux for 4 h. Then it was cooled down and extracted with Et_2O. The organic layers were combined, dried on Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 2:1) to give the pure product; yield: 0.67 g (46%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=2.88$ (s, 3H), 7.55 (t, $J=7.6$ Hz, 1H), 7.60 (d, $J=7.7$ Hz, 2H), 9.36 ppm (s, 2H).

***N*-(Quinazolin-8-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (23, 24)**: Compound **22** (0.20 g, 1.39 mmol) was used in procedure F to yield, via **23**, the crude product; yield: 0.15 g (25%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 0.03 (s, 3H), 0.05 (s, 3H), 0.87 (s, 9H), 1.34 (s, 3H), 1.35 (s, 3H), 2.88 (dd, $J=10.3$ Hz, $J'=2.2$ Hz, 1H), 3.16 (s, 1H), 4.68–4.74 (m, 1H), 7.62–7.68 (m, 1H), 7.79 (d, $J=7.6$ Hz, 1H), 8.13 (d, $J=6.8$ Hz, 1H), 9.30 (s, 1H), 9.36 ppm (s, 1H); ES$^+$-MS: *m/z*: 460.3 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(quinazolin-8-yl)methyl-D-ribitol·TFA (5a): Compound **24** (0.15 g, 0.35 mmol) was used in procedure E, and the crude product was purified by flash column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH, 85:15). The title compound was obtained as a TFA salt; yield: 0.136 g (99%). HPLC (II, $\lambda=214$ nm) $t_R=8.27$ min (100%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=3.42$ (d, $J=$ 12.5, 1H), 3.56 (dd, $J=12.6$ Hz, $J'=3.75$ Hz, 1H), 3.79 (m, 1H), 3. 87 (s, 2H), 4.16–4.30 (m, 2H), 4.83–4.95 (m, 1H), 5.28–5.38 (m, 1H), 7.81–7.85 (m, 1H), 8.20 (d, $J=7.0$ Hz, 1H), 8.25 (d, $J=8.2$ Hz, 1H), 9.35 (s, 1H), 9.61 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=57.9$, 58.7, 58.9, 70.3, 72.8, 73.1, 116.1 (q, CF_3CO_2H), 126.5, 128.7, 129.4, 131.2, 138.8, 149.7, 156.1, 162.6, 163.0 ppm (q, CF_3CO_2H); ES$^+$-MS: *m/z*: 276.1 $[M+H]^+$; LC–MS: $t_R=10.7$ min, *m/z*: 276.1 $[M+H]^+$.

8-Bromomethyl-4-chloroquinazoline (25). Compound **21** (0.89 g, 4.98 mmol) was used in procedure C and the crude residue was purified by flash column chromatography (SiO_2, hexane/EtOAc 9:1); yield: 0.42 g (33%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=5.10$ (s, 2H), 7.66 (t, $J=J'=7.9$ Hz, 1H), 8.04 (d, $J=7.1$ Hz, 1H), 8.20 (d, $J=$ 8.4 Hz, 1H), 9.09 ppm (s, 1H); ES$^+$-MS: *m/z*: 295.0 $[M+H]^+$.

***N*-(4-Chloroquinazolin-8-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (26):** Compound **25** (0.20 g, 0.79 mmol) was used in procedure D, and the crude residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 8:1); yield: 0.284 g (78%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.00$ (s, 3H), 0.02 (s, 3H), 0.84 (s, 9H), 1.30 (s, 3H), 1.54 (s, 3H), 2.83 (d, $J=9.6$ Hz, 1H), 3.11–3.16 (m, 2H), 3.65–3.78 (m, 2H), 4.50 (s, 2H), 4.57–4.66 (m, 2H), 7.67 (t, $J=J'=7.7$ Hz, 1H), 8.12 (d, $J=8.0$ Hz, 2H), 8.97 ppm (s, 1H); ES$^+$-MS: *m/z*: 464.1 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(quinazolon-8-yl)methyl-D-ribitol (4): Compound **26** (0.05 g, 0.10 mmol) was used in procedure E, and the crude residue was purified by flash column chromatography (SiO_2, $CHCl_3$/MeOH 6:1, 1% Et_3N); yield: 0.014 g, 50%. HPLC (II, $\lambda=$ 214 nm) $t_R=2.90$ min (100%); ^{1}H NMR (400 MHz, CD_3OD): $\delta=2.93$ (dd, $J=10.8$ Hz, $J'=3.8$ Hz, 1H), 3.18 (d, $J=7.3$ Hz, 1H), 3.73 (dd, $J=11.6$ Hz, $J'=3.6$ Hz, 1H), 3.84 (d, $J=11.8$ Hz, 1H), 3.99–4.02 (m, 1H), 4.10 (d, $J=4.6$ Hz, 1H), 4.21 (d, $J=12.9$ Hz, 1H), 4.85 (s, 2H), 7.53 (t, $J=J'=7.7$ Hz, 1H), 7.85 (d, $J=7.0$ Hz, 1H), 8.12 (s, 1H), 8.23 ppm (d, $J=7.9$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=57.7$, 59.0, 60.0, 70.9, 72.1, 73.3, 124.3, 128.1, 130.2, 132.2, 137.4, 146.7, 148.7, 163.3 ppm; ES$^+$-MS: *m/z*: 291.9 $[M+H]^+$; LC–MS: $t_R=$ 10.3 min, *m/z*: 291.9 $[M+H]^+$.

***N*-(4-Aminoquinazolin-8-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (27):** Compound **26** (0.15 g, 0.32 mmol) was used in procedure H, and the crude residue was purified by flash column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH, 95:5); yield: 0.039 g (27%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.04$ (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 1.36 (s, 3H), 1.59

(s, 3H), 2.89 (dd, J=10.3 Hz, J'=2.5 Hz, 1H), 3.16–3.18 (m, 1H), 3.19–3.23 (m, 1H), 3.71 (dd, J=10.7 Hz, J'=3.9 Hz, 1H), 3.82 (dd, J=10.8 Hz, J'=4.6 Hz, 2H), 4.50 (d, J=5.7 Hz, 1H), 4.65 (dd, J=6.5 Hz, J'=1.7 Hz, 1H),4.69–4.72 (m, 1H), 7.45 (t, J=J'=7.5 Hz, 1H), 7.59 (d, J=8.1 Hz, 1H), 8.00 (d, J=7.2 Hz, 1H), 8.60 ppm (s, 1H); ES^+-MS: m/z: 445.5 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-aminoquinazolin-8-yl)methyl-D-ribitol (5b): Compound **27** (0.039 g, 0.088 mmol) was used in procedure E, and the crude residue was purified by flash column chromatography (RPC_{18} H_2O to H_2O/MeCN, 2:8) to give a gum; yield: 0.014 g (55%). HPLC (II, λ=254 nm): t_R=6.55 min (98.44%); ^1H NMR (400 MHz, D_2O): δ=3.41 (d, J=12.9, 1H), 3.63 (d, J=13.2, 1H), 3.76 (s, 1H), 3.83 (s, 2H), 4.21 (d, J=4.1 Hz, 1H), 4.35 (s, 1H), 4.81 (d, J=13.4 Hz, 1H), 5.14 (d, J=13.4 Hz, 1H), 7.81 (t, J=J'=7.7 Hz, 1H), 8.17 (d, J=7.1 Hz, 1H), 8.38 (d, J=7.9 Hz, 1H), 8.49 ppm (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ=56.5, 57.3, 57.5, 68.8, 70.5, 70.8, 114.2, 118.7, 126.7, 128.9, 139.7, 144.5, 145.6, 156.7 ppm; ES^+-MS: m/z: 291.1 $[M+H]^+$; LC–MS t_R=10.8 min, m/z: 291.1 $[M+H]^+$.

5-Bromomethylquinoxaline (29): Compound **28** (0.30 g, 2.08 mmol) was used in procedure C, and the crude residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 6:4); yield: 0.19 g (41%). ^1H NMR (400 MHz, $CDCl_3$): δ=5.18 (s, 2H), 7.62–7.78 (m, 1H), 7.78–7.90 (m, 1H), 8.00–8.12 (m, 1H), 8.9 ppm (d, J=14.6 Hz, 2H).

***N*-(Quinoxaline-5-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (30):** Compound **29** (0.10 g, 0.35 mmol) was used in procedure D, and the crude residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 1:1); yield: 0.052 g (35%). ^1H NMR (400 MHz, $CDCl_3$): δ=0.00 (s, 3H), 0.02 (s, 3H), 0.84 (s, 9H), 1.30 (s, 3H), 1.53 (s, 3H), 2.82 (dd, J=10.3 Hz, J'=2.7 Hz, 1H), 3.10–3.15 (m, 1H), 3.15–3.23 (m, 1H), 3.69–3.78 (m, 1H), 3.75–3.83 (m, 1H), 4.50–4.60 (m, 2H), 4.62–4.70 (m, 1H), 7.68–7.75 (m, 1H), 7.90–7.97 (m, 2H), 8.75–8.81 ppm (m, 2H).

1,4-Dideoxy-1,4-imino-*N*-(quinoxalin-5-yl)-methyl-D-ribitol·TFA (6): Compound **30** (0.052 g, 0.12 mmol) was used in procedure E, and the crude residue was purified by flash column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH, 4:1); yield: 0.042 g (89%). HPLC (I, λ=254 nm): t_R=9.21 min (100%), and (I, λ=214 nm): t_R=9.21 min (100%); ^1H NMR (400 MHz, CD_3OD): δ=3.10–3.20 (m, 1H), 3.30–3.40 (m, 1H), 3,40–3.50 (m, 1H), 3.70–3.90 (m, 2H), 4.10–4.20 (m, 2H), 4.55–4.70 (m, 1H), 5.10–5.25 (m, 1H), 7.80–7.95 (m, 1H), 7.95–8.05 (m, 1H), 8.10–8.20 (m, 1H), 8.95 ppm (d, J=12.3 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ=57.5, 59.2, 59.5, 70.8, 72.5, 73.2, 116.0 (q, CF_3CO_2H), 131.3, 131.6, 133.6, 142.5, 144.2, 146.1, 147.1, 163.2 ppm (q, CF_3CO_2H); ES^+-MS: m/z: 276.0 $[M+H]^+$; LC–MS t_R=10.7 min, m/z: 276.0 $[M+H]^+$.

7-Methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (32): Compound **31** (2.0 g, 11.68 mmol) was used in the same procedure as for the synthesis of compound **20**; yield: 1.80 g (93%). ^1H NMR (400 MHz, $CDCl_3$): δ=2.45 (s, 3H), 7.52 (s, 1H), 8.22 ppm (s, 1H); ES^+-MS: m/z: 188.6 $[M+Na]^+$.

7-Bromomethylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (33): Compound **32** (0.50 g, 3.01 mmol) was used in procedure C. The organic solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 3:1); yield: 0.13 g (18%). ^1H NMR (400 MHz, $[D_6]$ DMSO): δ=4.81 (s, 2H), 8.27 (s, 1H), 8.34 ppm (s, 1H); ES^+-MS: m/z: 269.1 $[M+H]^+$, 247.1 $[M+Na]^+$.

***N*-(4(3*H*)-Thieno[3,2-*d*]pyrimidone-7-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (34):** Compound 33 (0.124 g, 0.51 mmol) was used in procedure D. The crude product was purified by flash column chromatography (hexane to hexane/EtOAc 1:1); yield: 0.18 g (78%). ^1H NMR (400 MHz, $CDCl_3$): δ=0.89 (s, 11H), 1.26 (s, 3H), 1.33 (s, 4H), 1.54 (s, 3H), 2.86 (dd, J=10.3 Hz, J'=1.9 Hz, 1H), 3.11 (s, 1H), 3.20–3.24 (m, 1H), 3.70 (dd, J=10.7 Hz, J'=4.4 Hz, 1H), 3.82 (dd, J=10.7 Hz, J'=4.2 Hz, 1H), 4.12 (dd, J=11.2 Hz, J'=4.0 Hz, 1H), 4.15–4.23 (m, 1H), 4.57 (dd, J=6.5 Hz, J'=2.0 Hz, 1H), 4.67–4.70 (m, 1H), 7.86 (s, 1H), 8.17 ppm (s, 1H); ES^+-MS: m/z: 452.5 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(7-thieno[3,2-*d*]pyrimidonyl)-methyl-D-ribitol·TFA (7): Compound **34** (0.18 g, 0.39 mmol) was used in procedure E to afford a gum; yield: 0.123 g (77%). HPLC (I, λ=254 nm): t_R=5.47 min (100%), and (I, λ=214 nm): t_R=5.39 min (100%); ^1H NMR (400 MHz, CD_3OD): δ=3.52 (s, 1H), 3.58 (d, J=4.1 Hz, 1H), 3.60–3.62 (m, 1H), 3.70–3.74 (m, 1H), 3.79 (s, 2H), 4.11 (s, 1H), 4.20–4.21 (m, 1H), 4.66 (d, J=13.5 Hz, 1H), 8.21 (s, 1H), 8.36 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ=57.9, 58.8, 67.9, 70.2, 71.8, 72.5, 116.4 (q, CF_3CO_2H), 125.4, 127.4, 139.5, 147.9, 157.5, 162.8 ppm (q, CF_3CO_2H); ES^+-MS: m/z: 298.1 $[M+H]^+$; LC–MS: t_R=10.7 min, m/z: 298.1 $[M+H]^+$.

4-Chloro-7-methylthieno[3,2-*d*]pyrimidine (35): Compound **32** (1.12 g, 6.77 mmol) was used in procedure A; yield: 1.15 g (92%). ^1H NMR (400 MHz, $[D_6]$ DMSO): δ=2.45 (s, 3H), 8.24 (s, 1H), 9.06 ppm (s, 1H); ES^+-MS: m/z: 184.9 $[M+H]^+$.

7-Methylthieno[3,2-*d*]pyrimidine (39): Compound **35** (2.30 g, 12.45 mmol), was dissolved in dioxane (40 mL). Then *p*-toluenesulfonylhydrazide (2.32 g, 12.45 mmol) was added.[24] The solution was stirred under reflux overnight. The dioxane was evaporated under reduced pressure. The crude product (4.35 g, 13.0 mmol) was dissolved in EtOH (20 mL), treated with 1 N NaOH (30 mL) and heated at reflux for 5 h. Then it was cooled and the EtOH was evaporated, and the produce was extracted with Et_2O. The organic layers were evaporated under reduced pressure, and the residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 1:1); yield: 0.30 g, 15%. ^1H NMR (400 MHz, $CDCl_3$): δ=2.49 (d, J=7.2 Hz, 3H), 7.59 (s, 1H), 9.19 ppm (s, 2H); ES^+-MS: m/z: 150.1 $[M+H]^+$.

***N*-(thieno[3,2-*d*]pyrimidin-7-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (40, 41):** Compound **39** (0.30 g, 2.0 mmol) was used in procedure F, and the crude residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 1:1); yield: 0.27 g, 30%. ^1H NMR (400 MHz, $CDCl_3$): δ=0.06 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 1.34 (s, 3H), 1.56 (s, 3H), 2.90 (dd, J=10.4 Hz, J'=2.1 Hz, 1H), 3.15 (s, 1H), 3.24 (dd, J=10.4 Hz, J'=5.4 Hz, 1H), 3.71 (dd, J=10.9 Hz, J'=4.3 Hz, 1H), 3.84 (dd, J=10.7 Hz, J'=4.2 Hz, 1H), 4.23–4.35 (m, 2H), 4.59 (d, J=6.5 Hz, 1H), 4.68–4.71 (m, 1H), 8.00 (s, 1H), 9.21 (s, 1H), 9.24 ppm (s, 1H); ES^+-MS: m/z: 436.2 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(thieno[3,2-*d*]pyrimidin-7-yl)methyl-D-ribitol·TFA (8a): Compound **41** (0.26 g, 0.61 mmol) was used in procedure E, and the crude residue was purified by flash column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH, 85:15) to give a syrup; yield: 0.09 g (37%). ^1H NMR (400 MHz, D_2O): δ=3.46 (dd, J=13.0 Hz, J'=3.1 Hz, 1H), 3.63 (dd, J=13.5 Hz, J'=5.0 Hz, 1H), 3.72–3.77 (m, 1H), 3.78–3.79 (m, 2H), 4.14–4.17 (m, 1H), 4.29–4.32 (m, 1H), 4.73 (s, 1H), 4.88 (d, J=13.6 Hz, 1H), 8.60 (s, 1H), 9.03 (s, 1H), 9.27 ppm (s, 1H); ^{13}C NMR (100 MHz, D_2O): δ=51.6, 57.0, 57.5, 68.8, 70.3, 71.0, 116.4 (q, CF_3CO_2 H), 124.1, 132.0, 143.6, 152.7, 153.5,

157.9, 162.8 ppm (q, CF_3CO_2H); ES^+-MS: *m/z*: 282.1 $[M+H]^+$, 304.1 $[M+Na]^+$.

***N*-(4-Chlorothieno[3,2-*d*]pyrimidin-7-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (37):** Compound **35** (0.51 g, 2.74 mmol) was used in procedure F and the crude residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc 1:1); yield: 0.32 g (25%). ES^+-MS: *m/z*: 492.2 $[M+Na]^+$.

***N*-(4-Aminothieno[3,2-*d*]pyrimidin-7-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (38):** Compound **37** (0.32 g, 0.68 mmol) was used in procedure H, and the crude residue was purified by flash column chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2 /MeOH 95:5); yield: 0.15 g (49%); ES^+-MS: *m/z*: 451.2 $[M+H]^+$, 473.2 $[M+Na]^+$.

1,4-Dideoxy-1,4-imino-*N*-(4-aminothieno[3,2-*d*]pyrimidin-7-yl)-methyl-D-ribitol (8b): Compound **38** (0.09 g, 0.20 mmol) was used in procedure E, and the crude residue was purified by flash column chromatography (RPC_{18}, H_20 to H_2O/MeCN, 2:8) to give a brown syrup; yield: 0.08 g (81%). HPLC (II, $\lambda = 214$ nm): $t_R = 7.68$ min (100%), (II, $\lambda = 254$ nm): $t_R = 7.71$ min (100%); ^{1}H NMR (400 MHz, D_2O): $\delta = 3.46$–3.67 (m, 2H), 3.75–3.77 (m, 1H), 3.81 (s, 2H), 4.20–4.21 (m, 1H), 4.35 (s, 1H), 4.74 (s, 1H), 4.88 (d, $J = 13.7$ Hz, 1H), 8.50 (s, 1H), 8.54 ppm (s, 1H); ^{13}C NMR (100 MHz, D_2O): $\delta = 51.7$, 56.9, 57.5, 68.7, 70.3, 71.0, 116.1, 124.5, 141.1, 149.6, 155.0, 155.9 ppm; ES^+-MS: *m/z*: 297.1 $[M+H]^+$, *m/z*: 319.1 $[M+Na]^+$; LC–MS: $t_R = 11.3$ min, *m/z*: 296.9 $[M+H]^+$.

2-(Boc)-amino-4-methylbenzothiazole (43): Compound **42** (1.00 g, 6.09 mmol) was dissolved in dioxane (20 mL) and Et_3N (0.62 g, 6.09 mmol) was added. The mixture was cooled in an ice bath, and di-*tert*-butyl dicarbonate (1.46 g, 6.70 mmol) was added. This was stirred overnight at room temperature. The starting material was not completely consumed, so another portion of di-*tert*-butyl dicarbonate (0.66 g, 3.04 mmol) and *N*,*N*-dimethylaminopyrimidine (0.15 g, 1.2 mmol) was added to speed up the reaction. The mixture was then heated at 40 °C in a water bath for an additional 12 h. Dioxane was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 2N HCl (2×70 mL) and brine (1×70 mL). After drying over Na_2SO_4, the organic layers were evaporated under reduced pressure, and the residue was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 9:1); yield: 0.86 g, 50%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.40$ (s, 9H), 2.65 (s, 3H), 7.12–7.19 (m, 2H), 7.59 ppm (d, $J = 7.4$ Hz, 1H); ES^+-MS: *m/z*: 287.2 $[M+Na]^+$.

2-(Boc)-amino-4-bromomethylbenzothiazole (44): Compound **43** (0.30 g, 1.07 mmol) was used in procedure C. The pure product was obtained after purification by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 9:1); yield: 0.26 g, 71%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.53$ (s, 9H), 4.92 (s, 2H), 7.23 (t, $J = J' = 7.7$ Hz, 1H), 7.44 (d, $J = 7.4$ Hz, 1H), 7.72 ppm (d, $J = 7.9$ Hz, 1H).

***N*-(2-(Boc)-amino-4-bromomethylbenzothiazole-4-yl)-5-*O*-(*tert*-butyldimethylsilyl)-1,4-dideoxy-1,4-imino-2,3-*O*-isopropylidene-D-ribitol (45):** Compound **44** (0.20 g, 0.58 mmol) was used in procedure D to afford the crude product (0.33 g, 0.90 mmol), which was purified by flash column chromatography (SiO_2, hexane to hexane/EtOAc, 3:1); yield: 0.14 g (43%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.05$ (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 1.57 (s, 9H), 2.86 (s, 1H), 3.13 (s, 2H), 3.76–3.82 (m, 2H), 4.26–4.52 (m, 1H), 4.58–4.70 (m, 2H), 7.24 (t, $J = J' = 7.8$, 1H),7.50 (d, $J = 7.3$, 1H), 7.66 ppm (d, $J = 7.6$, 1H); ES^+-MS: *m/z*: 550.2 $[M+H]^+$.

1,4-Dideoxy-1,4-imino-*N*-(2-amino-4-benzothiazoyl)-methyl-D-ribitol·TFA (9): Compound **45** (0.11 g, 0.29 mmol) was used in procedure E to afford a gum; yield: 0.088 g (73%). HPLC (I, $\lambda = 254$ nm): $t_R = 11.7$ min (100%), (I, $\lambda = 214$ nm): $t_R = 11.6$ min (100%); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 3.20$ (s, 2H), 3.39 (dd, $J = 12.7$ Hz, $J' = 4.0$ Hz, 1H), 3.56–3.60 (m, 1H), 3.74 (d, $J = 3.2$ Hz, 2H), 4.09–4.12 (m, 1H), 4.14 (d, $J = 2.4$, 1H), 4.50 (d, $J = 13.2$ Hz, 1H), 7.04 (t, $J = J' = 7.7$ Hz, 1H), 7.24 (d, $J = 7.4$ Hz, 1H), 7.60 ppm (d, $J = 7.80$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 57.9$, 58.6, 60.2, 70.8, 72.3, 72.8, 116.0 (q, CF_3CO_2H) 119.9, 123.0, 123.8, 128.6, 132.5, 151.4, 159.0 (q, CF_3CO_2H), 170.9 ppm; ES^+-MS: *m/z*: 295.8 $[M+H]^+$; LC–MS: $t_R = 4.9$ min, *m/z* 296.1 $[M+H]^+$.

Biochemistry

Enzyme inhibition studies on *T. vivax* IAG-NH: details of the assay and experimental determination of K_i were described previously.[16]

Enzyme inhibition studies on hPNP: hPNP was purchased at Sigma–Aldrich. Inhibitor dissociation constants were determined by measuring the rate of hydrolysis of a fixed concentration of inosine at variable (at least five) inhibitor concentrations, in a 50 mM phosphate buffer pH 7.0 at 35 °C. Inosine activities were measured in a xanthine-oxidase-coupled spectrophotometric assay, in which the further conversion of the product hypoxanthine to uric acid was monitored at 293 nm ($\varepsilon_{293} = 12.9\ mM^{-1}cm^{-1}$) with 60 milliunits of xanthine oxidase. K_i values were determined by fitting initial rates to the equation that describes competitive inhibition: $v_i = (k_{cat} \times S)/(K_M \times (1 + I/K_i) + S)$ in which v_i is the initial reaction rate, k_{cat} is the catalytic turnover number for inosine in the absence of inhibitor, K_M is the Michaelis constant for inosine, and K_i is the dissociation constant of enzyme–inhibitor complex; *I* is the inhibitor concentration, and *S* is the substrate concentration.

Acknowledgements

This work was funded by a research project from the Research Foundation Flanders (FWO-Vlaanderen). M.B. has a PhD grant from the Research Foundation Flanders (FWO-Vlaanderen). A.G. had a PhD grant from the Institute for the Promotion of Innovation through Science and Technology in Flanders. W.V. and P.VdV. have a postdoctoral grant from the Research Foundation Flanders (FWO-Vlaanderen).

Keywords: enzymes · inhibitors · metabolism · nucleoside hydrolase · purine salvage pathway · trypanosomiasis

[1] a) World Health Organization (WHO), *World Health Report 2002*: http://www.who.int/whr/2002/en/ (accessed November 25, 2008); b) Tropical Disease Research (TDR), **2005**: http://www.who.int/tdr/ (accessed November 25, 2008).
[2] M. H. el Kouni, *Pharmacol. Ther.* **2003**, *99*, 283–309.
[3] W. Versées, K. Decanniere, R. Pellé, J. Depoorter, E. Brosens, D. W. Parkin, J. Steyaert, *J. Mol. Biol.* **2001**, *307*, 1363–1379.
[4] D. W. Parkin, B. A. Horenstein, D. R. Abdulah, B. Estupinan, V. L. Schramm, *J. Biol. Chem.* **1991**, *266*, 20658–20665.
[5] L. Muzzolini, W. Versées, P. Tornaghi, E. Van Holsbeke, J. Steyaert, M. Degano, *Biochemistry* **2006**, *45*, 773–782.
[6] B. A. Horenstein, D. W. Parkin, B. Estupinan, V. L. Schramm, *Biochemistry* **1991**, *30*, 10788–10795.
[7] A. Fedorov, W. Shi, A. Kicska, E. Fedorov, P. C. Tyler, R. H. Furneaux, J. C. Hanson, G. J. Gainsford, J. Z. Larese, V. L. Schramm, S. C. Almo, *Biochemistry* **2001**, *40*, 853–860.

[8] W. Versées, J. Barlow, J. Steyaert, *J. Mol. Biol.* **2006**, *359*, 331–346.
[9] R. W. Miles, P. C. Tyler, G. B. Evans, R. H. Furneaux, D. W. Parkin, V. L. Schramm, *Biochemistry* **1999**, *38*, 13147–13154.
[10] G. B. Evans, R. H. Furneaux, G. J. Gainsford, J. C. Hanson, G. A. Kicska, A. A. Sauve, V. L. Schramm, P. C. Tyler, *J. Med. Chem.* **2003**, *46*, 155–160.
[11] H. R. Furneaux, V. L. Schramm, P. C. Tyler, *Bioorg. Med. Chem.* **1999**, *7*, 2599–2606.
[12] B. A. Horenstein, V. L. Schramm, *Biochemistry* **1993**, *32*, 7089–7097.
[13] W. Versées, K. Decanniere, E. Van Holsbeke, N. Devroede, J. Steyaert, *J. Biol. Chem.* **2002**, *277*, 15 938–15 946.
[14] A. Goeminne, M. McNaughton, G. Bal, G. Surpateanu, P. Van der Veken, S. De Prol, W. Versées, J. Steyaert, A. Haemers, K. Augustyns, *Eur. J. Med. Chem.* **2008**, *43*, 315–326.
[15] A. Goeminne, M. McNaughton, G. Bal, G. Surpateanu, P. Van der Veken, S. De Prol, W. Versées, J. Steyaert, L. Pieters, A. Haemers, K. Augustyns, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2523–2526.
[16] A. Goeminne, M. Berg, M. McNaughton, G. Bal, G. Surpateanu, P. Van der Veken, S. De Prol, W. Versées, J. Steyaert, A. Haemers, K. Augustyns, *Bioorg. Med. Chem.* **2008**, *16*, 6752–6763.
[17] G. W. J. Fleet, J. C. Son, *Tetrahedron* **1988**, *44*, 2637–2647.
[18] B. A. Horenstein, R. F. Zabinsky, V. L. Schramm, *Tetrahedron Lett.* **1993**, *34*, 7213–7216.
[19] M. G. Kayirere, A. Mahamoud, J. Chevalier, J.-C. Soyfer, A. Crémieux, J. Barbe, *Eur. J. Med. Chem.* **1998**, *33*, 55–63.
[20] J. Bergman, R. Engqvist, C. Stalhandske, H. Wallberg, *Tetrahedron* **2003**, *59*, 1033–1048.
[21] N. K. Dalley, G. Wue, J. Bradshaw, X. X. Zhang, R. G. Harrison, P. B. Savage, K. E. Krakowiak, R. M. Izatt, *J. Heterocycl. Chem.* **2001**, *38*, 1–9.
[22] G. B. Evans, R. H. Furneaux, D. H. Lenz, G. F. Painter, V. L. Schramm, V. Singh, P. C. Tyler, *J. Med. Chem.* **2005**, *48*, 4679–4689.
[23] L. Orfi, F. Waczek, J. Pato, I. Varga, B. Hegymegi-Barakonyi, R. A. Houghten, G. Kéri, *Curr. Med. Chem.* **2004**, *11*, 2549–2553.
[24] A. Gomtsyan, E. K. Bayburt, R. G. Schmidt, G. Z. Zheng, R. J. Perner, S. Didomenico, J. R. Koenig, S. Turner, T. Jinkerson, I. Drizin, S. M. Hannick, B. S. Macri, H. A. McDonald, P. Honore, C. T. Wismer, K. C. Marsh, J. Wetter, K. D. Stewart, T. Oie, M. F. Jarvis, C. S. Surowy, C. R. Faltynek, C.-H. Lee, *J. Med. Chem.* **2005**, *48*, 744–752.
[25] H. J. S. Machado, A. Hinchliffe, *J. Mol. Struct. THEOCHEM* **1995**, *339*, 255–258.
[26] P. E. Morris, Jr., G. A. Omura, *Curr. Pharm. Des.* **2000**, *6*, 943–959.
[27] G. B. Evans, R. H. Furneaux, A. Lewandowicz, V. L. Schramm, P. C. Tyler, *J. Med. Chem.* **2003**, *46*, 5271–5276.

Received: July 16, 2008
Revised: October 30, 2008
Published online on December 29, 2008

