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1,2,3-Triazolylalkylribitol derivatives as nucleoside hydrolase inhibitors

A. Goeminne,[a] M. McNaughton,[a,*] G. Bal,[a] G. Surpateanu,[a] P. Van der Veken,[a] S. De Prol,[b,c] W. Versées,[b,c] J. Steyaert,[b,c] S. Apers,[d] A. Haemers[a] and K. Augustyns[a,*]

[a]*Department of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium*
[b]*Department of Ultrastructure, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium*
[c]*Department of Molecular Interactions, VIB, Pleinlaan 2, B-1050 Brussels, Belgium*
[d]*Department of Pharmacognosy, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium*

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Abstract—A range of novel 1,2,3-triazolylalkylribitol derivatives were synthesized and evaluated as nucleoside hydrolase inhibitors. The most active compound (**11a**) has low micromolar potency and is structurally diverse from previously reported nucleoside hydrolase inhibitors, which, along with the simplicity of the chemistry involved in its synthesis, makes it a good lead for the further development of novel nucleoside hydrolase inhibitors.
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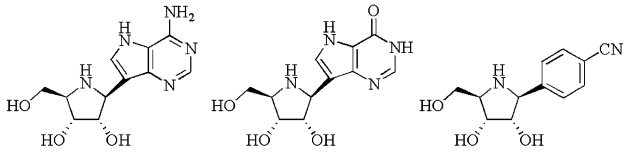
African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas' Disease) and leishmaniasis are infections caused by parasitic protozoa from the Trypanosomatidae family. These diseases continue to haunt developing countries causing in excess of one hundred thousand deaths annually.[1] In order to combat these infections, development of novel treatments is required.

As part of our ongoing research into novel agents for the treatment of trypanosomiasis we have been investigating the nucleoside hydrolase enzyme as a potential target. Parasitic protozoa are unable to synthesize purines de novo and are reliant on the purine salvage pathway to provide purinebases obtained from the nucleosides present in the host.[2] Nucleoside hydrolase (NH) is an essential enzyme in the purine salvage pathway. NH cleaves the *N*-glycosidic bond of nucleosides sequestered from the host to provide the purinebases which are necessary for the survival of the parasite. Based on the substrate specificity, four enzyme-types for NH are currently known.[3] Our target enzyme is isolated from the parasite *Trypanosoma vivax* and is an IAG-NH, with a high specificity for inosine, adenosine and guanosine.[4]

Iminoribitol molecules, **1** and **2**, originally developed by Schramm et al.,[5] have been reported with nanomolar activity against the IAG-NH isolated from the parasite *T. vivax*.[6] Schramm et al. synthesized a range of structurally less complex iminoribitol compounds and reported a K_i = 3 μM against the IAG-NH from *Trypanosoma brucei brucei* for the most active compound (**3**).[7] However, iminoribitol-based IAG-NH inhibitors are mostly compromised by their pronounced activity against nucleoside phosphorylase, another ribonucleoside cleaving enzyme. Moreover, there are a considerable number of synthetic steps required to synthesize such iminoribitol compounds. We were searching for IAG-NH inhibitors with good activities, structurally distinct from iminoribitol-based inhibitors and therefore synthetically easier to access.



1 K_i 0.0062 μM **2** K_i 0.0062 μM **3** K_i 3 μM

It was known from modelling studies within our group that ribitol derivatives with an aromatic or heteroaromatic substituent in the correct position can fit in the active site of the target enzyme (IAG-NH from *T. vivax*,

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* Corresponding authors. Tel.: +3238202735/17; fax: +3238202739; e-mail addresses: michael.mcnaughton@ua.ac.be; koen.augustyns@ua.ac.be

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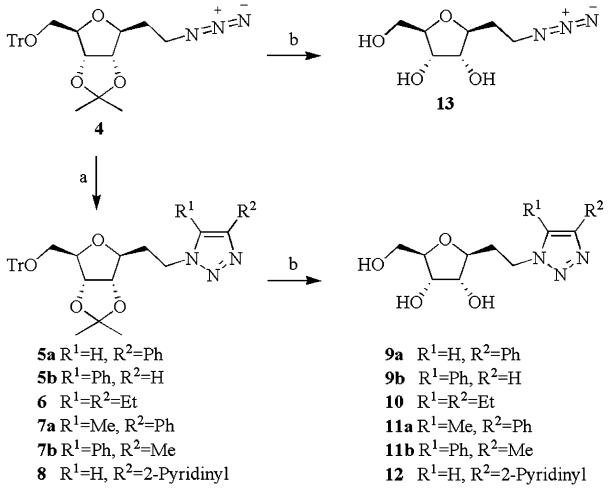
*Tv*NH) in an orientation that enables the aromatic substituent to participate in aromatic stacking interactions with two tryptophan residues in the active site of *Tv*NH. We considered the triazolyl group as a good candidate for these stacking interactions and in a search for biologically active non-purine C-nucleosides, we synthesized a series of 1,2,3-triazolylalkylribitol derivatives. A 1,3-dipolar cycloaddition reaction was used with an azidoalkylribitol as starting material.[8] This would allow for the possible creation of a wide range of ribitol derivatives through the addition of appropriate alkynes to the azidoalkylribitol. Herein we report our preliminary findings.

The synthesis of the ribitol derivatives started with the azide-alkyl-ribitol **4** (Scheme 1), which can be readily synthesized from D-ribose.[9] Initially, phenylacetylene was chosen as the first coupling partner for the azide. Treatment of **4** with phenylacetylene in toluene at 80 °C yielded the desired 1,2,3-triazole as its two possible regioisomers (**5a**:**5b**). At this stage the two isomers could be easily separated by column chromatography. Upon global deprotection of each isomer with trifluoroacetic acid, the desired ribitol compounds **9a** and **9b** were obtained. The assignment of regiochemistry of **9a** and **9b** was based on the literature where a difference in chemical shift of approximately 0.5 ppm was observed for the triazole-hydrogens of the different isomers.[10]

At this stage it was decided to test these two 1,2,3-triazoles for their activity as nucleoside hydrolase inhibitors in order to see if there is a difference in activity between the two isomers. It was revealed through the biochemical testing that there is a significant difference, with isomer **9a** being 30 times more active than **9b** as an inhibitor of IAG-NH (Table 1). A preliminary selection of commercially available alkynes was subsequently made in order to see if the activity could be improved.

It is widely known that the addition of CuCl to the reaction increases the yield of the desired 1,4-isomer in the case of terminal alkynes.[11] There are also reports that



Scheme 1. Reagents and conditions: (a) i—alkyne, toluene, 80 °C, 24 h or ii—alkyne, toluene, CuCl, 110–130 °C; (b) TFA/H_2O 1:1.

Table 1. Inhibition of *Tv*NH by the target compounds

Compound	Inhibition K_i (μM)
9a	$2.1 \times 10^1 \pm 0.6 \times 10^1$
9b	$6.8 \times 10^2 \pm 1.1 \times 10^2$
10	$6.3 \times 10^1 \pm 0.8 \times 10^1$
11a	2.3 ± 0.2
11b	$3.2 \times 10^1 \pm 0.3\ 10^1$
12	$2.3 \times 10^1 \pm 0.3 \times 10^1$
13	$5.6 \times 10^3 \pm 1.8 \times 10^3$
17a	$8.7 \times 10^1 \pm 1.2 \times 10^1$
17b	$5.2 \times 10^2 \pm 1.0 \times 10^2$

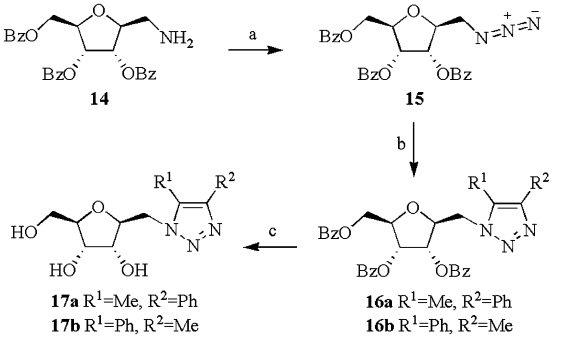
reaction times can be reduced with the addition of copper (I) salts to the reaction conditions.[11a]

Reaction of the selected alkynes with the azide **4** in the presence of CuCl furnished the compounds **6**–**8** (Scheme 1). Global deprotection with trifluoroacetic acid furnished the desired ribitol compounds **10**–**12**. Synthetically, the 1,4-isomer was the sole isomer isolated in the case of the terminal alkyne (**12**) as expected. The regiochemistry of **11a**–**b** and **12** was assigned using 2D NMR techniques (NOESY and HMBC experiments).

1,2,3-Triazole derivatives with only one carbon atom between the ribose and 1,2,3-triazole moieties were also synthesized to observe how this would influence IAG-NH inhibitor activities. The synthesis started with the synthetically available amine **14**[12] which underwent a diazo transfer reaction[13] to furnish the required azide (Scheme 2). Reaction with 1-phenyl-1-propyne yielded the desired 1,2,3-triazole as the two possible isomers which were deprotected with 7 N ammonia in methanol to furnish **17a** and **17b**. Assignment of the relative regiochemistry was done by comparison of the 1D NMR spectra of **17a**–**b** with those of **11a**–**b**.

Our target compounds were tested as inhibitors of *Tv*NH. A survey of the biochemical results as reported in Table 1 reveals that the 1,2,3-triazolylalkyl group on ribose considerably enhances inhibition of the nucleoside hydrolase compared to its free azide equivalent **13**.

It also appears from the biochemical results that the 4-phenyl-triazole is the more active regioisomer and that the addition of a methyl group at position 5 improves potency. The most active compound in this series,



Scheme 2. Reagents and conditions: (a) triflic azide, $NaHCO_3$, $CuSO_4 \cdot 5H_2O$, H_2O/toluene/MeOH; (b) alkyne, toluene, CuCl, 130 °C; (c) 7 N NH_3 in MeOH.

5-methyl-4-phenyl-1,2,3-triazolyliminoribitol derivative **11a**, shows a K_i value of 2.26 μM. Shortening the alkyl linker to one carbon, as in target compounds **17a**–**b**, considerably decreases potency.

In an attempt to find an explanation as to why the 4-phenyl isomer is more active than the 5-phenyl isomer we performed a molecular modelling study. In this study, inhibitors **9a**–**b** and **11a**–**b** were docked in the active site of the target enzyme, which was selected from the pdb structure of *Tv*NH co-crystallized with Immucillin-H (**2**) (pdb code 2FF2).[6] The structure of *Tv*NH with Immucillin-H is illustrated in Figure 1a.

Immucillin-H interacts with different active site residues of the enzyme through the hydroxyl groups and the ring-N of its iminoribitol moiety (Fig. 1a). The 9-deazahypoxanthine ring of Immucillin-H is orientated almost parallel between two Trp-residues (Trp83 and Trp260) of the active site, allowing for aromatic stacking interactions. The 9-deazahypoxanthine ring is also involved in several hydrogen bonds: a first hydrogen bond is formed between N7 and a water molecule in the active site, a second hydrogen bond is formed between the O6 carbonyl and amino acid residue Arg252, and a third hydrogen bond is formed between N3 and amino acid residue Asp40. The pK_a of this Asp40 was calculated to be approximately 7.2–7.8, making it mostly protonated in our experimental conditions (pH 7.0) and thus allowing a hydrogen bond with N3.[14]

An automatic docking protocol was used to position the inhibitors into the active site. During the docking experiments with our inhibitors, it was observed that the 5-phenyl isomers did not fit in the active site of the target enzyme, whereas the 4-phenyl isomers were able to fit within the active site. The compound with the best fit in the active site is the most potent inhibitor of this series, namely **11a**, and docking of this inhibitor is illustrated in Figures 1b and 2. The main interactions with the enzyme observed for our inhibitors are illustrated in Figure 1b and they include the interactions between the hydroxyl groups of the ribose moiety, similar to what was observed for Immucillin-H.

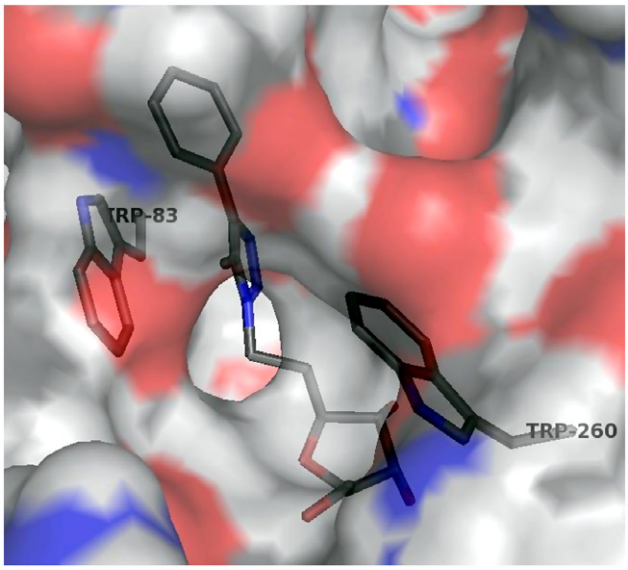


Figure 2. Binding of inhibitor **11a** in the active site of the target enzyme. The triazole moiety of the inhibitor is orientated parallel to Trp83, enabling aromatic stacking interaction. Carbons are grey, oxygens are red and nitrogens are blue. The colours of the enzyme surface are according to the elements. The image was obtained with PyMOL.[15]

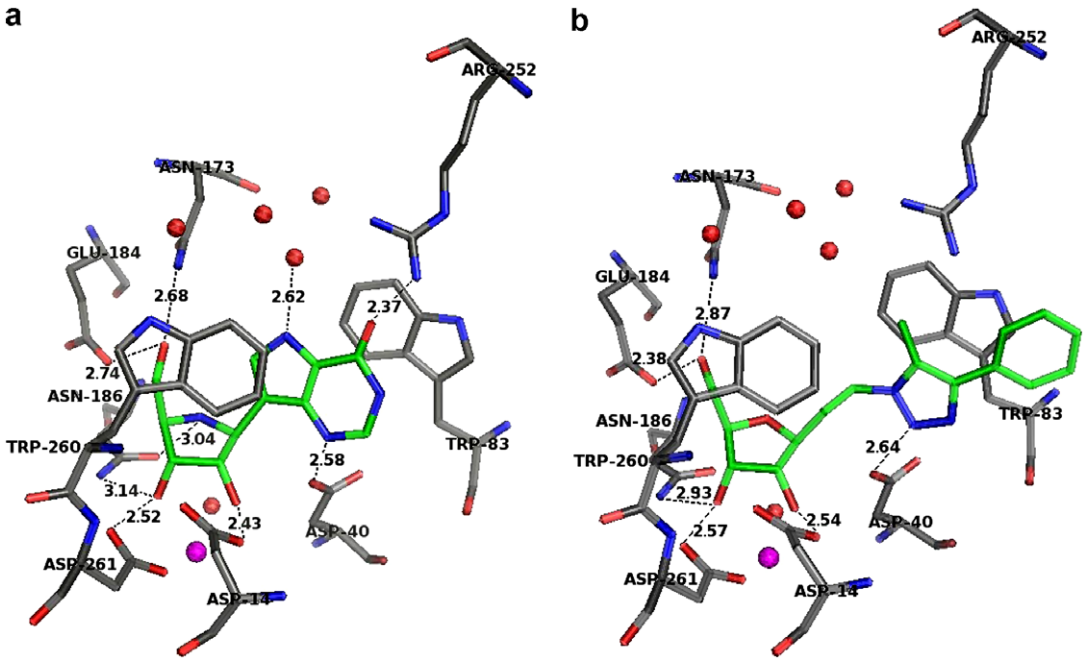


Figure 1. (a) The active site of *Tv*NH co-crystallized with Immucillin-H (**2**) (pdb code 2FF2). (b) Inhibitor **11a** in the active site of the target enzyme. For both images, the active site residues are depicted in grey (carbons), blue (nitrogens) and red (oxygens). The carbons of the inhibitors are depicted in green, the colour code for nitrogens and oxygens is the same as for the active site residues. Water molecules are depicted as red spheres, the Ca^{2+} ion is depicted as a magenta sphere. Distances are in Å. The images were made with PyMOL.[15]

From Figure 2 it is clear that the aromatic substituent (1,2,3-triazole moiety) is orientated parallel with one of the two Trp-residues from the enzyme, which suggests the possibility for aromatic stacking interactions. In the orientation of compound **11a**, as obtained during the docking experiments and illustrated in Figure 2, the triazole-nitrogens are pointing towards the protonated Asp40-residue of the active site. This enables an interaction between N2 from the triazole with Asp40.

Utilizing the commonly used 1,3-dipolar cycloaddition reaction we have synthesized a range of 1,2,3-triazolylalkylribitol derivatives with good to moderate activities as inhibitors of IAG-NH. We have confirmed that the 4-phenyl isomer is the more biochemically active of the two isomers. The most active compound, **11a** with $K_i = 2.3$ μM, is among the most active ribitol inhibitors for IAG-NH reported and is synthetically easier to access than the previously reported iminoribitol inhibitors. Compound **11a** therefore provides us with a good lead for the further development of non-iminoribitol IAG-NH inhibitors.

Acknowledgments

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Supplementary data

Detailed synthetic procedures and analytical data for the target compounds and biochemical protocol for K_i-determination are given.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.017.

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