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AdoHcy hydrolase of *Trichomonas vaginalis*: Studies of the effects of 5′-modified adenosine analogues and related 6-*N*-cyclopropyl derivatives

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ABSTRACT

Trypanosoma brucei and *Trichomonas vaginalis* are both parasitic protozoans that are known to share many similar biochemical pathways. Aristeromycin, as well as 5′-iodovinyl and 5′-oxime analogues of adenosine, are potent inhibitors of AdoHcy hydrolase in *T. brucei*, an enzyme that catalyses the hydrolysis of AdoHcy to adenosine and L-homocysteine. To help determine the role of this enzyme in *T. vaginalis*, we have tested a library of 5′-modified adenosine derivatives, including 5′-deoxy-5′-(iodomethylene)-adenosine and related 6-*N*-cyclopropyl analogues. Our results indicate that these inhibitors are effective at inhibiting the growth of *T. vaginalis*, by as much as 95%.

Trichomonas vaginalis is the causative agent of trichomoniasis, a common sexually-transmitted disease in humans. The current FDA-approved treatments for this disease are the compounds metronidazole and tinidazole. The medication is typically prescribed as a 2 g single dosage to be taken orally. However, approximately 2.5-5% of all reported cases are resistant to metronidazole with this percentage increasing.[1] The search for alternative new therapies for both nitroimidazole susceptible and resistant cases is imperative. *S*-Adenosylhomocysteine (AdoHcy) hydrolase functions as an essential catabolic enzyme that catalyses the hydrolytic cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy). It can also perform the reverse synthesis reaction. AdoHcy is formed after the donation of a methyl group by *S*-adenosylmethionine (AdoMet). AdoHcy hydrolase can perform the same reaction with different nucleotides and/or amino acids.[2]

In trypanosome protozoa, the pathways of methionine/AdoMet/decarboxylated AdoMet/AdoHcy and polyamine metabolism are closely related, and have both been successfully targeted in the design of trypanocides.[3] Hydrolytic cleavage of AdoHcy to adenosine and L-homocysteine requires AdoHcy hydrolase, which also acts as a potent feedback inhibitor of crucial transmethylation enzymes.

The 5′-deoxy-5′-(*E*)-(iodomethylene)adenosine **1a**, a known inhibitor of AdoHcy hydrolase, inhibited the growth of *Trypanosoma brucei* with an IC_{50} of 9 μg/mL. Interestingly, the 5′-deoxy-5′-(*Z*)-(iodomethylene)-adenosine analogue **1b** showed a much lower potency against *T. brucei* (IC_{50} of 45 μg/mL).[3] The different IC_{50} values betweens analogues **1a** and **1b** suggests that trypanosomes may have different versions of AdoHcy hydrolase.

The 6-*N*-cyclopropyl analogues **3**, **4** and **7b** did not exhibit inhibitory effects on human cells or malaria parasites. Nevertheless the 5′-fluorovinyl compound **3** displayed an IC_{50} value of 19 μg/mL against trypanosomes. However, compounds **4** and **7b** had no effect on inhibiting the growth of trypanosomes.[3]

This study looks at AdoHcy hydrolase as a possible candidate for drug design against the protozoan parasite, *T. vaginalis*. A CLUSTAL W alignment of AdoHcy hydrolase from *T. brucei, Trypanosoma cruzi, T.vaginalis* and humans has shown that this enzyme is conserved in its amino acid sequence between all four organisms (Fig. 1).

Primers to the putative *T. vaginalis* homologue of AdoHcy hydrolase (Accession No. XP_00132501) were designed and reverse transcription PCR[4] was performed on cells from the T1 strain of *T. vaginalis*. mRNA was detected in this strain, and GAPDH was used as control gene for the reaction (Fig. 2).

Given this gene expression information, we then tested a library of 5′-modified adenosine derivatives including, 5′-deoxy-5′-(halomethylene)adenosines, their 6-*N*-cyclopropyl derivatives and selected aristeromycin and 2′-deoxyeritadenine analogues to see what effect, if any, these would have on the growth of the organism.

The 5′-modified adenosine derivatives and their 6-*N*-cyclopropyl analogues **1**–**8** as well carbocyclic adenosine analogue aristeromycin, **9**, and aristeromycin-5′-carboxylic acid, **10** and acyclic

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Tbrucei          ------MTD---YKVRDISLAEWGRRELELAENEMPGLMELRREYGPSKPLKGAKIAGCL 51
Tcruzi           ------MTD---YKVRDISLAEWGRKAIEIAENEMPGLMELRREYSQSKPLKGAKIAGCL 51
Hsapiens         ------MSDKLPYKVADIGLAAWGRKALDIAENEMPGLMRMRERYSASKPLKGARIAGCL 54
Tvaginalis       MACKSPAGAPFEYRIADINLHVLGRKELTLAEKEMPGLMVLRERYSASKPLKGVRISGSL 60
                            *:: **.*   **: : :**:****** :*..*. ******.:*:*.*

Tbrucei          HMTMQTAVLIETLVELGAEVRWASCNIFSTQDHAAAAIAK---------RGIPVFAWKGE 102
Tcruzi           HMTVQTAVLIETLIQLGAEVRWSSCNIFSTQDNAAAAIAK---------RGIPVFAWKGE 102
Hsapiens         HMTVETAVLIETLVTLGAEVQWSSCNIFSTQDHAAAAIAK---------AGIPVYAWKGE 105
Tvaginalis       HMTVQTAVLIETLTALGADVRWASCNIFSTQDTAAAAIVVGPTGTPEKPAGIPVFAWKGE 120
                 ***::********  ***:*:*:********* *****.          ****:*****

Tbrucei          TEEEYMWCMKQTLKGFSGDGYPNMLLDDGGDLTNYVLD---------------------- 140
Tcruzi           TEEEYQWCIEQTLKGFSGDGFPNMILDDGGDLTNHVLD---------------------- 140
Hsapiens         TDEEYLWCIEQTLY-FK-DGPLNMILDDGGDLTNLIHT---------------------- 141
Tvaginalis       TLPEYWENTYRALTWPDGQGPQ-QVVDDGGDATLLISKGFEFETAGAVPEPTEADNLEYR 179
                 *  **     ::*   . :*    ::***** *  :

Tbrucei          ---------------ECKELDGKIYGVSEETTTGVKNLYKRLQRGKLTIPAMNVNDSVTK 185
Tcruzi           ---------------HCPHLVDKIYGISEETTTGVKNLYKRLQRGKLPIPAINVNDSVTK 185
Hsapiens         ---------------KYPQLLPGIRGISEETTTGVHNLYKMMANGILKVPAINVNDSVTK 186
Tvaginalis       CVLATLKQVFNQDKNHWHTVAAGMNGVSEETTTGVHRLYQLEKEGKLLFPAINVNDAVTK 239
                              .   :   : *:********:.**:   .* * .**:****:***

Tbrucei          SKFDNLYGCRESLVDGIKRATDVMIAGKTACVCGYGDVGKGCAAALRGFGARVVVTEVDP 245
Tcruzi           SKFDNLYGCRESLVDGIKRATDVMIAGKTACVCGYGDVGKGCAAALRAFGARVVVTEVDP 245
Hsapiens         SKFDNLYGCRESLIDGIKRATDVMIAGKVAVVAGYGDVGKGCAQALRGFGARVIITEIDP 246
Tvaginalis       SKFDNIYGCRHSLIDGINRASDVMIGGKTALVMGYGDVGKGCAQSLRGQGARVIITEVDP 299
                 *****:****.**:***:**:****.**.* * ********** :**. ****::**:**

Tbrucei          INALQAAMEGYQVLLVEDVVEEAHIFVTTTGNDDIITSEHFPRMRDDAIVCNIGHFDTEI 305
Tcruzi           INALQAAMEGYQVLLVEDIVEQAHIFVTTTGNDDIITAEHFPRMQDDAIVCNIGHFDTEI 305
Hsapiens         INALQAAMEGYEVTTMDEACQEGNIFVTTTGCIDIILGRHFEQMKDDAIVCNIGHFDVEI 306
Tvaginalis       ICALQAAMEGYQVRRIEEVVKDVDIFVTCTGNCDIISVDMMAQMKDKAIVGNIGHFDNEI 359
                 * *********:*  :::  :: .**** **  ***    : :*:*.*** ****** **

Tbrucei          QVAWLKAN-AKERVEVKPQVDRYTMANGRHIILLAEGRLVNLGCASGHPSFVMSNSFCNQ 364
Tcruzi           QVSWLKAN-AKERVEVKPQVDRYTMHNGRHIILLAEGRLVNLGCASGHPSFVMSNSFSNQ 364
Hsapiens         DVKWLNEN-AVEKVNIKPQVDRYRLKNGRRIILLAEGRLVNLGCAMGHPSFVMSNSFTNQ 365
Tvaginalis       DTDGLMKYPGIKHIPIKPEYDMWEFPDGHAILLLAEGRLLNLGCATGHPSFVMSMSFTNQ 419
                 :.  *    . ::: :**: * : : :*: *:********:***** ******** ** **

Tbrucei          VLAQIELWTNRDTGKYPRGAKAQVYFLPKKLDEKVAALHLGKLGAKLTKLTPKQAEYINC 424
Tcruzi           VLAQIELWTQRDSGKYPRGEKAQVYFLPKKLDEKVAALHLGKLGAKLTKLTAKQADYINC 424
Hsapiens         VMAQIELWTHPD--KYPVG----VHFLPKKLDEAVAEAHLGKLNVKLTKLTEKQAQYLGM 419
Tvaginalis       TLAQLDLYEKRG--KLEKK----VYTLPKHLDEEVARLHLGSLDVHLTKLTQKQADYINV 473
                 .:**::*: : .  *        *: ***:*** **  ***.*..:***** ***:*:.

Tbrucei          PVDGPFKPDHYRY 437
Tcruzi           PVDGPFKPDHYRY 437
Hsapiens         SCDGPFKPDHYRY 432
Tvaginalis       PVEGPYKSDAYRY 486
                 . :**:*.* ***
```

Figure 1. CLUSTAL W Alignment of AdoHcy hydrolase from *T. brucei*, *T. cruzi*, *T. vaginalis* and *H. sapiens*.

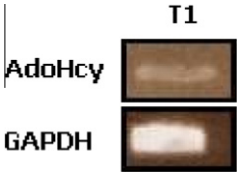

Figure 2. Reverse transcription PCR of AdoHcy hydrolase from *T. vaginalis*.

adenosine analogues **11** and **12** (Fig. 3), were tested against *T. vaginalis* in vitro.[5,6] The most potent inhibitors were aristeromycin **9**, (*E*) and (*Z*) 5′-deoxy-5′-(iodomethylene)adenosines **1a** and **1b**, and adenosine-5′-oxime **7a** (Table 1). These compounds have an IC_{50} of 10 μM, 60 μM, 76 μM, 40 μM, respectively. Metronidazole's IC_{50} value for the strain that these compounds were tested on was 0.72 μM (Table 2).

The 5′-iodovinyl compounds **1a** and **1b** showed similar inhibitory activity against both *T. vaginalis* and trypanosomes. However, even though the *N*-cyclopropyl 5′-iodovinyl analogue **2** showed antitrypanosomal activity, with an IC_{50} value of 12 μg/mL,[3] it was not as effective against *T. vaginalis*.

Interestingly, the *N*-cyclopropyl 5′-oxime analogue **7b** showed stronger inhibitory activity against *T. vaginalis* than trypanosomes.[3] Also, compounds **5** and **6** do not demonstrate significant inhibitory effects on *T. vaginalis*.

Adenosine 5′-carboxylic acid **8a** showed moderate potency higher than that of the corresponding methyl ester analogue **8b**. Furthermore, aristeromycin-5′-carboxylic acid **10**, was found to be less potent than the aristeromycin, **9**, itself. Moreover, open-chain carbocyclic analogues of adenosine **11** and **12** were inactive.

Aristeromycin **9**[7] as well 5′-iodovinyl **1a**/**1b**[6a] and 5′-oxime **7a**[6d] analogues of adenosine are potent inhibitors of AdoHcy hydrolase and the enzyme inhibitory potency of **1a**/**1b**[6a] and **7a**[6d] have been correlated with their antiviral and anticancer potencies. Our preliminary results presented here indicate that these AdoHcy

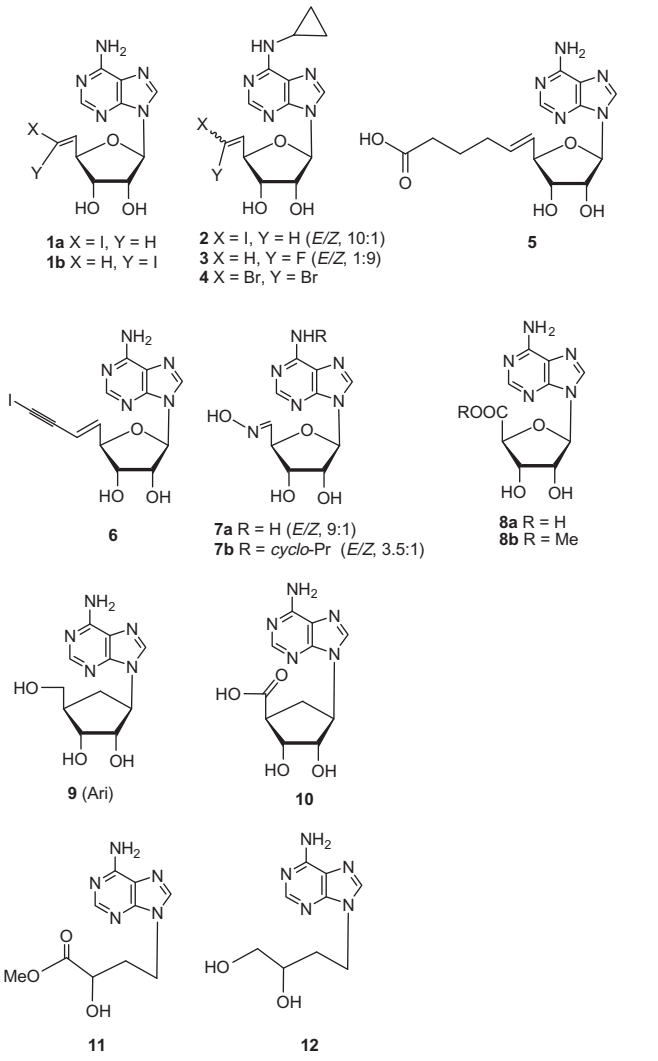

Figure 3. 5′-Modified adenosine and related 6-*N*-cyclopropyl analogues, as well as selected carbocyclic and acyclic adenine derivatives tested against *T. vaginalis*.

Table 1
Inhibitory activity of the 5′-modified adenosine analogues, their 6-*N*-cyclopropyl derivatives and other carbocyclic and acyclic adenine analogues against *T. vaginalis* T1 strain

Compound (100 μM)	% Inhibition (±SEM)
1a	85 (±2)
1b	81 (±3)
2	24 (±12)
3	10 (±10)
4	12 (±10)
5	22 (±15)
6	34 (±16)
7a	93 (±5)
7b	41 (±14)
8a	17 (±4)
8b	4 (±6)
9	95 (±2)
10	36 (±9)
11	16 (±3)
12	11 (±4)

inhibitors are also effective at inhibiting the growth of *T. vaginalis*. This raises the possibility of this enzyme having a significant role in this organism.

Table 2
Calculated IC_{50} values of the most effective compounds and comparison to metronidazole

Compound	IC_{50} value (μM)	IC_{50} value (μg/mL)
Metronidazole	0.72	0.12
1a	60	19
1b	76	24
7a	40	11
9	10	3

These results suggest that AdoHcy hydrolase enzyme plays a role in the growth of *T. vaginalis*. The degree of similarity of this enzyme between *T. brucei* and *T. vaginalis* supports the case for further determination of the role of AdoHcy hydrolase in *T. vaginalis* as a possible route for finding alternative forms of chemotherapy.

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4. *RNA isolation and reverse transcription PCR*: 1 μg of RNA isolated with Trizol reagent (Invitrogen) was added to 1 μl 10× DNase reaction buffer and 1 μl DNase I (ampicillin-grade). The volume was brought up to 10 μl of DEPC water and allowed to incubate for 15 min at room temperature. To the reaction, 1 μl 25 mM EDTA was added and heated to 65 °C for 10 min. After incubation, 1 μl oligo DT (diluted to 1:5) and 1 μl 10 mM RNA-work dNTPS were added. The reaction was set to incubate again at 65 °C for 5 min. After incubation, the reaction was placed on ice for 1 min. After being placed in a microcentrifuge for 1 min at 14,000 rpm, 4 μl 5× first strand buffer, 1 μl 0.1 M DTT, 1 μl RNase out were added. 1 μl superscript was then added to reaction; for a negative control, 1 μl of DEPC water was added. The reaction was mixed and then incubated at 50 °C for 1 h to allow the reaction to be performed. The reaction was inactivated by being incubated at 70 °C for 15 min. Samples of the cDNA were then used as template DNA for PCR, and run on 0.8% agarose gel, and compared to known standards (1 kb ladder, GeneRuler). GAPDH is used as a control.
5. *Inhibition assays*: cultures of the T1 strain of *T. vaginalis* were grown in 10 mL completed TYM Diamond's media in a 37 °C incubator for 24 h. 100 mM stocks of the compounds, dissolved in DMSO, were screened against the T1 and G3 strains of *T. vaginalis*. Cells untreated and inoculated with 10 μL DMSO are used as controls. 10 μL of 100 mM stocks of the compound library were inoculated against the various parasite strains for a final concentration of 100 μM. Results were calculated based off of counts utilized by a hemocytometer after 24 h.
6. The 5′-modified adenosine analogues were prepared as reported: **1a**,[6a] **1b**,[6a] **2**,[3] **3**,[3] **4**,[3] **5**,[6b] **6**,[6c] **7a**,[6d] **7b**,[3] **8a**,[6e] and **8b**.[6e] Aristeromycin 5′-carboxylic acid,[6f] **10**, was synthesized from aristeromycin[6g] **9** by the standard protection of 2′/3′ hydroxyls with the isopropylidene protection group, followed by oxidation of the 5′-hydroxyl group with potassium permanganate[6e] and acid-catalyzed removal of the isopropylidene group. (Fig. 3). Acyclic adenosine derivatives **11**[6h] and **12**[6i] were prepared as reported: (a) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **1994**, *37*, 3579; (b) Wnuk, S. F.; Sacasa, P. R.; Lewandowska, E.; Andrei, D.; Cai, S.; Borchardt, R. T. *Bioorg. Med. Chem.* **2008**, *16*, 5424; (c) Wnuk, S. F.; Lewandowska, E.; Sacasa, P. R.; Crain, L. N.; Zhang, J.; Borchardt, R. T.; De Clercq, E. *J. Med. Chem.* **2004**, *47*, 5251; (d) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem.* **1997**, *40*, 1608; (e) Wnuk, S. F.; Liu, S.; Yuan, C.-S.; Borchardt, R. T.; Robins, M. J. *J. Med. Chem.* **1996**, *39*, 4162; (f) Mahmoudian, M.; Rudd, B. A. M.; Cox, B.; Drake, C. S.; Hall, R. M.; Stead, P.; Dawson, M. J.; Chandler, M.; Livermore, D. G.; Turner, N. J.; Jenkins, G. *Tetrahedron* **1998**, *54*, 8171; (g) Yang, M.; Ye, W.; Schneller, S. W. *J. Org. Chem.* **2004**, *69*, 3993; (h) Zhang, Y.-M.; Ding, Y.; Tang, W.; Luo, W.; Gu, M.; Lu, W.; Tang, J.; Zuo, J.-P.; Nan, F.-J. *Bioorg. Med. Chem.* **2008**, *16*, 9212; (i) Holy, A. *Collect. Czech. Chem. Commun.* **1978**, *43*, 3444.
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