Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3505–3510

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF ADENOSINE ANALOGS AS INHIBITORS OF TRYPANOSOMAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE. MODIFICATIONS AT POSITIONS 5′ AND 8

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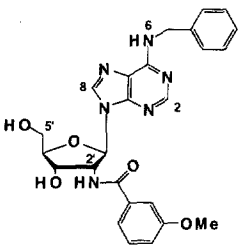
Received 18 August 1998; accepted 22 October 1998

Abstract: A number of 5′, N^6- and C^8, N^6-disubstituted adenosine analogs was synthesized and tested for inhibition of trypanosomal glyceraldehyde 3-phosphate dehydrogenase. The most active compound, N^6-(3-methyl-2-butenyl)-8-(2-thienyl)adenosine, had K_I of 9 μM and was marginally selective for the parasite enzyme.

Sleeping sickness continues to be a major health hazard in a number of areas in subsaharan Africa. Caused by protozoan parasite *Trypanosoma brucei*, it is fatal if left untreated.[1] Existing chemotherapy is unsatisfactory because of poor efficacy, host toxicity, and drug resistance.[2] Other diseases caused by related Trypanosomatidae include leishmaniasis (*Leishmania spp.*) and Chagas disease (*Trypanosoma cruzi*).

The bloodstream form of *Trypanosoma brucei* depends entirely on glycolysis for energy production.[3] Glycosomes, the single-membrane organelles, enclose the first seven glycolytic and two glycerol-metabolizing enzymes, generating 3-phosphoglycerate (3-PGA) from glucose.[4] Inhibiting glycolysis is therefore a paradigm for the development of antiparasitic agents. The work described herein is a continuation of our efforts in the design of antiparasitic glycolysis inhibitors. It is centered around the design of the inhibitors for trypanosomal glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The crystal structures of human and parasitic GAPDHs were shown to have distinct structural differences in the binding pocket for the adenosine moiety of NAD^+.[5-7]



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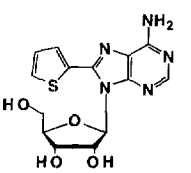
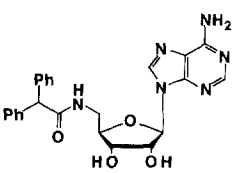


2



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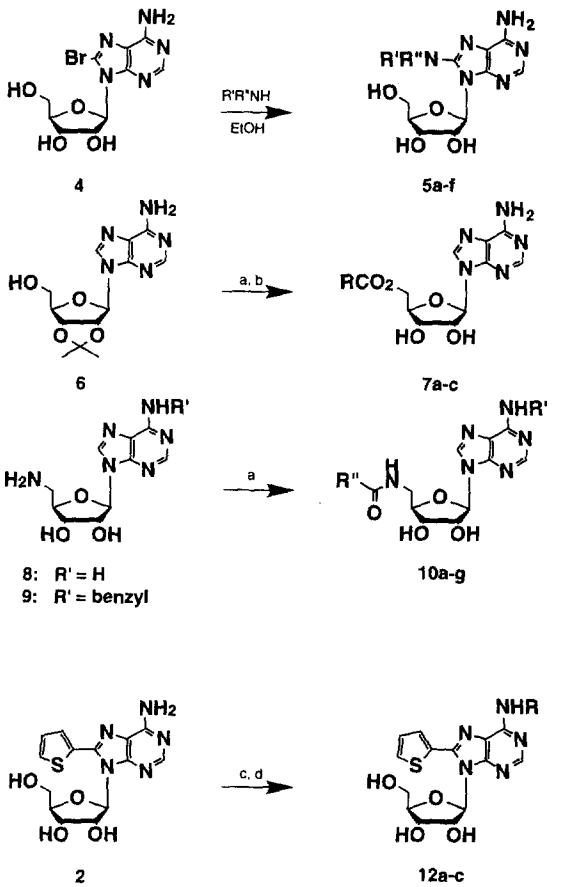
PII: S0960-894X(98)00635-0

Recently, we reported on the structure-based design of adenosine analogs as selective micromolar inhibitors of GAPDH that relied on a combined effect from 2′- and N^6-substituents (i.e., compound **1**).[8] Alternative substitution patterns have been reported earlier as well.[9,10] Among them are 8-substituted adenosine derivatives, where the introduction of a 2-thienyl group (**2**) led to a 100-fold or greater improvement in affinity over adenosine. A hydrophobic 5′-amido substitution (**3**) has also been tried and yielded 200- to 400-fold inhibition enhancement coupled with a mediocre selectivity gain. We decided to further explore the conformational space around these positions, possibly by combining them with the previously applied modifications.

Scheme 1

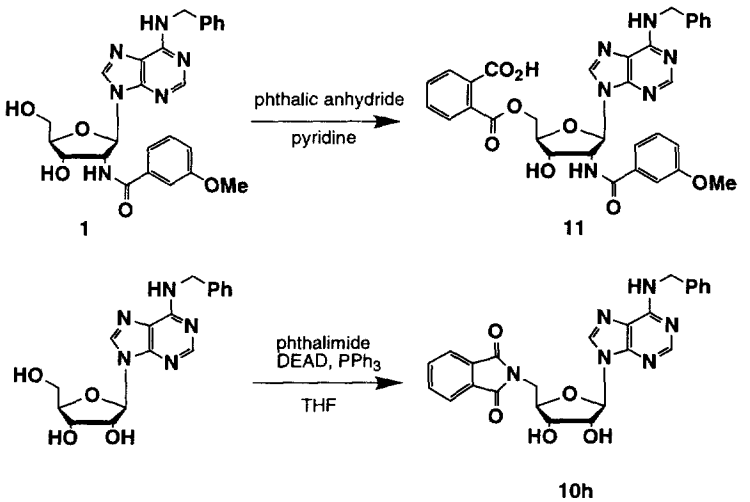


(a) ROCl, pyridine; (b) 10% HCO_2H, reflux; (c) RBr, DMF, 45 °C; then $iPrNH_2$/MeOH (1/3), reflux; (d) NH_4F, MeOH

Chemistry

C^8-aminoalkyl adenosines **5a-f** were prepared from 8-bromoadenosine **4** and the corresponding amines under the conditions described by Chattopadhyaya and Reese[11] in 80-100% yield (Scheme 1). 2′,3′-Isopropylidene adenosine **6** was acylated with acyl chlorides in high yield and subsequently deprotected to furnish 5′-esters **7a-c**.[12] 5′-Amino-5′-deoxyadenosine **8** was prepared as described[13] followed by benzoylation to give **10a**. N^6-Benzyl-5′-amino-5′-deoxyadenosine **9**[14] was acylated to yield **10b-g**.[15] To prepare **10d**, acetyl protection was used for the phenolic groups.[8] Mitsunobu coupling of phthalimide to N^6-benzyladenosine afforded **10h** (Scheme 2).[16] Compound **1** was acylated to furnish **11**. N^6-Benzyl-8-(2-thienyl)adenosine (**12a**), N^6-(2-methylbenzyl)-8-(2-thienyl)adenosine (**12b**), and N^6-(3-methyl-2-butenyl)-8-(2-thienyl)adenosine (**12c**) were prepared according to the earlier published procedure.[8]

Scheme 2



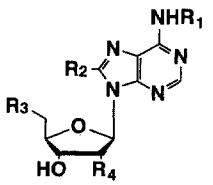
Results and Discussion

Adenosine analogs were tested as inhibitors of *Leishmania mexicana* GAPDH as described.[8] As expected based on the X-ray structure of the NAD^+:*Leishmania mexicana* GAPDH complex,[7] activity of 8-alkylamino modified adenosine analogs appeared to be very sensitive to the size of the substituent (Table 1). Cyclic amine derivatives **5a, b** as well as the benzylamine-containing **5d**, were inactive, possibly due to an increased preference for a *syn* orientation of the base relative to the ribose, while the analogs displaying smaller substituents showed improved potency. Isopropylamino-substituted **5f** was the most active compound in the series, its K_I value almost 20-fold lower than that of adenosine.

Our attempt to partially fill the large enzyme cavity proximal to the 5′-hydroxyl was only marginally successful. A benzoyloxy moiety appeared to potentiate activity by almost an order of magnitude, and the enzyme was insensitive to an ester/amide switch at the 5′ position (**7a**, **10a**). Introduction of 5′-carboxylate substituents was carried out since the cavity is normally occupied by the anionic pyrophosphate linker of NAD^+. However, these compounds, **7c**, **10e**, and **10f** are poor GAPDH inhibitors. Combining a well-tolerated N^6-benzyl group with a number of 5′-amides was expected to contribute to inhibitor potency; the K_I for phthalamide analog **10g** is 4-fold lower than for N^6-benzyladenosine. However, this substitution could not be combined with the N^6, 2′ combination, as the addition of the 5′-phthaloyloxy group onto **1** abrogated inhibitor activity (**11**).

In order to take advantage of the combined effect from binding at both C^8 and N^6 sites, compounds **12a-c** were synthesized. The two groups were introduced orthogonally with respect to each other, flanking the active site Leu 113 (*Leishmania mexicana* GAPDH). While the combination of two aromatic substituents in compounds **12a, b** resulted in loss of activity, most likely due to steric clashes with the Leu side chain, the introduction of a smaller 3-methyl-2-butenyl group at the N^6 position in **12c** appeared to satisfy the spacial requirements in that region of the active site (Fig. 1), resulting in 30-fold enhancement of inhibitor potency

Table 1. Inhibition of *Leishmania mexicana* GAPDH by Adenosine Analogs

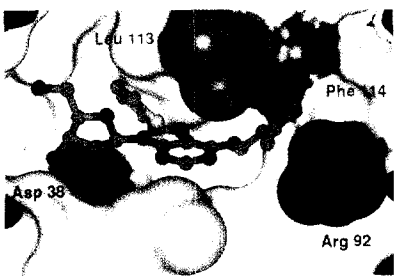


compd	R_1	R_2	R_3	R_4	K_i, mM
adenosine	H	H	OH	OH	25
N^6-benzyl adenosine	$PhCH_2$	H	OH	OH	2.8
5a	H	N-piperidinyl	OH	OH	>6.5 (100%)[a]
5b	H	N-pyrrolidinyl	OH	OH	>6.5 (100%)
5c	H	$(CH_3)_2N$	OH	OH	>6.5 (75%)
5d	H	$PhCH_2NH$	OH	OH	>6.5 (100%)
5e	H	$HOCH_2CH_2NH$	OH	OH	>6.5 (80%)
5f	H	$(CH_3)_2CHNH$	OH	OH	1.5
7a	H	H	benzoyloxy	OH	>4 (67%)
7b	H	H	β-naphthoyloxy	OH	>0.43 (100%)
7c	H	H	succinyloxy	OH	>2.6 (100%)
10a	H	H	benzamido	OH	3.3
10b	$PhCH_2$	H	phenylacetamido	OH	>0.45 (77%)
10c	$PhCH_2$	H	cyclohexylacetamido	OH	>0.24 (77%)
10d	$PhCH_2$	H	3,5-dihydroxybenzamido	OH	>0.26 (100%)
10e	$PhCH_2$	H	succinamido	OH	>0.97 (74%)
10f	$PhCH_2$	H	glutaramido	OH	>1 (79%)
10g	$PhCH_2$	H	phthalamido	OH	0.8
10h	$PhCH_2$	H	phthalimido	OH	4
11	$PhCH_2$	H	phthaloyloxy	MMBA[b]	0.59 (100%)
8-(2-thienyl) adenosine (2)	H	2-thienyl	OH	OH	0.3
N^6-(3-methyl-2-butenyl-adenosine	$(CH_3)_2C{=}CHCH_2$	H	OH	OH	>5.0 (84%)
12a	$PhCH_2$	2-thienyl	OH	OH	>0.1 (100%)
12b	$(2\text{-Me})C_6H_4CH_2$	2-thienyl	OH	OH	>0.1 (100%)
12c	$(CH_3)_2C{=}CHCH_2$	2-thienyl	OH	OH	0.009

[a] remaining enzyme activity at stated inhibitor concentration; [b] MMBA = (3-methoxy)-benzamido.

compared to **2**. The improvement over previously characterized N^6-(3-methyl-2-butenyl)adenosine[8] is a dramatic 2,000-fold. When tested against human GAPDH, compound **12c** exhibited a K_I of 18 μM, only two-fold higher than the K_I with *Leishmania mexicana* GAPDH. However, at the same NAD^+ concentration of 0.19 mM,[8] **12c** was over 10-fold selective for the parasitic enzyme versus the human counterpart. This can be explained by the difference in K_M values for NAD^+ (0.4 mM for *Leishmania mexicana* GAPDH versus 0.04 mM for human erythrocyte GAPDH).

Figure 1. Molecular modeling of inhibitor **12c** with *Leishmania mexicana* GAPDH.[17]



In summary, a series of adenosine analogs has been generated and tested for inhibition of *Leishmania mexicana* GAPDH. The most potent compound **12c** inhibits the enzyme at low micromolar concentrations, although the gain in selectivity over the human isozyme is modest. Further attempts of using a structure-based approach to designing adenosine analogs as anti-trypanosomatid agents are in progress.

Characterization data (active compounds only)

8-dimethylaminoadenosine (5c). ^{1}H NMR (MeOH-d_4) δ 2.7, 3.0 (2s, 6, 2 CH_3), 3.69-3.86 (m, 2, H5′, 5″), 4.12 (m, 1, H4′), 4.33 (dd, 1, H3′), 5.14 (dd, 1, H2′), 5.89 (d, 1, H1′), 8.01 (s, 1, H2).

8-(N-ethanolamino)adenosine (5e). ^{1}H NMR (MeOH-d_4) δ 3.18-3.40 (m, 4, 2 CH_2), 3.7-3.9 (m, 2, H5′, 5″), 4.18 (m, 1, H4′), 4.33 (dd, 1, H3′), 4.80 (dd, 1, H2′), 6.06 (d, 1, H1′), 7.98 (s, 1, H2).

8-isopropylaminoadenosine (5f). ^{1}H NMR (MeOH-d_4) δ 1.28-1.32 (dd, 6, 2 CH_3), 3.84 (m, 2, H5″, CH), 4.15 (m, 2, H4′,5′), 4.28 (dd, 1, H3′), 4.67 (dd, 1, H2′), 6.19 (d, 1, H1′), 8.19 (s, 1, H2).

5′-Benzoyl adenosine (7a). ^{1}H NMR (acetone-d_6) δ 4.40 (m, 1, H4′), 4.55-4.72 (m, 2, H5′, 5″), 4.75 (m, 1, H3′), 5.01 (m, 1, H2′), 6.10 (d, 1, H1′), 6.68 (br s, 2, NH_2), 7.50 (m, 2, H_m), 7.62 (m, 1, H_p), 8.03 (m, 3, H_o and H2), 8.19 (s, 1, H8).

5′-Benzamido-5′-deoxyadenosine (10a). ^{1}H NMR (acetone-d_6) δ 3.55 (m, 1, H5″), 4.06 (m, 1, H5′), 4.30 (m, 1, H4′), 4.41 (m, 1, H3′), 4.93 (m, 1, H2′), 5.91 (d, 1, H1′), 6.70 (br s, 2, NH_2), 7.46 (m, 2, 2 H_m), 7.52 (m, 1, H_p), 7.90 (m, 2, H_o), 7.96 (s, 1, H2), 8.18 (s, 1, H8).

N^6-Benzyl-5′-phenylacetamido-5′-deoxyadenosine (10b). ^{1}H NMR (DMSO-d_6) δ 3.38-3.50 (m, 4, H5′, 5″, CH_2), 3.96 (m, 1, H4′), 4.06 (m, 1, H3′), 4.70 (br s, 3, H2′, CH_2), 5.25 (d, 1, 3′-OH), 5.43 (d, 1, 2′-OH), 5.88 (d, 1, H1′), 7.15-7.35 (m, 10, aromatic protons), 8.22 (s, 1, H2), 8.35 (s, 1, H8).

N^6-Benzyl-5′-cyclohexylacetamido-5′-deoxyadenosine (10c). ^{1}H NMR (DMSO-d_6) δ 0.9-1.6 (m, 11, cyclohexyl), 1.95 (dd, 2, CH_2), 3.38-3.50 (m, 2, H5′, 5″), 3.94 (m, 1, H4′), 4.02 (m, 1, H3′), 4.68 (br s, 3, H2′, CH_2), 5.22 (d, 1, 3′-OH), 5.42 (d, 1, 2′-OH), 5.82 (d, 1, H1′), 7.15-7.35 (m, 5, benzyl), 8.11 (s, 1, H2), 8.33 (s, 1, H8).

N^6-Benzyl-5′-succinamido-5′-deoxyadenosine (10e). ^{1}H NMR (MeOH-d_4) δ 2.55 (m, 4, 2 CH_2), 3.35 (dd, 1, H5″), 3.81 (dd, 1, H5′), 4.18 (m, 2, H3′, 4′), 4.8-4.9 (m, 3, H2′, CH_2), 5.90 (d, 1, H1′), 7.2-7.4 (m, 5, benzyl), 8.22 (s, 1, H2), 8.32 (s, 1, H8).

N^6-Benzyl-5′-glutaramido-5′-deoxyadenosine (10f). ^{1}H NMR (MeOH-d_4) δ 1.90 (quintet, 1, β- CH_2), 2.32 (m, 4, 2 α-CH_2), 3.40 (dd, 1, H5″), 3.80 (dd, 1, H5′), 4.18 (m, 2, H3′, 4′), 4.80 (m, 3, H2′, CH_2), 5.90 (d, 1, H1′), 7.2-7.4 (m, 5, benzyl), 8.20 (s, 1, H2), 8.31 (s, 1, H8).

N^6-Benzyl-5′-phthalamido-5′-deoxyadenosine (10g). ^{1}H NMR (MeOH-d_4) δ 3.42 (dd, 1, H5″), 4.18 (dd, 1, H5′), 4.32 (m, 1, H4′), 4.42 (m, 1, H3′), 4.70 (br s, 2, CH_2), 4.90 (dd, 1, H2′), 5.84 (d, 1, H1′), 7.20-7.35 (m, 5, benzyl), 7.45-7.60 (m, 4, phthalamide), 7.92 (s, 1, H2), 8.15 (s, 1, H8).

N^6-Benzyl-5′-phthalimido-5′-deoxyadenosine (10h). ^{1}H NMR (DMSO-d_6) δ 3.83 (dd, 1, H5″), 3.95 (dd, 1, H5′), 4.11 (m, 1, H4′), 4.25 (dd, 1, H3′), 4.68 (br s, 2, CH_2), 4.76 (dd, 1, H2′), 5.29 (d, 1, 3′-OH), 5.48 (d, 1, 2′-OH), 5.84 (d, 1, H1′), 7.15-7.35 (m, 5, benzyl), 7.82 (m, 4, phthalimide), 7.97 (s, 1, H2), 8.36 (s, 1, H8).

N^6-(3-methyl-2-butenyl)-8-(2-thienyl)adenosine (12c). Yield 0.4 mg (10%). ^{1}H NMR (MeOH-d_4) δ 1.7 (br s, 6, 2 δ-CH_3), 3.78 (m, 1, H5′), 3.95 (m, 1, H5″), 4.25 (m, 1, H4′), 4.35 (m, 1, H3′), 4.25 and 4.45 (m, 2, α- CH_2), 5.22 (m, 1, H2′), 5.30 (t, 1, β-CH) 6.28 (d, 1, H1′), 7.30 (t, 1, H4″), 7.84 (d, 2, H3″, 5″), 8.27 (s, 1, H2).

Acknowledgements A.A. is the recipient of a Dow Chemical Fellowship Award. We wish to thank Prof. Wim Hol and Dr. Christophe Verlinde for valuable comments.

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15. Standard acylation conditions with an acyl chloride/pyridine mixture were used.
16. Procedure from ref. 13 was followed for N^6-benzyladenosine without significant changes, and the product **10h** was obtained in >95% yield.
17. Qualitative docking of compound **12c** was done using Insight II software (Molecular Simulations, Inc., San Diego, CA).