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Discovery of new *S*-adenosylmethionine decarboxylase inhibitors for the treatment of Human African Trypanosomiasis (HAT)

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ABSTRACT

Modification of the structure of trypanosomal AdoMetDC inhibitor **1** (MDL73811) resulted in the identification of a new inhibitor **7a**, which features a methyl substituent at the 8-position. Compound **7a** exhibits improved potencies against both the trypanosomal AdoMetDC enzyme and parasites, and better blood brain barrier penetration than **1**.

Sleeping sickness, or Human African Trypanosomiasis (HAT) is caused by the protozoan pathogen *Trypanosoma brucei*, which infects between 50,000 and 150,000 people each year across sub-Saharan Africa, and is fatal if left untreated.[1] Current drugs such as Melarsoprol or Eflornithine have either significant toxicity or highly impractical dosing regimens for rural Africa due to the requirement of extended intravenous infusion. New therapies are urgently needed, yet because of the extreme poverty in countries with endemic disease, for many years there has been little interest within the pharmaceutical industry in discovering and developing new drugs for this indication.

Polyamines, such as spermine and spermidine, are small cationic molecules that are critical to the survival of eukaryotes, including trypanosomes.[2,3] It has been postulated that the rate limiting step for the biosynthesis of polyamines is catalyzed by *S*-adenosylmethionine decarboxylase (AdoMetDC), which converts *S*-adenosylmethionine (AdoMet) to decarboxylated *S*-adenosylmethionine (dcAdoMet).[4,5] It has been reported that the *T. brucei* AdoMetDC differs substantially from the human host enzyme, and it is allosterically activated by heterodimer formation with an inactive paralog, termed prozyme.[6,7] This regulatory mechanism is found only in the trypanosomatids. In 1989, Casara et al. reported the discovery of a mechanism based inhibitor **1** (MDL73811) (Fig. 1), which potently inhibits AdoMetDC in vitro and notably improves the survival of *T. brucei* infected mice.[8,9] However, the pharmacokinetic and tissue distribution characteristics of compound **1**, especially its apparent low penetration of the blood brain barrier, are not adequate to meet the improved target product profile for a new anti-trypanosomal drug.

We undertook a medicinal chemistry effort to modify the structure of **1** with the goal of improving the potency and pharmacokinetic characteristics of the compound, especially the ability to cross the blood brain barrier. Because it was reported that the

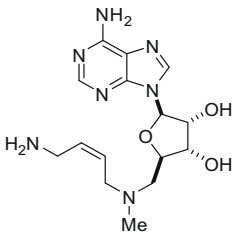

Figure 1. 1 (MDL73811).

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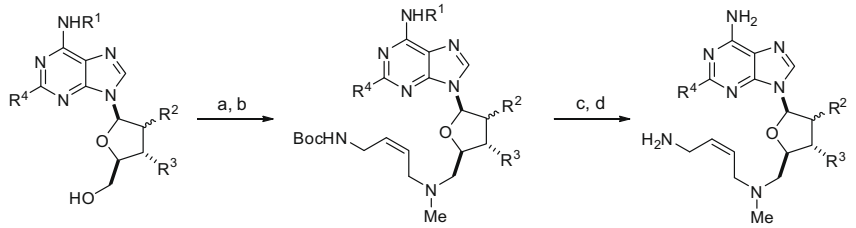
N-(Z)-4-aminobutenyl)-*N*-methyl group at the 5′-position is critical to the mechanism of AdoMetDC inhibition by forming a Schiff base with the pyruvoyl prosthetic group at the active site,[6] our efforts were mostly focused on the modification of the ribose and purine moieties. We reasoned that in order to increase penetration of the blood brain barrier, it would be critical to improve compound stability and increase exposure levels in the systemic circulation. To achieve this, substitution with a halogen, especially a fluorine atom, at the 2′-position of the ribose would be a reasonable measure to take, as such a change should have positive impact on the stability of the glycosidic bond. Other considerations include modifying the substitution pattern on the purine base of compound **1**. In 2001, Steven Ealick et al. reported an interesting discovery in their study of purine nucleoside inhibitors to human AdoMet DC, in which they revealed that such compounds adopt an unusual *syn*-conformation to bind to the enzyme.[10] Considering the similarity of human and trypanosomal AdoMetDCs, it is reasonable to assume that the inhibitor **1** might adopt the *syn*-conformation in the binding site too. Changes of the purine substitution pattern might not only decrease the susceptibility of the compounds to purine deaminase, but also alter their conformational preference. Particularly by adding a substituent at the 8-position, the energy penalty for adopting the *syn*-conformation could be reduced.

Here we report the discovery of a new compound **7a,** which features a methyl group at the 8-position of the purine. Compound **7a** demonstrates improved potency against AdoMetDC and is more effective in *T. brucei rhodeesiense* and *T. brucei brucei* growth inhibition assays than **1**. More interestingly, compound **7a** has a significantly improved ability to penetrate the blood brain barrier as demonstrated by its presence at higher concentrations in the mice brain than compound **1** following parenteral injection.

The synthetic methods used to construct derivatives of **1** with the desired changes on either the ribose or purine moieties are highlighted in Schemes 1 and 2. In Scheme 1, the purine nucleoside **2 (a–c)** was treated with either mesyl chloride or tosyl chloride in pyridine at 0 °C. The resulting 5′-mesyl or tosyl nucleosides underwent a displacement by (*Z*)-*tert*-butyl 4-(methylamino)but-2-enylcarbamate[11] in DMSO in the presence of triethyl amine to form compound **3** (**a**–**c**), which, in **3a** and **3b**, was subjected to deprotection of the N^6-benzoyl group using ammonia in methanol. Final deprotection of the Boc group with HCl in ethyl acetate produced compounds **4** (**a**–**c**). In Scheme 2, the starting nucleoside **5** (**a**–**d**) was reacted with thionyl chloride and pyridine in acetonitrile at 0 °C, followed by treatment with ammonium hydroxide in methanol.[12] The resulting 5′-chloro-adenosine derivatives were reacted with methylamine in ethanol at elevated temperature to produce **6** (**a**–**d**). N-alkylation of **6** (**a**–**d**) with (*Z*)-*tert*-butyl 4-chlorobut-2-enylcarbamate in the presence of potassium carbonate and a catalytic amount of sodium iodide in DMSO, followed by deprotection of the Boc group with HCl in either methanol or dioxane, gave the desired compound **7** (**a**–**d**).[8]

The preparation of some commercially unavailable starting nucleosides **5** (**b**–**d**) is illustrated in Schemes 3–5. 8-Ethyl-adenosine (**5b)** was prepared by a sequence highlighted in Scheme 3: 8-bromo-adenosine (**9**) was treated with hexamethyldisilazane (HMDS) in the presence of ammonium sulfate in dioxane, followed by reaction with triethylaluminum in the presence of palladium-

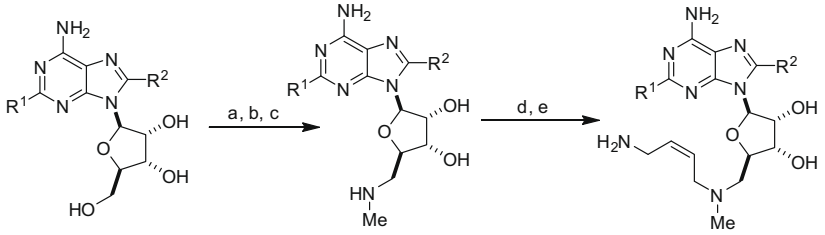


	R^1	R^2	R^3	R^4
2a[18]	Bz	α-F	OH	H
2b[19]	Bz	β-F	OH	H
2c[20]	H	R^2, R^3:α-acetonide		Cl

	R^1	R^2	R^3	R^4
3a	Bz	α-F	OH	H
3b	Bz	β-F	OH	H
3c	H	R^2, R^3:α-acetonide		Cl

	R^2	R^3	R^4
4a	α-F	OH	H
4b	β-F	OH	H
4c	α-OH	OH	Cl

Scheme 1. Reagents and conditions: (a) MsCl (or TsCl), pyridine, 0 °C; (b) (*Z*)-*tert*-butyl 4-(methylamino)but-2-enylcarbamate, Et_3N, DMSO, 65 °C (31–42%, two steps); (c, only for **3a**–**b**) NH_3, CH_3OH (65–95%); (d) HCl, EtOAc (35–53%). (see above-mentioned references for further information.)

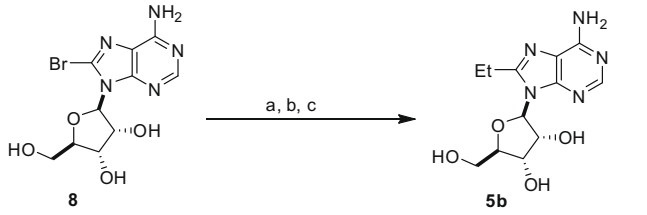


	R^1	R^2
5a[21]	H	Me
5b	H	Et
5c	H	n-Pr
5d	Me	H

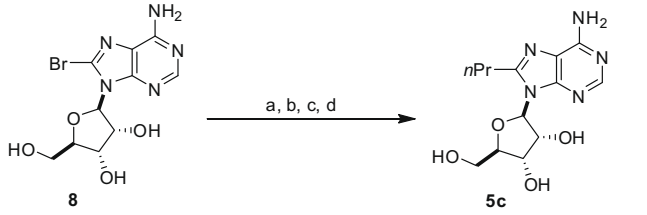
	R^1	R^2
6a	H	Me
6b	H	Et
6c	H	n-Pr
6d	Me	H

	R^1	R^2
7a	H	Me
7b	H	Et
7c	H	n-Pr
7d	Me	H

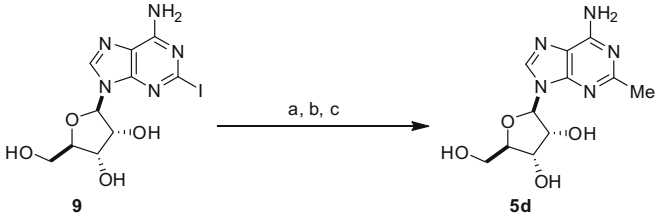
Scheme 2. Reagents and conditions: (a) $SOCl_2$, pyridine, ACN, 0 °C; (b) NH_4OH, CH_3OH/H_20 (35–75%, two steps); (c) CH_3NH_2, EtOH, 120 °C (50–55%); (d) (*Z*)-*tert*-butyl 4-chlorobut-2-enylcarbamate, NaI, K_2C0_3, DMSO, 65 °C; (e) HCl, CH_3OH or dioxane (38–65%, two steps). (see above-mentioned references for further information.)



Scheme 3. Reagents and conditions: (a) HMDS, $(NH_4)_2S0_4$; (b) Et_3Al, $Pd(PPh_3)_4$, THF, 70 °C; (c) NH_4Cl, $MeOH/H_20$ (74%, three steps).



Scheme 4. Reagents and conditions: (a) HMDS, $(NH_4)_2S0_4$; (b) allyltributyl tin, $Pd(PPh_3)_4$, NMP, μwave at 190 °C; (c) NH_4Cl, $MeOH/H_20$ (48%, three steps); (d) Pd/C, cat. TFA, $EtOH/H_2O$.



Scheme 5. Reagents and conditions: (a) HMDS, $(NH_4)_2S0_4$; (b) 2.0 equiv $AlMe_3$, $Pd(PPh_3)_4$, THF, 70 °C; (c) NH_4Cl, $MeOH/H_20$ (57%, three steps).

triphenylphosphine in THF at 70 °C. Cleavage of all TMS groups with ammonium chloride in methanol produced **5b**.[13] 8-Propyl-adenosine (**5c**) was synthesized from 8-bromo-adenosine (**9**) by the sequence highlighted in Scheme 4: (1) treatment with HMDS in dioxane in the presence of ammonium sulfate; (2) palladium triphenylphosphine mediated coupling with allyltributyl tin in NMP (*N*-methylpyrrolidinone);[14] (3) deprotection of TMS ethers with ammonium chloride and (4) hydrogenation using Pd on carbon in the presence of TFA in ethanol/water. 2-Methyl-adenosine (**5d**) was prepared from 2-iodo-adenosine (**9**) using the sequence highlighted in Scheme 5: (1) treatment with HMDS in the presence of ammonium sulfate; (2) reaction with excess of trimethylaluminum in the presence of palladium triphenylphosphine, and (3) deprotection with ammonium chloride.

Table 1
Activity against purified Tryp

Compound No.	k_{inact}/K_iapp[a] against Tryp *AdoMedDC/prozyme* (M^{-1} min^{-1})	*T. b. r.*[b] IC_{50}[d] (μM)	*T. b. b*[c] IC_{50}[d] (μM)
1 (MDL73811)	1.50	0.011	0.21
4a	0.19	1.4	>14
4b	0.19	2.6	>14
4c	0.02	1.7	7.27
7a	7.78	0.001	0.027
7b	ND	ND	0.90
7c	ND	ND	11.05
7d	ND	ND	>14

AdoMetDC and trypanosome parasites.
[a] Expression of kinetic enzyme inhibition. k_{inact}: first order of rate constant of inactivation of the enzyme; $K_i$app: determined by the equation of $k_{obs} = k_{inact}[I]/(K_i app + [I])$.[10]
[b] *T. brucei rhodeesiense.*
[c] *T. brucei brucei.*
[d] Compound concentration required to reduce parasite viability by 50%.[22]

Table 1 summarizes the inhibitory activities of these new 5′-(4-aminobutenyl-*N*-methylamino)-adenosine derivatives against the purified AdoMetDC enzyme and in growth inhibition assays with two trypanosomal strains, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei brucei.* Enzyme inhibition is expressed in k_{inac}/K_iapp, a method which measures kinetic inhibition of an enzyme, as first reported by Kitz and Wilson.[15] It is interesting to note that, although most changes on either the base or sugar moieties of the nucleoside resulted in decrease of activity, the addition of a methyl group at the C8-position of the purine (**7a**) significantly increased potency in both the AdoMetDC and parasite growth inhibition assays relative to **1**. A similar observation has been reported by Steven Ealick et al. in their recent study of human AdoMetDC inhibition.[16] A possible explanation has been postulated in the paper that a small substituent at the 8-position of purine results in favouring *syn*-conformation, and therefore increases the binding affinity for the active site of AdoMetDC.

We have also evaluated several compounds in mice plasma PK and assessed their ability to penetrate the blood brain barrier following parenteral administration. As indicated in Table 2, **7a** has a prolonged $t_{1/2}$ in circulating blood in comparison to **1**. More interestingly, it also exhibits increased blood brain penetration relative to compound **1** as evidenced by its higher brain C_{max} and AUC. At the present time, the reason for the improvements is not clear, though it is possible that the increased hydrophobicity of compound **7a** versus **1**[17], as well as its possibly altered conformational preference could be factors. Compound **7a** has subsequently been evaluated in vivo in *T. b. brucei* infected mice, and demonstrated greater efficacy than **1** in the reduction of parasitemia. A separate manuscript describing studies in the disease model has been submitted for publication (C. Bacchi, personal communication).

Table 2
PK parameters and brain penetration of **1** and **7a** following a single dose i.p administration in mice

Compound	AUC[a] in blood[b] (μg * h/mL)	$t_{1/2}$ (h)	AUC in brain (μg * h/mL)	AUC_{brain}/AUC_{blood}[c] (%)	C_{max}[d] in blood (μg/mL)	C_{max} in brain (μg/g)
1 (MDL 73811)	20.69	2.48	0.36	1.70	44.80	0.08
7a	17.92	7.43	1.30	7.28	36.90	0.66

[a] AUC: area under the curve.
[b] μg * hr/mL: microgram times hours/mL.
[c] AUC_{brain}/AUC_{blood} (%): area under the curve for brain divided by area under the curve for blood times 100.
[d] C_{max}: maximum concentration.

In conclusion, we have discovered a new trypanosomal AdoMetDC inhibitor **7a**, which has a methyl group at the 8-position of the purine. This substitution makes the compound **7a** a more potent inhibitor of AdoMetDC, and also increases its ability to penetrate the blood brain barrier penetration in mice. This discovery may indicate a new direction for further improvement of this class compounds as antitrypanosomal agents in the future.

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