SUPPORTING INFORMATION

Comparative study of adenosine analogs as inhibitors of protein arginine methyltransferases and a *Clostridioides difficile*-specific DNA adenine methyltransferase

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Table S1. Summary of X-ray data collection and refinement statistics* at SERCAT beamline 22ID, λ=1.0000 Å

Compound	**11a** (YD905)	**11b** (YD907)
PDB Code	8FS1	8FS2
Date Collected	2021-12-05	2021-12-05
Space group	$P2_12_12_1$	
Cell dimensions (Å)	80.91, 160.73, 229.62	81.26, 161.25, 229.60
α, β, γ (°)	90, 90, 90	
Resolution (Å)	44.95-2.74 (2.84-2.74)	44.16-2.59 (2.68-2.59)
[a] R_{merge}	0.415 (2.497)	0.270 (2.329)
R_{pim}	0.109 (0.776)	0.072 (0.722)
$CC_{1/2}$	0.984 (0.417)	0.995 (0.422)
[b] <I/σI>	9.9 (1.1)	10.2 (0.9)
Completeness (%)	98.9 (95.4)	98.0 (87.7)
Redundancy	13.7 (9.4)	12.9 (7.9)
Observed reflections	1,084,680	1,202,139
Unique reflections	79,171 (7780)	93,121 (8376)
Wilson B-factor (Å^2)	48.3	53.5
Refinement		
Resolution (Å)	2.74	2.59
No. reflections	78,515	92,780
[c] R_{work} / [d] R_{free}	0.172 / 0.214	0.173 / 0.207
No. Atoms		
Protein	13,309	13,318
DNA	1704	1704
Inhibitor	123	126
Solvent	517	513
B Factors (Å^2)		
Protein	54.2	61.7
DNA	61.3	71.0
Inhibitor	68.4	83.0
Solvent	43.5	51.4
R.m.s. deviations		
Bond lengths (Å)	0.006	0.008
Bond angles (°)	0.84	0.83

* Values in parenthesis correspond to highest resolution shell;

[a] $R_{merge}=\Sigma|I-<I>|/\Sigma I$, where I is the observed intensity and <I> is the averaged intensity from multiple observations; [b] <I/σI> = averaged ratio of the intensity (I) to the error of the intensity (σI);

[c] $R_{work}=\Sigma|Fobs-Fcal|/\Sigma|Fobs|$, where Fobs and Fcal are the observed and calculated structure factors, respectively; [d] R_{free} was calculated using a randomly chosen subset (5%) of the reflections not used in refinement.

Table S2. Inhibition reaction conditions at [I]=10 μM

Enzyme	Concentrations	t (min)	Buffer	Substrate (target A in red and underlined)
CamA	[E]=0.1 μM [SAM]=40 μM [S]=5 μM	1.5	50 mM Tris–HCl pH 7.5 100 mM NaCl 1 mM DTT 0.25% DMSO	5′-CGATTCAAAA**A**GTCCCAAG-3′ 3′-GCTAAGTTTTTCAGGGTTC-5′
CcrM		5		5′-CG**A**TTCAAAAAGTCCCAAG-3′ 3′-GCTA**A**GTTTTTCAGGGTTC-5′
DAM		1.5		5′-CCGCGG**A**TCCTGCT-3′ 3′-GGCGCCT**A**GGACGA-5′
MettL5-Trm112		60		5′-UCGUA**A**CAAGGUUU-3′
PCIF1		15		m7GpppA$_m$G (A_m=2′O-methyladenosine)
MettL3-MettL14	[E]=0.1 μM [SAM]=40 μM [S]=10 μM	10	50 mM HEPES pH 7.5 5 mM NaCl 1 mM DTT 0.25% DMSO	5′-AACAGAAUGGG**A**CUGUUC-3′
MettL16	[E]=0.4 μM [SAM]=160 μM [S]=5 μM	30	20 mM Tris–HCl pH 8.0 400 mM NaCl 1 mM DTT 0.25% DMSO	5′-GGUUGGCGUAGGCUAC**A**GAGAAGCCAACC-3′

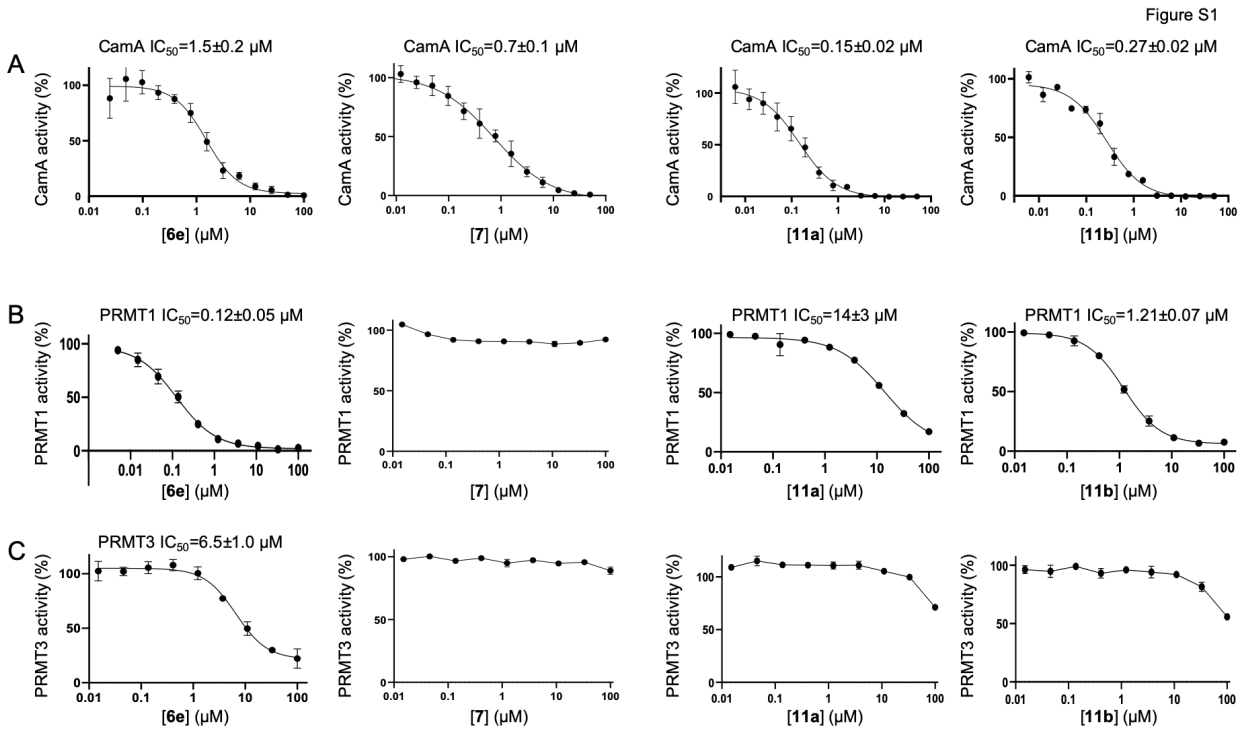
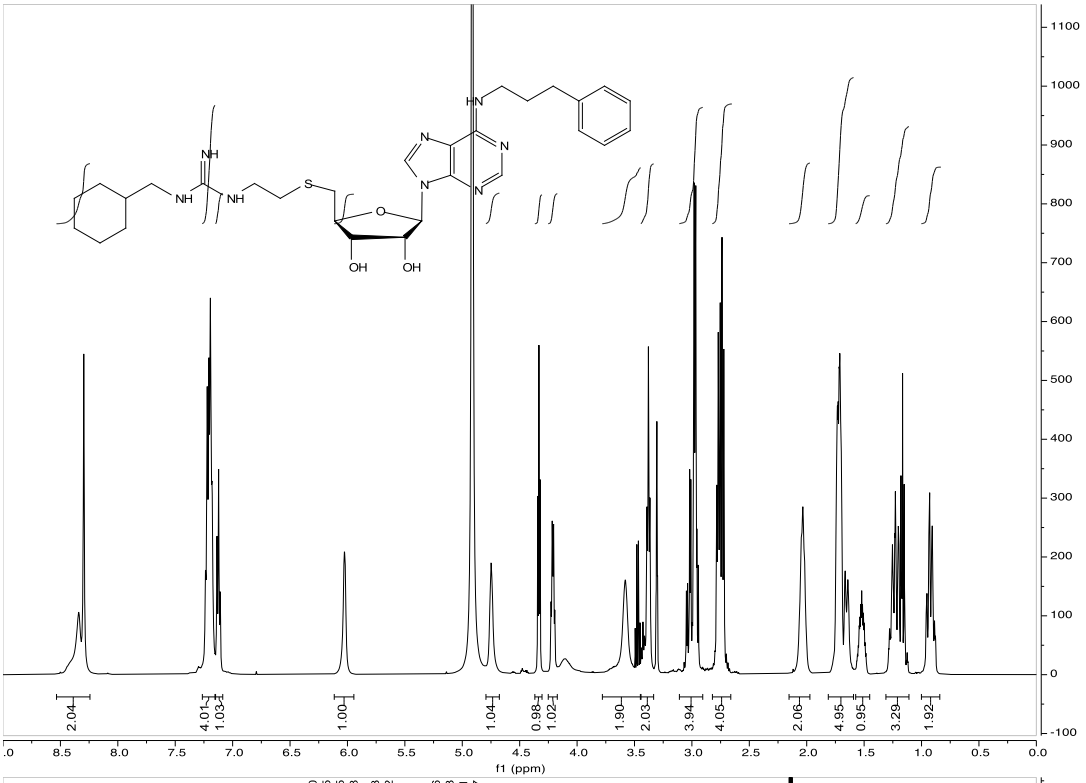


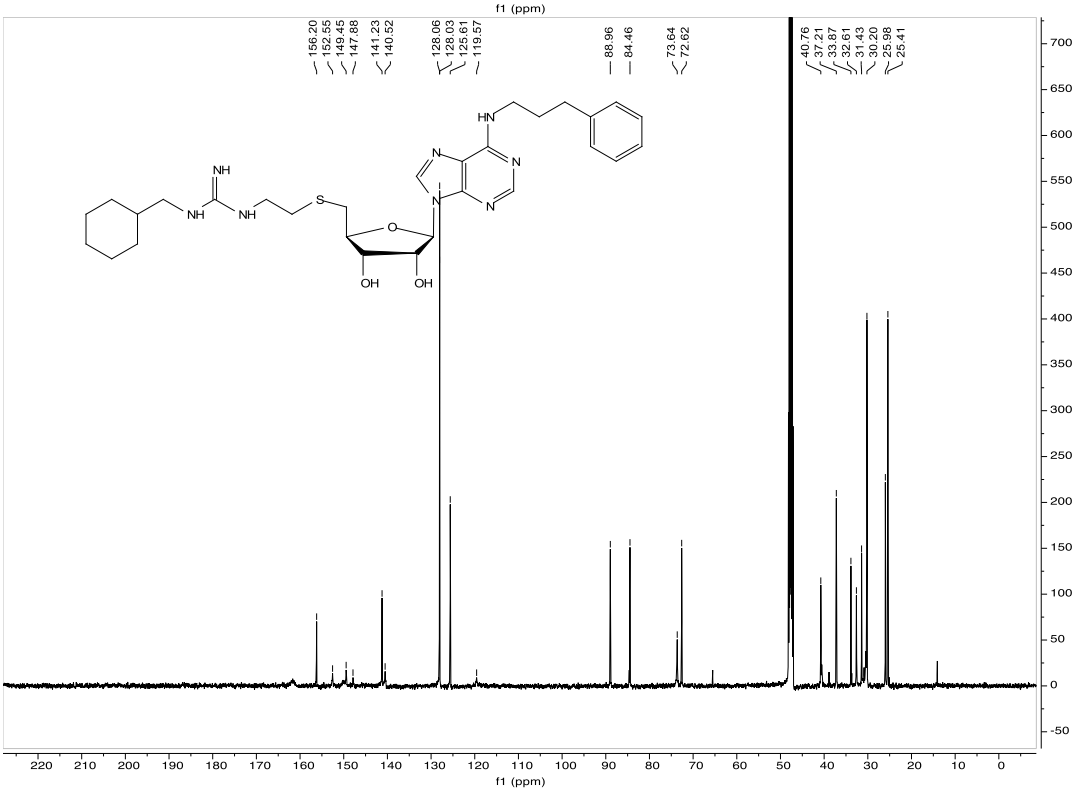
Figure S1. Related to Figure 2C, raw data plots of IC_{50} values for the four compounds (from left to right) **6e**, **7**, **11a**, and **11b** against (**A**) CamA, (**B**) PRMT1, and (**C**) PRMT3. Note the compound **7** for CamA is taken from reference [1].

Reference

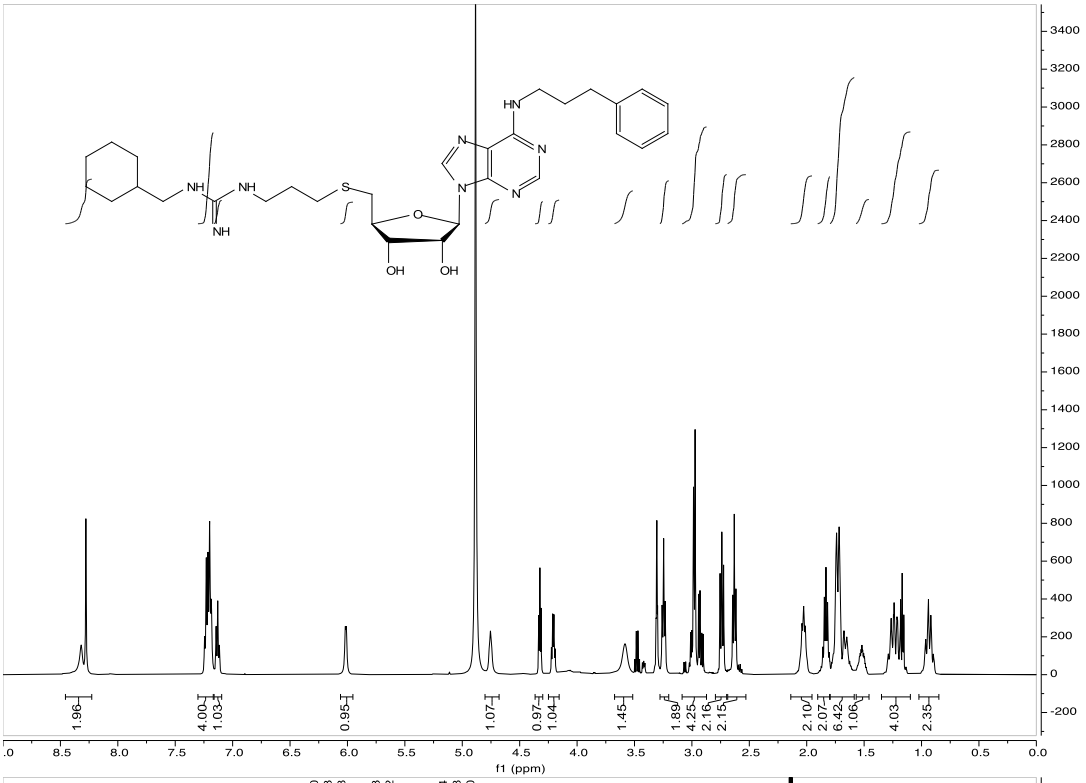
1. Zhou, J.; Horton, J. R.; Menna, M.; Fiorentino, F.; Ren, R.; Yu, D.; Hajian, T.; Vedadi, M.; Mazzoccanti, G.; Ciogli, A.; Weinhold, E.; Huben, M.; Blumenthal, R. M.; Zhang, X.; Mai, A.; Rotili, D.; Cheng, X., Systematic Design of Adenosine Analogs as Inhibitors of a Clostridioides difficile-Specific DNA Adenine Methyltransferase Required for Normal Sporulation and Persistence. *J Med Chem* **2023,** *66* (1), 934-950.

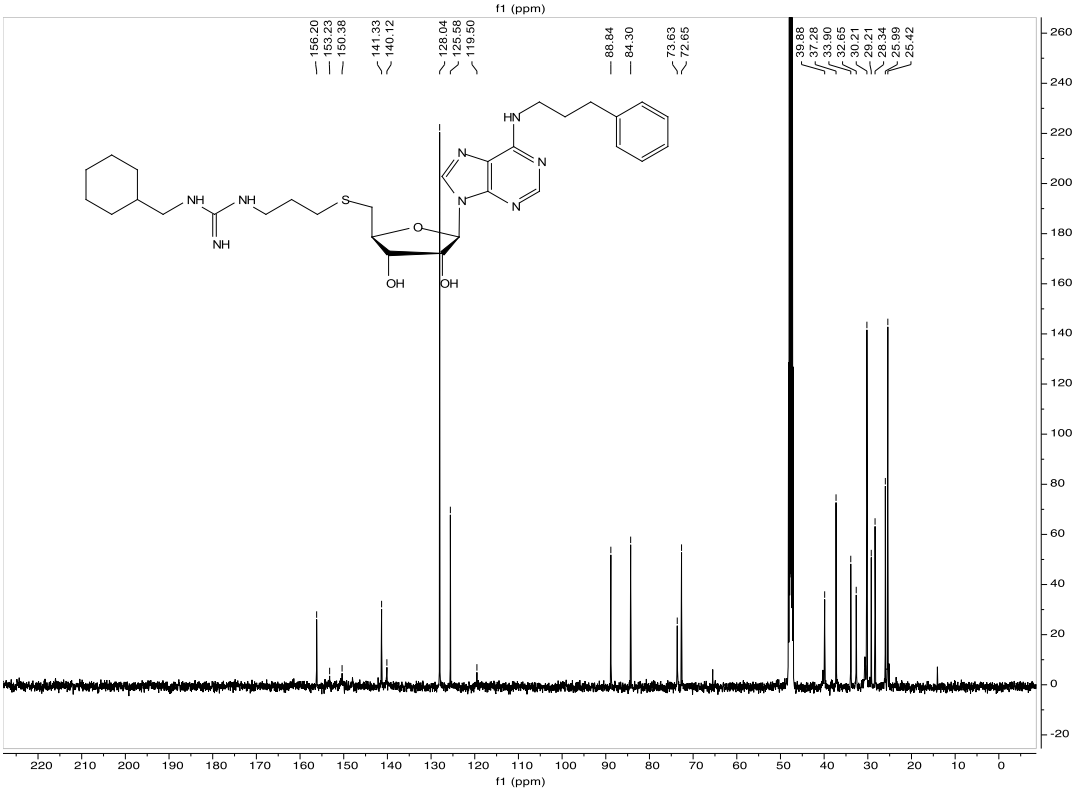
NMR spectra of compound 11a (YD9-05)



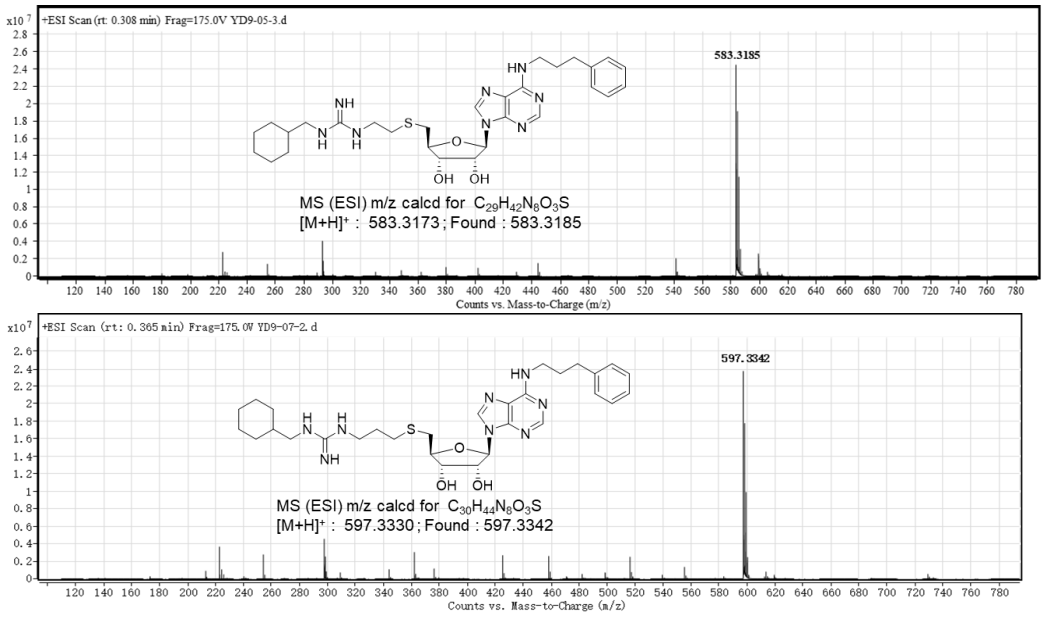


NMR spectra of compound 11b (YD9-07)





HRMS spectra of compounds (11a and 11b)



Purity of compounds 11a (YD9-05) and 11b (YD9-07) (>95%)