Transition state analogues of 5'-methylthioadenosine nucleosidase disrupt quorum sensing

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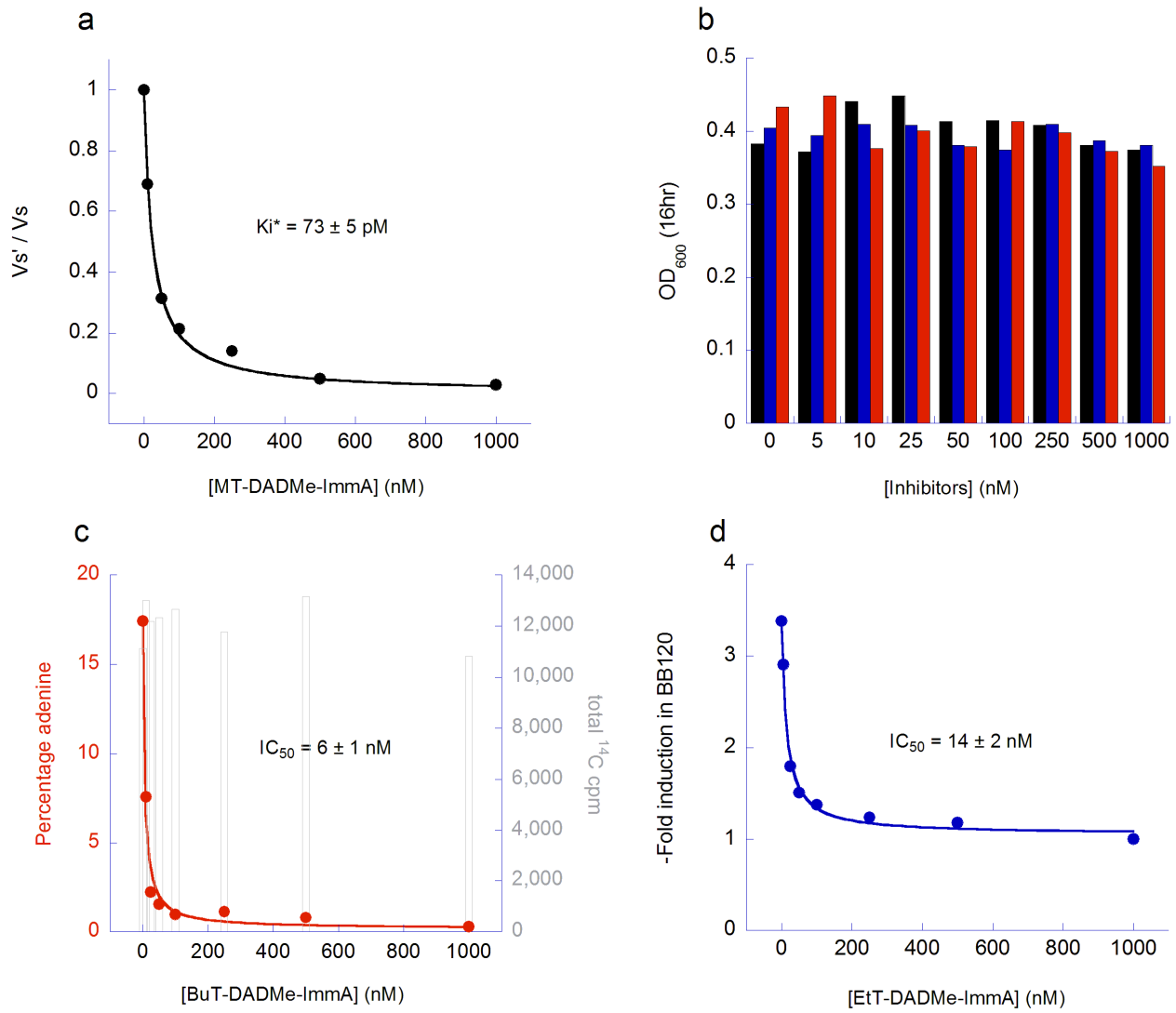
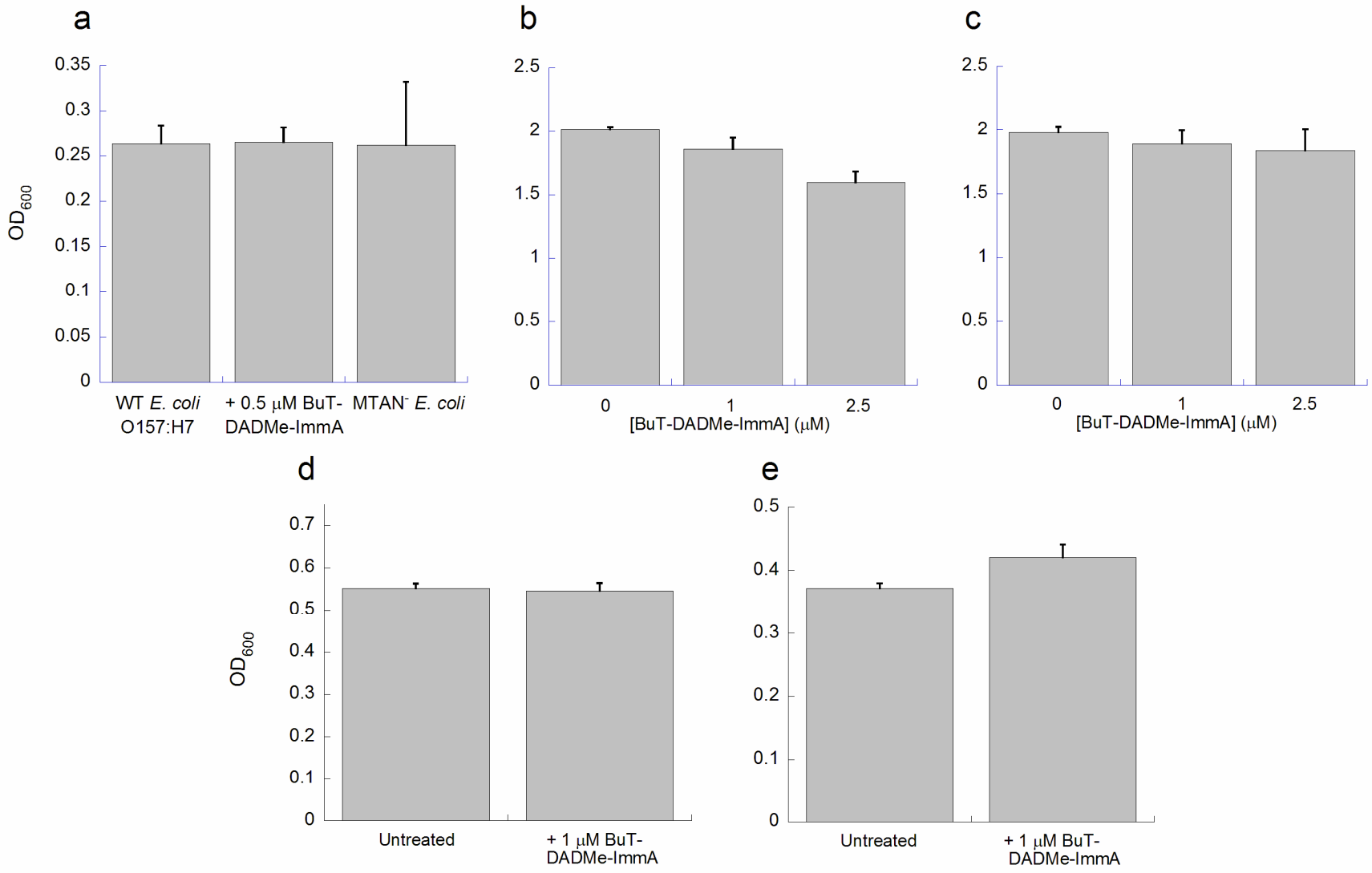


Supplementary Figure 1. Activity profiles as a function of inhibitor concentration all show dose-dependent inhibition. (**a**) Purified *Vc*MTAN inhibition assay with MT-DADMe-ImmA gave an overall dissociation constant K_i^* of 73 pM. (**b**) OD_{600} profile of *V. cholerae* N16961 grown in the presence of MT-DADMe-ImmA (■), EtT-DADMe-ImmA (■), and BuT-DADMe-ImmA (■), demonstrated nontoxicity. (**c**) MTAN activity assay in *V. cholerae* N16961 cells with BuT-DADMe-ImmA revealed inhibition of adenine production (●) and IC_{50} fit gave a value of 6 nM. Total ^{14}C counts (□) did not vary significantly showing efficient recovery of radiolabel. (**d**) Autoinducer production with EtT-DADMe-ImmA using luminescence induction assay in *V. harveyi* BB120 resulted in an IC_{50} of 14 nM. Complete set of inhibition constants are presented in **Table 1**.



Supplementary Figure 2. Effect of BuT-DADMe-ImmA on growth of pathogenic *E. coli* and *V. cholerae* upon short-term and long-term treatment with inhibitor, and under conditions that promote biofilm formation. OD_{600} measurements showed minimal effect on growth. (**a**) *E. coli* O157:H7 ± 0.5 μM BuT-DADMe-ImmA, and an $MTAN^-$ strain were grown static in AB medium for 16 hours (>5 generations) before assaying the spent medium for autoinducer-2 production. (**b**) *E. coli* O157:H7, and (**c**) *V. cholerae* N16961 grown shaken in LB for 26 generations. (**d**) *E. coli* O157:H7, and (**e**) *V. cholerae* N16961, ± 1 μM BuT-DADMe-ImmA grown static in LB medium at 25 ℃ for 24 hours on 96-well format for biofilm formation studies.

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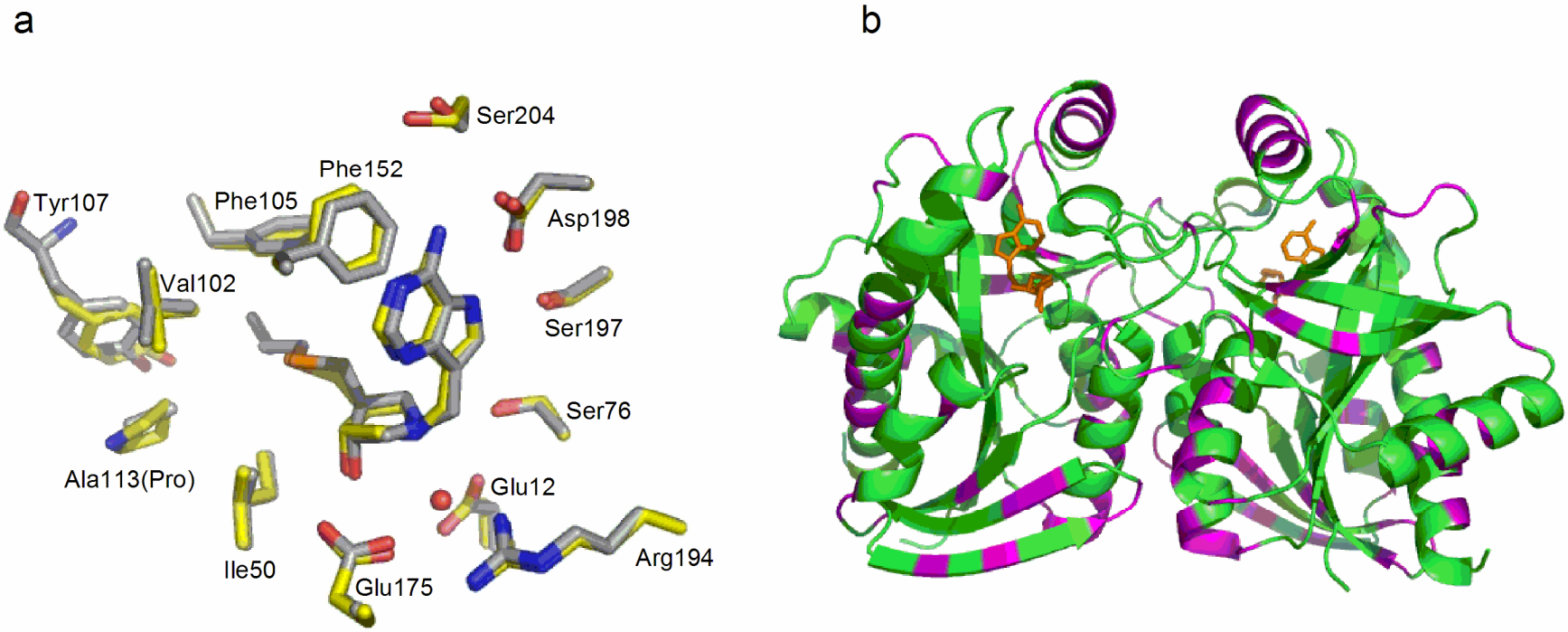
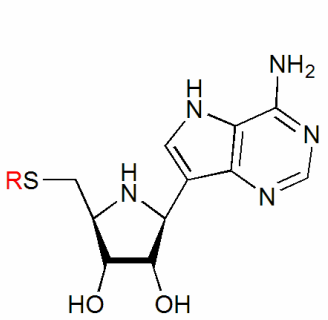
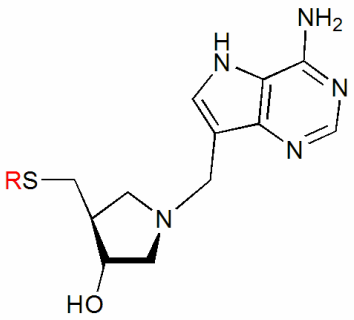


Supplementary Figure 3. Comparisons between *Ec*MTAN and *Vc*MTAN structures. (**a**) Active site superposition of *Vc*MTAN bound to BuT-DADMe-ImmA in grey compared to the active site of *Ec*MTAN bound to MT-DADMe-ImmA in yellow. (**b**) Overall structure of *Vc*MTAN in complex with BuT-DADMe-ImmA in green with mapped amino acid differences compared to *Ec*MTAN in magenta.

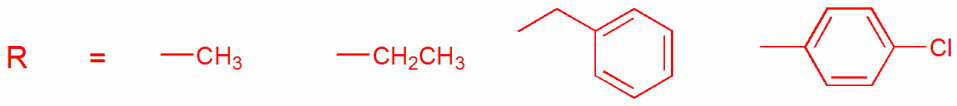
Supplementary Table 1. Dissociation constants of MT-, EtT-, BnT-, p-Cl-PhT-ImmA/DADMe-ImmA inhibitors with *Vc*MTAN.



ImmA



DADMeImmA



MT- EtT- BnT- p-Cl-PhT-

	K_d ImmA (nM)	K_d DADMe-ImmA (nM)	K^{ImmA}/K^{DADME}
MT-	10 ± 1	0.073 ± 0.005	137
EtT-	1.6 ± 0.3	0.070 ± 0.004	23
BnT-	2.1 ± 0.1	0.064 ± 0.006	33
p-Cl-PhT-	2.2 ± 0.3	0.33 ± 0.08	6.7

Supplementary Table 2. Data collection and refinement statistics for *Vc*MTAN - BuT-DADMe-ImmA complex.

Data collection	
Space group	P21
Cell dimensions	
a, *b*, *c* (Å)	67.74, 54.33, 69.79
α, β, γ (°)	90, 115.4, 90
Resolution (Å)	50 - 2.3 (2.83 - 2.3)*
R_{sym}	6.6 (18.8)
$I / \sigma I$	15.1 (4.4)
Completeness (%)	90.8 (79.9)
Redundancy	3.8 (2.9)
Refinement	
Resolution (Å)	50 - 2.3
No. reflections	18733
R_{work} / R_{free}	20.0 / 26.4
No. atoms	
Protein	3410
Ligand/ion	2
Water	69
Add. atoms	1 Iodine
B-factors	
Protein	45.95
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.319

* Values for the highest resolution shell are given in parentheses.

$R_{sym} = (\Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|) / \Sigma_{hkl}\Sigma_i I_i(hkl)$ for n independent reflections and observations of a given reflection, $\langle I(hkl)\rangle$ is the average intensity of the i observation.

Supplementary Methods

*Vc*MTAN expression and purification

The gene encoding MTAN in *V. cholerae* was synthesized and cloned into pDONR221 vector (DNA 2.0), along with an N-terminal thrombin-cleavable 6-His tag. The gene was placed into pBAD-DEST49 expression vector by Gateway cloning (Invitrogen), and was transformed into BL21 Star competent cells. Cells were grown at 37 °C with shaking to OD_{600} = 0.6, and induction was initiated with 0.05% arabinose and grown for another 4 hours. Harvested cells were lysed using a French press at 15K psi. Cell debris was removed by centrifugation, and the cleared supernatant was loaded on a Ni- Sepharose High Performance His-tag affinity column (GE Healthcare). His-tagged MTAN was eluted with a gradient of 0 – 250 mM imidazole, and buffer-exchanged into 100 mM HEPES at pH 7.0 prior to –80 °C storage.

RP-HPLC separation of ^{14}C-labeled adenine from unreacted MTA

The quenched reaction was first neutralized with potassium hydroxide, and rid of precipitates prior to HPLC separation. Carrier adenine and MTA were added to the cleared supernatant prior to loading on a C_{18} Luna HPLC column (Phenomenex). ^{14}C-Adenine product was separated from unreacted MTA using a gradient of 5 – 60% methanol in 25 mM ammonium acetate, pH 6, and 0.5 mM 1-octanesulfonic acid, and detected at 261 nm. Adenine eluted first (11 minutes), followed by MTA (14 minutes). Fractions were dried using speedvac, and

reconstituted in 1 mL deionized water prior to addition of 9 mL Liquiscint scintillation fluid (National Diagnostics). ^{14}C counts were determined on a liquid scintillation counter for 120 minutes per cycle for 2 cycles. Extent of reaction was determined as percentage ^{14}C-adenine counts of the total combined adenine and unreacted MTA counts. A control replaced cell lysate with lysis buffer prior to addition of radiolabeled substrate. Adenine ^{14}C counts appearing with the adenine carrier peak were used for correction of sample counts. The [8-^{14}C]MTA and [8-^{14}C]adenine showed efficient recovery of radiolabel in this system.

Autoinducer assay

V. cholerae N16961 cells were grown at 37 °C to stationary phase in LB medium for 16 hours in the absence and presence of 1 – 1000 nM MT-, EtT-, and BuT-DADMe-ImmA. The cells were centrifuged at 13K rpm for 30 minutes, and the supernatant was filtered through a 0.2 µm sterile syringe filter. *V. harveyi* BB120 and BB170 were grown overnight in autobioinducer (AB) medium at 30 °C, shaken at 225 rpm. The densely grown BB120 and BB170 cells were diluted 1:5000 in AB medium in a 96-well plate before addition of *V. cholerae* filtrate to 10% (v/v) of the total cell culture volume. The plates were incubated at 30 °C, and luminescence was measured on a Glomax luminometer (Promega). Maximum light response to exogenous AIs was observed after 4 hours of incubation, and was hence set as incubation time for all assays. AI background correction used sterile growth media treated as sample and light output from this incubation was used as blank.

A control experiment using untreated *V. cholerae* cell culture containing inhibitors exogenously added at concentrations corresponding to the treatment conditions was done to rule out any effect the inhibitors might have on the AIs already secreted in the media, and the latter's ability to induce bioluminescence in the reporter strains.