Electronic Supplementary Material (ESI) for ChemComm.

SUPPLEMENTARY INFORMATION

Potent inhibition of cyclic diadenylate monophosphate cyclase by the antiparasitic drug, suramin

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ADDITIONAL FIGURES

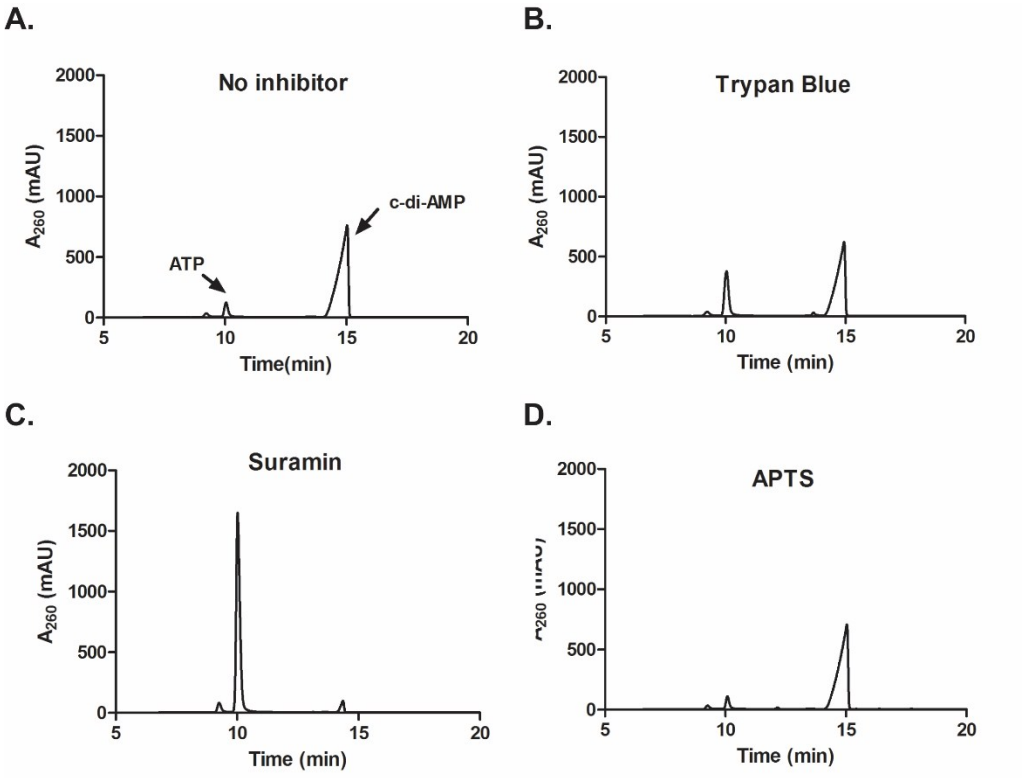

Fig. SI 1 HPLC analysis of DisA reactions. The results from further analysis of hits from coralyne assay showing reactions of 1 µM DisA with (A) No inhibitor, (B) 20 µM Trypan Blue and (C) 20 µM suramin. (D) The HPLC chromatogram of APTS reaction which was not tested by coralyne assay. Only suramin significantly inhibited DisA activity. ATP and c-di-AMP peaks with respective retention times of 10 min and 14.6 min are indicated with arrows on the No inhibitor chromatogram.

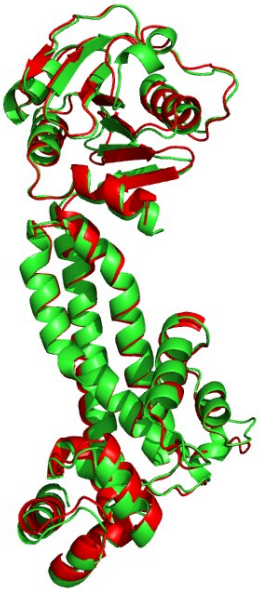

Fig. S2. Alignment of TmaDisA with computationally modelled *Bacillus subtilis* DisA. Phyre2 server was used to determine the 3D structure of DisA (red) from amino acid sequence. The modelled 3D structure of DisA was aligned with TmaDisA (PDB: 3C1Z; green).

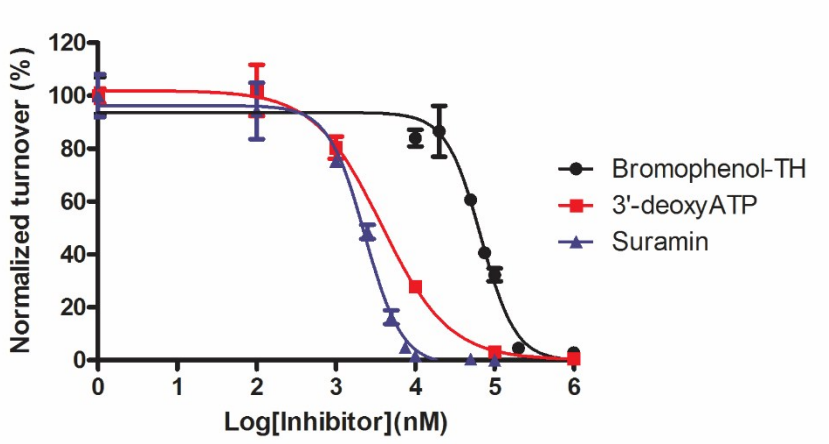

Fig SI 3. Inhibition profile of previously identified DisA inhibitors compared with suramin. The IC_{50} values of bromophenol thiohydantoin (BTH), 3'-deoxyATP and suramin were determined to be 67.2 µM, 3.8 µM and 2.3 µM respectively at 300 µM ATP and 1 µM DisA.

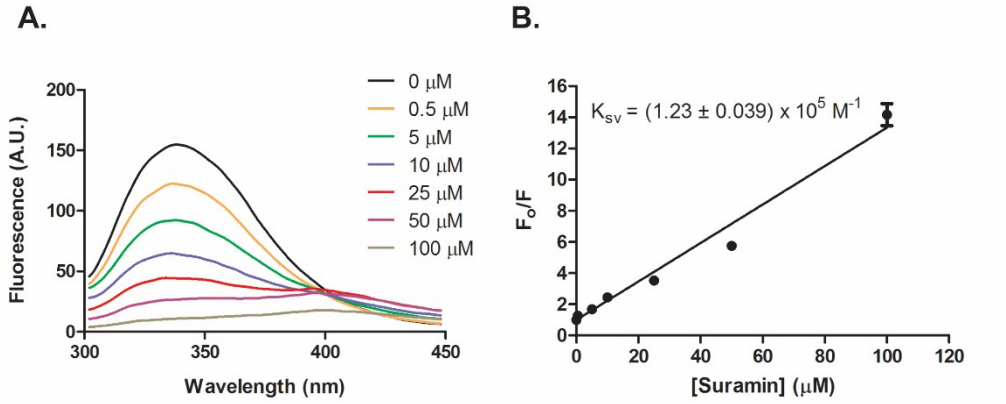

Fig. SI 4. Analysis of the binding interaction between DisA and suramin. (A) The fluorescence emission of DisA (5 μM) was observed to be quenched in the presence of increasing suramin concentration. (B) Stern-Volmer plot of the relative fluorescence (at 340 nm) as a function of suramin concentration. It was fitted to the linear equation[1]:

$$F_o/F = 1 + k_q\tau_o = 1 + K_{sv}[Q] \quad (2)$$

where F_o and F are respectively the fluorescence intensities of a biomolecule (DisA) without and with a quencher (suramin) at concentration Q, k_q is the quenching rate constant of the biomolecule, τ_o is the fluorescence lifetime of biomolecule (about 10^{-8} s)[2] and K_{sv} is the Stern-Volmer constant. K_{sv} was estimated to be $1.23 \pm 0.039) \times 10^5\ M^{-1}$ and so we determined k_q as $1.23 \times 10^{13}\ M^{-1}s^{-1}$. Since the estimated value of k_q is greater than the diffusion-controlled quenching rate, $2 \times 10^{10}\ M^{-1}s^{-1}$ a static quenching mechanism prevails in the DisA-suramin complex[2].

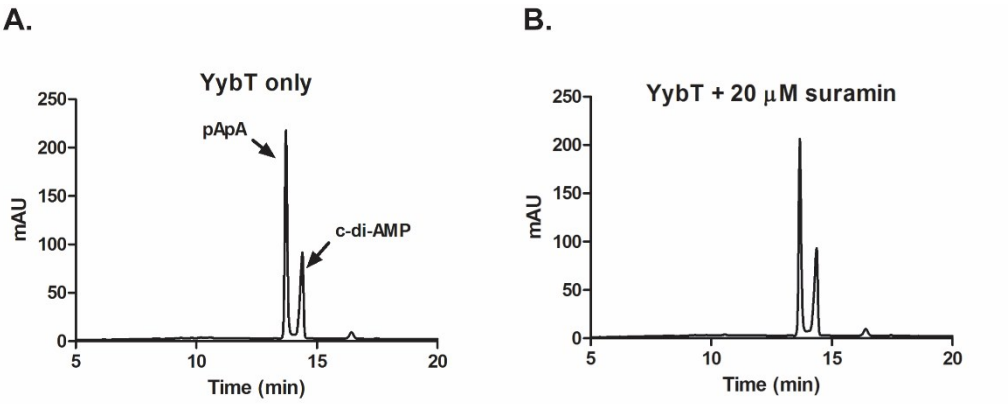

Fig. SI 5. HPLC analysis of YybT reactions. The effect of suramin on 1 μM YybT (A) without suramin and (B) with 20 μM suramin at 37 °C was analyzed by HPLC. There was no difference between the reaction in with and without suramin. The arrows point to the product, pApA and substrate, c-di-AMP which had retention times of 13.7 min and 14.4 min respectively.

EXPERIMENTAL SECTION

Protein expression and purification

E. coli BL21(DE3) cells transformed with plasmids of enzymes were grown at 37 °C in LB medium amended with 50 µg/mL Kanamycin. Cultures were grown to an OD_{600} of 0.6, and expression was induced by adding 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). YybT expression was induced for 6 h at 30 °C whilst DisA was induced for 18 h at 16 °C. Cell pellets were harvested by centrifugation at 4,000 rpm for 15 min and resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl for YybT and 50 mM sodium phosphate buffer, pH 8.0, 300 mM NaCl for DisA). The cells were lysed by sonication and the lysates were centrifuged at 25,000 rpm for 25 min at 4 °C to collect the supernatant. The hexahistidine-tagged proteins were purified from the supernatants by affinity chromatography using a GE HisTrap™ HP 1 mL column mounted onto a Bio-Rad NGC™ Chromatography System. Protein concentration was determined by measuring the UV absorbance at 280 nm and the purified proteins were stored with 10 % glycerol in their respective lysis buffers at - 80 °C.

The coralyne assay

The initial screening of the library was done at the Johns Hopkins University ChemCORE facility. Suramin and suramin-related compounds were stored as 10 mM stock solutions in DMSO. Triplicate 100 µL reactions were set up containing 300 µM ATP, 10 µM coralyne, 3 mM KI and 20 µM compound or DMSO in a 40 mM Tris-HCl pH 7.5, 100 mM NaCl and 10 mM $MgCl_2$ reaction buffer. The reactions were started by adding 1 µM DisA. The fluorescence of coralyne over the course of 30 min was measured on a Molecular Devices SpectraMax M5e microplate reader with λ_{ex}=420 nm and λ_{em}=475 nm at 30 °C.

HPLC analysis

Reactions containing 20 µM suramin or related compounds, 300 µM ATP and 1 µM DisA were set up and allowed to go for 30 min at 30 °C. The reactions were then terminated by heating at 95 °C for 5 min and the precipitated proteins were filtered off. Components of the filtrate were then analyzed on a COSMOSIL C18-MS-II Packed column (5 µm) using 0.1 M TEAA in water (Buffer A) and acetonitrile (Buffer B). The samples were eluted with 99 %→87 % Buffer A at 0 to 16 min, 87 %→10 % Buffer A at 16 to 22 min and kept at 10% Buffer A till 25 min, detecting signals at room temperature with a 260 nm UV detector. For the effect of suramin on YybT, HPLC reactions were set up containing 50 µM c-di-AMP and 1 µM YybT in the presence or absence of 20 µM suramin for 30 min at 37 °C in reaction buffer (100 mM Tris-HCl, pH 8.3, 20 mM KCl, 0.5 mM $MnCl_2$ and 1 mM DTT). The reaction was analyzed as described above.

IC_{50} determination

Half maximal inhibitory concentrations (IC_{50}) were determined in 10 µL reactions containing ATP (at either 100 µM, 300 µM, 500 µM or 1 mM), 11 nM ^{32}P-ATP and increasing concentrations of suramin mixed in reaction buffer (40 mM Tris-HCl pH 7.5, 100 mM NaCl and 10 mM $MgCl_2$). Reactions were initiated by adding 1 µM DisA at 30 °C for 1 hour. Afterwards, 0.4 µL aliquots of the reaction mixtures were spotted on TLC plates (EMD Millipore TLC Cellulose) and spot separation was achieved in a 1:1.5 (vol/vol) saturated $(NH_4)_2SO_4$ and 1.5 M KH_2PO_4 buffer.

Measurement of intrinsic fluorescence of DisA

DisA (5 µM) was incubated with various concentrations of suramin at 25 °C for 1 hour. Protein intrinsic fluorescence was measured on a Cary Eclipse Fluorescence Spectrophotometer (Agilent) with λ_{ex} = 290 nm and λ_{em} = 300 - 450 nm. Initially, apparent K_d (assuming 1:1 binding ratio) was calculated using fluorescence intensity at 340 nm according to the equation[3]:

$$F = F_o + \Delta F \frac{\left(K_d^{app} + P_t + Q_t\right) - \sqrt{\left(K_t^{app} + P_t + Q_t\right)^2 - 4P_tQ_t}}{2P_t} \quad (3)$$

Where F is the fluorescence intensity at 340 nm, F_o is the fluorescence intensity at 340 nm in the absence of ligand, ΔF is the change in fluorescence upon ligand binding, K_d^{app} is the apparent dissociation constant, P_t is the total protein (DisA) concentration and Q_t is the total ligand (suramin) concentration.

For Stern-Volmer analysis of the fluorescence emission spectra, the emission at 340 nm at each suramin concentration was used to generate the Stern-Volmer plots[1, 4] according to the equations (1) and (2) from which the Stern-Volmer constant, K_{sv} dissociation constant, K_d and number of binding site, n were estimated.

Molecular docking and computational modelling

We used Autodock Vina 1.1.1[5] to perform docking calculations. TmaDisA PDB file (3C1Z) was downloaded from RCBS Protein Data Bank and used as AutoDock protein. Suramin was converted into a PDB file using ChemDraw and used as AutoDock ligand. AutoDockTools was used to convert the PDB files to the required PDBQT file format. A grid box large enough to cover the globular nucleotide binding domain of TmaDisA (Center X = -20, Center Y = 10, Center Z = 75, Size X = 92, Size Y = 96, Size Z = 68) was selected. We set the exhaustiveness value to 32 and conducted the molecular docking experiment. The best pose was determined as the one with the highest binding affinity. PyMOL viewer version 1.3.49 was used to visualize the docking results. For computational modelling, the FASTA sequence of DisA was obtained from UniProt (A0A0C2Q1A6) and submitted to the Phyre2 web portal for protein modelling[6]. PyMOL was then used to align the 3D model with the structure of TmaDisA.

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