STL372167 targets *S. mutans* diadenylate cyclase

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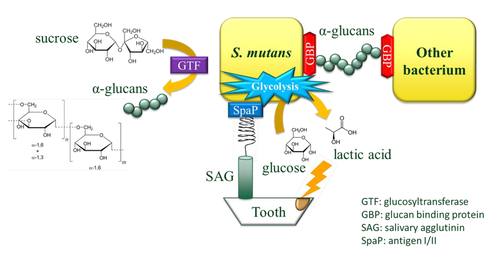
Introduction

Dental caries is the most prevalent infectious disease, affecting human oral health for millennia.

Oral microbiome consists of > 700 microorganisms, several possessing virulence factors and defense mechanisms which aid in the formation of robust biofilms during pathological conditions.

Streptococcus mutans, a gram-positive facultative anaerobe, is established as the primary etiological agent for the progression of dental caries.

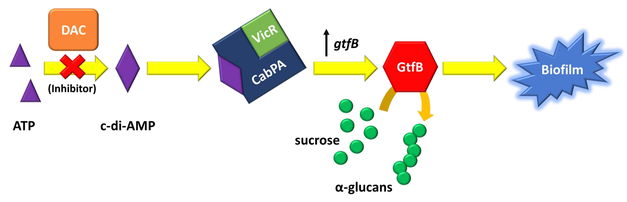


S. mutans synthesizes **glucosyltransferases (Gtfs)**, a family of extracellular enzymes which play a crucial role in the intricate architecture of oral biofilms.

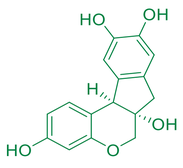
Gtfs convert sucrose into oligomeric chains of glucose, known as glucans, that link bacteria together via glucan binding proteins (GBPs). Gtf inhibitors have still not reached the clinic and there is still a demand for selectively targeting other biofilm-associated enzymes in *S. mutans*.

Diadenylate cyclases (DACs) is a family of enzymes present in bacteria which convert two molecules of ATP into cyclic di-AMP (c-di-AMP).

In *S. mutans*, intracellular **c-di-AMP** binds to the CabPA/VicR complex which consequently upregulates the expression of the *gtfB* gene. Therefore, the development of DAC (smDAC) inhibitors is a promising strategy for the selective inhibition of oral biofilms.



Prior Art



(+)-brazilin

***C. sappan* heartwood**

- ✓ DAC enzyme inhibitory IC_{50} = 25.1 ± 0.98 μM
- ✓ Michaelis-Menten K_i =140.0 ± 27.13 μM
- ✓ Binding-dissociation K_d = 11.87 μM
- ✓ Non-competitive inhibitor
- ✓ *S. mutans* biofilm inhibitory IC_{50} = 21.0 ± 0.60 μM
- ✓ > 90% glucan inhibition at 25 μM (+)-brazilin
- ✓ > 90% HA disc biofilm inhibition at 50 μM (+)-brazilin
- ✓ Selectivity for biofilm inhibition < 25 μM (+)-brazilin
- ✓ Favorable druglike properties
- ✓ Natural product
- ✓ Synthetic feasibility

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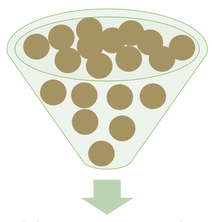
Methodology

I. *In silico* HTPS docking and filters

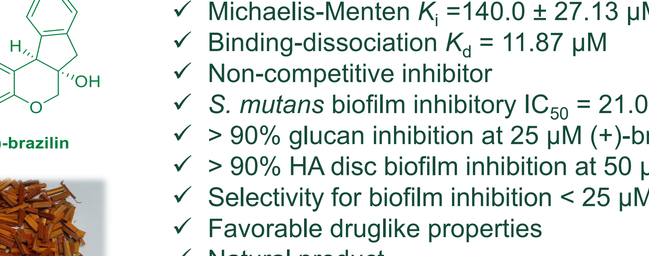
Leadlikeness	Druglikeness	Estimated Binding Affinity Range
• # Donors < 4 • # Acceptors < 7 • # Rotatable bonds < 8 • # Rings < 5 • 0 < # (Donors + Acceptors) < 10 • 10 ≤ # Heavy atoms ≤ 27 • 0 ≤ LogP ≤ 4	• # Donors ≤ 5 • # Acceptors ≤ 10 • MW ≤ 500 • LogP ≤ 5	• ≤ 10,000 nM • ≤ 1,000 nM

1.1M compound library



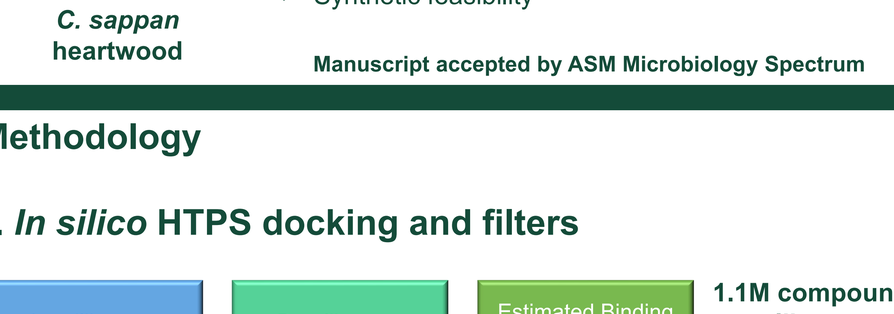
66 compounds purchased

II. HPLC Assay



Shimadzu HPLC

III. Microbiological assays



Specific Aims

I. Identify druglike small-molecule DAC inhibitors from *in silico* HTPS docking studies.

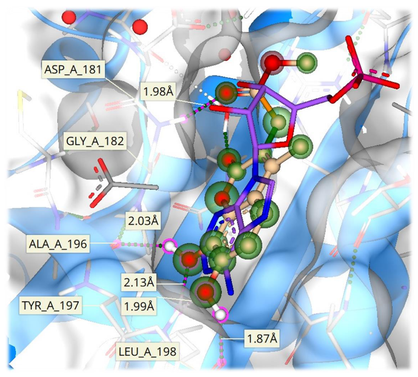
II. Screen the leads for DAC inhibition using HPLC and determine dose-dependent IC_{50}.

III. Evaluate the DAC inhibitors' growth and biofilm inhibitory activities using *in vitro* assays.

IV. Establish the DAC inhibitors' binding profiles using Michaelis-Menten kinetics studies.

Results

I. *In silico* HTPS screening identifies STL372167 as a potential inhibitor

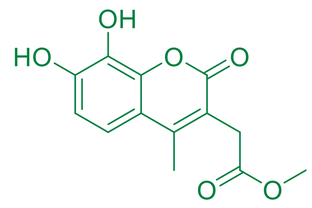


Druglike properties

- ✓ Estimated binding affinity within nM-μM range
- ✓ Minimal torsional strain
- ✓ Minimal intra- and inter-molecular clashes
- ✓ MW: 264.25 g/mol
- ✓ TPSA = 93.1
- ✓ LogP = 1.77

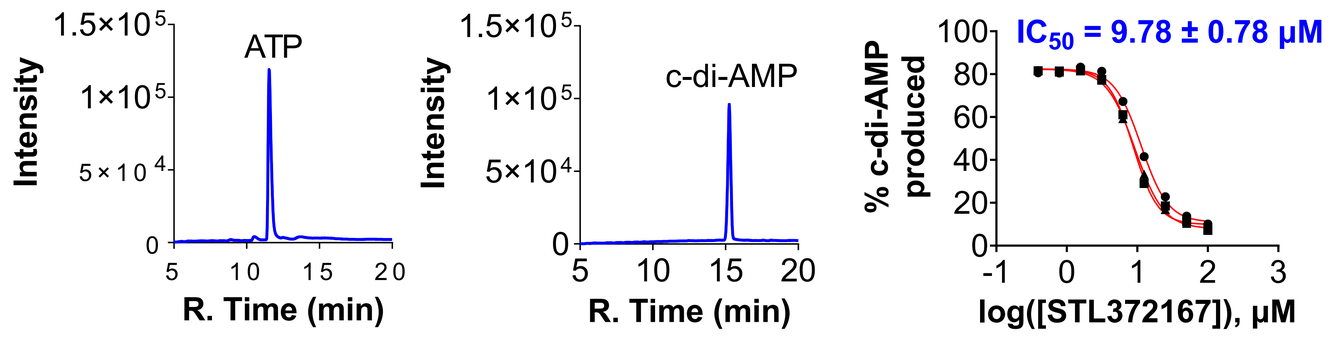
Favorable interactions

- ❖ **H-bonding:** Asp181, Gly182, Ala196, and Leu198
- ❖ **π-π stacking:** Tyr197

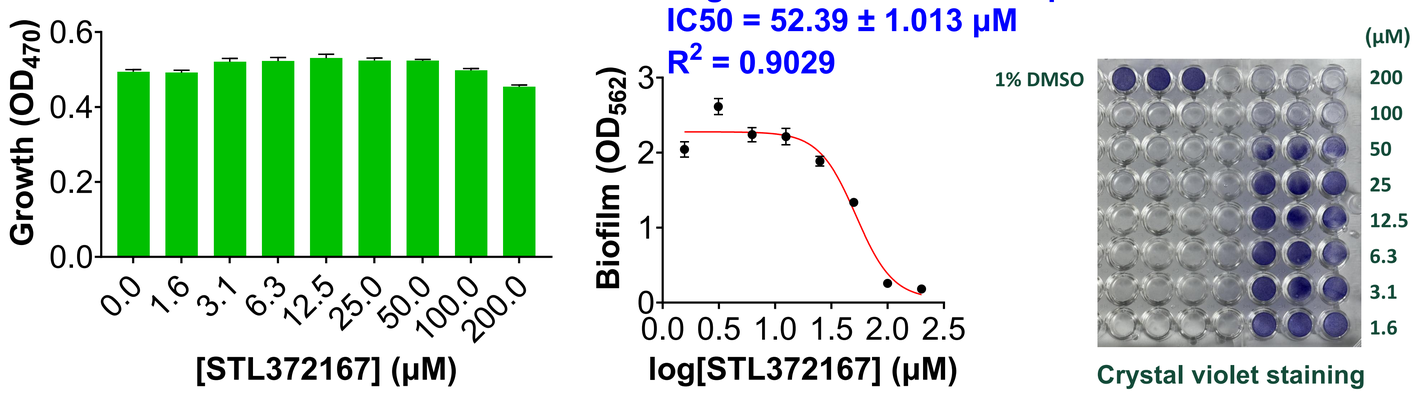


STL372167

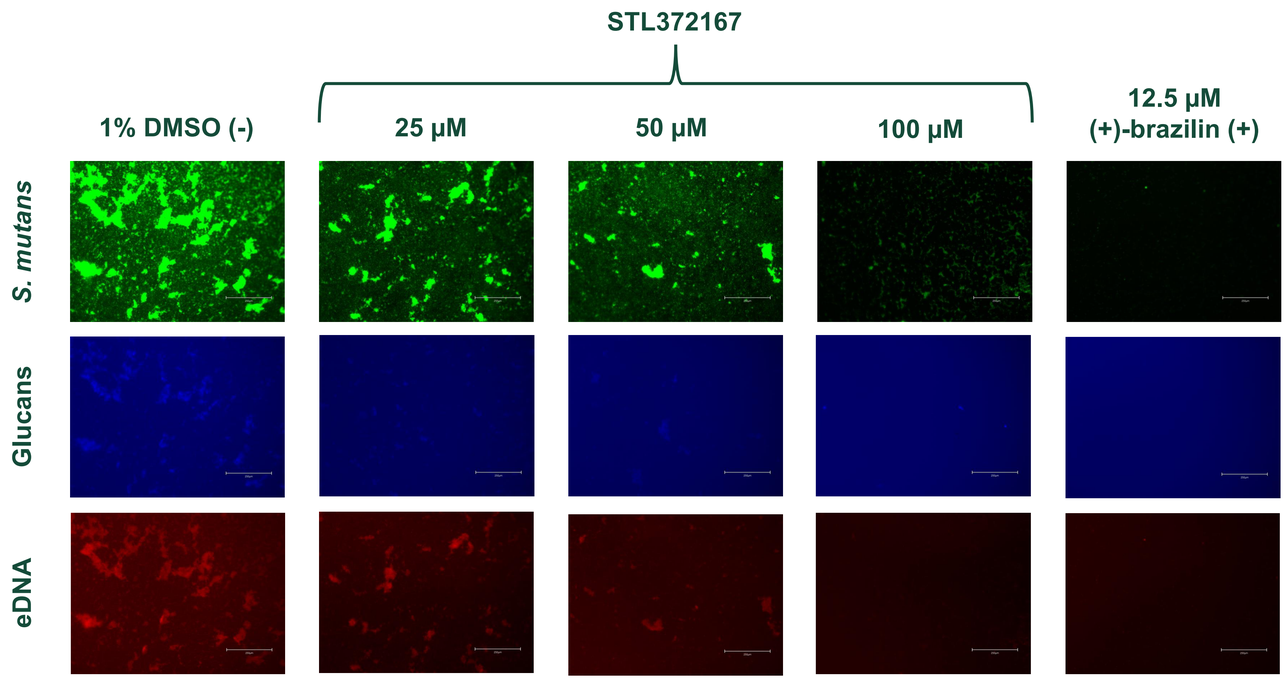
II. HPLC studies establishes STL372167 as a potent DAC enzyme inhibitor



III. STL372167 inhibits *S. mutans* biofilms while preserving the planktonic growth



IV. Fluorescence microscopy of *S. mutans* biofilms shows dose-dependent reduction in cells, glucans, and extracellular DNA (eDNA)



Conclusions and Future Work

- ❖ One of the three essential motifs for enzymatic activity amongst all DAC proteins is the **Asp-Gly-Ala triad** found in the loop between α-helix 3 and β-sheet 4. STL372167's strong H-bonding interactions with Asp181, Gly182, and Ala196 in the docking model is a potential mechanism for inhibiting DAC.
- ❖ STL372167 **inhibits DAC's enzymatic activity** in a dose-dependent manner with **2.6x more potency** than (+)-brazilin (non-competitive inhibitor, IC_{50} = 25.1 ± 0.98 μM) and we expect that STL372167 is a competitive inhibitor based on its nM-μM estimated binding affinity in our docking model.
- ❖ STL372167 **selectively inhibits *S. mutans* biofilms** while preserving its planktonic growth. Glucans and eDNA, both essential components of the biofilm matrix, were inhibited with increasing concentrations of STL372167.
- ❖ Hydroxyapatite disc (HA) assays, which constitute of a rougher and porous three-dimensional surface, will be performed to further establish its anti-biofilm effect in a more representative model of a tooth surface.
- ❖ Further studies will characterize the anti-biofilm and bactericidal effects of STL372167 against oral commensal streptococci.
- ❖ We plan to establish STL372167's binding kinetics and co-crystallize the inhibitor with DAC to elucidate its binding profile.

Acknowledgments

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The authors declare no conflict of interest.

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