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Inhibitory Effects of Novel Immucillin Analogues on *Borrelia burgdorferi* Bgp Nucleosidase

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Introduction

The pathogenic spirochaete *Borrelia burgdorferi* causes Lyme disease and is transmitted by deer ticks when they feed. Lyme disease is multisystemic—it adversely affects the heart, joints, and skin. Recent studies demonstrate that *B. burgdorferi* possesses three methylthioadenosine/S-adenosylhomocysteine (MTA/SAH) nucleosidases essential for the catabolic breakdown of both MTA and SAH. Both MTA and SAH are by-products of major pathways involving S-adenosylmethionine (SAM) and are kept at low micromolar concentrations due to their inhibitory activity.

This project examined the effect of transition state inhibitors on the surface binding *Borrelia* glycosaminoglycan-binding protein (Bgp) nucleosidase using recombinant Bgp and whole-cell *B. burgdorferi* activity assays. The transition state analogues are potent inhibitors of Bgp activity with K_i values ranging from 6pM-6nM. Bgp on the surface of live *B. burgdorferi* was also inhibited by treatment with low nanomolar concentrations of transition state analogues.

Hypothesis

Immucillin transition state analogues will be potent inhibitors of Bgp nucleosidase and antibiotics to treat Lyme disease.

Breakdown of Nucleosidase Substrate

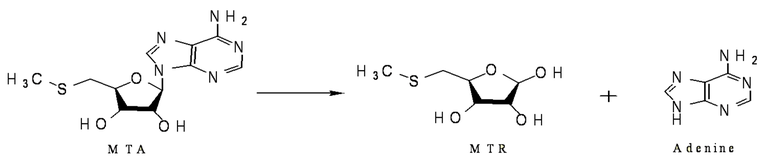

Figure 1 Catalysis of MTA to methylthioribose (MTR) and adenine (Ade)

Methods

- Recombinant Bgp was expressed and purified
- Enzyme purity was confirmed by *SDS-Polyacrylamide Gel Electrophoresis*
- Spectrophotometric analyses of Bgp activity were run using *Cary 50 and 100 UV-Visible Spectrophotometers*
- Using *Beer's Law* (A=clε), $\varepsilon = 1.6 mM^{-1}cm^{-1}$ determined concentration of nucleosidase substrate in assay
- Initial and delayed onset velocities (V_0, V_0', V*) were calculated with varying concentrations of inhibitor
- Using *IgorPro,* inhibition constants (K_i) were calculated by fitting data to the equation for competitive inhibition:

$$V_0'/V_0 = (K_m + [S])/\{(K_m+[S]+K_m[I]/K_i)\}$$

The Methionine Salvage Pathway

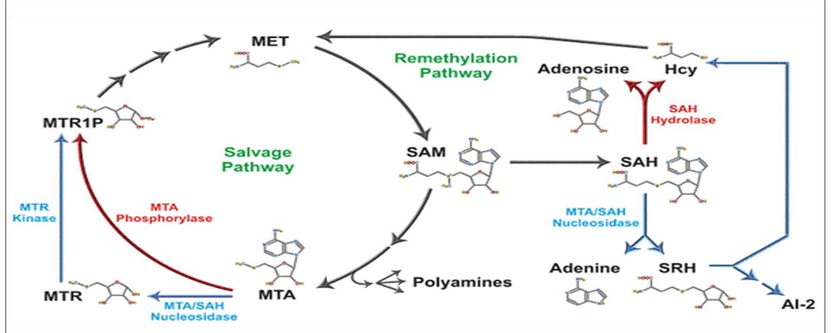

Figure 2 Biological pathway present in *B. burgdorferi* and target for drug testing

Enzyme Inhibition Assay

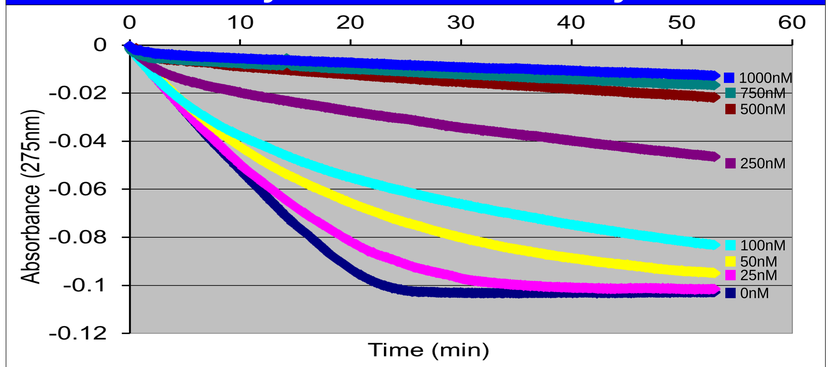

Figure 3 Spectrophotometric analysis (λ_{275}) of Bgp hydrolysis of MTA in the presence of varying concentrations (0-1000nM) of MT-ImmA analogue

Live Whole Cell Assay

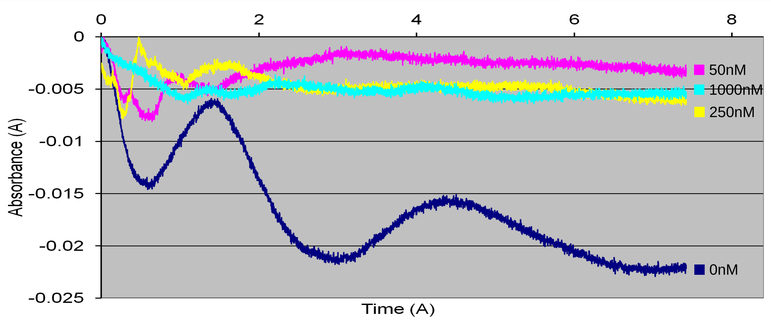

Figure 4 Nucleosidase activity assay (λ_{275}) of live *B. burgdorferi* whole cells with varying concentrations (0-1000nM) of MT-ImmA analogue

References

Parveen, N., & Cornell, K. A. (January 01, 2011). Methylthioadenosine/S-Adenosylhomocysteine nucleosidase, a critical enzyme for bacterial metabolism. *Molecular Microbiology, 79,* 1, 7-20

Lee, J. E., Settembre, E. C., Cornell, K. A., Riscoe, M. K., Sufrin, J. R., Ealick, S. E., & Howell, P. L. (January 01, 2004). Structural comparison of MTA phosphorylase and MTA/AdoHcy nucleosidase explains substrate preferences and identifies regions exploitable for inhibitor design. *Biochemistry, 43,* 18, 5159-69.

Summary of Analogue Inhibition Constants

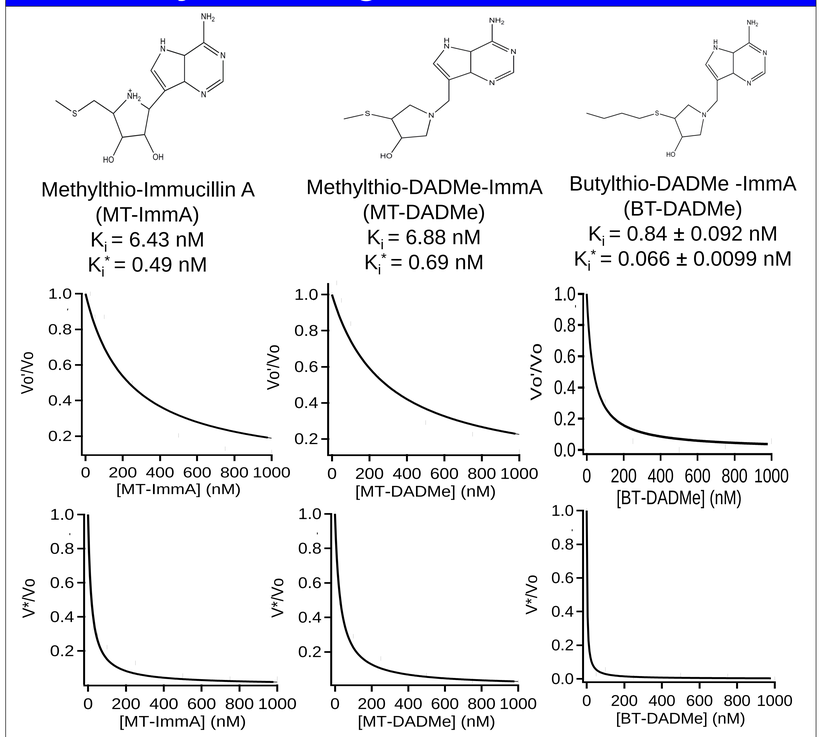

Figure 5 Illustrations of inhibitors and corresponding inhibition curves (both early onset, V_0'/V_0 and delayed onset, V^*/V_0)

Results

- Bgp is an active MTA nucleosidase found on the surface of *B. burgdorferi*
- Immucillin analogues were potent inhibitors of recombinant Bgp with subnanomolar to low nanomolar K_i values
- Bgp was readily inhibited in whole cell assays using low nanomolar concentrations of Immucillin analogues

Conclusion

The transition state analogues inhibit Bgp and live *B. burgdorferi* whole cells. Further studies can test inhibitors against other MTA/SAH nucleosidases and lead to the development of new drugs that combat Lyme disease.

Acknowledgements

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