Transition State Analogue Inhibitors of N-Ribosyltransferases: New Drugs by Targeting Nucleoside Processing Enzymes.

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ABSTRACT

The characterization of the transition state structure of a number of N-ribosyltransferases has enabled the design and synthesis of some extremely powerful inhibitors of these enzymes. We have three generations of inhibitors for some nucleoside processing enzymes which are therapeutic targets, and the potency of these compounds confers special advantages in their development as new drugs against cancer, autoimmune diseases, microbial infections and malaria.

INTRODUCTION

The allure of transition state analogue inhibitors is based on the proposal that the transition state of an enzyme catalyzed process is bound to the enzyme tighter than the substrate by a factor equal to the catalytic rate acceleration imposed by the enzyme. For many enzymes this predicts that the ideal inhibitor will bind with a $K_i \approx 10^{-10}$ M. While the ideal inhibitor will not be attainable as it will contain partial charges and bond orders, it will only be necessary to capture some of the features of the transition state to attain extremely potent inhibitors.

Kinetic isotope effects can be utilized to derive the transition state structure of an enzyme.¹ Subsequent inhibitor design incorporating important features of the transition state is expected to lead to more potent inhibitors than those available from ground state considerations.

RESULTS AND DISCUSSION

Human purine nucleoside phosphorylase (PNP) is recognised as a therapeutic target. Complete inhibition results in build-up of the substrate 2’-deoxyguanosine (dG). Human T-cells have a particular enzyme profile that ensures there is a resulting intracellular build-up of 2’-deoxyguanosine triphosphate (dGTP) in the T-cells. As a consequence T-cells stimulated into clonal expansion undergo apoptosis. Thus, PNP inhibitors which are potent enough to attain prolonged elevated blood levels of dG act to block T-cell proliferation.

The transition state structures of bovine and human PNP have been determined from kinetic isotope effects, and powerful inhibitors have been designed and synthesized.²⁻⁵ Three generations of inhibitors (eg 1 – 3) capture important features of the transition states and they are the most potent inhibitors known for these enzymes. Compounds 1 and 2 are in clinical trials for the control of T-cell cancers and autoimmune diseases.

The protozoan parasite Plasmodium falciparum is the causative agent for malaria. It is a purine auxotroph and is dependent on salvage enzymes to harvest purines from its host. PNP is an essential enzyme for this process and inhibitors of Pf PNP kill the organism in culture. The transition state of Pf PNP is similar to that of the human enzyme while there are differences in substrate specificity. We have designed and synthesized compounds that selectively inhibit Pf PNP as well as others that are potent inhibitors of Hs and Pf PNPs.⁶,⁷ These compounds are potential therapeutics for the control of malaria.

This approach for the design and synthesis of transition state analogue inhibitors has been extended to human Methylthioadenosine Phosphorylase (MTAP) and bacterial Methylthioadenosine/S-Adenosylhomocysteine nucleosidase (MTAN). These enzymes cleave adenine from the eponymous substrates by phosphorylisis and hydrolysis respectively and have similar transition state structures.

Inhibitors of MTAP slow down polamine biosynthesis and affect S-Adenosylmethionine levels. This results in anti-cancer activity against a number of human cancer cell lines, both in vitro and in vivo. MTAN inhibitors are expected to inhibit polamine biosynthesis, the salvage pathways for adenine and methionine and the synthesis of...
quorum sensing molecules involved in biofilm formation, 
exotoxin synthesis and antibiotic resistance factors.

We have selected compounds 4 and 5 for development as 
anti-cancer and anti-microbial agents respectively.

New results on inhibitor design will also be presented.

CONCLUSION

Knowledge of the transition state structure of an enzyme-
catalyzed reaction offers the opportunity to design 
inhibitors that capture features of the transition state. This 
can give rise to extremely powerful inhibitors. We have 
developed potent inhibitors of a number of nucleoside 
processing enzymes which offer promise as therapeutics.

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