

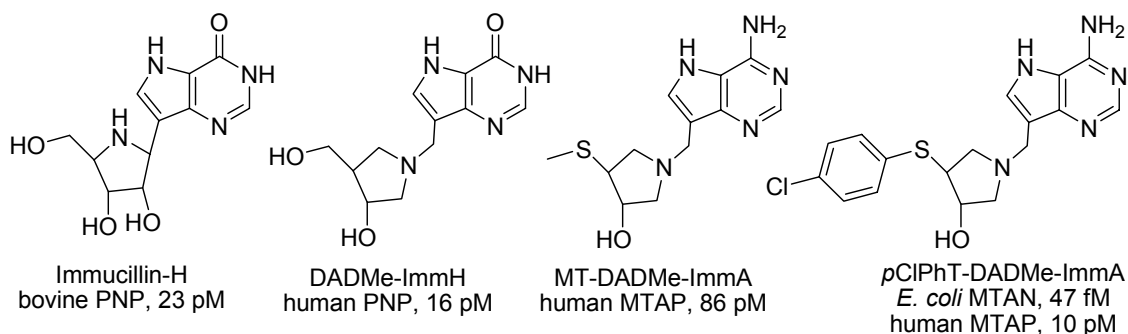
NEW DRUGS FROM TRANSITION STATE THEORY: THE IMMUCILLINS

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Chemically stable analogues of enzymatic transition states are the most powerful noncovalent inhibitors available for enzymatic reactions. The bond lengths and angles of reactants at specific enzymatic transition states can be established by a combination of kinetic isotope effects and computational chemistry. An early proof-of-concept for transition state analogue design was with bovine purine nucleoside phosphorylase (PNP). The partially dissociated ribooxacarbenium ion transition state of PNP is closely related to Immucillin-H. This 23 pM inhibitor binds 740,000 times more tightly than substrate. Human PNP has a transition state closely related to a fully dissociated ribooxacarbenium ion. DADMe-ImmH was synthesized to match this transition state and is a 16 pM inhibitor, binding 2.4 million times tighter than



substrate. DADMe-ImmH binds 8-fold more tightly to human PNP than to bovine PNP, demonstrating the ability of transition state design to distinguish between closely related enzymes. Immucillin-H is currently in Phase II clinical trials for T-cell cancers and DADMe-ImmH is in Phase I clinical trials for psoriasis. Polyamine synthesis is a validated anticancer target. We selected 5'-methylthioadenosine phosphorylase (MTAP) from the polyamine cycle as a unique target for inhibitor design. The transition state structure of human MTAP was determined to be highly dissociated. MT-DADMe-ImmA was designed to provide a geometric and electrostatic match of the MTAP transition state. It is an 86 pM inhibitor of the enzyme ($K_m/K_i = 58,000$) and shows antitumor activity in mice. Quorum sensing in bacteria is involved in biofilm and toxin formation. 5'-Methylthioadenosine nucleosidase (MTAN) chemistry is involved in two steps in the quorum sensing pathway. MTAN has a dissociated transition state with a hydrophobic pocket for the 5'-methylthio group. pClPhT-DADMe-ImmA matches both the transition state features and fills a hydrophobic pocket to give a dissociation constant of 47 fM. This affinity gives a K_m/K_i ratio of 91 million for *E. coli* MTAN, making this one of the most powerful inhibitors for any enzyme.