Table 15.6. Dissociation constants of transition-state analogs with HsPNP and His257 mutants

	DADMe-ImmH			ImmH		
PNP variant	K_d	K_m/K_d value	*x*-fold change	K_d	K_m/K_d value	*x*-fold change
Native	10.7 pM	3,700,000	1	57.9 pM	690,000	1
His257Asp	900 nM	1,500,000	2	86 nM	15,700	45
His257Phe	950 nM	337,000	11	172 nM	1,860	370
His257Gly	270 nM	2,800,000	1	11.0 nM	68,100	10

Notes: These dissociation constants are final, equilibrium constants after any slow-onset phase of inhibition, if applicable (those where $K_d < 1$ nM). Data adapted from Murkin et al.[41]

transition state generates a KIE. ImmH was found to yield a 5′-^{3}H BIE of 12.6%, and DADMe-ImmH gave an unprecedented, large 29.2% BIE (Table 15.7).[119] These values dwarf the 1.5% and 4.6% isotope effects resulting from binding of the substrate and from formation of the transition state, respectively.[41] Thus, much greater bond distortional forces are operative with the binding of transition-state analogs than in formation of the actual transition state.

These BIEs provide some insight regarding the nature of transition-state formation. In the thermodynamic model of transition-state theory, the transition state is viewed – at least conceptually – in equilibrium with the enzyme, to which it binds tightly. This theory would predict the magnitude of the transition-state analog BIEs to be similar to that of the KIE for formation of the transition state (~5%). A dynamic model of transition-state formation explains tight binding of analogs by a conversion of dynamic transition-state excursions into a more stable protein structure condensed around the chemically inert transition-state mimic. It is conceivable that this effect captures the ligand in a more bond-distorted form, giving rise to larger BIEs.

PHARMACOLOGICAL APPLICATIONS OF ImmH AND DADMe-ImmH

The rare genetic PNP deficiency is associated with T-cell immunodeficiency due to an accumulation of dGuo in blood, which ultimately causes inhibition of DNA replication through the inhibition of ribonucleotide reductase by dGTP in dividing T cells (Figure 15.10). PNP inhibitors such as the Immucillins could exploit this behavior in proliferative T-cell disorders by causing arrest of cell division specifically in T cells. Ongoing efforts have been made to study the in vivo effects of these potent PNP inhibitors.

In vivo studies with ImmH

Effects of ImmH on cultured human T cells

The effects of ImmH on the growth of the human T-cell culture lines CCRF-CEM and MOLT-4 were evaluated by treatment with varying concentrations of ImmH in the presence and absence of dGuo.[120] Inclusion of dGuo is required for the cellular accumulation of dGTP that occurs with PNP deficiency. Proliferation of both cell lines was selectively blocked by ImmH (IC_{50} = 0.4–5 nM) and only in the presence of dGuo (Figure 15.36). Inhibition of DNA synthesis by ImmH was also demonstrated by the reduced incorporation of [^{3}H]thymidine in its presence [Figure 15.36(b)]. At concentrations up to 50 μM, ImmH exhibited no toxic effects on a variety of non-T-cell tumors from

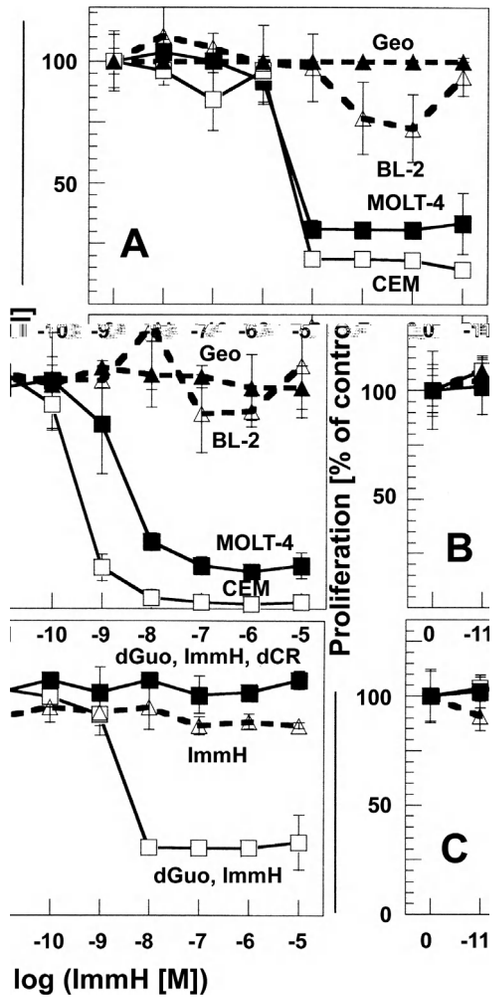

Figure 15.35. Inhibition of human T-cell leukemia cell lines by the joint action of ImmH and dGuo. Geo and BL-2 are human colon carcinoma and B-cell leukemia cell lines, respectively, while MOLT-4 and CEM are human T-cell leukemia cell lines. Cell lines were incubated with 20 μM dGuo and varying concentrations of ImmH and were analyzed for cell viability by (A) WST-1 and (B) incorporation of [^{3}H]thymidine. (C) Inhibition of proliferation only occurs when ImmH is treated in the presence of dGuo, but the activity can be regained by deoxycytidine (dCyd) rescue (dCR); dCyd, the preferred substrate for dCyd kinase, is converted to dCMP which inhibits the phosphorylation of dGuo, thereby preventing inhibition of ribonucleotide reductase by dGTP (see Figure 15.10). Reproduced from Kicska et al.[120]