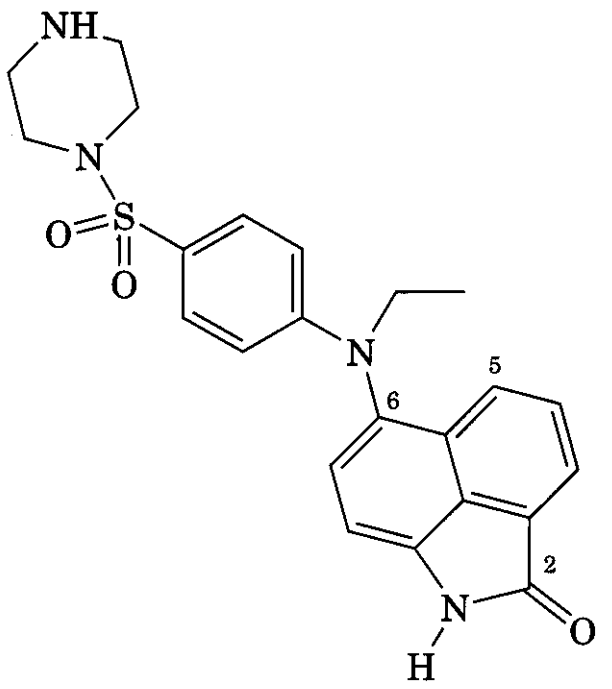
active site cavity, toward bulk solvent, resulted in (20). The use of an amine for the groups attached to position 6 of the benz-

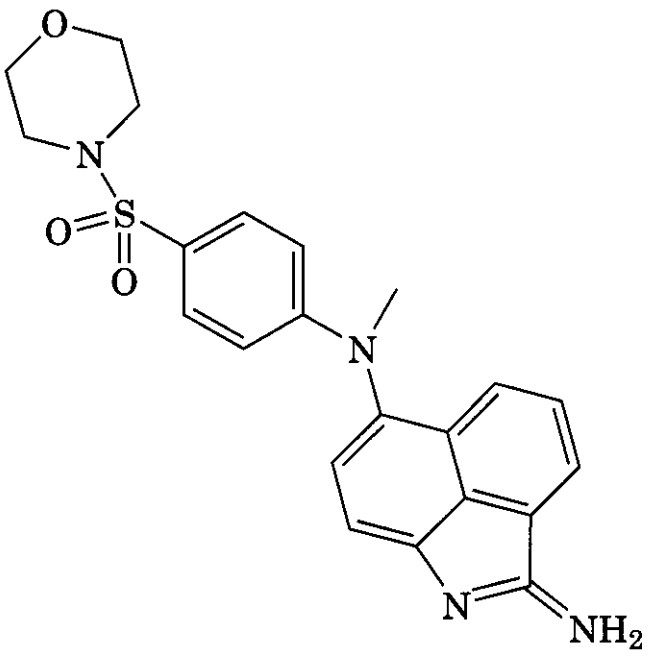


(20)

[*cd*]indole improved the synthetic ease for variation of these groups. Compound (20) had a K_{is} value of 3 μM for inhibition of human TS and was about 10-fold less potent against the bacterial enzyme.

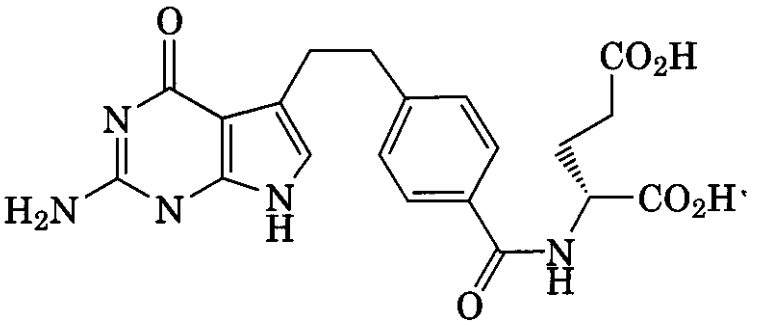
The X-ray structure of (20) bound to E. *coli* TS revealed that the compound actually binds more deeply into the active-site crevice than had been anticipated. Instead of interacting favorably with the enzyme-bound water indicated in Fig. 10.7, the oxygen at position 2 of the benz[*cd*]indole displaces it. This forced the Ala263 carbonyl oxygen to move by about 1 Å. Replacement of the oxygen at position 2 with nitrogen provided a significant increase in inhibitory potency. Structural studies revealed that this also resulted in recovery of the displaced water, and restoration of the original position of the Ala263 carbonyl oxygen. The substituents at position 5, on the tertiary amine nitrogen, and on the sulfonyl group were also varied during the iterative optimization process. The process yielded (21) (AG331), which has a K_{is} value of 12 nM for inhibition of human TS. Compound (21) entered clinical trials as an antitumor agent (71).

2.3.3 Clycinamide Ribonucleotide Formyltransferase. Glycinamide ribonucleotide formyltransferase (GARFT) catalyzes the N-formyla-

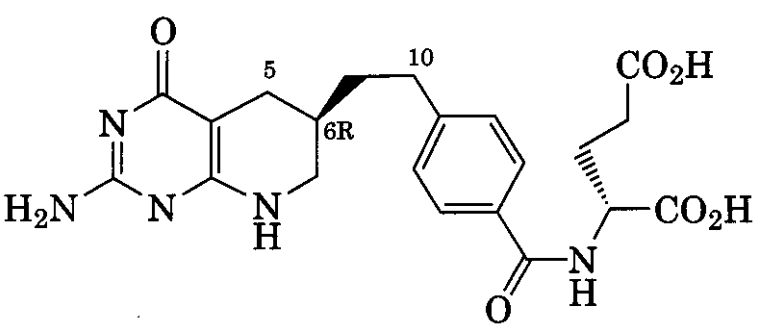


(21)

tion of glycinamide ribonucleotide, through use of *N*-10-formyltetrahydrofolate as the one-carbon donor. Because this is an essential step in the synthesis of purine nucleotides, GARFT is a target for blocking the proliferation of malignant cells. Several potent GARFT inhibitors, such as pemetrexed (22, ALIMTA,



(22) pemetrexed



(23) lometrexol

LY231514) and lometrexol (23, 5,10-dideazatetrahydrofolate, LY-264618), have been shown to be effective antitumor agents in clinical trials (71, 72).

These were designed through traditional medicinal chemistry approaches, in which an-