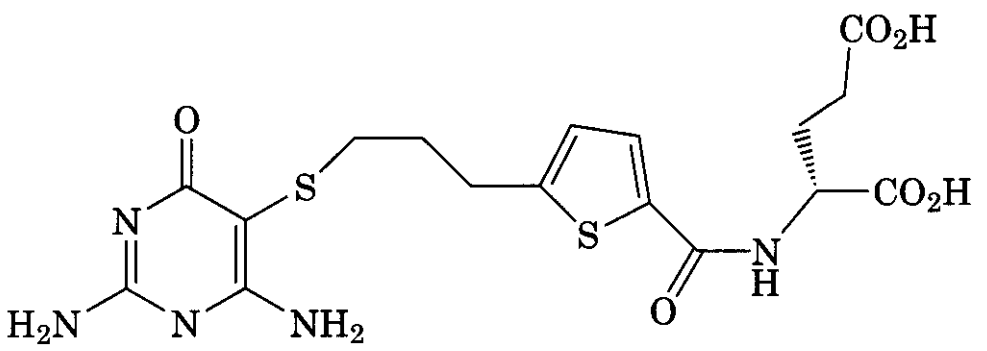
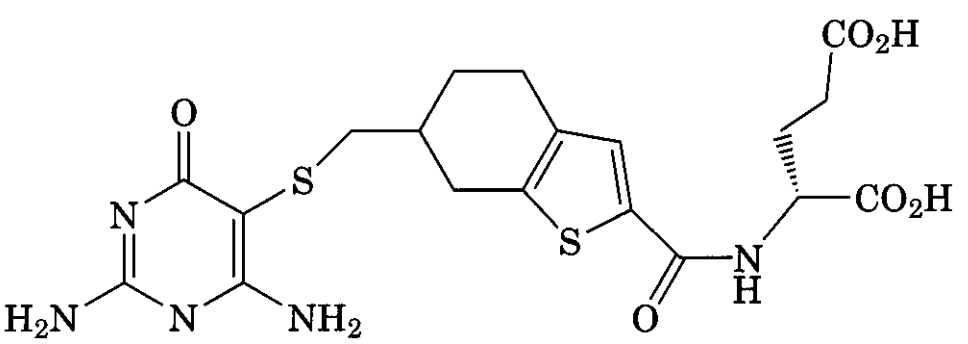


(24)

of (23), including some GARFT inhibitors in which the ring containing N5 was opened (80). Inspection of the structure of the bacterial GARFT–inhibitor complex revealed several important features. The pyrimidine portion of the pteridine was fully buried within the GARFT active site, forming many hydrogen bonds with conserved enzymic groups. The D-glutamate moiety was largely solvent exposed, with no immediately obvious potential for building additional interactions. Retention of the D-glutamate unmodified was also desirable for pharmacodynamic reasons. A significant opportunity was presented by the fact that the active site might accommodate a bulkier hydrophobic atom than the methylene group in 5-deazatetrahydrofolate that replaces the naturally occurring N5 in tetrahydrofolate. To test this idea, a series of 5-thiapyrimidinones were synthesized, including compound (24). These analogs were more readily prepared than the corresponding cyclic derivatives. This compound had a potency of 30–40 n*M* in both a cell-based antiproliferation assay and a biochemical assay for human GARFT inhibition. A crystal structure of human GARFT, complexed with (24) and glycinamide ribonucleotide, confirmed the structural homology between E. *coli* and human enzymes.

Compounds with one fewer methylene in the linker connecting the thiophenyl moiety to the 5-thia position were much less active. Several other analogs, such as (25), were made in attempts to fill the active site more fully, and to restrict the conformational flexibility of the linker. Molecular mechanics calculations failed to correctly predict the conformation on the 5-thiamethylene group of (25) bound to GARFT because of unforeseen conformational flexibility of the enzyme revealed by an X-ray structure of this complex. This again emphasizes the importance of interative experimental confirmation of molecular designs. Several functional criteria in addition to GARFT inhibition and cell-based assays were evaluated during the several cycles of optimization. These included the ability of exogenous purine to rescue cells (which indicates selective GARFT inhibition), and the ability of the inhibitors to function as substrates for enzymes involved in the transport and cellular accumulation of antifolate drugs. Balancing these criteria has resulted in the choice of compounds (26) and (27) (AG2034 and AG2037, respectively) for clinical development at Pfizer. (In 1999, Agouron Pharmaceuticals was acquired by Warner–Lambert, which was subsequently acquired by Pfizer.) It is as yet unclear whether the considerable toxicity of these and other GARFT inhibitors will allow these compounds to be acceptable as anticancer drugs.



(25)