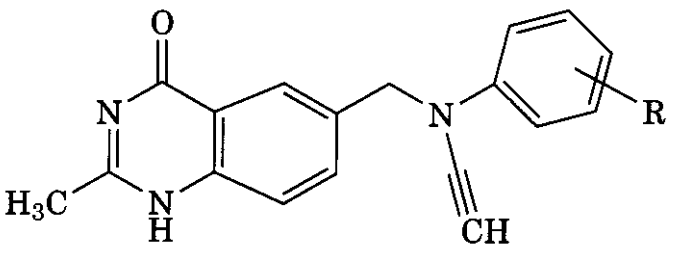
Table 10.1 **SAR for 2-Methyl-4-oxo-quinazoline Inhibitors of *TS*[a]**



(12)–(17)

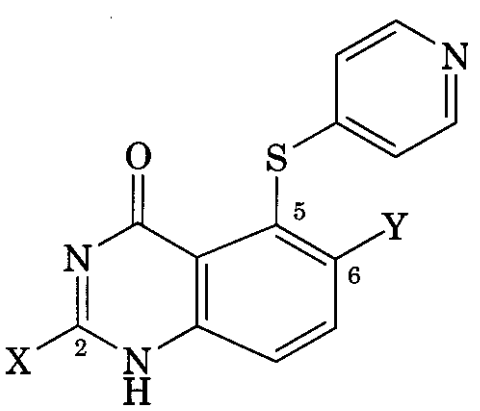
Compound	R	K_{is}, μM (*E. coli* TS)	K_{is}, μM (human TS)
(12)	para-CO-glutamate	0.005	0.009
(13)	H	4	2.2
(14)	*meta*-trifluoromethyl	0.45	0.4
(15)	para-SO,-phenyl	0.025	0.013
(16)	meta-trifluoromethyl, para-SO_2-phenyl	0.037	0.05
(17)	para-SO_2-(N-indolyl)	0.15	0.07

"From ref. 60.

date the best interaction for another. (This is a general problem for rigid scaffolds.) Compounds (**15–17**) had significant activity in ***in vitro*** cell-based assays, which could be reversed by exogenous thymidine. Compound (**17**) (AG85) was tested in human clinical trials for treatment of psoriasis (9).

The structure shown in Fig. 10.6 also suggested another approach to alter the structure of (12) to generate a lipophilic inhibitor of TS. The hydrophobic cavity filled by the aromatic ring of the para-aminobenzoyl group could be filled instead by a substituent attached to position 5 of the quinazoline nucleus. Four different 5-substituted 2-methyl-4-oxoquinazolines were made to test this idea, and one of these (**18**) was a 1 μM inhibitor of human TS (66).

The X-ray structure of the bacterial enzyme with (18) confirmed the hypothetical



(18) X = CH_3, Y = H
(19) X = NH_2, Y = CH_3

binding mode. Two dozen 5-substituted quinazolines were made to explore the SAR for this scaffold. However, the eventual clinical candidate (**19**) was only two steps away from (**18**). The methyl group at position 6 was incorporated for favorable interaction with Trp80. This also favorably restricted the torsional flexibility for the 5-substituent, and increased the inhibitory potency against human TS by 10-fold. The 2-methyl was replaced by an amino group, to create a hydrogen bond to a backbone carbonyl in the protein, and increased potency another sixfold. Compound (19)(AG337, also known as nolatrexed, and as the hydrochloride, Thymitaq) advanced into human testing and had progressed into later-stage clinical trials **as** an antitumor agent by 1996 (67).

2.3.2.2 De Novo Lead Generation: AG331. The ***de novo*** design effort was initiated through the use of a computational method, Goodford's GRID algorithm (68, 69), to locate a site favorable to the binding of an aromatic system within the TS active site (70). Using computer graphics, naphthalene was visualized and manipulated within this favorable site (Fig. 10.7). This facilitated alterations of the naphthalene scaffold to a benz[*cd*]indole to provide hydrogen-bonding groups to interact with the enzyme and a tightly bound water. Elaboration from the opposite edge of the naphthalene core to extend into the top of the