General Distance Geometry Three-Dimensional Receptor Model for Diverse Dihydrofolate Reductase Inhibitors

Arup K. Ghose and Gordon M. Crippen*

Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received April 29, 1983*

A common three-dimensional receptor model has been formulated for six different classes of rat liver dihydrofolate reductase inhibitors using the distance geometry approach. Altogether, 62 molecules of five different classes were used to generate the receptor model, which has 11 attractive site points and 5 repulsive ones. It gave a fit having a correlation coefficient of 0.949 and root mean square (rms) deviation of 0.527. The attractive site points of the model closely correspond to the one we reported earlier. The model successfully predicted the biological data of 33 molecules of 5 different classes, one molecule of which was a member of a new class not included in the original data set. Guidelines are put forth for the synthesis of improved inhibitors.

We have been developing a distance geometry model[1-4] for the inhibition of various dihydrofolate reductases by various inhibitors. In order to explore the site and the species specificity,[5] it is necessary to have inhibition data for the various enzymes for a set of compounds of varied structures. Only the recent data on rat liver dihydrofolate reductase are adequately diverse in inhibitor structures: 2,4-diamino-6,6-dimethyl-5-(substituted-phenyl)-5,6-dihydrotriazines,[6,7] quinazolines,[8-11] 2,4-diamino-5-substituted-benzyl)pyrimidines,[12-15] 2,4-diaminopyrroloquinazolines,[16] 2,4-diamino-6-[(substituted benzyl)amino]pyrido[2,3-*d*]pyrimidines,[9] pterins and pteridines.[10] In this work we extend our triazine- and quinazoline-based model[4] to include all the above molecules. Our methodology is much the same as in our earlier efforts, with one major exception. The usual distance geometry algorithm for finding the geometrically allowed binding modes[2] for a given site and a given molecule runs very fast because it represents the molecule only as a matrix of upper and lower bounds on the interatomic distances. However, this simplified representation permits testing of only *necessary* conditions for an allowed binding mode, although these conditions are not *sufficient*. Heretofore, this has caused no difficulty (the necessary conditions being practically correctly unless proposed binding modes are checked more thoroughly for geometric feasibility. The next section explains in detail the three-dimensional coordinate manipulation package we have devised to overcome the problem.

Methods

As before,[2,3] the basic procedure consists of the following steps: (1) Construct the molecules by joining their constituent fragments as obtained from crystallographic studies.[17] (2) Assume that the binding energy of the ligand comes from the interaction of various atoms of the ligand with some atoms of the receptor sites. Following this idea, make a working hypothesis of the binding modes of the various molecules in terms of which atoms of the ligand interact with which site points. (3) Deduce the site geometry from the structure of the ligand molecules. This problem can be solved in either of the following ways: (i) by using the distance geometry algorithm,[2,3] (ii) hypothesizing an active conformation derived from the conformational analysis of the strong and weak inhibitors,[18] or (iii) examining the crystallographic results on the receptor-bound ligand.[19] A site point either may represent the approximate location of an atom in the ligand when it binds to the site or it may represent the hypothetical or actual location of a site atom or group. Without high-resolution crystallographic data, the first possibility is straightforward, but the second requires the van der Waals radii of the ligand point and the site point. Even then it may be located anywhere on the sphere centered at the ligand point and whose radius is the sum of the van der Waals radii of the site point and the ligand point. However, some of the possibilities may be excluded on steric grounds. For example, if a chlorine atom attached to a benzene ring interacts with the receptor site, the latter should be away from the ring. Finding the exact location of the site has the advantage that even the distance dependencies of the interaction may be studied. (4) Determine the various geometrically possible binding modes, as

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Table I. Molecular Structure and the Observed and Calculated Rat Liver DHFR Inhibition Data of the Various Compounds Used to Construct the Model

no.	groups	log $1/C_{obsd}$	log $1/C_{calcd}$	$\Delta_{calcd-obsd}$
	Triazines (I)			
1	4′-CO_2CH_3	4.26	5.44	1.18
2	3′-SO_2NH_2	4.41	4.72	0.31
3	4′-SO_2NH_2	4.54	4.72	0.18
4	H	5.99	5.44	−0.55
5	4′-CF_3	6.06	6.46	0.40
6	4′-Br	6.24	6.24	0.00
7	3′-OCH_3	6.26	6.62	0.36
8	4′-OCH_3	6.26	6.26	0.00
9	4′-I	6.28	5.44	−0.84
10	4′-CH_3	6.41	6.17	−0.24
11	3′-F	6.42	6.42	0.00
12	4′-F	6.67	6.46	−0.21
13	3′-CH_3	6.81	6.62	−0.19
14	3′-Cl	6.93	6.93	0.00
15	3′-Br	7.06	7.06	0.00
16	3′-I	7.09	5.44	−1.65
17	3′-CF_3	7.10	6.91	−0.19
	Quinazolines (II)			
18	2,4-H_2	2.26	1.96	−0.30
19	2-SH, 4-NH_2	3.72	3.72	0.00
20	2,4-$(OH)_2$	3.89	3.90	0.01
21	2,4-$(NH_2)_2$, 5-$SO_2C_6H_3$-3′,4′-Cl_2	4.24	4.25	0.01
22	2,4-$(NH_2)_2$, 5-SOC_6H_3-3′,4′-Cl_2	4.26	4.26	0.00
23	2,4,6-$(NH_2)_3$	4.57	4.35	−0.22
24	2,4-$(NH_2)_2$, 6-Cl	5.40	5.40	0.00
25	4-NH_2, 6-SO_2-2′-$C_{10}H_7$	5.47	5.78	0.31
26	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-4′-OH	5.54	6.53	0.99
27	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-4′-Br	5.72	6.53	0.81
28	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-4′-F	5.96	6.53	0.57
29	2,4-$(NH_2)_2$, 6-$CH_2NHC_6H_2$-3′,4′,5′-$(OCH_3)_3$	6.18	7.33	1.15
30	2,4-$(NH_2)_2$, 6-$CH_2NHCOC_6H_4$-3′-CF_3	6.59	7.32	0.73
31	2-NH_2, 4-SH, 6-S-2′-$C_{10}H_7$	6.64	6.81	0.17
32	2,4-$(NH_2)_2$, 5-S-C_6H_3-3′,4′-Cl_2	6.80	7.54	0.74
33	2-NH_2, 4-OH, 6-S-C_6H_3-3′,4′-Cl_2	6.92	7.21	0.29
34	2-NH_2, 4-OH, 6-S-2′-$C_{10}H_7$	7.35	7.20	−0.15
35	2-NH_2, 4-OH, 6-SO-2′-$C_{10}H_7$	7.38	7.38	0.00
36	2,4-$(NH_2)_2$, 5-Cl, 6-$NHCO(CH_2)_2$-C_6H_4-4′-Cl	7.44	6.54	−0.90
37	2,4-$(NH_2)_2$, 5-CH_3, 6-$NHCOCH_2$-C_6H_4-4′-Br	7.49	6.53	−0.96
38	2-NH_2, 4-SH, 6-SO_2-2′-$C_{10}H_7$	7.60	7.43	−0.17
39	2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NHC_6H_3$-3′,4′-Cl_2	7.92	7.33	−0.59
40	2-NH_2, 4-OH, 6-SO_2-2′-$C_{10}H_7$	7.96	7.83	−0.13
41	2,4-$(NH_2)_2$, 6-S-C_6H_3-3′,4′-Cl_2	7.96	7.54	−0.42
42	2,4-$(NH_2)_2$, 5-CH_3, 6-$CH_2NHC_6H_4$-4′-$CONHCH(CO_2H)\cdot CH_2CH_2CO_2H$	8.13	8.13	0.00
43	2,4-$(NH_2)_2$, 6-S-2′-$C_{10}H_7$	8.15	7.53	−0.62
44	2,4-$(NH_2)_2$, 5-CH_3, 6-CH_2NH-C_6H_4-3′-Br	8.63	7.33	−1.30
	Pyrroloquinazolines (III)			
45	2,4-$(NH_2)_2$, 7-CH_3	5.82	6.33	0.57
46	2,4-$(NH_2)_2$, 7-$CH_2C_6H_3$-3′,4′-Cl_2	8.47	8.51	0.04
47	2,4-$(NH_2)_2$, 7-$CH_2C_6H_4$-4′-CH_3	9.05	8.50	0.55
	Pyrimidines (IV)			
48	3′,5′-$(OCH_3)_2$, 4′-C(=CH_2)CH_3	2.82	3.57	0.75
49	3′,5′-$(OCH_3)_2$, 4′-CO_2CH_3	3.15	3.57	0.42
50	3′,5′-$(OCH_3)_2$, 4′-$COCH_3$	3.15	3.57	0.42
51	3′,5′-$(OCH_3)_2$, 4′-CH_2OH	3.22	3.57	0.35
52	3′,4′,5′-$(OCH_3)_3$	3.50	3.57	0.07
53	4′-OCH_3	3.77	3.56	−0.21
54	4′-OH	3.80	3.56	−0.24
55	H	3.82	3.56	−0.26
56	4′-CH_3	4.00	3.56	−0.44
57	3′,4′-$(OCH_3)_2$, 5′-Cl	4.00	3.57	−0.43
58	4′-Cl	4.05	3.56	−0.49
59	3′,4′-$(OCH_3)_2$	4.15	4.29	0.14
60	3′-OCH_3	4.37	4.29	0.08
	Pyridopyrimidines (V)			
61	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_5$	6.27	6.28	0.01
62	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_3$-3′,4′-Cl_2	6.28	6.28	0.00

discussed in detail at the end of this section. (5) Finally, determine the interactions between the ligand points and the site points, and then determine the optimal binding mode of each molecule. Since each mode involves a specific

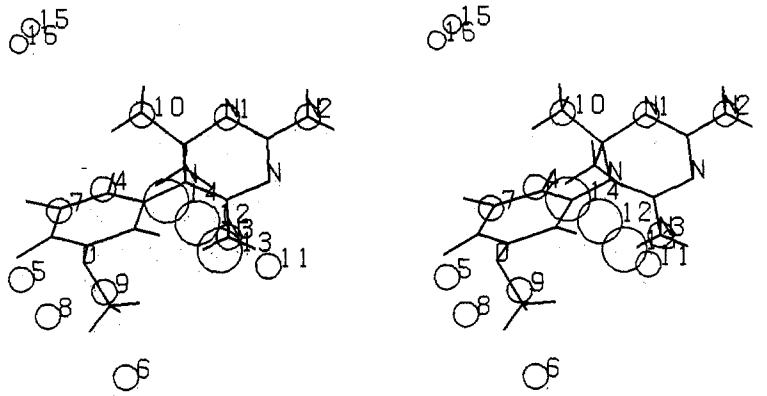

Figure 1. Stereo drawing of the site-bound conformation of 3′-methoxytriazine, molecule **7**. Except for sites 15 and 16, the size of the circles are proportional to their steric size. Sites 15 and 16 were given contracted size for clarity.

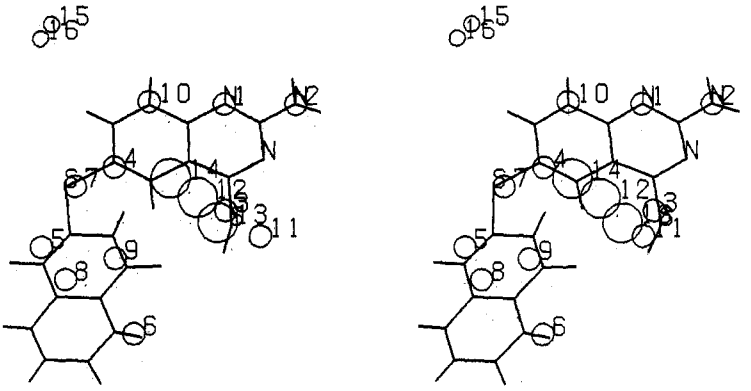

Figure 2. Stereo drawing of the site-bound conformation of a quinazoline, molecule **31**.

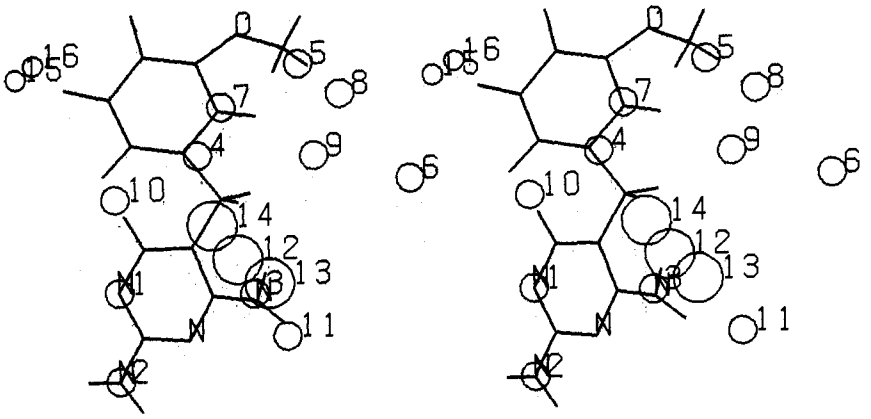

Figure 3. Stereo drawing of the site-bound conformation of a pyrimidine, molecule **60**.

conformation, the binding energy of a particular mode is

$$E_{\text{calcd}} = cE_c + \sum x_{t_i,t_m}$$

where E_c is the conformational energy, c is a coefficient to be determined, and the x's are the interactions of the site i with the bound ligand point m, which depend on their types, t. These interactions may either be evaluated by quadratic programming[2,20] or they can be correlated with some physicochemical parameters of the ligand points, as is done in Hansch's approach. We adjust the interaction energies by quadratic programming, rather than by simple least-square fitting, only because we do not know the binding mode of the molecules until we have the interaction energies, since among several geometrically allowed binding modes, the actual binding mode should be the one that is energetically most favorable. On the other hand, the interaction energies are evaluated on the basis of some supplied binding mode so as to give the least-squares deviation between the calculated inhibition of the supplied mode and the observed inhibition. This interlocking problem is solved by the procedure reported earlier.[3]

There are numerous binding modes of even a rigid molecule corresponding to combinations of translations or rotations, and many more for flexible molecules. Indeed, some variability of binding mode is observed experimentaly. However, we have to be somewhat conservative with our sketchy site model by assuming that many of the translations and rotations will be restricted by steric requirements or because such alternative modes will not lead to the biological response. One approach we have used is to construct a large repulsive sphere to define a general binding slot.[21] A more economical method we have employed in this study is to choose three noncolinear site

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Table II. Description of the Site Points[a]

site	correspondence with previous site points[b]	binds
1	1	N_1 of the key heterocyclic ring of the various inhibitors
2	2	the substituents at the 2-position of the key heterocyclic ring
3	3	the substituents at the 4-position of the key heterocyclic ring
4	4	the C'_6 of the Ph ring of the triazines or the C'_1 of the Ph ring of the pyrimidines; however, it cannot bind the 5-substituents of the quinazolines and related compds
5	5	4′-substituents of the triazines, C'_6 of the Ph ring of various quinazolines and related compds or the 2nd atom of the 3′-substituent of the pyrimidines
6	6	4′-substituents of the quinazolines and related compds
7	c	C'_5 of the triazines or C'_2 or 6-substituents of the quinazolines and related compds
8	8	the 2nd atom of the 3′- or 4-′substituents of the triazines
9	9	the 1st atom of the 3′-substituents of the triazines
10		6-substituents of the triazines or pyrimidine or the corresponding 2nd ring atom of the quinazolines and related compds
11		α-carbonyl group of the glutamic acid moiety of some quinazoline and pteridine derivatives

[a] Site points 12–16 are repulsive site points to exclude some binding modes of the pyrimidines. [b] Reference 4. [c] In our previous study we placed this site point so as to bind the C'_6 of the triazines.

Table III. Coordinates of the Site Points

site point	x, Å	y, Å	z, Å
1	0.000	0.000	0.000
2	2.811	0.000	0.000
3	0.037	3.967	0.000
4	−3.508	2.670	0.559
5	−5.833	4.260	−1.841
6	−2.882	5.508	−5.296
7	−4.749	3.084	0.042
8	−5.068	4.536	−3.471
9	−3.482	3.816	−3.340
10	−2.392	−0.074	0.042
11	1.141	0.558	−7.553
12	−0.851	1.187	−3.966
13	−0.221	2.116	−3.972
14	−1.716	0.519	−3.861
15	−5.505	−2.271	1.191
16	−5.854	−2.203	0.409

points and three corresponding rigid points in the ligand. The approximate superposition of these three ligand points on the specified site points by rigid translation and rotation leads to a basic mode. Each basic mode can represent many binding modes if there are rotatable bonds in the molecule. For each sterically allowed[2] conformation arising from the rotation of these bonds, the distances of the site points from each ligand point are evaluated. If a ligand point lies within some specified range of a site point, the points are considered to be in contact. If the site point represents the coordinates of the ligand point necessary to interact with the site, this range should vary from zero to some small positive value, δ, which represents the site flexibility. On the other hand, if the site point represents a real atom of the receptor site, then the range of binding should vary from $r_s + r_m - \delta$ to $r_s + r_m + \delta$, where r_s and r_m are the radii of closest approach of the site and ligand points, respectively. If the distance is lower than the lower limit, there should be strong repulsion, and the binding mode should be sterically disallowed. The distance range criterion for contact is computationally very simple and models the slight rearrangements the real site atoms and ligand atoms presumably make for optimal interaction. Here we allow the interaction of only one ligand point with any site point, although that could be easily modified. We neglect the conformation energy contribution to the binding energy, since Hopfinger[18] found that conformational energy does not have significant correlation to the observed binding energy.

The enormous number of possible conformations is the greatest problem in any three-dimensional structure directed QSAR. Molecules having three torsion angles or fewer can be analyzed easily, but the computer time required increases exponentially with the number of torsion angles and very rapidly with the number of values each torsion angle can assume. In order to keep the calculations to a manageable level, we held to the following guidelines. (i) If two molecules have some structurally comparable rotatable bonds, the selection of torsion angle values should be the same. This assumes consistency in the binding modes of similar molecules. (ii) For molecules with only two or fewer rotatable bonds and a large substituent like a benzene or naphthalene ring on one side, we used 10° increments in the torsion angles. (iii) For three rotatable bonds we used 20° increments: otherwise, we used 30° increments. (iv) Clearly an n-fold symmetric group, such as methyl ($n = 3$), needs only to be rotated over a total of $360/n$ degrees. (v) Since a peptide bond has a high 2-fold rotational barrier, only the cis and trans conformations were considered. An alternative approach that we did not pursue, would be to make thorough conformational analyses of the molecules and use only the low energy conformations in all successive steps.

Results and Discussion

The molecular structures of the 62 molecules used to construct the present model, together with their observed and calculated inhibition data, are given in Table I. Among these 62 molecules, the 17 triazines were reported by Dietrich et al.[6] and Hansch et al.;[7] the 27 quinazolines were reported by Fukunaga et al.,[8] Richter et al.,[9] McCormack et al.,[10] and Hynes et al.;[11] the pyrroloquinazoines were reported by McCormack et al.;[16] the pyridopyrimidines were reported by Richter et al.;[9] and the pyrimidines were reported by Roth et al.[13,14] and Kompis et al.[15] While searching for pyrimidine data, we found that the log $1/I_{50}$ for trimethoprim (**52**) differs considerably from author to author: Dietrich et al.[6] found 4.22 (4.17–4.26), where the range within parentheses is for the 95% confidence interval; Kompis et al.[15] found 3.30; Roth et al.[14] found 3.37–3.59 (range of six determinations over a period of time); and Hitchings et al.[12] found 3.59. The inhibition assay conditions were found to differ slightly but definitely with respect to the pH of the buffer, tem-

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Table IV. The Best Fitted Binding Modes

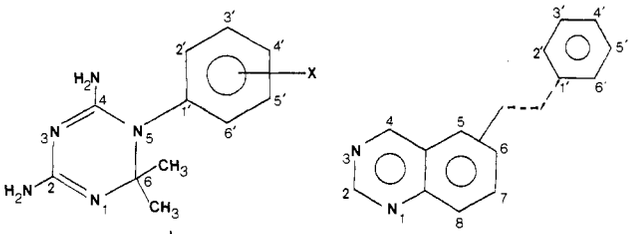
molecule[a]	site points										
	1	2	3	4	5	6	7	8	9	10	11
1	N_7	N_6	N_3	C_6	0	0	C'_5	E_4	0	C_{10}	0
2	N_7	N_6	N_3	0	0	0	C'_4	0	0	C_{10}	0
3[b]	N_7	N_6	N_3	0	D_4	0	C'_4	0	0	C_{10}	0
4	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
5	N_7	N_6	N_3	C'_6	B_4	0	C'_5	0	0	C_{10}	0
6	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
7	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	B_3	C_{10}	0
8	N_7	N_6	N_3	C'_6	A_4	0	C'_5	D_4	0	C_{10}	0
9	N_7	N_6	N_3	C'_6	0	0	C'_5	0	A_3	C_{10}	0
10	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
11	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
12	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
13	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
14	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
15	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	A_3	C_{10}	0
16	N_7	N_6	N_3	C'_6	A_4	0	C'_5	0	0	C_{10}	0
17	N_7	N_6	N_3	C'_6	A_4	0	C'_5	B_3	A_3	C_{10}	0
18	N_1	X_2	0	0	0	0	0	0	0	X_8	0
19	N_1	X_2	X_4	0	0	0	0	0	0	X_8	0
20	N_1	X_2	X_4	0	0	0	X_6	0	0	X_8	0
21	N_1	X_2	X_4	0	C'_3	0	X_6	0	0	X_8	0
22	N_1	X_2	X_4	0	C'_5	X'_3	X_6	C'_4	0	X_8	0
23	N_1	X_2	X_4	0	0	0	X_6	0	0	X_8	0
24	N_1	X_2	X_4	0	0	0	X_6	0	0	X_8	0
25[c]	N_1	X_2	X_4	0	C'_2	0	X_6	C'_3	C'_5	X_8	0
26[d]	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	H'_2	X_8	0
27[d]	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	H'_2	X_8	0
28[d]	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	H'_2	X_8	0
29	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	C'_3	0	X_8	0
30[e]	N_1	X_2	X_4	0	C	0	X_6	C'_1	C'_6	X_8	0
31	N_1	X_2	X_4	0	C'_2	X'_4	X_6	C'_3	C'_5	X_8	0
32	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	C'_3	X_8	0
33	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	C'_3	X_8	0
34[f]	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_5	X_8	0
35	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	0	X_8	0
36	N_1	X_2	X_4	0	C'_2	0	X_6	C'_3	C'_4	X_8	0
37	N_1	X_2	X_4	0	C'_2	0	X_6	C'_3	0	X_8	0
38[f]	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_5	X_8	0
39	N_1	X_2	X_4	0	C'_2	0	X_6	C'_6	X'_6	X_8	0
40[f]	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_5	X_8	0
41	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	C'_3	X_8	0
42[g]	N_1	X_2	X_4	0	H	0	X_6	0	C'_6	X_8	0
43[f]	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_5	X_8	0
44	N_1	X_2	X_4	0	C'_1	0	X_6	C'_6	X'_6	X_8	0
45	N_1	X_2	X_4	0	0	0	N_7	0	0	C_9	0
46	N_1	X_2	X_4	0	C'_2	0	N_7	C'_3	0	C_9	0
47	N_1	X_2	X_4	0	C'_2	0	N_7	0	0	C_9	0
48	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
49	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
50	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
51	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
52	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
53	N_1	X_2	X_4	C'_1	0	0	C'_2	0	0	H_6	0
54	N_1	X_2	X_4	C'_1	0	0	C'_2	0	0	H_6	0
55	N_1	X_2	X_4	C'_1	0	0	C'_2	0	0	H_6	0
56	N_1	X_2	X_4	C'_1	0	0	C'_2	0	0	H_6	0
57	N_1	X_2	X_4	0	0	0	0	0	Y'_3	H_6	0
58	N_1	X_2	X_4	C'_1	0	0	C'_2	0	0	H_6	0
59	N_1	X_2	X_4	C'_4	Y'_3	0	C'_2	0	0	H_6	0
60	N_1	X_2	X_4	C'_4	Y'_3	0	C'_2	0	0	H_6	0
61	N_1	X_2	X_4	0	C'_1	0	X_6	C'_6	X'_6	X_8	0
62	N_1	X_2	X_4	0	C'_1	0	X_6	C'_6	X'_6	X_8	0

[a] The molecules 1–17 correspond to reference structure VI, 18–44 and 61 and 62 correspond to reference structure VII, 45–47 correspond to reference structure VIII, and 48–60 correspond to reference structure IX. [b] D_4 represents one oxygen of the SO_2NH_2 group. [c] X_4 for this molecule stands for one hydrogen attached to the 4-NH_2 groups. [d] H'_2 represents the H atom attached to C'_2. [e] C represents the C atom of the CO group. [f] Y'_4 stands for the corresponding hydrogen attached to the second naphthalene ring. [g] H represents the hydrogen atom of the NH group and O represents the OH oxygen of the α-carbonyl group of the glutamic acid moiety.

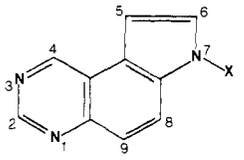
perature, substrate, and cofactor concentrations. We simply took the intermediate value where more than one datum was available for a single compound.

The molecular structures were generated from our

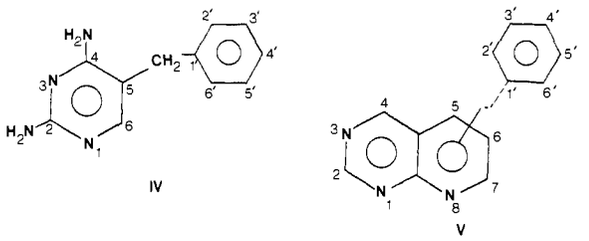
crystallographic fragment library as usual.[2,3] The pyrimidine ring structure was taken from Wheatley,[22] pyridopyrimidines were generated from the structure of quinazoline[23] and quinoline,[24] and pyrroloquinazolines were generated from the crystal structure of quinazoline[23] and indole.[25]



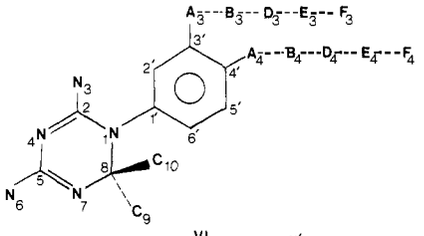
I II



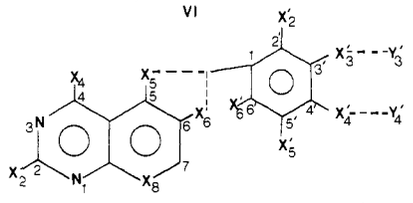
III



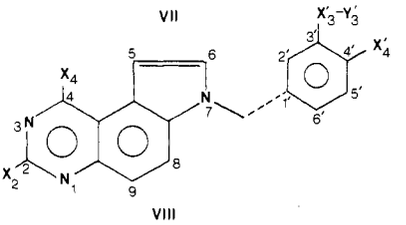
IV V



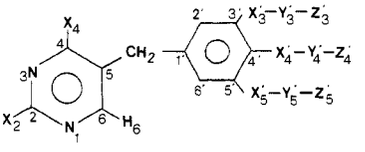
VI



VII



VIII



IX

Our line of reasoning for the roles and positions of the site points is as follows. We started with our previous site model[4] that accounted for the binding of triazines and quinazolines and introduced some pyrimidine derivatives (IV) which were a very weak inhibitors of rat liver dihydrofolate reductase, and pyrido[2,3-*d*]pyrimidines (V) and pyrroloquinazolines (III), which are structurally very similar to the quinazolines. The previous model showed that the two ring systems in the triazine derivatives (I) were almost perpendicular to each other, in accord with experiment[26,27] and theory.[18] Our site points represent the approximate locations and critical atoms in a ligand molecule bound at the receptor site. To generate the binding modes we placed the ring nitrogen (N1) at site point 1, the 2-substituent at site 2, and the 4-substituent at site 3. Stereo views of the site points and the placement of three representative triazine, quinazoline, and pyrimidine derivatives are shown in Figures 1–3. While calculating the binding modes of the pyrimidines, we noted that the phenyl ring and its substituents could easily reach the site points 5, 8 and 9. To avoid the mode and its much exaggerated binding energy, we added the three 1.25-Å sterically repulsive site spheres 12, 13, and 14 in the neighborhood of the 3′-hydrogen or other substituents of the corresponding conformation. Three were necessary, since the substituents can go to the site points 5, 8, and 9 even when the phenyl ring is somewhat tilted. Even after that we faced the problem that even then the phenyl ring for all the pyrimidines in the optimal conformation can reach site points 4 and 7, and the 3′-methoxy substituent can reach the site point 5, which gave all pyrimidines almost the same binding energy. However, the experimental binding energy of 3′,4′,5′-trimethoxypyrimidine is lower than that of the 3′-methoxypyrimidine, yet the 4′-methoxy has almost no effect on the binding energy. All these facts can be explained if we assume some additional sterically repulsive site points in the place of the 5′-substituent in the corresponding conformation. Hence, we generated two more repulsive site points, 15 and 16, both having a 1.25-Å steric radius. In order to account for the effect of the second ring of the quinazolines and various other dihydrofolate reductase inhibitors, we generated the attractive site point 10. Modeling the interaction of the amino acid residue in molecules **42** and **95** required another attractive site point, 11, placed according to the results of Piper et al.[28] They found that pigeon liver dihydrofolate reductase requires a free α-carboxyl or spatially equivalent free carboxyl in methotrexate analogues, whereas the free γ-carboxyl did not appear to be essential for the binding. We therefore placed site point 11 so as to bind the α-carboxyl group, assuming that the glutamic acid moiety maintains the same conformation as in the crystal structure of glutamic acid hydrochloride,[29] where for ligand **42** the benzene ring and the amide group all are planar and the CONR group is anti, since the cis conformation suffers from some steric overlap. The description of the site points is given in Table II, and the Cartesian coordinates are given in Table III. The binding modes were determined on the basis of a site flexibility of 0.7 Å.

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Table V. Apparent Interaction Energy[a] of the Dihydrofolate Reductase Site Points with the Various Molecular Points

ligand point type	site points 1	2	3	4	5	6	7	8	9	10	11
1. H											
2. C (sp^3)					0.724		0.803		1.179	1.880	
3. O[b]					0.820						
4. N[c]		0.121									
5. S[d]		1.761	1.543				1.001				
6. Cl						0.004	1.047		1.490		
7. F					1.021			0.286	0.980		
8. Br					0.800				1.620		
9. I											
10. C (sp^2)				0.724	2.167		0.442	0.009	0.01	1.959	
11. N[e]							1.979			1.705	
12. N[f]	2.273										
13. S[g]							1.187				
14. S[h]					*[k]		1.630		*[k]		
15. C[i]					*[k]						
16. O[j]			1.938								2.964

[a] All entries are in units of log $(1/C_{50})$. [b] Etheral. [c] Basic amino. [d] S or SH. [e] Nonbasic ring nitrogens. [f] Protonated positive nitrogen. [g] SO. [h] SO_2. [i] Carbonyl carbon. [j] Hydroxyl. [k] These interactions were set to the slightly repulsive value of −1.000 after the optimization procedure, which gave a better fit for the first three triazine molecules.

Table VI. Statistics of the Study

study	no. of compds	no. of interaction parameters	correl. coeffs	std deviation	max error
original data[a]	62	30	0.949	0.527	1.65
original data[b]	62	30	0.943	0.554	1.65
test data	33	0	0.844	0.700	1.81

[a] If the three interactions of Table V marked by an asterisk are made slightly repulsive. [b] If the three interactions of Table V marked by an asterisk are kept to the default attractive value of 0.001, as was obtained in the optimization procedure.

Table I shows that the model explained the data very well for the 17 triazines, except for compounds 1, **9**, and 16. Compounds **9** and 16 failed because their longer C–I bond placed the iodine far beyond the ordinary substituents, which interact with site points 5 and 9. If we modified our algorithm to take van der Waals radius into account during binding, the larger iodine atom would also interact.

Looking further in Table I, we see the agreement for the quinazolines (**18–44**) is good, except where there are 5-Cl or 5-CH_3 substituents. In these cases, the calculated binding energy is substantially underestimated, indicating a considerable interaction of these substituents with the receptor site. The pyrimidines (**48–60**) showed three levels of calculated binding energies, all of which are much lower than the triazine or quinazoline derivatives. The 3′,5′-disubstituted pyrimidines are expected to have a lower binding energy than 3′- or 3′,4′-disubstituted derivatives.

The binding modes of the triazines (Table IV, molecules 1–17) show a consistent pattern, except for the first three molecules, which satisfies the initial hypothesis of the binding of these molecules. In the first three molecules, the phenyl ring undergoes some rotation to avoid the repulsive interactions. Other minor deviations are seen in some molecules like **5**, where the second atom of the 4′-substituent binds to site point 5, or in molecule **7**, where the second atom of the 3′-substituent binds to site point 9.

Site points 4, 7, 8, and 9 were constructed for the triazines, but they happened to interact with quinazoline substituents. Site point 4 is unused by all quinazolines (see Table IV binding modes, molecules **18–44**) because the 5-substituent of the quinazoline is planar to the key heterocyclic ring, while the C'_6 of the triazines goes out of plane. We did not seek an intermediate position that can satisfy both the 5′-substituent of the quinazolines and the C'_6 of the triazines, although that would improve the calculated binding of the former. Site point 7 is always occupied by the 6-substituents. Site point 8 is found to be occupied by one carbon of the phenyl ring, which one being determined by the number of atoms separating the phenyl and the quinazoline ring as well as the place of substitution. If only one atom separates them and the substitution is at the 5-position of the quinazoline ring system, it is the para carbon (C'_4) of the phenyl ring that interacts. On the other hand, if the substitution is at the 6-position, it is the meta carbon (C'_3 or C'_5). If two atoms separate the rings and the substitution is at the 6-position, then it is the ortho carbon of the phenyl ring (C'_2 or C'_6); if three atoms separate them from the 6-position of the quinazoline ring, it is the first carbon (C'_1) of the phenyl ring. However, more than a three-atom separation does not throw the phenyl ring beyond this site point, since the torsion angles are not rigid. For example, in molecule **36** we find that the meta carbon (C'_5) binds to this site point. Site points 8 and 9 were generated to bind atoms that were separated by single bonds (of the 3′-substituents of triazines). We see from Table IV that although it is true in many cases, they can also bind atoms that are separated by two bonds. Site point 5 binds either the C'_1 atom or the ortho carbons (C'_2 or C'_6); in any case, it is the adjacent atom that is bound to site 8. In fact, site points 5, 8, and 9 bind the phenyl ring of the quinazolines, although the ring may have different relative orientations. Such differences in the relative orientations do not allow the consistent binding of site 6 with X'_4. The binding with X'_4 is possible only when site point 5 binds C'_2, site point 8 binds C'_3, and site point 9 binds C'_5. In molecules like **34**, **38**, **40**, and **43**, where the quinazoline and naphthalene rings are separated by one atom, the 4′-substituent can reach site point 6.

The binding modes of the pyrroloquinazolines (**45–47**) demonstrate that although the phenyl ring can reach site points 5 and 9, the substituents cannot. The binding of the pyridopyrimidines (**61** and **62**) are very similar to the quinazolines. The pyrimidines (**48–60**) show three distinct binding modes. The 3′,4′,5′-trisubstituted pyrimidines with a methoxy group at the 3′-position allowed the carbon of the methoxy group to reach site point without allowing the C'_1 and C'_2 carbons to interact with site points 4 and 7.

Table VII. Molecular Structure and the Observed and Calculated Rat Liver DHFR Inhibition Data of the Various Compounds Used to Test the Predictive Power of the Model

no.	groups	log $1/C_{obsd}$	log $1/C_{calcd}$	$\Delta_{calcd-obsd}$
	Triazines (I)			
63	4′-$CO_2C_2H_5$	4.44	5.44	1.00
64	4′-$COCH_3$	5.25	4.72	−0.53
65	3′-$COCH_3$	5.35	5.44	0.09
66	3′-$CO_2C_2H_5$	5.69	5.44	−0.25
67	4′-$C(CH_3)_3$	6.40	6.17	−0.23
68	3′-$C(CH_3)_3$	6.89	6.62	−0.27
	Quinazolines (II)			
69	4-NH_2, 6-S-2′-$C_{10}H_7$	4.68	5.15	0.47
70	4-NH_2, 6-SO-2′-$C_{10}H_7$	4.82	5.32	0.50
71	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-4′-CH_3	5.32	6.53	1.21
72	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-3′-OH	5.47	6.53	1.06
73	2-SH, 4-NH_2, 6-S-2′-$C_{10}H_7$	5.52	5.90	0.38
74	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-3′-CH_3	5.59	6.53	0.94
75	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-4′-Cl	5.66	6.53	0.87
76	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-2′-OH	5.72	6.53	0.81
77	2-OH, 4-NH_2, 6-S-2′-$C_{10}H_7$	5.77	5.14	−0.63
78	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_5$	5.82	6.53	0.71
79	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-2′-CH_3	5.89	7.70	1.81
80	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-3′,4′-Cl_2	6.10	6.53	0.43
81	2,4-$(NH_2)_2$, 6-$N(CH_3)$-2′-$C_{10}H_7$	6.10	6.52	0.42
82	2,4-$(NH_2)_2$, 6-$NHCH_2C_6H_4$-3′-Br	6.22	6.53	0.31
83	2,4-$(NH_2)_2$, 7-CH_3, 6-$NHCH_2C_6H_5$	6.40	6.53	−0.13
84	2,4-$(NH_2)_2$, 6-$CH_2NHC_6H_4$-3′-Br	6.60	7.33	0.73
85	2,4-$(NH_2)_2$, 5-CH_3, 6-$NHCH_2C_6H_3$-3′,4′-Cl_2	6.96	6.53	−0.43
86	2,4-$(NH_2)_2$, 6-$N(CH_3)$-1′-$C_{10}H_6$-2′-Cl	7.03	6.52	−0.51
87	2-NH_2, 4-OH, 6-$SO_2C_6H_3$-3′,4′-Cl_2	7.12	7.83	0.71
88	2,4-$(NH_2)_2$, 5-Cl, 6-$NHCH_2C_6H_5$	7.40	6.53	−0.87
89	2,4-$(NH_2)_2$, 6-SC_6H_4-3′-CF_3	7.44	7.53	0.09
90	2,4-$(NH_2)_2$, 6-$SO_2C_6H_4$-3′,4′-Cl_2	8.05	8.16	0.11
91	2,4-$(NH_2)_2$, 6-SO-2′-$C_{10}H_7$	8.15	7.72	−0.43
92	2,4-$(NH_2)_2$, 6-SO_2-2′-$C_{10}H_7$	8.40	8.16	−0.24
	Pyrroloquinazoline (III)			
93	2,4-$(NH_2)_2$, 7-$CH_2C_6H_2$-3′,4′,5′-$(OCH_3)_3$	8.35	9.69	1.34
	Pyrimidine (IV)			
94	3′,5′-$(OCH_3)_2$, 4-OH	4.00	3.56	−0.44
95	methotrexate	8.13	7.88	−0.25

The second mode is observed for the 4′-substituted pyrimidines. Here C'_1 goes to site 4 and C'_2 goes to site 7 (see Figure 1). Pyrimidines having a 3′-methoxy group but no 5′-substitution also have a similar mode, but, in addition, the carbon of the 3′-methoxy interacts favorably with the site point 5.

The interaction energies of the site points with various molecular points are given in Table V. All other interactions, either used or unused, were given the slightly attractive value of 0.001. The hydrogen atom interactions were held fixed at 0.001, and all other interactions appearing in the objective function were adjusted by the quadratic programming.[3] The qualitative picture of the interactions is very similar to the one we reported earlier.[4] Only when protonated does the ring nitrogen have a very large interaction with site point 1. The interactions of site point 2 differ from those reported earlier in that the basic amino group does not interact strongly, and the interaction of the hydroxyl group is even smaller. Although this is qualitatively comparable to our earlier studies, the high interaction of the SH group does not conform to our earlier report. Site point 3 showed large attractions for hydroxyl as well as thiol groups, but it is only feebly attractive to an amino group. This suggests that the interaction of the 2,4-diamino-substituted ring comes mainly from the interaction of the protonated ring nitrogen with site point 1. The intermediate activity of various non-diamino compounds is due to the absence of the interaction between the protonated nitrogen and site 1, which, however, is partly compensated by the presence of one interaction at site 2 or 3. Site point 5 interacts strongly with double-bonded carbons and only moderately with several other types of atoms. This feature suggests that biphenyl or 2-naphthyl derivatives of the triazines may have some enhanced activity. Since in all our studies we found a strong interaction of hydroxyl group with site 3, it is possible that 2-amino-4-hydroxytriazine derivatives may have some enhanced potency. Unlike other classes of compounds, the basicity of the ring nitrogen, N1, may not decrease by the replacement, especially since the triazine ring does not have the stable aromatic sextet. In fact we have undertaken an extensive molecular orbital calculation to examine the change of basicity of the N1 nitrogen by the different substituents. The strong interaction of the pyrrole ring nitrogen with site point 7 suggests synthesizing more derivatives of this class. Introduction of one more CH_2 group between the phenyl group and the pyrroloquinazoline group may help. The strong interaction of site 11 with the free α-carboxyl group of the glutamic acid moiety also suggests the introduction of this group in the 4′-position of the phenyl ring. The glutamic acid moiety may also be introduced at the 7-position of the naphthalene ring in 2-naphthyl derivatives of the triazines, as suggested earlier. The strong interaction of various ligand points with site 9 may be exploited by substitution at the phenyl ring. The place of substitution should be one

Table VIII. The Best Fitted Binding Modes of the Test Molecules

molecule	site points										
	1	2	3	4	5	6[a]	7	8	9	10	11
63	N_7	N_6	N_3	C'_6	0	0	C'_5	0	F_4	C_{10}	0
64	N_7	N_6	N_3	0	D_4	0	C'_4	0	0	C_{10}	0
65	N_7	N_6	N_3	C'_6	A_4	D_3	C'_5	0	A_3	C_{10}	0
66	N_7	N_6	N_3	C'_6	A_4	D_3	C'_5	B_4	A_3	C_{10}	0
67	N_7	N_6	N_3	C'_6	A_4	0	C'_5	B_4	A_3	C_{10}	0
68	N_7	N_6	N_3	C'_6	A_4	0	C'_5	D_3	A_3	C_{10}	0
69	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_3	X_8	0
70	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	0	X_8	0
71	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
72	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
73	N_1	X_2	X_4	0	C'_2	Y'_4	0	0	C'_3	X_8	0
74	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
75	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
76	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
77	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_3	X_8	0
78	N_1	X_2	X_4	0	C'_1	0	X_6	C'_6	X'_6	X_8	0
79	N_1	X_2	X_4	0	C'_1	0	X_6	0	X'_2	X_8	0
80	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
81	N_1	X_2	X_4	0	C'_6	0	X_6	0	0	X_8	0
82	N_1	X_2	X_4	0	C'_2	0	X_6	C'_3	0	X_8	0
83	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
84	N_1	X_2	X_4	0	C'_1	0	X_6	C'_6	X'_6	X_8	0
85	N_1	X_2	X_4	0	C'_2	0	X_6	0	C'_6	X_8	0
86	N_1	X_2	X_4	0	C'_2	0	X_6	0	0	X_8	0
87	N_1	X_2	X_4	0	C'_6	0	X_6	0	C'_3	X_8	0
88	N_1	X_2	X_4	0	C'_1	0	X_6	C'_2	0	X_8	0
89	N_1	X_2	X_4	0	C'_2	0	X_6	C'_5	0	X_8	0
90	N_1	X_2	X_4	0	C'_6	0	X_6	0	C'_3	X_8	0
91	N_1	X_2	X_4	0	C'_6	0	X_6	C'_5	0	X_8	0
92	N_1	X_2	X_4	0	C'_2	Y'_4	X_6	0	C'_5	X_8	0
93	N_1	X_2	X_4	0	C'_2	0	N_7	C'_3	Y'_3	C_9	0
94	N_1	X_2	X_4	C'_1	Z'_3	0	C'_2	0	0	H_6	0
95	N_1	X_2	X_4	0	0	0	X_6	0	C'_6	X_8	O[b]

[a] Y'_4 represents the H atom. [b] It is the oxygen of the α-carboxyl group in the glutamic acid moiety.

Table IX. Molecular Structure and the Observed and Calculated Rat Liver DHFR Inhibition Data of the Various Compounds the Model Failed To Predict Successfully

no.	groups	log $1/C_{obsd}$	log $1/C_{cald}$	Δ[a]
	Pteridines (X)			
96	2-NH_2, 4-OH, 6-$SCH_2C_6H_5$	4.00	6.94	2.94
97	2,4-$(NH_2)_2$, 6-$SCH_2C_6H_5$	4.15	7.28	3.13
98	2-NH_2, 4-OH, 6-SC_6H_4-4′-CH_3	4.60	6.94	2.34
99	2,4-$(NH_2)_2$, 6-SC_6H_5	4.70	7.29	2.59
100	2-NH_2, 4-OH, 6-SC_6H_5	4.89	6.95	2.06
101	2,4-$(NH_2)_2$, 6-SC_6H_4-4′-CH_3	5.04	6.28	1.24
102	2,4-$(NH_2)_2$, 6-SC_6H_4-2′-$CH(CH_3)_2$	6.26	7.28	1.02
	Quinazolines (II)			
103	2-SH, 4-OH, 6-S-2′-$C_{10}H_7$	5.00	7.84	2.84
104	2,4-$(SH)_2$, 2-S-2′-$C_{10}H_7$	5.05	7.44	2.39
105	2,4-$(OH)_2$, 2-S-2′-$C_{10}H_7$	5.07	7.08	2.01
106	2-OH, 4-SH, 6-S-2′-$C_{10}H_7$	5.24	6.68	1.44
107	2,4-$(NH_2)_2$, 6-$CH_2NHC_6H_4$-2′-Cl	6.62	8.81	2.19

[a] Calculated − observed.

carbon removed from the one that goes to site 5 (see Table IV).

The statistics of the fit of this model are given in Table VI. The correlation coefficient of the original data set is 0.949 and the root mean square (rms) deviation is 0.527. The fit, however, is slightly worse if the three repulsive interactions of Table V are given the attractive value of 0.001. Under that condition, the correlation coefficient is 0.943 and the rms deviation is 0.554. Table VI also gives the statistics of fit of the predicted molecules. The molecular structures of the 33 compounds that were successfully predicted by this model are presented in Table VII. These compounds showed a correlation coefficient of 0.844 and rms deviation of 0.700. The binding modes of these compounds are given in Table VIII. Comparing the calculated and observed binding energies, we find good agreement, except for molecules **79** and **93**. The calculated binding energies for these two molecules are greatly exaggerated. One reason for the low binding energies for these molecules may be that they are ortho-substituted benzene derivatives, which may make the conformation necessary for the binding mode energetically unfavorable, a feature that has been overlooked in the present algorithm. It would be, in fact, interesting to synthesize and check the activity of 3′-methoxybenzylpyrroloquinazoline of the type III, since the interaction of the 3′-methoxy group boosted its calculated binding energy. In that case no such conformational complication is expected.

The model, however, failed for some compounds (Table IX) falling into three major classes: 2,4-non-aminoquinazolines, 6-thio derivatives of pterines or pteridine, and 2′-substituted derivatives of quinazoline. Perhaps the non-aminoquinazolines bind in a different mode, due to unprotonated N1, so that the 6-substituents cannot reach the necessary site points. It is possible that the monoamino derivatives bind better due to partial protonation of the ring nitrogen. We did not take the interaction of protonated ring nitrogen with site 1, since our program fit only the binding of a single molecular species, and the experimental binding energy may be the average over two species. That is also in accord with our assumption that 2,4-diamino and the monoamino derivatives have the same binding mode. The reason behind the failure of the model for various 6-thiopteridines and -pterines may lie elsewhere. The presence of the 5-position ring nitrogen may

Table X. Conformational Results

no.	starting conformation angles, deg	energy, kcal/mol	minimized conformation angles, deg	energy kcal/mol
7[a]	(1) $\omega_1 = 90, \omega_2 = 0, \omega_3 = 90.1, \omega_4 = 0, \omega_5 = 0, \omega_6 = 45, \omega_7 = -60$	-6.52	$\omega_1 = 0, \omega_2 = 0, \omega_3 = 75, \omega_4 = 55, \omega_5 = 65, \omega_6 = 70, \omega_7 = 55$	-31.92
	(2) $\omega_1 = \omega_2 = \omega_3 = \omega_4 = \omega_5 = \omega_6 = \omega_7 = 45$	-1.93	$\omega_1 = 0, \omega_2 = 0, \omega_3 = 75, \omega_4 = 55, \omega_5 = 65, \omega_6 = 70, \omega_7 = 55$	-31.92
26[b]	(1) $\omega_1 = -35.8, \omega_2 = -13.4, \omega_3 = 261, \omega_4 = 73.5, \omega_5 = 199.2, \omega_6 = 0$	13.64	$\omega_1 = 155, \omega_2 = 165, \omega_3 = 30, \omega_4 = 165, \omega_5 = 90, \omega_6 = 0$	-2.86
	(2) $\omega_1 = \omega_2 = \omega_3 = \omega_4 = \omega_5 = \omega_6 = 90$	25.76	$\omega_1 = 155, \omega_2 = 165, \omega_3 = 40, \omega_4 = 55, \omega_5 = 60, \omega_6 = 180$	-3.59
	(3) $\omega_1 = \omega_2 = \omega_3 = \omega_4 = \omega_5 = \omega_6 = 45$	14.37	$\omega_1 = 155, \omega_2 = 165, \omega_3 = 35, \omega_4 = 55, \omega_5 = 55, \omega_6 = 180$	-3.60
60[c]	(1) $\omega_1 = 231.6, \omega_2 = 183.3, \omega_3 = 179, \omega_4 = 170.3, \omega_5 = -40.9, \omega_6 = 39.6$	329.48	$\omega_1 = 20, \omega_2 = 10, \omega_3 = 155, \omega_4 = 285, \omega_5 = 105, \omega_6 = 60$	-5.78
	(2) $\omega_1 = \omega_2 = \omega_3 = \omega_4 = \omega_5 = \omega_6 = 45$	2.26	$\omega_1 = 20, \omega_2 = 10, \omega_3 = 65, \omega_4 = 55, \omega_5 = 70, \omega_6 = 55$	-5.66
	(3) $\omega_1 = \omega_2 = \omega_3 = \omega_4 = \omega_5 = \omega_6 = 90$	16.43	$\omega_1 = 20, \omega_2 = 10, \omega_3 = 155, \omega_4 = 285, \omega_5 = 110, \omega_6 = 65$	-5.78

[a] ω_1 = N1-C2-N3-H, ω_2 = N4-C5-N6-H, ω_3 = C2-N1-C1′-C2′, ω_4 = N1-C8-C9-H, ω_5 = N1-C8-C10-H, ω_6 = C2′-C3′-A3-B3, and ω_7 = C3′-A3-B3-D3; for atom symbols, see structure VI. [b] ω_1 = C10-C4-X4-H, ω_2 = N3-C2-X2-H, ω_3 = C5-C6-X6(N)-C, ω_4 = C6-X6(N)-C-C1′, ω_5 = X6(N)-C-C1′-C2′, and ω_6 = C3′-C4′-X′4-H; for atom symbols, see structure VII. [c] ω_1 = C5-C4-X4-H, ω_2 = N3-C2-X2-H, ω_3 = C4-C5-C-C1′, ω_4 = C5-C-C1′-C2′, ω_5 = C2′-C3′-X′3-Y′3, ω_6 = C3′-X′3-Y′3-Z′3; for atom symbols, see structure IX.

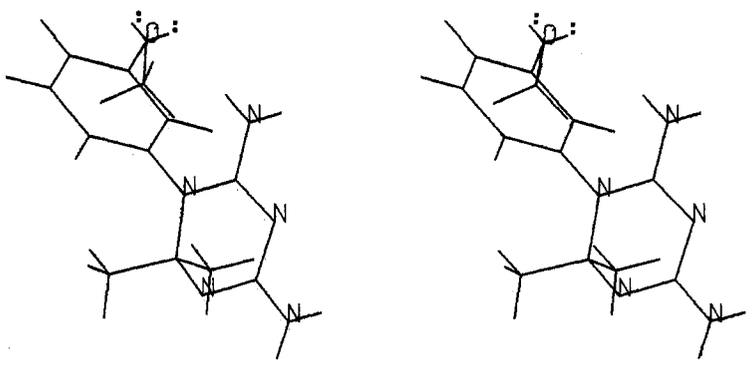

Figure 4. Stereo view of the minimum energy conformation of a triazine (7).

lead to easy hydrolysis of these compounds.[30] Such hydrolysis will lead to the elimination of the 6-substituents, thereby giving unusually low activity of these compounds. This may be a problem with any pterine or pteridine derivatives having an electronegative atom attached to the 6-position. The reason behind the failure of 2′-chloroquinazoline (**107**), as stated earlier, may be due to an unfavorable conformational energy of the necessary conformation.

Since our method represents a true 3-D QSAR, we can compare our site points with the X-ray crystal structure of dihydrofolate reductase. Unfortunately, no data on rat liver dihydrofolate reductase are available. Matthews et al. initially published the data on the binary complex of *Escherichia coli* dihydrofolate reductase and methotrexate[19] and the ternary complex consisting of *Lactobacillus casei* dihydrofolate reductase, methotrexate, and NADPH[31,32] at 2.5-Å resolution, but recently they have refined their data to 1.7 Å.[33,34] The vertebrate (avian) dihydrofolate reductase containing a phenyltriazine and NADPH was also reported from the same laboratory with 2.5-Å resolution. Refinement of the data to 1.7 Å is in progress.[32] On the other hand, Baker et al.[35] reported the data on *E. coli* dihydrofolate reductase and trimethoprim binary complex. All these observations are in general agreement that the 2,4-diamino-5,6-dihydrotriazine ring moiety of the phenyltriazine, the 2,4-diaminopyrimidine part of the methotrexate, and trimethoprim all bind in an equivalent position in spite of their chemical dissimilarities. This observation tempted us to devise a unique model that can explain the binding data of the various inhibitors in a particular dihydrofolate reductase. Baker et al.[35] observed that the 2,4-diaminopyrimidine ring in the *E. coli* methotrexate binary complex[19] is slightly tilted relative to that for trimethoprim. However, the recent report by Bolin et al.[33] stated that in the earlier work[19] they erroneously positioned the pteridine ring slightly to the left relative to their more refined work.[33] We are therefore not very sure about the difference between the two observations. Our model places the key heterocyclic ring in the same orientation and obtains a good correlation. Volz et al.[26] suggested that the triazine inhibitor's phenyl ring

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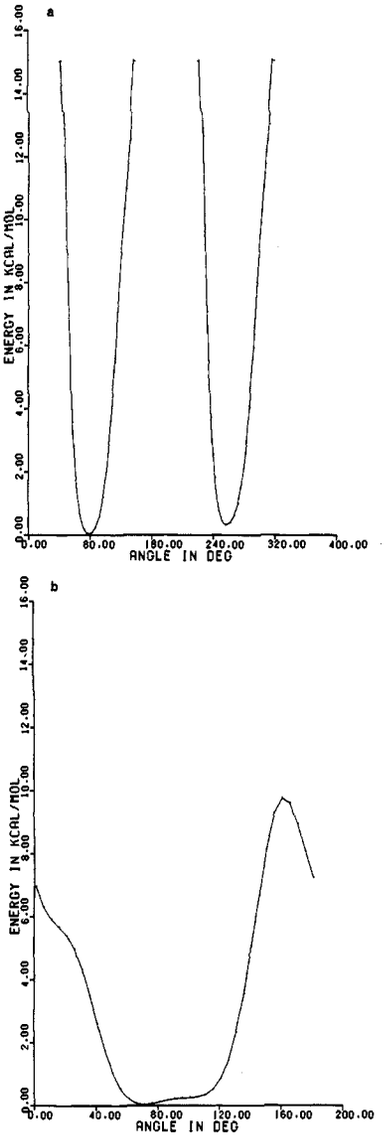

Figure 5. Triazines (7) energy relative to the global minimum: (a) varying ω_3 and (b) varying ω_6. All other dihedral angles are held fixed at the value for the global energy minimum.

occupies a space analogous to that utilized by the pyrazine and C_9–N_{10} portion of methotrexate. Although this is qualitatively very similar to our model, we have the phenyl ring perpendicular to the triazine ring, while for methotrexate the pyrazine ring is planar to the pyrimidine ring and the C_9–N_{10} bond is perpendicular to the pyrazine ring. The major discrepancy between our model and the X-ray results is in the binding of the benzene ring of methotrexate, since we have the benzene ring going in the opposite direction from the conformation shown by Bolin et al.[33] Although in our conformation the benzene ring will apparently hit the NADPH molecule, that may not actually be the case, since we are not sure about the handedness of our coordinate system. If we change the handedness, the benzene ring of methotrexate goes below the NADPH molecule. One observation that goes well with our model is that 5-substituted quinazolines bind equally well with the receptor site. Although in the present model we showed that monoaminoquinazolines binding can well be explained by the same binding mode, that does not necessarily exclude the possibility of an alternate binding mode. We explain the difference in binding energies by the difference in protonation of the ring nitrogen N_1. If the flipping of the ring is such that the 5- or 6-substituent of quinazoline derivatives does not change its place, that model will also explain all features explained by the present model. In addition we suggest that the non-amino derivatives of quinazoline have a completely different mode in which the 6-substituent goes to a place different from its usual position. The binding of the benzene ring of trimethoprim in *E. coli*[35] is somewhat different from our proposed mode. However, it should be remembered that trimethoprim binds very strongly with *E. coli* and only poorly with rat liver dihydrofolate reductase.

Although our binding modes were determined by examining all sterically allowed conformations,[2] the energy of the active conformation should be comparable to the global minimum. Note that any molecular mechanics calculation suffers from (i) lack of very reliable parameters for many heteroatoms, (ii) neglect of solvent effects, and (iii) lack of a practical but guaranteed global optimization technique. (We tried to handle the last problem by initiating the local minimization from several different points.) Using the van der Waals parameters of Allinger et al.,[36,37] a fixed valence geometry molecular mechanics representation, generated from our crystallographic fragment library,[2] formal atomic charges calculated by CNDO/2, and the torsional potentials taken from related molecules,[38–40] we minimized the energy by a pattern search technique[41] for three representative molecules (**7**, **26**, and **60**) from three different classes.

For the triazine **7** we started from two different conformations and obtained exactly the same minimum energy conformation (see Table X and Figure 4). The plots of energy vs. two important dihedral angles, ω_3 and ω_6, are shown in Figure 5. For these figures the other rotatable bonds were held fixed at their minimum energy values. There are two minima with respect to ω_3, one at 75° and the other at 255° (Figure 5a), in agreement with Hopfinger's values of 80 and 260°, respectively.[18] However, our calculation shows single sharp minima rather than double for each. Varying dihedral angle ω_6 (Figure 5b) has the energy minimum at 70°, but it can attain any conformation between 50 and 120° at the cost of 1 kcal/mol or less. The active conformation, for which $\omega_1 = \omega_2 = 0°$, $\omega_3 = -100°$, $\omega_4 = 55°$, $\omega_5 = 65°$, $\omega_6 = 76.4°$, and $\omega_7 = 60°$, has energy 0.38 kcal/mol above the global minimum.

For quinazoline **26** we started energy minimization from three different conformations and attained three different structures of comparable energy (see Table X), but two of these conformations were very close. The stereo picture of the lowest energy conformation is shown in Figure 6. The energetics of the three torsion angles between the quinazoline and the benzene rings are shown in Figure 7. The torsion angle ω_3 shows (Figure 7a) two sharp minima, one at 35° (global) and the other at 220°. On the other hand, ω_4 (Figure 7b) shows three minima. The global minimum lies at 55°, and and the other two lie at 165° and 235°. The energy barrier separating them is approximately 4.5 kcal/mol. However, energy increases sharply as the torsion angle goes below 25° or above 250°. The phenyl ring was rotated from 0° to 180° (Figure 7c), and we got

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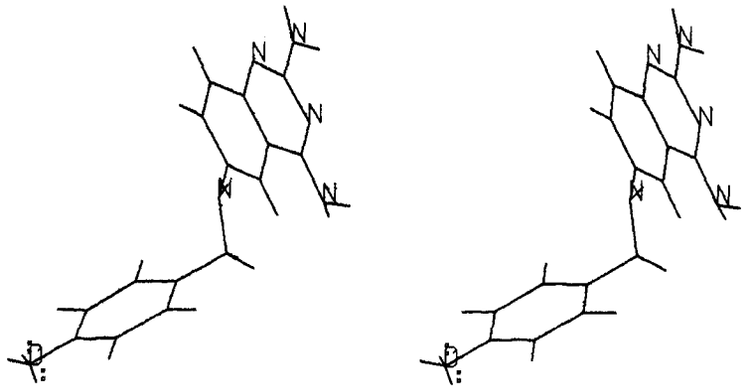

Figure 6. Stereo view of the minimum energy conformation of a quinazoline (**26**).

a single minimum at 55°. The energy of the active conformation (ω_1 = 155°, ω_2 = 165°, ω_3 = −26°, ω_4 = −118.5°, ω_5 = 97.8°, and ω_6 = 180°) is 11.59 kcal/mol above the global minimum.

For the pyrimidine **60** we started minimization from three different structures and here also arrived at three different conformations. All these conformations were energetically comparable, and two were also geometrically very similar (see Table X). The stereo picture of the minimum energy conformation is shown in Figure 8. The dependence of the energy on the two torsion angles, ω_3 and ω_4, between the pyrimidine and benzene rings is shown in Figure 9. For torsion angle ω_3 we found three minima at 95°, 155°, and 265°, of which the second one is the global minimum. The energy barrier separating the first two is 1.4 kcal/mol, while that between the second and third minima is 4.2 kcal/mol. The active conformation (ω_1 = 20°, ω_2 = 10°, ω_3 = −204°, ω_4 = −209°, ω_5 = 30.6°, and ω_6 = 45°) has an energy 12.03 kcal/mol above the global minimum.

Thus, the active conformation of triazine is very close to the global minimum, while the conformations of pyrimidine and quinazoline are not so close. However, we are not overly concerned because, for instance, Spark et al.[42] found that the methotrexate conformations at the receptor site reported by Matthews et al.[19,31] have energies 27 to 71 kcal/mol above the global minimum. The discrepancy may be due to the limited resolution X-ray data or inadequate parameters for molecular mechanics calculation. If we rely on molecular mechanics calculation, our active conformations are much better energetically than the crystallographic conformation!

Our distance geometry analysis is a true 3-D QSAR. Most conventional QSAR works in recent years[43] assume that the biological activity does not necessarily depend on the overall properties of the molecules but rather on the properties of some selected parts of the molecule. Although this approach is a powerful tool for mapping the receptor site, conclusions drawn for a conformationally flexible molecule may sometimes be logically faulty. For example, Fukunaga et al.,[8] seeing that SO_2NH_2 and $CONH_2$ decrease the activity of the 3′-substituted triazines, concluded that this part of the eceptor site is hydrophobic. However, if the effect was just from the interaction of the substituent with the corresponding receptor site, the phenyl ring may undergo 180° rotation to produce another equivalent and almost equally stable connormation having no such repulsive interaction, unless that conformation leads to some steric overlap with the receptor site. Such effects can be treated in the distance geometry approach, but not in the classical 2-D QSAR.

The necessity of the complex 3-D receptor model over simple correlation equations is obvious. If the key parts of the various classes of the inhibitors interact at the same receptor site, their substituents may not interact at the same region of the receptor site. Having a 3-D receptor model in hand, we may design a molecule of one class that can possibly interact with a region ordinarily used by members of another class.

Although the three-dimensional structure of drug molecules has been recognized[44] as a crucial factor in their binding, making use of molecular shape requires some clever scheme for dealing with the numerous molecular orientations and conformations. The first approach was by Marshall et al.,[45-47] where active molecules are superimposed by having corresponding important groups (the pharmacophore) coincide. The union of their volumes must be sterically allowed, and inactive compounds having the necessary pharmacophore often protrude beyond this allowed space. The results are qualitative (active vs. inactive), and require compounds homologous enough so that the superposition is unambiguous. The approach of Simon and co-workers[48] is less sophisticated in the treatment of steric effects and adds quantitative molecule–site interactions, but is based on the same superposition scheme. Hopfinger's method[18,49] quantifies the degree of volume overlap between superimposed molecules and uses this as another term in a Hansch analysis. Recently,[49] he has quantified the Marshall allowed-volume picture by calculating the molecular mechanics intermolecular potential field around a drug molecule, as an indication of how it may interact with the site. In contrast, the distance geometry approach focuses on building an explicit model of the site itself, rather than the commonality of active compounds. The site model is intentionally abstract and discretized in order to allow a more thorough and realistic examination of all modes of interaction with each drug molecule. The final interaction energies give quantitative agreement with the experimental binding data in spite of

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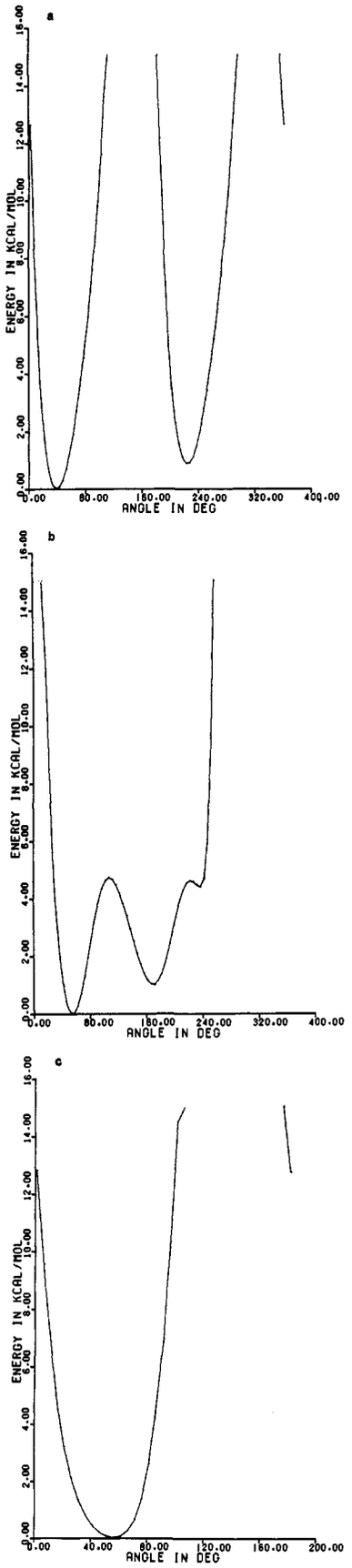

Figure 7. Quinazoline (**26**) energy relative to the global minimum: (a) varying ω_3, (b) varying ω_4, and (c) varying ω_5. All other dihedral angles are held fixed at the value for the global energy minimum.

the all-or-none interaction model we use to allow a full survey of possible binding modes.

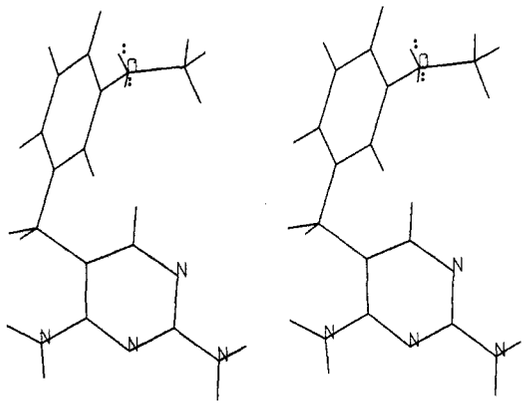

Figure 8. Stereo view of the minimum energy conformation of a pyrimidine (**60**).

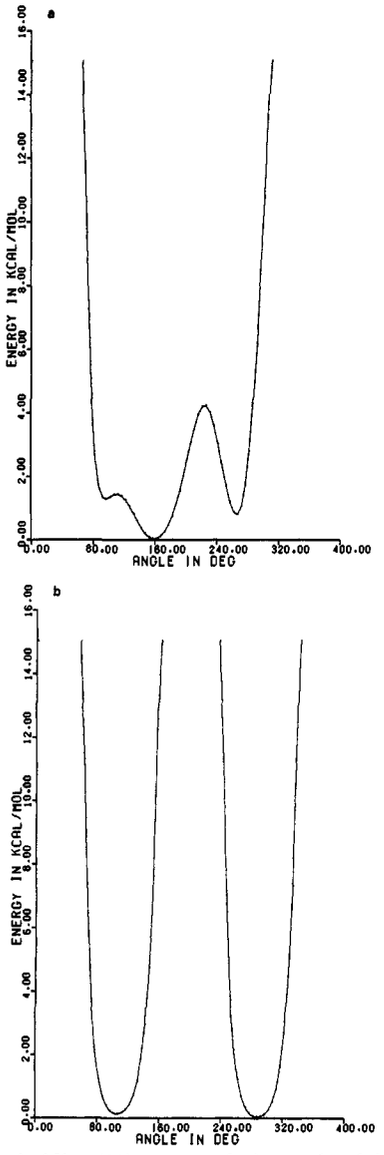

Figure 9. Pyrimidine (**60**) energy relative to the global minimum: (a) varying ω_3 and (b) varying ω_4. All other dihedral angles are held fixed at the value for the global energy minimum.

Conclusion

The present model is based on the idea that the 2,4-diamino heterocyclic ring of all the classes used in the data set, as well as the monoaminoquinazoline ring, binds in exactly the same orientation at the receptor site. Although

a large number of binding modes of the large substituents is possible, depending on the conformation of the substituents relative to the site points used to bind them, very few common site points are found to be capable of explaining the data set. In order to explain the low binding energy of the pyrimidines, it is necessary to assume a steric surface that will exclude some of the binding modes, since the substituted benzyl ring may otherwise occupy some common space occupied by the phenyl ring of the triazines. The model fails to predict the biological data of nonaminoquinazolines, thereby indicating the possibility of an alternate binding mode in which the 6-substituents cannot reach the necessary site points of the present model. The various interesting predictions regarding the molecular modifications to be made to get potent dihydrofolate reductase inhibitors merit actual synthesis and biological evaluation.

A three-dimensional structure directed QSAR is necessary for many reasons. Even when the X-ray crystallographic structure of an inhibitor-bound receptor is known, information that is seldom available, we are not sure about the quantitative energetics of the interaction of the ligand with the receptor site. Our calculations may be done not only when the crystallographic information is not available, but also to determine the interaction energies for a binding site of known structure. Crystallographic receptor structures at high resolution are, of course, the most desirable data on the drug-design problem. However, such studies suffer from the difficulty of crystallizing the binary or ternary complexes. Even a single complex takes considerable time and effort, so studies on several inhibitors or enzymes are unlikely in the near future. On the other hand, 3-D QSAR, as we have been developing, is much easier. Although the 2-D QSAR is even faster than the 3-D QSAR, it is limited to a single class of compounds and even may often lead to wrong conclusions for flexible molecules. 2-D QSAR may be recommended only as the first step of a 3-D QSAR. A 3-D QSAR, in theory, may suggest a new lead. However, extensive collaborative work between the experimental and theoretical medicinal chemistry groups is necessary in order to explore the possibilities completely.

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Notes

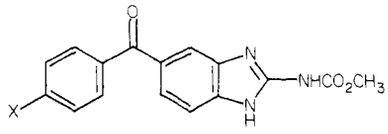
Synthesis of Potential Antifilarial Agents. 1. 1-(5-Benzoylbenzimidazol-2-yl)-3-alkyl- and -arylureas

Siya Ram,† Mike Skinner,† Doug Kalvin,† Dean S. Wise,† Leroy B. Townsend,*,† John W. McCall,‡ Don Worth,§ Don Ortwine,§ and Leslie M. Werbel§

Department of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, and Chemistry Department, Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, Michigan 48106. *Received June 9, 1983*

A series of 1-(5-benzoylbenzimidazol-2-yl)-3-substituted ureas have been synthesized by reacting an appropriate isocyanate with 2-amino-5-benzoylbenzimidazole or by reacting methyl (5-benzoylbenzimidazol-2-yl)carbamate with various amines. Several of the compounds have demonstrated antifilarial activity against *Brugia pahangi* and *Litomosoides carinii*.

Benzimidazole derivatives have exhibited a broad range of pharmacological actions, including anticonvulsant,[1-4] analgesic,[5] tranquilizing,[6] and paralyzing activities,[7] immunosuppression, and viral inhibition.[8] Recently, methyl (5-benzoylbenzimidazol-2-yl)carbamate (1, mebendazole)



1, X = H (mebendazole)
2, X = F (flubendazole)

and methyl [5-(*p*-fluorobenzoyl)benzimidazol-2-yl]carbamate (2, flubendazole) have demonstrated significant antifilarial activity[9] in addition to a broad spectrum of anthelmintic activity.[10,11] However, poor water solubility and

† University of Michigan.
‡ The University of Georgia.
§ Warner-Lambert Co.

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