bound to DHFR[6] as the shape reference compound for designing substituents on IV when in its active conformation. Further, some of the compounds in the original triazine data base[2] might be employed in such a study for purposes of evaluating polar/nonpolar binding constraints imposed on possible substituents by the second binding site. Such an investigation is now under consideration.

Acknowledgment. The theory and corresponding computer programs to calculate molecular shape descriptors have been developed under private funding. The application of MSA to the quinazolines was funded under a contract from the National Cancer Institute (Contract NO1-CP-75927) and a grant from the National Science Foundation (Grant ENV77-24061). The authors appreciate helpful discussions with Dr. J. Y. Fukunaga of Schering-Plough Corp. and R. Pearlstein and S. K. Tripathy of our laboratory during the course of this study.

Inhibition of Dihydrofolate Reductase: Structure-Activity Correlations of 2,4-Diamino-5-benzylpyrimidines Based upon Molecular Shape Analysis

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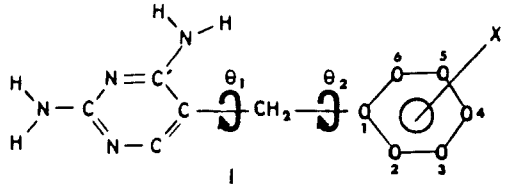
A quantitative structure-activity rleationship (QSAR) investigation of a set of 23 substituted 2,4-diamino-5-benzylpyrimidines spanning an activity range of 1.8 log $(1/C)$ units was carried out using molecular shape analysis (MSA). C is the molar concentration necessary for 50% inhibition of bovine liver dihydrofolate reductase (DHFR). The "active" shape of these compounds was deduced by comparing the change in conformational state to the activity of four compounds outside the data base described above. A correlation equation, analogous in descriptor form to those developed earlier for DHFR inhibition by substituted 2,4-diaminotriazines and -quinazolines, was constructed. The correlation coefficient, r, was 0.931 and the standard deviation of fit, s, was 0.137. The results suggest that these pyrimidines bind to DHFR with shape features different from both the triazines and quinazolines. It is postulated from the "active" shape of the pyrimidines that it is preferable to substitute at the meta position of the benzyl ring rather than at the para position.

In the preceding paper of this issue we developed a QSAR to describe the dihydrofolate reductase (DHFR) inhibition activity of a set of 2,4-diaminoquinazolines based upon molecular shape analysis (MSA).[1] Equivalent QSARs, based upon MSA, were also constructed for sets of 2,4-diaminotriazines which inhibit DHFR.[2,3] A methodology breakthrough in the potential design of new lead structures was realized in the investigation of the quinazolines. It was shown that the activities of these compounds could be explained by their shape similarity to a 2,4-diaminotriazine in its postulated active conformation.[1]

In this paper we report the results of a QSAR investigation, based upon MSA, of yet another set of DHFR inhibitors, substituted 5-benzyl-2,4-diaminopyrimidines (I). The importance of trimethoprim [2,4-diamino-5-



I

(3,4,5-trimethoxybenzyl)pyrimidine] as a broad-spectrum antibacterial agent[4-7] has spurred an interest in this class of compounds.

Method

(1) The Data Base. Blaney et al.[8] have put together a set of 23 5-(X-benzyl)-2,4-diaminopyrimidines whose inhibitory effect on bovine liver DHFR has been measured. The activity is expressed as log $(1/C)$, where C is the molar concentration of inhibitor which produces 50% inhibition. The compounds and corresponding activities are given as part of Table I. Blaney et al.[8] formulated correlation eq 1 to explain the DHFR inhibition

$$\log (1/C) = 0.622\pi_3 + 0.322\sum\sigma^+ + 4.99 \quad (1)$$

$$n = 23;\ r = 0.931;\ s = 0.146$$

activity. In eq 1, π_3 is the hydrophobic constant for substituents in position 3, and $\sum\sigma^+$ represents the summed electronic effect of 3, 4, and 5 substituents on position 1. $\sum\sigma^+$ is a somewhat surprising feature to find related to activity, especially since it does not appear in any other QSARs for DHFR inhibition developed by Hansch and co-workers for the triazines[9,10] and quinazolines.[11] However, the authors do point out that the $\sum\sigma^+$ term in eq 1 does not play too significant a role.[8]

(2) Conformational Analysis. A fixed valence geometry intramolecular conformational analysis of I was carried out using CHEMLAB (part of which was formerly known as CAMSEQ-II)[12] components. The bond rotation angles were explored at 30° resolution in the initial scan. The identified minima were then rigorously energy minimized.

(3) Molecular Shape Analysis. An "active" conformation of I was deduced with respect to the principle torsional degress of freedom θ_1 and θ_2. The manner of doing this is presented under Results. Each of the compounds in Table I can adopt the active

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Table I. The Set of 5-(Substituted-benzyl)-2,4-diaminopyrimidines Used in the Study and the Shape Descriptor, V_0, Using Compound 9, X = 4-$NHCOCH_3$, as the Shape Reference, the Sum of the 3- and 4-Position Hydrophobic Fragments ($\pi_3 + \pi_4$), and Observed, Predicted, and Differences in Log (1/C) Activity

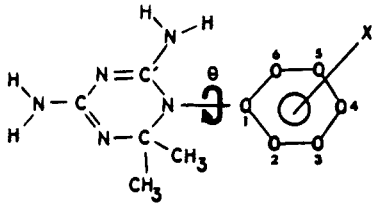
no.	compd	V_0, Å^3	$\pi_3 + \pi_4$	log (1/C) obsd	log (1/C) pred	Δ log (1/C), obsd − pred
1	3,4-$(OH)_2$	421.6	−1.24	4.30	4.36	−0.06
2	4-NH_2	414.0	−0.86	4.57	4.63	−0.06
3	4-$N(CH_3)_2$	446.5	0.29	4.76	4.90	−0.14
4	4-CH_3	433.8	0.56	4.80	5.05	−0.25
5	4-OCH_3	425.3	−0.02	4.92	4.86	0.06
6	4-OCF_3	426.2	0.34	4.99	5.01	−0.02
7	3-OCH_3 [a]	410.1	−0.02	5.02	5.06	−0.04
8	4-NO_2	416.7	−0.09	5.02	4.93	0.09
9	4-$NHCOCH_3$ [b]	486.9	−0.35	5.09	5.03	0.06
10	4-Cl	437.1	0.71	5.10	5.10	−0.00
11	3,4,5-$(OCH_3)_3$	405.6	0.04	5.10	5.17	−0.07
12	3,4-$(OCH_3)_2$	405.6	0.04	5.15	5.17	−0.02
13	3-NO_2, 4-$NHCOCH_3$ [b]	414.9	−0.38	5.16	4.83	0.33
14	4-Br	436.8	0.86	5.17	5.17	−0.00
15	4-F	417.3	0.14	5.18	5.02	0.16
16	H	395.7	0	5.19	5.36	−0.17
17	3-CH_3	406.5	0.56	5.22	5.38	−0.16
18	3-F	401.6	0.14	5.33	5.29	0.04
19	3-Cl	402.4	0.71	5.47	5.53	−0.06
20	3-CF_3	408.3	0.88	5.53	5.49	0.04
21	3-Br	401.5	0.86	5.54	5.62	−0.08
22	3-CF_3, 4-OCH_3	409.6	0.86	5.79	5.46	0.33
23	3-$OCH_2C_6H_5$	402.7	1.89	6.10	6.05	0.05

[a] Conformation has CH_3 of methoxy near the 4 position. [b] A cis amide geometry yields the best correlation.

conformation as a local minimum-energy conformer. Consequently, each compound was frozen into that conformation. Every stable conformer of the substituent(s) was treated as an individual entry in the pairwise shape analysis matrix. The common steric overlap volume, V_0,[2] between each pair entry in the shape analysis matrix, was determined as a function of the intermolecular geometry.

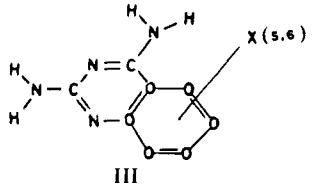
Results

The benzylpyrimidines offer a significantly greater challenge to analyze by MSA than the triazines (II) and



II

quinazolines (III). In the absence of a flexible substitu-

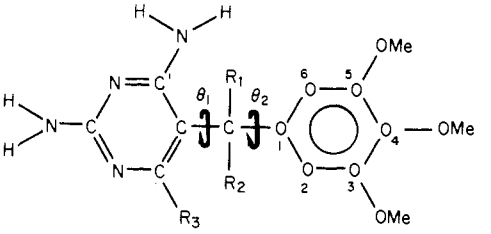


III

ent(s), the quinazolines possess no degree of conformational freedom and the triazines possess 1 degree of freedom, θ. The benzylpyrimidines have 2 intrinsic degrees of conformation freedom, θ_1 and θ_2.

We began our quest for the active conformation of I with respect to θ_1 and θ_2 by performing a conformational analysis of the most flexible member of the data base, X = H. Seven intramolecular minimum-energy conformers, close in energy, are identified. The number and relative stability of the conformer states are little affected by any of the substituents on the compounds in Table I. All of the compounds in Table I are quite flexible in comformation with respect to θ_1 and θ_2. Thus, in order to identify

Table II. The Four *E. coli* DHFR Benzylpyrimidine Inhibitors Having Substitutions at R_1, R_2, and/or R_3 Which Make It Possible to Deduce the Active Conformation ($\theta_1 = 90°$, $\theta_2 = 30°$)[a]



R_1	R_2	R_3	C_{50} (*E. coli*)	θ_1	θ_2	ΔE_1	ΔE_2
H	H	H	0.55[b]	90°	30°	0	1.9
H	H	Me	10[c]	60	30	0.6	1.2
H (+)	Me (−)	H	450[d]	60[e]	0[e]	1.4	1.7
H (−)	Me (+)	H	450[d]	0	60	4.3	0.3
OH (+)	Me (−)	H	>10 000[f]	60[e]	0[e]	1.2	1.6
OH (−)	Me (+)	H	>10 000[f]	0	60	4.4	0.2

[a] C_{50} is the molar concentration necessary for 50% inhibition. The reported θ_1 and θ_2 values define the change in conformation of the 90°, 30° "active" state due to the R substituents. $\Delta E_1 = E(\theta_1,\theta_2) - E(90°,30°)$ and $E_2 = E$ (global minimum) $- E(\theta_1,\theta_2)$. The energies, E, are in kcal/mol. [b] Compound 1, ref 13. [c] Compound 18, ref 14. [d] Compound 12, ref 13. [e] This is the more stable of the two conformers which emerge from the active conformation. The other conformer is at $\theta_1 = 30°$ and $\theta_2 = 90°$. [f] Compound 4, ref 13.

an active conformation we had to go outside the data base of Table I. *Escherichia coli* DHFR inhibition activity is available for the four compounds reported in Table II.[13,14] The locations of substituents on these benzylpyrimidines are critical to both activity and preferred conformation. The corresponding conformational energy maps of each

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of these compounds, including (±) isomers of R_1 and R_2, are shown in Figure 1a–f. It can be seen that the only two relative minima in the energy map of $R_1 = R_2 = R_3 = H$ (trimethoprin and compound 11 in Table I) which are altered due to substitutions are $\theta_1 = 90°$ and $\theta_2 = 30°$ or 210°. $\theta_2 = 30°$ and 210° are structurally degenerate upon complete energy minimization for X = 3,4,5-trimethoxy. Thus, there is only one conformer state which is altered due to substitutions R_1, R_2, and/or R_3. We arbitrarily denote this, the postulated "active" conformation, as the $\theta_1 = 90°$, $\theta_2 = 30°$ conformer state. The data in Table II suggest a direct qualitative relationship between C_{50} and the changes in θ_1, θ_2 from 90°, 30° as well as the corresponding ΔE_1. If, in fact, the H (+), Me (-) and OH (-), Me (+) isomers were dominant, these relationships would be quantitative. The active conformation for X = H is shown as a space-filling stereo model in Figure 2. An implicit assumption now made is that the *E coli* DHFR active conformation is also the active form for bovine liver DHFR inhibition.

A systematic series of correlation analyses were carried out to relate the MSA descriptors V_0, S_0, and L_0[2] and the substituent hydrophobic constants to the observed log $(1/C)$ measures. V_0 is the common steric overlap volume between pairs of molecules as a function of conformation and relative intermolecular geometry. $S_0 = V_0^{2/3}$ and $L_0 = V_0^{1/3}$. V_0 physically measures how much steric space a pair of molecules share under a prescribed intermolecular relationship.

The selection of these descriptors for generation of the QSAR was prompted by our previous findings[1-3] and those of Blaney et al.[8] The optimum correlation equation found in this study is eq 2.

$$\log (1/C) = -21.31V_0 + 2.39V_0^2 + 0.44(\pi_3 + \pi_4) + 52.23 \quad (2)$$

$n = 23$; $r = 0.931$; $s = 0.137$; ref compd X = *cis*-4-$NHCOCH_3$

The term $\pi_3 + \pi_4$ is the sum of the hydrophobic constants of the 3 and 4 position substituents using the fragment values given by Blaney et al.[8] However, the π value of the $OCH_2C_6H_5$ of compound **23** was assigned the computed value of 1.89 and not the reported value of 1.66. It is also important to note that the amide group in the cis form with respect to the amide bond for compounds **9** and **13** yields a better correlation equation than the trans structure. This was also found to be the case for the quinazolines.

The shape descriptor V_0 correlates with activity better than S_0 and L_0. This is somewhat surprising in that S_0 is the best correlating shape descriptor in the triazine and quinazoline QSAR analyses.[1-3] The correlation equation analogous to eq 2 using S_0, rather than V_0, has $r = 0.895$. Nevertheless, eq 2 is of the same general form as the QSAR developed for the triazines and quinazolines. In all cases the activity can be explained in terms of measures of molecular shape and hydrophobicity. Lastly, it was *not* possible to reliably account for the activities of the pyrimidines in Table I in terms of their shape similarity to a triazine or quinazoline DHFR inhibitor considered in the previous studies.[1-3] This is in contrast to the striking success of explaining the activity of the quinazolines by their shape similarity to 5-(3,4-dichlorobenzyl)-2,4-diaminotriazine!

Discussion

While eq 1 and 2 are about of the same quality, the physical model associated with each is different. Equation 1, development by Blaney et al.,[8] suggests that activity is enhanced by increasingly nonpolar 3 substituents and higher electron-withdrawing substituents at the 3, 4, and 5 positions. Equation 1 and its corresponding physicochemical model are considerably different from the correlation equations and models developed for triazine and quinazoline DHFR inhibitors by Hansch and co-workers.[9-11] In fact, the individual triazine and quinazoline QSARs of Hansch and co-workers are markedly different in descriptor form and physical meaning from each other. In contrast, eq 2 is identical in form with the QSARs derived to account for DHFR inhibition activities of the triazines[2,3] and quinazolines.[1] All of these QSARs account for activity solely in terms of molecular shape and substituent lipophilicity.

Equation 2 suggests that activity should increase with increasing lipophilicity of *both* the 3 and 4 position substituents. Inhibitory action is also predicted to increase as V_0 *decreases*. This latter finding is numerically different from the triazine and quinazoline QSARs in two ways. Firstly, V_0 is the preferred measure of shape similarity as opposed to S_0, as found in the earlier studies.[1-3] Secondly, activity can be *maximized* with respect to V_0 in the triazines and quinazolines. The optimum value of V_0 is about 715 Å^3 in each QSAR,[1-3] even though a different shape reference compound was employed in constructing each of these individual QSARs. In the pyrimidine QSAR, activity can be *minimized* with respect to V_0 [V_0 (min) = 445.8 Å^3 using X = *cis*-4-$NHCOCH_3$ as the shape reference molecule). Since most values of V_0 in Table I are less than 445.8 Å^3, it appears that the less a compound "looks like" the X = *cis*-4-$NHCOCH_3$ structure, with regard to shape, the more active it will be as a DHFR inhibitor. Equation 2 also predicts activity to increase for $V_0 > 445.8$ Å^3. However, the definition of V_0 limits its maximum value to the self-overlap volume of the common reference shape structure, which is 486.9 Å^3 in this case. Thus, while some increase in activity is predicted for $V_0 > 445.8$ Å^3, there is a definite limit in its upper value.

Possible conclusions to draw from eq 2 are that the binding shape and/or the binding orientation of the 2,4-diamino-5-(X-benzyl)pyrimidines may be different from that of 2,4-diaminotriazines and -quinazolines. The difference in binding is principally manifested through the relative orientation of the 5-benzyl ring relative to the pyrimidine ring. One might further speculate that increasing the "size" of V_0 diminishes activity. This would be consistent with the qualitative observation that 4-X substituents generate a larger V_0 than the corresponding 3-X substitutions and para-substituted pyrimidines are also somewhat less active; see Table I.

Lastly, it is pointed out that the global, free-space, intramolecular minimum-energy conformation with respect to θ_1 and θ_2 (see I) of each pyrimidine is *not* the postulated active conformation. This is also the case for the triazines[2,3] with respect to θ (see II). In each case the projected active conformation is a secondary minimum-energy conformer (about 0.3 to 1.5 kcal/mol less stable than the global minimum) which is identified by relating the change in activity to the change in conformation as a function of substitution.

This method of identifying active molecular shapes (conformations) contains an even more subtle and underlying assumption regarding MSA in drug design. It is implicitly accepted that one of the stable free-space, intramolecular conformers must be, in fact, the active state of a molecule. There is no reason for this to be true in general, even through it is a plausible concept. Preferential

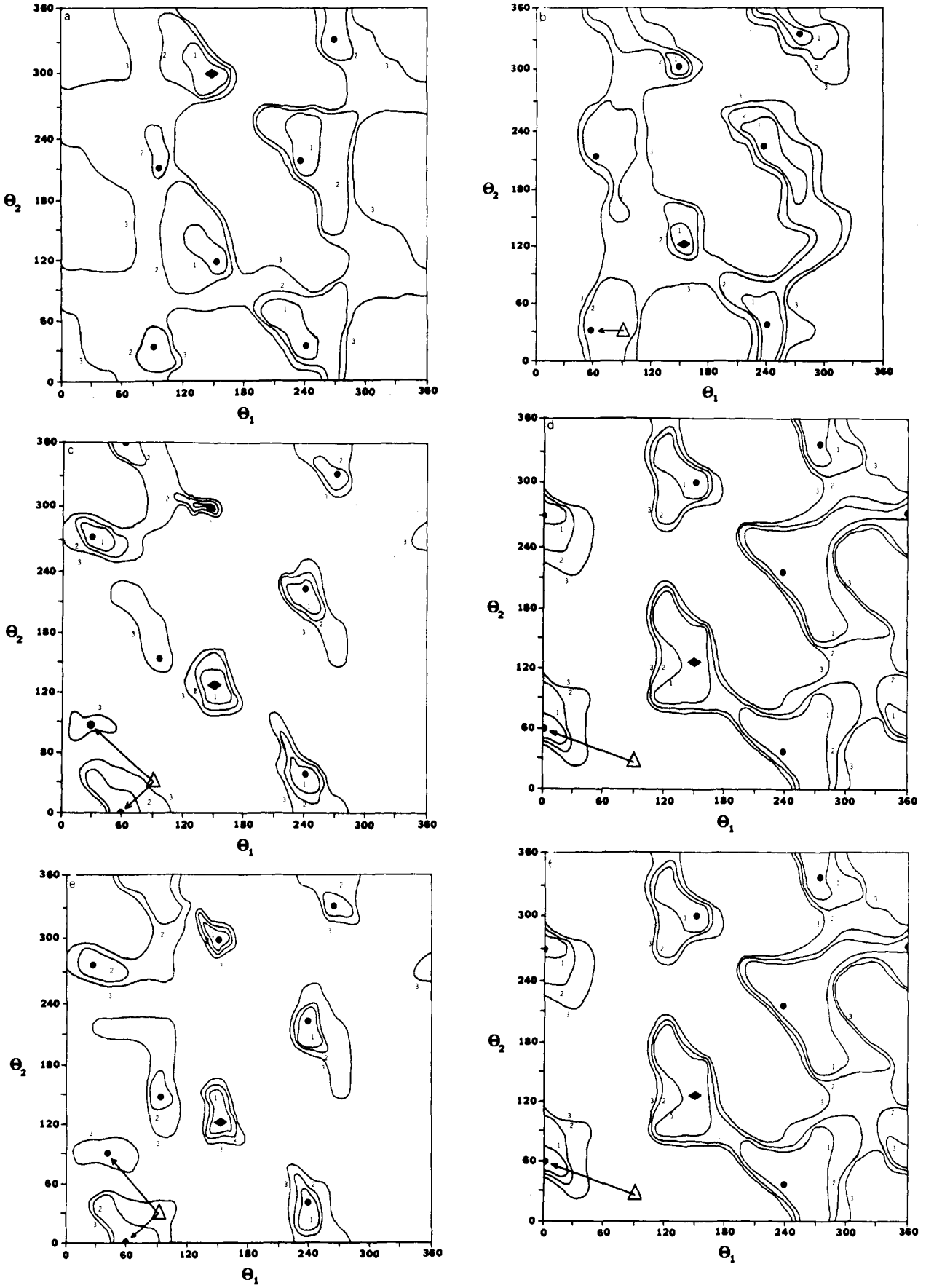

Figure 1. Free-space intramolecular conformational energy maps for the compounds reported in Table II. The energy contours are 1, 2, and 3 kcal/mol above the global minimum-energy conformer denoted by ♦. Relative minima (stable conformers) are denoted by ●. △ defines the location of the "active" conformation ($\Theta_1 = 90°$, $\Theta_2 = 30°$), and the arrow(s) indicate the relocation(s) of this conformer due to the substituents R_1, R_2, and/or R_3: (a) $R_1 = R_2 = R_3 = H$ (trimethoprim); (b) $R_1 = R_2 = H$, $R_3 = Me$; (c) $R_1 = H$ (+), $R_2 = Me$ (−), $R_3 = H$; (d) $R_1 = H$ (−), $R_2 = Me$ (+), $R_3 = H$; (e) $R_1 = OH$ (+), $R_2 = Me$ (−), $R_3 = H$; (f) $R_1 = OH$ (−), $R_2 = Me$ (+), $R_3 = H$.

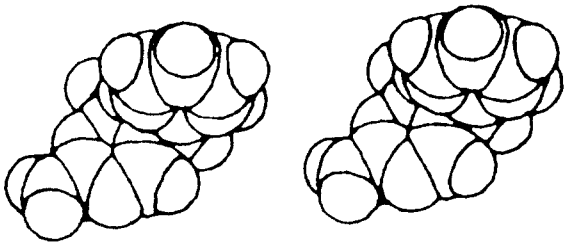

Figure 2. A space filling stereo molecular model of the active conformation for X = H.

receptor binding could certainly alter the conformation of a drug from any of the stable conformers realized in solution or free space. Nevertheless, the identification of a rigid active shape, in terms of θ (II) for the triazines and θ_1 and θ_2 (I) for the pyrimidines, leads to a very significant QSAR. In addition, for the triazines, quinazolines, and pyrmidines it is observed that one particular rotamer of a substituent, corresponding to a free-space minimum-energy conformer, correlates significantly better with observed activity than any other rotamer state of the substituent. The cis vs. trans amide substituents in the quinazoline and pyrimidine QSARs illustrate this point.

Thus, the hypothesis that one of the isolated stable conformers of a molecule corresponds to the active state is validated by the MSA QSARs developed for the triazine, quinazoline, and pyrimidine DHFR inhibitors. It is possible that some other conformer, probably "close" in shape to a postulated active conformer which is an isolated free-space relative minimum-energy state, could yield a better QSAR than presently found. This possibility can be tested using MSA. The hypohtesis of considering only stable intramolecular energy minima conformers in the development of a QSAR is only a working simplification and not a restriction of the MSA methodology. However, given the success we have enjoyed using this hypothesis, we plan to continue employing it to develop MSA QSARs on alternate data bases.

Acknowledgment. The theory and corresponding computer programs to calculate molecular shape descriptors have been developed under private funding. The application of MSA to the pyrimidines was funded under a contract from the National Cancer Institute (Contract NO1-CP-75927). The author appreciates helpful discussions with Dr. J. Y. Fukunaga of Schering-Plough Corp. concerning this work.

Effects of 1-Arylpyrroles and Naphthoflavones upon Cytochrome P-450 Dependent Monooxygenase Activities

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The inhibitions of cytochrome P-450 dependent monooxygenase activity in microsomes from rat liver by 1-phenylpyrrole, 1-(2-isopropylphenyl)pyrrole, 4(5)-phenylimidazole, and 1-(2-isopropylphenyl)imidazole have been compared. The results establish that the presence of an imidazole N-3 nitrogen substituent is not required to inhibit the monooxygenase activity measured by the deethylation of 7-ethoxycoumarin. The presence of an appropriately situated N-3 atom, however, as in 1-(2-isopropylphenyl)imidazole, significantly decreases both the K_i and αK_i of these mixed type inhibitors. The induction of 7-ethoxycoumarin deethylase activity in the microsomal fraction from rat liver by α-naphthoflavone, β-naphthoflavone, and 3-methylcholanthrene and the inhibition of these activities by flavone and α-, β-, and γ-naphthoflavone have also been examined. The results establish that α-naphthoflavone is the most effective in vitro inhibitor. The results also indicate that the microsomal monooxygenase activities induced in rat liver by α-naphthoflavone, β-naphthoflavone, and 3-methylcholanthrene are not equivalent. Based upon the observed results, it is concluded that differential effects of α- and β-naphthoflavone on aryl hydrocarbon skin tumorigenesis may be the result of differential enzyme induction rather than the result of differential enzyme inhibition.

In 1969, Johnson et al.[1] reported that 1-arylimidazoles were potent in vitro inhibitors of steroid 11β-hydroxylations catalyzed by the mitochondrial fraction of bovine adrenal cortex. Liebman and Ortiz[2] subsequently found that 1-(2-isopropylphenyl)imidazole and 1-(2-cyanophenyl)imidazole were inhibitors of the para-hydroxylation of aniline and acetanilide and of the N-demethylation of aminopyrine in rat liver microsomes. The 1-(2-isopropylphenyl)imidazole was also found to prolong and to intensify the in vivo action of hexobarbital in rats.[2] Other investigators,[3,4] especially Wilkinson and his co-workers,[5-9] have also investigated the inhibition of various cytochrome P-450 dependent monooxygenase activities by 1-arylimidazoles and related compounds.

Based upon the relative inhibition constants and the spectral dissociation constants of an extensive series of substituted imidazoles, Wilkinson and co-workers[6,7] proposed that the inhibition of cytochrome P-450 dependent monooxygenase activities by arylimidazoles is due to coordination of the nonbonded electrons of N-3 of the imidazole ring with the heme iron. Additional evidence for the binding of 1-arylimidazoles in the vicinity of the heme of cytochrome P-450 containing proteins has recently been provided by Swanson and Dus[10] who found that 1-(4-azidophenyl)imidazole can be used as a photoactive affinity probe of the heme site of the cytochrome P-450 dependent camphor hydroxylase of *Pseudomonas putida*.

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