Conformational Analysis of Antineoplastic Antifolates: The Crystal Structure of Trimetrexate and the Aminopterine Derivative 4-[*N*-[(2,4-Diamino-6-pteridinyl)methyl]amino]benzoic Acid

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The crystal structures of trimetrexate (TMQ) (2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline) and 4-[*N*-[(2,4-diamino-6-pteridinyl)methyl]amino]benzoic acid (PMAB) were determined to examine their conformational features with respect to the enzyme-bound form of methotrexate (MTX). TMQ and MTX are antineoplastic drugs that act by inhibiting the enzyme dihydrofolate reductase. The molecular conformation of TMQ is extended with the trimethoxyanilino ring twisted 89° from the quinazoline plane, and the molecular conformation of PMAB is completely planar. The geometry of the 2,4-diaminopteridine and 2,4-diaminoquinazoline rings are sensitive to protonation, and both TMQ and PMAB have geometries characteristic of a free base. TMQ crystallizes as a dimethyl sulfoxide hydrate. The quinazoline ring forms an antiparallel stacking arrangement in the lattice and forms a network of N···O hydrogen bonds with the solvent molecules. In PMAB there are both pteridine–benzoic acid (N···O) hydrogen bonds and pteridine–pteridine (N···N) hydrogen bonds. Although the molecular conformation of TMQ and PMAB differ from enzyme-bound MTX, rotational energy barriers calculated using CAMSEQ indicate that they can adopt a similar conformation to that seen for MTX complexed with dihydrofolate reductase. These energy calculations show that PMAB is quite flexible and further suggest that the 5-methyl in TMQ reduces its conformational flexibility in a different manner than the N(10)-methyl in MTX. These structural data also show that full geometry optimization and proper parameterization of electronic effects at N(10) are required to accurately represent antifolate conformational preferences for enzyme binding.

Folic acid is a vitamin that when reduced in vivo to tetrahydrofolate and dihydrofolate becomes a coenzyme in the biosynthetic pathway of pyrimidines, purines, serine, and glycine. Substitution of the 4-oxo position of folic acid with an amine yields aminopterin 1, which is a powerful inhibitor of tetrahydrofolate-mediated biosynthesis. The target of inhibition is dihydrofolate reductase (DHFR). Inhibitors of this enzyme were among the earliest drugs found useful for cancer chemotherapy. Methylation of the N(10) position of aminopterin yields one of the most widely used antineoplastic drugs, methotrexate (MTX, **2**). Although MTX has been used successfully for more than 30 years in the treatment of acute lymphoblastic leukemia in children and trophoblastic tumors in women,[1] its limitations include poor cellular uptake, toxicity, resistance by tumor cells, lack of activity against solid tumors, and inability to cross the blood–brain barrier.[2]

To overcome the limitations of MTX, numerous analogues of classical ((*p*-aminobenzoyl)glutamic acid derivatives) and "nonclassical" (lipophilic) antifolates have been designed. The lipophilic antifolates bypass the transport mechanism and thus enter tumor cells more effectively than classical antifolates. One example of a lipophilic antifolate is trimetrexate (TMQ, **4**), which is a potent inhibitor of DHFR and has recently entered phase II clinical studies as an antineoplastic drug.[3] Phase I studies showed it to have a wider range of tumor inhibition and to be longer acting than MTX. Being a lipophilic compound, TMQ has the ability to pass the blood–brain barrier, enter solid tumors,[4-6] and enter cells that are resistant to MTX by virtue of impaired drug transport.[7]

The design of new biologically active molecules is facilitated in part by accurately predicting the conformation of the inhibitor at the target site. The principle goal of this study is to evaluate the conformational, structural, and electronic characteristics of antifolates with respect to their active-site conformation. By studying the three-dimensional structures of antifolates, acceptable models for the study of rotational energy barriers can be obtained. Structural data for antifolates with a methylanilino side chain are reported for methotrexate[8] and quinespar[9] (QU, **5**). To fully evaluate conformational characteristics resulting from changes in the structure of these antifolates, data are needed for pteridines that vary in the 10-position and for quinazolines that vary in the 5- and 10-positions. We report here the crystal structures of two antifolates that have these desired substitution patterns, namely TMQ and an aminopterin derivative, 4-[*N*-[(2,4-diamino-6-pteridinyl)methyl]amino]benzoic acid (PMAB, **3**).

Since the crystal structure of DHFR has been determined with MTX bound to the active sites,[10] we are able to compare these structural data with the enzyme-bound conformation. Although quinazolines differ from pteridines by carbon substitution at two ring nitrogen positions, structural studies of enzyme-bound quinazolines and pteridines show that both ring types occupy the same location and make similar contacts with the enzyme.[10] The C(5)-methyl of TMQ can occupy a hydrophobic pocket when complexed with the enzyme. To expand on the antifolate structural data, a Cambridge Crystallographic

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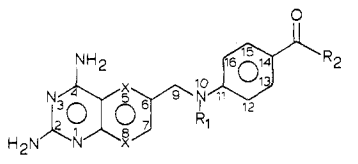
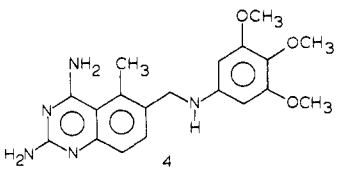


1. R_1=H, R_2=L-Glutamate, X=N
2. R_1=CH_3, R_2=L-Glutamate, X=N
3. R_1=H, R_2=OH, X=N
5. R_1=H, R_2=L-Aspartate diethyl ester, X=C-H



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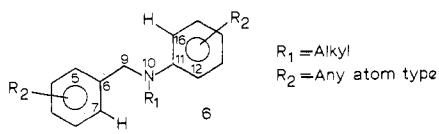

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R_1 = Alkyl
R_2 = Any atom type

Database[11] (CCD) search was made for compounds having a molecular fragment (**6**) representative of the methyleneanilino moiety of these antifolates. The systematic search of compounds with this molecular fragment provides information on conformational preferences that can assist in the design and evaluation of rotational energy strategies. Rotational energy studies using CAMSEQ[12] reveal that PMAB is quite flexible and further suggest that the 5-methyl in TMQ reduces its conformational flexibility in a different manner than the N(10)-methyl in MTX, yet TMQ is able to adopt the same conformation as enzyme-bound MTX.

Results

The final atomic coordinates and equivalent isotropic thermal parameters for TMQ and PMAB are listed in Tables I and II, respectively. The molecular conformation of TMQ (Figure 1) is extended with the trimethoxyanilino ring twisted 89° from the quinazoline ring. In contrast, the molecular conformation of PMAB is flat, with less than a 2° difference between the plane of the pteridine ring and *p*-aminobenzoic acid ring. In addition, the carboxylic acid of PMAB is coplanar with the benzene ring. There are steric effects that cause distortions in the TMQ molecule due to the proximity of N(4) and C(51). First, there are increases in the exocyclic bond angles at C(4), C(4a), and C(5) by 3–6° and second, there is a 2° twist in the quinazoline ring system. Similar distortions of antifolate geometry have been observed in 2,4-diamino-5-methyl-6-benzylpyrido[2,3-*d*]pyrimidine.[17]

Table I. Atomic Coordinates (×10⁴) and Isotropic Thermal Parameters (×10²) for Trimetrexate Dimethyl Sulfoxide Hydrate

atom	*x/a* (σ)	*y/b* (σ)	*z/c* (σ)	B_{eq} (σ),[a] Å²
C(2)	4848 (3)	4474 (2)	2307 (2)	276 (8)
C(4)	4863 (3)	3079 (2)	4094 (2)	283 (8)
C(4A)	5952 (3)	3648 (2)	4290 (2)	253 (8)
C(5)	6620 (3)	3253 (2)	5292 (2)	274 (8)
C(6)	7559 (3)	3964 (2)	5383 (2)	288 (8)
C(7)	7889 (3)	5019 (3)	4483 (2)	324 (9)
C(8)	7324 (3)	5378 (2)	3502 (2)	308 (9)
C(8A)	6336 (3)	4708 (2)	3387 (2)	266 (8)
C(9)	8204 (3)	3671 (3)	6456 (2)	340 (9)
C(11)	10235 (3)	2222 (2)	7424 (2)	276 (8)
C(12)	11584 (3)	1316 (2)	7375 (2)	297 (8)
C(13)	12371 (3)	932 (2)	8269 (2)	318 (9)
C(14)	11811 (3)	1412 (2)	9247 (2)	309 (9)
C(15)	10452 (3)	2295 (3)	9300 (2)	321 (9)
C(16)	9677 (3)	2713 (3)	8398 (2)	315 (9)
C(51)	6345 (4)	2084 (3)	6257 (3)	448 (11)
C(131)	14106 (4)	−800 (3)	7601 (4)	585 (14)
C(141)	12720 (5)	−191 (3)	10818 (3)	591 (14)
C(151)	8609 (5)	3616 (4)	10395 (3)	749 (17)
N(1)	5764 (2)	5128 (2)	2389 (2)	292 (7)
N(2)	4252 (3)	4860 (2)	1339 (2)	410 (9)
N(3)	4361 (2)	3478 (2)	3113 (2)	298 (7)
N(4)	4270 (3)	2121 (2)	4870 (2)	408 (9)
N(10)	9469 (3)	2598 (2)	6528 (2)	358 (8)
O(13)	13735 (2)	101 (2)	8256 (2)	469 (8)
O(14)	12617 (2)	1087 (2)	10116 (2)	398 (7)
O(15)	9964 (2)	2700 (2)	10293 (2)	472 (8)
C(171)	1020 (4)	3174 (3)	2591 (3)	542 (13)
C(172)	−201 (5)	1121 (4)	3368 (3)	672 (17)
O(171)	1483 (3)	1531 (2)	4553 (2)	486 (8)
S(17)	1493 (1)	1549 (1)	3339 (1)	409 (3)
O(W1)	6344 (3)	6691 (2)	76 (2)	657 (11)

[a] $B_{eq} = 4/3\sum_i\sum_j\beta_{ij}(a_i a_j)$.

Table II. Atomic Coordinates (×10⁴) and Isotropic Thermal Parameters (×10²) for 4-[*N*-[(2,4-Diamino-6-pteridinyl)methyl]amino]benzoic Acid

atom	*x/a* (σ)	*y/b* (σ)	*z/c* (σ)	B_{eq} (σ),[a] Å²
C(2)	4371 (3)	2045 (3)	1158 (2)	249 (8)
C(4)	5477 (3)	2691 (3)	38 (2)	233 (8)
C(4A)	6279 (3)	3872 (3)	503 (2)	218 (7)
C(6)	7910 (3)	5831 (3)	631 (1)	233 (7)
C(7)	7590 (3)	6010 (3)	1439 (2)	279 (8)
C(8A)	5987 (3)	4040 (3)	1302 (2)	230 (7)
C(9)	9021 (4)	6843 (3)	308 (2)	318 (9)
C(11)	10030 (3)	7374 (3)	−977 (2)	255 (8)
C(12)	10078 (3)	6990 (4)	−1798 (2)	325 (9)
C(13)	10980 (3)	7751 (4)	−2260 (2)	318 (9)
C(14)	11885 (3)	8937 (3)	−1946 (2)	248 (8)
C(15)	11861 (3)	9323 (3)	−1133 (2)	261 (8)
C(16)	10934 (3)	8569 (3)	−658 (2)	283 (8)
C(17)	12831 (3)	9687 (3)	−2485 (2)	272 (8)
N(1)	5027 (3)	3118 (3)	1644 (1)	238 (6)
N(2)	3425 (3)	1108 (3)	1471 (1)	319 (7)
N(3)	4529 (3)	1811 (3)	360 (1)	257 (6)
N(4)	5689 (3)	2488 (3)	−737 (1)	312 (7)
N(5)	7242 (3)	4758 (2)	166 (1)	238 (6)
N(8)	6649 (3)	5163 (3)	1783 (1)	271 (7)
N(10)	9142 (3)	6557 (3)	−530 (1)	305 (7)
O(17A)	13693 (2)	10784 (2)	−2160 (1)	313 (6)
O(17B)	12768 (2)	9346 (2)	−3209 (1)	372 (6)

[a] $B_{eq} = {}^4/_3\sum_i\sum_j\beta_{ij}(a_i a_j)$.

The orientation of the 13,14,15-trimethoxy groups in TMQ are −27°, −72°, and 1°, respectively, and are similar to values observed for trimethoxybenzoyl moieties found in the antibacterial trimethoprim antifolates.[18] Energy-minimization studies show that the orientation of the 13- and 15-methoxy positions can range from 0° to ±90° with

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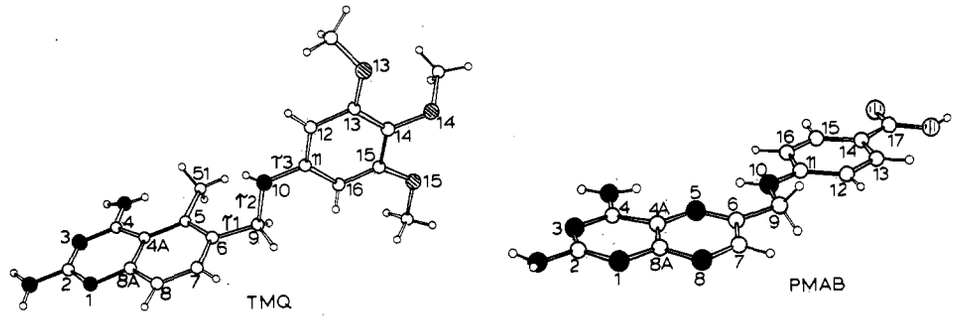

Figure 1. Atomic numbering and molecular conformation of TMQ and PMAB.

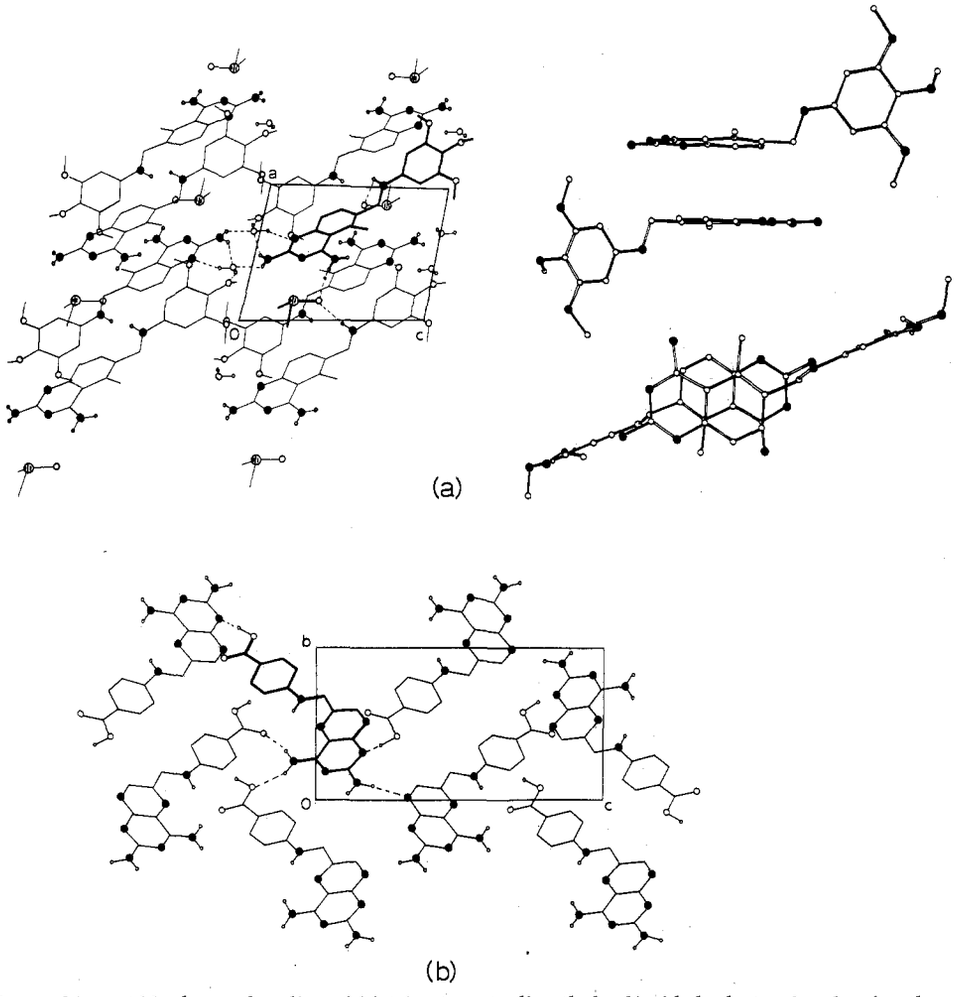

Figure 2. Molecular packing and hydrogen bonding of (a) trimetrexate dimethyl sulfoxide hydrate, also showing the stacking interactions, and (b) PMAB. The filled circles are nitrogen, larger open circles are oxygens, smaller open circles are hydrogens, and stippled circles are sulfur.

equal probability, while the low-energy conformation for the 14-position is ±90°.[19]

Hydrogen bonding (Figure 2) observed in the molecular packing of TMQ and PMAB differs from the usual base-paired type of hydrogen bonds between N(4)–H···N(3) found in other antifolate structures.[20,21] Instead, there is a strong preference for N···O interactions in the molecular packing of these structures. Trimetrexate dimethyl sulfoxide hydrate forms a network of N···O hydrogen bonds with the solvent molecules and the quinazoline rings from an antiparallel stacking arrangement that places C(4) within 3.36 Å of C(7) in a symmetry-related molecule (Figure 2a). The water molecule forms hydrogen bonds with N(1), N(2), and O(14), demonstrating that methoxy oxygens can be effective proton acceptors. The N(4) amino group utilizes only one of its hydrogens to form an interaction with dimethyl sulfoxide, which in turn hydrogen bonds to N(10). In general, the hydrogen-bonding pattern of TMQ is consistent with other quinazoline free base structures[9,22,23] in which there is at least one water of

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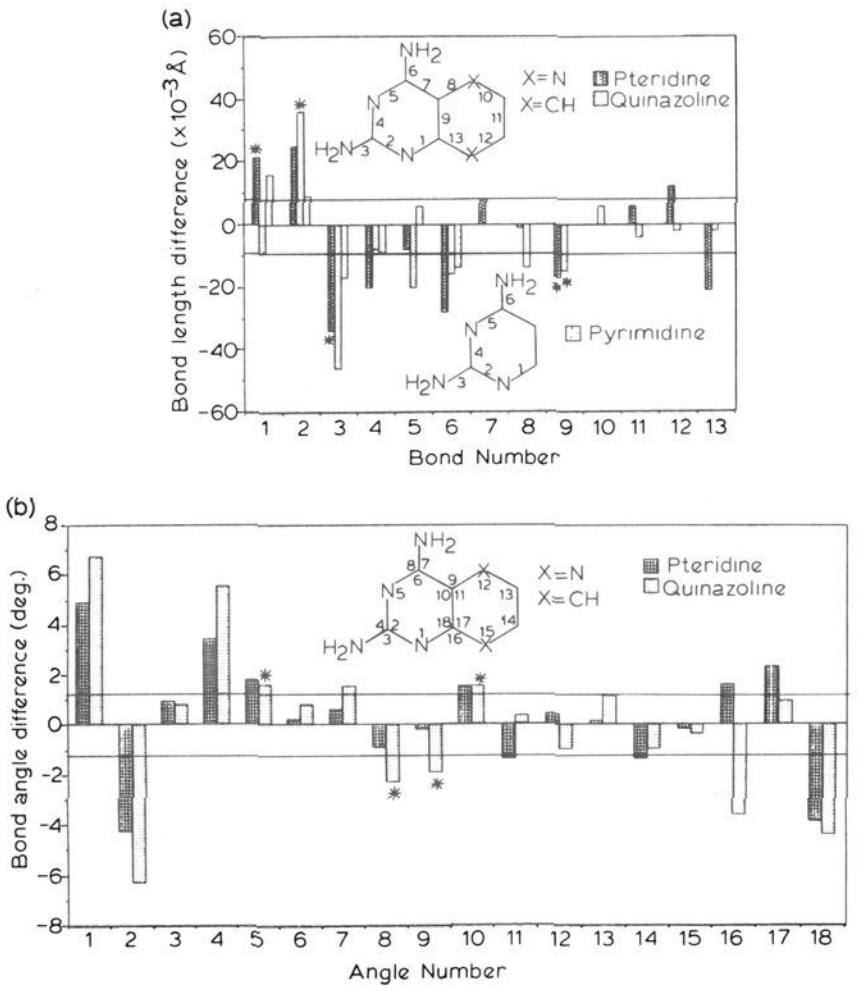

Figure 3. The differences obtained by subtracting the average protonated geometry from the average nonprotonated geometry for (a) bond lengths for 2,4-diaminopteridines, -quinazolines, and -pyrimidines, and (b) bond angles for 2,4-diamino-pteridines and -quinazolines (derived from ref 8, 9, 22, 23, 25–27). An analysis of variance using a statistical F test shows that there is insufficient evidence to indicate a difference for all values smaller than 0.009 Å and 1.3° for bond lengths and angles, respectively, and for those values labeled with an asterisk.

crystallization that hydrogen bonds to N(1) and where N(4) forms only one hydrogen bond because of the steric interactions of the quinazoline 5-substituent.

The molecular packing of PMAB mimics some interactions observed for MTX in the enzyme active site and in the single crystal structure of MTX.[8,10] The similarities are an acid O(17A)···N(1) interaction and two N(4)–H···O interactions (Figure 2b). Both H(N2A) and H(N10) are not involved in intermolecular hydrogen bonds. There is a close intramolecular contact between N(5)···N(10) of 2.707 Å, with a N(10)–H···N(5) distance of 2.274 Å.

Discussion

Previous observations of the effects of protonation on 2,4-diaminopyrimidine and 2,4-diaminopteridine bond lengths have shown that protonation decreases exocyclic C–N bond lengths.[21] The determination of additional pteridine and quinazoline crystal structures permits a more detailed analysis of these effects. A comparison of the average protonated and nonprotonated pteridine and quinazoline geometries (Figure 3) indicates that the geometry of these rings is more sensitive to the effects of protonation than 2,4-diaminopyrimidines. These data show that the largest changes in bond lengths involve

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Table III. Conformational Characteristics of Dihydrofolate Reductase Binders

torsion angle,[a] deg	TMQ	PMAB	QU	MTX	MTX-E[b]	FA[c]
τ_1	79	4	80	39	−146	31
τ_2	178	177	179	63	57	180
τ_3	170	180	169	−165	175	174

[a] τ_1 = N(5)[or C(5)]–C(6)–C(9)–N(10), τ_2 = C(6)–C(9)–N(10)–C(11), τ_3 = C(9)–N(10)–C(11)–C(12). [b] From *Lactobacillus casei*; see ref 10: There are three enzyme-bound conformations of MTX. The crystal structure of *Escherichia coli* DHFR has two molecules in the asymmetric unit, both of which have MTX bound to the active site. The conformation of these three enzyme-bound MTX structures differ by less than 5° among their respective values of τ. [c] Folic acid; from ref 24.

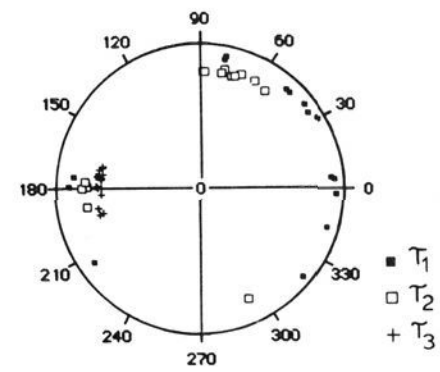

Figure 4. Distribution of τ's in the crystal structures obtained from the CCD search and those listed in Table III.

shortening of the exocyclic amino bonds and a lengthening of the adjacent endocyclic C–N bonds. Similarly, the largest angular changes are in the endocyclic angles at N(1) and C(2). The paucity of data in the pteridine and quinazoline groups precludes any quantitative judgements.

Since MTX crystallizes as a zwitterion[8] with N(1) protonated by its glutamic acid moiety, a zwitterion was expected in the PMAB crystal structure. A close contact between the benzoic acid and N(1) (O(17A)···N(1), 2.628 Å) suggested N(1) protonation. However, the presence of a proton on the benzoic acid moiety, supported by the differences in the two C–O bond lengths and observed geometric patterns in pteridine bond lengths and angles, indicates that the molecule is a free base.

The torsion angles that define the orientation of the C(6) side chain of antifolates with the methylene–amine bridge are compared in Table III. As shown, there are many accessible conformations for these antifolates. Only the conformation of TMQ and QU are similar. The principle differences are between τ_1 and τ_2, while τ_3 is consistently observed in a planar conformation due in part to the sp^2 hybridization at the N(10) position.

Although TMQ has a conformation similar to that of QU (Table III), its crystal structure differs in that QU forms N···N hydrogen bonds as observed in other antifolate structures,[20,21] while TMQ has only N···O interactions similar to MTX.[8] The observation that the hydrogen-bonding interactions of the 2,4-diaminoquinazoline of TMQ and 2,4-diaminopteridine of PMAB are similar indicates that a study of molecular packing may be relevant to interactions found in the enzyme active site.

To expand on the antifolate structural data, the crystallographic literature was searched for compounds containing structural fragments complimentary to the title compounds. The distribution of τ's in crystal structures with **6** as a molecular fragment is shown in Figure 4. Two important features about crystallographic data should be noted: first, crystals of nonchiral molecules have both enantiomorphs in the crystal lattice, and thus, both $\pm\tau$'s are present; second, ring numbering is ambiguous for τ_1

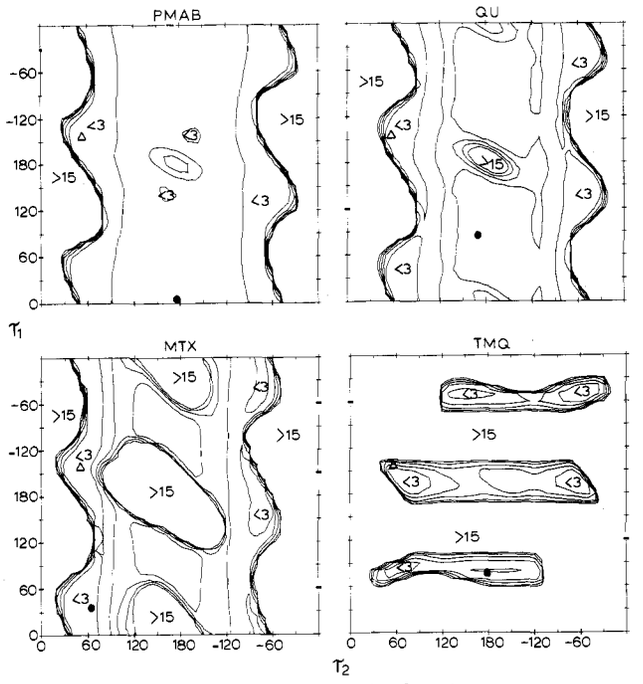

Figure 5. Two-dimensional CAMSEQ plots[1] of the principle torsion angles τ_1 and τ_2 using the molecular fragments in Figure 1 as the input structures. Each contour line represents an increase of 3 kcal/mol relative to their global minimum. The solid circle on each plot denotes the X-ray conformation for that compound, the open triangle represents enzyme bound MTX.

in structures with C–H at the 5- and 7-positions and for τ_3; thus alternative ring numbering adds 180° to these τ's. Values for τ_1 obtained by using the alternative ring numbering and alternative enantiomorph result in a distribution of τ_1 over the full 360° range. τ_2 has additional values generated by changing the structure's enantiomorph, yet its values still cluster near ±80° and 180°. These results indicate that τ_3 = 180° (or 0°) is a preferred conformation, independent of τ_1 and τ_2. These crystal structures located with search fragment **6** are representative of the antifolate conformations (Table III). The preference for a planar τ_3 further shows that there is an important electronic contribution to the conformation.

The conformational characteristics of these antifolates were defined by calculating the energy barriers of rotation around τ_1 and τ_2 with the program CAMSEQ. Since the orientation of τ_3 was found to be a consistent value, its crystallographically observed conformation was used. Two-dimensional contour maps of PMAB, QU, MTX, and TMQ (Figure 5) show that there is a progressive decrease in conformational flexibility upon substitution. Both PMAB and QU represent the minimal structures for pteridines and quinazolines, respectively. Since the crystal structure conformations of PMAB, QU, and TMQ are not within a <3 kcal/mol region on their energy contour maps (Figure 5), there may be electronic effects on τ_1 and/or τ_2 unaccounted for in these energy calculations. The contour maps are consistent with the results shown in Figure 4, where values of τ_1 adopt the full 360° range and values of τ_2 lie near ±80° or 180°. The apparent reduction of the energy barrier in TMQ at $\tau_1 = \tau_2$ = 180°, relative to QU, is a result of geometry distortions of TMQ due to the proximity of N(4) and C(51) and differences in the N-(10)-H geometry. One cannot simply construct TMQ from QU and generate the same map as that seen in the structure obtained through crystallographic studies.

Although enzyme-bound MTX (MTX-E) differs in conformation from the antifolate crystal structures (Table III), the contour maps reveal that its conformation is within 3 kcal/mol of the energy minimum of MTX, PMAB, and QU. The contour map of TMQ shows MTX-E to be in a region of >6 kcal/mol; however, since the value of τ_3 can influence the location and area of the energy wells in the contour map, a rotation of less than 10° can open the <3 kcal/mol area such that τ_1 and τ_2 of TMQ can adopt a similar conformation as that found for MTX-E. A structure with both a C(5)- and N(10)-methyl would have less flexibility than TMQ yet could adopt the MTX-E conformation. The contour map of this doubly substituted structure would have characteristics of both contour maps of TMQ and MTX (Figure 5), and would contain the same <3 kcal/mol regions as seen in the TMQ contour map.

These energy calculations are in general agreement with those reported for a variety of antifolates using molecular mechanics force field parameterizations (MM2P).[19] However, these force field studies reported the minimum energy conformation of MTX to be $\tau_1 = \tau_3$ = 90°, independent of τ_2, in contrast to observed crystallographic results (Table III, Figure 4) that show τ_3 = 180° is always preferred. However, neither CAMSEQ nor MM2P calculations incorporated the apparent underlying electronic effects of the aromatic N(10)-amine. Thus crystallographic data presented here extend the data base upon which precise parameterization of electronic structure is required for more sophisticated molecular mechanics calculations.

The CCD[11] contains over 50 000 crystal structures, yet only 11 structures have a molecular fragment and substitution pattern that is complementary to the antifolates of interest. In fact, the substitution pattern of MTX (N-(10)-alkyl and 5-nitrogen) and TMQ (C(5)-alkyl) are unique with respect to substitution on the search fragment **6**. Thus, the crystal structure of TMQ and PMAB provide a basis to evaluate the effects of substitution and protonation on conformation and electronic properties, which helps to further define the three-dimensional characteristics of antifolates. These results show that although the molecular conformations of TMQ and PMAB differ from that of MTX, both free and enzyme-bound, the range of τ_1 values observed in similar structural fragments shows that several low-energy conformations are accessible. The systematic search of similar molecular fragments such as those retrieved through a search of the CCD provide information on conformational preferences that can assist in the design of energy-minimization strategies. For example, the observation that τ_3 values are near 180° can reduce the conformational space over which calculations are made and also show that the electronic properties of this functional group are important in these calculations. The rotational-energy studies show that both TMQ and PMAB can adopt a conformation similar to that of MTX-E. The underlying differences in the maps suggest the importance of energy minimization when molecules are generated from "standard" geometry or molecular fragments.

Experimental Section

X-ray Studies on 2,4-Diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline. The free base of TMQ was a generous gift from Warner-Lambert. Crystals of TMQ were grown as the dimethyl sulfoxide (DMSO) hydrate from a 2-propanol solution where DMSO was present in a concentration equimolar to TMQ. The crystal used for data collection was rectangular with dimensions 0.06 × 0.40 × 0.58 mm. The crystal data show the following: space group $P\bar{1}$, triclinic, a = 9.423 (2) Å, b = 11.180 (4) Å, c = 12.399 (3) Å, α = 72.26 (2)°, β = 75.10 (2)°, γ = 74.13 (2)°, V = 1174.9 Å^3, Z = 2, D_{calcd} = 1.316 g/cm^{-1}. Data were collected on a Nicolet P3 diffractometer using Mo Kα

radiation. The crystal showed no deterioration during data collection. The data were corrected for Lorentz and polarization effects, but not for extinction or absorption effects. A total of 5440 unique reflections were collected to $2\theta = 130°$. The structure was solved by using the direct methods programs MULTAN[13] and NQEST.[14] Non-hydrogen atom thermal parameters were made anisotropic and refined by full-matrix least-squares techniques. Hydrogen atoms were located in Fourier difference maps and were given isotropic thermal parameters one unit greater than the heavy atoms to which they were bound; their thermal parameters were held constant during refinement. The structure converged to a final R index of 0.059 with 3335 ($I \geq 3\sigma(I)$) intensities.

X-ray Studies on 4-[*N*-[(2,4-Diamino-6-pteridinyl)methyl]amino]benzoic Acid. Crystals of PMAB (Sigma) were grown from an ethanol solution containing trace amounts of HCl. The crystal used for data collection was rectangular with dimensions 0.08 × 0.12 × 0.20 mm. The cell parameters for the monoclinic $P2_1/c$ lattice are as follows: $a = 9.182$ (2) Å, $b = 8.771$ (2) Å, $c = 16.561$ (3) Å, $\beta = 96.52$ (2)°, $Z = 4$, $V = 1325.2$ Å^3, $D_{calcd} = 1.560$ g/cm^{-1}. Intensities for 3086 independent reflections were measured on a Nicolet P3 diffractometer, using Mo Kα ($\lambda = 0.7069$) radiation. All non-hydrogen atoms were located by direct methods using MULTAN[13] and NQEST.[14] Acceptable atomic positions for all hydrogen atoms were located from Fourier difference maps. The structure was refined by full-matrix least-squares techniques. Hydrogen positional parameters were refined, while their isotropic thermal parameters were held constant at values one unit greater than the heavy atom to which they were bound. The structure converged to a final R index of 0.063 with 1787 ($I \geq 3\sigma(I)$ and $2\theta < 50°$) intensities.

Structural Studies. The data for structural comparisons were taken from the CCD.[11] Individual crystal structures were obtained by using the connectivity of 6 with the following restrictions: ring members were either carbon or nitrogen, except for positions 6, 7, 11, and 16, which were restricted to carbon; ring substitution was not restricted except for positions 7 and 16, which required a hydrogen; substitution on N(10) was restricted to alkyl substituents; position 9 was kept as a $-CH_2-$. A total of 11 independent conformations from nine crystal structures was found in the search.

Geometric and Conformational Analysis. The molecular geometry and rotational energies were evaluated on the National Institutes of Health PROPHET[15] system. Rotational energy barriers were calculated by using the program CAMSEQ.[12] The data from CAMSEQ were processed with the program CAMMAP and plots were generated with the program CONTOUR. This version of CAMSEQ is only dimensioned for structures the size of fragment 6; therefore, calculations were performed on molecular fragments derived from the X-ray coordinates of PMAB, QU, MTX, and TMQ, respectively.[16] All hydrogen atoms on carbons were placed in calculated positions. The energy surfaces were calculated at 10° intervals over the full 360° range. No energy minimization was performed.

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Supplementary Material Available: Tables of hydrogen-bonding geometries, anisotropic thermal parameters, hydrogen positional parameters, and torsional angle listing and references for fragment 6, and a figure of bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

Isothiourea Derivatives of 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole with Broad-Spectrum Anthelmintic Activity

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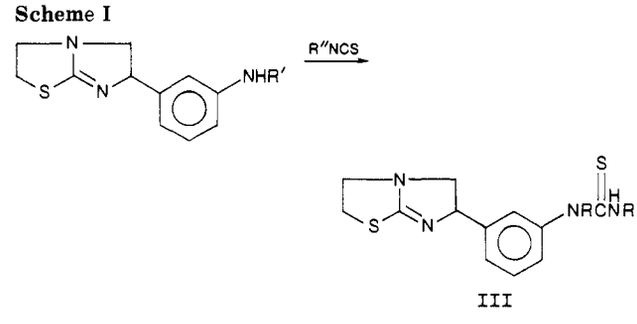
A series of isothiourea derivatives of 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (tetramisole) is described. The compounds are prepared by the S-alkylation of the thioureas that were obtained either by the reaction of an amine with 6-(3-isothiocyanatophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole or by the reaction of an isothiocyanate with 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole. These derivatives have an improved spectrum of activity over tetramisole and are active against nematodes, cestodes, and trematodes. The structure–activity relationships are discussed.

Since the discovery of the broad-spectrum nematode anthelmintic 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]-thiazole (tetramisole) (I) in 1966[1] many groups have prepared analogues with the aim of finding derivatives with improved activity and spectrum. While tetramisole is effective against a wide range of nematodes, it shows no activity against other important parasite species such as cestodes and trematodes.[2] Of the analogues of tetramisole reported in the literature, the 6-(3-aminophenyl) derivative (II) and its acyl derivatives[3] constitute an important group with improved activity against certain nematodes but no reported activity against either cestodes or trematodes.

We now report on the synthesis and anthelmintic activity of a series of 3′-isothioureido-substituted tetramisole

Scheme I



III

R′ = H or CH_3

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