# Crystal Structure of Quinespar, a Quinazoline Analogue of Methotrexate

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The molecular structure of quinespar, a quinazoline analogue of methotrexate and aminopterin, has been determined by X-ray crystallography. The molecule displays an extended conformation with the *p*-aminobenzoyl plane rotated 66° from the plane of the quinazoline. The orientation of the quinazoline ring relative to the rest of the molecule is intermediate between the orientations of the comparable pteridine rings in folic acid and in DHFR-bound methotrexate. Evidence is presented to suggest that 2,4-diaminoquinazolines bind to DHFR in the same manner as do 2,4-diaminopteridines.

Dihydrofolate reductase (DHFR) and thymidylate synthetase (TS) are two very important target enzymes for drug research in the field of cancer chemotherapy. These enzymes are linked in the biological production of thymidylate an essential component of DNA. DHFR reduces folic acid and dihydrofolate to tetrahydrofolate while TS transfers a methyl group from $N^5$,$N^{10}$-methylenetetrahydrofolate to deoxyuridylate to form deoxythymidylate, regenerating dihydrofolate in the process. Blockade of either DHFR or TS will lead to a deficit in thymidylate and eventually to cell death.

As folates and their reduced products are substrates for DHFR and cofactors for TS, folic acid has for many years been a model compound for the design of inhibitors of DHFR and TS which could be effective in cancer chemotherapy. Methotrexate, one of the most widely used anticancer drugs, is a close analogue of folic acid, differing only in replacement of the folic acid 4-carbonyl oxygen atom by an amino group and substitution of a methyl group at N(10) in place of hydrogen. These small differences are sufficient to cause methotrexate to bind very tightly to DHFR and inactive the enzyme.

However, like all cancer chemotherapeutic agents used to date, methotrexate is toxic to both normal and malignant cells. There are indications that small evolutionary or developmental changes in malignant cells result in altered uptake and transport characteristics for various drugs and alterations in enzyme–substrate affinities. A major emphasis in medicinal chemistry is to develop compounds which can exploit these small differences and selectively kill cancer cells with minimal damage to normal tissue.

Experiments aimed at developing new DHFR and TS inhibitors have led to the synthesis of a number of quinazolines, compounds that differ from pteridines such as folic acid and methotrexate by replacement of nitrogens 5 and 8 in the pteridine ring with carbons. Quinazoline analogues of both folic acid (2-amino-4-oxo compounds) and methotrexate (2,4-diamino compounds) have been examined as potential cancer chemotherapy agents. Several studies have shown that some 2,4-diaminoquinazolines are strong DHFR inhibitors, approximately equal to methotrexate in their binding strengths,[1] while 2-amino-4-oxo quinazolines have been found to bind very strongly to thymidylate synthetase.[2] Preliminary testing indicates that quinazolines have potential to augment methotrexate and the nucleotide metabolite of fluorouracil as DHFR and TS inhibitors and to become clinically useful anticancer drugs.[3]

We have begun a program of stereochemical examinations of quinazolines with the elucidation of the crystal structure of the prototype 2,4-diaminoquinazoline, quinespar. It is hoped that these studies will provide insight into the stereochemical determinants of substrate and inhibitor binding to both DHFR and TS, thus adding information useful for the systematic design of new antifolate anticancer drugs.

**Table I.** Crystal Data for $C_{24}H_{28}N_6O_5 \cdot 2H_2O$

| | |
|---|---|
| fw | 516.6 |
| $F(000)$ | 1064 |
| $a$, Å | 32.770 (15) |
| $b$, Å | 7.529 (9) |
| $c$, Å | 11.064 (3) |
| $\beta$, deg | 109.34 (2) |
| $V$, Å$^3$ | 2575.7 (4) |
| $d_{calcd}$, g cm$^{-3}$ | 1.332 (2) |
| space group | $C2$ |
| $Z$ | 4 |

## Experimental Section

A sample of *N*-[*p*-[[(2,4-diamino-6-quinazolinyl)methyl]-amino]benzoyl]aspartic acid diethyl ester (quinespar) was crystallized from a 50:50 water–ethanol solution by slow evaporation. X-ray intensity data were collected from a crystal 0.49 × 0.29 × 0.07 mm in size by using Ni-filtered Cu $K_\alpha$ X-radiation. Unit cell constants are given in Table I. The intensities of all independent reflections having $2\theta < 130°$ were measured on a Picker diffractometer using the $\theta$-$2\theta$ scan technique, with a scan range of 2° and stationary background counts at each end of the scan. The intensities were corrected for Lorentz and polarization factors, but no absorption corrections were made ($\mu = 8.2$ cm$^{-1}$). Three standard reflections monitored frequently showed no significant trends or abnormalities during data collection. A total of 2376 unique reflections were measured of which 1427 had $I > 2\sigma(I)$. Those reflections whose intensities were less than $2\sigma(I)$ were classified "unobserved" and not used in the structure refinement.

## Structure Determination

The structure factor data were converted to normalized values, $E$, and a multisolution process was used to determine phases of 245 reflections with $E > 1.50$. An $E$ map calculated from the set of phases with the next to lowest absolute figure of merit but best negative quartet value showed two molecular fragments totaling 21 atoms. A difference Fourier calculated with phases derived from these fragments revealed the other 16 atoms plus two other peaks later determined to be water molecules. After

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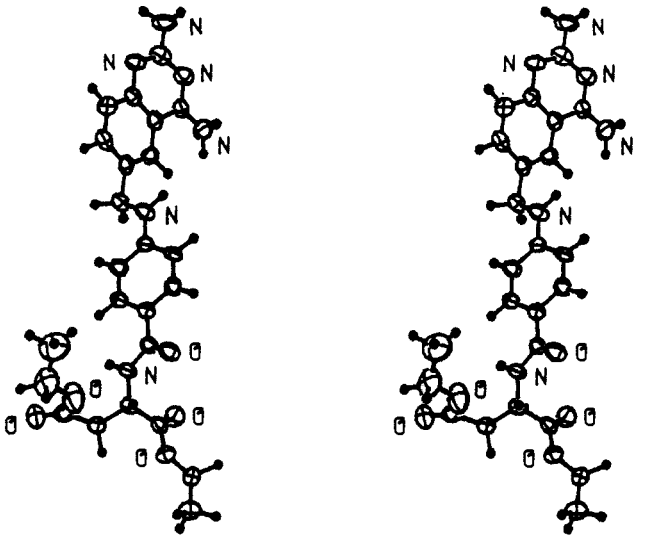

**Figure 1.** Stereoscopic view of quinespar (aspartic acid diethyl ester).

least-squares refinement had reduced $R(\sum||F_o| - |F_c||/\sum|F_o|)$ to 0.145 a difference map revealed positions for all 32 hydrogen atoms. Anisotropic temperature factors were assigned to the atoms of the quinazoline molecule only, keeping the water oxygens and all hydrogens isotropic. Full-matrix least-squares refinement of all non-hydrogen coordinates and thermal parameters gave a final $R$ of 0.079. Scattering factors for nitrogen, oxygen, and carbon and for hydrogen atoms were as cited.[4] Final atomic fractional coordinates and thermal parameters are available (see paragraph at the end of this paper concerning supplementary material).

## Results and Discussion

The 2,4-diaminoquinazoline title compound (quinespar) can be viewed as an analogue of methotrexate differing in that it has a hydrogen attached to N(10) and aspartic acid diethylester in place of glutamic acid. Figure 1 shows a stereoscopic view of the molecule. Quinespar is in an extended conformation with torsion angles: C(6)–C(9)–N(10)–C(11), 179° and C(14)–C(17)–N(18)–C(19), 176°.

The substituted quinazoline and *p*-aminobenzoyl groups are planar to within 0.016 and 0.007 Å, respectively, and the dihedral angle between their planes is 66°. The substituent atoms N(2), N(4), and C(9) are all less than 0.04 Å from the least-squares plane of the quinazoline; atoms N(10) and C(17) are 0.033 and 0.007 Å, respectively, out of the plane of the *p*-aminobenzoyl group. Bond distances and angles are given in Figure 2. The C(4A)–C(4) and C(4A)–C(5) bonds are significantly longer, and the C(5)–C(6) bond is significantly shorter than the C–C bond lengths expected in a delocalized $\pi$-electron ring system. The four chemically similar cyclic C–N bonds also show an alternation of short and long bond lengths. The observed distances are very likely indications of true bond order in the quinazoline ring as a similar bonding pattern has been observed in the reported structure of 2,4,6-triamino-5-chloroquinazoline.[5] The C–C bond involving the terminal methyl carbon C(28) deviates considerably from the expected $sp^3$ single bond value. The observed value (1.39 Å) is most likely shortened due to atom motion since C(28) exhibits a comparatively large thermal parameter.

**Table II.** Hydrogen Bond Distances (Å) and Angles (deg)

| | H···O(or N) | O(N)··· O(N) | <O(N)–H···O(N) |
|---|---|---|---|
| N(2)–H(N2,1)···N(3) | 2.03 | 3.04 | 163 |
| N(4)–H(N4,1)···O(W2) | 1.90 | 2.79 | 150 |
| O(W1)–H(W1)···N(1) | 1.95 | 2.83 | 170 |
| N(18)–H(N18)···O(W1) | 2.03 | 2.87 | 137 |
| O(W2)–H(W2)···O(25) | 1.97 | 2.91 | 137 |
| O(W2)–H(W2)···O(17) | 1.84 | 2.86 | 146 |

Figure 3 shows a perspective view of the unit cell contents. The hydrogen atoms are omitted for the sake of clarity. The quinazolines are associated with one another about the twofold symmetry axis forming hydrogen-bonded dimers. This interaction is clearly shown in the middle of the diagram where the N(2) amino donates one of its hydrogens to N(3) of a symmetry-related molecule. The other amino group (N(4)) forms a hydrogen bond with a water molecule (W2). W2 also hydrogen bonds the carbonyl oxygens O(17) and O(25) of translationally equivalent molecules forming an infinite chain along the *b* axis. The other water molecule (W1) donates a hydrogen to N(1) of a quinazoline and accepts a hydrogen from N(18) of a second molecule related by the $2_1$ symmetry axis. Table II lists the hydrogen bond distances and angles.

There are no indications of any stacking interactions involving the delocalized $\pi$-electron systems of either the quinazoline or *p*-aminobenzoyl groups.

An important question in the field of DHFR-inhibitor anticancer drugs has been whether the inhibitors bind to the enzyme in similar or different orientations from that of the folate substrates. Crystal structures analyses of two diastereoisomers of 5,10-methenyltetrahydrofolate,[6] of folic acid,[7] and of two methotrexate–DHFR complexes[8] and an NMR study of hydrogen transfer in the DHFR reduction of folic acid[9] have provided a valuable and consistent body of evidence on this question. They have shown that despite the close chemical similarities between folate substrates and 2,4-diaminofolate inhibitors of DHFR, the inhibitors bind to the enzyme with their pteridine ring rotated approximately 180° about the C(6)–C(9) bond away from the pteridine ring orientations of the enzyme-bound substrates. For example, in the crystal structure of folic acid the N(10)–H bond lies close to the plane of the pteridine ring and pointed toward the pteridine carbonyl oxygen. A water molecule hydrogen bonded to both the carbonyl oxygen and N(10)–H stabilizes this molecular conformation. In contrast, when bound to DHFR, methotrexate's N(10)–$CH_3$ bond is pointed away from the C(4)–$NH_2$ bond and a pteridine rotation of approximately 180° is necessary to bring it into near coincidence with the folic acid N(10)–H.

The orientation of the quinazoline ring in the present study lies between the pteridine orientations found in folic acid and in DHFR-bound methotrexate. The N(10)–H bond is roughly perpendicular to the quinazoline ring plane. The dihedral angle C(5)–C(6)–C(9)–N(10) is 79°;

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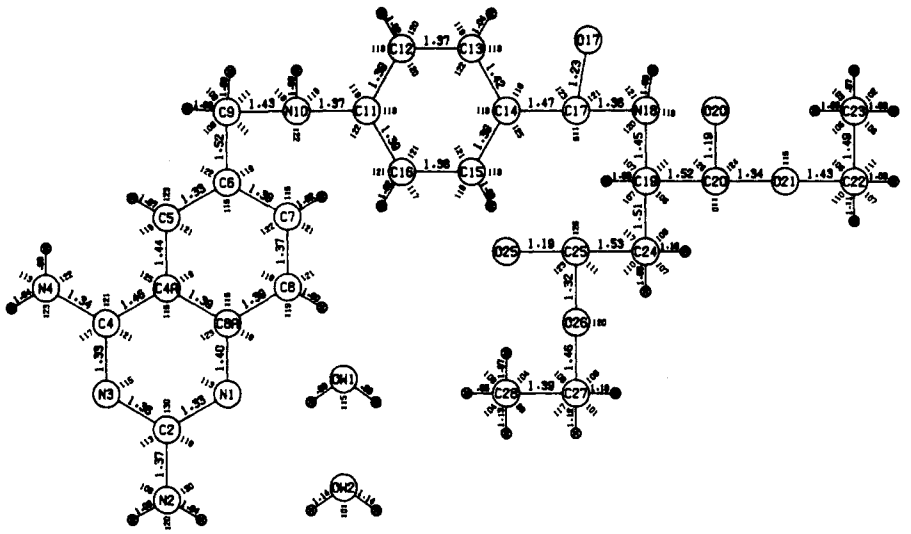

**Figure 2.** Bond distances (Å) and angles (°) for quinespar and water molecules. Additional angles not given in the figure for the tetrahedral carbon atoms range from 106° to 112°. Estimated standard deviations are 0.01 Å and 1.0°.

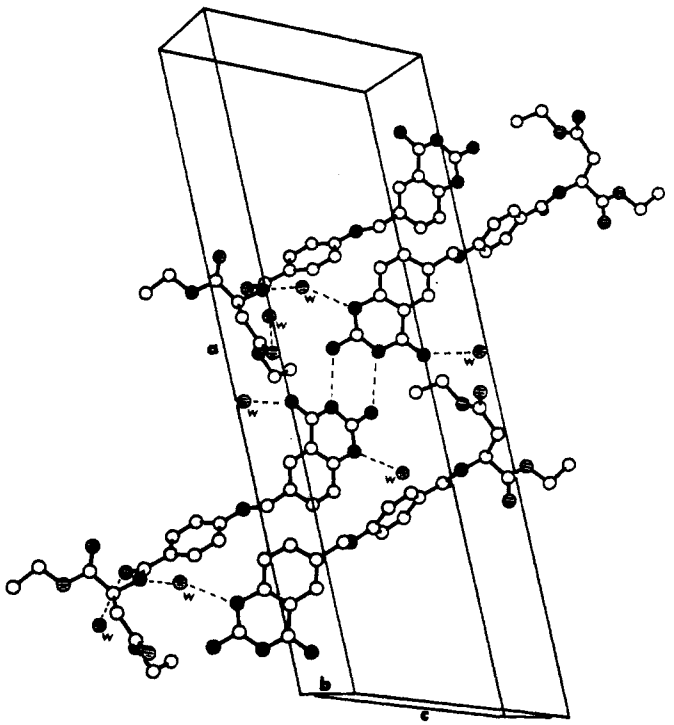

**Figure 3.** Unit cell contents and molecular packing of quinespar and water molecules (W). Nitrogen atoms are blackened and oxygen atoms striped. Hydrogen bonds are represented by dashed lines. Unit cell axes (*a*, *b*, *c*) are indicated.

the equivalent dihedral angles in folic acid and enzyme-bound methotrexate are 31° and −158°, respectively. Comparisons of the quinespar molecular conformation with those of folic acid and methotrexate are shown in Figure 4.

As the N(10)–H bond in the quinespar points neither toward nor away from the C(4)–$NH_2$ bond but assumes an intermediate position, the crystal structure results do not in themselves provide direct evidence for the quinazoline ring orientation when 2,4-diaminoquinazolines bind to and inhibit DHFR. However, examination of the crystal structure of methotrexate (Mastropaolo, Camerman, and Camerman, unpublished results) reveals that when in the solid state and not enzyme-bound, the molecular conformation of methotrexate is very similar to that of quinespar. The N(10)–$CH_3$ bond is approximately perpendicular to

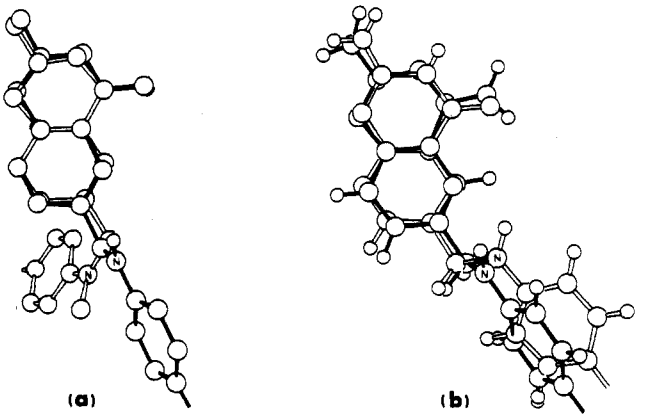


**Figure 4.** (a) Comparison of the quinespar (solid lines) structure with that of methotrexate bound to DHFR (hollow lines). (b) Comparison of the quinespar (solid lines) structure with that of folic acid. (Amino acid ends of the molecules are omitted).

the pteridine ring plane with N(5)–C(6)–C(9)–N(10) dihedral angles of 113° and −111° in the two methotrexate molecules in the crystal asymmetric unit. Thus it is logical to conclude that 2,4-diaminoquinazolines bind to DHFR in the same manner as do 2,4-diaminopteridines and the near perpendicular arrangement of the C(9)–N(10) bond relative to the heterocyclic ring planes in the crystal structures of unbound inhibitors either reflects a conformational preference in the isolated molecules or is due to local environmental factors.

In addition, although quinazolines have carbon instead of nitrogen atoms at positions 5 and 8, methotrexate can be replaced by a 2,4-diaminoquinazoline with identical conformation in the binding scheme proposed for the DHFR–methotrexate complex[10] with relatively little change in enzyme–inhibitor binding. The quinazoline could participate in five hydrogen bonds, vs. six proposed for methotrexate, and the one bond it could not form, that which involves N(8), is, in the methotrexate scheme, the weakest hydrogen bond with a water molecule serving as the donor group.

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**Registry No.** Quinespar, 18921-66-9.

**Supplementary Material Available:** Table of fractional atomic coordinates and thermal parameters (2 pages). Ordering information is given on any current masthead.