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The antifolate trimetrexate: observation of the enzyme-binding conformation

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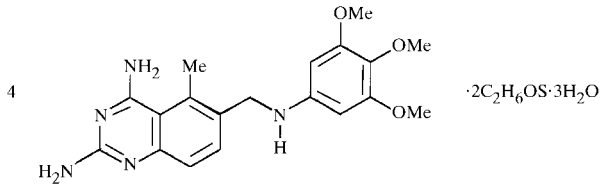
The crystal structure of the title compound contains four 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline molecules, two dimethyl sulfoxide molecules and three water molecules in the asymmetric unit, *i.e.* $4C_{19}H_{23}N_5O_3\cdot 2C_2H_6OS\cdot 3H_2O$. All four quinazoline molecules adopt *trans*,*gauche* conformations. An extensive hydrogen-bond network involving N···N base-pairing interactions, as well as the dimethyl sulfoxide and water molecules, stabilizes the crystal structure.

Comment

Trimetrexate (TMQ), a lipophilic antifolate, is a well known dihydrofolate reductase (DHFR) inhibitor and is widely used in cancer therapy and for the treatment of a variety of non-malignant disorders. We elucidated the present crystal structure of TMQ as a continuation of our studies into the effects of increased lipophilicity on the conformational properties and



(I)

stereochemical parameters in antifolates, which may indicate a direction for the development of new agents with better pharmacological profiles.

The asymmetric unit of the present crystal structure, (I), consists of four independent TMQ molecules, all adopting

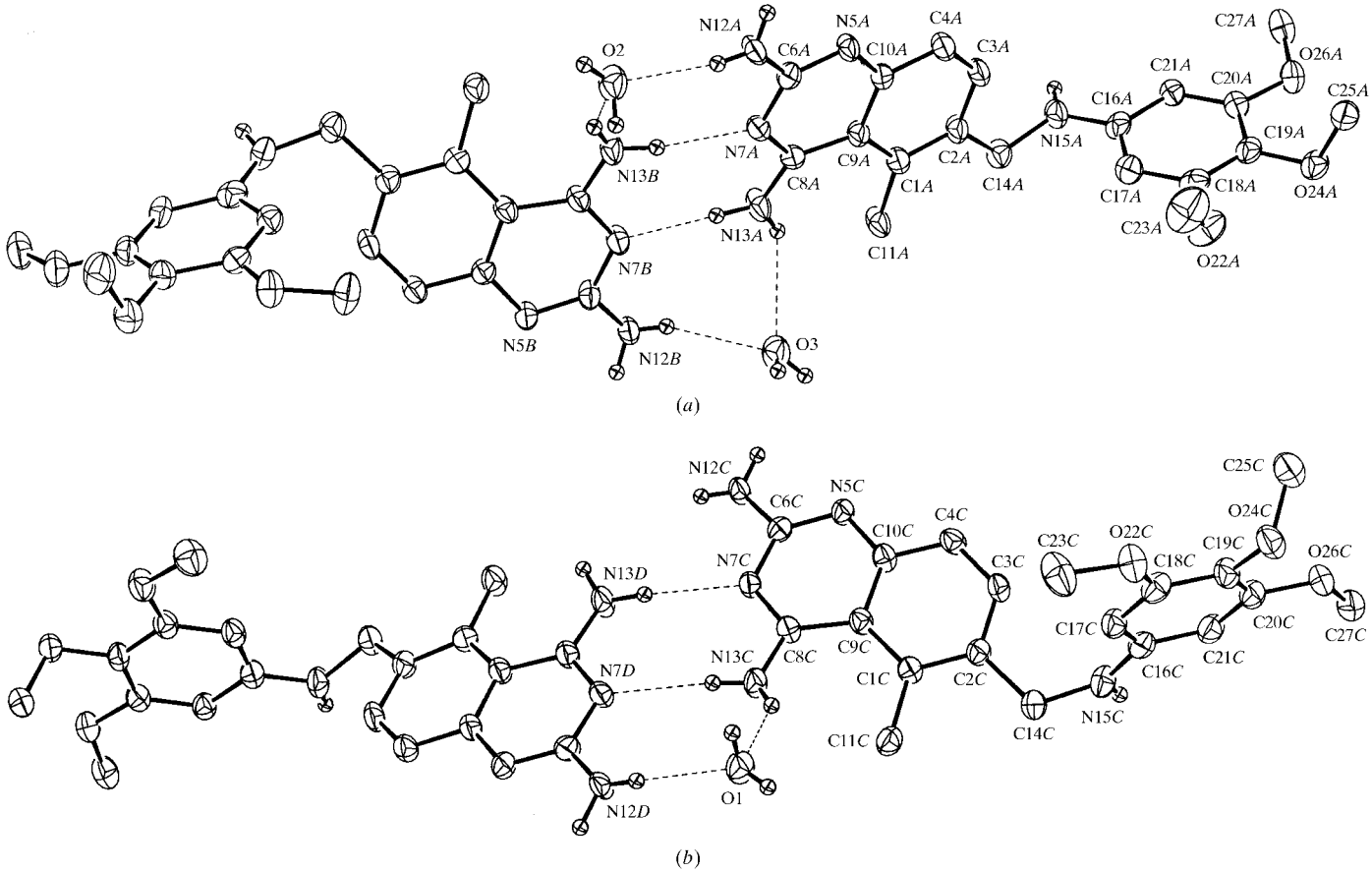


Figure 1
ORTEP-3 (Farrugia, 1997) view of the pairs of molecules [(*a*) *A*/*B* and (*b*) *C*/*D*] of similar conformation, showing 50% probability displacement elipsoids. For clarity, only H atoms taking part in hydrogen bonding are drawn as small circles of arbitrary radii.

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trans,gauche conformations [molecule *A*: τ_1 = 176.9 (3)° and τ_2 = 92.0 (4)°; molecule *B*: 178.8 (3) and 80.9 (4)°; molecule *C*: 174.2 (3) and 85.5 (4)°; molecule *D*: 164.9 (3) and 92.8 (4)°; the τ_1 and τ_2 torsion angles are defined by C1—C2—C14—N15 and C2—C14—N15—C16, respectively], two dimethyl sulfoxide molecules and three water molecules (Fig. 1). All four TMQ molecules show partially folded conformations, with the planes of the trimethoxyanilino rings twisted from those of the quinazoline rings by 84.3 (1), 86.7 (1), 89.8 (1) and 85.8 (1)° for molecules *A*, *B*, *C* and *D*, respectively. In previously reported crystal forms of trimetrexate (Sutton & Cody, 1987; Hempel *et al.*, 1988), the molecules exhibited *gauche,trans* conformations. There is substantial evidence, however, that the *trans,gauche* conformations observed for the first time in the present crystal structure is the one responsible for enzyme inhibition. The crystal structure of DHFR with methotrexate (MTX) (Bolin *et al.*, 1982) showed MTX to have a similar *trans,gauche* conformation when bound to the enzyme. Simulated annealing calculations performed for the solution structure of the complex of *Lactobacillus casei* DHFR with MTX (Gargaro *et al.*, 1998) showed that the stereochemistry of the binary complex in solution is very similar to that reported for the crystal structure. Similar calculations based on an analysis of the multidimensional NMR spectra of the complex of *Lactobacillus casei* DHFR with TMQ in solution have produced a family of 22 conformations of TMQ, all exclusively in the *trans,gauche* form, implying that this conformation of TMQ (molecules *A* or *B* in this report) is also that which is present in the active site of dihydrofolate reductase (Polshakov *et al.*, 1999).

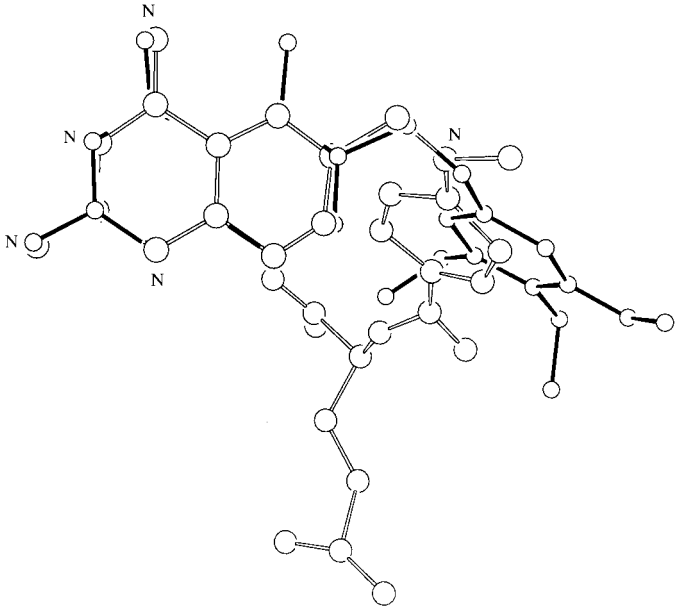

Figure 2
Superposition of the TMQ structure (molecule *A*, solid lines) with that of MTX in the complex with DHFR (hollow lines).

A conformational analysis of TMQ using empirical force-field and AM1 quantum mechanical methods concluded that TMQ prefers a *gauche,trans* rather than a *trans,gauche* conformation, in contrast with MTX which prefers an approximate *trans,gauche* conformation (Hoffman & Welsh, 1995). While this conclusion is in agreement with the conformations observed in the crystal structures of trimetrexate reported previously, it is not true for the crystal structure presented in this study, where TMQ molecules adopt the opposite *trans,gauche* form. A comparison of the stereochemistry of molecule *A* of TMQ with that of MTX bound to DHFR is shown in Fig. 2. The two structures are conformationally very closely related, indicating possible replacement of MTX by TMQ with little change in the stereochemistry of the enzyme–inhibitor binding.

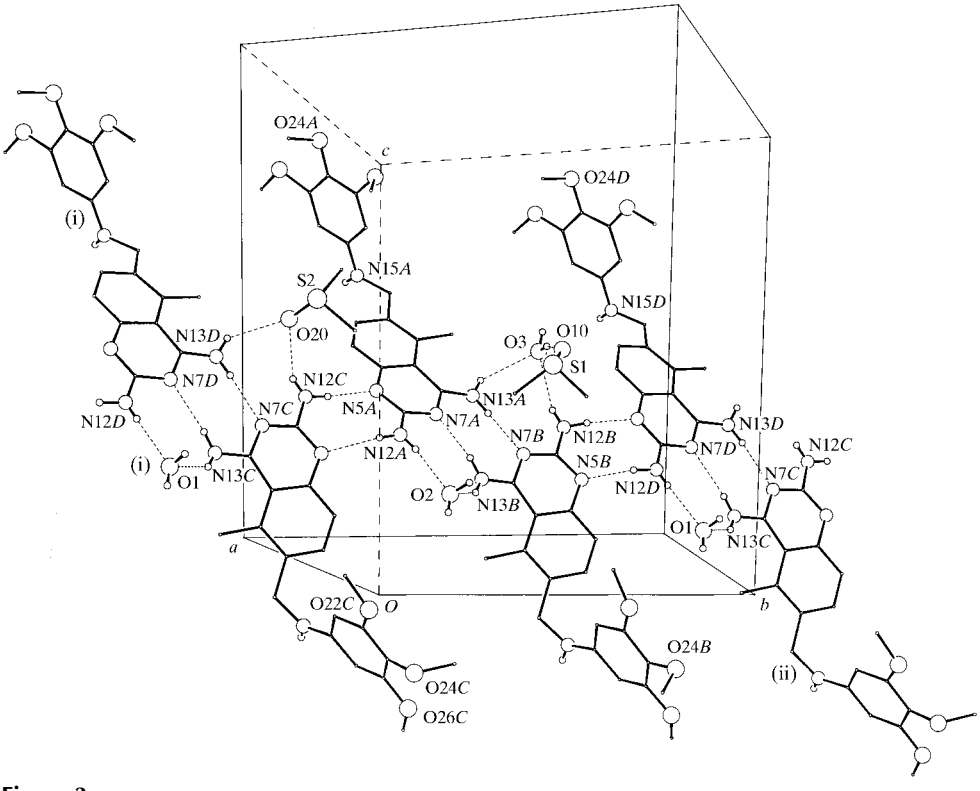

Figure 3
The crystal packing and hydrogen-bond geometry (dashed lines) in the title compound. Symmetry codes are the same as those given in Table 1.

The four independent TMQ molecules are hydrogen bonded among themselves through N···N base-pairing interactions involving the N7···N13 and

N5···N12 systems (Table 1 and Fig. 3). In other antifolate structures, only partial base-pairing interactions have been reported, *i.e.* N7···N13 for TMQ monoacetate monohydrate and the free base (Hempel *et al.*, 1988), N7···N12 for quinespar (Mastropaolo *et al.*, 1986) and no base-pairing in TMQ dimethyl sulfoxide hydrate (Sutton & Cody, 1987). Interestingly, no direct N···O hydrogen bonds exist between TMQ molecules in the crystal packing. The N···O bond occurs exclusively between TMQ and solvent (water and dimethyl sulfoxide) molecules. The water molecules connect TMQ molecules through hydrogen bonds to methoxy O atoms and the N12 and N13 atoms of the quinazoline. Curiously, the two potential hydrogen-bond donors (N15*B*—H15*B* and N15*C*—H15*C*), being surrounded by N13 and N7 atom pairs from different molecules, do not participate in classical hydrogen bonding due to longer than usual *D*···*A* distances.

Table 1
Hydrogen-bonding geometry (Å, °).

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N12*A*—H12*A*···N5*C*	0.83 (3)	2.26 (3)	3.081 (4)	168 (3)
N12*A*—H12*B*···O2	0.79 (3)	2.34 (3)	3.111 (4)	165 (3)
N12*B*—H12*C*···O3	0.77 (3)	2.28 (3)	3.016 (4)	160 (3)
N12*B*—H12*D*···N5*D*	0.90 (3)	2.10 (3)	2.979 (5)	167 (3)
N12*C*—H12*E*···N5*A*	0.88 (3)	2.08 (3)	2.950 (5)	172 (3)
N12*C*—H12*F*···O20	0.80 (3)	2.21 (3)	2.919 (4)	149 (3)
N12*D*—H12*G*···N5*B*	0.95 (3)	2.07 (3)	3.000 (5)	167 (3)
N12*D*—H12*H*···O1	0.72 (3)	2.33 (3)	3.038 (4)	165 (4)
N13*A*—H13*A*···N7*B*	0.92 (3)	2.07 (3)	2.983 (4)	173 (3)
N13*A*—H13*B*···O3	0.85 (3)	2.45 (3)	3.030 (4)	127 (3)
N13*B*—H13*C*···O2	0.74 (3)	2.37 (4)	2.976 (5)	140 (3)
N13*B*—H13*D*···N7*A*	0.89 (3)	2.07 (3)	2.955 (4)	169 (3)
N13*C*—H13*E*···N7*D*^{i}	0.85 (3)	2.24 (3)	3.091 (4)	173 (3)
N13*C*—H13*F*···O1^{i}	0.77 (3)	2.31 (4)	2.945 (5)	140 (3)
N13*D*—H13*G*···O20^{ii}	0.85 (3)	2.36 (3)	2.995 (4)	132 (3)
N13*D*—H13*H*···N7*C*^{ii}	0.88 (3)	2.15 (3)	3.028 (4)	171 (3)
N15*A*—H15*A*···N12*C*^{iii}	0.91 (3)	2.22 (3)	3.115 (5)	168 (3)
N15*D*—H15*D*···N12*B*^{iv}	0.85 (3)	2.58 (3)	3.411 (5)	167 (3)
O1—H11···O24*B*^{v}	0.84 (5)	2.16 (4)	2.929 (4)	152 (4)
O1—H12···O24*A*^{vi}	0.76 (4)	2.11 (4)	2.864 (4)	167 (5)
O2—H21···O24*D*^{vii}	0.75 (4)	2.10 (4)	2.839 (4)	170 (5)
O2—H22···O22*C*^{viii}	0.90 (5)	2.08 (5)	2.968 (4)	167 (4)
O3—H31···O10	0.98 (5)	1.85 (5)	2.803 (5)	165 (3)
O3—H32···O24*C*^{ix}	0.81 (3)	2.38 (3)	3.146 (3)	156 (5)
O3—H32···O26*C*^{ix}	0.81 (3)	2.52 (5)	3.130 (4)	132 (4)

Symmetry codes: (i) $1+x, y-1, z$; (ii) $x-1, 1+y, z$; (iii) $1-x, -y, 1-z$; (iv) $-x, 1-y, 1-z$; (v) $1-x, 2-y, -z$; (vi) $x, 1+y, z-1$; (vii) $1+x, y, z-1$; (viii) $2-x, 1-y, -z$; (ix) $x-1, y, 1+z$.

Experimental

Trimetrexate (NSC 249008-T) was obtained from the NCI Drug Synthesis and Chemistry Branch (NCI National Service Center). Crystals were obtained as pale yellow prisms by slow solvent evaporation from a water–dimethyl sulfoxide mixture.

Crystal data

$4C_{19}H_{23}N_5O_3\cdot2C_2H_6OS\cdot3H_2O$
$M_r = 1688.02$
Triclinic, $P\overline{1}$
$a = 16.124$ (2) Å
$b = 16.371$ (2) Å
$c = 17.766$ (1) Å
$\alpha = 81.676$ (3)°
$\beta = 66.289$ (2)°
$\gamma = 80.050$ (3)°
$V = 4214.4$ (8) Å^3
$Z = 2$
$D_x = 1.330$ Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from all reflections
$\mu = 0.142$ mm^{-1}
$T = 100$ (2) K
Prism, pale yellow
0.50 × 0.40 × 0.37 mm

Data collection

Nonius KappaCCD diffractometer
ω rotation scans
16 962 measured reflections
16 962 independent reflections
6946 reflections with $I > 2\sigma(I)$
$\theta_{max} = 26.42°$
$h = 0 \rightarrow 20$
$k = -19 \rightarrow 20$
$l = -20 \rightarrow 22$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)] = 0.065$
$wR(F^2) = 0.168$
$S = 0.877$
16 962 reflections
1174 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0735P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} = 0.001$
$\Delta\rho_{max} = 0.36$ e Å^{-3}
$\Delta\rho_{min} = -0.47$ e Å^{-3}

The H atoms bound to N atoms and those of the water molecules were found from the difference electron-density calculations and were refined independently [range of distances 0.72 (3)–0.98 (5) Å]. The remaining H atoms were calculated geometrically and were treated as riding atoms (range of distances 0.93–0.98 Å).

Data collection: *Nonius KappaCCD Server Software* (Nonius, 1997); cell refinement: *DENZO–SMN* (Otwinowski & Minor, 1997); data reduction: *DENZO–SMN*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*-3 (Farrugia, 1997); software used to prepare material for publication: *SHELXL*97.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: FG1597). Services for accessing these data are described at the back of the journal.

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