this publication does not necessarily reflect the views or policies of the Department of Health and Human Services nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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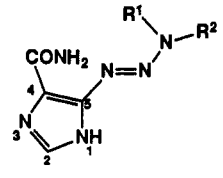
Antitumor Imidazotetrazines. 25.[1] Crystal Structure of 8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Temozolomide) and Structural Comparisons with the Related Drugs Mitozolomide and DTIC[†]

P. R. Lowe, C. E. Sansom, C. H. Schwalbe,* M. F. G. Stevens, and A. S. Clark

Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, U.K. *Received June 4, 1991*

The antitumor imidazotetrazinone, temozolomide (5), $C_6H_6N_6O_2$, forms crystals with unit cell dimensions a = 17.332 (3), b = 7.351 (2), c = 13.247 (1), β = 109.56 (1)° and space group $P2_1/c$. A doubly hydrogen-bonded dimer constitutes the asymmetric unit. One carboxamide group forms an additional intermolecular NH···O hydrogen bond; in both molecules the carboxamide group is coplanar with the heterocycle and its NH_2 group interacts with the imidazole nitrogen atom N(7). Molecular orbital calculations show the carbonyl carbon C(4) to be the most electron deficient atom, with relatively weak N(3)–C(4) and C(4)–N(5) bonds confirming that temozolomide should ring-open at this position in solution. The energy barrier to carboxamide group rotation of approximately 20 kJ mol^{-1} should permit interconversion between rotamers. In temozolomide and the related drug mitozolomide (4), N(7) is more negatively charged than N(1), which favors the formation of hydrogen bonds to the former atom in spite of their poor geometry. The relevance of these structural features to the action of temozolomide as a major-groove-directed prodrug of the alkylating agent MTIC (3) is discussed.

The antitumor drug 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide (1; DTIC) is used in the treatment of malignant melanoma,[2] a disease which is increasing in incidence particularly in Northern European races.[3] The bicyclic imidazotetrazinones mitozolomide (4)[4] and temozolomide (5)[5] have progressed to clinical trial as potential alternatives to DTIC based on their activity in in vivo murine[6] and human xenograft[7] tumor screens.



(1) $R^1=R^2=$ Me DTIC
(2) R^1=H, $R^2=(CH_2)_2Cl$ MCTIC
(3) R^1=H, R^2=Me MTIC
(4) R^1=H, $R^2=(CH_2)_2Cl$ Mitozolomide
(5) R^1=H, R^2=Me Temozolomide
(6) R^1=Me, R^2=Me

Clinical trials on 4 were compromised by the emergence in patients of a delayed and profound thrombocytopenia (damage to platelets).[4] This dose-limiting toxicity is probably elicited by a DNA cross-linking lesion which is forged subsequent to monoalkylation at the O(6) position of guanine residues[8] by the active cytotoxic species MCTIC (2), formed by the ring-opening of the prodrug mitozolomide at the electron-deficient C(4) position.[6,9] Clearly DTIC and temozolomide cannot induce DNA cross-linking and this is reflected in a different profile of toxicity with these two agents.

Structure–activity studies of antitumor imidazotetrazinones[10] substituted at the 8-position but retaining the C=O moiety show very good antitumor activity for several N-monosubstituted carboxamides. Aromatic substituents, and bulky substituents in general, are inimical to activity.[10] However, the *N*,*N*-dimethylcarboxamide derivative requires metabolic demethylation to become active, and a

[†] Contribution from the Joint Crystallography Unit, Universities of Aston and Birmingham.

(1) Part 24: Hepburn, P. A.; Tisdale, M. J. Growth Suppression by DNA from Cells Treated with Imidazotetrazinones. *Biochem. Pharmacol.* **1991**, *41*, 339–343.
(2) Comis, R. L.; Carter, S. K. Integration of Chemotherapy into Combined Modality Therapy of Solid Tumors. IV. Malignant Melanoma. *Cancer Treat. Rev.* **1974**, *1*, 285–304.
(3) Doll, R. Are We Winning the Fight against Cancer—an Epidemiologic Assessment. *Eur. J. Cancer* **1990**, *26*, 500–508.
(4) Newlands, E. S.; Blackledge, G.; Slack, J. A.; Goddard, C.; Brindley, C. J.; Holden, L.; Stevens, M. F. G. Phase I Clinical Trial of Mitozolomide. *Cancer Treat. Rep.* **1985**, *69*, 801–805.
(5) Slack, J. A.; Newlands, E. S.; Blackledge, G. R. P.; Quarterman, C. P.; Stuart, N. S. A.; Hoffman, R.; Stevens, M. F. G. Phase I Clinical Pharmacokinetics of Temozolomide. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 993.

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Table I. Fractional Coordinates of Temozolomide (5) ($\times 10^4$)[a]

primed molecule				unprimed molecule			
atom	x/a	y/b	z/c	atom	x/a	y/b	z/c
N(1)	9417 (2)	2375 (4)	3122 (2)	N(1′)	4153 (1)	−3571 (4)	−93 (2)
N(2)	10124 (2)	3105 (5)	3365 (2)	N(2′)	3462 (2)	−4343 (4)	−467 (2)
N(3)	10593 (2)	3365 (4)	4422 (2)	N(3′)	3107 (1)	−4599 (4)	−1554 (2)
C(31)	11413 (3)	4092 (8)	4580 (4)	C(31′)	2312 (2)	−5505 (6)	−1884 (3)
C(4)	10386 (2)	2897 (5)	5299 (3)	C(4′)	3424 (2)	−4095 (4)	−2331 (2)
O(4)	10801 (1)	3154 (4)	6209 (2)	O(4′)	3082 (1)	−4287 (3)	−3279 (2)
N(5)	9612 (1)	2096 (4)	4988 (2)	N(5′)	4186 (1)	−3282 (4)	−1885 (2)
C(6)	9184 (2)	1396 (5)	5596 (3)	C(6′)	4716 (2)	−2596 (5)	−2357 (3)
N(7)	8482 (2)	739 (4)	4994 (2)	N(7′)	5367 (1)	−1919 (4)	−1636 (2)
C(8)	8438 (2)	1013 (4)	3953 (2)	C(8′)	5271 (2)	−2145 (4)	−652 (2)
C(81)	7727 (2)	409 (5)	3035 (3)	C(81′)	5899 (2)	−1527 (4)	343 (2)
N(82)	7111 (2)	−355 (5)	3270 (3)	N(82′)	6527 (2)	−660 (4)	199 (2)
O(82)	7734 (1)	629 (4)	2121 (2)	O(82′)	5837 (1)	−1819 (3)	1231 (2)
C(8A)	9141 (2)	1844 (4)	3932 (2)	C(8A′)	4537 (2)	−3014 (4)	−791 (2)
H(1)[a]	1180 (3)	325 (7)	483 (3)	H(1′)	188 (3)	−456 (8)	−232 (4)
H(2)	1140 (3)	451 (7)	384 (4)	H(2′)	231 (3)	−644 (7)	−230 (4)
H(3)	1154 (3)	497 (7)	513 (4)	H(3′)	218 (3)	−588 (7)	−125 (4)
H(4)	941 (2)	145 (5)	637 (3)	H(4′)	458 (2)	−269 (4)	−312 (2)
H(5)	713 (2)	−49 (6)	395 (3)	H(5′)	652 (2)	−44 (6)	−51 (3)
H(6)	660 (2)	−78 (6)	269 (3)	H(6′)	693 (2)	−27 (6)	81 (3)

[a] All H atoms $\times 10^3$.

variety of esters are inactive. To account for these phenomena, and the known sequence preference for chloroethylation by mitozolomide in the major groove of DNA at runs of contiguous guanines,[11] we have proposed a new model (Figure 1) in which the imidazotetrazinone prodrugs

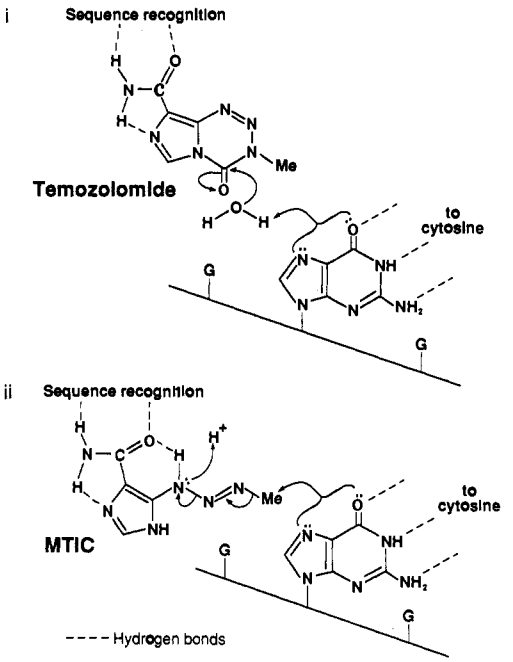


Figure 1. Schematic model of (i) the binding of temozolomide to GC-rich DNA with nucleophilic attack by an activated water molecule eventually forming MTIC, and (ii) subsequent alkylation of the DNA.

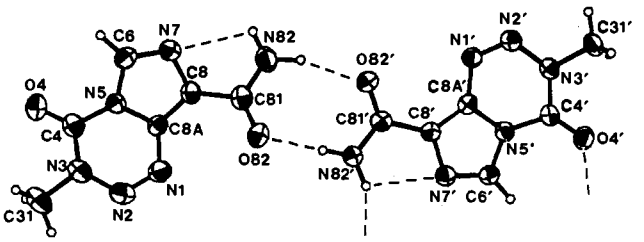


Figure 2. The two independent molecules in the asymmetric unit of temozolomide, showing the hydrogen-bonded dimer and possible intramolecular hydrogen bonding, and indicating the direction of approach of the intermolecular NH···O hydrogen bond between primed molecules. Thermal ellipsoids are drawn with ORTEP[19] at the 50% probability level.

(6) Hickman, J. A.; Stevens, M. F. G.; Gibson, N. W.; Langdon, S. P.; Fizames, C.; Lavelle, F.; Atassi, G.; Lunt, E.; Tilson, R. M. Experimental Antitumor Activity against Murine Tumor Model Systems of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Mitozolomide), a Novel Broad-Spectrum Agent. *Cancer Res.* **1985**, *45*, 3008–3013. Stevens, M. F. G.; Hickman, J. A.; Langdon, S. P.; Chubb, D.; Vickers, L.; Stone, R.; Baig, G. U.; Goddard, C.; Gibson, N. W.; Slack, J. A.; Newton, C. G.; Lunt, E.; Fizames, C.; Lavelle, F. Antitumor Activity and Pharmacokinetics in Mice of 8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (CCRG 81045; M & B 39831), a Novel Drug with Potential as an Alternative to Dacarbazine. *Cancer Res.* **1987**, *47*, 5846–5852.

(7) Fodstad, Ø.; Aamdal, S.; Pihl, A.; Boyd, M. R. Activity of Mitozolomide (NSC 353451), a New Imidazotetrazine, against Xenografts from Human Melanomas, Sarcomas, and Lung and Colon Carcinomas. *Cancer Res.* **1985**, *45*, 1778–1786.

(8) Gibson, N. W.; Hartley, J. A.; Barnes, D.; Erickson, L. C. Combined Effects of Streptozotocin and Mitozolomide against Four Human Cell Lines of the Mer$^+$ Phenotype. *Cancer Res.* **1986**, *46*, 4995–4998.

(9) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. Antitumor Imidazotetrazines. 1. Synthesis and Chemistry of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, a Novel Broad-Spectrum Antitumor Agent. *J. Med. Chem.* **1984**, *27*, 196–201.

(10) Lunt, E.; Newton, C. G.; Smith, C.; Stevens, G. P.; Stevens, M. F. G.; Straw, C. G.; Walsh, R. J. A.; Warren, P. J.; Fizames, C.; Lavelle, F.; Langdon, S. P.; Vickers, L. M. Antitumor Imidazotetrazines. 14. Synthesis and Antitumor Activity of 6- and 8-Substituted Imidazo[5,1-*d*]-1,2,3,5-tetrazinones and 8-Substituted Pyrazolo[5,1-*d*]-1,2,3,5-tetrazinones. *J. Med. Chem.* **1987**, *30*, 357–366. Horspool, K. R.; Stevens, M. F. G.; Newton, C. G.; Lunt, E.; Walsh, R. J. A.; Pedgrift, B. L.; Baig, G. U.; Lavelle, F.; Fizames, C. Antitumor Imidazotetrazines. 20. Preparation of the 8-Acid Derivative of Mitozolomide and its Utility in the Preparation of Active Antitumor Agents. *J. Med. Chem.* **1990**, *33*, 1393–1399.

(11) Hartley, J. A.; Gibson, N. W.; Kohn, K. W.; Mattes, W. B. DNA Sequence Selectivity of Guanine-N7 Alkylation by Three Antitumor Chloroethylating Agents. *Cancer Res.* **1986**, *46*, 1943–1947.

are preferentially ring-opened to their alkylating forms by water "activated" in the nucleophilic microenvironment of guanine-rich DNA sequences.[12] Sequence selectivity may arise either from (i) the participation of imidazotetrazinones in an initial noncovalent binding step probably involving their 8-substituent, or (ii) their activation followed by covalent bonding. Since all DNA bases irrespective of surroundings are likely to react readily with alkylating agents, selectivity is most probably achieved in advance of the covalent bonding step.

According to this model the imidazotetrazinones **4** and **5** should have an enhanced potential for sequence-selective interactions with DNA compared with DTIC. The latter drug requires prior metabolic demethylation in the liver to generate circulating levels of its active monomethyl metabolite MTIC (**3**), and its biological potency is subject to the vagaries of metabolism and distribution.[13] Temozolomide had superior antitumor activity to DTIC in preclinical screens,[6] possibly because conversion to its cytotoxic form MTIC is a controlled chemical process. Temozolomide shows clinical activity against malignant melanoma in humans,[14] and, surprisingly, has elicited responses in patients bearing brain gliomas when the drug is administered on a divided dose schedule by the oral route.[15]

Although their instability has precluded X-ray crystallographic analysis of the ring-opened triazenes **2** and **3**, the X-ray structure analyses of DTIC base[16] and hydrochloride salt,[17] and mitozolomide,[18] have been reported previously. We now report a crystal structure determination of temozolomide and a comparison of the structures of these three compounds which share a common lineage. In addition we have conducted a computer modeling study focusing on the dispositions of the crucial carboxamide groups and their involvement in intramolecular H-bonding interactions and potential for significant intermolecular H-bonding interactions within the major groove of DNA.

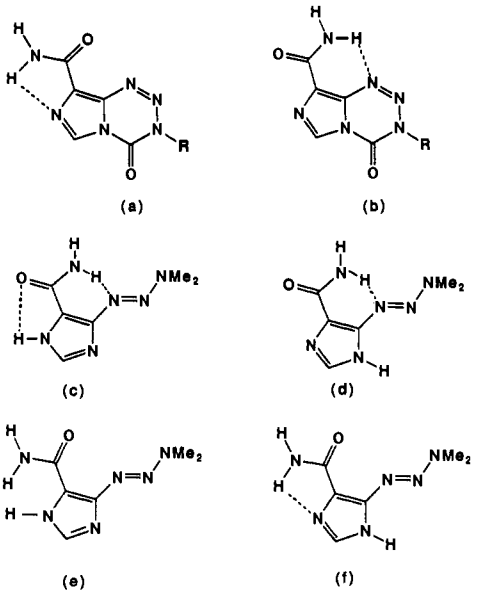


Figure 3. Rotamers with intramolecular hydrogen bonding patterns for temozolomide, mitozolomide, and DTIC: (a) found in the crystal structures of temozolomide and mitozolomide, (b) found in the crystal structure of mitozolomide only, (c) lower-energy 3*H*-tautomer found in the crystal structure of DTIC, (d) higher-energy 1*H*-tautomer found in the crystal structure of DTIC, (e) unfavorable 180° rotamer of the 3*H*-tautomer (c) of DTIC, (f) hypothetical 180° rotamer of the 1*H*-tautomer of DTIC, 7 kJ mol^{-1} more stable than (d) in vacuo.

Results and Discussion

Crystal Structure of Temozolomide. The two independent molecules in the asymmetric unit of **5** are depicted[19] with their numbering scheme in Figure 2. Fractional atomic coordinates are given in Table I. Unlike mitozolomide,[18] which exists as two discrete rotamers in the solid state, all molecules in temozolomide crystals have the same pseudo-tricyclic conformation (a in Figure 3). The independent molecules form a doubly hydrogen-bonded carboxamide dimer (Figure 2) around a pseudo-center of inversion. A carboxamide dimer also occurs in neutral DTIC[16] but not in protonated DTIC[17] nor in mitozolomide.[18] In the dimer of **5** both ring planes are nearly parallel to the (-5,4,1) plane, as is suggested by the exceptional strength of this reflection. The primed molecule deploys its remaining carboxamide H atom in the hydrogen bond from N(82′)–H(5′) to O(4′) transformed by $1 - x$, $^1/_2 + y$, $-^1/_2 - z$, which is not matched in the unprimed molecule and which constitutes the main difference between them. Chemically equivalent bond distances are virtually identical, except that the hydrogen bonded C(4′)=O(4′) is 0.009 Å (2 σ) longer than the free C(4)=O(4). The only significant discrepancies (3 to 6 σ) in bond angles involve these carboxamide groups. Hydrogen bonding satisfactorily explains the solid state (KBr) C=O stretching frequencies of temozolomide: 1755 cm^{-1} for the free C(4)=O(4), 1725 cm^{-1} for the hydrogen bonded C-

(12) Clark, A. S.; Stevens, M. F. G.; Sansom, C. E.; Schwalbe, C. H. Antitumor Imidazotetrazines. 21. Mitozolomide and Temozolomide—Probes for the Major Groove of DNA. *Anti-Cancer Drug Des.* **1990**, *5*, 63–68.

(13) Rutty, C. J.; Newell, D. R.; Vincent, R. B.; Abel, G.; Goddard, P. M.; Harland, S. J.; Calvert, A. H. The Species Dependent Pharmacokinetics of DTIC. *Br. J. Cancer* **1980**, *48*, 140. Tsang, L. L. H.; Quarterman, C. P.; Gescher, A.; Slack, J. A. Antitumor Imidazotetrazines. 23. Comparison of the Cytotoxicity in vitro of Temozolomide and Dacarbazine, Prodrugs of 3-Methyl-(triazen-1-yl)imidazole-4-carboxamide. *Cancer Chemother. Pharmacol.* **1991**, *27*, 342–346.

(14) Newlands, E. S.; Blackledge, G. R. P.; Slack, J. A.; Rustin, G. J. S.; Smith, D. B.; Stuart, N. S. A.; Quarterman, C. P.; Hoffman, R.; Stevens, M. F. G.; Brampton, M.; Gibson, A. C. Phase I Trial of Temozolomide (CCRG 81045: M & B 39831: NSC 362856). *Br. J. Cancer* **1992**, *65*, 287–291.

(15) O'Reilly, S. M.; Newlands, E. S.; Glaser, M. G.; Brampton, M.; Rice-Edwards, J. M.; Illingworth, R. D.; Richards, P. G.; Kennard, C.; Colquhoun, I. R.; Lewis, P.; Stevens, M. F. G. Temozolomide: a New Oral Cytotoxic Chemotherapeutic Agent with Promising Activity against Primary Brain Tumors. *Br. Med. J.*, in press.

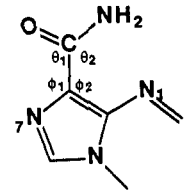
(16) Freeman, H. C.; Hutchinson, N. D. The Crystal Structure of the Anti-Tumor Agent 5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide (NSC-45388). *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1979**, *B35*, 2051–2054.

(17) Edwards, S. L.; Sherfinski, J. S.; Marsh, R. E. The Crystal Structure of the Antitumor Agent 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide Monohydrate Hydrochloride (NSC-45388). *J. Am. Chem. Soc.* **1974**, *96*, 2593–2597.

(18) Lowe, P. R.; Schwalbe, C. H.; Stevens, M. F. G. Antitumor Imidazotetrazines. Part 5. Crystal and Molecular Structure of 8-Carbamoyl-3-(2-chloroethyl)imidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Mitozolomide). *J. Chem. Soc. Perkin Trans. 2* **1985**, 357–361.

(19) Johnson, C. K. ORTEP. Oak Ridge Thermal-Ellipsoid Plot Program. Report ORNL-5136; Oak Ridge National Laboratories: Oak Ridge, TN, 1976.

Table II. Comparison of Crystallographic (experimental, e) and Optimized (theoretical, t) Carboxamide Bond Angles, and Calculated Partial Charges in DTIC[a] (1),[14] Mitozolomide (4)[16] and Temozolomide (5)



compd	φ1	φ2	θ1	θ2	q [N(1)]	q [N(7)]	q [C(4)]
1e	120.3	130.3	121.3	115.4			
1'e	120.7	133.4	120.5	115.8			
1t	120.7	133.7	120.4	119.4	−0.205	−0.245	
1't	124.4	126.2	122.6	118.0	−0.182	−0.258	
4e	119.9	130.4	120.2	116.4			
4'e	125.5	125.7	119.2	117.2			
4t	117.9	133.9	123.4	118.3	−0.146	−0.228	0.414
4't	121.9	129.9	123.1	118.1	−0.126	−0.250	0.416
5e	122.1[b]	128.2	119.4	116.5			
5'e	121.5	129.4	121.7	114.5			
5t	121.1	128.7	123.9	112.7	−0.114	−0.254	0.417
5't	120.7	129.2	124.2	112.7	−0.115	−0.255	0.417

[a] In DTIC, the primed molecule is protonated on the N atom next to the carboxamide group. [b] Estimated standard deviations for experimental bond angles of temozolomide are 0.3°.

(4')=O(4'), and 1675 cm^{-1} for the similarly hydrogen bonded C(81)=O(82) and C(81')=O(82').

Molecular Modeling. Partial atomic charges on equivalent atoms in the unprimed and primed molecules of 5 calculated by ab initio molecular orbital methods differ insignificantly, by <0.001 e. The greatest positive charge (+0.417) is found at carbonyl carbon atom C(4) (Table II), as it is in the congener 4 by ab initio (Table II) and semiempirical CNDO[12] techniques. Upon ab initio geometry optimization of 5, the most significant change in bond length was extension of the ring bonds N(3)–C(4) and C(4)–N(5). The relative weakness of these bonds implies that ring opening is likely to occur at C(4). Combined with experimental evidence that imidazotetrazinones are susceptible to nucleophilic attack and ring opening at this position,[6,9,20] these results support the theory that nucleophilic attack at C(4) by water molecules in the major groove of DNA may be responsible for the formation of cytoactive triazenes from imidazotetrazinone prodrugs.[12] Optimization of the isolated molecules equalized the C(4)=O(4) and C(4')=O(4') bond lengths, but the deviation of the carboxamide group from the bisector of the N(7)–C(8)–C(8A) angle persisted.

The energy of the two optimized molecules is identical within 0.2 kJ mol^{-1}. Rotation of the carboxamide group by 180° to give another pseudo-tricyclic conformation (b in Figure 3) equivalent to that of the unprimed molecule of mitozolomide,[18] with an intramolecular hydrogen bond to N(1), increased the energy by 8.4 (8.8) kJ mol^{-1} (values for the primed molecule in parentheses). Assuming that the carboxamide group is perpendicular to the heterocycle in the highest-energy conformation, the resulting barrier to rotation of this group of 22.0 (19.3) kJ mol^{-1} is insufficient to prevent free rotation in solution, yet large enough not to be easily overcome by intermolecular hydrogen bond formation. Thus 5 is likely to occupy the major groove of DNA as a nearly planar rotamer a or b.

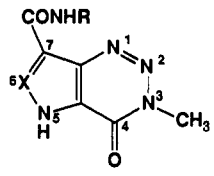
Triazenes 1–3 offer the same possibilities for carboxamide group rotation and an additional opportunity for tautomerism via exchange of a proton between N(1) and N(3) on the imidazole ring. In the crystalline 1 both tautomers are present;[16] in both, the carboxamide group exists as the opposite rotamer to that in 5 and engages in intramolecular hydrogen bonding to a triazene N atom (c and d in Figure 3). Single point ab initio calculations following optimization by semiempirical techniques predict that the 3*H*-tautomer (c) is more stable than the 1*H* (d) by 12.7 kJ mol^{-1}, and the 180° rotamers (e) and (f) are respectively 40.0 kJ mol^{-1} less stable and 7.0 kJ mol^{-1} more stable than (c) and (d). The different preferences of rotamer in the triazenes and the bicyclic systems can be attributed to three factors. (1) An intramolecular NH···N hydrogen bond from the carboxamide group achieves more favorable geometry when directed to the proximal N atom of the tetrazine ring or triazene chain than to imidazole ring atom N(7) or its equivalent. (2) In 5 the negative charge on N(7) exceeds that on the competing N atom sufficiently, by 0.140 e (Table II), to overcome the unfavorable geometry and stabilize an intramolecular hydrogen bond, while in the triazene 1, the differences in charge on the relevant N atoms are less, 0.040–0.076 e. (3) The 3*H*-tautomer of 1 gains extra stability in the observed conformation (c) from a weak intramolecular NH···O hydrogen bond to the carboxamide O atom which is changed to H···H steric clash in the 180° rotamer (e). Whereas the bicyclic systems and the 1*H*-tautomer of the triazenes are predicted to have more than one low-energy conformation, this 3*H*-tautomer of all the triazenes should have a well-defined conformational preference (c).

Two stereoelectronic features can be related to activity in this class of drugs. (1) The significance ascribed to hydrogen bonding by the carboxamide group in 4 and 5 is in accord with the requirement in active mitozolomide derivatives[10] for at least one amide H atom. If the substituent in such a monosubstituted carboxamide group is accommodated next to the heterocycle ("endo"), the remaining H atom points outward and is still able to form a hydrogen bond to the DNA target. Such a conformation becomes increasingly difficult to attain as the bulk of the

(20) Baig, G. U.; Stevens, M. F. G. Antitumor Imidazotetrazines. Part 12. Reactions of Mitozolomide and its 3-Alkyl Congeners with Oxygen, Nitrogen, Halogen, and Carbon Nucleophiles. *J. Chem. Soc. Perkin Trans. 1* **1987**, 665–670.

substituent increases. Alternatively, it is conceivable that an intramolecular hydrogen bond still maintains the pseudo-tricyclic conformation and presents the substituent to the surroundings ("exo"). The orientation of the substituent may determine whether a close approach to DNA is possible and could control the position of another linked moiety that enhances the sequence selectivity. To examine possible conformations the endo carboxamide hydrogen atom in rotamer **5a** was replaced by CH_3 (**6**, rotamer a) and the geometry was fully optimized by ab initio molecular orbital techniques. From the resulting structure the other, exo, methylated form of **6a** was generated and optimized. The exo form of **6a** is 28.6 kJ mol^{-1} more stable than *endo*-**6a**, but this difference may be compensated by intermolecular hydrogen bonding. Both endo- and exo-methylated forms of rotamer **6b** were generated, but a semiempirical energy calculation suggests that the endo conformation of **6b** is unlikely due to steric hindrance.

(2) The antitumor activity of analogs of **5** is strongly affected by the nature of the heterocycle. We postulate that the pyrrolotriazinone (**7**) and pyrazolotriazinone (**8**), in which the bridgehead N atom is replaced by C, should

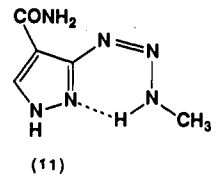


(7) X=CH, R=H

(6) X=N, R=Me

(9) R=Me

(10) R=$(CH_2)_2Cl$



(11)

no longer undergo facile ring opening since the calculated partial charge at C(4) of **7** is only +0.274. Preliminary tests on the pyrazolotriazinone **8** against Raji and GM892A cell lines indicate that this compound is not cytotoxic in vitro in concentrations <500 μM.[21] Likewise, a variety of benzotriazinone analogs of **4** and **5** in which the fused five-membered heterocycle is replaced by a fused benzene ring display no activity.[21] More subtly, the pyrazolotetrazinones (**9** and **10**) retain the bridgehead N atom, and the partial charge of +0.400 on C(4) of **9** implies a susceptibility to nucleophilic attack and ring opening similar to that of **5**. Perplexingly, **9** is reported to be devoid of activity against murine tumors sensitive to temozolomide[22] while **10** is very active. These observations can be rationalized if the replacement of N with CH at position 7 destabilizes a conformation analogous to a (Figure 3), required during the initial approach to DNA. The difference could also arise from the ring-opened pyrazolotriazene intermediate **11**, whose different charge distribution from that in **3** may favor a different tautomeric form of the heterocycle or the triazene chain, or a different rotamer of the chain. For a similar triazene tautomer to that in **1–3**, the structure displayed for **11** is preferred according to semiempirical calculations. Therefore **9** or **11** may not present the alkylating methyl group to the same region of the DNA as **3**. However, the longer and more flexible chloroethyl group of **10** may still be able to reach the target site, or may act by a different mechanism.

Experimental Section

Crystallographic Studies. $C_6H_6N_6O_2$ has M = 194.16. Crystals exhibit monoclinic symmetry, space group $P2_1/c$, with a = 17.332 (3), b = 7.351 (2), c = 13.247 (1) Å, β = 109.56 (1)°, V = 1590.4 (5) Å^3 (by least-squares refinement of setting angles of 25 reflections), Z = 8, D_{calc} = 1.622 g cm^{-3}, F (000) = 800, μ (MoKα) = 0.120 mm^{-1}. The specimen crystal was a colorless lath grown from acetone–water 3:1, dimensions 1.0 × 0.48 × 0.16 mm. Intensity data were collected on an Enraf-Nonius CAD4 four-circle diffractometer in the ω–2θ mode with ω scan width (0.95 + 0.35 tan θ)°, scan speed 0.95–4.0° min^{-1}, and graphite-monochromated MoKα radiation; 2643 reflections were measured ($\pm h$, $+k$, $+l$; $2 \leq \theta \leq 24°$), of which 2492 were unique (merging R = 0.030), 1749 considered observed with $I > 3\sigma(I)$. Intensity and orientation monitor reflections suggested no decomposition or movement of the crystal. Direct-phase determination and full-matrix least-squares refinement were carried out with SHELX.[23] Direct methods yielded only fragments of the molecules until the −5,4,1 reflection with its normalized structure factor of E = 7.305 was removed from the starting set, whereupon structure solution became routine. All non-hydrogen atoms were located in the E-map, and all H atoms appeared in difference Fourier syntheses. Positional and anisotropic thermal parameters were refined for non-hydrogen atoms, and positions and isotropic temperature factors for H atoms. Reflections were corrected empirically for absorption (DIFABS[24]) and extinction (SHELX[23]) and were assigned weights $w = k/[\sigma^2(|F_o|) + gF_o^2]$, where $\sigma(|F_o|)$ was obtained from counting statistics and an allowance for experimental instability, k refined to 6.3515, and g refined to zero. At termination no parameter shifted by more than 0.07 esd, the final R and R_g values reached 0.042 and 0.055, respectively, the observed and calculated structure amplitudes for the −5,4,1 reflection agreed within 2.5%, no peak or hole on a final difference electron density map exceeded 0.2 e Å^{-3}, and the SHELX variance[23] was only 0.83.

Molecular Modeling. The refined coordinates of the two independent molecules of temozolomide in the asymmetric unit were taken as the starting position for geometry optimization and charge calculation with the ab initio quantum mechanics program Gaussian 80,[25] using the STO-3G basis set. Similar calculations were performed on the rotamer of **6** analogous to a (Figure 3). For additional orientations of the carboxamide group in **5** and **6** generated with the molecular modeling package Chem-X,[26] the energy was evaluated by single-point calculations with Gaussian 80. As the semiempirical molecular orbital program MOPAC[27] gave similar results to Gaussian 80 for temozolomide, the geometry of all other molecules was optimized using MOPAC. Partial charges were then calculated with Gaussian 80. The analogs (**6–11**) were constructed from temozolomide within Chem-X using standard bond lengths and angles. All calculations were performed on a VAX 8650 computer.

(21) Clark, A. S.; Stevens, M. F. G., unpublished results.

(22) Chang, C. C.; Elslager, E. F.; Werbel, L. M.; Priebe, S. R.; Leopold, W. R. Pyrazole Derivatives. 5. Synthesis and Antineoplastic Activity of 3-(2-Chloroethyl)-3,4-dihydro-4-oxopyrazolo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide and Related Compounds. *J. Med. Chem.* **1986**, *29*, 1544–1547.

(23) Sheldrick, G. M. SHELX-76, Program for Crystal Structure Determination, University of Cambridge, 1976.

(24) Walker, N.; Stuart, D. An Empirical Method for Correcting Diffractometer Data for Absorption Effects. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A39*, 158–166.

(25) Chandra Singh, U.; Kollman, P. VAX Version of Gaussian 80 Package. *QCPE Bull.* **1982**, *2*, 117. Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN 47405.

(26) Davies, K. Chem-X, developed and distributed by Chemical Design Ltd., Oxford, UK, 1988.

(27) Stewart, J. J. P. MOPAC: a General Molecular Orbital Package. *QCPE Bull.* **1983**, *3*, 43. Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN 47405.

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Supplementary Material Available: Tables listing bond distances, bond angles, and hydrogen bond geometry (3 pages). Ordering information is given on any current masthead page.

Energy Aspects of Oil/Water Partition Leading to the Novel Hydrophobic Parameters for the Analysis of Quantitative Structure–Activity Relationships

Yong-Zhong Da, Katsuhiko Ito, and Hideaki Fujiwara*

Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-Oka, Suita, Osaka 565, Japan. *Received March 9, 1992*

Partition properties, that is partition coefficients and enthalpies ($\Delta H_p°$) and entropies ($\Delta S_p°$) of partition, have been measured for 50 benzoic acids in the 1-octanol/water system, and their role in QSAR (quantitative structure–activity relationship) analysis examined. The novel hydrophobic parameters have been introduced as a result of the separation of the Gibbs free energy term into the corresponding enthalpy and entropy terms. Application of these novel parameters to some available biological activity data supported the usefulness of these parameters in QSAR analysis. Relative contributions of the enthalpy and entropy terms are also discussed.

Introduction

The optimization between biological activity and structure in a series of drugs by variation of the substitution pattern is an important aim in quantitative structure–activity relationship (QSAR) analysis. A rational approach to this was first proposed by Hansch in 1962.[1] In the Hansch approach, hydrophobic, electronic, and steric substituent constants[2,3] are the three major factors used for a regression-analysis to determine QSAR. Electronic and steric substituent parameters can be estimated by such calculation methods.[4] On the other hand, hydrophobic parameters (log P) are difficult to estimate exactly for different kinds of compounds, despite the considerable efforts hitherto devoted to doing so. As of yet, most of the partition coefficients (P) have been obtained in the 1-octanol/water system, and the logarithms of P have been confirmed to be related to the logarithms of bioactive concentrations of drugs.[2-4] Now, these results are discussed widely for the development of new medicines with superb consequences, stimulating studies about hydrophobic factors from various points of view.

We have been studying partition properties especially from an energy standpoint, using various partition systems such as micelle/water, liposome/water, and so on.[5-7] The log P term reflects the Gibbs free energy of transfer ($\Delta G_p°$) for partition and includes the enthalpy term ($\Delta H_p°$) as well as the entropy term ($\Delta S_p°$):

$$\log P = -\Delta G_p°/(2.303RT) = -\Delta H_p°/(2.303RT) + \Delta S_p°/(2.303R) \quad (1)$$

where R is the gas constant and T is the absolute temperature. Hitherto, $\Delta H_p°$ and $\Delta S_p°$ have been reported for several solutes in several partition systems.[8-11] How-

(1) Hansch, C. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1971; Vol. 1.
(2) *Partition Coefficient. Determination and Estimation*; Dunn, W. J., Block, J. H., Pearlman, R. S., Eds.; Pergamon: New York, 1979.
(3) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley: New York, 1979.
(4) Franke, R. In *Theoretical Drug Design Method*; Nauta, W. Th., Rekker, R. F., Eds.; Elsevier: Amsterdam, 1984; Pharmacochemistry Library Vol. 7.
(5) Fujiwara, H.; Ohtaku, I.; Miyagi, R.; Sasaki, Y. Thermodynamic Properties of Solution of Nonspherical Molecules Determined from Gas–Liquid Chromatography and Calculated by the Scaled Partical Theory Using the Kihara Potential. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3426–3432.
(6) Fujiwara, H.; Yoshikawa, H.; Murata, S.; Sasaki, Y. Thermochemical Aspects of Partition of Methyl- and Halogen-Substituted Alcohols in 1-Octanol/Water and a Novel Regression Analysis of Alcohol Toxicities. *Chem. Pharm. Bull.* **1991**, *39*, 1095–1098.
(7) (a) Fujiwara, H.; Da, Y.-Zh.; Ito, K.; Takagi, T.; Sasaki, Y. The Energy Aspect of Oil/Water Partition and Its Application to the Analysis of Quantitative Structure–Activity Relationships. Aliphatic Alcohols in the Liposome/Water Partition System. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3707–3712. (b) Fujiwara, H.; Da, Y.-Zh.; Ito, K. The Energy Aspects of Oil/Water Partition. Proposition of Novel Hydrophobic Parameters for the Treatment of Quantitative Structure–Activity Relationships. *Chem. Lett.* **1992**, 215–218.

0022-2623/92/1835-3382$03.00/0