in the culture supernatant for p25gag viral protein by an antigen capture assay (ELISA). The antiviral activity was expressed as the dose which reduced by 50% (IC_{50} in μM) the amount of p25gag viral protein in the supernatant fluid of the infected-treated cells as compared to the infected-untreated cells. Cytotoxicity was expressed as the dose which inhibited the replication of the host cell by 50% ($TCID_{50}$).

These compounds were also evaluated against Rauscher-Murine leukemia virus (R-MuLV) in cell culture by similar methodology as previously described.[10]

Procedures for Stability Determinations. The stability of each compound was determined by incubating a 2 mg/mL solution in either phosphate-buffered saline (pH 7.4) or 0.01 N HCl (pH 2) at 37 °C. At various times, a 20-μL aliquot of the incubation was analyzed on an 8 mm × 10 cm μBondapak C-18 Radial-Pak Column (Waters), by employing a linear gradient of 0-30% CH_3CN into 0.01 M $(NH_4)OOCCH_3$ (pH 5.5) at 3.0 mL/min over 30 min. The column effluent was monitored at 254 nm with an Altex Model 153 UV detector. The chromatographic peaks were integrated on a Model 621 Data Master System (Gilson Electronics) and the $t_{1/2}$ was determined from a plot of peak area vs time of incubation. Peaks were identified on the basis of comparison with retention times of the 2,5'-anhydro compounds, parent (nonanhydro) compounds, and the respective pyrimidine bases summarized as follows:

compd	t_R, min	compd	t_R, min
1	16.3	5-bromouracil	6.1
13	13.1	**4**	20.1
thymine	5.9	**16**	16.8
2	13.1	5-iodouracil	7.6
14	9.2	**5**	11.8
uracil	3.1	**17**	10.7
3	18.3	**18**	12.8
15	14.3		

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Registry No. 1, 30516-87-1; **2**, 84472-85-5; **3**, 105784-82-5; **4**, 85236-92-6; **5**, 3416-05-5; **6**, 64638-13-7; **7**, 100898-89-3; **8**, 120966-76-9; **9**, 120966-77-0; **10**, 120966-78-1; 11, 120966-79-2; **12**, 120966-80-5; **13**, 106060-85-9; **14**, 120826-45-1; **15**, 120826-43-9; **16**, 120826-42-8; **17**, 120966-81-6; **18**, 108441-50-5.

On the Structure Selectivity Problem in Drug Design. A Comparative Study of Benzylpyrimidine Inhibition of Vertebrate and Bacterial Dihydrofolate Reductase via Molecular Graphics and Quantitative Structure-Activity Relationships

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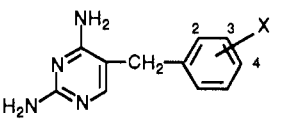
Quantitative structure-activity relationships (QSAR) have been derived for the action of 68 5-(substituted benzyl)-2,4-diaminopyrimidines on dihydrofolate reductase (DHFR) from *Lactobacillus casei* and chicken liver. The QSAR are analyzed with respect to the stereographics models of the active sites of the enzymes and found to be in good agreement. Using these QSAR equations, we have attempted to design new trimethoprim-type antifolates having higher selectivity for the bacterial enzyme. The general problem of developing selective inhibitors is discussed.

DHFR is a ubiquitous enzyme that is present in bacteria, protozoa, plants, and mammals. It catalyzes the reduction of dihydrofolate to tetrahydrofolate in the presence of the coenzyme NADPH. In its tetrahydro form this vitamin performs many vital functions, most of which involve the transfer of single-carbon units necessary for nucleic acid and certain amino acid synthesis. Inhibition of this enzyme results in cessation of DNA synthesis and eventually cell death.[1] DHFR inhibitors such as methotrexate and trimethoprim are used extensively as antineoplastic and antibacterial agents, respectively.[2] Trimethoprim is a potent and selective inhibitor of bacterial DHFRs as opposed to the mammalian enzymes.[3,4] Its toxicity and selectivity have provided the impetus for further study of the binding of the (2,4-diaminobenzyl)pyrimidine nucleus to DHFRs from various species.

In continuing our study of the comparative inhibition of dihydrofolate reductase (DHFR) from various sources by substituted (2,4-diaminobenzyl)pyrimidines (I) via



I

molecular graphics and quantitative structure-activity relationships (QSAR), new data are presented on the inhibition of the enzyme from *Lactobacillus casei*.[5-7] The structure-activity relationships of binding of this class of

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(1) Gready, J. E. *Adv. Pharmacol. Chemother.* **1980**, *17*, 37.
(2) Blakley, R. L. *Folates and Pterins, Chemistry and Biochemistry of Folates*; Blakley, R. L., Benkovic, S. J., Eds.; Wiley-Interscience: New York, 1985; Vol. 1, Chapter 5.
(3) Volz, K. W.; Matthews, D. A.; Alden, R. A.; Freer, S. T.; Hansch, C.; Kaufman, B. T.; Kraut, J. *J. Biol. Chem.* **1982**, *257*, 2528.
(4) Stone, S. R.; Morrison, J. F. *Biochim. Biophys. Acta* **1986**, *869*, 275.
(5) Hansch, C.; Li, R. L.; Blaney, J. M.; Langridge, R. *J. Med. Chem.* **1982**, *25*, 777.
(6) Li, R. L.; Hansch, C.; Matthews, D.; Blaney, J. M.; Langridge, R.; Delcamp, T. J.; Susten, S. S.; Freisheim, J. H. *Quant. Struct.-Act.* **1982**, *1*, 1.
(7) Blaney, J. M.; Hansch, C.; Silipo, C.; Vittoria, A. *Chem. Rev.* **1984**, *84*, 333.

0022-2623/89/1832-1895$01.50/0

antifolates to this enzyme are also analyzed.

Our previous QSAR for benzylpyrimidines acting on *L. casei* DHFR is shown in eq 1.[5] In this expression the

$$\log (1/K_i) = 0.89MR'_{3,4} + 0.31\pi_{3,4} - 0.91 \log [\beta(10^{\pi_{3,4}}) + 1] - 0.22\sigma^-_R + 5.31 \quad (1)$$

$$n = 42 \quad r = 0.889 \quad s = 0.214$$

$$\log \beta = -1.34 \quad \pi_0 = 1.05$$

subscripts 3 and 4 refer to substituents in the 3- and 4-positions of the benzyl ring. The prime with MR indicates that MR (MR is scaled by 0.1) has been truncated at the value of 0.79. That is, the maximum value of MR′ for any monosubstituent is 0.79, regardless of the actual value for MR. For simultaneous substitution of the 3- and 4-positions the maximum value is 2 × 0.79 (i.e., 1.58). The minimum value for disubstitution is thus 0.2 (i.e., 2 × MR_H). Since MR is primarily a measure of substituent volume,[8] it appears that parts of the groups (having MR > 0.79) do not effectively contact the enzyme. This cutoff point for the effectiveness of substituents in promoting inhibitor potency was empirically derived, and it is assumed to model a positive steric effect not accounted for in the hydrophobic effect modeled by $\pi_{3,4}$. The role of hydrophobicity on inhibitory potency is accounted for in eq 1 by using the bilinear model of Kubinyi, in which potency first rises linearly with slope of 0.31π to π_0 and then decreases linearly with slope of −0.60 (0.31–0.91).[9] Reasons for such breaks in slope include the following: In one case substituents of great length project beyond the enzyme. In this situation the slope of the right-hand side of the bilinear curve tends to approach zero. In the present case with the isolated receptor, the slope is strongly negative, which suggests that this is the result of an unfavorable steric effect. A third possibility could involve binding by long superoptimal carbon chains via hydrophobic and/or dispersion forces in such a way that binding by the critical pharmacophore (in this case, the 2,4-diaminopyrimidine moiety) to its receptor sites is diminished. This would allow for easy displacement of the inhibitor by dihydrofolic acid, since competition would ensue between the pharmacaphore for its proper site and the lipophilic substituents for their preferred hydrophobic environment.

The single most important term in eq 1 is $MR'_{3,4}$, which we believe models a positive steric effect and/or a dispersion interaction. The importance of steric bulk suggests the occurrence of a slight conformational change in the enzyme which increases the difficulty of binding for the substrate and/or its cofactor. This dependence of bacterial DHFR inhibition on MR′ represents one of the most significant differences in the way the benzylpyrimidines interact with bacterial and vertebrate DHFR. It accounts in part for the great selectivity of trimethoprim for procaryotic enzymes.[7] In the case of the vertebrate DHFR it is the hydrophobicity of the substituents modeled by π that is the dominant factor in the inhibitory action of the benzylpyrimidines.[10] The σ^-_R term is of marginal importance. It is a measure of the resonance interaction of the substituents with the π electrons of the benzene ring.[8] In all, 42 congeners (n) were used to formulate eq 1; r is the correlation coefficient, s is the standard deviation, and π_0 is the optimum value for $\pi_{3,4}$. The disposable parameter β is evaluated via an iterative procedure.[9] In the development of eq 1, we were not able to determine any role for 5-substituents other than that accounted for by σ^-_R. 5-Substituents were defined as those present in 3,5-disubstituted congeners, and since in all examples only identical substituents were present in both positions, there was no ambiguity about them. All mono meta compounds were considered to be 3-substituted, irrespective of their individual hydrophobicities.

It is noteworthy that both hydrophilic and hydrophobic 3-substituents were parameterized by π and MR. It seemed somewhat surprising that hydrophilic 3-X ($\pi < 0$) would bind in hydrophobic space when a simple 180° rotation of the phenyl ring would place them in hydrophilic space. Yet highly hydrophilic groups such as 3-CH_2OH, 3-OH, 3-OSO_2CH_3, and 3-CH_2OCH_3 were all reasonably well fit by this type of parameterization. Their negative hydrophobic interaction was offset by their positive MR interaction. However, the effects were not large, and since there was only 100-fold range in K_i, we were considering less than 3 kcal change in the free energy of binding between the strongest and weakest inhibitor.

We have observed other instances where both π and MR are required to account for effects of substituents in certain positions.[11,12] In one instance the coefficient with MR is positive,[11] and in one, it is negative.[12] A negative coefficient with MR suggests the occurrence of a dominating steric interaction. The positive MR term might be associated with producing or maintaining a favorable change in the conformation of the receptor or the position of the substrate. It appears that the substituents in the 3- and 4-positions have the same optimum π values.

With an extended set of congeners we can now make a more vigorous evaluation of eq 1 and the structure–activity relationships of the benzylpyrimidines.

Materials and Methods

DHFR from *L. casei* was purchased from Biopure Fine Chemicals, Inc. (Boston, MA) as a lyophilized powder. This was dissolved in 0.05 M Tris buffer, pH 7.20, to yield about 0.5 enzyme unit/mL. Aliquots were stored at −20 °C and were thawed just before using. The buffer contained 50 mM mercaptoethanol to prevent oxidation. The solutions of dihydrofolic acid and NADPH were kept at 0 °C. The inhibitors were solubilized with DMSO such that the final concentration of DMSO did not exceed 0.5%. DHFR activity, with and without added inhibitor, was determined by the spectrophotometric method which measures the oxidation of NADPH to $NADP^+$ and the reduction of dihydrofolic to tetrahydrofolic acid at 340 nm by using a Durrum stopped-flow spectrophotometer.[13] The K_i, log $(1/K_i)$, and 95% confidence limits were determined by using the jackknife procedure.[13]

Materials. The syntheses of most of these benzylpyrimidines have been previously reported: compounds **17**, **20**, **37**, **39**, **50**, **51**, **55–61** and **68**;[14] compounds **10**, **11**, **32–35**, **43–45**, **47**, **49**, and **63**;[15] compounds **18**, **31**, **36**, **48**, **54**, **65**, and **66**;[16] compounds **9**, **29**, and **30**;[17] compounds **12**, **15**, **19**, and **64**;[10] and compounds **13**, **14**, **22–27**,

(8) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.
(9) Kubinyi, H.; Kehrhahn, O. H. *Arzneim.-Forsch.* **1978**, *28*, 598.
(10) Selassie, C. D.; Fang, Z. X.; Li, R. L.; Hansch, C.; Klein, T.; Langridge, R.; Kaufman, B. T. *J. Med. Chem.* **1986**, *29*, 621.
(11) Ames, M. M.; Selassie, C. D.; Woodson, L. C.; van Loon, J. A.; Hansch, C.; Weinshilboum, R. M. *J. Med. Chem.* **1986**, *29*, 354.
(12) Hansch, C.; Fukunaga, J. Y.; Jow, P. Y. C.; Hines, J. B. *J. Med. Chem.* **1977**, *20*, 96.
(13) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205.
(14) Blaney, J. M.; Dietrich, S. W.; Reynolds, M. A.; Hansch, C. *J. Med. Chem.* **1979**, *22*, 614.
(15) Li, R. L.; Dietrich, S. W.; Hansch, C. *J. Med. Chem.* **1981**, *24*, 538.
(16) Li, R. L.; Hansch, C.; Kaufman, B. T. *J. Med. Chem.* **1982**, *25*, 435.
(17) Coats, E. A.; Genther, C. S.; Selassie, C. D.; Strong, C. D.; Hansch, C. *J. Med. Chem.* **1985**, *28*, 1910.

52, and **53**.[18] Compounds 4–8 were generously provided by Dr. H. Gutman and P. Weber (Hoffman La Roche), and compounds **1**, **3**, **38**, **40**, 41, and **46** were a gift from Dr. B. Roth, (Burroughs, Wellcome, Research Triangle Park). Compound **2** was provided by Dr. H. Kubinyi, and compounds **16**, **28**, **42**, and **62** were a generous gift from Dr. T. Sweeny, Division of Medicinal Chemistry, Walter Reed Medical Center, Washington, DC. Compound **67** was synthesized as follows.[49]

Synthesis of 2,4-Diamino-5-(4-phenylbenzyl)pyrimidine. To a mixture of 4-phenylbenzaldehyde (6.0 g; 33 mM), β-anilinopropionitrile (6.0 g; 40 mM), and DMSO (40 mL), which had been heated on a steam bath, was added a slurry of sodium methoxide (2 g) in DMSO (50 mL). The suspension was heated for 2 h and then poured into a mixture of ice–H_2O. The solution was cooled, and the yellow solid that precipitated was collected. The crude 4-phenyl-β-cyano-*N*-phenylcinnamylaniline (9.0 g; 30 mM) was used in the next step without further purification. It was heated at reflux with sodium methoxide (2.4 g) and guanidine (9.8 g; 100 mM) in ethanol (100 mL) for 24 h. The ethanol was removed, and the solid was washed with H_2O (50 mL). The yellow product was collected and recrystallized from 95% ethanol (yield = 48%). The analytical sample was recrystallized from acetonitrile, mp 235–236 °C. Anal. Calcd for $C_{17}H_{16}N_4$: C, 73.89; H, 5.83. Found: C, 73.97; H, 5.88.

QSAR. The π constants of most of these substituents have been taken from the benzene system, which has been well documented.[8] The partition coefficients of some of these analogues have been previously measured.[10] Newly measured partition coefficients are in Table I, where the π values are collected as outlined.

(1) For **1**:

$$\pi_3 = \pi_4 = \pi_5 = \pi_{sum}/3 = 0.86$$

(2) For **2–9**:

$$\log P[X = 3,5\text{-}(OCH_3)_2] = 1.58 \text{ (ref 19)}$$

$$\log P(X = H) = 1.58 \text{ (ref 20)}$$

$$\therefore \pi[3,5\text{-}(OCH_3)_2] = 1.58 - 1.58 = 0$$

$$\therefore \pi(3\text{-}OCH_3) = \pi(5\text{-}OCH_3) = 0$$

$$\therefore \pi_4 = \pi_{sum} - (\pi_3 + \pi_5)$$

(3) For **11**:

$$\log P[X = 3,5\text{-}(OCH_3)_2] = 1.58 \text{ (ref 19)}$$

(4) For **12**:

$$\log P[X = 3,5\text{-}(OCH_2CH_3)_2] = 2.52 \text{ (ref 20)}$$

(5) For **13–15**, log *P* values from benzene system were used.

(6) For **16–30** (3,4-disubstitution), the difference in the π values for the same substituents in the benzene system and the benzylpyrimidine system were divided by 2 and applied equally to the 3- and 4-positions. For **23**:

$$\pi[(3\text{-}OCH_3,4\text{-}OH\text{-}benzyl)pyrimidine] = 0.64 - 1.58 = -0.94$$

$$\pi(3\text{-}OCH_3,4\text{-}OH\text{-}benzene) = -0.69$$

$$\therefore \pi_{diff} = -0.69 + 0.94 = -0.25$$

$$\therefore \pi_{diff}/2 = -0.125$$

$$\therefore \pi(3\text{-}OCH_3) = -0.02 - 0.125 = -0.145$$

$$\therefore \pi(4\text{-}OH) = -0.67 - 0.125 = -0.795$$

Results

In formulating QSAR for the interactions of ligands with purified enzymes whose X-ray crystallographic structures are known, it is apparent that a problem is encountered with hydrophilic meta substituents. They could bind in hydrophobic space or avoid such contact by a 180° rotation of the phenyl ring to which they are attached.[22,23] This maneuver could place them in hydrophilic space. Such a situation also appears to occur with an enzyme whose X-ray crystallography structure is not known; with thiopurine *N*-methyltransferase, both π and MR effects seem to occur in a hydrophobic cleft of somewhat similar nature to that of *L. casei* DHFR.[11]

With the present, larger data set (Table II), it seemed important to ascertain whether or not the hydrophobicity of the regions where 3- and 5-substituents interact with DHFR are nonequivalent. With this analysis of the data, certain assumptions were made. The π values of 3-substituents whose intrinsic π values are <0 were set equal to 0. This implied that they did not bind in 3-space, but in a possibly more hydrophilic 5-space. These substituents were then assigned an MR'_5 value to test for the possibility that interaction in 5-space could be polar or steric in nature. Such substituents were also assigned a π_5 value. Thus, hydrophobic substituents were aligned in 3-space and hydrophilic in 5-space. Where two equivalent substituents were present in the 3- and 5-positions with $\pi < 0$, one was forced into the 3-position and the other was assigned to the 5-position. It was also assumed that 180° rotation of the phenyl ring did not alter the binding position of 4-substituents. Analysis with these restrictions yielded eq 2e with the attendant line of development shown in eq 2a–d. The results of eq 2a–e are significant at the 0.95 level of significance.

Inhibition of *L. casei* DHFR at pH 7.20:

$$\log (1/K_i) = 0.81(\pm 0.32)MR'_4 + 5.82(\pm 0.19) \quad (2a)$$

$$n = 65 \quad r = 0.539 \quad s = 0.425 \quad F_{1,63} = 25.83$$

$$\log (1/K_i) = 0.96(\pm 0.29)MR'_4 + 0.66(\pm 0.29)MR'_3 + 5.48(\pm 0.22) \quad (2b)$$

$$n = 65 \quad r = 0.687 \quad s = 0.370 \quad F_{1,62} = 21.24$$

$$\log (1/K_i) = 0.97(\pm 0.28)MR'_4 + 0.63(\pm 0.28)MR'_3 + 0.36(\pm 0.55)MR_5 - 0.06(\pm 0.32)MR_5^2 + 5.33(\pm 0.22) \quad (2c)$$

$$n = 65 \quad r = 0.744 \quad s = 0.345 \quad F_{2,60} = 5.51 \quad MR^\circ_5 = 2.98$$

$$\log (1/K_i) = 1.18(\pm 0.23)MR'_4 + 0.59(\pm 0.23)MR'_3 + 0.39(\pm 0.44)MR_5 - 0.10(\pm 0.26)MR_5^2 + 0.36(\pm 0.19)\pi_4 - 0.90(\pm 0.33) \log [\beta_4(10^{\pi_4}) + 1] + 5.39(\pm 0.18) \quad (2d)$$

$$n = 65 \quad r = 0.854 \quad s = 0.276 \quad F_{3,57} = 12.34 \quad MR^\circ_5 = 2.05 \quad \log \beta_4 = -0.830 \quad \pi^\circ_4 = 0.66$$

$$\log (1/K_i) = 1.24(\pm 0.21)MR'_4 + 0.52(\pm 0.27)MR'_3 + 0.42(\pm 0.45)MR_5 - 0.13(\pm 2.26)MR^2_5 + 0.46(\pm 0.21)\pi_4 + 0.31(\pm 0.23)\pi_3 - 0.92(\pm 0.31) \log [\beta_4(10^{\pi_4}) + 1] - 0.71(\pm 0.36) \log [\beta_3(10^{\pi_3}) + 1] + 5.45(\pm 0.17) \quad (2e)$$

$$n = 65 \quad r = 0.894 \quad s = 0.245 \quad F_{3,54} = 6.14 \quad \pi^\circ_4 = 0.49 \quad \log \beta_4 = -0.501 \quad \pi^\circ_3 = 1.33 \quad \log \beta_3 = -1.431 \quad MR^\circ_5 = 1.66$$

(18) Fang, Z. X.; Li, R. L.; Jiang, Y. M.; Gao, T. N. *Yaoxue Xuebao*, in press.
(19) Seiler, P.; Bischoff, O.; Wagner, R. *Arzneim.-Forsch.* **1982**, *32*, 711.
(20) Pomona College Medchem Databank.
(21) Calas, M.; Barbier, A.; Giral, L.; Balmayer, B.; Despaux, E. *Eur. J. Med. Chem.* **1982**, *17*, 497.
(22) Carotti, A.; Smith, R. N.; Wong, S.; Hansch, C.; Blaney, J. M.; Langridge, R. *Arch. Biochem. Biophys.* **1984**, *229*, 112.
(23) Carotti, A.; Ragueso, C.; Hansch, C. *Chem.-Biol. Interact.* **1985**, *52*, 279.

The squared correlation matrix for the variables in eq 2e is

	MR'_3	MR'_4	MR_5	π_3	π_4
MR'_3	1.00	0.05	0.00	0.25	0.00
MR'_4	0.05	1.00	0.00	0.08	0.05
MR_5	0.00	0.00	1.00	0.05	0.02
π_3	0.26	0.08	0.05	1.00	0.01
π_4	0.00	0.05	0.02	0.01	1.00

The eigenvalues and the corresponding percent explained variance (in parentheses) are 1.74 (35), 1.23 (25), 0.92 (18), 0.70 (14), and 0.41 (18). All of the terms in eq 2e are justified by the F statistic, the variables are reasonably orthogonal, and the eigenvalues appear to justify five parameters. Equation 2e is extremely complex; including the intercept, it contains 11 disposable parameters. However, the problem is not its complexity, but rather its simplicity.

Considering the horrendous difficulties of accounting for all the structural variations of 68 complex inhibitors, eq 2e is not without merit. It explains almost 80% of the variance in log $(1/K_i)$, and it does provide insight lacking in eq 1. In our earlier work we were unable to establish any role for 5-substituents. Equation 2e reveals a role for these hydrophilic substituents which in the present data set comprise the following: H, OCH_3, OC_2H_5, OH, NO_2, CH_2OH, $OCH_2CH_2OCH_3$, OSO_2CH_3, OCH_2CONH_2, and CH_2OCH_3.

If the bilinear relationship is the result of large substituents projecting beyond the enzyme, a slope of near 0 is expected, since there is no hydrophobic effect for that portion of the substituent projecting beyond hydrophobic space. However, this condition does have its limitations, particularly if a highly polar part of the side chain falls into aqueous space.[10] With alkyl side chains of various lengths where MR and π are collinear it appears that when the slope of the right side of the bilinear part of the hydrophobic curve is substantial, unfavorable steric interactions are operative.

From these equations, it is apparent that steric factors represented by the various MR terms account for about 50% of the variance in the data. However, the role of hydrophobicity in the interactions of these benzylpyrimidines and the bacterial enzyme cannot be minimized. Although it does incorporate some steric components the hydrophobic contribution to inhibition of *L. casei*, DHFR varies, depending on the substituent position. The slope of the bilinear equation in the 3-positions (0.31) indicates that the substituent is partially desolvated and that it encounters a steric problem after approximately three carbon atoms ($\pi^\circ_3 = 1.33$). The 0.46 coefficient with π_4 indicates that the substituent also binds on the enzyme surface where it is exposed to solvent. Once again any substituent larger than two carbon atoms experiences steric interference from other residues in the active site of the enzyme. Substituents in the 5-position interact with a polar surface region of the enzyme. To a certain extent, these aspects of eq 2e are supported by the graphics analysis (see below). In eq 2e, the σ^-_R term of eq 1 is not present, which suggests that this weak parameter may be an artifact.

In conclusion, since we obtain a better correlation by assigning hydrophilic meta substituents to 5-space and hydrophobic meta substituents to 3-space than by doing the reverse, it is thus assumed that the flexible phenyl ring rotates 180° to orient polar substituents in 5-space.

Three compounds have not been included in the derivation of eq 4. These include the 3-OCH_3,4-OH (**23**), 3-$CH_2OC_4H_9$ (**43**), and 4-OH (**53**) analogues, which are 6 times more active, 5 times less active, and 6 times more active, respectively, than predicted. Inclusion of all data points yields the equation:

$$\log (1/K_i) = 1.13(\pm0.23)MR'_4 + 0.47(\pm0.30)MR'_3 + 0.53(\pm0.51)MR_5 - 0.19(\pm0.29)MR^2_5 + 0.34(\pm0.30)\pi_3 + 0.25(\pm0.18)\pi_4 - 0.62(\pm0.42) \log [\beta_3(10^{\pi_3}) + 1] - 0.78(\pm0.34) \log [\beta_4(10^{\pi_4}) + 1] + 5.43(\pm0.19) \quad (2f)$$

$$n = 68 \quad r = 0.852 \quad s = 0.283$$
$$\pi^\circ_3 = 1.09 \quad \log \beta_3 = -1.02$$
$$\pi^\circ_4 = 0.65 \quad \log \beta_4 = -0.98$$
$$MR^\circ_5 = 1.43$$

Chicken Liver DHFR. High homology exists among the vertebrate DHFR enzymes, which contain approximately 189 residues and an active site glutamate, as opposed to 160 residues and an active site aspartate in the known bacterial DHFRs. The latter enzymes also show low homology. A comparison of the vertebrate enzymes via X-ray crystallography coordinates of the ternary complexes of chicken DHFR[24] and mouse L1210 lymphoma DHFR[25] indicates strong homology between these two enzymes. Equation 3 has been previously formulated for chicken DHFR.[10] In the development of eq 3 π_3 is the most important parameter. The MR_3 term is barely significant, and the negative coefficient with MR_5 appears to be associated with a steric effect between the substituent and a tyrosine residue. Again, as in the case of the *L. casei* enzyme, the opposite signs with π_5 and MR_5 mean that for alkyl groups and OC_2H_5, but not OCH_3, the hydrophobic effect is neutralized by the steric effect. One of our early thoughts several years ago was to invert the $-OCH_3$ to $-CH_2OH$, which would yield the same MR value but a much lower π value and hence should give a good ratio between inhibition of chicken and *L. casei* DHFR. The activity of 3,5-(CH_2OH) is well predicted by eq 2e and 4e. The log $(1/K_i)$ for chicken DHFR of 3.23 is indeed the lowest of any benzylpyrimidines. log $(1/K_i)$ with *L. casei* DHFR is only moderately high, 5.73; however, the difference between the two values (the therapeutic index) is 2.50. TMP has a difference of 3.00 and is about 1000 times more active against *L. casei* DHFR.

$$\log (1/K_i) = 0.43\pi_3 - 1.13 \log [\beta_3(10^{\pi_3}) + 1] + 0.59\pi_4 - 0.63 \log [\beta_4(10^{\pi_4}) + 1] + 0.48\pi_5 - 0.63MR_5 + 0.14MR_3 + 4.87 \quad (3)$$

$$n = 53 \quad r = 0.921 \quad s = 0.208$$

$$\pi^\circ_3 = 2.00 \quad \log \beta_3 = -2.21 \quad \pi^\circ_4 = 1.53 \quad \log \beta_4 = -0.40$$

With the larger and more varied data set (particularly, more 3,5-disubstituted analogues) the inhibition of chicken liver DHFR was reevaluated (Table III). As in our previous analysis, all meta substituents were assumed to be binding in hydrophobic 3-space. With 3,5-disubstituted analogues, the 5-substituent was forced into 5-space even though steric constraints due to Tyr-31 prevailed at this position. The following equations were obtained for the inhibition of chicken DHFR by the benzylpyrimidines:

(24) Matthews, D. A.; Bolin, J. T.; Burridge, J. M.; Filman, D. J.; Volz, K. W.; Kaufman, B. T.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Kraut, J. *J. Biol. Chem.* **1985**, *260*, 381.

(25) Stammers, D. K.; Champness, J. N.; Dann, J. G.; Beddell, C. R. *Chemistry and Biology of Pteridines*; Blair, J. A., Ed.; de Gruyter: Berlin and New York, 1983; p 567.

$$\log (1/K_i) = 0.41(\pm 0.13)\pi_3 - 1.18(\pm 0.81) \log [\beta_3(10^{\pi_3}) + 1] + 4.45(\pm 0.09) \quad (4a)$$

$$n = 65 \quad r = 0.614 \quad s = 0.366 \quad F_{3,61} = 12.29$$

$$\pi^\circ_3 = 2.57 \quad \log \beta_3 = -2.84$$

$$\log (1/K_i) = 0.40(\pm 0.13)\pi_3 + 0.30(\pm 0.16)\pi_4 - 1.17(\pm 0.76) \log [\beta_3(10^{\pi_3}) + 1] - 0.42(\pm 0.45) \log [\beta_4(10^{\pi_4}) + 1] + 4.43(\pm 0.09) \quad (4b)$$

$$n = 65 \quad r = 0.730 \quad s = 0.326 \quad F_{3,58} = 6.43$$

$$\pi^\circ_3 = 2.62 \quad \log \beta_3 = -2.90 \quad \pi^\circ_4 = 2.17 \quad \log \beta_4 = -1.79$$

$$\log (1/K_i) = 0.45(\pm 0.10)\pi_3 + 0.50(\pm 0.23)\pi_4 + 0.57(\pm 0.17)MR_5 - 1.16(\pm 0.45) \log [\beta_3(10^{\pi_3}) + 1] - 0.45(\pm 0.32) \log [\beta_4(10^{\pi_4}) + 1] + 4.71(\pm 0.13) \quad (4c)$$

$$n = 65 \quad r = 0.856 \quad s = 0.248 \quad F_{1,57} = 42.93$$

$$\pi^\circ_3 = 2.37 \quad \log \beta_3 = -2.56 \quad \pi^\circ_4 \simeq 3.0 \quad \log \beta_4 = -0.24$$

$$\log (1/K_i) = 0.43(\pm 0.09)\pi_3 + 0.43(\pm 0.20)\pi_4 - 0.65(\pm 0.15)MR_5 + 0.42(\pm 0.20)\sigma - 1.08(\pm 0.39) \log [\beta_3(10^{\pi_3}) + 1] - 0.32(\pm 0.28) \log [\beta_4(10^{\pi_4}) + 1] + 4.67(\pm 0.11) \quad (4d)$$

$$n = 65 \quad r = 0.894 \quad s = 0.217 \quad F_{1,56} = 18.46$$

$$\pi^\circ_3 = 2.39 \quad \log \beta_3 = -2.56 \quad \pi^\circ_4 \simeq 3.00 \quad \log \beta_4 = -0.29$$

$$\log (1/K_i) = 0.39(\pm 0.09)\pi_3 + 0.44(\pm 0.21)\pi_4 - 0.75(\pm 0.17)MR_5 + 0.44(\pm 0.19)\sigma - 1.04(\pm 0.42) \log [\beta_3(10^{\pi_3}) + 1] + 0.37(\pm 0.29)\pi_5 - 0.32(\pm 0.28) \log [\beta_4(10^{\pi_4}) + 1] + 4.70(\pm 0.12) \quad (4e)$$

$$n = 65 \quad r = 0.906 \quad s = 0.207 \quad F_{1,55} = 6.39$$

$$\pi^\circ_3 = 2.45 \quad \log \beta_3 = -2.69 \quad \pi^\circ_4 \simeq 3.00 \quad \log \beta_4 = -0.18$$

The squares correlation matrix for the variables in eq 4e is

	π_3	π_4	π_5	MR_5	σ
π_3	1.00	0	0.04	0	0
π_4	0	1.00	0	0	0.03
π_5	0.04	0	1.00	0.23	0.01
MR_5	0	0	0.23	1.00	0.08
σ	0	0.03	0.01	0.08	1.00

The eigenvalues and the percent of the variance (in parentheses) accounted for by each eigenvalue are 1.65 (33), 1.12 (22), 1.01 (20), 0.78 (16), and 0.44 (9). Inclusion of all data points yields the equation:

$$\log (1/K_i) = 0.41(\pm 0.11)\pi_3 + 0.42(\pm 0.23)\pi_3 - 0.69(\pm 0.19)MR_5 + 0.34(\pm 0.22)\sigma - 1.09(\pm 0.50) \log [\beta_3(10^{\pi_3}) + 1] + 0.47(\pm 0.32)\pi_5 - 0.30(\pm 0.32) \log [\beta_4(10^{\pi_4}) + 1] + 4.66(\pm 0.13) \quad (4f)$$

$$n = 68 \quad r = 0.871 \quad s = 0.243$$

$$\pi^\circ_3 = 2.46 \quad \log \beta_3 = -2.68 \quad \pi^\circ_4 \simeq 3.00 \quad \log \beta_4 = -0.24$$

Equation 4e is similar to eq 3 that was previously obtained, with the exception of the addition of the σ term. The increased electron density in the phenyl ring leads to greater antiavian inhibitory potency. Three compounds were not included in the derivation of the equations—3,4,5-$(C_2H_5)_3$ (**1**), 3-OH (**35**), and 4-OCF_3 (**62**)—which are 5 times more active, 4 time less active, and 4 times less active, respectively, than predicted. In the stepwise development of eq 4e, eq 4a–e are significant at the 0.95 level of significance. There are two bilinear parts to eq 4e, which establish the optimum hydrophobicity for substituents in the 3- and 4-positions. The initial slopes of π_3, π_4, and π_5 are about 0.5, which suggests partial desolvation of X on the enzyme surface. The right-hand slopes of the bilinear equation for π_3 and π_4 are −0.65 and 0, respectively. The sharp descending slope with π_3 suggests a possible steric effect in the 3-position, where unfavorable contact with Val-115 causes the drop in activity after π_0 of 2.50 has been attained. The flat slope ~ 0 of π_4 indicates that hydrophobic substituents larger than hexyloxy do not contact the enzyme but project into aqueous space. The high negative coefficient (−0.75) with MR_5 suggests strong steric hindrance to binding due to the presence of Tyr-31. Tyr-31 in its normal position packs against the polypeptide backbone atoms of residues 26–28 and the side chain of Leu-22 to reveal a hydrophobic surface where substituents in the 4- and 5-positions can bind.[24] However, if the substituents in the 4- and 5-positions are relatively large, then the tyrosine residue undergoes a conformational change and rotates 180° from its normal position to an alternate position.[26]

From a molecular graphics analysis of the ternary complex of TMP–NADPH–DHFR and the isopropenyl derivative [X = 3,5-$(OCH_3)_2$,4-$C(CH_3)=CH_2$] (**7**), it appears that most hydrophobic substituents in the para position would retain Tyr-31 in its normal position (as with MTX or the triazines) while hydrophilic substituents would do the opposite. This type of behavior, which would affect the binding of both 4,5- and 3,4,5-substituted benzylpyrimidines, is at best difficult to parameterize.

Matthews et al. have demonstrated that the 3,4,5-triethyl analogue (**1**) causes the side chain of Tyr-31 to undergo a conformational change on the formation of the ternary complex like it does with TMP despite the possibility of hydrophobic interaction between the 4-ethyl and the side chains of Leu-22 and Tyr-31.[26] Crystallographic evidence suggests that there are slight geometrical differences in the orientation of the benzyl side chains of TMP and its triethyl congener. In particular, one of the *m*-ethyl groups positioned at the back of the binding cavity appears to be in a different conformation than that of the corresponding methoxy group of TMP. The triethyl compound turns out to be one of the most active compounds in Table III and is not well predicted by eq 4e in contrast to TMP and the isopropenyl derivative (**7**).

There is good agreement between our mathematical model and the computer graphics analysis. The complexity of the equation is dictated by the geometry of the active site and the wide diversity in substituent type. The equation is such that it delineates most of the interactions involved at the three different positions and may simplify the task of designing an effective inhibitor of DHFR. To attain selectivity, we need to maximize binding with *L. casei* DHFR and minimize binding with chicken liver DHFR since the QSAR of the latter is very similar to that of human Wilz DHFR.

Molecular Graphics Analysis

Our previous molecular graphics studies based on X-ray crystallographic coordinates of *L. casei* DHFR with bound inhibitors allow the following interpretation of eq 2e.[5,7] The present equation provides information about substituent interactions in three specific binding areas of 3-, 4-, and 5-substituents.

On the basis of the optima for π_3 (1.33) we would assume that one of the meta substituents can contact a moderately

(26) Matthews, D. A.; Bolin, J. T.; Burridge, J. M.; Filman, D. J.; Volz, K. W.; Kraut, J. J. *J. Biol. Chem.* **1985**, *260*, 392.

Table I. Partition Coefficients. Calculation of Individual π Values

no.	X	log P	ref	π_{sum}	π_3	π_4	π_5
1	3,4,5-$(CH_2CH_3)_3$	1.55A[a]	20	2.58	0.86	0.86	0.86
2	3,5-$(OCH_3)_2$,4-$OCH_2CH_2OCH_3$	−1.81A	20	−0.78	0	−0.78	0
3	3,4,5-$(OCH_3)_3$	−1.55A	20	−0.52	0	−0.52	0
4	3,5-$(OCH_3)_2$,4-$N(CH_3)_2$	1.51B	20	−0.07	0	−0.07	0
5	3,5-$(OCH_3)_2$,4-Br	2.01B	19	0.43	0	0.43	0
6	3,5-$(OCH_3)_2$,4-SCH_3	1.54B	19	−0.04	0	−0.04	0
7	3,5-$(OCH_3)_2$,4-$C(CH_3)=CH_2$	2.07B	20	0.49	0	0.49	0
8	3,5-$(OCH_2CH_3)_2$,4-pyrryl	2.90B	20	1.32	0.47	0.38	0.47
9	3,5-$(OCH_3)_2$,4-OC_8H_{17}			3.78	0	3.78	0
10	3,5-$(CH_2OH)_2$			−2.06	−1.03	0	−1.03
11	3,5-$(OCH_3)_2$	1.58B	19	0	0	0	0
12	3,5-$(OCH_2CH_3)_2$	2.52B	20	0.94	0.47	0	0.47
13	3-OCH_2CH_3,5-$OCH_2CH_2CH_3$			1.52	1.05	0	0.47
14	3,5-$(OCH_2CH_2CH_3)_2$			2.10	1.05	0	1.05
15	3,5-$(CH_3)_2$			1.12	0.56	0	0.56
16	3,4-$(OH)_2$	2.08A	20	−1.05	0	−0.52	−0.52
17	3-NO_2,4-$NHCOCH_3$	−2.23A	20	−1.20	0	−0.26	−0.94
18	3,4-$(OCH_2CH_2OCH_3)_2$	1.89A	20	−0.86	0	−0.43	−0.43
19	3,4-OCH_2O	1.50B	19	−0.08	0	−0.04	−0.04
20	3,4-$(OCH_3)_2$	0.97B	19	−0.61	0	−0.31	−0.31
21	3-OH,4-OCH_3	0.74B	19	−0.84	0	−0.09	−0.75
22	3-OCH_3,4-OSO_2CH_3	0.60B	20	−0.98	0	−0.92	−0.06
23	3-OCH_3,4-OH	0.64B	19	−0.94	0	−0.80	−0.14
24	3-OCH_3,4-$OCH_2CH_2OCH_3$	−1.87A	20	−0.84	0	−0.56	−0.28
25	3-OCH_3,4-$OCH_2C_6H_5$	2.66B	19	1.08	0	1.38	−0.30
26	3-OSO_2CH_3,4-OCH_3	−1.92A	20	−0.89	0	−0.01	−0.88
27	3-$OCH_2C_6H_5$,4-OCH_3	2.44B	20	0.86	1.27	−0.41	0
28	3-CF_3,4-OCH_3	−0.18A	20	0.85	0.87	−0.02	0
29	3-OC_8H_{17},4-OCH_3	2.54A	20	3.57	3.69	−0.12	0
30	3-OCH_2CH_3,4-$OCH_2C_6H_5$	1.00A	20	2.03	0.38	1.65	0
31	3-OCH_2CONH_2			−1.37	0	0	−1.37
32	3-CH_2OH			−1.03		0	−1.03
33	3-OSO_2CH_3			−0.88	0	0	−0.88
34	3-CH_2OCH_3			−0.78	0	0	−0.78
35	3-OH	−1.34A	20	−0.31	0	0	−0.31
36	3-$OCH_2CH_2OCH_3$	−1.33A	20	−0.30	0	0	−0.30
37	3-OCH_3	1.54B	19	0.04	0	0	0.04
38	3-F	−0.80A	20	0.23	0.23	0	0
39	3-CH_3	−0.51A	20	0.52	0.52	0	0
40	3-Cl	−0.36A	20	0.67	0.67	0	0
41	3-Br			0.86	0.86	0	0
42	3-CF_3			0.88	0.88	0	0
43	3-$CH_2OC_4H_9$	2.88B	20	1.30	1.30	0	0
44	3-I			1.12	1.12	0	0
45	3-OC_4H_9			1.55	1.55	0	0
46	3-$OCH_2C_6H_5$	0.53A	20	1.56	1.56	0	0
47	3-OC_6H_{13}			2.63	2.63	0	0
48	3-OC_7H_{15}			3.23	3.23	0	0
49	3-OC_8H_{17}			3.79	3.79	0	0
50	4-NH_2	0.22B	19	−1.36	0	−1.36	0
51	4-$NHCOCH_3$	−1.94A	20	−0.91	0	−0.91	0
52	4-OSO_2CH_3	0.60B	20	−0.98	0	−0.98	0
53	4-OH	−1.47A	20	−0.44	0	−0.44	0
54	4-$OCH_2CH_2OCH_3$			−0.30	0	−0.30	0
55	4-NO_2	−1.03A	20	0	0	0	0
56	4-OCH_3	1.34B	21	−0.20	0	−0.20	0
57	4-F			0.14	0	0.14	0
58	4-$N(CH_3)_2$	1.82B	20	0.24	0	0.24	0
59	4-CH_3			0.56	0	0.56	0
60	4-Cl			0.71	0	0.71	0
61	4-Br			0.86	0	0.86	0
62	4-OCF_3	0.06A	20	1.09	0	1.09	0
63	4-OC_4H_9			1.55	0	1.55	0
64	4-$OCH_2C_6H_5$			1.66	0	1.66	0
65	4-OC_6H_{13}			2.63	0	2.63	0
66	4-OC_7H_{15}			3.23	0	3.23	0
67	4-C_6H_5	0.98A	20	2.01	0	1.96	0
68	4-H	1.58B	20	0	0	0	0
		1.54B	21	0	0	0	0
		−1.03A	20	0	0	0	0

[a] A = 0.1 N HCl. B = 0.01 N NaOH.

large hydrophobic area. The π_0 for 3-substituents would suggest that this region could accommodate between two and three carbons, while the coefficients of 0.31 for π_3 suggest that only partial desolvation of substituents occurs.[22,23] This implies that the substituent is not completely engulfed in the cavity as it would be in partitioning into octanol. The molecular graphics analysis confirms these results. The 3-substituents extend into a fairly extensive hydrophobic area where binding occurs on the surface of the enzyme active site in the region of Leu-19.

Table II. Parameters Used To Derive Equation 2e for the Inhibition of *L. casei* DHFR by Benzylpyrimidines I

no.	X	log $(1/K_i)$ obsd	log $(1/K_i)$ calcd	MR'_4	MR'_3	MR_5	π_3	π_4
1	3,4,5-$(CH_2CH_3)_3$	6.88	7.25	0.79	0.79	1.03	0.86	0.86
2	3,5-$(OCH_3)_2$,4-$OCH_2CH_2OCH_3$	6.26	6.71	0.79	0.79	0.79	0.00	−0.78
3	3,4,5-$(OCH_3)_3$	6.88	6.81	0.79	0.79	0.79	0.00	−0.52
4	3,5-$(OCH_3)_2$,4-$N(CH_3)_2$	6.45	6.96	0.79	0.79	0.79	0.00	−0.07
5	3,5-$(OCH_3)_2$,4-Br	7.28	7.04	0.79	0.79	0.79	0.00	0.43
6	3,5-$(OCH_3)_2$,4-SCH_3	7.25	6.97	0.79	0.79	0.79	0.00	−0.04
7	3,5-$(OCH_3)_2$,4-$C(CH_3)=CH_2$	7.34	7.04	0.79	0.79	0.79	0.00	0.49
8	3,5-$(OCH_2CH_3)_2$,4-C_4H_4N	7.33	7.21	0.79	0.79	1.25	0.38	0.56
9	3,5-$(OCH_3)_2$,4-$O(CH_2)_7CH_3$	5.70	5.78	0.79	0.79	0.79	0.00	3.78
10	3,5-$(CH_2OH)_2$	5.73	5.76	0.10	0.72	0.72	−1.03	0.00
11	3,5-$(OCH_3)_2$	6.42	6.12	0.10	0.79	0.79	0.00	0.00
12	3,5-$(OCH_2CH_3)_2$	6.41	6.32	0.10	0.79	1.25	0.47	0.00
13	3-OC_2H_5,5-OC_3H_7	6.23	6.42	0.10	0.79	1.25	1.05	0.00
14	3,5-$(OC_3H_7)_2$	6.19	6.45	0.10	0.79	1.71	1.05	0.00
15	3,5-$(CH_3)_2$	5.87	6.10	0.10	0.57	.57	0.56	0.00
16	3,4-$(OH)_2$	5.84	5.69	0.29	0.10	.29	0.00	−0.53
17	3-NO_2,4-$NHCOCH_3$	6.00	6.27	0.79	0.10	0.74	0.00	−0.94
18	3,4-$(OCH_2CH_2OCH_3)_2$	6.51	6.58	0.79	0.10	1.93	0.00	−0.43
19	3,4-OCH_2O	6.34	6.10	0.45	0.10	0.45	0.00	−0.04
20	3,4-$(OCH_3)_2$	6.92	6.53	0.79	0.10	0.79	0.00	−0.31
21	3-OH,4-OCH_3	6.59	6.45	0.79	0.10	0.29	0.00	−0.09
22	3-OCH_3,4-OSO_2CH_3	6.44	6.29	0.79	0.10	0.79	0.00	−0.92
23	3-OCH_3,4-OH	6.47	5.72	0.29	0.10	0.79	0.00	−0.80
24	3-OCH_3,4-$OCH_2CH_2OCH_3$	6.54	6.44	0.79	0.10	0.79	0.00	−0.56
25	3-OCH_3,4-$OCH_2C_6H_5$	6.50	6.49	0.79	0.10	0.79	0.00	1.38
26	3-OSO_2CH_3,4-OCH_3	6.67	6.71	0.79	0.10	1.70	0.00	−0.01
27	3-$OCH_2C_6H_5$,4-OCH_3	6.91	6.88	0.79	0.79	0.10	1.27	−0.41
28	3-CF_3,4-OCH_3	7.10	6.81	0.79	0.50	0.10	0.87	−0.02
29	3-$O(CH_2)_7CH_3$,4-OCH_3	6.50	6.29	0.79	0.79	0.10	3.69	−0.12
30	3-OCH_2CH_3,4-$OCH_2C_6H_5$	6.51	6.64	0.79	0.79	0.10	0.38	1.65
31	3-OCH_2CONH_2	5.96	5.86	0.10	0.10	1.60	0.00	0.00
32	3-CH_2OH	5.67	5.75	0.10	0.10	0.72	0.00	0.00
33	3-OSO_2CH_3	5.92	5.86	0.10	0.10	1.70	0.00	0.00
34	3-CH_2OCH_3	5.64	5.83	0.10	0.10	1.21	0.00	0.00
35	3-OH	5.82	5.62	0.10	0.10	0.29	0.00	0.00
36	3-$OCH_2CH_2OCH_3$	6.12	5.85	0.10	0.10	1.93	0.00	0.00
37	3-OCH_3	5.93	5.92	0.10	0.79	0.10	0.04	0.00
38	3-F	5.38	5.61	0.10	0.09	0.10	0.23	0.00
39	3-CH_3	5.78	5.93	0.10	0.57	0.10	0.52	0.00
40	3-Cl	5.90	5.98	0.10	0.60	0.10	0.67	0.00
41	3-Br	6.23	6.11	0.10	0.79	0.10	0.86	0.00
42	3-CF_3	6.16	5.96	0.10	0.50	0.10	0.88	0.00
43	3-$CH_2OC_4H_9$	5.49	6.15	0.10	0.79	0.10	1.30	0.00
44	3-I	6.67	6.14	0.10	0.79	0.10	1.12	0.00
45	3-$O(CH_2)_3CH_3$	6.13	6.14	0.10	0.79	0.10	1.55	0.00
46	3-$OCH_2C_6H_5$	6.15	6.14	0.10	0.79	0.10	1.56	0.00
47	3-$O(CH_2)_5CH_3$	5.77	5.87	0.10	0.79	0.10	2.63	0.00
48	3-$O(CH_2)_6CH_3$	5.60	5.65	0.10	0.79	0.10	3.23	0.00
49	3-$O(CH_2)_7CH_3$	5.30	5.43	0.10	0.79	0.10	3.79	0.00
50	4-NH_2	5.47	5.58	0.54	0.10	0.10	0.00	−1.36
51	4-$NHCOCH_3$	6.05	6.08	0.79	0.10	0.10	0.00	−0.91
52	4-OSO_2CH_3	6.49	6.05	0.79	0.10	0.10	0.00	−0.98
53	4-OH	6.38	5.65	0.29	0.10	0.10	0.00	−0.44
54	4-$OCH_2CH_2OCH_3$	6.05	6.32	0.79	0.10	0.10	0.00	−0.30
55	4-NO_2	6.00	6.34	0.74	0.10	0.10	0.00	0.00
56	4-OCH_3	6.25	6.35	0.79	0.10	0.10	0.00	−0.20
57	4-F	5.67	5.56	0.09	0.10	0.10	0.00	0.14
58	4-$N(CH_3)_2$	6.17	6.45	0.79	0.10	0.10	0.00	0.24
59	4-CH_3	5.83	6.19	0.57	0.10	0.10	0.00	0.56
60	4-Cl	6.19	6.22	0.60	0.10	0.10	0.00	0.71
61	4-Br	6.21	6.43	0.79	0.10	0.10	0.00	0.86
62	4-OCF_3	6.30	6.38	0.79	0.10	0.10	0.00	1.09
63	4-$O(CH_2)_3CH_3$	6.37	6.22	0.79	0.10	0.10	0.00	1.55
64	4-$OCH_2C_6H_5$	6.35	6.17	0.79	0.10	0.10	0.00	1.66
65	4-$O(CH_2)_5CH_3$	5.73	5.74	0.79	0.10	0.10	0.00	2.63
66	4-$O(CH_2)_6CH_3$	5.38	5.46	0.79	0.10	0.10	0.00	3.23
67	4-C_6H_5	6.41	6.02	0.79	0.10	0.10	0.00	2.01
68	H	5.20	5.55	0.10	0.10	0.10	0.00	0.00

Thus both π_0 and the coefficient with π_3 seem reasonable in the light of past experiences with other enzymes.

The interactions of 5-substituents with *L. casei* DHFR are of a more complex nature. With this larger data set, we can now define binding in this area as being associated with MR_5, that is, with substituent bulk and/or dispersion forces. Hydrophobic interactions are not apparent in this position. Graphics analysis reveals this region to be polar in nature. The binding of 5-X is fit by a parabolic model since the dearth of large substituents at this position

Table III. Parameters Used To Derive Equation 4f for the Inhibition of Chicken Liver DHFR by Benzylpyrimidines I

no.	X	log $(1/K_i)$ obsd	log $(1/K_i)$ calcd	π_3	π_4	π_5	MR_5	σ
1	3,4,5-$(CH_2CH_3)_3$	5.25	4.58	0.86	0.86	0.86	1.03	−0.29
2	3,5-$(OCH_3)_2$,4-$OCH_2CH_2OCH_3$	3.64	3.74	0.00	−0.78	0.00	0.79	0.00
3	3,4,5-$(OCH_3)_3$	3.98	3.88	0.00	−0.52	0.00	0.79	0.07
4	3,5-$(OCH_3)_2$,4-$N(CH_3)_2$	4.15	4.26	0.00	−0.07	0.00	0.79	0.57
5	3,5-$(OCH_3)_2$,4-Br	4.54	4.36	0.00	0.43	0.00	0.79	0.47
6	3,5-$(OCH_3)_2$,4-SCH_3	4.29	4.13	0.00	−0.04	0.00	0.79	0.25
7	3,5-$(OCH_3)_2$,4-$C(CH_3)=CH_2$	4.17	4.26	0.00	0.49	0.00	0.79	0.22
8	3,5-$(OCH_2CH_3)_2$,4-C_4H_4N	4.33	4.38	0.38	0.56	0.38	1.25	0.57
9	3,5-$(OCH_3)_2$,4-$O(CH_2)_7CH_3$	4.77	4.59	0.00	3.78	0.00	0.79	−0.08
10	3,5-$(CH_2OH)_2$	3.23	3.30	−1.03	0.00	−1.03	0.72	0.00
11	3,5-$(OCH_3)_2$	4.12	4.14	0.00	0.00	0.00	0.79	0.24
12	3,5-$(OCH_2CH_3)_2$	4.14	4.13	0.47	0.00	0.47	1.25	0.20
13	3-OC_2H_5,5-OC_3H_7	4.36	4.35	1.05	0.00	0.47	1.25	0.20
14	3,5-$(OC_3H_7)_2$	4.13	4.22	1.05	0.00	1.05	1.71	0.20
15	3,5-$(CH_3)_2$	4.61	4.56	0.56	0.00	0.56	0.57	−0.14
16	3,4-$(OH)_2$	3.59	4.03	−0.53	−0.53	0.00	0.10	−0.28
17	3-NO_2,4-$NHCOCH_3$	4.34	4.41	−0.26	−0.94	0.00	0.10	0.71
18	3,4-$(OCH_2CH_2OCH_3)_2$	3.91	4.17	−0.43	−0.43	0.00	0.10	−0.14
19	3,4-OCH_2O	4.68	4.38	−0.04	−0.04	0.00	0.10	−0.32
20	3,4-$(OCH_3)_2$	4.46	4.27	−0.31	−0.31	0.00	0.10	−0.12
21	3-OH,4-OCH_3	3.85	4.16	−0.75	−0.09	0.00	0.10	−0.15
22	3-OCH_3,4-OSO_2CH_3	4.59	4.39	−0.06	−0.92	0.00	0.10	0.48
23	3-OCH_3,4-OH	4.31	4.09	−0.14	−0.80	0.00	0.10	−0.25
24	3-OCH_3,4-$OCH_2CH_2OCH_3$	4.19	4.19	−0.28	−0.56	0.00	0.10	−0.12
25	3-OCH_3,4-$OCH_2C_6H_5$	4.72	4.68	−0.30	1.38	0.00	0.10	−0.11
26	3-OSO_2CH_3,4-OCH_3	4.53	4.26	−0.88	−0.01	0.00	0.10	0.12
27	3-$OCH_2C_6H_5$,4-OCH_3	4.74	4.82	1.27	−0.41	0.00	0.10	−0.15
28	3-CF_3,4-OCH_3	4.99	4.95	0.87	−0.02	0.00	0.10	0.16
29	3-$O(CH_2)_7CH_3$,4-OCH_3	4.71	4.78	3.69	−0.12	0.00	0.10	−0.17
30	3-OCH_2CH_3,4-$OCH_2C_6H_5$	4.74	4.97	0.38	1.65	0.00	0.10	−0.13
31	3-OCH_2CONH_2	4.27	4.07	−1.37	0.00	0.00	0.10	0.12
32	3-CH_2OH	4.31	4.15	−1.03	0.00	0.00	0.10	0.00
33	3-OSO_2CH_3	4.33	4.38	−0.88	0.00	0.00	0.10	0.39
34	3-CH_2OCH_3	4.37	4.26	−0.78	0.00	0.00	0.10	0.02
35	3-OH	3.87	4.48	−0.31	0.00	0.00	0.10	0.12
36	3-$OCH_2CH_2OCH_3$	4.83	4.48	−0.30	0.00	0.00	0.10	0.10
37	3-OCH_3	4.45	4.57	0.04	0.00	0.00	0.10	0.00
38	3-F	4.70	4.79	0.23	0.00	0.00	0.10	0.34
39	3-CH_3	4.72	4.72	0.52	0.00	0.00	0.10	−0.07
40	3-Cl	5.01	4.97	0.67	0.00	0.00	0.10	0.37
41	3-Br	5.03	5.06	0.86	0.00	0.00	0.10	0.39
42	3-CF_3	4.92	5.08	0.88	0.00	0.00	0.10	0.43
43	3-$CH_2O(CH_2)_3CH_3$	5.17	5.05	1.30	0.00	0.00	0.10	0.02
44	3-I	4.79	5.13	1.12	0.00	0.00	0.10	0.35
45	3-$O(CH_2)_3CH_3$	5.22	5.17	1.55	0.00	0.00	0.10	0.10
46	3-$OCH_2C_6H_5$	5.63	5.18	1.56	0.00	0.00	0.10	0.12
47	3-$O(CH_2)_5CH_3$	5.67	5.33	2.63	0.00	0.00	0.10	0.10
48	3-$O(CH_2)_6CH_3$	4.79	5.17	3.23	0.00	0.00	0.10	0.10
49	3-$O(CH_2)_7CH_3$	5.08	4.88	3.79	0.00	0.00	0.10	0.10
50	4-NH_2	3.73	3.72	0.00	−1.36	0.00	0.10	−0.66
51	4-$NHCOCH_3$	4.26	4.21	0.00	−0.91	0.00	0.10	0.00
52	4-OSO_2CH_3	4.31	4.34	0.00	−0.98	0.00	0.10	0.36
53	4-OH	4.17	4.23	0.00	−0.44	0.00	0.10	−0.37
54	4-$OCH_2CH_2OCH_3$	4.26	4.34	0.00	−0.30	0.00	0.10	−0.24
55	4-NO_2	4.37	4.90	0.00	0.00	0.00	0.10	0.78
56	4-OCH_3	4.29	4.37	0.00	−0.20	0.00	0.10	−0.27
57	4-F	4.79	4.62	0.00	0.14	0.00	0.10	0.06
58	4-$N(CH_3)_2$	4.01	4.25	0.00	0.24	0.00	0.10	−0.83
59	4-CH_3	4.56	4.63	0.00	0.56	0.00	0.10	−0.17
60	4-Cl	4.83	4.83	0.00	0.71	0.00	0.10	0.23
61	4-Br	4.79	4.86	0.00	0.86	0.00	0.10	0.23
62	4-OCF_3	4.32	4.95	0.00	1.09	0.00	0.10	0.35
63	4-$O(CH_2)_3CH_3$	4.67	4.72	0.00	1.55	0.00	0.10	−0.32
64	4-$OCH_2C_6H_5$	4.83	4.78	0.00	1.66	0.00	0.10	−0.23
65	4-$O(CH_2)_5CH_3$	4.71	4.86	0.00	2.63	0.00	0.10	−0.32
66	4-$O(CH_2)_6CH_3$	4.73	4.93	0.00	3.23	0.00	0.10	−0.32
67	4-C_6H_5	5.26	4.92	0.00	2.01	0.00	0.10	−0.01
68	H	4.71	4.55	0.00	0.00	0.00	0.10	0.00

precludes the use of the bilinear model. MR_5 has not been truncated as have MR_3 and MR_4. The optimum with MR_5 suggests the existence of a fairly large polar area for binding, which corresponds in part to the surface of the 2′-hydroxyl of the coenzyme.

In the case of 4-substituents, a π_0 of 0.49 and a coefficient of 0.46 with π_4 are obtained. In studying the graphics surface, one would expect a slope for π_4 of 0.5 since the hydrophobic surface which is delineated by Phe-49 and Leu-19 seems rather flat and open until it gets bumpy in

the region of Pro-61. This appears to come into play at about a few carbons chain length in the 4-position. The right-hand side of the π_4 bilinear curve yields a negative slope of −0.46 which we believe is indicative of a marginal steric effect attributed to the presence of Pro-61. Past analyses of animal studies in vivo have indicated that the negative character of the descending bilinear curve can be attributed to the random walk process, which is clearly absent in the case of isolated, purified enzymes. In the latter case, one expects a slope of zero when substituents extent beyond the enzyme surface into aqueous space.

A procedure that is increasingly gaining support in structure–activity studies is the calculation of electrostatic surface potentials of both ligands and proteins.[27-30] In our studies with surface potentials of various enzymes and ligands, we have not been able to devise a satisfactory procedure of quantifying the differences in potentials so that this information can be utilized in our multivariate analyses. However, our previous procedure of factoring the enzyme surface into color-coded surfaces (red, yellow, and blue) is a crude attempt at recognizing three types of surface polarity.[6] Although it would be beneficial to categorize more surface types, it would nonetheless create an additional problem of testing many new surface probes (substrates or inhibitors) in order to instill confidence in the added parameters.

Equation 4e, pertaining to chicken liver DHFR QSAR, reiterates our previous QSAR findings and is well augmented by our molecular graphics analysis.[10] The substituents on the inhibitor phenyl ring are accommodated in a hydrophobic environment comprised of five hydrophobic residues (Phe-34, Met-52, Ile-60, Leu-67, and Val-115) and the side-chain methyl of Thr-56.[24] The initial slopes of π_3, π_4, and π_5 are all about 0.5, which once again suggests ~50% desolvation of X in partitioning onto the enzyme. Molecular graphics analysis also indicates that hydrophobic 3-substituents can bind to the enzyme surface up to $\pi_0 = 2.5$. Then binding diminishes considerably due to the presence of Val-115. In our previous work this residue was mistakenly identified as Pro-61.[10] The strong descending slope of the bilinear equation (−0.65) is thus attributed to a steric interaction with this bulky residue.

In the para position, substituents larger than hexyloxy project beyond the enzyme surface into aqueous space, hence the flat slope (+0.10). The area available for binding of 4-substituents is located behind the Tyr-31 residue and is quite extensive as suggested by the optimum π_4 (3.0). It is delineated by residues Leu-67 and Phe-34.

The bulk of Tyr-31 precludes binding in this region by monosubstituted 3- and 4-analogues and disubstituted 3,4-analogues. With 3,5-disubstituted benzylpyrimidines the absence of a substituent in the para position alleviates the steric crowding to some extent and increases the accessibility of the substituent to the hydrophobic space behind the Tyr-31 residue. However, with the trisubstituted analogues, forcing of the 5-substituent into the limited space available for both the 4- and 5-substituents behind the Tyr-31 residue induces some constraints in the overall binding process. Thus the positive hydrophobic interaction is offset by the marked, negative steric interaction, which is denoted by the negative MR_5 term in eq 4e.

Discussion

One of the most important questions in antifolate chemotherapy concerns the tremendous selectivity of the (diaminobenzyl)pyrimidines for bacterial enzymes compared to vertebrate enzymes. Trimethoprim is over 100000 times as potent an inhibitor against *Escherichia coli* DHFR compared with human DHFR.[5,6] A comparison of the unsubstituted benzylpyrimidine (X = H) and TMP shows log $(1/K_i)$ values of 3.94, 5.20 (X = H) and 3.77, 6.88 [X = 3,4,5-$(OCH_3)_3$] for human[6] and *L. casei* DHFR, respectively. The parent analogue is 20 times more active, while TMP is 1300 times more active, against *L. casei* DHFR compared to human Wilz DHFR. What factors determine the differences in substituent effects between the two enzymes?

In our earlier analysis of the problem,[32] it was apparent that binding interactions of the substituents with vertebrate enzymes were mainly hydrophobic in nature while those with bacterial enzyme were steric dispersion. Our later studies have substantiated these results.

Equations 2e and 4e are intricate expressions and would need to be more complex in order to yield a more encompassing analysis of these two types of DHFRs. They serve to illustrate why the design of selective bacterial antifolates with minimal mammalian toxicity is so cumbersome, complex, and difficult. The inhibition of DHFR by benzylpyrimidines constitutes one of the most thoroughly studied QSAR in which all of the state of the art computer, crystallographic, and graphic techniques have been applied.[7,10,33-41] Some of these studies are briefly reviewed.

In a SAR study of rat liver DHFR, Hyde and Roth[33] realized that the hydrophobic interactions of the substituents were of paramount importance while with *E. coli* DHFR only steric factors came into play. Their results confirmed our earlier findings.[32] Recently, Hopfinger et al. have extended his earlier QSAR for the benzylpyrimidine inhibition of bovine liver DHFR to *E. coli* DHFR.[38] Molecular shape analysis indicated that vertebrate DHFR required hydrophobic parameters for rationalization but that bacterial DHFR did not. The simple relationship in eq 5 correlated 18 out of 44 compounds. It

$$\log (1/K_i) = 0.0413V_0 - 0.396 \qquad (5)$$

$$n = 18 \quad r = 0.98$$

seems rather unlikely that a single variable encompassing a number of hidden parameters can effectively handle the

(27) Warshel, A. *Acc. Chem. Res.* **1981**, *14*, 284.
(28) Politzer, P., Trunlar, D., Eds. In *Chemical Applications of Atomic and Molecular Electrostatic Potentials*; Plenum Press: New York, 1981.
(29) Weinstein, R.; Rabinowitz, J.; Liebman, M. N.; Osman, R. *Environ. Health Perspect.* **1985**, *61*, 147.
(30) Weiner, S.; Kollman, P.; Case, D.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 785.
(31) Hansch, C.; Klein, T. E. *Acc. Chem. Res.* **1986**, *19*, 392.
(32) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205.
(33) Hyde, R. M.; Roth, B. In *Strategy in Drug Research*; Buisman, J. A. K., Ed.; Elsevier: Amsterdam, 1982; p 385.
(34) Hopfinger, A. J. *J. Med. Chem.* **1981**, *24*, 818.
(35) Hopfinger, A. J. *J. Med. Chem.* **1983**, *26*, 990.
(36) Motoc, I.; Marshall, G. R. *Z. Naturforsch.* **1985**, *407*, 1114.
(37) Ghose, A. K.; Crippen, G. M. *J. Med. Chem.* **1985**, *28*, 333.
(38) Mabilia, M.; Pearlstein, R. A.; Hopfinger, A. J. *Eur. J. Med. Chem.* **1985**, *20*, 163.
(39) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Fenne, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E. A.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1982**, *25*, 1120.
(40) Simon, Z.; Badilescu, I.; Racovitan, T. J. *J. Theor. Biol.* **1977**, *66*, 485.
(41) Roth, B.; Rauckman, B. S.; Ferone, R.; Baccanari, D. P.; Champness, J. N.; Hyde, R. M. *J. Med. Chem.* **1987**, *30*, 348.

Table IV

X	chicken	*L. casei*	TI[a]	log *P*
3,4,5-$(OCH_3)_3$	3.98	6.88	2.90	−1.55[b]
3,4-(OCH_3),2,4-$OCH_2CH_2OCH_3$	3.64	6.26	2.62	−1.81[b]
3-C_2H_5,4,5-$(CH_2OH)_2$	(3.58)	(6.71)	3.13	−2.23[c]
3-C_2H_5,4-CH_2OH,5-$NHCOCH_3$	(3.46)	(6.82)	3.36	−2.17[c]

[a] Toxicity index. [b] 0.1 N HCl/octanol. [c] Calculated log *P* values.

SAR problem for diversely substituted benzylpyrimidines. Studies in progress of *E. coli* DHFR indicate that although steric factors heavily affect the interactions of the benzylpyrimidines, hydrophobic nuances are quite discernible in the inhibitory process.

Ghose and Crippen[37] in a unique approach combined the data of the inhibition of *E. coli* DHFR by two different sets of antifolates—benzylpyrimidines and triazines. Using distance geometry combined with substituent constants (atomic hydrophobicity, molar refractivity, charge density), they formulated a model that could account for the activity of 30/36 benzylpyrimidines and 19/42 triazines. However, this sophisticated approach entailed the combination of precise data on purified *E. coli* DHFR (K_i values for benzylpyrimidines) and marginal data (I_{50} values for triazines) from crude *E. coli* enzyme preparations. In order to correlate the 49 data points, a large number of disposable parameters were employed: 19 binding sites and 3 physicochemical parameters. It is worthwhile to note that both hydrophobic and steric constants were needed in the final formulation of the mathematical model. Motoc and Marshall[36] have also tackled the benzylpyrimidine–*E. coli* problem by utilizing a three-dimensional, conformation-dependent molecular shape descriptor. In assessing the potencies of 22/36 pyrimidines, they obtained a correlation equation with $r = 0.95$ using two overlap volume descriptors which were akin to MR'_4 and MR'_{35} in our earlier work. No role was found for any hydrophobic interactions. From the work of different groups using four different approaches to this SAR problem, it is apparent that the steric effects of the substituents in the benzylpyrimidine nucleus are of utmost significance for the inhibition of bacterial DHFR.

Utilizing eq 2e and 4e, we have designed two new benzylpyrimidines which we compare in Table IV to trimethoprim and tetroxoprim.

These two hypothetical examples serve to demonstrate the potential utility of multiple-regression equations in guiding drug modification. The new derivatives are predicted to be more selective than the currently used clinical drugs. Equation 2e suggests that they might be slightly more potent against bacterial DHFR in addition to their better selectivity. It is unlikely that such derivatives would have been selected by the traditional methods of drug modification. One of the advantages of the use of substituent constants and regression analysis is that they guide you systematically down new avenues of thought.

In designing new congeners for therapeutic use, one must not minimize the importance of partition coefficients in the overall pharmacokinetics of the drugs. The two new molecules have calculated log *P* values only slightly different from that of tetroxoprim so that it is quite likely that they will distribute in a similar fashion in vivo.

The complexity of obtaining a selective drug is well illustrated by eq 2e and 4e. Even with the QSAR for the two systems, it is not easy to select the best derivatives from the point of view of maximizing potency in one system while minimizing it in another, keeping log *P* in the appropriate range and designing a molecule with a minimal degree of synthetic difficulty. Most medicinal chemists are painfully familiar with such problems, yet decisions on how to move in this complex game are made intuitively. In this case, the constraints of the correlation equations focus one's thinking and allow one to more clearly understand the difficulties.

The crux of the matter in the present case is that in order to make more selective bacterial antifolates, one needs to synthesize unsymmetrical benzylpyrimidines with a lipophilic substituent in the 3-position with $\pi > 1$ and a polar substituent in the 5-position with MR near 0.80. The more polar the para substituent is, the better the selectivity. However, polar groups also tend to reduce antibacterial as well as antiavian potency. It is not surprising, then, that obtaining more effective compounds calls for making unusual derivatives as we have seen before.[42,43] The easy synthetic problems are always the first to be explored; to gain the extra kick needed for a potent, selective inhibitor one must ferret out the small relative differences at the various points of substitution in the parent and maximize the positive aspects while at the same time maintaining proper overall hydrophobicity and avoiding structural changes that leave the derivative vulnerable to metabolic attack. Such multivariate optimization calls for multivariate analysis in order to design a drug that will not be easily displaced by a better derivative.

In making such modifications, it is virtually impossible to exactly obtain the log *P* one would like and still attain all of the other desirable features. It is our belief that when in doubt one should err on the side of hydrophilicity for two reasons. One is that *other factors being equal* microsomes appear to metabolize organic compounds in direct proportion to their log *P*.[44] The second reason is that *other factors being equal* increasing lipophilicity in a series generally increases toxicity.[45] It has been well established that log *P* of about 2 seems ideal for entry into the central nervous system.[46]

A final caveat is that for QSAR, or any other means of predicting biological activity, one cannot expect good results in predicting the activity of derivatives that are beyond explored data space.[47] In the above examples predicted to be more selective than trimethoprim, we have not employed novel substituents but have used substituents previously studied.

In summary, the central theme of this study, that the major difference in the substituent effect of the benzylpyrimidines on bacterial and vertebrate DHFR resides in the dominance of the hydrophobic interaction for the vertebrate enzyme and the dominance of the steric effect with the bacterial DHFR, was evident in our first comparative study.[32] In somewhat different terms it has been confirmed by Hyde and Roth.[48] It is hoped that further testing of eq 2e and 4e will lead to a more precise understanding of why the steric effect seems to be so important for the bacterial DHFR but is not of paramount importance for the vertebrate enzyme.

(42) Hansch, C.; McClarin, J.; Klein, T.; Langridge, R. *Mol. Pharmacol.* **1985**, *27*, 493.
(43) Kim, K. H.; Hansch, C.; Fukunaga, J. Y.; Steller, E. E.; Jow, P. Y. C.; Craig, P. N.; Page, J. *J. Med. Chem.* **1979**, *22*, 366.
(44) Hansch, C. *Drug Metab. Rev.* **1972**, *1*, 1.
(45) Hansch, C.; Dunn, W. J., III. *J. Pharm. Sci.* **1972**, *61*, 1.
(46) Hansch, C. *Drug. Inf. J.* **1984**, *18*, 115.
(47) Hansch, C. In *On the predictive value of QSAR in biological activity and chemical structure*; Buisman, J. A. K., Ed.; Elsevier: Amsterdam, 1977; p 47.
(48) Hyde, R. M.; Roth, B. In *Strategy in drug research*; Buisman, J. A. K., Ed.; Elsevier: Amsterdam, 1982; p 385.
(49) Poe, M.; Ruyle, W. U.S. Patent. 1981, 4,258,045. *Chem. Abstr.* *95*, 810125.

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Nucleoside Peptides. 10. Synthesis and T-Cell Immunostimulatory Properties of Certain Peptide Derivatives of 6-Azacadeguomycin[1]

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Several amino acid and peptide conjugates of 6-azacadeguomycin (6-amino-1-β-D-ribofuranosyl-4,5-dihydro-4-oxopyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid, **2**) have been prepared in good yields, via a two-step procedure involving 1-hydroxybenzotriazole and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride mediated coupling of **2** with an appropriately protected amino acid or peptide, followed by ammonolysis. Thus, condensation of **2** with L-phenylalanine methyl ester, glycine ethyl ester, and L-glutamic acid diethyl ester gave the corresponding protected linear nucleoside peptides (**3**, **5**, and **7**, respectively). Subsequent ammonolysis of **3**, **5**, and **7** furnished L-phenylalanine amide (**4**), glycine amide (**6**) and L-glutamic acid diamide (**8**) conjugates of 6-azacadeguomycin, respectively. Saponification of **7** gave the corresponding L-glutamic acid derivative **9**. A similar coupling of **2** with L-phenylalaninyl-N^t-nitro-L-arginine methyl ester trifluoroacetate and subsequent ammonolysis (after catalytic hydrogenation) gave L-phenylalaninyl-L-arginine amide conjugate (**12**) of 6-azacadeguomycin. Compounds **2**, **4**, **6**, **8**, **9**, and **12** were evaluated for their ability to potentiate T-cell responses to plant mitogens, in comparison with cadeguomycin (**1**). Compounds **4**, **6**, and **9** exhibited an increase in the T-cell proliferation in a dose-dependent manner.

Interest in the nucleoside peptides was rekindled largely due to the recent isolation of several new naturally occurring peptidyl nucleoside antibiotics, e.g. arginomycin,[2] chryscandin,[3] and A201A.[4] The nucleoside and nucleotide peptides isolated from various sources differ markedly in structure and length of nucleotide and peptide chain, as well as in the nature of the peptide linkage.[5-8] Such variance of type of linkage and position of peptide attachment may be correlated with different reactivity and biological function.[5] Certain nucleotide peptides which readily bind to DNA and inhibit nucleic acid synthesis are suggestive of a regulatory function.[9] Gabbay and coworkers[10] have shown that peptides containing aromatic amino acids readily interact with DNA and the aromatic residue of the peptide is partially inserted between base pairs. This intercalation is rather specific and shows an affinity for A:T binding sites.[11] Sequence-specific DNA binding proteins regulate gene expression and also serve structural and catalytic functions in other cellular processes.[12,13] Considerable evidence is now accumulating indicating that various peptides and proteins are linked to certain types of viral DNA[14] and RNA.[15,16] It is of particular interest that certain DNA-binding oligopeptides exhibit remarkable antiviral activity,[17] e.g. netropsin[18] and

(1) For part 9, see: Ramasamy, K.; Robins, R. K.; Revankar, G. R. *Tetrahedron* **1988**, *44*, 1023.
(2) Argoudelis, A. D.; Baczynskyj, L.; Kuo, M. T.; Laborde, A. L.; Sebek, O. K.; Truesdell, S. E.; Shilliday, F. B. *J. Antibiot.* **1987**, *40*, 750.
(3) Yamashita, M.; Tsurumi, Y.; Hosoda, J.; Komori, T.; Kohsaka, M.; Imanaka, H. *J. Antibiot.* **1984**, *37*, 1279.
(4) Kirst, H. A.; Dorman, D. E.; Occolowitz, J. L.; Jones, N. D.; Paschal, J. W.; Hamill, R. L.; Szymanski, E. F. *J. Antibiot.* **1985**, *38*, 575.
(5) Shabarova, Z. A. *Prog. Nucleic Acid Res. Mol. Biol.* **1970**, *10*, 145.
(6) Uramoto, M.; Kobinata, K.; Isono, K.; Higashijima, T.; Miyazawa, T. *Tetrahedron* **1982**, *38*, 1599.
(7) Yamaguchi, H.; Sato, S.; Yoshida, S.; Takada, K.; Itoh, M.; Seto, H.; Otake, N. *J. Antibiot.* **1986**, *39*, 1047.
(8) Castro-Pichel, J.; Garcia-Lopez, M. T.; De las Heras, F. G. *Tetrahedron* **1987**, *43*, 383.
(9) Islam, M. N.; Kay, E. R. M. *Arch. Biochem. Biophys.* **1972**, *150*, 250.
(10) Gabbay, E. J.; Sanford, K.; Baxter, C. S. *Biochemistry* **1972**, *11*, 3429.
(11) Gabbay, E. J.; Sanford, K.; Kapicak, L. *Biochemistry* **1973**, *12*, 4021.
(12) Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem.* **1984**, *53*, 293.
(13) Dervan, P. B. *Science* **1986**, *232*, 464.
(14) Hyman, R. N.; Richards, J. C.; Kudler, L. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 522.
(15) Flanigan, J. B.; Peterson, R. F.; Ambros, V.; Hewlett, M. J.; Baltimore, D. *Proc. Natl. Acad. Sci. U.S.A* **1977**, *74*, 961.
(16) Lee, Y. F.; Nomoto, A.; Detjen, B. M.; Wimmer, E. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 59.
(17) Goodsell, D.; Dickerson, R. E. *J. Med. Chem.* **1986**, *29*, 727.

0022-2623/89/1832-1905$01.50/0