mL), and THF (1 mL) was stirred overnight at 25 °C. The mixture was acidified with 1 N HCl (0.5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over Na_2SO_4 and filtered, and the filtrate was evaporated. The residue was recrystallized from CH_3CN to yield 0.065 g (77%) of **84**, mp 175.5–176 °C. Anal. ($C_{15}H_{14}O_3S$) C, H, S.

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Quantitative Structure–Activity Relationship of 5-(X-Benzyl)-2,4-diaminopyrimidines Inhibiting Bovine Liver Dihydrofolate Reductase

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The inhibitory effect for a set of 23 5-(X-benzyl)-2,4-diaminopyrimidines acting on bovine liver dihydrofolate reductase (DHFR) has led to the following quantitative structure–activity relationship (QSAR): $\log 1/C = 0.62\pi_3 + 0.33\sum\sigma + 4.99$, where $r = 0.931$ and $s = 0.146$. C in this expression is the molar concentration of inhibitor producing 50% inhibition, π_3 is the hydrophobic parameter for substituents on the 3 position of the phenyl moiety, and $\sum\sigma$ is the sum of the Hammett σ constants for the 3, 4, and 5 substituents of the phenyl ring.

The selective inhibition of dihydrofolate reductase (DHFR) continues to be one of the most promising leads for the medicinal chemist seeking means for controlling bacterial and parasitic diseases as well as cancer. One of the most interesting features of this enzyme is the great variation in inhibition by nonclassical inhibitors that one finds with enzyme from different sources. Burchall[1] has shown the variability of the inhibitory power of tri-

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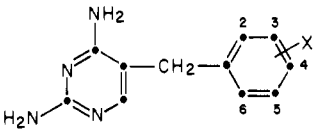
Table I. Inhibition Constants and Physicochemical Parameters Used for Deriving Equations 2 and 3 for Inhibition of Bovine Liver DHFR by Pyrimidines of Type I

no.	X	log $1/C$[a] obsd[b]	log $1/C$[a] calcd[c]	$\lvert\Delta\rvert$	π_3	$\Sigma\sigma$
1	3,4-$(OH)_2$	4.30 (4.25-4.35)	4.52	0.22	-0.62[d]	-0.28
2	4-NH_2	4.57 (4.53-4.61)	4.78	0.21	0.00	-0.66
3	4-$N(CH_3)_2$	4.76 (4.72-4.80)	4.72	0.04	0.00	-0.83
4	4-CH_3	4.80 (4.78-4.82)	4.94	0.14	0.00	-0.17
5	4-OCH_3	4.92 (4.87-4.98)	4.90	0.02	0.00	-0.27
6	4-OCF_3	4.99 (4.97-5.02)	5.11	0.12	0.00	0.35
7	3-OCH_3	5.02 (4.99-5.04)	5.02	0.00	-0.02	0.12
8	4-NO_2	5.02 (4.99-5.05)	5.25	0.23	0.00	0.78
9	4-$NHCOCH_3$	5.09 (5.03-5.15)	4.99	0.10	0.00	0.00
10	4-Cl	5.10 (5.07-5.14)	5.07	0.03	0.00	0.23
11[e]	3,4,5-$(OCH_3)_3$	5.10 (5.05-5.15)	5.00	0.10	-0.02[f]	0.07
12	3,4-$(OCH_3)_2$	5.15 (5.11-5.18)	4.98	0.17	0.04[d]	-0.12
13	3-NO_2, 4-$NHCOCH_3$	5.16 (5.13-5.19)	5.06	0.10	-0.28	0.71
14	4-Br	5.17 (5.13-5.21)	5.07	0.10	0.00	0.23
15	4-F	5.18 (5.15-5.21)	5.01	0.17	0.00	0.06
16	H	5.19 (5.15-5.23)	4.99	0.20	0.00	0.00
17	3-CH_3	5.22 (5.17-5.26)	5.32	0.10	0.56	-0.07
18	3-F	5.33 (5.31-5.35)	5.19	0.14	0.14	0.34
19	3-Cl	5.47 (5.43-5.50)	5.56	0.09	0.71	0.37
20	3-CF_3	5.53 (5.48-5.57)	5.68	0.15	0.88	0.43
21	3-Br	5.54 (5.50-5.58)	5.66	0.12	0.86	0.39
22	3-CF_3, 4-OCH_3	5.79 (5.76-5.81)	5.60	0.19	0.88	0.16
23	3-$OCH_2C_6H_5$	6.10 (6.07-6.12)	6.07	0.03	1.66	0.12

[a] $C = I_{50}$ = molar concentration of inhibitor which causes 50% inhibition of the enzyme. [b] Values in parentheses are 95% confidence intervals for log $1/C$. [c] Calculated using eq 3. [d] $\pi_3 = {}^1/_2\pi_{3,4\text{-}(OR)_2}$, for R = H or CH_3. [e] Trimethoprim. [f] For X = 3,4,5-$(OCH_3)_3$, assumed that $\pi_3 = \pi_{OCH_3}$ and not that $\pi_3 = {}^1/_3\pi_{3,4,5\text{-}(OCH_3)_3}$; i.e., assumed interactive effects of 3-OCH_3 and 5-OCH_3 are to reduce π_4, with π_3 and π_5 essentially unaffected.

methoprim with enzyme from different microorganisms as well as mammalian sources. We have been studying QSAR for a variety of DHFR inhibitors: 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(X-phenyl)-*s*-triazines,[2] 2,4-diaminoquinazolines,[3] and 5-(X-benzyl)-2,4-diaminopyrimidines.[3b] In an analysis of a study by Hitchings et al.[4] on the inhibition of DHFR (*Escherichia coli*) by pyrimidines of type I, we formulated[3b] correlation eq 1. In



I

$$\log 1/C = -1.12\Sigma\sigma_R^+ + 5.54 \quad (1)$$

$$n = 10;\ r = 0.986;\ s = 0.182$$

this expression, C is the molar concentration of inhibitor producing 50% inhibition of enzyme and $\Sigma\sigma_R^+$ is the Taft resonance parameter for the effect of substituents (X) in the 3–5 positions on the electron density in the 2 position of I. Groups increasing the electron density in the 2 position via through resonance increase inhibitory power. X represents multiple substitution with 1 to 3 substituents in the 3–5 positions. For eq 1, n represents the number of data points, r is the correlation coefficient, and s is the standard deviation from the regression.

Although Hitchings et al.[4] tested the pyrimidines used to formulate eq 1 on rat liver enzyme, we were unable to formulate a satisfactory QSAR on these data.[3b] One of the reasons was that there is relatively little spread in the activity of the congeners of I against rat liver enzyme (about 40-fold). Hence, unless quite accurate data are taken, the experimental "noise" tends to obscure the QSAR. We have therefore undertaken the task of extending the work of Hitchings et al. to a larger and better designed set of congeners and to test these on highly purified enzyme.

In this first study of the benzylpyrimidines we have elected to examine the inhibition of bovine liver DHFR and thus assess the structural requirements for activity of this class of inhibitors with a mammalian enzyme. Values for the parameters π, σ, and MR were taken from our previous compilations.[5]

Results and Discussion

We have formulated eq 2 and 3 from the data in Table

$$\log 1/C = 0.677(\pm0.17)\pi_3 + 5.01(\pm0.09) \quad (2)$$

$$n = 23;\ r = 0.874;\ s = 0.190$$

$$\log 1/C = 0.622(\pm0.13)\pi_3 + 0.332(\pm0.18)\Sigma\sigma + 4.99(\pm0.07) \quad (3)$$

$$n = 23;\ r = 0.931;\ s = 0.146$$

I. C in these equations is the molar concentration of inhibitor producing 50% inhibition, π_3 is the hydrophobic constant for substituents in position 3, and $\Sigma\sigma$ represents the summed electronic effect of 3, 4, and 5 substituents on position 1. Attempts to find a role for through resonance electronic effects of the type in eq 1 resulted in poorer correlation, which establishd a completely different electronic effect for the interaction of the benzyl moiety with bovine liver enzyme. Equation 2, showing the importance of hydrophobic interaction of 3 substituents, is highly significant ($F_{1,21} = 67.9$; $F_{1,21(\alpha=0.001)} = 14.6$). Adding a term in $\Sigma\sigma$ as in eq 3 improves the correlation significantly ($F_{1,20} = 15.5$; $F_{1,20(\alpha=0.001)} = 14.8$). However, the electronic effect in eq 3 is opposite to that of eq 1. The positive coefficient with $\Sigma\sigma$ in eq 3 indicates that electron-withdrawing substituents increase inhibitory power. In deriving eq 3 we have used π constants derived from the benzene system[5] and applied them to substituents associated with a benzyl moiety. Since it is known[6] that in different systems π is dependent on σ, the term in $\Sigma\sigma$ of eq 3 might be a correction on π. We feel that this is unlikely because the hydrophobic character of alkyl moieties (in this case, the CH_2 of benzyl) is insensitive to

Table II. 2,4-Diamino-5-(X-benzyl)pyrimidines (I)[a]

no.	X	mp, °C obsd	mp, °C lit.	formula[b]
1[c,d]	3,4-$(OH)_2$	269-271		
2[e]	4-NH_2	224-225 dec		$C_{11}H_{13}N_5$
3	4-$N(CH_3)_2$	240-241	231-235[f]	$C_{13}H_{17}N_5$
4	4-CH_3	202.5-203.5	166-171[f]	$C_{12}H_{14}N_4$
5	4-OCH_3	218.5-219.5	198-202 dec[f]	$C_{12}H_{14}N_4O_1$
6[c]	4-OCF_3	170-171	174-175[g]	
7	3-OCH_3	221-223	219-220[h]	$C_{12}H_{14}N_4O_1$
8[i]	4-NO_2	242-243 dec	238-239[f]	$C_{11}H_{11}N_5O_2$[j]
9[k]	4-$NHCOCH_3$	259.5-260.5		$C_{13}H_{15}N_5O_1$[j]
10	4-Cl	221-223	215-217[l]	$C_{11}H_{11}Cl_1N_4$
11[c]	3,4,5-$(OCH_3)_3$	198.5-200	199[h]	
12	3,4-$(OCH_3)_2$	234.5-237	228-233 dec[f]	$C_{13}H_{16}N_4O_2$
13[m]	3-NO_2, 4-$NHCOCH_3$	198-199		$C_{13}H_{14}N_6O_3$
14	4-Br	233-235		$C_{11}H_{11}Br_1N_4$
15	4-F	220.5-223		$C_{11}H_{11}F_1N_4$
16	H	199-200	196[l]	$C_{11}H_{12}N_4$
17	3-CH_3	192-193		$C_{12}H_{14}N_4$
18[c]	3-F	236.5-238 dec		$C_{11}H_{11}F_1N_4$
19[c]	3-Cl	228-231.5 dec		$C_{11}H_{11}Cl_1N_4$
20[c]	3-CF_3	164.5-165	163-166[g]	
21[c]	3-Br	206.5-209 dec		$C_{11}H_{11}Br_1N_4$
22[c]	3-CF_3, 4-OCH_3	194-195	198-200[g]	
23[c]	3-$OCH_2C_6H_5$	156.5-157.5 dec		$C_{18}H_{18}N_4O_1$

[a] Prepared by method A, unless otherwise indicated. [b] Analyzed for C and H, unless otherwise indicated. [c] See acknowledgment for source. [d] Hydrochloride monohydrate. [e] Prepared by method C. [f] Reference 10. [g] Reference 11. [h] Reference 12. [i] Prepared by method B. [j] Analyzed for C, H, and N. [k] Prepared by method D. [l] Reference 13. [m] Prepared by method E.

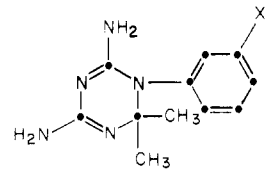
σ effects.[7] The role of $\sum\sigma$ in eq 3 is small in any case, since it accounts for only a 10% reduction in the variance of log $1/C$ compared to 76% for π_3. Lack of orthogonality between π_3 and $\sum\sigma$ is not a problem, since $r_{\pi_3,\sum\sigma}{}^2 = 0.05$ for this data set. Neither $\sum\pi_{3-5}$ nor $\sum MR_{3-5}$ were of significant value in correlating the inhibiting effect of the benzylpyrimidines. In the formulation of eq 3 we have assigned π values of 0.0 to substituents in the 4 and 5 positions; this suggests that, at least for the present data, they do not contact the enzyme in a meaningful way. If 4 substituents are parameterized by adding a π_4 or MR_4 term to eq 3, a slight improvement in correlation is found ($r = 0.935$ and $s = 0.145$ for MR_4; $r = 0.932$ and $s = 0.149$ for π_4). However, neither addition is significant statistically. It is likely that with larger substituents in position 4 a role could be found for MR_4 or π_4.

Equation 3 can be compared with eq 4, which we have

$$\log 1/C = 1.05\pi_3 - 1.21 \log(\beta \times 10^{\pi_3} + 1) + 6.64 \quad (4)$$

$n = 28$; $r = 0.955$; $s = 0.210$; $\pi_0 = 1.56$; $\log \beta = -0.736$

found for inhibitors of type II acting on bovine liver



II

DHFR.[2c] Equation 4 is based on Kubinyi's bilinear model,[8] which gives a better fit of the data than the parabolic model (i.e., $\pi + \pi^2$). The coefficient of π_3 in eq 4 is considerably larger than that of π_3 in eq 3. Apparently, X in II fits into the hydrophobic pocket of the enzyme better than 3-X in I. Equation 4 does not contain a term in σ, although adding a term in σ to eq 4 marginally improves it. The coefficient with such a σ term is positive, showing that for congeners of type II electron withdrawal slightly increases inhibitory power. Comparison of the intercepts of eq 3 and 4 indicates that II is intrinsically a more potent class of inhibitors of the bovine enzyme than I.

We are now undertaking a study of the inhibitory effect of I on bacterial DHFR in order to provide a direct comparison of our set of benzylpyrimidines with those upon which eq 3 is based.

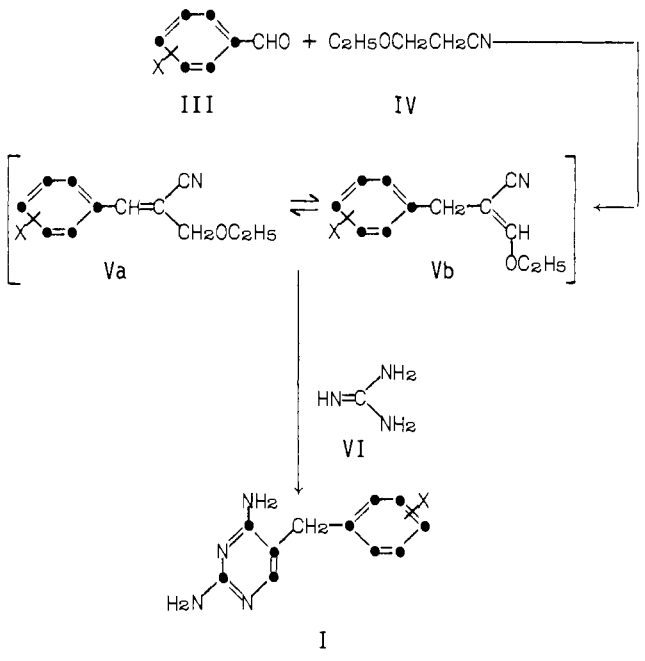
Experimental Procedures

Synthesis of Pyrimidine Inhibitors. Most of the 2,4-diamino-5-(X-benzyl)pyrimidines I were prepared by the general synthetic procedure of Stenbuck et al.:[9] the appropriately substituted benzaldehyde III was condensed with β-ethoxypropionitrile (IV) using NaOEt; distillation provided a mixture of the crude benzal nitriles Va and Vb, which were reacted with guanidine (VI) to provide the desired pyrimidines I; see method A below and Scheme I. Compounds 8, 2, 9, and 13 (Table II) were prepared by sequential nitration, reduction, acetylation, and nitration of compound 16 (Table II); see methods B–E below.

Melting points (Buchi capillary apparatus) are uncorrected. Microanalyses were performed by C. F. Geiger, Ontario, Calif., and are within ±0.4% of the theoretical values. TLC (precoated qualitative silica gel or alumina plates; UV visualization) was routinely used to check the purity of the benzal nitriles Va and Vb and pyrimidines I and to analyze column chromatography elutent fractions. ^1H NMR spectra (Varian Model A-60 spectrometer; Me_2SO-d_6 with Me_4Si as internal standard) of compounds 2, 4, 5, 8, and 13 (Table II) were consistent with the assigned structures.

Method A. To 50 mmol of Na metal in 100 mL anhydrous EtOH there were added 100 mmol of the benzaldehyde III and 100 mmol of β-ethoxypropionitrile (IV). The mixture was refluxed for 2–5.5 h, removing about 40 mL of distillate per hour with addition to the reaction mixture of an equal volume of anhydrous EtOH. The solvent was removed and the resulting oil partitioned between H_2O and ether. The organic phase was washed with saturated aqueous sodium bisulfite (until a clear aqueous phase was obtained) and then with H_2O and was then dried (Na_2SO_4). The solvent was removed and the resulting oil vacuum distilled to give a mixture of Va and Vb, which sometimes partially crystallized. The first distillation fraction was discarded, and subsequent fractions were tested with 2,4-dinitrophenylhydrazine and inspected by TLC for purity. Boiling point ranges were very broad (usually a 20 °C range due to the Va + Vb mixture),

Scheme I



normally between 90 and 180 °C at 1–3 mmHg. The yield of crude Va + Vb was 35–70%.

To 300 mmol of Na metal in 125 mL of anhydrous MeOH there was added 300 mmol of guanidine hydrochloride (VI·HCl) in 75 mL of anhydrous MeOH. NaCl was removed by filtration and the filtrate diluted to 210 mL with anhydrous MeOH.

Crude Va + Vb, 100 mmol, and methanolic guanidine solution, 140–210 mL (200–300 mmol of VI), were refluxed for 7.5–44 h. After cooling the mixture, the solvent was removed to give an oil (which was dissolved in $CHCl_3$ and washed with H_2O; evaporation of the $CHCl_3$ provided the crude I) or a solid residue (which was washed with H_2O, MeOH, or ether and then recrystallized from MeOH or MeOH/H_2O to give the crude I), 12–78% crude I from the crude Va + Vb. The crude I was purified by column chromatography (Alumina), eluting with $CHCl_3$ and MeOH. Evaporation of solvent from the appropriate elutent fractions provided the purified I, 5–24% yield from the crude Va + Vb, 3–17% overall yield from the benzaldehyde III.

Method B. To 269 mmol of 16 (Table II) dissolved in 410 mL concentrated H_2SO_4 and cooled to 0 °C there was added 269 mmol of finely powdered KNO_3 in 1 h, keeping the temperature below 5 °C. After allowing to warm to room temperature, the reaction mixture was heated to 50 °C for 1 h, let cool to room temperature, poured over 1100 g of ice, stored at 5 °C for 3 days, and filtered to give the sulfate salt of 8 (Table II). This was suspended in 320 mL of 20% aqueous NH_3, stirred for 30 min, filtered, washed with H_2O, and then dissolved in 950 mL of 8.5 N HOAc. After filtering to remove insoluble material, the pH was adjusted to 6 with 20% NaOH. After storing the mixture at 5 °C overnight, the crystalline product was collected by filtration and recrystallized from EtOH/H_2O, 52% yield.

Method C. 8, 23.2 mmol (Table II), suspended in 100 mL of EtOH, 100 mL of H_2O, and 25 mL of concentrated HCl was hydrogenated (Parr hydrogenation apparatus) at room temperature with 750 mg of 10% Pd/C. The catalyst was removed by filtration and the filtrate reduced in volume to 100 mL by rotary evaporation. Adjusting the pH to 10 with 10% aqueous NaOH and then filtering gave a solid which was dissolved in 350 mL of hot MeOH and then passed over a short alumina column. After evaporation of the solvent, the solid residue was crystallized from MeOH, decolorizing with carbon, 58% yield.

Method D. To 41.8 mmol of 2 (Table II) suspended in 85 mL of H_2O there was added alternately, with stirring, in 1 h 68 mL (721 mmol) of Ac_2O and enough dilute aqueous NH_3 to maintain the pH at 8.0. After adjusting the pH to 9 with dilute aqueous NH_3, the mixture was cooled and filtered. The crude product was recrystallized from boiling H_2O, decolorizing with carbon, 86% yield.

Method E. 9, 1.94 mmol (Table II), dissolved in 5 mL of concentrated H_2SO_4, and KNO_3, 1.98 mmol, were reacted and the reaction then worked up as per method B, except the heating to 50 °C was omitted, 34% yield. 13 can also be prepared by reacting 9 (1.94 mmol) with 4 mL of concentrated HNO_3 (sp gr 1.45–1.50) at 20 °C for 1 h, with subsequent workup as in method B, 88–94% crude yield, but purity lower than with the above procedure.

Inhibition Assay. Assays were performed as described in our previous study:[2c] 1.40×10^{-5} M dihydrofolic acid and 1.00×10^{-4} M NADPH in 100 mM phosphate buffer, pH 6.25, and 0.05 M in 2-mercaptoethanol at 25 °C for the final assay solution. Inhibitor samples were prepared by dissolving in Me_2SO and then diluting with buffer, such that [Me_2SO] in final assay solution was 0–0.18%, v/v. Me_2SO was not found to have any inhibitory effects on the bovine liver DHFR in this concentration range.

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