of **5b** as green fine crystals; mp 227.6 °C dec; IR ν_{max} 3300 (NH), 3085 (aromatic CH), 1599 (C=N), 1568, 1553, 1541, 1518, 1488, and 1450 (aromatic C=C), 1270 and 1233 (CN). Anal. ($C_{20}H_{12}Cl_6N_4$) C, H, N.

2,4-Dichlorophenylglyoxal Bis(2,5-Dichlorophenylhydrazone) (5c). By the method used to synthesize **5a**, 4.27 g (0.22 mol) of 2,5-dichlorophenylhydrazine hydrochloride and 5.16 g (0.02 mol) of 2,2,2',4'-tetrachloroacetophenone gave, after recrystallizing from benzene–Skellysolve B, 2.24 g (27% of theory) of **5c** as orange fine crystals: mp 208.2 °C; IR ν_{max} 3350 and 3355 (NH), 3100 (aromatic CH), 1590 (C=N), 1547, 1523, 1500, 1474, and 1457 (C=C stretch), 1264, 1255, and 1241 (CN). Anal. ($C_{20}H_{12}Cl_6N_4$) C, H, N.

***m*-Chlorobenzoyl Chloride 2,4,6-Trichlorophenylhydrazone (1ss).** To a suspension of 11.52 g (0.05 mol) of *m*-chlorobenzaldehyde phenylhydrazone in 300 mL of glacial HOAc at ambient temperature was added 11.7 mL (0.25 mol) of Cl_2 with stirring. An exothermic reaction ensued. The mixture was cooled to 20 °C, stirred for 0.5 h, and then filtered. The crude product was crystallized from Skellysolve B to afford 13.4 g (73% of theory) of **1ss** as white crystals, mp 127–128 °C. Anal. ($C_{13}H_7Cl_5N_2$) C, H, Cl, N.

***p*-Toluoyl Chloride Phenylhydrazone (1a).** To 5.89 g (0.026 mol) of *p*-toluic acid phenylhydrazide in 100 mL of CCl_4 was added 6.25 g (0.03 mol) phosphorus pentachloride. The mixture was cautiously heated to reflux. After 2 h, the mixture was cooled and then chilled in an ice bath. To this mixture was added dropwise 13.18 g (0.14 mol) of phenol in 60 mL of CCl_4 with stirring over 0.5 h. Stirring was continued at 0 °C for 0.75 h, and then the mixture was concentrated in vacuo to dryness. The residue was suspended in cold (0 °C) MeOH and filtered. The crude product was dissolved in 25 mL of hot CH_2Cl_2, diluted with 25 mL of Skellysolve B, and concentrated to 30 mL total volume. Cooling gave yellow plates: yield 4.36 g (68% of theory); mp 134–136 °C. Anal. ($C_{14}H_{13}ClN_2$) C, H, Cl, N.

Anthelmintic Evaluations. The various compounds were evaluated for preliminary anthelmintic activity in mice and, if active, also in dogs and sheep.

Mice naturally parasitized with *Syphacia obvelata* were experimentally infected with *Nematospiroides dubius* and *Hymenolepis nana*. After a prepatency period of 2 weeks, the mice were allotted to treated and control groups. The compounds were formulated for intraperitoneal and oral treatments by grinding the material with a mortar and pestle and then suspending the finely ground material in a sterile aqueous vehicle consisting of 0.10% carboxymethylcellulose, 0.04% polysorbate 80, and 0.0042% polyparaben. The treatment(s) was then administered to the animals on a milligram per kilogram of body weight basis. Each suspension 0.1 mL, was orally administered via cannula or intraperitoneally administered via a 20-gauge hypodermic needle; each mouse received the treatment once a day for 4 days. Two days after the final treatment, all of the mice were necropsied and examined for the presence or absence of the parasites. Ratios were developed for the treated and control mice based on the absence of the three parasites. Activity was then determined by comparing the ratios of the treated mice and the nontreated controls. The percent clearance was found by subtracting the percent clearance, if any, of the controls from the apparent percent clearance of the treatment groups. A compound found to give 50% or greater clearance of one or more of the mouse parasites was further evaluated for activity in dogs and/or lambs.

Compounds were evaluated in naturally parasitized lambs. Anthelmintic activity was determined by the clinical test method (i.e., significant decrease in posttreatment helminth egg counts). The McMaster technique was used to determine the number of eggs per gram (EPG) of fecal material from each animal. Three pretreatment and three posttreatment egg count determinations were made on each animal. One animal was used for each compound and/or for each dosage regimen for each compound.

Each compound was finely ground with a mortar and pestle and then suspended by sonication in approximately 50 mL of the sterile vehicle as described previously. The suspension was administered orally using a stomach tube. Alternatively, the finely ground test compound was weighed and placed in gelatin capsules. The capsules were administered orally via a balling gun. Compounds active against one or more of the parasites of mice were also evaluated against hookworms and ascarids of dogs. Dogs naturally infected with *Toxascaris leonina* (ascarids) were experimentally infected with a mixed culture of *Ancylostoma caninum* and *Uncinaria stenocephala* larva. Test compounds were finely ground, weighed, and placed in gelatin capsules for oral administration. Three pre- and three posttreatment egg count determinations were made during the course of each experiment. A significant decrease in the posttreatment egg counts was the criteria used to determine activity.

Acknowledgment. We thank N. H. Knight and associates of The Upjohn Co. for performing the elemental analyses.

Supplementary Material Available: Mouse anthelmintic data for phenylhydrazone series **2**, **3**, and **4** are listed (3 pages). Ordering information is given on any current masthead page.

Quantitative Structure–Selectivity Relationships. Comparison of the Inhibition of *Escherichia coli* and Bovine Liver Dihydrofolate Reductase by 5-(Substituted-benzyl)-2,4-diaminopyrimidines

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In our previous publication (Blaney, J. M.; Dietrich, S. W.; Reynolds, M. A.; Hansch, C. *J. Med. Chem.* **1979**, *22*, 614), correlation equations were presented for the inhibition of bovine liver and *Escherichia coli* dihydrofolate reductase (DHFR) by 5-(substituted benzyl)-2,4-diaminopyrimidines. These equations brought out differences in the way these two enzymes interact with substituents, which explain the high selectivity of drugs like trimethoprim. We have tested and further developed these equations in this report. It is of particular interest that our previously published correlation equation for *E. coli* DHFR accurately predicted the potency of a commercial competitor of trimethoprim (tetroxoprim) now in clinical use. We believe that new and effective competitors for trimethoprim can be designed by means of the two correlation equations.

In our recent study[2,3] of the inhibition of DHFR from bovine liver and *E. coli* by benzylpyrimidines I, we formulated the quantitative structure–activity relationships (QSAR) of eq 1 and 2. *C* in these equations is the molar

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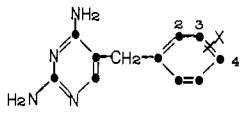


I

Bovine DHFR

$$\log (1/C) = 0.62\ (\pm 0.13)\ \pi_3 + 0.33\ (\pm 0.18)\ \Sigma\sigma + 4.99 \quad (1)$$

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

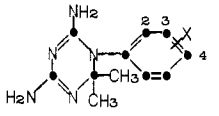
E. coli DHFR

$$\log (1/C) = 1.38\ (\pm 0.30)\ MR'_{3,5} + 0.82\ (\pm 0.35) MR'_4 + 5.77 \quad (2)$$

$$n = 23;\ r = 0.918;\ s = 0.250$$

concentration of inhibitor causing 50% inhibition, the figures in parentheses are for construction of the 95% confidence intervals, n represents the number of data points, r is the correlation coefficient, and s is the standard deviation from the regression. X of I represents mono-, di-, and trisubstitution. In the bovine equation, π_3 is the hydrophobic constant,[4] and the subscript indicates that this applies only to substituents in position 3 of the benzyl moiety. For substituents in the 4 and 5 positions, π is set equal to 0. The Hammet σ constants[4] are selected with respect to their effect on the point of CH_2 attachment and $\Sigma\sigma$ represents the sum of σ for all substituents.

Equation 1 was a surprise to us because it indicated that 4-substituents do not engage in hydrophobic interaction with the DHFR. Since triazines II have a pronounced



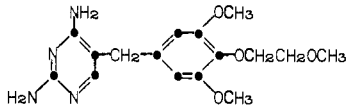
II

dependence of inhibition on 4-substituents,[5] it seemed likely that one could expect the same for I.

Equation 2, for the inhibition of *E. coli* DHFR, is completely different from eq 1 for the bovine enzyme. Binding by X to the *E. coli* enzyme does not appear to be mediated by a typical hydrophobic interaction modeled by π. Instead, molar refractivity[4] is the parameter best suited for correlation. The subscripts in eq 2 indicate the position of attachment of X, and MR′ has a severe limitation. The value of MR′ (scaled by 0.1) is limited to 0.79 at each of the positions 3, 4, and 5. Hence, insofar as we could tell for 23 data points, the most active possible compounds would have log $(1/C) = 8.59$ (i.e., $1.38 \times 2 \times 0.79 + 0.82 \times 0.78 + 5.77$). These results lead us to assume that groups with larger (assuming MR to be primarily related to molar volume) values of MR than OCH_3 (MR = 0.79) extend beyond the enzyme and that part of the substituent extending beyond the enzyme would have no inhibitory action.

The two correlation equations clearly bring out differences in enzymatic space in a mammalian and bacterial enzyme. It is the difference that makes trimethoprim [I, X = 3,4,5-$(OCH_3)_3$] such a selective and valuable drug.

In order to firm up eq 1 and 2 and to assess their predictive value, we have made 12 new congeners of I. In addition, we have tested a new variation of I, tetroxoprim (III), now used clinically in Europe.



III

In selecting an additional set of substituents to study the predictive value of eq 1 and 2, as well as to map more of the substituent binding space on the two different enzymes, there are a number of problems that had to be considered. Our main concern in formulating eq 1 and 2 was to establish the importance or lack of importance of the electronic effect of substituents.[2] In checking out the electronic effects we decided to employ mostly small substituents to minimize steric problems. Our finding that σ is only of marginal importance in eq 1 and of no value for eq 2 allows us to neglect this parameter in the present study. There are a number of difficulties in selecting 13 new congeners (1–13 in Table I). In the first place, many benzylpyrimidines are hard to make and purify. At best, yields are low. This common constraint in drug modification restricted the variety of substituents we would like to have had; another constraint was the need to obtain derivatives which would, insofar as possible, validate and extend two quite different equations—one heavily dependent on π, the other having a specialized dependence on MR. Still lacking much feeling for steric constraints, we decided to concentrate on variations primarily at the 3 position, since this appeared to be essential to obtain good variance in K_i for eq 1. The new set of $\pi_{3,5}$ values ranges from −2.06 to 3.79, almost six powers of 10, which gives us a good test and extension of eq 1. Of course this variation in π is of importance in confirming the lack of importance of hydrophobic effects in eq 2.

The role of MR in eq 2 is unusual in our experience. Substituents with MR > 0.79/position appeared to make no contribution to inhibitory potency for that part of the substituent larger than 0.79. Congeners **7** and **9–13** now test this point more fully, since they have MR values much greater than 0.79.

Only two new derivatives were tested which check the role of large substituents in position 4. Compound **3**, the clinically important tetroxoprim, has a large MR value, as does compound **8**. The π value for 4-$OCH_2CH_2OCH_3$ in compound **3** is low, but the π value for compound **8** [4-$O(CH_2)_3CH_3$] is rather high.

Results and Discussion

***E. coli* DHFR QSAR.** From the data in Table I we have refit our old data and the new data to eq 2 to obtain eq 3. The parameters of eq 3 are quite close to those of

$$\log (1/K_{i\,app}) = 1.33\ (\pm 0.24)\ MR'_{3,5} + 0.94\ (\pm 0.31)\ MR'_4 + 5.69\ (\pm 0.24) \quad (3)$$

$$n = 34;\ r = 0.904;\ s = 0.281$$

eq 2, indicating the predictive value of eq 2 except for two points, 3,5-$(CH_2OH)_2$ and 3-$O(CH_2)_7CH_3$ (1 and **9**, respectively, Table I), which were so badly fit they were not used to derive eq 3. Log $(1/K_{i\,app})$ is equivalent to log $(1/C)$ in this equation. The most important variable in eq 3 is $MR'_{3,5}$; $F_{1,32}$ for this term is 47.3. The addition of MR'_4

(1) Visiting Professor from Beijing Medical College, Beijing, China.
(2) Blaney, J. M.; Dietrich, S. W.; Reynolds, M. A.; Hansch, C. *J. Med. Chem.* **1979**, *22*, 614.
(3) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205.
(4) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979.
(5) Hansch, C.; Dietrich, S. W.; Fukunaga, J., unpublished results.

Table I. Parameters Used in the Derivation of Equations 3-8

		log $(1/K_{i\,app})$												
		bovine			*E. coli*									
no.	X	obsd	calcd[a]	|Δ|	obsd	calcd[b]	|Δ|	$\pi_{3,5}$	$\Sigma\sigma$	I	MR'$_{3,5}$	MR$_{3,5}$	MR'$_4$	MR$_4$
1	3,5-$(CH_2OH)_2$	4.30[c] ± 0.04[d]	4.48	0.18	5.31[c] ± 0.03	7.70	2.39	−2.06	0.00	1	1.44	1.44	0.10	0.10
2	3-OH	4.88[c] ± 0.04[d]	5.27	0.39	6.47 ± 0.03	6.30	0.17	−0.67	0.12	1	0.39	0.39	0.10	0.10
3	3,5-$(OCH_3)_2$, 4-$O(CH_2)_2OCH_3$	4.88[c] ± 0.03[d]	5.73	0.85	8.35 ± 0.08	8.52	0.17	−0.04	0.00[e]	1	1.57	1.57	0.79	1.93
4	3-CH_2OH	5.20 ± 0.04[d]	5.04	0.16	6.28 ± 0.03	6.88	0.60	−1.03	0.00	1	0.82	0.82	0.10	0.10
5	3-CH_2OCH_3	5.49 ± 0.03[d]	5.18	0.31	6.59 ± 0.03	6.97	0.38	−0.78	0.02	1	0.89	1.31	0.10	0.10
6	3,5-$(OCH_3)_2$	5.51 ± 0.04[d]	5.71	0.20	8.38 ± 0.08	7.88	0.50	0.08	0.24	1	1.57	1.57	0.10	0.10
7	3-OSO_2CH_3	5.58 ± 0.03[d]	5.23	0.35	6.92 ± 0.03	6.97	0.05	−0.88	0.39	1	0.89	1.80	0.10	0.10
8	4-$O(CH_2)_3CH_3$	5.74 ± 0.04[d]	5.50	0.24	6.89 ± 0.03	6.71	0.18	0.00	−0.32	1	0.21	0.21	0.79	2.17
9	3-$O(CH_2)_7CH_3$	5.78 ± 0.04[d]	5.77	0.01	6.25[c] ± 0.04	6.97	0.72	3.79	0.10	1	0.89	4.07	0.10	0.10
10	3-$CH_2O(CH_2)_3CH_3$	5.86 ± 0.03[d]	6.04	0.18	6.55 ± 0.03	6.97	0.42	0.84	0.02	1	0.89	2.71	0.10	0.10
11	3-I	6.15 ± 0.02[d]	6.28	0.13	7.23 ± 0.04	6.97	0.26	1.12	0.35	1	0.89	1.50	0.10	0.10
12	3-$O(CH_2)_5CH_3$	6.39 ± 0.04[d]	6.43	0.04	6.86 ± 0.03	6.97	0.11	2.67	0.10	1	0.89	3.17	0.10	0.10
13	3-$O(CH_2)_3CH_3$	6.48 ± 0.03[d]	6.38	0.10	6.82 ± 0.03	6.97	0.15	1.55	0.10	1	0.89	2.27	0.10	0.10
14	3,4-$(OH)_2$	4.30 ± 0.05	4.53	0.23	6.46 ± 0.07	6.48	0.02	−0.67	−0.28	0	0.39	0.39	0.29	0.29
15	4-NH_2	4.57 ± 0.04	4.78	0.21	6.30 ± 0.01	6.48	0.18	0.00	−0.66	0	0.21	0.21	0.54	0.54
16	4-$N(CH_3)_2$	4.76 ± 0.04	4.73	0.03	6.78 ± 0.03	6.71	0.07	0.00	−0.83	0	0.21	0.21	0.79	1.56
17	4-CH_3	4.80 ± 0.02	4.92	0.12	6.48 ± 0.02	6.51	0.03	0.00	−0.17	0	0.21	0.21	0.57	0.57
18	4-OCH_3	4.92 ± 0.05	4.89	0.03	6.82 ± 0.02	6.71	0.11	0.00	−0.27	0	0.21	0.21	0.79	0.79
19	3-OCH_3	5.02 ± 0.03	5.00	0.02	6.93 ± 0.02	6.97	0.04	−0.02	0.12	0	0.89	0.89	0.10	0.10
20	4-NO_2	5.02 ± 0.03	5.20	0.18	6.20 ± 0.06	6.66	0.46	0.00	0.78	0	0.21	0.21	0.74	0.74
21	4-Cl	5.10 ± 0.03	5.04	0.06	6.45 ± 0.01	6.53	0.08	0.00	0.23	0	0.21	0.21	0.60	0.60
22	3,4-$(OCH_3)_2$	5.15 ± 0.04	4.96	0.19	7.72 ± 0.07	7.62	0.10	0.04	−0.12	0	0.89	0.89	0.79	0.79
23	3-NO_2, 4-$NHCOCH_3$	5.16 ± 0.03	5.03	0.13	6.97 ± 0.02	7.55	0.58	−0.28	0.71	0	0.84	0.84	0.79	1.49
24	4-Br	5.17 ± 0.04	5.04	0.13	6.82 ± 0.01	6.71	0.11	0.00	0.23	0	0.21	0.21	0.79	0.89
25	4-F	5.18 ± 0.03	4.99	0.19	6.35 ± 0.03	6.05	0.30	0.00	0.06	0	0.21	0.21	0.09	0.09
26	4-OCF_3	5.42 ± 0.03[d]	5.70	0.28	6.57 ± 0.01	6.71	0.14	0.00	0.35	1	0.21	0.21	0.79	0.79
27	3-Cl	5.47 ± 0.04	5.46	0.01	6.65 ± 0.00	6.73	0.08	0.71	0.37	0	0.71	0.71	0.10	0.10
28	3,4,5-$(OCH_3)_3$	5.51 ± 0.02[d]	5.59	0.08	8.87 ± 0.05	8.52	0.35	−0.04	0.07	1	1.57	1.57	0.79	0.79
29	3-CF_3	5.53 ± 0.05	5.56	0.03	7.02 ± 0.01	6.60	0.42	0.88	0.43	0	0.61	0.61	0.10	0.10
30	3-Br	5.54 ± 0.04	5.54	0.00	6.96 ± 0.03	6.97	0.01	0.86	0.39	0	0.89	0.99	0.10	0.10
31	H	5.67 ± 0.02[d]	5.59	0.08	6.18 ± 0.05	6.07	0.11	0.00	0.00	1	0.21	0.21	0.10	0.10
32	3-F	5.67 ± 0.02[d]	5.77	0.10	6.23 ± 0.03	6.05	0.18	0.14	0.34	1	0.20	0.20	0.10	0.10
33	3-CH_3	5.71 ± 0.02[d]	5.87	0.16	6.70 ± 0.02	6.68	0.02	0.56	−0.07	1	0.67	0.67	0.10	0.10
34	4-$NHCOCH_3$	5.83 ± 0.02[d]	5.59	0.24	6.89 ± 0.00	6.71	0.18	0.00	0.00	1	0.21	0.21	0.79	1.49
35	3-CF_3, 4-OCH_3	6.27 ± 0.02[d]	6.10	0.17	7.69 ± 0.08	7.24	0.45	0.88	0.16	1	0.61	0.61	0.79	0.79
36	3-$OCH_2C_6H_5$	6.53 ± 0.03[d]	6.43	0.10	6.99 ± 0.05	6.97	0.02	1.66	0.12	1	0.89	3.28	0.10	0.10

[a] Calculated using eq 7. [b] Calculated using eq 3. [c] These points not used in the formulation of eq 3 or 7 as the case may be. [d] Tested at pH 7.2. [e] Estimated value.

results in a significant improvement: $F_{1,31} = 37.2$. The $(CH_2OH)_2$ analogue is of particular interest because it is so very badly mispredicted. We expected this congener to be about as active as 3,5-$(OCH_3)_2$, since the two compounds have essentially the same MR′ values. The reason for the poor fit is not clear. The most likely bet is that the high hydrophilicity of these two substituents holds the molecule in the aqueous phase and hinders normal binding. The 3-CH_2OH congener is also poorly fit, although much less so. The OH functions of the CH_2OH units may also interact in some way with enzymatic space to produce the deleterious effect. Simple polarity alone is not the problem, since NH_2, OH, OSO_2CH_3, and $NHCOCH_3$ attached directly to the benzene ring are well fit. We are now attempting to make a wider range of derivatives containing more polar groups and hydrogen bonding groups in an attempt to better understand the behavior of the 3,5-$(CH_2OH)_2$ congener.

Another poorly fit molecule is the 3-$O(CH_2)_7CH_3$ analogue. This compound is about five times less active than expected. Shorter side chains, even 3-$O(CH_2)_5CH_3$ (compound **12**), are well fit. It is possible that such a long side chain as octyloxy may at first not contact the enzyme but is long enough to reach an area of contact in such a way as to weaken binding by the pyrimidine moiety. This would not have to be a strong interaction; a binding energy of approximately 1 kcal would produce a change in K_i of a factor of 5.

Including all points in the regression analysis produces eq 4. The single-variable equation in $MR'_{3,5}$ is quite

$$\log (1/K_{i\,app}) = 1.06\ (\pm 0.40)\ MR'_{3,5} + 1.07\ (\pm 0.53)\ MR'_4 + 5.75\ (\pm 0.40) \quad (4)$$

$$n = 36;\ r = 0.721;\ s = 0.484$$

significant with $F_{1,34} = 13.2$ and $F_{1,33} = 16.6$ for the additional term in MR'_4. Of real interest is the fact that compound **7**, tetroxoprim, a compound of great clinical importance, is well predicted. Of the 13 new compounds tested, four (**4**–**6** and **9**) are misfit by more than the standard deviation of eq 3. Only compound **1** is seriously mispredicted by a factor of 250. The next most serious failure is compound **9**, which is off by a factor of 5. $1/K_{i\,app}$ values have a range of 3400-fold.

In our first study of the benzylpyrimidines we were primarily concerned with electronic effects of substituents; hence, we did not test many large groups. More large groups have been included in this set and we are impressed that groups in the 4 position as large as $OCH_2CH_2OCH_3$ and $OCH_2CH_2CH_2CH_3$ are well fit, showing that the last three carbon atoms do not appear to contact the enzyme.

Rather small groups (OCH_3 or smaller) or the equivalent portion of a larger group contact the enzyme, but not in a typical hydrophobic manner. It may be that by a kind of "breathing" action DHFR opens up to accommodate substituents in the 3, 4, and 5 positions of the benzyl moiety. These trapped fragments would tend to distort the enzyme and hold the ligand and enzyme together. There is now abundant evidence of the flexibility of enzymes.[6]

As we have noted before, correlation equations fail sooner or later as greater and greater structural changes are made; however, it is at these points of failure that the next advances in understanding the overall SAR are most likely to be made.

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Bovine Liver DHFR QSAR. Equations 5–8 for the inhibition of bovine DHFR by benzylpyrimidines, when compared with eq 3, bring out the differences in the character of enzymatic space in the region of the active sites of the two enzymes. One data point (no. 3) has been omitted in the formulation of eq 5–8. If this is included, we obtain essentially the same correlation parameters, except that r is lower and s higher than for eq 8 [i.e., $0.54\pi_{3,5} + 0.31\sum\sigma + 0.58I - 1.36 \log (\beta \cdot 10^{\pi_{3,5}} + 1) + 4.97$; $r = 0.929$, $s = 0.224$]. Tetroxoprim is 7 times less inhibitory than eq 8 predicts. There are some significant differences between eq 8 and eq 1. In eq 8, π for both 3- and 5-substituents is included in $\pi_{3,5}$. In our previous study, only one congener was present containing a 5-substituent. We now have four such examples (**1**, **3**, **6**, and **28**) in Table I. The largest π value in our earlier study for a 3-substituent was that of 1.66 for $OCH_2C_6H_5$. We have now made some much more lipophilic derivatives, especially 3-OC_6H_{17} and 3-OC_6H_{13}, which can be well fit using the bilinear model and which allows us to define π_0 as 2.2 for the bovine enzyme. In our earlier study[5] of inhibitors (II) reacting with bovine DHFR, we found π_0 for 3-substituents to be 1.6, which would *suggest* that the 3-substituents of I and II are more or less binding in the same fashion to the bovine hydrophobic pocket. However, the correlation equation for 3-X-II binding to bovine DHFR was found as shown in eq 9. The initial slope of 1.05 in this equation is much greater than that of 0.54 for eq 7 or 8, which definitely shows that a different type of hydrophobic effect is involved in each case. Moreover, 4-substituents of II show a strong hydrophobic effect (unpublished results), while 4-groups show no such interaction for I. No doubt quite different binding by congeners I and II is occurring with the bovine enzyme.

$$\log (1/K_{i\,app}) = 0.36\ (\pm 0.14)\ \pi_{3,5} + 5.32\ (\pm 0.15) \quad (5)$$

$$n = 35;\ r = 0.670;\ s = 0.424;\ F_{1,33} = 26.8$$

$$\log (1/K_{i\,app}) = 0.32\ (\pm 0.11)\ \pi_{3,5} + 0.56\ (\pm 0.23)\ I + 5.01\ (\pm 0.17) \quad (6)$$

$$n = 35;\ r = 0.832;\ s = 0.322;\ F_{1,32} = 25.3$$

$$\log (1/K_{i\,app}) = 0.56\ (\pm 0.10)\ \pi_{3,5} + 0.63\ (\pm 0.15)\ I - 1.32\ (\pm 0.41) \log (\beta \cdot 10^{\pi_{3,5}} + 1) + 4.99\ (\pm 0.11) \quad (7)$$

$$n = 35;\ r = 0.935;\ s = 0.213;\ F_{2,30} = 21.5;\ \pi_0 = 2.16;\ \log \beta = -2.28$$

$$\log (1/K_{i\,app}) = 0.54\ (\pm 0.09)\ \pi_{3,5} + 0.62\ (\pm 0.14)\ I - 1.31\ (\pm 0.38) \log (\beta \cdot 10^{\pi_{3,5}} + 1) + 0.30\ (\pm 0.21)\ \sum\sigma + 4.98\ (\pm 0.10) \quad (8)$$

$$n = 35;\ r = 0.950;\ s = 0.191;\ F_{1,29} = 8.24;\ \pi_0 = 2.19;\ \log \beta = -2.34$$

The indicator variable in eq 7 and 8 accounts for two different sets of experimental conditions. In our first studies with bovine enzyme, the assays were made at pH 6.25. We have recently found[5] (eq 10) in a study of tri-

$$\log (1/K_{i\,app})_{7.2} = 1.03\ (\pm 0.05)\ [\log (1/K_{i\,app})]_{6.25} + 0.29\ (\pm 0.25) \quad (10)$$

$$n = 13;\ r = 0.997;\ s = 0.102$$

azines 2-X-II inhibiting bovine DHFR a close parallel between inhibition at the two different pH's. We have made a similar study (eq 11) of eight (compounds **26**, **28**,

$$\log (1/K_{i\,app}) = 1.05\pi_3 - 1.21 \log (\beta \cdot 10^{\pi_3} + 1) + 6.64 \quad (9)$$

$$n = 28;\ r = 0.955;\ s = 0.210;\ \pi_0 = 1.56;\ \log \beta = -0.733$$

$$[\log (1/K_{i\,app})]_{7.2} = 1.01\ (\pm 0.13)\ [\log (1/K_{i\,app})]_{6.25} + 0.38\ (\pm 0.71) \quad (11)$$

$$n = 8;\ r = 0.992;\ s = 0.053$$

and **31–36**) of the 23 benzylpyrimidines used to formulate eq 1 [4-OCF_3, H, 3,4,5-$(OCH_3)_3$, 3-CH_3, 3-F, 3-CF_3-4-OCH_3, 4-$NHCOCH_3$, 3-$OCH_2C_6H_5$]. The new benzylpyrimidines reported in this paper were all assayed at pH 7.2.

We find a slope of essentially 1 for both eq 10 and 11 with the difference in testing at the two pH's showing up in the intercepts. Activity is higher at the higher pH in both series of inhibitors. To us, these results imply that, since the pH change does not alter the QSAR, there is probably no significant change in the overall structure of the enzyme. Accordingly, we have used an indicator variable (I) in eq 6–8 which takes the value of 1 for those compounds tested at pH 7.2 (21) and a value of 0 for those tested at pH 6.25 (15).

The $\sum\sigma$ term in eq 8 is of marginal value but does bring out the fact that electron-deficient rings make slightly better inhibitors.

The right-hand portion of the bilinear curve of eq 8 is much steeper (0.54 − 1.31 = −0.77) than for eq 9 (1.05 − 1.21 = −0.16). The strong negative slope of eq 8 is heavily influenced by one data point [3-$O(CH_2)_7CH_3$]. We are now making a variety of highly lipophilic congeners to firm up this part of the QSAR.

Of the 13 new benzylpyrimidines tested against bovine DHFR, seven (**2**, **3**, **5–8**, and **26**) are mispredicted by an amount equal to or greater than the standard deviation. The poorest fit is tetroxoprim (**3**), which is about 7 times less inhibitory than eq 7 estimates. None of the other six is off by as much as twice the standard deviation. There is a rather small range of 150-fold in $1/K_{i\,app}$ for the bovine enzyme.

Equation 7 has now been shown to be linear with respect to $\pi_{3,5}$ up to values of 2 (π_0). Thus, linearity covers a range of π of 4 (i.e., 10000-fold in P). Since our primary objective in this report was to more clearly define the role of 3-substituents for the two quite different enzymes, we did not attempt to make a selection of 3,5-substituted derivatives. Of the four such examples (**1**, **3**, **6**, and **28**), all except one contain two OCH_3 groups whose π value is ~0. Although this is not a good test of $\pi_{3,5}$, the fact that the highly hydrophilic 3,5-$(CH_2OH)_2$ congener is so well fit by eq 7 cannot be taken lightly, especially since the coefficients with π in eq 1 and 7 are almost identical. At some point a set of unsymmetrically substituted 3,5-analogues must be studied, even though these are very time-consuming molecules to synthesize.

In studying the action of the benzylpyrimidines on *E. coli* DHFR, all kinds of equations involving π, MR, and σ taken singly, as well as in combination, were examined. The bilinear model of Kubinyi was also studied. In the end we could not find a better correlation than eq 3. Viewing the $MR'_{3,5}$ and the MR'_4 values in Table I on which this equation is based, one would like to see a greater range in MR. This is not possible because of the nature of the interaction between the inhibitors and the *E. coli* enzyme. There simply is a cutoff in activity at MR of about 0.79/position. To better understand this effect, we have included in Table I reference MR values for comparison with MR′. There are five examples (**3**, **8**, **16**, **23**, and **34**) for the 4 position where $MR_4 > MR'_4$. Except for the disubstituted compound **23**, all are very well fit, including the rather large 4-substituents of compounds **3** and **8**, by eq 7. While there is perforce limited variation in MR'_4 between 0.10 of H and 0.79, compounds **14**, **15**, **17**, **21**, and **25** are reasonably well fit. Thus, we believe that MR'_4 is best justified by the fact that large substituents are fit by an MR of 0.79 rather than by a good fit of an even distribution of MR'_4 values between 0.10 and 0.79.

The situation with $MR'_{3,5}$ is much the same. We do not have any congeners with both 3- and 5-substituents >0.79. Eventually, such congeners must be tested. We do feel that the evidence in hand is good for using MR'_3. The following examples have MR_3 values significantly larger than 0.79: compounds **5**, **7**, **9–13** and **36**. In the formulation of eq 2 we had only compound **36**; nevertheless, eq 2 does a reasonable job of predicting the seven new congeners with $MR_3 > 0.79$. Only compound **9** is mispredicted by more than 2 times the standard deviation. Considering the heterogeneity of the substituents and of the enzyme's surface with which these substituents come into contact, the results are surprisingly good. If π were the parameter involved, the results would be less surprising because we assume hydrophobic space does not contain a high percentage of polar residues with the attendant possibilities for hydrogen bonding and dipolar interactions.

One feels more secure with eq 7 and 8 for the bovine enzyme than with eq 3 for the bacterial enzyme. In the SAR model for *E. coli* enzyme we have defined the perimiter around the 3, 4, and 5 positions of the benzyl moiety within which a substituent can make effective contact with the enzyme as being equivalent to an MR of 0.79. MR being in one sense a "corrected" molar volume term, our definition of effective MR space is a sharp demarkation which is unlikely to correspond to reality and is in need of better delineation.

In conclusion, we can say that by means of eq 7 or 8 and eq 3 a reasonable model is available for the design of a more selective inhibition of bacterial enzyme as compared to bovine enzyme. We feel that the most serious shortcoming of the model is in the treatment of the CH_2OH group reaction with the bacterial enzyme. We hope that work in progress will enable us to understand the unexpectedly low potency of this congener against *E. coli* DHFR.

It must be borne in mind that in making drugs for in vivo use one must consider the overall log P value. Effective drugs could be designed using equations obtained from isolated enzyme studies only if the log P of the drug were suitable for animal systems. In this sense it is of interest to note that log P (between octanol and 0.1 N HCl) for trimethoprim is −1.55, close to that for tetroxoprim (−1.81). These drugs would be about 50% ionized at physiological pH so that the distribution coefficient would be higher than the partition coefficient of the protonated species.

Experimental Section

Synthesis of Pyrimidine Inhibitors (I). All of the new 2,4-diamino-5-(substituted benzyl)pyrimidines (I), except one (**10**, Table III), were prepared by the general synthetic procedure of Stenbuck et al.:[7] the corresponding substituted benzylaldehyde

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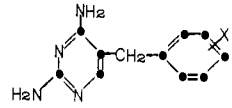
Table II. Benzal Nitriles VIa and VIb

no.	X	corresponding compd no. from Table I	yield, %	bp (mmHg), °C
1	3,5-$(CH_2OH)_2$	1	95[a]	
2	3-$O(CH_2)_7CH_3$	9	61.2	170–190 (0.15)
3	3-CH_2OH	4	48.6	140–165 (0.6)
4	3-OH	2	60.0	175–200 (2.5)
5	3-$CH_2O(CH_2)_3CH_3$	10	66.9	140–170 (0.7)
6	3-CH_2OCH_3	5	77.9	125–150 (0.7)
7	3-$O(CH_2)_3CH_3$	13	26.4	155–182 (1.1)
8	3-$O(CH_2)_5CH_3$	12	70.9	165–190 (5)
9	4-$O(CH_2)_3CH_3$	8	45.4	140–170 (0.8)
10	3-I	11	35.6	155–178 (0.8)
11	3,5-$(OCH_3)_2$	6	57.0	155–185 (0.5)

[a] Purified by column chromatography (alumina), due to its high boiling point, using 25% CH_3OH in $CHCl_3$ as an eluent solvent.

Table III. 2,4-Diamino-5-(substituted benzyl)pyrimidines[a]



no. (Table I)	X	mp, °C	yield,[b] %	formula[c]
1	3,5-$(CH_2OH)_2$	175.5–177.5	5.2	$C_{13}H_{16}N_4O_2$
9	3-$O(CH_2)_7CH_3$	114–115.5	17.4	$C_{19}H_{28}N_4O$
4	3-CH_2OH	200–202	1.7	$C_{12}H_{14}N_4O$
2	3-OH	249.5–252	2.8	$C_{11}H_{12}N_4O$
10	3-$CH_2O(CH_2)_3CH_3$	103–105	6.0	$C_{16}H_{22}N_4O$
5	3-CH_2OCH_3	164.5–166.5	12.3	$C_{13}H_{16}N_4O$
13	3-$O(CH_2)_3CH_3$	141.5–143	3.9	$C_{15}H_{20}N_4O$
12	3-$O(CH_2)_5CH_3$	111.5–113	11.4	$C_{17}H_{24}N_4O$
8	4-$O(CH_2)_3CH_3$	175–176	8.2	$C_{15}H_{20}N_4O$
7	3-OSO_2CH_3[d]	176.5–179	41.6	$C_{12}H_{14}N_4O_3S$
11	3-I	220–222[e]	8.6	$C_{11}H_{11}N_4I$
6	3,5-$(OCH_3)_2$	164.5–166.5	10.2	$C_{13}H_{17}N_4O_2$

[a] Prepared by method A, unless otherwise noted. [b] Yield of pure material calculated on the amount of benzaldehyde used. [c] Analyzed for C and H. [d] Prepared by method B. [e] Reference 8.

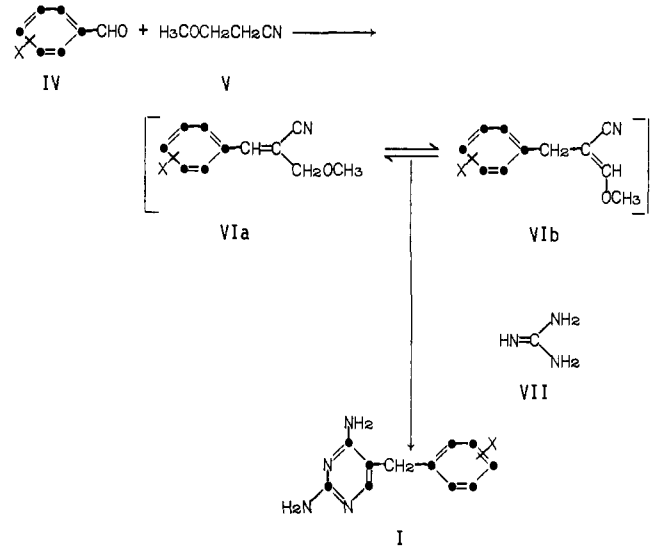
(IV) was condensed with β-methoxypropionitrile (V) using sodium methoxide; vacuum distillation provided a mixture of the crude benzal nitriles, VIa and VIb, which were reacted with guanidine (VII) to provide the desired pyrimidines I; see method A below and Scheme I.

Melting points (Buchi capillary apparatus) are uncorrected. Microanalyses were performed by C. F. Geiger, Ontario, CA, and are within ±0.4% of theoretical values. TLC (precoated qualitative alumina plate; UV visualization) was routinely used to check the purity of the pyrimidine I and to analyze column chromatography eluent fractions.

Method A. Ten volumes of anhydrous methanol containing 0.5 mol of sodium was used for each gram of benzaldehyde and a molar equivalent of β-methoxypropionitrile V. The mixture was refluxed for 6 to 7 h, during which time about 30 mL per hour was removed by distillation and replaced with an equivalent amount. The solvent was then vacuum evaporated, and the remaining oil partitioned between water and ether. The organic phase was washed with aqueous saturated sodium bisulfite until a clear aqueous phase was obtained. The ether solution was then dried over anhydrous sodium sulfate. Evaporation of the ether and distillation of the residue gave a mixture of VIa and VIb. The first small amounts of distillate gave a positive test with 2,4-dinitrophenylhydrazine and were discarded. Boiling ranges were broad, usually 20–30 °C. The yields of benzal nitriles are given in Table II.

A solution (3 mL of methanol/g of hydrochloride) of 3 equiv of guanidine hydrochloride per equivalent of benzal nitrile to be employed was combined with 3 equiv of sodium dissolved in methanol (8 mL of methanol/g of benzal nitrile). The two solutions were mixed and, after a few minutes, the precipitate of sodium chloride was removed by filtration. One equivalent of

Scheme I



benzal nitrile was then added and the mixture refluxed for 48 h. The mixture was then vacuum evaporated and the remaining oil dissolved in chloroform. The chloroform solution was washed with water and then evaporated to yield crude I. The benzylpyrimidine I was purified by column chromatography using chloroform and methanol for elution. In those instances where crude I crystallized, it was washed with water and methanol and then recrystallized from methanol or methanol–water. Several crystallizations were necessary to obtain a pure product.

Method B. For compound 4 of Table III, 0.7 g (3.2 mmol) was dissolved in 10 mL of 2 N KOH solution and stirred with 1.1 g

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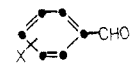
Table IV. Benzaldehydes Prepared for the Synthesis of Pyrimidines I



no.	X	no. from Table I	bp (mmHg) or mp, °C obsd	bp (mmHg) or mp, °C lit.	yield, %	method of synthesis[a]
1	3,5-$(CH_2OH)_2$	1	112-113		14.3	A
2	3-$O(CH_2)_2CH_3$	9	130-132 (0.45)	166-168 (0.05)[b]	100.0	B
3	3-CH_2OH	4	95-96 (2)		57.6	C
4	3-$CH_2O(CH_2)_3CH_3$	10	110 (0.7)		74.5	D
5	3-CH_2OCH_3	5	75-78 (1.1)	76.5 (0.4)[c]	86.7	D
6	3-$O(CH_2)_3CH_3$	13	110-113 (2.5)	142-143 (14)[d]	53.4	B
7	3-$O(CH_2)_5CH_3$	12	128-129 (0.7)	110 (0.01)[b]	33.2	B
8	4-$O(CH_2)_3CH_3$	8	125-128 (1.6)	148-149 (10)[e]	78.4	B
9	3-I	11	59.5-61	58[f]	12.8	E

[a] A: The diethyl ester of 5-nitrophthalic acid was reduced by $LiAlH_4$ in THF to give 5-amino-1,3-bis(hydroxymethyl)benzene (mp 99-100 °C). The amine was diazotized and treated with $Cu_2(CN)_2$ to produce the 5-cyano alcohol (mp 141.5-143.5 °C), which was treated with Raney Ni in 75% formic acid to produce 3,5-bis(hydroxymethyl)benzaldehyde.[14] B: Hydroxybenzaldehyde was refluxed with appropriate alkyl bromide in ethanolic KOH solution. C: α-Bromo-*m*-tolunitrile was hydrolyzed by $AgNO_3$ in 50% acetone (H_2O) to give *m*-(hydroxymethyl)benzonitrile, which was then reduced by Raney Ni in 75% formic acid to give *m*-(hydroxymethyl)benzaldehyde. D: α-Bromo-*m*-tolunitrile reacted with appropriate sodium alkoxide to give the corresponding *m*-(alkoxymethyl)benzonitrile. The benzonitrile was reduced by Raney Ni (Al-Ni) in 75% formic acid to give the corresponding benzaldehyde.[14] E: Direct iodination of benzaldehyde.[9] [b] Reference 12. [c] Reference 10. [d] Reference 11. [e] Reference 13. [f] Reference 9.

(9.6 mmol) of CH_3SO_2Cl in 5 mL of anhydrous benzene with cooling in an ice bath. The crude product (compound **10** in Table III) was removed by filtration and washed with 2 N KOH and then with water, mp 169-178 °C. This crude product was then recrystallized twice from methanol.

The benzaldehydes prepared for the synthesis of the pyrimidines are listed in Table IV.

Enzymatic Assay. The procedure for determining $K_{i\,app}$ and its confidence interval is that given in our recent publication.[3]

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Inhibition of Bovine and Rat Liver Dihydrofolate Reductase by 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(4-substituted-phenyl)-*s*-triazines

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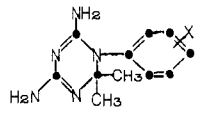
Quantitative structure–activity relationships (QSAR) have been formulated for the inhibition of purified bovine liver and rat liver dihydrofolate reductase (DHFR) by a series of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(4-X-phenyl)-*s*-triazines. The derived QSAR equations indicate that the interactions of the smaller 4-X substituents with both enzymes are hydrophobic, although size-limited, in nature. Further studies are suggested for elucidation of the specific interactions (hydrophobic or otherwise) of larger 4-X substituents with DHFR from mammalian sources.

Continuing our studies[1-5] of the inhibition of DHFR from various sources by triazines of type I, we report now



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on the inhibition of this enzyme from two mammalian sources (bovine and rat liver) by a series of 4-X substituted I. The impetus for this work is our desire to gain a general understanding of the parameters of importance (and hence the physical and chemical properties they model) for the interaction of ligands with enzymes and especially to develop the techniques for designing inhibitors which would be selective for enzyme from one source. We formulated eq 1 and 2 in an initial investigation[5] of inhibitors of type

Inhibition of Bovine Liver DHFR

$$\log (1/C) = 1.05\ (\pm 0.14)\ \pi_3 - 1.21\ (\pm 0.20) \log (\beta \cdot 10^{\pi_3} + 1) + 6.64\ (\pm 0.11) \quad (1)$$

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

Inhibition of Rat Liver DHFR

$$\log (1/C) = 1.12\ (\pm 0.15)\ \pi_3 - 1.34\ (\pm 0.26) \log (\beta \cdot 10^{\pi_3} + 1) + 6.28\ (\pm 0.12) \quad (2)$$

$n = 18$; $r = 0.977$; $s = 0.171$; $\pi_0 = 1.68$; $\log \beta = -0.978$

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