crystallized from 380 mL of ethanol to give 29 g of product, which was dissolved in 300 mL of hot ethanol and filtered and the filtrate was treated with 65 mL of concentrated hydrochloric acid to give 20 g of crystalline product, mp 247–251 °C. This was suspended in 350 mL of ethanol and the resulting mixture was heated to boiling and water was added just to solution. This was filtered, the filtrate was treated with 30 mL of concentrated HCl, and the solution was cooled quickly to give 13.7 g (29%) of product, mp 248–252 °C. Anal. ($C_7H_{11}N_5 \cdot 2HCl$) C, H, N, Cl.

1-Amino-2-(pyrid-2-ylmethyl)guanidine Dihydrochloride. A mixture of 46.6 g (0.2 mol) of *S*-methylthiosemicarbazide hydriodide, 43.0 g (0.4 mol) of 2-(aminomethyl)pyridine, and 200 mL of ethanol was heated under reflux for 2.5 h, then cooled, and treated with 85 mL of concentrated HCl followed by cooling to give 65 g of crystalline solid. A solution of this solid in 500 mL of boiling ethanol and 50 mL of water was filtered, the filtrate was treated with 25 mL of concentrated HCl, and the solution was cooled to give 30 g of crystalline product, mp 210–213 °C. This solid was dissolved in a hot solution of 400 mL of ethanol and 15 mL of water, treated with 20 mL of concentrated HCl, and cooled quickly to obtain 24.5 g (54%) of crystalline product, mp 213–216 °C. Anal. ($C_7H_{11}N_5 \cdot 2HCl$) C, H, N, Cl.

1-Amino-2-(pyrid-3-ylmethyl)guanidine Trihydrochloride. A mixture of 46.6 g (0.2 mol) of *S*-methylthiosemicarbazide hydriodide and 43.0 g (0.4 mol) of 3-(aminomethyl)pyridine in 200 mL of ethanol was heated under reflux for 2.5 h and then cooled overnight to give 37 g of crystalline product. This solid was dissolved in 400 mL of hot ethanol, the solution was filtered, and the filtrate was treated with 50 mL of concentrated HCl and then cooled to give a gummy oil, which crystallized: yield 29.5 g. A solution of this material in 400 mL of boiling ethanol and 15 mL of water was cooled to about 50 °C and treated with 20 mL of concentrated HCl to give 17.5 g of product. The process was repeated to give 8.9 g of product. All of these materials had unsatisfactory melting points and/or analyses. This material was dissolved in 10 mL of water and treated with 20 mL of concentrated HCl and then with 55 mL of ethanol to obtain 2.5 of crystalline product, mp 250–255 °C. Anal. ($C_7H_{11}N_5 \cdot 3HCl$) C, H, N, Cl.

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Inhibition by 5-(Substituted-benzyl)-2,4-diaminopyrimidines of Murine Tumor (L5178Y) Cell Cultures Sensitive to and Resistant to Methotrexate.[1] Further Evidence for the Sensitivity of Resistant Cells to Hydrophobic Drugs

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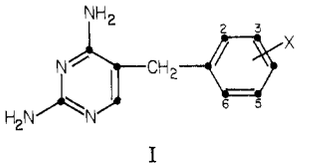
Forty-three 5-(substituted-benzyl)-2,4-diaminopyrimidines have been studied as inhibitors of murine tumor cell cultures (L5178Y). Two types of cells were used—one resistant to methotrexate and one sensitive to methotrexate. The formulation of quantitative structure–activity relationships showed that the methotrexate-resistant cells are more sensitive to the more hydrophobic congeners. π_0 for the sensitive cells is about 1.4, while π_0 for the methotrexate-resistant cells is above 3. These results are similar to those found for 2,4-diaminotriazines (Selassie, C. D.; Guo, Z. R.; Hansch, C.; Khwaja, T. A.; Pentecost, S. *J. Med. Chem.* **1982**, *25*, 157).

The great success of the inhibitors of dihydrofolate reductase (DHFR) as antibacterials (trimethoprim, tetroxoprim) and antitumor agents [methotrexate (MTX), Baker's antifols] is a fascinating chapter in medicinal chemistry. Although the general mechanism of action of the antifols is understood,[3] the details of how these compounds achieve their success are still unclear. Why is trimethoprim so selective to bacterial enzyme as compared to human reductase? Although MTX shows little, if any, selectivity for DHFR from different sources, why is it so remarkably effective in the treatment of a variety of cancers,[4-8] as well as other diseases?[9] We believe that much improved and more selective drugs for many diseases can be discovered by gaining a clearer understanding of the details of how ligands interact with various DHFR's. For this reason we have been systematically studying the inhibitory action of two classes of drugs (I and II) on purified DHFR.[10-13] The quantitative structure–activity relationships (QSAR) formulated from these investigations provide ideas for the synthesis of new analogues.

While the inhibition constants one finds for isolated DHFR are a good measure of the intrinsic activity of an inhibitor, we cannot yet predict with much assurance how



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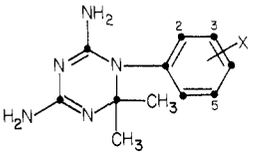

II

such inhibitors will behave in cell cultures or, especially, in animals. In order to gain some general knowledge of

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use in drug design, we have initiated a program for testing inhibitors at three levels of complexity: isolated enzyme, cell culture, and whole animal. In this report we consider the action of 5-(substituted-benzyl)-2,4-diaminopyrimidines on murine tumor cells (L5178Y) which are either sensitive to or resistant to methotrexate. These results are then compared with the action of triazines II on the same cell systems.

Results and Discussion

Table I lists the log $1/C$ values for 50% inhibition of cell culture growth, along with the necessary substituent constants for the formulation of correlation eq 1–5. In these

Inhibition of L5178Y Cells Sensitive to MTX

$$\log 1/C = 0.13\ (\pm 0.08)\ \Sigma\pi + 5.20\ (\pm 0.11) \quad (1)$$

$$n = 42;\ r = 0.479;\ s = 0.327;\ F_{1,40} = 11.9$$

$$\log 1/C = 0.30\ (\pm 0.07)\ \Sigma\pi - 0.10\ (\pm 0.03)\ \Sigma\pi^2 + 5.33\ (\pm 0.08) \quad (2)$$

$$n = 42;\ r = 0.803;\ s = 0.225;\ \pi_0 = 1.47\ (1.20\text{–}1.89);\ F_{1,39} = 45.6$$

$$\log 1/C = 0.30\ (\pm 0.07)\ \Sigma\pi - 0.12\ (\pm 0.03)\ \Sigma\pi^2 + 0.10\ (\pm 0.07)\ MR_3 + 5.27\ (\pm 0.09) \quad (3)$$

$$n = 42;\ r = 0.842;\ s = 0.206;\ \pi_0 = 1.24\ (1.01\text{–}1.55);\ F_{1,38} = 8.55$$

$$\log 1/C = 0.29\ (\pm 0.07)\ \Sigma\pi - 0.11\ (\pm 0.03)\ \Sigma\pi^2 + 0.09\ (\pm 0.07)\ MR_3 + 0.18\ (\pm 0.21)\ \Sigma\sigma + 5.26\ (\pm 0.08) \quad (4)$$

$$n = 42;\ r = 0.855;\ s = 0.200;\ \pi_0 = 1.29\ (1.04\text{–}1.64);\ F_{1,37} = 3.00$$

$$\log 1/C = 0.38\ (\pm 0.09)\ \Sigma\pi - 0.79\ (\pm 0.24)\ \log(\beta \cdot 10^{\Sigma\pi} + 1) + 0.08\ (\pm 0.08)\ MR_3 + 0.18\ (\pm 0.23)\ \Sigma\sigma + 5.23\ (\pm 0.09) \quad (5)$$

$$n = 42;\ r = 0.837;\ s = 0.215;\ \pi_0 = 1.38;\ F_{1,36} = 2.31$$

expressions, C is the molar concentration of I necessary to reduce the rate of cell growth by 50% in 48 h, $\Sigma\pi$ is the sum of the hydrophobic constants[14] for all substituents on the benzyl moiety, $\Sigma\sigma$[14] for all substituents is selected with respect to the CH_2 connection, MR_3 (scaled by 0.1 to make it more nearly equiscalar with π) is the molar refractivity[14] of substituents in position 3, n represents the number of data points used in constructing the correlation equation, r is the correlation coefficient, s is the standard deviation from the regression equation, F is the F statistic for the significance of the addition of each variable, and π_0 is the optimum value for hydrophobicity for maximum inhibitory potency, other factors being constant. In eq 5, which is based on the bilinear model of Kubinyi,[15] β is a disposable parameter derived by an iterative procedure via the computer.

While the stepwise development of eq 4 shows that all terms are significant at $\alpha = 0.10$ or better, MR_3 and especially σ are not very important. The term MR_3 refers only to the larger of the substituents in the 3 and 5 positions of the benzyl group (i.e., CH_3 rather than H); in cases where two identical groups are present in these positions, MR for only one of them is employed. Attempts to find other significant MR terms failed.

The correlation in terms of r is not as high as one would like; however, in terms of s it is about as good as one can expect with systems of this complexity. We assume that the variance which is not accounted for is due primarily to small difficult-to-assess steric effects between ligand and the enzyme receptor region. Hopfinger[16] has recently addressed this problem with the benzylpyrimidines and DHFR using molecular shape analysis.[17] His studies emphasize the importance of substituent shape for ligand interaction with DHFR but do not completely solve the problem. Elucidation of the DHFR structure via X-ray crystallography should shed some light on this difficulty.[18,19]

Although the MR_3 term is not of great importance, it is of interest because we also find it to be significant in the QSAR for isolated mammalian DHFR.[13]

While the bilinear model of eq 5 does not give as good a correlation as eq 4, even though it contains an additional adjustable parameter (β), the results are very similar to eq 4. The two equations point to an optimum view of π_0 near 1.35. The right-hand portion of the bilinear part of eq 5 has a slope of −0.41 (0.38–0.79) which brings out the symmetrical dependence of activity on π, just as in eq 4.

Kubinyi has presented a large amount of experimental data, as well as theoretical reasons, which support the idea that in the random-walk process of drugs through biological material to their sites of action, concentration at the active site should increase in a linear fashion with respect to lipophilic character until an optimum (log P_0 or π_0) is reached; after this point, activity should decline in a linear manner.[15,20,21] While there is much evidence to support this bilinear model, it is often found that the "parabolic" model[22] ($\pi + \pi^2$ as in eq 4) gives a better fit of the data, especially with the more complex systems. We believe that our studies with DHFR in vitro and in situ are beginning to shed light on this problem. Our finding that the bilinear model is most effective in the correlation of inhibition of isolated DHFR is clear from a number of examples.[12,23] With the isolated DHFR, inhibitory potency ($1/K_i$) first increases linearly with respect to π with a slope of about 0.5 or 1.0 until a break occurs at π_0, when the line becomes essentially flat ($\sim$0). One would expect a more symmetrical bilinear model (so-called tepee model) in cell-culture studies where equilibrium is not attained and especially in animal studies where activity is even more time dependent. If, indeed, the bilinear model is the true general case, rather than the parabola, then the expectation for QSAR in animals would be a set of bilinear equations which would include one equation for each process. Thus, one would obtain different bilinear models for reaction at the receptor, for the random-walk process, as well as for the various types of metabolism, excretion, etc. The net result of this may well be that a parabola is the best approximation to a set of different bilinear models, and for this reason the parabolic model works better than the Kubinyi model in complex situations. This may account

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Table I. Parameters Used in the Derivation of Equations 1-8

		L5178Y/S log 1/C			L5178Y/R log 1/C					
no.	group	obsd	calcd[a]	Δ	obsd	calcd[b]	Δ	$\Sigma\pi$	MR_3	$\Sigma\sigma$
1	3,5-$(OCH_3)_2$, 4-$O(CH_2)_2OCH_3$	4.20 ± 0.11[c]	5.07	−0.87	3.16 ± 0.14	3.39	−0.23	−0.72	0.79	0.0
2	3,5-$(CH_2OH)_2$	3.79 ± 0.15	4.22	−0.43	2.49 ± 0.14	2.50	−0.01	−2.06	0.72	0.0
3	3,5-$(OCH_3)_2$	5.47 ± 0.09	5.37	0.10	3.83 ± 0.06	3.85	−0.02	0.08	0.79	0.24
4	3,4,5-$(OCH_3)_3$	5.19 ± 0.08	5.13	0.06	3.73 ± 0.06	3.46	0.27	−0.60	0.79	0.07
5	3,4-$(OH)_2$	5.06 ± 0.06	4.68	0.38	4.94 ± 0.20[d]	2.99	1.95	−1.34	0.29	−0.25
6	3,4-$(OCH_2CH_2OCH_3)_2$	5.06 ± 0.06	5.16	−0.10	3.28 ± 0.12	3.34	−0.06	−0.80	1.93	−0.14
7	3-NO_2, 4-$NHCOCH_3$	5.11 ± 0.10	4.79	0.32	3.20 ± 0.12	3.05	0.15	−1.25	0.74	0.71
8	3,4-$(OCH_3)_2$	5.41 ± 0.07	5.37	0.04	3.54 ± 0.09	3.85	−0.31	0.08	0.79	−0.15
9	3-CF_3, 4-OCH_3	5.85 ± 0.17	5.49	0.36	4.39 ± 0.15	4.26	0.13	0.86	0.50	0.16
10	3-OCH_2CONH_2	4.91 ± 0.20	4.80	0.11	3.14 ± 0.08	2.97	0.17	−1.37	1.60	0.12
11	3-CH_2OH	4.97 ± 0.06	4.91	0.06	2.98 ± 0.12	3.20	−0.22	−1.03	0.72	0.0
12	3-OSO_2CH_3	5.28 ± 0.10	5.09	0.19	3.34 ± 0.09	3.29	0.05	−0.88	1.70	0.39
13	3-CH_2OCH_3	5.28 ± 0.06	5.09	0.19	3.65 ± 0.04	3.35	0.30	−0.78	1.21	0.02
14	3-OH	4.89 ± 0.07	5.05	−0.16	3.09 ± 0.17	3.42	−0.33	−0.67	0.29	0.12
15	3-$OCH_2CH_2OCH_3$	5.20 ± 0.08	5.33	−0.13	3.70 ± 0.07	3.58	0.12	−0.40	1.93	0.10
16	3-OCH_3	5.06 ± 0.12	5.34	−0.28	3.63 ± 0.07	3.80	−0.17	−0.02	0.79	0.12
17	H	5.24 ± 0.06	5.28	−0.04	3.88 ± 0.10	3.81	0.07	0.0	0.10	0.0
18	3-F	5.27 ± 0.16	5.32	−0.05	3.61 ± 0.09	3.89	−0.28	0.14	0.09	0.34
19	3-CH_3	5.50 ± 0.07	5.46	0.04	4.15 ± 0.12	4.11	0.04	0.56	0.57	−0.07
20	3-Cl	5.39 ± 0.06	5.48	−0.09	3.85 ± 0.15	4.18	−0.33	0.71	0.60	0.37
21	3-$CH_2O(CH_2)_3CH_3$	5.74 ± 0.08	5.71	0.03	3.93 ± 0.11	4.25	−0.32	0.84	2.60	0.02
22	3-Br	5.52 ± 0.05	5.53	−0.01	4.12 ± 0.09	5.26	−0.14	0.86	0.89	0.39
23	3-CF_3	5.63 ± 0.06	5.49	0.14	4.37 ± 0.08	4.27	0.10	0.88	0.50	0.43
24	3-I	5.86 ± 0.07	5.60	0.26	4.36 ± 0.11	4.38	−0.02	1.12	1.39	0.35
25	3-$O(CH_2)_3CH_3$	5.74 ± 0.04	5.67	0.07	4.99 ± 0.05	4.58	0.42	1.55	2.17	0.10
26	3-$OCH_2C_6H_5$	5.91 ± 0.05	5.76	0.15	4.70 ± 0.14	4.62	0.08	1.66	3.17	0.12
27	3-$O(CH_2)_5CH_3$	5.30 ± 0.09	5.53	−0.23	5.16 ± 0.18	5.01	0.15	2.67	3.07	0.10
28	3-$O(CH_2)_6CH_3$	5.10 ± 0.16	5.35	−0.25	4.61 ± 0.36	5.19	−0.58	3.23	3.52	0.10
29	3-$O(CH_2)_7CH_3$	5.12 ± 0.31	5.09	0.03	4.92 ± 0.19	5.34	−0.42	3.79	3.97	0.10
30	4-NH_2	4.65 ± 0.07	4.73	−0.08	3.58 ± 0.12	3.07	0.52	−1.23	0.10	−0.66
31	4-$NHCOCH_3$	5.12 ± 0.14	4.88	0.24	3.23 ± 0.12	3.23	0.00	−0.97	0.10	0.00
32	4-$OCH_2CH_2OCH_3$	4.77 ± 0.13	5.14	−0.37	3.08 ± 0.07	3.58	−0.50	−0.40	0.10	−0.24
33	4-NO_2	5.18 ± 0.08	5.18	0.00	3.67 ± 0.16	3.65	0.02	−0.28	0.10	0.78
34	4-OCH_3	5.10 ± 0.07	5.27	−0.17	3.52 ± 0.14	3.80	−0.28	−0.02	0.10	−0.27
35	4-F	5.48 ± 0.05	5.32	0.16	3.90 ± 0.06	3.89	0.01	0.14	0.10	0.06
36	4-$N(CH_3)_2$	4.95 ± 0.06	5.33	−0.38	4.02 ± 0.09	3.91	0.11	0.18	0.10	−0.83
37	4-CH_3	5.26 ± 0.06	5.41	−0.15	4.21 ± 0.07	4.11	0.10	0.56	0.10	−0.17
38	4-Cl	5.37 ± 0.05	5.43	−0.06	4.56 ± 0.09	4.18	0.38	0.71	0.10	0.23
39	4-Br	5.38 ± 0.07	5.44	−0.06	3.98 ± 0.12	4.26	−0.28	0.86	0.10	0.23
40	4-OCF_3	5.24 ± 0.05	5.46	−0.22	4.46 ± 0.12	4.34	0.12	1.04	0.10	0.35
41	4-$O(CH_2)_3CH_3$	5.37 ± 0.11	5.45	−0.08	4.83 ± 0.10	4.58	0.25	1.55	0.10	−0.32
42	4-$O(CH_2)_5CH_3$	5.47 ± 0.05	5.22	0.25	5.48 ± 0.13	5.01	0.47	2.67	0.10	−0.32
43	4-$O(CH_2)_6CH_3$	5.13 ± 0.22	4.99	0.14	5.67 ± 0.13	5.19	0.48	3.23	0.10	−0.32

[a] Calculated using eq 3. [b] Calculated using eq 8. [c] Not used in eq 3. [d] Not used in the formulation of eq 8.

for the less sharp result of eq 5 compared to eq 4.

Again we find that the parabolic model (eq 8) and the bilinear model give almost equivalent results; however, eq 7 and 8 are not much improvement over the simple linear relationship in eq 6. Not enough data points with π values

Inhibition of L5178Y Cells Resistant to MTX

$$\log 1/C = 0.49\ (\pm 0.07)\ \Sigma\pi + 3.76\ (\pm 0.09) \quad (6)$$

$$n = 42;\ r = 0.916;\ s = 0.288;\ F_{1,40} = 209$$

$$\log 1/C = 0.58\ (\pm 0.08)\ \Sigma\pi - 2.24\ (\pm 1.28)\ \log\ (\beta \cdot 10^{\Sigma\pi} + 1) + 3.78\ (\pm 0.08) \quad (7)$$

$$n = 42;\ r = 0.937;\ s = 0.256;\ \pi_0 = 3.02;\ F_{2,38} = 12.4$$

$$\log 1/C = 0.56\ (\pm 0.09)\ \Sigma\pi - 0.04\ (\pm 0.04)\ \Sigma\pi^2 + 3.81\ (\pm 0.10) \quad (8)$$

$$n = 42;\ r = 0.925;\ s = 0.277;\ \pi_0 = 6.96\ (3.9\text{–}234);\ F_{1,39} = 4.3$$

greater than π_0 are available, so that confidence intervals cannot be placed on π_0 of eq 7. The confidence limits on π_0 for eq 8 are so large that little importance can be attached to the figure of 6.96. All three equations tell the same story—that activity is essentially linearly dependent on π for almost all of the compounds in Table I.

Unfortunately, we cannot firmly establish a value for π_0; however, from an inspection of the data, we believe that it is at least 3, which is much higher than the values obtained for eq 4 or 5. The more lipophilic drugs are more effective against the MTX-resistant tumor cells in culture. We have found similar results with triazines II acting on the two L5178Y cell culture systems, although the disparity between the two π_0 values is even greater[24] with the triazines. For sensitive tumor cells, $\pi_0 = 0.8$, while for the resistant cells, $\pi_0 = \sim 6$. Again, it is difficult to get an accurate reading of π_0 on the resistant cells because not enough molecules with π greater than π_0 have been tested. Moreover, we are approaching the limit of water solubility in the range of π_0 with both the benzylpyrimidines and the triazines. Such compounds are, at best, difficult and often impossible to assay.

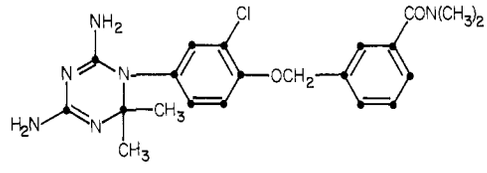
In deriving eq 1–5, one data point—that of tetroxoprim (1)—has been omitted from the correlation; it is considerably less active than predicted by eq 4 or 5. Including this point in eq 3 gives essentially the same parameters but a slightly poorer correlation ($r = 0.803$; $s = 0.245$). One

(24) Selassie, C. D.; Guo, Z. R.; Hansch, C.; Khwaja, T. A.; Pentecost, S. *J. Med. Chem.* **1982**, *25*, 157.

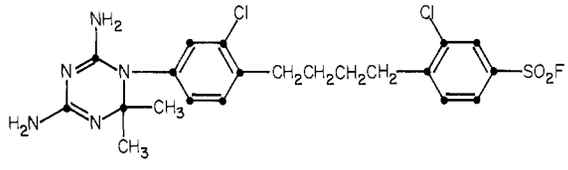
data point was dropped [3,4-$(OH)_2$] in the derivation of eq 6–8; it is more active than expected. Including this point in eq 8 yields a little changed equation with $r = 0.839$ and $s = 0.400$.

A crucial question which we can now begin to analyze is: What is log P_0 for these two series and how can we make use of this figure in the design of better antitumor antifols? Log P for the parent form of II in neutral solution or at physiological pH is very difficult to measure because the compound is so highly hydrophilic in this completely protonated form. To circumvent this problem, we measured the log P for 4-C_6H_5-II,[14] and from this value of −1.08, we subtracted a $\pi_{C_6H_5}$ of 1.96 to obtain a log P of −3.04 for the parent triazine. Adding a π_0 of 0.8 to this for cells sensitive to MTX or 6.0 for resistant cells yields the respective log P_0 values of −2.24 and 2.96.

These values can be compared with log P_0 for two of Baker's antifols (III and IV) which are not in clinical trials.



III



IV

Compound III has a measured value of −2.46,[14] which is (possible coincidentally) quite close to what the ideal value should be to inhibit the growth of the sensitive cell cultures. Since the cell culture is grown in fetal calf serum as nutrient, it may be a fair model for the whole animal. The log P_0 values of −2.24 and −2.46 are extremely low; in fact, about the lowest we have found. However, hydrophilic drugs are the rule rather than the exception in antitumor drugs, which, up to now, have been selected largely on the basis of their effectiveness against leukemia.[25]

Baker's antifol IV is now attracting attention because it appears to be effective against some solid tumors.[26] The calculated log P for IV is 2.4. The figure of 2.4 is not surprising because we have found that the ideal log P for penetration into the CNS by neutral compounds is ~2,[22] which suggests that log P for neutral compounds of ~2 is required for general perfusion into lipophilic cavities in the body, including those in tumor masses. Thus, we would expect Baker's antifol IV to penetrate solid tumors more effectively than antifol III because of its greater lipophilicity, and, in addition, it should be much more effective against cells resistant to MTX.

An important conclusion from our findings is that in clinical work one should combine a much more lipophilic antifol, such as IV, with MTX to prevent the growth of cells resistant to MTX.

The problem of exactly what is the optimum hydrophobicity of congeners I is more complex, because at physiological pH, trimethoprim and its benzyl-substituted congeners are about 50% ionized. Since it is still not clear which form is the active one in vivo, it is not obvious which form should be used as the parent compound in calculating log P_0. We have measured log P for the parent form of I (X = H) and found it to be −1.03 using octanol/0.1 N HCl. The neutral form has a log P of 1.58 as ascertained by partitioning between octanol and 0.1 N NaOH. Hence, log P_0 would lie between the values of 0.37 and 2.98 (log $P + \pi_0$) for the sensitive cell culture, while it would be between 2.0 and 4.6 for the resistant cell culture.

Regardless of the value of log P_0, our results suggest that combination chemotherapy is called for to prevent resistance from developing in clinical work. Trimethoprim has a log P for the protonated form of −1.55 and a log P of 0.82 for the neutral form. We suggest that combining trimethoprim with a benzylpyrimidine having a log P of 2–3 would be effective in preventing the rise of resistance.

Our studies with antifolates and bacterial as well as mammalian cells resistant to MTX lead us to believe that both types of cells have some mechanism for excluding hydrophilic antifols. Our results might be interpreted in other ways; for example, several colleagues have suggested that we might be involved with the inhibition of another enzyme in the MTX-resistant cells. This problem has been considered by Chello and his colleagues;[27] in particular, they showed that while MTX is an effective inhibitor of thymidylate synthetase ($K_i = 4.5 \times 10^{-5}$ M), Baker's antifol (III) at 10^{-3} M had no effect on thymidylate synthetase. Hence, we believe that it is unlikely that our antifols are inhibiting thymidylate synthetase. Moreover, it seems unlikely to us that, with the wide range of substituent changes we have made of congeners I and II, such complex compounds would interact with a completely different enzyme to give similar QSAR. This would imply that such an enzyme would have an action rather similar to DHFR in many ways.

In conclusion, the thought occurs to us that cells may have a general mechanism of excluding toxic hydrophilic substances by the erection of a barrier to such compounds. We believe that this idea is worth testing by investigating the effect of lipophilic drugs on cells which have become resistant to hydrophilic toxicants.

Experimental Section

Chemistry. The syntheses of the benzylpyrimidines used in this study have been reported previously.[13]

Biology. The original L5178Y/S and L5178Y/R cell lines were kindly provided by Dr. J. R. Bertino, Department of Pharmacology, Yale University, School of Medicine, New Haven, CT. For routine pasage and during dose–response experiments, the murine leukemia cells were maintained in asynchronous logarithmic growth at 37 °C in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum and 1% (v/v) penicillin–streptomycin. The population doubling times of the L5178Y/S and L5178Y/R were 11–12 and 15–18 h, respectively. Twice a week, the cells in the mid to late logarithmic stage of growth were diluted (v/v) 1:10- and 1:20-fold with fresh medium in order to maintain a portion of the cell stock in the logarithmic stage of growth at all times. The stock solutions of the benzylpyrimidines were made with dimethyl sulfoxide and unsupplemented medium such that the final concentration of Me_2SO in the microtitre plate was always less than 2%. Appropriate controls with Me_2SO were utilized in the assay.

Cell cultures were seeded at $4.0–6.0 \times 10^4$ cells/mL in duplicate for each drug concentration in a plastic microtitre plate. The benzylpyrimidines which were added to the cell cultures in 1:10 dilution to achieve the desired drug concentration were tested at a minimum of six different concentrations. After 48 h of continuous drug exposure in a humidified incubator supplied with 95% air and 5% carbon dioxide, the cells were harvested and

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counted using a Coulter counter. A control untreated set of cultures and Me_2SO-treated cultures were included for each separate dose–response experiment. Duplicate counts were taken on each well and were usually in agreement with each other (±10%).

From the data obtained, a dose–response curve was drawn, and the ID_{50} and its confidence limits were calculated as in our previous studies.[24]

Substituent Constants. The values for the substituent constants in Table I were taken from our recent compilation.[14]

Collinearity of Variables. The following squared correlation matrix shows that there is little collinearity among the variables used to formulate eq 1–8.

	π	MR_3	$\Sigma\sigma$
π	1	0.17	0.00
MR_3		1	0.03
$\Sigma\sigma$			1

Synthesis and Biological Evaluation of Novel Pyrimidine Nucleoside Analogues of 1,4-Oxathiane, 1,4-Dithiane, and 1,4-Dioxane

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Nine pyrimidine nucleoside analogues, in which the group attached at N-1 is a six-membered ring containing two heteroatoms, have been synthesized using the Vorbrüggen and Bennua (Vorbrüggen, H.; Bennua, B. *Tetrahedron Lett.* **1978**, 1339) coupling procedure. These are 1-(1,4-oxathian-3-yl)-5-fluorouracil (**8**), 1-(4-oxo-1,4-oxathian-3-yl)-5-fluorouracil (two stereoisomers **9** and **10**, resolved by silica gel column chromatography), 1-(1,4-oxathien-3-yl)-5-fluorouracil (**11**), 1-(1,4-oxathian-2-yl)-5-fluorouracil (**12**), 1-(1,4-dithian-2-yl)-5-fluorouracil (**15**), 1-(1,4-dithian-2-yl)uracil (**16**), 1-(1,4-dithian-2-yl)thymine (**17**), and 1-(1,4-dioxan-2-yl)-5-fluorouracil (**20**). All of the analogues were tested for cell-growth inhibition using mouse and human tumor cell lines. The ID_{50} values of all of the analogues are greater than 10^{-4} M, except in the case of **11** using the L1210 cell line. The most active analogues found are compounds **11** and **17**, which were found to be 100 and 200 times less active, respectively, than 5-fluorouracil in the human erythroleukemia cell line, K-562.

The fluorinated pyrimidines were first demonstrated as potentially useful chemotherapeutics by Heidelberger et al. in 1957.[2] Since that time, many derivatives of the clinically useful drug 5-fluorouracil (5-FUra) have been synthesized in the hope of discovering compounds having lower toxicity and improved antitumor activity than does 5-FUra.[3] Many of these derivatives have been shown to slowly release 5-FUra in vivo, and thus they function as relatively nontoxic reservoirs of 5-FUra.[3c,4] In addition to the reduced toxicity, some of these derivatives, including N^1-acetyl-N^3-*o*-toluyl-5-fluorouracil,[3c] 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil, and 1-(tetrahydro-2-furanyl)-5-fluorouracil (Ftorafur),[4a] offer the convenience of oral administration; also, their relatively long half-lives within the animal body allow the achievement of long lasting and much higher blood and tissue concentrations of 5-FUra than is possible with 5-FUra itself, which is administered by continuous intravenous infusion.[5] Improved tumor affinity appears to have been achieved with 1,3-bis(tetrahydro-2-furanyl)-5-fluorouracil which, compared to Ftorafur, gives not only much higher tissue concentration of 5-FUra but also a relatively higher concentration of 5-FUra in tumor tissue than in normal tissue.[4a] More recently, 5′-deoxy-5-fluorouridine, a new orally active antitumor agent, was reported[3b,6] to offer significant advantages in terms of activity and toxicity over 5-FUra, Ftorafur, and 5-FdUrd.

In contrast to the large number of derivatives of 5-FUra, in which the group attached to N-1 contains a five-membered ring,[3-6] there have been relatively few derivatives, synthesized and screened for activity, in which this group contains a six-membered ring.[3j] The observation that various purines substituted at N-9 with 1,4-dithiane, 1,4-dioxane, or 1,4-oxathiane possessed significant antitumor activity[7] prompted the preparation of 5-FUra derivatives containing these heterocyclic rings. A recent communication has described[8] the preparation of compounds **12** and **20** by the lewis-acid-catalyzed condensation of trimethylsilyloxyalkanal dialkyl acetals with 2,4-bis(trimethylsilyl)-5-fluorouracil, although no data were reported on their biological activities. The present article describes the synthesis of various such compounds and their effects on the growth of a variety of tumor cells in tissue culture.

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