removed under reduced pressure, and the residue was dissolved in 0.05 N sodium acetate buffer, pH 4.5, for injection. Aliquots were removed for radio-TLC analysis on silica gel (CH_2Cl_2–EtOH, 90:10) to detect residual [^{18}F]-3 (R_f of 3 was 0.42, R_f of 4 was 0.00); radio-TLC purity was >99%. See Figure 2 for radio-HPLC purity. Specific activity, which was 500–1200 mCi/mmol, was determined by UV/radio-HPLC.

Tissue Distribution Study. This was performed in two male mongrel dogs (20.1 and 20.4 kg). Animals received a bolus intravenous injection (cephalic vein) of 2 mCi of [^{18}F]-4 in 2.0 mL of sterile 0.150 M acetate buffer (pH 4.5). The dogs were sacrificed 1 h later by rapid iv injection of sodium pentobarbital. Duplicate tissue samples (15–80 mg) of organs were excised, washed free of blood with 0.9% saline solution, blotted dry, quickly weighed, and counted on a Packard 5780 autogamma counter for 1 min. The technique for isolating adrenomedullary tissue has been described previously.[13] To normalize for differences in animal weights, tissue concentrations are expressed in terms of percent kilogram dose per gram (% kg dose/g).[23] Radioactive concentrations in Tables II–IV are decay corrected.

Pharmacological Studies. The selective uptake of [^{18}F]-4 and [^{3}H]NE was inhibited in female Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 230–290 g, by the ip injection of desmethylimipramine hydrochloride (Revlon Care Group, Tuckahoe, NY), 10 mg/kg.[24] The [^{3}H]NE (*levo*-[7-^{3}H]), specific activity 15–20 Ci/mmol, was obtained from Du Pont NEN, Wilmington, DE. Significance levels were determined by the Student's *t* test.

The adrenergic neurons of the rat heart were impaired in rats (Sprague–Dawley) by the ip injection of 6-hydroxydopamine hydrobromide (Aldrich Chemical Co., Milwaukee, WI) freshly dissolved in physiological saline, 100 mg/kg, 5 days prior to the tracer experiments.[25]

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Quantitative Structure–Activity Relationships for the Inhibition of *Escherichia coli* Dihydrofolate Reductase by 5-(Substituted benzyl)-2,4-diaminopyrimidines

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Quantitative structure–activity relationships for the inhibition of *Escherichia coli* (MB 1428) dihydrofolate reductase (DHFR) by 61 5-(substituted benzyl)-2,4-diaminopyrimidines are reported and analyzed. The 61 compounds include 17 congeners whose activities have not been previously reported, five of which have a 5′-substituent larger than a methoxy group. The correlation equations indicated that the molar refractivity (MR) values of the 5′-substituent, just as with the 3′- and 4′-substituents, contributed maximally at the value of 0.79 with no increment of binding for compounds with MR larger than 0.79 (which corresponds to a 5′-methoxy substitution). This experimental result is in agreement with the crystal structure of the *Escherichia coli* DHFR–trimethoprim complex, which shows a reasonably large trimethoprim-binding site. The inhibition of *E. coli* (MB 1428) DHFR by nine of the 17 new benzylpyrimidines is at lower concentrations than for trimethoprim. However, all 17 are much less potent than trimethoprim in inhibition of growth of *E. coli* (1515).

Dihydrofolate reductase (DHFR) plays a crucial role in DNA synthesis; its inhibition can be used to control growth in any organism: animal, plant, insect, or microorganism. By studying DHFR from host and from pathogen, one can establish selectivity in growth inhibition (a therapeutic index) before commencing expensive animal testing.

Trimethoprin [5-(3′,4′,5′-trimethoxybenzyl)-2,4-diaminopyrimidine, 44] is a potent dihydrofolate reductase inhibitor and is widely used as bacteriostatic agent in combination with sulfamethaxazole.[1] Trimethoprim is a selective inhibitor of DHFR. Burchall[2] has shown the variability of the inhibitory power of trimethoprim with DHFR from different microorganisms, as well as mammalian sources. The apparent K_i values in nanomolar obtained for trimethoprim are 1.35 (*Escherichia coli* DHFR),[3] 132 (*Lactobacillus casei* DHFR),[4] 170 000 (human lymphoblast DHFR),[5] and 7900 (bovine liver DHFR).[6] Thus, trimethoprim binds from 60 to 100 000 times more tightly to the bacterial enzymes than to the mammalian enzymes.

Because of the clinical success of the antimicrobial agent trimethoprim, the structure–activity relationships for 5-

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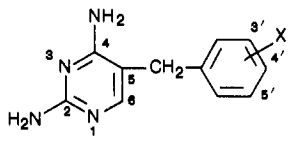
(substituted benzyl)-2,4-diaminopyrimidines (I) have been intensively studies.[3-8] The best correlation equation for



I

44, X = 3′,4′,5′—$(OCH_3)_3$

the inhibition of *E. coli* DHFR by benzylpyrimidines obtained in previous studies is[4]

$$\log 1/K_i(\text{app}) = 0.43\pi_{3',4',5'} + 1.23\text{MR}'_{3',5'} + 0.80\text{MR}'_{4'} - 0.88 \log (\beta \cdot 10^{\pi_{3',4',5'}} + 1) - 0.45\sigma_R^- + 5.81 \quad (1)$$

$$n = 43,\ r = 0.923,\ s = 0.263,\ \log \beta = -0.67,\ \pi_0 = 0.64\ (\pm 0.64),\ F_{1,36} = 8.88$$

In eq 1, K_i(app) is the apparent inhibitory constant, n represents the number of data points used to derive eq 1, r is the correlation coefficient, and s is the standard deviation from regression. The figures in parentheses are 95% confidence limits. MR is molar refractivity of substituents, defined as $\text{MR} = (n^2 - 1)[\text{MW}/(n^2 + 2)d]$, where n is the index of refraction, MW the molecular weight, and d the density. This definition makes MR a corrected molar volume, thus representing the substituent volume; the values of MR are multiplied by 0.1 to make their magnitudes comparable to π and σ. π is the hydrophobic constant of substituents. The subscript on π and MR indicates the position of substituents on the benzyl moiety. The electronic parameter σ_R^- is defined as $\sigma_R^- = \sigma^- - \tau$[9] and thus represents only the resonance effect of the substituents. The hydrophobic constant π in eq 1 is fit to a Kubinyi bilinear model.[10] The prime symbol on MR indicates a nonstandard, scaled use of this parameter. The limiting value for this parameter has been concluded to be 0.79, which is the MR value of OCH_3, from a computerized series of trial and error calculations. Larger substituents having greater values of MR at position 3′, 4′, and 5′ are predicted to be no more effective at enhancement of DHFR inhibition than a methoxy substituent. However, the series of benzylpyrimidines utilized in the derivation of eq 1 do not include any compounds with a MR value of its 5′-substituent larger than 0.79. Therefore, it is hard to be completely confident that eq 1 will hold true for I with a 5′-substituent larger than OCH_3. In order to confirm eq 1, a few congeners of I with a 5′-substituent larger than a methoxy group have been synthesized[11] and tested against *E. coli* DHFR. The largest 5′-substituent is a benzyloxy group, which has a MR value of 3.17 (when scaled by 0.1). All of the 17 new congeners of I have been correlated with the 41 benzylpyrimidines used in the derivation of eq 1 by utilizing a similar calculational approach.[4-10]

Results and Discussion

The inhibition of *E. coli* DHFR by the 17 new congeners are tested according to reference 12. Equations 2–7

$$\log 1/K_i(\text{app}) = 1.391\ (\pm 0.51)\text{MR}'_{3',5'} + 6.307\ (\pm 0.45) \quad (2)$$

$$n = 60,\ r = 0.585,\ s = 0.908,\ F_{1,58} = 30.25$$

$$\log 1/K_i(\text{app}) = 0.722\ (\pm 0.31)\text{MR}'_{3',5'} + 1.773\ (\pm 0.32)I + 6.316\ (\pm 0.26) \quad (3)$$

$$n = 60,\ r = 0.889,\ s = 0.517,\ F_{1,57} = 123.60$$

$$\log 1/K_i(\text{app}) = 1.000\ (\pm 0.29)\text{MR}'_{3',5'} + 0.942\ (\pm 0.38)\text{MR}'_4 + 1.525\ (\pm 0.29)I + 5.765\ (\pm 0.31) \quad (4)$$

$$n = 60,\ r = 0.924,\ s = 0.434,\ F_{1,56} = 25.28$$

$$\log 1/K_i(\text{app}) = 1.131\ (\pm 0.25)\text{MR}'_{3',5'} + 0.918\ (\pm 0.32)\text{MR}'_{4'} + 1.081\ (\pm 0.49)\pi_{3',4',5'} - 1.336\ (\pm 0.57) \log (\beta \cdot 10^{\pi_{3',4',5'}} + 1) + 1.494\ (\pm 0.25)I + 6.983\ (\pm 0.57) \quad (5)$$

$$n = 60,\ r = 0.949,\ s = 0.367,\ F_{3,53} = 24.39,\ \log \beta = 0.71,\ (\pi_{3',4',5'})_0 = -0.08$$

$$\log 1/K_i(\text{app}) = 0.942\ (\pm 0.27)\text{MR}'_{3',5'} + 0.765\ (\pm 0.34)\text{MR}'_{4'} + 0.380\ (\pm 0.19)\pi_{3',4',5'} - 0.769\ (\pm 0.31) \log (\beta \cdot 10^{\pi_{3',4',5'}} + 1) - 0.43\ (\pm 0.30)\sigma_R^- + 1.384\ (\pm 0.25)I + 6.054\ (\pm 0.27) \quad (6)$$

$$n = 60,\ r = 0.954,\ s = 0.351,\ F_{1,52} = 6.10,\ \log \beta = -0.46,\ (\pi_{3',4',5'})_0 = 0.45$$

$$\log 1/K_i(\text{app}) = 1.282\ (\pm 0.41)\text{MR}'_{3',5'} + 1.157\ (\pm 0.50)\text{MR}'_{4'} + 1.393\ (\pm 0.64)\pi_{3',4',5'} - 1.681\ (\pm 0.76) \log (\beta \cdot 10^{\pi_{3',4',5'}} + 1) - 0.09\ (\pm 0.44)\sigma_R^- + 1.483\ (\pm 0.38)I + 6.953\ (\pm 0.75) \quad (7)$$

$$n = 61,\ r = 0.909,\ s = 0.542,\ \log \beta = 0.61,\ (\pi_{3',4',5'})_0 = 0.074$$

have been derived from the results summarized in Table I, with symbols as in eq 1. In eq 2–6, F is the test of significance of the regression analysis. The first subscript number is the number of terms added in the equation, and the second number is the degrees of freedom.

Equation 6 is the best correlation equation, i.e., it has the highest r. Equations 2–5 are the stepwise-developed equations that lead to eq 6. Compound 1 of Table I is not used in the derivation of eq 2–6. The K_i for compound 1 is 3236-fold larger than its predicted value. It is interesting that the inhibitory activities for many kinds of DHFR by compound 1 are all much larger than predicted values; the reason for this disparity has not yet been clarified. Including all points in the regression analysis gives eq 7, which has a smaller correlation coefficient r (0.909 vs 0.954 in eq 6) and a multiplier for the σ_R^- term insignificantly different from zero. In eq 2–6, I is an indicator variable, indicative of two different methods of enzyme testing. $I = 1$ indicates the compounds are tested as described in ref 12, while $I = 0$ indicates the compounds are tested as described in ref 3.

The results summarized by eq 6 show that compounds with a 5′-substituent with MR′ larger than 0.79, the MR′ of a methoxy group, did not fit the correlation equation

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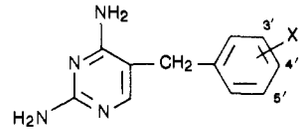
Table I. Data Used in the Derivation of Eq 2–8



no.	X	log $1/K_i$(app) obsd	log $1/K_i$(app) calcd[i]	$\|\Delta\|$	MR′$_{3',5'}$	MR′$_{4'}$	$\pi_{3'4'5'}$	σ_{R^-}	I
1	3′,5′-$(OH)_2$[j]	3.04	6.55	3.52	0.58	0.10	−1.34	−0.90	0
2	4′-$O(CH_2)_6CH_3$	5.60	6.13	0.53	0.21	0.79	3.23	−0.42	0
3	4′-$O(CH_2)_5CH_3$	6.07	6.37	0.30	0.21	0.79	2.63	−0.42	0
4	H	6.18	6.23	0.05	0.21	0.10	0	0	0
5	4′-NO_2	6.20	6.47	0.27	0.21	0.74	0[e]	0.57	0
6	3′-F	6.23	6.42	0.19	0.20	0.10	0.23[e]	−0.38	0
7	3′-$O(CH_2)_7CH_3$	6.25	6.03	0.22	0.89	0.10	3.79	−0.42	0
8	3′-CH_2OH	6.28	6.47	0.19	0.82	0.10	−1.03	0.08	0
9	4′-NH_2	6.30	6.23	0.07	0.21	0.54	−1.32[e]	−0.17	0
10	3′,5′-$(CH_2OH)_2$	6.31	6.63	0.32	1.44	0.10	−2.06	0.16	0
11	4′-F	6.35	6.41	0.06	0.21	0.09	0.14	−0.38	0
12	3′-$O(CH_2)_6CH_3$	6.39	6.25	0.14	0.89	0.10	3.23	−0.42	0
13	4′-$OCH_2CH_2OCH_3$	6.40	6.87	0.47	0.21	0.79	−0.30[e]	−0.42	0
14	4′-Cl	6.45	6.70	0.25	0.21	0.60	0.71	−0.14	0
15	3′,4′-$(OH)_2$	6.46	6.52	0.06	0.39	0.29	−1.34	−0.90	0
16	3′-OH	6.47	6.41	0.06	0.39	0.10	−0.67	−0.45	0
17	4′-CH_3	6.48	6.68	0.20	0.21	0.57	0.56	−0.11	0
18	3′-$OCH_2CH_2OCH_3$	6.53	6.98	0.45	0.89	0.10	−0.30[e]	−0.42	0
19	3′-$CH_2O(CH_2)_3CH_3$	6.55	6.87	0.33	0.89	0.10	0.84	0.01	0
20	3′-OCH_2CONH_2	6.57	6.63	0.06	0.89	0.10	−1.37	−0.42	0
21	4′-OCF_3	6.57	6.78	0.21	0.21	0.79	1.04	−0.11	0
22	3′-CH_2OCH_3	6.59	6.65	0.06	0.89	0.10	−0.78	0.01	0
23	3′-Cl	6.65	6.78	0.13	0.70	0.10	0.67[e]	−0.14	0
24	3′-CH_3	6.70	6.75	0.05	0.67	0.10	0.52[e]	−0.11	0
25	4′-$N(CH_3)_2$	6.78	6.89	0.11	0.21	0.79	0.24[e]	−0.22	0
26	4′-Br	6.82	6.83	0.01	0.21	0.79	0.86	−0.16	0
27	4′-OCH_3	6.82	6.90	0.08	0.21	0.79	−0.20[e]	−0.42	0
28	3′-$O(CH_2)_3CH_3$	6.82	6.88	0.06	0.89	0.10	1.55	−0.42	0
29	3′-$O(CH_2)_5CH_3$	6.86	6.48	0.38	0.89	0.10	2.63	−0.42	0
30	4′-$O(CH_2)_3CH_3$	6.89	6.76	0.13	0.21	0.79	1.55	−0.42	0
31	4′-$NHCOCH_3$	6.89	6.61	0.28	0.21	0.79	−0.91[e]	−0.26	0
32	3′-OSO_2CH_3	6.92	6.73	0.19	0.89	0.10	−0.88	−0.26	0
33	3′-OCH_3	6.93	6.71	0.22	0.89	0.10	0.11[e]	−0.42	0
34	3′-Br	6.96	6.95	0.01	0.89	0.10	0.86	−0.16	0
35	3′-NO_2, 4′-$NHCOCH_3$	6.97	6.86	0.11	0.84	0.79	−1.19[e]	0.31	0
36	3′-$OCH_2C_6H_5$	6.99	6.51	0.48	0.89	0.10	1.56[e]	−0.42	0
37	3′-CF_3	7.02	6.49	0.53	0.67	0.10	0.88	0.27	0
38	3′,4′-$(OCH_2CH_2OCH_3)_2$	7.22	7.52	0.30	0.89	0.79	−0.86[e]	−0.84	0
39	3′-I	7.23	6.86	0.37	0.89	0.10	1.12	−0.10	0
40	3′-CF_3, 4′-OCH_3	7.69	7.18	0.52	0.61	0.79	1.05[e]	−0.15	0
41	3′,4′-$(OCH_3)_2$	7.72	7.61	0.11	0.89	0.79	−0.58[e]	−0.84	0
42	3′,5′-$(OCH_3)_2$, 4′-$OCH_2CH_2OCH_3$	8.35	8.38	0.03	1.58	0.79	−0.78[e]	−1.26	0
43	3′,5′-$(OCH_3)_2$	8.38	7.89	0.49	1.58	0.10	0.02[e]	−0.84	0
44	3′,4′,5′-$(OCH_3)_3$	8.87	8.46	0.41	1.58	0.79	−0.52[e]	−1.26	0
45	3′-$OCH_2C_6H_5$, 4′-OCH_3[b]	10.01[d]	9.12	0.89	0.89	0.79	1.00[f]	−0.84	1
46	3′-OCH_3, 4′-$OCH_2CH_2OCH_3$[b]	9.25[d]	8.87	0.38	0.89	0.79	−0.96[f]	−0.84	1
47	3′-OCH_3, 4′-OH[b]	9.05[d]	8.63	0.42	0.89	0.29	−0.56	−0.87	1
48	3′-OCH_3, 4′-$OCH_2C_6H_5$[b]	9.43[d]	9.10	0.33	0.89	0.79	1.10[f]	−0.84	1
49	3′,5′-$(OCH_2CH_2CH_3)_2$[b]	8.80[d]	8.91	0.11	1.58	0.79	2.06[g]	−0.84	1
50	3′-OCH_2CH_3, 5′-$O(CH_2)_2CH_3$[b]	8.49[d]	9.11	0.62	1.58	0.10	1.50[g]	−0.84	1
51	3′-OSO_2CH_3, 4′-OCH_3[b]	8.96[d]	8.75	0.21	0.89	0.79	−1.08	−0.68	1
52	4′-OSO_2CH_3[b]	8.15[d]	8.00	0.15	0.21	0.79	−0.88	−0.26	1
53	4′-$OCH_2C_6H_5$[a]	8.70[d]	8.11	0.59	0.21	0.79	1.66	−0.42	1
54	3′,4′-OCH_2O-[a]	7.92[d]	8.37	0.45	0.55	0.79	−0.06[e]	−0.84	1
55	3′,5′-$(OCH_2CH_3)_2$[a]	9.11[d]	9.26	0.15	1.58	0.10	0.94[e]	−0.84	1
56	3′-$O(CH_2)_2OCH_3$, 4′-OCH_3[b]	8.74[d]	8.90	0.16	0.89	0.79	−0.86[f]	−0.84	1
57	3′-OCH_3, 4′-$O(CH_2)_3CH_3$[b]	9.01[d]	9.10	0.09	0.89	0.79	1.10[f]	−0.84	1
58	3′,5′-$(OCH_2C_6H_5)_2$[b]	8.77[d]	8.48	0.29	1.58	0.10	3.18[h]	−0.84	1
59	3′,5′-$(CH_3)_2$[a]	8.05[d]	8.54	0.49	1.14	0.10	1.12	−0.22	1
60	3′-Br, 4′-OCH_3, 5′-$O(CH_2)_2CH_3$[c]	8.96[d]	9.66	0.70	1.58	0.79	1.64[f]	−1.00	1
61	3′-Br, 4′-OCH_3, 5′-$O(CH_2)_3CH_3$[c]	8.96[d]	9.45	0.49	1.58	0.79	2.20[f]	−1.00	1

[a] Reference 11a. [b] Reference 11b. [c] Reference 11c. [d] New compounds tested following methods reported in reference 11. [e] Data from reference 11a. [f] Calculated on the basis of 3′,4′-$(OCH_3)_2$. [g] Calculated on the basis of 3′,5′-$(OC_2H_5)_2$. [h] Calculated on the basis of 3′,5′-$(OCH_3)_2$. [i] Calculated with eq 6. [j] Not used in the derivation of eq 2–6.

well unless their MR′ was set at 0.79. For compounds with a 5′-substituent larger than a methoxy group, the unscaled MR values and scaled values larger than 0.79 (e.g. 1.25, 1.00, 0.89) were used in correlation equations, but none of the correlation coefficients with these equations were better than the equations that had 0.79 as the maximum

MR′. A computer graphics model indicates[4] that the trimethoprim binding site of *E. coli* DHFR is reasonably tight in the immediate vicinity of the trimethoxyphenyl portion of trimethoprim but that the active site widens considerably near the entrance to the trimethoprim binding site. This could explain why there appears to be an upper limit on MR (MR′) for inhibition of *E. coli* DHFR. The quantitative structure–activity relationship (QSAR) equation indicates that there is essentially no additional interaction for substituents larger than a methoxy group at the 3′-, 4′-, or 5′-position; only the first two atoms of the substituent contribute to enzyme binding. The portion of substituents beyond the first two atoms will be located in the widened region of the trimethoprim binding site of the enzyme where there is no opportunity for tight contacts.

Compound **45** (Table I) is about 7.8-fold more potent than its predicted activity. The difference between observed and calculated activities is more than twice the standard deviation of eq 6. If this compound is omitted, the correlations do not significantly improve. If the actual MR values of 3′-, 4′-, and 5′-substituents are used in the derivation of QSAR analysis (see eq 8), compound **45** will

$$\log 1/K_i(\text{app}) = 0.348\ (\pm 0.15)\text{MR}_{3',5'} + 0.236\ (\pm 0.16)\text{MR}_{4'} + 0.273\ (\pm 0.17)\pi_{3',4',5'} - 1.144\ (\pm 0.40)\log(\beta \cdot 10^{\pi_{3',4',5'}} + 1) - 0.73\ (\pm 0.32)\sigma_R^- + 1.367\ (\pm 0.30)I + 6.374\ (\pm 0.22) \quad (8)$$

$$n = 60,\ r = 0.938,\ s = 0.407,\ \log\beta = -0.98,\ (\pi_{3',4',5'})_0 = 0.48$$

fit the correlation equation better than when using MR′ of the substituents. However, a poorer overall fit is made when MR is used instead of MR′; i.e., the correlation coefficient of eq 6 is smaller and the standard deviation of eq 8 is larger than eq 6. In eq 6, MR rather than π is the principal correlate of the inhibitory activities, although inclusion of the π constant of 3′-, 4′-, and 5′-substituents bilinearly improves the correlation of MR′. Molecular modeling reveals that only a small portion of each 3′-, 4′-, and 5′-substituent is buried within a hydrophobic region, with most of the substituent solvated.[4] This explains why the π constant of these substituents is not the primary parameter determining the inhibitory activity against *E. coli* DHFR.

By comparison of eq 6 with eq 1 it can be seen that the coefficients of each corresponding term in the two equations are nearly the same. Therefore, it appears that there is no substantial difference between these two equations, except for the indicator variable term. The coefficient of I in eq 6 means that the K_i values tested as in ref 12 are about 24-fold smaller than the K_i values measured as in ref 3. The difference in K_i values using these two methods is much larger than the experimental error. Trimethoprin has been tested by these two different methods; its K_i is 1.2 nM (by ref 12) and 1.35 nM (by ref 3), a 1.12-fold difference. There may be some as yet unrevealed factors concealed in the I term of eq 6, which may be revealed in future studies.

It is noteworthy that nine compounds (**45–48**, **51**, **55**, **57**, **60**, and **61**) are more potent inhibitors of *E. coli* (MB 1428) DHFR than trimethoprim. The most potent one is about 12-fold more potent than trimethoprim. We have tested these nine compounds as inhibitors of growth of *E. coli* (1515) to see whether they are more potent than trimethoprim in growth inhibition. The results (Table II) show that they are all less potent than trimethoprim. For example, **45** is 125-fold less potent than trimethoprin in growth inhibition.

Table II. Inhibitory Activities of Some 5-(Substituted benzyl)-2,4-diaminopyrimidines against the Activity of *E. coli* MB 1428 Dihydrofolate Reductase and the Growth of *E. coli* 1515 and *E. coli* MB 1428

no.	X	log $1/K_i$-(app)	log $1/C$[a] 1515	log $1/C$[a] 1428
44	3′,4′,5′-$(OCH_3)_3$[b]	8.92	7.06	5.22
45	3′-$OCH_2C_6H_5$, 4′-OCH_3	10.01	4.96	5.02
46	3′-OCH_3, 4′-$OCH_2CH_2OCH_3$	9.25	5.82	4.30
47	3′-OCH_3, 4′-OH	9.05	6.45	3.51
48	3′-OCH_3, 4′-$OCH_2C_6H_5$	9.43	4.96	5.02
51	3′-OSO_2CH_3, 4′-OCH_3	8.96	6.42	4.13
55	3′,5′-$(OCH_2CH_3)_2$	9.11	6.54	4.77
57	3′-OCH_3, 4′-$O(CH_2)_3CH_3$	9.01	5.93	4.98
60	3′-Br, 4′-OCH_3, 5′-$O(CH_2)_2CH_3$	8.96	4.91	6.07
61	3′-Br, 4′-OCH_3, 5′-$O(CH_2)_3CH_3$	8.96	5.02	6.24

[a] C is the molar concentration of benzylpyrimidine that inhibits growth by 80%. The column labeled 1515 is the logarithm of the inverse of the concentration of the compound listed in column 1 that inhibited growth of *E. coli* 1515 by 80%. The column labeled 1428 is analogously defined for *E. coli* MB 1428. [b] Trimethoprin.

Equation 9 is the correlation equation describing the inhibition of growth of *E. coli* (1515) bacteria by compounds **45–48**, **51**, **55**, **57**, **60**, and **61** in Table II.[11c]

$$\log 1/C = 0.079\text{MR}_{3',4',5'} - 1.876\log(\beta \cdot 10^{\text{MR}_{3',4',5'}} + 1) + 5.554 \quad (9)$$

$$n = 20,\ r = 0.840,\ s = 0.345,\ F_{3,16} = 20.29,\ \log\beta = -2.17,\ (\text{MR}_{3',4',5'})_0 = 1.95$$

Equation 9 is based on a Kubinyi bilinear formulation of $\text{MR}_{3',4',5'}$; the optimum value of $\text{MR}_{3',4',5'}$ is 1.95. The $\text{MR}_{3',4',5'}$ values of compounds **45**, **46**, **48**, **51**, **55**, **57**, **60**, and **61** are larger and the $\text{MR}_{3',4',5'}$ of compound **47** is smaller than the optimum value. The compounds in Table II are all less potent than trimethoprim in inhibition of growth of *E. coli* (1515). The *E. coli* DHFR used in enzyme testing came from a different species of *E. coli*; therefore, it is not possible to directly compare eq 6 with eq 9. Coats et al.[13] studied the QSAR of the inhibition of growth of *E. coli* (MB 1428) by 5-(substituted benzyl)-2,4-diaminopyrimidines and derived eq 10.

$$\log 1/C = 1.25\text{MR}'_{3',4',5'} + 0.35\pi_{3',4',5'} + 2.11 \quad (10)$$

$$n = 26,\ r = 0.0969,\ s = 0.238,\ F_{1,23} = 87.3$$

In eq 10, $\text{MR}'_{3',4',5'}$ and $\pi_{3',4',5'}$ are defined as in eq 1 [see references 3–8]. When the predicted bacteriostatic activities of the nine compounds of Table II are calculated from eq 10, the calculated growth inhibitory activities do not parallel DHFR inhibitory activities. The calculated activities of compounds **60** and **61** are higher than that of trimethoprim because they have more favorable $\text{MR}'_{3',4',5'}$ and $\pi_{3',4',5'}$ values. The above results indicate that the most potent inhibitor of DHFR is not necessarily the most potent bacteriostatic agent. Clearly, in the evaluation of DHFR inhibitors as antibacterial agents, it is necessary to test with whole bacteria.

Experimental Section

Enzymatic Assay. Buffer R was 80 mM KCl, 20 mM Tris, 10 mM KH_2PO_4, and 5 mM $MgCl_2$ adjusted to pH 7.2 with 2 M HCl. *E. coli* (MB 1428) DHFR, NADPH, and H_2-folate were prepared and standardized as in ref 12. Inhibitors were accurately weighed and dissolved in 1.00 mL of $(CH_3)_2SO$ to make a 500–2000 μM solution. For assay, there was added 10 μL of H_2-folate solution (9.4 mM) to 990 μL of a mixture of 9.90 mL of buffer

(13) Coats, E. A.; Genther, C. S.; Selassie, C. D.; Strong, C. D.; Hansch, C. *J. Med. Chem.* **1985**, *28*, 1910.

R and 0.10 mL of 5.8 mM NADPH in a cuvette in the sample chamber of a Cary 118 UV-vis spectrometer set at 340 nm at room temperature. Then, an aliquot of inhibitor was added, followed by 10 μL of DHFR. The initial steady rate of A(340) decrease was measured.

Calculation of K_i. The following equation was used: $K_i(app) = i_{50}[1/(1+(s/K_s))]$, where s was the concentration of H_2-folate in M in cuvette, K_s was the Michaelis constant for H_2-folate (440 nM), and $i_{50} = i[1/([v(i = o)/v(i = i)]) - 1)]$ where i is the concentration of inhibitor in nM, i_{50} is the concentration of inhibitor that gave 50% inhibition of DHFR activity, $v(i = o)$ is the rate of decrease of A(340) without inhibitor, and $v(i = i)$ is the rate of decrease of A(340) in the presence of inhibitors at concentration i. DHFR assays and calculations for each inhibitor were repeated three to seven times until the standard error in K_i was less than 20%.

QSAR Parameters. All parameters of Table I come from the compilation used in ref 9 unless noted.

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Registry No. 1, 80407-57-4; 2, 80407-60-9; 3, 80407-61-0; 4, 7319-45-1; 5, 69945-52-4; 6, 69945-57-9; 7, 77113-60-1; 8, 77113-56-5; 9, 69945-50-2; 10, 77113-54-3; 11, 836-06-6; 12, 80407-62-1; 13, 80407-59-6; 14, 18588-43-7; 15, 71525-05-8; 16, 77113-55-4; 17, 46726-70-9; 18, 80416-29-1; 19, 77113-61-2; 20, 80407-58-5; 21, 49561-94-6; 22, 77113-57-6; 23, 69945-58-0; 24, 69945-56-8; 25, 69945-51-3; 26, 69945-55-7; 27, 20285-70-5; 28, 77113-63-4; 29, 77113-62-3; 30, 77113-59-8; 31, 69945-53-5; 32, 77113-58-7; 33, 59481-28-6; 34, 69945-59-1; 35, 69945-54-6; 36, 69945-60-4; 37, 50823-94-4; 38, 73356-41-9; 39, 30077-60-2; 40, 50823-96-6; 41, 5355-16-8; 42, 53808-87-0; 43, 20344-69-8; 44, 738-70-5; 45, 78233-99-5; 46, 107697-99-4; 47, 73356-40-8; 48, 83158-06-9; 49, 107698-00-0; 50, 7334-22-7; 51, 111743-19-2; 52, 107698-01-1; 53, 49873-11-2; 54, 13932-40-6; 55, 100515-03-5; 56, 107697-98-3; 57, 80267-19-2; 58, 111743-20-5; 59, 100515-04-6; 60, 107697-96-1; 61, 107697-97-2; DHFR, 9002-03-3.

N-Aryl 3-Halogenated Azetidin-2-ones and Benzocarbacephems, Inhibitors of β-Lactamases

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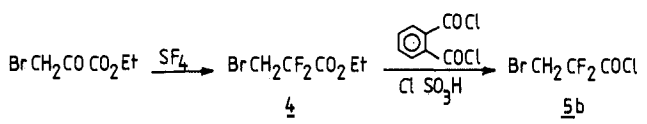
N-(3-Carboxy-6-methylphenyl)-3-fluoroazetidin-2-one and a series of related *N*-aryl-3-halo- and -3,3-dihaloazetidinones **3**, in which the halo substituent is a fluorine or a bromine atom, were prepared, by using the Wasserman procedure of cyclization of β-bromopropionamides as a key step. Their affinities for the TEM-1 β-lactamase were determined and compared with those of a series of tricyclic azetidinones, the benzocarbacephems **2**, and known β-lactamase inhibitors. The β-lactams **2** and **3** behave as competitive inhibitors and not as substrates of the enzyme; neither halogen substitution (series **3**) nor ring strain (series **2**) induces enzymatic hydrolysis.

In spite of the introduction in therapy of innovative β-lactams with expanded antibacterial activity and improved β-lactamase stability, bacteria continue to exhibit resistance. Therefore enzymatic inactivation of the newer molecules is still a major problem. A recent outbreak of bacteria carrying plasmid-mediated β-lactamases markedly active against third-generation cephalosporins has renewed the interest in β-lactamase inhibitors.[1] Clavulanic acid, sulbactam, and related compounds[2] are β-lactamase inhibitors which potentiate the antibacterial activity of various β-lactams, but the need for β-lactamase inhibitors of new chemical structures and new biological properties remains a subject of very high interest.

We have previously shown that some *N*-arylazetidinones, and particularly the 3-carboxy-6-methyl-substituted one, **1a** (Figure 1), are interesting competitive inhibitors of β-lactamases. However, they are not detectable substrates of the enzymes.[3] Their bromomethylated analogues such as **1b** are not suicide inhibitors,[3] for ring opening is a prerequisite for the design of new suicide inhibitors of β-lactamases.[2]

To increase the reactivity of the β-lactam ring toward enzymatic ring opening, two ways were considered: cyclization to give strained tricyclic β-lactams **2** and halogen substitution α to the carbonyl to give compounds of type **3** (vide infra). We have already described the synthesis

Scheme I



of the benzocarbacephems **2** (Y = H, Cl, or F).[4] We report here the results of their biological activity together with the synthesis and properties of the *N*-aryl 3-halogenated and 3,3-dihalogenated azetidinones **3** (X^1, X^2 = H, Br, or F).

The presence of one or two halogen substituents α to the carbonyl should increase the $\nu_{c=o}$ IR stretching frequency, one of the criteria of the reactivity of the β-lactam ring.[5] In this respect, fluorine substitution, which will not

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