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Quantitative Structure–Activity Relationships for 5-Substituted 8-Hydroxyquinolines as Inhibitors of Dental Plaque

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Fourteen 8-hydroxyquinolines were tested for antiplaque activity by measuring their minimum inhibitory concentrations [MIC (M)] against *Streptococcus mutans* No. 6715. Linear regression analysis was conducted with the MIC (M) values and hydrophobic (log *P*), electronic (σ, pK_a^{OH}, pK_a^{N}), and steric [molar refractivity (MR), molecular weight (mol wt)] parameters. The best correlation ($r^2 = 0.90$) was obtained with MR, log *P*, and σ. The smaller the steric contribution of the 5-substituent, the more active the compound. The parent 8-hydroxyquinoline was the most active. The negative contribution toward activity by 5-substituents larger than hydrogen can be overcome by the positive contributions of groups that are lipophilic and electron withdrawing; for example, the 5-chloro derivative is as active as the parent 8-hydroxyquinolines.

Predicting the activity of a compound has been a primary goal of structure–activity relationship (SAR) studies for many years. When quantitative biological data are available for a series of congeners, then quantitative structure–activity relationship (QSAR) methods may be useful. There are three major approaches in the study of QSAR: (1) the semiempirical linear free-energy related model proposed by Hansch,[1] (2) the empirical mathematical model proposed by Free and Wilson,[2] and (3) quantum chemical approaches.[3]

In order to make the problem manageable in the linear free-energy approach, it is customary to study a congeneric series of compounds in which a parent molecule is modified by the presence of one or more substituents. Implicit in this approach is the assumption that all members of the series act on the biological system by the same mechanism and only their quantitative potency is modified by the substituents. The appearance over the last 10 years of a large number of successful SAR correlations in the literature supports this assumption. The biological activity data must be obtained under uniform conditions, and the biological response should be of low complexity.[4]

The bacteria *Streptococcus mutans* No. 6715 play a major role in the formation of dental plaque. Dental plaque in turn is the primary cause of caries and periodontal disease. One approach for the prevention of these diseases has involved use of antibacterial agents. 8-Hydroxyquinolines have been shown to be some of the most potent in vitro inhibitors of *S. mutans* No. 6715.[5-7] Initial studies indicated that 8-hydroxyquinolines with log *P* values between 1 and 4 display the best activity against this organism.[5] As part of our continuing studies of dental plaque inhibitors, we synthesized and tested a series of 5-substituted 8-hydroxyquinolines.[6,7] These analogues were chosen since the substituent would be some distance from the portion of the molecule involved in chelation. It has been shown that chelation with iron or copper ions is required for antibacterial activity. In the absence of these ions, the 8-hydroxyquinolines are not toxic to microorganisms. Substitution near the nitrogen atom or the phenolic group reduces or eliminates biological activity and the ability of the compound to chelate metal ions.[8,9] We concluded from the preliminary results that the use of the single parameter, log *P*, was not adequate to accurately predict antiplaque activity. For example, 5-methoxymethyl-8-hydroxyquinoline and 5-cyanomethyl-8-hydroxyquinoline have log *P* values which differ by only 0.03; yet, the compounds show[6] 20 and 80% inhibition of *S. mutans* at 10^{-4} M, respectively.

To delineate the structural requirements for optimal antibacterial activity, we have investigated 14 compounds as inhibitors of *S. mutans* No. 6715. The antibacterial activities were measured by determining the minimum molar concentrations of the agents required for total inhibition of bacterial growth. These are recorded as the MIC (M) values in Table I. In Table I are also given the

experimentally determined log P values (octanol–water), the log P values corrected for ionization, and the pK_a values of the substituents in the 5 position (pK_a^R), the 8-hydroxyl (pK_a^{OH}), and the quinoline nitrogen (pKa^N). Literature values of the Hammett σ, the fragment molar refractivity (MR),[10] and the fragment molecular weight (mol wt) values of the 5-substituents are also given.

Experimentally determined log P values were used rather than literature π values from substituted phenols or benzenes (vide infra). Using linear regression analysis, equations were generated to correlate the minimum inhibitory concentrations of the 5-substituted 8-hydroxyquinolines with hydrophobic (log P), electronic (σ, pK_a^{OH}, pK_a^N), and steric (MR and mol wt)[10] parameters (see eq 1–13 in Table II). In the single-parameter equations (eq 1–6 of Table II), the best correlation was obtained with fragment molar refractivity (MR) (eq 6). This correlation indicates the prime importance of the size of the 5-substituents as a determinant of biological activity. Although mol wt is also a steric parameter, the correlation obtained with it was not as good (eq 5). This is substantiated by the low squared correlation coefficient of 0.53 between MR and mol wt (Table III). Equations 1–4 involving lipophilic and electronic parameters account for less than 10% of the variance of the data. In the regression equations (Tables II and IV), n represents the number of data points used in the regression, r is the correlation coefficient, and s is the standard deviation. Values in parentheses after each variable are the 95% confidence intervals.

In contrast to hydrophobic and electronic constants, the choice of a proper steric constant is difficult. Until quite recently, little use has been made of MR in quantitative terms. However, it is increasingly evident that MR can be an important parameter in QSAR.[11-13] Although MR models interactions in nonhydrophobic space or steric bulk, MR is sometimes highly collinear with π especially for apolar groups;[11,13] this is because both MR and π depend to a certain extent on molar volume.[14] In such cases, it is difficult to distinguish the interactions between π or log P (those in hydrophobic space) from MR (nonspecific interactions in polar space or steric interactions). It is only with a proper selection of substituents that one can separate the nonhydrophobic and/or the steric effects of MR from the hydrophobic contribution of MR. In the present study, however, the correlation between MR and log P is so low ($r^2 = 0.26$) that one can have much more confidence than where there exists a high collinearity.

Using MR and a second parameter gave eq 7–10. The log of the minimum inhibitory concentration correlates best with MR and log P as shown by eq 10. This equation has high statistical significance and satisfies the F test at the 99% level with an F value of 17.48 ($F_{1,11(\alpha=0.01)} = 9.65$) and accounts for 72% ($r^2 = 0.72$) of the variance in the data. Equations 11–13 are three-parameter equations involving MR and log P with one of the three electronic parameters (σ, pK_a^{OH}, or pK_a^N). All give similar high correlations. That eq 12 and 13 are almost equivalent is not surprising because of the high squared correlation coefficient between σ and pK_a^{OH} (see Table III). The squared correlation coefficients between σ (or pK_a^{OH}) and pK_a^N are not as high, but it is clear that an electronic parameter is important. The stepwise application of the F statistic indicates that each of the terms in eq 11–13 is valid. Comparing these three equations with eq 6, one obtains $F_{2,10} = 15.11$ for eq 11; $F_{2,10} = 20.65$ for eq 12; and $F_{2,10} = 32.14$ for eq 13, and $F_{2,10(\alpha=0.001)} = 14.91$. Comparing these three equations with eq 10, we obtain $F_{1,10} = 5.53$, $F_{1,10(\alpha=0.05)} = 4.97$ for eq 11; $F_{1,10} = 9.81$, $F_{1,10}$-

Table I. Physicochemical Data for 5-Substituted 8-Hydroxyquinolines[a]

5-Substituent	Log P_{obsd}	Log P_{cor}[b]	pK_a^{OH}[c]	pK_a^N[c]	pK_a^R[c]	σ	MR	Mol wt	MIC (M)[d] obsd	Log 1/MIC (M)[e] Obsd	Calcd	Deviation
H	1.94 (0.34)	1.95	9.64 (0.02)	5.14 (0.01)	0.00	0.00[f]	1.03[f]	1.0	2	−0.301	−0.686	0.385
Cl	2.91 (0.09)	2.94	8.70 (0.03)	3.80 (0.07)	0.00	0.23[f]	6.03[f]	35.4	2	−0.301	−0.090	−0.211
NO_2	−1.17 (0.07)	0.11[g]	5.97 (0.02)	3.68 (0.20)	0.00	0.78[f]	7.36[f]	46.0	80	−1.903	−1.703	−0.200
$C(=O)CH_3$	1.80 (0.01)	1.96	7.62 (0.01)	4.07 (0.05)	0.00	0.50[f]	11.18[f]	43.1	4	−0.602	−1.007	0.405
CH_3	2.38 (0.02)	2.38	9.92 (0.04)	5.25 (0.02)	0.00	−0.17[f]	5.65[f]	15.0	20	−1.301	−1.039	−0.262
OCH_3	2.10 (0.08)	2.10	9.96 (0.03)	4.84 (0.03)	0.00	−0.27[f]	7.87[f]	59.0	40	−1.602	−1.615	0.013
$CH_2N(CH_3)_2$	1.06 (0.03)	2.37[g]	9.46 (0.06)	4.47 (0.12)	8.66 (0.03)	0.01[f]	18.74[f]	58.1	40	−1.602	−2.104	0.502
$CH_2N(C_2H_5)_2$	1.41 (0.08)	4.41[g]	9.19 (0.01)	4.48 (0.12)	9.32 (0.12)	0.01[h]	28.04[i]	86.1	40	−1.602	−1.423	−0.179
CH_2OH	1.00 (0.01)	1.00	9.53 (0.04)	4.93 (0.04)	0.00	0.00[f]	7.19[f]	31.0	100	−2.000	−2.048	0.048
CH_2CN	1.81 (0.08)	1.82	9.25 (0.02)	4.30 (0.06)	0.00	0.01[f]	10.11[f]	40.0	60	−1.778	−1.679	−0.099
$CH_2CO_2CH_3$	1.59 (0.03)	1.59	9.46 (0.05)	4.72 (0.04)	0.00	−0.07[j]	16.06[k]	73.1	800	−2.903	−2.561	−0.342
$CH_2CO_2C_2H_5$	2.26 (0.01)	2.27	9.40 (0.03)	4.72 (0.03)	0.00	−0.07[j]	25.36[l]	87.1	800	−2.903	−2.951	0.048
I	3.27 (0.05)	3.29	8.31 (0.07)	4.43 (0.08)	0.00	0.18[f]	13.94[f]	126.9	4	−0.602	−0.671	0.069
CHO	1.63 (0.01)	2.07	7.01 (0.04)	4.42 (0.08)	0.00	0.42[f]	6.88[f]	29.0	6	−0.778	−0.602	−0.176

[a] All determinations were done in triplicate; values in parentheses indicate standard deviations. [b] Values corrected for ionization (see Experimental Section). [c] pK_a values were determined at 10^{-3} M. [d] Antibacterial testing was performed on HCl salts; MIC (M) data expressed as 10^{-6} M. [e] Log 1/MIC (M) calculated values were obtained from eq 13. [f] Reference 10. [g] $\alpha_{NO_2} = 0.945$; $\alpha_{CH_2NMe_2} = 0.954$; $\alpha_{CH_2NEt_2} = 1.01$; since $\alpha_{CH_2NEt_2}$ was calculated to be greater than 1, we assumed $\alpha_{CH_2NEt_2} = 0.999$ for the calculation of log P_{cor}. [h] Value was assumed to be equal to that for 5-CH_2NMe_2.[10] [i] $MR_{CH_2NEt_2} = MR_{CH_2NMe_2} (18.74) + 2 \times [MR_{C_2H_5} (10.30) - MR_{CH_3} (5.65)] = 28.04$. [j] Value was assumed to be equal to that for 5-CH_2CO_2H.[10] [k] $MR_{CH_2CO_2Me} = MR_{CO_2Me} (12.87) + [MR_{CH_2NMe_2} (18.74) - MR_{NMe_2} (15.55)] = 16.06$. [l] $MR_{CH_2CO_2Et} = MR_{CH_2CO_2Me} (16.06) + 2 \times [MR_{C_2H_5} (10.30) - MR_{CH_3} (5.65)] = 25.36$.

Table II. Correlation Equations of Minimum Inhibitory Concentrations (M) of a Series of 5-Substituted 8-Hydroxyquinolines against *S. mutans* No. 6715 with Lipophilic, Electronic, and Steric Parameters

Eq no.	Equation	n	s	r	r^2
1	log 1/MIC = 0.259 (±0.508) log P − 2.001 (±1.204)	14	0.846	0.305	0.093
2	log 1/MIC = 0.916 (±1.790) σ − 1.543 (±0.531)	14	0.845	0.306	0.094
3	log 1/MIC = −0.166 (±0.441) pK_a^{OH} + 0.022 (±3.916)	14	0.864	0.231	0.053
4	log 1/MIC = 0.331 (±1.146) pK_a^N + 0.054 (±5.200)	14	0.874	0.179	0.032
5	log 1/MIC = −0.008 (±0.016) mol wt − 1.019 (±0.948)	14	0.844	0.311	0.097
6	log 1/MIC = −0.058 (±0.058) MR − 0.758 (±0.818)	14	0.754	0.528	0.279
7	log 1/MIC = −0.055 (±0.062) MR − 0.109 (±0.404) pK_a^{OH} + 0.173 (±3.552)	14	0.775	0.549	0.302
8	log 1/MIC = 0.053 (±0.061) MR + 0.627 (±1.667) σ − 0.882 (±0.900)	14	0.764	0.567	0.321
9	log 1/MIC = −0.059 (±0.016) MR − 0.394 (±0.842) pK_a^N + 1.040 (±4.029)	14	0.762	0.570	0.324
10	log 1/MIC = −0.101 (±0.045) MR + 0.655 (±0.345) log P − 1.667 (±0.719)	14	0.489	0.850	0.722
11	log 1/MIC = −0.106 (±0.038) MR + 0.702 (±0.297) log P − 0.591 (±0.560) pK_a^N + 0.962 (±2.565)	14	0.412	0.906	0.821
12	log 1/MIC = −0.102 (±0.034) MR + 0.773 (±0.273) log P − 0.284 (±0.202) pK_a^{OH} + 0.596 (±1.698)	14	0.365	0.927	0.860
13	log 1/MIC = −0.100 (±0.028) MR + 0.788 (±0.227) log P + 1.362 (±0.702) σ − 2.120 (±0.508)	14	0.303	0.950	0.903
14	log 1/MIC = −0.054 (±0.054) MR + 0.491 (±0.509) π_{Bz} + 0.984 (±1.510) σ − 0.995 (±0.799)	14	0.662	0.732	0.536
15	log 1/MIC = −0.063 (±0.067) MR + 0.308 (±0.764) π_{Ph} + 0.473 (±1.745) σ − 0.832 (±0.927)	14	0.771	0.610	0.372
16	log 1/MIC = −0.094 (±0.033) MR + 0.760 (±0.378) log P + 1.626 (±0.976) σ − 2.111 (±0.853)	12	0.313	0.956	0.915
17	log 1/MIC = −0.075 (±0.044) MR + 0.441 (±0.340) π_{Bz} + 2.355 (±1.292) σ − 0.794 (±0.571)	12	0.413	0.923	0.851
18	log 1/MIC = 0.096 (±0.058) MR + 0.368 (±0.542) π_{Ph} + 1.843 (±1.628) σ − 0.542 (±0.715)	12	0.526	0.871	0.759

Table III. Squared Correlation Coefficients

	pK_a^{OH}	pK_a^N	σ	MR	Mol wt
log P	0.116	0.009	0.126	0.258	0.192
pK_a^{OH}		0.560	0.939	0.025	0.000
pK_a^N			0.616	0.004	0.034
σ				0.039	0.005
MR					0.534

$_{(\alpha=0.025)}$ = 6.94 for eq 12; and $F_{1,10}$ = 18.68, $F_{1,10(\alpha=0.01)}$ = 10.04 for eq 13. Equation 13 satisifes the F test at the 99% level and accounts for 90% of the variance of the data.

Equations 11–13 show that all three parameters (lipophilic, electronic, and steric) are important for determining activity. Examination of eq 13 shows a positive coefficient for log P and indicates that increased lipophilicity results in increased activity. The positive coefficient for the electronic term (σ) suggests that electron-withdrawing substituents in the 5 position improve the activity against *S. mutans* No. 6715 for this series of 8-hydroxyquinolines. The negative coefficient for MR indicates that bulky substituents in the 5 position will result in decreased activity.

The calculated 1/MIC (M) values shown in Table I were derived from eq 13, and there is a reasonable agreement between observed and calculated values.

Because of the uncertainty of the interrelationship between electronic factors and solubility in the 5-substituted 8-hydroxyquinolines, we used experimentally determined log P values for the lipophilic parameter. It has been noted in other heteroaromatic systems (e.g., benzothiadiazines)[15] that literature π values do not correlate well with experimentally determined log P values. This was also found with the compounds of this study (see eq 13–18 in Table II); the most notable discrepancies occur with the 5-nitro and 5-diethylaminomethyl derivatives (see Table V).

Equations 13–18 (Table II) are three-parameter (lipophilic, MR, and σ) equations and allow one to compare the effects of using observed log P, π obtained from substituted phenols (π_{Ph}), and π derived from substituted benzenes (π_{Bz}) for the lipophilic parameter. Equations 14 and 15 (in which all 14 compounds were included) show that neither π_{Ph} nor π_{Bz} gave better correlations than were obtained using observed log P values.

The 5-nitro and 5-diethylaminomethyl substituents showed the greatest deviation in π values (π_{obsd}) calculated from observed log P values from π_{Ph} or π_{Bz} (Table V). Elimination of the two compounds with these substituents from the regression analysis gave eq 16–18 (Table II). While good correlations were obtained with the literature π values (eq 17 and 18), the best correlation is still obtained with the experimental log P values (eq 16). A priori, one would expect π_{Ph} to give a better correlation than π_{Bz} for the compounds of this study. However, it was noted that π_{Bz} gave the better correlation (compare eq 14, 15, 17, and 18). Equations of Table IV show the correlations between these three different lipophilic parameters (π_{obsd}, π_{Bz}, and π_{Ph}). Even with 12 data points (without the two extreme points, 5-nitro and 5-diethylaminomethyl) the correlation between π_{obsd} and π_{Bz} or π_{Ph} is poor (eq 3 and 4 of Table IV). Equation 5 shows a similar poor correlation between the two literature values (π_{Bz} and π_{Ph}). It has been shown[16] that the deviation between π derived from the phenol system and that derived from the benzene system correlates well with σ (r = 0.94). Thus the reference system used for the determination of π is important; this observation supports the desirability of using experimentally determined log P values for the compounds of this study.[17]

Table IV. Correlations between Lipophilic Parameters of Table V

Eq no.	Equation	n	s	r	r^2
1	$\pi_{obsd} = 0.663\ (\pm 0.821)\ \pi_{Ph} + 0.030\ (\pm 0.588)$	14	0.933	0.453	0.205
2	$\pi_{obsd} = 1.113\ (\pm 0.665)\ \pi_{Bz} + 0.180\ (\pm 0.420)$	14	0.721	0.725	0.525
3	$\pi_{obsd} = 0.829\ (\pm 0.655)\ \pi_{Ph} + 0.104\ (\pm 0.490)$	12	0.692	0.666	0.443
4	$\pi_{obsd} = 1.019\ (\pm 0.520)\ \pi_{Bz} + 0.295\ (\pm 0.352)$	12	0.544	0.810	0.656
5	$\pi_{Ph} = 0.845\ (\pm 0.390)\ \pi_{Bz} + 0.250\ (\pm 0.246)$	14	0.422	0.807	0.650

Table V. Comparison of Observed π Values with Literature Values

Compd no.	5-Substituent	π_{obsd}[a]	π_{Bz}	$\lvert\Delta\pi_{Bz}\rvert$[b]	π_{Ph}	$\lvert\Delta\pi_{Ph}\rvert$[c]
1	H	0.00	0.00[d]	0.00	0.00[e]	0.00
2	Cl	0.99	0.71[d]	0.28	0.93[e]	0.06
3[f]	NO_2	−1.84	−0.28[d]	1.56	0.50[e]	2.34
4	$COCH_3$	0.01	−0.55[d]	0.56	−0.11[e]	0.12
5	CH_3	0.43	0.56[d]	0.13	0.48[e]	0.05
6	OCH_3	0.15	−0.02[d]	0.17	−0.12[e]	0.27
7	CH_2NMe_2	0.42	−0.15[d]	0.57	−0.44[g]	0.86
8[f]	CH_2NEt_2	2.46	0.85[h]	1.61	0.56[i]	1.90
9	CH_2OH	−0.95	−1.03[d]	0.08	−1.26[e]	0.31
10	CH_2CN	−1.13	−0.57[d]	0.44	0.12[j]	0.25
11	CH_2CO_2Me	−0.36	−0.30[k]	0.06	0.19[l]	0.55
12	CH_2CO_2Et	0.32	0.70[m]	0.38	1.19[n]	0.87
13	I	1.34	1.12[d]	0.22	1.46[e]	0.12
14	CHO	0.12	−0.65[d]	0.77	0.33[o]	0.21

[a] Calculated from log P_{cor} values of Table I. [b] $\lvert\Delta\pi_{Bz}\rvert = \lvert\pi_{obsd} - \pi_{Bz}\rvert$. [c] $\lvert\Delta\pi_{Ph}\rvert = \lvert\pi_{obsd} - \pi_{Ph}\rvert$. [d] Reference 10. [e] T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964). [f] These values were not used for deriving eq 16-18. [g] $\pi_{Ph}^{CH_2NMe_2} = \log P^{C_6H_5CH_2NMe_2}$ (1.98) + [log $P^{p\text{-}HO\text{-}C_6H_4\text{-}NH_2}$ (0.04) − log $P^{C_6H_5NH_2}$ (0.98)] − log $P^{C_6H_5OH}$ (1.48) = −0.44. [h] $\pi_{Bz}^{CH_2NEt_2} = \pi_{Bz}^{CH_2NMe_2}$ (−0.15) + 2 × CH_2 (2 × 0.5) = 0.85. [i] $\pi_{Ph}^{CH_2NEt_2} = \pi_{Ph}^{CH_2NMe_2}$ (−0.44) + 2 × CH_2 (2 × 0.5) = 0.56. [j] $\pi_{Ph}^{CH_2CN} = \log P^{p\text{-}HO\text{-}C_6H_4\text{-}CN}$ (1.60) + [log $P^{C_6H_5CH_2CN}$ (1.56) − log $P^{C_6H_5CN}$ (1.56)] − log $P^{C_6H_5OH}$ (1.48) = 0.12. [k] $\pi_{Bz}^{CH_2CO_2Me} = \log P^{C_6H_5CH_2CO_2Me}$ (1.83) − log $P^{C_6H_6}$ (2.13) = −0.3. [l] $\pi_{Ph}^{CH_2CO_2Me} = \log P^{p\text{-}HO\text{-}C_6H_4CO_2Me}$ (1.96) + [log $P^{C_6H_5CH_2CO_2Me}$ (1.83) − log $P^{C_6H_5CO_2Me}$ (2.12)] − log $P^{C_6H_5OH}$ (1.48) = 0.19. [m] $\pi_{Bz}^{CH_2CO_2Et} = \pi_{Bz}^{CH_2CO_2Me}$ (−0.3) + 2 × CH_2 (2 × 0.5) = 0.7. [n] $\pi_{Ph}^{CH_2CO_2Et} = \pi_{Ph}^{CH_2CO_2Me}$ (0.19) + 2 × CH_2 (2 × 0.5) = 1.19. [o] $\pi_{Ph}^{CHO} = \log P^{o\text{-}HO\text{-}C_6H_5\text{-}CHO}$ (1.81) − log $P^{C_6H_5OH}$ (1.48) = 0.33.

In conclusion, we have found a high inverse correlation between antibacterial activity against the plaque-forming organism *S. mutans* No. 6715 with the size of the substituents in the 5 position of a series of 5-substituted 8-hydroxyquinolines. As single parameters, lipophilicity or electronic terms are not so important, but the combination of these two (e.g., log P and σ) is nearly as important as MR in accounting for the variance of the data; r^2 for a two-parameter equation with log P and σ is 0.29 (not shown in Table II) compared to $r^2 = 0.28$ for the single-parameter equation with MR (eq 6 of Table II). From these correlations, the most potent inhibitors of *S. mutans* No. 6715 would be predicted to contain a relatively small 5-substituent that is lipophilic and electron withdrawing. The fact that compounds **1** and **2** show equally high activity is well explained by this conclusion. 8-Hydroxyquinoline (**1**) has no steric bulk at position 5 and the 5-chloro group in compound **2** compensates for its bulk by proper contributions from lipophilicity and electron withdrawal.

Experimental Section

Materials. The following compounds were commercially available from the indicated sources: 1–3 (Aldrich); 4 (Alfred Bader). Compounds **5**–**14**[7,10] were prepared as previously described.

Antibacterial Activity. To 7.8 ml of sterile trypticase broth, 1.0 ml of an aqueous solution of the hydrochloride salt of the test compound and 1.0 ml of 50% sterile sucrose solution were added. The media were inoculated with 0.20 ml of a 24-h culture of *S. mutans* No. 6715, a pure strain of plaque-forming bacteria isolated at and made available by the National Institute of Dental Research. This mixture was incubated under anaerobic conditions (BBL-Gaspak, BBL, Division of Bioquest, Cockeyville, Md.) at 37 °C. Bacterial growth was determined after 24 and 48 h spectrophotometrically using a Coleman Jr. spectrophotometer. Once the range for 100% inhibition and 0% inhibition was established for each compound, a series of dilutions was made and each dilution was run in sets of five. The set of tubes containing the smallest amount of drug for which % T readings were above 60 % T after 24 h was considered the MIC of that particular agent. Repeated determinations gave the same MIC values.

Partition Coefficients. The compounds were partitioned between 1-octanol saturated with distilled water and distilled water saturated with octanol. Usually, 50–150-ml portions of octanol and water were used. In partitioning these compounds, gentle shaking for 90 min was carried out at room temperature (25 ± 5 °C). The volume ratio of the two phases and the amount of sample were chosen so that, in most cases, the absorbance of the sample from the test layer after partitioning had a value between 0.2 and 0.9 using a 1-cm cell. Only the concentration of the sample in one layer was determined and in the other was obtained by difference. Analyses of the concentrations of the partitioned substances were made using a Beckman DB-G recording spectrophotometer. The partition coefficient was calculated as $P = C_{octanol}/C_{water}$. Each determination was done in at least triplicate using different amounts of sample and the average value for log P was reported. The log P_{cor} values were calculated from the following equation.[18]

$$P_{cor} = \frac{[C]_{octanol}}{[C]_{water} \times (1 - \alpha)}$$

The term, α, is the fraction of drug ionized at the pH at which partitioning was carried out. The Henderson–Hasselbalch equation was used to determine this value.

Ionization Constants.[19] Ionization constants were determined potentiometrically using an Orion four-place electronic digital pH meter with a full-range Corning combination electrode. A water-jacketed 200-ml beaker was used with a constant temperature circulating water bath set to control the temperature of the test solution at 25 ± 0.1 °C. Test solutions were prepared at a concentration of 10^{-3} M with varying amounts of ethanol (95%) and water. A volume of 100 ml of test solution was used

for each titration. A magnetic stirrer was used for mixing and nitrogen was bubbled through the test solution. Both the stirring and nitrogen flow were stopped to permit pH readings to stabilize after each aliquot of 0.25 ml of titrant was added. The electrode was cleaned periodically by alternate soaking in dilute NaOH and dilute HCl for 5–10-min periods followed by thorough rinsing with distilled water. To titrate the acidic function, 0.02 N KOH was used, and for the basic function, 0.02 N HCl was used. Distilled, deionized water was boiled and used for all dilutions. The use of 10-ml burets permitted aliquots of 0.25 ml of titrant to be added accurately and the KOH buret was fitted with a $Ca(OH)_2$ tube to eliminate CO_2. Between five and ten pK_a readings were used to obtain the average ionization constant. All values determined in ethanol were normalized to 100% aqueous solutions.

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Quantitative Structure–Activity Relationships of Antimalarial and Dihydrofolate Reductase Inhibition by Quinazolines and 5-Substituted Benzyl-2,4-diaminopyrimidines[1]

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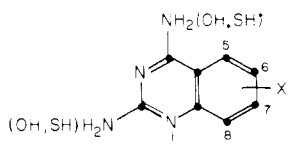
A quantitative structure–activity relationship (QSAR) for the inhibition of dihydrofolate reductase from *S. faecium* by quinazolines has been formulated. This is compared with a QSAR for inhibition of *E. coli* dihydrofolate reductase by 2,4-diamino-5-benzylpyrimidines. The QSAR for inhibition of bacterial enzyme is compared with QSAR for mammalian enzyme inhibition. A QSAR has also been formulated for the antimalarial action of quinazolines against *P. berghei* in mice. The antimalarial QSAR is consistent with that of the in vitro bacterial study.

We have been interested in the quantitative structure–activity relationships (QSAR) of enzyme inhibitors and their use as starting points in drug development.[2-6] Dihydrofolate reductase inhibition is of particular interest since such inhibitors have already proved to be of value as antibacterial, antimalarial, and antitumor agents. The success of dihydrofolate reductase inhibitors as antimicrobial agents depends on the great differences in this enzyme from mammalian and microbial sources.[7] We are concerned in the present report with the comparative QSAR of quinazolines (I) and benzylpyrimidines (II) acting as inhibitors of mammalian and bacterial dihydrofolate



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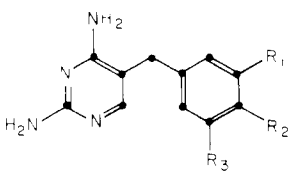

II

reductase. The QSAR of the isolated enzymes is compared with the antimalarial action of the quinazolines.

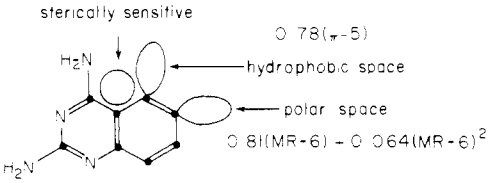

Figure 1.

Using the results of Hynes et al., the QSAR of eq 1 was obtained for enzyme from rat liver.[6] We have now formulated eq 2 for bacterial (*Streptococcus faecium*) enzyme from other data from the laboratories of Freisheim and Hynes.[8c] Equation 3, based on data for antimalarial activity, is from the work of Elslager et al.[9] The necessary biological activity and physicochemical parameters are given in the section on Method.

Method. π and MR constants were taken from our compilation[10] or calculated as before.[3,5] π constants were determined for seven new groups by measuring the following log P values.