Articles

Inhibition of Rat Hepatic Microsomal Aminopyrine *N*-Demethylase Activity by Benzimidazole Derivatives. Quantitative Structure-Activity Relationships

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Eighty-two benzimidazole derivatives have been prepared and tested for the ability to inhibit cytochrome P-450 mediated enzyme activity (aminopyrine *N*-demethylase) from phenobarbitone-induced rat hepatic microsomes. Using physicochemical parameters and multiple regression analysis, we derived a quantitative structure-activity relationship (QSAR) that describes up to 87% of the data variance in terms of hydrophobic and electronic effects and the molar refractivity of the substituent in the 2-position of the benzimidazole ring.

The hepatic microsomal mixed-function oxidase (MFO) system is active in the oxidative metabolism of xenobiotics to more polar substances.[3] The system is also implicated in the activation of polycyclic aromatic hydrocarbons to proximate carcinogens.[4] Several classes of compounds, including methylenedioxyphenyl compounds,[5] imidazoles and related compounds,[6-11] SKF 525-A,[12] benzimidazoles and related compounds,[13-15] and 2,6- and 2,4-dihydroxyphenylalkyl ketones,[16] are established inhibitors of the MFO activities of various species.

Although the structure-activity relationships of some of these groups of inhibitors have been discussed, relatively few attempts have been made to quantify these relationships. This paper presents the results of QSAR studies of a series of substituted benzimidazoles and identifies some of the physicochemical parameters that are important for the inhibition of aminopyrine *N*-demethylase (APDM) activity from phenobarbitone-induced rat liver microsomes.

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Results and Discussion

Table I shows the inhibitory potency of 82 benzimidazole derivatives toward APDM activity in hepatic microsomes from phenobarbitone-induced rats. All compounds inhibited APDM activity with I_{50} values ranging from 1.1×10^{-5} M (compounds **70** and **71**) to 125×10^{-5} M (compound 17). Several equations were evaluated in attempts to correlate inhibitory activity with physicochemical parameters describing the hydrophobic, electronic, and steric character of the compounds (Table II).

Equations 7-11 (Table II) were evaluated from the inhibition data of all 82 compounds. However, it was apparent from these data that six compounds (**77-82**) were consistent outliers of the correlation analyses, and their exclusion from the analyses resulted in an improvement in the correlations. This can be clearly seen by comparison of the correlations in eq 7-11 with those in the corresponding eq 1-6. Thus, when the r^2 value of eq 1 is compared with that of eq 7, a 7% improvement is shown, and eq 3 and 5 account for an additional 14% of the data variance over eq 8 and 10, respectively. The omission of compounds **77-82** is justified on the basis that they are either polycyclic aromatics (**81** and **82**) or compounds with relatively large carbocylic substituents in the 4- or 5-positions of the ring (**77-80**). Since these compounds consistently exhibited greater potency than predicted by the regression equations, it appears that they possess additional properties that favor binding to and/or interaction with the active center of cytochrome P-450.

The fundamental importance of hydrophobicity to inhibitory potency is clearly demonstrated by eq 1 (Table II), which successfully explains 65% of the data variance in terms of log P alone. This is not entirely unexpected, since the primary function of the MFO system is to convert lipophilic molecules to more hydrophilic metabolites, and inhibitors, like substrates, must presumably possess sufficient lipophilic character to penetrate to and interact with the active center of cytochrome P-450. In previous studies with a series of 1-alkylimidazoles,[8] an apparent parabolic relationship between π and inhibitory potency toward aldrin epoxidation was suggested to indicate the existence of a hydrophobic patch of limited size in the vicinity of cytochrome P-450. In the present study the inclusion of a $(\log P)^2$ term into the regression analysis led to a statistically significant improvement (compare eq 1 and 2; Table II) in the correlation. Even though this inclusion

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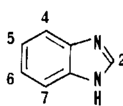
Table I. Inhibition of Aminopyrine *N*-Demethylase Activity by Benzimidazole Derivatives and Parameters Used in the Derivation of Regression Equations



no.	groups	pI_{50}[a] obsd	pI_{50}[a] calcd[b]	$\lvert\Delta pI_{50}\rvert$	log P[c]	MR_2[d]	MR_{2^*}[d]	$\Sigma\sigma$[e]
1[f]		3.01	3.21	0.20	1.38	0.103	0	0
2[f]	2-CH_3	2.97	3.26	0.29	1.43	0.565	0	−0.17
3[f]	2-C_2H_5	3.17	3.51	0.34	1.93	1.030	0	−0.15
4[f]	2-*n*-C_3H_7	3.52	3.75	0.23	2.43	1.496	0	−0.13
5[f]	2-*i*-C_3H_7	3.27	3.74	0.47	2.41	1.498	0	−0.15
6	2-c-C_3H_5	3.60	3.58	0.02	2.02	1.353	0	−0.21
7[f]	2-*n*-C_4H_9	3.92	3.94	0.02	2.93	1.959	0	−0.16
8[f]	2-*i*-C_4H_9	3.80	3.95	0.15	2.91	1.963	0	−0.15
9	2-*tert*-C_4H_9	3.52	3.92	0.40	2.86	1.962	0	−0.20
10[f]	2-*n*-C_5H_{11}	4.30	4.13	0.17	3.43	2.425	0	−0.16
11[f]	2-*n*-C_6H_{13}	4.49	4.30	0.19	3.93	2.890	0	−0.16
12[f]	2-c-C_6H_{11}	4.04	4.15	0.11	3.39	2.669	0	−0.22
13[f]	2-*n*-C_7H_{15}	4.59	4.44	0.15	4.43	3.350	0	−0.16
14[f]	2-*n*-C_8H_{17}	4.54	4.57	0.03	4.93	3.820	0	−0.16
15[f]	2-*n*-$C_{11}H_{23}$	4.82	4.82	0.00	6.43	5.215	0	−0.16
16[f]	2-*n*-$C_{13}H_{27}$	4.64	4.88	0.24	7.43	6.145	0	−0.16
17	2-CH_3, 5-$NHCOCH_3$	2.90	3.01	0.11	0.61	0.565	0	−0.07
18	2-*n*-C_5H_{11}, 5-$NHCOCH_3$	3.80	4.01	0.21	2.61	2.425	0	−0.05
19	2-CH_3, 5-NHCO-*n*-C_4H_9	3.38	3.50	0.12	2.11	0.565	0	−0.07
20	2-CH_3, 4-$NHCOCH_3$, 6-Cl	3.51	3.37	0.14	1.27	0.565	0	0.24
21	2-CH_3, 5-$NHCOC_6H_5$	3.52	3.67	0.15	3.19	0.565	0	−0.26
22	2-CH_3, 5-NO_2	3.66	3.64	0.02	1.69	0.565	0	0.58
23	2-CH_3, 4-NO_2, 6-Cl	4.00	4.09	0.09	3.06	0.565	0	0.88
24	4-NO_2, 6-Cl	3.89	4.05	0.16	3.01	0.103	0	1.05
25	5-NO_2	3.51	3.59	0.08	1.64	0.103	0	0.75
26	4-NO_2	3.33	3.70	0.37	2.01	0.103	0	0.75
27	5-Cl	3.80	3.62	0.18	2.38	0.103	0	0.30
28	2-CH_3, 5-Cl	3.82	3.66	0.16	2.43	0.565	0	0.13
29	5,6-Cl_2	4.09	3.91	0.18	3.17	0.103	0	0.60
30	2-CH_3, 5,6-Cl_2	4.09	3.94	0.15	3.22	0.565	0	0.43
31	4,6-Cl_2	4.27	3.92	0.35	3.26	0.103	0	0.60
32	2-CH_3, 4,6-Cl_2	4.00	3.96	0.04	3.31	0.565	0	0.43
33	5-F	3.36	3.49	0.13	2.03	0.103	0	0.20
34	2-(2'-pyridyl)	4.19	4.02	0.17	2.38	2.303	2.303	0.17
35	2-(3'-pyridyl)	3.70	3.84	0.14	1.70	2.303	2.303	0.17
36	2-CH_2(1'-naphthyl)	4.60	4.74	0.14	4.80	4.542	4.542	−0.09
37	2-CH_2(2'-naphthyl)	4.74	4.74	0.00	4.79	4.542	4.542	−0.09
38	2-$CH_2C_6H_5$, 5-Cl	4.64	4.52	0.12	4.44	3.001	3.001	0.21
39	2-$CH_2C_6H_5$, 5,6-$(CH_3)_2$	4.27	4.33	0.06	5.23	3.001	3.001	−0.35
40	2-$CH_2C_6H_5$, 4,6-Cl_2	4.82	4.67	0.15	5.32	3.001	3.001	0.51
41	2-CH_2Cl	3.85	3.38	0.47	1.15	1.068	0	0.12
42	2-CH_2NH_2	3.34	2.83	0.51	−0.06	0.909	0	0
43	2-CF_3	3.59	3.80	0.21	2.39	0.502	0	0.54
44[f]	5-CH_3	3.24	3.32	0.08	1.89	0.103	0	−0.12
45	4-CH_3	3.28	3.31	0.03	1.86	0.103	0	−0.12
46[f]	2,5-$(CH_3)_2$	3.32	3.37	0.05	1.94	0.565	0	−0.29
47[f]	2-C_2H_5, 5-CH_3	3.57	3.60	0.03	2.44	1.030	0	−0.27
48	2-*n*-C_7H_{15}, 5-CH_3	4.38	4.43	0.05	4.94	3.355	0	−0.27
49	5,6-$(CH_3)_2$	3.74	3.39	0.35	2.35	0.103	0	−0.26
50	2,5,6-$(CH_3)_3$	3.43	3.43	0.00	2.40	0.565	0	−0.43
51	2-C_2H_5, 5,6-$(CH_3)_2$	3.74	3.64	0.10	2.90	1.030	0	−0.43
52	2-*n*-C_3H_7, 5,6-$(CH_3)_2$	3.96	3.83	0.13	3.40	1.496	0	−0.43
53	2-*n*-C_4H_9, 5,6-$(CH_3)_2$	4.26	3.99	0.27	3.90	1.959	0	−0.43
54	2-*n*-C_7H_{15}, 5,6-$(CH_3)_2$	4.35	4.37	0.02	5.40	3.355	0	−0.43
55[f]	2,4,5-$(CH_3)_3$	3.60	3.43	0.17	2.36	0.565	0	−0.41
56	2-C_6H_5	4.26	4.11	0.15	2.87	2.536	2.536	−0.01
57	2-(±)-$CH(OH)C_6H_5$	3.80	3.86	0.06	1.47	3.152	3.152	−0.03
58[f]	2-$CH_2C_6H_5$	4.04	4.28	0.24	3.46	3.001	3.001	−0.09
59	2-$(CH_2)_2C_6H_5$	4.41	4.43	0.02	3.96	3.465	3.465	−0.12
60	2-$(CH_2)_3C_6H_5$	4.72	4.58	0.14	4.46	3.929	3.929	−0.12
61	2-$(CH_2)_4C_6H_5$	4.82	4.70	0.12	4.96	4.393	4.393	−0.12
62	2-$CH_2OC_6H_5$	4.16	4.23	0.07	2.66	3.219	3.219	0.04
63	2-$(CH_2)_2OC_6H_5$	4.60	4.36	0.24	3.16	3.684	3.684	−0.12
64	2-$CH_2OC_6H_3$-3',5'-$(CH_3)_2$	4.59	4.58	0.01	3.50	4.143	4.143	0.04
65	2-$CH_2OC_6H_3$-3',4'-$(CH_3)_2$	4.59	4.56	0.03	3.40	4.143	4.143	0.04
66	2-$CH_2OC_6H_2$-3',5'-$(CH_3)_2$-4-Cl	4.52	4.79	0.27	4.43	4.643	4.643	0.04
67	2-$CH_2OC_6H_4$-4'-C_2H_5	4.17	4.59	0.42	3.59	4.146	4.146	0.04
68	2-$CH_2OC_6H_4$-4'-Br	4.66	4.59	0.07	3.77	4.004	4.004	0.04
69	2-$CH_2OC_6H_4$-2'-Br	4.92	4.55	0.37	3.51	4.004	4.004	0.04

Table I (*Continued*)

no.	groups	pI_{50} [a] obsd	pI_{50} calcd [b]	$\lvert\Delta pI_{50}\rvert$	log P [c]	MR_2 [d]	MR_{2*} [d]	$\Sigma\sigma$ [e]
70	$2\text{-}CH_2OC_6H_3\text{-}2',4'\text{-}Br_2$	4.96	4.82	0.14	4.52	4.789	4.789	0.04
71	$2\text{-}CH_2OC_6H_3\text{-}2',6'\text{-}Br_2$	4.96	4.82	0.14	4.42	4.789	4.789	0.04
72	$2\text{-}CH_2OC_6H_4\text{-}3'\text{-}CH_3$	4.54	4.42	0.12	3.14	3.681	3.681	0.04
73	$2\text{-}CH_2OC_6H_4\text{-}4'\text{-}CH_3$	4.35	4.42	0.07	3.12	3.681	3.681	0.04
74	$2\text{-}CH_2OC_6H_4\text{-}2'\text{-}Cl$	4.57	4.46	0.11	3.33	3.719	3.719	0.04
75	$2\text{-}CH_2OC_6H_4\text{-}4'\text{-}Cl$	4.60	4.50	0.10	3.59	3.719	3.719	0.04
76	$2\text{-}CH_2OC_6H_3\text{-}2',3'\text{-}Cl_2$	4.55	4.69	0.14	4.26	4.219	4.219	0.04
77 [g]	$4\text{-}NHCOC_6H_5$, 6-Cl	4.57	3.66	0.91	2.68	0.103	0	0.22
78 [g]	$5\text{-}COC_6H_5$	4.70	3.67	1.03	2.43	0.103	0	0.39
79 [g]	$5\text{-}OC_2H_5$	4.17	3.34	0.83	1.88	0.103	0	−0.07
80 [g]	$2\text{-}CH_3$, $5\text{-}OC_2H_5$	4.25	3.38	0.87	1.93	0.565	0	−0.24
81 [g]	naphtho[1,2-*d*]imidazole	4.27	3.61	0.66	2.72	0.103	0	0.08
82 [g]	phenanthro[9,10-*d*]imidazole	4.52	3.86	0.66	4.06	0.103	0	0.16

[a] pI_{50} = negative logarithm (base 10) of molar I_{50} value. [b] Calculated using eq 5 (Table II). [c] Log P values for compounds 1, 2, 25, 43, and 49 taken from ref 21. Log P values for other compounds were calculated from appropriately substituted aniline log P data (also from ref 21) using the additivity principle. Log P values were assumed to increment by $0.50/CH_2$ unit. In the case of 2-[(aryloxy)alkyl]benzimidazoles (62–76), log P values were calculated using available log P data for substituted phenols as appropriate.[21] [d] Molar refractivity values were taken from ref 22 and 23. When the additivity principle was employed, MR was assumed to increment by $0.565/CH_2$ unit. MR_2 and MR_{2*} were scaled by 0.1 to yield more manageable coefficients. [e] Since benzimidazole (when no 1-substituent is present) can exist in two tautomeric forms, $\Sigma\sigma$ was calculated as the average of the total substituent Hammett constants for both tautomers. The four carbocylic positions in benzimidazole were assigned Hammett constants as follows: 4, para; 5, meta; 6, para; 7, meta. A slightly, though not significantly, better fit was obtained when σ_{para} and not σ_{meta} constants were used for the 2-position substituent. [f] Data originally presented in ref 13. [g] The data for these compounds were used to derive eq 7–11 but not equations 1–6. These compounds were exposed as statistical outliers using the Student's t distribution ($p < 0.05$) of the unit normal deviate form of the residuals. Calculated pI_{50} values for these compounds were evaluated from eq 5 (Table II).

Table II. Parameter Coefficients of Regression Analyses for Aminopyrine *N*-Demethylase Inhibition by Benzimidazoles

eq [a,b]	log P	$(\log P)^2$	MR_2	MR_{2*}	$\Sigma\sigma$	intercept	r	s	F	r^2, %
1	0.335 (0.0283) [c]					2.982 (0.0962)	0.809	0.323	140	65
2	0.592 (0.0953)	−0.0372 (0.0132)				2.604 (0.163)	0.830	0.308	81	69
3	0.463 (0.0752)	−0.0430 (0.0101)	0.181 (0.0250)			2.680 (0.125)	0.905	0.236	109	82
4	0.385 (0.0771)	−0.168 (0.0103)		0.122 (0.0163)		2.851 (0.127)	0.908	0.233	113	82
5	0.440 (0.0652)	−0.0409 (0.0088)	0.207 (0.0222)		0.394 (0.0786)	2.664 (0.108)	0.931	0.204	115	87
6	0.386 (0.0750)	−0.0156 (0.0101)		0.121 (0.0159)	0.193 (0.0846)	2.835 (0.124)	0.915	0.226	91	84
7	0.318 (0.0300)					3.076 (0.101)	0.764	0.348	112	58
8	0.516 (0.0960)	−0.0449 (0.0131)	0.119 (0.0300)			2.724 (0.160)	0.827	0.307	56	68
9	0.422 (0.0948)	−0.0233 (0.0128)		0.103 (0.0199)		2.884 (0.157)	0.847	0.291	66	72
10	0.491 (0.0905)	−0.0426 (0.0124)	0.143 (0.0290)		0.369 (0.109)	2.717 (0.151)	0.852	0.288	51	73
11	0.418 (0.0927)	−0.0214 (0.0125)		0.102 (0.0195)	0.222 (0.104)	2.871 (0.153)	0.856	0.284	53	73

[a] Compounds 1–76 were used to calculate eq 1–6; compounds 1–82 were used to calculate eq 7–11. [b] Different parameter formats were compared using F ratios: $F_{1,73}$ = 8.1 (eq 1 and 2); $F_{1,72}$ = 51.8 (eq 3 and 2); $F_{1,72}$ = 55.6 (eq 4 and 2); $F_{1,71}$ = 25.4 (eq 5 and 3); $F_{1,71}$ = 5.6 (eq 6 and 4), (from tables, $F_{1,60}$ = 4.00). [c] Values in parentheses are standard deviations of parameter coefficients derived using multiple regression analysis.

of a $(\log P)^2$ term was statistically justified, no obvious parabolic relationship between pI_{50} and log P was observed. Thus, with the series of 2-alkylbenzimidazoles (2–16; Table I) inhibitory potencies increase with increasing chain length and appear to reach a maximum in compounds containing seven to eight carbon atoms; thereafter pI_{50}'s remain relatively constant through the 2-undodecyl compound (16). Although the latter compound is somewhat more potent than the 2-undecyl derivative (15), it is unlikely that this indicates the attainment of an optimal log P value. Further evidence for the absence of an obvious parabolic relationship is evident from the widely varying log P_0 values (7.96, 5.38, 11.45, 5.37, and 12.37) obtained by partial differentiation of eq 2–6, respectively. It is

Table III. Squared Correlation Matrices (r^2) for Collinearity among Variables in Equations 5 and 6

	Equation 5			
	log P	$(\log P)^2$	$\Sigma\sigma$	MR_2
log P	1.000	0.828	0.005	0.061
$(\log P)^2$		1.000	0.002	0.004
$\Sigma\sigma$			1.000	0.056
MR_2				1.000
	Equation 6			
	log P	$(\log P)^2$	$\Sigma\sigma$	MR_{2*}
log P	1.000	0.915	0.000	0.129
$(\log P)^2$		1.000	0.003	0.068
$\Sigma\sigma$			1.000	0.002
MR_{2*}				1.000

possible that the bilinear model of Kubinyi[17] may eventually be useful in describing the relationship between the hydrophobicity of benzimidazoles and their potency as inhibitors of APDM activity. However, at this stage, too few compounds with sufficiently large log P values have been prepared and tested to justify a bilinear analysis.

It has been established that many nitrogen-containing ligands, especially nitrogen heterocycles, can produce a type II spectral change with oxidized cytochrome P-450.[8,11,18] This spectral change is associated with a shift in the high spin/low spin equilibrium in ferric P-450 toward the low-spin component. It is widely held that this is due to direct heme binding caused by the nitrogen-containing molecule acting as the sixth axial ligand of ferric P-450. The current hypothesis of the mechanistic sequence in P-450 function suggests that an increase in the low-spin component prevents oxidation of substrate from occurring.[19] A close relationship between inhibition of aldrin epoxidation and type II binding has been observed for a series of 1-alkylimidazoles.[8]

Because of the apparent importance of type II binding in the mechanism of inhibition by certain nitrogen heterocycles, it was considered important to attempt to relate inhibitory potency to the electronic character of the benzimidazole imino nitrogen. Substituent electronic effects were described by the term $\sum\sigma$, as defined in the footnotes to Table I.

The inclusion of $\sum\sigma$ significantly increased r^2 (compare eq 5 and 3; Table II). The positive coefficients of $\sum\sigma$ in all equations suggest that electron-withdrawing groups enhance inhibitory effectiveness. It is possible, therefore, that withdrawal of electron density from the benzimidazole imino nitrogen promotes inhibition and results in a shorter heme iron to imidazole nitrogen ligand distance.

It is important to recognize that not all benzimidazoles give rise to the type II spectral changes in oxidized microsomes. Certain derivatives with bulky 2-substituents actually produce reverse type I or type I spectral changes.[14,20] Neither of these spectral types have been as well accounted for as the type II change but they are not thought to involve direct heme binding. Consequently, it must be concluded, on the basis of spectral evidence, that more than one mode of reversible inhibition of cytochrome P-450 by benzimidazoles may be involved in the present data set.

In view of the established importance of steric parameters in the inhibition of drug oxidation by several substituted imidazoles,[9] it was of considerable interest to evaluate whether the size of substituents in the 2-position of benzimidazole played any role in their inhibitory potency. For this purpose, the terms MR_2 and MR_{2*} (denoting, respectively, the molar refractivity of 2-substituents and the molar refractivity of any 2-substituent that contains an additional aromatic center) were used to model 2-substituent size. The inclusion of these parameters clearly led to improved correlations (compare eq 3 and 4 with eq 2; Table II) and indicates that increasing the size of the 2-substituent improves inhibitory potency.

A number of benzimidazoles that are included in this study contain large 2-substituents. The isomeric naphthylmethyl derivatives (**36** and **37**) are particularly potent inhibitors of APDM and contain bulky aromatic groupings that may be rotated. Other inhibitors in this category are the 2-[(aryloxy)alkyl]benzimidazoles (**62**–**76**), although the most potent compounds tested were molecules in which a lesser degree of rotation is possible in the aryl-containing side chain. These were the dibromo derivatives, **70** and **71**, which possess at least one bromine atom ortho to the ether oxygen in the 2-position. The possibility exists to prepare potent inhibitors that possess an even lower degree of flexibility than the 2-[(2′-bromoaryloxy)alkyl]benzimidazoles. Such an approach may obviate the possibility of bulky side chains being coordinated away from the substrate binding site, which could lead to less effective inhibition.

At present no information is available as to whether an optimal 2-substituent size exists for anti-APDM potency. The evaluation of candidate inhibitor molecules with larger 2-substituents may eventually provide statistically justified regression equations which include molar refractivity terms in a higher power (perhaps a squared MR term). In such a case, partial differentiation with respect to MR would lead to an estimate of the optimal size for 2-substituents for most effective anti-APDM inhibition.

In the context of the present QSAR analysis of the inhibition of APDM activity by derivatives of benzimidazole, it is concluded that inhibitor lipophilicity is of primary importance. This property determines the effective penetration of the inhibitor into the catalytic site of cytochrome P-450, and then electronic and steric factors modify the binding at and/or near the heme iron of the cytochrome. The importance of the molar refractivity term for the 2-substituent position in benzimidazoles indicates that the size of these substituents determines to a great extent the ability of the inhibitor to compete with the cytochrome P-450 substrate (in this case aminopyrine) for effective binding.

Of particular interest was the observation that outlier compounds were usually those with relatively large carbocyclic substituents in the 4- and 5-positions of benzimidazole. The preparation of further derivatives with large carbocyclic substituents may lead to a greater understanding of why compounds **77**–**82** were unusually potent. It may eventually be possible to account for these data in terms of specific physicochemical properties.

Experimental Section

Preparation of Microsomal Fraction. White, male Wistar rats (100–150 g) were given phenobarbitone (60, 75, 85, and 100 mg/kg ip) on consecutive days before sacrifice. The animals were killed by cerebral occlusion, and their livers were removed, rinsed with potassium phosphate buffer (0.1 M, pH 7.4), weighed, and homogenized (Waring blender) in 7 volumes of buffer. The homogenate was centrifuged at 1000g for 10 min and then at 9000g for 10 min. The resultant supernatant was then centrifuged at 105000g for 60 min. Microsomal pellets were resuspended in phosphate buffer so that 1 mL contained microsomal protein derived from 1 g of wet liver. Protein was determined by the method of Robinson and Hodgson.[24]

Aminopyrine *N*-Demethylase (APDM) Activity. The assay medium for APDM activity determinations contained aminopyrine (1.61 μmol), $MgCl_2$ (10 μmol), NADP (1.4 μmol), glucose 6-phosphate (15.6 μmol), glucose-6-phosphate dehydrogenase (1 unit), semicarbazide hydrochloride (25 μmol), and microsomal protein (5.6 mg) in potassium phosphate buffer (0.1 M, pH 7.4

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Table IV. Properties of Some Benzimidazole Derivatives

no.	yield,[a] %	mp,[b] °C	recrystn solvent [c]	formula	anal. [d]
9	70	227	A	$C_{11}H_{14}N_2$	C, H
18	80	203-206	B	$C_{14}H_{19}N_3O$	C, H, N
19	80	197-200	B	$C_{13}H_{17}N_3O$	C, H, N
20	75	250-251 [e]	C	$C_{10}H_{10}ClN_3O \cdot 0.5H_2O$	C, H, N
23	75	210	D	$C_8H_6ClN_3O_2$	C, H, N
28	82	195-198	D	$C_8H_7ClN_2$	C, H, N
36	41	232-235	E	$C_{18}H_{14}N_2$	C, H, N
37	44	202-204	E	$C_{18}H_{14}N_2$	C, H, N
40	85	220-221	F	$C_{14}H_{10}Cl_2N_2$	C, H, N
47	70	168-169	G	$C_{10}H_{12}N_2$	C, H, N
51	78	236	C	$C_{11}H_{14}N_2$	C, H, N
52	72	167	C	$C_{12}H_{16}N_2$	C, H
53	65	116	H	$C_{13}H_{18}N_2$	C, H
54	60	112-115	H	$C_{16}H_{24}N_2$	C, H
60	51	149-151	E	$C_{16}H_{16}N_2$	C, H, N
61	42	193-196	E	$C_{17}H_{18}N_2$	C, H, N
63	25	156-158	C	$C_{15}H_{14}N_2O$	C, H, N
65	66	165-166	F	$C_{16}H_{16}N_2O$	C, H, N
66	65	172-173	F	$C_{16}H_{15}ClN_2O$	C, H, N
67	68	188-189	C	$C_{16}H_{16}N_2O$	C, H, N
70	25	199-202	E	$C_{14}H_{10}Br_2N_2O$	C, H, N
71	12	176-179	E	$C_{14}H_{10}Br_2N_2O$	C, H, N
76	10	206-208	F	$C_{14}H_{10}Cl_2N_2O$	C, H, N
77	90	290-292	C	$C_{14}H_{10}ClN_3O$	C, H, N
78	20	143-145	G	$C_{14}H_{10}N_2O$	C, H, N
79	78	116	I	$C_9H_{10}N_2O$	C, H
80	76	137-140	I	$C_{10}H_{12}N_2O$	C, H

[a] Yields are presented for the final step of a reaction sequence. [b] Melting points were determined on a Reichert block calibrated against known standards. [c] A = ethanol; B = acetone–hexane; C = aqueous ethanol; D = acetone–light petroleum ether; E = ethyl acetate–cyclohexane; F = acetone–cyclohexane; G = ethyl acetate–light petroleum ether; H = methanol; I = toluene–hexane. [d] All analyses were within ±0.4% of calculated values. [e] Isolated as the hemihydrate.

to 3.0 mL). Incubation time was 15 min, and the extent of reaction was quantified according to the method of Nash,[25] based on the Hantzsch reaction. Control APDM activity was 4.9 ± 0.2 nmol of formaldehyde formed per milligram of protein per minute.

Inhibitors were added directly in dimethyl sulfoxide (100 μL) or 0.07 M HCl (100 μL). Dimethyl sulfoxide produced 30% inhibition of enzyme activity, but I_{50}'s for 2-*n*-butylbenzimidazole were identical whether the compound was added in Me_2SO or 0.07 M HCl. I_{50}'s were determined using seven inhibitor concentrations. Solvent was added to control incubations. I_{50} analyses were replicable to within ±10% of the stated mean values.

Chemistry. Compound **43** was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, and compounds **48** and **55** were supplied by Merck, Sharp and Dohme (Australia) Pty. Ltd. Biochemicals were purchased from Boehringer Mannheim (Australia) Pty. Ltd., and all other chemicals were analytical grade.

Chemical Syntheses. General Method for Substituted Benzimidazoles. The method of Pool et al.[26] was used for the general synthesis of substituted benzimidazoles. Compounds **1–16**, **22–28**, **34–39**, **41**, **42**, **44**, **46**, **47**, **49–54**, **56–61**, **78**, **81**, and **82** were prepared by this method. Compounds **29–33**, **40**, and **45** were prepared by the method of Pool et al. after prior reduction of an appropriately substituted nitroaniline derivative in a Parr hydrogenation apparatus using Raney nickel as catalyst and ethanol as solvent.

Spectral data (^1H NMR, MS, and IR) of all compounds were in agreement with their assigned structures, and melting point data for known compounds were consistent with observed values.

Amidobenzimidazoles. Compounds **17–21** and **77** were synthesized from a nitroaniline derivative already prepared using the method of Pool et al., as above. The example of compound **77** is considered. 6(5)-Chloro-4(7)-nitrobenzimidazole (5.1 mmol) was reduced in a Parr hydrogenator using Raney nickel as catalyst in ethanol. After the uptake of hydrogen had ceased, the reaction solution was filtered and the ethanol was removed under reduced pressure. The solid residue was dissolved in dry dimethylformamide (10 mL) containing 2 equiv of pyridine. Benzoyl chloride (10.2 mmol) was added dropwise, and the temperature was maintained below 10 °C. After standing overnight at room temperature, the mixture was diluted, neutralized with solid sodium bicarbonate, and then extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate, and then the chloroform was removed under reduced pressure. The solid residue recrystallized from ethanol–water, affording a 90% yield of **77**: mp 290–292 °C. Anal ($C_{14}H_{10}ClN_3O$) C, H, N.

2-[(Aryloxy)alkyl]benzimidazoles. These compounds (**62–76**) were prepared from *o*-phenylenediamine and an appropriate aryloxyalkanoic acid. In the case of compounds **62** and **64–76**, substituted phenoxyacetic acids, prepared by the method of Vogel,[27] were refluxed for 2 to 24 h in 2 M HCl. Highly substituted derivatives required longer reflux times.

The reaction mixture was neutralized with concentrated ammonia solution, and the crude benzimidazole was isolated by filtration. Yields and recrystallization solvents, in the case of novel compounds, are recorded in Table IV.

Compound **63**, 2-(2′-phenoxyethyl)benzimidazole, was prepared from *o*-phenylenediamine and 3-phenoxypropionic acid. The latter was synthesized in 15% yield by refluxing phenol (0.11 mol) and 3-chloropropionic acid (0.15 mol) in 50% KOH solution (40 mL) for 2 h. Following the isolation of 3-phenoxypropionic acid, cyclization was effected by refluxing with o-phenylenediamine in 4 M HCl for 6 h. 2-(2′-Phenoxyethyl)benzimidazole was isolated as for other 2-[(aryloxy)alkyl]benzimidazoles in 25% yield: mp 156–158 °C. Anal ($C_{15}H_{14}N_2O$) C, H, N.

5(6)-Ethoxy-2-methylbenzimidazole (80). 4-Ethoxyacetanilide (0.63 mol) was dissolved in glacial acetic acid (190 mL) and water (136 mL) and warmed to 45 °C with stirring. After the addition of nitric acid (*d* 1.41, 100 mL) the temperature of the reaction was kept at 70 °C for 10 min, then lowered to 25 °C over 10 min, and then kept at 4 °C for 3 h. The product was removed by filtration (yield 92%) and recrystallized from aqueous ethanol to give yellow crystals of 4-ethoxy-2-nitroacetanilide (mp 103 °C). 4-Ethoxy-2-nitroacetanilide (0.11 mol) was reduced in

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a Parr hydrogenation apparatus using palladium on charcoal as catalyst in ethyl acetate. After the uptake of hydrogen had ceased, the solution was filtered and the solvent was removed under reduced pressure. The crude 2-amino-4-ethoxyacetanilide was recrystallized from aqueous ethanol to give white needles: yield 100%; mp 141 °C. 2-Amino-4-ethoxyacetanilide (0.052 mol) was refluxed for 24 hours in 4 M HCl (30 mL). Cooling and neutralization with 10% KOH solution precipitated a solid, which was isolated by filtration and recrystallized from toluene–hexane: yield 76%; mp 137–140 °C. Anal ($C_{10}H_{12}N_2O$) C, H.

5(6)-Ethoxybenzimidazole (79). 2-Amino-4-ethoxyacetanilide (0.052 mol) was refluxed with 20% KOH solution (100 mL) for 2 h until hydrolysis was complete. The product, 4-ethoxy-o-phenylenediamine (0.052 mol) was refluxed with formic acid (20 mL) in 4 M HCl (80 mL) for 4 h and isolated as for compound **80**: yield 78%; mp 116 °C. Anal ($C_9H_{10}N_2O$) C, H.

Quantitative Structure–Activity Relationship by Distance Geometry: Quinazolines as Dihydrofolate Reductase Inhibitors

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This is a reinvestigation of 68 quinazoline inhibitors of dihydrofolate reductase. As in the earlier study, the binding data fitted to an 11-point model of the site, but improved computer algorithms resulted in a much better overall fit (correlation coefficient 0.95, standard deviation 0.727 kcal) and a more accurate fit for some very loosely bound 2,4-diaminoquinazolines. Removal of two of the site points (numbers 5 and 9) gave an even better fit than the original 11 site points. However, deleting a third one (number 8) worsened the calculated binding energies of the loosely bound 2,4-diaminoquinazolines. The results lead to predictions of chemical modifications of the quinazolines that should improve their biological activity.

This is the fifth paper in the series[1-4] on the distance geometry approach to quantitative structure–activity relationships. The energetic treatment in this approach to QSAR resembles a linear free energy model or Free–Wilson analysis; the added feature is the geometric constraints during the fitting of the data so that the ultimate outcome is a geometric interpretation of the biological activity. Ideally one should treat all aspects of drug action, i.e., transport of the drug molecule from point of administraiton to the receptor site, in vivo chemical midification along the way, free-energy changes within a flexible drug molecule to adopt the conformation required by the active site, free-energy changes within the site to adapt to the bound molecule, the energetics of the molecule–site interaction, and finally the production of the biological response. Since the first two and the last steps are complex and poorly understood, we make no attempt to take them into account and cosider only in vitro binding assays. We further simplify the situation by assuming the site is relatively rigid and that the intramolecular energy change upon binding is small compared to the interaction energy between site and drug molecule.

Methods

The calculations proceed as follows: (1) The molecules are constructed from the crystallographic data[5] on their constituent fragments. (2) For each molecule, we calculate the matrix of upper and lower bounds on the distances between its atoms over all sterically allowed conformations. In order to take only the physically acceptable conformations of low energy into consideration, slight penetration beyond the van der Walls radii or the radii of closest approach[6,7] may be allowed, although a better approach would be to select only those conformations having energies close to the global minimum. (3) In order to reduce the computer time required for the subsequent steps, some atoms of each molecule must be deleted. (4) Next, a plausible binding mode for each molecule is selected. A binding mode specifies which molecular point should be bound to which site point. Examples are included in Table IV. Often for a data set having varied molecular structures it is preferable to specify initially the binding modes of only those molecules having similar structures and high biological activity. (5) On the basis of the specified binding modes, the site point distance bounds are evaluated, using the earlier reasoning[1] that each intersite point distance should lie in the range common to the corresponding interatomic distances in all the molecules. In many cases no such overlapping range may exist, for instance when the highest of the lower bounds (l) is slightly higher than the least of the upper (u) distance bounds. Under that condition, the two site points may still bind the respective molecular points if it is assumed that the site point distance is intermediate between these two limits and the site flexibility, $\delta \geq (l-u)/2$. However, the binding modes should be chosen such that the value of δ is minimal. (6) The site point distance limits evaluated in this way have been selected quite independently of one another. However, the intersite point distances may be expressed by only $3n-6$ coordinates, where n is the number of site points, and there are $n(n-1)/2$ intersite distances. It is therefore obvious that such distances or distance limits cannot hold in three-diminensional space in general. The structures having the closest fit with these distance bounds are determined by the method of Crippen et al.[8] (7) Returning to the problem of step 5, since there is no guarantee in these derived coordinates that the site point distances will be exactly at the mean of the respective two limits, it is necessary to reevaluate the final value of δ corresponding to these coordinates. (8) In case some molecules have ambiguities about the molecular points to be bound with some site points, the site point coordinates, δ, and the intramolecular distance bounds may be used to select which molecular points will best fit these site points. (9) After the site point coordinates, δ, and fixed binding modes from the above algorithm are selected, it is next necessary to check whether these fixed modes are all geometrically acceptable and have no unfavorable forced contacts.[1] This step is very important, since in the next step the various interaction energy parameters are adjusted such that this mode becomes the energetically optimal binding mode, which must also be geometrically feasible. (10) Finally, the interaction parameters are evaluated by quadratic programming,[9]

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