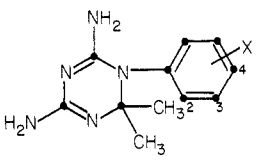
Inhibition of Human Dihydrofolate Reductase by 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(substituted-phenyl)-*s*-triazines. A Quantitative Structure-Activity Relationship Analysis

Bruce A. Hathaway, Zong-ru Guo,† Corwin Hansch,* Tavner J. Delcamp, Sandra S. Susten, and James H. Freisheim

Department of Chemistry, Pomona College, Claremont, California 91711, and Department of Biological Chemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267. *Received October 25, 1982*

The inhibitory activity of 101 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(substituted-phenyl)-*s*-triazines against purified dihydrofolate reductase from human lymphoblastoid cell (WIL 2) has been studied. From the obtained $K_{i\,app}$ values, quantitative structure-activity relationships (QSAR) have been derived. The QSAR from human dihydrofolate reductase are compared with QSAR for triazines inhibiting bovine and murine tumor DHFR, as well with QSAR for their inhibitory action on murine tumor cell culture.

The search for more selective inhibitors of the enzyme dihydrofolate reductase (DHFR) continues to attract many laboratories. While most of the effort is directed toward finding more selective antitumor and antimicrobial agents, interest in using the approach for the development of pesticides is also developing. The key to success in uncovering such agents is to discover compounds that have inhibitory power against DHFR from the undesirable cells while being relatively noninhibitory to cells from humans. Hence, a most important base of reference for such work is an extensive set of K_i values for inhibitors of the human enzyme. In our first effort to develop such a data base, we tested a set of 40 5-(substituted-benzyl)-2,4-diaminopyrimidines against human DHFR and established a quantitative structure–activity relationship.[1] In this study we report on the inhibition of human DHFR by 101 triazines of structure I.



I

There is abundant evidence[2] from studies of the amino acid sequences in DHFR from various sources that it is an enzyme of great variability. This is also evident when enzymes from different sources are probed with inhibitors.[3-7] Also, the new molecular graphics techniques using X-ray crystallographic data[8,9] are beginning to clarify differences in DHFR from different sources.[1,10] What needs more clarification is how slight differences in structure affect the binding of ligands and how such differences might be exploited to yield even better drugs. Recalling that $\Delta G° = -1.37 \log K_i$, we see that if $\Delta\Delta G$ for the binding of an inhibitor differs by only 4 kcal for two forms of DHFR, this would represent about a 1000-fold difference in the K_i. The free energy involved in hydrogen bond formation is 3–5 kcal; hence, the difference in making a poor or a good hydrogen bond with one form of the DHFR and not the other could yield a quite significant therapeutic advantage. Small differences in steric effects could be even more important. Hence, taking advantage of the knowledge of small differences in structural details of different forms of DHFR obtained from the study of enzymes with molecular probes (inhibitors) and X-ray crystallography should help in the more rational design of bioselective compounds.

A striking example of the design of a new highly potent DHFR inhibitor based on a knowledge of the structural details obtained from X-ray crystallography has been presented by Kuyper et al.[11]

In line with these objectives, we report our results and QSAR for the inhibition of DHFR from human lymphoblastoid cell line (WIL2) using inhibitors I.

Results and Discussion

In the study of inhibitors I we have factored our results into two sets: those with substituents in position 3 and those with substituents in position 4 of I. There are salient differences in the way these two groups of inhibitors interact with human DHFR.

Equations 1–4 correlate 4-substituted I, and equations 5–8 correlate 3-substituted congeners.

QSAR for 4-Substituted I

$$\log 1/K_i = 1.78\ (\pm 0.56)\ I + 5.28\ (\pm 0.30) \quad (1)$$

$$n = 35,\ r = 0.746,\ s = 0.739,\ F_{1,33} = 41.5$$

$$\log 1/K_i = 0.27\ (\pm 0.11)\ \pi'_4 + 1.50\ (\pm 0.45)\ I + 5.04\ (\pm 0.26) \quad (2)$$

$$n = 35,\ r = 0.860,\ s = 0.575,\ F_{1,32} = 22.5$$

$$\log 1/K_i = 0.95\ (\pm 0.29)\ \pi'_4 - 0.94\ (\pm 0.38) \log (\beta \cdot 10^{\pi'_4} + 1) + 1.21\ (\pm 0.36)\ I + 5.41\ (\pm 0.24) \quad (3)$$

$$n = 35,\ r = 0.927,\ s = 0.435,\ F_{1,30} = 12.9,\ \log \beta = -0.463$$

(1) Li, R. L.; Hansch, C.; Matthews, D. A.; Blaney, J. M.; Langridge, R.; Delcamp, T. J.; Susten, S. S.; Freisheim, J. H. *Quant. Struct.-Act. Relat.* **1982**, *1*, 1.
(2) Kumar, A. A.; Blankenship, D. T.; Kaufman, B. T.; Freisheim, J. H. *Biochemistry* **1980**, *19*, 667.
(3) Hitchings, G. H.; Smith, S. L. *Adv. Enzyme Regul.* **1980**, *18*, 349.
(4) Kim, K. H.; Dietrich, S. W.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1248.
(5) Li, R. L.; Dietrich, S. W.; Hansch, C. *J. Med. Chem.* **1981**, *24*, 538.
(6) Gutteridge, W. E.; Jaffee, J. J.; McCormack, Jr., J. J. *Biochim. Biophys. Acta* **1969**, *191*, 753.
(7) Burchall, J. J. *J. Infect. Dis.* **1972**, *128*, 437S.
(8) Langridge, R.; Ferrin, T. E.; Kuntz, I. D.; Connolly, M. L. *Science* **1981**, *211*, 661.
(9) Feldman, R. J.; Bing, D. H.; Furie, B. C.; Furie, B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5409.
(10) Hansch, C.; Li, R. L.; Blaney, J. M.; Langridge, R. *J. Med. Chem.* **1982**, *25*, 777.
(11) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E. A.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1982**, *25*, 1120.

† Visiting Scientist at Pomona College from the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China.

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$$\log 1/K_i = 0.78\ (\pm 0.20)\ \pi'_4 - 0.78\ (\pm 0.29) \log (\beta \cdot 10^{\pi'_4} + 1) + 1.26\ (\pm 0.32)\ I - 0.88\ (\pm 0.45)\ \nu + 5.83\ (\pm 0.34) \quad (4)$$

$$n = 35,\ r = 0.953,\ s = 0.361,\ F_{1,29} = 14.7,\ \log \beta = -0.926,\ \pi_0 = 3.43$$

$$\log 1/K_i = 0.57\ (\pm 0.21)\ \pi'_4 - 0.61\ (\pm 0.39) \log (\beta \cdot 10^{\pi'_4} + 1) + 1.28\ (\pm 0.48)\ I - 1.16\ (\pm 0.57)\ \nu + 5.85\ (\pm 0.45) \quad (4a)$$

$$n = 38,\ r = 0.909,\ s = 0.521,\ \log \beta = -1.839,\ \pi_0 = 2.91\ (\pm 4.26)$$

The above equations present the stepwise development of eq 4, the "best" QSAR. The most important single variable is the indicator variable I, which is given the value of 1 for congeners containing the OCH_2, SCH_2, or the CH_2S bridge between the phenyl group in I and a second phenyl group. This variable is only used when the second group on the bridge is a phenyl ring. It is not used for OR groups or for $OCH_2CH_2OC_6H_4$-4′-NH_2. The OCH_2 bridge strongly increases binding beyond that contributed by the hydrophobicity of the phenyl moiety alone. The bridged phenyl unit may bind somewhat like the $CH_2NHC_6H_4CONH$ moiety of folic acid.

The second most important variable in reducing variance is π'_4, as seen in eq 12. The prime with π indicates that for groups of the type $ZCH_2C_6H_4$-Y or CH_2S-C_6H_4-Y (where Z = O or S), π_Y is set equal to zero. That is, $\pi_{OCH_2C_6H_4\text{-}Y} = \pi_{OCH_2C_6H_5}$. Since all of the bridged congeners having the same Z have essentially the same K_i (except $OCH_2C_6H_4$-3′,4′-Cl_2) whether or not Y is hydrophobic, hydrophilic, large, or small, it was assumed that Y did not contact the enzyme. The next most significant term is the bilinear term[12] in π'_4, and the final term to enter eq 4 is the steric parameter ν developed by Charton.[13] While Charton's parameter is based on the van der Waals radius of substituents, he has shown it to be rather closely related to Taft's experimentally determined steric constant E_s. We have employed Charton's values because a much more extensive set is available.

From equation 4 an estimate can be made of π_0 (the ideal value for hydrophobicity of X); however, so few superoptimal π values were studied that confidence limits cannot be placed on π_0.

In the above equations, n represents the number of data points used to derive the equation, r is the correlation coefficient, and s is the standard deviation. β is a disposable parameter derived by an iterative procedure, and F is the F statistic for significance of each additional variable.

Equation 4 shows that inhibitory potency first increases linearly with increases in hydrophobicity up to a π of 3.4, and then the relationship becomes flat (0.78 − 0.78 = 0 for the slope of the right-hand side of the bilinear curve). This is a rough approximation that works best for the small substituents, such as the first 16 molecules in Table I. The relationship is not so well obeyed by the long alkyl and alkoxy groups.

Three compounds are so poorly fit by eq 4 that they have not been employed in its derivation: 4-$COOCH_2CH_3$, 4-CN, 4-$OCH_2CON(CH_2CH_2)_2O$. We have found the ester group to be less active than expected in both the 3- and 4-positions with human DHFR, as well as with DHFR from other sources.[14,15] The 4-CN is considerably less active than expected, and the 3-CN (see below) is about 10 times more active than expected.[14,15] The morpholino derivative is especially interesting, since it is about 25 times as active as eq 4 predicts. No doubt some special interaction not parameterized in eq 4 is operative. We shall have to await crystallographic results before this is understood.

Equations 5–8 show the development of the "best" correlation equation for 3-substituted triazines.

QSAR for 3-Substituted I

$$\log 1/K_i = 0.11\ (\pm 0.09)\ \pi'_3 + 6.49\ (\pm 0.23) \quad (5)$$

$$n = 60,\ r = 0.317,\ s = 0.617,\ F_{1,58} = 6.49$$

$$\log 1/K_i = 0.98\ (\pm 0.18)\ \pi'_3 - 1.12\ (\pm 0.22) \log (\beta \cdot 10^{\pi'_3} + 1) + 6.24\ (\pm 0.15) \quad (6)$$

$$n = 60,\ r = 0.832,\ s = 0.367,\ \pi'_0 = 1.83\ (\pm 0.37),\ \log \beta = -0.975,\ F_{2,56} = 53.9$$

$$\log 1/K_i = 1.02\ (\pm 0.23)\ \pi'_3 - 1.06\ (\pm 0.27) \log (\beta \cdot 10^{\pi'_3} + 1) + 0.46\ (\pm 0.19)\ I + 6.32\ (\pm 0.15) \quad (7)$$

$$n = 60,\ r = 0.876,\ s = 0.322,\ \pi'_0 = 1.83\ (\pm 0.59),\ \log \beta = -0.452,\ F_{1,55} = 17.8$$

$$\log 1/K_i = 1.07\ (\pm 0.23)\ \pi'_3 - 1.10\ (\pm 0.26) \log (\beta \cdot 10^{\pi'_3} + 1) + 0.50\ (\pm 0.19)\ I + 0.82\ (\pm 0.66)\ \sigma + 6.07\ (\pm 0.21) \quad (8)$$

$$n = 60,\ r = 0.890,\ s = 0.308,\ \pi'_0 = 2.10\ (\pm 0.87),\ \log \beta = -0.577,\ F_{1,54} = 6.04$$

$$\log 1/K_i = 0.53\ (\pm 0.12)\ \pi'_3 - 0.56\ (\pm 0.18) \log (\beta \cdot 10^{\pi'_3} + 1) + 0.61\ (\pm 0.24)\ I + 0.60\ (\pm 0.80)\ \sigma + 5.82\ (\pm 0.24) \quad (8a)$$

$$n = 64,\ r = 0.871,\ s = 0.404,\ \log \beta = -1.604,\ \pi'_0 = 2.83\ (\pm 1.11)$$

In eq 5–8 the variables have the same meaning as in eq 1–4. Equation 8 is the "best" equation, with all terms justified by the F test. The most difficult difference between eq 4 and 8 to rationalize is the presence of the σ term in eq 8 and its absence in eq 4. As we have noted before,[16] it is difficult (although not impossible) to rationalize an electronic effect from a meta position without having a corresponding effect from the para position. In fact, one might dismiss σ in eq 8 as an artifact if it were not for its presence in other QSAR (see below).

The rather small difference in the initial slopes of eq 4 and 8 (0.8 vs. 1.1) is not of much significance; however, the large difference between π'_0 values of 3.43 and 2.10 is important, especially since we cannot place confidence limits on the 3.43 figure. With more data on more lipophilic analogues, a firm figure for π'_0 of 4-substituted I would probably be even higher.

The most significant difference between the two equations is the steric parameter of eq 4, which has no counterpart in eq 7 or 8. A very small improvement in eq 8 can be obtained with Austel et al.'s steric parameters,[17] but no significant improvement was obtained with ν. Thus, it seems clear that the 4-substituted encounters steric

(12) Kubinyi, H. *Drug Res.* **1979**, *23*, 97.
(13) Charton, M. In "Design of Biopharmaceutical Properties through Prodrugs and Analogs"; Roche, E. B., Ed.; American Pharmaceutical Association: Washington, DC, 1977; p 228.
(14) Dietrich, S. W.; Smith, R. N.; Fukunaga, J. Y.; Olney, M.; Hansch, C. *Arch. Biochem. Biophys.* **1979**, *194*, 600.
(15) Guo, Z. R.; Dietrich, S. W.; Hansch, C.; Dolnick, B. J.; Bertino, J. R. *Mol. Pharmacol.* **1981**, *20*, 649.
(16) Unger, S. H.; Hansch, C. *J. Med. Chem.* **1973**, *16*, 745.
(17) Austel, V.; Kutter, E.; Kalbfleisch, W. *Arzneim.-Forsch.* **1979**, *29*, 585.

Table I. Parameters Used in the Derivation of Equations 1-4

no.	group	log $1/K_{i\ app}$ obsd (95% CI)	pred	$\lvert\Delta \log 1/K_{i\ app}\rvert$	π'	I	ν
1	H	5.78 (5.74-5.82)	5.79	0.01	0.00	0.00	0.00
2	4-SO_2NH_2	3.81 (3.77-3.84)	3.54	0.27	−1.82	0.00	0.99
3	4-SO_2CH_3	4.08 (4.04-4.11)	3.69	0.39	−1.63	0.00	0.99
4	4-$CONH_2$	3.64 (3.59-3.69)	4.03	0.39	−1.49	0.00	0.72
5	4-$COCH_3$	4.50 (4.47-4.53)	4.76	0.26	−0.55	0.00	0.72
6	4-$COOCH_3$	3.85 (3.80-3.89)	4.46	0.61	−0.01	0.00	1.51
7	4-$COOCH_2CH_3$	3.46 (3.42-3.50)	4.79	1.33	0.51	0.00	1.51
8	4-OH	4.57 (4.54-4.60)	5.02	0.45	−0.67	0.00	0.32
9	4-NH_2	4.65 (4.62-4.68)	4.56	0.09	−1.23	0.00	0.35
10	4-$NHCOCH_3$	4.23 (4.18-4.27)	4.27	0.04	−0.97	0.00	0.91
11	4-CF_3	5.58 (5.55-5.60)	5.50	0.08	0.88	0.00	0.91
12	4-F	6.15 (6.11-6.18)	5.65	0.50	0.14	0.00	0.27
13	4-Cl	6.20 (6.18-6.22)	5.74	0.46	0.71	0.00	0.55
14	4-BR	5.76 (5.72-5.79)	5.72	0.04	0.86	0.00	0.65
15	4-I	5.51 (5.48-5.54)	5.70	0.19	1.12	0.00	0.78
16	4-CN	3.69 (3.65-3.73)	5.02	1.33	−0.57	0.00	0.40
17	4-OCH_2CO-morpholine	5.66 (5.61-5.71)	4.20	1.46	−1.39	0.00	0.62
18	4-$O(CH_2)_2OC_6H_4$-4'-NH_2	6.00 (5.97-6.03)	5.54	0.46	0.45	0.00	0.61
19	4-CH_3	5.97 (5.93-6.00)	5.69	0.28	0.56	0.00	0.52
20	4-$(CH_2)_3CH_3$	6.27 (6.24-6.31)	5.93	0.34	2.13	0.00	0.68
21	4-$(CH_2)_8CH_3$	6.52 (6.45-6.59)	5.94	0.58	4.83	0.00	0.68
22	4-$C(CH_3)_3$	5.66 (5.62-5.70)	5.43	0.23	1.98	0.00	1.24
23	4-$C{\equiv}CC_6H_5$	5.42 (5.37-5.46)	5.92	0.50	2.65	0.00	0.70
24	4-OCH_3	5.31 (5.28-5.34)	5.46	0.15	0.02	0.00	0.36
25	4-$O(CH_2)_2CH_3$	5.57 (5.54-5.59)	5.85	0.28	1.05	0.00	0.58
26	4-$O(CH_2)_5CH_3$	5.53 (5.49-5.57)	6.00	0.47	2.62	0.00	0.61
27	4-$O(CH_2)_{10}CH_3$	5.68 (5.64-5.72)	5.97	0.29	5.37	0.00	0.65
28	4-$O(CH_2)_{11}CH_3$	5.85 (5.80-5.89)	5.97	0.12	5.91	0.00	0.65
29	4-$OCH_2C_6H_5$	6.93 (6.88-6.98)	7.18	0.25	1.66	1.00	0.65
30	4-$OCH_2C_6H_3$-3',4'-Cl_2	6.46 (6.39-6.53)	7.18	0.72	1.66	1.00	0.65
31	4-$OCH_2C_6H_4$-4'-SO_2NH_2	7.21 (7.18-7.24)	7.18	0.03	1.66	1.00	0.65
32	4-$OCH_2C_6H_4$-4'-$CONH_2$	7.23 (7.19-7.26)	7.18	0.05	1.66	1.00	0.65
33	4-$OCH_2C_6H_4$-4'-CH_2OH	7.12 (7.09-7.15)	7.18	0.06	1.66	1.00	0.65
34	4-$CH_2SC_6H_5$	7.33 (7.31-7.34)	7.07	0.26	2.30	1.00	0.82
35	4-$CH_2SC_6H_4$-2'-CH_3	7.18 (7.16-7.19)	7.07	0.11	2.30	1.00	0.82
36	4-$CH_2SC_6H_4$-3'-CH_3	7.22 (7.18-7.25)	7.07	0.15	2.30	1.00	0.82
37	4-$SCH_2C_6H_5$	7.01 (6.97-7.05)	6.78	0.23	2.30	1.00	1.15
38	4-$SCH_2C_6H_4$-4'-Cl	6.97 (6.95-6.99)	6.78	0.19	2.30	1.00	1.15

effects not encountered by the 3-substituent.

Another difference between the two QSAR is the coefficient with I, which is much larger for 4-substituted than for 3-substituted I. The bridged phenyl moiety, which may bind where the aminobenzoic acid moiety of folic acid binds, provides greater binding capacity when it is attached to the 3-position of I.

Although the intercepts for eq 4 and 8 are different, considering the size of the 95% confidence limits on these parameters, the difference is not significant.

In the development of the QSAR for 3-substituted I, the following four data points seemed to behave in unique ways and were omitted in the development of eq 8: 3-$COOC_2H_5$, 3-$CH(OH)C_6H_5$, 3-$O(CH_2)_2CH_3$, and 3-$CH_2N(CH_3)_3{}^+Cl^-$. We have known for some time that branching next to the phenyl ring produces a bad steric effect;[18,19] therefore, as usual, the first two of the above substituents exhibit much lower than expected activity. We find no obvious explanation for the low activity of 3-$O(CH_2)_2CH_3$. We did not expect the charged ammonium ion to fit our QSAR, since it has long been known that charged groups are poorly behaved in correlation analysis.[20] This is especially true of π constants, since the concentration of the counterion and the ionic strength of medium greatly affect π values. The inhibitory potency of the ammonium ion is about 1100 times greater than eq 7 predicts; however, this may be due to an effective π constant higher than −5.5, which was determined by extrapolation of partitioning experiments to infinite dilution. The higher than expected activity may also be due to the interaction of the positive charge with the active site of DHFR.

Equations 7 and 8 for the action of 3-substituted I can be compared with DHFR from two other mammalian sources. Equations 9 and 10 are for bovine DHFR, and eq 11 is for murine tumor reductase.

3-Substituted I Inhibition of Bovine DHFR[22]

$$\log 1/K_i = 1.08\pi'_3 - 1.19 \log (\beta \cdot 10^{\pi'_3} + 1) + 7.27 \quad (9)$$

$n = 38$, $r = 0.903$, $s = 0.288$, $\pi_0 = 1.62$, $\log \beta = -0.656$

$$\log 1/K_i = 1.10\pi'_3 - 1.23 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.61\sigma + 7.08 \quad (10)$$

$n = 38$, $r = 0.914$, $s = 0.277$, $\pi_0 = 1.72$, $\log \beta = -0.789$

3-Substituted I Inhibition of Murine Tumor DHFR[22]

$$\log 1/K_i = 1.19\pi'_3 - 1.38 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.50I + 0.90\sigma + 6.20 \quad (11)$$

$n = 38$, $r = 0.935$, $s = 0.289$, $\pi_0 = 1.56$, $\log \beta = -0.750$

(18) Silipo, C.; Hansch, C. *J. Am. Chem. Soc.* **1975**, *97*, 6849.
(19) Dietrich, S. W.; Smith, R. N.; Brendler, S.; Hansch, C. *Arch. Biochem. Biophys.* **1979**, *194*, 612.
(20) Hoefnagel, A. J.; Hoefnagel, M. A.; Wepster, B. M. *J. Org. Chem.* **1978**, *43*, 4720.
(21) Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.
(22) Khwaja, T. A.; Pentecost, S.; Selassie, C. D.; Guo, Z. R.; Hansch, C. *J. Med. Chem.* **1982**, *25*, 153.

Table II. Parameters Used in the Derivation of Equations 5-8

no.	group	log $1/K_{i\,app}$ calcd (95% CI)	log $1/K_{i\,app}$ pred	$\lvert\Delta \log 1/K_{i\,app}\rvert$	π'	I	σ
1	H	5.78 (5.74-5.82)	5.96	0.18	0.00	0.00	0.00
2	3-SO_2NH_2	4.55 (4.50-4.59)	4.49	0.06	−1.82	0.00	0.46
3	3-$CONH_2$	4.64 (4.62-4.67)	4.70	0.06	−1.49	0.00	0.28
4	3-$COCH_3$	5.46 (5.44-5.48)	5.76	0.30	−0.55	0.00	0.38
5	3-$COOCH_2CH_3$	4.95 (4.93-4.97)	6.62	1.67	0.51	0.00	0.37
6	3-OH	5.53 (5.48-5.59)	5.42	0.11	−0.67	0.00	0.12
7	3-CF_3	6.67 (6.64-6.70)	6.84	0.17	0.88	0.00	0.43
8	3-F	6.61 (6.58-6.64)	6.35	0.26	0.14	0.00	0.34
9	3-Cl	7.03 (7.01-7.04)	6.72	0.31	0.71	0.00	0.37
10	3-Br	7.21 (7.17-7.25)	6.80	0.41	0.86	0.00	0.39
11	3-I	7.17 (7.13-7.20)	6.84	0.33	1.12	0.00	0.35
12	3-NO_2	6.09 (6.05-6.13)	6.29	0.20	−0.28	0.00	0.71
13	3-CN	6.30 (6.27-6.32)	5.88	0.42	−0.57	0.00	0.56
14	3-$CH_2N(CH_3)_3^+Cl^-$	3.55 (3.53-3.57)	0.50	3.05	−5.50	0.00	0.40
15	3-CH_3	6.74 (6.71-6.76)	6.29	0.45	0.56	0.00	−0.07
16	3-CH_2CH_3	6.93 (6.89-6.98)	6.47	0.46	1.03	0.00	−0.07
17	3-$(CH_2)_5CH_3$	7.02 (7.00-7.04)	6.54	0.48	3.21	0.00	−0.08
18	3-$(CH_2)_8CH_3$	6.66 (6.61-6.70)	6.48	0.18	4.83	0.00	−0.08
19	3-$(CH_2)_{11}CH_3$	6.52 (6.49-6.54)	6.43	0.09	6.45	0.00	−0.08
20	3-$C(CH_3)_3$	6.39 (6.36-6.43)	6.54	0.15	1.98	0.00	−0.10
21	3-*dl*-$CH(OH)C_6H_5$	5.56 (5.54-5.59)	6.30	0.74	0.54	0.00	−0.04
22	3-OCH_3	5.78 (5.72-5.84)	6.04	0.26	0.02	0.00	0.12
23	3-OCH_2CH_3	5.66 (5.64-5.69)	6.32	0.66	0.38	0.00	0.10
24	3-$O(CH_2)_2CH_3$	5.68 (5.65-5.70)	6.61	0.93	1.05	0.00	0.10
25	3-$O(CH_2)_3CH_3$	6.08 (6.05-6.10)	6.69	0.61	1.54	0.00	0.10
26	3-$O(CH_2)_4CH_3$	6.09 (6.07-6.12)	6.71	0.62	2.08	0.00	0.10
27	3-$O(CH_2)_5CH_3$	6.12 (6.10-6.14)	6.70	0.58	2.62	0.00	0.10
28	3-$O(CH_2)_8CH_3$	6.78 (6.76-6.86)	6.65	0.13	4.29	0.00	0.10
29	3-$O(CH_2)_{10}CH_3$	6.61 (6.58-6.63)	6.61	0.00	5.37	0.00	0.10
30	3-$O(CH_2)_{11}CH_3$	6.69 (6.66-6.71)	6.60	0.09	5.91	0.00	0.10
31	3-$O(CH_2)_{12}CH_3$	6.54 (6.51-6.56)	6.58	0.04	6.45	0.00	0.10
32	3-$O(CH_2)_{13}CH_3$	6.34 (6.24-6.44)	6.56	0.22	6.99	0.00	0.10
33	3-$O(CH_2)_2OC_6H_5$	6.82 (6.80-6.84)	6.70	0.12	1.68	0.00	0.10
34	3-$O(CH_2)_2OC_6H_4$-3'-CF_3	6.92 (6.89-6.95)	6.70	0.22	2.56	0.00	0.10
35	3-$O(CH_2)_4OC_6H_5$	6.94 (6.91-6.96)	6.70	0.24	2.71	0.00	0.10
36	3-$O(CH_2)_4OC_6H_4$-3'-CF_3	6.90 (6.89-6.92)	6.67	0.23	3.50	0.00	0.10
37	3-$OCH_2C_6H_5$	6.72 (6.70-6.74)	7.19	0.47	1.66	1.00	0.10
38	3-$OCH_2C_6H_3$-3',4'-Cl_2	6.83 (6.79-6.86)	7.19	0.36	1.66	1.00	0.10
39	3-$OCH_2C_6H_4$-4'-$CONH_2$	6.95 (6.92-6.98)	7.19	0.24	1.66	1.00	0.10
40	3-OCH_2-1-adamantyl	6.11 (6.07-6.14)	6.67	0.56	3.61	0.00	0.10
41	3-CH_2O-c-C_6H_{11}	6.64 (6.61-6.67)	6.65	0.01	1.43	0.00	0.06
42	3-$CH_2NHC_6H_3$-3',5'-$(CONH_2)_2$	6.78 (6.77-6.80)	7.07	0.29	1.00	1.00	0.06
43	3-$CH_2NHC_6H_4$-4'-SO_2NH_2	7.20 (7.18-7.23)	7.07	0.14	1.00	1.00	0.06
44	3-$CH_2OC_6H_5$	7.23 (7.21-7.26)	7.16	0.07	1.66	1.00	0.06
45	3-$CH_2OC_6H_4$-3'-Cl	7.44 (7.41-7.48)	7.16	0.28	1.66	1.00	0.06
46	3-$CH_2OC_6H_4$-3'-CN	7.44 (7.41-7.48)	7.16	0.28	1.66	1.00	0.06
47	3-$CH_2OC_6H_4$-3'-OCH_3	7.33 (7.28-7.38)	7.16	0.17	1.66	1.00	0.06
48	3-$CH_2OC_6H_4$-3'-CH_2OH	7.04 (7.00-7.08)	7.16	0.12	1.66	1.00	0.06
49	3-$CH_2OC_6H_4$-3'-CH_3	7.22 (7.17-7.27)	7.16	0.06	1.66	1.00	0.06
50	3-$CH_2OC_6H_4$-3'-CH_2CH_3	7.37 (7.33-7.40)	7.16	0.21	1.66	1.00	0.06
51	3-$CH_2OC_6H_4$-3'-$CH(CH_3)_2$	7.15 (7.09-7.20)	7.16	0.01	1.66	1.00	0.06
52	3-$CH_2OC_6H_4$-3'-$C(CH_3)_3$	7.47 (7.44-7.50)	7.16	0.31	1.66	1.00	0.06
53	3-$CH_2OC_6H_4$-3'-C_6H_5	7.14 (7.12-7.15)	7.16	0.02	1.66	1.00	0.06
54	3-$CH_2OC_6H_4$-3'-$NHCOCH_3$	7.30 (7.23-7.36)	7.16	0.14	1.66	1.00	0.06
55	3-$CH_2OC_6H_4$-3'-$NHCSNH_2$	7.16 (7.13-7.19)	7.16	0.00	1.66	1.00	0.06
56	3-$CH_2OC_6H_4$-3'-$NHCONH_2$	7.39 (7.38-7.41)	7.16	0.23	1.66	1.00	0.06
57	3-$CH_2OC_6H_4$-4'-$(CH_2)_4CH_3$	6.73 (6.70-6.76)	7.16	0.43	1.66	1.00	0.06
58	3-CH_2O-2-naphthyl	7.12 (7.09-7.16)	7.16	0.04	1.66	1.00	0.06
59	3-CH_2O-1-naphthyl	6.89 (6.86-6.93)	7.16	0.27	1.66	1.00	0.06
60	3-$CH_2SC_6H_5$	6.93 (6.91-6.94)	7.17	0.24	2.30	1.00	0.06
61	3-$CH_2SC_6H_4$-3'-CH_3	7.12 (7.11-7.12)	7.17	0.05	2.30	1.00	0.06
62	3-$CH_2SeC_6H_5$	7.52 (7.47-7.57)	7.17	0.35	2.37	1.00	0.06
63	3-$SCH_2C_6H_5$	7.37 (7.31-7.42)	7.14	0.23	2.30	1.00	0.03
64	3-$SCH_2C_6H_4$-4'-Cl	7.20 (7.11-7.30)	7.14	0.06	2.30	1.00	0.03

The parameters of eq 9 correspond rather closely with those of eq 7, and eq 10 is quite similar to eq 8, except that we could not find a role for I in the bovine QSAR. The lower intercept with human DHFR indicates that it is about 10 times more resistant to the triazine inhibitors than bovine enzyme. Of definite interest is the significance of the term σ in eq 10. While the addition of this term does not make a large improvement in the QSAR, it is justified by the F test. Except for the difference in the intercepts and the I term, eq 10 and 11 agree quite well. On the average, the murine DHFR is 10 times more resistant to the inhibitors than bovine enzyme and about the same difficulty to inhibit as human DHFR.

The fact that σ turns out to be significant in both eq 10 and 11, as well as eq 8, convinces us that there must be a small electronic role for substituents in the 3-position

Table III

substituents with unknown ν	model substituent	ν
SO_2NH_2	SO_3^-	0.99
SO_2CH_3	SO_3^-	0.99
$CONH_2$	$COCH_3$	0.72
$COOC_2H_5$	$COOCH_3$	1.51
$NHCOCH_3$	$NHCH(CH_3)_2$	0.91
$OCH_2CON(CH_2CH_2)_2O$	$OCH_2CH(CH_3)CH_2CH_3$	0.62
$C{\equiv}CC_6H_5$	$CH_2CH_2C_6H_5$	0.70
$OCH_2C_6H_4$-Y	OCH_2-c-C_6H_{11}	0.65
$SCH_2C_6H_5$	$SCH_2CH(CH_3)CH_2CH_3$	1.15
$OCH_2CH_2OC_6H_5$	$O(CH_2)_7CH_3$	0.61

of which the exact nature is unclear. One might expect this to be a correction of π values from the benzene system, since π does vary from system to system;[21] however, if this were true, then it should also occur with 4-substituted I congeners. There is no doubt that 3-substituted I congeners are interacting in enzymic space of a different nature from that of 4-substituted I, and it may be that a polar interaction correlated with σ could account for the importance of this term for 3- but not 4-substituted analogues.

Equation 11 for the action of 3-substituted I on purified enzyme can be compared with the inhibition of murine tumor cell culture via eq 11 and 12.

3-Substituted I Inhibition of Murine Tumor Cell Culture[22]

$$\log 1/C = 1.34\pi - 1.69 \log (\beta \cdot 10^{\pi} + 1) + 0.58I + 0.75\sigma + 7.87 \quad (12)$$

$$n = 37,\ r = 0.942,\ s = 0.254,\ \pi_0 = 0.82,\ \log \beta = -0.249$$

In eq 12 it is necessary to use π for the whole $ZCH_2CH_6H_4$-Y fragment, since movement of the drugs in the cells is highly dependent on overall hydrophobicity. Optimal hydrophobicity (π_0) is considerably lower for the cell culture than for purified DHFR. Also, the slope of the right-hand portion of the bilinear model (1.34 − 1.69 = −0.35) is significantly different from zero. This brings out the role of hydrophobicity in the random walk process.[12] Of greatest interest to us are the terms in I and σ, which are important in rationalizing inhibitor potency against purified DHFR, as well as DHFR in vivo. Correlation analysis will, we believe, be of great help in determining whether or not enzymes behave in the same way in living cells as they do in isolated form in buffer solution.[23] For example, quite a different QSAR is obtained for the inhibition by 3-substituted I of murine tumor cells resistant to MTX.[24]

In conclusion, we wish to emphasize that the QSAR for triazines and pyrimidines[1] inhibiting human DHFR should provide useful guidelines in studying DHFR from other sources in the quest for better drugs and pesticides.

Experimental Section

The inhibition assays and the calculation of $K_{i\,app}$ were performed as in our previous studies.[25] The confidence limits on $\log 1/K_i$ and π_0 were calculated by the jackknife procedure.[26] It must be emphasized that $\log 1/K_i$ values approaching that of methotrexate (7.65) will not be reliable because of the problem of stoichiometric inhibition and tight binding.[27,28]

Two parameters were investigated to account for the steric effects of substituents: MR and Charton's ν constants. For the substituents in Table III, ν has not been determined, so that it was necessary to use ν for known substituents similar to our unknown substituent. The values for substituent constants were taken from our recent compilation.[29]

The synthesis of many of the triazines has been reported.[14,15] The synthesis of the new analogues will be reported elsewhere. The procedure for the isolation of purified human DHFR from lymphoblastoid cell line (WIL2) is described elsewhere.[30]

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Registry No. I (X = 4-SO_2NH_2), 90-08-4; I (X = 4-SO_2CH_3), 74798-28-0; I (X = 4-$CONH_2$), 87871-34-9; I (X = 4-$COCH_3$), 85304-88-7; I (X = 4-$COOCH_3$), 85304-87-6; I (X = 4-$COOCH_2CH_3$), 17740-29-3; I (X = 4-OH), 74798-26-8; I (X = 4-NH_2), 87871-35-0; I (X = 4-$NHCOCH_3$), 74798-27-9; I (X = 4-CF_3), 47071-11-4; I (X = 4-F), 1542-59-2; I (X = 4-Cl), 516-21-2; I (X = 4-Br), 3567-84-8; I (X = 4-I), 46781-41-3; I (X = 4-CN), 17711-68-1; I [X = 4-(methoxycarbonyl)morpholine], 50574-87-3; I (X = 4-$O(CH_2)_2OCOH_4$-4′-NH_2), 87871-36-1; I (X = 4-CH_3), 15233-37-1; I [X = 4-$(CH_2)_3CH_3$], 4653-73-0; I [X = 4-$(CH_2)_8CH_3$], 87739-87-5; I [X = 4-$C(CH_3)_3$], 4653-75-2; I (X = 4-$C{\equiv}{\equiv}CC_6H_5$), 87871-37-2; I (X = 4-OCH_3), 21316-30-3; I [X = 4-$O(CH_2)_2CH_3$], 87739-96-6; I [X = 4-$O(CH_2)_5CH_3$], 4653-85-4; I [X = 4-$O(CH_2)_{10}CH_3$], 87739-97-7; I [X = 4-$O(CH_2)_{11}CH_3$], 79515-25-6; I (X = 4-$OCH_2C_6H_5$), 17944-10-4; I (X = 4-$OCH_2C_6H_3$-3′,4′-Cl_2), 85304-89-8; I (X = 4-$OCH_2C_6H_4$-4′-SO_2NH_2), 87739-88-6; I (X = 4-$OCH_2C_6H_4$-4′-$CONH_2$), 87739-89-7; I (X = 4-$OCH_2C_6H_4$-4′-CH_2OH), 87739-90-0; I (X = 4-$CH_2SC_6H_5$), 87739-93-3; I (X = 4-$CH_2SC_6H_4$-2′-CH_3), 87739-94-4; I (X = 4-$CH_2SC_6H_4$-3′-CH_3), 87739-95-5; I (X = 4-$SCH_2C_6H_5$), 87739-91-1; I (X = 4-$SCH_2C_6H_4$-4′-Cl), 87739-92-2; I (X = 3-SO_2NH_2), 70579-32-7; I (X = 3-$CONH_2$), 70579-33-8; I (X = 3-$COCH_3$), 70579-34-9; I (X = 3-$COOCH_2CH_3$), 70650-62-3; I (X = 3-OH), 70579-35-0; I (X = 3-CF_3), 1492-81-5; I (X = 3-F), 3850-94-0; I (X = 3-Cl), 13351-02-5; I (X = 3-Br), 24849-96-5; I (X = 3-I), 51012-14-7; I (X = 3-NO_2), 17711-74-9; I (X = 3-CN), 70743-55-4; I (X = 3-$CH_2N(CH_3)_3{}^+Cl^-$), 87739-86-4; I (X = 3-CH_3), 4038-60-2; I (X = 3-CH_2CH_3), 87739-76-2; I (X = 3-$(CH_2)_5CH_3$), 70650-60-1; I (X = 3-$(CH_2)_8CH_3$), 87739-77-3; I (X = 3-$(CH_2)_{11}CH_3$), 87739-78-4; I [X = 3-$C(CH_3)_3$], 70579-36-1; I [X = 3-*dl*-$CH(OH)C_6H_5$], 75153-70-7; I (X = 3-OCH_3), 17711-73-8; I (X = 3-OCH_2CH_3), 46985-98-2; I [X = 3-$O(CH_2)_2CH_3$], 14052-49-4; I [X = 3-$O(CH_2)_3CH_3$], 70606-63-2; I [X = 3-$O(CH_2)_4CH_3$], 87739-98-8; I [X = 3-$O(CH_2)_5CH_3$], 74798-21-3; I [X = 3-$O(CH_2)_8CH_3$], 70579-30-5; I [X = 3-$O(CH_2)_{10}CH_3$], 70579-55-4; I [X = 3-$O(CH_2)_{11}CH_3$], 70579-29-2; I [X = 3-$O(CH_2)_{12}CH_3$], 79508-88-6; I [X = 3-$O(CH_2)_{13}CH_3$], 70579-52-1; I [X = 3-$O(CH_2)_2OC_6H_5$], 19161-84-3; I [X = 3-$O(CH_2)_2OC_6H_4$-3′-CF_3), 70579-37-2; I [X = 3-$O(CH_2)_4OC_6H_5$], 70579-31-6; I [X = 3-$O(CH_2)_4OC_6H_4$-3′-CF_3), 70579-41-8; I (X = 3-$OCH_2C_6H_5$), 70579-38-3; I (X = 3-$OCH_2C_6H_3$-3′,4′-Cl_2), 70579-39-4; I (X = 3-$OCH_2C_6H_4$-4′-$CONH_2$), 87739-82-0; I (X = 3-OCH_2-1-adamantyl), 87871-38-3; I (X = 3-OCH_2O-c-C_6H_{11}), 87871-39-4; I [X = 3-$CH_2NHC_6H_3$-3′,5′-$(CONH_2)_2$], 70579-40-7; I (X = 3-$CH_2NHC_6H_4$-4′-SO_2NH_2), 70579-42-9; I (X = 3-$CH_2OC_6H_5$), 79508-78-4; I (X = 3-$CH_2OC_6H_4$-3′-Cl), 79508-79-5; I (X = 3-$CH_2OC_6H_4$-3′-CN), 79519-97-4; I (X = 3-$CH_2OC_6H_4$-3′-OCH_3), 79508-80-8; I (X = 3-$CH_2OC_6H_4$-3′-CH_2OH), 79508-81-9; I (X = 3-$CH_2OC_6H_4$-3′-CH_3), 79508-82-0; I (X = 3-$CH_2OC_6H_4$-3′-CH_2CH_3), 79508-83-1; I [X = 3-$CH_2OC_6H_4$-3′-$CH(CH_3)_2$], 79508-84-2; I [X = 3-$CH_2OC_6H_4$-3′-$C(CH_3)_3$], 79508-85-3; I (X = 3-$CH_2OC_6H_4$-3′-C_6H_5), 87739-80-8;

(23) Coats, E. A.; Genther, C. S.; Dietrich, S. W.; Guo, Z. R.; Hansch, C. *J. Med. Chem.* **1981**, *24*, 1422.
(24) Selassie, C. D.; Guo, Z. R.; Hansch, C.; Khwaja, T. A.; Pentecost, S. *J. Med. Chem.* **1982**, *25*, 157.
(25) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205.
(26) Dietrich, S. W.; Dreyer, N. D.; Hansch, C.; Bentley, D. L. *J. Med. Chem.* **1980**, *23*, 1201.
(27) Morrison, J. F. *Trends Biochem. Sci.* **1982**, *7*, 102.
(28) Williams, J. W.; Duggleby, R. G.; Cutler, R.; Morrison, J. F. *Biochem. Pharmacol.* **1980**, *29*, 589.
(29) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979.
(30) Delcamp, T. J.; Susten, S. S.; Blankenship, D. T.; Freisheim, J. H. *Biochemistry*, in press.

I (X = 3-$CH_2OC_6H_4$-3′-$NHCOCH_3$), 79508-86-4; I (X = 3-$CH_2OC_6H_4$-3′-$NHCSNH_2$), 79508-87-5; I (X = 3-$CH_2OC_6H_4$-3′-$NHCONH_2$), 70579-43-0; I [X = 3-$CH_2OC_6H_4$-4′-$(CH_2)_4CH_3$], 87739-81-9; I [X = 3-(methoxycarbonyl)-2-naphthyl], 87871-40-7; I [X = 3-(methoxycarbonyl)-1-naphthyl], 87871-41-8; I (X = 3-$CH_2SC_6H_5$), 80239-83-4; I (X = 3-$CH_2SC_6H_4$-3′-CH_3), 87739-85-3; I (X = 3-$CH_2SeC_6H_5$), 87739-79-5; I (X = 3-$SCH_2C_6H_5$), 87739-83-1; I (X = 3-$SCH_2C_6H_4$-4′-Cl), 87739-84-2; DHFR, 9002-03-3.

Adrenal Medulla Imaging Agents: A Structure–Distribution Relationship Study of Radiolabeled Aralkylguanidines[1]

Donald M. Wieland,* Thomas J. Mangner, Muthiah N. Inbasekaran, Lawrence E. Brown, and Jiann-long Wu[†]

Division of Nuclear Medicine, The University of Michigan Medical Center, Ann Arbor, Michigan 48109.
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Fourteen ^{125}I-labeled aralkylguanidines were synthesized and evaluated as potential imaging agents for the adrenal medullae and tumors of adrenomedullary origin. These guanidines are radiotracer analogues of guanethidine, an antihypertensive agent thought to mediate neuron blockade by uptake into adrenergic nerves. Dog adrenal medullae were used as a model to test radiotracer affinity for catecholamine storage tissue. Tissue distribution studies revealed that a number of radioiodinated guanidines showed pronounced localization in the adrenal medullae following intravenous injection, in certain cases exceeding that of either (–)-[^3H]norepinephrine or [^{14}C]guanethidine. (*m*-[^{125}I]Iodobenzyl)guanidine (*m*-IBG, **2b**) gave the best combination of high concentration and selectivity. The low adrenomedullary affinity observed with [^{14}C]guanidine and *m*-[^{125}I]iodobenzylamine demonstrates the uniqueness of the aralkylguanidine structure. Preliminary evidence suggests that **2b** is a storage analogue of norepinephrine. [^{125}I]**2a** is now being used clinically in imaging and radiotherapy of catecholamine tumors, such as pheochromocytoma.

An imaging agent for the adrenal medulla and its diseases has been actively pursued for more than a decade.[2-13] Recent efforts in our laboratory to develop a clinically useful agent have focused on radioiodinated analogues of the antihypertensive drug guanethidine. This drug inhibits the release of norepinephrine from adrenergic nerve endings, as well as depletes neuronal stores of norepinephrine.[14,15] Both of these effects involve the direct action of guanethidine on the adrenergic nerves.[16] If the adrenal medulla is considered a specialized sympathetic ganglion,[17] then compounds known to have an affinity for adrenergic nerves might be expected to localize in the adrenal medulla.

Studies in dogs in the early 1960's, however, showed that pharmacological doses of guanethidine, although rapidly depleting the heart and spleen of norepinephrine, had little effect on the catecholamine content of the adrenal medulla.[18,19] Nonetheless, our initial studies revealed that [^{14}C]guanethidine had a high affinity for the dog adrenal medulla. Although guanethidine is not readily labeled with a γ-emitting radionuclide suitable for use in scintigraphy, pharmacologically active analogues such as benzylguanidines can be readily labeled by substitution of radioiodine on the aromatic ring. In the benzylguanidine series, Short and Darby[20] have shown that lipophilic aromatic substituents (e.g., CF_3, Br, I) can, in certain cases, enhance neuron blocking potency. However, since pharmacological activity may not be the best correlate of adrenal medulla uptake, we report here a structure–distribution relationship (SDR) study of 14 ^{125}I-labeled (iodoaralkyl)guanidines and 3 [^{14}C]guanidines in dogs. This study focuses on the structural elements of aralkylguanidines necessary for maximum adrenomedullary uptake and retention. One of the most promising compounds, (*m*-iodobenzyl)guanidine (*m*-IBG, **2a**), when radiolabeled with γ-emitting isotopes ^{131}I or ^{123}I, has shown recent clinical success in imaging diseases of the adrenal medulla.[21,22]

Chemistry. At the outset of this investigation, attempts were made to synthesize the (iodoaralkyl)guanidines by reaction of the appropriate amine with 2-methyl-2-thiopseudourea sulfate.[23,24] It was subsequently found that reaction of the appropriate amine hydrochloride with molten cyanamide gave consistently higher yields of

[†]Present address: Medi-Physics, Emeryville, CA 94608.

(1) A brief report of part of the present study has appeared: Wieland, D. M.; Brown, L. E.; Mangner, T. J.; et al. Proceedings of the 3rd International Symposium on Radiopharmaceutical Chemistry, *J. Labelled Cmpd. Radiopharm.* **1981**, *18*, 122.
(2) Morales, J. O.; Beierwaltes, W. H.; Counsell, R. E.; et al. *J. Nucl. Med.* **1967**, *8*, 800.
(3) Lieberman, L. M.; Beierwaltes, W. H.; Varma, V. M.; et al. *J. Nucl. Med.* **1969**, *10*, 93.
(4) Anderson, B. G.; Beierwaltes, W. H.; Harrison, T. S.; et al. *J. Nucl. Med.* **1973**, *14*, 781.
(5) Fowler, J. S.; Ansari, A. N.; Atkins, H. L.; et al. *J. Nucl. Med.* **1973**, *14*, 867.
(6) Fowler, J. S.; Wolf, A. P.; Christman, R. D.; et al. In "Radiopharmaceuticals", Subramanian, G.; Rhodes, B. A.; Cooper, J. T., et al., Eds.; Society of Nuclear Medicine: New York, 1975; p 196.
(7) Ice, R. D.; Wieland, D. M.; Beierwaltes, W. H.; et al. *J. Nucl. Med.* **1975**, *16*, 1147.
(8) Fowler, J. S.; MacGregor, R. R.; Wolf, A. P. *J. Med. Chem.* **1976**, *19*, 356.
(9) Counsell, R. E.; Yu, T.; Ranade, V. V.; et al. *J. Med. Chem.* **1973**, *16*, 1038.
(10) Korn, N.; Buswink, A.; Yu, T.; et al. *J. Nucl. Med.* **1977**, *18*, 87.
(11) Wieland, D. M.; Swanson, D. P.; Brown, L. E.; et al. *J. Nucl. Med.* **1979**, *20*, 155.
(12) Wieland, D. M.; Wu, J. L.; Brown, L. E.; et al. *J. Nucl. Med.* **1980**, *21*, 349.
(13) Wieland, D. M.; Brown, L. E.; Tobes, M. C.; et al. *J. Nucl. Med.* **1981**, *22*, 358.
(14) Chang, C. C.; Chang, J. C.; Su, C. Y. *Br. J. Pharmacol.* **1967**, *30*, 213.
(15) Giachetti, A.; Hollenbeck, R. A. *Br. J. Pharmacol.* **1976**, *58*, 497.
(16) For an excellent review, see: Maxwell, R. A.; Wastila, W. B. *Handb. Exp. Pharmacol.* **1977**, *39*, 161.
(17) Stjarne, L. *Handb. Exp. Pharmacol.* **1972**, *33*, 231.
(18) Athos, W. J.; McHugh, B. P., Fineberg, S. E.; et al. *J. Pharmacol.* **1962**, *137*, 229.
(19) Cass, R.; Kuntzman, R.; Brodie, B. B. *Proc. Soc. Exp. Biol. Med.* **1960**, *103*, 871.
(20) Short, J. H.; Darby, T. D. *J. Med. Chem.* **1967**, *10*, 833.
(21) Sisson, J. C.; Frager, M. S.; Valk, T. W.; et al. *N. Engl. J. Med.* **1981**, *305*, 12.
(22) Valk, T. W.; Frager, M. S.; Gross, M. D.; et al. *Ann. Int. Med.* **1981**, *94*, 762.

0022-2623/84/1827-0149$01.50/0