Structural Features of Imidazole Derivatives That Enhance Styrene Oxide Hydrolase Activity in Rat Hepatic Microsomes

Margaret O. James* and Kenneth B. Sloan

Department of Medicinal Chemistry, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610.
Received August 23, 1984

The present study is an investigation of the in vitro effects of several model imidazole compounds and three antifungal drugs on styrene oxide hydrolase activity in hepatic microsomes from control, PB-induced, and 3MC-induced rats. We first determined the influence of the position of substitution of a phenyl group in the imidazole or imidazoline ring on the ability of 10^{-3} M concentration of the imidazole derivative to enhance microsomal epoxide hydrolase activity. This study showed that 1-phenylimidazole enhanced epoxide hydrolase activity to the greatest extent and that, for any one imidazole derivative, the extent of enhancement was similar for microsomes from untreated, PB-induced, or 3MC-induced rats. We next studied the potency and maximum effectiveness of several N-1-substituted imidazole derivatives with differing lipophilicities and pK_a values. Hansch analysis showed that potency for activating epoxide hydrolase correlated with both lipophilicity and electron-withdrawing effects of imidazole ring substituents. The most potent compounds had aryl or aralkyl substitutents at the 1-position of imidazole and pK_a in the range 7–9 and were clotrimazole, ketoconazole, miconazole, 1-benzylimidazole, and 1-(4-hydroxyphenyl)imidazole. These compounds enhanced styrene oxide hydrolase by 100% at concentrations $<2 \times 10^{-4}$ M. 1-Benzylimidazole increased both K_m and V_{max} and abolished the inhibition observed at high concentrations of styrene oxide.

Molecules containing an imidazole or imidazoline ring may have a variety of biological activities: these include antimycotic activity,[1,2] antiinfective activity,[3] histamine H_2 receptor antagonist activity,[4] adrenergic antagonist activity,[5] and antidepressant activity.[6] In addition, imidazole-containing molecules affect pathways of xenobiotic biotransformation and may therefore affect the disposition and duration of action of coadministered drugs. The ability of imidazole derivatives to inhibit some (but not all) cytochrome P-450 dependent monooxygenase activities has been previously studied,[7-11] and some structure–activity relationships are known. James and Little have recently shown[12] that some imidazole derivatives modify benzo[*a*]pyrene (BaP) metabolism by rat hepatic microsomes differently depending on the rat pretreatment. In 3-methylcholanthrene (3MC) treated rats, simple imidazole derivatives did not inhibit overall BaP metabolism but did enhance epoxide hydrolase activity with BaP 9,10-oxide so that the proportion of 9,10-diol formed was increased, whereas in control and phenobarbital (PB) treated rats the predominant effect of the imidazole derivative was to inhibit overall monooxygenase activity without affecting the proportion of individual metabolites formed. Other laboratories[13,14] have reported that certain imidazole derivatives can enhance epoxide hydrolase activity, but no systematic study of this effect has been undertaken. The present study is an investigation of the in vitro effects of several model imidazole compounds and three antifungal drugs on styrene oxide hydrolase activity in hepatic microsomes from control, PB-induced, and 3MC-induced rats. We first determined the influence of the position of substitution of a phenyl group in the imidazole or imidazoline ring on the ability of a fixed concentration of the imidazole derivative to enhance microsomal epoxide hydrolase activity. This study showed that 1-phenylimidazole enhanced epoxide hydrolase activity to the greatest extent. We therefore studied the potency and maximum effectiveness of several N-1-substituted imidazoles with differing lipophilicities and pK_a values.

Table I. Enhancement of Styrene Oxide Hydrolase Activity in Hepatic Microsomes from Male Wistar Rats by Phenylimidazole Isomers and 2-Phenylimidazoline

	enhancement[a]		
compd added (10^{-3} M)	untreated	PB treated	3MC treated[b]
1-phenylimidazole	3.34 ± 0.22	3.15, 3.21	3.75 ± 0.18
2-phenylimidazole	2.00 ± 0.06	1.99	2.09 ± 0.07
4(5)-phenylimidazole	2.30 ± 0.19	2.45 ± 0.26	2.61 ± 0.08
2-phenylimidazoline	1.19 ± 0.05	1.11 ± 0.03	1.15

[a] In each case, enhancement was the ratio of (activity in presence of added imidazole derivative)/(activity in absence of imidazole derivative). Results shown are mean ± SD for n = three or four individuals or the individual results. The incubation pH was 8.7, and tubes were incubated at 37 °C for 10 min. Microsomal epoxide hydrolase activities (nmol min^{-1} (mg of protein)$^{-1}$) in the absence of imidazole derivatives were as follows: untreated, 12.9 ± 1.8 (n = 4); PB treated, 27.2 ± 4.8 (n = 4), 3MC treated, 12.5 ± 1.9 (n = 4). [b] The rats were injected ip with 80 mg/kg of PB or 40 mg/kg of 3MC daily for 3 days before sacrifice and preparation of washed hepatic microsomes. The cytochrome P-450 contents and BaP hydroxylase activities of the rat hepatic microsomes were in agreement with literature values for similarly pretreated animals.

Results and Discussion

The extent to which a fixed concentration of the hydrochloride salts of several phenylimidazole isomers and 2-phenylimidazoline enhanced styrene oxide hydrolase activity in hepatic microsomes prepared from untreated,

(1) Borgers, M. *Rev. Infect. Dis.* **1980**, *2*, 520.
(2) Bianchi, A.; deCarneri, I.; Trane, F. *Eur. J. Med. Chem.—Chim. Ther.* **1982**, *17*, 139.
(3) Jolles, G. E. "Metronidazole"; Finegold, S. M., Ed.; Excerpta Medica: Amsterdam, 1977; p 3.
(4) Ganellin, C. R. *J. Med. Chem.* **1981**, *24*, 913.
(5) Mimnaugh, M.; Gearin, J. E. "Principles of Medicinal Chemistry"; Foye, W. O., Ed.; Lea and Febinger: Philadelphia, 1981; Chapter 16.
(6) Caroon, J. M.; Clark, R. D.; Kluge, A. F.; Olah, R.; Repke, D. B.; Unger, S. H.; Michel, A. D.; Whiting, R. L. *J. Med. Chem.* **1982**, *25*, 666.
(7) Wilkinson, C. F.; Hetnarski, K.; Cantwell, G. P.; DiCarlo, F. J. *Biochem. Pharmacol.* **1974**, *23*, 2377.
(8) Rogerson, T. D.; Wilkinson, C. F.; Hetnarski, K. *Biochem. Pharmacol.* **1977**, *26*, 1039.
(9) Little, P. J.; Ryan, A. D. *J. Med. Chem.* **1982**, *25*, 622.
(10) Murray, J.; Ryan, A. J.; Little, P. J. *J. Med. Chem.* **1982**, *25*, 887.
(11) Little, P. J.; James, M. O.; Bend, J. R.; Ryan, A. *Biochem. Pharmacol.* **1982**, *30*, 2876.
(12) James, M. O.; Little, P. J. *Drug Metab. Dispos.* **1983**, *11*, 350.
(13) Leibman, K. C.; Ortiz, E. *Drug Metab. Dispos.* **1973**, *1*, 775.
(14) Kahl, R.; Friederici, D. E.; Kahl, G. F.; Ritter, W.; Krebs, R. *Drug Metab. Dispos.* **1980**, *8*, 191.

0022-2623/85/1828-1120$01.50/0

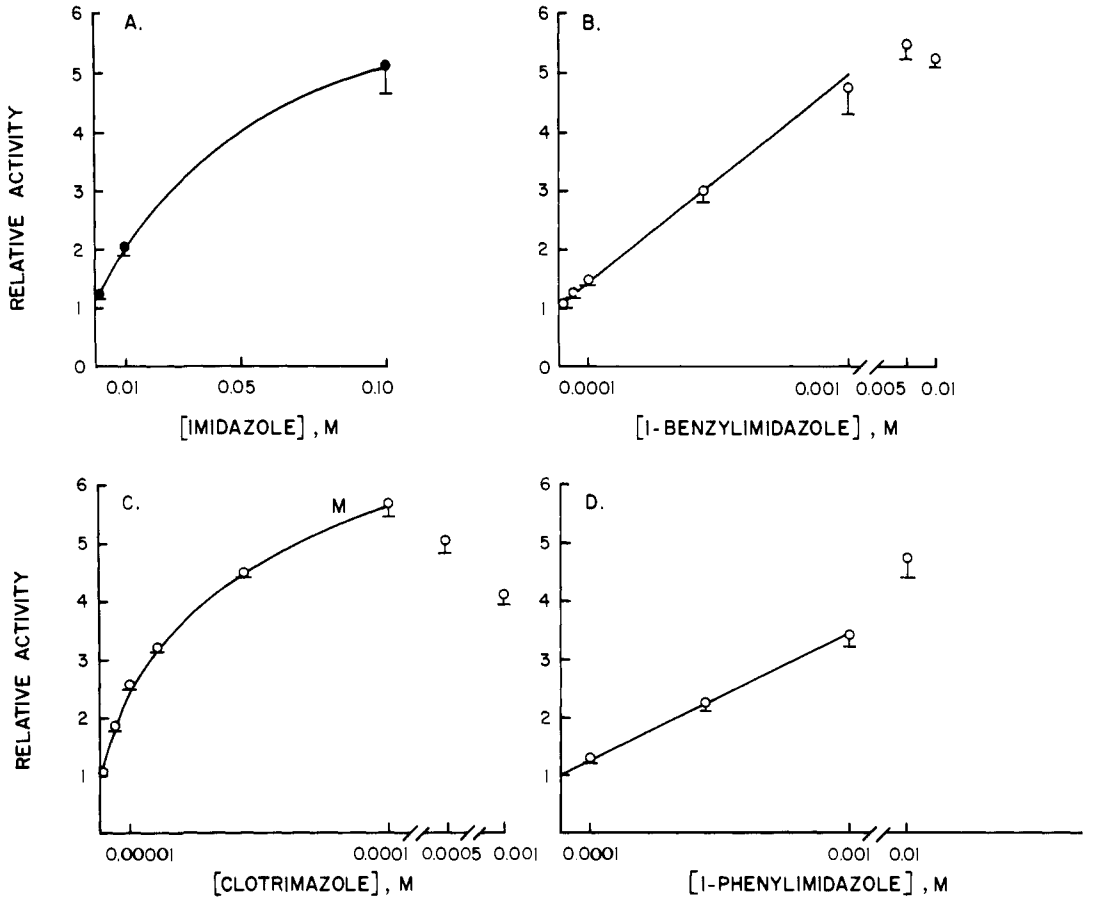


Figure 1. Concentration–effect plots for selected 1-substituted imidazole derivatives of low (A, imidazole), intermediate (B, 1-benzylimidazole, D, 1-phenylimidazole), and high (C, clotrimazole) potency. The relative activity is (activity in presence of imidazole derivative)/(activity in absence of imidazole derivative).

PB-treated, and 3MC-treated male Wistar rats is shown in Table I. The concentration of imidazole derivative used (10^{-3} M) was equal to the concentration of styrene oxide substrate. As expected, base-line hydrolase activities were similar in untreated and 3MC-treated rats and more than twofold higher in PB-treated rats.[15] For any one compound, the extent of enhancement of epoxide hydrolase activity at pH 8.7 was similar, regardless of whether microsomes were prepared from untreated or induced rats. Thus, the epoxide hydrolase enzyme induced by PB is similar to that present in untreated animal in its response to imidazole derivatives and 3MC administration does not affect the response. The most effective compound was 1-phenylimidazole, and the least effective was 2-phenylimidazoline. In further studies, microsomes from untreated rats were used to determine the effect of substituting different groups at the 1-position of imidazole.

Several model compounds and three antifungal drugs that are 1-substituted imidazoles were studied for their effect on microsomal epoxide hydrolase activity. Potency was compared in terms of the concentration of drug needed to produce a 100% enhancement of epoxide hydrolase activity (the E_{100} concentration). Maximum effectiveness was the maximum enhancement of epoxide hydrolase activity observed for a given imidazole derivative. The results are presented in Table II. The concentration–effect relationships for four selected compounds of high, intermediate, and low potencies are shown in Figure 1. For most compounds studied, the concentration–effect relationship was linear up to the concentration needed to produce a 100% enhancement of activity (relative activity of 2) and thereafter gradually reached a plateau. The E_{100} concentrations shown in Table II were obtained from plots

(15) Oesch, F. *Xenobiotica* **1973**, *3*, 305.

Table II. Enhancement of Styrene Oxide Hydrolase Activity by 1-Substituted Imidazoles

no.	imidazole substit	E_{100},[a] M	max enhancement (concn required, M)
1	none	$8.8 \pm 2.0 \times 10^{-3}$ (6)[b]	5.1 ± 0.5 (10^{-1})
2	1-methyl	$1.2 \pm 0.2 \times 10^{-2}$ (3)	3.9 ± 0.2 (5×10^{-2})
3	1-ethyl	$2.8 \pm 0.5 \times 10^{-3}$ (3)	4.6 ± 0.3 (5×10^{-2})
4	1-phenyl	$3.3 \pm 0.7 \times 10^{-4}$ (3)	4.7 ± 0.4 (10^{-2})
5	1-(4-fluorophenyl)	$5.5 \pm 1.5 \times 10^{-4}$ (6)	4.4 ± 0.3 (5×10^{-3})
6	1-(4-hydroxyphenyl)	$2.4 \pm 0.4 \times 10^{-4}$ (3)	3.8 ± 0.2 (10^{-3})
7	1-(4-nitrophenyl)	[c]	1.3 ± 0.1 (10^{-3})
8	1-benzyl	$2.3 \pm 0.5 \times 10^{-4}$ (4)	5.7 ± 0.3 (5×10^{-3})
9	1-benzyl-4-nitro	$5.9 \pm 1.2 \times 10^{-3}$ (4)	1.9 ± 0.4 (5×10^{-3})
10	1-benzyl-2-methyl-4-nitro	[c]	1.5 ± 0.0 (2×10^{-3})
11	clotrimazole[d]	$6.2 \pm 0.8 \times 10^{-6}$ (4)	5.4 ± 0.5 (10^{-4})
12	miconazole[d]	$8.4 \pm 2.0 \times 10^{-5}$ (4)	3.1 ± 0.4 (5×10^{-4})
13	ketoconazole[d]	$5.4 \pm 0.6 \times 10^{-5}$ (4)	3.3 ± 0.5 (10^{-3})

[a] E_{100} is the concentration of imidazole derivative required in incubation tube to enhance epoxide hydrolase activity by 100% at pH 8.7. [b] Mean ± SD for (n) individual rats. [c] These compounds did not cause a 100% enhancement of epoxide hydrolase at pH 8.7 at any concentration tested. We were unable to test concentrations greater than those indicated due to the lower water solubility of these compounds. E_{100} values calculated by extrapolation from the linear portion of the dose–response plot were as follows: compound **7**, $3.9 \pm 0.3 \times 10^{-3}$ M; compound **10**, $3.4 \pm 0.6 \times 10^{-3}$ M. [d] Clotrimazole: 1-[(2-chlorophenyl)diphenylmethyl]imidazole. Miconazole: 1-[2,4-dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl]-imidazole. Ketoconazole: *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxalan-4-yl]methoxy]-phenyl]piperazine.

Table III. QSAR for Enhancement of Epoxide Hydrolase Activity by 1-Substituted Imidazoles

no.[a]	log $1/E_{100}$,[a] obsd	log P[b]	$\sum\sigma$[c]	log $1/E_{100}$,[d] predicted
1	2.05	−0.42	0	1.81
2	1.94	0.28	−0.04	2.46
3	2.55	0.81	−0.03	2.85
4	3.48	1.48	+0.03	3.24
5	3.26	1.67	+0.09	3.29
6	3.62	0.94	−0.34	3.28
7	2.40	1.19	+0.73	2.29
8	3.64	2.01	−0.05	3.64
9	2.23	1.93	+1.21	2.23
11	5.21	6.14	−0.02	4.69
12	4.08	5.84	−0.01	4.68
13	4.27	3.73	+0.08	4.24

[a] See Table II for structures and observed potencies (E_{100}), The "observed" value for compound 7 was extrapolated from the linear portion of the dose–response plot. [b] Log P values were calculated from values in ref 18. [c] $\sum\sigma$ values were calculated from values in ref 19. [d] Predicted value derived from the Hansch equation (ref 17): $\log 1/E_{100} = k_1 \log P - k_2(\log P)^2 + k_3\sum\sigma + K_4$. Values for k_1–k_4 were derived from multiple linear regression analysis (ref 20) and are listed below. The overall fit of the data (R^2) was 0.891 and the root mean square was 0.394. The significance shown was computed from the F statistic value derived from error mean square values for each parameter (ref 20). Parameter, estimate (mean ± SE), significance: k_1, 0.845 ± 0.217, 0.005; k_2, 0.072 ± 0.034, 0.067; k_3, −1.089 ± 0.304, 0.007; k_4, 2.181 ± 0.226, 0.0001.

of at least three concentrations of imidazole derivative above and below the E_{100} concentration.

The maximum enhancement of epoxide hydrolase activity found (Table II) was related to potency and water solubility for all the imidazole derivatives studied. For most of the compounds (Table II), the maximum observable enhancement was about fivefold and was measured at concentrations in the range 10–30 times the E_{100} concentration. Further increases in concentration of the added imidazole derivative did not cause further activation of epoxide hydrolase (see also Figure 1). Lesser maximum enhancements were found with compounds that were poorly water soluble and formed precipitates in the incubation tube at concentrations 10–30 times the E_{100} concentration, e.g., miconazole and ketoconazole, or at concentrations lower than the expected E_{100} concentration, e.g., nitro derivatives. It was very important to add the imidazole derivatives to incubation tubes from aqueous solution: if acetone solutions were used, the enhancement was diminished or abolished. Acetone itself slightly (up to 1.3-fold) enhances epoxide hydrolase activity[16] and disrupts the microsomal membrane, suggesting that the enhancement caused by imidazole derivatives requires an intact microsomal membrane.

Of the compounds studied, imidazole and 1-methylimidazole were the least potent enhancers of epoxide hydrolase activity and clotrimazole the most potent. Of the model compounds, 1-benzylimidazole was the most potent but the ability of 1-benzylimidazole to enhance styrene oxide hydrolase could be markedly reduced by substituting a nitro group at the 4-position of the imidazole ring. A related compound, 1-benzyl-2-methyl-4-nitroimidazole also had very low potency and effectiveness for enhancing epoxide hydrolase activity.

Correlation of the observed potencies shown in Table II with physicochemical parameters of the imidazole derivatives was undertaken by the method of Hansch[17] (see Table III). The log P values are lipophilicity of the unionized form, calculated from the fragmentation contacts

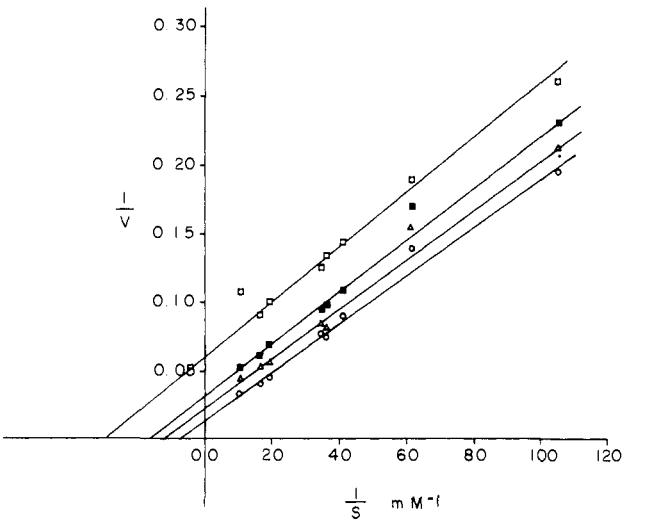


Figure 2. Effect of 1-benzylimidazole on styrene oxide hydrolase activity in rat hepatic microsomes. Values shown are the mean of duplicate determinations with microsomes from a single rat. The experiment was repeated with similar results. For each concentration of 1-benzylimidazole, Michaelis–Menton kinetic parameters were derived with data points from eight concentrations of styrene oxide by the method of Cleland.[31] The values shown are the mean ± SE: (□) no addition, K_m = 0.034 ± 0.003 mM, V_{max} = 16.79 ± 0.72 nmol min^{-1} (mg of protein)$^{-1}$, V_{max}/K_m = 4.92 ± 0.23; (■) 1 × 10^{-4} M 1-benzylimidazole, K_m = 0.058 ± 0.005 mM, V_{max} = 30.56 ± 1.51 nmol min^{-1} (mg of protein)$^{-1}$, V_{max}/K_m = 5.29 ± 0.23; (△) 2 × 10^{-4} M 1-benzylimidazole, K_m = 0.076 ± 0.011 mM, V_{max} 42.24 ± 3.72 nmol min^{-1} (mg of protein)$^{-1}$, V_{max}/K_m = 5.55 ± 0.36; (○) 6 × 10^{-4} M 1-benzylimidazole, K_m = 0.123 ± 0.023 mM, V_{max} = 70.48 ± 8.91 nmol min^{-1} (mg of protein)$^{-1}$, V_{max}/K_m = 5.71 ± 0.36.

of Rekker,[18] and the $\sum\sigma$ values for electronic effects of substituents are Hammet σ constants as compiled by Perrin.[19] We did not include 1-benzyl-2-methyl-4-nitroimidazole in the analysis because the influence of the 2-methyl group on effectiveness is unknown and because the E_{100} for this compound was obtained by extrapolation (Table II). The values used and the results obtained from multiple regression analysis[20] are shown in Table III. The value for 1-(4-nitrophenyl)imidazole was obtained by extrapolation and inclusion or omission of this compound had little effect on the results. The correlation coefficient (R^2) for an equation containing terms in log P, log P^2, and $\sum\sigma$ was 0.891. Although the log P^2 term was small and of low statistical significance (p = 0.067, see Table III), omission of the log P^2 term from the model caused the correlation coefficient (R^2) to decrease to 0.829 and the root mean square to increase to 0.46 (data not shown). This indicated that inclusion of a term in log P^2 improved the model. Equations containing only terms in log P or only terms in $\sum\sigma$ gave much lower correlation coefficients (R^2 < 0.72) and higher root mean square values (>0.60).

The Hansch analysis showed that the enhancement of epoxide hydrolase activity was dependent on the electronic effects of substituents, which in turn affect the pK_a of the imidazole derivatives as well as their lipophilicity. The pK_a of 1-benzylimidazole is 7.3, so at pH 8.7, 3.8% will be protonated. The nitroimidazoles, with pK_a = −0.1 (1-benzyl-4-nitroimidazole) and 3.5 (1-benzyl-2-methyl-4-

(16) Seidegard, J.; DePierre, J. W. *Eur. J. Biochem.* **1980**, *112*, 643.
(17) Hansch, C.; Lien, E. J. *J. Med. Chem.* **1971**, *14*, 653.
(18) Rekker, R. F. "The Hydrophobic Fragmental Constant"; Elsevier: Amsterdam, 1977; Chapter 3.
(19) Perrin, D. D. "Physical-Chemical Properties of Drugs"; Yalkowsky, C. H., Sinkala, A.A., Velvani, S. C., Eds.; Marcel Dekker: New York, 1980; Chapter 1.
(20) "SAS Users Guide: Statistics"; SAS Institute, Cary, NC, 1982.

nitroimidazole) are both virtually unionized at pH 7.0 or 8.7. It appears that the electronic requirement for potency in enhancing styrene oxide hydrolase activity is a pK_a within 2 units of the assay pH. The imidazole derivative will then be partly unionized at the pH of the assay and will penetrate membranes well but will also be readily protonated. Since the virtually unionized nitro derivatives are inactive, it appears that the ability to retain protons at the assay pH is important for the enhancement.

Studies of the kinetics of enhancement of epoxide hydrolase activity were conducted at pH 8.7 with three concentrations of 1-benzylimidazole and seven concentrations of styrene oxide. Lineweaver–Burk plots of the data are parallel (Figure 2) and show that 1-benzylimidazole increased both K_m and V_{max}, giving a "mixed" activation.[21] In the absence of 1-benzylimidazole we observed inhibition at the highest concentration of styrene oxide. The results obtained provided clues to the mechanism of enhancement. The kinetic analysis is consistent with the active site being modified so as to prevent the observed substrate inhibition. We have shown that the most potent imidazoles have lipophilic substituents at N-1 and pK_a such that N-3 can readily exchange protons at the assay pH. Others have demonstrated that a histidine at the active site of epoxide hydrolase acts as a proton donor/acceptor during epoxide hydrolysis:[22,23] possibly the imidazole derivatives that enhance epoxide hydrolase do so by similarly participating in base-catalyzed hydrolysis at or near the active site of epoxide hydrolase.

Significance

These studies define some of the structural features of imidazole-containing molecules that are needed for enhancement of styrene oxide hydrolase activity. A mechanism of enhancement is proposed but awaits validation by further biochemical studies.

Potency in activating epoxide hydrolase correlated with both lipophilicity and electron-withdrawing effects of imidazole ring substituents, and we derived parameters that will be of predictive value in determining the potency of other imidazole derivatives for enhancement of styrene oxide hydrolase activity. The most potent compounds we studied ($E_{100} < 2 \times 10^{-4}$ M for assay at pH 8.7) have aryl or aralkyl substituents at the 1-position of imidazole and pK_a in the range 7–9. Three antifungal drugs fall into this category. Their potency is such that an interaction with epoxide hydrolase may be expected at therapeutic concentrations of drug, at least in the case of ketoconazole.[24]

The ability of imidazole derivatives to inhibit certain cytochrome P-450 dependent monoxygenase reactions has been correlated with lipophilicity and molecular size,[7-10] and it is clear that there is considerable overlap in the structural requirements for cytochrome P-450 inhibition and epoxide hydrolase activation.

Previous studies have shown that not all epoxide substrates are susceptible to increased microsomal hydrolysis in the presence of imidazole derivatives: BaP 4,5-oxide or BaP 7,8-oxide hydrolysis was not increased in the presence of 1-phenylimidazole, 4(5)-phenylimidazole, benzimidazole, or naphthimidazole although BaP 9,10-oxide hydrolysis was increased.[12] No studies have yet been conducted with different epoxide substrates to determine what structural requirements are needed for the epoxide hydrolysis to be enhanced by imidazoles.

In general, epoxides are toxic metabolites that are detoxified by hydrolysis or conjugation with glutathione, so the ability of imidazole-containing drugs to stimulate epoxide hydrolase would in most cases be a desirable interaction. A well-documented exception is BaP 7,8-oxide, whose hydrolysis product, BaP 7,8-dihydrodiol, is further metabolized by cytochrome P-450 to the proximate carcinogen BaP 7,8-diol 9,10-oxide.[25] Our present findings contribute to an understanding of the interaction of imidazole-containing drugs with epoxide hydrolase and how this will affect the fate of other drugs metabolized by the epoxide–diol pathway.

Experimental Section

Synthesis. 1-Benzyl-2-methyl-4-nitroimidazole (mp 105–107 °C, lit.[26] mp 106 °C from toluene) and 1-benzyl-4-nitroimidazole (mp 78–80 °C, lit.[26] mp 76 °C from benzene) were prepared under basic conditions (anhydrous K_2CO_3) in dry acetone from the reaction of benzyl iodide, generated in situ from benzyl chloride and anhydrous NaI, with 2-methyl-4-nitroimidazole and 4-nitroimidazole, respectively.[27]

Source of Chemicals. Imidazole, clotrimazole, miconazole, and buffer chemicals were obtained from Sigma Chemical Co., St. Louis, MO. Compounds 2–8 (Table II) were obtained from Trans-World Chemical Co., Washington, DC. Ketoconazole was a generous gift of Janssen Pharmaceutica, Piscataway, NJ. [8-^{14}C]Styrene oxide was obtained from New England Nuclear.

Tissue Preparation. Male Wistar rats, body weight 200 ± 20 g, were sacrificed by decapitation. Their livers were removed immediately, rinsed three times in ice-cold 0.15 M KCl in 0.05 M potassium phosphate, pH 7.4, and used to prepare washed microsomes as described previously.[12] For some experiments, rats were treated with 3-methylcholanthrene (40 mg/kg) or phenobarbital (80 mg/kg) daily for 3 days before sacrifice. The washed hepatic microsomes were suspended in a buffer containing 0.25 M sucrose, 0.01 M Hepes, pH 7.4, 5% glycerol, 0.1 M EDTA (1 mL/2 g of wet weight liver) and stored in 1-mL portions at −20 °C until use.

Assays. Styrene oxide hydrolase activity was measured as described previously.[12] In a 0.145-mL final volume, tubes contained 0.25 M Tris-Cl, pH 8.7, 0.03% Tween-80, microsomal protein (0.1–0.2 mg), and test compound or solvent. The imidazole derivatives were added from aqueous solutions of their hydrochloride salts, except for the 4-nitroimidazole derivatives, which were dissolved in the minimum volume of dimethyl sulfoxide/water (1:1). Tubes were placed in a water bath at 37 °C and the reaction started by adding 1 mM [^{14}C]styrene oxide (0.005 mL in acetonitrile solution). After 10 min the reaction was stopped by addition of hexanes (3 mL) and vortex mixing as described previously.[12] In all cases, duplicate tubes were assayed at each concentration of imidazole derivative. The reference tubes contained diluted HCl or solvent. Blanks for measuring nonenzymatic hydrolysis of styrene oxide contained no tissue. Evaporative losses of styrene oxide did not occur with this short incubation period and the ^{14}C added could be quantitatively accounted for after the 10 min of incubation.

Protein content of hepatic microsomes was measured by the method of Lowry et al.[28]

Benzo[*a*]pyrene hydroxylase activity[29] and cytochrome P-450

(21) *Int. Union Biochem. Arch. Biochem. Biophys.* **1983**, *224*, 732.
(22) Dubois, G. C.; Apella, E.; Levin, W.; Lu, A. Y. H.; Jeriona, D. M. *J. Biol. Chem.* **1978**, *253*, 2932.
(23) Armstrong, C. N.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1980**, *255*, 4698.
(24) Brass, C.; Galgiani, J. N.; Blaschke, T. F.; Defelice, R.; O'Reilly, R. A.; Stevens, D. A. *Antimicrob. Agents Chemother.* **1982**, *21*, 1951.
(25) Kapitulnik, J.; Levin, W.; Conney, A. H.; Yagi, M.; Jerina, D. M. *Nature (London)* **1977**, *266*, 378.
(26) Cosar, C.; Crisan, C.; Hosclois, R.; Jacob, R. M.; Robert, J.; Tchelitcheff, S.; Vaupre, R. *Arzheim. Forsch.* **1966**, *16*, 23.
(27) Grimson, A.; Ridd, J.; Smith, B. V. *J. Chem. Soc.* **1960**, 1352.
(28) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
(29) DePierre, J. W.; Moron, M. S.; Johannesen, K. A. M.; Ernster, L. *Anal. Biochem.* **1975**, *63*, 470.
(30) Omura, T.; Sato, R. *J. Biol. Chem.* **1964**, *239*, 2370.
(31) Cleland, W. W. *Methods Enzymol.* **1979**, *63*, 103.

content[30] of hepatic microsomes were measured in induction experiments in order to verify that the expected response to 3MC and PB was elicited.

Acknowledgment. We thank Gary Myers for excellent technical assistance and Dr. Mike Conlon and Ned Phillips for assistance with the multiple regression analysis. This research was supported by an NIH biomedical research grant to the College of Pharmacy.

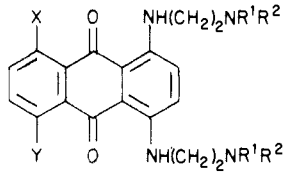
Synthesis and Antineoplastic Evaluations of 5,8-Bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones

A. Paul Krapcho,* John J. Landi, Jr., Miles P. Hacker, and John J. McCormack

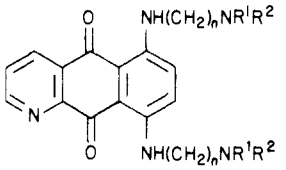
Vermont Regional Cancer Center and Departments of Chemistry and Pharmacology, The University of Vermont, Burlington, Vermont 05405. *Received December 14, 1984*

Several 5,8-bis[(aminoalkyl)amino]-1-azaanthracene-9,10-diones have been synthesized and evaluated for antitumor activity against L1210 leukemia both in vitro and in vivo. Comparisons are made to the corresponding carbocyclic analogues. One of the aza analogues showed modest in vivo activity.

Recently the synthesis and antineoplastic evaluation of a number of symmetrical 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones related to **1** have been reported.[1]



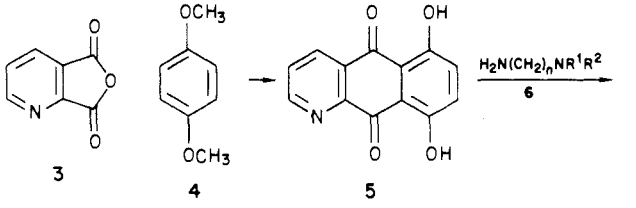
1a, X = Y = R^1 = H; R^2 = $(CH_2)_2OH$
b, X = Y = OH; R^1 = H; R^2 = $(CH_2)_2OH$
c, X = Y = H; R^1 = R^2 = CH_3
d, X = Y = H; R^1 = R^2 = CH_2CH_3



2

Compounds **1a** (AQ, ametantrone) and **1b** (mitoxantrone, DHAQ or DHAD) are representative of this relatively new class of antineoplastic compounds. In particular, **1b** has shown outstanding activity and is in clinical trials.[1c,d,h] The cardiotoxicities of both AQ and DHAQ have been studied, and AQ is reported to be 10-fold less toxic than DHAQ in a rat cardiotoxic model system.[2] It has also been reported that DHAQ on intraperitoneal administration to non-tumor-bearing mice caused a delayed lethality while **1a** (AQ) did not.[3] The 5,8-dihydroxy groups were suggested as being implicated in this lethality since **1a** did not cause delayed deaths. The myocardial effects of DHAQ and doxorubicin have recently been suggested as being quite similar in the mouse and guinea pig.[4] The search for new analogues with antitumor activity but without the undesirable cardiotoxic effects is of extreme interest.

Scheme I



3 **4** **5** **2**

a, n = 2; R^1 = R^2 = CH_3
b, n = 2; R^1 = R^2 = CH_2CH_3
c, n = 3; R^1 = R^2 = CH_3
d, n = 2 R^1 = H; R^2 = $COCH_3$

Table I. Activity of Azaanthracene-9,10-diones and Their Carbocyclic Analogues against L1210 Cells in Vitro

compd	ID_{50}, μg/mL	compd	ID_{50}, μg/mL
5	0.18	**2d**	>10
2a	0.16	**1c**	0.08
2b	2.4	**1d**	0.30
2c	1.7		

As part of a drug development program dealing with the structure–activity relationships of anthracene-9,10-diones and the preparation of less cardiotoxic compounds, we have explored the synthesis of heterocyclic analogues **2**, related to **1** in which a CH group is replaced by a nitrogen atom. It was anticipated that the ease of electron addition to an azaanthracene-9,10-dione would be more facile than the corresponding anthracene-9,10-dione. In addition, the heterocyclic models would probably be more soluble in aqueous media and more readily metabolized and excreted from the body.

A French group has recently reported that the substitution of a nitrogen atom for an aromatic CH group in an [(aminoalkyl)amino]ellipticine increased the antitumor activity for various neoplasms.[5] Several recent papers have recently appeared describing the synthesis of azaanthracene-9,10-diones that might be useful in the preparations of analogues for biological evaluation.[6] In

(1) (a) Cheng, C. C.; Zee-Cheng, R. K. Y. In "Progress in Medicinal Chemistry"; Ellis, G. P., West, G. B., Eds.; Elsevier: Amsterdam, 1983; pp 20, 83, and references cited therein. (b) Zee-Cheng, R. K. Y.; Cheng, C. C. *Drugs Future*, **1983**, *8*, 229. (c) Durr, F. E.; Wallace, R. E.; Citarella, R. V. *Cancer Treat. Rev.* **1983**, *10* (Suppl B), 3. (d) Stuart-Harris, R. C.; Bozek, T.; Pavlidis, N. A.; Smith, I. E. *Cancer Chemother. Pharmacol.* **1984**, *12*, 1. (e) Zee-Cheng, R. K. Y.; Podrebarac, E. G.; Menon, C. S.; Cheng, C. C. *J. Med. Chem.* **1979**, *22*, 501. (f) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291. (g) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024. (h) Wallace, R. E.; Murdock, K. C; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570. (i) Durr, F. E.; Murdock, K. C., American Cyanamid Co., U.S. Patent 4 197 249 (Cl. 260-380; CO7C97126), 08 Apr 1980; *Chem. Abstr.* **1980**, *93*, 713968.

(2) Zbinden, G.; Beilstein, A. K. *Toxicol. Lett.* **1982**, *11*, 289.

(3) Corbett, T. H.; Griswold, D. P., Jr.; Roberts, B. J.; Schnabel, F. M., Jr. *Cancer Chemother. Pharmacol.* **1981**, *6*, 161.

(4) Perkins, W. E.; Schroeder, R. L.; Carrano, R. A.; Imondi, A. R. *Cancer Treat. Rep.* **1984**, *68*, 841.

(5) Ducrocq, C.; Wendling, F.; Tourbez-Perrine, M.; Rivalle, C.; Tambourin, P.; Pochon, F.; Bisagni, E.; Chermann, J. C. *J. Med. Chem.* **1980**, *23*, 1212.

(6) (a) Croisey-Delcey, M.; Bisagni, E. *J. Chem. Soc., Chem Commun.* **1984**, 897. (b) Potts, K. T.; Bhattacharjee, D. *Synthesis* **1983**, 31. (c) Potts, K. T.; Bhattacharjee, D.; Walsh, E. B. *J. Chem. Soc., Chem. Commun.* **1984**, 114.

(7) Raudnitz, H. *Chem. Ber.* **1929**, *62*, 938.

0022-2623/85/1828-1124$01.50/0