Inhibitors of Hepatic Mixed-Function Oxidases. 4.[1] Effects of Benzimidazole and Related Compounds on Aryl Hydrocarbon Hydroxylase Activity from Phenobarbitone and 3-Methylcholanthrene Induced Rats

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A series of 2-*n*-alkylbenzimidazoles inhibited cytochrome P-450 dependent aryl hydrocarbon hydroxylase (AHH) and aminopyrine *N*-demethylase (APDM) activities in phenobarbitone (PB) induced rat liver microsomes. 2-Undecylbenzimidazole was the most potent compound in the series, having I_{50} values of 1.8×10^{-5} and 1.5×10^{-5} M against AHH and APDM activities, respectively. Inhibitory activity increased with increasing carbon chain length of the 2-substituent. Regression analysis showed that there was an apparent relationship between inhibitory activity and hydrophobicity (expressed as the octanol/water partition coefficient) for the inhibition of both AHH and APDM activities in PB-induced rat liver microsomes. In contrast, these compounds showed little or no inhibitory activity toward cytochrome P-448 dependent AHH activity in hepatic microsomes from 3-methylcholanthrene (3-MC) treated rats. Two 5,6-dimethylbenzimidazoles showed slight inhibitory activity and naphtho[2,3:4′,5′]imidazole was only threefold less active toward 3-MC-induced ($I_{50} = 2.6 \times 10^{-4}$ M) than PB-induced ($I_{50} = 8.4 \times 10^{-5}$) AHH activity. These results suggest that for nitrogen heterocycles there may be a relationship of increasing polycyclic size and increasing inhibitory activity toward AHH activity in 3-MC-induced rat liver microsomes.

Hepatic microsomal mixed-function oxidase (MFO) activity mediated by cytochrome P-450 in untreated and phenobarbitone (PB) treated rats may be inhibited by a range of compounds, such as methylenedioxyphenyl derivatives,[2] phenolic ketones,[3] 2-(dimethylamino)ethyl - 2,2-diphenylvalerate hydrochloride (SKF 525-A), and imidazole derivatives,[4-8] including benzimidazoles;[9] the activity (as aniline *p*-hydroxylase) may also be stimulated by acetone,[10] 2,2′-biphenylpyridyl,[11] and 2-methyl- and 2,5-dimethylbenzoxazole.[9] Treatment of mammals with polycyclic hydrocarbons, such as 3-methylcholanthrene (3-MC) and benzo[*a*]pyrene (BP), leads to the formation of cytochrome P-448[12] in the endoplasmic reticulum of hepatocytes and a consequent increase in some metabolic oxidations, particularly aryl hydrocarbon hydroxylase (AHH) activity.[13,14] The importance of AHH activity resides in its association with the initiation of cancers by polycyclic aromatic hydrocarbons which require metabolic activation by microsomal oxidases to form the proximate carcinogen.[15] AHH activity, per se, has been intensively studied; the inhibition or stimulation of this activity has not been greatly explored, but known inhibitors include 5,6- and 7,8-benzoflavone and some of their derivatives,[16] as well as the recently described ellipticines.[17-19] Inhibitors may play a part in establishing the role of microsomal oxidases in the process of chemical carcinogenesis.

A series of benzimidazole derivatives and related compounds has previously been shown to inhibit hepatic microsomal aniline *p*-hydroxylase (AH) and aminopyrine N-demethylase (APDM) activities in hepatic microsomes from PB-treated rats.[9] This report describes the effects of some additional benzimidazole derivatives on APDM activity and further describes the effects of 12 nitrogen heterocycles and SKF 525-A on AHH activity in hepatic microsomes from PB- and 3-MC-treated rats.

Results

Table I shows the molar I_{50} values for the inhibition of aryl hydrocarbon hydroxylase and aminopyrine N-demethylase activities in hepatic microsomes from phenobarbitone-treated rats by ten benzimidazole derivatives, naphtho[2,3:4′,5′]imidazole, and two reference compounds, SKF 525-A and 4(5)-phenylimidazole. For the 2-*n*-alkylbenzimidazole homologous series (compounds **1–7**) as inhibitors of AHH activity, the potency increased 60 times with increasing carbon chain length and, therefore, increasing hydrophobicity of the compounds. The results of regression analysis of the apparent relationship between hydrophobicity and inhibitory potency are shown in Table II. For seven 2-*n*-alkylbenzimidazole derivatives (compounds **1–7** inclusive), the first- and second-order regression equations for the correlation of pI_{50} with log P yielded statistically significant ($p < 0.05$) correlation coefficients of 0.955 and 0.969, respectively. Although inclusion of the

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Table I. Inhibition of Aryl Hydrocarbon Hydroxylase (AHH) and Aminopyrine N-Demethylase (APDM) Activities in Hepatic Microsomes from Phenobarbitone-Treated Rats

		$I_{50} \times 10^5$, M	
no.	compd	AHH	APDM[a]
1	benzimidazole	110	98
2	2-methylbenzimidazole	67	108
3	2-ethylbenzimidazole	63	67
4	2-*n*-propylbenzimidazole	48	30
5	2-*n*-pentylbenzimidazole	8.2	5.0
6	2-*n*-heptylbenzimidazole	2.4	2.6
7	2-undecylbenzimidazole	1.8	1.5
8	5,6-dimethylbenzimidazole	9.7	18
9	2,5,6-trimethylbenzimidazole	17	37
10	5,6-dimethyl-2-ethylbenzimidazole	11	18
11	naphtho[2,3:4',5']imidazole	8.4	5.3
12	2-(diethylamino)ethyl 2,2-diphenylvalerate hydrochloride (SKF 525-A)	2.2	0.82
13	4(5)-phenylimidazole	3.3	0.74

[a] APDM I_{50} values for compounds **1-7** and **12** have been reported in a previous communication from this laboratory.[9] Control values ± standard errors (number of observations) were 4.64 ± 0.75 nmol of formaldehyde formed (mg of protein)$^{-1}$ min^{-1} ($N = 20$) for APDM activity and 0.17 ± 0.01 nmol of 3-hydroxybenzo[*a*]pyrene fluorescence equivalents (mg of protein)$^{-1}$ ($N = 20$) for AHH activity in hepatic microsomes from PB-treated rats.

Table II. Regression Equations for the Analysis of the Inhibition of Aryl Hydrocarbon Hydroxylase Activity in Hepatic Microsomes from Phenobarbitone-Treated Rats by Benzimidazole Derivatives

$$pI_{50} = k_0 + k_1 \log P + k_2 (\log P)^2$$

compd	k_0	k_1	k_2	r[a]	n[b]	s[c]	eq
1-7	2.47	0.368	0	0.955	7	0.242	1
1-7	1.97	0.684	−0.0387	0.969	7	0.225	2
1-10	2.68	0.339	0	0.888	10	0.300	3
1-10	2.04	0.725	−0.0472	0.914	10	0.284	4

[a] Multiple correlation coefficient. [b] Number of compounds in analysis. [c] Standard deviation from the regression. Partition coefficients ($\log P_{octanol/water}$) were derived or calculated from the literature[31] and are given in Table III.

$(\log P)^2$ term into the first-order analysis produced a reduction in the variance, the improvement was not significant at $p = 0.10$. The first- and second-order equations explained 91 and 94%, respectively, of the variance of pI_{50} in the equations. Inclusion of the three 5,6-dimethylbenzimidazole derivatives (**8-10**) into the analysis gave lower correlation coefficients for both linear and parabolic models. However, the correlation coefficients were statistically significant ($p < 0.05$), and eq 3 and 4 explained 79 and 84%, respectively, of the variance of the data from the proposed models. Although other substituent constants may have improved this fit of the data, it was considered that the substituents of the present series of compunds could only yield information on the role of hydrophobicity in determining inhibitory potency. Table III shows the log P values calculated for compounds **1-10** and the observed and calculated (eq 4, Table II) pI_{50} values for each benzimidazole derivative as an inhibitor of AHH activity in PB-induced rat liver microsomes. The analysis of the data for the inhibition of APDM activity by alkylbenzimidazoles has been described elsewhere.[9]

Table IV shows the effect of seven benzimidazole derivatives, naphtho[2,3:4′,5′]imidazole, and two reference compounds (**12** and **13**) on AHH activity in hepatic mi-

Table III. Log P Values and Observed and Calculated pI_{50} Values for Benzimidazole Derivatives as Inhibitors of Aryl Hydrocarbon Hydroxylase Activity in Phenobarbitone-Induced Rat Liver Microsomes

		pI_{50}	
no.	log P[a]	obsd	calcd
1	1.35	2.96	2.93
2	1.85	3.17	3.22
3	2.35	3.20	3.48
4	2.85	3.32	3.72
5	3.85	4.09	4.13
6	4.85	4.62	4.45
7	6.85	4.75	4.79
8	2.35	4.01	3.48
9	2.85	3.77	3.72
10	3.35	3.96	3.94

[a] Log P values were derived and calculated from ref 31 and 32. [b] Calculated pI_{50} values using eq 4, Table II.

Table IV. Effect of Benzimidazole and Related Compounds on Aryl Hydrocarbon Hydroxylase Activity in Hepatic Microsomes from 3-Methylcholanthrene-Treated Rats

no.	concn, M	control act.,[a] %
1	1.2×10^{-3}	101 ± 2[b]
4	5.9×10^{-4}	99 ± 1
5	3.6×10^{-4}	89 ± 6
6	1.7×10^{-4}	93 ± 11
7	5.0×10^{-5}	103 ± 1
8	8.0×10^{-4}	69 ± 3
9	2.3×10^{-4}	77 ± 6
11	2.6×10^{-4}	50[c]
12	5.2×10^{-5}	105 ± 5
13	4.3×10^{-4}	83 ± 6

[a] Control activity: mean ± standard error (number of observations). 3-MC-induced rat liver microsomes, 0.53 ± 0.02 nmol of 3-hydroxybenzo[*a*]pyrene fluorescence equivalents (mg of protein)$^{-1}$ min^{-1} ($N = 21$). [b] Mean ± standard error of three determinations of activity at the indicated concentration of compound, which was approximately the maximum solubility in the incubation medium. Except for compound **11**, the degree of inhibition at the concentration tested was insufficient to allow for the reliable determination of I_{50} values. [c] I_{50}.

crosomes from 3-MC-treated rats. Only compounds **8**, **9**, **11**, and **13** showed any inhibitory activity.

In order to investigate the possible mechanism of the selectivity of the present compounds for the inhibition of AHH activity in PB- and 3-MC-induced microsomes, we examined the cytochrome P-450-binding properties of some of the compounds. An initial study by the method of Mailman et al.[20] of the difference spectra resulting from the binding of 4(5)-phenylimidazole and benzimidazole to the P-450-type cytochromes of 3-MC- and PB-induced microsomes showed that, at saturating concentrations, the compounds produced type II difference spectra which were qualitatively similar for both microsomal preparations. Table V shows the binding type[21] and K_s values for the binding of compounds **1**, **2**, **8**, **9**, **11**, and **13** and an additional compound, 5(6)-methylbenzimidazole (**14**), to the P-450-type cytochrome of 3-MC- and PB-induced microsomes. A comparison of the binding type of the ligands showed that six of the compounds (**1**, **2**, **8**, **11**, **13**, and **14**) yielded the same type of difference spectrum with both

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Table V. Interaction of Benzimidazole Derivatives with Oxidized P-450-Type Cytochromes in Hepatic Microsomes from 3-Methylcholanthrene (3-MC) and Phenobarbitone (PB) Treated Rats

	3-MC-induced microsomes		PB-induced microsomes	
no.	binding type [a]	K_s, M	binding type [a]	K_s, M
1	II (429, 394)	3.2×10^{-3}	II (430, 394)	1.1×10^{-4}
2	RI (420, 394)	5.1×10^{-4}	RI (424, 386)	4.4×10^{-4}
8	II (431, 398)	3.2×10^{-4}	II (429, 398)	6.2×10^{-5}
9	I (388, 419)	2.8×10^{-5}	RI (425, 396)	1.0×10^{-4}
11	II (434, 400)	8.2×10^{-5}	II (431, 404)	1.5×10^{-5}
13	II (433, 399)	1.7×10^{-6}	II (433, 406)	1.1×10^{-6}
14 [b]	II (429, 394)	2.1×10^{-4}	II (430, 397)	6.2×10^{-5}

[a] Binding type followed by wavelength (nanometers) of peak and trough, respectively. [b] 5(6)-Methylbenzimidazole. Microsomes were resuspended and diluted to 1 mg of protein/mL. The test compounds were added in microliter volumes from stock solutions in 0.07 N HCl. Equal volumes of 0.07 N HCl were added to the reference cuvette. Peak and trough wavelengths were determined with respect to characteristic absorbances of holmium oxide.

microsomal preparations but that 2,5,6-trimethylbenzimidazole (compound **9**) gave a distinct type I (peak 388 nm, trough 419 mm) difference spectrum with 3-MC-induced microsomes and a type RI spectrum with PB-induced microsomes as would be predicted for a 2-methylbenzimidazole derivative from the studies of Dickens et al.[22]

Discussion

This study shows that the hydrophobic character of the 2-*n*-alkylbenzimidazole homologous series is apparently of major importance in determining the potency of these compounds as inhibitors of AHH activity in PB-induced rat liver microsomes. Inhibitory activity would therefore appear to be related to the ability of the compounds to penetrate lipophilic barriers and/or the interaction of the inhibitors with a hydrophobic binding site at the target enzyme. A similar relationship between inhibitory activity and partition coefficient has been previously described for alkylbenzimidazoles as inhibitors of APDM activity in PB-induced microsomes.[9] Although for the inhibition of AHH activity the linear analysis in log P was the statistically preferred relationship (Table II, eq 1), evidence from examination of larger series of compounds as inhibitors of APDM activity[9] would suggest that inclusion of compounds of higher log P values may have established the statistical preference for the parabolic relationship described by eq 2 or 4 (Table II) for the inhibition of AHH activity.

For the three 5,6-dimethylbenzimidazole derivatives (**8**–**10**), changes of structure resulted in similar changes of inhibitory potency toward both cytochrome P-450 mediated APDM and AHH activities, although each compound was approximately twofold more potent as an inhibitor of AHH activity (Table I). The mechanism of action of these nitrogen heterocycles as inhibitors of APDM and AHH activities is presently unknown. However, the similarities of the I_{50} values and pI_{50} vs. log P regression equations and of the response to the three 5,6-dimethylbenzimidazoles suggest that alkylbenzimidazoles act to inhibit a pathway or a catalytic site which is common to the oxidation of both aminopyrine and benzo[*a*]pyrene in hepatic microsomes from PB-treated rats.

In contrast to the inhibitory activity shown against PB-induced AHH activity, five 2-*n*-alkylbenzimidazole derivatives (**1** and **4**–**7**) and the two reference compounds (**12** and **13**) were much less inhibitory or totally inactive toward the inhibition of AHH activity in hepatic microsomes from 3-MC-treated rats (Table IV). 3-Methylcholanthrene-induced rat liver microsomes show three times greater AHH activity than PB-induced microsomes (on a per milligram of protein basis) due to the formation of a new hemoprotein (cytochrome P-448) which is different from the native or PB-inducible hemoprotein (cytochrome P-450).[12] 4(5)-Phenylimidazole is representative of the imidazole derivatives which have been shown to have submicromolar I_{50} values against a number of microsomal mixed-function oxidase activities;[4,6] this compound was only very weakly inhibitory at 4.3×10^{-4} M (17% inhibition) against AHH activity in 3-MC-induced microsomes. The lack of inhibitory activity shown by SKF 525-A, 4-(5)-phenylimidazole, and the benzimidazole derivatives toward AHH activity in hepatic microsomes from 3-MC-treated rats suggests that the susceptible catalytic site of cytochrome P-448 (3-MC-induced microsomes) is structurally different from that of cytochrome P-450 (PB-induced microsomes).

Not all compounds studied were inactive as inhibitors of AHH activity in hepatic microsomes from 3-MC-treated rats. Compounds **8** and **9** possess adjacent methyl groups on the benzo ring of the benzimidazole nucleus, which may be considered to form the "commencement" of a third ring on the molecule, and each showed slight inhibitory activity. Compound **11**, a tricyclic nitrogen heterocycle in which a third ring is fully formed, showed significant inhibitory activity toward 3-MC-induced AHH activity having an I_{50} value (2.6×10^{-4} M) that was only three times greater than the I_{50} value (8.4×10^{-5} M) against PB-induced AHH activity. The inhibition shown by compound **11** is possibly due to the increase in the size of the nucleus and not simply the increase in the partition coefficient. The partition coefficient of **11** (log P = 2.73) is less than that of 2-*n*-propylbenzimidazole (log P = 2.85) and less than that of the longer-chain benzimidazole derivatives (Table III, compounds **5**–**7**), which caused no inhibition of 3-MC-induced AHH activity. Thus, in contrast to the hydrophobicity-dependent relationship described for the inhibition of PB-induced AHH activity, **11** was considerably more potent as an inhibitor of 3-MC-induced AHH activity than were more hydrophobic benzimidazole derivatives. Accordingly, based on the trend demonstrated by our results (i.e., increasing inhibitory activity toward a cytochrome P-448 mediated reaction as the basic structure tends to form an additional carbocyclic ring), the lack of inhibitory potency demonstrated for the imidazole derivative (**13**), and the established potency of the tetracyclic ellipticines,[17-19] we speculate that there may be a basic relationship of increasing polycyclic size and increasing inhibitory potency toward AHH activity in 3-MC-induced rat liver microsomes. This relationship appears to be distinct from and more important in determining inhibitory potency than is the increased hydro-

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phobicity associated with increased polycyclic size.

Studies on MFO inhibitors have often included an investigation of the optical difference spectra resulting from the interaction of the inhibitors with the cytochrome P-450 of the appropriate microsomal preparation.[6-8,17] The spectral evidence of binding has been considered to be essential to, or associated with, the mechanism of inhibition.[6,17] Comparisons of pK_s with pI_{50} values and comparisons of pK_s and pI_{50} values with log P[6] for inhibitors of microsomal mixed-function oxidases have shown close similarities and significant correlations and are responsible for suggestions that binding is closely associated with inhibition.[6,17] In the present study, 4(5)-phenylimidazole was shown to elicit a type II difference spectrum with both PB- and 3-MC induced rat liver microsomes. K_s values were similar, being 1.7×10^{-6} M with 3-MC-induced microsomes and 1.1×10^{-6} M with PB-induced microsomes. Rogerson et al.[8] determined a K_s value of 5.2×10^{-6} M for the interaction of 4(5)-phenylimidazole with hepatic microsomes from untreated rats. Although the binding of **13** was qualitatively and quantitatively similar with both rat liver microsomal preparations used in the present study, this compound selectively inhibited the AHH activity in the hepatic microsomes from PB-treated rats. The lack of inhibition of **13** toward AHH activity in 3-MC-induced microsomes may have been related to the possibility that the substrate, BP, had a higher affinity for cytochrome P-448 than for cytochrome P-450; BP did not produce a large enough difference spectrum with either microsomal preparation to allow for the determination of the K_s values. Additionally, in studies with preparations which are now known to be heterogeneous in terms of P-450-type cytochromes,[23] it is possible that K_s values may be determined by a form of cytochrome P-450 which is not the major catalytic form for the metabolic oxidation being studied. A lack of inhibitory activity concomitant with a low K_s value has been previously described for related nitrogen heterocycles (oxazoles and thiazoles[24]), and our results support the conclusion[24] that high-affinity type II binding as measured in microsomal preparations may not necessarily indicate that inhibition of microsomal oxidation will be observed.

Results of binding studies with benzimidazole derivatives (Table IV) did not disclose any relationships between binding and inhibitory activity. Each of the three binding types[21] was encountered (types I, II, and RI), and the relationship between these different binding types and the magnitude of K_s is not presently understood.

Conclusion

As inhibitors of AHH activity in hepatic microsomes from PB-treated rats, 2-alkylbenzimidazoles followed a relationship of increasing inhibitory potency with increasing hydrophobicity; similar relationships have been described for the inhibition of a number of microsomal oxidations by benzimidazoles[9] and other nitrogen heterocycles.[6] 2-Alkylbenzimidazoles, however, showed little or no inhibitory activity toward cytochrome P-448 mediated AHH activity in hepatic microsomes from 3-MC-treated rats. Consideration of the inhibitory activities observed in the series of compounds, 4(5)-phenylimidazole, 5,6-dimethylbenzimidazole, and naphtho[2,3:4′,5′]imidazole, and of the known inhibitory activity of the tetracyclic ellipticines[17-19] lead to the proposal that there may be a relationship of increasing inhibitory activity toward cytochrome P-448 mediated oxidations and increasing polycyclic size of the inhibitor.

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Experimental Section

Chemicals. 4(5)-Phenylimidazole was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Compounds **8–10** were synthesized by a standard procedure involving condensation of 1,2-diamino-4,5-dimethylbenzene with formic, acetic, and propionic acids, respectively.[25] Recrystallization from aqueous ethanol gave pure products. **8**: mp 209–211 °C (lit.[26] mp 199–201 °C). Anal. ($C_9H_{10}N_2$) C, H, N. **9**: mp 235 °C (lit.[27] mp 233–234 °C). Anal. ($C_{10}H_{12}N_2$) C, H, N. **10**: mp 236 °C. Anal. ($C_{11}H_{14}N_2$) C, H, N. Compound **11** was synthesized by the condensation of 2,3-diaminonaphthalene (Aldrich Chemical Co.) with formic acid in 4 N HCl; the product was recrystallized from aqueous ethanol: mp 218–220 °C (lit.[28] mp 218 °C). Anal. ($C_{11}H_8N_2$) C, H, N. Other compounds for evaluation were prepared or obtained as previously described.[9] Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and benzo[*a*]pyrene were obtained from Sigma Chemical Co., St. Louis, MO, and all other chemicals and reagents were of analytical reagent grade.

Enzyme Preparation. Microsomes were prepared as previously described,[9] except that potassium phosphate buffer (0.1 M, pH 7.4) was used instead of sodium phosphate buffer. Immature male Wistar rats (100–150 g) were injected with either PB [80 (mg/kg)/day, ip] for 3 days or with a single dose of 3-MC (25 mg/kg in corn oil, ip) 3 days before sacrifice; rats were starved for 24 h prior to sacrifice. Protein was determined by the biuret method using bovine serum albumin as standard.[29]

Aryl Hydrocarbon Hydroxylase Activity. Incubation mixtures contained BP [285 nmol (for PB-induced microsomes) or 357 nmol (for 3-MC-induced microsomes)], $MgCl_2$ (3.0 μmol), NADPH (0.50 μmol), and microsomal protein (1.2 mg) in potassium phosphate buffer (0.1 M, pH 7.4, final vol 3.0 mL). The test compounds were added to the incubation mixtures from stock solutions in 0.07 N HCl or Me_2SO–water (4:1, v/v) and solvent was added to control incubations. Incubation times were 10 and 6 min at 37 °C for PB- and 3-MC-induced microsomal preparations, respectively; the formation of product by each preparation was linear over this period. AHH activity was followed by the formation of fluorescent phenols.[30] Fluorescence was measured at 396-nm excitation and 522-nm emission in an Aminco-Bowman spectrophotofluorimeter using quinine sulfate to standardize the instrument. I_{50} values were determined by linear regression analysis of the results of experiments with five to eight concentrations of inhibitor. Stated I_{50} values were means of duplicate estimations which varied by less than 8% from the mean.

Aminopyrine N-Demethylase Activity. The microsomal N-demethylation of aminopyrine was followed by the formation of formaldehyde as previously described.[9]

Binding Studies. Optical difference spectra were recorded with an Aminco DW-2 spectrophotometer (American Inst. Co., Silver Springs, MD). Spectral dissociation constants (K_s) were determined in duplicate from the abscissal intercepts of double-reciprocal plots of ΔOD (peak–trough) vs. compound concentration (five to eight concentrations) at a microsomal protein concentration of 1 mg/mL.

Regression Analyses. Structure–activity relationships were examined by multiple linear regression analysis using the Cyber

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72 computer at the (Sydney) University Computer Centre. Partition coefficients (log $P_{octanol/water}$) were derived or calculated from the literature[31,32] by addition of the summed hydrophobic substituent (π) values to the log P values of the parent structure as described.[31] Log P values used in calculations are shown in Table III.

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Carbocyclic Analogue of 3-Deazaadenosine: A Novel Antiviral Agent Using *S*-Adenosylhomocysteine Hydrolase as a Pharmacological Target

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The carbocyclic analogue of 3-deazaadenosine (3-deaza-C-Ado) has been synthesized and found to have antiviral activity in cell culture against herpes simplex virus type 1, vaccinia virus, and HL-23 C-type virus. It is relatively noncytotoxic at effective antiviral concentrations and is not subject to deamination or phosphorylation. It acts as a competitive inhibitor of *S*-adenosyl-L-homocysteine hydrolase, is at best a poor substrate, and does not inactivate the enzyme significantly. 3-Deaza-C-Ado may cause a selective inhibition of the methylation of the polynucleotide 5′ cap of viral mRNA via higher cellular concentrations of *S*-adenosyl-L-homocysteine, resulting from the inhibition of *S*-adenosylhomocysteine hydrolase in infected cells, since increases in the intracellular level of *S*-adenosylhomocysteine, but no effects on DNA or RNA synthesis, were observed after incubation of these cells with it.

S-Adenosylmethionine (AdoMet) dependent methylation reactions exhibit a wide range of sensitivity toward inhibition by *S*-adenosyl-L-homocysteine (AdoHcy), one of the products of methylation reactions.[1-3] Because of this variable sensitivity, analogues of adenosine or AdoHcy have been synthesized and tested for their ability to inhibit AdoHcy hydrolase,[1-3] which hydrolyzes AdoHcy to homocysteine and adenosine. Although the equilibrium of the reaction favors synthesis, physiologically the reaction proceeds in the hydrolytic direction because adenosine and homocysteine are removed by metabolism. When AdoHcy hydrolase is inhibited, cellular accumulation of AdoHcy takes place. The pharmacological consequence is a disruption of the cellular ratio of AdoMet/AdoHcy, leading to a selective perturbation of methylation reactions.[4,5] Until now, one of the more potent inhibitors found for AdoHcy hydrolase is 3-deazaadenosine,[1-5] which, depending on species and organs, can also serve as a substrate for AdoHcy hydrolase, generating 3-deaza-AdoHcy.[1-3] It is difficult to determine whether AdoHcy, 3-deaza-AdoHcy, or the combination is the pharmacological agent responsible for the biochemical and biological effects observed. These effects are inhibition of phospholipid methylation and creatine biosynthesis in vivo,[4,5] inhibition of protein carboxymethylation,[6] inhibition of chemotaxis and phagocytosis,[7,8] inhibition of histamine release by human basophils,[9] inhibition of lymphocyte-mediated cytolysis,[10] antimalarial effect in vitro,[11] conversion of 3T3-L1 fibroblasts to fat cells,[12] and antiviral effects.[13-15]

The concept of exploiting AdoHcy hydrolase as a pharmacological target for chemotherapeutic purposes has been proposed.[1-5] Particularly notable is the observation that 3-deazaadenosine is a potent antiviral agent against a variety of DNA and RNA viruses.[13-15] The RNA viruses that are affected are Rous sarcoma virus, vesicular stomatitis, Sindbis, Newcastle disease,[13,14] and HL-23.[15] The finding that 9-(±)-[(1α,2β,3β,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]adenine, the carbocyclic analogue of adenosine (C-Ado), is the most potent inhibitor for AdoHcy hydrolase thus far studied, with a K_i of 5 nm,[16]

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