Mechanism-Based Inactivation of *N*-Arylhydroxamic Acid *N,O*-Acyltransferase by 7-Substituted-*N*-hydroxy-2-acetamidofluorenes

Virginia C. Marhevka, Nancy A. Ebner, Russell D. Sehon, and Patrick E. Hanna*

Departments of Medicinal Chemistry and Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.
Received July 26, 1984

N-Arylhydroxamic acid *N,O*-acyltransferase (AHAT) catalyzes the transfer of the *N*-acetyl group from *N*-arylhydroxamic acids to arylamines. In the absence of an arylamine acceptor, AHAT catalyzes the conversion of *N*-arylhydroxamic acids to reactive electrophilic intermediates that become irreversibly bound to cellular nucleophiles, including those present on AHAT itself. As part of an investigation of the AHAT-catalyzed bioactivation process, a series of 7-substituted analogues of *N*-hydroxy-2-acetamidofluorene (1) was synthesized and evaluated in vitro as substrates and inactivators of a partially purified hamster hepatic AHAT preparation. All of the compounds functioned as acetyl donors in the AHAT-catalyzed transacetylation of 4-aminoazobenzene (AAB) and all of them were inactivators of AHAT. The inactivation process exhibited apparent first-order kinetics, and the 7-methoxy compound exhibited the largest inactivation rate constant. Quantitative structure–activity analysis provided support for the concept that positively charged species are involved in the inactivation of AHAT by this series of compounds. Results of experiments in which nucleophilic trapping agents such as glutathione, cysteine, methionine, guanosine phosphate, and tRNA were included in incubation mixtures with AHAT and the *N*-arylhydroxamic acids indicated that electrophiles which diffuse away from the enzyme active site participate in the inactivation process.

The concept that numerous drugs, pesticides, and other xenobiotics produce untoward effects in mammalian tissues only after metabolic conversion to reactive electrophilic intermediates is widely accepted.[1] Indeed, the reactive intermediate hypothesis accounts for much of the data relating to the mechanism of action of chemical mutagens and carcinogens.[2] Among the latter groups are certain *N*-arylhydroxamic acids which are biotransformed to electrophilic species that form covalent adducts with nucleophilic functional groups on cellular macromolecules.[2]

The enzyme systems that are capable of activation of *N*-arylhydroxamic acids exhibit considerable tissue specificity, and the reactive intermediates that are generated include both free radicals and positively charged species.[3,4] *N*-Arylhydroxamic acid *N,O*-acyltransferase (AHAT), a cytosolic enzyme that is present in tissues of numerous mammalian species, catalyzes the conversion of certain *N*-arylhydroxamic acids to *N*-acetoxyarylamines (I, Scheme I) which undergo heterolytic decomposition to yield resonance-stabilized nitrenium–carbenium ion intermediates that react with biologically important nucleophiles.[5,6] It is also conceivable that, in some instances, N–O bond breakage in I may occur as a result of either the attack of a nucleophile on the nitrogen atom or subsequent to the attack of a nucleophile on the aromatic ring in an S_N2' type reaction. Recently it was reported from this laboratory that some *N*-arylhydroxamic acids, including the carcinogen *N*-hydroxy-2-acetamidofluorene (1, Table I), function as mechanism-based irreversible inhibitors (suicide substrates) for rat and hamster hepatic AHAT.[7a] The data derived from that study provided evidence for an association of hamster hepatic AHAT activity with certain acetyl-CoA-dependent transacetylation activities (EC 2.3.1.5), a result that is consistent with the findings of Glowinski et al., who demonstrated that these activities

Scheme I

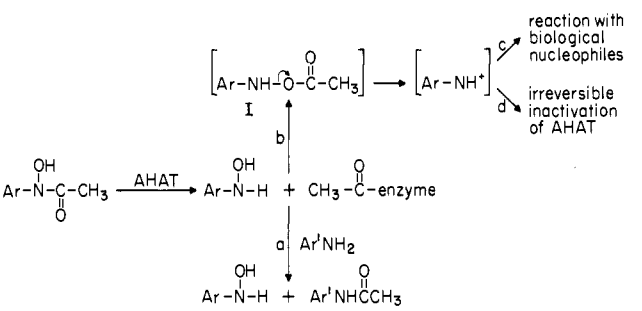
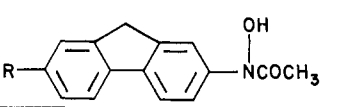


Table I. 7-Substituted-*N*-hydroxy-2-acetamidofluorenes



no.	R	mp, °C	yield, %	reduction[a] time, h	formula
1	H	149–150[b]	28[c]	1.5	$C_{15}H_{13}NO_2$
2	F	158–158.5[d]	39	1.5	$C_{15}H_{12}FNO_2$
3	Cl	167.5–168	54	3	$C_{15}H_{12}ClNO_2$
4	Br	181–182	31	3	$C_{15}H_{12}BrNO_2$
5	I	174–175	37	2	$C_{15}H_{12}INO_2$
6	CN	179–181	28	1	$C_{16}H_{12}N_2O_2$
7	CH_3CO	179–180	33	1.5[e]	$C_{17}H_{15}NO_3$
8	CH_3O	155–156	28	1	$C_{16}H_{15}NO_3$
9	CH_3CH_2O	164–165	11	1	$C_{17}H_{17}NO_3$
10	$CH_3(CH_2)_3O$	151–152	6	1.5	$C_{19}H_{21}NO_3$

[a] Length of reaction time used for reduction of the 7-substituted-2-nitrofluorenes. See Experimental Section. [b] Lotlikar, P. D.; Miller, E. C.; Miller, J. A.; Margeth, A. *Cancer Res.* **1965**, *25*, 1743. [c] Compounds **1–6**, **8** were recrystallized from benzene. Compounds **7**, **9**, **10** were recrystallized from $CHCl_3$–petroleum ether (60–70 °C). [d] Miller, E. C.; Lotlikar, P. D.; Pitot, H. C.; Fletcher, T. L.; Miller, J. A. *Cancer Res.* **1966**, *26*, 2239. [e] The ketal derivative **15** was used in the reduction reaction. See Experimental Section.

are properties of the same enzyme in rabbit liver.[8]

The finding that the kinetic characteristics of AHAT inactivation by 1 are consistent with a suicide mechanism offered a promising approach to obtaining information about the nature of the reactive intermediates generated during AHAT-catalyzed bioactivation processes and about the structural properties that influence the likelihood that an *N*-arylhydroxamic acid will serve as a substrate or in-

(1) Nelson, S. D. *J. Med. Chem.* **1982**, *25*, 753.
(2) Miller, E. C. *Cancer Res.* **1978**, *38*, 1479.
(3) Floyd, R. A. *Radiat. Res.* **1981**, *86*, 243.
(4) Miller, E. C.; Miller, J. A. *Cancer* **1981**, *47*, 2327.
(5) Bartsch, H.; Dworkin, M.; Miller, J. A.; Miller, E. C. *Biochim. Biophys. Acta* **1972**, *286*, 272.
(6) King, C. M. *Cancer Res.* **1974**, *34*, 1503.
(7) (a) Hanna, P. E.; Banks, R. B.; Marhevka, V. C. *Mol. Pharmacol.* **1982**, *21*, 159. (b) A preliminary report on portions of this research has been presented. Hanna, P. E.; Marhevka, V. C.; Elfarra, A. A.; Ebner, N. A.; Sehon, R. D. "Abstracts of Papers", 183rd National Meeting of the American Chemical Society Las Vegas, NV, April, 1982; American Chemical Society: Washington, DC, 1982; MEDI 89.
(8) Glowinski, I. B.; Weber, W. W.; Fysh, J. M.; Vaught, J. B.; King, C. M. *J. Biol. Chem.* **1980**, *255*, 7883.

0022-2623/85/1828-0018$01.50/0

activator of AHAT. Therefore, a series of 7-substituted-N-hydroxy-2-acetamidofluorenes were synthesized and evaluated as substrates and inactivators of hamster hepatic AHAT. The results, which are described in the present paper, indicate that the electronic properties of the N-arylhydroxamic acids are important determinants of their effectiveness as suicide inhibitors of AHAT.[7b]

Synthesis. The hydroxamic acids 1–10 (Table I) were prepared from the corresponding 7-substituted-2-nitrofluorenes by reduction of the nitro compound to the hydroxylamine and subsequent acetylation with acetyl chloride.[9,10] In order to obtain satisfactory yields, the time periods of the reduction reactions were varied as indicated in Table I. The attempted conversion of 7-acetyl-2-nitrofluorene (14) to hydroxamic acid 7 by the above described method was not successful. Therefore, 14 was converted to its ethylene ketal derivative 15, which was reduced and acetylated, followed by hydrolysis of the ketal function, to yield the desired hydroxamic acid 7.

Those 7-substituted-2-nitrofluorenes that were not commercially available were prepared from 2-nitrofluorene as described in the Experimental Section.

Biological Results

Transacetylation Assay. In addition to being transformed to N-acetoxyarylamines, those N-arylhydroxamic acids that are substrates for AHAT can also serve as acetyl donors in the AHAT-catalyzed transacetylation of arylamines as illustrated in pathway a of Scheme I. Compounds 1–10 were evaluated, therefore, for their relative abilities to serve as acetyl donors in the enzymatic transacetylation of 4-aminoazobenzene (AAB) according to the method of Booth.[11] Hamster hepatic AHAT was partially purified by ammonium sulfate fractionation and was used for the transacetylation rate studies as well as for all other experiments described in this paper. Substrate concentrations, protein concentrations, and incubation times were adjusted as indicated in the Experimental Section in order to obtain a linear response for each compound.

The 7-fluoro compound 2 exhibited a somewhat higher AAB transacetylation rate than the unsubstituted parent compound 1 (Table II). The 7-chloro (3) and 7-bromo (4) compounds were equally effective acetyl donors as 1, whereas the 7-cyano (6) and 7-acetyl (7) derivatives had activities that were 78% and 66%, respectively, of that of 1. The 7-methoxy (8) and 7-ethoxy (9) compounds each exhibited approximately one-half the transacetylation rate of 1, and the 7-iodo (5) and 7-butoxy (10) analogues were 25% and 22% as active as 1, respectively. The range of AAB transacetylation rates for this group of compounds was only sixfold, indicating that the properties of the 7-substituents exert a limited amount of influence on the process.

Inactivation of AHAT. The approach to studying the inactivation of AHAT by compounds 2–10 was similar to that reported previously for 1 and several other N-arylhydroxamic acids.[7,9,14] Various concentrations of compounds 2–10 were preincubated with partially purified AHAT for 2 min, and the remaining AAB transacetylation activity was measured at the end of the preincubation period. The extent of inhibition of AHAT was proportional to inhibitor concentration and the inhibition process was saturable at higher concentrations of each compound (data not shown). The inhibition was maximal at an inhibitor concentration of 0.05 mM for all compounds except the 7-methoxy (8) and 7-ethoxy (9) derivatives, which exhibited maximal inhibition at 0.005 mM, and the 7-iodo analogue, which required a concentration of 0.5 mM for maximal inhibitory activity. Several concentrations of each compound were incubated with the enzyme preparation for various time periods and linear semilog plots of percent remaining activity vs. time were obtained. First-order inactivation rate constants (k_{obsd}) were calculated from the slopes of the lines, and limiting rate (k_i) and dissociation constants (K_D) were obtained from the double-reciprocal plots of k_{obsd} vs. inhibitor concentration according to the method of Kitz and Wilson.[15] The results are shown in Table II.

Table II. 4-Aminoazobenzene Transacetylation Rates and Kinetic Constants for N-Arylhydroxamic Acid N,O-Acyltransferase Inactivation

no.	rate,[a] nmol (mg of protein)$^{-1}$ min^{-1}	k_i,[b] min^{-1}	K_D, μM	r[c]	k_i/K_D, s^{-1} M^{-1}
1	20.9 ± 0.7	1.95	88.8	0.98	360.4
2	26.8 ± 0.4	2.59	128.6	0.98	334.4
3	20.1 ± 0.9	0.76	48.8	0.87	258.2
4	20.5 ± 0.5	0.22	5.9	0.93	620.3
5	5.3 ± 0.2	0.07	0.4	0.95	2900.0
6	16.5 ± 0.6	0.09	40.0	0.98	37.5
7	13.7 ± 0.4	0.25	0.4	0.98	10400.0
8	10.9 ± 0.7	3.73	35.1	0.98	1774.3
9	9.6 ± 0.8	0.67	3.8	0.93	2936.8
10	4.6 ± 0.5	0.58	5.7	0.98	1694.7

[a] Activity was measured as the AAB transacetylation rate (mean ± SE; N = 15–30) with partially purified hamster hepatic enzyme. The procedure is described in the Experimental Section. [b] Double-reciprocal plots of the k_{obsd} values vs. inhibitor concentration were used to calculate the apparent inactivation rate constants (k_i) and apparent dissociation (binding) constants (K_D). The k_{obsd} values were determined for five concentrations of each compound after five preincubation periods. Each k_{obsd} value was determined in three experiments with each incubation being done in triplicate. Partially purified hamster hepatic AHAT was used. [c] Correlation coefficients for the fit of the points to the lines generated in the double-reciprocal plots.

There was a 53-fold range in k_i values, with the 7-methoxy compound (8) exhibiting the fastest rate of inactivation and the 7-iodo (5) and the 7-cyano (6) analogues being the slowest inactivators. Compounds 1–10 displayed a 320-fold range in K_D values. The 7-acetyl (7) and the 7-iodo derivatives had the greatest apparent affinities for AHAT, whereas the 7-fluoro compound (2) appeared to have the least affinity. Thus, those compounds having low K_D values do not exhibit the highest inactivation rate constants, and there is no obvious correlation between the apparent dissociation constants, K_D, and the pseudo-first-order inactivation rate constants, k_i, for the series of compounds.

Also shown in Table II are the k_i/K_D values for compounds 1–10. This ratio is a bimolecular rate constant for the inactivation process and it represents the efficiency, or potency, of the inactivators. According to this criterion, the 7-acetyl compound 7 is the most potent AHAT inactivator whereas the 7-cyano derivative 6 has the lowest k_i/K_D value. Thus, both the most potent and least potent

(9) Yeh, H-M.; Hanna, P. E. *J. Med. Chem.* **1982**, *25*, 842.
(10) Smissman, E. E.; Corbett, M. D. *J. Org. Chem.* **1972**, *37*, 1847.
(11) Booth, J. *Biochem. J.* **1966**, *100*, 745.
(12) Hansch, C. In "Advances in Pharmacology and Chemotherapy"; Garattini, S., Goldin, A., Hawking, F., Kopin, I. J., Eds.; Academic Press: New York, 1975; Vol. 13, p 16.
(13) (a) Hansch, C. In "Correlation Analysis in Chemistry"; Chapamn, N. B., Shorter, J., Eds.; Plenum Press: New York, 1978; p 406. (b) Coats, E. A.; Genther, C. S.; Dietrich, S. W.; Guo, Z.; Hansch, C. *J. Med. Chem.* **1981**, *24*, 1422.
(14) Mangold, B. L. K.; Hanna, P. E. *J. Med. Chem.* **1982**, *25*, 630.
(15) Kitz, R. J.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.

Table III. Inactivation of Hamster Hepatic AHAT by 7-Substituted-*N*-hydroxy-2-acetamidofluorenes: Protection by Nucleophiles

	% protection[a]				
no.	glutathione	L-cysteine	L-methionine	guanosine phosphate	tRNA
1	15.7 ± 6.1	39.1 ± 3	23.1 ± 4	34.9 ± 3	1.9[b]
2	55.2 ± 3	54.5 ± 3	38.2 ± 4	58.4 ± 6	17.6 ± 3
4	34.7 ± 4	31.1 ± 11	6.6 ± 2	26.9 ± 9	0
6	30.5 ± 7	35.9 ± 4	8.0 ± 3	5.9 ± 0.4	2.9 ± 2
7	26.3 ± 4	32.4 ± 5	0.3 ± 0.3	1.4 ± 1	8.3 ± 4
8	46.1 ± 2	47.6 ± 15	28.3 ± 5	54.9 ± 6	32.2 ± 0.5
9	39.4 ± 9	34.2 ± 8	43.3 ± 4	21.3 ± 9	19.2 ± 2
10	6.0 ± 3	9.3 ± 4	8.1 ± 1	18.9 ± 0.6	18.0 ± 6

[a] Percent protection = the difference between the percentage remaining AAB transacetylation activity after preincubation with the 7-substituted-*N*-hydroxy-2-acetamidofluorenes in the presence and absence of added nucleophile. The results are presented as the mean ± SE for three experiments. [b] Mean of two experiments.

compounds in the series have substituents that are relatively hydrophilic and have strong electron-withdrawing properties, as judged by their Hansch π constants and Hammett σ constants, respectively.[16] Although the 7-alkoxy compounds **8–10** have k_i and K_D values that vary over ranges of 6- and 9-fold, respectively, their k_i/K_D values are very similar, indicating similar potencies as inactivators of AHAT. The 7-H, 7-F, 7-Cl, and 7-Br derivatives have similar potencies, while the efficiency of the 7-iodo analogue **5** falls within the range of those of the 7-alkoxy compounds.

Previously it was demonstrated that inactivation of AHAT by **1** and structurally related compounds was irreversible.[7a,14] In order to confirm that the inactivation produced by the 7-substituted-fluorenylhydroxamic acids was also irreversible, selected members of the series (compounds **4**, **7**, and **8**) were incubated with hamster hepatic AHAT preparations, and the reaction mixtures were subjected to dialysis as described in the Experimental Section. In no case was AHAT transacetylation activity restored by dialysis, indicating that the inactivation was indeed irreversible (data not presented).

Inactivation in the Presence of Nucleophiles. The process whereby an enzyme undergoes inactivation by mechanism-based inhibitors may include reaction of the enzyme with electrophiles that have been released from the active site as well as reaction with electrophilic species before they diffuse away from the enzyme active site. The inclusion of nucleophiles in the incubation mixture can yield information with regard to the contribution to the inactivation process made by reactants that escape from the active site because nucleophiles can react with, or trap, the inactivating species before they become covalently bound to the enzyme.[17] If electrophiles in solution contribute significantly to inactivation of the enzyme, the presence of nucleophilic agents would be expected to alter the rate or extent of enzyme inactivation by a mechanism-based inhibitor.

It was demonstrated previously that the inclusion of low-molecular-weight nucleophiles, such as glutathione or cysteine, in incubation mixtures with hamster hepatic AHAT and **1** resulted in partial protection of the enzyme from inactivation, whereas a macromolecule, tRNA, did not protect the enzyme from inactivation by **1**.[7a] In the present study, the *N*-arylhydroxamic acids listed in Table III were incubated with hamster hepatic AHAT in the presence of glutathione, cysteine, methionine, guanosine phosphate, and tRNA. The percent protection from inactivation by a given nucleophile was calculated as the difference between the percentage of remaining enzyme activity after preincubation with the inactivator in the presence and absence of a nucleophile.

The results listed in Table III indicate that reactive species which diffuse away from the active site of AHAT contribute to the inactivation activity of each of the fluorenylhydroxamic acids studied. The sulfhydryl nucleophiles, cysteine and glutathione, provided 15–55% protection of AHAT from inactivation by all the compounds except the 7-butoxy analogue (**10**). In the latter case, glutathione and cysteine provided only 6% and 9% protection, respectively. Although both glutathione and cysteine provided 30% or greater protection from inactivation by **6** and **7**, which have the most electronegative of the substituents studied, none of the other nucleophiles provided more than 8% protection from these two compounds. L-Methionine provided 23–43% protection from inactivation by **1**, **2**, **8**, and **9**, but the extent of AHAT inactivation by compounds **4**, **6**, **7**, and **10** was reduced by 8% or less in the presence of this nucleophile. The inactivation was reduced 18–58% by guanosine phosphate in the presence of all compounds except those with the most electronegative 7-substituents (**6**, **7**), for which the extent of protection was less than 6%.

As noted above, tRNA does not provide a significant amount of protection of AHAT from inactivation by **1**. Similar results were obtained with tRNA and compounds **4**, **6**, and **7**, each of which has a relatively strong electron-withdrawing 7-substituent. On the other hand, tRNA protected AHAT from inactivation by compounds **2**, **8**, **9**, and **10** to the extent of 17–32%; compounds **2**, **8**, **9**, and **10** contain 7-substituents that have a significant amount of electron-donating resonance capacity.

Discussion

The mechanistic aspects of the interaction of *N*-arylhydroxamic acids with AHAT are of considerable interest because of the ability of this enzyme system to convert such compounds to potentially mutagenic or carcinogenic reactants.[5,6] In the present investigation, compounds **1–10** were first examined for their abilities to serve as acetyl donors in the AHAT-catalyzed transacetylation of AAB. The results (Table II) indicate that electronic properties of the 7-substituents are not major determinants of the transacetylation rates of this group of compounds. This point is exemplified by compounds **6** and **8** which contain strongly electron-withdrawing and electron-donating substituents, respectively, but which had similar AAB transacetylation rates. The absence of a detectable electronic effect of the 7-substituents on the AAB transacetylation process may indicate that there is little development of charge in the transition state of the acyl transfer reaction.

In general, those compounds with the largest 7-substituents, such as **5**, **9**, and **10** were less effective acetyl donors than the analogues that have smaller 7-substituents. Linear regression analysis revealed an inverse relationship

(16) Martin, Y. C. "Quantitative Drug Design"; Marcel Dekker: New York, 1978; p 377.
(17) Walsh, C. *Horiz. Biochem. Biophys.* **1977**, *3*, 36.

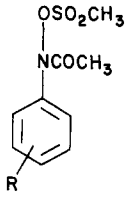
Table IV. Quantitative Structure–Activity Results and Physicochemical Constants

no.	log k_i		log $1/K_D$		σ^c	π	0.1 MR
	obsd	calcd[a]	obsd	calcd[b]			
1	0.29	0.39	−1.95	−2.27	0	0	0.103
2	0.41	0.31	−2.11	−2.29	0.06	0.14	0.092
3	−0.12	−0.27	−1.69	−1.35	0.23	0.71	0.600
4	−0.66	−0.46	−0.77	−0.81	0.23	0.86	0.890
5	−1.16	−0.70	0.40	0.11	0.18	1.12	1.390
6	−1.05	−0.96	−1.60	−1.30	0.66	−0.57	0.630
7	−0.60	−1.02	0.40	−0.39	0.50	−0.55	1.120
8	0.57	0.38	−1.55	−1.00	−0.27	−0.02	0.790
9	−0.17	0.04	−0.58	−0.15	−0.24	0.38	1.250
10	−0.24	−0.43	−0.75	1.56	−0.32	1.03	2.170

[a] Calculated with eq 2. [b] Calculated with eq 4. [c] Physicochemical constants were taken from ref 16. The squared correlation coefficients between variables were 0.21, 0.10, 0.24 for $\sigma - \pi$, σ − 0.1 MR, and π − 0.1 MR, respectively. The MR values were scaled by 0.1 for the correlation analyses as suggested by other authors.[13b]

between the AAB transacetylation rates of **1–10** and the molar refractivities (MR) of the 7-substituents (r = 0.87). Hansch and co-workers have suggested that since MR is largely a measure of volume, such an inverse relationship may indicate steric hindrance due to the volume of the substituent.[12,13] The narrow range of transacetylation rates exhibited by compounds **1–10**, however, precludes a more detailed structure–activity analysis.

The observation that **1** functions as a mechanism-based inactivator of AHAT suggested the use of analogues of **1** to investigate the properties and reactivities of the electrophilic species generated when *N*-hydroxy-2-acetamidofluorenes are activated by this enzyme system. Early studies with in vitro chemical model systems led to the proposal that the electrophilic intermediates formed in the AHAT-catalyzed bioactivation of *N*-arylhydroxamic acids are resonance-stabilized nitrenium–carbenium ions derived from the heterolytic decomposition of the *N,O*-acyltransfer product I (Scheme I).[5] Theoretical calculations indicate that arylnitrenium ions exist preferentially as ground-state singlets as a result of delocalization of the positive charge to the aromatic ring system and transfer of electrons from the ring to the nitrogen atom.[18] It would be expected that electron-donating substituents would facilitate the formation of such cationic species and might thereby enhance the rate of inactivation of AHAT. Such a result would be analogous to that obtained by Gassman and Granrud, who found that the rates of thermal rearrangement of a series of *N*-arylhydroxamic acid *O*-methanesulfonates (**11**) were



11

dependent upon the electron-donating properties of the ring substituents, indicating heterolytic cleavage of the N–O bond and the generation of *N*-acylarylnitrenium ion intermediates.[19] Thus, the finding that the 7-methoxy congener **8** exhibited the largest k_i values for inactivation of AHAT was not unexpected. On the other hand, the relatively low k_i values found for the 7-ethoxy (**9**) and 7-butoxy (**10**) compounds are not consistent with the involvement of positively charged intermediates in the inactivation process.

In order to determine whether the steric bulk or hydrophobicity of the 7-substituents might be contributing to the unexpectedly low k_i values of **9** and **10**, correlations of the rate constants with the physicochemical properties of the substituents were examined by multiple regression analysis. The correlation with the Hammett σ constant, an electronic descriptor, is shown in eq 1. In eq 1, n is

$$\log k_i = -1.163\ (\pm 1.143)\ \sigma - 0.152\ (\pm 0.372) \quad (1)$$
$$n = 10,\ r = 0.64,\ s = 0.48$$

the number of data points, r is the correlation coefficient, and s is the standard deviation. The numbers in parentheses are the 95% confidence intervals. When the correlation between log k_i and either 0.1 MR or the hydrophobic constant π was examined, equations were obtained that had r values of 0.41 and 0.13, respectively. Thus, electronic properties appear to be more important than either the steric bulk or the hydrophobicity of the 7-substituents in determining k_i for compounds **1–10**. The negative coefficient for σ in eq 1 indicates that electron-donating substituents enhance the inactivation rate, as would be expected if the key intermediate is a positively charged species.

The inclusion of both π and σ in the correlation resulted in little statistical improvement over eq 1, but the inclusion of both σ and 0.1 MR produced a much more satisfactory correlation, as shown in eq 2.

$$\log k_i = -1.537\ (\pm 0.739)\ \sigma - 0.638\ (\pm 0.388)\ 0.1\ \text{MR} + 0.463\ (\pm 0.438) \quad (2)$$
$$n = 10,\ r = 0.90,\ s = 0.29,\ F_{1,7} = 15.16$$

The log k_i values predicted by eq 2 are listed in Table IV along with the physicochemical constants for the substituents in compounds **1–10**. The negative coefficient of 0.1 MR in eq 2 may be interpreted to indicate that large 7-substituents will reduce the rate of AHAT inactivation. Because of the likelihood of the involvement of positively charged reactants in the inactivation process, equations in which the resonance descriptor R was substituted for σ were derived. These equations were, however, less satisfactory than eq 1 and 2.

Attempts to correlate the K_D values with σ, π, and 0.1 MR were less successful than the correlations involving k_i. The best single parameter equation was that obtained with 0.1 MR (eq 3), but the 7-butoxy compound **10** was very poorly predicted. The correlation was improved by omission of **10** from the analysis (eq 4), but no significant improvement could be obtained by inclusion of σ or π.

$$\log 1/K_D = 1.012\ (\pm 0.863)\ 0.1\ \text{MR} - 1.935\ (\pm 0.932) \quad (3)$$
$$n = 10,\ r = 0.69,\ s = 0.70$$

(18) Ford, G. P.; Scribner, J. D. *J. Am. Chem. Soc.* **1981**, *103*, 4281.
(19) Gassman, P. G.; Granrud, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 1498.

$$\log 1/K_D = 1.854\ (\pm 0.849)\ 0.1\ MR - 2.464\ (\pm 0.746) \quad (4)$$
$$n = 9,\ r = 0.89,\ s = 0.47$$

The positive coefficient of 0.1 MR in eq 4 indicates that 7-substituents with large 0.1 MR values should enhance the apparent affinity of the compounds for AHAT. However the very poor prediction of the K_D of compound **10** by eq 4 (Table IV) may indicate that the 7-butoxy group exceeds the optimal steric bulk requirements for interaction with AHAT. Unfortunately, compounds with 7-substituents larger then *n*-butoxy were not available to test this possibility, and the equation obtained by including a squared term in 0.1 MR was not statistically satisfactory to demonstrate a parabolic dependence on 0.1 MR. The dissociation constant of the 7-acetyl compound **7** also is not well predicted by eq 4. The reason for the apparent high affinity of **7** for AHAT is not obvious, but it is clear that it is the unexpectedly low K_D value which is responsible for this compound's large k_i/K_D ratio. Attempts to correlate the k_i/K_D values with π, σ, and 0.1 MR did not yield statistically acceptable equations.

The results of previous experiments with *N*-arylhydroxamic acids indicated that inactivation of AHAT involves a contribution by electrophilic species that diffuse away from the active site prior to becoming covalently bound to the enzyme.[7a,14] In certain cases, the reactants which become covalently bound after diffusion into the surrounding medium appeared to play a more prominent role in the inactivation process than those which react while remaining complexed with the active site.[14] Information can be obtained about the contribution of solution electrophiles to the inactivation process by the inclusion of nucleophilic agents in the incubation mixtures. If electrophiles in solution contribute to the inactivation process, the rate or extent of enzyme inactivation may be reduced in the presence of nucleophiles because of the ability of the nucleophiles to trap the electrophilic reactants before they become covalently bound to the enzyme.

The data listed in Table III demonstrate that although the extent of AHAT inactivation by compounds **1–10** can be reduced by biological nucleophiles, the amount of protection appears to depend upon the chemical properties of both the nucleophiles and the inactivators. The thiols, glutathione and cysteine, were the most effective protective agents, although they provided less than 10% protection from inactivation by the 7-butoxy compound **10**, and glutathione protected AHAT from inactivation by **1** to an extent of only 15%. The latter result is similar to that of Mulder and co-workers, who found that cysteine was twice as effective as glutathione in the prevention of covalent binding to protein by electrophiles generated from the *N*,*O*-sulfate ester of *N*-hydroxy-2-acetamidofluorene.[21]

In contrast to glutathione and cysteine, the thio ether methionine provided less than 10% protection from inactivation by **6** and negligible protection from inactivation by **7**. Compounds **6** and **7** contain the strongest electron-withdrawing substituents among the inactivators studied. It should be noted, however, that the low levels of protection of AHAT from inactivation by **6** and **7** do not necessarily mean that methionine does not react with the electrophiles produced from these substances. Indeed, compounds **6** and **7** gave the highest yields of methylthio adducts when radiolabeled methionine was used as a trapping agent in a related investigation of the bioactivation of 7-substituted-*N*-hydroxy-2-acetamidofluorenes by hamster hepatic AHAT.[20] Thus, a low level of protection from inactivation provided by a given nucleophile in the presence of a specific inactivator may reflect the inability of the nucleophile to trap those electrophiles that participate in the enzyme inactivation process, but it cannot necessarily be concluded that the nucleophile will fail to form adducts with activated forms of the compounds.

Guanosine phosphate behaved somewhat similarly to methionine in the protection experiments in that guanosine phosphate provided negligible protection from inactivation by **6** and **7**, although guanosine phosphate was more effective than methionine in protecting AHAT from inactivation by the 7-bromo and 7-butoxy analogues. Guanosine phosphate was approximately as effective as glutathione and cysteine with regard to its ability to prevent inactivation by the 7-bromo compound **4**, but it provided greater protection than either of the thiols against inactivation by the 7-butoxy analogue **10**.

Of particular interest is the behavior of the macromolecule tRNA as a nucleophilic protecting agent. It was reported previously that tRNA did not protect AHAT from irreversible inactivation by either *N*-hydroxy-2-acetamidofluorene (**1**) or *N*-hydroxy-4-acetamidobiphenyl, and the suggestion was made that, although tRNA is an effective trap for electrophiles generated from arylhydroxamic acids, the inability of tRNA to protect AHAT might be related to its relatively large size which could limit its access to electrophiles near the enzyme surface.[7a] Alternatively, the nucleophilic groups of tRNA might not be capable of competing for those electrophiles in solution that participate in the inactivation of AHAT. In the present study, tRNA provided protection ranging from 17% to 32% for compounds **2**, **8**, **9**, and **10**, the analogues which contain those 7-substituents that are most capable of electron donation via resonance. Although it might be reasonable to conclude that those 7-substituents which facilitate the development and stabilization of positively charged intermediates also facilitate reaction with the nucleophilic target sites of nucleic acids, experiments which quantify the extent of reaction of the activated forms of **1–10** with nucleic acids will be required in order to determine whether this interpretation is correct.

Although guanosine residues have been identified as the predominant sites of reaction of bioactivated forms of *N*-arylhydroxamic acids with tRNA, guanosine phosphate and tRNA did not exhibit identical patterns of protective effects in the present experiments.[22] Both guanosine phosphate and tRNA provided substantial protection of AHAT from inactivation by **2**, **8**, **9**, and **10**, whereas neither of them protected significantly against the effects of **6** and **7**, the compounds with the strongest electron-withdrawing substituents. On the other hand, guanosine phosphate provided partial protection of AHAT from inactivation by the 7-H compound **1** and the 7-bromo compound **4**, but tRNA was ineffective against both **1** and **4**. Presumably, the large differences in the physical properties of guanosine phosphate and tRNA are responsible for these differences in their behaviors in the protection experiments.

In conclusion, the results of the present investigation support the concept that positively charged, resonance-stabilized nitrenium–carbenium species are responsible for the loss of enzyme activity that occurs subsequent to the AHAT-catalyzed bioactivation of *N*-hydroxy-2-acetamidofluorenes. The results also indicate that electrophiles which diffuse away from the enzyme active site participate

(20) Hanna, P. E.; Elfarra, A. A., unpublished results.
(21) Mulder, G. J.; Hinson, J. A.; Gillette, J. R. *Biochem. Pharmacol.* **1978**, *27*, 1641.
(22) Kriek, E. *Prog. Clin. Biol. Res.* **1983**, *132B*, 161.

in the inactivation process and that the effectiveness of nucleophilic protective agents is related to the chemical characteristics of both the nucleophile and the inactivating agent.

Experimental Section

Synthesis. Melting points were determined in open capillary tubes with either a Thomas-Hoover or a Mel-Temp apparatus and are uncorrected. IR spectra (KBr) were obtained with a Perkin-Elmer 281 or a Beckman 237B spectrophotometer. Mass spectra were obtained with an Associated Electronic Industries MS-30 mass spectrometer in the University of Minnesota Mass Spectrometry Laboratory, Department of Chemistry; samples were introduced by direct inlet. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN. Elemental analyses for all compounds were within 0.4% of theoretical values, and the IR and mass spectra were consistent with the assigned structures.

7-Chloro-2-nitrofluorene (12). Compound **12** was prepared according to the procedure of Gutman and Ray in 47% yield: mp 235–235.5 °C (lit.[23] mp 237–238 °C).

7-Iodo-2-nitrofluorene (13). To 2-nitrofluorene (3.2 g, 0.015 mol) in 100 mL of glacial acetic acid was added iodine (1.87 g, 0.007 mol). The solution was stirred at room temperature for 10 min, after which were added 10 mL of concentrated H_2SO_4 and $NaNO_2$ (1.1 g, 0.015 mol). The solution was heated under reflux for 30 min and poured onto 100 g of ice, and the yellow solid that formed was collected by filtration (4.91 g). Recrystallization from glacial acetic acid afforded 4.6 g (93%) of **13**: mp 242–243.5 °C (lit.[24] mp 244–245 °C).

7-Acetyl-2-nitrofluorene (14). Compound **14** was prepared according to the method of Oehlschlaeger and MacGregor in 64% yield: mp 227–228 °C (lit.[25] mp 228 °C).

7-Acetyl-2-nitrofluorene 1,2-Dioxoethyl Ketal (15). 7-Acetyl-2-nitrofluorene (2.0 g, 0.008 mol) in 400 mL of dry benzene was heated under reflux. 4-Toluenesulfonic acid (0.02 g, 0.12 mmol) and ethylene glycol (0.50 g, 0.008 mol) were added, and water (approximately 0.2 mL) was collected in a Dean–Stark trap while the mixture was heated under reflux for 18 h. The reaction mixture was cooled to room temperature, extracted with two 50-mL portions of saturated $NaHCO_3$, washed with water (25 mL), and dried ($MgSO_4$). Evaporation of the benzene afforded a yellow solid (2.2 g) that was recrystallized from benzene to yield **15** (1.15 g, 48%): mp 168–170 °C. The ketal **15** was used without further purification for the preparation of **7**.

7-Hydroxy-2-nitrofluorene (16). A solution of 3-chloroperbenzoic acid (46.8 g, 0.29 mol) in $CHCl_3$ (500 mL) was added to a solution of **14** (28.8 g, 0.114 mol) in 1.5 L of $CHCl_3$. The reaction mixture was stirred vigorously for 15 min and then allowed to stand in the dark for 10 days. The $CHCl_3$ was evaporated to yield 56.6 g of a yellow solid that was dissolved in a solution of 95% EtOH (500 mL) and concentrated HCl (500 mL). The reaction mixture was heated under reflux for 3.5 h, filtered, and allowed to cool to room temperature. The yellow crystals that formed were collected by filtration and recrystallized from 95% EtOH to yield **16** (19.2 g, 77%): mp 248–249 °C (lit.[26] mp 248–250 °C).

7-Alkoxy-2-nitrofluorenes 17–19. Compound **16** (4.7 g, 0.02 mol) was dissolved in acetone (250 mL). Solid K_2CO_3 (2.8 g, 0.02 mol) was added and the mixture was stirred and heated under reflux. The alkyl halide (0.02 mol) was added dropwise over 10 min. The reaction mixture was heated under reflux for 3 h, cooled to room temperature, and poured into water (500 mL). The yellow solid that precipitated was extracted with five 50-mL portions of CH_2Cl_2 which were combined, washed with 20 100-mL portions of 1% NaOH and two portions of H_2O, and dried ($MgSO_4$). Evaporation of the solvent afforded the 7-alkoxy-2-nitrofluorenes which were recrystallized once from acetone–H_2O and twice from benzene.

7-Methoxy-2-nitrofluorene (17): yield 50%; mp 213–214 °C (lit.[27,28] mp 215 °C).

7-Ethoxy-2-nitrofluorene (18): yield 41%; mp 161–161.5 °C. Anal. ($C_{15}H_{13}NO_3$) C, H, N.

7-*n*-Butoxy-2-nitrofluorene (19): yield 42%; mp 120–120.5 °C. Anal. ($C_{17}H_{17}NO_3$) C, H, N.

7-Cyano-2-nitrofluorene (20). 7-Iodo-2-nitrofluorene (**13**; 2.5 g, 0.007 mol) was dissolved in 300 mL of DMF to which was added CuCN (0.66 g, 0.007 mol). The mixture was stirred and heated under reflux for 4 h, filtered while hot, and poured onto 400 g of ice. A tan solid (2.6 g) was collected by filtration and was chromatographed on silica gel (85 g, CH_2Cl_2) to afford **20** (0.7 g, 30%): mp 274–276 °C (lit.[29,30] mp 281–282 °C); IR (KBr) 2225 cm^{-1}; EI MS (70 eV), m/e (236) (M^+).

7-Substituted-*N*-hydroxy-2-acetamidofluorenes 1–10 (Table I). The synthesis of **8** is representative. 7-Methoxy-2-nitrofluorene (**17**; 1.04 g, 0.004 mol) was dissolved in DMF (200 mL) and H_2O (20 mL) with stirring under a nitrogen atmosphere. The solution was cooled to 0 °C and ammonium chloride (0.92 g, 0.016 mol) was added in one portion followed by powdered zinc (1.13 g, 0.016 mol). After 1 h of stirring, the mixture was filtered and 40 mL of H_2O was added to the filtrate. The resulting aqueous suspension was extracted with eight 100-mL portions of Et_2O which were combined and dried ($MgSO_4$). To the Et_2O solution at 0 °C were added 1.0 mL of saturated $NaHCO_3$ solution and acetyl chloride (0.004 mol) in 5 mL of Et_2O. After 1 h of stirring, the mixture was extracted with five 100-mL portions of 1% NaOH which were combined and adjusted to pH 7 with NaH_2PO_4 (for the preparation of **7**, the basic solution was adjusted to pH 4–5 with concentrated HCl in order to hydrolyze the ketal). The aqueous solution was extracted with four 75-mL portions of $CHCl_3$ which were combined, dried ($MgSO_4$), and concentrated with a stream of nitrogen to a volume of 60 mL. The solution was brought to the cloud point with petroleum ether (30–60 °C), and the resulting crystals were collected by filtration and recrystallized as indicated in Table I.

Enzymatic Studies. Male golden syrian hamsters (70–80 g) were obtained from Charles River Farms (Wilmington, MA). Ultracentrifugation was performed on a refrigerated Beckman L5-65 untracentrifuge and a Beckman J-21B instrument was used for low-spin centrifugation. Incubations were performed in a Dubnoff metabolic shaking incubator, and enzyme activities were measured with a Beckman Model 34 spectrophotometer. The following reagents were obtained from the commercial sources listed: 4-aminoazobenzene (AAB) (Eastman); sodium pyrophosphate (Mallinckrodt); trichloroacetic acid (MCB); ammonium sulfate (Baker); glutathione, L-methionine, L-cysteine, guanosine 2′- and 3′-phosphate, dithiothreitol (DTT), type X tRNA, and cellulose dialysis tubing (10 × 0.62 in.) (Sigma). The dialysis tubing was rinsed with distilled water and with dialysis buffer prior to use.

Enzyme Preparation. Animals were lightly anesthetized with ether and were decapitated. The livers were removed, the gall bladders were excised, and the livers were minced and homogenized in 1 mL of cold sodium pyrophosphate buffer (0.05 M, pH 7, 1 mM DTT) per gram of liver in a Potter type homogenizer with a motor-driven Teflon pestle. The homogenate was centrifuged at 105000*g* for 60 min. The supernatant was diluted with an equal volume of cold sodium pyrophosphate buffer and was purified two- to three-fold by ammonium sulfate fractionation as described by King.[6] The ammonium sulfate pellets were stored at −70 °C and were dissolved in 0.05 M sodium pyrophosphate buffer (pH 7, 1 mM DTT) prior to use. Protein concentrations were determined by the method of Lowry et al.[31]

AAB Transacetylation Assay. Incubation mixtures contained 1.0 mL of 0.05 M sodium pyrophosphate buffer (pH 7, 1

(23) Gutman, H. R.; Ray, F. E. *J. Am. Chem. Soc.* **1951**, *73*, 4033.
(24) Weisburger, E. K. *J. Am. Chem. Soc.* **1950**, *72*, 1758.
(25) Oehlschlaeger, H. F.; MacGregor, I. R. *J. Am. Chem. Soc.* **1949**, *71*, 3223.
(26) Friess, S. L. *J. Am. Chem. Soc.* **1949**, *71*, 14.
(27) Weisburger, J. H.; Weisburger, E. K. *J. Chem. Soc.* **1954**, 758.
(28) Andrews, H. F.; Campbell, N.; Craig, J. T.; Nichol, K. J. *J. Chem. Soc. C* **1968**, 1761.
(29) Vink, J. A.; Verheizdt, P. L.; Comelisse, J.; Havinga, E. *Tetrahedron* **1972**, *29*, 5081.
(30) Hayashi, M.; Komiya, H.; Ishikawa, N.; Zitsu, Y. *Yuki Gosei Kagaku Kyokaishi* **1955**, *13*, 171.
(31) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.

mM DTT), 0.03–0.1 mL of enzyme solution, 0.1 mL of substrate solution, and enough 1.15% KCl to bring the final volume to 2.5 mL. A 2-min temperature equilibration period was initiated by addition of the enzyme solution to 25-mL Erlenmeyer flasks, containing KCl and buffer, in a 37 °C shaker bath. Reactions were started by the addition of the substrate solution and were carried out at 37 °C in air. Reactions were terminated by the addition of 2.5 mL of 20% (w/v) trichloroacetic acid in 50% (v/v) EtOH/H_2O, and the resulting mixture was centrifuged for 20 min in a desk-top centrfuge. The supernatants were analyzed spectrophotometrically at 497 nm.[11] *N*-Arylhydroxamic acids were omitted for the incubation mixtures that were used as reference standards. Incubations were performed in triplicate.

The substrate solutions consisted of the *N*-arylhydroxamic acid and AAB dissolved in either 95% EtOH (compounds **1** and **2**) or Me_2SO/$MeOCH_2CH_2OH$ (1:1). The final protein concentration was 1.0 mg/mL except for assays with **5** (0.5 mg/mL) and **7** (0.6 mg/mL). The final *N*-arylhydroxamic acid concentration was 1.0 mM except for **5** (0.5 mM) and **7** (0.6 mM). The final AAB concentration was 0.15 mM except for assays with **5** and **7** in which it was 0.075 and 0.10 mM, respectively. The incubation time for assays with **5** was 3.5 min; for all other compounds it was 2.0 min.

Kinetics of AHAT Inactivation. The preincubation mixtures contained 1 mL of sodium pyrophosphate buffer (pH 7, 1 mM DTT), *N*-arylhydroxamic acid (0.005–1.0 mM final concentration) dissolved in 0.05 mL of 95% EtOH or 0.05 mL of Me_2SO/$MeOCH_2CH_2OH$ (1:1), 0.03–0.1 mL of hamster hepatic AHAT preparation (protein concentration as described under transacetylation assay), and sufficient 1.15% KCl to give a final volume of 2.45 mL. Control flasks contained 0.05 mL of the appropriate solvent instead of the *N*-arylhydroxamic acid solution. The preincubations were started by the addition of enzyme and were carried out at 37 °C in air. At the end of the preincubation periods (1–15 min), 0.05 mL of a substrate solution consisting of the appropriate *N*-arylhydroxamic acid and AAB were added as described under the description of the transacetylation assay in order to measure the amount of remaining enzyme activity. The mixtures were then incubated in air at 37 °C for either 2 or 3.5 min (compound **5**) and the reactions were terminated and the mixtures were assayed as described above. The calculation of the kinetic constants was accomplished as reported previously, and is summarized under Biological Results.[7a,15]

Inactivation of AHAT in the Presence of Nucleophiles. Incubations were conducted in the same manner as in the kinetic studies except each flask contained 10 mM of a low-molecular-weight nucleophile (L-methionine, L-cysteine, glutathione, guanosine phosphate) or 1 mg/mL of tRNA. A saturating concentration of inactivator (*N*-arylhydroxamic acid) was used and preincubation times (2–20 min) were adjusted such that maximum inactivation of the enzyme occurred. Control flasks contained nucleophiles, but 0.05 mL of the appropriate solvent was added instead of the inhibitor solution. The presence of the nucleophiles did not significantly affect the AAB transacetylion rates in the control flasks. Preincubations with inhibitors were initiated by addition of enzyme. At the end of the preincubation period, 0.05 mL of substrate solution (*N*-arylhydroxamic acid and AAB) was added in order to measure the remaining enzyme activity. The incubations were carried out and terminated as described above. The method of determination of the amount of protection of AHAT from inactivation by the *N*-arylhydroxamic acids was described previously and is summarized under Biological Results.[7a]

Dialysis Experiments. The incubations were run on a scale 8–16 times that of a standard incubation. The 16× standard incubation mixture contained 16.0 mL of 0.05 M sodium pyrophosphate buffer (pH 7, 1 mM DTT), 0.1 mM *N*-arylhydroxamic acid (dissolved in 0.8 mL of Me_2SO/$MeOCH_2CH_2OH$, 1:1), 0.48–1.6 mL of enzyme solution (1 mg/mL final protein concentration) and sufficient 1.15% KCl to give a 40-mL final incubation volume. The control flasks contained 0.8 mL of solvent instead of the *N*-arylhydroxamic acid solution. The incubation period was initiated by the addition of *N*-arylhydroxamic acid solution. The mixture was incubated in air at 37 °C. The incubation times (5–40 min) were adjusted to obtain maximum inactivation of the enzyme. At the end of the incubation period, samples (2.45 mL) were removed for AHAT activity assay. The remaining incubation mixture was dialyzed against four 800-mL portions of cold 0.05 M sodium pyrophosphate buffer (pH 7, 1 mM DTT) containing 2.04% Me_2SO/$MeOCH_2CH_2OH$, 1:1). The dialysis buffer solutions were changed four times during the 4-h dialysis period. Nitrogen was bubbled through the buffer before and during the dialysis. At the end of the dialysis period, samples (2.45 mL) were removed and assayed for activity. The substrate solution used to initiate the transacetylation assay contained AAB and the same *N*-arylhydroxamic acid used used in the preincubation. The concentrations of substrates and the solvents used are given under the description of the transacetylation assay.

Acknowledgment. This investigation was supported in part by NIH Grant CA-24427.

Registry No. **1**, 53-95-2; **2**, 2508-18-1; **3**, 92901-04-7; **4**, 92901-05-8; **5**, 70952-93-1; **6**, 92901-06-9; **7**, 92901-07-0; **8**, 92901-08-1; **9**, 92901-09-2; **10**, 92901-10-5; **12**, 6939-05-5; **13**, 23055-47-2; **14**, 34172-49-1; **15**, 92901-11-6; **16**, 6633-40-5; **17**, 54961-21-6; **18**, 92901-12-7; **19**, 92901-13-8; **20**, 39150-37-3; AHAT, 52660-15-8; 2-nitrofluorene, 607-57-8; ethylene glycol, 107-21-1; 3-chloroperbenzoic acid, 937-14-4; glutathione, 70-18-8; L-cysteine, 52-90-4; L-methionine, 63-68-3; guanosine 2′-phosphate, 130-50-7; guanosine 3′-phosphate, 117-68-0.

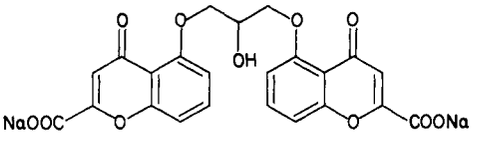
[1,4]Benzoxazine-2,3-diones as Antiallergic Agents

Bernard Loev,† Howard Jones,† Richard E. Brown,† Fu-chih Huang,*† Atul Khandwala,‡ Mitchell J. Leibowitz,§ and Paula Sonnino-Goldman§

Departments of Medicinal Chemistry, Biochemistry, and Pharmacology, Revlon Health Care Group, Research and Development Division, Tukahoe, New York 10707. *Received December 27, 1983*

The synthesis of a series of [1,4]benzoxazine-2,3-diones and a new class of compounds, benzobisoxazinetetrones, is described. These compounds were evaluated for their effect in the rat mast cell (RMC) test passively sensitized in vitro with rat antiovalbumin serum and for their effect in inhibitory passive cutaneous anaphylaxis (PCA) in the rat. Some of these compounds are of the same potency level as disodium cromglycate in the RMC test and some are effective orally in PCA.

Disodium cromoglycate (DSCG, **1**[1]) is the only compound on the market that is indicated for prophylactic treatment of certain types of extrinsic asthma. It is claimed to act by inhibiting the release of mediators stimulated by antibody–antigen interactions.[2] Because



1

DSCG is effective in asthma only when inhaled,[3] a search

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.
§ Department of Pharmacology.

0022-2623/85/1828-0024$01.50/0