protein and 100 μM NADPH in phosphate buffer containing glucose 6-phosphate (2.5 mM) and glucose-6-phosphate dehydrogenase (264 units/mL); inhibitors were added as 10 μL/mL from stock solutions made in DMSO. Different concentrations of inhibitor were used. DMSO alone was added for the controls.

Microsomes, inhibitors, and the cofactors were preincubated together during different times (up to 10 min). Then 10 μL of an ethanolic solution of [1β,2β-^{3}H]androstenedione was added to a $10K_m$ substrate concentration. Tritium-release incubations were then conducted at 37 °C for 10 min as described above. Background radioactivity was determined in incubations lacking enzyme.

The two methods described by Nelson[30] for plotting the ln of the percent of activity remaining versus time were used. In method 1, which was used to calculate K_I and k_{inact} values, the percent of the activity remaining was determined by dividing the activity in the presence of inhibitor after preincubation by the activity in the presence of inhibitor at the beginning of the preincubation ($t = 0$) and multiplying by 100. The values of k_{obs} were obtained by plotting the ln of the percent activity remaining versus time of preincubation for each concentration of inhibitor. Least square analysis of the double-reciprocal plots of k_{obs} versus inhibitor concentrations allowed the K_I and k_{inact} values to be determined. In method 2, the percent of activity remaining was determined by dividing the activity in the presence of inhibitor after preincubation by the activity present at $t = 0$ with no inhibitor added and multiplying by 100.

Experiments without NADPH. Time-dependent experiments were done as described above, except the cofactor NADPH was omitted in the preincubation but was given at the same time as the substrate.

β-Mercaptoethanol (β-MSH) and Substrate Protection Experiments. They were conducted as described above for the time-dependent experiments including β-MSH (0.5 and 10 mM) or substrate (0.5 and 1 μM of nontritiated androstenedione) in the preincubation buffer. The results are expressed as described above and compared to controls (without β-MSH or without substrate in the preincubation).

Irreversibility of Inhibition Caused by 14. In 5 mL of total volume, at 37 °C, 10 mg of microsomal protein was incubated in phosphate buffer with 0.5 and 0.05 μM 14 in the presence of 5 mM NADPH; G6P (50 mM) and G6PDase (264 IU/mL) were added to ensure against depletion of reduced cofactor. After 2 h, each incubation was stopped in ice and an aliquot was checked for aromatase activity. The rest was dialyzed (4 °C) against 500 mL of incubating phosphate buffer. This was replaced every 2 h by fresh buffer and an aliquot was withdrawn for aromatase assay after 8 and 24 h of dialysis. The results are expressed as percent of control (obtained with no inhibitor present).

In Vivo Experiments. Female Wistar rats from IFFA-CREDO (Lyon, France) were fed an ad libitum diet and housed under conditions of controlled temperature and humidity. They were maintained on a 12 h light/12 h dark cycle. Female rats 60 days old were injected with PMSG (100 IU/rat every 2 days for 10 days). On day 11, compound 13 or 4-OH-A (1) was given subcutaneously (50 mg/kg), intravenously (5 and 2.5 mg/kg), or orally (50 mg/kg). 2 h later, the animals were sacrificed by decapitation. Total trunk blood was collected and the estradiol measured by RIA. The ovaries were collected and ovarian microsomes were prepared as described.[6] Aromatase activity was assessed as with human placental microsomes. (*$P < 0.05$, **$P < 0.01$[47]).

Acknowledgment. We wish to thank our colleagues and their collaborators in the Physical Chemistry Department and the Analytical Laboratory for their help in recording and interpreting the spectra and performing the elemental analysis, respectively. We thank Dr. J. Leclaire for stimulating discussions. We are grateful to the Maternité des Lilas for providing the placentae.

(47) Dunnett, C. W. New Tables for Multiple Comparisons with a Control. *Biometrics* 1964, *20*, 482-491.

Novel Anthraquinone Inhibitors of Human Leukocyte Elastase and Cathepsin G[1]

David E. Zembower,† Chih-Min Kam, James C. Powers, and Leon H. Zalkow*

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332. *Received January 24, 1991*

A large series of variously substituted anthraquinones has been synthesized and assayed for inhibitory capacity against human leukocyte elastase (HLE) and cathepsin G (CatG), two serine proteinases implicated in diseases characterized by the abnormal degradation of connective tissue, such as pulmonary emphysema and rheumatoid arthritis. It was found that 2-alkyl-1,8-dihydroxyanthraquinone analogues are competitive inhibitors of HLE with IC_{50} values ranging from 4 to 10 μM, and also inhibit CatG with IC_{50} values ranging from 25 to 55 μM. Consequently, analogues containing the 2-alkyl-1-hydroxy-8-methoxyanthraquinone substitution pattern inhibit HLE to the same magnitude as for the compounds above, but show very little inhibition of CatG. Anthraquinones containing long, hydrophobic *n*-butyl carbonate moieties in the 1- and 8-positions in conjunction with a third hydrophobic substituent in the 2- or 3-position are highly selective for HLE, with K_i values in the range of 10^{-7} M. All of the inhibitors described are completely reversible, with no evidence of acyl-enzyme formation detected.

Introduction

Human leukocyte elastase (HLE) and cathepsin G (CatG) are two serine proteinases derived from the azurophil granules of human neutrophils and have been implicated in various pathological states associated with abnormal degradation of connective tissue, such as rheumatoid arthritis, adult respiratory distress syndrome, and pulmonary emphysema.[2] Both of these enzymes have been shown to attack lung elastin, which is the basis of their implication in abnormal connective tissue degradation. The most widely accepted explanation of the observed pathogenesis is the proteinase–antiproteinase imbalance[3] postulate, which reasons that a deficiency in the naturally occurring inhibitor, α-1-proteinase inhibitor (α-1-antitrypsin), allows extracellular proteinases to act unchecked, thus resulting in uncontrolled connective tissue destruction. This postulate is supported by the fact that persons with genetic deficiencies in the α 1-PI gene are strongly associated with development of pulmonary emphysema. Additionally, it has been shown[4] that the oxi-

† Current address: Division of Developmental Oncology Research, Mayo Clinic and Foundation, Rochester, MN 55905.

(1) This material was presented in April 1991 at the 201st National Meeting of the American Chemical Society in Atlanta, Georgia (Division of Medicinal Chemistry Abstract #36).

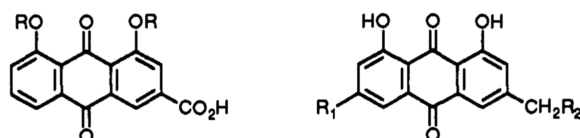
(2) Mittman, C., Taylor, J. C., Eds. *Pulmonary Emphysema and Proteolysis*; Academic Press: New York, 1988; Vol. 2.
(3) Robbins, S. L.; Kumar, B. W. B. *Basic Pathology*, 4th ed.; Saunders Company: Philadelphia, 1987; pp 417-422.

0022-2623/92/1835-1597$03.00/0 © 1992 American Chemical Society

dants in cigarette smoke inactivate α 1-PI, further supporting this view.

On the basis of the proteinase–antiproteinase imbalance postulate, much research has been conducted with the goal of developing a synthetic inhibitor of HLE which could be useful as a chemotherapeutic agent in the treatment of the aforementioned diseases. Many potent inhibitors of HLE have been reported,[5] but there is still the need for inhibitors that are HLE-specific, potent at low concentrations, biologically stable, and exhibit low toxicity at therapeutic doses.

Rhein (**1a**) and diacetylrhein (**1b**) have been shown to be useful antiinflammatory agents in the treatment of rheumatoid arthritis.[6] In 1982 it was reported[7] that rhein and several of its 1,8-diacyl analogues (**1b–d**) inhibit several proteases such as pepsin, trypsin, carboxypeptidase A, and elastase in the range of 10^{-4}–10^{-5} M inhibitor concentrations. More recently[8] it was shown that rhein inhibits pancreatic kallikrein; in addition, emodin (**2**) was reported to strongly inhibit pancreatic kallikrein, trypsin, and lipase, and aloe-emodin (**3**) was reported to inhibit pancreatic kallikrein and elastase. On the basis of this information, we decided to explore the possible utility of these and related anthraquinone analogues as inhibitors of HLE. We report on a series of novel reversible, competitive inhibitors of HLE, some of which also show significant activity against CatG.



(4) Gadek, J. E.; Fells, G. A.; Crystal, R. G. Cigarette Smoking Induces Functional Antiprotease Deficiency in the Lower Respiratory Tract of Humans. *Science* **1979**, *206*, 1315–1316.

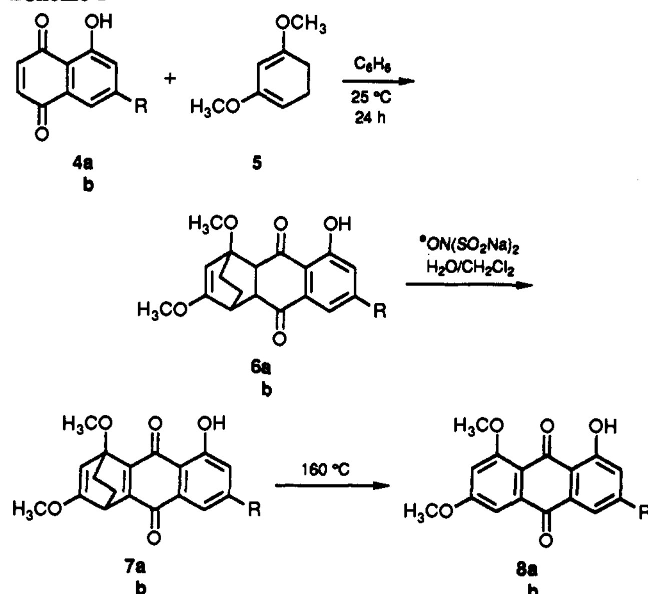
(5) (a) Bode, W.; Meyer, E.; Powers, J. C. Human Leukocyte and Porcine Pancreatic Elastase: X-Ray Crystal Structures, Mechanism, Substrate Specificity, and Mechanism-Based Inhibitors. *Biochemistry* **1989**, *28*, 1951–1963. (b) Baici, A.; Pelloso, R.; Horler, D. The Kinetic Mechanism of Inhibition of Human Leukocyte Elastase by MR889, a New Cyclic Thiolic Compound. *Biochem. Pharmacol.* **1990**, *39*, 919–924. (c) Krantz, A.; Spencer, R. W.; Tam, T. F.; Liak, T. J.; Copp, L. J.; Thomas, E. M.; Rafferty, S. P. Design and Synthesis of 4*H*-3,1-Benzoxazin-4-ones as Potent Alternate Substrate Inhibitors of Human Leukocyte Elastase. *J. Med. Chem.* **1990**, *33*, 464–479. (d) Groutas, W. C.; Brubaker, J. J.; Stanga, M. A.; Castrisos, J. C.; Crowley, J. P.; Schatz, E. J. Inhibition of Human Leukocyte Elastase by Derivatives of N-Hydroxysuccinimide. A Structure–Activity-Relationship Study. *J. Med. Chem.* **1989**, *32*, 1607–1611. (e) Miyano, M.; Deason, J. R.; Nakao, A.; Stealey, M. A.; Villamil, C. I.; Sohn, D. D.; Mueller, R. A. (Acyloxy)benzophenones and (Acyloxy)-4-pyrones. A New Class of Inhibitors of Human Neutrophil Elastase. *J. Med. Chem.* **1988**, *31*, 1052–1061. (f) For a review, see Groutas, W. C. Inhibitors of Leukocyte Elastase and Leukocyte Cathepsin G. Agents for the Treatment of Emphysema and Related Ailments. *Med. Res. Rev.* **1987**, *7*, 227–241.

(6) Friedmann, C. A. Carboxy Anthraquinones for Treatment of Arthritis. U.S. Patent 4,346,103; 24 August 1982.

(7) Raimondi, L.; Banchelli Soldani, G.; Buffoni, F.; Ignesti, G.; Massacesi, L.; Amaducci, L.; Friedmann, C. A. Rhein and Derivatives. In Vitro Studies on Their Capacity to Inhibit Certain Proteases. *Pharmacol. Res. Commun.* **1982**, *14*, 103–112.

(8) Cai, Y.; Chen, Q. Biochemical Study of Chinese Rhubarb. XXVII. Inhibitory Effects of Anthraquinone Derivatives on Four Pancreatic Enzymes. *Shengwu Huaxue Yu Shengwu Wuli Xuebao* **1989**, *21*, 338–343.

Scheme I[a]



[a] **a**, R = CH_3; **b**, R = H.

Chemistry and Structure–Activity Relationships

In order to construct analogues for a structure–activity relationship we first required a ready supply of the polyhydroxylated anthraquinone systems of interest. Because of the great expense of commercially available emodin (**2**), we utilized the synthetic route described by Krohn[9] to prepare emodin on a large scale (Scheme I). The Diels–Alder cyclization of 7-methyljuglone (**4a**)[10] with 1,3-dimethoxy-1,3-cyclohexadiene (**5**)[11] afforded the unstable enol ether **6a**, which was easily hydrolyzed in the presence of a catalytic amount of acid. Therefore, **6a** was immediately oxidized with an aqueous solution of disodium nitrosodisulfonate[12] to the more stable quinone **7a**, as described by Birch and Powell.[13] Pyrolysis of **7a** at 160 °C afforded emodin 1,8-dimethyl ether (**8a**), which was efficiently deprotected to emodin (**2**) with 48% aqueous HBr in refluxing acetic acid. Attempts to deprotect **8a** with HBr in glacial acetic acid provided the partially deprotected analogue physcion (**9**, Table I). The route described in Scheme I was also used to prepare 1,3-dimethoxy-8-hydroxyanthraquinone (**8b**) using commercially available juglone (**4b**) instead of **4a**.

To obtain an initial structure–activity relationship, we decided to evaluate the relative importance of the phenolic hydroxyl groups, as well as the oxidation state (e.g. methyl, hydroxymethyl, carboxylic acid) and position of the alkyl

(9) Krohn, K. Synthetische Anthracyclinone. XVI. Synthesese Hydroxylierter Anthrachinone Durch Regioselektive Diels-Alder-Reaktion. *Tetrahedron Lett.* **1980**, *21*, 3557–3560.

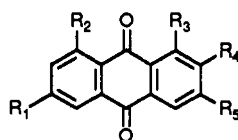
(10) Krohn, K.; Broser, E. Synthetic Anthracyclinones. 25. An Improved Route to 8-Demethoxyaranciamycinone and Synthesis of the α-L-Daunosamine Glycosides. *J. Org. Chem.* **1984**, *49*, 3766–3771.

(11) Birch, A. J.; Shoukry, E. M. A.; Stansfield, F. The Base-Catalysed Isomerisation of Some 3-alkyldihydroanisoles. *J. Chem. Soc.* **1961**, 5376–5380.

(12) Wehrli, P. A.; Pigott, F. Oxidation With the Nitrosodisulfonate Radical. I. Preparation and Use of Disodium Nitrosodisulfonate: Trimethyl-*p*-benzoquinone. In *Organic Syntheses*; Noland, W. E., Ed.; Wiley: New York, 1988; Collect. Vol. VI, pp 1010–1013.

(13) (a) Powell, V. H. Application of Fremey's Salt for the Syntheses of Polycyclic Quinones. *Tetrahedron Lett.* **1970**, 3463–3465. (b) Birch, A. J.; Powell, V. H. Synthesis of Some Polycyclic Quinones Through 1-Methoxycyclohexa-1,3-dienes. *Ibid.* **1970**, 3467–3470.

Table I. Initial Protease Assays of Anthraquinone Derivatives



R_1	R_2	R_3	R_4	R_5	no.	[I] (μM)	% inhibition[a] PPE	HLE	ChT	CatG
OH	OH	OH	H	CH_3	**2**	53	NI[b]	55 (52)[c]	2	42
OCH_3	OH	OH	H	CH_3	**9**	65	NI	100 (6.2)	Act[d]	39
OPr	OH	OH	H	CH_3	**10**	62	NI	100 (5.9)	Act	35
OAc	OAc	OAc	H	CH_3	**11**	62	22	79 (13)	3	NI
OAc	OAc	OAc	H	CH_2Br	**12**	28	NI	28	3	9
OH	OH	OH	H	CH_2OH	**13**	62	4	26	NI	22
OH	OH	OH	H	CO_2H	**14**	58	9	21	3	14
OCH_3	OCH_3	OCH_3	H	CH_3	**15**	30	4	16	6	NI
OCH_3	OCH_3	OCH_3	H	CH_2Br	**16**	29	NI	NI	NI	14
OCH_3	OCH_3	OCH_3	H	$CHBr_2$	**17**	30	NI	61 (17)	Act	24
H	OH	OH	H	CH_3	**18**	28	NI	79 (18)	NI	39
H	OAc	OAc	H	CH_3	**19**	56	NI	NI	NI	NI
H	OAc	OAc	H	CH_2Br	**20**	59	NI	33	Act	Act
H	OAc	OAc	H	CH_2OAc	**21**	61	NI	30	3	NI
H	OH	OH	H	CH_2OH	**3**	63	18	21	13	3
H	OAc	OAc	H	CO_2H	**1b**	70	NI	NI	NI	5
H	OH	OH	H	CO_2H	**1a**	86	NI	NI	NI	22
OCH_3	OCH_3	OH	CH_3	H	**22**	27	NI	80 (13)	7	4
OH	OH	OH	CH_3	H	**23**	55	NI	100 (21)	5	13
OAc	OAc	OAc	CH_3	H	**24**	52	NI	96 (12)	NI	Act

[a] Percentage of inhibition was measured in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer containing 9–11% DMSO at 25 °C. [b] NI = no inhibition. [c] Numbers in parentheses correspond to IC_{50} values in μM concentrations. [d] Act = activation of enzyme relative to controls.

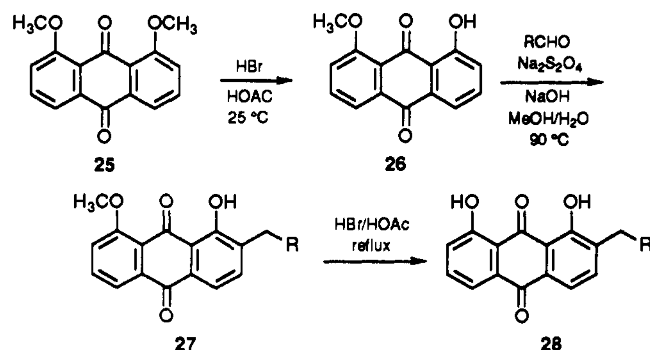
group. Selective alkylation of the 3-hydroxyl group with 1-bromopropane in DMF at 100 °C provided 3-*O*-propylemodin (**10**) in 31% yield. Preparation of triacetylemodin (**11**) with acetic anhydride/pyridine followed by bromination with *N*-bromosuccinimide in refluxing benzene[14] afforded a mixture of the monobromide **12** and its corresponding dibromide in a 71:29 ratio, with purification of the monobromide easily achieved by several recrystallizations from acetone. Analogous bromination of emodin trimethyl ether (**15**) provided the monobromide **16** and the dibromide **17** in approximately the same ratios as described above, which were separated via silica gel chromatography.[15] Treatment of **12** with sodium acetate followed by acid-catalyzed hydrolysis[16] provided the natural product citreorosein (**13**). Chromic acid oxidation of **11** followed by base-catalyzed hydrolysis[17] afforded the natural product emodic acid (**14**). These same reaction pathways were utilized with diacetylchrysophanol (**19**) to provide analogous derivatives of chrysophanol (**18**). To ascertain the importance of the methyl group's position, "isoemodin" (**23**) was prepared via Marschalk alkylation[18] of 1,3-dimethoxy-8-hydroxyanthraquinone (**8b**) with formaldehyde to provide compound **22** followed by HBr deprotection to provide the desired isomer **23**.

These initial analogues were assayed against four related proteases in order to determine a structure–activity relationship and their relative degree of selectivity: HLE, CatG, porcine pancreatic elastase (PPE), and chymotrypsin (ChT). The assays were conducted as previously described,[19] and the results are presented in Table I. We arbitrarily chose to concentrate on compounds that showed 50% or greater enzyme inhibition at an inhibitor concentration of 60 μM or less, and the IC_{50} values for these compounds are reported in parentheses next to the percentage of inhibition in Table I.

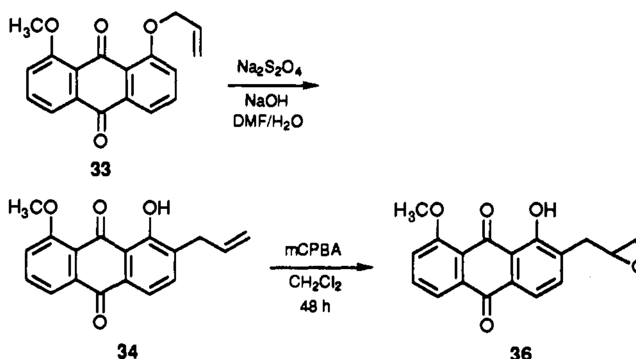
It is quite apparent that these compounds are selective for HLE and CatG, with the degree of inhibition greater for HLE. The first point to recognize is the marked increase in inhibition upon the blocking or removal of the hydroxyl group at R_1. Emodin (**2**), which has a free hydroxyl group at R_1, shows moderate inhibition against HLE, with an IC_{50} of 52 μM. However, blocking the R_1 hydroxyl as a methyl ether (physcion, **9**) or a propyl ether (**10**) increases the degree of inhibition by nearly 10-fold, with the IC_{50} values of these two compounds 6.2 and 5.9 μM, respectively. Similarly, chrysophanol (**18**), which only contains a hydrogen at R_1, shows an IC_{50} value of 18 μM, nearly a 3-fold increase in inhibition over emodin. Clearly

(14) Thiem, J.; Wessel, H. P. Synthesis of Emodin Aldehyde. *Z. Naturforsch., B: Chem. Sci.* **1985**, *40B*, 422–425.
(15) Koyama, M.; Takahashi, K.; Chou, T. C.; Darzynkiewicz, K.; Kapuscinski, J.; Kelly, T. R.; Watanabe, K. A. Intercalating Agents with Covalent Bond Forming Capability. A Novel Type of Potential Anticancer Agents. 2. Derivatives of Chrysophanol and Emodin. *J. Med. Chem.* **1989**, *32*, 1594–1599.
(16) Rajagopalan, T. R.; Seshadri, T. R. Synthesis of Citreorosein and Aloe-Emodin. *Proc. Indian Acad. Sci., Chem. Sci.* **1956**, *44A*, 418–422.
(17) Anslow, W. K.; Breen, J.; Raistrick, H. Studies in the Biochemistry of Micro-organisms. 64. Emodic Acid (4,5,7-Trihydroxyanthraquinone-2-carboxylic acid) and ω-Hydroxyemodin (4,5,7-Trihydroxy-2-(hydroxymethyl)-anthraquinone), Metabolic Products of a Strain of *Penicillium cyclopium* Westling. *Biochem. J.* **1940**, *34*, 159–168.
(18) Marschalk, C.; Koenig, F.; Oursoussoff, N. A New Method of Introducing Side Chains into the Anthraquinone Nucleus. *Bull. Soc. Chim. Fr.* **1936**, *3*, 1545–1575.
(19) (a) Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. Mapping the Extended Binding Site of Cathepsin G and Human Leukocyte Elastase. *J. Biol. Chem.* **1979**, *254*, 4027–4032. (b) Powers, J. C.; Tanaka, T.; Harper, J. W.; Minematsu, Y.; Barker, L.; Lincoln, D.; Crumley, D. V.; Fraki, F. E.; Schechter, N. M.; Lazarus, G. G.; Nakajima, D.; Nakashino, K.; Neurath, H.; Woodbury, R. G. Mammalian Chymotrypsin-Like Enzymes. Comparative Reactivities of Rat Mast Cell Proteases, Human and Dog Skin Chymases, and Cathepsin G with Peptide 4-Nitroanilide Substrates and with Peptide Chloromethyl Ketone and Sulfonyl Fluoride Inhibitors. *Biochemistry* **1985**, *24*, 2048–2058.

Scheme II



Scheme III



the presence of a free hydroxyl group at R_1 hinders the degree of inhibition against HLE. Emodin, in addition to being a poorer inhibitor of HLE in comparison with the above-mentioned compounds, was also found to show slow-binding kinetics.

Another important observation is the increase in inhibition upon moving the methyl group from R_5 (emodin, **2**) to R_4 (isoemodin, **23**), with the IC_{50} decreasing from 52 μM to 21 μM. This is interesting in that isoemodin, which contains a free hydroxyl group at R_1, is essentially twice as active as emodin; the only difference between the two compounds is the position of the methyl group, which must be the determining factor of the increased inhibition. Oxidation of the R_5 methyl group to a hydroxymethyl group (**13** and **3**) or a carboxylic acid (**14** and **1a**) only led to decreased activity relative to their counterparts containing the methyl group at R_5; it is interesting to note that neither rhein (**1a**) nor diacetylrhein (**1b**) showed any inhibition in our assays at concentrations up to 90 μM, as these were the inhibitors reported by Raimondi et al.[7] which initiated our interest in this project. The monobromides **12**, **16**, and **20** showed only little if any activity, but the dibromo analogue **17** was quite active, with an IC_{50} of 17 μM. This was surprising in that its analogous monobromide **16** was completely inactive against HLE.

On the basis of the observations discussed above, we decided to prepare anthraquinone analogues consisting of the 2-alkyl-1,8-dihydroxyanthraquinone nucleus. This series of compounds combines the observation that inhibition is increased in the absence of a hydroxyl group at R_1 with the observation that inhibition is increased with an alkyl functionality in the R_4 position relative to the R_5 position. These compounds were obtained via Marschalk alkylation of 8-hydroxy-1-methoxyanthraquinone (**26**), prepared from 1,8-dimethoxyanthraquinone (**25**)[20] by partial deprotection with HBr,[15] with various aldehydes to provide the 8-O-methylated analogues **27a–g**; subsequent deprotection of the 8-methyl ether moiety with HBr in refluxing acetic acid provided the desired analogues **28a–g** (Scheme II). This reaction proceeded cleanly with the higher aldehydes (propyl, *n*-butyl, isobutyl, etc.), but alkylation with formaldehyde gave only a low yield of **27a**, and attempted alkylation with acetaldehyde gave very low mass recoveries which contained several components by TLC and ^{1}H NMR analyses. Marschalk alkylation of 1-hydroxyanthraquinone[21] with benzaldehyde and formaldehyde provided compounds **37** and **38**, respectively. The 2-allyl analogue **34** was prepared by the reductive Claisen rearrangement[22] of 1-(allyloxy)-8-methoxyanthraquinone (**33**), which was efficiently epoxidized with mCPBA to the epoxide **36** (Scheme III). These and related congeners were assayed as above, and were found to possess quite interesting activities with regard to HLE and CatG (Table II). None of the compounds in Table II showed any significant activity toward either PPE or ChT,

Table II. IC_{50} Values of HLE and CatG Inhibitors

R_1	R_2	R_3	no.	IC_{50} (μM)[a] HLE	IC_{50} (μM)[a] CatG
OCH_3	OH	CH_3	**27a**	19	NI[b]
OCH_3	OH	$(CH_2)_2CH_3$	**27b**	8.7	>60
OCH_3	OH	$(CH_2)_3CH_3$	**27c**	5.9	55
OCH_3	OH	$CH_2CH(CH_3)_2$	**27d**	11	NI
OCH_3	OH	CH_2Ph	**27e**	6.7	NI
OCH_3	OH	$(CH_2)_4CH_3$	**27f**	6.1	>60
OCH_3	OH	$(CH_2)_5CH_3$	**27g**	6.0	>60
OH	OH	CH_3	**28a**	9.9	55
OH	OH	$(CH_2)_2CH_3$	**28b**	4.2	25
OH	OH	$(CH_2)_3CH_3$	**28c**	6.4	24
OH	OH	$CH_2CH(CH_3)_2$	**28d**	8.0	37
OH	OH	CH_2Ph	**28e**	13	56
OH	OH	$(CH_2)_4CH_3$	**28f**	3.9	33
OH	OH	$(CH_2)_5CH_3$	**28g**	4.2	28
OCH_3	OAc	$CH_2CH(CH_3)_2$	**29**	Act[c]	NI
OAc	OAc	$CH_2CH(CH_3)_2$	**30**	Act	Act
OCH_3	OAc	CH_2Ph	**31**	NI	NI
OAc	OAc	CH_2PH	**32**	Act	Act
OCH_3	OH	$CH_2CH{=}CH_2$	**34**	11	>60
OCH_3	OCH_3	$CH_2CH{=}CH_2$	**35**	≫60	NI
OCH_3	OH	$CH_2\underset{O}{CHCH_2}$	**36**	NI	NI
H	OH	CH_2Ph	**37**	6.6	NI
H	OH	CH_3	**38**	11	>60
H	OCH_3	CH_3	**39**	>60	NI
H	H	CH_3	**40**	39	NI
H	H	CH_2CH_3	**41**	Act	NI

[a] IC_{50} values were determined in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer containing 9–11% DMSO at 25 °C. [b] NI = no inhibition at concentrations up to 60 μM. [c] Act = activation of enzyme.

(20) Oesterle, O. A.; Haugseth, A. R. Certain Derivatives of Rhein. *Arch. Pharm. (Weinheim, Ger.)* **1915**, *253*, 330–334.
(21) 1-Hydroxyanthraquinone was prepared from 1-aminoanthraquinone using the procedure described in Dimroth, K.; Berndt, A.; Perst, H.; Reichardt, C. 2,4,6-Triphenylphenoxyl. In *Organic Syntheses*; Baumgarten, H. E., Ed.; John Wiley & Sons, Inc.: New York, 1973; Collect. Vol. V, pp 1130–1134.
(22) (a) Boddy, I. K.; Boniface, P. J.; Cambie, R. C.; Craw, P. A.; Larsen, D. S.; McDonald, H.; Rutledge P. S.; Woodgate, P. S. Reductive Claisen Rearrangements of Anthraquinone Allyl Ethers. *Tetrahedron Lett.* **1982**, *23*, 4407–4408. (b) Takahashi, I.; Terashima, S. Studies on the Synthesis of 4-[1,8-Dioxygenated-9,10-dioxoanthracen(or anthracen)-2-yl]butanoic Acid Derivatives. *Chem. Pharm. Bull.* **1982**, *30*, 4539–4544.

which exemplifies the significant level of specificity observed for these inhibitors.

Of first importance is the specificity of compounds **27a–g** for HLE over CatG. While the IC_{50} values range from 6.0 to 19 μM for HLE, these compounds only inhibit CatG slightly, if any, at inhibitor concentrations up to 60 μM. On the other hand, the deprotected analogues **28a–g** show essentially the same magnitude of inhibition against HLE as do the 8-O-methylated analogues **27a–g** (IC_{50}'s range from 3.9 to 9.9 μM), but are also moderately active against CatG, with IC_{50} values ranging from 24 to 56 μM. This is significant in that it provides a series of compounds that are specific inhibitors of HLE (**27a–g**) and a closely related series of analogues that show comparable inhibition against HLE *and* CatG (**28a–g**) simply by the inclusion or exclusion of a methyl ether, respectively. In both cases the mode of inhibition was reversible, but the compounds **28a–g** showed slow-binding kinetics with respect to CatG.

There appears to be little preference for a particular size of the 2-alkyl chain with respect to inhibition of either HLE or CatG. Although the inhibitors containing the longer 2-alkanes (*n*-propyl to *n*-hexyl) showed slightly greater inhibition against both enzymes than the smaller 2-methyl-substituted analogues (**27a** and **28a**), all of the compounds within a given series inhibited within the same order of magnitude. There also appears to be no preference for a saturated versus an unsaturated 2-alkyl group, because the 2-allyl analogue **34** shows essentially the same inhibition as the saturated 2-*n*-propyl analogue **27b** (IC_{50} values of 11 and 8.7 μM, respectively). The requirements of the hydroxyl groups are much more striking, though, as it appears that CatG requires a free hydroxyl group at both R_1 and R_2; the inhibitors **28a–g**, which possess free hydroxyl groups at R_1 and R_2, are relatively good inhibitors of CatG, but the analogues **27a–g**, which have the hydroxyl at R_1 blocked as a methyl ether, show only slight activity against CatG at concentrations up to 60 μM. Additionally compounds **37** and **38**, which contain only a hydrogen at R_1, also show little if any inhibition against CatG at inhibitor concentrations up to 60 μM. It is apparent that a free hydroxyl at R_1 is not necessary to maintain inhibition against HLE, though, because compounds **27a–g**, **37**, and **38**, with either a methyl ether or a hydrogen at R_1, all show inhibition against HLE. It does appear that a free hydroxyl at R_2 is necessary to maintain inhibition against HLE because the compounds **29–32**, which have either methoxy or acetoxy groups at positions R_1 and R_2, are completely inactive against either enzyme. Additionally, compounds **35** and **39**, which both contain methoxy substituents at position R_2, show greatly decreased activity relative to their counterparts containing free hydroxyl groups at R_2 (**34** and **38**, respectively). One anomaly to this observation is 2-methylanthraquinone (**40**) which, although it does not contain a phenolic group, still shows inhibition against HLE, with an IC_{50} of 39 μM. This is surprising in that neither 2-ethylanthraquinone (**41**) nor anthraquinone itself, two closely related analogues of **40**, showed any activity at all.

These compounds act as competitive, reversible inhibitors and show no time dependence other than the slow-binding kinetics toward CatG. Because the analogues containing large alkyl groups at R_3 (e.g. **28f** and **28g**) show essentially the same degree of inhibition as the compounds containing smaller groups at R_3 (e.g. **28b** and **28c**), it is unlikely that these alkyl chains are occupying the S_1 binding site; it is known that HLE prefers substrates which contain small, aliphatic P_1 residues, such as valine. However, Stein[23] reported that the specificity of the S_1 site is highly dependent upon the size of the substrate, with the degree of specificity decreasing proportionally with the size of the substrate. Because the inhibitors described above are rather small molecules, it is conceivable that the R_3 alkyl chain could fit into the S_1 pocket. Groutas[5d] recently described inhibitors of HLE consisting of *N*-hydroxysuccinimide derivatives, in which he postulates that substituents as large as isopentyl fit into the S_1 subsite. One important observation that does suggest the S_1 pocket as the site being occupied by the R_3 alkyl chain is the fact that the allyl analogue **34**, which inhibits HLE with an IC_{50} of 11 μM, completely loses all activity when oxidized to the epoxide **36**. This is somewhat analogous to the observation that oxidation of the Met358 residue of α1-PI to its sulfoxide completely inactivates the inhibitory capacity of α1-PI.[24] *However, we cannot state with any degree of confidence that this is the mode of interaction of the R_3 alkyl group, as we have no evidence concerning the mode of binding for these inhibitors*; suffice it to say that the R_3 alkyl substituent forms stabilizing hydrophobic interactions with the enzyme. In addition, the presence of at least one free hydroxyl group increases the inhibitor–enzyme interaction, presumably through hydrogen-bonding; while anthraquinone itself shows no activity against either HLE or CatG, 1,8-dihydroxyanthraquinone (chrysazin) is moderately active, with an IC_{50} of 42 μM for HLE. As a representative example of this series, the enzyme–inhibitor dissociation constant (K_i) was determined for compound **28c** from a standard Dixon plot and found to be 0.60 μM against HLE.

Table III. K_i and IC_{50} Values of Anthraquinone Poly(*n*-butyl carbonates) for HLE

$H_3C(CH_2)_3O_2CO$, O, $OCO_2(CH_2)_3CH_3$, R_2, R_1, R_3, O

R_1	R_2	R_3	no.	K_i (μM)[a]	IC_{50} (μM)[a]
H	H	H	**42**	7.5	12
CH_3	H	H	**43**	1.9	5.1
CH_3	H	OCH_3	**44**	2.8	2.5
CH_3	H	$O(CH_2)_2CH_3$	**45**	0.15	1.8
CH_3	H	$OCO_2(CH_2)_3CH_3$	**46**	ND[b]	1.3
H	$(CH_2)_5CH_3$	H	**47**	0.43	2.1

[a] K_i and IC_{50} values were determined in 0.1 M HEPES, 0.5 M NaCl, pH 7.5 buffer containing 9–11% DMSO at 25 °C. [b] K_i could not be determined for **46** due to mixed inhibition.

(23) (a) Stein, R. L. Catalysis by Human Leukocyte Elastase: III. Steady-State Kinetics for the Hydrolysis of p-Nitrophenyl Esters. *Arch. Biochem. Biophys.* **1985**, *236*, 677–680. (b) Stein, R. L.; Strimpler, A. M.; Hori, H.; Powers, J. C. Catalysis by Human Leukocyte Elastase: Mechanistic Insights into Specificity Requirements. *Biochemistry* **1987**, *26*, 1301–1305.

(24) (a) Beatty, K.; Mattheson, N.; Travis, J. Kinetic and Chemical Evidence for the Inability of Oxidized α-1-Proteinase Inhibitor to Protect Lung Elastin from Elastolytic Degradation. *Hoppe Zeyler's Z. Physiol. Chem.* **1984**, *365*, 731–736. (b) Johnson, D.; Travis, J. Structural Evidence for Methionine at the Reactive Site of Human α-1-Proteinase Inhibitor. *J. Biol. Chem.* **1978**, *253*, 7142–7144. (c) One reviewer pointed out that the change in conformation upon oxidation of **34** to **36**, due to hydrogen bonding, could account for the absence of activity for compound **36**. We agree that this is a possibility, and only offer the comparison with α1-PI to suggest the possibility of binding in the S_1 pocket. However, as stated in the text, we do not claim to know the exact mode of binding of these inhibitors.

(25) Brew, E. J. C.; Thompson, R. H. Naturally Occurring Quinones. Part XX. Anthraquinones in *Digitalis purpurea*. *J. Chem. Soc. C* **1971**, 2007–2010.

Upon reviewing the increase in activity of triacetylemodin (11, Table I) and triacetylisoemodin (24) relative to their non-acetylated counterparts (2 and 23, respectively), we noted two important points: first, the dramatic increase in activity, and second, the greater degree of selectivity for HLE over CatG. We therefore decided to explore the possibility that anthraquinones containing several large hydrophobic substituents could provide potent, selective inhibition of HLE over CatG. The simplest route to construct molecules of this sort was to form long-chain alkyl carbonates of existing polyhydroxylated anthraquinones, which was readily achieved via acylation of the parent anthraquinone with *n*-butyl chloroformate.

The data in Table III verifies that these compounds are indeed potent inhibitors of HLE, with K_i values ranging from 7.5 μM (compound **42**) to 0.15 μM (compound **45**). It is quite apparent that the magnitude of inhibition is dependent directly upon the number of long-chain hydrophobic substituents. Compound **42** (IC_{50} = 12 μM), which has only two hydrophobic chains, is about as active as triacetylemodin (**11**, IC_{50} = 13 μM) and triacetylisoemodin (**24**, IC_{50} = 12 μM) which both contain three smaller acetyl substituents. Addition of one (compound **43**) or two (compound **44**) small hydrophobic substituents increases the inhibition, with the K_i value dropping from 7.5 μM (compound **42**) to the range of 1.9–2.8 μM. The presence of three long-chain hydrophobic substituents (compounds **45–47**) leads to a further increase in activity by 1 order of magnitude, with the K_i values dropping into the sub-micromolar range (0.15–0.43 μM). The K_i value for compound **46** could not be determined because this inhibitor appeared to exhibit mixed modes of inhibition. It is interesting that the placement of the third long-chain hydrophobic substituent seems to have little effect on the magnitude of inhibition; compound **45**, which has the third hydrophobic substituent meta to one of the *n*-butyl carbonate moieties, shows essentially the same magnitude of inhibition as compound **47**, which has a long-chain alkyl substituent ortho to an *n*-butyl carbonate moiety. This is interesting because of the dramatic preference for the alkyl group in the ortho position for the inhibitors described in Tables I and II. Of additional interest is the specificity of these inhibitors for HLE: assays with PPE and ChT at a 30 μM inhibitor concentration showed no inhibition of enzymatic activity, and assays with CatG showed either no inhibition (compounds **42–44**) or only slight inhibition (compounds **45–47**, less than 20% inhibition) at 30 μM inhibitor concentration.

To determine the mode of inhibition we evaluated the possibility that one of the carbonate moieties is transferring an acyl group to the enzyme. Dialysis of a HEPES buffer solution (containing 10% DMSO) of HLE and compound **45**, at such concentrations that HLE was inhibited by 89%, at 5 °C for 24 h resulted in the enzyme regaining 55% of its activity. These results could be interpreted in two ways: first, compound **45** rapidly acylates HLE (because no time dependence is observed), followed by rapid deacylation during the dialysis procedure. Second, these inhibitors operate through simple hydrophobic interactions with the enzyme, combined with possible hydrogen bonding. We feel that the latter is most likely the case, because we have observed no evidence for the formation of an acyl-enzyme.

Conclusions

We have presented a series of anthraquinone congeners which show selective inhibition of human leukocyte elastase and cathepsin G (see Table IV for physical data). These inhibitors are quite selective for the human neutrophil enzymes over their porcine counterparts (porcine pancreatic elastase and chymotrypsin, respectively). The inhibitors presented in Table II are extremely interesting in that the inclusion (**47a–g**) or exclusion (**48a–g**) of a simple methyl ether provides selective inhibitors that are active against HLE (**47a–g**) or against both HLE and CatG (**48a–g**). Additionally, the inhibitors presented in Table III represent simple molecules which show surprisingly selective and potent inhibition of HLE, presumably through hydrophobic interactions with the enzyme.

Table IV. Chemical Data for Anthraquinone Congeners

compd[a]	% yield[b]	recryst solvent	mp (°C)	formula[c]
27a	14	MeOH	200–201	NA
27b	35	MeOH	165	$C_{18}H_{16}O_4$
27c	78	MeOH	103	$C_{19}H_{18}O_4$
27d	62	MeOH	129–130	$C_{19}H_{14}O_4$
27e	81	MeOH	192	$C_{22}H_{16}O_4$
27f	30	MeOH	104	$C_{20}H_{20}O_4$
27g	13	MeOH	120–121	$C_{21}H_{22}O_4 \cdot {}^1/_5H_2O$
28a	87	MeOH	168–169	NA
28b	51	MeOH	135	$C_{17}H_{14}O_4$
28c	71	MeOH	120	$C_{19}H_{16}O_4$
28d	82	MeOH	147	$C_{18}H_{16}O_4$
28e	86	HOAc	190	$C_{21}H_{14}O_4$
28f	75	MeOH	118	$C_{19}H_{18}O_4$
28g	78	MeOH	102	$C_{20}H_{20}O_4$
29	100	MeOH	179	$C_{21}H_{20}O_5$
30	100	MeOH	184	$C_{22}H_{20}O_6$
31	100	MeOH	178	$C_{24}H_{14}O_5 \cdot {}^1/_4H_2O$
32	100	MeOH	196–197	$C_{25}H_{18}O_6 \cdot H_2O$
37	14	MeOH	155	NA
38	51	MeOH	181	NA
39	73	EtOH	163–164	NA
42	39	EtOH	104	$C_{24}H_{24}O_8$
43	49	EtOH	126	$C_{25}H_{26}O_8$
44	54	MeOH	124	$C_{26}H_{28}O_9$
45	67	EtOH	106	$C_{28}H_{32}O_9$
46	55	EtOH	98	$C_{30}H_{34}O_{11}$
47	55	MeOH/H_2O	39	$C_{20}H_{36}O_8$

[a] All compounds displayed ^{1}H NMR, EIMS, and FTIR spectra consistent with the assigned structure. Full spectral data are available as supplementary material. [b] Yield reported represents yield after recrystallization. [c] Elemental analyses (±0.4% of theoretical value) were obtained for C and H for all new compounds.

Experimental Section

Melting points were determined on a Koffler hot-stage equipped with a digital thermometer and are corrected. ^{1}H NMR spectra were recorded on a Varian Gemini-300 (300 MHz) spectrometer. All ^{1}H chemical shifts are reported downfield (δ) in ppm relative to a tetramethylsilane internal standard. Mass spectra were recorded on a VG Analytical 70-SE mass spectrometer equipped with a 11-250J data system, and all exact mass determinations were recorded at 10000 resolution. FTIR spectra were recorded on a Nicolet 520 FTIR spectrophotometer as either thin film, Nujol mull, or 5% $CHCl_3$ solution cell. All microanalyses were performed by Atlantic Microlab, Norcross, GA, and are within 0.4% of theoretical values. Dry THF was obtained by refluxing over sodium/benzophenone under an argon atmosphere. Enzymatic assays were performed as previously described[19] and monitored with a Varian DMS-90 spectrophotometer or a Nicolet 520 spectrophotometer. TLC was conducted on Merck Kieselgel 60 F_{254} precoated silica gel plates. Column chromatography was conducted using Davison Chemical S-704 silica gel or equivalent. Human leukocyte elastase and cathepsin G were provided by Dr. James Travis, University of Georgia, Athens. Chymotrypsin was purchased from Sigma Chemical Co. and porcine pancreatic elastase was purchased from Research Organics, Inc. Suc-Ala-Ala-Ala-pNA was purchased from Peninsula Laboratories. 2-Methylanthraquinone (**40**) and 2-ethylanthraquinone (**41**) were purchased from Aldrich Chemical Co., and the former was recrystallized from methanol before use; juglone (**4a**) was purchased from Aldrich Chemical Co. and used without further purification.

1,4-Dihydro-1,3-dimethoxy-8-hydroxy-6-methyl-1,4-ethano-9,10-anthraquinone (7a). A solution of 7-methyl-

juglone[10] (**4a**, 1.88 g, 0.10 mmol) and 1,3-dimethoxy-1,3-cyclohexadiene[11] (**5**, 70% pure, 3.0 g, 0.15 mmol) in benzene (50 mL) was stirred at room temperature for 24 h. The solvent was evaporated, and the residue (crude **6a**) was dissolved in 0.5 N methanolic KOH (50 mL) and heated on a steam bath for 10 min. Methylene chloride (100 mL) was added, then a solution of sodium nitrosodisulfonate (Fremey's salt), generated electrochemically[12] (ca. 0.4 M, 75 mL), was added, and the solution was stirred at room temperature for 2 h. The solution was diluted with chloroform (100 mL), the lower organic phase was collected, and the aqueous phase was extracted with chloroform (2 × 100 mL). The combined organic extracts were washed with saturated brine (200 mL) and water (200 mL), dried over magnesium sulfate, filtered, and evaporated to afford a dark brown oil. The oil was dissolved in ether and cooled in an ice bath, upon which time a dark orange solid precipitated. The solid was collected via vacuum filtration, washed with cold petroleum ether, and air-dried to afford 1.85 g (57%) of the desired product. An analytical sample was obtained via silica gel chromatography ($CHCl_3$). Notes: It was sometimes necessary to add a small amount of petroleum ether to the crude product while it was dissolved in ether to induce precipitation of the product. Yields of this reaction varied, and the use of greater than 1.2 equiv of diene **5** led to marked decreases in yield: mp 124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (m, 1 H), 1.86 (m, 3 H), 2.40 (s, 3 H), 3.54 (s, 3 H), 3.68 (s, 3 H), 4.25 (m, 1 H), 5.19 (d, 1 H, J = 2.5 Hz), 7.03 (d, 1 H, J = 1.4 Hz), 7.42 (d, 1 H, J = 1.4 Hz), 12.34 (s, 1 H); EIMS m/e 326.1174 (M^+, requires 326.1154), 298, 280, 269, 252; FTIR ($CHCl_3$) 1650, 1633, 1587 cm^{-1}.

Emodin 1,3-Dimethyl Ether (8a). Into a small Erlenmeyer flask was placed 500 mg (1.5 mmol) of the bridged compound **7a**, and the flask was heated to 160 °C with an oil bath. After several minutes the solid melted and then quickly resolidified as a dark brown solid. After heating for 30 min, the flask was cooled, and the solid was recrystallized from methanol/chloroform (9:1) to provide dark orange crystals (420 mg, 92%): mp 207 °C (lit.[9] mp 204–205 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (s, 3 H), 4.00 (s, 3 H), 4.04 (s, 3 H), 6.80 (d, 1 H, J = 2.5 Hz), 7.09 (d, 1 H, J = 1.5 Hz), 7.48 (d, 1 H, J = 2.5 Hz), 7.59 (d, 1 H, J = 1.5 Hz), 13.11 (s, 1 H); EIMS m/e 298.0840 (M^+, requires 298.0841), 280, 269, 252; FTIR ($CHCl_3$) 1631, 1596, 1561 cm^{-1}.

Emodin (2). A solution of **8a** (780 mg, 2.6 mmol) in glacial acetic acid (80 mL) and HBr (30% aqueous, 80 mL) was refluxed overnight. Upon cooling, a precipitate formed which was collected by vacuum filtration, washed neutral with water, and air-dried. Recrystallization from chloroform provided emodin as orange needles, identical with an original sample.

3-*O*-Propylemodin (10). A solution of **2** (100 mg, 0.37 mmol), 1-bromopropane (50 mg, 0.41 mmol), and K_2CO_3 (300 mg, 2.2 mmol) in DMF (15 mL) was stirred at 100 °C overnight. After cooling, the solution was poured into dilute HCl (200 mL), and the brick-orange precipitate was collected by vacuum filtration, washed with water, and air-dried. The crude solid (77 mg) was dissolved in boiling methanol, stirred with a small amount of Norite, and filtered while hot. Evaporation of the filtrate afforded an orange powder, which showed two spots on TLC (silica gel, $CHCl_3$, R_f = 0.64, 0.49). The components were separated via silica gel chromatography ($CHCl_3$); the lead component (R_f = 0.64) provided the desired compound as an orange powder (36 mg, 31%), while the trailing component (R_f = 0.49) corresponded to unreacted emodin: mp 172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, 3 H, J = 7.4 Hz), 1.86 (m, 2 H), 2.45 (s, 3 H), 4.06 (t, 1 H, J = 6.5 Hz), 6.67 (d, 1 H, J = 2.5 Hz), 7.08 (b s, 1 H), 7.36 (d, 1 H, J = 2.5 Hz), 7.63 (b s, 1 H), 12.14 (s, 1 H), 12.31 (s, 1 H); EIMS m/e 312.0996 (M^+, requires 312.0998), 270, 242, 213; FTIR ($CHCl_3$) 1676, 1627, 1611, 1566 cm^{-1}. Anal. ($C_{18}H_{16}O_5 \cdot {}^2/_5H_2O$) C, H.

1,4-Dihydro-8-hydroxy-1,3-dimethoxy-1,4-ethano-9,10-anthraquinone (7b): prepared as described above for **7a** except using juglone (**4b**, 1.1 g, 6.1 mmol) instead of **4a**; yield 960 mg (51%); mp 152 °C (pyrolyzes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (m, 1 H), 1.88 (m, 3 H), 3.54 (s, 3 H), 3.68 (s, 3 H), 4.27 (m, 1 H), 5.20 (d, 1 H, J = 2.5 Hz), 7.24 (dd, 1 H, J = 7.9 Hz, 1.5 Hz), 7.59 (t, 1 H, J = 7.9 Hz), 7.59 (dd, 1 H, J = 7.9 Hz, 1.5 Hz), 12.38 (s, 1 H); IR ($CHCl_3$) 1652, 1636, 1593 cm^{-1}; exact mass calcd for $C_{18}H_{16}O_5$ 312.0998, found 312.1012.

1,3-Dimethoxy-8-hydroxy-9,10-anthraquinone (8b): prepared as described above for **8a**, except using **7b** (100 mg, 0.32 mmol) in place of **7a**; yield 78 mg (85%); mp 218–219 °C (lit.[13b] mp 215–216 °C) ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 3 H), 4.03 (s, 3 H), 6.78 (d, 1 H, J = 2.5 Hz), 7.28 (dd, 1 H, J = 8 Hz, 1.3 Hz), 7.45 (d, 1 H, J = 2.5 Hz), 7.59 (t, 1 H, J = 8 Hz), 7.74 (dd, 1 H, J = 8 Hz, 1.3 Hz), 13.14 (s, 1 H); EIMS m/e 284.0680 (M^+, requires 284.0685), 266, 255, 238, 223; IR ($CHCl_3$) 1639, 1604 cm^{-1}. Anal. ($C_{16}H_{12}O_5$) C, H.

6,8-Dimethoxy-1-hydroxy-2-methyl-9,10-anthraquinone (22). To a solution of 1.5% aqueous NaOH (100 mL) and methanol (100 mL) was added **8b** (1.50 g, 5.3 mmol), and the solution was purged with nitrogen for 15 min. The solution was heated to 70 °C, sodium dithionite (1.86 g, 10.6 mmol) was added, and the solution was stirred for 10 min. Formaldehyde (37% aqueous, 30 mL) was added, and the solution was stirred at 70 °C under a nitrogen atmosphere overnight. The solution was cooled to room temperature, acidified with HCl, and then cooled at 3 °C for several hours. The resulting orange crystalline solid was collected by vacuum filtration, washed with water, and air-dried (1.29 g, 82%). An analytical sample was obtained via column chromatography (silica, $CHCl_3$) to provide a bright orange crystalline solid: mp 219–220 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3 H), 4.00 (s, 3 H), 4.04 (s, 3 H), 6.80 (d, 1 H, J = 2.5 Hz), 7.46 (d, 1 H, J = 7.6 Hz), 7.48 (d, 1 H, J = 2.5 Hz), 7.69 (d, 1 H, J = 7.6 Hz); EIMS m/e 298.0847 (M^+, requires 298.0841), 280, 269, 252; FTIR ($CHCl_3$) 1668, 1628, 1596, 1457 cm^{-1}. Anal. ($C_{17}H_{14}O_5$) C, H.

2-Methyl-1,6,8-trihydroxy-9,10-anthraquinone (Isoemodin, 23). A solution of **22** (1.2 g, 4.4 mmol) in glacial acetic acid (100 mL) and HBr (48% aqueous, 50 mL) was refluxed for 3 h, then additional HBr (50 mL) was added, and the solution was refluxed overnight. The solution was diluted with 50 mL of water and cooled, and the resulting tan solid was collected by vacuum filtration, washed with water, and air-dried. Recrystallization from methanol provided orange needles (0.90 g, 76%): mp 287 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.35 (s, 3 H), 6.68 (d, 1 H, J = 2.3 Hz), 7.29 (d, 1 H, J = 2.3 Hz), 7.67 (d, 1 H, J_{AB} = 7.7 Hz), 7.80 (d, 1 H, J_{AB} = 7.7 Hz), 12.21 (s, 1 H), 12.55 (s, 1 H); EIMS m/e 270.0522 (M^+, requires 270.0528), 253, 241, 224, 213; IR (Nujol) 3375, 1613, 1577 cm^{-1}. Anal. ($C_{15}H_{10}O_5 \cdot {}^1/_{10}H_2O$) C, H; H: calcd, 3.71; found, 4.19.

2-Methyl-1,6,8-triacetoxy-9,10-anthraquinone (24). A solution of **23** (500 mg, 1.8 mmol) in acetic anhydride (50 mL) and pyridine (3 mL) was heated at 90 °C for 90 min. The solvent was evaporated to give the desired product in quantitative yield. Recrystallization from methanol afforded light yellow needles: mp 199 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3 H), 2.36 (s, 3 H), 2.45 (s, 3 H), 2.48 (s, 3 H), 7.24 (d, 1 H, J = 2.5 Hz), 7.65 (d, 1 H, J = 7.5 Hz), 7.95 (d, 1 H, J = 2.5 Hz), 8.12 (d, 1 H, J = 7.5 Hz); EIMS m/e 397.0945 (M^+, requires 397.0923), 354, 312, 270, 241; FTIR ($CHCl_3$) 1776, 1677, 1634, 1600 cm^{-1}. Anal. ($C_{21}H_{17}O_8$) C, H.

8-Hydroxy-1-methoxy-9,10-anthraquinone (26). A solution of 1,8-dimethoxy-9,10-anthraquinone[20] (**25**, 16.4 g, 61.2 mmol) in glacial acetic acid (500 mL) and HBr (30% in acetic acid, 40 mL) was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was triturated with water. The fluffy crystalline solid was collected with a Buchner funnel, washed well with water, and air-dried to provide the desired product in quantitative yield. Recrystallization from methylene chloride/ether provided fluffy orange needles: mp 198 °C (lit.[22b] mp 200–201 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.08 (s, 3 H), 7.30 (dd, 1 H, J = 8 Hz, 1 Hz), 7.37 (dd, 1 H, J = 8 Hz, 1 Hz), 7.63 (t, 1 H, J = 8 Hz), 7.76 (t, 1 H, J = 8 Hz), 7.79 (dd, 1 H, J = 8 Hz, 1 Hz), 7.98 (dd, 1 H, J = 8 Hz, 1 Hz), 12.96 (s, 1 H); EIMS m/e 254.0572 (M^+, requires 254.0579), 236, 208, 168, 139; FTIR ($CHCl_3$) 1672, 1635, 1588, 1458 cm^{-1}.

General Procedure for the Marschalk Alkylation of 26 with Various Aldehydes. 2-*n*-Butyl-1-hydroxy-8-methoxy-9,10-anthraquinone (27c). To a solution of 5% NaOH in methanol/water (1:1), which had been deaerated with nitrogen for 30 min, was added **26** (1.50 g, 5.91 mmol) and sodium dithionite (6.0 g), and the resulting orange solution was heated at 70 °C under nitrogen for 15 min. Butyraldehyde (4.25 g, 59.1 mmol) was added, and the solution was stirred at 90 °C under nitrogen overnight. The solution was cooled while stirring open to the air and then acidified with HCl. The dark-orange precipitate thus formed was

collected via vacuum filtration, washed with a large volume of water, and air-dried. Recrystallization from methanol provided bright orange needles (78%): mp 103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, 3 H, J = 7.5 Hz), 1.42 (sextet, 2 H, J = 7.5 Hz), 1.64 (m, 2 H), 2.76 (t, 2 H, J = 7.5 Hz), 4.08 (s, 3 H), 7.36 (d, 1 H, J = 8 Hz), 7.49 (d, 1 H, J = 8 Hz), 7.72 (d, 1 H, J = 8 Hz), 7.74 (t, 1 H, J = 8 Hz), 7.97 (d, 1 H, J = 8 Hz), 12.51 (s, 1 H); EIMS m/e 310.1212 (M^+, requires 310.1205), 295, 281, 267, 250; FTIR ($CHCl_3$) 1667, 1631, 1589, 1465 cm^{-1}. Anal. ($C_{19}H_{18}O_4$) C, H.

For the analogues which precipitated upon acidification of the reaction mixture, one or two recrystallizations from methanol was usually sufficient to obtain analytically pure material. However, several of the analogues did not precipitate upon acidification, and the material was worked up by extraction with chloroform, drying the organic extracts over sodium sulfate, and evaporation to provide a thick orange oil. Column chromatography over silica gel ($CHCl_3$) provided the desired compounds as orange powders, which were then recrystallized from methanol. Analogously, compounds **37** and **38** were prepared from 1-hydroxyanthraquinone with formaldehyde and butyraldehyde, respectively, as described above.

General Procedure for HBr-Mediated Deprotection of 27 to 28. 2-*n*-Butyl-1,8-dihydroxy-9,10-anthraquinone (28c). A solution of **27c** (155 mg, 0.50 mmol) in glacial acetic acid (15 mL) and HBr (30% in HOAc, 3 mL) was refluxed for 3 h. The solvent was evaporated, and the residue was chromatographed through a short silica gel column ($CHCl_3$) to provide the corresponding analogue **28c** as an orange powder. Recrystallization from methanol afforded light orange needles (105 mg, 71%): mp 120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, 3 H, J = 7.5 Hz), 1.42 (sextet, 2 H, J = 7.5 Hz), 1.65 (m, 2 H), 2.77 (t, 2 H, J = 7.5 Hz), 7.30 (dd, 1 H, J = 8 Hz, 1 Hz), 7.55 (d, 1 H, J = 7.5 Hz), 7.68 (t, 1 H, J = 8 Hz), 7.79 (d, 1 H, J = 7.5 Hz), 7.84 (dd, 1 H, J = 8 Hz, 1 Hz), 12.13 (s, 1 H), 12.46 (s, 1 H); EIMS m/e 296.1052 (M^+, requires 296.1049), 278, 267, 254, 225; FTIR ($CHCl_3$) 3116, 1670, 1623, 1598 cm^{-1}. Anal. ($C_{18}H_{16}O_4$) C, H.

General Procedure for the Acetylation of Hydroxyanthraquinones. 1-Acetoxy-2-isobutyl-8-methoxy-9,10-anthraquinone (29). A solution of **27d** (25.5 mg, 0.082 mmol) in acetic anhydride (10 mL) and pyridine (1 mL) was heated at 90 °C overnight. The solvent was evaporated to provide the desired product in quantitative yield as a light yellow solid. An analytical sample was obtained via recrystallization from methanol: mp 179 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (d, 6 H, J = 7 Hz), 1.95 (heptet, 1 H, J = 7 Hz), 2.52 (s, 3 H), 2.54 (s, 2 H), 4.01 (s, 3 H), 7.32 (dd, 1 H, J = 8 Hz, 1 Hz), 7.57 (d, 1 H, J = 8 Hz), 7.68 (t, 1 H, J = 8 Hz), 7.89 (dd, 1 H, J = 8 Hz, 1 Hz), 8.11 (d, 1 H, J = 8 Hz); EIMS m/e 352.1311 (M^+, requires 352.1311), 310, 295, 281, 267; FTIR ($CHCl_3$) 1785, 1673, 1588, 1468 cm^{-1}. Anal. ($C_{21}H_{20}O_5$) C, H. Analogously, **30**, **31**, and **32** were prepared from **28d**, **27e**, and **28e**, respectively.

1-(Allyloxy)-8-methoxy-9,10-anthraquinone (33). To a solution of **26** (10.0 g, 39 mmol), potassium carbonate (16 g), and potassium iodide (6.5 g) in 1 L of acetone was added allyl bromide (19 g, 16 mmol), and the solution was stirred at reflux for 48 h. The solvent was evaporated under reduced pressure to approximately 200 mL and then poured into 1 L of water. The yellow crystalline solid was collected via vacuum filtration, washed well with water, and air-dried to provide 11 g (95%) of desired product, which could be used without further purification. Recrystallization from methanol provided shiny yellow plates: mp 177 °C (lit.[22b] mp 174–175 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.02 (s, 3 H), 4.78 (d, 2 H, J = 4.9 Hz), 5.37 (dd, 1 H, J = 9.1 Hz, 1.5 Hz), 5.58 (dd, 1 H, J = 17.2 Hz, 1.5 Hz), 6.13 (ddd, 1 H, J = 17.2 Hz, 9.1 Hz, 1.5 Hz), 7.29 (d, 1 H, J = 8 Hz), 7.31 (d, 1 H, J = 8 Hz), 7.61 (t, 1 H, J = 8 Hz), 7.64 (t, 1 H, J = 8 Hz), 7.84 (d, 1 H, J = 8 Hz), 8.85 (d, 1 H, J = 8 Hz); EIMS m/e 294.0884 (M^+, requires 294.0892), 279, 265, 253, 237, 209; FTIR ($CHCl_3$) 1672, 1588, 1466, 1319 cm^{-1}.

2-Allyl-1-hydroxy-8-methoxy-9,10-anthraquinone (34). To a solution of DMF/H_2O (1:1, 150 mL) were added sodium dithionite (1.2 g, 6.8 mmol) and **34** (1.0 g, 3.4 mmol), and the solution was heated to 70 °C under nitrogen. Several (3–5) pellets of solid sodium hydroxide were added, and the resulting bright orange solution was stirred under nitrogen at 70 °C for 2 h. The solution was oxygenated with a steady stream of oxygen for 30 min, then poured into 50 mL water, and acidified with HCl. The resulting orange solid was collected via vacuum filtration, washed with water, and air-dried. The solid was chromatographed on silica gel ($CHCl_3$) to provide 640 mg (64%) of desired product as a bright orange solid. An analytical sample was obtained via recrystallization from ethanol: mp 175 °C (lit.[22b] mp 172 °C, EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53 (d, 1 H, J = 6.7 Hz), 4.08 (s, 3 H), 5.15 (d, 1 H, J = 10.4 Hz), 5.16 (d, 1 H, J = 14.6 Hz), 6.03 (ddd, 1 H, J = 14.6 Hz, 10.4 Hz, 6.7 Hz), 7.37 (d, 1 H, J = 8.4 Hz), 7.51 (d, 1 H, J = 7.7 Hz), 7.74 (d, 1 H, J = 7.7 Hz), 7.76 (t, 1 H, J = 8.4 Hz), 7.98 (d, 1 H, J = 8.4 Hz); EIMS m/e 294.0894 (M^+, requires 294.0892), 279, 264, 251; FTIR ($CHCl_3$) 1669, 1631, 1588, 1465 cm^{-1}.

2-Allyl-1,8-dimethoxy-9,10-anthraquinone (35). To a solution of **34** (610 mg, 2.1 mmol) in 100 mL of acetone were added potassium carbonate (695 mg, 5.0 mmol) and 1 mL of dimethyl sulfate, and the mixture was stirred at reflux for 21 h. The solution was filtered while hot and evaporated under reduced pressure, and the residue was crystallized from 95% ethanol to provide 460 mg (72%) as fluffy yellow needles: mp 135 °C (lit.[22b] mp 135.0–135.5 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.56 (d, 2 H, J = 6.6 Hz), 3.97 (s, 3 H), 4.03 (s, 3 H), 5.12 (dd, 1 H, J = 8.8 Hz, 1.6 Hz), 5.16 (dd, 1 H, J = 2.8 Hz, 1.6 Hz), 5.99 (m, 1 H), 7.32 (d, 1 H, J = 7.7 Hz), 7.55 (d, 1 H, J = 7.7 Hz), 7.66 (t, 1 H, J = 8 Hz), 7.86 (d, 1 H, J = 8 Hz), 7.97 (d, 1 H, J = 8 Hz); EIMS m/e 308.1047 (M^+, requires 308.1049), 293, 277, 267, 263; FTIR ($CHCl_3$) 1673, 1589, 1468 cm^{-1}.

2-(2′,3′-Epoxypropyl)-1-hydroxy-8-methoxy-9,10-anthraquinone (36). A solution of **34** (515 mg, 1.75 mmol) and 3-chloroperbenzoic acid (606 mg, 3.50 mmol) in CH_2Cl_2 (50 mL) was stirred under a nitrogen atmosphere at room temperature for 48 h. The solution was washed with 5% $NaHCO_3$ (2 × 50 mL) and water (50 mL), dried over sodium sulfate, filtered, and evaporated to provide an orange solid. Recrystallization from methanol afforded the desired product as orange needles (438 mg, 81%): mp 201–202 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60 (dd, 1 H, J = 4.7 Hz, 2.6 Hz), 2.83 (dd, 1 H, J = 4.7 Hz, 4.7 Hz), 2.93 (dd, 1 H, J = 14.6 Hz, 6.1 Hz), 3.12 (dd, 1 H, J = 14.6 Hz, 4.7 Hz), 3.31 (m, 1 H), 7.38 (d, 1 H, J = 7.7 Hz), 7.60 (d, 1 H, J = 7.7 Hz), 7.75 (d, 1 H, J = 7.7 Hz), 7.77 (t, 1 H, J = 7.7 Hz), 7.98 (d, 1 H, J = 7.7 Hz); EIMS m/e 310.0841 (M^+, requires 310.0841), 292, 279, 266, 249, 238, 189; FTIR ($CHCl_3$) 1690, 1631, 1588, 1466 cm^{-1}. Anal. ($C_{18}H_{14}O_5 \cdot {}^1/_4H_2O$) C, H.

1-Methoxy-2-methyl-9,10-anthraquinone (39). Prepared from **38** as described above for compound **35**. Recrystallization from 95% ethanol provided bright yellow plates in 73% yield: mp 163–164 °C (lit.[26] mp 152–153 °C, benzene); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (s, 3 H), 3.95 (s, 3 H), 7.62 (d, 1 H, J = 8.0 Hz), 7.78 (m, 2 H), 8.07 (d, 1 H, J = 8.0 Hz), 8.27 (m, 2 H); EIMS m/e 252.0791 (M^+, requires 252.0786), 237, 223; FTIR ($CHCl_3$) 1673, 1592, 1322 cm^{-1}.

General Procedure for Preparation of Poly(butyl carbonates) 42–47. 1,8-Bis[(*n*-butoxycarbonyl)oxy]-6-methyl-3-propoxy-9,10-anthraquinone (45). To a solution of **10** (28 mg, 0.09 mmol) in dry THF (15 mL) containing several drops of triethylamine at 0 °C was added n-butyl chloroformate (30 mg, 0.22 mmol) dissolved in 5 mL dry THF, at which time the initial light-red color of the solution disappeared. The solution was allowed to warm to room temperature and then was stirred for 15 min. The solvent was evaporated, and the residue was recrystallized from 95% ethanol to afford light yellow needles (31 mg, 67%): mp 106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (t, 6 H, J = 7.3 Hz), 1.07 (t, 3 H, J = 7.4 Hz), 1.49 (sextet, 4 H, J = 7.3 Hz), 1.79 (m, 4 H), 1.86 (m, 2 H), 2.51 (s, 3 H), 4.10 (t, 2 H, J = 6.5 Hz), 4.32 (t, 2 H, J = 6.6 Hz), 6.97 (d, 1 H, J = 2.7 Hz), 7.30 (b s, 1 H), 7.68 (d, 1 H, J = 2.7 Hz), 8.03 (b s, 1 H); EIMS m/e 512.2037 (M^+, requires 512.2046), 412, 312, 270, 242, 213; FTIR ($CHCl_3$) 1767, 1674, 1663, 1609, 1346 cm^{-1}. Anal. ($C_{28}H_{32}O_9$) C, H.

Acknowledgment. We express our sincere gratitude to Dr. James Travis (University of Georgia, Athens) for

(26) Eckert, A.; Endler, G. Some Derivatives of 2-Methylanthraquinones. *J. Prakt. Chem.* **1921**, *102*, 332–337.

the human leukocyte elastase and cathepsin G used in this study. Special thanks is extended to Mr. Masaru Yamamoto (Georgia Institute of Technology) for his preparation of MeO-Suc-Ala-Ala-Pro-Val-pNA used in the HLE assays, and to Mr. Jeffrey Mathys (University of Akron) who helped in the initial preparations of several anthraquinone analogues as part of the Undergraduate Research Program at Georgia Institute of Technology. This project was financially supported through a Graduate Research Fellowship graciously donated by Union Camp.

Registry No. **2**, 518-82-1; **3**, 481-72-1; **4a**, 14787-38-3; **4b**, 481-39-0; **5**, 28495-22-9; **6a**, 139565-29-0; **7a**, 139565-30-3; **7b**, 139565-31-4; **8a**, 5018-84-8; **8b**, 28785-82-2; **9**, 521-61-9; **10**, 139565-32-5; **11**, 6030-60-0; **12**, 84993-87-3; **13**, 481-73-2; **14**, 478-45-5; **15**, 6414-42-2; **16**, 60699-01-6; **17**, 121210-61-5; **18**, 481-74-3; **19**, 18713-45-6; **20**, 65615-59-0; **21**, 25395-11-3; **22**, 139565-33-6; **23**, 19228-39-8; **24**, 19228-40-1; **25**, 6407-55-2; **26**, 5539-66-2; **27a**, 51996-00-0; **27b**, 139565-34-7; **27c**, 139582-75-5; **27d**, 139565-35-8; **27e**, 139565-36-9; **27f**, 139565-37-0; **27g**, 139565-38-1; **28a**, 34425-60-0; **28b**, 106491-48-9; **28c**, 139565-39-2; **28d**, 76643-51-1; **28e**, 139565-40-5; **28f**, 139565-41-6; **28g**, 139565-42-7; **29**, 139565-43-8; **30**, 139565-44-9; **31**, 139565-45-0; **32**, 139565-46-1; **33**, 85313-87-7; **34**, 85313-88-8; **35**, 85313-89-9; **36**, 139565-47-2; **37**, 54454-84-1; **38**, 6268-09-3; **39**, 20460-44-0; **40**, 84-54-8; **41**, 84-51-5; **42**, 69595-67-1; **43**, 139565-48-3; **44**, 139565-49-4; **45**, 139565-50-7; **46**, 139565-51-8; **47**, 139565-52-9; CatG, 56645-49-9; HLE, 109968-22-1; serine proteinase, 37259-58-8; butyraldehyde, 123-72-8; 1-hydroxyanthraquinone, 129-43-1; propanal, 123-38-6; isobutyraldehyde, 78-84-2; benzaldehyde, 100-52-7; pentanal, 110-62-3; hexanal, 66-25-1; 1-bromopropane, 106-94-5; allyl bromide, 106-95-6; butyl chloroformate, 592-34-7.

Supplementary Material Available: Full spectral characterization for the compounds reported in Table IV, including 300 MHz ^{1}H NMR, EIMS, FTIR, and elemental analyses (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Human Purine Nucleoside Phosphorylase. Synthesis of Pyrrolo[3,2-*d*]pyrimidines, a New Class of Purine Nucleoside Phosphorylase Inhibitors as Potentially T-Cell Selective Immunosuppressive Agents. Description of 2,6-Diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one[1]

Jagadish C. Sircar,*[†] Catherine R. Kostlan,[†] Richard B. Gilbertsen,[‡] Mary K. Bennett,[‡] Mi K. Dong,[‡] and W. J. Cetenko[†]

Departments of Chemistry and Immunopathology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105. *Received October 7, 1991*

Purine nucleoside phosphorylase (PNP) is a purine-metabolizing enzyme in the purine cascade and has been a target for drug design for sometime. A series of potent human PNP inhibitors, pyrrolo[3,2-*d*]pyrimidines (9-deazaguanines), has been synthesized and evaluated in the enzyme assay and in the cell line assay using MOLT-4 (T-cell) and MGL-8 (B-cell) lymphoblasts for selectivity. One of the compounds, 2,6-diamino-3,5-dihydro-7-(3-thienylmethyl)-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (11c; CI-972), was found to be moderately potent, competitive, and reversible inhibitor of PNP with $K_i = 0.83$ μM. It was also found to be selectively cytotoxic to MOLT-4 lymphoblasts ($IC_{50} = 3.0$ μM) but not to MGL-8 lymphoblasts and was evaluated further. Compound 11c (CI-972) is under development in the clinic.

Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) is an essential enzyme of the purine salvage pathway and has been a target for drug design for sometime. Other enzyme inhibitors of the purine cascade, such as the adenosine deaminase inhibitor 2′-deoxycoformycin (pentostatin; DCF)[2] and the xanthine oxidase inhibitor allopurinol, have proven to be useful drugs, but no PNP inhibitor has yet been developed.

PNP is a purine-metabolizing enzyme which catalyzes the reversible phosphorylosis of inosine, deoxyinosine, guanosine, and deoxyguanosine to the corresponding purines, hypoxanthine and guanine. PNP deficiency in children causes profound impairment in T-cell function with minimal or no effect on B-cell function.[3] Thus, it is theorized that a potent PNP inhibitor could be a potentially useful immunosuppressive agent in the treatment of T-cell-dependent diseases, such as rheumatoid arthritis and psoriasis, and in T-cell leukemia and lymphomas.[4] PNP inhibitors should also be efficacious in the treatment of metabolic disorders such as hyperuricemia because of their ability to block the degradation of nucleosides which are precursors of uric acid. Numerous other potential indications have been postulated.[4]

Previously, we described the synthesis and biological activity of the PNP inhibitor 2,8-diamino-9-(2-thienylmethyl)guanine (1; PD 119229; CI-950).[5,6] Although 1 (CI-950) is an extremely potent PNP inhibitor ($K_i = 0.067$ μM),[7] physical properties of this compound limited its

[†] Department of Chemistry.
[‡] Department of Immunopathology.

(1) Preliminary reports of this work were presented at the 7th International/3rd European Joint Symposium on Purine and Pyrimidine Metabolism in Man, Bournemouth, GB, June 30–July 5, 1991.

(2) Cheson, B. D.; Martin, A. Clinical Trials in Hairy Cell Leukemia. Current Status and Future Directions. *Ann. Internal Med.* 1987, *1*, 871–878.

(3) Giblett, E. R.; Ammann, A. J.; Wara, D. W.; Sandman, R.; Diamond, L. K. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet* 1975, *1*, 1010–1013.

(4) Sircar, J. C.; Gilbertsen, R. B. Selective immunosuppressive agents. *Drug News Perspect.* 1990, *3*, 213–217.

(5) Sircar, J. C.; Kostlan, C. R.; Pinter, G. W.; Suto, M. J.; Bobovski, T. P.; Capiris, T.; Schwender, C. F.; Dong, M. K.; Scott, M. E.; Bennett, M. K.; Kossarek, L. M.; Gilbertsen, R. B. 8-Amino-9-substituted guanines: Potent purine nucleoside phosphorylase (PNP) inhibitors. *Agents Actions* 1987, *21*, 253–256.

(6) Gilbertsen, R. B.; Scott, M. E.; Dong, M. K.; Kossarek, L. M.; Bennett, M. K.; Schrier, D. J.; Sircar, J. C. Preliminary report on 8-amino-9-(2-thienylmethyl)guanine (PD 119229), a novel and potent purine nucleoside phosphorylase inhibitor. *Agents Actions* 1987, *21*, 272–274.

(7) The K_i was determined at a 50 mM inorganic phosphate concentration. At this phosphate concentration, the putatively most potent PNP inhibitor, acyclovir diphosphate, has a K_i of 0.51 μM.

 © 1992 American Chemical Society