Brief Articles

Structure−Activity Relationship Studies of Nordihydroguaiaretic Acid Inhibitors toward Soybean, 12-Human, and 15-Human Lipoxygenase

Stephanie Whitman,[†] Mikail Gezginci,[‡] Barbara N. Timmermann,*[,‡] and Theodore R. Holman*[,†]

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, and Department of Pharmacology and Toxicology, Division of Medicinal and Natural Product Chemistry, College of Pharmacy, University of Arizona, P.O. Box 210207, 1703 E. Mabel Street, Tucson, Arizona 85721-0207

Received March 20, 2002

Lipoxygenase (LO) is a biological target for many diseases such as asthma, atherosclerosis, and cancer. Our labs have synthesized and investigated nordihydroguaiaretic acid (NDGA) derivatives and have established that the reductive inhibition of soybean and 15-human LO can be affected by the strength of the electron-withdrawing substituents on the phenyl rings of NDGA. In addition, we have determined that hydrophobic NDGA derivatives activate 15-HLO, suggesting a hydrophobic allosteric site.

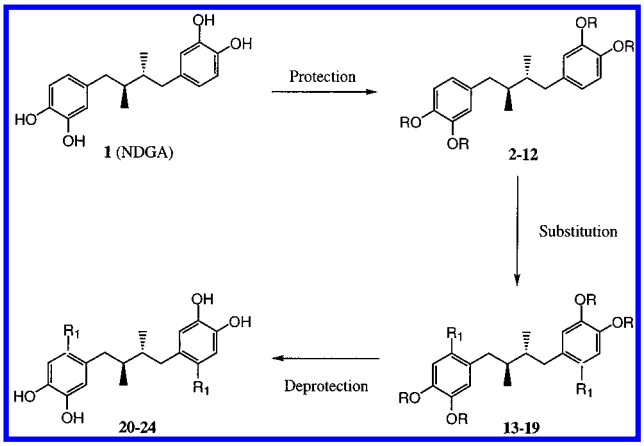
Introduction

Lipoxygenases are implicated as key biological players in asthma, heart disease, and cancer, which make them logical targets for inhibitory therapeutics.[1−4] Specific human lipoxygenases, such as 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase, are associated with particular disease states, and therefore, development of selective inhibitors is critical. To develop potent, selective inhibitors to lipoxygenase, we have synthesized and assayed a number of nordihydroguaiaretic acid (NDGA) derivatives to determine their inhibitor constraints toward soybean lipoxygenase-1 (SLO), human platelet 12-lipoxygenase (12-HLO), and human reticulocyte 15-lipoxygenase (15-HLO).

Lipoxygenases are a class of non-heme iron enzymes that catalyze the incorporation of dioxygen into 1,4-*cis*,*cis*-pentadiene containing fatty acids (e.g., linoleic and arachidonic acids) to form hydroperoxide products.[5−7] The essential iron atom is in the inactive ferrous oxidation state, as isolated, and is activated by 1 equiv of hydroperoxide product ((9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid (HPOD)), which oxidizes the iron to the ferric state. The most widely accepted reaction mechanism for the hydroperoxidation is radical-based, where the fatty acid is oxidized by the ferric iron to form a fatty acid radical and a ferrous iron. This ferrous/substrate radical intermediate is then attacked regio- and stereospecifically by dioxygen to form only the *S*-configured product.[8−11]

NDGA is a major natural product from the creosote bush (*Larrea tridentata* Cav. family Zygophyllaceae) and has been studied extensively for its inhibition of soybean lipoxygenase,[12] purified 5-HLO,[13,14] and 15-HLO.[15] The principal mechanism of action for NDGA is the reduction of the ferric iron to the inactive ferrous form, and the catechol substituent in NDGA is oxidized to the semiquinone.[12] This is an inhibitory mechanism similar to that seen for catechol derivatives, which have been shown to reduce the ferric center in the soybean enzyme, but there is no correlation between their reduction potential and the relative inhibitor strength, presumably because of sterics.[16] In the current study, we investigated a number of NDGA derivatives that had both their reduction potentials and steric bulk modified. These NDGA derivatives were tested against SLO, 12-HLO, and 15-HLO and determined that electronics and sterics could be modified to improve their inhibition strength and selectivity.

Scheme 1



Results and Discussion

The general synthetic pathway and structures for the NDGA derivatives (Scheme 1, Table 1) was described previously and is reiterated in the Supporting Information.[17] The IC_{50} data are presented in Table 2, and the inhibitors are grouped by their relative potencies. The complexes that have their phenolic oxygen atoms modi-

* To whom correspondence should be addressed. For B.N.T.: phone, 520-626-2481; fax, 520-626-2515; email, btimmer@pharmacy.arizona.edu. For T.R.H.: phone, 831-459-5884; fax, 831-459-2935; e-mail, tholman@chemistry.ucsc.edu.
[†] University of California, Santa Cruz.
[‡] University of Arizona.

10.1021/jm0201262 CCC: $22.00
Published on Web 05/04/2002

Table 1

compound	R	R1	formula[a]
1	H		$C_{18}H_{22}O_4$
2	Me		$C_{22}H_{30}O_4$
3	Ac		$C_{26}H_{30}O_8$
4	propionyl		$C_{30}H_{38}O_8$
5	butyryl		$C_{34}H_{46}O_8$
6[b]	isobutyryl		$C_{34}H_{46}O_8$
7	valeryl		$C_{38}H_{54}O_8$
8	hexanoyl		$C_{42}H_{62}O_8$
9	monoacetonide (mixt)[i]		$C_{21}H_{26}O_4$
10	monomethyl (mixt)[j]		$C_{19}H_{24}O_4$
11	dimethyl (mixt)[k]		$C_{20}H_{26}O_4$
12	pivaloyloxymethyl		$C_{42}H_{62}O_{12}$
13[c]	Me	Cl	$C_{22}H_{28}Cl_2O_4$
14	Me	Br	$C_{22}H_{28}Br_2O_4$
15	Me	NO_2	$C_{22}H_{28}N_2O_8$
16[d]	Me	Ac	$C_{26}H_{34}O_6$
17	Me	NH_2	$C_{22}H_{32}N_2O_4$
18[e]	Me	AcNH	$C_{26}H_{36}N_2O_6$
19	propionyl	NO_2	$C_{30}H_{36}N_2O_{12}$
20	H	Cl	$C_{18}H_{20}Cl_2O_4$
21	H	Br	$C_{18}H_{20}Cl_2O_4$
22[f]	H	NO_2	$C_{18}H_{20}N_2O_8$
23[g]	H	Ac	$C_{22}H_{26}O_6$
24[h]	H	Et	$C_{22}H_{30}O_4$

[a] Analyses were within 0.4% of the calculated values except where noted (footnotes b−h). [b] H: calcd, 7.96; found, 8.42. [c] C: calcd, 61.83; found, 62.40. [d] C: calcd, 70.56; found, 69.93. [e] C: calcd, 66.08; found, 65.32. [f] C: calcd, 55.10; found, 53.13. N: calcd, 7.14; found, 6.09. [g] C: calcd, 68.38; found, 67.71. [h] C: calcd, 73.71; found, 71.05. H: calcd, 8.44; found, 7.34. [i] The mixt refers to the fact that an acetonide moiety is equally distributed over the two phenyl alcohols of only one of the catechol groups. [j] The mixt refers to the fact that a methyl moiety is equally distributed over the two phenyl alcohols of only one of the catechol groups. [k] The mixt refers to the fact that a methyl moiety is equally distributed over the two phenyl alcohols of both of the catechol groups.

fied into ethers (**2**−**8**, **12**−**19**) are not inhibitors to any of the lipoxygenases SLO, 12-HLO, and 15-HLO. The derivatives that are potent inhibitors have free phenolic hydroxyl functional groups and reduce lipoxygenase, as seen by a fluorescence decrease for SLO (Table 2).[18] This result indicates that the primary potency of these NDGA derivatives comes from their ability to reduce the ferric ion and not from tight binding to the active site, such as in competitive inhibition.[12] The inhibitors can be segregated into two categories: those with partially masked phenolic groups (**9**−**11**) and those with substituted phenyl rings (**20**−**24**). The phenyl-substituted inhibitors follow a general trend in which their potency decreases relative to the electron-withdrawing properties of the substituent, as seen by the Hammett values in Table 2.[19] Inhibitors with Hammett values up to 0.31 reduce SLO, but inhibitors with values above 0.44 do not. This general trend is also seen for 15-HLO; however, 12-HLO has notable exceptions.

1 and **24** are less potent inhibitors than **20** and **21** for SLO, 12-HLO, and 15-HLO, even though their Hammett values are less, suggesting the halide functionality groups induce a better fit in the catalytic cavity than the smaller proton of **1** and the larger ethyl group of **24**. Compounds **22** and **23** do not inhibit or reduce SLO but do inhibit 12-HLO and 15-HLO, which may be indicative of a change in the reduction potential of the iron in 12-HLO and 15-HLO to a value lower than that of SLO (+600 mV).[20] This variation of the reduction potential of different lipoxygenases may possibly be a method for selectively inhibiting a particular lipoxygenase over another and will need to be investigated further.[20] For 12-HLO, **22** is unusual because its Hammett value is markedly greater than that of **23** but its inhibitor potency is ∼10-fold greater. Sterics cannot account for this difference because their phenyl substituents are of comparable size, so the polarity of the functionality appears to be the distinction.

The other class of potent inhibitors is the partially masked phenolic derivatives **9**−**11,** which retain free phenolic functional groups and thus can reduce SLO, as seen by fluorescence. For SLO, 15-HLO, and 12-HLO, **9** is the strongest inhibitor while **10** is weaker and **11** is the weakest. This is probably due to sterics because **10** and **11** have methoxy groups next to the active phenol group, which could sterically hinder the inner-sphere reduction of the iron (both **10** and **11** have a mixture of methoxy functionalities on either the para or the meta position but not on both positions of a single phenyl of the molecule). **11** is less potent than **10** because it has a methoxy group on both ends of the NDGA derivative while **10** has a free catechol group on one end, which is not sterically blocked. **9** is more potent than **10** probably because it has both alcohol groups masked on one side of the compound, which converts the hydrophilic tail to a more hydrophobic one. This architecture of a polar headgroup and a hydrophobic tail is typical of previously isolated lipoxygenase inhibitors and may indicate an improved hydrophobic interaction in the active site.[4]

The selectivity of the NDGA derivatives against 12-HLO and 15-HLO has been investigated with the current data (Table 2). The majority of the NDGA derivatives preferentially inhibit 15-HLO with the best (**11**) having a 12-HLO/15-HLO ratio of 72. This result suggests a larger pocket near the iron for 15-HLO than for 12-HLO, which allows for the inner-sphere reduction despite the presence of the adjacent methoxide moiety. This implies that 12-HLO may be more sterically constricted at the iron site, which could hamper a close approach by the inhibitor. Compound **22** has the best

Table 2[a]

compound	Hammett	IC_{50}(15-HLO)	IC_{50}(12-HLO)	12-HLO/15-HLO	IC_{50}(SLO)	reduction
1	0	0.11 ± 0.01	5.1 ± 1	46	0.18 ± 0.02	yes
9	0	0.085 ± 0.008	2.2 ± 0.2	26	0.14 ± 0.006	yes
10	0	0.25 ± 0.02	14 ± 2	56	0.17 ± 0.009	yes
11	0	0.58 ± 0.3	42 ± 12	72	3.1 ± 0.3	yes
20	0.3	0.065 ± 0.009	0.36 ± 0.03	5.5	0.065 ± 0.005	yes
21	0.31	0.083 ± 0.008	0.43 ± 0.02	5.2	0.057 ± 0.004	yes
22	0.75	0.35 ± 0.05	0.36 ± 0.06	1	no effect	no
23	0.44	0.38 ± 0.2	26 ± 6	68	no effect	no
24	−0.11	0.16 ± 0.02	3.1 ± 0.7	19	0.14 ± 0.009	yes

[a] All IC_{50} values are expressed in micromolar units.

ratio of all the derivatives against 12-HLO, and yet this cannot be considered selective because the inhibition is equally effective for both enzymes (12-HLO/15-HLO = 1). It is unclear what specific physical properties make NDGA and its derivatives less potent against 12-HLO than 15-HLO, and more study is required.

As stated previously, the blocked phenolic derivatives do not inhibit any of the lipoxygenases; however, a select group does activate 15-HLO but not SLO or 12-HLO: **4**, $AC_{50} = 0.3 \pm 0.1\ \mu M$, 22% increase in activity; **5**, $AC_{50} = 0.3 \pm 0.1\ \mu M$, 77% increase; **6**, $AC_{50} = 0.5 \pm 0.1\ \mu M$, 70% increase; **7**, $AC_{50} = 0.2 \pm 0.05\ \mu M$, 110% increase; **8**, $AC_{50} = 0.2 \pm 0.05\ \mu M$, 70% increase ($AC_{50}$ is defined as the activation concentration at 50% activation). The effect is not due to a nonspecific detergent interaction because this is particular to only a few NDGA derivatives of this class and because standard detergents, such as cholate ($IC_{50} = 1000\ \mu M$) and Triton X-100 ($IC_{50} = 15\ \mu M$), inhibit 15-HLO. The percent activation of 15-HLO with NDGA derivatives increases for all activators in the absence of cholate, and yet their potency decreases (data not shown). This is suggestive of a competition between cholate and the activators for the same site on 15-HLO and is consistent with our previous results, which indicate a unique allosteric binding site in 15-HLO.[21,22] These activators can now be investigated in whole cell assays in order to determine a possible biological role for the activation of 15-HLO.

Conclusion

Our diverse collection of NDGA derivatives have clearly established that phenolic alcohols are essential for inhibition of both 12- and 15-HLO, and if they are masked, all inhibitory properties are lost. For SLO and 15-HLO, the inhibitor potency can generally be affected by the strength of the electron-withdrawing substituent on the phenolic ring of NDGA, but there are exceptions for 12-HLO. The NDGA derivatives, especially **11**, are preferentially selective against 15-HLO over 12-HLO, which suggests that they are a good class of molecules for selective inhibition against 15-HLO. Finally, particular NDGA ester derivatives activate 15-HLO, indicating a unique allosteric site that may be related to the biological role of 15-HLO.

Experimental Section

The synthetic procedures for the novel compounds are discussed in more detail in the Supporting Information.[17] The kinetic and fluorescence experimental details were published previously, and any modifications are discussed in the Supporting Information section.[4]

Acknowledgment. T.R.H. gratefully thanks C. D. Funk for the cDNA of 12-HLO and the ACS and NIH for financial support (ACS Grant RPG-00-219-01-CDD; NIH Grant GM-56062-01). B.N.T. thanks the Arizona Disease Control Research Commission (ADCRC Contract No. 20009) and NIH (NIH Grant 5U01TW00316-09).

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Samuelsson, B.; Dahlen, S. E.; Lindgren, J. A.; Rouzer, C. A.; Serhan, C. N. Leukotrienes and Lipoxins: Structures, Biosynthesis, and Biological Effects. *Science* **1987**, *237*, 1171−1176.
(2) Dailey, L. A.; Imming, P. 12-Lipoxygenase: Classification, Possible Therapeutic Benefits from Inhibition and Inhibitors. *Curr. Med. Chem.* **1999**, *6*, 389−398.
(3) Steele, V. E.; Holmes, C. A.; Hawk, E. T.; Kopelovich, L.; Lubet, R. A.; Crowell, J. A.; Sigman, C. C.; Kelloff, G. J. Lipoxygenase inhibitors as potential cancer chemopreventives. *Cancer Epidemiol., Biomarkers Prev.* **1999**, *8*, 467−483.
(4) Carroll, J.; Jonsson, E. N.; Ebel, R.; Hartman, M.; Holman, T. R.; Crews, P. Probing Sponge-Derived Terpenoids for Human 15-Lipoxygenase Inhibitors. *J. Org. Chem.* **2001**, *66*, 6847−6851.
(5) Veldink, G. A.; Vliegenthart, J. F. G. Lipoxygenases, Nonheme Iron-Containing Enzymes. *Adv. Inorg. Biochem.* **1984**, *6*, 139−161.
(6) Nelson, M. J.; Seitz, S. P. The Structure and Function of Lipoxygenase. *Curr. Opin. Struct. Biol.* **1994**, *4*, 878−884.
(7) Solomon, E. I.; Zhou, J.; Neese, F.; Pavel, E. G. New Insights from Spectroscopy into the Structure/function Relationships of Lipoxygenases. *Chem. Biol.* **1997**, *4*, 795−808.
(8) DeGroot, J. J. M. C.; Aasa, R.; Malmstrom, B. G.; Slappendel, S.; Veldink, G. A.; Vliegenthart, J. F. G. Demonstration by EPR Spectroscopy of the Functional Role of Iron in Soybean Lipoxygenase-1. *Biochim. Biophys. Acta* **1975**, *377*, 71−79.
(9) Gardner, K. A.; Mayer, J. M. Understanding C−H bond oxidations: hydrogen and hydride transfer in the oxidation of toluene by permanganate. *Science* **1995**, *269*, 1849−1851.
(10) Rickert, K. W.; Klinman, J. P. Nature of Hydrogen Transfer in Soybean Lipoxygenase-1: Separation of Primary and Secondary Isotope Effects. *Biochemistry* **1999**, *38*, 12218−12228.
(11) Knapp, M.; Seebeck, F. P.; Klinman, J. Steric Control of Oxygenation Regiochemistry in Soybean Lipoxygenase-1. *J. Am. Chem. Soc.* **2001**, *123*, 2931−2932.
(12) Kemal, C.; Louis-Flamberg, P.; Krupinski-Olsen, R.; Shorter, A. Reductive Inactivation of Soybean Lipoxygenase-1 by Catechols. *Biochemistry* **1987**, *26*, 7064−7072.
(13) Reddanna, P.; Rao, K. M.; Reddy, C. C. Inhibition of 5-Lipoxygenase by Vitamin E. *FEBS Lett.* **1985**, *193*, 39−43.
(14) McMillan, R. M.; Masters, D. J.; Sterling, W. W.; Bernstein, P. R. *Biosynthesis of Leukotriene B4 in Human Leukocytes*; Plenum: New York, 1985; pp 655−668.
(15) Ishiura, S.; Yoshimoto, T.; Villee, C. A. Reticulocyte lipoxygenase, ingensin, and ATP-dependent proteolysis. *FEBS Lett.* **1986**, *201*, 87−93.
(16) Nelson, M. J. Catecholate Complexes of Ferric Soybean Lipoxygenase-1. *Biochemistry* **1988**, *27*, 4273−4278.
(17) Gezginci, M. H.; Timmermann, B. N. A short synthetic route to nordihydroguaiaretic acid (NDGA) and its stereoisomer using Ti-induced carbonyl-coupling reaction. *Tetrahedron Lett.* **2001**, *42*, 6083−6085.
(18) Agro, A. F.; Avigliano, L.; Egmond, M. R.; Veldink, G. A.; Vliegenthart, J. F. G. Fluorescence Perturbation in Soybean Lipoxygenase-1. *FEBS Lett.* **1975**, *52*, 73−76.
(19) Hansch, C.; Leo, A.; Taft, R. W. A Survey of Hammett Substituent Constants and Resonance and Field Parameters. *Chem. Rev.* **1991**, *91*, 165−195.
(20) Holman, T. R.; Zhou, J.; Solomon, E. I. Spectroscopic and Functional Characterization of a Ligand Coordination Mutant of Soybean Lipoxygenase: First Coordination Sphere Analogue of Human 15-Lipoxygenase. *J. Am. Chem. Soc.* **1998**, *120*, 12564−12572.
(21) Mogul, R.; Johansen, E.; Holman, T. Oleyl Sulfate Reveals Allosteric Inhibition of Soybean Lipoxygenase-1 and Human 15-Lipoxygenase. *Biochemistry* **2000**, *39*, 4801−4807.
(22) Mogul, M.; Holman, T. R. Inhibition Studies of Soybean and Human 15-Lipoxygenase with Long-Chain Alkenyl Sulfate Substrates. *Biochemistry* **2001**, *40*, 4391−4397.

JM0201262