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CATECHOL BASED INHIBITORS OF 15-LIPOXYGENASE

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Abstract: A potent 15-lipoxygenase (15-LO) inhibitor, compound **6**, was identified by mass screening the Parke-Davis compound portfolio. The active moiety of compound **6** was determined to be the catechol functionality. Additional analogs were prepared and analyzed for inhibitory activity against 5-, 12-, and 15-lipoxygenase.

Introduction:

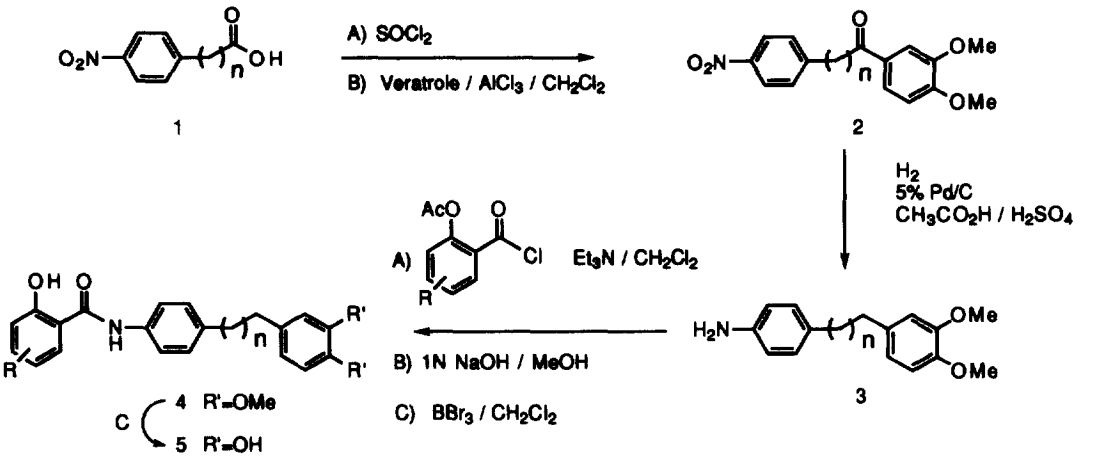
Modification of low density lipoproteins (LDL) by products derived from oxidative processes is believed to play a role in atherogenesis.[1,2] A current hypothesis relating to this holds that reactive aldehydes, generated as the end products of lipid peroxidation, derivatize lysine residues on the apolipoprotein-B-100 moiety of the LDL particles. These modified lipoprotein particles are taken up in an unregulated fashion by the so-called scavenger receptor which is found on the surface of monocyte/macrophages.[3] When macrophages are incubated in vitro with modified LDL they accumulate massive amounts of cholesteryl ester, as do the foam cells of the atherosclerotic lesion.[4] Oxidative modification of LDL can occur non-enzymatically via incubation with redox active transition metals or by incubation with certain types of cells found in the artery wall. A number of cultured cells have been shown to possess the capacity to modify LDL via oxidative mechanisms, including endothelial cells, monocytes and smooth muscle cells.[5,6,7] Several investigators have shown that incubation of LDL with 15-lipoxygenase (15-LO) can produce an oxidatively modified LDL particle which mimics cell mediated modification.[8] In order to determine the relevance of 15-LO-mediated modification of LDL in atherosclerosis, we undertook a search of the Parke-Davis compound collection to discover inhibitors of this enzyme.

A spectrophotometric microtiter-based assay, which detects the formation of hydroperoxy derivatives of linoleic acid formed by the action of 15-LO, was employed to screen our compound portfolio.[9] Appropriate controls were used to eliminate general antioxidants which this assay will also detect. One compound which showed considerable 15-LO inhibitory activity on initial testing was catechol **6**. To further evaluate the structure activity relationships (SAR) in this series, structurally similar compounds from the collection were tested. Compound **6** was also used as a point of departure for the synthesis of novel analogs.

Chemistry:

A general synthesis of the catechol derivatives is shown in Scheme 1.[10] The carboxylic acid **1** was converted into the acid chloride using thionyl chloride and the crude acid chloride reacted with veratrole under standard Friedel Crafts conditions to give nitroketone **2** (83%). Reduction of both the nitro and ketone functionalities was accomplished by hydrogenation over 5% palladium on carbon to give amine **3** (40-65%). Conversion of the amine to the amide was accomplished by reaction with the appropriate acid chloride and triethyl amine. The acetyl protecting group was removed with base to give amide **4** (80-95%). Removal of both methyl ethers using BBr_3 in CH_2Cl_2 (45-95%) gave the targeted catechol derivatives.

Scheme 1

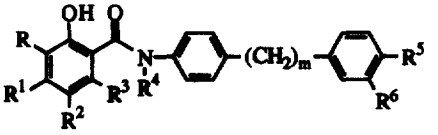


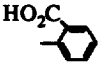
Results and Discussion:

All compounds were tested for inhibitory activity against rabbit reticulocyte 15-LO by measuring inhibition of the conversion of linoleic acid to its corresponding hydroperoxide.[9] The more interesting agents were also tested against human platelet 12-LO[11] and rat basophilic leukemia cell 5-LO[12] to determine the specificity of lipoxygenase inhibition by the compounds.

The parent compound (**6**) showed good inhibitory activity (IC_{50} = 0.3 μM) against 15-LO (Table 1). Since the catechol moiety is known to be a pharmacophore which imparts 5-LO activity,[13] we prepared both the mono (**9**) and dimethylated (**7**) analogs and confirmed the importance of the catechol for 15-LO inhibition in this series. The inhibitory activity of these compounds was reduced by two orders of magnitude, when compared to the parent catechol (**6**). We were interested in the importance of the salicylic acid hydroxyl group for activity. Thus the deshydroxyl derivative (**22**)[14] was prepared by reaction of benzoyl chloride and compound 3 (n=1). The activity of the deshydroxy moiety (**22**) decreased slightly to 1.3uM. The length of the chain connecting the catechol and aromatic ring was also investigated and found to have no significant effect on inhibitory activity when varied from two to four carbons (**6, 11, 12**). Methylation of the amide nitrogen significantly reduced inhibitory activity (**8** vs **10**).

Table 1



Compound	R	R^1	R^2	R^3	R^4	R^5	R^6	m	15-LO IC_{50}(uM)
6	H	H	H	H	H	OH	OH	2	0.3
7	H	H	H	H	H	OMe	OMe	2	>25
8	H	Me	H	H	H	OH	OH	2	0.4
9	H	Me	H	H	H	OH	OMe	2	>25
10	H	Me	H	H	Me	OH	OH	2	5
11	H	H	H	H	H	OH	OH	3	0.3
12	H	H	H	H	H	OH	OH	4	0.3
13	Cl	H	H	H	H	OH	OH	2	1.2
14	CO_2H	H	H	H	H	OH	OH	2	>25
15	HO_2C 	H	H	H	H	OH	OH	2	>25
16	H	OH	H	H	H	OH	OH	2	5
17	H	Cl	H	H	H	OH	OH	2	0.4
18	H	Ph	H	H	H	OH	OH	2	>25
19	H	H	Br	H	H	OH	OH	2	1.5
20			H	H	H	OH	OH	2	0.4
21	H	H			H	OH	OH	2	0.6

The salicylic acid moiety was modified to determine substituent effects on activity. Carboxylic acid analog **14** and benzoic acid analog **15** profoundly reduced the 15-LO inhibitory activity, whereas the addition of chlorine to this position (**13**) resulted in a modest four-fold reduction. A variety of substitutions were prepared at the 3-position (R^1). The hydroxyl derivative (**16**) was ten fold less active than the parent, while the methyl and chloro-derivatives (**8, 17**) were equipotent with parent (**6**). Phenyl substitution (**18**) was detrimental to inhibitory activity with an IC_{50} >25uM. Replacement of the phenyl with naphthalene (**20, 21**) had no significant effect on the activity.

To determine the specificity of this class of compounds, the more active compounds were assayed for inhibitory activity against human platelet 12-LO and rat basophilic leukemia cell 5-LO. The activity for compound **6** is representative of the general trend in this class of compounds (15-LO = 0.3uM, 12-LO = 5uM, 5-LO = 0.28uM). In general, whereas none of the compounds exhibited significant specificity for 15-LO over 5-LO, all were able to discriminate between 15-LO and 12-LO. As a reference we prepared and tested nordihydroguaiaretic acid(NDGA)[13]. Nordihydroguaiaretic acid had an IC_{50} of 4.9uM against 15-LO, 5uM against 12-LO, and 0.4uM against 5-LO.

In summary, the use of a microtiter assay to find 15-LO inhibitors has proven to be an effective means of discovering new leads. The catechol moiety was critical for retaining lipoxygenase activity. None of the analogs significantly improved the inhibitory activity of the parent compound (**6**), although a number of substitutions caused a dramatic reduction in 15-LO inhibitory activity. Mass screening our compound portfolio has provided us with a series of novel 15-LO inhibitors which will serve as a tool for further investigation of the role of 15-LO in atherosclerosis.

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14. Compound 22 is N-{4-[2-(3,4-Dihydroxy-phenyl)-ethyl]-phenyl}-benzamide.

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