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4-[5-(2,3-DIHYDROXYPHENYL)PENTYLOXY]-2-HYDROXY-3-PROPYLBENZOIC ACID (RO 24-0553) : AN ORALLY ACTIVE 5-LIPOXYGENASE INHIBITOR WITH ANTIINFLAMMATORY ACTIVITY

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Abstract. The chemical synthesis and pharmacological properties of 4-[5-(2,3-dihydroxyphenyl)pentyloxy]-2-hydroxy-3-propylbenzoic acid (Ro 24-0553, **1**), a novel 5-lipoxygenase (5-LO) inhibitor with *in vivo* activity appropriate for development as a therapy for inflammatory bowel disease, are described

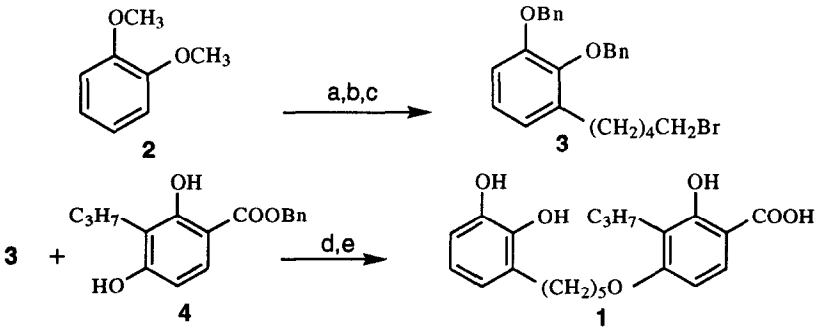
Oxidative metabolism of arachidonic acid by the 5-lipoxygenase (5-LO) pathway leads to the formation of the peptidoleukotrienes, LTC_4 and LTD_4, and to leukotriene B_4 (LTB_4). Inhibition of the 5-LO enzyme is thus an attractive pharmacological approach to a number of diseases where these leukotrienes are believed to play major roles. LTB_4 in particular is proposed as a major mediator in inflammatory bowel disease (IBD), rheumatoid arthritis and psoriasis.[1,2] LTB_4, a potent chemotactic agent, is thought to be responsible for the recruitment of PMN's to sites of inflammation.[3] Reactive oxygen metabolites produced by inflammatory cells have also been suggested to be involved in IBD.[4] In fact, two drugs used in IBD therapy, Sulfasalazine and 5-aminosalicylic acid (5-ASA) function as free radical scavengers *in vitro*[5] and 5-ASA may act in this manner *in vivo* in IBD patients.[6] Recent studies have also found increased IL-1 production and release in the mucosa of IBD patients.[7] The effectiveness of an IL-1 receptor antagonist in immune complex colitis in rabbits[8] and in acetic acid-induced colitis in rats[9] suggests that drugs which reduce or block IL-1 effects may be useful in IBD.

In this communication we report on the synthesis and biological profile of 4-[5-(2,3-dihydroxyphenyl)pentyloxy]-2-hydroxy-3-propylbenzoic acid (Ro 24-0553), **1**, a potent inhibitor of 5-LO with free radical scavenging properties and the ability to reduce IL-1 biosynthesis. We began this work with the knowledge that nordihydroguaiaretic acid (NDGA) was a potent inhibitor of 5-LO[10] with *in vivo* activity in animal models of inflammation[11] but exhibited poor oral bioavailability. Various substituted catechols, some of which are extremely active 5-LO inhibitors, have been reported.[12] Salicylates are readily absorbed drugs with therapeutically useful analgesic and antipyretic properties. It was envisioned that linking a catechol with a salicylic acid moiety might provide a compound which was a potent 5-LO inhibitor with significant oral antiinflammatory activity.

Functionalization of veratrole **2** at the 3-position by lithiation with butyl lithium in tetrahydrofuran followed by treatment with 1,5-dibromopentane under described conditions[13] provided the required dimethoxybromide in

33 % yield. The corresponding benzyl ether **3** was obtained from the bromide in an overall yield of 54 % by cleavage of the methyl ethers with boron tribromide in methylene chloride at -72 °C, followed by benzylation of the crude catechol (benzyl bromide, K_2CO_3, toluene), utilizing TDA-1 as a phase transfer catalyst to solubilize the potassium salt of the catechol. The benzyl ester **4** was prepared from methyl 2,4-dihydroxybenzoate by allylation (67 % yield), Claisen rearrangement (185-210 °C, 46 % yield), catalytic hydrogenation (94 % yield) and finally base hydrolysis followed by benzylation (70 % yield).[14] This sequence of reactions has been reported for the preparation of the corresponding methyl ester of **4**.[15] Alkylation of **4** with **3** provided the benzyl-protected intermediate as a solid, mp 67-69 °C, in 86 % yield. Removal of the benzyl groups by catalytic hydrogenolysis afforded **1**, mp 153-155 °C, in 90 % yield.

Scheme I[a]



[a]Reagents: (a) C_4H_9Li, $Br(CH_2)_5Br$, THF; (b) BBr_3, CH_2Cl_2; (c) BnBr, K_2CO_3, TDA-1, toluene; (d) K_2CO_3, NaI, acetone; (e) H_2, Pd, THF

1 inhibited 5-LO from rat basophilic leukemia (RBL-1) cells with an IC_{50} of 6 nM under optimized assay conditions.[16] It also was found to inhibit LTB4 (IC_{50} = 0.6 μM) and PGE_2 (35 μM) biosynthesis in ionophore stimulated rat peritoneal macrophages [17] and LTB4 biosynthesis in human neutrophils (IC_{50} = 1 μM).[18] It did not inhibit ram seminal vesicle cyclooxygenase at 100 μM indicating selective inhibition of the 5-LO pathway of arachidonate metabolism. **1** also inhibited the superoxide-dependent, iron promoted peroxidation of rat cardiac membrane liposomes[19] with an IC_{50} of 0.4 μM demonstrating its ability to act as a free radical scavenger. Under our assay conditions, NDGA gave an IC_{50} of 10 nM for inhibition of 5-LO and an IC_{50} of 0.1 μM for inhibition of lipid peroxidation. Zileuton, a 5-LO inhibitor which has exhibited some beneficial clinical effects in ulcerative colitis[20,21] has been reported to inhibit 5-LO with an IC_{50} of 0.5 μM.[22]

IL-1β biosynthesis by isolated human mononuclear cells[23] was inhibited by 40 ± 13 % with 10 μM **1** *in vitro*. NDGA, which contains two catechol moieties, has been reported to inhibit IL-1 production by LPS-stimulated human monocytes with an IC_{50} of 4-8 μM.[24] Since IL-1 can stimulate the production of tumor necrosis factor (TNF) and other cytokines, [25] the ability of **1** to reduce IL-1β biosynthesis may augment its inhibition of 5-LO by reducing the production of proinflammatory cytokines, as well as leukotrienes.

In addition to these *in vitro* studies, compound **1** was also examined *in vivo* in three animal models of inflammation. Two models commonly used to assess compounds for utility in topical treatment of inflammatory skin conditions are the 12-O-tetradecanoylphorbol acetate (TPA) and the arachidonic acid (AA) mouse ear inflammation tests. A topical dose of 0.4 mg of **1** caused 26 ± 9 % inhibition of the TPA-induced edema[26] and a 1 mg dose caused 47 ± 7 % inhibition of AA-induced edema.[27] Myeloperoxidase activity, a measure of neutrophil infiltration, was inhibited by 88 ± 5 % at the 0.4 mg dose in the TPA treated mice and by 86 % at a 0.1 mg dose in the AA treated mice. Acetic acid-induced colitis in the rat was used as an acute model of intestinal inflammation.[28] When **1** was administered orally twice daily for two days before induction of colitis, it reduced neutrophil accumulation with a minimum effective dose of 100 μg/kg. (38 ± 12 % reduction of neutrophils). At 1 mg/kg neutrophil accumulation was reduced by 88 ± 16 %. In the same model **1** reduced erythema by 55 ± 15 % and necrosis by 77 ± 23 % at 1 mg/kg. NDGA was inactive in the rat colitis model when tested orally at 10 mg/kg however at a dose of 100 mg/kg it gave 70 ± 20 % inhibition of neutrophil infiltration. Zileuton, the only selective 5-LO inhibitor which has been tested clinically in inflammatory bowel disease, was ineffective at 1 mg/kg but gave 47 ± 25 % inhibition at an oral dose of 10 mg/kg in this model. Zileuton also exhibited fewer and less consistent beneficial effects on gross pathology and histopathology at 10 mg/kg than did **1** at 1 mg/kg. Sulphasalazine, a drug commonly used to treat IBD, was ineffective in the rat colitis model at 30 mg/kg but gave 75 ± 19 % reduction of neutrophils and comparable efficacy to **1** at 100 mg/kg.

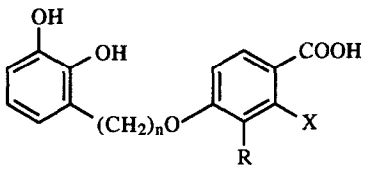
Our structure-activity study, which led to selection of **1** for further development, included the preparation and testing of analogs (Table 1) with a tether of from two to ten carbon atoms (**1a-g**). All of these were potent inhibitors of 5-LO and of rat cardiac membrane peroxidation with the activity falling off somewhat with the less lipophilic analogs **1a** and **1b**. In order to determine if the salicylic acid moiety was contributing to the activity, analogs **1h-j** were prepared. The salicylic acid hydroxyl seems unimportant for 5-LO activity and the most lipophilic of the three, **1i**, was the most active. The less lipophilic **1h** and **1j** were also less active as inhibitors of lipid peroxidation. Earlier workers also found that lipophilicity was an important factor determining 5-LO activity of various catechol derivatives.[12] Although all members of the series exhibited *in vivo* activity in the acetic acid-induced colitis model when tested at 10 mg/kg, **1** showed the best profile of high activity combined with good protective effects on the colon tissue of test animals.

Recent studies on the mechanism of inhibition of soybean lipoxygenase by NDGA and other catechols have demonstrated that they function by reducing the catalytically active ferric enzyme to the catalytically inactive ferrous form.[10] An antioxidant or free radical scavenging mechanism has also been proposed for 5-LO inhibition by catechols.[29] In fact it was recently suggested that the activity of NDGA in the rat acetic acid colitis model is due to radical scavenging of oxygen derived free radicals.[11] Therefore the mechanism by which **1** inhibits 5-LO *in vitro* and exhibits activity in the *in vivo* models described could involve either radical scavenging or reduction of the ferric enzyme.

In conclusion, we have described a novel compound with an *in vitro* and *in vivo* profile superior to several standard drugs and which seems a likely candidate for clinical trials in IBD and inflammatory skin diseases.

Unlike NDGA, **1** demonstrates good oral activity and bioavailability as shown by antiinflammatory activity in animal models of skin and intestinal inflammation. Furthermore, the ability of **1** to inhibit IL-1 production is likely to be important in reducing inflammation since it is likely that multiple mediators are responsible for the symptoms of IBD and other inflammatory diseases.

Table 1



Compound	n	R	X	Inhib of 5-LO IC_{50} (μM)	Inhib of Lipid Perox IC_{50} (μM)	Rat Colitis % Inhib at 10 mg/kg po
1a	2	n-C_3H_7	OH	0.25	17 % at 1μM	Not tested
1b	3	n-C_3H_7	OH	0.17	35 % at 1μM	54 ± 13
1c	4	n-C_3H_7	OH	0.09	0.5	73 ± 16
1	5	n-C_3H_7	OH	0.006	0.4	88 ± 16 (1 mg/kg)
1d	6	n-C_3H_7	OH	0.02	0.3	89 ± 8
1e	7	n-C_3H_7	OH	0.005	0.5	Not tested
1f	8	n-C_3H_7	OH	0.09	0.3	35 ± 36
1g	10	n-C_3H_7	OH	0.02	0.4	Not tested
1h	6	H	OH	0.3	33 % at 1μM	95 ± 10 (1 mg/kg)
1i	6	n-C_3H_7	H	0.02	0.68	Not tested
1j	6	H	H	0.2	9 % at 1μM	89 ± 11

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PBMC was 83 ± 10% as determined by giemsa staining. PBMC in RPMI medium with 2 % heat-inactivated AB serum (1.5 x 10^6 cells) were incubated at 37 °C with drug or vehicle (10 % DMSO) for 30 min. At this time LPS (*E. coli* O55:B5, Sigma) was added to 25 ng/mL and incubated for 24 h. Cell viability was assessed by trypan blue exclusion and MTT assay. After incubation, PBMC were associated) was measured by radioimmunoassay as described by: Youngman, K. R.; Simon, P. L.; West, G. A.; Cominelli, F.; Rachmilewitz, D.; Klein, J. S.; Fiocchi, C. *Gastroenterology* **1993**, 104, 749. The limit of detection of IL-1β was 40-60 pg/mL.

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26. The TPA mouse ear inflammation test was a modification of that described by: Young, J. M.; Wagner, B. M.; Spires, D. A. *J. Invest. Derm.* **1983**, 80, 48. Compound **1** was dissolved in pyridine:water:diethyl ether (20:5:75) and 0.4 mg in 10 μL was applied to the inside of the right ear of 3-5 week old CD-1 mice n = 8). TPA (0.25 μg/10 μL), dissolved in the same vehicle was applied to the outside of the same ear 30 min later. The animals were sacrificed 6 h after TPA application and ear biopsy punches (6 mm) were removed, weighed and assayed for MPO activity using the method of: Bradley, P. P.; Priebat, D. A.; Christensen, R. D. Rothstein, G. *J. Invest. Derm.*, **1982**, 78, 206. The wet weight of the biopsy punches was used as a measure of ear edema. The data are expressed as percent inhibition (mean of 4 experiments ± SEM) relative to control groups and were analyzed for statistical significance using Students t-test. For MPO assay the ear punches from each group of animals were pooled and analyzed in duplicate.

27. The AA mouse ear inflammation test was a modification of that described by: Young, J. M.; Spires D. A.; Bedford, C. J.; Wagner, B.; Ballaron, B. S.; De Young, L. M. *J. Invest. Derm.*, **1984**, 367. Compound **1** (1 mg in 25 μL of acetone) was applied to the right ear of 3-4 week old CD-1 mice (n = 8). AA (0.5 mg in 25 μL of acetone) was applied to the outside of the same ear 30 min later. The animals were sacrificed and biopsy punches were removed 1 h after AA application. Since 0.5 mg of AA did not produce an increase in MPO activity, the concentration of AA was increased to 1 mg/10 μL and the measurement time to 6 h after AA application, for the MPO determination. The data are expressed as percent inhibition (mean of 4 experiments ± SEM) relative to the control group.

28 The rat acetic acid colitis test was a modification of that described by: Sharon, P.; Stenson, W. F. *Gastroenterology*, **1985**, 88, 55. Rats were pretreated for two days with oral doses of **1** in DMSO at 8 AM and 4 PM. Colitis was induced on the third day with a 2 mL enema of 3.5% (v/v) acetic acid followed 10 sec later with a 3 mL phosphate buffered saline (pH 7.4). Changes in pathology and neutrophil accumulation were evaluated 24 h later. Neutrophil accumulation was estimated by measuring myeloperoxidase.activity. Pathology was quantified using standard scores assigned by at least two blind observers.

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