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BENZENEPROPANOIC ACIDS CONTAINING CHROMANONE OR NAPHTHALENONE MOIETIES ARE POTENT AND ORALLY ACTIVE LEUKOTRIENE B_4 ANTAGONISTS

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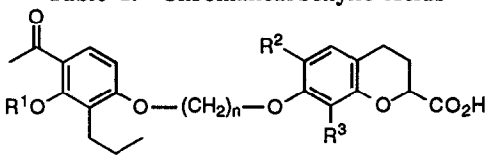
Abstract. Systematic structural modification of peptidoleukotriene antagonists of the o-hydroxyacetophenone class has led to the discovery of certain [[(3,4-dihydro-4-oxo-8-propyl-2H-1-benzopyran-7-yl)oxy]alkyl]benzenepropanoic acids and related compounds (**7**), which appear to be potent and selective antagonists of the proinflammatory mediator leukotriene B_4.

In recent years, intensive research efforts have focused on the development of leukotriene B_4 receptor antagonists as novel antiinflammatory agents with the result that several second generation members of this class, with improved potency relative to earlier compounds, are now entering clinical trials.[1] We wish to disclose in this Letter the results of preliminary studies which have led to the discovery of a promising class of acidic chromanones and isosteric naphthalenones represented by structure **7** (Table 2), possessing potent LTB_4 antagonist properties.

Our efforts in this area originated with the observation that the peptidoleukotriene antagonist **1** (Table 1) inhibited LTB_4-induced bronchoconstriction in guinea pigs when administered by the aerosol route.[2] Subsequently, it was found that this o-hydroxyacetophenone inhibited binding of LTB_4 to its receptor on isolated human neutrophils.[3] In a related study, workers at G. D. Searle reported that phenolic methylation of the peptidoleukotriene antagonist **3** afforded an LTB_4 antagonist **4**, no longer possessing peptidoleukotriene antagonist properties.[4] It is interesting to note that while methylation of **3** produces a substantial increase in LTB_4 binding potency, the improvement in binding potency obtained through the analogous methylation of **1** (cf. **2**) is much more modest.

Given these observations, and in an attempt to build upon the lead represented by chromancarboxylic acids **1-4**, we decided to convert the o-alkoxy acetophenone unit into a cyclic structure, specifically a chromanone system. Such a modification results in restriction of conformations available to the carbonyl group with unknown consequences regarding interactions with the LTB_4 receptor. To this end, the chromanone **5** was synthesized.[5] Evaluation of this compound in the binding assay[3] revealed an encouraging, ca. 2-fold potency enhancement relative to "open" analog **2**. In carrying out variations of the acidic region, it soon became apparent that the chromancarboxylic acid feature was not required for high affinity binding. Thus the straight-chain acid **6**[6], in which the distance between the carboxyl moiety and chromanone unit is approximately the same as that in **5**, exhibits very similar binding affinity. When the linking chain was modified by the incorporation of an o-substituted aromatic ring as in compounds **7a-c** (Table 2), binding affinity was significantly improved; however, the most potent analogs in this series were obtained when a second acidic chain was attached to the phenyl ring at C-5 or C-6. It can be seen that diacids **7d-j**

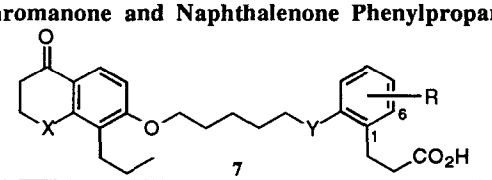
Table 1. Chromancarboxylic Acids



Cpd	n	R^1	R^2	R^3	K_i (nM)[a]
1(±) (Ro 23-3544)	5	H	CH_3CO	H	320
2	5	CH_3	CH_3CO	H	210
3	3	H	H	n-C_3H_7	3700
4(±) (SC-41930)	3	CH_3	H	n-C_3H_7	93

[a]Inhibition of LTB_4 binding to its receptor on intact human neutrophils (binding inhibition constant; all data from this study).[3]

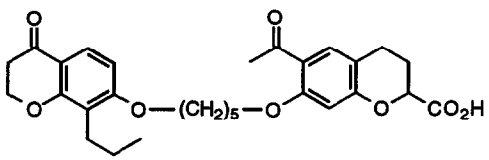
Table 2. Chromanone and Naphthalenone Phenylpropanoic Acids



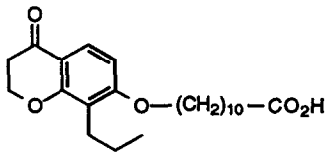
7

Cpd[a]	mp °C.	X	Y	R	K_i (nM)[b]	% Control, 0.1 mg/kg, p.o.[c]	% Control, 1.0 mg/kg, p.o.[d]
7a	82-85[e]	O	O	H	29	N.T.[h]	N.T.
7b	98-99[e]	O	CH_2	H	10	88±9	N.T.
7c	90-92[e]	CH_2	O	H	78	N.T.	N.T.
7d	119-120[e]	O	CH_2	5-[O(CH_2)$_3$$CO_2$H]	1	58±20	45±4
7e	116-117[f]	O	CH_2	6-[O(CH_2)$_3$$CO_2$H]	1	25±5	20±5
7f	103-104[e]	O	CH_2	6-[O(CH_2)$_4$$CO_2$H]	1	35±9	26±8
7g	85-87[f]	O	CH_2	6-[O(CH_2)$_5$$CO_2$H]	1	21±7	21±7
7h	91-93[e]	O	CH_2	6-[O(CH_2)$_6$$CO_2$H]	2	41±10	73±8
7i	79-81[e]	O	CH_2	6-[O(CH_2)$_7$$CO_2$H]	1	31±8	27±7
7j	63-65[e]	O	CH_2	6-[O(CH_2)$_8$$CO_2$H]	3	38±11	24±7
7k	163-164[g]	O	O	4-[O(CH_2)$_3$$CO_2$H]	88	N.T.	N.T.
7l	117-118[f]	CH_2	CH_2	6-[O(CH_2)$_3$$CO_2$H]	5	56±7	19±5
7m	100-101[e]	CH_2	CH_2	6-[O(CH_2)$_5$$CO_2$H]	3	28±8	25±5
4					93	91±10	N.T.
LY223982					11	88±18	N.T.
Ono-4057					7	101±24	N.T.
RP 69698					10	18±5	15±6
SC-50605					5	60±8	36±6
CGS-25019C					1	80±13	N.T.
RG 14893					2	94±16	71±10
LY293111					2	79±12	N.T.

[a]All new compounds provided satisfactory (±0.4%) C,H combustion analyses, compatible 400 MHz ^{1}H NMR, and low resolution mass spectra; [b]Inhibition of LTB_4 binding to its receptor on intact human neutrophils (binding inhibition constant);[3] [c]Percent of control LTB_4-induced bronchoconstriction in guinea pigs - 2 hr pretreatment at 0.1 mg/kg;[7] [d]Percent of control LTB_4-induced bronchoconstriction in guinea pigs - 20 hr pretreatment at 1.0 mg/kg;[7] [e]Recrystallized from hexane-ethyl acetate; [f]Recrystallized from acetonitrile; [g]Recrystallized from ethyl acetate; [h]Not Tested.



5(±); mp 148-151 °C.; K_i 100 nM

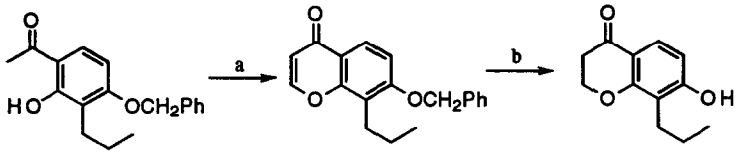


6; mp 101-102 °C.; K_i 130 nM

and **7l,m** exhibit low nanomolar inhibition constants in the binding assay. Apparently, the binding site is tolerant of size modifications in the substituent R since compounds of varying chain length (**7e-j**) show very similar binding potencies; however, when the second acid chain was attached at C-4 (**7k**), a substantial reduction in binding affinity was observed relative to the other diacids. Both naphthalenones and chromanones exhibited high affinity binding. The straightforward syntheses of all of these analogs is summarized in Schemes 1 and 2.

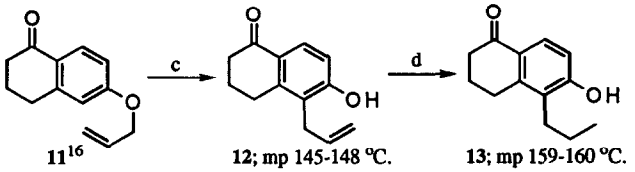
Certain members of this series were also evaluated in an LTB_4-induced, guinea pig bronchoconstriction model.[7] Test compounds were administered by the oral route at a dose of 0.1 mg/kg, with a 2 hr pretreatment time and, in order to estimate duration of action, at 1.0 mg/kg with a 20 hr pretreatment. In addition, several reported LTB_4 antagonists were evaluated for comparison including **4**,[4] LY223982,[8] Ono-4057,[9] RP 69698,[10] SC-50605,[11] CGS-25019C,[1h] RG 14893,[12] and LY293111.[13] The diacids **7** tested generally show >50% inhibition of bronchoconstriction (<50% of control bronchoconstriction) at both the 2 hr and 20 hr pretreatment points and appear superior, in this regard, to all of the standard antagonists with the exception of RP 69698.

Scheme 1



8[15] **9**; mp 87-89 °C. **10**; mp 126-129 °C.

Reagents: (a) 1. $HC(OMe)_2NMe_2$, 120-160 °C.; 2. p-TsOH (1.1 eq), EtOH, reflux, 87%, cf. ref. 14; (b) H_2, Pd/C, MeOH-EtOAc, R.T., 1 atm., 61%

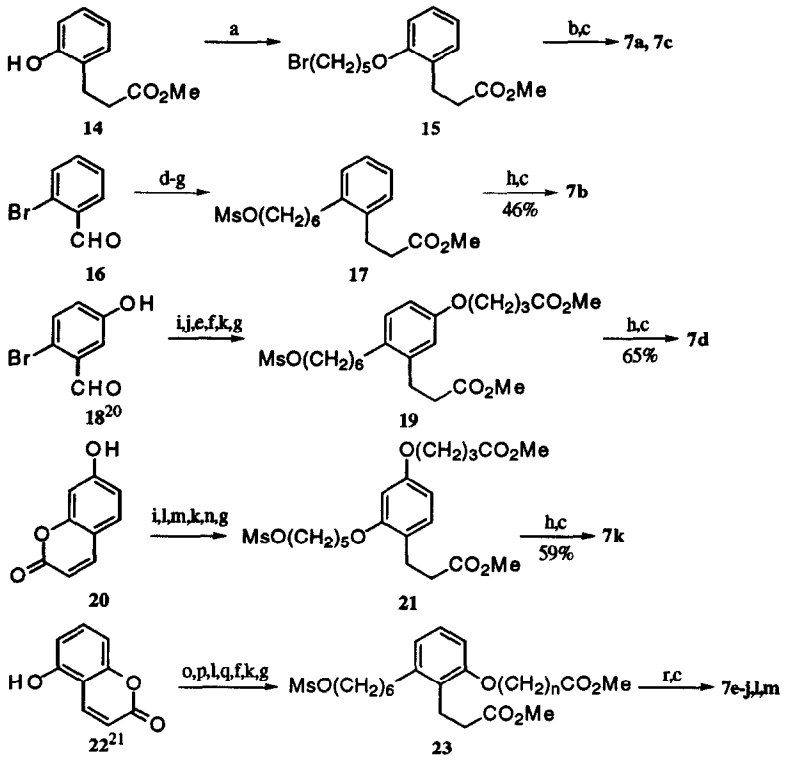


11[16] **12**; mp 145-148 °C. **13**; mp 159-160 °C.

(c) 1. Δ, N,N-Diethylaniline, 200-220 °C., 23 hr; 2. Recrystallize from EtOAc to remove 7-allyl isomer, 60%; (d) H_2, Pd/C, EtOH, R.T., 1 atm., quant.

Diacid 7g (Ro 25-4094) was selected for further evaluation. In the bronchoconstriction assay, this compound exhibited ED_{50} values of of 0.07 mg/kg, i.v. (1 min pretreatment) and 0.4 mg/kg, p.o. (20 hr pretreatment). It inhibited LTB_4-induced calcium flux[17] and chemotaxis[18] with IC_{50} values of 2 and 9 nM, respectively but showed no affinity for LTD_4 or PAF receptors.[19] Thus diacid **7g** appears to be a potent, selective, and orally bioavailable LTB_4 antagonist which should prove useful in elucidating the role of this lipid mediator in inflammatory conditions. Further studies involving **7g** and other analogs in this series will be reported in future communications.

Scheme 2



Reagents: (a) 1. $Br(CH_2)_5Br$ (8 eq.), K_2CO_3, MeCN, reflux 24 hr; 2. p-TsOH, MeOH, reflux, 17 hr, 74%; (b) **10** or **13**, K_2CO_3, DMF-acetone, reflux, 5 hr; (c) LiOH (4-5 eq.), THF-H_2O, R.T.; (d) 5-Hexyn-1-ol, $PdCl_2(PPh_3)_2$ (cat.), CuI, Et_3N, 90 °C., 3 hr, 60%; (e) $Ph_3P{=}CHCO_2Me$, toluene, reflux, 4hr; (f) H_2, 10% Pd/C, MeOH, R.T., 1 atm., 78% for 2 steps; (g) MsCl, Et_3N, EtOAc, 0 °C., quant.; (h) **10**, K_2CO_3, TDA-1 (cat.), toluene, reflux, 6 hr; (i) $Br(CH_2)_3CO_2Et$, K_2CO_3, DMSO, R.T., 24 hr, 80%;(j) $THPO(CH_2)_4C{\equiv}CH$, $PdCl_2(PPh_3)_2$ (cat.), CuI, Et_3N, 90 °C., 3 hr, 94%; (k) p-TsOH, MeOH, reflux, 24 hr; (l) 25% NaOMe, MeOH, reflux 24 hr; (m) $Br(CH_2)_5OAc$,[5] K_2CO_3, DMSO, R.T., 20 hr,quant.; (n) H_2, 10% Pd/C, MeOH-EtOAc 1:1, R.T., 1 atm., quant.; (o) Tf_2O, Py, CH_2Cl_2, 0 °C.-R.T., 88%; (p) $THPO(CH_2)_4C{\equiv}CH$, $PdCl_2(PPh_3)_2$ (cat.), CuI, Et_3N, DMF, 100 °C., 24 hr, 67%; (q) $Br(CH_2)_nCO_2Et$, K_2CO_3, DMSO, R.T., 24 hr, quant.; (r) **10** or **13**, K_2CO_3, NaI, CH_3CN, reflux or **10** or **13**, K_2CO_3, TDA-1 (cat.), toluene, reflux

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3. [^{3}H]LTB_4 binding to human neutrophils: Assays were performed in 96 well microtiter plates. Each well contained 150 µl isolated human neutrophils ($7.5x10^6$ cells/ml) and antagonists in 5 µl DMSO or 5 µl DMSO alone. 20 µl [^{3}H]LTB_4 was diluted in 50 mM Hepes (pH 7.4) containing 10 mM $MgCl_2$ and added to give a final assay volume of 0.175 ml. All incubations were done at 4 °C. and assays were terminated by rapid filtration through Whatman GF/C glass fiber filters using a Brandel Cell Harvester. Filters were washed three times with 4 ml ice-cold Tris buffer (10 mM $MgCl_2$ and 50 mM Tris, pH 7.4). The radioactivity retained by each filter was measured by liquid scintillation spectroscopy at a counting efficiency of 43%. Specific binding was determined as the difference between [^{3}H]LTB_4 bound in the absence and bound in the presence of 1 µM unlabeled LTB_4. Each assay point was tested in duplicate. Non-linear analysis of the binding data was performed using LIGAND (Munson, P.J.; Rodbard, D. *Endocrinology* **1979**, *105*, 1377-1381). K_i values were determined using the Cheng-Prusoff relationship (Cheng, Y.C.; Prusoff, W.H. *Biochemistry* **1973**, *12*, 2612-2619).

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5. Hydroxychromanone **10** (Scheme 1) was alkylated with rac-6-acetyl-7-[[5-[(methylsulfonyl)oxy]pentyl]oxy]-3,4-dihydro-2H-1-benzopyran-2-carboxylic acid methyl ester using potassium carbonate and TDA-1 in refluxing toluene followed by saponification: See Manchand, P.S.; Micheli, R.A.; Saposnik, S.J. *Tetrahedron* **1992**, *43*, 9391-9398.

6. Prepared by alkylation of **10** with methyl 11-bromoundecanoate (K_2CO_3, DMF-acetone, reflux) followed by saponification.

7. LTB_4-induced bronchoconstriction: Male Hartley guinea pigs weighing 300-500 g were anesthetized with urethane (2 g/kg, i.p.) and cannulated in the jugular vein for drug administration. Tracheal pressure was recorded from a tracheal cannula connected to a Gould P231D pressure transducer. Animals were paralyzed with succinylcholine (1.2 mg/kg, i.v.) and mechanically respirated (40 breaths/minute, 2.5 ml tidal volume). Propranolol (0.1 mg/kg) was given 5 min prior to the LTB_4 challenge (100 µg/kg, i.v.); antagonists were administered 1 min (i.v.), 2 hr or 20 hr (p.o.) prior to LTB_4. Data is expressed as the percent of control LTB_4-induced bronchoconstriction for n=6 animals per group. Thus smaller values represent greater inhibition of the bronchoconstriction. ED_{50} values were determined by non-linear analysis of competition curves containing 5-6 separate doses, n=6 animals per dose.

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17. Calcium flux assay: LTB_4-induced changes in intracellular calcium concentration were measured using Fura 2 labeled human neutrophils. Cells at concentrations of 2-5 $x10^6$/ml were labeled with 5 μM Fura 2 for 15 min in HBSS without calcium or magnesium. The cells were washed and resuspended in Gey's solution at final concentrations of 1-2 x 10^7 cells/ml. Calcium fluxes were initiated by the addition of 2.4 nM LTB_4. Antagonists at appropriate concentrations were added to the cells just prior to the addition of LTB_4. IC_{50} values were determined as the concentration of antagonist required to give 50% inhibition of the LTB_4-induced calcium flux. Fluorescence measurements were made in a Perkin Elmer model LS-5B spectrofluorometer at 37 °C. and calcium concentrations were determined using the ratio method.

18. Chemotaxis assay: Chemotaxis was measured using ^{51}Cr labeled human neutrophils. Cells were incubated with ^{51}Cr (2 μCi/1x10^6 cells) for 30 min at 37 °C. and were washed twice with Gey's salt solution. 1-5 x 10^6 labeled cells in 100 μl Gey's salt solution were placed in the upper wells of a 6.5 mm Transwell chamber. 500 μl of LTB_4 (2.4 nM) in Gey's salt solution was placed in the lower wells. Antagonists were also placed in the lower wells at 6-7 concentrations and were tested in duplicate. After 90 min at 37 °C. in 5% CO_2, the cells were removed from the lower well and counted by scintillation spectroscopy. IC_{50} values were determined to be the concentration of antagonist required to give 50% inhibition of LTB_4-induced chemotaxis.

19. Screening studies were carried out at NovaScreen®, 7170 Standard Drive, Hanover MD 21076.

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