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trans-3-BENZYL-4-HYDROXY-7-CHROMANYLBENZOIC ACID DERIVATIVES AS ANTAGONISTS OF THE LEUKOTRIENE B_4 (LTB_4) RECEPTOR

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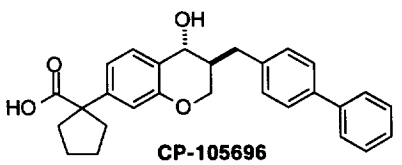
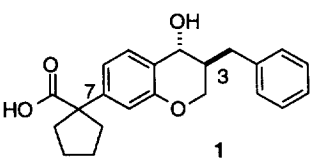
Abstract: The SAR of a series of 2-(7-chromanyl)benzoic acids has been investigated with the aim of identifying potent and selective LTB_4 receptor antagonists that maintain potency in complex biological fluids. We found optimal activity in derivatives with electron-withdrawing groups in the benzoic acid ring and with an unsubstituted C-3 benzyl group on the chromanol nucleus. While compounds containing a 3-(4-phenyl)benzyl chromanol substituent were potent LTB_4 receptor antagonists, the increased lipophilicity imparted by the additional phenyl substituent led to decreased potency in the presence of plasma proteins. From among the potent compounds identified, CP-195543, the 5′-trifluoromethyl 3-benzyl chromanol, was selected for development.

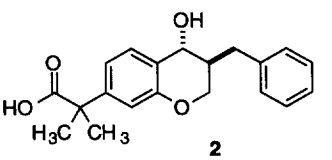
Over the past few years we have reported on the activity and SAR of a series of 7-chromanylacetic acids, which display activity in a spectrum of assays that involve LTB_4 as a prime mediator.[1–3] CP-105696, the clinical candidate derived from this series, has shown efficacy in several preclinical animal disease models including collagen-induced arthritis[4] and experimental allergic encephalomyelitis in mice[5] and antigen-induced airway hyper-responsiveness in monkeys.[6] These results, in addition to extensive literature from other groups,[7–10] suggest that LTB_4, which is a potent chemoattractant and activator of neutrophils, eosinophils, monocytes and lymphocytes, plays an important role in the pathology of a number of inflammatory diseases. LTB_4 receptor antagonists are therefore anticipated to be useful therapeutics for the treatment of such diseases.



CP-105696



1



2

During our studies on 7-chromanylacetic acid-based antagonists, we noted in the X-ray crystal structure of a closely related compound, the C-3 benzyl derivative **1**, that the carboxylic acid function was approximately perpendicular to the plane of the chromanol ring system.[2] Furthermore, for **1**, calculations showed that such a conformation, with the carboxylic acid perpendicular to and either above or below the plane, was greatly preferred. In contrast, calculations on the less active α,α-dimethylacetic acid analog **2** showed that such a conformation was not as likely to be significantly populated.[11] These results suggested that the orientation of the carboxylate function in **1** and CP-105696 was an important contributor to the potent activity of these cyclopentylacetic acid derived compounds. This suggestion led us to consider other systems in which a carboxylate function would be similarly oriented. In this regard, compounds in which an *ortho*-carboxyphenyl

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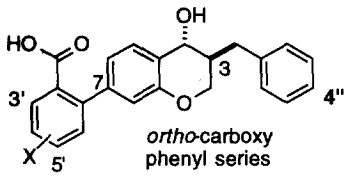
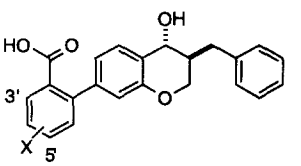
functionality replaces the α,α-disubstituted acetic acid were considered prime targets since the propensity for the rings in an *ortho*-substituted biphenyl system to exist in an orthogonal conformation is well known.



In order to assess whether an *ortho*-carboxyphenyl group could effectively replace the cyclopentylacetic acid moiety of CP-105696 we first prepared a set of three compounds: the unsubstituted, 5′-methoxy- and 5′-chlorobenzoic acid derivatives **3–5**, respectively.[12] Evaluation of these compounds as inhibitors of [^{3}H]-LTB$_4$ binding to LTB$_4$ receptors on guinea pig spleen membranes[13] showed that the compounds were indeed receptor antagonists (see Table 1). While the best of the three, the 5′-chloro derivative **5**, was an order of magnitude less potent than CP-105696, this level of activity was still better than that of most of the compounds in our 7-chromanylacetic acid-based series. In addition, when assessed as inhibitors of [^{3}H]-LTB$_4$ binding to LTB$_4$ receptors on human neutrophils, **5** was found to be nearly as potent as CP-105696 (see Table 2). We were further encouraged by the functional activity displayed by **5** in the human neutrophil chemotaxis assay[3] (see Table 2). These results were clearly sufficient to lead us to pursue this series further. Our goal was to identify a compound with potency similar to that of CP-105696 (i.e., not more than ~5-fold less active in binding to LTB$_4$ receptors on human neutrophils nor more than ~10-fold less potent in inhibiting the LTB$_4$-induced chemotaxis of human neutrophils), but with less pronounced plasma protein binding. The high plasma protein binding of CP-105696 is believed to be responsible for the exceedingly long half-life of CP-105696 in humans[14] and is reflected by a ~180-fold shift in CD11b up-regulation inhibition when assayed in whole blood versus when assayed on isolated human neutrophils.[3]

Table 1a



Compd	X	isomer[b]	GPS IC$_{50}$ (nM)[c]
3	H	+/-	120 ± 31
4	5′-OCH$_3$	+/-	160
5	5′-Cl	+/-	59 ± 29
CP-105696			6.4 ± 2.2

a values are either individual determinations or mean ± SD of two or more assays.
b +/- indicates racemic compound.
c inhibition of LTB$_4$ binding to receptors on guinea pig spleen membranes, ref 13.

The observation that the 5′-methoxy derivative **4** was the weakest binder in the guinea pig spleen membrane assay while the 5′-chloro analog **5** was the most potent demonstrated that the binding affinity was dependent on the electron density of the arylcarboxylic acid system and presumably manifested in the pK_a of the system. As a result, we focused on compounds containing electron withdrawing groups in the benzoic acid ring and prepared the 5′-fluoro-, 4′-fluoro-, 3′-fluoro-, and 5′-trifluoromethyl- analogs **8**, **11**, **12**, and **15**, respectively. All of these racemates displayed potent activity against the human neutrophil receptor (Table 2) and the potency was within the range sought (<40 nM). With the exception of the 4′-fluoro derivative **11**, the receptor antagonism of the compounds was reflected in their activity as inhibitors of human neutrophil chemotaxis which was also within the desired range (<50 nM).

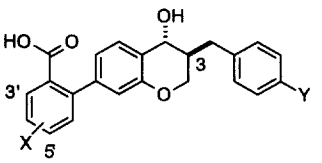
The level of potency displayed by the racemates **5**, **8**, **12**, and **15** warranted their being separated into enantiomers. This revealed that the dextrorotatory compounds were uniformly more active than the corresponding levorotatory derivatives. The difference in binding activities ranged from 8-fold (**16** vs. **17**) to 22-fold (**13** vs. **14**). Such a difference in binding activity for pairs of enantiomers stands in contrast to what we observed in the α,α-disubstituted acetic acid series.[2] In the latter, little difference in activity between the isomers was found, with the largest difference being only ~2-fold.[15] Owing to the high potency imparted by a 5′-fluoro substituent even the levorotatory isomer of this set of enantiomers displayed activity within the desired potency range.

Other dextrorotatory isomers that were prepared and examined include the 3′,5′-difluoro-, 3′-trifluoromethyl-, 5′-carboxy-, and 5′-*N*-methylcarboxamidobenzoates, **18**–**21**, respectively. Of these, the 3′,5′-difluoro- and 3′-trifluoromethyl-derivatives had the requisite level of activities.

As indicated above, a critical component of activity that we sought was potent inhibition of LTB_4-mediated CD11b up-regulation in whole blood. Potent activity in whole blood and a small shift in the IC_{50}'s determined in whole blood versus those determined on isolated neutrophils would suggest that protein binding effects during subsequent in vivo evaluation would be minimal. A difference between whole blood and isolated neutrophil IC_{50}'s of less than ~2-fold was deemed desirable as was potency on isolated neutrophils similar to that of CP-105696 (not more than ~2-fold less). Examination of **6**, **9**, **10**, **13**, **16**, **18**, and **19**, all of which possess the requisite binding and chemotactic potency, revealed that all except **10**, the only levorotatory compound with potent LTB_4 receptor antagonism, have the desired level of potency when assayed against isolated neutrophils. The absolute potency of these compounds as inhibitors of CD11b up-regulation on isolated neutrophils was considerably less than their potency as inhibitors of LTB_4 binding. This observation is in accordance with what was found with CP-105696 and is presumed to reflect a difference in affinities for the low and high affinity LTB_4 receptors.[3] Comparison of the IC_{50}'s determined against isolated neutrophils versus those determined in whole blood showed **6** to be clearly outside the desired range (>4-fold shift) while all of the other six compounds met our criteria with potency shifts of ~2.5- to 0.7-fold.

In the α,α-disubstituted acetic acid chromanol series, the introduction of a phenyl substituent at C-4″ of the C-3 benzyl group led to a nearly two orders of magnitude increase in binding potency.[2] Introduction of a C-4″ phenyl group in the present series, however, did not affect activity nearly so dramatically. With the exception of the levorotatory 5′-chloro derivative **23**,[16] the additional phenyl substituent had relatively little affect on potency giving rise to both modest increases and decreases in potency (see compounds **22**–**27**, Table 2). The potency increases were greatest for the levorotatory enantiomers while two of the dextrorotatory enantiomers actually decreased in potency with the addition of the C-4″ phenyl group. These changes in relative potencies essentially eliminated the potency differences that were observed for enantiomeric pairs in the parent series. As a result, all of the compounds in the set, both dextro- and levorotatory isomers, displayed binding and chemotactic activity within the desired range. Additionally, all six derivatives displayed potency as inhibitors of CD11b up-regulation on isolated neutrophils similar to that of CP-105696. In whole blood, however, the potency of most of these dropped significantly. Since the lipophilicity of the 4″-phenyl series, as measured by clogP's, was ~1.5 to 1.9 log units higher than in the parent series, increased levels of plasma protein binding would be expected. Thus, the observed decrease in potency in whole blood is not surprising. Of this set, only **24** remained within the range of desired potencies.

Table 2[a]



Compd	X	Y	isomer[b]	PMN IC_{50} (nM)[c]	CTX IC_{50} (nM)[d]	CD11b (IC) IC_{50} (nM)[e]	CD11b (WB) IC_{50} (nM)[f]	ratio[g]	clogP
CP-105696				8.4 ± 0.3	5.0 ± 2.0	430 ± 130	76700 ± 10400	180	5.53
5	5′-Cl	H	+/-	12	26 ± 4.0	-	-	-	-
6	5′-Cl	H	+21.3°	14 ± 8.5	30 ± 14	265 ± 35	1300 ± 170	4.9	4.50
7	5′-Cl	H	-	200	83 ± 68	-	-	-	-
8	5′-F	H	+/-	12 ± 7.5	5.0 ± 2.2	-	-	-	-
9	5′-F	H	+24.5°	3.0 ± 1.0	14 ± 9.0	240 ± 66	600 ± 510	2.5	3.94
10	5′-F	H	-23.0°	27 ± 7.0	5.0 ± 1.7	3000	3500 ± 1400	1.2	3.94
11	4′-F	H	+/-	24 ± 5.0	300	-	-	-	-
12	3′-F	H	+/-	22 ± 21	10 ± 1.5	-	-	-	-
13	3′-F	H	+22.7°	11 ± 7.5	40 (5)	400 ± 225	1000 ± 670	2.5	3.58
14	3′-F	H	-	240	650	-	-	-	-
15	5′-CF_3	H	+/-	13 ± 5.8	7.0 ± 3.6	-	-	-	-
16	5′-CF_3	H	+20.2°	6.8 ± 0.75	2.4 ± 1.6	280 ± 60	660 ± 60	2.3	4.74
17	5′-CF_3	H	-20.7°	56 ± 31	2.0 ± 1.2	-	-	-	-
18	3′,5′-F_2	H	+	5.0 ± 1.6	40 ± 22	300	200	0.7	3.75
19	3′-CF_3	H	+	46 ± 19	24 ± 8.7	750 ± 47	880 ± 900	1.2	3.94
20	5′-CO_2H	H	+	>10000	-	-	-	-	-
21	5′-$CONHCH_3$	H	+	4700	-	-	-	-	-
22	5′-Cl	Ph	+	4.0 ± 0.6	6.0 ± 1.7	520	1700 ± 1100	3.3	6.39
23	5′-Cl	Ph	-	8.0 ± 6.6	26 ± 18	590	3900 ± 1800	6.6	6.39
24	5′-F	Ph	+	4.0 ± 2.2	56 ± 40	280 ± 310	700 ± 200	2.5	5.82
25	5′-F	Ph	-11.6°	14 ± 6.5	5.0 ± 1.7	700 ± 170	5100 ± 2400	7.3	5.82
26	5′-CF_3	Ph	+	38 ± 15	6.0 ± 2.0	120 ± 64	1900 ± 1300	15.8	6.23
27	5′-CF_3	Ph	-	27 ± 19	7.0 ± 3.5	820 ± 880	4000 ± 750	4.9	6.23
28	5′-CF_3	Cl	+	+	70 ± 18	58 ± 20	-	-	-
29	5′-Cl	F	+/-	+/-	54	400	-	-	-
30	5′-Cl	CF_3	+/-	+/-	400	9000	-	-	-
31	5′-Cl	[h]	+/-	+/-	100	2250	-	-	-
32	5′-F	(2-propyl)	+	+	190 ± 87	210 ± 47	-	-	-
33	5′-F	[i]	+/-	+/-	35	32 ± 24	-	-	-
34	5′-F	CF_3	+/-	+/-	54	1800	-	-	-

a see footnote a, Table 1.

*b +/- indicates racemic compound, + (or –) indicates compound inferred to be dextrorotatory (or levorotatory) through correlation of the relative TLC mobility of its corresponding t-BOC-L- or D-tryptophan ester derivative with that for a compound of known rotation, numerical values are optical rotations of pure isomers, all optical rotations c = 1.0 (MeOH) except **9** c = 0.61 and **10** c = 0.76.*

c inhibition of LTB_4 binding to receptors on isolated human neutrophils, ref 3.

d inhibition of LTB_4-induced chemotaxis of isolated human neutrophils, ref 3.

e inhibition of LTB_4-induced CD11b up-regulation on isolated human neutrophils, ref 3.

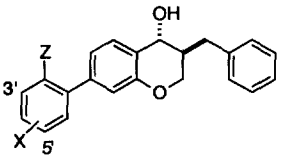
f inhibition of LTB_4-induced CD11b up-regulation on human neutrophils in whole blood, ref 3.

g ratio of CD11b (WB) IC_{50} to CD11b (IC) IC_{50}.

*h in place of a C-3 benzyl substituent, **31** contains a C-3 2-naphthylmethyl substituent.*

*i in place of a C-3 benzyl substituent, **33** contains a C-3 2-phenethyl substituent.*

Table 3[a]



Compd	X	Z	isomer[b]	PMN IC_{50} (nM)[c]	CTX IC_{50} (nM)[d]	CD11b (IC) IC_{50} (nM)[e]	CD11b (WB) IC_{50} (nM)[f]	ratio[g]
35	5'-Cl	$-CO_2CH_3$	+	1700 ± 290	6400	-	-	-
36	5'-CF_3	$-CH_2OH$	+/-	1700 ± 730	9[h]	-	-	-
37	5'-F	5-tetrazolyl-	+/-	20 ± 9.0	110	-	-	-
38	5'-F	$-NHSO_2CF_3$	+	3.8 ± 3.4	15 ± 11	360 ± 0	330 ± 15	0.9
39	5'-F	$-NHSO_2CF_3$	-	50	44 ± 12	>3000	>3000	-
40	5'-F	$-SO_2NH_2$	+	37	17	540	>10000	-

[a] *see footnote a, Table 1.* [d] *see footnote d, Table 2.* [g] *see footnote g, Table 2.*
[b] *see footnote b, Table 2.* [e] *see footnote e, Table 2.* [h] *see ref 17.*
[c] *see footnote c, Table 2.* [f] *see footnote f, Table 2.*

With the unique exception of a C-4″ phenyl group, in the α,α-disubstituted acetic acid series we had found that introduction of substituents onto the C-3 benzyl group or replacing the benzyl group led to reductions in activity.[2] This experience was mirrored in the present series (see compounds **28–34**, Table 2). Simple substituents such as 4″-chloro, 4″-fluoro, 4″-trifluoromethyl or 4″-isopropyl all led to reductions in activity such that none of these derivatives, **28–30**, **32** and **34**, had activity within the desired range. Replacing the C-3 benzyl group with the larger 2-naphthylmethyl moiety as in **31** was also detrimental to activity. The C-3 phenethyl derivative **33** was the only compound from this set that had close to the desired levels of activity, but this was clearly less active than the corresponding racemic C-3 benzyl derivative **8**.

Replacement of the carboxy group on the C-7 phenyl group with either an ester, as in **35**, or an alcohol, as in **36**, led to loss of binding activity (see Table 3). Replacing this carboxy group with acidic surrogates, such as a tetrazole, a triflamide, or a sulfonamide, led to compounds with binding activity in the desired range. Of these, only the tetrazole **37** did not inhibit neutrophil chemotaxis with sufficient potency. Examination of **38–40** for their abilities to inhibit CD11b up-regulation revealed that only the dextrorotatory triflamide **38** had the potency required on isolated neutrophils and retained it in whole blood.

Of the compounds prepared, seven (**9**, **13**, **16**, **18**, **19**, **24**, and **38**) met the established activity criteria. Clearly the replacement of the α,α-disubstituted acetic acid with an *ortho*-carboxyphenyl group was successful. In fact, the replacement gave a series of compounds that were generally more active than the original series. Given that many compounds met the criteria for activity as receptor binding antagonists and as chemotaxis inhibitors, we were able to select only those with the most promising levels and ratios of activities as inhibitors of CD11b up-regulation for further study. From these studies, CP-195543, **16**, emerged as the compound with the best overall profile of properties including a very small shift in its activities in the presence of plasma proteins.[18] This compound has been advanced into clinical trials.

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15. The differences in activity observed between the enantiomer pairs in the present series vs. that in the α,α-disubstituted acetic acid series could also be related to the assays used in the two studies. In the human neutrophil-based assay used in the present study, **1** is 12-fold more potent than its enantiomer. This stands in contrast to only a 1.4-fold difference in potency observed for this pair in the guinea pig spleen membrane-based assay suggesting that the differences between the present and our earlier work reflects the change in assays. However, CP-105696 and its enantiomer have similar potency in both assays with the levorotatory isomer being 1.6-fold more potent in the human neutrophil-based assay and 2.0-fold more potent in the guinea pig spleen membrane-based assay. Similar comparative data for other enantiomer pairs which might help resolve this point do not exist.
16. The 25-fold increase in potency of **23** over **7** brings the potency of **23** only to the same level as other analogs in this series since **7** was a notably weak inhibitor of LTB_4 binding.
17. The potent chemotactic activity of **36** is presumed to derive from neutrophil-mediated oxidative metabolism, yielding **15**, under the assay conditions at 37 °C. In contrast, the binding assay is performed at 4 °C, which would inhibit the neutrophils' metabolic activity. The formation of **15** during the assaying of **36** was not, however, specifically examined.
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