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DISCOVERY OF L-740,515, A POTENT THIENOPYRIDINE cysLT$_1$ RECEPTOR (LTD$_4$ RECEPTOR) ANTAGONIST

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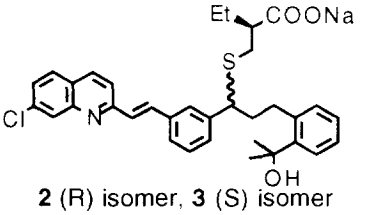
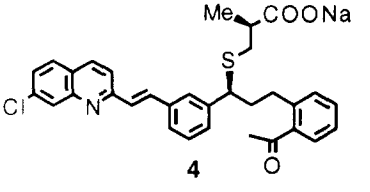
Abstract. Structure-activity studies leading to the discovery of a new series of non-quinoline cysLT$_1$ receptor (LTD$_4$ receptor) antagonists are described. These studies demonstrated that the quinoline ring system of montelukast (**5**) may be replaced by an appropriately substituted thienopyridine system, yielding potent compounds. Two other molecular features of montelukast, the terminal phenyl ring substitution and the vinyl link, were also reevaluated. These studies led to the identification of **1** (**L-740,515**), a compound with optimized *in vitro* and *in vivo* biological profiles.

A number of specific and orally active cysLT$_1$ receptor (leukotriene D$_4$ receptor) antagonists have demonstrated useful activity in human bronchial asthma.[1] We have recently described new series of styrylquinoline thioethers, exemplified by compounds **2**, **3**[2], **4**,[3,4] and **5**,[5] which were potent cysLT$_1$ receptor antagonists and were orally active in animal models of asthma. All these compounds, including **5**[6] (Singulair®, montelukast, MK-0476, L-706,631) and its predecessor verlukast (**6**), had in common the chloroquinoline ring system. In the search for a backup compound for montelukast, we focused our structure activity relationship (SAR) studies on finding an alternative for this quinoline moiety. These studies led to the identification of **1** (**L-740,515**).



2 (R) isomer, **3** (S) isomer



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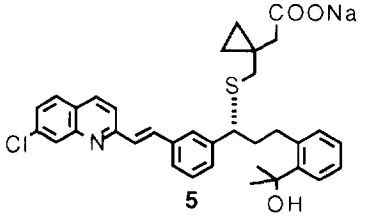

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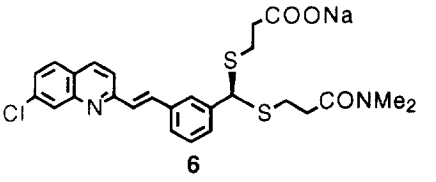

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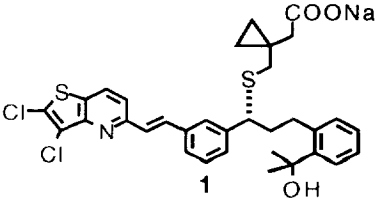
The strategy used in evaluating the compounds consisted of three steps.

First, the *in vitro* potency on the guinea pig receptor in the presence and absence of added human serum albumin (HSA) was determined. The ratio of binding activity with HSA over that without HSA (the protein shift) was found to correlate to the effectiveness of the compound *in vivo* in the squirrel monkey model[8] and ultimately in humans. Compounds with smaller shifts have shown superior *in vivo* activity relative to more shifted compounds, presumably due to less protein binding. Compounds were then evaluated for potency on the human receptor (dU937 cell membranes), followed by an evaluation of pharmacokinetics in the squirrel monkey. Compounds satisfying on the above criteria were finally tested in the peroxisomal enzyme induction assay in mice[9] and in the LTD$_4$ challenged conscious squirrel monkey model[9].

Amongst the various quinoline surrogates studied[9], the thienopyridine ring system consistently yielded potent compounds and thus was examined more extensively. Table 1 shows the effect of halogen substitution on the thienopyridine group. On the human cysLT$_1$ receptor,[10] intrinsic potency appeared to parallel the electron withdrawing ability of the substituents on the thiophene ring. This trend was not obvious from the guinea pig lung membrane assay results, but a correlation was observed when the assay was performed

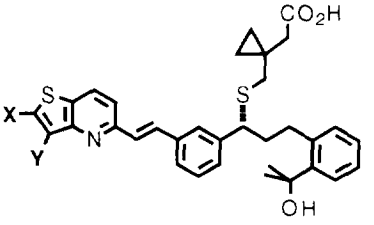


1

in the presence of 0.05% human serum albumin (HSA). The IC_{50} of the unsubstituted compound **7** was *17-fold less* potent in the presence of 0.05% HSA, whereas the dihalo compounds **1** and **10** were *3-fold more* potent in the presence of 0.05% HSA. This shift of potency in the presence of added protein appeared to correlate with enhanced *in vivo* efficacy, as exemplified by **8** and **1**, in the blockade of LTD_4-induced bronchoconstriction in squirrel monkeys with 4h predosing of drug. The protein shifted compound **8** caused a 66% (n=2) inhibition of LTD_4 response at 10μg/kg p.o., whereas the inversely shifted compound **1** caused a 90% (n=5) inhibition at 3μg/kg p.o., under the otherwise identical conditions.

The two best compounds of Table 1, **10** and **1**, differed in two aspects. First, the terminal half-life of **10** was shorter than that of **1**, which was also reflected in the clearance rates. Second, and perhaps more importantly, the difluoro compound **10** was susceptible to attack by nucleophiles such as hydroxide and alkoxide. This intrinsic instability of the difluoro thienopyridine made the preparation of pure carboxylic acid difficult by synthetic routes which involved hydroxide, such as a saponification, and implied potential reactivity towards biological nucleophiles. For these reasons, the dichlorothienopyridine group was selected for completing the SAR studies in this series.

TABLE 1. Effect of the substitution on the thienopyridine ring system



	X	Y	Guinea pig IC_{50}[a] (nM)	Guinea pig HSA IC_{50}[b] (nM)	Human IC_{50}[c] (nM)	Monkey pharmacokinetics CL[d]	$t_{1/2}$[e]	F[f]
7	H	H	1.31±0.16	20.4±5.00	8.49±3.4	-	-	-
8	H	Cl	0.54±0.20	1.58±0.40	1.91±0.29	0.39	-	13%
9	Cl	H	0.72±0.24	0.57±0.15	1.16±0.33	1.2	-	31%
1	Cl	Cl	0.89±0.26	0.34±0.07	0.55±0.28	0.57	4.2	29%
10	F	F	0.42±0.17	0.26±0.03	0.76±0.19	1.61	2.5	25%

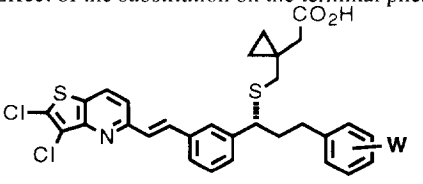
a) Inhibition of binding of [^{3}H]LTD_4 to guinea pig lung membrane.[11] Values are mean ± S.E.M. or individual determinations. **b)** Binding assay performed as in a), but the incubation mixture is supplemented with 0.05% HSA. **c)** Inhibition of binding of [^{3}H]LTD_4 to DMSO differentiated U937 cell membranes.[10] Values are mean ± S.E.M. or individual determinations. **d)** Clearance in ml/min.•Kg, estimated from the area under the curve (AUC) of the drug concentration vs. time graph, after 5 mg/kg i.v. dosing in two squirrel monkeys. **e)** Terminal half-life in hours, estimated by fitting the i.v. curve to the four parameters equation of the two-compartment model $C = Ae^{-ax} + Be^{-bx}$ and calculating $t_{1/2} = \ln(2)/b$. **f)** Bioavailability, calculated from the ratio of the p.o. and i.v. drug concentration curve AUC's, after dosing 4 squirrel monkeys at 5 mg/kg.

Using the dichlorothienopyridine group, the optimal terminal phenyl ring substitution was then investigated (Table 2). In general, for a given substituent, the ortho, meta or para substitution pattern had little effect on intrinsic potency, shift and bioavailability (compare **1** and **11**; **12**, **13** and **14**; **18** and **19**, **20** and **21**). However, the substitution pattern had an important influence on the clearance rates. Ortho substituted compounds were consistently cleared at slower rates than the corresponding meta and para regioisomers, and were therefore more desirable as drug candidates.

Unlike the substitution pattern, the nature of the substituent on the terminal phenyl ring had a marked effect on all the parameters shown in Table 2. In receptor binding assays, the compounds containing an alcohol group (**1**, **11-16**) tended to be more potent, with the exception of the very acidic bistrifluoromethylalcohol compound **15**. Initially, it appeared that potency could be related to hydrogen bond acceptor ability of the substituent. This hypothesis however was invalidated by compound **17**, which was very potent in spite of the absence of substitution on the ring.

The pharmacokinetic behavior of the compounds was also apparently affected by the nature of the terminal ring substituent. The lipophilic substituents such as Br (**18**, **19**) exhibited long half-life, but tended to be less bioavailable. An alcohol substituent seems to be optimal in providing the proper balance between clearance rate and bioavailability.

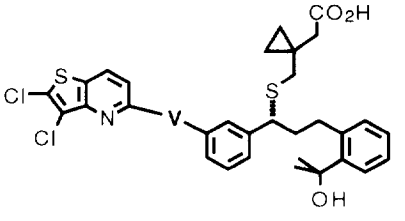
TABLE 2. Effect of the substitution on the terminal phenyl ring



	W	Guinea pig IC_{50}[a] (nM)	Guinea pig HSA IC_{50}[b] (nM)	Human IC_{50}[c] (nM)	Monkey pharmacokinetics CL[c]	$t_{1/2}$[d]	F[e]
1	o- $C(CH_3)_2OH$	0.89±0.26	0.34±0.07	0.55±0.28	0.57	4.2	29%
11	p- $C(CH_3)_2OH$	0.56±0.39	0.20±0.05	0.38±0.11	6.6		35%
12	o-1,1-c-BuOH	0.74±0.08	0.34±0.04	1.05±0.13	1.0		22%
13	m-1,1-c-BuOH	0.44±0.10	0.24±0.04	0.47±0.07	2.6		13%
14	p-1,1-c-BuOH	0.58±0.13	0.29±0.02	0.65±0.25	4.7	2.0	15%
15	o- $C(CF_3)_2OH$	4.12±1.3	1.21±0.28	6.42±1.4	0.9	11	2%
16	o- CH_2OH	0.48±0.26	0.48±0.21	0.72±0.10	3.5		
17	H	0.34±0.09	0.37±0.03	1.63±0.50	1.8	4.0	13%
18	o-Br	2.60±1.2	1.93±0.40	2.43±1.2	0.46	8.8	3%
19	p-Br	1.95±0.39	1.03±0.04	4.70±1.3	1.6	5.1	3%
20	o-CF_3	1.95±0.22	0.87±0.01	3.36±1.3	0.4	6.1	1%
21	p-CF_3	3.84±0.82	1.43±0.26	8.67±0.84	1.4		4%
22	p-F	1.09±0.50	0.65±0.11	2.16±0.63	1.8		5%

For table footers, see Table 1.

TABLE 3. Effect of the thienopyridine-phenyl link



Compound	V	Guinea pig IC_{50}[a] (nM)	Guinea pig HSA IC_{50}[b] (nM)	Human IC_{50}[c] (nM)
1	-CH=CH-	0.89±0.26	0.34±0.07	0.55±0.28
23	$-CH_2O-$	0.42±0.09	2.20±0.6	2.01±0.46
24	(E)-1,2-c-Pr	10.9±2.6	9.12±1.7	15.7±2.6
25	$-CH_2CH_2-$	0.42±0.04	3.21±1.1	1.06±0.17
26	$-OCH_2-$	>150	>150	>150,>150

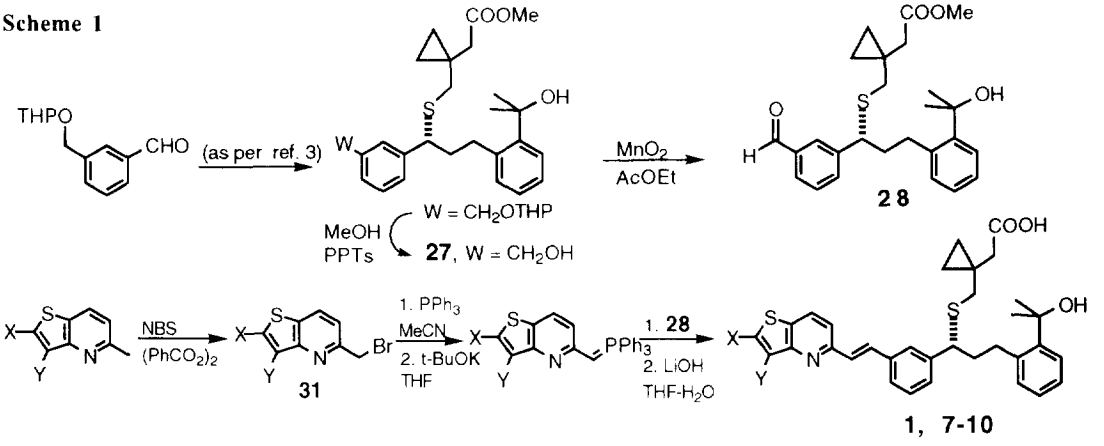
For table footers, see Table 1.

The effect of changing the vinyl link between the thienopyridine and the right hand portion of the molecule was also investigated (Table 3), keeping the dichlorothienopyridine and the ortho tertiary alcohol constant. While **23** and **25** bound to the receptor with potencies comparable to that of **1**, they were significantly shifted in the presence of HSA. It was also interesting to note that **26**, a subtle change from **23**, lost practically all affinity for the cysLT$_1$ receptor. This may relate to the drastic effect of such a change on the basicity of the thienopyridine nitrogen.

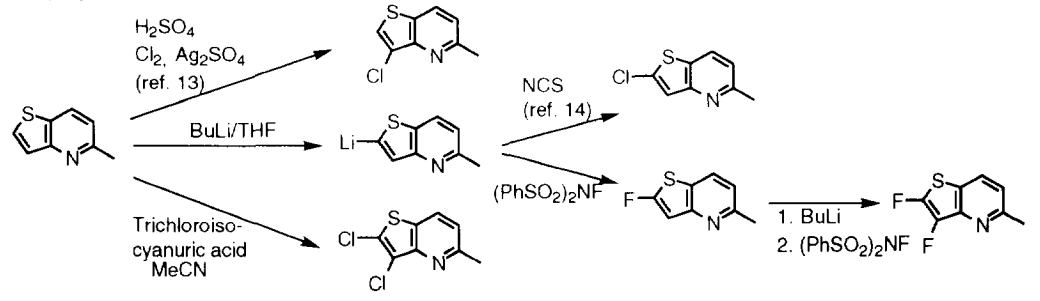
L-740,515: pharmacological profile. In the guinea-pig lung membrane binding assay[10], **1** competed with [^{3}H]leukotriene D$_4$ with an IC$_{50}$ of 0.89 ± 0.26 nM. In the presence of 0.05 % HSA added to the binding assay, the IC$_{50}$ was 0.34 ± 0.07 nM. In LTC$_4$ and LTB$_4$ binding assays with dU937 and PMN membranes respectively, IC$_{50}$ values of >46 μM and 3 μM were observed. Oral administration of 0.003 mg/kg of **1** as the sodium salt in 1.0% methocel to squirrel monkeys,[8] followed 4 h later by a LTD$_4$ challenge, produced a 90% ($p<0.01$) inhibition of the increase in specific airway resistance, relative to those produced by a control leukotriene D$_4$ challenge in the same animals treated with vehicle alone. Under a more stringent assay protocol, namely with a 24 h drug pretreatment before LTD$_4$ challenge, a 0.03 mg/kg dose of **1** produced a 100% (n=2) inhibition of the increase in specific airway resistance. Treatment of mice for 4 days with either **1** at 400 mg/kg p.o. or vehicle alone, followed by weighing of the livers and assay of the FACO activity[4] showed no statistically significant difference between the two groups.

Chemistry[12]. The compounds of Table 1 were prepared as shown in Schemes 1 and 2. The approach involved coupling the various thienopyridines with the constant "right hand" portion of the molecule **28** through a Wittig reaction. The thienopyridine ring system was prepared as described in the literature[13], and functionalized as described in Scheme 2.

Scheme 1



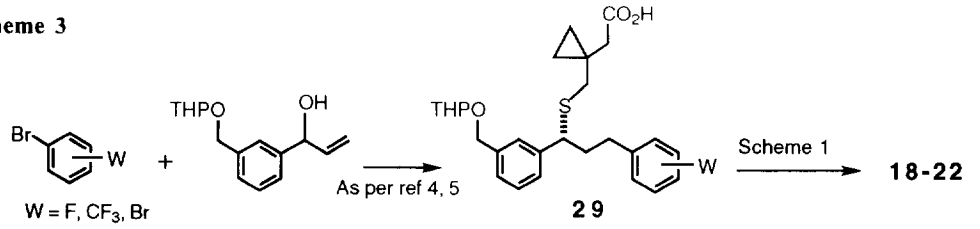
Scheme 2



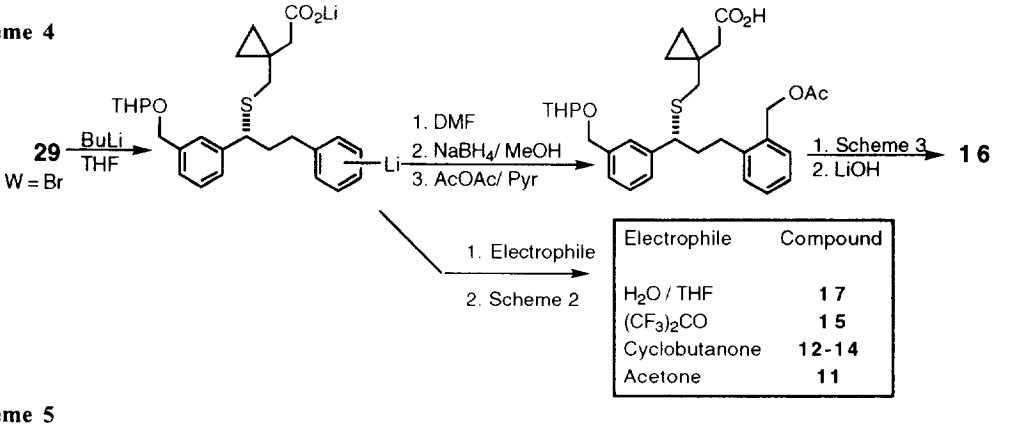
Schemes 3 and 4 describe the synthetic strategy used in the preparation of compounds with various terminal phenyl ring substituents. Some substituents could be carried through the synthesis (Scheme 3), which allowed the use of the route described in Scheme 1. For the other compounds, the bromo intermediates **29** were

found to be critical building blocks, leading to several derivatives (Scheme 4) after a metalation step with butyllithium.

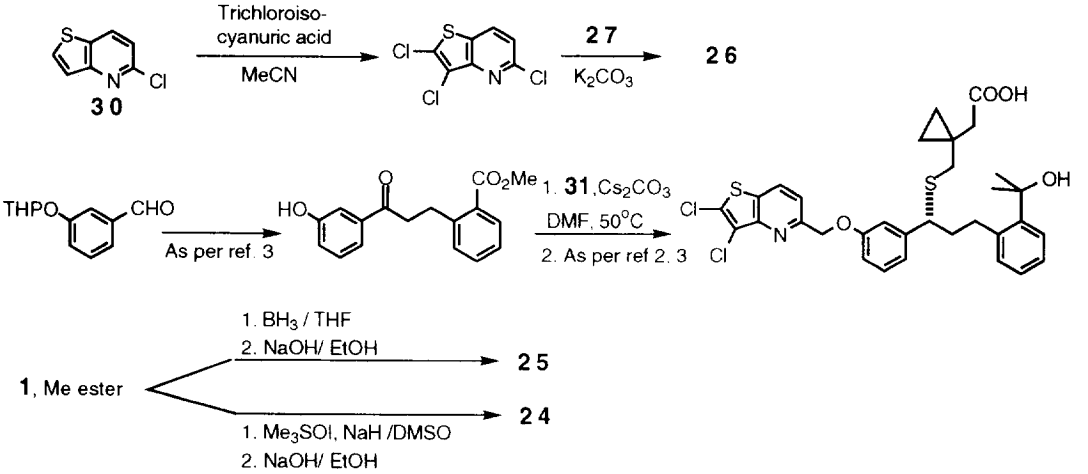
Scheme 3



Scheme 4



Scheme 5



Scheme 5 describes the preparation of the compounds of Table 3, where the thienopyridine-phenyl link was modified. The two ether compounds were prepared from **30**[15] and intermediates described in other compounds, whereas the cyclopropane and ethylidene linked compounds were prepared from the methyl ester of the olefinic compound **1**.

Conclusion. A new series of potent, selective, orally active thienopyridine cysLT$_1$ receptor antagonists devoid of a quinoline ring system has been discovered. SAR studies showed that dichloro substitution on the thiophene ring of the thienopyridine gave very potent compounds with advantageous pharmacokinetic properties. The t-alcohol group on the terminal phenyl ring was found to be optimal for proper balance of

pharmacodynamics and pharmacokinetics. The olefin link between the thienopyridine and the central phenyl ring gave the most interesting compounds. This study yielded **1** (**L-740,515**) as an optimized candidate.

References and Notes.

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