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# A SERIES OF NON-QUINOLINE cysLT$_1$ RECEPTOR ANTAGONISTS: SAR STUDY ON PYRIDYL ANALOGS OF SINGULAIR®

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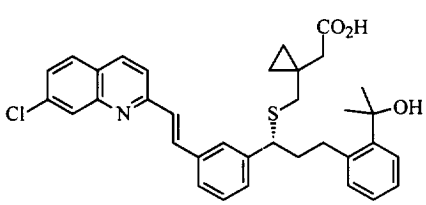
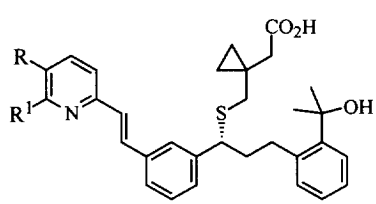
**Abstract:** The structure–activity relationship of a series of styrylpyridine analogs of MK-0476 (montelukast, Singulair®) is described. This work has led to the identification of a number of potent and orally active cysLT$_1$ receptor (LTD4 receptor) antagonists including **2ab** (**L-733,321**) as an optimized candidate.


Peptidoleukotrienes were long believed to be important biological mediators in several hypersensitivity and allergic diseases, such as asthma.[1] In particular, LTD4 has been shown to cause a prolonged constriction of the respiratory smooth muscle.[2] These compounds also play a fundamental role in mucus production and changes in vascular permeability. These findings, amongst others, have prompted intense research in the scientific community that resulted in the identification, by us[3,4] and others,[5] of a number of potent cysLT$_1$ receptor antagonists. Thus, a few years ago we reported Singulair® (**1**) (montelukast) as a very potent, orally active cysLT$_1$ receptor antagonist in animal models[4] and in man.[6] Ultimately, the benefits of cysLT$_1$ receptor antagonist therapy for the treatment of human bronchial asthma were recognized with the recent market approval of Accolate® and of Singulair®.

While Singulair® clinical trials were underway, we decided to focus our attention on finding other potential development candidates. Our search for a backup compound was guided by the following criteria: the compound should be structurally different to **1** and with at least an equivalent overall profile in our *in vitro* and *in vivo* assays. Part of our investigation focused on finding a surrogate for the 7-chloroquinoline moiety which has been a constant motif of all our clinical candidates so far[3] and some of this work has already been reported.[7] Herein we describe the structure–activity relationship (SAR) of a series of pyridine analogs of Singulair®.




Singulair® (**1**)




**2**

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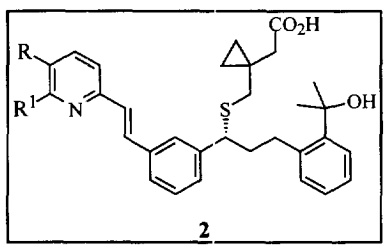
**Results and discussion.** The *in vitro* potency of the compounds was initially measured as their binding affinities ($IC_{50}$) on the receptor from guinea pig lung membranes[8] with or without added human serum albumin (HSA). The difference between these two $IC_{50}$'s was taken as a indirect evaluation of the potential for protein binding in blood (or protein shift). As already reported,[7b] compounds with smaller shifts have a superior *in vivo* profile than those substantially shifted in the presence of HSA. *In vitro* potency was also evaluated on the human receptor in a DMSO differentiated U937 cell line.[9] The most potent antagonists were also subjected to pharmacokinetic evaluation in both rats and in squirrel monkeys and the best compounds were tested for *in vivo* activity in the LTD4 challenged conscious squirrel monkey model.

As shown in Table 1, the unsubstituted pyridine (**2a**) was only moderately potent and introduction of a polar or bulky substituent at the 5-position of the heterocycle (**2b–c**) did not improve the *in vitro* potency. Smaller groups and simple primary alkyl substituent (**2d–f**) resulted in an increase of potency by approximately

**Table 1.** *In vitro* potency of monosubstituted pyridines.




**2**

| Compound | R | $R^1$ | Guinea Pig $IC_{50}$[(a)] (nM) |
|---|---|---|---|
| **2a** | H | H | 194 ± 3.5 |
| **2b** | $CO_2H$ | H | 421 |
| **2c** | Ph | H | 410 |
| **2d** | $CF_3$ | H | 25 |
| **2e** | $CH_3O$ | H | 12.7 ± 0.2 |
| **2f** | *n*-Bu | H | 11.8 ± 2.5 |
| **2g** | H | Cl | 22.8 ± 1.7 |
| **2h** | H | *n*-Pr | 1.2 ± 0.3 |
| **2i** | H | *n*-Bu | 2.4 ± 0.3 |
| **2j** | H | *i*-Pr | 2.4 ± 0.1 |
| **2k** | H | *c*-Bu | 0.8 ± 0.1 |

**Table 2.** *In vitro* potency of disubstituted pyridines.

| Compound | R | $R^1$ | Guinea Pig $IC_{50}$[(a)] (nM) | Guinea Pig HSA $IC_{50}$[(b)] (nM) |
|---|---|---|---|---|
| **2l** | $CF_3$ | Et | 25.2 ± 8.6 | 36.9 ± 0.9 |
| **2m** | Cl | Et | 1.2 ± 0.3 | 1.7 ± 0.2 |
| **2n** | Et | $CH_3$ | 2.7 ± 0.4 | 50.4 ± 3.2 |
| **2o** | $CH_3$ | $CH_3$ | 0.9 ± 0.1 | 24.8 ± 1.5 |
| **2p** | $CH_3$ | Et | 0.8 ± 0.3 | 3.4 ± 0.4 |
| **2q** | $CH_3$ | *i*-Pr | 4.9 ± 1.1 | 4.7 ± 0.1 |
| **2r** | $CH_3$ | *i*-Bu | 1.4 ± 0.2 | 1.2 ± 0.1 |
| **2s** | $CH_3$ | *n*-Pr | 0.5 ± 0.1 | 0.7 ± 0.1 |
| **2t** | $CH_3$ | *n*-Bu | 0.9 ± 0.2 | 0.5 ± 0.1 |

(a) Inhibition of [$^3$H]$LTD_4$ binding to guinea pig lung membrane.[8] Values are individual determinations or mean ± average deviation where $n \geq 2$. (b) As in a), but 0.05% (w/v) HSA is added to the incubation mixture.

tenfold. Another order of magnitude was gained when the small alkyl substituent was moved from the 5- to the 6-position of the pyridine ring as in **2h–k**. The next logical step was then to investigate 5,6-disubstituted pyridine analogs and this lead to a series of very potent antagonists where several interesting trends were found. The 5-ethyl-6-methyl pyridine analog **2n** had an average $IC_{50}$ of 2.7 nM on the guinea pig receptor but was shifted to 50 nM in the presence of HSA. The 5,6-dimethyl compound **2o** was more potent but still suffered a large shift by HSA (0.9 nM/24 nM) whereas the 5-methyl-6-ethyl analog **2p** had an $IC_{50}$ of 0.8 nM, and was only shifted to 3.4 nM with added protein. This and other examples (**2q–2t**) indicated that whereas substitution at both positions increases the potency, the combination of a relatively small nonpolar group at the 5-position and a larger lipophyllic group at the 6-position gives compounds which retain the potency with less shift associated with protein binding.

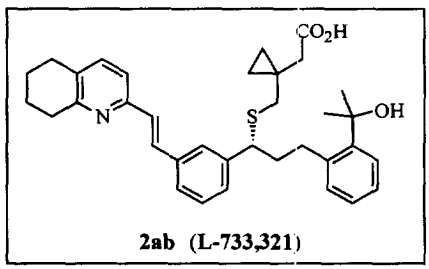
Joining the 5- and 6-substituents together gave 5,6-ring fused pyridine derivatives **2u–ae** (Table 3). All of the five-, six-, and seven-membered fused ring systems prepared were found to be very potent *in vitro*. Many of these exhibited subnanomolar $IC_{50}$'s on the human receptor. Their pharmacokinetic parameters in rat and in squirrel monkey were evaluated (data not shown). The compound with the best overall profile, particularly in terms of bioavailability and terminal half life, was the unsubstituted 5,6-cyclohexenopyridine analog **2ab**.

**Table 3**. *In vitro* potency of 5,6-ring fused pyridines

| Compound | R —— $R^1$ | Guinea pig $IC_{50}$[(a)] (nM) | Guinea pig HSA $IC_{50}$[(b)] (nM) | Human $IC_{50}$[(c)] (nM) |
|---|---|---|---|---|
| **2u** | $(CH_2)_3$ | 0.5 ± 0.04 | 4.5 ± 0.6 | 0.44 |
| **2v** | $SCH_2CH_2$ | 0.74 ± 0.1 | 4.8 ± 0.4 | 2.9 ± 0.2 |
| **2w** | $CH_2SCH_2$ | 0.21 ± 0.02 | 2.1 ± 0.3 | 0.61 ± 0.07 |
| **2x** | $CH_2CH_2CH(CH_3)$ | 0.73 ± 0.16 | 1.35 ± 0.05 | 1.7 ± 0.1 |
| **2y** | $CH_2C(CH_3)_2CH_2$ | 0.34 ± 0.08 | 0.43 ± 0.07 | 0.56 ± 0.07 |
| **2z** | CH2CH2C(CH3)2 | 0.87 ± 0.14 | 0.35 ± 0.03 | 2.0 ± 0.2 |
| **2aa** | $(CH_2)_5$ | 0.58 ± 0.08 | 1.8 ± 0.4 | 1.1 |
| **2ab** | $(CH_2)_4$ | 0.19 ± 0.05 | 1.8 ± 0.6 | 0.25 ± 0.11 |
| **2ac** | $(CH_2)_3CH(CH_3)$ | 0.51 ± 0.03 | 0.63 ± 0.02 | 0.47 |
| **2ad** | $(CH_2)_2C(CH_3)_2CH_2$ | 0.19 ± 0.03 | 0.17 ± 0.04 | 0.42 ± 0.17 |
| **2ae** | $(CH_2)_3C(CH_3)_2$ | 2.6 ± 0.7 | 0.75 ± 0.17 | 5.0 |
| **(1)** | **Singulair®** | 0.64 ± 0.36 | 0.43 ± 0.17 | 0.78 ± 0.35 |

For footers a and b see table 2. (c) Inhibition of $[^3H]LTD_4$ binding to DMSO differentiated U937 cell membranes.[9]

**Pharmacological profile of L-733,321**. As shown in Table 3, **L-733,321** (**2ab**) has an $IC_{50}$ of 0.2 nM on the guinea pig receptor with a ninefold shift in the presence of 0.05% HSA, and on the human receptor the average $IC_{50}$ is 0.25 nM. For Singulair® (**1**), the $IC_{50}$ values were 0.64 nM, 0.42 nM, and 0.78 nM, respectively. Excellent pharmacokinetics were found for compound **2ab** in a number of animal species. Bioavailability was 39% and 40% in the rat and in the squirrel monkey respectively, compared to 29% and 42% for **1**. The plasma concentrations following a 10 mg/kg oral administration of **2ab** in squirrel monkey were

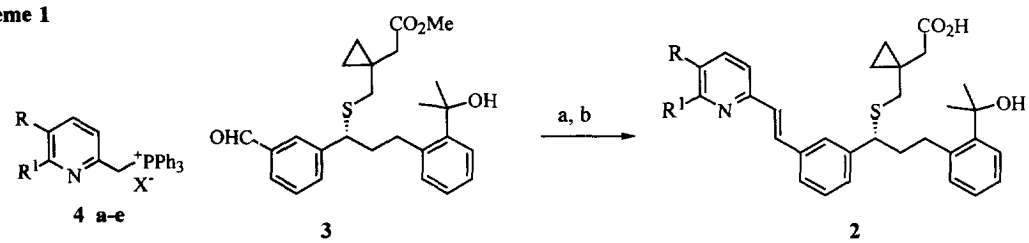



**2ab (L-733,321)**

excellent with a $C_{max}$ at 2 h post dose of 33.2 μg/mL and with a terminal half-life of at least 6 h compared to 4 h for **1**.[4a] In our conscious squirrel monkey *in vivo* model,[10] an oral dose of 0.01 mg/kg (4 h pretreatment) of **L-733,321** (**2ab**) produced a 72% inhibition of the increase in pulmonary airflow resistance and a 90% inhibition of the decrease in dynamic compliance induced by an areosol LTD4 challenge. These figures are 52% and 78%, respectively, in the case of Singulair® (**1**).[4b] Clearly, according to our *in vitro* and *in vivo* models, **L-733,321** is a potent antagonist of the $cysLT_1$ receptor.

**Chemistry.**[11] Synthesis of the various styryl pyridine analogs **2** is outlined in Scheme 1. The strategy involved a Wittig-type condensation of the readily available aldehyde intermediate **3**[7a] with the corresponding pyridine phosphonium salts **4a–ae** to give exclusively the *trans*-styrylpyridine derivatives. Saponification of the methyl ester gave the desired carboxylic acids **2.**

**Scheme 1**

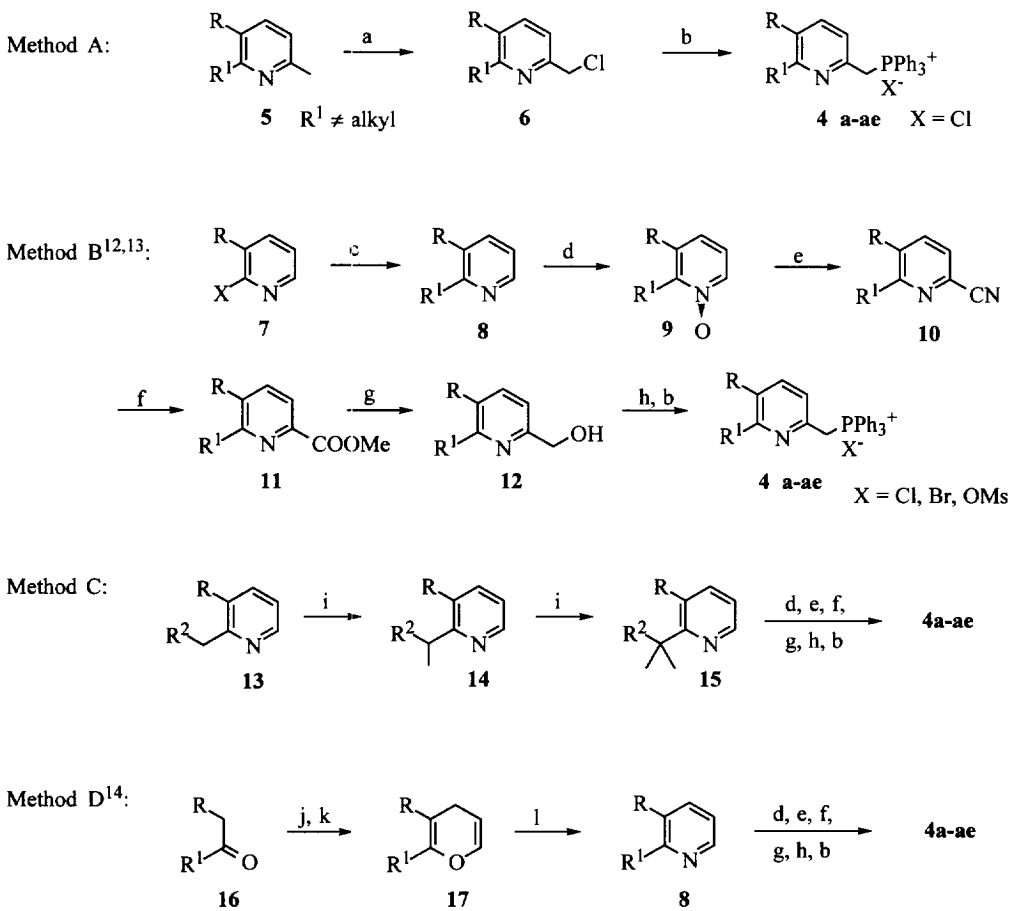



**4 a-e** **3** **2**

Conditions: (a) *n*-BuLi or *t*-BuOK, THF, 0 $^0$C to rt; (b) NaOH aq, MeOH, THF

The Wittig reagents **4** were prepared according to one of the methods shown in Scheme 2. For most of the 6-alkyl and 5,6-dialkylpyridine derivatives, the phosphonium salts were prepared by method B. Introduction of a primary alkyl group could be easily achieved by the nickel catalyzed cross-coupling reaction[12] of a Grignard reagent to the available fluoro-, chloro-, or bromopyridines **7** to give the 6-primary alkylpyridines **8**. Oxidation using either *m*-chloroperbenzoic acid or monoperoxyphthalic acid magnesium salt (hexahydrate) gave the pyridine N-oxides **9**, which were treated with dimethylcarbamyl chloride and trimethylsilyl cyanide[13] to give the 2-cyanopyridines **10**. Acid methanolysis of the nitriles afforded the corresponding methyl esters **11** that could be reduced to the primary alcohols **12** by treatment with diisobutyl-aluminium hydride. Then, under standard conditions, these alcohols were transformed to mesylates, chlorides, or bromides, which upon treatment with triphenylphosphine yielded the desired Wittig reagents **4a–ae**. In order to prepare pyridine analogs bearing a secondary or tertiary alkyl substituent at the 6-position, the methylene group of intermediate **13** could be methylated once or twice to give **14** and **15**, respectively (method C). Further transformation as described above furnished the corresponding phosphonium salts **4a–ae**. Finally, in some cases the pyridine ring had to be prepared from the ethanone precursors **16** (method D).[14] Formation of its trimethylsilyl enolether was followed by lanthanide catalyzed hetero Diels–Alder reaction with acrolein to yield the disubstituted 4H-pyran derivatives **17**. Then, treatment with ammonium acetate and cupric acetate gave the 2,3-dialkylpyridine compounds **8**.

**Scheme 2**




Conditions: (a) $POCl_3$, $Et_3N$, $CH_2Cl_2$; (b) $Ph_3P$, $CH_3CN$ or tol., Δ; (c) $R^1MgX$, $NiCl_2$(dppp) cat., $Et_2O$, 0 °C; (d) *m*-CPBA, $CH_2Cl_2$ or MMPP•$6H_2O$, MeOH/$CH_2Cl_2$; (e) $Et_2NCOCl$, TMSCN, $ClCH_2CH_2Cl$, rt; (f) MeOH, dry HCl, Δ; (g) DIBAH, $CH_2Cl_2$, -78 °C to rt; (h) $Ph_3P$•$Br_2$ or MsCl, $Et_3N$ or $SOCl_2$, $CH_2Cl_2$; (i) LDA, MeI; (j) LDA, TMSCl, THF, -78 °C to 0 °C; (k) acrolein, Yb(fod)$_3$, $ClCH_2CH_2Cl$; (l) $NH_4OAc$, AcOH, Cu(OAc)$_2$, Δ.

**Conclusion**. A new series of potent and selective cysLT$_1$ receptor antagonists has been discovered by replacing the 7-chloroquinoline moiety in **1** by a variety of 5,6-disubstituted pyridines. The compound with the best overall profile in this class, **L-733,321** (**2ab**) is very active orally in blocking the LTD4-induced bronchoconstriction in the squirrel monkey.

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