Bioorganic & Medicinal Chemistry Letters, Vol.2, No.12, pp. 1699-1702, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

DEVELOPMENT OF L-689,065 - THE PROTOTYPE OF A NEW CLASS OF POTENT 5-LIPOXYGENASE INHIBITORS

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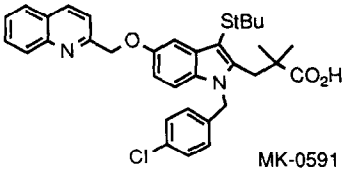
(*Received* 22 *September* 1992)

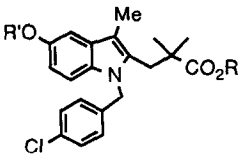
Abstract: The combination of a 5-phenylpyridine substituent and a novel, substituted thiopyrano[2,3,4-c,d]indole ring system yields a potent and selective 5-lipoxygenase inhibitor, L-689,065.

It has recently been established that 5-lipoxygenase (5-LO) inhibitors[1] and leukotriene D_4 antagonists[2] are clinically effective in treating asthma. In addition, leukotrienes have been implicated in other disease states[3] such as inflammatory bowel disease, psoriasis and rheumatoid arthritis. Thus the inhibition of the biosynthesis of leukotrienes by blocking the action of 5-LO, the first dedicated enzyme in the leukotriene cascade, may also provide a therapy for these diseases. Specific inhibitors of leukotriene biosynthesis can be divided into two classes: those that act by inhibiting 5-LO (direct inhibitors)[4] and those that act by binding to 5-lipoxygenase activating protein (FLAP)[5] thereby inhibiting the translocation of 5-LO to the membrane (indirect inhibitors). We have pioneered the latter approach which has led to the development of MK-0591[6], a potent and orally active FLAP inhibitor which is currently undergoing clinical evaluation. During the development of MK-0591 it was noticed that, in addition to binding to FLAP , some analogues were weak inhibitors of 5-LO activity. In this paper we describe how the judicious combination of structural features has led to the evolution of a new class of selective, *direct* 5-LO inhibitors from our *indirect* FLAP series of leukotriene biosynthesis inhibitors. This new class of 5-LO inhibitors are potent, bioavailable and functionally active.

The SAR investigations of the quinoline-indole class of FLAP inhibitors[7] (typified by MK-0591) focused on several areas. Two key modifications were the replacement of the quinoline group with other heterocycles and the investigation of lipophilic substituents at C-3 and C-4 of the indole ring. The initial studies into the substitution of the quinoline for other heterocycles were carried out on compounds containing a methyl group at C-3 of the indole. This was due, in part, to the ease of preparation and availability of the starting phenol 1. Among the various heterocyclic analogues prepared were the quinoline 2 and the 5-phenylpyridine derivative 3. As can be seen from the Table, the quinoline 2 is very potent in the FLAP binding assay[8] with an IC_{50} of 2.8 nM but it is essentially inactive as an inhibitor of rat 5-LO [9]. The phenylpyridine 3 is considerably less potent at binding to FLAP (IC_{50}=9.3 μM) but, more interestingly, showed weak activity (IC_{50}≈0.7 μM) when screened against the rat 5-LO enzyme. Presumably the increased ability to inhibit the 5-LO enzyme is largely associated with the 5-phenyl pyridine moiety.



MK-0591



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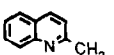
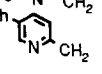
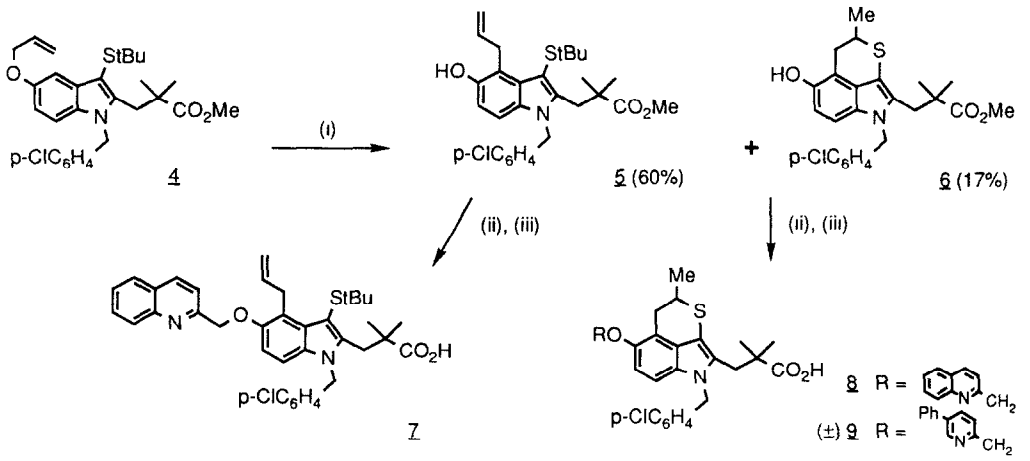
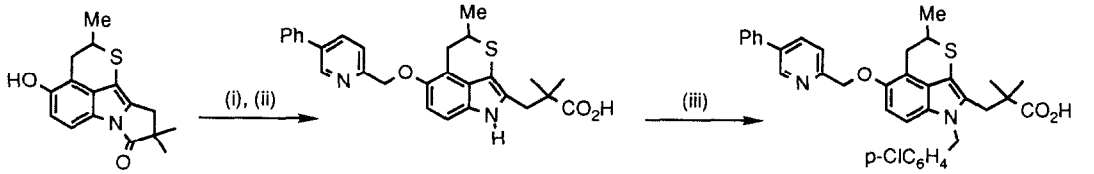
2 R = H, R' = 

3 R = H, R' = 



Scheme 1 Reagents. (i) Δ / 1,2-$Cl_2C_6H_4$, (ii) RCl/Cs_2CO_3/DMF,80% (iii) LiOH/THF/MeOH/H_2O,90%



Scheme 2 Reagents: (i) 5-Ph-$C_5H_3NCH_2Cl$/Cs_2CO_3/DMF;78% (ii) LiOH/THF;98% (iii) p-$ClC_6H_4CH_2Cl$/NaH/DMF;40%

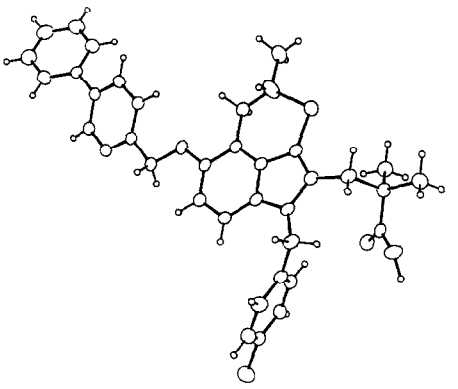


Figure 1 ORTEP Drawing of L-689,065 (With 20% Ellipsoids)

Entry	Compd.	FLAP binding (IC_{50})	5-LO (IC_{50})
1	MK0591	2.3 nM	>2 μM
2	2	2.8 nM	>5 μM
3	3	9.3 μM	0.7 μM
4	7	27nM	>8 μM
5	8	19nM	0.3 μM
6	(±) 9	22 μM	60 nM
7	(+) 9	>10 μM	150 nM
8	(-) 9	>10 μM	40 nM

Table

Concurrent with these studies, investigations were also underway to examine the effect of lipophilic substituents on the indole skeleton. We were aware from our previous work on an indole series of FLAP inhibitors[10] that a lipophilic substituent at C-3 (e.g. StBu) is required for optimum activity. This led us to consider the effect of substitution at the C-3 and C-4 positions of the current series. Functionalization at C-4 was achieved by a Claisen rearrangement of the allyl ether 4 (Scheme 1) which afforded the desired C-4 allyl indole 5 as well as the thiopyrano[2,3,4-c,d]indole 6[11]. The phenols of both products were alkylated with 2-chloromethylquinoline followed by hydrolysis of the methyl esters to provide the quinolines 7 and 8 respectively. When tested in the FLAP binding assay both 7 and 8 were found to be less potent than MK-0591 (see Table). The allyl indole 7 was also inactive as a 5-LO inhibitor at 8 μM. As before, we were intrigued by the observation that the quinoline 8 possessed modest 5-LO inhibitory properties with an IC_{50} of 0.3 μM and we attributed this to the thiopyran portion of the molecule.

The observation of the weak direct 5-LO inhibitory effect of both the phenylpyridine 3 and the thiopyranoindole 8 presented us with an opportunity to study the effect of combining the key portions of each molecule which appeared to be contributing to this activity. Thus alkylation of the phenol 6 with 2-chloromethyl-5-phenylpyridine and subsequent hydrolysis yielded the desired hybrid compound (±) 9, designated L-689,065[12]. Gratifyingly, this compound proved to be considerably more potent as a 5-LO inhibitor than either of its progenitor compounds. In fact, with an IC_{50} in the rat 5-LO assay of 60 nM[13], L-689,065 is more potent than A-64077 (Zileuton; IC_{50}=200nM)[14] which has shown efficacy in clinical trials for asthma[1a]. Another noteworthy point is the remarkable ability to target FLAP or 5-LO activity selectively by choosing either a quinoline or 5-phenylpyridine substituent (Table, entries 5 and 6).

Since L-689,065 contains the unprecedented thiopyrano[2,3,4-c,d]indole ring system, the crystal structure was obtained by single crystal X-ray diffraction analysis[15]. The ORTEP structure is given in Figure 1 and it confirms the structural assignment. Interestingly, the compound is relatively flat with the p-chlorobenzyl and acid groups lying below the plane. As L-689,065 contains a chiral centre we were interested to determine if the enzyme discriminated between the individual enantiomers. Resolution of the enantiomers was achieved through the HPLC separation of the tetracycle 10 (Scheme 2) using a chiral column (Chiralcel OD column, Daicel Chemical Industries, Ltd). The individual enantiomers of 10 thus obtained were then converted to (+) 9 and (-) 9 as shown. Analysis of the enantiomers in the 5-LO assay showed that (-) 9 is 3 to 4 times more potent than (+) 9 (40 nM versus 150 nM) - the enzyme is indeed able to discriminate between the two enantiomers (both inactive versus FLAP binding). The enzyme also requires the acid group for binding as witnessed by the fact that the methyl ester of L-689,065 is inactive. In light of these results it is interesting to speculate that L-689,065 is acting as a substrate mimic for the 5-LO enzyme. Arachidonic acid could adopt a conformation wherein superposition of the acid groups allows the C-20 chain to partially overlap the thiopyranoindole ring system.

Preliminary biological studies show that when L-689,065 (sodium salt) was given to rats at a dose of 20mg/kg P.O. (1% methocel) and 5mg/kg I.V.(PEG 200) the compound was well absorbed (C_{max}=5 μM, t_{max}=2-4 hr) and bioavailable (AUC=37%). Also the compound was found to be active in the hyperreactive rat model[16] with 55% inhibition of dyspnea at 0.5 mg/kg P.O.(1% methocel; 4 hour pretreatment time).

In summary, we have described the discovery of a new class of direct 5-LO inhibitors which contain a 5-phenylpyridine moiety appended to a novel thiopyrano[2,3,4-c,d]indole ring system. The prototype of this class,

L-689,065, is potent, well absorbed, bioavailable and functionally active in the rat. In future publications we will report on biochemical and pharmacological studies on L-689,065 as well as SAR investigations in this series.

Acknowledgements: The authors wish to thank Dr.D.Macdonald for the preparation of 10.[17]

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12. L-689,065, (±) 9, chemical name: 3-[1-(4-chlorobenzyl)-4-methyl-6-(5-phenylpyridin-2-ylmethoxy)-4,5-dihydro-1H-thiopyrano[2,3,4-c,d]indol-2-yl]-2,2-dimethylpropanoic acid. ^{1}H NMR (300MHz, CDCl3) ∂:1.29(3H,s), 1.33(3H,s), 1.49(3H,d,J=6.7Hz), 2.89(1H,dd,J=16.3,9.7Hz), 2.93(1H,d,J=15Hz), 3.02(1H,d, J=15Hz), 3.37(1H,m), 3.51(1H,dd,J=16.3,2.9Hz), 5.17-5.27(4H,m), 6.7(4H,m), 7.13(2H,d, J=8.3Hz), 7.4-7.6(5H,m), 7.67(1H,d,J=8.2Hz), 7.96(1H,dd,J=8.2,2.2Hz), 8.84(1H,d,J=2.2Hz); m.p. 214-215°C.

13. Mechanistic studies show that (±) 9 inhibits both the oxygenase and pseudoperoxidase activities of human 5-LO and does not function as a reducing substrate for the enzyme. Riendeau,D.; Falgueyret ,J.-P.,unpublished results

14. Carter,G.W.; Young,P.R.; Albert,D.H.; Bouska,J.; Dyer,R.; Bell,R.L.; Summers,J.B.; Brooks,D.W *J Pharmacol. Exper Ther.*, **1991**,*256*,929. The IC50 we report is the result obtained in our own assay.

15. Crystal structure details: $C_{35}H_{33}ClN_2O_3S$, M_r = 597.18, monoclinic, $P2_1/n$, a = 5.877 (3), b = 29.467 (4), c = 17.340 (4) Å, ß = 90.49 (3)°, V = 3002 Å^3, Z = 4, D_x = 1.329 g cm^{-3}, monochromatised radiation λ(Cu K∝) = 1.54184 Å, μ = 2.08 mm^{-1}, $F(000)$ = 1256, T = 296 K. Data collected on an Enraf-Nonius CAD4 diffractometer to a 2Θ limit of 140° with 2441 observed, $I \geq 3\sigma(I)$, reflections out of 6389 measured. Structure solved by direct methods and refined using full-matrix least-squares on F using 379 parameters. All non-hydrogen atoms refined with anisotropic thermal displacements. Hydrogen atom contributions included in calculations. Final agreement statistics are: R = 0.058, wR = 0.053, S = 2.21, $(\Delta/\sigma)_{max}$ = 0.02. Weighting scheme is $1/\sigma^2(F)$. Max peak height in final difference Fourier map 0.39(5) eÅ^{-3} with no chemical significance.

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17. Preparation of 10 is described in European Patent to be published December 1992.