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SUBSTITUTED INDOLES AS POTENT AND ORALLY ACTIVE 5-LIPOXYGENASE ACTIVATING PROTEIN (FLAP) INHIBITORS

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Abstract: This paper reports on the SAR investigation of inhibitors of 5-lipoxygenase activating protein (FLAP) based on MK-0591. Emphasis was made on modifications to the nature of the link between the indole and the quinoline moieties, to the substitution pattern around the two heterocycles and to possible replacements of the quinoline moiety. Lead optimization culminated in (3-[1-(4-chlorobenzyl)-3-(*t*-butylthio)-5-(pyridin-2-ylmethoxy)-indol-2-yl]-2,2-dimethylpropanoic acid (**18k**), as a potent inhibitor of leukotriene biosynthesis that is well absorbed and active in functional models.

Introduction. Leukotrienes (LTs) have been implicated in a variety of diseases such as asthma, ulcerative colitis, and rhinitis.[1] Leukotrienes are important biological mediators derived from arachidonic acid through the action of 5-lipoxygenase (5-LO).[2] An essential component for this process in intact cells is the presence of 18kD membrane bound protein, 5-lipoxygenase activating protein (FLAP).[3] Upon cellular activation, 5-LO has been shown to translocate to the nuclear membrane[4] where it presumably can interact with FLAP and perhaps also phospholipase A_2 to produce LTs.[5] Methods that inhibit the biosynthesis of LTs have good potential as therapies for the diseases mentioned above.

Our group has shown in clinical studies that MK-0591 (**1**) demonstrated efficacy for the FLAP modality of LT intervention.[6] In the same structural series, Abbott's group attempted to optimize the *in vivo* pharmacological properties, suitable for clinical development, by inserting an oxime moiety in the 2-alkylcarboxylate substituent of **1**, resulting in a similar *in vitro* inhibitory potency in the assays studied.[7] This paper describes the continuation of our SAR investigations into the MK-0591 series with particular emphasis on modifications to the nature of the link, to the substitution pattern around the indole and the quinoline rings and finally to the possible heterocyclic replacements of the quinoline moiety.

Chemistry and Biology

In Vitro Studies. All of the compounds prepared were evaluated as inhibitors of LTB_4 biosynthesis using Ca^{2+} ionophore activated human polymorphonuclear (HPMN) leukocytes.[8] As previously reported for MK-0591, the series of compounds reported here had poor direct 5-LO catalytic inhibitory activity.[8] The drug dependent inhibition of LTB_4 biosynthesis in human whole blood (HWB) activated with the ionophore A23187 was assessed using previously described methodology.[8] The FLAP activity was evaluated by measuring the affinity of leukotriene synthesis inhibitors for FLAP, using human leukocyte membranes as the source of FLAP and a radioiodinated leukotriene synthesis inhibitor, [^{125}I]-L-691,831, as ligand.[9]

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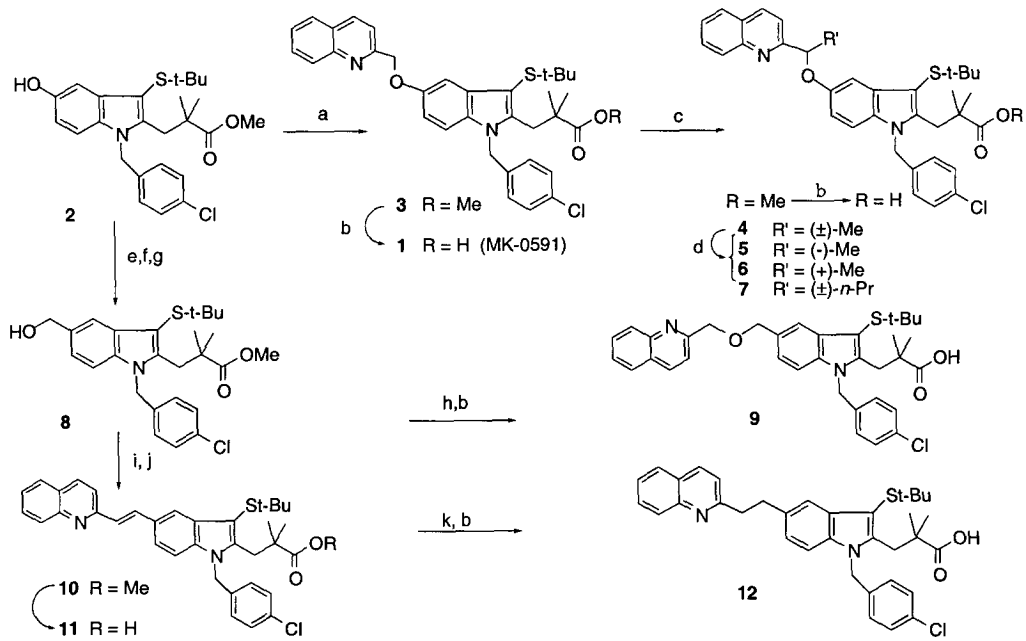
Nature of the link. Our first investigation focused on the modification of the link between the indole and the quinoline moities, the rational being to improve potency by modifying the electron density on the indole ring. Starting from the known phenol **2**,[10] we were able to prepare in a straightforward manner various types of links (Scheme 1). The results are reported in Table 1.

Scheme 1.

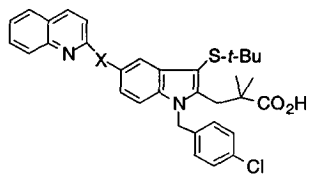


Reagents: (a) 2-bromomethyl quinoline, K_2CO_3, DMF, rt, 24 h; (b) aq LiOH, THF/MeOH, reflux, 3 h; (c) KHMDS, R'-I, THF, -78 °C to rt, 2 h; (d) Chiracel OD chiral column 50 x 2cm, hexane:*i*-PrOH (60:40); (e) Tf_2O, 2,6-di-*t*-butylpyridine, CH_2Cl_2, 0 °C to rt, 2 h; (f) CO, $Pd(OAc)_2$, dppf, DMF, 70 °C, 4 h; (g) BH_3-THF, -10 °C to rt, 18 h; (h) NaH, 2-bromomethyl quinoline, DMF, rt, 5 h; (i) MnO_2, EtOAc, rt, 18 h; (j) 2-methyl quinoline phosphonium bromide, BuLi, THF, -78 °C to rt, 30 min; (k) BH_3-THF, -10 °C to rt, 18 h.

Link substituted analogs **4-7** were obtained by alkylation of the methyl ester of MK-0591 (**3**) with alkyl iodides. Racemic α-methylated compound **4** was less active than MK-0591. Resolution with a chiral column at the ester stage afforded, after hydrolysis, the two enantiomers **5** and **6**. The (-)-isomer **5** was found to be 20 times more active than the (+)-isomer **6** in the FLAP binding assay. This position does not tolerate large groups since lower *in vitro* activities were observed in the case of the *n*-propyl analog **7**. Using palladium-mediated hydroxycarbonylation methodology,[11] we have also prepared from phenol **2**, the alcohol **8** which gives access to the extended ether linked analog **9**. Lastly, the olefinic ester **10** became the precursor to the two analogs **11** and **12** which showed reasonable HPMN and FLAP activities but poor HWB activity.

Thus, varying the nature of the link did not improve the overall activities and even the insertion of a small group such as a methyl on the ether link lowered the FLAP potency. The methyleneoxy link of MK-0591 is the optimum link identified.

Table 1. Nature of the Link

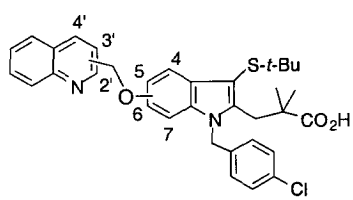


Compound	X	HPMN (IC_{50}, nM)	FLAP (IC_{50}, nM)	HWB (IC_{50}, nM)
1 (MK-0591)	$-CH_2O-$ $-CH(CH_3)O-$	4	2	500
4	(±)	9	16	803
5	(-)	5	7	362
6	(+)	32	116	>3000
7	(±) $-CH(CH_2CH_2CH_3)O-$	>52	350	>3000
9	$-CH_2OCH_2-$	>18	26	>3000
11	$-CH_2=CH_2-$	12	11	>3000
12	$-CH_2CH_2-$	4	16	>3000

Substitution around the indole and the quinoline moities. Another avenue of investigation was to vary the position of the substitution on the indole and the quinoline moities. Firstly, we moved the quinol-2-ylmethoxy moiety around the indole ring. Using Fischer indole synthesis methodology already reported to prepare this series,[10] the condensation of the appropriate methoxyphenylhydrazine with the methyl 5-(*tert*-butylthio)-2,2-dimethyl-4-oxopentanoate produced the desired analogs.

From the 3-methoxyphenylhydrazine, we obtained a (1:4) mixture of **13** and **14** (separable on flash silica gel after the benzylation step). The quinoline derivative **15** was obtained from the 2-methoxyphenylhydrazine. Based on the FLAP binding assay (Table 2), the indole substituted at the 5-position is highly favoured (see **1**), whereas the sterically conjested 7-substituted analog is highly disfavoured (see **15**). Secondly, with the 5-position fixed on the indole, we varied the position of the substitution on the quinoline moiety. The analogs **16** and **17** were obtained by reacting **2** with the 3- and the 4-bromomethyl quinolines, respectively (see Scheme 1, step a). From Table 2, we can see that the modifications at these positions on the quinoline are important (compare **1**, **16** and **17**) and still favours the 2'-position. These results confirm that the pattern of substitution found in MK-0591 is optimal.

Table 2. Substitution Around the Indole and the Quinoline Rings

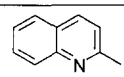
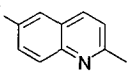
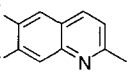
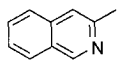
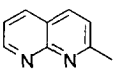
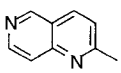
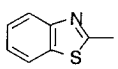
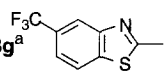
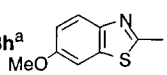
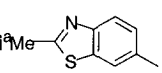
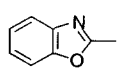
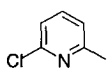
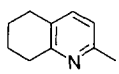
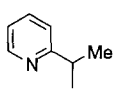
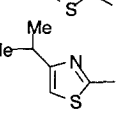
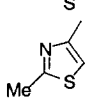


Compound	Substitution	FLAP (IC_{50}, nM)
1	5,2'	2
13	4,2'	85
14	6,2'	428
15	7,2'	1955
16	5,3'	48
17	5,4'	62

Alternative heteroarylmethoxy substituents. A series of analogs of **1** bearing alternative heteroarylmethoxy substitutents on the indole template were prepared by the alkylation of **2** (Scheme 2). At this stage, we selected active compounds by their ability to inhibit the production of LTB_4 in the HWB assay (Table 3). The only bicyclic heterocycle substituent showing improved *in vitro* potency over **1** is the benzothiazolyl derivative **18f**. In the pyridine series, the position of the substitution is important as shown by **18k**, **18l** and **18m.** Compounds **18k, 18n,** and **18q** showed the best *in vitro* profiles in this series. As observed in the

α-methylated quinoline series (see **5**), one enantiomer (**18q**) showed a better *in vitro* profile than the other one (**18r**). The two thiazolyl derivatives **18v** and **18w** also showed a better *in vitro* profile than **1.**

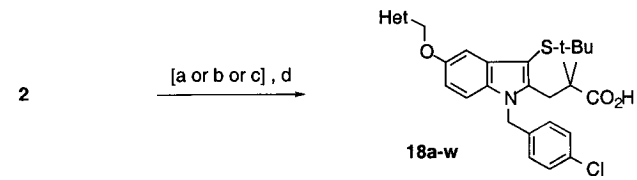
Table 3. 5-Heteroarylmethoxy Substituted Indoles (18a-w)

Compd.[i]	Het.	HPMN (IC_{50}, nM)	FLAP (IC_{50}, nM)	HWB (IC_{50}, nM)
1[a]		4	2	500
18a[a]		2	2	880
18b[a]		7	14	>3000
18c[b]		3	10	>3000
18d[b]		6	2	>3000
18e[b]		3	2	980
18f[b]		3	2	260
18g[a]		22	186	>3000
18h[a]		8	73	670
18i[a]		6	40	>3000
18j[a]		<7	5	2500
18k[b]		2	3	130
18l[b]		8	59	>3000
18m[b]		6	48	>3000
18n[b]		3	8	410
18o[c]		3	8	2890
18p[c] (RS)		4	8	270
18q[c] (R)		2	6	280
18r[c] (S)		3	12	470
18s[b]		4	7	400
18t[a]		4	70	>3000
18u[b]		3	13	>3000
18v[b]		3	5	270
18w[b]		2	3	130

i. coupling step a or b or c from Scheme 2.

In Vivo Studies. The most promising compounds from the preceeding *in vitro* SAR studies were evaluated *in vivo* in the rat as their corresponding sodium salts. For each compound, the plasma level profile (pharmacokinetics) was obtained, its functional activity in a hyperreactive rat model of dyspnea [12] was assessed as well as its effect on *in vivo* LTB_4 biosynthesis in a rat pleurisy model.[13] All the compounds reported in Table 4 display superior bioavailabilities and plasma levels at 2 h than MK-0591. In spite of this, variable activities are observed in the hyperreactive rat model.

Scheme 2.



Reagents: (a) Het-CH_2-Br, Cs_2CO_3, DMF/CH_3CN, rt, 3 h; (b) Het-CH_2-Cl, Cs_2CO_3, DMF/CH_3CN, 70 °C, 4 h; (c) Het-CH_2-OMs, Cs_2CO_3, CH_3CN, 70 °C, 2 h; (d) aq LiOH, THF/MeOH, reflux, 3 h.

From the combined *in vitro* and *in vivo* data presented in the Tables 2, 3, and 4, compound **18k** stands out as having the best overall profile. It was found to be well absorbed in rat (48% bioavailability) and showed high plasma levels at 2 h (9 uM). This compound showed the best activity in the hyperreactive rat model with 77% inhibition of dyspnea with a 2 h pretreatment time. The effect of **18k** in the rat pleurisy model was evaluated. Using a 3 h pretreatment time, it was effective at inhibiting LTB_4 biosynthesis with an ED_{50} of 0.30 mg/kg (n = 6). However, when the pretreatment time was increased to 6 h, it showed higher efficacy with an ED_{50} of 0.09 mg/kg. Using similar pretreatment times, 2 h or 6 h, MK-0591 had ED_{50} values of 0.51 and 0.15 mg/kg, respectively.[8]

Table 4. *In Vivo* Profile of Substituted Indoles in Rat

compound	rat plasma levels[a] F[b]	C_{2h}[c]	hyperreactive rat[d] (%)	rat pleurisy model ED_{50} mg/kg (pretreat.)[e]
1 (MK-0591)	24%	2.5uM	51	0.51 (2h) 0.15 (6h)
5	48%	4.2 uM	45	1.0 (3h)
18f	81%	6.0 uM	45	0.85 (3h)
18k	48%	9.0 uM	77	0.30 (3h) 0.09 (6h)
18n	37%	3.5 uM	34	----
18q	60%	13.0 uM	64	0.16 (3h)
18v	98%	8.0 uM	61	0.12 (3h)
18w	98%	8.0 uM	48	0.15 (3h)

[a]20 mg/kg PO in 1% methocel; 5 mg/kg IV in PEG400. [b]Bioavailability $AUC_{PO}/4$ X AUC_{IV}. [c]Plasma conc at 2 h. [d]Inhibition of dyspnea (0.5 mg/kg PO in 1% methocel, 2 h pretreatment, n = 6). [e]Inhibitor dissolved in 1% methocel and administrated orally either 2 or 3 or 6 h before interpleural injection with ionophore A23187.

Due to the *in vitro* and *in vivo* profile of **18k**, it was further evaluated in two other animal models of asthma. Squirrel monkeys were challenged with an aerosol of ascaris antigen and changes in pulmonary functions were monitored as previously described.[14] A significant inhibition of the induced bronchoconstriction was observed at 1 mg/kg PO in 1% methocel suspension (n = 5) with an increase in resistance (R_L = 57%) and a decrease in

dynamic compliance (C_{dyn} = 54%). Under the same conditions, comparable activities were observed for MK-0591 with inhibition of 55% for both R_L and C_{dyn}.

The therapeutic potential of **18k** was also assessed in the nonsensitised anaesthetised dog model.[15] The dogs were infused with the inhibitor and its capacity to inhibit the LTs was evaluated by the measurement of the urinary LTE_4 excretion as well as the *ex vivo* inhibition of LTB_4 biosynthesis in A23187-challenged dog whole blood. With an intravenous infusion of 2.5 ug/kg/min, compound **18k** showed 62% inhibition of *ex vivo* LTB_4 in whole blood and a 94% inhibition of urinary LTE_4 excretion where MK-0591, at the same dose, showed respectively 94% and 71%.

In summary we have identified a new inhibitor of leukotriene biosynthesis in the MK-0591 series, **18k**, which is highly potent, well absorbed and active in functional models. The *in vitro* and *in vivo* profile of **18k** is comparable or superior to MK-0591, which has showed biochemical efficacy in inhibiting *ex vivo* LTB_4 biosynthesis and urinary LTE_4 excretion in clinical trials.

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