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Potent and selective 5-LO inhibitor bearing benzothiophene pharmacophore: Discovery of MK-5286 ☆

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ABSTRACT

The strategy and SAR studies that led to the discovery of a novel potent and orally available 5-lipoxygenase (5-LO) inhibitor 3-(4-fluorophenyl)-6-({4-[(1*S*)-1-hydroxy-1-(trifluoromethyl)propyl]-1*H*-1,2,3-triazol-1-yl}methyl)-1-benzothiophene-2-carboxamide ((*S*)-**2l** or **MK-5286**) were described.

Leukotrienes, a group of locally acting hormones produced in living systems from arachidonic acid metabolism, are potent contractile and inflammatory mediators in various diseases.[1] The biosynthesis of these leukotrienes, including leukotriene B_4 (LTB_4), LTC_4, LTD_4, and LTE_4, begins with the epoxidation of arachidonic acid by 5-lipoxygenase (5-LO) enzyme[2] and 5-lipoxygenase-activating protein (FLAP)[3] to produce leukotriene A4 (LTA_4). LTA_4 is then converted to the other leukotrienes by subsequent enzymatic steps. Indeed, inhibition of leukotriene biosynthesis by a 5-LO inhibitor has been a validated strategy in searching for novel therapeutic agents to treat various inflammatory conditions.[4] Furthermore, it has been reported that 5-LO may be an important contributor to the atherogenic process and cancer,[5] and other oxidative stress-linked pathological processes.[6] Thus, a potent and selective 5-LO inhibitor might also be useful for treatment of diseases related to these pathogenic processes. Although there is currently one marketed 5-LO inhibitor, Zileuton (ZYFLO®), for the treatment of asthma,[7] the search for 5-LO inhibitor that targets better safety and efficacy profile never stopped. Herein we disclose our work that led to the discovery of a potent and orally available 5-LO inhibitor bearing a benzothiophene scaffold.

As part of our previous efforts[8] for the 5-LO inhibitor program, we established that compounds with a common structure as represented by **1a** exhibited 5-LO inhibitory activity (Fig. 1). In addition, we also observed that the 3-fluoro-5-(4-methoxytetrahydro-2*H*-pyran-4-yl)phenol ether moiety in **1a** had serious liabilities related to oxidative metabolism resulting in high level of covalent protein binding. We then found that this moiety could be replaced by various groups that were more resistant to oxidative metabolism and one such group was a triazole moiety as shown in **1b**. Previous studies also showed that the 5-LO inhibitory activity associated with **1b** mainly resided on the enantiomer with an (*S*)-configuration at the carbon center bearing a hydroxyl group.[9] Thus, our further efforts were focused on identifying a suitable aryl (Ar) group in **1b** that enabled potent 5-LO inhibitory activity and

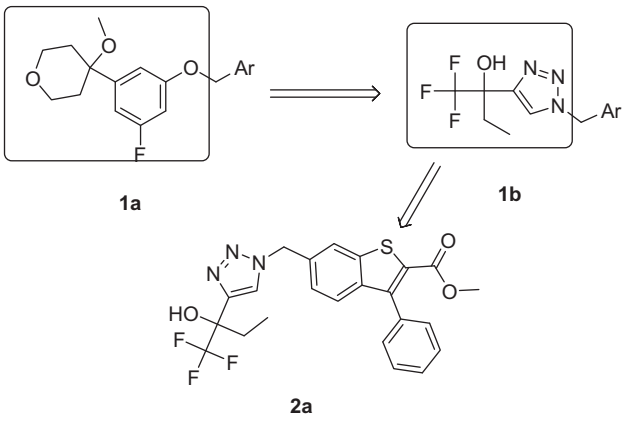

Figure 1. The lead generation.

☆ In memory of legacy *Merck Frosst Centre for Therapeutic Research*.

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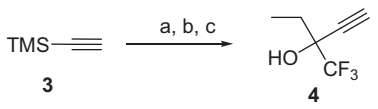
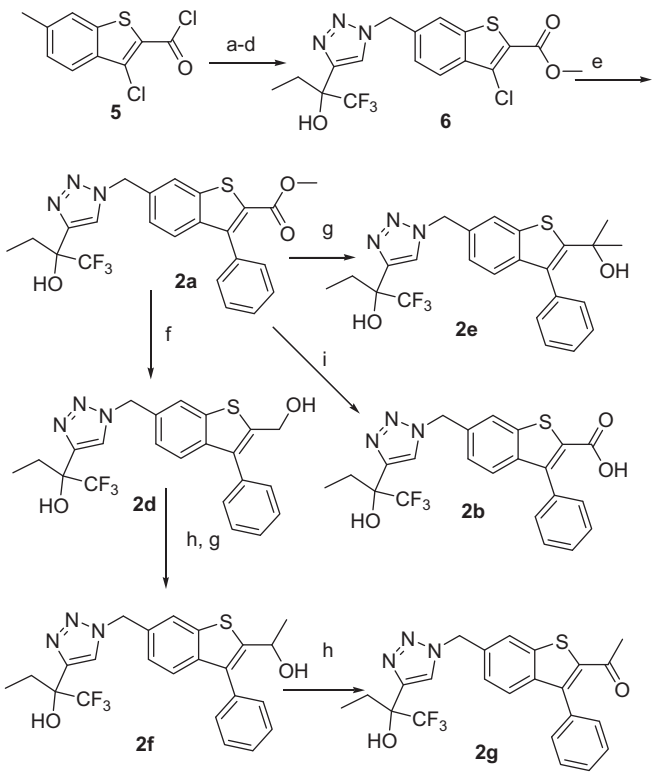
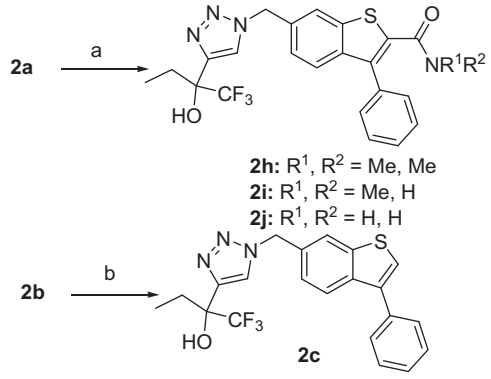


Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; (b) $EtCOCF_3$, THF, −78 °C to rt; (c) TBAF, THF, rt.



Scheme 2. Reagents and conditions: (a) MeOH, DMAP, DCM; (b) NBS, BzOOBz, CCl_4, 80 °C; (c) NaN_3, DMF, rt; (d) **4**, CuI, DIPEA, THF, rt; (e) $PhB(OH)_2$, $Pd(OAc)_2$, DavePhos, dioxane/H_2O, rt; (f) DIBAL, THF, −78 °C; (g) MeLi, THF, −78 °C; (h) MnO_2, DCM; (i) NaOH, THF/MeOH/H_2O, reflux.



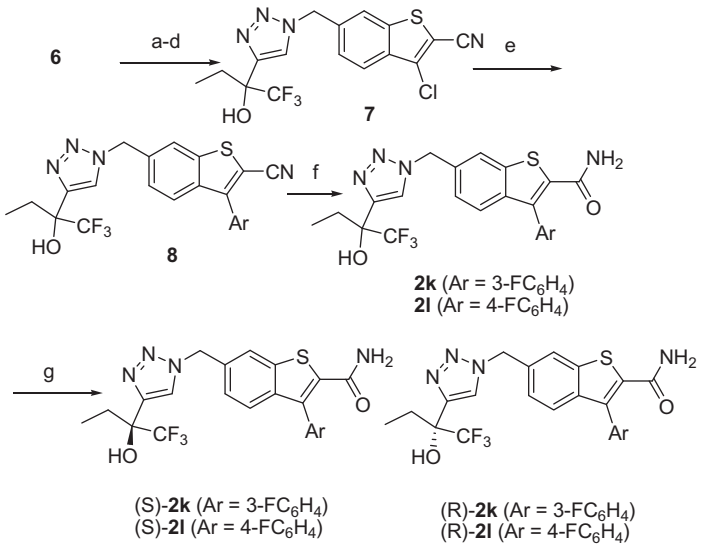
Scheme 3. Reagents and conditions: (a) Me_3Al, NH_4Cl ($MeNH_2$ or Me_2NH), C_6H_6, 80 °C; (b) Cu (powder), quinoline, 140 °C.

had other desirable properties such as high bioavailability and good metabolic stability. After a quick screen of several heteroaryl groups, we identified a benzothiophene scaffold as represented in compound **2a** as the best Ar group in **1b**. Thus compound **2a** served as a prototype for our subsequent SAR studies which were focused on the substituents of the benzothiophene scaffold.

The 5-LO inhibitors described in this study were prepared as shown in Schemes 1–4.[10] The synthesis of alcohol **4** was achieved by the addition of [(trimethylsilyl)ethynyl]lithium, which was formed in situ by reaction of **3** with *n*-BuLi, to 1,1,1-trifluorobutan-2-one (Scheme 1). The corresponding alcohol thus formed was treated with TBAF to remove the TMS protective group to provide the desired alcohol **4**. Compound **5**, the starting material used to build the advanced intermediate **6**, was prepared according to a literature procedure (Scheme 2).[11] Compound **5** was esterified with MeOH in the presence of DMAP to form the corresponding ester. The ester thus formed was subjected to benzylic bromination reaction with NBS, followed by a reaction with sodium azide to provide an azide intermediate, which then underwent a click reaction with compound **4** to give compound **6**. The Suzuki coupling between **6** and phenylboronic acid catalyzed by a palladium catalyst formed in situ from $Pd(OAc)_2$ and DavePhos yielded the prototype compound **2a**. Compound **2a** is a potent 5-LO inhibitor and could also serve as a starting material for the preparation of other 5-LO inhibitors with a range of different functional groups substituted at the 2-position of the benzothiophene scaffold (Scheme 2). First, **2a** was reduced by DIBAL-H to form **2d**. Oxidation of **2d** with MnO_2, followed by a reaction with methyllithium provided compound **2f**. Compound **2f** was further transformed to compound **2g** through oxidation with MnO_2. Carboxylic acid **2b** was obtained by hydrolysis of **2a** with aqueous NaOH solution. Tertiary alcohol **2e** was formed by a reaction of **2a** with an excess amount of MeLi in THF at low temperature. The 2-carboxamide derivatives, **2h, 2i**, and **2j**, were synthesized by reacting **2a** with an aluminum amide intermediate formed in situ from Me_3Al with the corresponding amine source such as ammonium chloride, methylamine, or dimethylamine, respectively (Scheme 3). Decarboxylation of **2b** promoted by copper powder in hot quinoline provided compound **2c**. Alternatively, intermediate **6** was transformed into nitrile **7** by a reaction sequence including reduction with DIBAL-H, oxidation with MnO_2, oxime formation with hydroxylamine hydrogen chloride, and dehydration with CDI (Scheme 4). The Suzuki coupling between compound **7** and a suitable arylboronic acid under the above-mentioned Suzuki coupling conditions provided compound **8**, which can be further hydrolyzed to form amide **2k** and **2l**. When the racemic mixture of **2j**, **2k**, or **2l** was submitted to chiral separation on a Chiralpak AD column, each of them was resolved to provide the desired enantiomer (*S*)-**2j**, (*S*)-**2k**, or (*S*)-**2l**, respectively.

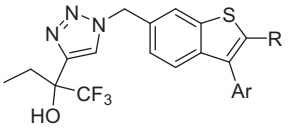
The 5-LO inhibitors reported herein were evaluated for their potency to inhibit the oxidation of arachidonic acid by recombinant human 5-LO (H5-LO),[8] and the production of LTB_4 in calcium ionophore-stimulated HWB.[12] To address the hERG binding issue observed in other related series of 5-LO inhibitors, all compounds were also evaluated for its potential to block the voltage-gated potassium channel encoded by the human ether-a-go-go gene (hERG) as measured in vitro by using a MK-499 displacement binding assay. As shown in Table 1, compound **2a** exhibits good and moderate activities (entry 1) in human 5-LO enzyme assay and LTB_4 whole blood assay, respectively. However, it also shows moderate activity in the hERG binding assay. Our goal of SAR study was to find a compound that was potent in both human 5-LO enzyme assay and LTB_4 whole blood assay but with diminished hERG potassium channel binding activity. Numerous compounds were prepared in the series and data in Table 1 only summarizes the results of representative compounds that illustrated the SAR trends and key compounds that were selected for further evaluation. Among all of the substituents made to the benzothiophene pharmacophore, the most impact on structure–activity relationship (SAR) came from substituents at the 2-position.

The apparent liability of compound **2a** is its instability towards hydrolysis, either chemically or enzymatically. The corresponding carboxylic acid **2d**, the more stable hydrolysis product, was found



Scheme 4. Reagents and conditions: (a) DIBAL, THF, −78 °C; (b) MnO_2, DCM; (c) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, rt; (d) DCI, DCM, rt; (e) $ArB(OH)_2$, $Pd(OAc)_2$, DavePhos, dioxane/H_2O, rt; (f) $Na_2CO_3{\cdot}1.5H_2O_2$, acetone, reflux; (g) Chiralpak AD.

Table 1
In vitro activity and hERG binding affinity of selected analogs of 5-LO inhibitors **2**



Entry	Compd	Ar	R	IC_{50}[a] (nM)		hERG K_i[a] (μM)
				Human 5-LO	HWB	
1	**2a**	Ph	–COOMe	7.9	260	6.3
2	**2b**	Ph	–COOH	>10,000	>20,000	>20
3	**2c**	Ph	–H	40	598	n/a
4	**2d**	Ph	$–CH_2OH$	19	64	13
5	**2e**	Ph	$–C(OH)Me_2$	198	392	2.6
6	**2f**	Ph	–CH(OH)Me	88	161	3.8
7	**2g**	Ph	–COMe	11	84	4.4
8	**2h**	Ph	$–CONMe_2$	1890	1840	>20
9	**2i**	Ph	–CONHMe	223	263	>20
10	**2j**	Ph	$–CONH_2$	55	70	17
11	(*S*)-**2j**	Ph	$–CONH_2$	28	32	16
12	(*S*)-**2k**	3-F-phenyl	$–CONH_2$	39	31	14
13	(*S*)-**2l**[b]	4-F-phenyl	$–CONH_2$	55	21	13
14	(*R*)-**2l**	4-F-phenyl	$–CONH_2$	1160	154	11
15	(*S*)-**2m**	4-F-phenyl	–COOH	>10,000	>20,000	>20

[a] IC_{50} and K_i values represent an average of at least three independent titrations.
[b] The compound was assigned as **MK-5286**.

to be completely inactive. The complete removal of substituent at the 2-position led to compound **2c** with more than two-fold loss of 5-LO activity when compared to **2a**, indicating that a substituent at the 2-position is helpful in improving 5-LO inhibiting activity. Therefore, we prepared compounds **2d**, **2e**, **2f**, and **2g** in hope to identify the intrinsic SAR trend associated with the series. The analysis of the SAR trend of these compounds clearly indicated that a small substituent bearing polar group, as shown by **2d**, was both beneficial to the improved 5-LO inhibiting activity and diminished hERG potassium channel binding activity. The good 5-LO inhibiting activity by **2g** suggests that a planar carbonyl group also helps. These observations prompted us to prepare three amide bearing compounds **2h**, **2i**, and **2j**. This led to the identification of primary amide group as a superior substituent for both high 5-LO inhibiting activity and low hERG potassium channel binding affinity. As we expected, enantiomer (*S*)-**2j** possesses most of 5-LO inhibitory activity as compared to the racemate **2j**. To follow up on this, we carried out further SAR study by introducing substituent on the phenyl group substituted at the 3-position of the benzothiophene scaffold. The purpose of this practice is two-fold: (1) to further improve the 5-LO inhibition potency and (2) to minimize the potential oxidative metabolism at this part of the molecules. As shown in Table 1 (entries 12 and 13), introduction of a fluorine at the 3- or 4-position of the phenyl moiety slightly improved the 5-LO inhibiting activity but showed little impact on hERG potassium channel binding affinity. Based on the HWB activity, (*S*)-**2l** was selected for further evaluation. (*S*)-**2l** shows no activity against 12-LO, 15-LO, and FLAP (IC_{50} >20 μM), and retain low affinity to hERG potassium channel. These properties of (*S*)-**2l** prompted us to perform intensive PK studies on it. We found that (*S*)-**2l** exhibits a good pharmacokinetic profile in pre-clinical species such as rat (5 mg/kg IV, 20 mg/kg PO, F = 114% and $t_{1/2}$ = 7.9 h, C_{max} = 19.4 μM), dog

(5 mg/kg IV, 4 mg/kg PO, $F = 120\%$ and $t_{1/2} = 29$ h, $C_{max} = 6.7$ μM), and rhesus monkey (5 mg/kg IV, 4 mg/kg PO, $F = 66\%$ and $t_{1/2} = 22$ h, $C_{max} = 1.4$ μM), respectively. It is worth mentioning that the vehicle used for PO administration of (*S*)-**2l** was 50% PEG 400 at 4 mL/kg/day. With this formulation, inhibitor (*S*)-**2l** was dosed to studied animals as a clear solution. Attempts to administer the inhibitor as a fine suspension in 0.5% methocel and other vehicles resulted in <10% bioavailability across all studied species. In the PK studies, we also observed the formation of a circulating metabolite (*S*)-**2m** in all studied species. Thus (*S*)-**2m** was prepared[13] and evaluated. As presented in Table 1, (*S*)-**2m** shows no measurable activities to 5-LO and hERG. Both inhibitor (*S*)-**2l** and metabolite (*S*)-**2m** were also evaluated in the MDS Pharma PanLab Screen, in vitro and in vivo covalent protein binding assay, ancillary pharmacology study (CV dog at PO 3 and 10 mg/kg dose; mouse CNS at PO 100 mg/kg dose). No signal that might suggest potential liability was identified. Based on all these findings inhibitor (*S*)-**2l** was selected to advance into pre-clinical development.

To conclude, we discovered that substituted benzothiophene was able to serve as a novel scaffold of 5-LO inhibitors. Through extensive SAR studies on the series, a potent and selective 5-LO inhibitor 3-(4-fluorophenyl)-6-({4-[(1*S*)-1-hydroxy-1-(trifluoromethyl)propyl]-1*H*-1,2,3-triazol-1-yl}methyl)-1-benzothiophene-2-carboxamide ((*S*)-**2l** or **MK-5286**) was identified.

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