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Discovery of selective imidazole-based inhibitors of mammalian 15-lipoxygenase: Highly potent against human enzyme within a cellular environment

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Abstract—A series of 2,4,5-tri-substituted imidazoles has proven to be highly potent in inhibiting mammalian 15-lipoxygenase (15-LO) with excellent selectivity over human isozymes 5- and P-12-LO. Non-symmetrical sulfamides (e.g., **21a**–**n**) were found to be suitable replacements for the earlier arylsulfonamide-containing members of this series (e.g., **2**, **14a**–**p**). Several members of these series also demonstrated potent inhibition of human 15-LO in a cell-based assay.

The lipoxygenase family of enzymes has received considerable attention for its role in the etiology of human disease. For example, as a mediator of leukotriene biosynthesis, 5-lipoxygenase (5-LO) has been an active therapeutic target for the control of asthma.[1] Human 15-lipoxygenase (h15-LO) has also emerged as an interesting target for drug discovery, with reports implicating its role in the progression of numerous diseases including cancer,[2] Alzheimer's disease,[3] and, most notably, cardiovascular disease.[4] However, convincing pre-clinical or clinical evidence for therapeutic effects as a result of 15-LO inhibition has yet to be reported. Also, recent reports suggesting anti-inflammatory and anti-neoplastic roles for 15-LO cast some uncertainty on the potential for this target in treating human disease.[5,6] Herein we describe our continued efforts to discover potent, selective, and orally bioavailable inhibitors of h15-LO, the identification of which is expected to assist in elucidating the role of this enzyme in disease progression.

We previously reported the discovery of a series of tryptamine and homotryptamine-based sulfonamides as inhibitors of mammalian (rabbit) 15-LO.[7] This series of compounds potently inhibited the enzyme with high selectivity over the closely related isozymes 5- and platelet-derived 12-LO (P-12-LO). The *p*-methoxyphenyl-substituted tryptamine derivative **1** (Fig. 1) was previously reported to inhibit rabbit reticulocyte-derived 15-LO with IC_{50}'s of 37 nM and 164 nM, employing linoleic acid (LA) or arachidonic acid (AA) as enzyme substrates, respectively.[8] Despite the favorable inhibition potency and selectivity profiles of this lead series, the generally unfavorable physical chemical properties (solubility, log *P*) of its members generally precluded their evaluation in vivo. A campaign was thus initiated to improve these properties while retaining the activity profile of the lead series. To this end, it was found that the diaryl histamine-based sulfonamide **2**, conceptually derived from **1** by fragmentation of its indole core and introduction of one nitrogen atom, showed improved inhibitory potency against 15-LO while maintaining

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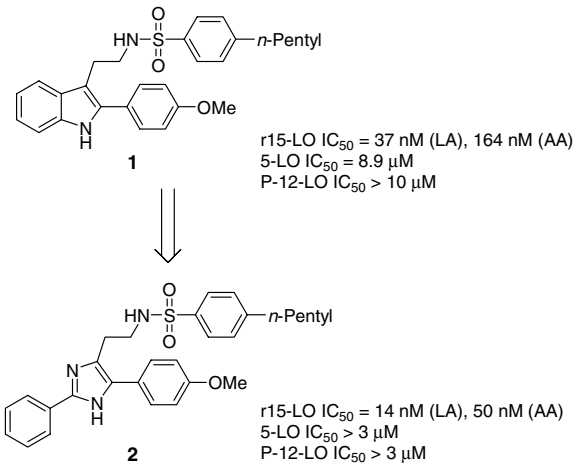
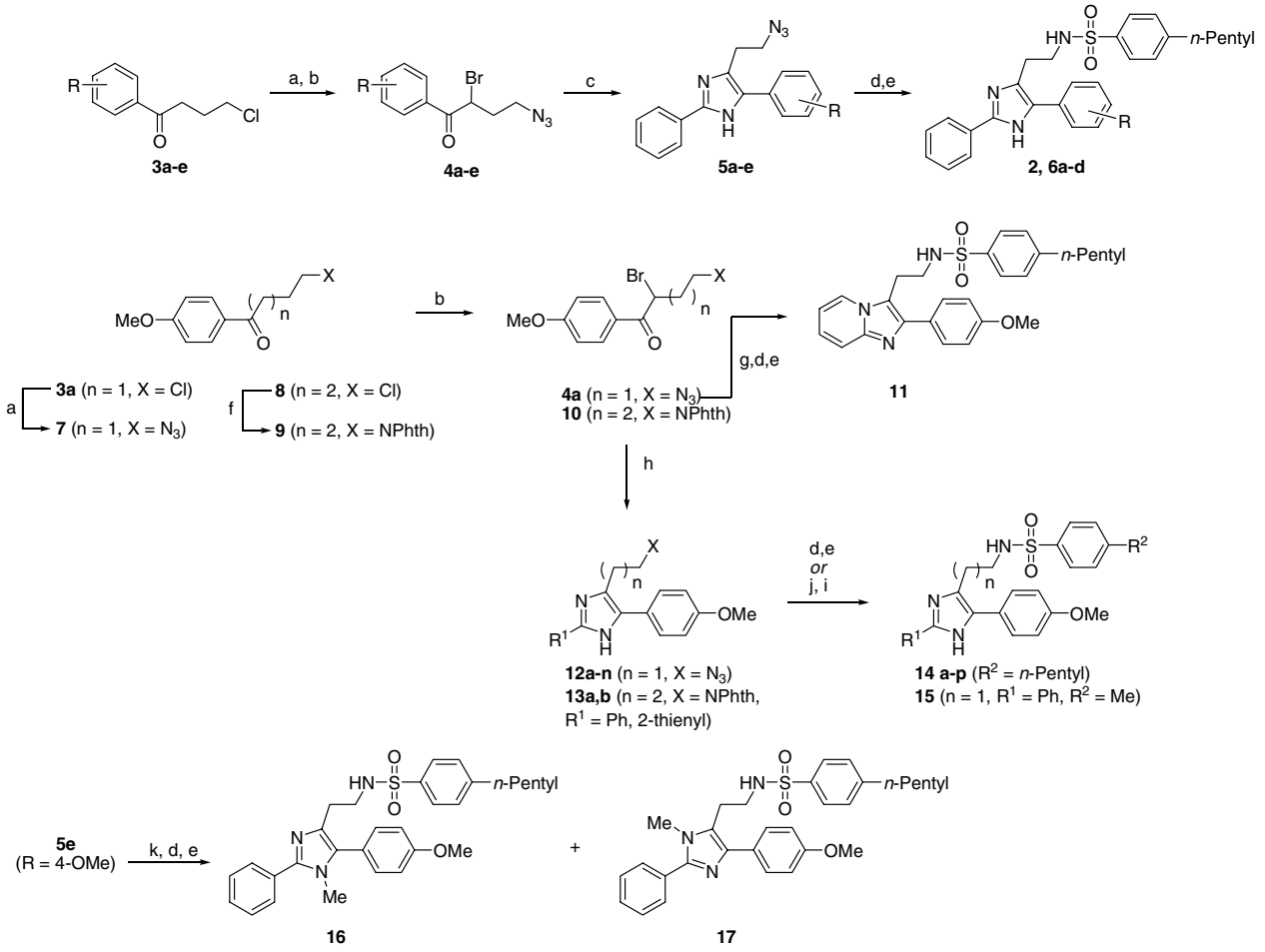


Figure 1. Tryptamine-derived sulfonamide **1** and 2,5-diarylhistamine sulfonamide **2**.

selectivity over 5- and P-12 LO's. In addition, **2** demonstrated potent inhibition of 15-LO in Chinese hamster ovary (CHO) cells overexpressing human recombinant enzyme (vide infra).

An initial survey of substituted phenyl groups at C-5 of the imidazole was carried out. Compounds were prepared as described in Scheme 1. Treatment of the commercially available γ-chloro butyrophenones **3a**–**e** with sodium azide, followed by α-bromination of the resulting ketones, gave bromo azides **4a**–**e**. Imidazole formation was accomplished by condensation with benzamidine hydrochloride to provide the imidazole azides **5a**–**e**, which were subsequently hydrogenated to the primary amines and converted to the 4-*n*-pentyl benzene sulfonamides **2** and **6a**–**d**. The 15-LO inhibitory activities of the differentially substituted C-5 phenyl imidazoles are depicted in Table 1. The diphenyl imidazole **6a** proved to be two- to threefold less potent than **2**. Electron-withdrawing groups at the *para* position of the C-5 phenyl ring decreased potency relative to methoxy, as evidenced by the trifluoromethyl and fluoro-containing compounds **6b** and **6c**, respectively. Placement of the methoxy group in the meta position of the C-5 phenyl appendage (**6d**) also led to a significant loss in potency.

A series of sulfonamide analogs of the imidazole **2**, maintaining the *p*-methoxyphenyl group at C-5, was



Scheme 1. Reagents and conditions: (a) NaN_3, DMF (95%); (b) Br_2, 1,4-dioxane (99%); (c) benzamidine hydrochloride, potassium carbonate, CH_3CN (35–54%); (d) H_2, Pd/C (10% w/w), MeOH (100%); (e) 4-*n*-pentylbenzenesulfonyl chloride, triethylamine, CH_2Cl_2 (90–95%); (f) potassium phthalimide, DMF (81%); (g) 2-aminopyridine, EtOH (99%); (h) $R^1C(=N)NH_2$ hydrochloride, potassium carbonate, DMF; (i) 4-$MePhSO_2Cl$, triethylamine, CH_2Cl_2 (90–95%); (j) hydrazine hydrate, THF (60%); (k) MeI, NaH, DMF (96%).

Table 1. 15-LO inhibitory activities of sulfonamides **2**, **6a–d**

Compound	R	r15-LO IC_{50} (μM)	
		LA[a]	AA[b]
2	4-OMe	0.014	0.05
6a	H	0.033	0.162
6b	4-CF_3	0.072	0.372
6c	4-F	0.059	0.261
6d	3-OMe	0.082	0.531

[a] Enzyme inhibition measured in the presence of linoleic acid as substrate.
[b] Enzyme inhibition measured in the presence of arachidonic acid as substrate.

then prepared according to the chemistry described in Scheme 1. Treatment of γ-chloro *p*-methoxy butyrophenone **3a** with sodium azide provided azide **7**. The one-carbon homolog, phthalimide analog of **7** (**9**), was prepared by treatment of chloride **8** with potassium phthalimide. Treatment of the ketones with bromine provided bromides **4a** and **10**. The azide **4a** was converted to imidazo [1,2-*a*]pyridine **11** by condensation with 2-aminopyridine followed by reduction of the azide and subsequent sulfonylation with 4-pentylbenzene sulfonyl chloride. Bromides **4a** and **10** were condensed with the appropriate amidines to provide the imidazole azides **12a–n** and phthalimides **13a,b**. Hydrogenation of the azide or hydrazinolysis of the phthalimide afforded the primary amines which were then converted to the sulfonamides **14a–p** and **15**. The regioisomeric *N*-methyl imidazoles **16** and **17** were prepared by methylation of the intermediate imidazole **5e** with methyl iodide, followed by subsequent separation of the regioisomers, hydrogenation of the azides, and sulfonylation of the resultant amines.

The requirement for the imidazole N-1 hydrogen was addressed by comparison of imidazo[1,2-*a*]pyridine **11** and *N*-methylated regioisomers **16** and **17** with the related indole **1** and imidazole **2** . Removal of the N–H pharmacophore resulted in a dramatic loss in potency ($IC_{50} > 10$ μM) versus 15-LO. A similar loss of activity was observed upon methylation of the previously reported tryptamine-based series of 15-LO inhibitors[7] and is consistent with the requirement for a hydrogen-bond donor at this position of the molecule.

A variety of groups were explored at the imidazole C-2 position (Table 2). Little tolerance was observed for substitution at the *para* position of the C-2 phenyl group. Introduction of methoxy or methyl groups (**14a** and **14b**, respectively) led to a significant loss of inhibitory activity. Some tolerance was noted for *para*-chlorophenyl and *meta*-nitrophenyl groups (**14c** and **14d**, respectively). The C-2 unsubstituted compound **14e** proved to be inactive as did the C-2 methyl and cyclopropyl analogs (**14f** and **14g**). Incorporation of the *tert*-butyl group (**14h**) resulted in only micromolar potency. Several heterocyclic replacements for the C-2 phenyl group were also explored. While the 4-pyridyl analog **14i** maintained some of the inhibitory potency

Table 2. 15-LO Inhibitory activities of sulfonamides **2, 14a–p**, **15**

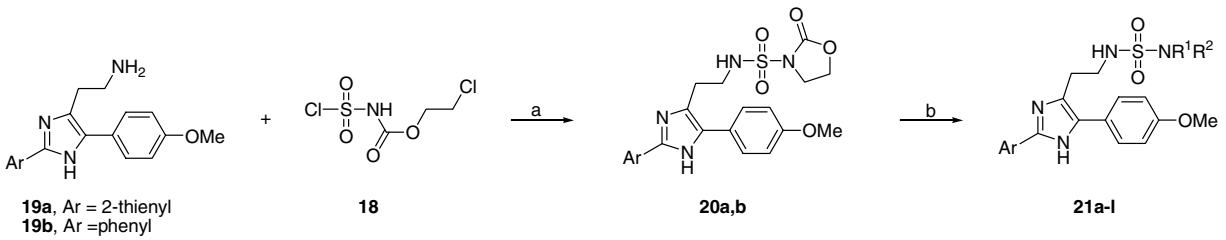
Compound	*n*	R^1	r15-LO IC_{50} (μM)	
			LA[a]	AA[b]
2	1	Ph	0.014	0.05
14a	1	4-OMe-Ph	>10	—
14b	1	4-Me-Ph	>3	—
14c	1	4-Cl-Ph	0.21	1.1
14d	1	3-NO_2-Ph	0.099	1.1
14e	1	H	>10	—
14f	1	Me	>10	—
14g	1	Cyclopropyl	>10	>10
14h	1	*tert*-Butyl	1.92	>10
14i	1	4-Pyridyl	0.087	3211
14j	1	2-Pyridyl	0.97	5322
14k	1	3-Pyridyl	0.56	—
14l	1	2-Pyrazinyl	2.81	—
14m	1	4-(2-Me)-thiazoyl	0.72	—
14n	1	2-Thienyl	0.006	0.013
14o	2	Ph	0.140	0.914
14p	2	2-Thienyl	0.053	0.396
15	1	Ph (R^2 = Me)	0.364	2.80

[a] Enzyme inhibition measured in the presence of linoleic acid as substrate.
[b] Enzyme inhibition measured in the presence of arachidonic acid as substrate.

of **2** (approximate 7 fold loss in activity with LA as substrate, 60 fold loss with AA), the corresponding 2- and 3- pyridyl isomers (**14j** and **14k**), however, were less tolerated. The 2-pyrazinyl analog **14l** and 4-(2-methyl)thiazolyl analog **14m** also showed reduced potency versus **2**. Isosteric replacement of the C-2 phenyl group with 2-thienyl gave **14n**, which proved to be most potent within this series, providing single digit nanomolar inhibition of 15-LO (LA as substrate). In addition, **14n** maintained selectivity over 5-LO ($IC_{50} > 10$ μM) and P-12-LO ($IC_{50} = 9.7$ μM). The effect of one-carbon homologation of the imidazole-sulfonamide linker was also studied. In contrast to previous observations in the tryptamine-based series,[7] homologation of the ethylene chain adjacent to the sulfonamide nitrogen proved deleterious. Thus, the one-carbon homologs of **2** and **14n** (**14o** and **14p**, respectively) were both an order of magnitude less potent than their ethylene-linked counterparts.

Despite its promising potency and isozyme selectivity, **14n** did little to enhance the physicochemical limitations of the tryptamine-based series, having both suboptimal aqueous solubility and hydrophilicity (<1 μg/mL at pH 6.5, $\log D > 6$). Not surprisingly, a single, 7 mg/kg oral dose of **14n** to male Sprague Dawley rats resulted in limited systemic plasma exposure ($C_{max} = 0.6$ μM, AUC = 1.60 μM h).

It is also noteworthy that in contrast to the previously reported indole-based series,[7] the presence of the *p*-methoxyphenyl group at C-5 of the imidazole core of **2** (analogous to the C-2 indole substituent of **1**) did not allow for a significant reduction in the size of the aryl sulfonamide moiety. Thus, the *p*-tolyl sulfonamide **15** proved to be significantly less potent than the corre-

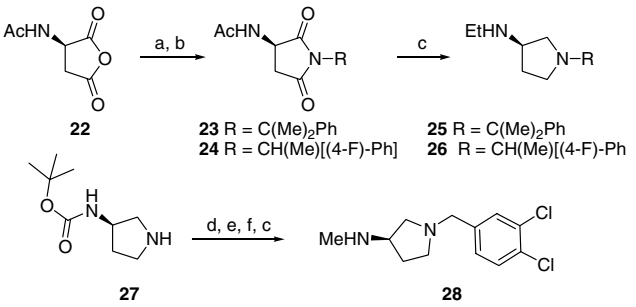


Scheme 2. Reagents and condition: (a) Et_3N, CH_2Cl_2; (b) NHR^1R^2, CH_3CN (75–98% for two steps).

sponding *n*-pentyl benzene sulfonamide **2**. On the basis of these findings, significant additional modifications would need to be incorporated into the imidazole-based series to further improve its physicochemical properties.

A significant improvement in the physicochemical properties of the lead series was realized by incorporation of tertiary amine-containing sulfamides as replacements for the sulfonamide functionality in **2**. Non-symmetrical sulfamides were prepared by condensation of carbamoyl chlorosulfamate **18** with either amine **19a** or **19b** in the presence of triethylamine to give the sulfamoyl oxazolidinones **20a,b** (Scheme 2). Displacement of the oxazolidinone leaving group with either primary or secondary amines then provided the sulfamides **21a**–**l**.[9] The 3-aminopyrrolidines employed for the preparation of examples **21j**–**l** were prepared as described in Scheme 3. Thus, *N*-acetyl-D-aspartic acid anhydride **22** was condensed with either cumylamine (for **21k**) or 1-(4-fluorophenyl)ethanamine (for **21l**) to provide the imides **23** and **24**.[10] Reduction of the imides provided the 3-ethylaminopyrrolidines **25** and **26**. For the preparation of **21j**, alkylation of (3*R*)-(+)-3-(Boc-amino)pyrrolidine **27** with 3,4-dichlorobenzyl bromide in the presence of potassium carbonate, followed by removal of the Boc group and reductive methylation, provided the homochiral 3-methylaminopyrrolidine **28**.

N-Alkyl-substituted piperazine sulfamides were first explored as replacement for the phenyl sulfonamides (Table 3). The 4-*n*-butyl piperazine **21a** provided potent inhibition of the enzyme, with a significant loss in potency observed upon chain elongation to the 4-*n*-pentyl piperazine **21b**. As compared to phenyl sulfonamide



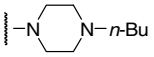
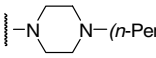
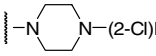
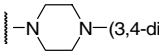
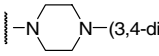
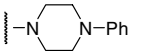
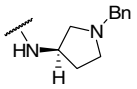
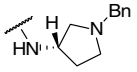
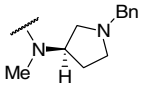
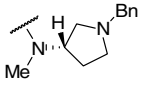
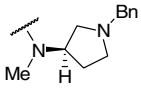
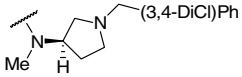
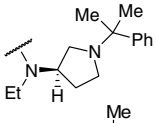
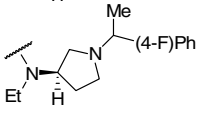
Scheme 3. Reagents and conditions: (a) NHR, acetone; (b) Ac_2O, reflux (85% for two steps); (c) LAH, THF (74%); (d) 3,4-dichlorobenzyl bromide, K_2CO_3, DMF (98%); (e) TFA, CH_2Cl_2 (95%); (f) methyl chloroformate, K_2CO_3, CH_2Cl_2, H_2O (65%).

14n, an improvement in systemic plasma exposure after a single 7 mg/kg oral dose of **21a** to rats was observed (C_{max} = 1.7 μM, AUC = 4.4 μM h) in part attributed to an increased aqueous solubility of **21a** by the presence of the sulfamide group. The chlorinated phenyl piperazines **21c,d** proved to be of similar potency to alkyl piperazines **21a,b** and more potent than the 2-phenyl substituted imidazoles **21e** and **21f**. Given the promising activity of the piperazine sulfamides, a solution-phase library of sulfamides utilizing amine **19b** and diamines (primary or secondary amines bearing pendant tertiary amines) was prepared. From this work, it was determined that sulfamides derived from 1-benzyl-3-aminopyrrolidines (**21g**–**i**) were found to be suitable replacements for the piperazine sulfamides, with some preference for the *R* isomer observed. The sulfamide **21g** derived from *R*-1-benzyl-3-aminopyrrolidine proved to be threefold (with both substrates) more potent than the corresponding *S* isomer **21h**. An additional threefold boost in potency was observed upon methylation of the exocyclic amine with maintained preference for the *R* enantiomer (**21i**) over the *S* isomer (**21j**). Replacement of the phenyl substituent at the imidazole C-2 of **21i** with thiophene (**21k**) maintained activity, consistent with observations in the sulfonamide series (cf., **14n** and **2**). Incorporation of chlorine atoms at the 3- and 4-positions of the benzylic phenyl group (**21l**) continued to improve potency by another 3-fold over the simple benzyl pyrrolidine **21k**. Bearing in mind the propensity of benzylic amines to undergo metabolic oxidative de-alkylation, the *gem*-dimethyl benzyl pyrrolidine **21m** and the *p*-fluoro phenethyl pyrrolidine **21n** were prepared. **21n** proved to be the most potent 15-LO inhibitor in the series, with an IC_{50} of 3 nM (LA).

Representative sulfonamide and sulfamide-bearing compounds were assayed for inhibitory activity over human 5- and P-12-LOs (Table 4). Only modest inhibition of these isozymes with select compounds at relatively high concentrations (10 or 3 μM) was observed, consistent with the selectivity noted for the tryptamine series of 15-LO inhibitors.[7]

Given the promising potencies of these histamine-based sulfonamides and sulfamides in inhibiting isolated rabbit-derived 15-LO enzyme, select compounds were also assayed for their abilities to inhibit the activity of the human enzyme in a cellular assay (Table 5). Chinese hamster ovary (CHO) cells overexpressing recombinant h15-LO were employed in this study, with enzymatic inhibition measured by reverse-phase HPLC

Table 3. 15-LO inhibitory activities of sulfamides **21a–l**

Compound	Ar[a]	NR^1R^2	r-15-LO IC_{50} (μM)	
			LA[b]	AA[c]
21a	A		0.014	0.05
21b	A		0.091	0.646
21c	A		0.045	0.132
21d	A		0.036	0.133
21e	B		0.055	0.378
21f	B		0.212	3.45
21g	B		0.083	0.719
21h	B		0.272	2.41
21i	B		0.031	0.186
21j	B		0.080	0.822
21k	A		0.038	0.375
21l	A		0.011	0.083
21m	A		0.010	0.132
21n	A		0.003	0.075

[a] A, 2-thienyl; B, phenyl.
[b] Enzyme inhibition measured in the presence of linoleic acid as substrate.
[c] Enzyme inhibition measured in the presence of arachidonic acid as substrate.

quantitation of the extent of generation of 15-HETE from exogenous AA.[11] The sulfonamides **2** and **14n** both provided potent (submicromolar) inhibition of human 15-LO in this cellular context. The sulfamides **21i**, **21d**, and **21n** also provided potent inhibition in the CHO assay, with **21n** proving the most potent, consistent with its high inhibitory activity against the isolated rabbit enzyme.

Table 4. Selectivity data for selected compounds

Compound	% Inhibition[a]	
	5-LO	P-12-LO
2[b]	15	45
6a	39	32
6c	45	47
14n	41	50
21l	67	42

[a] Measured at 10 μM inhibitor concentration, unless specified otherwise.
[b] Measured at 3 μM inhibitor concentration.

Table 5. CHO cell 15-hLO inhibitory activities of selected compounds

Compound	15-LO inhibition	
	IC_{50} (μM)	% Inhibition (10 μM)
2	0.402	83
14n	0.147	98
21i	3.13	70
21d	0.433	84
21n	0.051	96

In conclusion, a series of 2,4,5-tri-substituted imidazoles evolved from previously described tryptamine-based compounds[7] has provided potent inhibitors of mammalian 15-LO. Replacement of the aryl sulfonamide moiety with sulfamides was well tolerated, retaining potency of the former while showing promise in improving physicochemical and pharmacokinetic properties. As observed in our previous work,[7] inhibitor potency depends on the choice of substrate employed in the assay (LA vs AA). The K_m values reported for both substrates against 15-LO isolated from human cultured keratinocytes are very similar (10.6 μM and 9.5 μM for AA and LA, respectively),[12] making it difficult to reconcile the difference in inhibitor potency based on these values alone. Additional studies, beyond the scope of this investigation, will be required to adequately explain these findings. Selectivity over isozymes 5- and P-12-LO was also demonstrated. Importantly, representative examples proved to be potent in inhibiting h15-LO in a cellular assay. The ability of these compounds to inhibit both the human and rabbit enzymes is consistent with their overall sequence homology (80%).[13] While an X-ray crystal structure of h15-LO has yet to be reported, the structural similarity of the rabbit and human enzymes is also supported by a recent report which demonstrates the utility of a homology model based on the crystal structure of rabbit-derived 15-LO in identifying inhibitors of the human enzyme.[14] The identification of potent and selective inhibitors of 15-LO may enable better validation of this target and ultimately new therapeutic treatments for human disease.

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8. See footnote 17 of Ref. 7 for a description of the 15-LO enzyme inhibition colorimetric assay.
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11. Stably transfected Chinese hamster ovary (CHO) cells overexpressing human 15-lipoxygenase cDNA were generated. In a 48-well format (triplicate wells per data point), CHO/15-LO cells were pretreated with compound, supplemented with arachidonic acid (5 μM final concentration), whereupon the 15-HETE released by the cell into the surrounding serum-free medium was analyzed by reverse-phase HPLC on a Waters 600 chromatography system using a Luna C18 column (2×50 mm). The column was developed at a flow rate of 0.4 ml/min by a gradient program using two solvents (A and B) set at 70% B for 0–6 min, 100% B for 7–13.5 min, where A was 50% MeOH/H_2O/0.1%TFA/0.05%TEA and B was 90%MeOH/H_2O/0.1%TFA/0.05%TEA. The HPLC eluate was monitored using a Waters 996 photodiode array (PDA) detector set at 235 nm for monoHETE. In order to record the UV spectra for each individual peak, the PDA was set for data acquisition from 200 to 300 nm. Products were quantitated based on absorbance at 235 nm using Millennium[32] (Waters) software.
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