Bioorganic & Medicinal Chemistry Letters 21 (2011) 4141–4145

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pyrazole-based sulfonamide and sulfamides as potent inhibitors of mammalian 15-lipoxygenase

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ARTICLE INFO

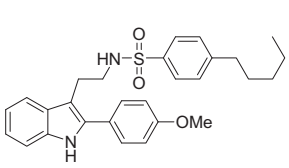
Article history:
Received 5 April 2011
Revised 25 May 2011
Accepted 26 May 2011
Available online 6 June 2011

Keywords:
15-Lipoxygenase
Inhibitors
Pyrazole
Sulfonamides
Sulfamides
Arachidonic acid
Linoleic acid

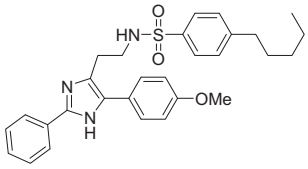
ABSTRACT

A series of inhibitors of mammalian 15-lipoxygenase (15-LO) based on a 3,4,5-tri-substituted pyrazole scaffold is described. Replacement of a sulfonamide functionality in the lead series with a sulfamide group resulted in improved physicochemical properties generating analogs with enhanced inhibition in cell-based and whole blood assays.

Lipoxygenases (LOs) are non-heme iron containing enzymes that catalyze the oxidation of polyunsaturated fatty acids and esters to a hydroperoxy derivative.[1] These enzymes can be found in both plant and animal kingdoms.[2] In humans, lipoxygenases are named according to the position at which a key substrate, arachidonic acid (AA), is oxidized. Members of this class are 5-lipoxygenase (5-LO), 12-lipoxygenase (12-LO) and 15-lipoxygenase (15-LO).[1,3a] These enzymes are further subdivided. 12-LO (with platelet-type and leukocyte-type form) and 15-LO (15-LO-1 the reticulocyte or leukocyte-type and 15-LO-2 the epidermis-type).[3b] These enzymes have received a great deal of attention because of their involvement in a variety of diseases. In humans, 5-LO is involved in cancer[4,5] and asthma[6] and 12-LO has been implicated in immune disorders[7] and breast cancer.[5,8,9] In addition to cancer,[10] 15-LO has been shown to play a significant role in the progression of Alzheimer's disease[11] and most notably cardiovascular disease.[12] There have been several reports in the literature describing 15-LO inhibitors[13] including inhibitors from our own laboratories based on tryptamine[14] **I** and histamine[15] **II** scaffolds.



I



II

Herein we report a pyrazole-derived series of highly potent and selective inhibitors of 15-LO which may potentially be of therapeutic value for the treatment of atherosclerosis. A summary of in vitro SAR studies involving replacement of a sulfonamide group with a sulfamide functionality leading to the discovery of analogs with enhanced inhibition in cell-based and whole blood assays is described.

The general synthetic route for pyrazole derived sulfonamides is described in Scheme 1. 4-Chlorobutyryl chloride (**1**) was readily transformed to the Weinreb amide **2** which upon treatment with appropriate Grignard reagents yielded the corresponding ketones **3**. Conversion of **3** to azides **4** was achieved by displacing the chloro group via treatment with sodium azide in DMF at 90 °C. Transformation of the azido ketones **4** to the β-diketones **5** was carried out by treatment with lithium bis(trimethylsilyl)amide in THF at −78 °C followed by quenching the resulting anion with

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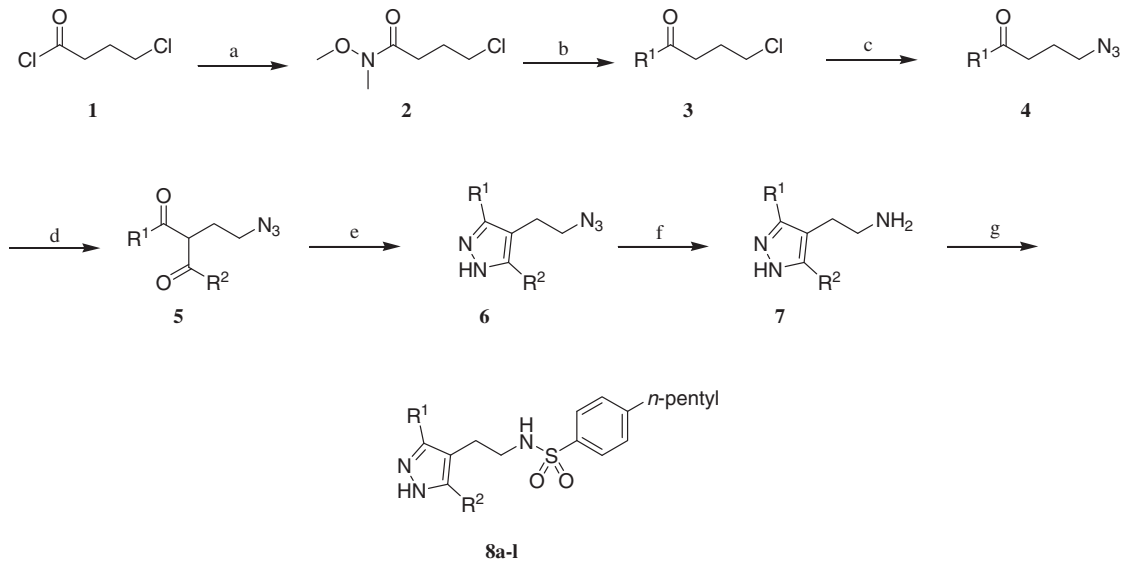
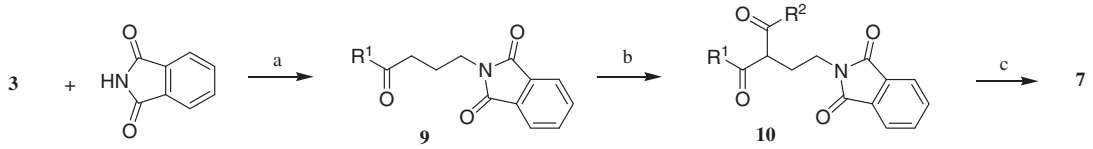
doi:10.1016/j.bmcl.2011.05.107



Scheme 1. Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, *N*,*N*-diisopropylethylamine, THF, 85%; (b) R^1MgBr, THF, 0 °C 70–85%; (c) NaN_3, DMF, 90 °C, 75–83%; (d) lithium bis(trimethylsilyl)amide, R^2COCl, THF, −78 °C, 40–91%; (e) NH_2NH_2, MeOH, 70 °C, 80–95%; (f) triphenylphosphine, H_2O/THF, 100 °C, 85–95%; (g) 4-*n*-pentylphenylsulfonyl chloride, Et_3N, CH_2Cl_2, 75–85%.

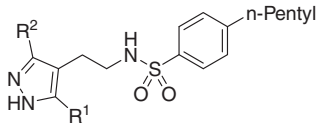
various acyl halides. Condensation of the diketones **5** with hydrazine furnished pyrazoles **6**. Reduction of the azido group was accomplished by treatment with triphenylphosphine in water–THF at 100 °C which generated the corresponding primary amines **7**. Finally, sulfonylation of **7** using 4-*n*-pentylphenylsulfonyl chloride in the presence of triethylamine in dichloromethane, generated the target analogs **8a**–**l**.

The intermediate primary amines **7** can also be obtained (Scheme 2) by alkylation of phthalimide with chlorobutyroketones **3** yielding **9**. The β-diketones **10**, prepared from **9** as described in Scheme 1,



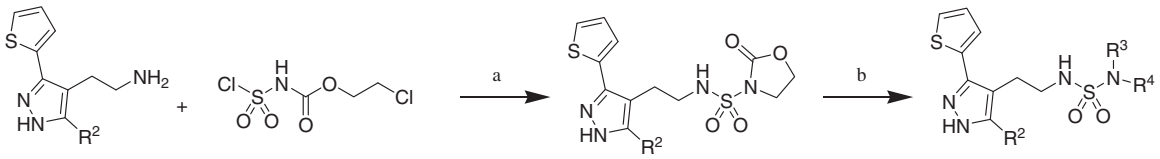
Scheme 2. Reagents and conditions: (a) K_2CO_3, NaI, DMF, 90 °C, 35–45%; (b) lithium bis(trimethylsilyl)amide, R^2COCl, THF, −78 °C, 45–50%; (c) NH_2NH_2, HOAc, MeOH, 70 °C, 20–35%.

Table 1
15-LO inhibitory activities of sulfonamides **8a**–**8l**



Compound	R^1	R^2	15-LO IC_{50} (nM)		15-LO CHO cell inhibition (% at 10 μM)
			LA[a]	AA[b]	
8a	(4-OCH_3)Ph	CH_3	3980	NT	NT
8b	(4-OCH_3)Ph	Isobutyl	305	NT	NT
8c	(4-OCH_3)Ph	Ph	150	1560	NT
8d	(2-OCH_3)Ph	Ph	1220	NT	NT
8e	(3-OCH_3)Ph	Ph	68	524	NT
8f	Ph	Ph	35	313	NT
8g	Ph	Benzyl	158	918	NT
8h	Thien-2-yl	Ph	11	28	21
8i	Thien-2-yl	Thien-2-yl	4	19	54
8j	Thien-2-yl	(3-OCH_3)Ph	9	53	29
8k	Thien-2-yl	5-Me-isoxazol-3yl	10	36	2.8
8l	Thien-2-yl	Pyrazin-2-yl	12	65	25

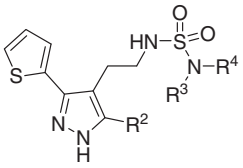
NT = not tested.
[a] Enzyme inhibition measured using linoleic acid as substrate.
[b] Enzyme inhibition measured using arachidonic acid as substrate.

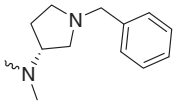
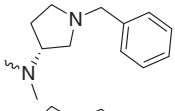
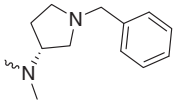
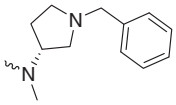
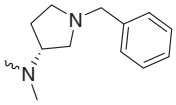
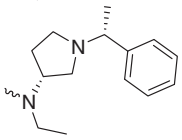
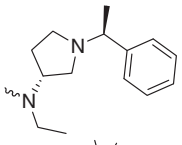
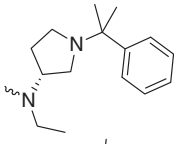
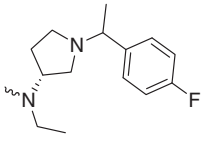


7 13 14 15

Scheme 3. Reagents and condition: (a) Et_3N, CH_2Cl_2; (b) HNR^3R^4, CH_3CN (75–90% two steps).

Table 2
15-LO inhibitory activities of sulfamide **15a–15i**



Compound	R^2	NR^3R^4	*c* log *P*	Aqueous solubility (μg/mL)	15-LO IC_{50} (nM)		15-LO CHO cell (% inhibition)	Rabbit whole blood assay (% inhibition)
					LA[a]	AA[b]		
8i	—	—	7.11	<1	4	19	54 at 10 μM	25 at 10 μM
15a	Ph		5.21	NT	3.6	7.5	68 at 10 μM	44 at 10 μM
15b	(3-OMe)Ph		5.21	NT	1.5	4.3	51 at 1 μM	43 at 10 μM
15c	Thien-2-yl		5.09	NT	2	7	79 at 1 μM	40 at 3 μM
15d	5-Me-Isoxazol-3yl		4.23	NT	4.2	15	62 at 10 μM	71 at 10 μM
15e	Pyrazin-2-yl		3.14	79	3	7	81 at 1 μM	51 at 3 μM
15f	Pyrazin-2-yl		3.97	71	5.3	8.2	61 at 3 μM	55 at 10 μM
15g	Pyrazin-2-yl		3.97	76	1.8	2.4	94 at 3 μM	69 at 3 μM 39 at 1 μM
15h	Pyrazin-2-yl		4.37	71	2.7	4.1	83 at 3 μM	63 at 10 μM
15i	Pyrazin-2-yl		4.12	129	1.4	3.1	IC_{50} = 51 nM	73 at 3 μM 45 at 1 μM

NT = not tested.
[a] Enzyme inhibition measured using linoleic acid as substrate.
[b] Enzyme inhibition measured using arachidonic acid as substrate.

were readily transformed to the pyrazolyl amines **7** by treatment with hydrazine in the presence of acetic acid in methanol.

The various analogs thus prepared were tested for in vitro activity against mammalian 15-LO isolated from rabbit reticulocyte using either arachidonic acid (AA) or linoleic acid (LA) as substrates. Inhibition was measured using a standard colorimetric assay monitoring the formation of the lipid hydroperoxide product of either arachidonic acid or linoleic acid [15-hydroperoxyeicosatetraenoic acid (15-HPETE) or 13-hydroperoxyoctadecadienoic acid (13-HPODE), respectively].[13] Compound **8a** displayed modest potency against 15-LO with an IC_{50} value of 3.98 μM using LA as the substrate (Table 1). Replacement of the methyl group of **8a** with isobutyl generated compound **8b** with submicromolar potency. Further modification of isobutyl to phenyl resulted in a twofold increase in potency (compound **8c**, IC_{50} = 150 nM). Additional SAR was carried out by keeping the R^2 group constant as phenyl while modifications were made to the R^1 group. The unsubstituted phenyl analog **8f** (R^1 = Ph) displayed superior potency against 15-LO both with AA and LA as substrates compared to analogs with methoxyphenyl groups (**8c**–**e**). While replacement of the R^2 phenyl with benzyl (**8g**) resulted in fivefold loss in potency, introduction of a thien-2-yl group led to compound **8h** which was highly potent in both the LA and AA based-assays (IC_{50} = 11 and 28 nM, respectively). The preference for a small aromatic ring at R^1 dictated an additional SAR investigation using the thien-2-yl group as a constant while further modifications were made to R^2. Replacement of the R^2 phenyl with heteroaryls (e.g. isoxazolyl, thienyl, pyrazinyl) led to potent 15-LO inhibitors with low double and single digit nanomolar enzyme inhibitory activities. In particular, the symmetrical thien-2-yl analog **8i**, with 4 and 19 nM inhibitory activities respectively in the LA and AA based-assays, was determined to be the most potent analog within this series.

Select compounds were tested in a cell based-assay using Chinese hamster ovary (CHO) cells stably over expressing human recombinant 15-LO.[15] With the exception of compound **8i**, which exhibited only modest activity (54% inhibition at 10 μM, Table 1), most compounds in Table 1 displayed relatively poor activity in the cell-based assay. In addition, the various sulfonamides described herein were exceedingly hydrophobic thus displaying poor aqueous solubilities (e.g. compound **8i**: pH 6.5, solubility = 0.2 μg/mL, *c* log *P* = 7.1). Efforts to introduce more polar functionalities centered around the optimization of thienyl analogs **8h** and **8i**. In our most recent communication,[15] we demonstrated that replacement of the sulfonamide group with sulfamide functionality resulted in compounds with lower *c* log *P* values and increased aqueous solubilities. These sulfamides **15** were generated by derivatization of amines **7** with 2-chloroethyl chlorosulfonylcarbamate (**13**) in the presence of triethylamine to afford the oxazolidinones **14** (Scheme 3) which could readily be converted to the target sulfamides **15** via treatment with various secondary amines.[15]

Biological evaluation of compounds **15** revealed further improvement in enzyme inhibitory activities. As outlined in Table 2, in addition to excellent potency in the LA based assay, most sulfamides also displayed improved activity in the AA based-assay, exhibiting similar single digit nanomolar potencies. The baseline *N*-benzylpyrrolidine analog **15a** displayed 3.6 and 7.5 nM inhibition respectively in the LA and AA based-screens. Introduction of a 3-OMe group at the phenyl group (**15b**) resulted in a twofold improvement in potency. Replacement of the R^2 phenyl with heteroaryls (compounds **15c**–**15e**) retained single digit nanomolar enzyme activities. Introduction of small hydrophobic groups (Me, F) at the benzylamine site was well tolerated. As hoped, introduction of the more polar pyrrolidinyl sulfamide group had a major impact on the overall polarity (*c* log *P*) and aqueous solubility of the target molecules. This resulted in a significant enhancement of inhibitory activity in the cell based assay. For example, the bis-thienyl sulfamide **15c** with a *c* log *P* value of 5.09, displayed 79% inhibition at 1 μM, and was significantly more potent in cells than the corresponding sulfonamide **8i** (*c* log *P* = 7.11, 54% inhibition at 10 μM). Introduction of a more polar pyrazinyl ring (**15e**–**15i**) resulted in dramatic improvement in polarity and aqueous solubility (e.g. compound **15e**: *c* log *P* = 3.14, aqueous solubility = 79 μg/mL) as well as, in most cases, a significantly enhanced inhibitory activity in the cells (Table 2). For example, the pyrazinyl analog containing a 4-fluorophenyl group (compound **15i**) with a *c* log *P* value of 4.12 shows a marked improvement in aqueous solubility (129 μg/mL at pH 6.5) and significantly improved inhibitory activity in CHO cells (IC_{50} = 51 nM).

Table 3
Selectivity data for selected compounds

Compound	Inhibition[a]		
	15-LO IC_{50} (nM)	5-LO[b]	12-LO[b] (%)
8i	19	NT	35
15f	8.2	68%	38
15g	2.4	5.5 μM	32
15h	4.1	60%	35
15i	3.1	NT	16

NT = not tested.
[a] Enzyme inhibition measured using arachidonic acid as substrate.
[b] Displayed data represent IC_{50} values or % inhibition at 10 μM.

In order to identify potential in vivo candidates, select compounds were evaluated in a rabbit whole blood assay measuring inhibition of 15-HETE production.[16,17] Inhibitory activity was measured at 1, 3 or 10 μM concentrations and is outlined in Table 2. As predicted by the CHO cell activity, the relatively hydrophobic and poorly soluble *n*-pentylphenyl analog **8i** (*c* log *P* 7.11, aqueous solubility < 1 μg/mL) displayed weak inhibition in the whole blood assay (25% at 10 μM). In contrast, and not unexpectedly, the more polar analogs (**15a**–**15i**) with generally improved CHO cell activity were also significantly more potent in the whole blood assay. Among the best was compound **15i**: CHO cell IC_{50} of 51 nM and 73% inhibition of 15-HETE in the whole blood assay at 3 μM concentration.

Several compounds were evaluated for inhibition selectivities versus the 5- and 12-lipoxygenase isoforms. As outlined in Table 3, all compounds tested showed excellent selectivity with only modest inhibition of 5-LO or 12-LO at relatively high concentrations (10 μM).

In conclusion, we initially identified a novel series of pyrazoyl sulfonamides as potent inhibitors of the mammalian 15-LO. While the enzyme activity of this series could be significantly improved, most sulfonamides displayed poor activity in cell-based assays. Extensive SAR studies resulted in the discovery of the pyrrolidinyl sulfamide series with significantly improved aqueous solubility and enzyme and cell activity. Additionally, as demonstrated by compound **15i** (CHO cell IC_{50} = 51 nM, whole blood inhibition 73% at 3 μM), compounds with enhanced cell activity also displayed good inhibition in the whole blood assay. As such, many of these analogs appeared to exhibit adequate in vitro potency for testing in vivo. Unfortunately these analogs exhibited poor pharmacokinetics in rodent models[18] upon oral dosing and were deemed not suitable for pharmacodynamic evaluation.

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17. (a) Phenylhydrazine treated rabbits: A 1.5% phenylhydrazine (Sigma, St. Louis, MO) solution was prepared fresh daily using 1 M HEPES (pH 7.4)-buffered water (ratio of 1:4). Eight-month-old New Zealand White Rabbits were given subcutaneous injections of phenylhydrazine (6 mg/kg/day). After 5 days of consecutive phenylhydrazine treatment a heparinized blood sample was obtained from an ear artery of each rabbit. A portion of the blood sample was utilized to determine the reticulocyte count and hematocrit. (b) Inhibition of 15-HETE production in rabbit reticulocyte-rich whole blood: Freshly drawn blood was obtained from phenylhydrazine-treated rabbits and collected into 10-cc glass tubes containing sodium heparin. Fresh blood samples were divided into 1 mL aliquots and either vehicle or compound (prepared in 100% DMSO) were added. The blood sample was subsequently stimulated with the addition of calcium ionophore A23187 and arachidonic acid, final concentrations were 30 and 10 μM, respectively. The samples were then incubated for 30 min at 37 °C with continuous gentle mixing. Extraction and RP-HPLC quantitation of 15-HETE presence were as previously described [Surette, M. E.; Odeimat, A.; Palmantier, R.; Marleau, S.; Poubelle, P. E.; Borgeat, P. Reverse-phase high-performance liquid chromatography analysis of arachidonic acid metabolites in plasma after stimulation of whole blood ex vivo. *Anal. Biochem.* **1994**, *216*, 392].
18. As an example, compound **15g** produced poor plasma exposures upon dosing in mice at an 8 mpk oral dose with an AUC of 83 nM h.