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Discovery of novel and potent aryl diamines as leukotriene A_4 hydrolase inhibitors

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ABSTRACT

The synthesis and biological evaluation of a series of aryl diamines as inhibitors of LTA_4-h inhibitors are described. The optimization which led to the identification of the optimal *para*-substitution on the diphenyl ether moiety and diamine spacer is discussed. The resulting compounds such as **3l** have excellent enzyme and cellular potency as well as desirable pharmacokinetic properties.

Leukotriene B_4 (LTB_4) is a potent pro-inflammatory activator of inflammatory cells, including neutrophils, eosinophils, monocytes, macrophages, T cells, and B cells, which is a functional link between early innate and late adaptive immune responses.[1] There is substantial evidence that LTB_4 plays a significant role in the amplification of many inflammatory disease states[2] including asthma,[3] inflammatory bowel disease (IBD),[4] chronic obstructive pulmonary disease (COPD),[5] arthritis,[6] psoriasis,[7] and atherosclerosis.[8] LTB_4 also stimulates the production of various cytokines and may play a role in immunoregulation.[9] Therefore, a therapeutic agent that inhibits the biosynthesis of LTB_4 may be useful for the treatment of these inflammatory conditions.

Three enzymes are involved in the biosynthesis of LTB_4 from arachidonic acid (AA): phospholipase A_2 (PLA_2) to release AA from the membrane lipids; 5-lipoxygenase (5-LO) to form the unstable epoxide leukotriene A_4 (LTA_4); and leukotriene A_4 hydrolase (LTA_4-h) to form LTB_4.[10] In addition to being the precursor to LTB_4, LTA_4 is a substrate of LTC_4 synthase to yield the pro-inflammatory cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4[11] and a substrate of lipoxygenases to give the anti-inflammatory mediators, lipoxins A_4 (LXA_4), and B_4 (LXB_4).[12] Thus, the targeting of LTA_4-h would leave other immune response pathways intact.

LTA_4-h is a monomeric, soluble 69-kDa zinc metallohydrolase of the M1 class which has sequence homology to aminopeptidases M and B. It can act as a non-specific peptidase but has no known physiological substrate. The bifunctional nature of LTA_4-h allows for the determination of inhibition constants using either a peptidase or a hydrolase assay.[13–15] Since LTA_4-h is an intracellular enzyme, inhibitors were additionally characterized in a cellular human whole blood assay (WBA).[13] Data from the hydrolase and WBA are presented in Tables 1 and 2. In the end, the WBA is presumed to be more relevant, but initially the hydrolase assay was used for optimization. LTA_4-h is considered to be a druggable target with reports of high-resolution crystal structures with bound inhibitors.[16] The search for inhibitors has attracted attention,[14,17] including our report on *N*-alkyl glycine amides.[18] In this letter, we describe the transition of *N*-alkyl glycine amide **1** to the aryl diamine template of potent, selective, and orally available inhibitors of LTA_4-h.

As part of a general strategy to decrease the molecular weight, basicity, and overall length of the compound, we reduced the central diamine group in our *N*-alkyl glycine analog series to yield our aryl diamine template described here. The transformation is outlined in Figure 1 with truncation of the glycine group in **1** by removal of the aminomethyl group to give **2**. The 6-fold loss in activity could be reversed by further truncation of **2** to give an aryl diamine analog represented by **3**. Unfortunately, we were unable to obtain X-ray crystal structures of our inhibitors in LTA_4-h and relied on SAR to direct our optimization effort.

The preparation of analogs in the aryl diamine series is shown with the synthesis of **3l** (Scheme 1). Conversion of 4-carboxyani-

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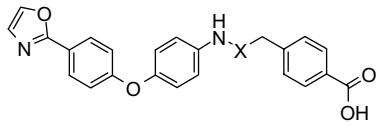
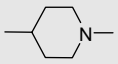
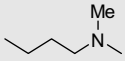
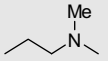
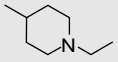
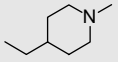
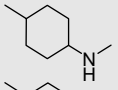
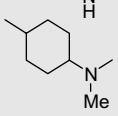
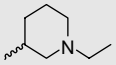
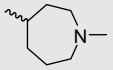
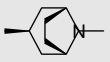
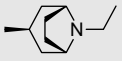
Table 1
Optimization of spacer of 4-(2-oxazolyl)phenyl phenyl ether analogs



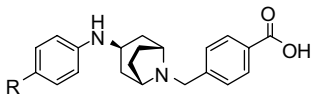
Compound	X	LTA_4-h IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
3a		30	80
3b		3	18
3c		130	560
3d		3	50
3e		5	1500
3f		11	220
3g		9	100
3h		60	200
3i		2	70
3j		7	60
3k		15	30
3l		5	40
3m		1	40

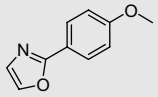
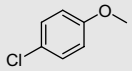
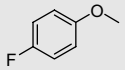
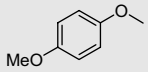
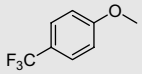
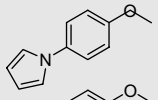
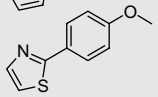
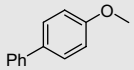
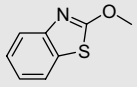
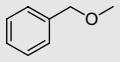
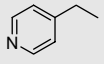
[a] Values are means of at least two experiments.

sole, **6**, to the corresponding acid chloride with $POCl_3$ followed by treatment with aminoacetaldehyde dimethyl acetal gave the amide intermediate **7**. Cyclization using Eaton's reagent to the desired oxazole followed by treatment with an aqueous methanolic sodium hydroxide solution gave the desired 4-(2-oxazolyl)phenol, **8**. Nucleophilic displacement of the fluorine on nitrofluorobenzene by **8** gave aminodiphenyl ether **9** after hydrogenolysis. Reductive alkylation of **9** with *N*-Boc-nortropinone followed by removal of the Boc protecting group with 4.0 M HCl solution in THF gave a single *endo* diastereomer, **10**. Alkylation of **10** with methyl bromomethylbenzoate followed by saponification gave **3l**.

Since the *N*-methyl tertiary aniline analog of **2** was inactive in the hydrolase assay (IC_{50} >1000 nM), the piperidine group on **2** was systematically modified to optimize the inhibitor to LTA_4-h interactions (Table 1). Several cyclic, bicyclic, and acyclic amines were prepared and assayed to rank the importance of the distance between the aromatic groups and the placement of the amine in the chain. When a flexible chain is used as in the acyclic series, the optimal distance between the aromatic groups is set at 6-atoms, **3b**, with a 40-fold decrease in activity seen with the corresponding 5-atom chain **3c**. In the piperidine series, the addition of a carbon to either end of the piperidine group increased the potency at least 6-fold, **3d** and **3e**, compared to **3a**. Shifting of the nitrogen out of the ring, as seen in the aminocyclohexane analogs **3f** and **3g**, resulted in a loss of activity compared to **3d**. Shifting of the anilino group from C-4 to C-3 of the piperidine, **3h**, resulted in a 2-fold drop in activity compared to **3a**, but a similar shift in the

Table 2
Optimization of phenoxy moiety of **3l**



Compound	R	LTA_4-h IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
3l		5	40
4a		14	20
4b		6	310
4c		320	870
4d		40	30
4e		6	40
4f		23	170
4g		13	230
4h		4	30
4i		8	19
4j		5	25
4k		9	100
4l		47	220
4n		3	40
4o		75	440

[a] Values are means of at least two experiments.

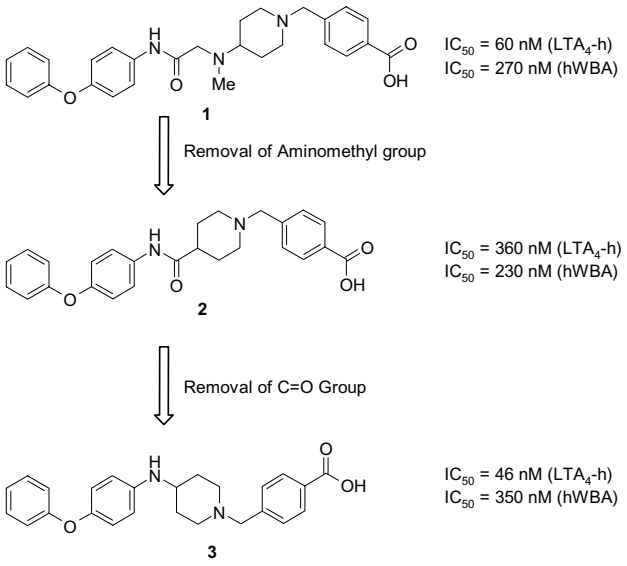
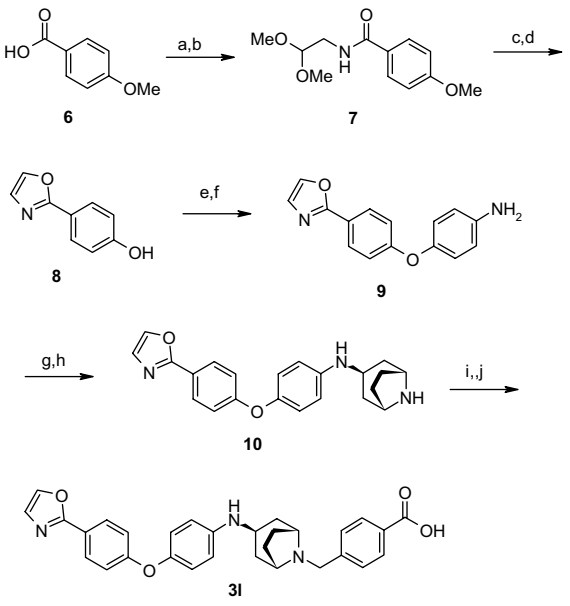


Figure 1. Optimization strategy.



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF; (b) protected aminal, CH_2Cl_2; (c) Eaton's reagent, 180 °C; (d) NaOH, MeOH–H_2O; (e) 4-nitrofluorobenzene, DIEA, DMF; (f) H_2, Pd/C, MeOH; (g) *N*-Boc-nortropinone, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (h) 4.0 M HCl, THF; (i) methyl 4-bromomethylbenzoate, K_2CO_3, MeCN; (j) $LiOH{\cdot}H_2O$, H_2O–THF, reflux.

one-carbon extension series, **3i**, resulted in an equipotent analog to **3d**. Evidence for a specific interaction between the amine and LTA_4-h is further demonstrated with the 5- and 7-membered ring analogs of **3a**, **3j**, and **3k**, having similar potency. Although the comparison is complicated by the introduction of a chiral center caused by the change in the substitution pattern and the testing of the compounds as racemic mixtures, the data clearly supports similar activity for the analogs. The 6-fold increase in the potency of bicyclic amine **3l** over **3a** and the 3-fold increase of **3m** over **3d** suggest the amine group binds in a sterically less-demanding pocket of LTA_4-h.

With nortropane as the amine, we explored the substitution on the phenoxy group (Table 2). The unsubstituted analog, **4a,** has

Table 3
DMPK profile of selected inhibitors

Compound	Cl (mL/min/kg)	C_{max} (μg/mL)	$t_{1/2}$ (h)	%F	Metabolic stability[a]		
					Rat	Dog	Human
3a	1	37	1.5	99	91	81	92
3b	23	4	1.9	67	81	75	81
3l	6	7	2.9	99	96	91	82
4a	36	1	0.9	62	90	91	94
4b	4	2	12	96	100	100	99
4d	9	2	4.8	99	99	94	97

[a] metabolic stability; % remaining after 1.0 h in the corresponding liver microsomes.

similar activity to **3l**. Introduction of chlorine at the *para* position, **4b**, did not improve potency, whereas at the *ortho* position, **4c,** was 50-fold less potent. The potency of the corresponding fluorine analogs, **4d** and **4e**, reversed the trend and suggests steric bulk is favorable at the *para* position but not at the *ortho* position. The steric argument is supported by the lack of a significant impact of shifting from electron-donating substituent as methoxy (**4f**) to an electron-withdrawing substituent, as trifluoromethyl (**4g**) or by substitution of a pyrrole (**4h**), thiazole (**4i**), or phenyl (**4j**) group. Although the benzothiazole (**4k**) group maintained potency, the benzyl analog (**4l**) was about 10-fold less potent and may indicate the importance of the shape of the diphenyl ether group to binding in the hydrophobic pocket. The increase in potency upon substitution of carbon for the ether oxygen, **4n** versus **4a**, suggests that the oxygen does not interact with the protein. The loss of activity upon introduction of a pyridine group, **4o**, suggests that polar groups are not tolerated in the hydrophobic pocket.

The WBA does not follow the activity in the hydrolase assay due to the need for analogs to enter the cell to inhibit the target. Cellular uptake favors the less polar analogs as **4h**, **4i**, and **4j** over polar substituents as methoxy (**4f**) or trifluoromethyl (**4g**). A similar effect was seen with the pharmacokinetic profile (PK) of the analogs after additional factors such as molecular rigidity and accessibility are considered. Flexible analogs such as **3b** had higher clearance and shorter half-lives (data not shown) than the *endo*-nortropane analogs **3l**, **4b**, and **4d** (Table 3) which had low clearance, significant C_{max}, long half-lives, metabolic stability, and good oral bioavailability.

In summary, a series of aryl diamines were designed and prepared which showed significant activity in both the enzyme and cell-based assays. The optimum amine, nortropane group, and the optimum phenoxy substituent, 2-oxazole, were combined in analog **3l**. Further studies are planned and will be reported on in due course.

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