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Synthesis of *N*-alkyl glycine amides as potent inhibitors of leukotriene A_4 hydrolase

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ABSTRACT

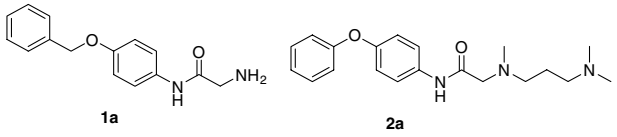
The synthesis and biological evaluation of a series of *N*-alkyl glycine amide analogs as LTA_4-h inhibitors and the importance of the introduction of a benzoic acid group to the potency and pharmacokinetic parameters of our analogs are described. The lead compound in the series, **4q**, has excellent potency and oral bioavailability.

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 simulates 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 lipooxygenases 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 1–3. 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] In this letter, we describe our effort to identify a potent, selective, and orally available inhibitor of LTA_4-h.

Starting from HTS hit **1a** with an IC_{50} of 0.3 μM, a series of analogs were prepared which contained either a benzyloxy or phenoxy substituent on the aniline and either a cyclic or acyclic diamine. One of the most potent hits was **2a** with IC_{50} value of 0.16 μM. Compound **2a** was used as the starting point for further optimization.



1a **2a**

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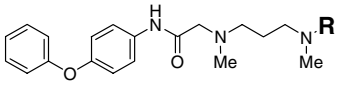
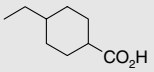
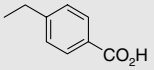
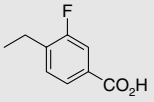
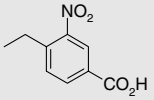
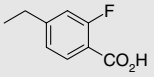
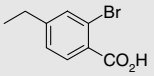
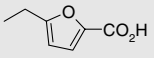
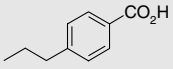
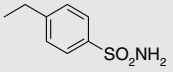
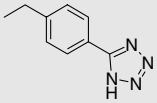
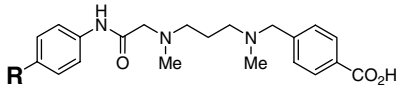
Table 1
Substitution on amine

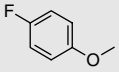
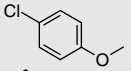
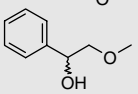
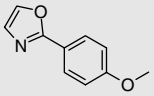


Compound	R	Hydrolase IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
2a	–Me	160	135
2b	$-CH_2Ph$	90	350
2c	$-(CH_2)_3CO_2H$	110	1500
2d	$-(CH_2)_4CO_2H$	18	230
2e		25	430
2f		6	120
2g		7	160
2h		15	290
2i		6	340
2j		10	360
2k		28	440
2l		3	80
2m		33	>1000
2n		4	250

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

Table 2
Variation of the biphenyl ether moiety



Compound	R	Hydrolase IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
2f		6	120
3a		16	230
3b		3	70
3c		6	120
3d		17	90
3e		19	210
3f		5	60

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

The synthesis of analogs is shown in Scheme 1 using **4q** as an example for analog preparation using a chiral reagent. Phenoxyaniline is acylated in quantitative yield with bromoacetyl bromide and one equivalent of diethylaniline in methylene chloride to give **5**. Alkylation of 1,1-dimethylethyl (2*S*)-2-(aminomethyl)-pyrrolidinecarboxylate with **5** using Hunig's base in acetonitrile, followed by methyl iodide using Hunig's base in DMF gave the desired tertiary amine **6** in a 25% yield over the two steps. The *N*-Boc group was removed with trifluoroacetic acid and the resulting amine was alkylated with methyl 4-(bromomethyl)benzoate in 40% yield. Saponification using sodium hydroxide in a mixture of aqueous methanol and THF then provided **4q** in 60% yield. Analog **4p** was prepared similarly to **4q**. The enantiomeric pairs **4n** and **4o**, **4r** and **4s**, and **4t** and **4u** were prepared by separating the racemic mixtures by chiral chromatography without assignment of absolute configuration.

To further optimize the interactions between **2a** and LTA_4-h, we sought to mimic the proposed binding of LTA_4 to LTA_4-h[16] through the addition of a carbon chain terminating with a carboxylic acid (Table 1). Alkyl carboxylates having a chain length over three carbons (**2d** and **2e** vs **2c**) were more potent in the hydrolase assay than **2a**, but not as measured in the WBA. Substitution of a benzene ring, **2f**, for the alkyl chain further increased the potency of the analog and resulted in a 25-fold increase in the potency in the hydrolase assay over **2a**

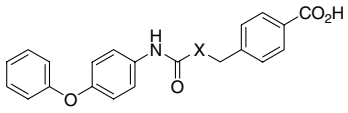
Although we were unable to obtain crystal structures of bound inhibitors in this series by either soaking or co-crystallization experiments, a model of **2f** bound to LTA_4-h was proposed (Fig. 1). The key interactions are between the diphenyl ether group and the hydrophobic pocket, the diamine group with Gln_{136} and Glu_{296}, and the benzoic acid group and Arg_{563} and Lys_{565}. The modeled structure rationalizes the 15-fold increase in potency of **2f** over the corresponding phenyl analog, **2b** and the importance of the carboxylate group.

In an attempt to increase the potency further, a variety of substituents were added to the benzoic acid (Table 1). No significant improvement in potency in either the hydrolase or WB assays was observed upon substitution of the benzoic acid ring for **2g**, **2h**, **2i**, and **2j**. Replacement of the benzene ring with a heterocycle, **2k** or replacement of the carboxylic acid with a sulfonamide, **2m**, showed a slight decrease in potency relative to **2f**. The tetrazole analog, **2n**, was equipotent with **2f**. The most potent compound was the extended linker analog, **2l**. None of these modifications had a significant effect on potency in the WBA, which presumably reflects the difficulty in uptake of charged compounds into cells.

The steric requirement of the hydrophobic pocket was explored using the linear diamine and modifying the diphenyl ether (Table 2). Neither substitution on the diphenyl ether, **3a**, **3c**, and **3d**, nor replacement of the ether linkage with a methylene, **3b**, resulted in a significant change in potency compared with **2f**. Increasing the size of the group by the addition of a heterocycle, **3f**, or extension of the linker, **3e**, did not significantly affect the activity in either assay.

The optimization of the interaction of the diamine group with the two aspartic acid residues near the zinc was explored next (Ta-

Table 3
Variation of the central diamine group



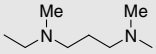
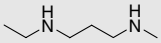
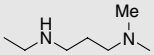
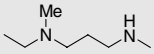
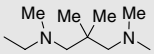
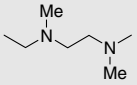
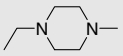
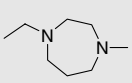
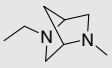
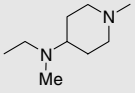
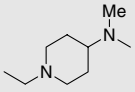
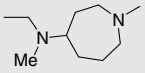
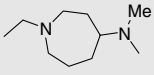
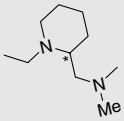
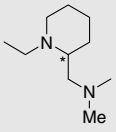
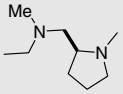
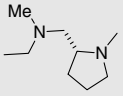
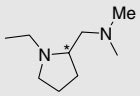
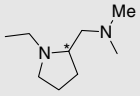
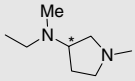
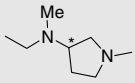
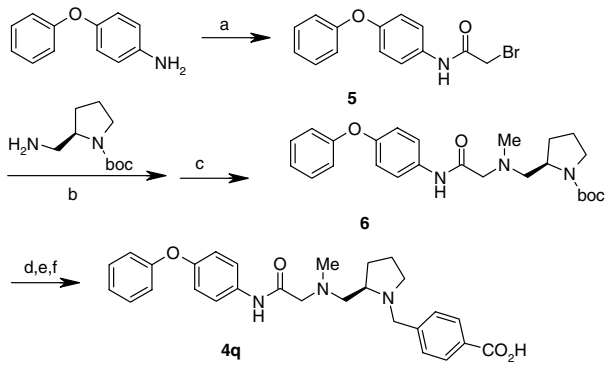
Compound	X	Hydrolase IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
2f		6	120
4a		370	>1000
4b		330	>1000
4c		19	420
4e		7	70
4f		15	140
4g		1000	>1000
4h		70	180
4i		44	240
4j		60	270
4k		490	260
4l		160	380
4m		140	220
4n		17	100
4o		16	60
4p		100	200
4q		7	30

Table 3 (*continued*)

Compound	X	Hydrolase IC_{50}[a] (nM)	WBA IC_{50}[a] (nM)
4r		26	60
4s		17	60
4t		120	180
4u		57	220

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



Scheme 1. Synthesis of **4q**. Reagents and conditions: (a) Bromoacetyl bromide, diethyl aniline, CH_2Cl_2; (b) *i*PrNEt$_2$, CH_3CN; (c) MeI, PrNEt$_2$, DMF; (d) TFA, CH_2Cl_2; (e) Methyl 4-(bromomethyl)benzoate, K_2CO_3, DMF; (f) 1 N NaOH, MeOH/THF.

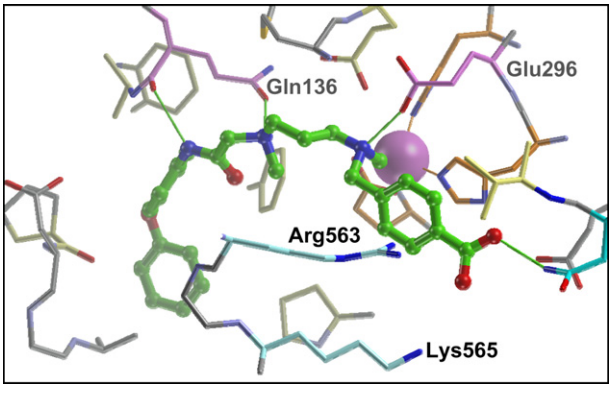


Figure 1. Model of **2f** bound to LTA4-h.

ble 3). The relative importance of the *N*-methyl groups on **2f** to binding was explored with nor-analogs, **4a**, **4b**, and **4c.** Removal of the *N*-methyl from the glycine amino group resulted in a 50+-fold drop in binding as seen in **4a** and **4b**, but only a 3-fold drop with removal of the *N*-methyl from the benzyl amino group, **4c**. The importance of flexibility and the distance between nitrogen atoms is seen in the 60-fold increase in activity seen by opening up the piperazine ring, **4g**, to the linear ethylene diamine, **4f** or the 10-fold increase in expanding the piperazine ring, **4g**, to a

homo-piperazine ring, **4h**. The use of cyclic amines introduces asymmetry into many of the compounds, but potentially allows for improved binding. For example, the methylaminopiperidine group can be inserted in two different orientations, **4j** and **4k**, with an 8-fold difference in potency in the hydrolase assay. This difference in potency with orientation was not seen with the 7-membered ring analogs **4l** and **4m**. The additional diversity element of stereochemistry is added with the pyrrolidine or piperidine analogs. Although the importance of the chiral center was minimal in most cases, as seen in the pairs **4n** and **4o**, **4r** and **4s**, and **4t** and **4u**, the 10-fold difference in activity between **4p** and **4q** is the exception and suggests a tight-binding orientation for the *R*-isomer, **4q**.

In parallel to the potency assays, the ADME profile of key compounds was evaluated. An added benefit of the benzoic acid group was the increased stability in rat microsomes from less than 33% remaining at 1 h for **2a** and **2b** to >90% remaining at 1 h for **2f**. The clearance value of 38 mL/min/kg and half-life of 0.9 h after iv administration for **2f** was not improved by substitution on the benzoate ring, **2g** to **2j**, replacement with heterocycles, **2k**, or substitution on the phenoxy group, **3a** through **3f** (data not shown). Insertion of a pyrrolidine group in the chain did improve the half-life of for **4q**, 2.6 h after iv administration, but not the clearance value, 28 mL/min/kg. The % F value for both **2f** and **4q** was similar at around 45% compared to the negligible oral bioavailability of **1a** or **2a**.

In this letter, we have described the optimization of a series of diamines as inhibitors of LTA_4-h. Starting with **1a**, we have extended the compound through the addition of a benzoic acid group to give **4q** with increased potency and oral bioavailability.

References and notes

1. Goodarzi, K.; Goodarzi, M.; Tager, A. M.; Luster, A. D.; von Andrian, U. H. *Nat. Immunol.* **2003**, *4*, 965.
2. Lewis, R. A.; Austen, K. F.; Soberman, R. J. *N. Engl. J. Med.* **1990**, *323*, 645.
3. Munafo, D. A.; Shindo, K.; Baker, J. R.; Bigby, T. D. *J. Clin. Invest.* **1994**, *93*, 1042.
4. Sharon, P.; Stenson, W. F. *Gastroenterology* **1984**, *86*, 453.
5. Barnes, P. J. *Respiration* **2001**, *68*, 441.
6. Griffiths, R. J.; Pettipher, E. R.; Koch, K.; Farrell, C. A.; Breslow, R.; Conklyn, M. J.; Smith, M. A.; Hackman, B. C.; Wimberly, D. J.; Milici, A. J.; Scampoli, D. N.; Cheng, J. B.; Pillar, J. S.; Pazoles, C. J.; Doherty, N. S.; Melvin, L. S.; Reiter, L. A.; Biggars, M. S.; Falkner, F. C.; Mitchell, D. Y.; Liston, T. E.; Showell, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 517.
7. Ikai, K. *J. Dermatol. Sci.* **1999**, *21*, 135.
8. Helgadöttir, A. Manolescu, A.; Helgason, A.; Thorleifsson, G.; Thorsteinsdöttir, U.; Gudbjartsson, D. F.; Gretarsdöttir, S.; Magnusson, K. P.; Gudmundsson, G.; Hicks, A.; Jonsson, T.; Grant, S. F. A.; Sainz, J.; O'Brien, S. J.; Sveinbjornsdöttir, S.; Valdimarsson, E. M.; Matthiasson, S. E.; Levey, A. I.; Abramson, J. L.; Reilly, M. P.; Vaccarino, V.; Wolfe, M. L.; Gudnason, V.; Quyyumi, A. A.; Topol, E. J.; Rader, D. J.; Thorgeirsson, G.; Gulcher, J. R.; Hakonarson, H.; Kong, A.; Stefansson, K. *Nat. Genet.* **2006**, *38*, 68.
9. Ford-Hutchinson, W. *Crit. Rev. Immunol.* **1990**, *10*, 1.
10. Ford-Hutchinson, A. W.; Gresser, M.; Young, R. N. *Annu. Rev. Biochem.* **1994**, *63*, 383.
11. Aharony, D. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, S214.
12. Serhan, C. N. *Prostaglandins* **1997**, *53*, 107.
13. Penning, T. D. *Curr. Pharm. Des.* **2001**, *7*, 163.
14. Penning, T. D.; Russell, M. A.; Chen, B. B.; Chen, H. Y.; Liang, C.-D.; Mahoney, M. W.; Malecha, J. W.; Miyashiro, J. M.; Yu, S. S.; Askonas, L. J.; Gierse, J. K.; Harding, E. I.; Highkin, M. K.; Kachur, J. F.; Kim, S. H.; Villani-Price, D.; Pyla, E. Y.; Ghoreishi-Haack, N. S.; Smith, W. G. *J. Med. Chem.* **2002**, *45*, 3482.
15. Liang, A. M.; Claret, E.; Ouled-Diaf, J.; Jean, A.; Vogel, D.; Light, D. R.; Jones, S. W.; Guilford, W. J.; Parkinson, J. F.; Snider, R. M. *J. Biomol. Screen.* **2007**, *12*, 536.
16. Thunissen, M. M. G. M.; Nordlund, P.; Haeggström, J. Z. *Nat. Struct. Biol.* **2001**, *8*, 131.
17. Kirkland, T. A.; Adler, M.; Bauman, J. G.; Chen, M.; Haeggström, J. Z.; King, B.; Kochanny, M. J.; Liang, A. M.; Mendoza, L.; Phillips, G. B.; Thunnissen, M.; Trinh, L.; Whitlow, M.; Ye, B.; Ye, H.; Parkinson, J.; Guilford, W. J. *Bioorg. Med. Chem.* **2008**, *16*, 4963.