Bioorganic & Medicinal Chemistry Letters 20 (2010) 2851–2854

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel leukotriene A_4 hydrolase inhibitors based on piperidine and piperazine scaffolds

Vincent Sandanayaka [a], Bjorn Mamat [b], Nikhil Bhagat [a], Louis Bedell [a], Gudrun Halldorsdottir [b], Heida Sigthorsdottir [b], Þorkell Andrésson [b], Alex Kiselyov [a], Mark Gurney [a,b], Jasbir Singh [a,*]

[a] Medicinal Chemistry Department, deCODE Chemistry, Inc. 2501 Davey Road, Woodridge, IL 60517, United States
[b] deCODE Genetics, Inc., Sturlugata 8, IS-101, ReykjaVik, Iceland

ARTICLE INFO

Article history:
Received 27 December 2009
Revised 7 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Keywords:
Human LTA4H Inhibitors
LTA4 hydrolase inhibitors
Structure-based design
Piperidine and piperazine derivatives

ABSTRACT

Novel piperidine and piperazine derivatives have been designed and tested as inhibitors of LTA_4 hydrolase (LTA_4H). Most potent compounds showed good potency in both enzymatic and functional human whole blood assay. Crystallography studies further confirmed observed structure–activity relationship and LTA_4H binding mode for analogs from the piperidine series.

Leukotriene A_4 hydrolase (LTA_4H) is a key enzyme in the leukotriene pathway, which hydrolyzes leukotriene A_4 (LTA_4) to leukotriene B_4 (LTB_4), a proinflammatory mediator.[1] There is substantial evidence that LTB_4 plays a significant role in many diseases such as inflammatory bowel disease (IBD),[2] rheumatoid arthritis,[3] psoriasis,[4] gout,[5] and inflammatory lung diseases including asthma.[6] In addition, LTB_4 also stimulates the production of cytokines and may play a role in immunoregulation.[7]

We became interested in this target as a result of our human population genetic studies, which indicated LTA_4H as a potential therapeutic target for cardiovascular (CV) disease including acute myocardial infarction (AMI) and stroke.[8] A number of LTA_4H inhibitors have been reported over the past two decades.[9] Design of the earlier agents was based on the substrate, LTA_4 while later molecules were directed towards Zn-chelation as exemplified by **1**,[10] **3**,[11] and **4**.[12] A non-peptidic molecule **2** was introduced as a non-Zn chelating LTA_4H inhibitor.[13]

Crystal structure of LTA_4H has been reported;[14] however, there were no systematic studies elaborating on the potential of LTA_4H active site in the drug design context. Considering the significance of our genetic findings and potential broader applications of LTA_4H inhibitors in multiple disease areas, we used both X-ray crystallography and ligand data from the literature to identify novel chemotypes active in both enzymatic and functional assays.

Based on our internal structural data,[15] the active site of LTA_4H is buried deep in the protein, and a small opening is exposed to the solvent (Fig. 1A). Several key residues, namely *Gln*-134, *Gln*-136, and *Tyr*-267 in the hydrophobic and linker regions could be accommodated in the ligand design process, as shown in Figure 1. Thus, our design exploited several unique features of the active site, namely: (a) large hydrophobic pocket, (b) three tightly bound, structurally conserved water molecules residing in the hydrophobic site, (c) hydrophilic pocket containing catalytic Zn^{2+} ion, and (d) L-shaped junction featuring ca. 90° angle that separates the hydrophobic and hydrophilic sites (Fig. 1B). These structural data combined with the structure–activity relationship (SAR) reported by our group earlier led to the identification of a lead series based on prolinol template[15,16] and a Phase II clinical candidate DG-051 (**5**). This agent displayed IC_{50} values of 87 nM in the LTA_4H enzyme and 449 nM in a physiologically relevant secondary functional assay, where human whole blood (hWB) was used to assess inhibition (IC_{50}) of LTB_4 production after stimulation with Ca^{2+} ionophore. In our subsequent efforts to identify additional potent chemical series based on the fragment-based approach,[15,17] we turned our attention to the six-membered alicyclic templates including piperidine and piperazine exemplified by **6** (Scheme 1).

In designing a feasible synthetic route to the targeted molecules, we used cyclic sulfamidate analog **11a** (Y = CH_2, Scheme 2) available either directly from **8a** or from the corresponding sulfoxide **9a** (Scheme 1). Compound **11a** was reacted with a series of phenols **10** followed by N-alkylation of piperidine moiety with R^2X to furnish derivatives **13**–**34**. In order to further expand the

* Corresponding author. Tel.: +1 630 904 2218.
E-mail addresses: jsingh@decode.com, Jasbir@Jasinds.com (J. Singh).

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.03.047

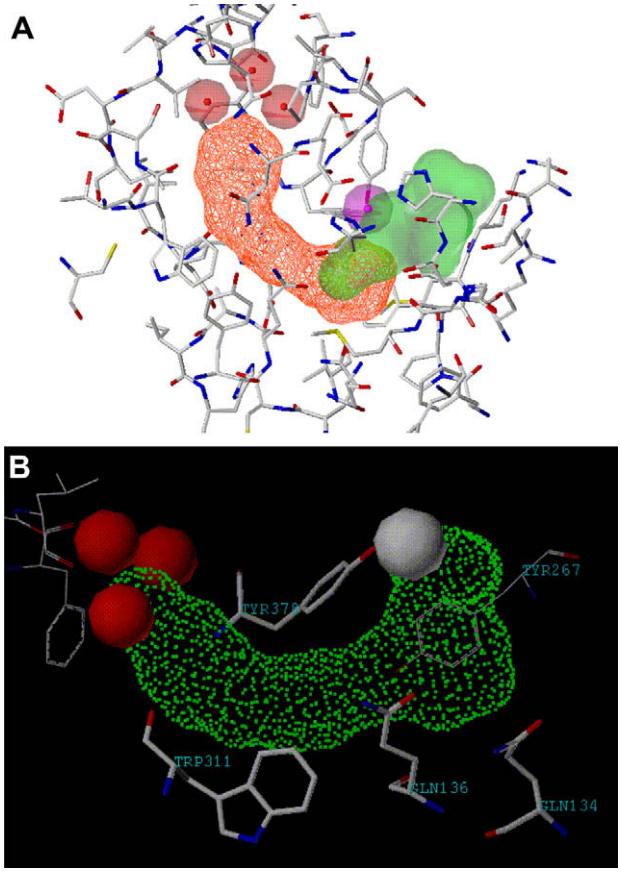


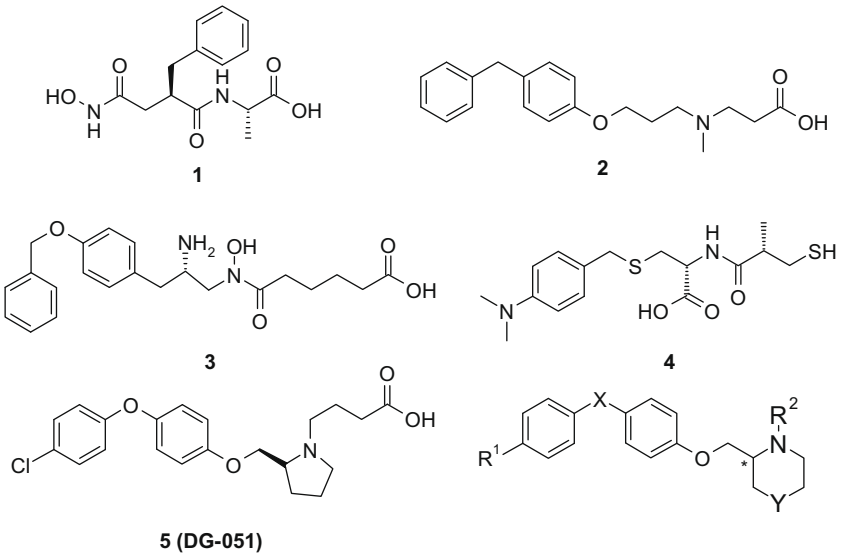
Figure 1. (A) Surface representation of LTA_4H showing large solvent-accessible cavity and narrow hydrophobic pocket; area associated with peptidase activity (green) and hydrophobic region related to hydrolase activity (red) are highlighted. (B) Active site with surface targeted in our drug discovery effort; dot surface shown is for compound **5** (DG-051), the crystallographic waters and zinc ion are shown as CPK (Corey, Pauling and Koltun) rendering in red and white, respectively; key residues are highlighted.

diversity within our structure–activity relationship (SAR) studies, we also prepared several relevant piperazine analogs **35** and **36** from **8b** following similar synthetic route.

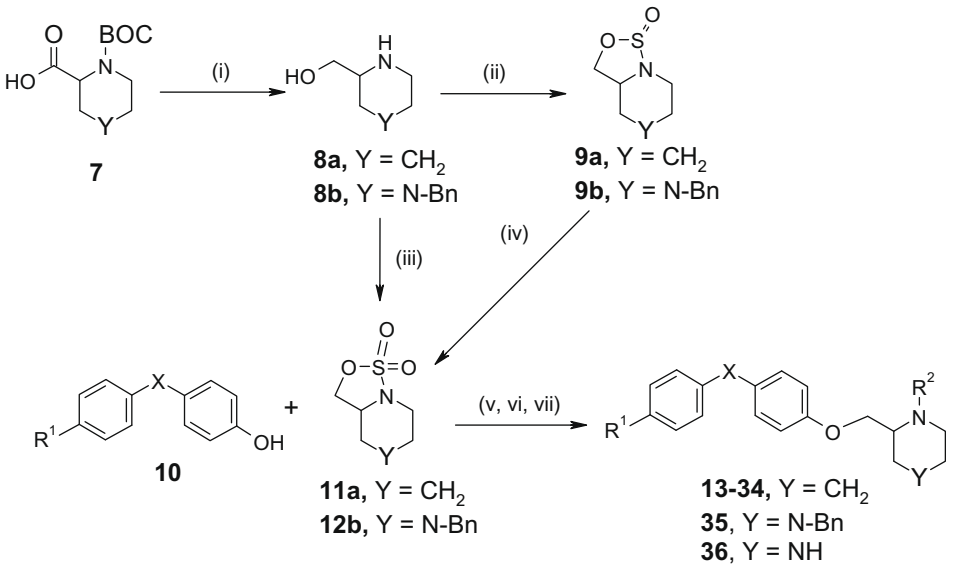
Selected compounds from these series were evaluated in the recombinant human LTA_4H enzyme assay and functional human whole blood (hWB) LTB_4 production assay (Table 1). Early racemic compounds **13**–**14** displayed comparable potency in both enzyme and hWB assays. Enantiomers containing either methylene or ether bridge (**15**–**17**) featured very good activity in these assays. Biphenyl ether analogs **16** and **17** were two-fold more active than the respective biphenyl methylene compond **15**. When a phenyl group was introduced at the *para*-position of the terminal phenyl ring (e.g., **18**, **19**), enzymatic activity dropped, whereas hWB potency improved. A variety of other *para*-substituents were well tolerated yielding molecules (**22**–**24**) active in both enzymatic and functional settings. Similar to unsubstituted phenyl analogs, 4-chlorophenyl derivatives afforded better activity in biphenyl series featuring ether versus methylene linker (compare **13** vs. **14** and **23** vs. **24**).

In order to optimize the position of CO_2H group complexing to Zn^{2+}, we varied acyclic alkyl chain between piperidine nitrogen and carboxylic acid (**25**–**28**) using **24** as a template. Oxadizole **29** was about a log order less active compared with the carboxylic acid analog **24**. Ethylene linker yielded four-fold more potent compound (**26**) than the corresponding methylene derivative (**25**). Butyric acid derivatives (**27**, **28**) displayed further enhancement in potency. The *R*-enantiomer **28** exhibited an IC_{50} of 56 nM in the enzymatic and 6 nM in the hWB assays. We believe, this profile is due to the optimized interactions of the piperidine template with both *Gln*-134 and *Gln*-136 residues. Encouraged by this result, we further investigated the left hand side of the molecule by incorporating substituents at the terminal phenyl ring. Our rationale for this synthetic step was to displace structurally conserved water molecules and thereby increase the potency of the resulting molecules. Specifically, we prepared several compounds containing five-membered heterocycles at the *para*-position of the phenyl ring (**30**–**33**). Notably, these analogs showed good potency against the enzyme, with compounds **32** and **33** featuring IC_{50} values of 1 and 6 nM, respectively, in the hWB assay. A co-crystal structure of **33** with LTA_4H unequivocally showed displacement of the central water molecule with the 3-thienyl substituent, thus confirming our design hypothesis.[16]

We also prepared racemic piperazine derivatives **34** and **35** as described above (Scheme 2). These compounds were generally less potent compared to piperidine analogs with the enzymatic poten-

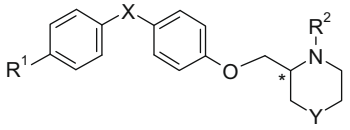


Scheme 1. Previously reported LTA_4H inhibitors and new series (**6**).



Scheme 2. Reagents and conditions: (i) (a) BH_3·THF; (b) 4 M HCl, *p*-dioxane, 0–5 °C to rt, 2 h (89–99%); (ii) $SOCl_2$, Et_3N, CH_2Cl_2, 0–5 °C, 45 min (36–95%); (iii) SO_2Cl_2, Et_3N, CH_2Cl_2, −78 °C,18 h then at rt, 2 h (39%); (iv) $NaIO_4$, $RuCl_3$, 0–5 °C (10 min), then rt for 20 min (50–76%); (v) K_2CO_3, DMF, 50 °C, 18 h, 65 °C, 7 h (20–54%); (vi) R^2X, Et_3N, rt to 30 °C, 6–18 h (35–60%); (vii) for ester hydrolysis step: 12 N HCl *p*-dioxane, 55 °C, 5 h (90–99%).

Table 1
In vitro assay results for piperidine and piperazine analogs



Compound	R^1	X	Y	*	R^2	IC_{50} (μM) [a] ($hLTA_4H$)	IC_{50} (μM) [a] (hWB)
13	H	CH_2	CH_2	*R/S*	H	0.134	0.105
14	H	O	CH_2	*R/S*	H	0.070	0.207
15	H	CH_2	CH_2	*R*	H	0.094	0.060
16	H	O	CH_2	*R*	H	0.048	0.126
17	H	O	CH_2	*S*	H	0.044	0.286
18	Ph	O	CH_2	*R*	H	0.096	0.029
19	Ph	O	CH_2	*S*	H	0.114	0.043
20	CF_3	O	CH_2	*R*	H	0.093	0.173
21	F	O	CH_2	*S*	H	0.174	0.090
22	Cl	CH_2	CH_2	*R*	H	0.315	ND
23	Cl	CH_2	CH_2	*S*	H	0.438	ND
24	Cl	O	CH_2	*S*	H	0.110	0.040
25	Cl	O	CH_2	*S*	CH_2CO_2H	0.880	ND
26	Cl	O	CH_2	*S*	$(CH_2)_2CO_2H$	0.212	ND
27	Cl	O	CH_2	*S*	$(CH_2)_3CO_2H$	0.106	0.023
28	Cl	O	CH_2	*R*	$(CH_2)_3CO_2H$	0.053	0.006
29	Cl	O	CH_2	*S*	CH_2(1,2,4-oxadiazole)	6.900	ND
30	Thiophene-2-yl	CH_2	CH_2	*S*	H	0.056	ND
31	Thiophene-3-yl	CH_2	CH_2	*S*	H	0.060	ND
32	Thiophene-3-yl	O	CH_2	*S*	H	0.093	0.001
33	Thiophene-3-yl	CH_2	CH_2	*R*	$(CH_2)_3CO_2H$	0.049	0.006
34	H	CH_2	NBn	*R/S*	H	0.660	ND
35	H	CH_2	NH	*R/S*	H	0.342	ND

[a] Assay was performed as reported previously, see Ref. 16 for details.

cies of 660 nM and 350 nM for **34** and **35**, respectively. This chemotype was not pursued further.

In summary, we have discovered a novel series of LTA_4H inhibitors containing piperidine and piperazine ring systems. Complimentary to the five-membered prolinol series, the piperidine analogs showed excellent potency in both enzyme and hWB assays. Our findings are likely to provide additional understanding of ligand binding modes to LTA_4H. In vivo data for selected best analogs from the piperidine series will be reported elsewhere in a future Letter.

References and notes

1. Samuelsson, B.; Funk, C. D. *J. Biol. Chem.* **1989**, *264*, 19469.
2. Sharon, P.; Stenson, W. F. *Gastroenterology* **1984**, *86*, 453.
3. Iverson, L.; Kragballe, K.; Ziboh, V. A. *Skin Pharmacol.* **1997**, *10*, 169.
4. Klickstein, L. B.; Shapleigh, C.; Geotzl, E. J. *J. Clin. Invest.* **1980**, *66*, 1166.
5. Rae, S. A.; Davidson, E. M.; Smith, M. J. H. *Lancet* **1982**, *11*, 1122.
6. Lewis, R. A.; Austen, K. F.; Soberman, R. J. *N. Engl. J. Med.* **1990**, *232*, 645.
7. Ford-Hutchinson, A. W. *Immunology* **1990**, *10*, 1.
8. (a) Helgadottir, A.; Manolescu, A.; Helgason, A.; Thorleifsson, G.; Thorsteinsdottir, U.; Gudbjartsson, D. F.; Gretarsdottir, S.; Magnusson, K. P.; Gudmundsson, G.; Hicks, A.; Jonsson, T.; Grant, S. F. A.; Sainz, J.; O'Brien, S. J.;

Sveinbjornsdottir, S.; Valdimarsson, E. M.; Matthiasson, S. E.; Levey, A. I.; Abramson, J. L.; Reilly, M.; Vaccarino, V.; Wolfe, M.; 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; (b) Helgadottir, A.; Manolescu, A.; Thorleifsson, G.; Jonsdottir, H.; Thorsteinsdottir, U.; Samani, N. J.; Gretarsdottir, S.; Johannsson, H.; Gudmundsson, G.; Grant, S. F. A.; Sveinbjornsdottir, S.; Valdimarsson, E. M.; Matthiasson, S. E.; Gudmundsdottir, O.; Gurney, M.; Sainz, J.; Thorhallsdottir, P.; Andresdottir, M.; Frigge, M. L.; Gudnason, V.; Kong, A.; Topol, E. J.; Thorgeirsson, G.; Gulcher, J. R.; Hakonarson, H.; Stefansson, K. *Nat. Genet.* **2004**, *36*, 233.

9. Penning, T. D. *Curr. Pharm. Des.* **2001**, *7*, 163.
10. Penning, T. D.; Askonas, L. J.; Djuric, S. W.; Haack, R. A.; Yu, S. S.; Michener, M. L.; Krivi, G. G.; Pyla, E. Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2517.
11. Hogg, J. H.; Ollmann, I. R.; Haeggström, J. Z.; Wetterholm, A.; Samuelsson, B.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 1405.
12. Tsuji, F.; Miyake, Y.; Enomoto, H.; Horiuchi, M.; Mita, S. *Eur. J. Pharmacol.* **1998**, *346*, 81.
13. 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. A.; Kachur, J. F.; Kim, S. H.; Price, D. V.; Pyla, E. Y.; Ghoreishi-Haack, N. S.; Smith, W. G. *J. Med. Chem.* **2002**, *45*, 3482.
14. Thunnissen, M. M. G. M.; Nordlund, P.; Haeggström, J. Z. *Nat. Struct. Biol.* **2001**, *8*, 131.
15. Davies, D.; Mamat, B.; Magnusson, O. T.; Christensen, J.; Haraldsson, M. H.; Mishra, R.; Pease, B.; Hansen, E.; Singh, J.; Zembower, D.; Kim, H.; Kiselyov, A. S.; Burgin, A.; Gurney, M. E.; Stewart, L. J. *J. Med. Chem.* **2009**, *52*, 4694.
16. Sandanayaka, V.; Mamat, B.; Mishra, R.; Winger, J.; Krohn, M.; Zhao, L.-M.; Kevan, M.; Enache, L.; Sullins, D.; Onua, E.; Zhang, J.; Halldorsdottir, G.; Sigthorsdottir, H.; Thorlaksdottir, A.; Sigthorsson, G.; Thorsteinnsdottir, M.; Davies, D.; Stewart, L.; Zembower, D.; Andresson, T.; Kiselyov, A.; Singh, J.; Guney, M. *J. Med. Chem.* **2010**, *53*, 573–585.
17. Structures of several previously reported LTA4H bound fragments were used for the design of new ligand series, including the ones reported here. In particular, fragments **4**, **14** and **16** from Ref. 15 were used for this piperidine series.