Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2265–2268

5-Lipoxygenase inhibitors with histamine H_1 receptor antagonist activity

Timothy A. Lewis,* Lynn Bayless, Joseph B. Eckman, James L. Ellis, Gurmit Grewal, Lyn Libertine, Jean Marie Nicolas, Ralph T. Scannell, Bruce F. Wels, Karen Wenberg and Donna M. Wypij

UCB Research, 840 Memorial Dr., Cambridge MA 02139, USA

Received 4 December 2003; accepted 2 February 2004

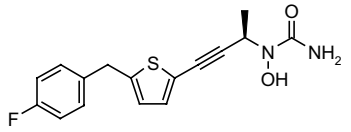
Abstract—A series of novel compounds with both 5-lipoxygenase (5-LO) inhibitory and histamine H_1 receptor antagonist activity were designed for the treatment of asthma. These dual-function compounds were made by connecting 5-LO and H_1 pharmacophores, *N*-hydroxyureas and benzhydryl piperazines, respectively. A range of in vitro activities was observed, with the furan analog **10** demonstrating both activities in an animal model. The activities observed were compared to single-function drugs.

Asthma is a complex respiratory disease involving some 50 mediators.[1] Targeting more than one of these mediators may provide greater benefits for asthmatics. Clinical data in asthmatics demonstrated that those patients treated with a combination of an LTD_4 receptor antagonist (zafirlukast) and an antihistamine H_1 receptor antagonist (loratidine) responded better than those patients treated with a single drug.[2] Similarly, the combination of montelukast (LTD_4 receptor antagonist) and cetirizine (H_1 receptor antagonist) has proven equally effective as corticosteroids for asthmatics.[3]

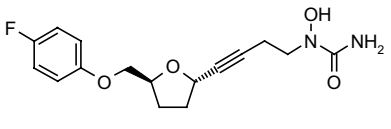
Leukotriene D_4 is one of the several peptidoleukotrienes, which are potent, pro-inflammatory mediators responsible for bronchoconstriction during an asthmatic attack.[4] Biosynthesis of the leukotrienes begins with the oxidation of arachidonic acid by 5-lipoxygenase (5-LO); hence 5-LO inhibitors (e.g., zileuton) have been investigated for the treatment of asthma.[5] To our knowledge, no clinical studies have been performed with a combination of antihistamines and 5-LO inhibitors, though mizolastine, an antihistamine, is reported to demonstrate 5-LO inhibition in vitro.[6] Our goal was instead to discover compounds acting as both 5-lipoxygenase inhibitors and as H_1 receptor antagonists.[7,8]

Several groups have investigated the use of *N*-hydroxyureas as 5-LO inhibitors, working to improve the potency and pharmacokinetics of zileuton. Abbott studied a number of furans and thiophenes such as ABT-761,[9] while others focused on tetrahydrofurans such as CMI-977.[10]

Our strategy to simultaneously target both an intracellular enzyme (5-LO) and a cell surface receptor (H_1) was to link together two distinct pharmacophores. To maintain 5-LO activity, we retained the ring-butynyl-hydroxyurea portion of the above 5-LO inhibitors. To incorporate the H_1 receptor antagonist activity, the fluorophenyl group was removed from the above



ABT-761



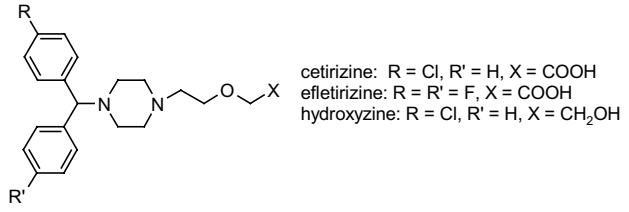
CMI-977

* Corresponding author. Tel.: +1-617-547-0033x262; fax: +1-617-547-8481; e-mail: timothy.lewis@ucb-group.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.02.005

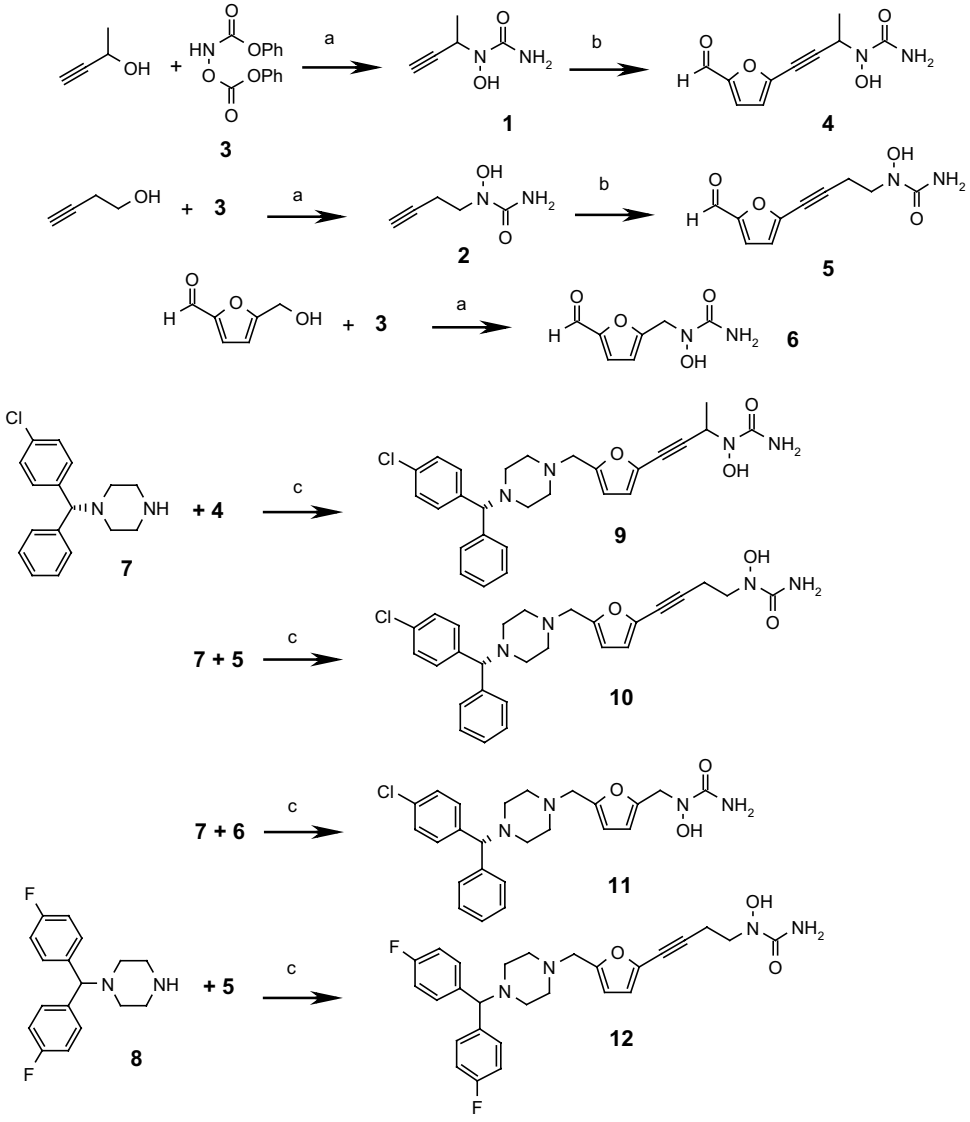
structures and replaced with benzhydryl piperazines, the H_1 pharmacophore of the antihistamines cetirizine[11] and efletirizine.[12]

Any number of dual-function hybrid compounds are feasible. Carboxylates were not included in our hybrid structures for two reasons: as 5-LO is an intracellular enzyme, *N*-hydroxyureas need a certain degree of lipophilicity to penetrate cells and get to the active site. Secondly, the carboxylate moiety is not necessary for H_1 activity, as illustrated by the potent antihistamine hydroxyzine.[11]

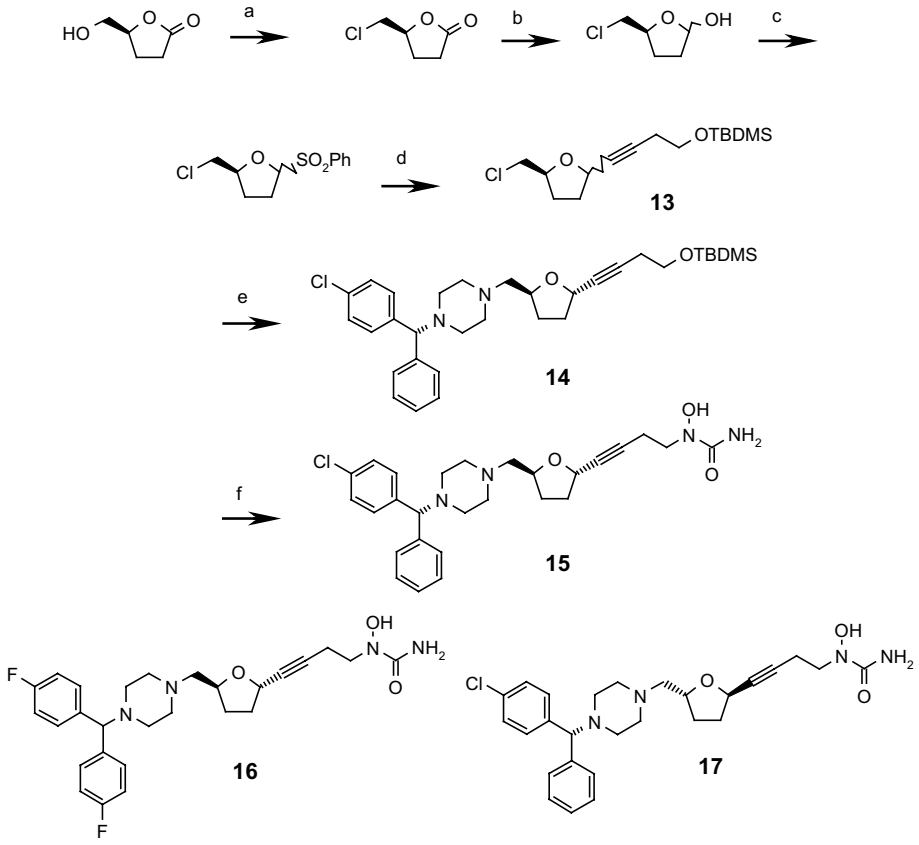


Dual-function furans were synthesized as in Scheme 1. Conversion of either terminal butynol to the corresponding *N*-hydroxyureas **1**[13] and **2** was performed by treating the alcohols with *N*,*O*-(bisphenoxycarbonyl)hydroxylamine **3**[14] under Mitsunobu conditions, followed by treatment with ammonia. Palladium catalyzed coupling of **1** and **2** to 5-bromo-2-furaldehyde gave aldehydes **4** and **5**, respectively. Aldehyde **6**, with no alkyne, was prepared by reacting **3** with 5-(hydroxymethyl)furfural, followed by treatment with ammonia. Reductive aminations of **4**, **5**, and **6** were performed with either benzhydryl piperazine **7** or **8**. Enantiomerically pure **7** has the same stereochemistry as the more potent isomer of cetirizine.[11] Compound **9** was tested as a mixture of diastereomers.[15]

Tetrahydrofuran synthesis began with commercially available, optically pure 5-(*S*)-hydroxymethyl-γ-butyrolactone (Scheme 2). The alcohol was converted to the chloride with thionyl chloride, and the lactone reduced to the lactol. Condensation with benzenesulfinic



Scheme 1. (a) 1. PPh_3, DIAD/THF, 2. NH_3/water/THF (10–50%); (b) $PdCl_2(CH_3CN)_2$, CuI, PPh_3, Et_3N/DMF (25–67%); (c) $NaBH_3CN$, HOAc/MeOH (20–67%).



Scheme 2. (a) $SOCl_2$/pyridine (90%); (b) DIBAL-H/CH_2Cl_2 −78 °C, (84%); (c) $PhSO_2H$, $CaCl_2/CH_2Cl_2$ (91%); (d) $HCCCH_2CH_2OTBDMS$, EtMgBr, $ZnBr_2$/THF (78%); (e) **7**, NaI, K_2CO_3/DMF/toluene, reflux (36%), column chromatography; (f) 1. TBAF/THF, 2. **3**, PPh_3, DIAD/THF, 3. NH_3/MeOH (36%, three steps).

acid gave the sulfone. The sulfone was displaced with the acetylenic Grignard reagent derived from TBDMS-protected 3-butyn-1-ol giving compound **13** as a mixture of stereoisomers.[16] This mixture was treated with amine **7** to give a mixture of *cis* and *trans* isomers of **14**. These isomers were separated by column chromatography and the relative stereochemistry determined by NOE and COSY ^{1}H NMR (400 MHz) experiments. Previous work on *N*-hydroxyureas containing 2,5-disubstituted tetrahydrofuran rings consistently demonstrated better 5-LO activity for the *trans* isomers than for the *cis*, hence only the *trans* isomer of **14** was carried further.[17] The silyl group was removed with TBAF and the resulting alcohol converted to the *N*-hydroxyurea **15**. Compound **13** was also treated with amine **8** and the sequence continued to give **16**. Repeating the entire synthesis with 5-(*R*)-hydroxymethyl-γ-butyrolactone and amine **7** gave **17**, with the opposite (*R*,*R*) configuration about the THF ring.

Compounds **9**–**12** and **15**–**17** were tested in vitro for both 5-LO inhibitory activity and H_1 receptor antagonist activity using standard assays (Table 1). 5-LO activity was tested in a human whole blood (HWB) assay monitoring the inhibition of LTB_4 formation using zileuton as a reference standard.[18] Human H_1 receptor binding was performed using CHO-K1 cells expressing recombinant human H_1 receptor with cetirizine as the positive control.[19]

Table 1. 5-LO and H_1 activities of dual-function molecules[a]

Compound	H_1 binding (K_i, nM)	5-LO activity (HWB, IC_{50}, nM)
9	190	3500 ± 2100 (2)
10	150	420 ± 360 (5)
11	4	1700
12	550	170 ± 50 (3)
15	50 ± 20 (5)	310 ± 180 (3)
16	660	140
17	180	510
Zileuton	—	873 ± 391 (118)
Cetirizine	14 ± 6 (60)	—

[a] Mean ± SD (*n*); otherwise $n = 1$.

The furan series displayed greater variance among the analogs tested. Structural changes near the *N*-hydroxyurea affected both the 5-LO and the H_1 activity of the molecules. Compound **11** has antihistaminergic activity similar to cetirizine, but with poor 5-LO activity. Moving the hydroxyurea moiety further from the furan ring improves the 5-LO activity with concomitant loss of H_1 activity (**11** vs **10** and **12**). Compounds **10** and **12**, with the terminal hydroxyurea, both gave increased 5-LO activity over the α-substituted hydroxyurea **9**. The difluoro analog **12** had the highest H_1 binding constant, but the best 5-LO potency in the furan series. This trend was seen in the tetrahydrofuran series as well (**16** vs **15**

and **17**). Compound **15**, with the same (*S*,*S*) stereochemistry about the THF ring as CMI-977, had better activities than its diastereoisomer **17**. For comparison, the reported 5-LO IC_{50}'s in human whole blood for ABT-761 and for CMI-977 are 160 nM[9] and 117 nM,[10] respectively.

Compounds **10** and **12** were further tested for 5-LO inhibitory activity using an RBL-2H3 cell lysate 5-LO assay;[5,18] both had IC_{50}'s <200 nM. Compound **17** was tested using human recombinant 5-lipoxygenase,[20] the IC_{50} of 420 nM agrees well with its HWB activity (510 nM). These results further confirm that the compounds are binding to the lipoxygenase active site.

Analogs were then screened for their in vitro pharmacokinetic properties. Compound **10** showed an acceptable metabolic stability when incubated with NADPH-fortified rat liver microsomes (Cl_{int} 33 μL/min/mg protein) and a high permeability in Caco-2 cells (Papps: 6×10^{-6} cm/sec). Because of its overall promising in vitro profile, compound **10** was selected for testing in 5-LO and H_1 animal models (guinea pig, 2 mg/kg, po, $t = 3$ h, $n \geqslant 4$). The 5-LO ex vivo activity was evaluated by monitoring calcium ionophore-induced LTB_4 formation in whole blood samples,[21] 46% inhibition was observed. In the same model, zileuton demonstrated 82% inhibition at 5 mg/kg. H_1 activity of **10** was evaluated by monitoring the inhibition of histamine-induced bronchoconstriction using the Konslett–Rossler protocol,[22] 86% inhibition was observed. Cetirizine displayed essentially complete inhibition, 96%, at a lower dose level (0.5 mg/kg). Overall, the oral activity of compound **10** on both targets agrees with its in vitro profile.

In conclusion, starting with known 5-LO and H_1 pharmacophores, we have made a novel, orally active, dual-function compound.[23] Further optimization of the spacer linking these two pharmacophores led to compounds with improved in vitro and in vivo activities. These results shall be reported soon.

References and notes

1. Barnes, P. J.; Chung, K. F.; Page, C. P. *Pharmacol. Rev.* **1998**, *50*, 515.
2. Roquet, A.; Dahlen, B.; Kumlin, M.; Ihre, E.; Gudrun, A.; Binks, S. *Am. J. Respir. Crit. Care. Med.* **1997**, *155*, 1856.
3. Wilson, A. M.; Orr, L. C.; Sims, E. J.; Dempsey, O. J.; Lipworth, B. J. *Am. J. Respir. Crit. Care Med.* **2000**, *162*, 1297.
4. Bernstein, P. R.; Bird, T. G.; Brewster, A. G. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1997; Vol. 5, pp 405–488.
5. Carter, G. W.; Young, P. R.; Albert, D. H.; Bouska, J.; Dyer, R.; Bell, R. L.; Summers, J. B.; Brooks, D. W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929.
6. Sudo, K.; Nagai, K.; Yamada, N. *Jpn. Pharmacol. Ther.* **1998**, *26*(Suppl), 155.
7. For an appraisal of dual acting anti-inflammatory drugs Bertolini, A.; Ottani, A.; Sandrini, M. *Pharmacol. Res.* **2001**, *44*, 437.
8. For other H_1/5–LO molecules see Kawasaki, N.; Miyataka, H.; Matsumoto, H.; Yamashita, N.; Sakane, T.; Mizushima, Y.; Satoh, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 19.
9. Brooks, C. D. W.; Stewart, A. O.; Basha, A.; Bhatia, P.; Ratajczyk, J. D.; Martin, J. G.; Craig, R. A.; Kolasa, T.; Bouska, J. B.; Lanni, C.; Harris, R. R.; Malo, P. E.; Carter, G. W.; Bells, R. L. *J. Med. Chem.* **1995**, *38*, 4768.
10. Gurjar, M. K.; Murugaiah, A. M. S.; Padhakrishna, P.; Ramana, C. V.; Chorghade, M. S. *Tetrahedron: Asymmetry* **2003**, *14*, 1363.
11. Gillard, M.; Van Der Perren, C.; Moguilevsky, N.; Massingham, R.; Chatelain, P. *Mol. Pharmacol.* **2002**, *61*, 391.
12. Leeson, G. A.; Castaner, R. *Drugs Future* **1997**, *22*, 626.
13. Thomas, A. V.; Patel, H. H.; Reif, L. A.; Chemburkar, S. R.; Sawick, D. P.; Shelat, B.; Balmer, M. K.; Patel, R. R. *Org. Process Res. Dev.* **1997**, *1*, 294.
14. Stewart, A. O.; Brooks, D. W. *J. Org. Chem.* **1992**, *57*, 5020.
15. All compounds tested were characterized by ^{1}H NMR, mass spectral, and IR analysis; purity was >95% as determined by HPLC analysis with UV detection at 254 nM and tandem mass spectral detection.
16. Brown, D. S.; Bruno, M.; Davenport, R. J.; Ley, S. V. *Tetrahedron* **1989**, *45*, 4293.
17. Unpublished results, CytoMed Inc.
18. Sirois, P.; Borgeat, P.; Lauziere, M.; Dube, L.; Rubin, P.; Kesterson, J. *Agents Action* **1991**, *34*, 117.
19. Moguilevski, N.; Varsalona, F.; Noyer, M.; Gillard, M.; Guillaume, J. P.; Garcia, L.; Szpirer, C.; Szpirer, J.; Bollen, A. *Eur. J. Biochem.* **1994**, *224*, 489.
20. Zhang, Y. Y.; Lind, B.; Radmark, O.; Samuelsson, B. *J. Biol. Chem.* **1993**, *268*, 2535.
21. Konsett, H.; Rossler, R. *Arch. Exp. Pathol. Pharmakol.* **1940**, *195*, 71.
22. Spaethe, S. M.; Snyder, D. W.; Pechous, P. A.; Clarke, T.; VanAlstyne, E. L. *Biochem. Pharmacol.* **1992**, *43*, 377–382.
23. This work was presented by T. Lewis at the ACS 224th National Meeting, Boston, MA, August 18, 2002, MEDI 15.