

Available online at www.sciencedirect.com



Bioorganic & Medicinal Chemistry Letters

Bioorganic & Medicinal Chemistry Letters 15 (2005) 1083-1085

5-Lipoxygenase inhibition by N-hydroxycarbamates in dual-function compounds

Timothy A. Lewis,^{a,*} Lynn Bayless,^a Alan J. DiPesa,^a Joseph B. Eckman,^a Michel Gillard,^b Lyn Libertine,^a Ralph T. Scannell,^a Donna M. Wypij^a and Michelle A. Young^a

^aUCB Research, 840 Memorial Dr., Cambridge, MA 02139, USA ^bIn Vitro Pharmacology, UCB Pharma SA, Chemin du Foriest, B-1420 Braine l'Alleud, Belgium

> Received 9 October 2004; revised 6 December 2004; accepted 9 December 2004 Available online 12 January 2005

Abstract—A series of *N*-hydroxycarbamates containing a histaminergic H_1 receptor antagonist pharmacophore was synthesized. In vitro assays determined the compounds had both histaminergic binding and 5-lipoxygenase inhibiting activities comparable to the corresponding *N*-hydroxyurea analog. Animal models demonstrated antihistaminergic and the 5-lipopxygenase inhibitory activity, with the *N*-hydroxyurea analog having a better overall profile. © 2004 Elsevier Ltd. All rights reserved.

Histamine plays a role in the pathophysiology of asthma alongside its key role in allergic response. Clinical studies have demonstrated that asthmatics treated simultaneously with an H_1 receptor antagonist and a LTD_4 receptor antagonist experienced less airway obstruction than those patients treated with either drug alone.¹ A similar combination proved to be as efficacious as a corticosteroid in treating the airway symptoms of allergic rhinitis and asthma.² Inhibition of 5-lipoxygenase (5-LO) is potentially a more efficacious treatment than antagonism of leukotriene receptors, as biosynthesis of all the pro-inflammatory leukotrienes would be diminished.

Our work has focused on dual-function antihistaminergic/5-LO inhibiting compounds such as the *N*-hydroxyurea UCB 62045 (1), which demonstrates both activities in vitro and in vivo.³ UCB 62045 is effective in reducing ovalbumin-induced bronchoconstriction in the guinea pig bronchoconstriction model with oral dosing, and has been investigated as a treatment for asthma and allergic rhinitis. Reports on the 5-LO inhibitory effect of *N*-hydroxycarbamates⁴ supported investigating this moiety in similar dual-function molecules.



Synthesis of 1 began by reacting 4-iodophenol with 1,4dibromobutane to give 2 (Scheme 1). Palladium catalyzed coupling of 2 with 3-butyn-1-ol gave 3. N-Alkylation of 1-bis-(4-fluorophenyl)methyl piperazine with 3 gave alcohol 4, which was converted to N-hydroxyurea 1 using a literature procedure.⁵ Synthesis of the carbamate analogs began with alcohol 4, which was converted into hydroxylamine 5 via mesylation and displacement with aqueous hydroxylamine. Treatment of 5 with ethyl chloroformate gave the desired N-hydroxycarbamate 6. However, treatment of 5 with isopropyl chloroformate gave predominately the undesired N,O-bis-acylated product 7 in 43% yield. Treatment of 7 with KOH/ i-PrOH hydrolyzed only the carbonate group to afford *N*-hydroxycarbamate 8. This two step procedure, alkyl chloroformate addition to 5 making the diacyl product followed by basic hydrolysis, also proved more convenient for the preparation of the methyl, isobutyl, and benzyl *N*-hydroxycarbamates **9–11**.⁶

Human H_1 receptor binding was performed using CHO-K1 cells expressing the recombinant H_1 receptor⁷ (Table

Keywords: 5-LO inhibitor; N-Hydroxycarbamate; Antihistamine.
* Corresponding author. Tel.: +1 508 460 5099; e-mail: timlewis1201@ comcast.net

⁰⁹⁶⁰⁻⁸⁹⁴X/\$ - see front matter @ 2004 Elsevier Ltd. All rights reserved. doi:10.1016/j.bmcl.2004.12.023



Scheme 1. Reagents and conditions: (a) 1,4-dibromobutane, K_2CO_3/DMF (83%); (b) 3-butyn-1-ol, (Ph₃P)₂PdCl₂, CuI, Et₃N/CH₂Cl₂ (76%); (c) 1-[bis-(4-fluorophenyl)methyl]piperazine, K_2CO_3/DMF (77%); (d) (1) PPh₃, DIAD, PhOCO₂NHCOOPh/THF; (2) NH₃/MeOH (25%); (e) (1) MeSO₂Cl, Et₃N/CH₂Cl₂; (2) NH₂OH (aq), THF/MeOH (39%); (f) for **6**: EtoCOCl, Et₃N/CH₂Cl₂ (39%); (g) (1) ROCOCl, Et₃N/CH₂Cl₂; (2) KOH/ ROH (**8**–21%, **9**–23%, **10**–32%, **11**–15%, two steps).

1). Hydroxyurea 1 displayed the best binding,⁸ better than the reference standard, cetirizine. In the carbamate series, the compounds with the smaller alkoxy groups (OR) displayed better H_1 binding, the methyl carbamate 9 binding better than cetirizine, the ethyl carbamate 6 binding equally, and all others binding with lower affinity. The overall variation in H_1 binding was not pronounced throughout the entire series.

Inhibition of 5-LO was tested with human recombinant 5-LO (HR),⁹ and a human whole blood (HWB) assay, which monitors calcium ionophore-induced LTB₄ for-

Table 1. In vitro activities of dual-function compounds^a

Compound	H_1 binding $(K_i nM)^b$	5-LO inhibition (HR, IC ₅₀ , nM) ^c	5-LO inhibition $(HWB, IC_{50}, nM)^d$
1	3.63 ± 0.52	193 ± 76	89 ± 34
6	5.89 ± 0.49	365 ± 55	72 ± 40
8	9.55 ± 0.62	335 ± 115	102 ± 69
9	5.01 ± 0.59	170 ± 60	90 ± 11
10	7.59 ± 0.66	420 ± 30	176 ± 116
11	17.8 ± 0.60	350 ± 20	150 ± 56
Cetirizine	5.89 ± 0.81	_	_
Zileuton	_	518 ± 146^{b}	$873 \pm 391^{b,e}$

^a Mean \pm SD.

^b n ≥ 6.

 ${}^{c}n \ge 2.$ ${}^{d}n \ge 3.$

^e Lit. value: 900.¹⁰

mation¹⁰ (Table 1). All the compounds tested were more potent than zileuton, the reference standard, with little variation occurring between the carbamate analogs.



N-Hydroxyurea 1 and *N*-hydroxycarbamates 6 and 8 were tested in animal models with oral dosing (2 mg/kg). While methyl carbamate 9 was more potent in the H₁ and 5-LO (HR) assays, analogs 6 and 8 were expected to have better metabolic stability. Compounds 6 and 8 are both orally active antihistamines with 5-LO inhibitory activity for up to 6 h.

The H_1 antagonist activity was determined using the Konzett–Rössler protocol, which monitors histamineinduced bronchoconstriction (Fig. 1).¹¹ Antihistaminergic activity profiles of the three compounds were different; hydroxyurea **1**, after a slow onset, displayed nearly complete inhibition of bronchoconstriction at 3 and 6 h. Ethyl carbamate **6** had a faster onset and increasing activity with time, while isopropyl carbamate **8** had slow onset and the least activity at 3 and 6 h. None of the



Figure 1. The effect of dual-function compounds on histamine-induced bronchoconstriction in guinea pigs (1, 6, and 8, 2 mg/kg, po: cetirizine, 0.5 mg/kg, po, n = 3 animals/timepoint).

dual-function compounds are as active as cetirizine, which displayed nearly complete inhibition of histamine-induced bronchoconstriction at all time points with a lower dose (0.5 mg/kg).

The ex vivo 5-LO assay monitors the inhibition of LTB₄ production after calcium ionophore-induced stimulation¹² (Fig. 2). Zileuton was tested alongside the dualfunction compounds as a reference. Inhibition of 5-LO activity by the three dual-function molecules was detected up to six hours after challenge. *N*-Hydroxyurea **1** has better activity than the two *N*-hydroxycarbamates tested, and the activity increases with time. The *N*hydroxycarbamate activities are fairly constant in this assay, or decrease with time.

In conclusion, in a dual-function antihistaminergic system, *N*-hydroxycarbamates demonstrate 5-LO inhibitory activity, both in vitro and in vivo with oral dosing. However, the ex vivo 5-LO activities of the *N*hydroxycarbamates are lower at 1, 3, and 6 h than the analogous *N*-hydroxyurea. Further work is required to



Figure 2. The effect of dual-function compounds and of zileuton on the inhibition of A-23187-stimulated LTB₄ production in guinea pigs (for 1, 6, 8, 2 mg/kg, po; zileuton, 5 mg/kg, po, n = 3 animal/ timepoint).

fully determine the therapeutic potential of *N*-hydroxycarbamates.¹³

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl. 2004.12.023.

References and notes

- Roquet, A.; Dahlen, B.; Kumlin, M.; Ihre, E.; Gudrun, A.; Binks, S. Am. J. Resp. Crit. Care Med. 1997, 155, 1856.
- Wilson, A. M.; Orr, L. C.; Sims, E. J.; Dempsey, O. J.; Lipworth, B. J. Am. J. Resp. Crit. Care Med. 2000, 162, 1297.
- Lewis, T. A.; Bayless, L.; Eckman, J. B.; Ellis, J. L.; Grewal, G.; Libertine, L.; Nicolas, J. M.; Scannell, R. T.; Wels, B. F.; Wenberg, K.; Wypij, D. M. *Bioorg. Med. Chem. Lett.* 2004, 14, 2265; Lewis, T. A.; Young, M. A.; Arrington, M. P.; Bayless, L.; Cai, X.; Collart, P.; Eckman, J. B.; Ellis, J. L.; Ene, D. G.; Libertine, L.; Nicolas, J.-M.; Scannell, R. T.; Wels, B. F.; Wenberg, K.; Wypij, D. M. *Bioorg. Med. Chem. Lett.* 2004, 14, 5591; Selig, W. M.; Bayless, L.; Libertine, L.; Eckman, J. B.; Wypij, D. M.; Wels, B. F.; Eckert, M.; Young, M. A.; Nicolas, J.-M.; Scannell, R. T.; Ellis, J. L. *Chest* 2003, 123, 371; Scannell, R. T. et al. *Inflamm. Res.* 2004, 53(Supplement 1), S33.
- Surman, M. D.; Mulvihill, M. J.; Miller, M. J. J. Org. Chem. 2002, 67, 4115; Yatabe, T.; Kawai, Y.; Oku, T.; Tanaka, H. Chem. Pharm. Bull. 1998, 46, 966; Connolly, P. J.; Wetter, S. K.; Beers, K. N.; Hamel, S. C.; Chen, R. H. K.; Wachter, M. P.; Ansell, J.; Singer, M. M.; Steber, M.; Ritchie, D. M.; Argentieri, D. C. Bioorg. Med. Chem. Lett. 1999, 9, 979.
- 5. Stewart, A. O.; Brooks, D. W. J. Org. Chem. 1992, 57, 5020.
- 6. All compounds tested were characterized by ¹H 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. Recrystallization from EtOAc gave analytically pure material as determined by combustion analysis (C, H, N \pm 0.4% of calculated values).
- Gillard, M.; Van Der Perren, C.; Moguilevsky, N.; Massingham, R.; Chatelain, P. Mol. Pharmacol. 2002, 61, 391.
- 8. The discrepancy between the H_1 value for 1 reported here and in Refs. 3b,c is due to testing at two different laboratories using different cell lines with different batches of 1.
- Brooks, C. D.; 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. C.; Bell, R. L. J. Med. Chem. 1995, 38, 4768.
- 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.
- 11. Konzett, H.; Rössler, R. Naonyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 1940, 195, 71.
- 12. Spaethe, S. M.; Snyder, D. W.; Pechous, P. A.; Clarke, T.; VanAlstyne, E. L. *Biochem. Pharmacol.* **1992**, *43*, 377.
- 13. Experimental procedures for the synthesis of all intermediates and final compounds, plus the biological experimental procedures can be found in the Supplementary material.