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

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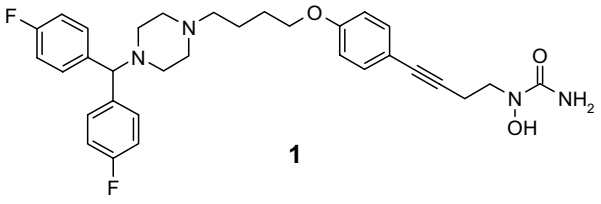
Abstract—A series of *N*-hydroxycarbamates containing a histaminergic H_1 receptor antagonist pharmacophore was synthesized. In vitro assays determined the compounds had both histaminergic binding and 5-lipoxygenase inhibiting activities comparable to the corresponding *N*-hydroxyurea analog. Animal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

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*-hydroxycarbamates[4] supported investigating this moiety in similar dual-function molecules.



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Synthesis of **1** began by reacting 4-iodophenol with 1,4-dibromobutane 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 receptor[7] (Table

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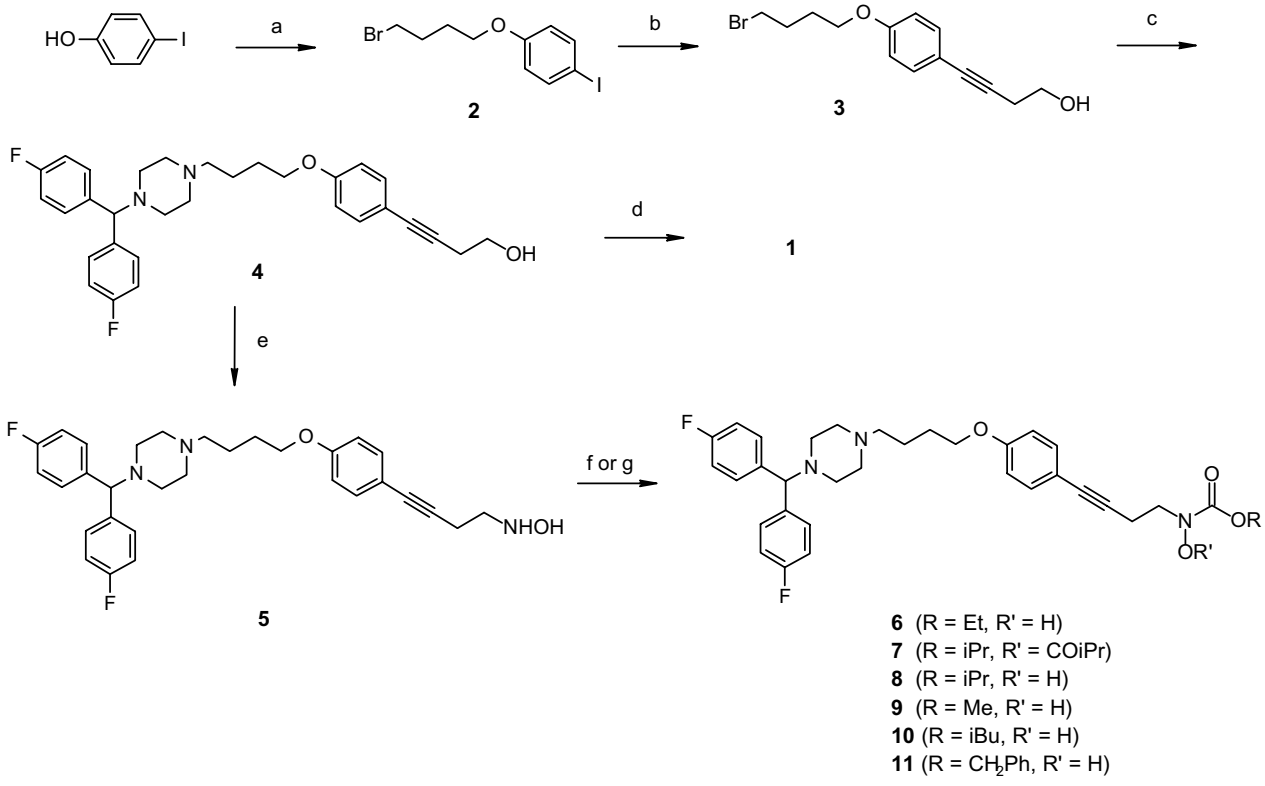


Scheme 1. Reagents and conditions: (a) 1,4-dibromobutane, K_2CO_3/DMF (83%); (b) 3-butyn-1-ol, $(Ph_3P)_2PdCl_2$, CuI, Et_3N/CH_2Cl_2 (76%); (c) 1-[bis-(4-fluorophenyl)methyl]piperazine, K_2CO_3/DMF (77%); (d) (1) PPh_3, DIAD, $PhOCO_2NHCOOPh$/THF; (2) NH_3/MeOH (25%); (e) (1) $MeSO_2Cl$, Et_3N/CH_2Cl_2; (2) NH_2OH (aq), THF/MeOH (39%); (f) for **6**: EtOCOCl, Et_3N/CH_2Cl_2 (39%); (g) (1) ROCOCl, Et_3N/CH_2Cl_2; (2) KOH/ROH (**8**—21%, **9**—23%, **10**—32%, **11**—15%, two steps).

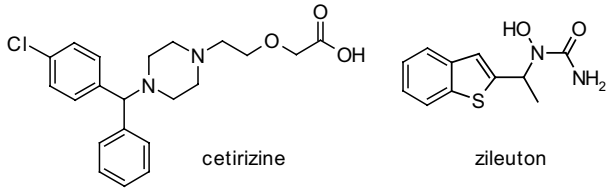
1). Hydroxyurea **1** displayed the best binding,[8] better than the reference standard, cetirizine. In the carbamate series, the compounds with the smaller alkoxy groups (OR) displayed better H_1 binding, the methyl carbamate **9** binding better than cetirizine, the ethyl carbamate **6** binding equally, 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 LTB_4 formation[10] (Table 1). All the compounds tested were more potent than zileuton, the reference standard, with little variation occurring between the carbamate analogs.

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 ± 391[b,e]

[a] Mean ± SD.
[b] $n \geqslant 6$.
[c] $n \geqslant 2$.
[d] $n \geqslant 3$.
[e] Lit. value: 900.[10]



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 H_1 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 histamine-induced 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

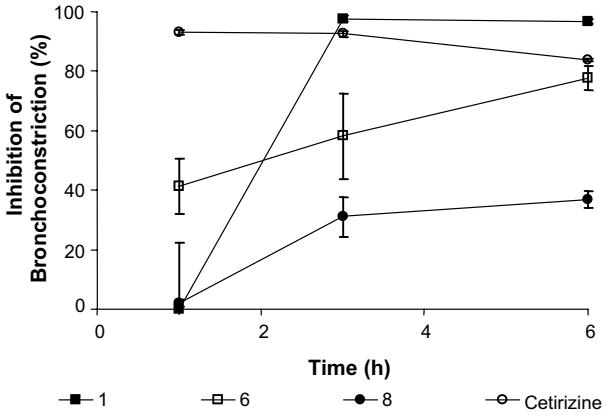


Figure 1. The effect of dual-function compounds on histamine-induced bronchoconstriction in guinea pigs (**1**, **6**, and **8**, 2 mg/kg, po: cetirizine, 0.5 mg/kg, po, $n = 3$ animals/timepoint).

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 LTB_4 production after calcium ionophore-induced stimulation[12] (Fig. 2). Zileuton was tested alongside the dual-function 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 fully determine the therapeutic potential of *N*-hydroxycarbamates.[13]

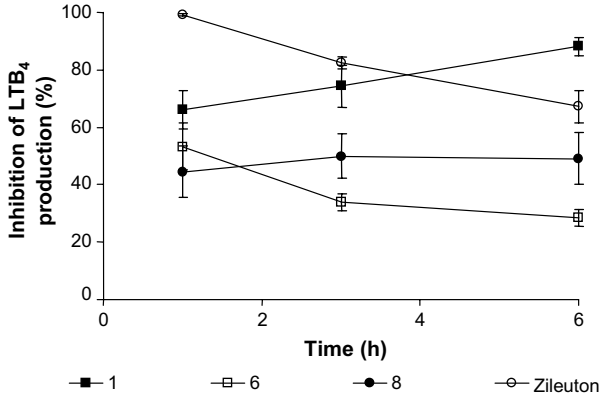


Figure 2. The effect of dual-function compounds and of zileuton on the inhibition of A-23187-stimulated LTB_4 production in guinea pigs (for **1**, **6**, **8**, 2 mg/kg, po; zileuton, 5 mg/kg, po, $n = 3$ animal/timepoint).

Supplementary data

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

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