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Cetirizine and loratadine-based antihistamines with 5-lipoxygenase inhibitory activity

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Abstract—A series of compounds possessing both H_1 histamine receptor antagonist and 5-lipoxygenase (5-LO) inhibitory activities was synthesized. The H_1-binding scaffolds of cetirizine, efletirizine, and loratadine were linked to a lipophilic *N*-hydroxyurea, the 5-LO inhibiting moiety of zileuton. Both activities were observed in vivo, as was increased CYP3A4 inhibition compared to their respective single-function drugs. Selected analogs in the series were shown to be orally active in guinea pig models.
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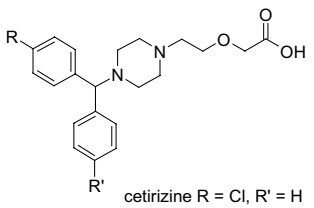
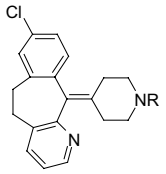
Asthma is characterized by bronchial hyperresponsiveness and airway inflammation, with more than 50 mediators involved.[1] Not surprisingly, monotherapy does not provide full control of the disease. The combination of an inhaled corticosteroid with a beta-2 agonist is among the best available treatment options.[2] Histamine and leukotrienes are key mediators in the pathogenesis of asthma, being involved in both the early and late phase asthmatic reactions.[3] Clinical trials have demonstrated the additive benefit of a combined leukotriene (LTD_4) receptor antagonist and H_1 receptor antagonist treatment.[3,4] A 5-lipoxygenase (5-LO) inhibitor, which decreases the biosynthesis of all the leukotrienes, may be more effective than a LTD_4 receptor antagonist when used concurrently with an antihistamine. An orally active dual 5-LO inhibitor/H_1 antagonist would provide a valuable alternative to the currently available therapies, circumventing the poor compliance usually associated with multiple and/or inhalation therapies.

Balancing H_1 receptor antagonist activity and 5-LO inhibition in a single drug represents a significant challenge. Such dual-active compounds were predicted to show an unfavorable physicochemical profile due to high molecular weight. The lipophilicity of the compounds must not be such as to prevent acceptable oral bioavailability, yet a certain degree of lipophilicity is required for cell penetration to reach the intracellular 5-LO target.

We have previously reported the design of dual-function compounds[5] in which the H_1 scaffold of cetirizine was linked to an *N*-hydroxyurea, the 5-LO inhibiting moiety of zileuton.[6] A furan-based linker resulted in a compound demonstrating dual activities both in vitro and in vivo. Similar results are obtained replacing the furan ring with the more metabolically favorable phenyl ring. In this communication, we report the synthesis of dual-function analogs in which the same *N*-hydroxyurea scaffold was attached to three different H_1 scaffolds by alkyl linkers of varying length. We used the H_1 scaffolds of the



cetirizine R = Cl, R' = H
efletirizine R = R' = F



loratadine R = COOEt
desloratadine R = H

Keywords: H_1 antihistamine; 5-LO inhibitor.
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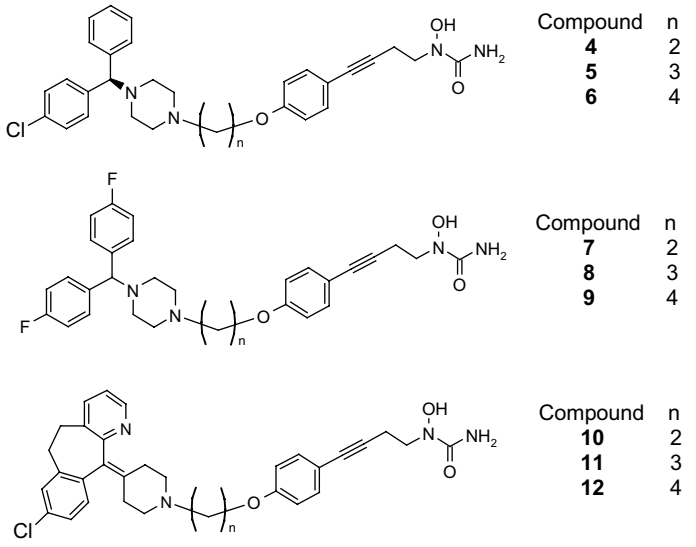
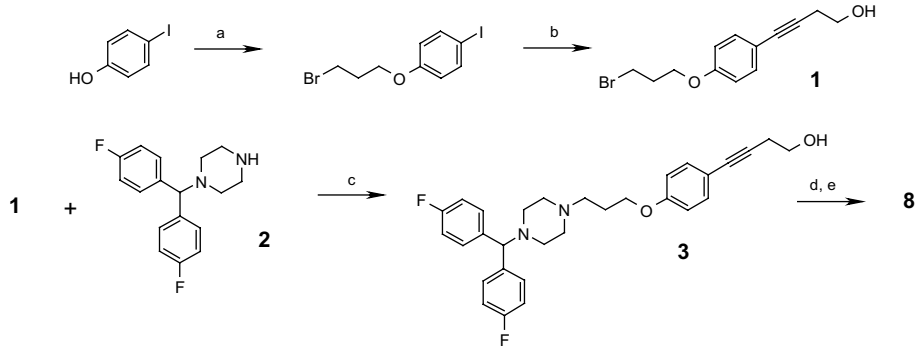


Figure 1. Dual-function H_1/5-LO compounds.



Scheme 1. Reagents and conditions: (a) $BrCH_2CH_2CH_2Br$, K_2CO_3, DMF (52%); (b) 3-butyn-1-ol, $PdCl_2(PPh_3)_2$, CuI, Et_3N, CH_2Cl_2 (56%); (c) K_2CO_3, DMF (64%); (d) *N*,*O*-(bisphenoxycarbonyl)hydroxylamine, DIAD, PPh_3, THF; (e) NH_3/MeOH (60%, two steps).

second-generation antihistamines cetirizine,[7] efletirizine,[8] and loratadine/desloratadine.[9]

The synthetic routes for compounds **4**–**12** (Fig. 1) were similar, a representative example is shown in Scheme 1. Alkylation of 4-iodophenol with 1,3-dibromopropane followed by Sonogashira coupling with 3-butyn-1-ol gave **1**. Compound **1** was then treated with amine **2** to give **3**. Conversion of alcohol **3** to the *N*-hydroxyurea **8** was performed using a literature procedure.[10] For the cetirizine analogs, enantiomerically pure (*R*)-1-[(4-chlorophenyl)-phenyl-methyl]-piperazine[11] was used for the *N*-alkylation step, the (*R*)-stereochemistry corresponding to the eutomer of cetirizine, that is, levocetirizine.[7] Desloratadine, obtained by basic hydrolysis of loratadine,[12] was alkylated with **1** and its analogs to provide the loratadine-based compounds. Yields for the synthesis of the other compounds were similar to those in Scheme 1, except for the alkylation of 4-iodophenol with 1,2-dibromoethane in which the yield was typically 10–15%.[13]

Compounds **4**–**12** were tested in vitro for both 5-LO inhibitory activity and H_1 receptor antagonist activity using standard assays (Table 1). 5-Lipoxygenase activity was evaluated in a human whole blood (HWB) assay monitoring the inhibition of calcium ionophore-induced LTB_4 formation with zileuton as the positive control.[14] The three series showed potent inhibitory activity with IC_{50} values ranging from 48 to 213 nM, that is, at least

Table 1. In vitro H_1 and 5-LO activities[a]

Compound	H_1 binding (K_i, nM)	5-LO activity (HWB, IC_{50}, nM)
4	305 ± 121 (11)	213 ± 89 (10)
5	9 ± 6 (7)	96 ± 101 (4)
6	30 ± 16 (2)	68
7	295 ± 78 (2)	48
8	109 ± 50 (4)	90 ± 34 (6)
9	26 ± 26 (25)	89 ± 34 (12)
10	19 ± 14 (8)	185 ± 81 (10)
11	14 ± 14 (8)	194 ± 37 (2)
12	50	57
Zileuton	NT	873 ± 391 (118)
Cetirizine	14 ± 6 (60)	NT
Loratadine	414 ± 131 (7)	NT
Desloratadine	0.97 ± 0.23 (6)	NT

[a] Mean ± SD (*n*); otherwise *n* = 1, NT = not tested.

Table 2. In vitro pharmacokinetic properties

Compound	Caco-2 permeability ($\times 10^{-6}$ cm/s)		CYP3A4 inhibition IC_{50} (μM)	Cl_{int} in liver microsomes (μL/min/mg protein)		
	A > *B*	*B* > *A*		Rat	Guinea pig	Human
4	1.9	1.5	18	48	19	2
5	2.3	4.6	8	108	99	0
6	1.9	4.2	6	NT	NT	NT
9	3.4	5.4	6	138	111	13
10	1.4	8.6	6	101	105	15
12	2.6	8.1	NT	NT	NT	NT

NT = not tested.

Table 3. In vivo pharmacokinetic and activity profile

Compound	Rat oral/iv dosing at 2 mg/kg			Guinea pig, 3 h after oral dosing at 2 mg/kg		
	C_{max} (ng/mL)	AUC (ng h/mL)	*F* (%)	Inhibitory activity (%)		Plasma levels (ng/mL; μM)
				Bronchoconstriction[a]	LTB_4 production[b]	
4	66	300	23	62 ± 4	41 ± 5	187; 350
10	30	83	8	65 ± 5	84 ± 4	72; 129

[a] % Inhibition of bronchoconstriction after histamine-induced challenge.
[b] % Inhibition of calcium ionophore (A-23187) induced LTB_4 formation.

4-fold more potent than zileuton in this assay. Compounds **4**, **9** (**9** as the fumarate salt), and **10** were further assessed using the RBL-2H3 cytosolic 5-LO assay.[6] The IC_{50} values (85 ± 11 nM, $n = 4$; 68 ± 54 nM, $n = 3$; 170, $n = 1$, respectively) were found to be in broad agreement with those measured in intact cells. These findings demonstrated that the compounds were inhibiting the intracellular lipoxygenase enzyme.

Human H_1 receptor binding was performed using CHO-K1 cells expressing the recombinant human H_1 receptor.[15] Cetirizine, loratadine, and desloratadine were used as the positive controls, our data agreeing with the literature values.[7,8] The three series showed potent H_1 binding activity with K_i values ranging from 9 nM (**5**) to 305 nM (**4**). In the cetirizine/efletirizine series, the 2-carbon linker (**4/7**) consistently led to lower H_1 binding affinity than the 3- or 4-carbon linkers (**5/6**, **8/9**). This contrasts with the loratadine-containing analogs (**10**–**12**) where linker length had minimal effect.

The next area of focus was to investigate the in vitro pharmacokinetic properties of these dual-function analogs (Table 2). Intestinal permeability was the primary concern because of their unfavorable physicochemical properties (MW > 500, $c\mathrm{Log}P \geqslant 5$, up to 13 rotable bonds). All the tested analogs demonstrated acceptable apical-to-basolateral permeability in Caco-2 cells ($>1 \times 10^{-6}$ cm/s).[16] However, those derived from the loratadine scaffold (**10**, **12**) showed polarized transport. This finding might be related to the ability of loratadine to act as a P-glycoprotein substrate.[17] The dual-function analogs were found to inhibit human CYP3A4.[18] Analog **4** was the least inhibitory, with an IC_{50} of 18 μM. The IC_{50} values for cetirizine, loratadine, and zileuton were >100, 32, and >100 μM, respectively. Finally, the analogs were assessed for their in vitro metabolic stability in NADPH-fortified liver microsomes from different species.[19] All of the analogs tested showed higher metabolic clearance (Cl_{int}) in rat and guinea pig than in human. Analog **4** showed the best in vitro metabolic stability in all three species.

Compounds **4** and **10** were selected for a preliminary evaluation of their in vivo pharmacokinetic profile and their oral efficacy after single dosing at 2 mg/kg (Table 3). As anticipated from the in vitro data, when compared to **10**, the cetirizine-based analog **4** demonstrated somewhat higher exposure and improved oral bioavailability in the rat. Three hours after single oral dosing to male guinea pig, both **4** and **10** significantly inhibited histamine-induced bronchoconstriction (Konzett–Rössler protocol[20]) and ex vivo LTB_4 production (calcium ionophore-stimulated whole blood[21]). The mean plasma levels were in the range of the concentrations active in vitro, suggesting that the measured activities directly derived from the parent drugs. With respect to their H_1 activity, compounds **4** and **10** were found to be less potent than cetirizine (90% inhibition of histamine-induced bronchocontriction at 0.5 mg/kg).

In conclusion, a series of compounds has been developed combining the scaffolds of various H_1 receptor antagonists with that of zileuton, a reference 5-LO inhibitor. The resultant dual-function analogs showed potent in vitro activities. Compounds **4** and **10** were demonstrated to be bioavailable and orally active in animal models of respiratory inflammation.

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