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Design, Synthesis and Biological Evaluation of novel desloratadine derivatives with anti-inflammatory and H_1 antagonize activities

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Abstract

To improve the anti-inflammatory activity of desloratadine, we designed and synthesized a series of novel desloratadine derivatives. All compounds were evaluated for their anti-inflammatory and H_1 antagonistic activities. Among them, compound **2c** showed the strongest H_1 antagonistic and anti-inflammatory activity. It also exhibited promising pharmacokinetic profiles and low toxicity. All these results suggest that compound **2c** as a novel anti-allergic agent is worthy of further investigation.

Keywords: anti-inflammatory; H_1 antagonize; anti-allergic; desloratadine

Allergic diseases, such as acute and chronic urticaria, allergic rhinitis, allergic ophthalmia, allergic skin disease etc., have severely influenced the quality of people's life [1-2]. It has been confirmed that the main effect of H_1 antihistamines is related to the blockade of H_1 receptors, thus executing their antiallergic function [3]. Hence antihistamines, such as loratadine, desloratadine, fexofenadine, cetirizine (shown in **Fig 1**) have been approved to treat allergic diseases. Although they were efficacious and a lot of patients have benefited from these drugs, they still have some side-effects such as sleepiness [4], nasal congestion, dry mouth and headache [5]. To avoid these drawbacks, it is urgent to develop novel antihistamines drugs or new therapeutic strategies.

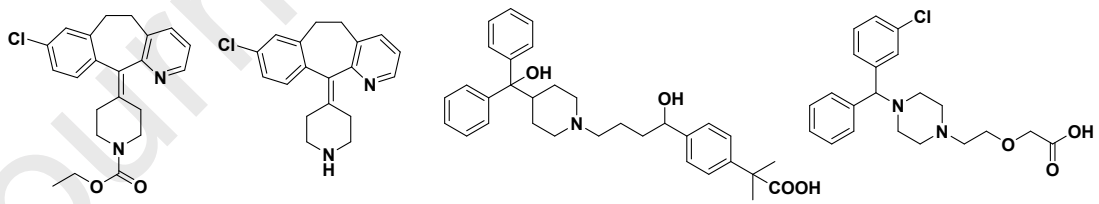


Fig. 1. Structure of second-generation antihistamines commonly used in clinical practice

Allergy and inflammation are intertwined in many pathological processes. For instance, chronic spontaneous urticaria (CSU) is characterized by the combined effects of allergy and chronic inflammation, where patients benefit poorly from single anti-allergic or anti-inflammatory therapy. Moreover, it has long been established that inflammation is a major consequence of allergic reactions, hence the term "allergic inflammation" [6,7]. Unfortunately, despite their strong anti-allergic potency, the new generation of H_1 antihistamines have weak anti-inflammatory efficacy [8-

[9]. Therefore, development of drugs with dual antihistamine and anti-inflammatory functionalities could represent a viable approach towards the treatment of allergy-related disease where inflammation also plays a critical role [10]. The aim of this study was to design, synthesize and evaluate novel desloratadine analogs for their H_1 antagonistic activity and anti-inflammatory potential.

Some studies found that guaiacol has anti-inflammation effect [11-12]. For example, natural product extract containing guaiacol inhibits NF-κB and MAPK in lipopolysaccharide-stimulated RAW264.7 cells and reduces cellular inflammatory response [13]. Based on the above studies, two compounds (**1c-2c**) were initially designed by combining the structures of desloratadine and guaiacol through a linker, as shown in Fig 2. Then, based on their activities, compound **2c** was chosen as the lead compound for further modification. Compounds **3c-15c** were subsequently designed and synthesized in the search for more potent compounds and to establish a structure-activity relationship (Fig 2).

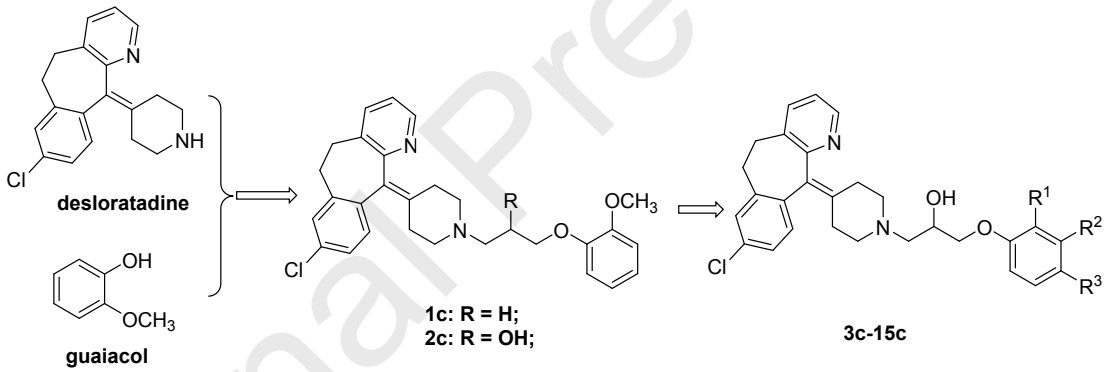
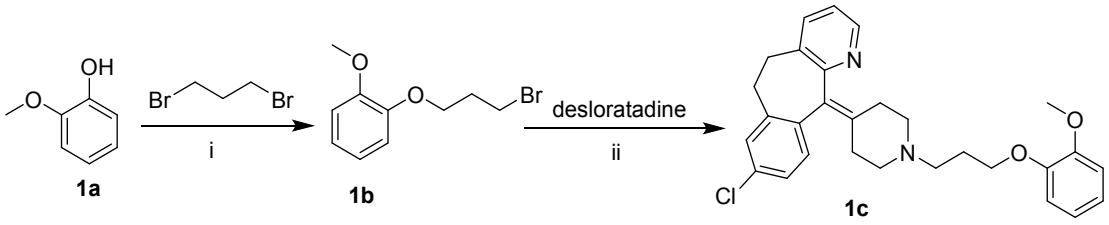


Fig. 2. Design of target compounds

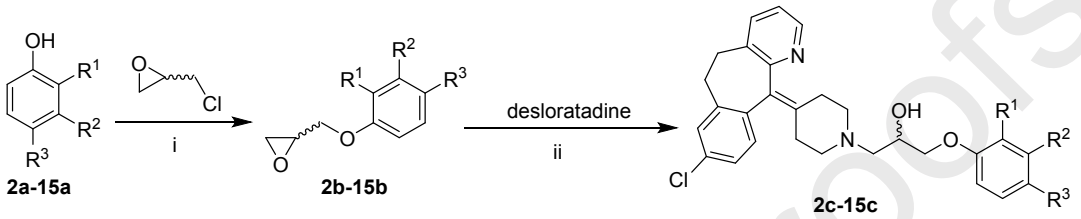
The synthesis of target compound **1c** is described in **scheme 1**. Reaction of guaiacol (**1a**) with 1,3-dibromopropane afforded intermediate **1b** that was then condensed with desloratadine to afford the target compound **1c**.

The preparation of compounds **2c-15c** is described in **Scheme 2**. Reaction of (chloromethyl)oxirane with corresponding compounds **2a-15a** in NaOH aqueous afforded intermediates **2b-15b** that were condensed with desloratadine to afford the target compounds **2c-15c**.

The preparation of chiral isomers **16c** and **17c** is described in **scheme 3**. Guaiacol **1a** reacted with R- or S-(chloromethyl) oxirane to give the intermediates **16b** or **17b** that were condensed with desloratadine respectively to give the target compounds **16c** and **17c**.

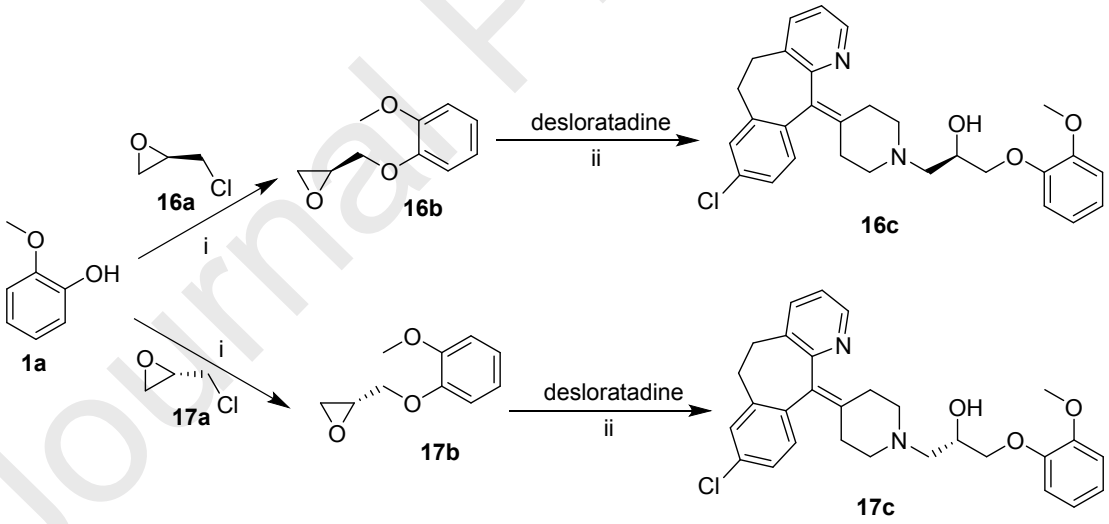


Scheme 1. Reagents and conditions: (i) DMF, K_2CO_3, r t, 2 h; (ii) DMF, K_2CO_3, 60℃, 6 h。



2a, 2b, 2c:	$R^1 = OCH_3$,	$R^2 = R^3 = H$;	9a, 9b, 9c:	$R^1 = Br$,	$R^2 = R^3 = H$;
3a, 3b, 3c:	$R^1 = R^2 = R^3 = H$;		10a, 10b, 10c:	$R^1 = NO2$,	$R^2 = R^3 = H$;
4a, 4b, 4c:	$R^1 = OCH_2CH_3$,	$R^2 = R^3 = H$;	11a, 11b, 11c:	$R^1 = CF3$,	$R^2 = R^3 = H$;
5a, 5b, 5c:	$R^1 = CH_3$,	$R^2 = R^3 = H$;	12a, 12b, 12c:	$R^1 = COCH_3$,	$R^2 = R^3 = H$;
6a, 6b, 6c:	$R^1 = OCF_3$,	$R^1 = R^3 = H$;	13a, 13b, 13c:	$R^2 = OCH_3$,	$R^1 = R^3 = H$;
7a, 7b, 7c:	$R^1 = F$,	$R^2 = R^3 = H$;	14a, 14b, 14c:	$R^3 = OCH_3$,	$R^1 = R^2 = H$;
8a, 8b, 8c:	$R^1 = Cl$,	$R^2 = R^3 = H$;	15a, 15b, 15c:	$R^1 = OCH3$,	$R^2 = R^3 = F$;

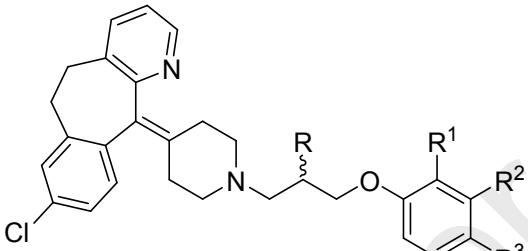
Scheme 2. Reagents and conditions: (i) NaOH, 50℃, 6 h; (ii) isopropanol, 90℃, 6 h;



Scheme 3. Reagents and conditions: (i) NaOH, 50℃, 6 h; (ii) isopropanol, 90℃, 6 h.

1c and **2c** were firstly designed to assess whether a three-carbon linker is appropriate. H_1 antagonistic activities of both **1c** and **2c** were maintained (IC_{50} values of 9.28 nM and 2.95 nM, respectively) suggesting incorporating this linker does not damp ligand binding to H_1 receptor. Moreover, **2c** is more potent than **1c**, indicating a hydroxyl group on the linker is beneficial. Next, effects of the substituents on the distal benzene ring were examined. The results showed that neither

removal (**3c**) nor replacement (**4c-12c**) of the -OCH_3 group on the benzene ring with other electron-donating or electron-withdrawing groups led to an increase in H_1 antagonistic activity. Only compounds **3c** (IC_{50} = 6.23 nM), **5c** (IC_{50} = 8.90 nM) and **7c** (IC_{50} = 4.74 nM) exhibited H_1 antagonistic activities similar to **2c**. These results indicated that -OCH_3 is the optimal substituent at this position. Moreover, H_1 antagonistic activity slightly decreased when the -OCH_3 group was switched to *meta*- or *para*- position (**13c-14c**). It was also found that diflourine-substituted analogue **15c** did not exhibit better activity.



1c-15c

Table 1 Activities of the target compounds against H_1 receptor

Compd.	R	R^1	R^2	R^3	IC_{50} (nM)
1c	H	-OCH_3	H	H	9.28±1.86
2c	OH	-OCH_3	H	H	2.95±0.79
3c	OH	H	H	H	6.23±0.84
4c	OH	-OCH_2CH_3	H	H	17.65±2.28
5c	OH	-CH_3	H	H	8.90±1.51
6c	OH	-OCF_3	H	H	36.71±6.38
7c	OH	-F	H	H	4.74±1.01
8c	OH	-Cl	H	H	28.41±2.41
9c	OH	-Br	H	H	40.22±2.00
10c	OH	-NO_2	H	H	52.85±4.96
11c	OH	-CF_3	H	H	81.78±5.55
12c	OH	-$COCH_3$	H	H	20.98±2.64
13c	OH	H	H	-OCH_3	8.14±1.06
14c	OH	H	-OCH_3	H	11.97±2.26
15c	OH	-OCH_3	-F	-F	27.88±3.66
desloratadine					1.84±0.19

Based on the above results, compounds **1c-3c**, **5c**, **7c** and **13c** were chosen to evaluate their anti-inflammatory activities in 2,4-dinitrofluorobenzene (DNFB)-induced mouse ear edema model. As shown in **Table 2**, compared with the desloratadine (35.6%), all compounds exhibited higher inhibitory activity (43-59.5%) at the dose of 5 mg/kg except compound **7c**. This result indicates that incorporating guaiacol structure into desloratadine could indeed enhance its anti-inflammatory

activity, which further supports our design rationale.

Table 2 Effect of DNFB-induced swelling and swelling inhibition of mouse ear (Mean±SD, n=8)

Compd.	Swelling（mg）	inhibition(%)@5mg/kg
model	17.4±3.6	-
Desloratadine	11.2±3.2△△	35.6
1c	7.1±4.3△△#	59.5
2c	7.1±2.4△△#	59.2
3c	7.1±2.9△△#	59.1
5c	7.1±2.9△△#	59.1
7c	12.9±3.2△	26.0
13c	9.9±3.0△△	43.1

$^{\triangle}P<0.05$ $^{\triangle\triangle}P<0.01$ vs model group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs Desloratadine

As pro-inflammatory mediators (such as TNF-α) play a key role in inflammatory diseases, [14-16] compounds **1c, 2c, 3c**, **5c**, **7c** and **13c** were selected to evaluate their inhibitory effects on TNF-α via enzyme-linked immunosorbent assay (ELISA) at a dosage of 5 mg/kg in BALB/C mice (shown in **Figure 3**). Compared with the model group, all compounds and desloratadine can significantly inhibit the release of TNF-α ($P<0.05$). Among them, compounds **1c**, **2c** and **3c** demonstrated higher potency than desloratadine in TNF-α inhibition ($P<0.05$), suggesting that these compounds have more pronounced immunosuppressive activity compared with desloratadine.

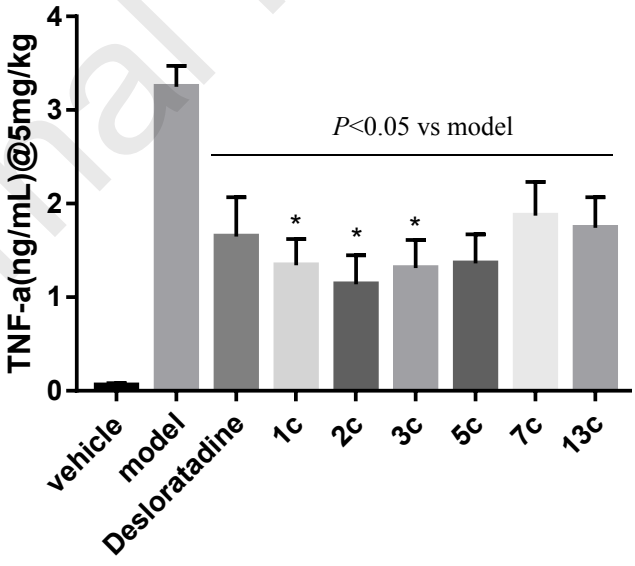


Fig3. LPS induced inflammatory factor release in BALB/C mice ($^{*}P<0.05$ vs desloratadine)

Since compound **2c** showed both the strongest activity against H_1 receptor and promising anti-inflammatory effects, it was subjected to further study. Firstly, the *R*- and *S*-enantiomers (**16c** and **17c**) were prepared individually and evaluated for their H_1 antagonistic activity and anti-

inflammatory activity. As shown in **Table 3**, compare with **2c**, enantiomers **16c** and **17c** showed nearly identical H_1 antagonistic activity with IC_{50} values of 3.67 nM and 4.56 nM, respectively. No deviation is anti-inflammation activity was observed either, which means there is no significant biological discrepancy between the two enantiomers.

Table 3 H_1 antagonize and anti-inflammatory activity of **2c** and its optical isomer

compound	Stereo configuration	H_1 antagonize activity IC_{50} (nM)	TNF-α (ng/mL, n=10)
2c	Racemate	2.95	1.14±0.32
16c	R	3.67	1.21±0.33
17c	S	4.56	1.18±0.28

Next, the pharmacokinetics of **2c** was studied and the results showed that **2c** has longer half-life in rats (iv. 2 mg/kg) ($T_{1/2}$=0.9 h) than in mice (iv. 2.5 mg/kg) ($T_{1/2}$=0.47 h). Meanwhile, good plasma exposure was observed in rats after oral administration (10 mg/kg) and the oral bioavailability of **2c** in rats is 18% (see Supporting Information Table 1). The metabolic stability and the potential metabolites of **2c** were investigated by incubating it with microsomes from different species (human, mouse, rat, dog and monkey) for 6 h and the major metabolites were identified by LC/MS (see Supporting Information Table 2). The results showed that compound **2c** exhibited the greatest stability in dog liver microsomes (DLM). The major metabolic pathway for **2c** was the N-dealkylation of the piperidinyl moiety leading to the formation of desloratadine (**M-1,** 24%). As, *O*-dealkylation constitutes the second major metabolic pathway, leading to **M-2** (9.2%). Similar to desloratadine, the tricyclic structure is readily hydroxylated leading to the hydroxylated product (**M-3**, 3.5%). (see Supporting Information Figure 1**)**.

To further evaluate the safety profile of **2c**, additional maximum tolerated dose (MTD) determination test was conducted. The results showed that compound **2c** exhibited significantly low toxicity with MTD values over 750 mg/kg, which is significantly higher than desloratadine (LD_{50} = 250 mg/kg).

In this study, a series of novel derivatives were designed and synthesized to optimize the anti-inflammatory activity of desloratadine. All compounds were evaluated for their H_1 antagonize and some compounds with single-digit nanomole IC_{50} values were evaluated for their anti-inflammatory

activities. Among them, racemate **2c** exhibited the most promising anti-inflammatory and H_1 antagonistic activities. Moreover, pharmacokinetic profiling of **2c** demonstrated that it possesses high stability *in vivo* and satisfying oral bioavailability and low toxicity. Currently, compound **2c** is undergoing further investigation in our lab.

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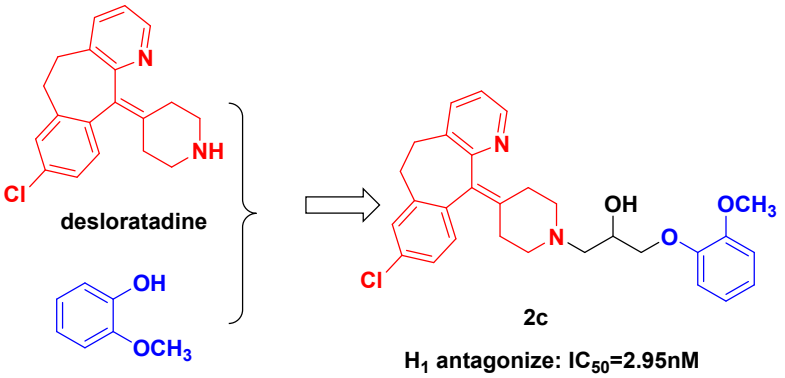
Graphical Table of Contents

Graphical Abstract



H_1 antagonize: IC_{50}=2.95nM

Swelling inhibition:59.2% @5mg/kg

LPS-induced release of TNF-α： 1.14±0.32ng/mL

To improve the anti-inflammatory activity of desloratadine, we designed and synthesized a series of novel desloratadine derivatives. All compounds were evaluated for their anti-inflammatory and H1 antagonistic activities. Among them, compound 2c showed the strongest H1 antagonistic and anti-inflammatory activity. It also exhibited promising pharmacokinetic profiles and low toxicity. All these results suggest that compound 2c as a novel anti-allergic agent is worthy of further investigation.