Discovery of *S*-(2-Guanidylethyl)-isothiourea (VUF 8430) as a Potent Nonimidazole Histamine H_4 Receptor Agonist

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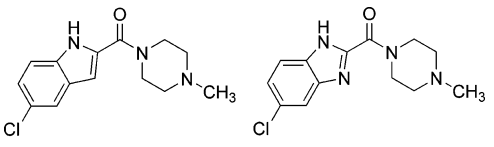
Abstract: During an in-house database screen, we identified *S*-(2-guanidylethyl)-isothiourea as a high affinity agonist for the histamine H_4 receptor, with a 33-fold selectivity over the histamine H_3 receptor and negligible affinity for the other histamine receptor subtypes. This nonimidazole ligand is introduced as a useful and complementary pharmacological tool that enables further unraveling of the physiological roles of the H_4 receptor.

The human histamine H_4 receptor (H_4R) is a $G_{i/o}$ protein-coupled receptor that was identified and cloned in 2000.[1] Several lines of evidence indicate that this receptor plays important roles in the immune system. Activation of the receptor leads to chemotaxis of mast cells and eosinophils[2,3] and mediates the production of inflammatory mediators, such as IL-16 and leukotriene B_4.[4,5] These data suggest that H_4R antagonists have potential as drugs to treat inflammatory diseases, such as asthma and allergy.[6] To validate the H_4R as a drug target, pharmacological tools such as selective agonists and antagonists are needed. A few selective H_4R antagonists have been published in literature, most notably **1** and **2** (Figure 1).[7,8]

The H_4R agonist OUP-16 described earlier shows only moderate affinity for the H_4R ($pK_i = 6.9$).[9] Recently, we have described 4-methylhistamine (**4**) as a selective human H_4R agonist ($pK_i = 7.3$) that shows >100-fold selectivity over the human H_1R, H_2R, and H_3R.[10] In the same publication, the H_2R agonist and H_3R antagonist dimaprit (**5**) was also identified as an H_4R agonist with moderate affinity (Figure 2).[10] Here we describe a focused screening effort of close analogues of dimaprit (**5**) taken from our proprietary compound collection, using SK-N-MC cells stably expressing the human H_4R.[11] The affinity of the ligands for the human H_4R was determined by displacement of [^{3}H]histamine binding, as described previously.[10]

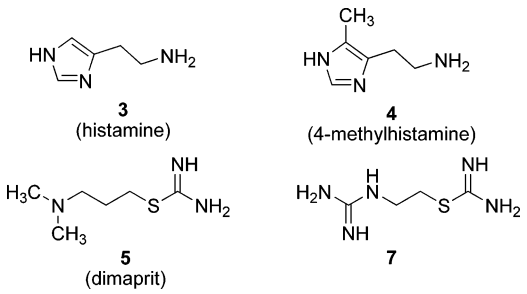
Substitution of the tertiary amine group of dimaprit (**5**) by a guanidine group (compound **6**, Table 1) results in a dramatic decrease in affinity. However, shortening the spacer of **6** that connects the isothiourea and guanidine groups from a propylene to an ethylene moiety leads to excellent H_4R affinity (compound **7**, *S*-(2-guanidylethyl)-isothiourea dihydrobromide, (VUF8430)). This compound has a pK_i of 7.5, which is almost as high as that of histamine (**3**; $pK_i = 7.9$; Figure 3 and Table 1).

The two chemically different basic moieties of **7** are key for affinity, as the corresponding compound with two isothiourea groups and the compound with two guanidine groups (**8** and **9**, respectively) have almost 10-fold lower affinity. Analogues separating the guanidine and isothiourea moieties by a longer carbon spacer have reduced affinity for the human H_4R (compare **10** and **11**). This shows that the ethylene spacer of **7** is optimal for interaction with the human H_4R.



1 (JNJ 7777120) **2** (VUF 6002)

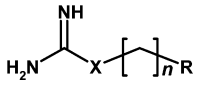
Figure 1. Structures of reference H_4R antagonists.



3 (histamine) **4** (4-methylhistamine) **5** (dimaprit) **7**

Figure 2. Structures of H_4R agonists.

Table 1. pK_i Values of Dimaprit (**5**) Analogues at the Human H_4R, as Determined by Displacement of [^{3}H]Histamine Binding[a]



compound[b]	X	n	R	pK_i
3 (histamine)				7.9 ± 0.1
5 (dimaprit)	S	3	*N*,*N*-dimethyl	6.5 ± 0.1
6	S	3	guanidine	5.1 ± 0.1
7	S	2	guanidine	7.5 ± 0.1
8	S	2	isothiurea	6.6 ± 0.1
9	NH	2	guanidine	6.4 ± 0.1
10	S	4	guanidine	5.5 ± 0.1
11	S	6	guanidine	5.4 ± 0.1

[a] Data shown are the mean ± SEM of at least three independent experiments. [b] Histamine was used as the dihydrochloride salt, all other compounds in Table 1 were used as the dihydrobromide salts.

Compound **7** was originally derived from the H_2R agonist dimaprit (**5**), but it is poorly active at the H_2R as determined at the right atrium of the guinea pig ($pD_2 = 3.8$, $\alpha = 0.4$).[12] In a binding assay, **7** shows only minimal inhibition of [^{125}I]-iodoaminopotentidine binding at the human H_2R expressed in CHO cells (Figure 3A). Likewise, **7** displays minimal inhibition of [^{3}H]pyrilamine binding to the human H_1R expressed in COS-7 cells (Figure 3A). Furthermore, it shows only moderate affinity ($pK_i = 6.0 \pm 0.1$) at the human H_3R, which is the closest relative of the human H_4R, as determined in a [^{3}H]N^{α}-methylhistamine displacement binding assay on the human H_3R stably expressed in SK-N-MC cells (Figure 3).

Compound **7** exerts agonistic activity ($pEC_{50} = 7.3 \pm 0.1$) at the human H_4R, which is determined as the inhibition of forskolin-induced cAMP-mediated increase in β-galactosidase activity. This inhibition reaches the same level as that exerted by histamine (**3**). Therefore, **7** is a full agonist (intrinsic activity $\alpha = 1$). Furthermore, the activity of **7** is dose-dependently shifted rightward by the H_4R-antagonist **1** (Figure 3B). Schild plot analysis of the antagonism of **1** against **7** results in a pA_2 value of 7.8, with a slope of 0.95 ± 0.05, which is in accord with the previously described pA_2 of **1** against histamine (**3**).[10]

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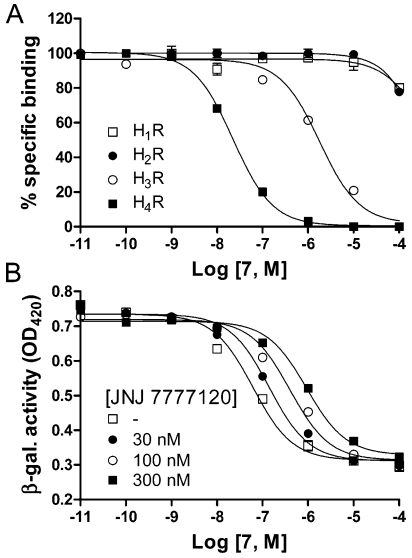
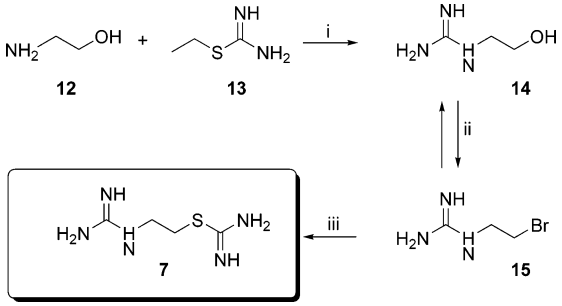


Figure 3. Pharmacological characteristics of compound **7**. (A) Displacement of radioligands bound at the human H_1R, H_2R, H_3R, and H_4R by different concentrations of **7**. (B) Competitive antagonism by **1** of H_4R agonism by **7**, as measured by the inhibition of forskolin-induced CRE-β-galactosidase activity.

Scheme 1. Synthesis of *S*-(2-Guanidylethyl)-isothiourea (**7**)[a]



[a] Reagents and conditions: (i) ethanol, reflux; (ii) 48% HBr, microwave 130 °C, 20 min (3×); (iii) thiourea, ethanol, microwave 125 °C 15 min.

Compound **7** also exerts full agonistic activity ($pEC_{50} = 6.5 \pm 0.1$, $\alpha = 1$) at the human H_3R in CRE-β-galactosidase assay performed in SK-N-MC cells. Interestingly, at the highest tested concentration (100 μM), **7** shows no agonistic activity at the human H_1R and only 50% agonistic activity at the human H_2R (data not shown). The latter agrees with the result reported previously for H_2R activity evaluated in the right atrium of guinea pig.[12]

The synthesis of *S*-(2-guanidylethyl)-isothiourea (**7**) has been described in literature.[13,14] According to this procedure, aminoalcohol **12** (Scheme 1) is treated with *S*-ethylisothiourea hydrobromide **13**, and in a one-pot procedure, the resulting alcohol **14** was treated with thiourea and concentrated HBr (48%). However, the isolated yield of this original procedure is very poor (10%). By iterative treatment of **14** with HBr under microwave conditions, isolation of bromide **15** and subsequent formation of the isothiourea moiety, a considerable increase in isolated yield (72%) can be obtained, making this compound readily available.

In conclusion, we have discovered a new potent H_4R agonist that shows a different pharmacological profile than that of the previously described human H_4R agonist 4-methylhistamine (**4**). Therefore, these two compounds may complement each other in their use as H_4R pharmacological tools. Additionally, we report an improved, high-yield synthesis of this ligand that gives easy access to this novel pharmacological tool. The compound is currently being used to further characterize the H_4R in vivo.

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Supporting Information Available: Experimental protocols and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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