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Synthesis and evaluation of a 2-benzothiazolylphenylmethyl ether class of histamine H4 receptor antagonists

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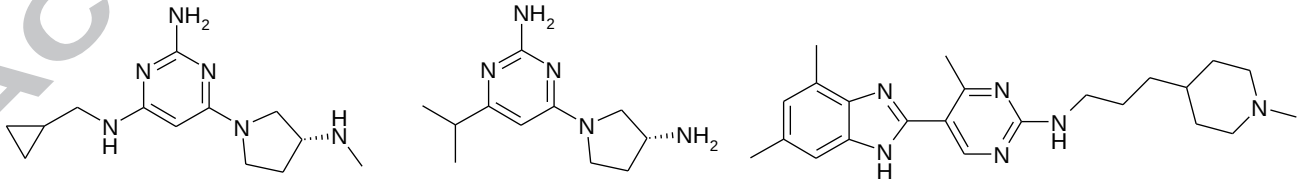
Abstract:
Synthesis and biological evaluation of a new class of histamine H_4 receptor ligands, distinct from the previously reported chemotypes, are described. A virtual screening of our corporate compound collection identified a hit with an undesired dual H3R/H4R activity. Chemical exploration led to the discovery of a more potent and selective 2-benzothiazolylphenylmethyl ether lead compound.

Keywords: Histamine, Histamine H_4 receptor, asthma, atopic dermatitis

Since its characterization and cloning in 2000 by Nakamura et al.[1] and Liu et al.[2] the histamine H4 (H4R) receptor has been widely studied and reviewed.[3] This G-protein-coupled receptor is mainly expressed on a variety of immune cells such as eosinophils, dendritic cells, mast cells and leukocytes[4] and represent an interesting pharmacological target for autoimmune or inflammatory disorders. Figure 1 describes a selection of H4R ligands that already entered clinical trials for the treatment of asthma, rheumatoid arthritis, atopic dermatitis and psoriasis.[5]



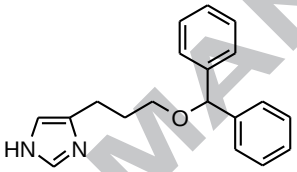
PF-3893787/ZPL-3893787
Phase II

JNJ-39758979
Phase II (discontinuated)

Toreforant/JNJ-38518168
Phase II

Figure 1. A selection of histamine H_4 ligands in clinical trials.

In this paper we describe the discovery of a new type of H4R ligands, distinct from the previously reported chemotypes.[6] We started our research program by a virtual screening of our corporate compound collection based on a 3D model of the receptor.[7] Representative elements of the more promising series were then evaluated in an in vitro hH4R [35S]GTPγS binding assay and among several hits we identified compound **1** (Kb= 19 nM) (Figure 2) as a very interesting starting point for a medicinal chemistry program. We first disclosed this 3-(1H-imidazol-4-yl)propyldiphenylmethyl ether in 1996 as a selective (over histamine H1 and H2 receptors) and potent histamine H3 receptor antagonist (hH3 binding Ki= 17 nM).[8] The fourth histamine receptor was unknown at this time. A dual H3R/H4R activity is not surprising since these two paralogues are found to share the highest degree of homology within the histamine receptor family.[1] Several other research groups also identified H3R ligands with H4R affinity.[9]

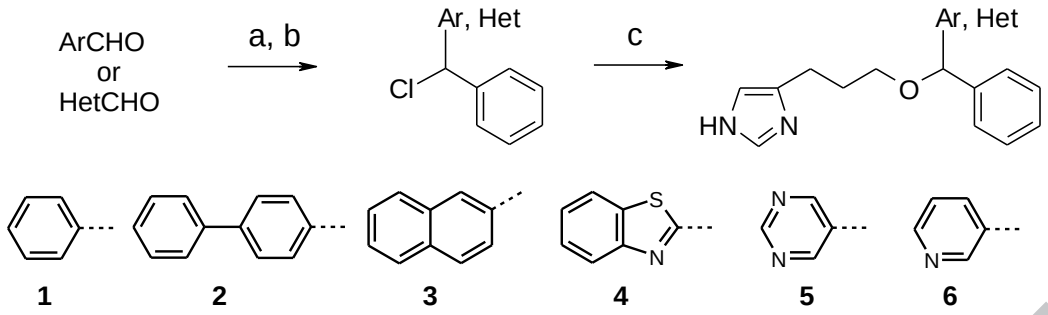


Compound 1

hH_4R K_b= 19 nM
hH_3R K_i= 17 nM
selectivity: 0.9

Figure 2. Hit compound

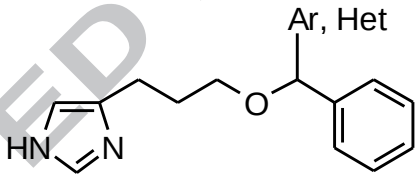
Here we report our efforts to design potent histamine H4 ligands with a better selectivity over the histamine H3 receptor. We first investigated the replacement of one phenyl group by a small selection of aryl or hetaryl rings. Thus we prepared different benzydrol analogs by condensation of various aryl carboxaldehydes with aryl or hetaryl lithium or Grignard reagents according to standard methods. These intermediates were chlorinated with $SOCl_2$ and the resulting chlorides treated with 3-(1*H*-imidazol-4-yl)propan-1-ol hydrochloride[10] to afford compounds **2-6** (Scheme 1).

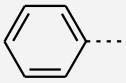
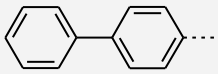
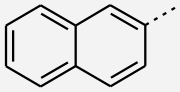


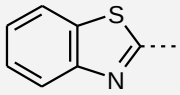
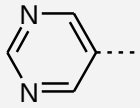
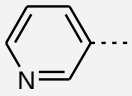
Scheme 1. Synthesis of compounds **1-6**. Reagents and conditions: (a) PhMgCl, THF, rt or 40°C, 1h or benzothiazole/BuLi, THF, -78°C, 2h (b) $SOCl_2$, DCM, rt, 2h; (c) 3-(1*H*-imidazol-4-yl)propanol hydrochloride, ACN, reflux, 24h.

Histamine H_4 receptor binding affinities of compounds **1-6** were evaluated in a [35S]GTPγS binding assay (stimulation with the hH4R agonist Imetit) on membranes of SH-SY5Y cells stably expressing the human H_4 receptor. Results are reported in Table 1. In this test, ligands **1-15** proved to be neutral antagonists (intrinsic activities of zero).

Table 1
hH4R binding data for the aryl or hetaryl selection (compounds **1-6)**.



Compound	Ar, HetAr	hH_4R K_b (nM)
1		19
2		17
3		100

4		1.1
5		29
6		32

Among the first synthesized ligands, the 2-benzothiazolyl group (compound **4**) proved to be the more promising hetaryl ring with a tenfold improved affinity for H4R in comparison with the phenyl of the hit compound. Figure 3 describes the possible binding mode of **4** at the H4R. The imidazole could form a salt bridge with Aspartate 94 of TM3, the oxygen of the linker a H-bond with the phenol moiety of Tyrosine 95 (TM3) and the nitrogen atom of benzothiazolyl group would establish another H-bond with Tyrosine 319 (TM6).

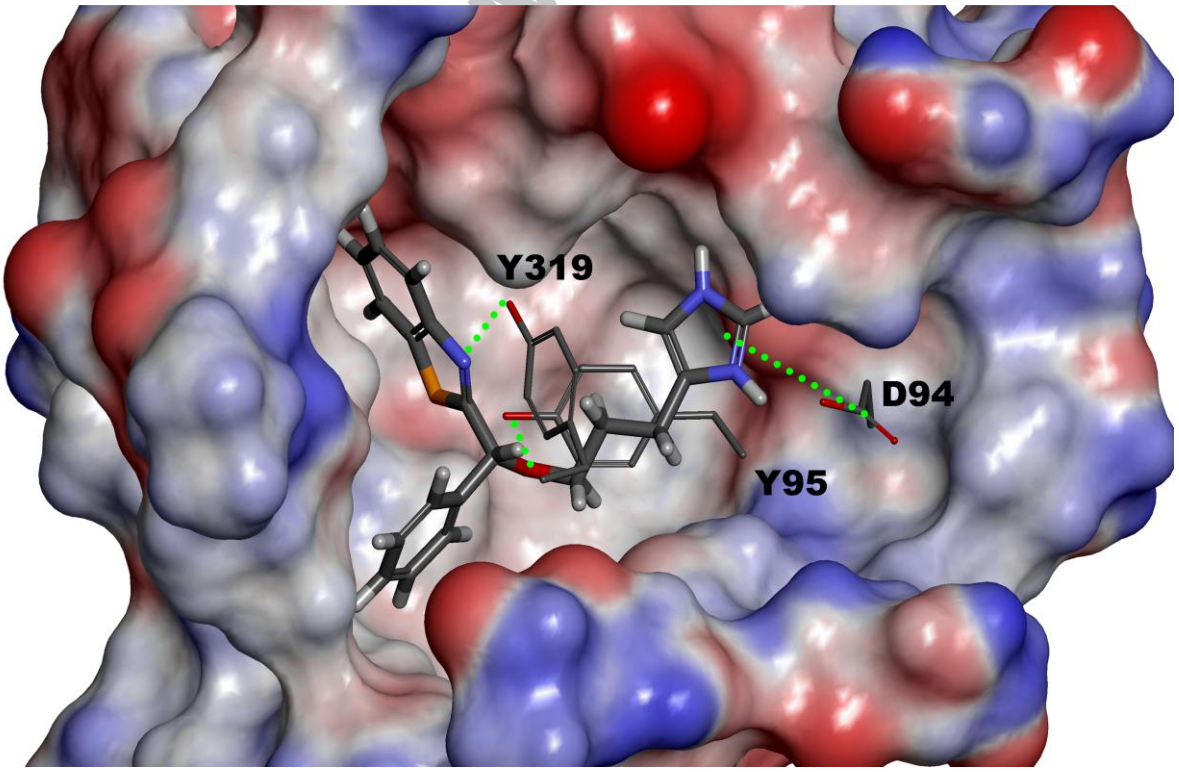


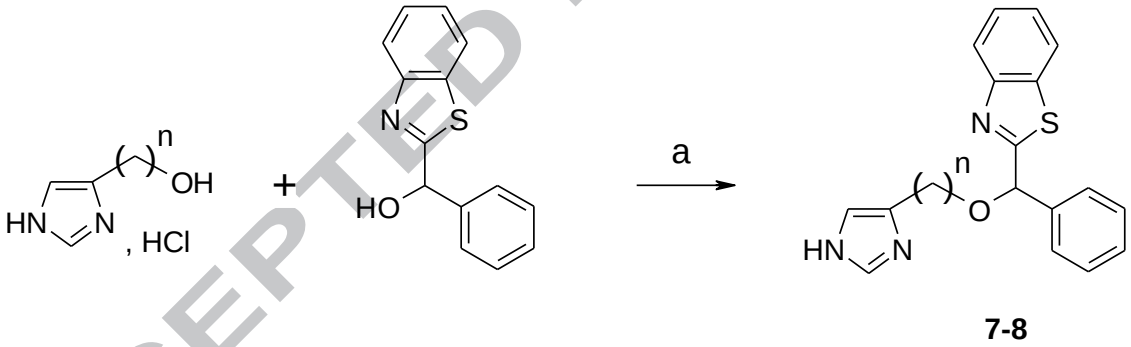
Figure 3. Detail of the predicted binding mode of compound **4.** The binding site is represented by its electrostatic potential on the **Connoly** surface. Several residues are hidden to improve the visibility.

We logically kept this benzothiazole to explore the western part of the scaffold. We investigated a shorter linker (compounds **7** and **8**), N- or C-methylated imidazoles (compounds **9-12**) and N-imidazole branched linkers (compounds **13-15**).

The synthesis of compounds **7** and **8** are described in scheme 2. Etherification of commercially available (1*H*-imidazol-4-yl)methanol and 2-(1*H*-imidazol-4-yl)ethan-1-ol with benzothiazol-2-yl(phenyl)methanol was performed in the presence of *p*-toluenesulfonic acid and Dean-Stark apparatus.

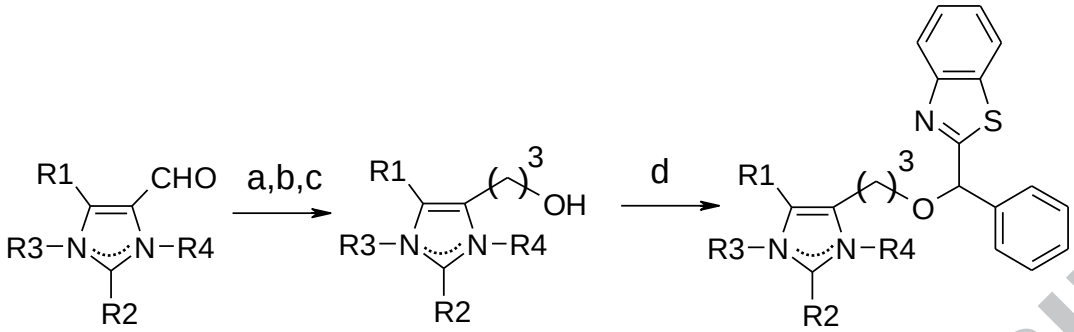
Compounds **9-12** were prepared analogously to **7** and **8**. Starting N(1), N(3), C(2) or C(5) methyl-imidazol-4-yl-propan-1-ols were synthesized in a 3-step procedure from the corresponding imidazole-carboxaldehydes (Scheme 3).

Ligands **13-15** were obtained by alkylation with imidazole of chlorinated intermediates (Scheme 4).



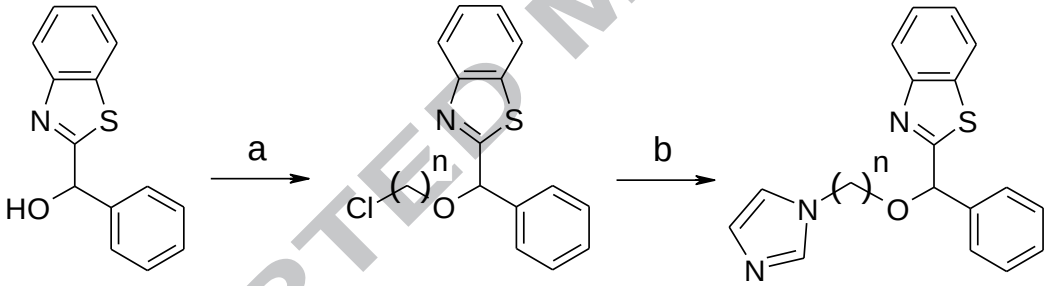
7-8

Scheme 2. Synthesis of compounds **7-8**. Reagents and conditions: (a) APTS, toluene, reflux with Dean-Stark apparatus, 20h.



9 (R1=Me, R2=H)
10 (R1=H, R2=Me)
11 (R3=Me, R4=/)
12 (R3=/, R4=Me)

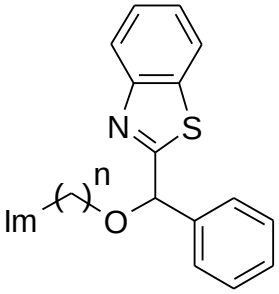
Scheme 3. Synthesis of compounds **9-12**. Reagents and conditions: (a) (Carbethoxymethylene)triphenylphosphorane, toluene, reflux, 6h (b) H_2, Pd/C 10%, MeOH, rt, 15h (c) $LiAlH_4$, THF, reflux, 3h (d) benzothiazol-2-yl(phenyl)methanol, APTS, toluene, reflux with Dean-Stark apparatus, 20h.

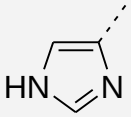
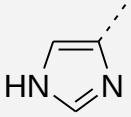
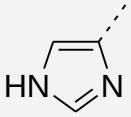
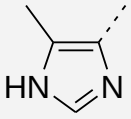
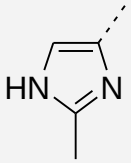
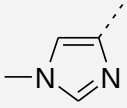
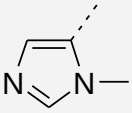
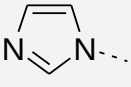


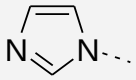
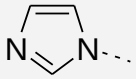
13-15

Scheme 4. Synthesis of compounds **13-15**. Reagents and conditions: (a) 2-Chloroethan-1-ol (n=2) or 3-chloropropan-1-ol (n=3) or 4-chlorobutan-1-ol (n=4), APTS, toluene, reflux with Dean-Stark apparatus, 20h; (b) imidazole, DMF, 3-15h

Table 2
hH4R binding data for compounds **7-16**.

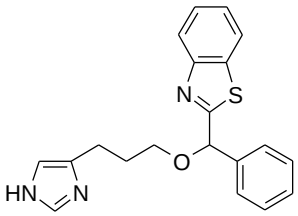


Compound	Im	n	hH_4R K_b (nM)
7		1	399
8		2	3.7
4		3	1.1
9		3	1.6
10		3	> 10 000
11		3	> 10 000
12		3	> 10 000
13		2	> 10 000

14		3	> 10 000
15		4	> 10 000

Reduced affinities of compounds **7** and **8** versus ligand **4** illustrate the progressive loss of the salt bridge interaction of imidazole with D94 when linker is shortened. C5-methylated imidazole (compound **9**) displayed equivalent potency whereas the substitution of C2 is sterically unfavorable (compound **10**).[11] What is more surprising is the loss of affinity for N-methylated compounds **11** and **12**. It is reminiscent of the discrepancy between binding interactions of histamine (HA) and its metabolite tele-methylhistamine (tMeHA) at the Histamine H3 receptor (Ki = 12.8nM for HA and Ki = 20µM for tMeHA)[12] or at the Histamine H4 receptor (Ki = 1.6nM for HA and Ki = 11µM for tMeHA).[13] Analogously N-imidazole branched linkers (compounds **13-15**) are totally inactive at the H4R whatever the alkyl length.

The Histamine H_3 receptor binding affinity of the more promising ligand (compound **4** – **BP1.2106**) was evaluated by displacement of [^{125}I]-iodoproxyfan (IPX) binding to membranes of HEK-293 cells stably expressing the human H_3 receptor[14] (Figure 4). Ligand **4** retained nearly comparable H3R binding affinity (34.0 nM versus 18.7 nM for hit compound) but displayed improved hH3R/hH4R selectivity (31 versus 0.9).



Compound 4 (BP1.2106)

hH_4R K_b= **1.1** nM
hH_3R K_i= 34 nM
selectivity: **31**

Figure 4. hH3R/hH4R selectivity for compound **4**

In summary, we have discovered a new class of histamine H4 receptor antagonists, distinct from the previously reported chemotypes. Starting from a hit suffering from a dual H3R/H4R activity, introduction of 2-benzothiazolyl group allowed to reach a nanomolar hH4R affinity and an improved selectivity over hH3R. Thus compound **4** (**BP1.2106)** was selected as a very promising starting point for lead generation. Further improvements will be reported in due course

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Dermatitis (NCT02424253) (www.clinicaltrials.gov); (f) A Study to Determine the Efficacy of ZPL-3893787 in Subjects With Plaque Psoriasis (NCT02618616) (www.clinicaltrials.gov).

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[11] See the supplementary material for details of the binding mode of imidazole moiety.

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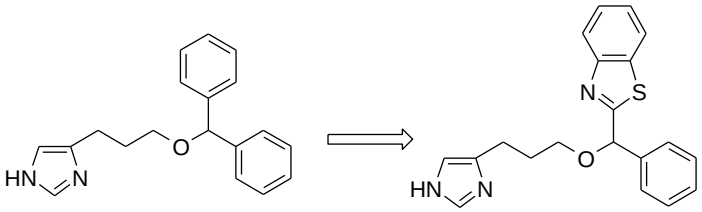

Hit Compound

hH_4R K_b= 19 nM
hH_3R K_i= 17 nM
selectivity: 0.9

Compound 4

hH_4R K_b= **1.1** nM
hH_3R K_i= 34 nM
selectivity: **31**