Characterization of the Histamine H_4 Receptor Binding Site. Part 1. Synthesis and Pharmacological Evaluation of Dibenzodiazepine Derivatives

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A series of dibenzodiazepine derivatives was synthesized to probe the binding site of the recently discovered histamine H_4 receptor (H_4R). Optimization of the lead structure clozapine (**2**) resulted in (*E*)-7-chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b*,*f*][1,4]oxazepine (**7j**), a potent H_4R agonist (H_4R, $pK_i = 7.6$). Pharmacological data suggests that the series of nonimidazole compounds can be used to describe the orthosteric binding site of the H_4R because both **2** and **7j** displace [^{3}H]histamine in a competitive manner. Furthermore, it is demonstrated that the effects of **7j** are competitively antagonized by the selective H_4R antagonist JNJ 7777120 (**1**), indicating considerable overlap of their binding sites. On the basis of the derived structure−activity relationships and additional pharmacological results, a pharmacophore model was constructed, which will be the premise for the design of novel H_4R ligands.

Introduction

Histamine is an extensively studied biogenic amine that is present in a variety of tissues. Its physiological role is mediated through the activation of four G-protein coupled receptors (GPCRs) that significantly differ in location, (patho)physiology and ligand binding. The histamine H_4 receptor (H_4R) has only recently been discovered independently by several research groups.[1−5] This receptor is mainly expressed in eosinophils, T cells, dendritic cells, basophils, and mast cells. The administration of selective H_4R antagonist **1** (Figure 1) inhibits the H_4R-mediated calcium influx and the chemotaxis of mast cells.[6] Furthermore, it has been shown that zymosan-induced neutrophil recruitment from the bone marrow can be blocked by thioperamide, a potent inverse agonist at both the H_4R and H_3R subtypes.[7] Together with the reports on the role of histamine as a leukocyte chemoattractant, these findings suggest an important role for the H_4R for the treatment of inflammatory diseases such as asthma, inflammatory bowel disease, and several dermatological disorders.[8−13] It has been suggested that the H_4R is involved in the histamine-induced itch, and H_4R antagonists could potentially lead to the first effective treatment of pruritis.[14,15]

The human H_4R is most closely related to the human H_3R. The two proteins have a sequence identity of 31% overall and 54% in the transmembrane domains where the orthosteric binding sites of the class A aminergic GPCRs are located. The orthosteric binding site of the H_4R is the site where its natural ligand histamine binds. It is not surprising, therefore, that most imidazole-containing ligands (agonists and antagonists) that have been developed for the H_3R also display considerable affinity for the H_4R.[13,16] Remarkably, the more recently developed nonimidazole H_3R inverse agonists and antagonists, compounds that have a high clincial potential in H_3R-mediated diseases, all lack H_4R affinity.[17]

A nonimidazole reference compound that has considerable affinity for the H_4R is the widely used antipsychotic drug clozapine (**2**) (Figure 2).[1] The binding of **2** to other GPCRs has been extensively studied, and many receptors tolerate its tricyclic dibenzodiazepine structure to some extent.[18,19] Because **2** acts as an antagonist on most GPCRs we were intrigued to find that it acts as a full agonist at the human H_4R ($\alpha = 1$, $pEC_{50} = 6.7$; $pK_i = 6.8$).[16] Taking into account the novelty of the target and the size and rigidity of tricyclic ligand **2**, we consider this compound to be an interesting starting point to explore the H_4R binding site. Therefore, a series of dibenzodiazepine analogues were synthesized to explore the structure−activity relationship (SAR) of this class of compounds at the human H_4R.

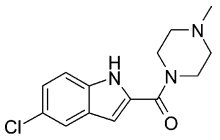

Figure 1. JNJ 7777120 (**1**).

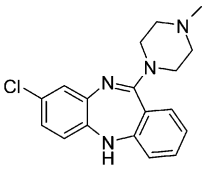

Figure 2. Clozapine (**2**).

Chemistry. We synthesized several different clozapine analogues using a modified method described by Nagarajan et al. (1974) (Scheme 1).[20] First, substituted chloro or fluorobenzoic acids were converted to their corresponding acid chlorides by $SOCl_2$. These acid chlorides were then added to a solution of different substituted *o*-aminophenols and triethylamine in THF to obtain the respective amide intermediates. Ring closure of the amides was achieved with NaOH in DMF. $POCl_3$ was used to convert the benzoxazepines in situ to iminochlorides that could then react with different cyclic amines. Compound **14** was synthesized according to a literature procedure.[21] Most of the compounds obtained were then converted to fumaric acid salts to improve handling of the compounds.

Pharmacology. Radioligand Displacement Studies. Homogenates of SK-N-MC cells stably expressing human H_3Rs or H_4Rs were used to determine ligand affinities for the H_3R or H_4R. These methods have been described previously.[16] An

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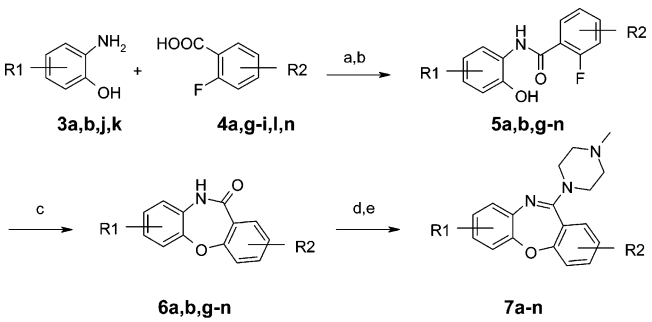
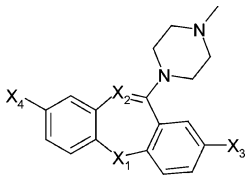
Scheme 1. General Synthesis of Clozapine Analogues[a]



[a] Reagents and Conditions: (a) $SOCl_2$, Reflux; (b) Et_3N, THF, 0 °C−RT; (c) NaOH, DMF, 120 °C; (d) $POCl_3$, Reflux (e) *N*-methylpiperazine, Toluene, Reflux

Table 1. H_4 Receptor Affinity of Clozapine Analogues with Alterations in the Tricyclic Framework as Determined by the Displacement of [^{3}H] Histamine



compd	X_1	X_2	X_3	X_4	pK_i ± SEM
2	NH	N	H	Cl	6.75 ± 0.1
8	S	N	H	Cl	5.69 ± 0.1
9	N-CH_3	N	H	Cl	5.85 ± 0.2
7a	O	N	H	Cl	7.37 ± 0.1
10	CH_2	N	H	H	5.32 ± 0.2
7b	O	N	H	H	6.88 ± 0.1
11	O	N	Cl	H	5.34 ± 0.0
12	O	CH_2	Cl	H	<5
1[a]					7.80 ± 0.1

[a] Structure as depicted in Figure 1.

analysis of ligand binding to the H_1R and H_2R was performed according to a literature procedure.[22]

Colorimetric cAMP Assay. SK-N-MC cells stably expressing the H_3R or the H_4R with a CRE-β-galactosidase reporter gene were employed to determine the functional acivity of either the H_3R or the H_4R. This assay has been described previously.[16]

NFκB-Luciferase Reporter Assay. COS-7 cells transiently transfected with the H_1R were used for the determination of the functional activity at the H_1R according to literature.[22]

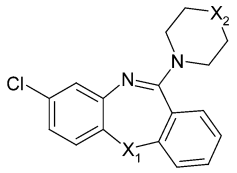
Results and Discussion

Initially, we optimized the tricyclic carbon framework of clozapine for H_4R affinity.

As can be seen in Table 1, the replacement of the X_1 nitrogen of clozapine (**2**) by a sulfur atom or methylamine group (compare **2**, **8**, and **9** with **7a**) or a carbon atom (compare **10** and **7b)**, is detrimental to H_4R affinity. In contrast, the substitution of the X_1 position with oxygen increases H_4R affinity 4-fold (**7a**). Liégois and co-workers have recently reported crystallographic data showing that the nature of the atom that fuses the phenyl rings determines the dihedral angle between the planes of both aromatic rings.[23] This angle, therefore, strongly determines the 3D structure of the molecules. Although an electronic or steric effect might also explain the increased receptor binding, conformational differences of the minimized structures can also be seen in the clozapine analogue series (data not shown).

Changing the piperazine substituent in **7a** from methyl to any other substituent decreased affinity for the H_4R in all cases

Table 2. H_4 Receptor Affinity of Clozapine Analogues with Alterations in the Piperazine Side Chain as Determined by the Displacement of [^{3}H] Histamine



compd	X_1	X_2	pK_i ± SEM
7c	O	NH	6.49 ± 0.1
7a	O	N-CH_3	7.37 ± 0.1
7d	O	N-CH_2CH_3	6.54 ± 0.1
7e	O	N-*i*-Propyl	5.97 ± 0.1
13	NH	N-CH_2CH_2OH	5.33 ± 0.2
2	NH	N-CH_3	6.75 ± 0.1
14	NH	CH_2	<5
7f	NH	O	<5

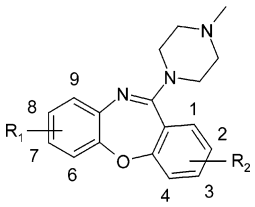
(Table 2). The distal nitrogen in unsubstituted compound **7c** is less basic than its methylated analogue **7a.** The same holds true for ethylated analogue **7d**, yet no increase in H_4R affinity can be observed compared to **7c**. A steric component might, therefore, also play a role in the binding of these compounds. A further increase in the size of this methyl substituent decreases the H_4R affinity even more. The absence of the distal nitrogen atom in the piperazine side chain, as can be seen in the piperidine (**14**) and morpholine (**7f**) analogues, even leads to a dramatic loss of H_4R affinity.

Changing the R_1 chlorine from the 8- to the 7-position (compounds **7a** and **7j**) gave a slight increase in affinity. Changing this substituent at the 7-position to a methyl group as in analogue **7k** made the affinity drop slightly. The unsubstituted compound **7b** also showed a decrease in affinity compared to that of **7a**, indicating the importance of a lipophilic substituent on the left aromatic ring. An investigation of the SAR of the right aromatic ring showed that a chlorine substituent at the 2- (compare **7b** and **11**) and 3-positions (compare **7h** and **7a**) was detrimental to affinity, whereas a chlorine substituent at the 4-position was tolerated (**7l**). Fluorine substitution at the 2-position also decreased H_4R affinity (compare **7a** and **7g**). Fluorine substitution at the 3- and 4-positions was allowed, but these compounds were no more potent than, for example, the unsubstituted 7-chloro analogue **7j**. The functional profile of the compounds in Table 3 was also studied to see if they would retain the full agonistic behavior that was found for clozapine. Interestingly, all of the compounds in Table 3 were full agonists except for **11**, which acted as a partial agonist. Chlorine substitution at the 2-position appeared to be detrimental for H_4R affinity as well as for the full agonistic profile of the clozapine analogues.

Table 3 contains several tricyclic structures that bind to the H_4R with nanomolar affinity, and of these, compound **7j** has been taken as a model compound to study the affinity and functional behavior at the other histamine receptors.

These data show that **7j** has about a 5-fold higher affinity for the H_1R than that for the H_4R and is 330 times more selective for the H_4R over the H_3R. These data suggest that a compound can be designed that is relatively inactive at the H_2R and the H_3R, while binding in the lipophilic domains of the H_1R and H_4R active sites. In fact, dual action ligands acting as both H_1R and H_4R antagonists or inverse agonists have been suggested to be a potential new way to treat inflammatory diseases.[10] Although one group reported known H_1R ligands (doxepine, cinnarazine, and promethazine) to be active at the H_4R[3] we were

Table 3. H_4 Receptor Affinity of Clozapine Analogues with Varying Aromatic Substituents as Determined by the Displacement of [^{3}H] Histamine



compd	R_1	R_2	pEC_{50}	α	$pK_i \pm$ SEM
7b	H	H	6.52 ± 0.19	1	6.88 ± 0.1
11	H	2-Cl	6.66 ± 0.04	0.5	5.34 ± 0.0
g	8-Cl	2-F	7.09 ± 0.11	1	6.72 ± 0.1
7h	8-Cl	3-Cl	6.99 ± 0.11	1	6.51 ± 0.1
7i	8-Cl	4-F	7.83 ± 0.11	1	7.56 ± 0.1
7a	8-Cl	H	7.55 ± 0.12	1	7.37 ± 0.1
7j	7-Cl	H	7.70 ± 0.10	1	7.55 ± 0.1
7k	7-CH_3	H	6.98 ± 0.08	1	7.10 ± 0.1
7l	7-Cl	4-Cl	7.83 ± 0.07	1	7.34 ± 0.1
7m	7-Cl	4-F	7.69 ± 0.08	1	7.51 ± 0.1
7n	7-Cl	3-F	7.41 ± 0.02	1	7.41 ± 0.0

Table 4: Affinity and Functional Profile of **7j** at the Different Histamine Receptors

	compd **7j**		
receptor	$pK_i \pm$ SEM	pEC_{50}	α
H_1	8.11 ± 0.10	8.17 ± 0.14	−1
H_2	5.06 ± 0.05	n.d.[a]	n.d.[a]
H_3	5.04 ± 0.14	n.d.[a]	n.d.[a]
H_4	7.55 ± 0.09	7.70 ± 0.10	1

[a] Not determined.

unable to reproduce those results to combine them with the SAR found for the clozapine analogues at the H_4R.[16]

Clozapine (**2**) and its metabolites have been reported to have affinity for the D_1-D_4 receptors, several 5-HT subreceptors, the α_1-adrenoreceptor, the H_1R, and the muscarinic receptor subtypes. Compound **2** and one of its metabolites have also been reported to act as allosteric modulators of GPCRs, although evidence for this is sometimes contradictory.[18,19] Therefore, we investigated whether **2** and **7j** bind to the H_4R as orthosteric or allosteric ligands by looking at the B_{max} value of the H_4R in the presence of increasing concentrations of **2** and **7j** (Figure 3).

The B_{max} value for [^{3}H]histamine did not change significantly when increasing concentrations of the selected compounds were used. In contrast, the K_d value for [^{3}H]histamine increased upon increasing concentrations **2** and **7j**. These data indicate that **2** and **7j** compete for the same binding site as [^{3}H]histamine in the H_4R. Compounds **2** and **7j**, therefore, do not act as allosteric modulators on the recombinant H_4R system but as orthosteric ligands.

We could also show that known H_4R antagonist **1** competitively antagonized the agonistic effect of **7j** (Figure 4). Schild plot analysis resulted in a pA_2 value of 7.8 for **1**, which nicely correlates with the H_4R affinity of pK_i 7.8.[16]

When the structure of **1** is compared to that of **7j**, several similarities can be noticed. Most strikingly, both ligands have a piperazine moiety linked to an aromatic ring system. For both classes of compounds, it has been shown that the basic nitrogen of this piperazine is ideally substituted by a methyl group, whereas other substituents lead to a drop in affinity.[24,25] Furthermore, halogenation of the aromatic rings lead to similar subtle differences in affinity. On the basis of these similarities in structure and SAR and because the pharmacological results suggest that **7j** and **1** compete for the same H_4R binding site, we were tempted to perform preliminary modeling studies to investigate the structural similarities of these compounds, even though **1** is an (neutral) H_4R antagonist and **7j** is a full H_4R agonist.[16]

For these similarity studies, the procedure as described by Labute and Williams was used.[26] Calculations were carried out using the flexible alignment module of Molecular Operating Environment (MOE), employing the MMFF94 force field. The best scoring fit (both in terms of similarity and objective function; similarity $F = 104.6195$, objective function $S = 187.6832$, $dU = 0.7498$ kcal/mol) is shown in Figure 5. In this superposition, the key structural elements are perfectly superposed. The chlorinated aromatic ring of **7j** is superimposed on the phenyl ring of **1** with the chlorine atoms pointing in the same direction. The carbonyl group in **1** is flexible enough to give a good overlay of the protonated nitrogens in both compounds. The methyl substituents also overlap perfectly, whereas the bulkier piperazine moiety in compound **1** and **7j** occupy approximately the same space. Obviously, this qualitative pharmacophore model is unable to explain the mode of action of the ligands (agonism for **7j**, neutral antagonism for **1**). With the limited number of structurally diverse classes of

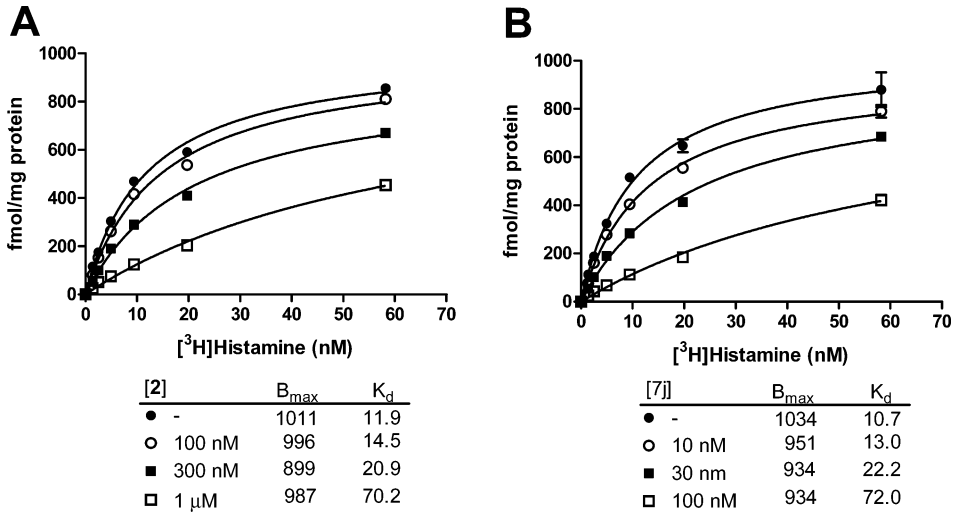


Figure 3. Potential orthosteric binding of **2** and **7j** by saturation binding analysis. Binding of [^{3}H]histamine to the H_4R. The presence of **2** (A) and **7j** (B) dose-dependently increases the K_d value without affecting the B_{max} of [^{3}H]histamine binding, suggesting that **2** and **7j** displace [^{3}H]histamine in a competitive manner.

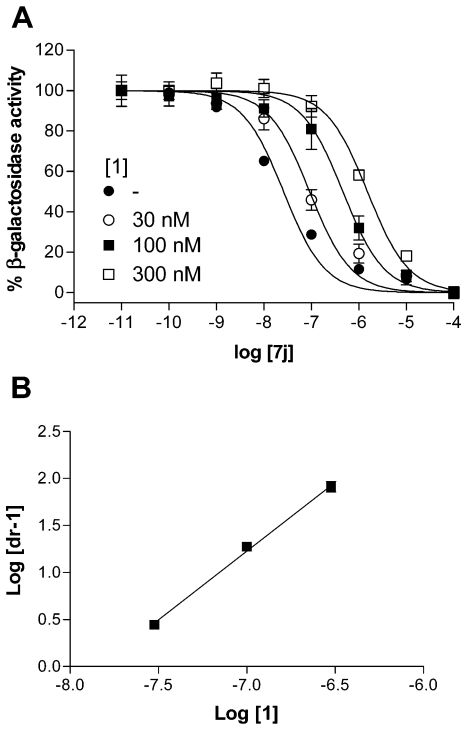


Figure 4. Selective histamine H_4 antagonist **1** competitively antagonizes the H_4R effects of **7j** activity. (A) Compound **1** shifts the dose−response curve of **7j** to the right in a dose-dependent manner. (B) Schild plot analysis resulted in a pA_2 value of 7.8 for JNJ 7777120 (**1**).

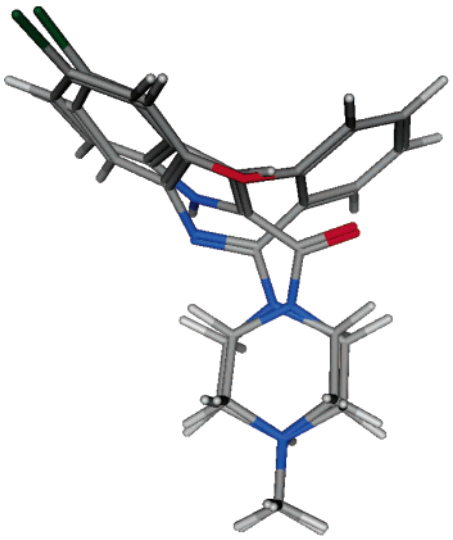


Figure 5. Alignment of **1** and **7j**. The superpostion shown was selected on the basis of the highest similarity score (*F*) and the highest objective function score (*S*).

H_4R ligands reported to date and without taking into account the receptor protein environment, it will be difficult to address the functional activity of the ligands. It has already been shown that for the histamine H_3R subtype, subtle changes in the chemical structures of the H_3R ligands can have a major impact on functional activity. Even within a single class of ligands, close homologues can vary in H_3R activity from agonist to neutral antagonist and inverse agonist.[27] Nevertheless, we consider this preliminary pharmacophore a useful working model for H_4R affinity.

In conclusion, probing the H_4R pharmacophore, we have optimized the dibenzodiazepine structure of clozapine (**2**), resulting in the identification of **7j** as a high-affinity full H_4R agonist. Compound **7j** was found to displace [^{3}H]histamine from its orthosteric binding site in the H_4R. Given the structural similarities of **7j** and **1**, we have conducted preliminary modeling studies and propose a model that gives a description of the H_4R binding site of **7j**. The results from these modeling studies are currently being used to design new chemical entities that will fit the H_4R binding site. In the search for new antipsychotic drugs, many analogues of **2** have been made in the past that also seem to fit the H_4R pharmacophore. The fact that many of these analogues can modulate the H_4R and cause a possible clinically relevant but yet unknown response mediated by this histamine receptor should, therefore, be taken into consideration.

Experimental Section

General Remarks. Compounds **2** and **11** were purchased from Sigma RBI (U.S.A.). Compounds **8**, **9**, **10**, and **13** were donated by Dr. Aebischer from the Sandoz research institute in Bern. Clozapine analogue **12** was purchased from Tocris Cookson, Ltd. (U.K.).

The THF and toluene used in the reactions were freshly distilled from lithium aluminum hydride and calcium hydride, respectively. Other chemicals and reagents were obtained from commercial suppliers and were used without further purification. The yields given are isolated yields unless otherwise mentioned and were calculated on the free base after purification by flash chromatography. Flash column chromatography was carried out on an Argonaut Flashmaster II flash chromatography system, using prepacked Isolute Flash Si II columns with the UV detector operating at 254 nm. All melting points are uncorrected and were measured on an IA9200 electrothermal meltingpoint apparatus. All ^{1}H NMR and ^{13}C NMR spectra were measured on a Brüker 200. Analytical HPLC-MS analyses were conducted using a Shimadzu LC-8A preparative liquid chromatograph pump system with a Shimadzu SPD-10AV UV−Vis detector set at 254 nm, with the MS detection performed with a Shimadzu LCMS-2010 liquid chromatograph mass spectrometer. The analyses were performed using the following two conditions. Condition *I*: an Alltima(C18) 5 μm column (150 mm × 4.6 mm) with the following two solvents: solvent A, 10% MeOH− 90% H_2O− 0.1% formic acid; solvent B, 95% MeOH− 5% H_2O− 0.1% formic acid; flow rate = 1.0 mL/min; start: 100% A, linear gradient time is 15 min, then 10 min at 100% B, then 15 min at 100% A. The total run time was 40 min. Condition *II*: Xbridge(C18) 5 μm column (100 mm × 6 mm), isocratic elution with varying percentages of acetonitrile−H_2O containing 0.1% formic acid; flow rate = 1.0 mL/min. The total run time was 40 min. Compounds that were isolated as fumaric acid salts all showed an extra peak around 4.1 min under condition *I* (fumaric acid was found to have t_R= 4.1 min in this system). Under condition *II*, fumaric acid blancs were used to determine the t_R of fumaric acid. Purities calculated are based on RP HPLC−UV peak surface area of the compounds, taking the fumaric acid peaks into account.

Methods. General Procedure. The synthesis of **7b** from precursors **6b** and **5b** described below is a general procedure that has been used for the synthesis of all tricyclic oxazepines.

2-Fluoro-*N*-(2-hydroxyphenyl)-Benzamide (5b). To 2-fluorobenzoic acid (5.0 g, 36 mmol) was added thionyl chloride (10 mL). The solution was refluxed for 2 h; after which the excess of thionyl chloride was removed under reduced pressure, and the residual traces were then removed by coevaporation with dry toluene. The acid chloride was then added dropwise to a solution of 2-aminophenol (3.9 g, 36 mmol) and Et_3N (10 mL, 72 mmol) in dry THF (75 mL) at 0 °C. After the addition of the acid chloride, the mixture was allowed to warm up to room temperature overnight while stirring under a nitrogen atmosphere. After stirring overnight, the mixture was diluted with water (400 mL) and neutralized with 4% HCl to pH 7. The precipitated amide was then filtered off over a glass filter and washed with 4% HCl and copious amounts of water. The washed product was dried in vacuo overnight in the presence of P_2O_5 and was used in the next step without further

purification. Yield 6.19 g (76%). Mp 144−147 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 10.10 (s, 1H), 9.48 and 9.43 (2s, 1H, rotamers), 8.17−8.05 (m, 1H), 7.94−7.87 (m, 1H), 7.72−7.52 (m, 1H), 7.45−7.24, (m, 3H), 7.03−6.80 (m, 2H).

Dibenzo[*b,f*][1,4]oxazepin-11(10*H*)-one (6b). 2-Fluoro-*N*-(2-hydroxyphenyl)-benzamide (**5b**) (500 mg, 2.16 mmol) was dissolved in DMF (10 mL), and one equivalent of freshly powdered NaOH was added. The resulting mixture was heated at reflux for 5 h After cooling to room temperature, the mixture was diluted with water (100 mL), and the precipitated product was filtered over a glass filter and washed with 5% NaOH and then water to remove the unreacted starting material. The solid residue was dried overnight under vacuum and was used in the next step without further purification. Yield 320 mg (70%). Mp 210−212 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 10.55 (s, 1H), 7.78 (dd, J = 1.6 Hz, J = 7.7 Hz, 1H), 7.66−7.58 (m, 1H,), 7.37−7.28 (m, 3H), 7.17−7.11 (m, 3H).

11-(4-Methylpiperazin-1-yl)-dibenzo[*b,f*][1,4]oxazepine (7b). 10*H*-Dibenzo[*b,f*][1,4]oxazepin-11-one (**6b**) (1.0 g, 4.7 mmol) was added to $POCl_3$ (5 mL) and heated at reflux overnight. Excess $POCl_3$ was evaporated under reduced pressure yielding the crude iminochloride. Dry toluene (10 mL) and *N*-methylpiperazine (5.3 mL, 47 mmol) were added to the iminochloride, and the mixture was heated at reflux. After 2 h, the solvent was allowed to cool to room temperature, and the mixture was added to EtOAc and water. The aqueous layer was then extracted with EtOAc, and the combined organic layers were dried over Na_2SO_4. Evaporation of the solvent yielded the crude product, which was purified by flash chromatography (100% EtOAc) to give the free base as a light yellow oil. Yield 472 mg (34%); ^{1}H NMR ($CDCl_3$) δ (ppm) 7.45−6.99 (m, 8H), 3.65 (m, 4H), 2.52 (m, 4H), 2.34 (s, 3H); ^{13}C NMR (DMSO-d_6) δ (ppm) 160.82, 160.20, 152.00, 140.44, 132.45, 129.42, 126,78, 125.36, 124.63, 124.00, 123.48, 121.11, 120.03, 54.84, 47.13, 46.02; MS (ESI) m/z 294 $(M + H)^+$.

Acknowledgment. We greatly acknowledge Dr. T. Lovenberg (Johnson & Johnson Pharmaceutical Research and Development, L.L.C., San Diego, CA) for the gift of the cells expressing the human H_4R and H_3R. Compounds **3**, **5**, **7**, and **13** were generously donated by Dr. Aebischer from the Sandoz research institute in Bern. Thanks also goes out to Andrea van de Stolpe for expert technical assistance.

Supporting Information Available: LCMS purity data for compounds **7a**−**n** and **14** and experimental details for compounds **5**−**7a**, **7c**−**f**, **14**, **5g**−**n**, **6g**−**n**, and **7g**−**n**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Liu, C.; Ma, X.-J.; Jiang, X.; Wilson, S. J.; Hofstra, C. L.; Blevitt, K.; Li, X.; Chai, W.; Carruthers, N.; Lovenberg, T. W. Cloning and pharmacological characterization of a fourth histamine receptor (H_4) expressed in bone marrow. *Mol. Pharmacol.* **2001**, *59*, 420−426.

(2) Morse, K. L.; Behan, J.; Laz, T. M.; West, R. E., Jr.; Greenfender, S. A.; Anthes, J. C.; Umland, S.; Wan, Y.; Hipkin, R. W.; Gonsiorek, W.; Shin, N.; Gustafson, E. L.; Qiao, X.; Wang, S.; Hedrick, J. A.; Green, J.; Bayne, M.; Monsma, F. J., Jr.; Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 1058−1066.

(3) Nguyen, T.; Shapiro, D. A.; George, S. R.; Setola, V.; Lee, D. K.; Cheng, R.; Rauser, L.; Lee, S. P.; Lynch, K. R.; Roth, B. L.; O'Dowd, B. F. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol.* **2001**, *59*, 427−433.

(4) Oda, T.; Morikawa, N.; Saito, Y.; Masuho, Y.; Matsumoto, S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000**, *275*, 36781−36786.

(5) Zhu, Y.; Michalovich, D.; Wu, H.-L.; Tan, K. B.; Dytko, G. M.; Mannan, I. J.; Boyce, R.; Alston, J.; Tierney, L. A.; Li, X.; Herrity, N. C.; Vawter, L.; Sarau, H. M.; Ames, R. S.; Davenport, C. M.; Hieble, J. P.; Wilson, S.; Bergsma, D. J.; Fitzgerald, L. R. Cloning, expression and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol.* **2001**, *59*, 434−444.

(6) Thurmond, R. L.; Desai, P. J.; Dunford, P. J.; Fung-Leung, W. P.; Hofstra, C. L.; Jiang, W.; Nguyen, S.; Riley, J. P.; Sun, S.; Williams, K. N.; Edwards, J. P.; Karlsson, L. A potent and selective histamine H4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 404−413.

(7) Takeshita, K.; Bacon, K. B.; Ganter, F. Critical role of L-selectin and histamine H_4 receptor in Zymosan-induced neutrophil recruitment from the bone marrow: comparison with carrageenan. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 272−280.

(8) Buckland, K. F.; Williams, T. J.; Conroy, D. M. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. *Br. J. Pharmacol.* **2003**, *140*, 1117−1127.

(9) Ling, P.; Ngo, K.; Nguyen, S.; Thurmond, R. L.; Edwards, J. P.; Karlsson, L.; Fung-Leung, W. P. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol.* **2004**, *1*, 161−171.

(10) Daugherty, B. L. Histamine H_4 antagonism: a therapy for chronic allergy? *Br. J. Pharmacol.* **2004**, *142*, 5−7.

(11) Jablonowski, J. A.; Carruthers, N. I.; Thurmond, R. L. J. A. The histamine H_4 receptor and potential therapeutic uses for H_4 ligands. *Mini Rev. Med. Chem.* **2004**, *4*, 993−1000.

(12) Fung-Leung, W. P.; Thurmond, R. L. J. A.; Ling, P.; Karlsson, L. W. P. Histamine H_4 receptor antagonists: the new antihistamines? *Expert Opin. Invest. Drugs* **2004**, *5*, 1174−1183.

(13) de Esch, I. J. P.; Thurmond, R. L. J. A.; Jongejan, A.; Leurs, R. The histamine H_4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol. Sci.* **2005**, *26*, 462−469.

(14) Bell, J. K.; McQueen, D. S.; Rees, J. L. Involvement of histamine H_4 and H_1 receptors in scratching induced by histamine receptor agonists in BalbC mice. *Br. J. Pharmacol.* **2004**, *142*, 374−380.

(15) Hagermark, O. Peripheral and central mediators of itch. *Skin Pharmacol.* **1992**, *5*, 1−8.

(16) Lim, H. D.; van Rijn, R. M.; Ling, P.; Bakker, R. A.; Thurmond, R. L.; Leurs, R. Evaluation of histamine H_1-, H_2-, and H_3-receptor ligands at the human histamine H_4 receptor: Identification of 4-methylhistamine as the first potent and selective histamine H_4 receptor agonist. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1310−1321.

(17) Celanire, S.; Wijtmans, M.; Talaga, P.; Leurs, R.; de Esch, I. J. P. Histamine H_3 receptor antagonists reach out for the clinic. *Drug Discovery Today* **2005**, *10*, 1613−1627.

(18) Michal, P.; Lysikova, M.; El-Fakahany, E. E.; Tucek, S. Clozapine interaction with the M2 and M4 subtypes of muscarinic receptors. *Eur. J. Pharmacol.* **1999**, *376*, 119−125.

(19) Zorn, S. H.; Jones, S. B.; Ward, K. M.; Liston, D. R. Clozapine is a potent and selective muscarinic M_4 receptor agonist. *Eur. J. Pharmacol.* **1994**, *269*, R1−R2.

(20) Nagarajan, K.; Kulkarni, L.; Venkateswarlu, A.; Shah, R. Condensed heterotricycles: Dibenz[*b,f*][1,4]oxazepin-11(10H)-thiones, 11-substituted dibenz[*b,f*][1,4]oxazepines & dibenz[*b,f*][1,4]thiazepines analogues. *Indian. J. Chem.* **1974**, *12*, 258−262.

(21) Wander, A. Patent DE2316438, 1966.

(22) Bakker, R. A.; Weiner, D. M.; ter Laak, T.; Beuming, T.; Zuiderveld, O. P.; Edelbroek, M.; Hacksell, U.; Timmerman, H.; Brann, M. R.; Leurs, R. 8R-lisuride is a potent stereospecific histamine H1-receptor partial agonist. *Mol. Pharmacol.* **2004**, *65*, 538−549.

(23) Liegeois, J. F.; Eyrolles, L.; Ellenbroek, B. A.; Lejeune, C.; Carato, P.; Bruhwyler, J.; Geczy, J.; Damas, J.; Delarge, J. New pyridobenzodiazepine derivatives: modifications of the basic side chain differentially modulate binding to dopamine (D(4.2), D(2L)) and serotonin (5-HT(2A)) receptors. *J. Med. Chem.* **2002**, *45*, 5136−5149.

(24) Jablonowski, J. A.; Grice, C. A.; Chai, W.; Dvorak, C. A.; Venable, J. D.; Kwok, A. K.; Ly, K. S.; Wei, J.; Baker, S. M.; Desai, P. J.; Jiang, W.; Wilson, S. J.; Thurmond, R. L.; Karlsson, L.; Edwards, J. P.; Lovenberg, T. W.; Carruthers, N. I. The first potent and selective nonimidazole human histamine H4 receptor antagonists. *J. Med. Chem.* **2003**, *19*, 3957−3960.

(25) Terzioglu, N.; van Rijn, R. M.; Bakker, R. A.; de Esch, I. J. P.; Leurs, R. Synthesis and structure−activity relationships of indole- and benzimidazole piperazines as histamine H_4 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5251−5256.

(26) Labute, P.; Williams, C.; Feher, M.; Sourial, E.; Schmidt, J. M. Flexible Alignment of small molecules. *J. Med. Chem.* **2001**, *44*, 1483−1490.

(27) Wieland, K.; Bongers, G.; Yamamoto, Y.; Hashimoto, T.; Yamatodani, A.; Menge, W. M.; Timmerman, H.; Lovenberg, T. W.; Leurs, R. Constitutive activity of histamine h(3) receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H(3) antagonists. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 908−914.

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