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Synthesis and Pharmacological Activity of Fluorescent Histamine H_2 Receptor Antagonists Related to Potentidine[1]

Liantao Li,[a] Julia Kracht,[b] Shiqi Peng,[a] Günther Bernhardt,[b] Sigurd Elz[b] and Armin Buschauer[b,*]

[a]*College of Pharmaceutical Sciences, Peking University, 100083, Beijing, China*
[b]*Institute of Pharmacy, University of Regensburg, D-93040 Regensburg, Germany*

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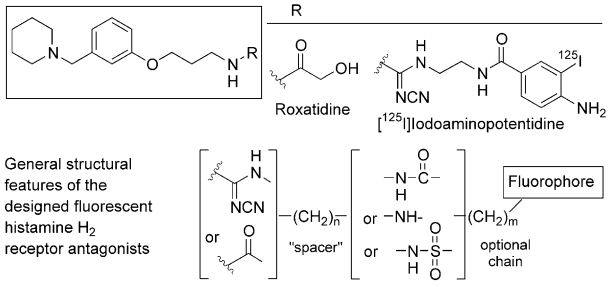
Abstract—Fluorescently labeled histamine H_2 receptor antagonists were synthesized starting from *N*-cyano-*N′*-[3-(3-piperidin-1-ylmethylphenoxy)propyl]guanidines with an additional *N″*-ω-aminoalkyl substituent (chain lengths 2–8 methylene groups) or from 3-(3-piperidin-1-ylmethylphenoxy)propylamine. The primary amino group was derivatized with various fluorophores (fluorescein, acridine, dansyl, nitrobenzoxadiazole (NBD), indolo[2,3-*a*]quinolizine). On the isolated spontaneously beating guinea pig right atrium most of the fluorescent probes were only weakly active, however, the NBD-labeled substances proved to be potent histamine H_2 receptor antagonists achieving pA_2 values in the range of 7.5–8.0, comparable to the activity of famotidine.

As part of a program to develop fluorescence-based methods for the study of ligand receptor interactions at G-protein coupled receptors (GPCRs) we have recently demonstrated that the affinity of agonists and antagonists can be determined by flow cytometry under equilibrium conditions by using cyanine5-labeled neuro peptide Y.[2] This approach is very promising in case of peptides, and it could generally be a very attractive alternative to radioligand binding if it were also applicable to the investigation of small molecules acting at GPCRs such as biogenic amines and their antagonists. Therefore, suitable fluorescent probes are needed to investigate the applicability of such methods. We selected histamine receptors[3,4] as a model to study low molecular weight ligands which interact with GPCRs. Very recently, we reported on fluorescent histamine H_1 receptor antagonists related to mepyramine.[5] In the present study fluorescently labeled H_2 antagonists are described.[1] As spacefilling residues such as radiolabeled partial structures including spacer groups are tolerated in the aminopotentidine series,[6] the title compounds were designed by analogy with the approach that has been successfully applied to the development of the high affinity radioligand for the H_2 receptor, [^{125}I]iodoaminopotentidine[6,7] (Scheme 1).



Scheme 1.

Chemistry

The fluorescent histamine H_2 receptor antagonists **7a**,**c**, **8a**,**c**, **9a**, **10a**, **11a**–**g**, **12a** were synthesized as outlined in Scheme 2. 3-(3-Piperidin-1-ylmethylphenoxy)propylamine (**2**) was prepared in three steps from 3-hydroxybenzaldehyde (**1**) as described elsewhere.[8] The synthesis of the ω-aminoalkyl-substituted cyanoguanidines **5a**–**g** from **2** was accomplished by analogy with the procedures described in the literature[6,8] using diphenoxymethylenecyanamide (**3**) and the pertinent diamines of various chain lengths (**4a**–**g**). The primary amino group was conjugated to fluorescent dyes by treating **5a**–**g** with the corresponding succinimidyl esters (**6A**/**B**, **6D**) or acid chlorides (**6C**, **6F**) or with 4-chloro-7-nitrobenzo[2,1,3]oxadiazole (**6E**).

*Corresponding author. Tel.: +49-941-9434827; fax: +49-941-9434820; e-mail: armin.buschauer@chemie.uni-regensburg.de

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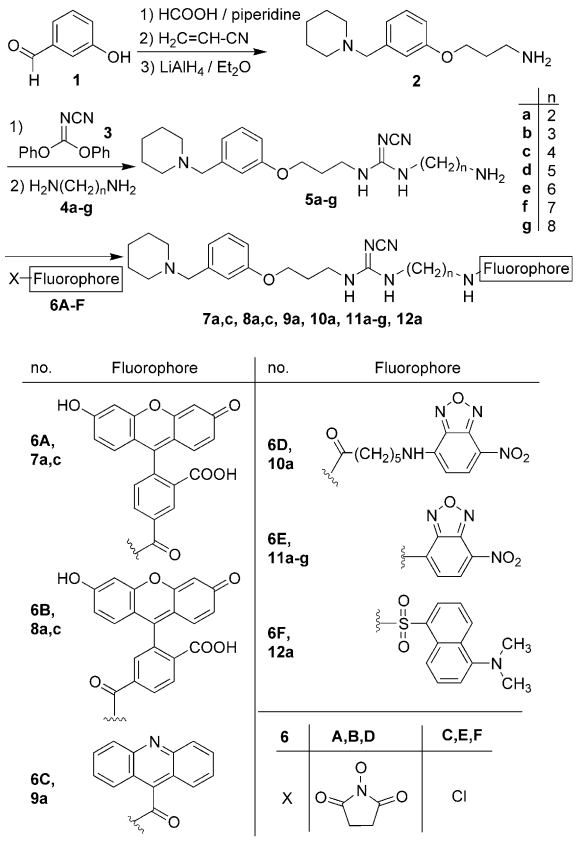
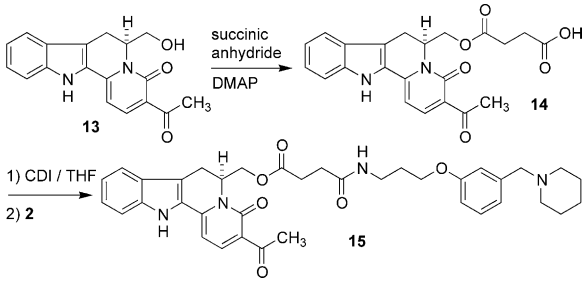


Scheme 2.

The coupling reactions with the succinimidyl esters of 5-/6-carboxyfluorescein (commercially available mixture of the isomers **6A** and **6B**) and the NBD-substituted aminohexanoic acid **6D** were carried out in anhydrous dichloromethane/DMSO in the dark.[9] Subsequently, the isomers **7a**/**8a** and **7c**/**8c** were separated by HPLC on a semipreparative scale. Analogously, the derivatization of the amines with acridine-9-carbonyl chloride (**6C**) NBD-Cl (**6E**), and dansyl chloride (**6F**) was accomplished in anhydrous solvents in the presence of triethylamine or diisopropylethylamine (DIPEA).

In addition to the preparation of the cyanoguanidines the amine **2** was converted to the amide **15** with succinic acid monoester **14** after activation with carbonyldiimidazole (Scheme 3). Compound **14** was obtained from (*S*)-tryptophane by a multistep procedure via **13** as described[10] followed by conversion of the alcohol to **14** with succinic anhydride in the presence of 4-dimethylaminopyridine.



Scheme 3.

Table 1. Histamine H_2 receptor antagonistic activities of fluorescent ligands and reference compounds on the isolated guinea pig right atrium

Compd	λ_{max} Ex (nm)[a]	λ_{max} Em (nm)[a]	Guinea pig atrium[b] $pA_2 \pm$SEM
Cimetidine			6.00±0.06[c]
Ranitidine			6.77±0.10[d]
Roxatidine acetate			7.41±0.06[e]
Famotidine			7.74[f]
5a			5.96±0.11
7a	n.d.	n.d.	4.18±0.13
7c	n.d.	n.d.	5.05±0.09
8a	497	525	4.35±0.37
8c	n.d.	n.d.	5.00±0.18
9a	360	433	6.74±0.08
10a	482	539	7.59±0.09
11b	483	539	7.43±0.15
11e	482	539	7.49±0.12
11g	483	539	7.96±0.05[g]
12a	333	539	5.71±0.16
15	421[h]	513[h]	5.74±0.02

[a]Longest wavelength absorption and fluorescence emission maximum determined with a Kontron UV–Vis-Spectrometer and a Perkin–Elmer LS-50B spectrofluorimeter, the compounds were dissolved in a buffer (NaCl 120 mM, KCl 5 mM, $MgCl_2$ 2 mM, $CaCl_2$ 1.5 mM, Hepes 25 mM, glucose 10 mM, adjusted to pH 7.4 with NaOH); n.d.: not determined.
[b]Inhibition of histamine-stimulated positive chronotropic response; pA_2: mean values calculated from the rightward-shift of isometrically recorded cumulative concentration–response curves for histamine in the absence and presence of the H_2 receptor antagonists at 32.5 °C; $n = 2$–10; antagonist concentrations from 0.1 to 100 μM (depending on the activity the test compound). *Schild*-plots were constructed for cimetidine, roxatidine acetate and **11g**.
[c]$n = 16$, slope: 0.85±0.04.
[d]Slope not significantly different from unity.
[e]$n = 15$; slope: 0.85±0.04; ref 13: $pA_2 = 6.56$.
[f]Ref 14.
[g]cf. Fig. 1.
[h]In MeOH: λ_{max} Ex: 420 nm, λ_{max} E_m: 495 nm.

Pharmacology

The fluorescent compounds were investigated for histamine H_2 receptor antagonistic activity on the isolated spontaneously beating guinea pig right atrium[11] according to standard experimental protocols.[12] The results are summarized in Table 1. Representative concentration-response curves and a *Schild*-plot are shown for compound **11g** in Figure 1.

Results and Discussion

Amide derivatives of compound **2**, such as roxatidine, as well as related compounds having *N*,*N*′-disubstituted cyanoguanidine or nitroethenediamine partial structures are known as rather potent histamine H_2 antagonists (for review see ref 15). It is characteristic of these 3-(3-piperidin-1-ylmethylphenoxy)propylamine derivatives that relatively bulky residues may be tolerated without loss of activity. Depending on their structure the substituents may even confer additional H_2 receptor affinity, as demonstrated by [^{125}I]iodoaminopotentidine, the high affinity radioligand for the histamine H_2 receptor.[6,7] Therefore, by analogy with the design of the latter, except compound **18**, the fluorescent probes for the H_2 receptor described in this study were structurally

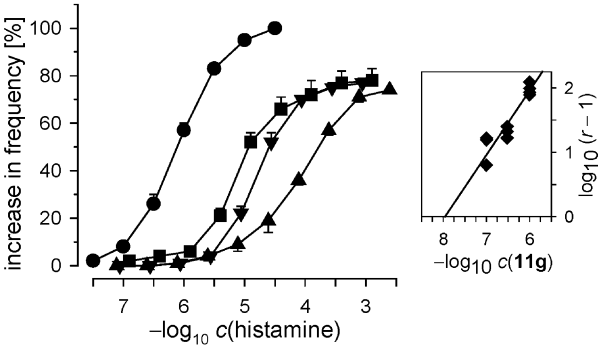


Figure 1. Positive chronotropic effect of histamine in isolated, isometrically set up (resting tension 5 mN), spontaneously beating guinea-pig right atria in the absence (●, $n=10$) and presence of compound **11g**: 0.1 μM (■, $n=3$, E_{max} of histamine 78±5%), 0.3 μM (▼, $n=3$, 77±2%), and 1 μM (▲, $n=4$, 74±1%). (±)-Propranolol (0.3 μM) was present throughout the experiment. Symbols represent the arithmetic mean±SEM. Inset: *Schild*-plot regression yielded a pA_2 value of 7.96±0.05 ($n=10$, 95% confidence limits: 7.84–8.09) and a slope not significantly different from unity (0.93±0.13, $p>0.5$, two-tailed *t*-test).

derived from aminoalkyl-substituted *N*-[3-(3-piperidin-1-ylmethylphenoxy)propyl]cyanoguanidine.

The results of the investigation for H_2 antagonism on the isolated guinea pig right atrium (Table 1) show that the nitrobenzoxadiazole group was by far the best one to obtain compounds with reasonable antagonistic activity. These NBD derivatives achieve activities in the range of pA_2 7.5–8.0. A spacer with 6–8 methylene groups proved to be favorable, whereas an additional chain as in compound **10a** ($CO(CH_2)_5$) did not increase the antagonistic activity further. For instance, the pA_2 value of 7.96 found for compound **11g** on the guinea pig atrium (Fig. 1) is comparable to that of famotidine, which is the most potent therapeutically used H_2 antagonist. Obviously, the fluorophore contributes to the increase in the receptor affinity of the ligand by interaction with an extra binding site, as the primary amines used as starting material were only weak or moderately active H_2 antagonists (e.g., **5a**).

All the other fluorophores were inappropriate to confer high histamine H_2 receptor antagonistic activity. This is in contrast to a series of H_1 antagonists related to mepyramine, where both the NBD as well as the carboxyfluorescein moiety were found to have an activity-enhancing effect. As the NBD group is the smallest among the fluorescent labeling agents tested in this study, it may be speculated that the bulk of the fluorophore and not the length of the connecting chains is the limiting factor in case of the H_2 antagonists related to iodoaminopotentidine.

Conclusions

The results summarized in Table 1 show that potent fluorescence-labeled H_2 antagonists of the *N*-[3-(3-piperidin-1-ylmethylphenoxy)propyl]cyanoguanidine type can be obtained by derivatization of corresponding primary amines having appropriate spacer lengths with NBD dyes. Such fluorescent probes may be useful to study the GPCRs in tissue preparations and on cells. Although, due to its spectral properties and low quantum yield in physiological buffers, NBD is not an ideal fluorophore, we demonstrated by the strategy described in this paper, that it is possible to obtain fluorescent histamine H_2 receptor ligands which should be useful pharmacological tools to investigate ligand receptor interactions, for example in fluorimetric binding assays and functional studies.

Acknowledgements

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9. Labeling of **5a–g**. (a) Fluorescein-labeled compounds **7a**,**c**,**8a**,**c**: To the solution of **5a**,**c** (0.023 mmol) in 1.5 mL of anhydrous CH_2Cl_2 9.0 mg (0.019 mmol) of **6A**/**B**, dissolved in 200 μL of anhydrous DMSO, were added and the mixture was stirred at room temperature in the dark for 24 h. After evaporation of the solvent the isomers were chromatographed analytically and separated on a semipreparative scale by RP-HPLC according to a previously described method.[5] **7a** and **8a**: Yield: 90%; $^+$FAB-MS (Varian MAT 95, xenon, MeOH-glycerol): *m*/*z* (%) = 717 ($[M+H]^+$, 100); HR-$^+$FAB-MS $C_{40}H_{41}N_6O_7$, calcd: 717.3037, found: 717.3005; **7c** and **8c**: Yield: 87%; $^+$FAB-MS: *m*/*z* (%) = 745 ($[M+H]^+$, 100), HR-$^+$FAB-MS: $C_{42}H_{45}N_6O_7$, calcd: 745.3350, found: 745.3333.
(b) *N*-[2-(Acridine-9-carbonyl)aminoethyl)]-*N*′-cyano-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]guanidine (**9a**) was synthesized from **6C** (0.41 mmol) and **5a** (130 mg, 0.36 mmol) in 15 mL of anhydrous $CHCl_3$ in the presence of 200 μL

DIPEA (N_2 atmosphere). The product was isolated by chromatography (Chromatotron™ model 8924 (Harrison Research), rotors with 4 mm layers of silica gel PF_{254} containing gypsum, EtOAc:MeOH, gradient 1:0 to 1:1). Yield 85%, mp 115 °C (from MeOH). ESI-MS (MeOH + 1% HOAc): m/z (%) = 564 ($[M+H]^+$, 100), 282 ($[M+2H]^{2+}$, 95); IR (KBr): ν (cm^{-1}) = 2168 (C≡N), 1586 (C=N). Analysis ($C_{33}H_{37}N_7O_2 \cdot H_2O$ (581.7)) calcd: C 68.14, H 6.76, N 16.86; found: C 68.05, H 6.78, N 16.40.

(c) *N*-Cyano-*N*′-{2-[5-(7-nitrobenzoxadiazol-4-yl)aminopentyl)carbonylaminoethyl]-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]guanidine (**10a**): The succinimidyl ester **6D** (24 mg, 0.066 mmol), dissolved in 200 μL of DMSO, was added to the solution of **5a** (20 mg, 0.06 mmol) in 2 mL of anhydrous CH_2Cl_2. The mixture was stirred at room temperature for 24 h in the dark and chromatographed (Chromatotron, $CHCl_3$:MeOH = 3:1) to obtain **10a** as orange powder (yield 56%). HR-$^+$FAB-MS: $C_{31}H_{43}N_{10}O_5$, calcd: 635.3417, found: 635.3417.

(d) *N*-Cyano-*N*′-[ω-(7-nitrobenzoxadiazol-4-yl)aminoalkyl]-*N*″-[3-(3-piperidin-1-ylmethylphenoxy)propyl]guanidines **11a**–**g**: 22 mg (0.11 mmol) of **6E** was added to the solution of 0.1 mmol of **5a**–**g** and 30 μL of Et_3N in 5 mL of anhydrous $CHCl_3$. The mixture was stirred at room temperature for 10 h in the dark. After evaporation of the solvent the residue was chromatographed (Chromototron, eluent: $CHCl_3$: MeOH = 3:1). **11a**: Yield: 23%; HR-$^+$FAB-MS: $C_{25}H_{32}N_9O_4$, calcd: 522.2577, found: 522.2572. IR (KBr): ν(cm^{-1}) = 2169 (C≡N), 1297 (NO_2). **11b**: Yield: 38%; HR-$^+$FAB-MS: $C_{26}H_{34}N_9O_4$, calcd: 536.2733, found: 536.2726. **11c**: Yield: 37%; HR-$^+$FAB-MS: $C_{27}H_{36}N_9O_4$, calcd: 550.2890, found: 550.2870. **11d**: Yield: 44%; HR-$^+$FAB-MS: $C_{28}H_{38}N_9O_4$, calcd: 564.3046, found: 564.3033. **11e**: Yield: 26%; HR-$^+$FAB-MS: $C_{29}H_{40}N_9O_4$, calcd: 578.3203, found: 578.3179. **11f**: Yield: 49%; HR-$^+$FAB-MS: $C_{30}H_{42}N_9O_4$, calcd: 592.3359, found: 592.3334. **11g**: Yield: 37%; HR-$^+$FAB-MS: $C_{31}H_{44}N_9O_4$, calcd: 606.3516, found: 606.3515; ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.47 (d, J = 8.7 Hz, 1H), 7.21 (t, J = 8.0 Hz, 1H), 6.95–6.73 (m, 4H), 7.18 (d, J = 8.7 Hz, 1H), 5.71 (t, J = 5.1 Hz, 1H), 5.36 (t, J = 5.1 Hz, 1H), 4.08 (t, J = 5.5 Hz, 2H), 3.50–3.44 (m, 6H), 3.16–3.08 (q, J = 6.7 Hz), 2.39 (s, 4H), 2.12–2.00 (m, J = 5.8 Hz, 2H), 1.85–1.25 (m, 18H). IR (KBr): ν(cm^{-1}) = 2165 (C≡N), 1585 (C=N), 1297 (NO_2).

(e) *N*-Cyano-*N*′-[2-(5-dimethylaminonaphthalene-1-sulfo-nyl) aminoethyl] - *N*″ - [3 - (3 - piperidin - 1 - ylmethylphenoxy)propyl]-guanidine (**12a**): The mixture of **5a** (55.6 mg, 0.155 mmol), **6F** (50 mg, 0.185 mmol) and Et_3N (66 μL) in 2 mL of CH_2Cl_2 was stirred at room temperature for 14 h and the product was isolated with a Chromatotron ($CHCl_3$: MeOH = 95:5). Yield 78%, mp 67 °C. HR-$^+$FAB-MS: $C_{31}H_{41}N_7SO_3$, calcd: 592.3070 found: 592.3088.

N-[3-(3-Piperidin-1-ylmethylphenoxy)propyl]succinamic acid (6*S*)-3-acetyl-4-oxo-4,6,7,12-tetrahydroindolo[2,3-a]quinolizin-6-ylmethyl ester (**15**). Succinic anhydride (80 mg, 0.8 mmol) was added to the solution of **13** (80 mg, 0.26 mmol) and DMAP (80 mg, 0.65 mmol) in 5 mL CH_2Cl_2. After stirring at room temperature for 2 h the reaction was quenched with MeOH (0.6 mL), diluted with CH_2Cl_2, and neutralised with cold 10% aqueous citric acid. The organic layer was washed with water, saturated aqueous NaCl, dried over Na_2SO_4 and filtered. After evaporation of the solvent **14** was obtained as yellow powder (yield 85%), mp 94 °C (decomp.). $^+$FAB-MS: m/z (%) = 409 ($[M+H]^+$, 31), 309 (100). IR (KBr): ν (cm^{-1}) = 1734 (ester C=O), 1653 (C=O). Analysis (C,H,N): $C_{22}H_{20}N_2O_6$ (408.4), calcd C 64.70, H 4.94, N 6.86, found C 64.59, H 4.88, N 6.80. $[\alpha]_D^{25} = -2°$ (c = 0.1 g; DMSO). After activation of **14** (140 mg, 0.34 mmol) with CDI (56 mg, 0.34 mmol) in 5 mL of anhydrous THF, the solution of **2** (140 mg, 0.556 mmol) in 5 mL of dry THF was added and the mixture was stirred overnight. After evaporation in vacuo the residual solid was triturated with water for 4 h and chromatographed (Chromatotron; $CHCl_3$: MeOH = 90:10). Yield 81% **15** as yellow solid, mp 73–75 °C (MeOH). ESI-MS (MeOH + 1% HOAc): m/z (%) = 639 ($[M+H]^+$, 100); ^{1}H NMR (DMSO-d_6): δ (ppm) = 11.90 (s, 1H), 8.12 (d, J = 7.9 Hz, 1H), 7.88 (t, J = 5.5 Hz, 1H), 7.63 (d, J = 7.9 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.26 (2dd, J = 6.7/1.2 Hz, 1H), 7.16 (t, J = 7.9 Hz, 1H), 7.08 (2dd, J = 6.7/0.8 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.81–6.78 (m, 2H), 6.75 (dd, J = 7.9/2.4 Hz, 1H), 5.68 (q, J = 6.3 Hz, 1H), 4.17 (dd, J = 11.1/7.13 Hz, 1H), 4.00 (dd, J = 11.1/6.34 Hz, 1H), 3.91 (t, J = 6.3 Hz, 2H), 3.33 (s, 2H), 3.27–3.12 (m, 4H), 2.57 (s, 3H), 2.40–2.16 (m, 8H), 1.80 (m, J = 6.6 Hz, 2H), 1.52–1.28 (m, 6H). Analysis [$C_{37}H_{42}N_4O_6 \cdot H_2O$ (656.8)] calcd: C 67.66, H 6.75, N 8.53, found: C 67.75, H 6.69, N 8.43; $[\alpha]_D^{25} = -19°$ (c = 0.1, DMSO).

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