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Fluorescent non-imidazole histamine H_3 receptor ligands with nanomolar affinities

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Abstract—ω-Piperidinoalkanamine derivatives with fluorescent moieties (2-cyanoisoindol-1-yl, 7-nitrobenzofurazan-4-yl) have been synthesized starting from piperidine in three steps. The compounds display moderate to good histamine hH_3 receptor affinities with K_i values ranging from 178 to 11 nM. The new compounds may act as tools for identification and understanding of the binding site on the histamine H_3 receptor.

Within the four known histamine receptors, the H_3 subtype is predominantly expressed in the brain.[1] The histamine H_3 receptor has been shown to inhibit the histamine synthesis in and release from histaminergic neurons via a negative feedback loop, but also to modulate the release of other neurotransmitters.[2] Actually there is great effort in the development potential of highly potent and selective antagonists due to their discussed importance in the treatment of various diseases, e.g., schizophrenia, Alzheimer's disease, obesity and attention-deficit hyperactivity disorder (ADHD).[1]

The development of H_3 receptor antagonists opens a wide range of compounds differentiated into two main classes. Almost all potent antagonists published before 1999 were imidazole derivatives monosubstituted in the 4(5)-position (Fig. 1; **A**).[3] By exchange of the imidazole moiety to piperidine, pyrrolidine, piperazine and related structures an additional class of non-imidazole antagonists has been described (Fig. 1; **B**).[1,2,4]

Fluorescent compounds for G protein-coupled receptors may be useful research tools for non-radioactive binding assays or for investigations on the structural properties of these receptor–ligand interactions.[6] Such ligands may offer a multiplicity of information such as the mechanism of ligand binding,[7] localization, movement and internalisation of receptors in living cells.[8] They can give hints on the environment of receptor binding sites, because some fluorophores show excitation and emission wavelengths depending on the surrounding, lipophilicity, pH, temperature, solvent, etc.[9,10] A commercially available fluorescent histamine derivative (BODIPY® FL histamine; Molecular Probes) is able to show lysosomal localization,[11] but to our knowledge receptor binding properties have not been described. Based on the recent results on structure–activity relationships[1–4,12] we have designed and prepared some novel non-imidazole histamine H_3 receptor ligands which possess a fluorescent chromophore in the lipophilic part of a general blueprint for antagonist structures.[1] Very recently during the preparation of this project related

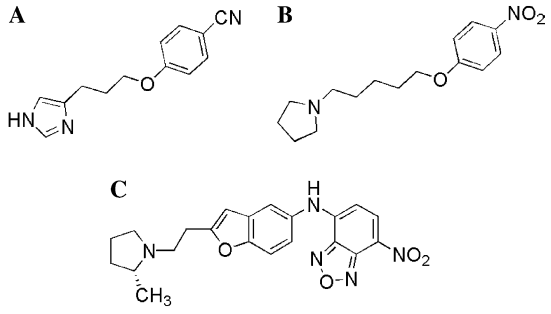

Figure 1. Different structurally related lead structures for histamine H_3 receptor antagonists.[3–5]

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fluorescent histamine H_3 receptor ligands of a benzofuran series have been presented (Fig. 1; **C**).[5]

The compounds were synthesized in three steps according to the scheme shown in Figure 2: ***1***. Alkylation of piperidine with ω-bromoalkannitrile (65 – 85% yields);[12] ***2***. Reduction of the nitrile group with Raney-nickel/hydrogen at room temperature (90–95% yields);[13] ***3***. Reaction of amine compound with phthaldialdehyde/NaCN[14] or 4-chloro-7-nitrobenzofurazan (4-chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole)[15] resulting in the final compounds (25–85% yields).

All excitation and emission data were measured with an Amico-Bowman Series 2 Spectrometer. The fluorescent compounds were measured at a concentration of 10^{-5} M in absolute spectroscopic ethanol at room temperature. Emission spectra were recorded at the excitation max. wavelength (Figs. 3 and 4).

Pharmacological data of histamine H_3 receptor affinities were obtained by [^{125}I]iodoproxyfan binding assay on CHO-K1 cells stably expressing the human H_3 receptor (Table 1). [16]

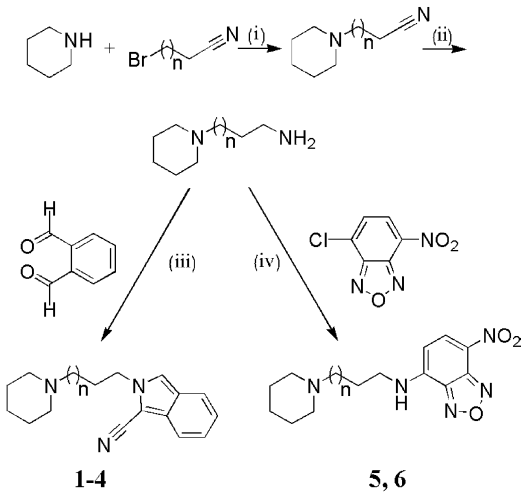

Figure 2. Synthesis of compounds **1**–**6**. (i) K_2CO_3, KI, abs acetonitrile, 18 h, rt; (ii) MeOH/ NH_3, Raney-nickel, H_2, 12 h, rt; (iii) NaCN, MeOH, $NaBH_4$, 18 h, rt; (iv) abs dioxane, 18 h, rt.

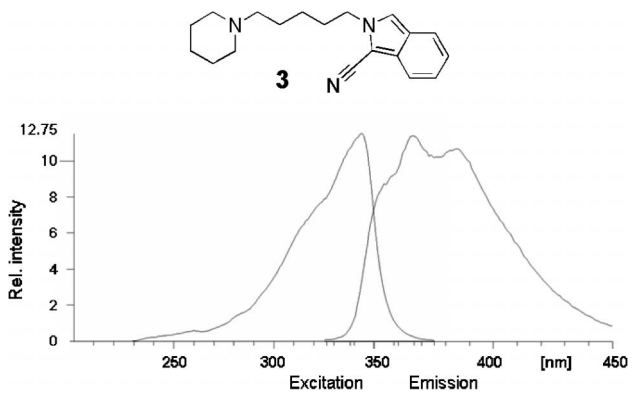

Figure 3. Excitation and emission spectra of the prominent pentamethylene derivative **3** of isoindole series.

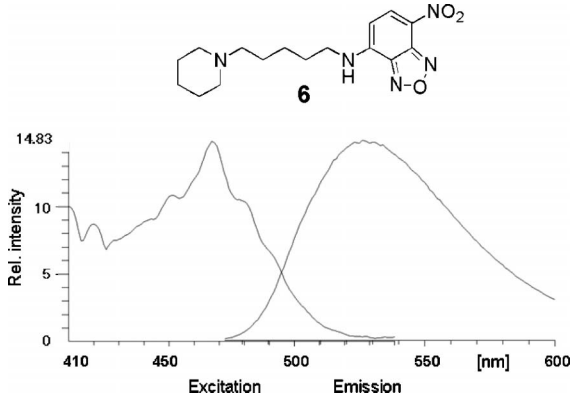

Figure 4. Excitation and emission spectra of the prominent pentamethylene derivative **6** of 4-nitrobenzofuran series.

Table 1. Binding affinities of histamine H_3 receptor ligands (cf. Fig. 2): isoindole (**1**–**4**), 4-nitrofurazan (**5**, **6**) and reference compounds[3–5]

Compound	*n*	$K_i \pm$ SEM[a] (nM)	*C*log *P*[b]	Excitation λ_{max} (nm)	Emission λ_{max} (nm)
1	1	351 ± 6	3.50	343	359
2	2	178 ± 41	3.70	350	366
3	3	31 ± 10	3.50	344	366
4	4	31 ± 5	4.75	342	357
5	2	170 ± 34	3.84	503	526
6	3	11 ± 1	4.37	467	526
A		12 ± 3[c]	1.89		
B		39[d]	3.82		
C		0.10[e]	5.76	465[e]	535[e]

[a] [^{125}I]Iodoproxyfan binding on CHO-K1 cells stably expressing the hH_3 receptor.[16]

[b] The *n*-octanol/water partition coefficient based on established chemical interactions; calculated with ChemDraw Ultra 7.0.[17]

[c,d] K_i values obtained in experiments of [^{3}H]histamine release from rat cerebral cortex synaptosomes see, respectively.[3,4]

[e] Value from Ref. 5.

All tested compounds could be easily obtained by the method described. They showed moderate to good affinities for histamine H_3 receptors in the nanomolar concentration range (Table 1).

In the series of fluorescent isoindole compounds **1**–**4** the highest binding affinities (e.g., 'lowest K_i values') were obtained with compounds having a chain length of five (**3**) to six (**4**) methylene groups as spacer. Therefore, in the 4-nitrobenzofurazan series the related spacer lengths from basic amine to aromatic residue have only been considered. The lipophilicities of all compounds are in acceptable calculated range of *C*log *P* values below 5[17]. All compounds showed an acceptable difference of excitation to emission maximal wavelengths (Stokes shift). Especially the most potent compound **6** showed the highest Stokes shift of 59 nm. The wavelengths for excitation vary from 342 to 503 nm, whereas those for emission vary from 377 to 526 nm.

It was demonstrated that it is possible to obtain fluorescent histamine H_3 receptor ligands by application of the general blueprint and knowledge on structure–activity relationships. The fluorescent compounds represent a

new series of potent compounds, which may be utilized for further in vivo or cell studies using modern imaging techniques and have the potential for useful pharmacological tools to investigate receptor–ligand interactions, for example, in fluorimetric binding assays or functional studies. The moderate to good affinities and maximal emission wavelengths open a wide area for further optimization on these fluorescent histamine H_3 receptor ligands.

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14. Compound **1**: 2-(3-Piperidin-1-ylpropyl)-2*H*-isoindol-1-carbonitrile was synthesized from (3-aminopropyl)piperidine with 1,2-phthaldialdehyde (10 mmol), NaCN (12 mmol) and sodium tetraborate (15 mmol) in methanol (20 mL). After stirring in the dark for over 18 h at room temperature, the reaction mixture was added to ice/water. Filtration, drying, and purification by column chromatography using CH_2Cl_2-MeOH, NH_3 saturated (95:5) resulted in the final product. Yield: 65%, mp 141 °C; ESI-MS $C_{17}H_{21}N_3$ calcd: 267.38, found: 267.9; ^{1}H NMR (DMSO): δ (ppm) 7.91 (s, 1H), 7.74 (d, J = 13.2 Hz, 1H), 7.65 (d, J = 7.5 Hz, 1H), 7.28 (dd, J = 7.0 Hz, 1H), 7.10 (dd, J = 7.8 Hz, 1H), 4.46 (t, J = 7.8 Hz, 2H), 3.34 (t, J = 4.9 Hz, 2H), 2.94–3.04 (4H), 2.29 (t, J = 7.1 Hz, 2H), 2.01 (dt, J = 7.2 Hz, 4H), 1.69 (t, J = 4.6 Hz, 2H). Elemental analysis, calcd: C, 59.36; H, 6.82; N, 10.93. Found: C, 59.27; H, 6.58; N, 10.90. Compound **2**: 2-(4-Piperidin-1-ylbutyl)-2*H*-isoindol-1-carbonitrile, yield: 91%, mp 139 °C; ESI-MS $C_{18}H_{23}N_3$ calcd: 281.40, found: 281.9; ^{1}H NMR (DMSO): δ (ppm) 7.69 (s, 1H), 7.66 (d, J = 10.6 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.09 (t, J = 6.9 Hz, 1H), 4.47 (t, J = 6.9 Hz, 2H), 3.47 (t, J = 6.9 Hz, 2H), 3.13 (t, J = 8.4 Hz, 2H), 2.81–2.92 (4H), 2.03 (t, J = 7.6 Hz, 2H), 1.76–1.84 (4H), 1.49–1.52 (2H). Elemental analysis, calcd: C, 62.40; H, 6.94; N, 10.92. Found: C, 62.75; H, 6.79; N, 10.54. Compound **3**: 2-(5-Piperidin-1-ylpentyl)-2*H*-isoindol-1-carbonitrile. Yield: 98%, mp 140 °C; ESI-MS $C_{19}H_{25}N_3$ calcd: 295.43, found, 295.8; ^{1}H NMR (DMSO): δ (ppm) 7.84 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.26 (t, J = 7.4 Hz, 1H), 7.08 (t, J = 6.1 Hz, 1H), 4.38 (t, J = 6.9 Hz, 2H), 2.95–3.01 (4H), 2.89 (t, J= 8.4 Hz, 4H), 1.68 (t, J = 7.4 Hz, 4H), 1.46–1.50 (4H), 1.27 (dd, J = 7.4 Hz, 2H). Elemental analysis, calcd: C, 63.71; H, 6.85; N, 10.41. Found: C, 63.81; H, 6.92; N, 10.05. Compound **4**: 2-(6-Piperidin-1-ylhexyl)-2*H*-isoindol-1-carbonitrile. Yield: 97%, mp 141 °C; ESI-MS $C_{20}H_{27}N_3$ calcd: 309.46, found: 310.0; ^{1}H NMR (DMSO): δ (ppm) 7.88 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.24 (t, J = 6.7 Hz, 1H), 7.08 (t, J = 8.1 Hz, 1H), 4.36 (t,J = 6.9 Hz, 2H), 2.90–3.03 (4H) 2.81 (t, J = 7.9 Hz, 4H), 1.88 (t, J = 6.8 Hz, 2H), 1.66 (t, J = 5.2 Hz, 4H), 1.56–1.60 (4H), 1.45–1.47 (4H), 1.26–1.28 (2H). Elemental analysis, calcd: C, 65.41; H, 7.36; N, 10.40. Found: C, 65.39; H, 7.33; N, 10.14.
15. Compound **5**: 7-Nitro-*N*-(4-(piperidin-1-yl)butyl)-benzo[*c*][1,2,5]oxadiazol-4-amine was synthesized from (4-aminobutyl)piperidine (20 mmol) and 4-chloro-7-nitro-benzo[*c*][1,2,5]oxadiazole (10 mmol) in abs. dioxane (20 mL) in the dark over 17 h at room temperature. Purification was performed after evaporation in vacuum by column chromatography using CH_2Cl_2-MeOH, NH_3 saturated (95:5). Yield: 65%, mp 157 °C; ESI-MS $C_{15}H_{21}N_5O_3$ calcd: 319.16, found; 319.8; ^{1}H NMR (DMSO): δ (ppm) 8.49 (d, J = 8.9 Hz, 1H), 6.42 (d, J = 9.0 Hz, 1H), 3.94 (s, 1H, NH), 3.43 (t, J = 6.9 Hz, 2H), 2.81–3.15 (6H), 1.65–1.85 (8H), 1.48–1.54 (2H). Elemental analysis, calcd: C, 48.38; H, 5.43; N, 15.94. Found: C, 48.55; H, 5.48; N, 15.63. Compound **6**: 7-Nitro-*N*-(5-(piperidin-1-yl)pentyl)benzo[*c*][1,2,5]oxadiazol-4-amine. Yield: 62%, mp 186 °C; ESI-MS $C_{16}H_{23}N_5O_3$ calcd: 333.39; found: 333.9; ^{1}H NMR (DMSO): δ (ppm) 8.50 (d, J = 8.9 Hz, 1H), 6.42 (d, J = 9.0 Hz, 1H), 3.96 (s, 1H, NH), 3.42 (t, J = 7.0 Hz, 2H), 3.02–3.08 (2H), 1.69–1.75 (6H), 1.45–1.55 (8H), 1.27–1.29 (2H). Elemental analysis, calcd: C, 49.15; H, 5.66; N, 15.24. Found: C, 49.26; H, 5.80; N, 15.00.
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