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Synthesis and Pharmacological Activity of Fluorescent Histamine H_1 Receptor Antagonists Related to Mepyramine

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Abstract—Fluorescently labeled histamine H_1 receptor antagonists were synthesized starting from N-demethylmepyramine by introduction of ω-aminoalkyl chains (2–8 methylene groups in length) followed by derivatization of the terminal NH_2 group with various fluorophores (fluorescein, naphthofluorescein, rhodamine, tetramethylrhodamine, BODIPY, dansyl, and nitrobenzoxadiazole (NBD)). On the isolated guinea pig ileum and in a Ca^{2+} assay on U373MG human glioblastoma cells the highest H_1 antagonistic activities were found in 5- and 6-carboxyfluorescein labeled compounds with hexa- and octamethylene spacers and in an analogous NBD-aminohexanoyl derivative (pA_2 or pK_B values in the range: 8.3–9.0; compared to 9.3–9.4 for mepyramine).
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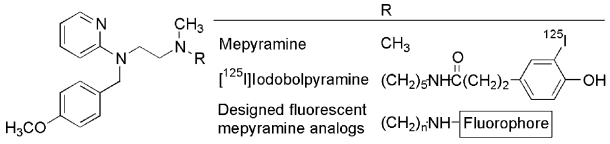
Fluorescent probes offer attractive alternatives to the application of radioligands, for example to detect receptors in cells and tissues, and to study drug receptor interactions on soluble or cell-bound targets.[1,2] Recently, we have demonstrated by using cyanine 5-labeled neuropeptide Y, that the affinity of agonists and antagonists acting at G-protein coupled receptors (GPCRs) can be determined under equilibrium conditions by flow cytometry.[3,4] Generally, this method cannot be easily transferred to small molecules as ligands of GPCRs since the labeling of such compounds, for example biogenic amines and their antagonists, with bulky fluorescent dyes usually leads to a much more pronounced decrease in activity than in the case of peptide ligands. Thus, suitable fluorescent probes are required to further develop the aforementioned fluorescence-based methods for the investigation of GPCRs. In continuation of our studies we selected histamine H_1 receptors[5] as a model.

The design of the fluorescent H_1 antagonists followed an approach that has been successfully applied for the development of the high affinity radioligand [^{125}I]iodobolpyramine,[6,7] a Bolton–Hunter-modified analogue of the H_1 receptor antagonist mepyramine (Scheme 1).[5] To obtain fluorescent analogues, *N*-demethylmepyramine ((**3**), *N*-(4-methoxybenzyl)-*N*′-methyl-*N*-(2-pyridyl)-1,2-ethanediamine, normepyramine) was selected as the active moiety to be conjugated with various fluorophores (**10A–I**) by different functional groups and through spacers of various lengths (Schemes 2 and 3).



Scheme 1.

Chemistry

2-(4-Methoxybenzylamino)pyridine **1** was treated with bromoacetyl bromide and then with methylamine to obtain amide **2** which was reduced with lithium–aluminium hydride to give the central building block **3** (Scheme 2). The phthalimidoalkanoic acids **4a–d**, prepared from phthalic anhydride and ω-aminoalkanoic acids, were activated with carbonyldiimidazole (CDI) at room temperature in anhydrous THF and allowed to react with **3** to give the amides **5a–d**. Deprotection with hydrazine hydrate gave the aminoalkanamides **6a–d**, which were treated with $LiAlH_4$ to yield **7a–d** (Scheme 2). The compounds **7a**,**b** were also obtained from **3** by alkylation with the ω-bromonitriles **8a**,**b** in toluene at 100 °C, followed by reduction of the nitriles **9a**,**b**.

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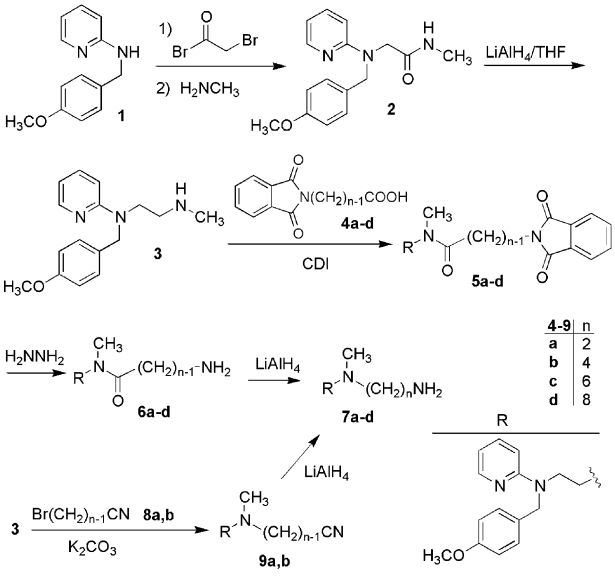
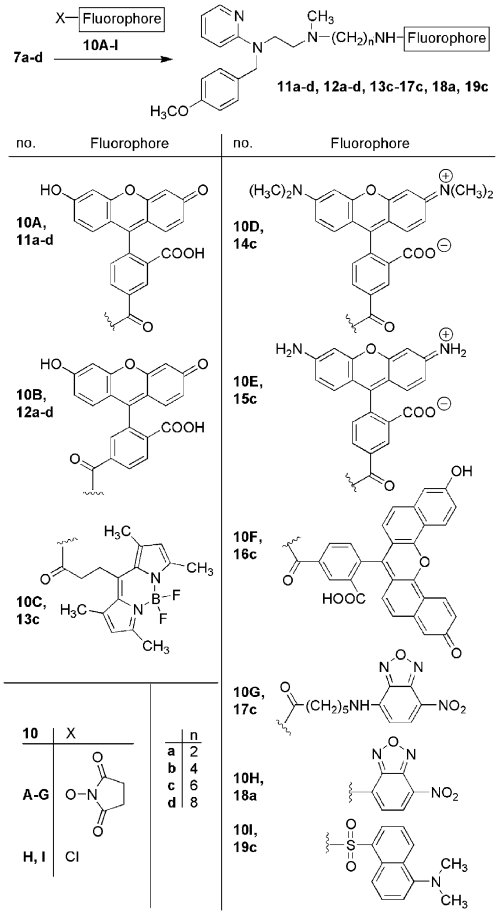
The coupling reactions of **7a–d** (Scheme 3) with carboxyfluorescein were carried out with a commercially available mixture of the 5- and 6-isomers **10A**,**B** (succinimidyl ester) in anhydrous dichloromethane/DMSO in the dark.[8] The separation of the derivatives **11a–d** and **12a–d** was performed by HPLC on a semi-preparative scale. Analogously, **7c** was labeled with the succinimidyl esters **10A–G** or chloronitrobenzoxadiazole (**10H**), and **7a** was derivatized with dansyl chloride (**10I**) to give the corresponding fluorescent ligands **13c–17c**, **18a** and **19c**, respectively (Scheme 3).[9]



Scheme 2.



Scheme 3.

Pharmacology

The fluorescent compounds were investigated for histamine H_1-receptor antagonistic activity at the isolated guinea pig ileum according to known experimental protocols.[10] Additionally, the histamine H_1 receptor antagonism was determined on Fura-2-AM loaded U373MG human glioblastoma cells with a fluorimetric calcium assay[11] (Fura assay) following the general procedure described elsewhere.[12,13] The results are summarized in Table 1.

Results and Discussion

Small changes in the typical H_1 antihistamine structure of mepyramine may lead to compounds with considerably reduced H_1 antagonist activity on the guinea

Table 1. Histamine H_1 receptor antagonistic activities determined on the isolated guinea pig ileum and on U373MG human glioblastoma cells

Compd	λ_{max} Ex (nm)[a]	λ_{max} Em (nm)[a]	Guinea pig ileum[b] $pA_2 \pm$SEM	U373MG cells (Ca^{2+} assay)[c] $pK_B \pm$SEM
Mepyramine			9.42±0.15[d]	9.30±0.20
3			8.18±0.07	8.12±0.04
7a			7.88±0.19	7.84±0.09
7b			7.65±0.19	7.61±0.07
7c			7.89±0.09	8.03±0.07
7d			8.01±0.35	8.06±0.15
11a	493	522	7.16±0.13	7.21±0.08
11b	493	521	8.35±0.29	8.47±0.11
11c	493	523	7.67±0.02	8.17±0.15
11d	496	524	8.47±0.10	8.94±0.16
12a	494	516	6.84±0.05	6.93±0.05
12b	494	521	7.50±0.06	7.81±0.12
12c	500	520	7.58±0.04	8.40±0.19
12d	499	520	8.30±0.08	8.53±0.17
13c	501	512	7.83±0.17	7.85±0.06
14c	555	576	7.48±0.06	7.54±0.02
15c	501	527	7.58±0.13	7.68±0.28
16c	nd	nd	7.44±1.33	7.18±0.13
17c	483	536	8.71±0.26	9.05±0.20
18a	486	540	8.33±0.24	8.12±0.09
19c	321	536	7.75±0.15	7.76±0.35

[a]Longest wavelength absorption and fluorescence emission maximum determined with a Kontron UV–visspectrometer and a Perkin–Elmer LS-50B spectrofluorimeter, using solutions of the compounds 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), which was also used for the Ca^{2+} assay; nd: not determined.

[b]pA_2 values: calculated from the rightward shift of isotonically recorded cumulative concentration-response curves (n: 6–12) for histamine in the absence and presence of the putative antagonist (15 min incubation at 30 °C).

[c]Fura-2-loaded U373MG cells[11,12] were incubated with varying concentrations of the antagonists (n=2–4) for 1 min prior to stimulation with 30 μM histamine (producing submaximal increase in $[Ca^{2+}]_i$). pK_B values: calculated from IC_{50} values with the Cheng–Prusoff equation.[14]

[d]Ref. 7: pA_2=8.89.

pig ileum.[7] The removal of a methyl group from the tertiary amino group in mepyramine, as well as the replacement of the methyl with an aminoalkyl group, results in a pronounced decrease in activity (**3**, **7a–d**). However, compared to the non-labeled building blocks **7a–d**, some of the fluorescent probes are more potent H_1 blockers, for example, depending on the chain length of the spacer, suitable fluorophores may confer additional H_1 receptor affinity. Comparing the activities of 5- and 6-carboxyfluorescein-labeled H_1 antagonists **11a–d** and **12a–d** on the ileum there is a spacer-dependent increase with highest activity residing in the compounds with more than 2 methylene groups. Though the pA_2 or pK_B values of mepyramine were not achieved, a number of the fluorescent probes listed in Table 1 were found to have H_1 antagonistic activities in the range of commercially available drugs such as pheniramine or diphenhydramine (pA_2 values in the range of 8.1–9.0). The derivatives **13–16c** and **19c**, which have the same chain length but are labeled with different dyes, were found to be less active than mepyramine by 1 to 2 orders of magnitude. Compound **18a**, which contains only a short chain (CH_2CH_2) connecting the affinity-conferring partial structure and the NBD dye, was found to be about half as potent as **17c**, which contains a long spacer group ($(CH)_6NHCO(CH_2)_5$).

The H_1 antagonistic activities found on the guinea pig ileum are in rather good agreement with the results from U373MG human glioblastoma cells for the non-labeled compounds and for most of the fluorescent ligands. There are only a few exceptions among the investigated compounds showing discrepancies between the two pharmacological test models >0.6 log units, which may be associated with the distinct incubation periods (15 min (ileum) vs 1 min (U373MG)) or the different accessibility of the H_1 receptor (diffusion and/or enrichment of the compounds in the tissue and the cells). Fortunately, the Fura assays (ratiometric method, λ_{ex}: 340 and 380 nm, λ_{em}: 510 nm) was not impaired by the fluorescent H_1 antagonist, which have excitation maxima at longer wavelengths. This finding is of particular importance with respect to the development of multiparametric cellular assays for the determination of receptor binding and functional data.

Bulky fluorophores are tolerated when linked to the affinity-conferring partial structure by an appropriate spacer group. It is conceivable that the various bulky dyes are not interacting with amino acids of the transmembrane H_1 receptor domains but are extending into the extracellular space. The contribution of the fluorophore to the receptor affinity could result from its interaction with extracellular loop regions.

Conclusions

The results summarized in Table 1 show that rather potent fluorescence-labeled H_1 antagonists are obtained by connecting normepyramine and fluorescein or NBD via spacer groups of appropriate length. In principle, high affinity fluorescent ligands of biogenic amine receptors may be obtained by this approach, however, predictions of the best suited fluorophore are difficult. The fluorescent properties of the H_1 antagonists are not detrimental to the application of the Fura assay. This gives further support to the idea that it may be possible to combine fluorescence-based methods for (a) functional studies (e.g., Ca^{2+} assay) and (b) the determination of binding constants of GPCR ligands. The combination of both may be potentially useful in high-throughput screening to get information on affinities and agonistic/antagonistic properties of potential drug candidates in one assay. Moreover, fluorescent probes such as the fluorescein-labeled mepyramine analogues may be useful to study the GPCRs in tissue preparations and on cells.

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References and Notes

1. McGrath, J. C.; Arribas, S.; Daly, C. J. *Trends Pharmacol. Sci.* **1996**, *17*, 393.
2. Mellentin-Michelotti, J.; Evangelista, L. T.; Swartzman, E. E.; Miraglia, S. J.; Werner, W. E.; Yuan, P.-M. *Anal. Biochem.* **1999**, *272*, 182.
3. Mayer, M.; Li, L.; Kracht, J.; Bernhardt, G.; Buschauer, A. *Arch. Pharm.-Pharm. Med. Chem.* **2000**, *333* (Suppl. 2), 51.
4. Mayer, M. PhD Thesis, University of Regensburg, 2002.
5. Hill, S. J.; Ganellin, C. R.; Timmerman, H.; Schwartz, J. C.; Shankley, N. P.; Young, J. M.; Schunack, W.; Levi, R.; Haas, H. L. *Pharmacol. Rev.* **1997**, *49*, 253.
6. Körner, M.; Bouthenet, M. L.; Ganellin, C. R.; Garbarg, M.; Gros, C.; Ife, R. J.; Sales, N.; Schwartz, J. C. *Eur. J. Pharmacol.* **1986**, *120*, 151.
7. Blakemore, R. C.; Ganellin, C. R.; Garbarg, M.; Gros, C.; Ife, R. J.; Körner, M.; Ruat, M.; Schartz, J.-C.; Tertiuk, W.; Theobald, C. J. *Eur. J. Med. Chem.* **1987**, *22*, 91.
8. General procedure for preparation of the *N*′-[ω-(fluorescein - 5(and - 6) - carbonyl)aminoalkyl] - *N* - (4 - methoxybenzyl)-*N*′-methyl-*N*-(pyridin-2-yl)-1,2-ethanediamines **11a–d** and **12a–d**. To the solution of **7a–d** (0.02 mmol) in 2 mL anhydrous dichloromethane, the solution of 10.0 mg (0.021 mmol) of 5- and 6-carboxyfluorescein succinimidyl ester (commercially available mixture of **10A/10B** with **10A** as the main isomer) in 600 μL of anhydrous DMSO was added. The mixture was stirred at room temperature in the dark for 24 h. After concentration in vacuo the residue was chromatographed analytically (column (250×4 mm, 5 μm) and precolumn: Eurospher 100-C18 (Knauer); Kontron Instruments: HPLC Pump 420; UV-detector: HPLC Detector 430; Fluorescence detector: Merck-Hitachi F1000) eluent: MeOH/0.1% TFA (50/50), flow rate: 1 mL/min; UV detection: 254 nm, fluorescence detection: λ_{ex}: 490 nm, λ_{em}: 520 nm). Then portions of the crude mixture of isomers (sufficient for pharmacological investigations) were purified with HPLC on a semipreparative scale (column (500×16 mm, 7 μm): Nucleosil-100-C18 (Macherey & Nagel), ALTEX Model 110A pump, Merck-Hitachi UV Monitor 655A-22; eluent: MeOH/0.1% TFA, 50:50 (**11a**,**c**, **12a**,**c**), 55:45 (**11b**, **12b**), 60:40 (**11d**, **12d**), flow rate 6 mL/min; UV detection: 254

nm; retention times (min): **11a**: 106/**12a**: 71/**11b**: 72/**12b**: 59, **11c**: 41/**12c**: 37, **11d**: 41/**12d**: 36). Yields: **11a**/**12a** 26%, **11b**/**12b**: 30%, **11c**/**12c**: 47%, **11d**/**12d**: 37%; 5- to 6-isomer ratio ca. 2:1. The obtained solution was concentrated in vacuo (<40 °C) and lyophilized to obtain the 5-isomers **11a–d** and 6-isomers **12a–d** as yellow powder.

11a: $^{+}$FAB-MS (Varian MAT 95, $^{+}$FAB-MS, xenon, MeOH/glycerol): *m/z* (%) = 673 ([M + H]$^{+}$, 10) 241 (20), 121 (100); $^{+}$FAB–HRMS: $C_{39}H_{37}N_4O_7$; calcd: 673.2662 found: 673.2668; **11b**: $^{+}$FAB-MS: *m/z* (%) = 701 ([M + H]$^{+}$, 10), 241 (24), 121 (100); $^{+}$FAB-HRMS: $C_{41}H_{41}N_4O_7$, calcd: 701.2975, found: 701.2960; **11c**: $^{+}$FAB-MS: *m/z* (%) = 729 ([M + H]$^{+}$, 100); ^{1}H NMR (250 MHz; CD_3OD): δ (ppm) = 8.43 (d, *J* = 1.6 Hz, 1H), 8.19 (dd, *J* = 7.9 and 1.6 Hz, 1H), 8.13 (dd, *J* = 5.2 and 1.9 Hz, 1H), 7.68 (dd, *J* = 7.1 and 1.8 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 5.5 Hz, 1H), 6.75 (s, 2H), 6.64 (d, *J* = 8.7 Hz, 2H), 6.57 (dd, *J* = 8.7 and 2.4 Hz, 2H), 4.70 (s, 2H), 3.97 (t, *J* = 5.9 Hz, 2H), 3.75 (s, 3H), 3.43 (t, *J* = 6.9 Hz, 2H), 3.20 (t, *J* = 7.9 Hz, 2H), 2.95 (s, 3H), 1.82–1.60 (m, 4H), 1.53–1.36 (s, 4H); **11d**: $^{+}$FAB-MS: *m/z* (%) = 757 ([M + H]$^{+}$, 7), 241 (39), 121 (100); $^{+}$FAB-HRMS: $C_{45}H_{49}N_4O_7$: calcd: 757.3601, found: 757.3580; **12a**: $^{+}$FAB-MS: *m/z* (%) = 673 ([M + H]$^{+}$, 10), 241 (17), 121 (100). **12b**: $^{+}$FAB-MS: *m/z* (%) = 701 ([M + H]$^{+}$, 11), 241 (26), 121 (100). **12c**: $^{+}$FAB-MS: *m/z* (%) = 729 ([M + H]$^{+}$, 66), 241 (100); $^{+}$FAB-HRMS: $C_{43}H_{45}N_4O_7$: calcd: 729.3288, found: 729.3259. **12d**: FAB-MS: *m/z* (%) = 757 ([M + H]$^{+}$, 7), 241 (28), 121 (100).

9. The compounds **13–17c** and **19c** were obtained by analogy with the procedure described for **11a–d**, **12a–d**[8]: **7c** was labeled with either BODIPY 493/503 SE (**10C**), 5(6)-TAMRA SE (**10D**), Rhodamine Green™ carboxylic acid succinimidyl ester (5(6)-CR110, SE, (**10E**)), 5(6)-carboxynaphthofluorescein SE (**10F**) (**10D–F**: commercially available mixtures of the 5- and 6-isomer; **14c–16c** (the main isomers) were obtained after chromatographic separation by analogy with the method described for **11a–d**), NBD-x SE (**10G**), or dansyl chloride (**10I**). **18c** was obtained by treating a solution of **7c** (20 mg, 0.06 mmol) and 100 μL of triethylamine in 2 mL of abs. chloroform with NBD-Cl (**10H**; 12 mg, 0.06 mmol). The mixture was stirred at room temperature in the dark for 2 h. After concentration of the solution **18c** was purified chromatographically (Chromatotron 8294; (Harrison Research), rotors with 4 mm layers of silica gel PF_{254}, containing gypsum (Merck), eluent $CHCl_3$: MeOH, 3:1).

13c: yield: 72% $^{+}$FAB-MS: *m/z* (%) = 673 ([(M + H]$^{+}$, 100); $^{+}$FAB-HRMS: $C_{38}H_{52}BF_2N_6O_2$, calcd: 673.4249, found: 673.4245. **14c**: yield: 42%; $^{+}$FAB-MS: *m/z* (%) = 783 ([(M + H]$^{+}$, 100); $^{+}$FAB-HRMS: $C_{47}H_{55}N_6O_5$, calcd: 783.4234, found: 783.4202. **15c**: yield: 35%; FAB-MS: *m/z* (%) = 727 ([(M + H]$^{+}$, 100); $^{+}$FAB-HRMS: $C_{43}H_{47}N_6O_5$, calcd: 727.3608, found: 727.3607. **16c**: FAB-MS: *m/z* (%) = 829 ([(M + H]$^{+}$, 100); $^{+}$FAB-HRMS: $C_{51}H_{49}N_4O_7$: calcd: 829.3601, found: 829.3623. **17c**: yield: 31%; FAB-MS: *m/z* (%) = 647 ([(M + H]$^{+}$, 54), 241 (58), 121 (100); $^{+}$FAB-HRMS: $C_{34}H_{47}N_8O_5$, calcd: 647.3669, found: 647.3685. **18c**: yield: 23%; FAB-MS: *m/z* (%) = 478 ([M + H]$^{+}$, 1), 241 (32), 121 (100); ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.43 (d, *J* = 8.7 Hz, 1H), 8.11 (dd, *J* = 5.2 and 2.0 Hz, 1H), 7.41 (dd, *J* = 7.1 and 2 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 6.65–6.54 (m, 2H), 7.12 (m, 1H), 4.61 (s, 2H), 4.40–3.60 (m, 11H), 3.37 (s, 3H). **19c**: yield: 69%; $^{+}$FAB-MS: *m/z* (%) = 604 ([(M + H]$^{+}$, 100); $^{+}$FAB-HRMS: $C_{34}H_{46}N_5O_3S$: calcd: 604.3312; found: 604.3320.

10. Buschauer, A.; Friese-Kimmel, A.; Baumann, G.; Schunack, W. *Eur. J. Med. Chem.* **1992**, *27*, 321.
11. Kracht, J.; Meister, A.; Bernhardt, G.; Buschauer, A. *Arch. Pharm.-Pharm. Med. Chem.* **2000**, *333* (Suppl 2), 48.
12. Kracht, J. PhD Thesis, University of Regensburg, 2001.
13. Müller, M.; Knieps, S.; Gessele, K.; Dove, S.; Bernhardt, G.; Buschauer, A. *Arch. Pharm. Pharm. Med. Chem.* **1997**, *330*, 333.
14. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.