N-[ω-[4-(2-Methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamines as High-Affinity Fluorescent 5-HT1A Receptor Ligands

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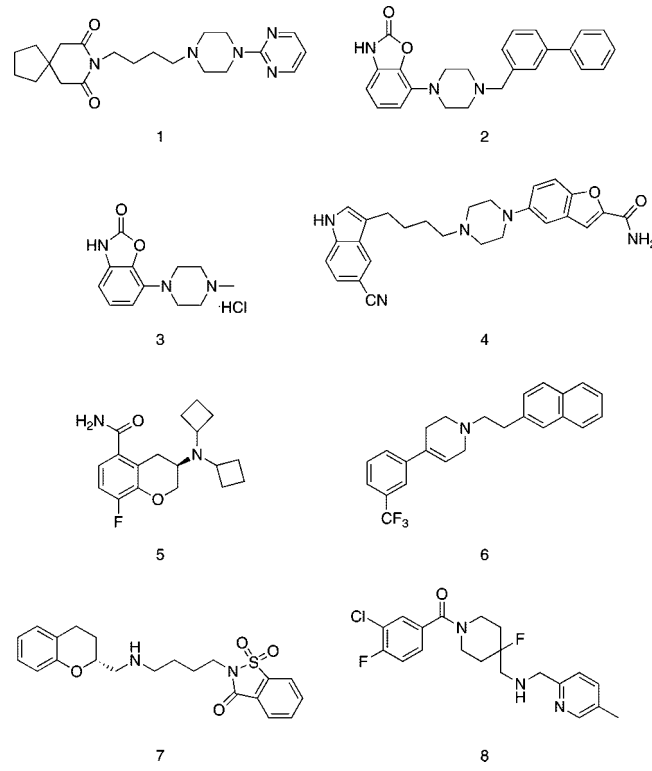
We here report on the design, synthesis, binding affinities, and fluorescent properties of a series of serotonin 5-HT_{1A} receptor ligands, with *N*-[ω-[4-(2-methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamine structure. Several of the new ligands displayed nanomolar affinity at 5-HT_{1A} receptor and good fluorescent properties. In particular, derivative **24** showed a favorable combination of 5-HT_{1A} receptor affinity (K_i = 0.4 nM), Stokes shift (excitation wavelength = 381 nm, emission wavelength = 455 nm), and quantum yield in ethanol (Φ = 0.45).

Introduction

Fluorescence spectroscopy is becoming a valuable addition to the array of techniques available for real time direct visualization of fluorescent ligand–receptor interactions in biological systems. For instance, scanning confocal microscopy,[1–3] fluorescence correlation spectroscopy,[4,5] and two-photon fluorescence laser scanning microscopy[6,7] allow the noninvasive imaging and quantification of these interactions in single living cells under a wide variety of experimental conditions. Moreover, fluorescence polarization techniques have been used in analytical and clinical chemistry as versatile screening alternatives with greatly reduced environmental and cost impacts compared with radioligand assays.[8]

The serotonin-1A (5-HT_{1A}) receptor is an important member of the large family of serotonin receptors, and it is perhaps the most extensively studied for a number of reasons. One of the main reasons is the availability of the selective agonist 8-OH-DPAT[a] that has allowed extensive biochemical, physiological, and pharmacological characterization of the receptor. The 5-HT_{1A} receptor was the first among all the serotonin receptors to be cloned and sequenced. The human, rat, and mouse 5-HT_{1A} receptors have been cloned and their amino acid sequences deduced. More importantly, the receptor has been stably expressed in a number of neural and non-neural cell lines. Furthermore, it was the first serotonin receptor for which polyclonal antibodies were obtained, allowing their visualization at the subcellular level in various regions of the brain.[9] Starting in the mid-1980s, the 5-HT_{1A} receptor has been the object of intense study which culminated in the introduction into the market of the 5-HT_{1A} receptor partial agonist buspirone (**1**) as an anxiolytic (Chart 1).[10] Other 5-HT_{1A} agents have demonstrated a role for the 5-HT_{1A} receptor in various pathologies. For instance, the mixed 5-HT_{1A}/D_2 agents bifeprunox (**2**)[11] and SLV308 (**3**)[12] have been studied for the treatment of schizophrenia and Parkinson's disease, respectively; vilazodone (**4**),[13] a 5-HT_{1A} partial agonist/serotonin reuptake inhibitor, has been proposed as a fast-acting antidepressant, as well as the 5-HT_{1A} antagonist robalzotan (**5**);[14] xaliproden (**6**)[15] and repinotan (**7**)[16] have been investigated as neuroprotective agents. Very recently,

Chart 1. Structures of 5-HT_{1A} Receptor Agents



the 5-HT_{1A} receptor agonist F 13640 (**8**) underwent phase II clinical trials for the treatment of severe, chronic pain.[17] Therefore, after two decades of research, the 5-HT_{1A} receptor is still under active investigation.

Several structurally different compounds are known to bind 5-HT_{1A} receptor.[18] Among these, a large number of N1-substituted N4-arylpiperazines (the so-called "long-chain" arylpiperazines) have been extensively studied. The general formula of these compounds presents an 1-arylpiperazine linked through an alkyl chain of variable length to a terminal fragment, belonging to one of the following four structural classes: (i) imides, (ii) amides, (iii) alkyl, arylalkyl, or heteroarylalkyl derivatives, and (iv) tetralins.[19] During the course of our studies we have published several papers dealing with the synthesis and structure-affinity relationships elucidation of "long-chain" arylpiperazines as 5-HT_{1A} receptor ligands.[20,21]

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[a] Abbreviations: 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropylaminotetraline; GPCR, G-protein-coupled receptor; BODIPY, dipyrromethene boron difluoride.

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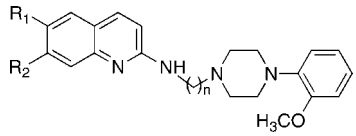
Table 1. Binding Affinities of Ligands **9**–**22**, and **24** for Serotonin 5-HT_{1A} Receptor and Their Fluorescence Properties in Ethanol



compd	R_1	R_2	n	K_i, nM ± SEM[a]	excitation λ_{max} (nm)	emission λ_{max} (nm)	Φ
9	NO_2	H	2	14.2 ± 2.7[b]	271	450	0.09
10	H	H	2	33 ± 4	343	406	0.17
11	H	H	3	13 ± 1	342	405	0.30
12	H	H	4	9.7 ± 0.3	341	390	0.46
13	H	H	5	0.030 ± 0.008	347	392	0.35
14	OCH_3	H	2	28 ± 2	362	405	0.52
15	OCH_3	H	3	22.0 ± 0.8	360	408	0.52
16	OCH_3	H	4	0.20 ± 0.05	359	405	0.54
17	OCH_3	H	5	2.6 ± 0.8	360	410	0.53
18	H	OCH_3	2	2.1 ± 0.4	361	381	0.14
19	H	OCH_3	3	0.20 ± 0.06	358	380	0.47
20	H	OCH_3	4	4.6 ± 0.2	365	382	0.49
21	H	OCH_3	5	0.50 ± 0.05	362	380	0.45
22	CH_3	H	4	0.54 ± 0.01	346	400	0.20
24	NH_2	H	4	0.38 ± 0.02	381	455	0.45
8-OH-DPAT				5.1 ± 0.5			
2-aminopyridine					290	353	0.37

[a] The values are the mean ± SEM from three independent experiments in triplicate. [b] Data taken from ref 28.

Recently, we embarked on a research program to develop high affinity fluorescent ligands for certain GPCR,[22] including the 5-HT_{1A} receptor.

Fluorescent probes have been prepared by labeling a ligand with a fluorophore, such as fluorescein,[3] BODIPY,[23] coumarin,[24] and dansyl,[25] into an area of the structure that would have minimal influence on biological activity. However, this structural modification has been reported to cause variations of lipophilicity, affinity, selectivity, and intrinsic activity.[26,27]

For our purpose, we have envisaged a different strategy by including the fluorescent core into a framework endowed with affinity for the target receptor. In this way, it is possible overcome the above-mentioned shortcomings and allow access to an entire series of fluorescent ligands with the possibility to optimize the fluorescence properties and receptor affinity at the same time.[22]

We have focused our attention on *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-6-nitro-2-quinolinamine (**9**, Table 1) that we reported as mixed serotonin transporter/5-HT_{1A} receptor ligand (5-HT_{1A} K_i = 14.2 nM).[28] In particular, we wondered if **9** was fluorescent because the 2-quinolinamine moiety closely resembles that of 2-aminopyridine, which is one of the fluorescent standards used for quantum yield determination (Table 1). Actually, **9** demonstrated to be fluorescent (Table 1), and therefore, it represented our starting point to develop fluorescent 5-HT_{1A} receptor ligands. Subsequent structural modifications of **9** were performed with the twofold aim to increase both 5-HT_{1A} receptor affinity and fluorescent emission.

Chemistry

The target compounds were prepared according to Scheme 1. Final compounds **10**–**22** were prepared by condensing 2-chloroquinolines **25a**−**d** with the appropriate amine **26a**−**d**. Target compound **24** was obtained as follows: 2-chloro-6-nitroquinoline (**25e**) reacted with amine **26d** to give nitro derivative **23**, which was hydrogenated to afford **24**. The starting materials were purchased from commercial sources or prepared according to literature, as detailed in the Experimental Section.

Results and Discussion

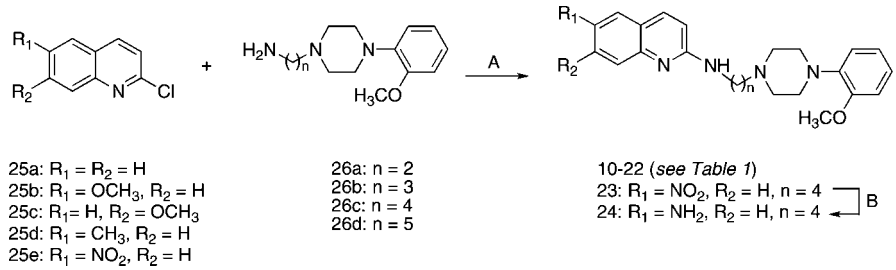
The affinity values for serotonin 5-HT_{1A} receptor and fluorescent properties of the target compounds are displayed in Table 1. Derivative **9** possessed good binding affinity for 5-HT_{1A} receptor and quite low fluorescent emission. Therefore, we performed structural modifications with the aim to improve both properties. It is well documented that the fluorescence emission of aromatic substances containing a nitro group is generally weak, primarily as a result of large non radiative decay from the excited state.[29] On the other hand, it has been reported that an electron-donating group such as methoxy (having inductive $+I$ and resonance $+M$ effects) can increase fluorescence emission.[30] On the basis of such considerations, the unsubstituted quinoline **10** was prepared as well as the 6-methoxy- and 7-methoxyquinolines **14** and **18**, respectively. These latter compounds would allow us to evaluate the effect of conjugation on fluorescence emission. As far as the affinity for 5-HT_{1A} receptor was concerned, we tested derivatives **10**, **14**, and **18** and their homologues **11**–**13**, **15**–**17**, and**19**–**21** bearing an alkyl chain from three to five methylene units because optimization of the intermediate alkyl chain length is a key step when studying "long-chain" arylpiperazines. Considering the fluorescent properties of **10**, it can be observed that removal of nitro group from **9** resulted in an enhancement of fluorescent emission. The nitro group replacement in **9** by the methoxy substituent (compound **14**) resulted in a considerable increase of Φ value (0.09 vs 0.52). Shifting of the methoxy from 6- to 7-position (compounds **14** and **18**) diminished fluorescence emission (Φ = 0.52 and 0.14, respectively). Elongation of the alkyl chain length of **10**, **14**, and **18** increased fluorescence emission, which was more evident for the unsubstituted quinolines **11**–**13** and for the 7-methoxyquinolines **19**–**21**. These results confirmed that introduction of a methoxy group on the aromatic ring can increase fluorescence, especially when conjugation of π-system was extended. Moreover, compounds with a four methylene chain (**12**, **16**, and **20**) showed higher quantum yields than their respective homologues. Finally, compounds **10**–**21** showed high difference of excitation to emission maximal wavelengths (Stokes shift).

As far as the 5-HT_{1A} receptor affinity is concerned, the analogues **10** and **14** displayed similar affinity as **9**, whereas **18** was slightly more potent than the nitro derivative **9**. Elongation of the alkyl chain of **10**, **14**, and **18** had a beneficial effect on 5-HT_{1A} receptor affinity. In fact, compounds **13** (n = 5), **16** (n = 4), **19** (n = 3), and **21** (n = 5) displayed affinities in the subnanomolar range. However, a general trend did not emerge because maximum 5-HT_{1A} receptor affinity was shown by compounds with different linker length.

Taken together, 5-HT_{1A} receptor affinity data and fluorescence properties indicated that the pursued optimization strategy was successful, especially for derivative **16** which showed the highest Φ value within this series and very high 5-HT_{1A} receptor affinity (K_i = 0.20 nM).

Finally, we wanted to explore if other electron donating substituents in 6-position (i.e., methyl or amino) would have beneficial effect on fluorescence. Methyl derivative **22** showed worst fluorescence properties than **16** because of lower excitation and emission wavelengths and lower quantum yield, whereas the amino derivative **24** (Figure 1) retained acceptable quantum yield and displayed excitation and emission maximum at higher wavelengths than **16**. Moreover, compounds **22** and **24** demonstrated to be as potent as **16** at 5-HT_{1A} receptor.

Scheme 1[a]



[a] Reagents: (A) heated at 150 °C; (B) H_2, Pd/C 10%.

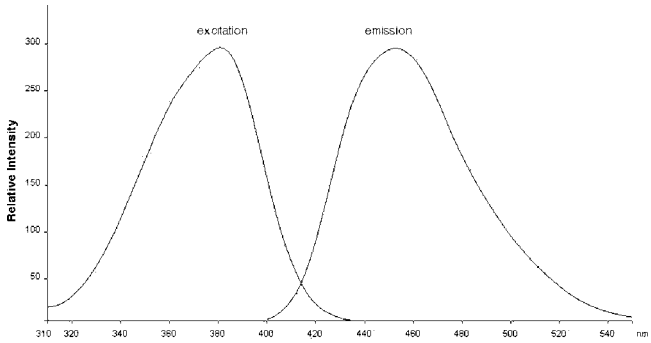


Figure 1. Excitation and emission spectra of compound **24** in ethanol.

Next, we studied the intrinsic activity of compounds **16** and **24** because fluorescent agonists or antagonists provide different information on receptor trafficking. The intrinsic activity at 5-HT_{1A} receptor of **16** and **24** was assessed in an isolated guinea-pig ileum assay.[31] Both compounds displayed agonistic properties at 5-HT_{1A} receptor. In fact, the derivative **16** acted as a full agonist ($EC_{50} = 34.4 \pm 7.0$ μM), and its activity was reverted by the 5-HT_{1A} receptor antagonist WAY-100635 in a dose-dependent manner ($pA_2 = 7.94 \pm 0.79$), whereas **24** behaved as a partial agonist (81% of the maximal response, $EC_{50} = 62.1 \pm 3.5$ μM).

Conclusions

We have identified a series fluorescent ligands for 5-HT_{1A} receptors from the structural modifications of the weakly fluorescent 5-HT_{1A} ligand *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-6-nitro-2-quinolinamine (**9**). Several of the newly prepared ligands displayed nanomolar affinity at 5-HT_{1A} receptor and fluorescent properties suitable for use in two-photon microscopy, being their excitation wavelengths within the excitation range of lasers currently available (720–880 nm). In particular, the fluorescent ligand **24** showed a favorable combination of 5-HT_{1A} receptor affinity ($K_i = 0.4$ nM), Stokes shift (excitation wavelength = 381 nm, emission wavelength = 455 nm), and quantum yield in ethanol ($\Phi = 0.45$). Fluorescence visualization experiments in cell lines expressing 5-HT_{1A} by two photon laser microscopy are in due course.

Experimental Section

General Procedure for the Preparation of Derivatives 10–23. A mixture of chloro derivative **25a**−**e** (3.6 mmol) and amine **26a**−**d** (5.4 mmol) was heated in a closed glass tube at 150 °C for 5 h (24 h when **25b** was used). After cooling, the reaction mixture was diluted with CH_2Cl_2 and washed with a 20% aqueous solution of Na_2CO_3. The separated organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by chromatography ($CHCl_3/CH_3OH$, 19:1 as eluent) giving the expected compound as pale yellow oil.

***N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]-6-methoxy-2-quinolinamine (16):** 22% yield; GC−MS *m/z* 421 ($M^+ + 1$, 4), 420 (M^+, 8), 258 (100), 227 (30); ^{1}H NMR ($CDCl_3$) δ 1.71–1.74 (m, 4H), 2.52 (t, 2H, $J = 6.9$ Hz), 2.71 (br s, 4H), 3.14 (br s, 4H), 3.45–3.51 (m, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 5.19 (br s, 1H, D_2O exchanged), 6.64 (d, 1H, $J = 8.8$ Hz), 6.85–7.03 (m, 5H), 7.20 (dd, 1H, $J = 2.7$ Hz, 9.1 Hz), 7.61 (d, 1H, $J = 9.1$ Hz), 7.74 (d, 1H, $J = 8.8$ Hz). The hydrochloride salt melted at 94–97 °C (from CH_3OH/Et_2O). Anal. ($C_{25}H_{32}N_4O_2 \cdot 4HCl$) C, H, N.

6-Amino-*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-2-quinolinamine (24). Nitro derivative **23** (0.32 g, 0.73 mmol) was dissolved in ethanol and hydrogenated at normal pressure and room temperature in the presence of 10% Pd/C (0.1 g) until the uptake ceased. The catalyst was removed by filtration through Celite, and the solvent was evaporated in vacuo to give a crude residue that was chromatographed ($CHCl_3/CH_3OH$, 9:1, as eluent) to provide pure compound **24** as a pale-brown solid (0.140 g, 47% yield): GC-MS *m/z* 405 (M^+, 3), 243 (100), 212 (27), 159 (36); ^{1}H NMR ($CDCl_3$) δ 1.74 (app t, 4H), 2.53 (app t, 2H), 2.74 (br s, 4H), 3.15 (br s, 4H), 3.46–3.49 (m, 2H), 3.65 (br s, 2H, D_2O exchanged), 3.86 (s, 3H), 5.60 (br s, 1H, D_2O exchanged), 6.65 (d, 1H, $J = 9.1$ Hz), 6.81–7.05 (m, 6H), 7.57 (d, 1H, $J = 8.8$ Hz), 7.68 (d, 1H, $J = 8.8$ Hz); mp 97 °C dec (from $CHCl_3$/*n*-hexane). Anal. ($C_{24}H_{31}N_5O$) C, H, N.

Fluorescence Spectroscopy. Emission and excitation spectra of compounds **9**−**22** and **24** were recorded as detailed in the Supporting Information. Fluorescence quantum yields were calculated in reference to that of 2-aminopyridine in ethanol as a standard (excitation wavelength 285 nm; $\Phi = 0.37$),[29] according to Demas et al.[32]

Supporting Information Available: Spectral data of compounds **10**–**15**, **17**–**23**. Biological methods and statistical analysis. Elemental analysis data of compounds **10**–**22** and**24**. Experimental procedure for fluorescence spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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