Bioorganic & Medicinal Chemistry Letters, Vol.3, No.10, pp. 1935-1940, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

Fluorescent Probes for 5-HT_{1A} Receptors: Synthesis and Characterization of 5-Methoxy-3-[*n*-propyl-(4-*n*-aminobutyl)] amino-3,4-dihydro-2*H*-1-benzopyran Derivatives

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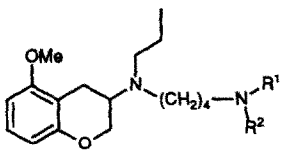
(*Received in Belgium* 4 *March* 1993; *accepted* 16 *July* 1993)

Abstract: Fluorescent ligands of 5-HT_{1A} receptors have been synthesized. Their affinity and specificity for these sites are reported.

Drugs interacting with the 5-HT_{1A} subtype[1] of serotonine (5-HT) receptors are of potential clinical interest in the treatment of behavioral disorders, such as depression, anxiety and psychosis[2], and physiological phenomena like apetite, memory, thermoregulation and sexual behavior[3]. The regional distribution of 5-HT receptors has been characterized by autoradiographic techniques using high specific radioligands[4]. However, in order to more fully understand the nature and functioning of the 5-HT_{1A} receptor, it is necessary to localize the distribution of this receptor at the cellular/subcellular level and to also determine their mobility in the membranes in normal and diseased states[5]. One approach, which has been increasingly successful in this direction, involves the light microscopy study of the membrane-bound receptors using fluorescently labeled ligands, at a resolution higher than that permitted by autoradiographic techniques[6]. This method has been fruitful to varying degrees in the study of insulin[7], cholinergic[8], opioid[9], glucagon[10], β-adrenergic[11], dopamine[12] and serotonine[13] receptors.

We recently published the synthesis of powerful and selective ligands for serotoninergic receptors whose 5-HT_{1A} affinity was equal or superior to the reference derivatives (buspirone, serotonine, 8-OH-DPAT)[14,15]. All these compounds possess a basic 3,4-dihydro-3-amino-2*H*-1-benzopyran structure (Scheme 1).



Scheme 1

As a continuation of our work, we now describe the synthesis, fluorescent properties and pharmacological characterization of two fluorescent probes with high affinity for 5-HT_{1A} receptors.

Design and chemistry.

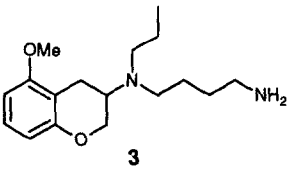
First, efficient fluorescent moieties were chosen, then a ligand moiety possessing a reactive and appropriate functional group reacting with the fluorescent entities, and finally this stepwise procedure led to the design and development of fluorescent probes for 5-HT_{1A} receptors.

The fluorescent moieties chosen were the 7-nitrobenzo-2-oxa-1,3-diazol-4-yl (NBD)[12,13,16] and the 2,3,6,7-tetrahydro-11-oxo-1*H*,5*H*,11*H*-[1]benzopyrano[6,7,8-*i*,*j*]quinolizin-9-carboxamido groups, which were respectively introduced by commercially available NBD-Cl **1** and the 2,3,6,7-tetrahydro-11-oxo-1*H*,5*H*,11*H*-[1]benzopyrano[6,7,8-*i*,*j*]quinolizin-9-carboxylic acid **2**[17], respectively (Scheme 2).

1 **2**

Scheme 2

The ligand was then chosen on the basis of the high affinity and selectivity observed with the substituted benzopyrans previously described[14c,15]. In this way we prepared the primary amine **3** that was likely to react with both derivatives **1** and **2** (Scheme 3).



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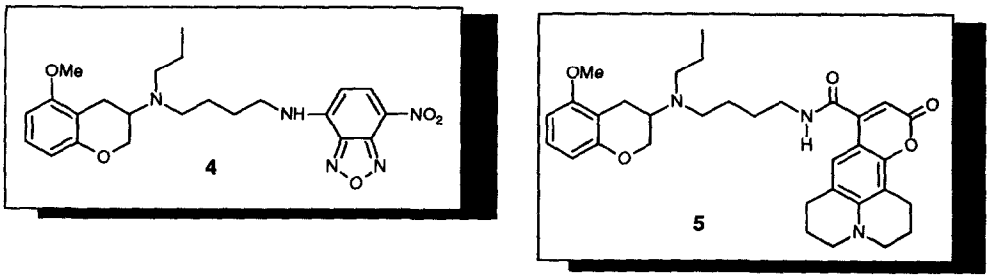
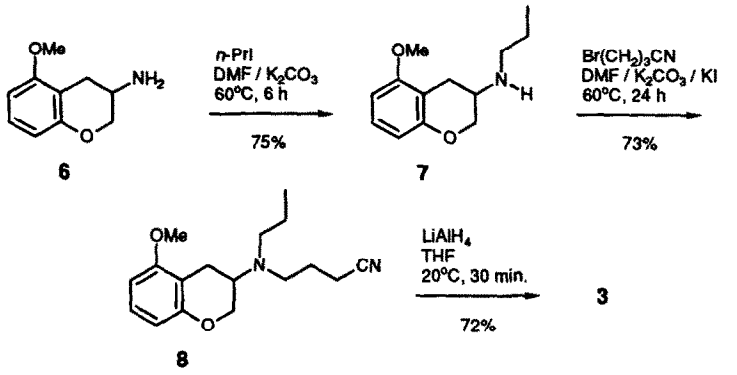
Scheme 3

The required probes **4** and **5** resulted from the coupling of the amino derivative **3** and the fluorescent entities **1** and **2** (Scheme 4).



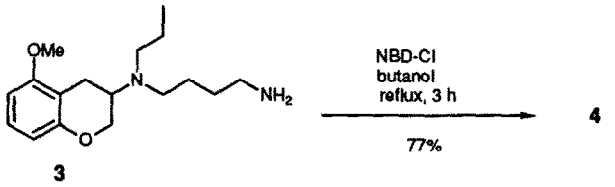
Scheme 4

The 5-methoxy-3-[*n*-propyl-(4-*n*-aminobutyl)]amino-2*H*-1-benzopyran **3** was prepared as described in Scheme 5. The primary amine **6** was first treated with 1-iodopropane and potassium carbonate in DMF to provide the 5-methoxy-3-*n*-propylamino-2*H*-1-benzopyran **7**. The compound **8** was obtained by alkylation of compound **7** with 4-bromobutyronitrile in the presence of potassium carbonate and potassium iodide. Reduction of the product **8** was carried out at room temperature using lithium aluminum hydride in tetrahydrofuran (Scheme 5).



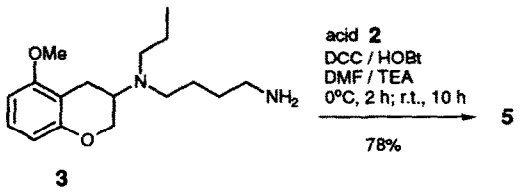
Scheme 5

The NBD probe **4**[18,19] was obtained by reaction of the amine **3** with NBD-Cl **1** using a procedure described by Bakthavachalam *et al*[12,13] (Scheme 6).



Scheme 6

The coumarinyl conjugate **5**[18,19] was prepared by treatment of the amine **3** with the acid **2** in DMF in the presence of dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) (Scheme 5).

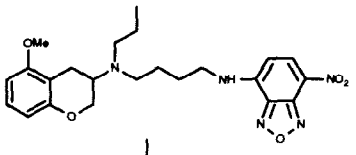
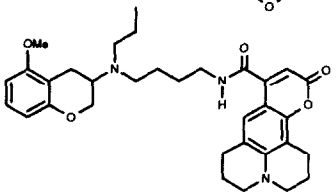


Scheme 7

Fluorescent properties

As expected the fluorescent properties shown in Table 1 permit the use of the two ligands in light microscopy studies. They possess emission wavelenghts near 550 nm and showed a small overlapping of the absorption and emission spectra.

Table 1. Absorption and fluorescence data[a] of ligands **4** and **5**.

N°	Products	λ_{ex} (nm)	log ε	λ_{em} (nm)	φ[b]
4		464	4,02	525	<0,01
5		410	4,21	550	<0,01

[a] No modification of the fluorescent properties were observed when the oxalate salts of **4** and **5** were tested.
[b] Calculated as described in previous papers[17,20].

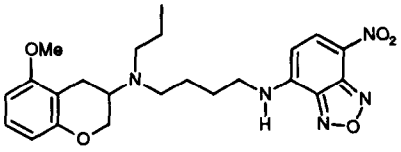
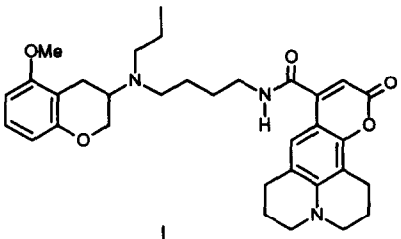
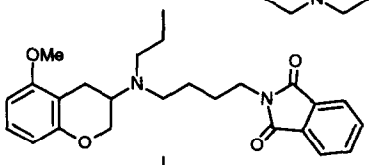
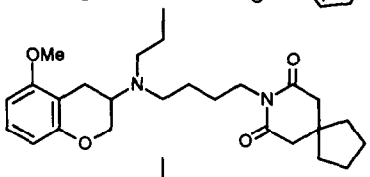
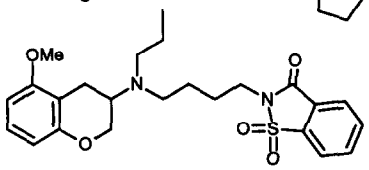
Pharmacological characterization

The pharmacological characterization of the fluorescent ligands was carried out by measuring the ability of these compounds to displace [^{3}H]8-OH-DPAT, [^{3}H]serotonin and [^{3}H]ketanserine from 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptor sites in cellular membranes of perfectly defined brain structures[21]. The affinities (IC_{50}) were determined and are shown in Table 2. Also included are data of non-fluorescent parents ligands.

By comparison with parent products, we observed that coupling the coumarinyl moiety to the amine **3** results in altering the specificity of this ligand **5** for the 5-HT_{1A} receptor subclass. Its good affinity for the 5-HT_{1B} receptor subtype implies a lower specificity as that expected. In contrast the NBD substituted ligand **4** binds to the 5-HT_{1A} receptor with good affinity and convenient selectivity.

In conclusion, the fluorescent ligand **4** constitutes the best candidate for the development of light microscopic studies and should enable the localization of 5-HT_{1A} receptors in brain membranes by fluorescence detection.

Table 2. Binding values of compounds **4** and **5**.

N°	Products[a,b]	IC_{50} 5-HT_{1A}	5-HT_{1B}	5-HT_2
4		10^{-10}	$3{,}4.10^{-7}$	$1{,}8.10^{-5}$
5		3.10^{-10}	9.10^{-9}	$1.5\ 10^{-6}$
S20006		5.10^{-8}	$4\ 10^{-7}$	3.10^{-5}
S20244		2.10^{-10}	5.10^{-6}	10^{-6}
S20393		10^{-9}	10^{-6}	10^{-5}

[a] All ligands tested were racemic forms and used as oxalate salts.
[b] The 5-HT_{1D} and 5-HT_3 affinity of compounds **4** and **5** were also determined. Values are between 10^{-5} and 10^{-6} for **4** and near 10^{-8} for **5**.

Acknowledgment: We thank *I. R. I. SERVIER* for multiform support.

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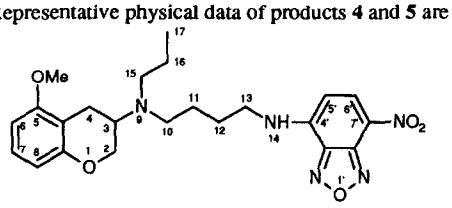
(17) Compound **2** was obtained by hydrolysis of the corresponding methyl ester described in: Besson, T.; Coudert, G.; Guillaumet, G. *J. Heterocycl. Chem.* **1991**, *28*, 1517.

(18) All compounds exhibited satisfactory ^{1}H-NMR, IR, mass spectra and elemental analysis.

(19) Representative physical data of products **4** and **5** are as follows.



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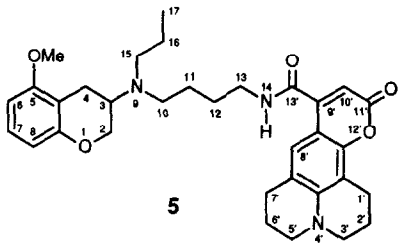

5

Compound 4: Oil; IR (film): ν = 3020 (NH), 1260 (ether) cm^{-1}; MS (CI) m/z = 456 (M+1); ; ^{1}H-NMR ($CDCl_3$): δ = 0.78 (t, J = 7.32 Hz, 3H, $CH_3{}^{17}$), 1.45-1.98 (m, 6H, $CH_2{}^{11}$ $CH_2{}^{12}$ $CH_2{}^{16}$), 2.56-2.73 (m, 5H, $CH_2{}^{15}$, $CH_2{}^{10}$, H^4), 2.85 (dd, J = 1.67 Hz and J = 5.59 Hz, 1H, H^4), 3.19-3.31 (m, 1H, H^3), 3.35-3.45 (m, 2H, H^{13}), 3.78 (s, 3H, OCH_3), 3.91 (dd, $J_1 = J_2 = 9.83$ Hz, 1H, H^2), 4.21-4.29 (m, 1H, H^2), 6.11 (dd, J = 8.71 Hz, 1H, $H^{5'}$), 6.37 (d, J = 8.17 Hz, 1H, H_{arom}), 6.46 (d, J = 8.17 Hz, 1H, H_{arom}), 7.02 (t, J = 8.17 Hz, 1H, H^7), 8.49 (d, J = 8.71 Hz, 1H, $H^{6'}$); *Anal.* ($C_{23}H_{29}N_5O_{43}$) C, H, N.

Compound 5: Oil; IR (film): ν = 3020 (NH), 1710 (C=O), 1260 (ether) cm^{-1}; MS (CI): m/z = 460 (M+1); ^{1}H-NMR ($CDCl_3$): δ = 0.77 (t, J = 7.32 Hz, 3H, $CH_3{}^{17}$), 1.39-1.73 (m, 6H, H^{11}, H^{12}, H^{16}), 1.88-2.00 (m, 4H, $H^{2'}$, $H^{6'}$), 2.46-2.86 (m, 10H, $H^{1'}$, H^4, $H^{7'}$, H^{10}, H^{15}), 3.07-3.18 (m, 1H, H^3), 3.21-3.29 (m, 4H, $H^{3'}$, $H^{5'}$), 3.42-3.54 (m, 2H, H^{13}), 3.79 (t, 9.93 Hz, 1H, H^2), 3.82 (s, 3H, OCH_3), 4.20-4.27 (m, 1H, H^2), 5.97 (s, 1H, $H^{10'}$), 6.40 (d, J = 8.09 Hz, 1H, H_{arom}), 6.46 (d, J = 8.09 Hz, 1H, H_{arom}), 6.70 (brs, 1H, NH), 7.03 (t, J = 8.09 Hz, 1H, H^7), 7.16 (s, 1H, $H^{8'}$); *Anal.* ($C_{33}H_{41}N_3O_{23}$) C, H, N.

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