ACS Medicinal Chemistry Letters
Letter
pubs.acs.org/acsmedchemlett

Original Design of Fluorescent Ligands by Fusing BODIPY and Melatonin Neurohormone

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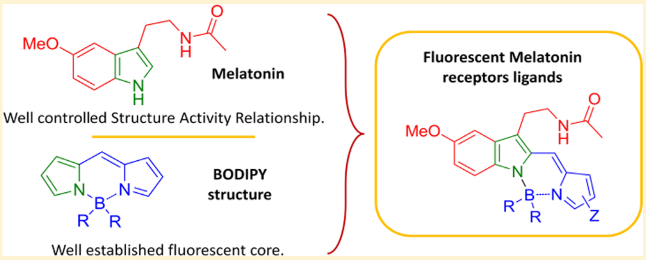
Supporting Information

ABSTRACT: An original design and synthesis of fluorescent ligands for melatonin receptor studies is presented and consists in the fusion of the endogenous ligand with the fluorescent BODIPY core. Probes **I–IV** show high affinities for MT_1 and MT_2 melatonin receptors and exhibit fluorescence properties compatible with cell observation.



KEYWORDS: *GPCRs, melatonin, fluorescence, molecular probes, BODIPY*

Fluorescence is one of the most sensitive spectroscopic methods and many fluorescent ligands have been reported for locating G-protein-coupled receptors (GPCRs), for studying ligand/receptor interactions, and more generally for better understanding their pharmacology and physiological process.[1−4] The history of fluorescent ligands is linked to the development of commercially available fluorophores. Organic dyes have been designed and synthesized to exhibit excitation and emission wavelengths, which are compatible with biological observation and are associated to ligands in conjugation reactions. The addition of such a distinct fluorescent molecule may alter both the chemical (while remaining within a spectrum of lipophilicity to hydrophilicity) and pharmacological (affinity, functionality, etc.) properties of the resulting fluorescent ligand that will modify its cellular behavior.

The melatonin receptors MT_1 and MT_2 are members of the GPCR family. They are involved in the regulation of the circadian rhythm and seasonal functions in mammals. They are also implicated in many biological processes ranging from anti-inflammatory to antioxidant effects including anti-Parkinson effects[5,6] and were recently reported as part of the mechanism of action of the antidepressant agomelatine, an MT_1 and MT_2 receptor agonist and 5-HT_{2C} antagonist.[7,8] Despite the discovery of the high affinity agonist and nonselective 2-[^{125}I]-MLT radioligand[9] research on the pharmacology and the functionality/physiological impact of melatonin receptors suffers from the lack of selective probes for these receptors due to their very low level of expression. Moreover, the main disadvantages of this method are the radioactive hazards and the limitations of studying the molecular dynamics of receptor activation. To offer alternative probes, we have developed a concept aiming at using the aromatic core of an endogenous ligand as the source of fluorescence after slight chemical modification and without loss of biological activity. Herein, we report the design and synthesis of fluorescent ligands for melatonin receptor studies thanks to the fusion of the endogenous ligand with the fluorescent BODIPY core.

Melatonin presents in its chemical structure an indole ring possessing fluorescent properties that are unfortunately inappropriate for biological analysis due to interferences from other biochromophores (such as tryptophan).[10,11] Our expertise on the melatonin structure/activity relationship[12−16] prompts us to investigate the extension of the π-conjugation of the indole scaffold at position C-2 in order to obtain biologically compatible photophysical properties. The original idea consists in the fusion of the pyrrole ring of melatonin with one of the pyrrole rings of the highly stable and bright difluoroboraindacene (BODIPY) fluorophore[17−19] (Figure 1). The fluorescence of the resulting indole-based BODIPY was expected as recently described by Zhao, Zhu, and coworkers.[20,21]

To attempt the fused melatonin-BODIPY core, 2-iodomelatonin **1**,[22−24] was converted into 2-formylmelatonin **2** by a palladium catalyzed carbonylative coupling reaction in the presence of tributyltin hydride in good yields using the Fukuyama procedure.[25] Condensation of the pyrroles with **2**

Received: October 15, 2013
Accepted: November 20, 2013
Published: November 20, 2013

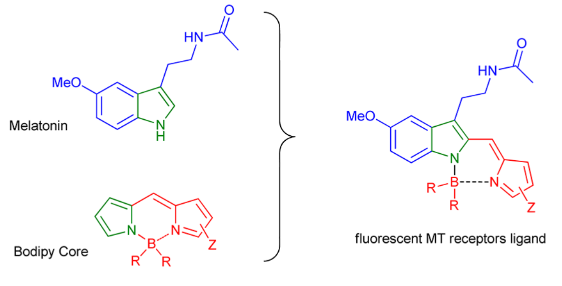
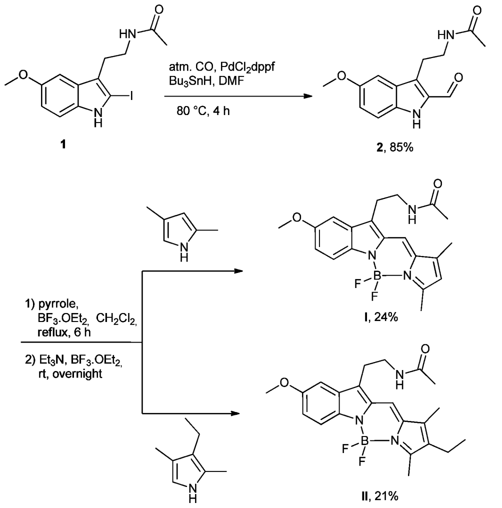


Figure 1. Fused melatonin-BODIPY.

under traditional experimental conditions using acidic catalyst ($POCl_3$, HBr) was unable to furnish the desired product. Considering the final difluoroborane complex, we envisaged the direct activation of the carbonyl function in compound **2** with boron trifluoroborate etherate.[26] The Lewis acid was added slowly at low temperature to the solution of 2-formylmelatonin **2**, and after 15 min the pyrrole was added. Synthesis of the final boron complex was achieved by adding triethylamine with six extra equivalents of $BF_3{\cdot}Et_2O$. The first two desired structures **I** and **II** were isolated in 21% and 24% yield, respectively (Scheme 1).

Scheme 1. Synthetic Pathway to Molecules I and II

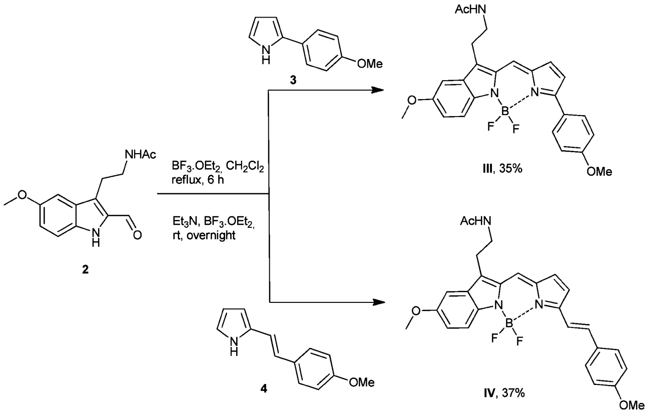


Accentuation of the conjugation in BODIPY is known to induce a red-shift in the excitation and emission wavelengths, which is more compatible for biological observations.[17−19,27,28] Such a π extension can be performed by introducing an aryl or a hetaryl group at the 3 and/or 5 positions of the pyrrole moiety using metal catalyzed reactions,[29−32] the Knoevenagel reaction,[33,34] vicarious nucleophilic substitution (VNS),[35] or by modifying the substituents linked to the boron atom.[36] For our purpose, aryl and styryl groups were envisaged and pyrroles **3** and **4** were first synthesized. Condensation with 2-formylmelatonin according to the previous protocol finally afforded the desired boron complexes **III** and **IV** with extended π-delocalization in 35 and 37% yield, respectively (Scheme 2).

The photophysical properties of these new dyes were measured in dimethylsulfoxide. Absorption (Figure 2) and molar extinction coefficients of compounds **I**−**IV** are reported in Table 1.

All four boron complexes show fluorescent properties. The emission spectra (Figure 3) for **I** and **II** are around 490−540 nm. Compounds **III** and **IV**, with extra π-conjugation, show excitation and emission bands at lower energy as expected. These new indole-based BODIPYs present Stokes shifts between 21 and 111 nm comparable with standard BODIPY dye values.[17−19]

Scheme 2. Synthetic Pathway to Molecules III and IV



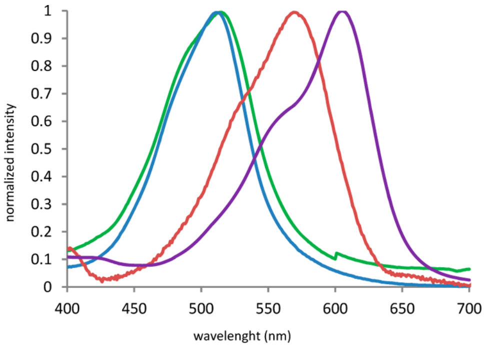


Figure 2. Normalized absorption of compounds **I** (green), **II** (blue), **III** (red), and **IV** (purple) in DMSO.

Table 1. Absorption Wavelengths and Molar Extinction Coefficients of Compounds I−IV in DMSO

compd	I	II	III	IV
abs_{max} (nm)	515	512	569	604
$\varepsilon\lambda_{max}$ ($L{\cdot}mol^{-1}{\cdot}cm^{-1}$)	31094	32970	29544	31230

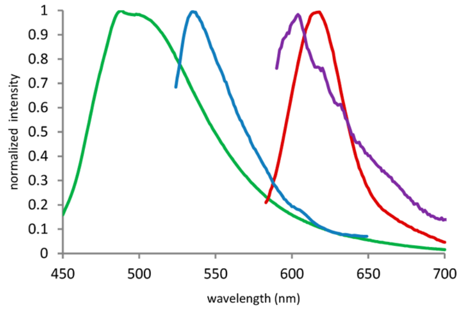


Figure 3. Normalized fluorescence emission spectra properties of compounds **I** (green), **II** (blue), **III** (red), and **IV** (purple) in DMSO (for λ_{exc}, see Table 2).

The binding affinities of the four fluorescent derivatives **I**−**IV** were evaluated (Table 3) on human MT_1 and MT_2 receptors. They all show good affinities: ligands **I** and **IV** present affinities in the range of tens of nanomolar concentrations for the two

Table 2. Photophysical Properties of Compounds I−IV in DMSO

compd	I	II	III	IV
λ_{exc} (nm)	426	424	573	583
λ_{em} (nm)	493	535	616	604
Stokes shift (nm)	67	111	43	21

Table 3. Binding Affinity of Compounds I−IV on Human MT_1 and MT_2 Receptors

compd	MT_1 K_i ± SEM (nM)	MT_2 K_i ± SEM (nM)
I	32 ± 5	10 ± 0.7
II	256 ± 40	96 ± 20
III	49 ± 15	315 ± 9
IV	71 ± 15	26 ± 1

receptors, while ligand **II** is more selective for the MT_2 receptor, and ligand **III** displays a larger MT_1 receptor selectivity.

In conclusion, by fusing the endogenous ligand of melatonin receptors with the well-known and efficient fluorescent BODIPY core, we have been able to design and isolate four new condensed fluorescent probes with good melatonin receptor affinities. Extension of the π-conjugation of ligands **I** and **II** by coupling with an aryl (ligand **III**) or a styryl group (ligand **IV**) induces a bathochromic shift with slight impact on the affinity. Cellular imaging studies are currently under way.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the synthesis and the characterization of ligand **I**−**IV** and intermediates, spectroscopic data, and pharmacology. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was supported by La Région Centre (APR2009-LOIREMEL). During this study, J.T. and J.M. were supported (Ph.D grants) by the Conseil Général du Loiret (J.T.) and La Région Centre (J.M., APR2012-LIFERMEL)

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Dr. S. Poupart for her advice.

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