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THE DEVELOPMENT OF A CHARGED MELATONIN RECEPTOR LIGAND

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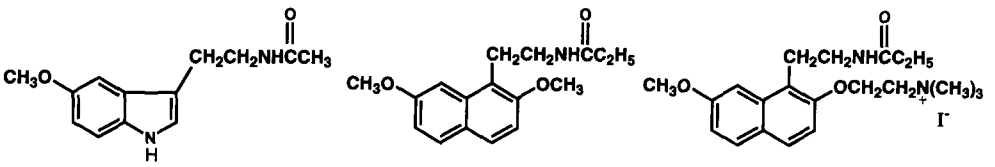
Abstract: We report the synthesis and radioligand binding analysis of a novel charged melatonin receptor ligand, N-[2-(2-Trimethylammoniumethyleneoxy-7-methoxy)ethyl]propionamide iodide. The charged ligand has potential in determining whether internalization of the melatonin receptor occurs following melatonin exposure.

Melatonin is a pineal hormone that is involved in the regulation of circadian rhythms, seasonal breeding, cardiovascular function, retinal function, and oncogenesis.[1-6] Melatonin receptors are classified into either the ML_1 or ML_2 types based on pharmacological profiles.[5] Cloning of the human melatonin receptors has revealed the existence of two subtypes, Mel_{1a}[7] and Mel_{1b},[8] which belong to the ML_1 class of melatonin receptors. Melatonin receptors are highly regulated throughout the day and following melatonin exposure. For example, the density and affinity of melatonin receptors decrease during the night and following melatonin exposure.[2,3,9-11] Perhaps, critical to the normal functioning of melatonin within the body is its ability to "turn off" or desensitize its receptors. One component of desensitization, that is, internalization may be one of the mechanisms by which melatonin receptors become refractory to melatonin. Internalization is the process by which receptors are sequestered and removed from the membrane surface following agonist exposure. This form of desensitization is utilized by many other G-protein-coupled receptors including muscarinic cholinoceptors[12,13] and beta-adrenoceptors.[14] Whether or not melatonin receptors internalize following melatonin exposure has not been determined due to the unavailability of specific probes, that is, charged melatonin receptor ligands that are unable to penetrate membranes and bind only to surface melatonin receptors. The development and use of this ligand would greatly enhance our understanding of melatonin's actions at the level of the receptor.

Recently, we have synthesized a series of quinolinic analogs of melatonin that exhibit high affinity (<1 nM affinity) to both the human Mel_{1a} and Mel_{1b} melatonin receptors. However, the N-methylated analogs of the quinolinic derivatives displayed very low (>1 μM affinity) for both melatonin receptors.[15] We postulated that the incorporation of a positive charge into the quinolinic (aromatic) nucleus was detrimental to the ability of these analogs to bind to the melatonin receptors. Thus, we designed a melatonin receptor ligand that contains a

charge away from the aromatic nucleus where it remains neutral. Recently, it was reported[16] that N-[2-(2,7-dimethoxynaphthyl)ethyl]propionamide **2** is a melatonin receptor ligand that binds melatonin receptors with high affinity ($K_i = 0.07 \pm 0.004$ nM);

Figure 1 Structures of Melatonin and Related Analogs



Melatonin = 1 **N-[2-(2,7-dimethoxynaphthyl)ethyl] propionamide = 2** **TMEPI = 3**

It was postulated that the 2-methoxy group binds to the accessory binding site of the receptor. Thus, we designed and synthesized N-[2-(2-Trimethylammoniumethyleneoxy-7-methoxy)ethyl]propionamide iodide (**3=TMEPI**) as a charged melatonin receptor ligand.

Figure 2 outlines the synthesis of target compound **TMEPI**. 7-Methoxy-2-naphthol **4** was used as a starting material. Selective formylation of the 1-position of compound **4** was accomplished by reaction with chloroform in the presence of NaOH (Reimer-Tiemann reaction), yielding the desired phenolic aldehyde **5**. The structure of **5** was confirmed unambiguously by ^{1}H NMR spectra in which 4 protons have big coupling of 9.0 Hz and the proton of OH moved downfield to 13.14 ppm. Protection of phenol **5** as benzyl ether by reaction with benzyl bromide using K_2CO_3 as a base gave compound **6** (100%). Condensation of aldehyde **6** with nitromethane in the presence of NH_4OAc afforded nitroalkene **7** (96.5%). Reduction of **7** with $LiAlH_4$ followed by acylation with propionyl chloride furnished amide **8** (56.2% based on **7**). Cleavage of the benzyl ether in compound **8** by hydrogenation gave the phenol **9** (100%). Reacting **9** with 2-iodoethanol yielded the primary alcohol **10**. The alcohol **10** was converted to the iodide **11** with PPh_3/I_2. The tertiary amine **12** was obtained by reacting **11** with dimethylamine. The target compound **3=TMEPI** was obtained by reacting **12** with methyl iodide. Compounds **2, 13** and **15** were synthesized by reacting **9** with the respective alkyl iodides. Selected compounds were tested for their ability to bind to the melatonin receptors as described previously [17] and as shown in Table 1.

Figure 2: Synthesis of TMEPI

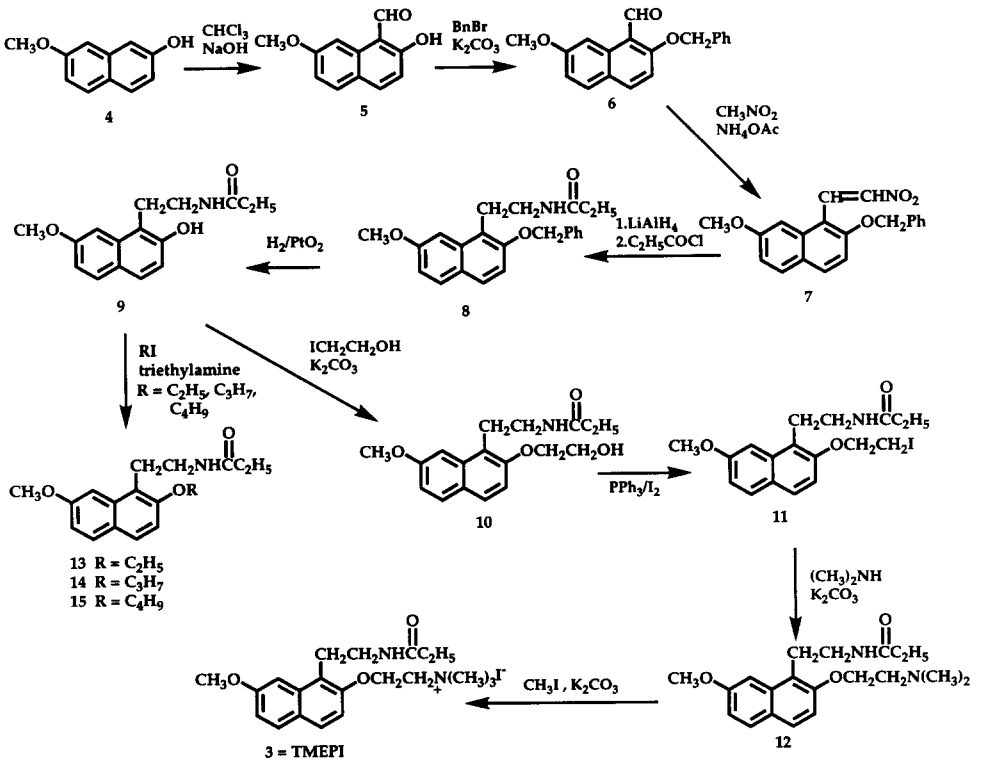
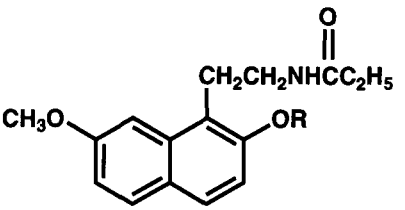


Table 1. Competition of Melatonin and Napthalenic Analogs for 2-[^{125}I]-Iodomelatonin Binding to Human Mel_{1a} or Mel_{1b} Melatonin Receptors Stably Expressed in CHO Cells



		K_i(nM) (range of SEM)	
Compound	R	Mel_{1a}	Mel_{1b}
2	CH_3	0.099 (0.09-0.10)	0.38 (0.35-0.41)
3	$CH_2CH_2N(CH_3)_3^+I^-$	93 (45-100)	23 (22-25)
9	H	2.2 (1.7-2.9)	1.2 (1.16-1.19)
10	CH_2CH_2OH	4.3 (2.9-6.6)	1.3 (1.2-1.5)
11	CH_2CH_2I	100 (76-300)	73 (54-97)
12	$CH_2CH_2N(CH_3)_2$	42 (23-74)	10 (8-13)
13	C_2H_5	0.39 (0.33-0.45)	1.2 (1.1-1.2)
14	C_3H_7	8.1 (4.2-6.7)	16 (11-21)
15	C_4H_9	7.7 (2.9-21)	6.7 (4.4-10)
Serotonin		> 100,000	> 100,000
Dopamine		7100 (6500-7800)	9100 (3200-26,000)
Melatonin		0.20 (0.11-0.23)	0.29 (0.18-0.49)

All competition binding experiments were performed on CHO whole cell lysates using 80-100 pM 2-[^{125}I]-iodomelatonin (NEN/DuPont, Boston, MA; 2200 Ci/mmol) at room temperature. The affinity of 2-[^{125}I]-iodomelatonin for Mel_{1a} and Mel_{1b} melatonin receptors was 80 pM and 150 pM, respectively.

Several 2-substituted (both hydrophobic and hydrophilic) napthalenic analogs of melatonin were synthesized and their binding affinities tested. Within the hydrophobic series of analogs, it was found that those analogs that contained a small substituent (i.e., compounds **2** and **13**) displayed higher affinity for the melatonin receptors than those with a larger substituent (i.e., compounds **14** and **15**). Similarly, in the hydrophilic series of analogs (i.e., compounds **9**, **10**, **12**, and **TMEPI**), those analogs with smaller substituents (compounds **9** and **10**) exhibited higher affinity for the melatonin receptor compared to those with larger substituents (compounds **12** and **TMEPI**). In addition, comparison of analogs with hydrophobic substituents to analogs with hydrophilic substituents of similar size, affinity for the melatonin receptors was consistently less for the hydrophilic analogs. However, **TMEPI**, an analog with a hydrophilic substituent, still exhibited an affinity and selectivity for melatonin receptors. Competition of either serotonin or dopamine for 2-[^{125}I]-iodomelatonin binding to Mel_{1a} or Mel_{1b} melatonin receptors expressed in Chinese Hamster Ovary cells displayed at least 100-fold lower affinity for melatonin receptors compared to **TMEPI**. Here, we report the development of the first permanently charged melatonin receptor ligand that displays nanomolar affinity for the melatonin receptors where use of this ligand will aid in our understanding of melatonin receptor function.

Acknowledgment

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