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Heterocyclic Aminopyrrolidine Derivatives as Melatoninergic Agents

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Abstract—A series of chiral heterocyclic aminopyrrolidine derivatives was synthesized as novel melatoninergic ligands. Binding affinity assays were performed on cloned human MT_1 and MT_2 receptors, stably expressed in NIH3T3 cells. Compound **16** was identified as an orally bioavailable agonist at MT_1 and MT_2 melatonin receptors with low vasoconstrictive activity.

The neurohormone melatonin (*N*-acetyl-5-methoxytryptamine) (Fig. 1), first isolated from bovine pineal gland extracts in 1958,[1] is present in all mammalian species. It is synthesized and secreted primarily by the pineal gland in a circadian manner that closely follows the daily light/dark cycle.[2,3] It plays a central role in the regulation of circadian rhythms, the modulation of retinal physiology, and the control of seasonal cycles in vertebrates. Melatonin alleviates jet lag by decreasing sleep latency, improving sleep quality, and reducing fatigue, while accelerating the resynchronization of circadian rhythms associated with transmeridian travel.[4–7] In addition, melatonin has been shown to have antitumor properties and has been implicated in immune system responsiveness.[8]

It has been demonstrated that many of the effects of melatonin are mediated through G-protein coupled receptors expressed primarily in the brain, retina, pituitary, and blood vessels.[9] Cloning of several G-protein coupled melatonin receptor genes has revealed at least three melatonin receptor subtypes, two of which are defined as MT_1 and MT_2 and have been found in mammals.[10]

As a pharmacological tool and therapeutic entity, melatonin is not ideal because of its short biological half-life (about 19 min in rat), contractile effects on vascular smooth muscle, low aqueous solubility, and poor oral bioavailability. In an attempt to develop novel chemical tools to overcome the liabilities of melatonin, as well as understand melatonin receptor function, we report here the synthesis and biological activity of heterocyclic aminopyrrolidine derivatives as novel melatoninergic agents. These derivatives were designed to be melatoninergic ligands at melatonin receptors. In addition, they have the potential for improved metabolic stability, increased oral bioavailability, and reduced vasoconstrictive activity due to modification of the aromatic methoxy group and amido side chain.

Initially, pyrrolidine **1** (Fig. 1) was prepared to determine the effect of conformationally-constrained structures on the binding affinity. Evaluation of **1** showed good affinity for MT_1 and MT_2 receptors, $K_i = 3.7$ and 2.6 nM, respectively. Unfortunately, this compound had the same vasoconstrictive activity as melatonin itself in assays conducted with rat caudal arteries. In an effort to

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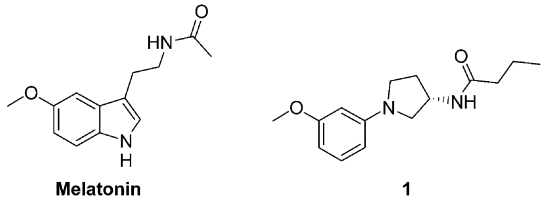
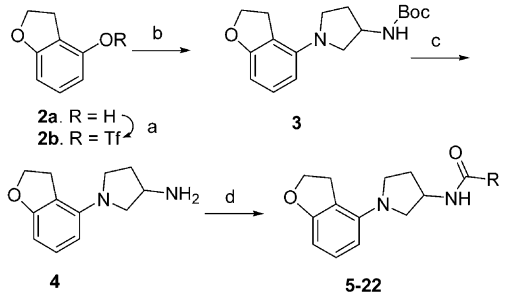


Figure 1.

overcome this liability, the arylalkoxy moiety in **1** was replaced with a dihydrobenzofuran motif. Thus, the targets were redesigned as shown in Scheme 1.[11]

As depicted in Scheme 1, 4-hydroxydihydrobenzofuran (**2a**) was prepared by Parham cycloalkylation of the 2-bromoresorcinol.[11] Phenol **2a** was converted to triflate **2b** by treatment with triflic anhydride in the presence of pyridine. Palladium-catalyzed coupling of triflate **2b** with (*R* or *S*)-pyrrolidin-3-yl-carbamic acid *tert*-butyl ester in the presence of $Pd(OAc)_2$ and BINAP using Cs_2CO_3 as a base in toluene under reflux furnished carbamate **3**. The yield of the palladium-catalyzed amination step was affected by the percentage of $Pd(OAc)_2$ and BINAP. When $Pd(OAc)_2$ (3 mol%) and BINAP (5 mol%) were used, the yield was only 35%. However, when $Pd(OAc)_2$ and BINAP were increased to 12 mol% and 18 mol%, respectively, the yield was dramatically increased to 86%. Deprotection of **3** with HCl in either ethyl acetate or dioxane gave amine **4** in quantitative yield. The amine **4** was acylated using acid chlorides or reacted with isocyanates to give the desired amide and urea products **5**–**22**.[11]

The K_i values of compounds **5**–**22** for human MT_1 and MT_2 melatonin receptor subtypes were determined in binding assays using 2-[^{125}I]-iodomelatonin with the described assay method[12] and the results are reported in Table 1. The MT_1 and MT_2 affinity values identified some compounds with excellent affinity but most of the compounds had little selectivity between MT_1 and MT_2 receptors. Cyclopropylcarbamide **9**, in particular, had the highest selectivity for the MT_1 receptor with potent affinity ($MT_2/MT_1 = 9$). The data also demonstrated clearly that the affinities of amides **5**–**14** were related to the absolute stereochemistry: (*S*)-enantiomers were more potent than (*R*)-enantiomers. For example, (*R*)-acetamide **5** had only modest affinity for both MT_1 ($K_i = 202$ nM) and MT_2 ($K_i = 121$ nM) receptors. However, the (*S*)-enantiomer **10** had excellent affinity for both MT_1 and MT_2 receptors ($K_i = 1.5$ and 1 nM, respectively). Furthermore, the binding potency also depended on the length or size of the amido chain. Replacement of the acetyl group by propanoyl or butanoyl increased the affinity of the compounds. In contrast to the amide derivatives, the affinity of urea analogues **15**–**22** was only slightly influenced by the stereochemistry and the length of the alkyl chain, as (*R*)-enantiomers of urea derivatives except (*R*)-urea **15**, which was less active than (*R*)-urea **19**, were slightly more potent than the (*S*)-enantiomers.



Scheme 1. (a) Tf_2O, pyridine, 0–25 °C, 90%; (b) pyrrolidin-3-yl-carbamic acid *tert*-butyl ester, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 86%; (c) HCl/dioxane, 100%; (d) RCOCl, Et_3N, CH_2Cl_2 or RNCO, benzene.

Table 1. Binding affinity of compounds **5**–**22** for human MT_1 and MT_2 melatonin receptors stably expressed in NIH3T3 cells. Values represent mean from experiments performed in duplicate

Compd	R	Chirality	MT_1 K_i (nM)	MT_2 K_i (nM)
Mel	—	—	0.4	0.3
1	—	S	3.7	2.6
5	Me	R	202	121
6	Et	R	49.4	39.3
7	*n*Pr	R	67	22.3
8	*i*Pr	R	129	85
9	*c*Pr	R	3.9	35
10	Me	S	1.5	1.0
11	Et	S	0.18	0.4
12	*n*Pr	S	0.14	0.31
13	*i*Pr	S	6.0	6.4
14	*c*Pr	S	1.2	2.8
15	NH-Me	R	2.2	4.6
16	NH-Et	R	0.65	0.42
17	NH-*n*Pr	R	4.4	2.6
18	NH-*c*Pr	R	6.6	10.8
19	NH-Me	S	0.90	0.94
20	NH-Et	S	7.0	2.1
21	NH-*n*Pr	S	17.7	5.8
22	NH-*c*Pr	S	21	19

The three most active compounds emerging from this series, **11**, **12**, and **16** were evaluated in our advanced profiling assays. It has been reported[13] that melatonin has a marked ability to enhance α-adrenoceptor-mediated vasoconstriction of the rat tail artery. Thus, the effect of these compounds on vascular smooth muscle was evaluated using the method already described.[14] Compared to melatonin, only compound **16** showed significantly reduced vasoconstrictive activity in assays conducted with rat caudal arteries (0.48 relative to melatonin), whereas **11** and **12** were 1.2 and 0.83, respectively, relative to melatonin. These studies led to the identification of **16** as a potent and promising ligand for MT_1 and MT_2 receptors. Compound **16** was further tested for functional activity in NIH3T3 cells expressing melatonin MT_1 or MT_2 receptor using the method already described[14] and found to be a full agonist at both MT_1 and MT_2 receptors. As Figure 2 shows, compound **16** exhibited dose-dependent inhibition of forskolin-stimulated cAMP accumulation in NIH3T3 cells stably expressing the human MT_1 or MT_2 receptor with a maximum inhibition similar to melatonin.

To determine the selectivity for melatonin receptors over a large number of other hormone or neurotransmitter receptors, enzymes, and ion channels, com-

pound **16** was tested through a commercial screen of radioligand binding panels.[15] At a concentration of 10 μM, **16** was found to have no significant affinity to all binding sites examined. For these purposes, significant activity was defined as ≥50% inhibition of corresponding binding at 10 μM.

Compound **16** was characterized in several pharmacokinetic studies (Table 2). The oral bioavailability was significant in both rat and dog (49% and 77%, respectively), which is superior to the oral bioavailability of melatonin at the same dose (24% and 17%, respectively). This oral bioavailability is presumably due to its good absorption, consistent with the excellent Caco-2 permeability (Pc 544 nm/s) and moderate clearance. The effective absorption was rapid in the rat, with peak concentration occurring within 30 min, whereas the compound was slowly absorbed in the dog. As a prelude to evaluating compound **16** in animal models of sleep disorder, the ability of compound **16** to enter rat brain following intravenous administration was determined. Table 3 shows the plasma and brain concentrations, as well as brain/plasma ratios at 20 min and 2 h after administration of an IV bolus dose (5 mg/kg). Compound **16** was shown to have moderate brain penetration based on the samples taken at 20 min and 2 h. Using ultrafiltration (Amicon Centrifree® cones), the plasma protein binding of compound **16** to freshly prepared rat and human plasma was determined at 0.2 and 1.0 g/mL. Compound **16** was not highly protein bound, with values ranging from 21–56% in rat plasma and 47–72% in human plasma.

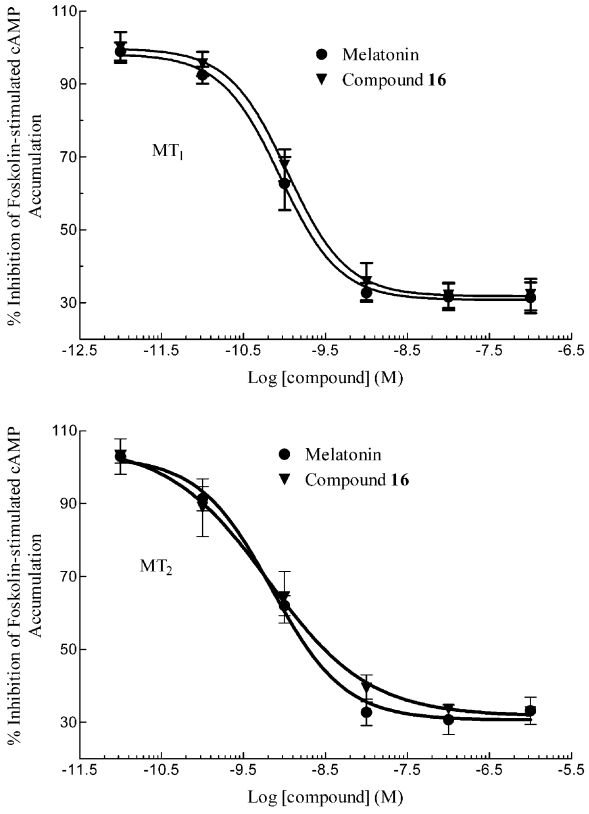


Figure 2. The effect of melatonin and compound **16** on forskolin-induced cAMP accumulation in NIH3T3 cells stably expressing the human MT_1 (upper graph) or the human MT_2 receptor (lower graph). Data is a composite of 3–6 separate experiments performed in duplicate. Non-linear regression analysis yielded for the MT_1 receptor, melatonin $pEC_{50} = 10.1 \pm 0.1$ (0.09 nM), $E_{max} = 69 \pm 2\%$, and intrinsic activity = 1, compound **16** $pEC_{50} = 9.9 \pm 0.1\%$ (0.13 nM), $E_{max} = 66 \pm 3\%$, and intrinsic activity = 1; for the MT_2 receptor, melatonin $pEC_{50} = 9.1 \pm 0.1$ (0.76 nM), $E_{max} = 69 \pm 2\%$, and and intrinsic activity = 1, compound **16** $pEC_{50} = 9.1 \pm 0.24$ (1.1 nM), $E_{max} = 68\% \pm 2\%$, and intrinsic activity = 1.

Table 2. Pharmacokinetic parameters of **16**

PK parameters	Rat[a]	Dog[b]
IV		
Dose (mg/kg)	1	1
$t_{1/2}$ (h)	0.9	1
Cl (mL/min/kg)	15	8.8
Vd (L/kg)	0.7	0.5
PO		
Dose (mg/kg)	1	1
F (%)	49	77

[a]Compound dosed in rats as a solution in PEG-400.
[b]Compound dosed in male beagle dogs as a solution in 50% PEG-400/50% H_2O.

The effects of compound **16** on circadian phase shift were also investigated using the method already described[14] (Fig. 3). Vehicle injection gave no significant phase advance, while melatonin (1 mg/kg) gave a sig-

Table 3. Brain and plasma concentration after IV administration of **16** to rats[a]

Time	Plasma (ng/mL)	Brain (ng/mL)	B/P ratio
20 min	5547	2014	0.36
2 h	1002	250	0.3

[a]Compound dosed as a solution in PEG-400 at 5 mg/kg, po ($n = 3$).

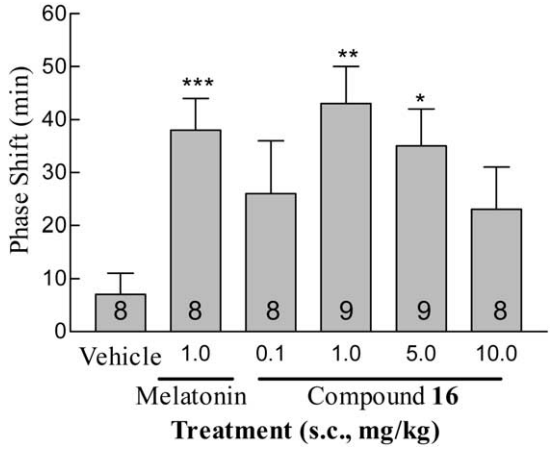


Figure 3. Acute effects of vehicle (5%DMSO, 45% PEG-400, 50% saline), melatonin (1 mg/kg), and **16** (0.1, 1, 5, and 10 mg/kg) on circadian phase shift. Vehicle vs. dose: $*p = 0.005$, $**p = 0.0003$, $***p = 0.0002$. The number of rats per treatment is shown within each bar. Rats showing disrupted circadian rhythms were not included in the study. One outlier from the 1.0 mg/kg group and one from the 10 mg/kg were excluded using two standard deviations from the mean.

nificant phase advance of 38 min. Compound **16** at doses from 0.1 to 10 mg/kg produced phase shifts that were not significantly different from that produced by melatonin. The shift in the onset of activity produced by administration of compound **16** at 1.0 and 5.0 mg/kg was significantly different from the small change observed in the vehicle-treated group.

In conclusion, this work has identified novel melatonin agonists with high affinity for MT_1 and MT_2 receptors. Compound **16** is a full agonist that produces circadian phase advances similar to that produced by melatonin. In addition, compound **16** is orally bioavailable and possesses low vasocontrictive activity in vitro in rat tail artery. Furthermore, compound **16** was chosen as a potential clinic trial candidate.

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