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N-{2-[2-(4-Phenylbutyl)benzofuran-4-yl]cyclopropylmethyl}-acetamide: an orally bioavailable melatonin receptor agonist

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Abstract—N-{2-[2-(4-Phenylbutyl)benzofuran-4-yl]cyclopropylmethyl}acetamide **3a** was synthesized as an orally bioavailable agonist at MT_1 and MT_2 melatonin receptors with significantly low vasoconstrictive activity.

Sleep disorders have an estimated prevalence of 15–27% in the adult population.[1] Insomnia, the most common sleep disorder, affects 20–40% of American adults[2] with the incidence increasing significantly with age. While benzodiazepines have been the most widely prescribed pharmacologic agents for the short-term treatment of sleep disorders, these compounds have profound effects on the CNS with the common side effects of residual daytime sedation, rebound insomnia, amnesia, irritability, and dependence, alterations in sleep architecture and synergistic interaction with alcohol.[3] Insomnia has a myriad of causes, one of which is disruption of the normal circadian sleep–wake cycle. Dysynchrony in the sleep–wake cycle can result from physiological changes. A therapeutic potential for the treatment of such disorders is resynchronization of the sleep–wake cycle via modulation of the melatoninergic system. The hormone melatonin (*N*-acetyl-5-methoxytryptamine) (Fig. 1) is synthesized and released primarily by the pineal gland in a circadian manner that closely follows the daily light/dark cycle.[4,5] It plays a major role in the regulation of circadian rhythms and the control of seasonal cycles.[6,7] Melatonin alleviates jet lag, regulates delayed sleep phase syndrome,[8] and induces sleep.[9] 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.[10] The recent cloning of several G-protein-coupled melatonin receptor genes has revealed at

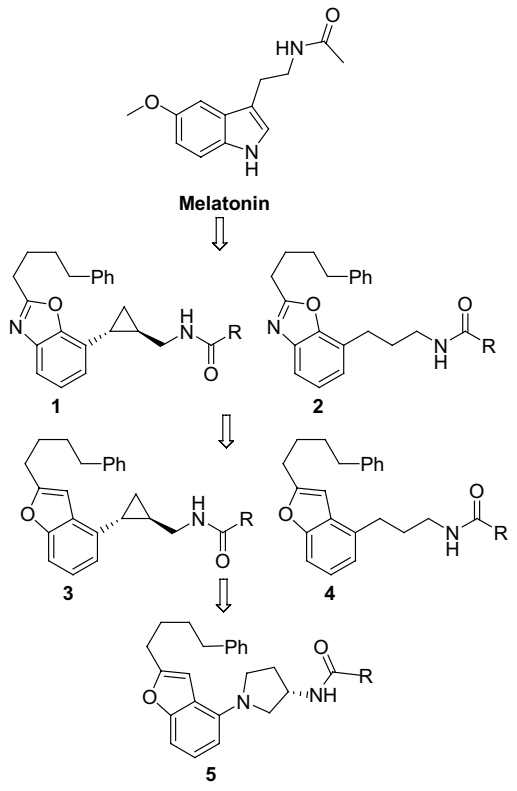

Figure 1.

Keywords: Melatonin; Melatonin receptors; Benzofuran; Agonist.
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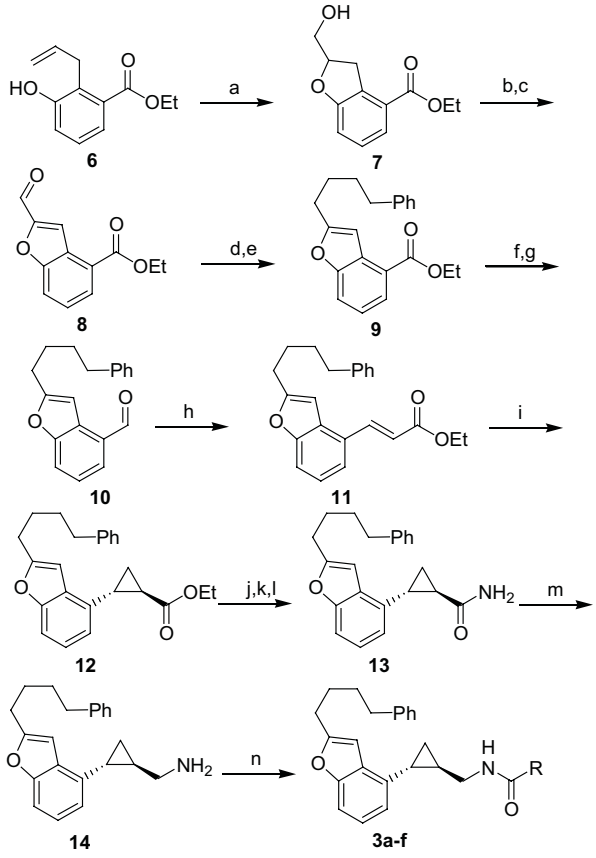
least three melatonin receptor subtypes, two of which are defined as MT_1 and MT_2 and are found in mammals.[11]

As a pharmacological tool and therapeutic entity, melatonin is less than ideal due to several major handicaps: the short biological half-life (about 19 min in rat),[12] contractile effects on vascular smooth muscle,[13] low aqueous solubility, and poor oral bioavailability. Therefore, a need exists for the development of its surrogates to overcome these liabilities of the endogenous ligand.

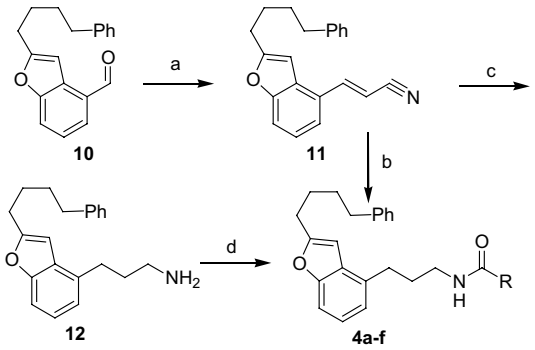
Recently, we reported the discovery of the benzoxazole nucleus as a melatoninergic pharmacophore, which served as an isosteric replacement for the previously established alkoxyaryl core.[14,15] It is of particular interest to note that compounds from this series bearing a 4-phenylbutyl chain at the 2-position of the benzoxazole moiety, **1** and **2** (Fig. 1), exhibited the highest binding affinity at both human MT_1 and MT_2 receptor subtypes, and the highest selectivity (up to 35-fold) for MT_1 with subnanomolar activity against MT_2, respectively. Given the increased activity and selectivity of compounds incorporating a 4-phenylbutyl moiety at the 2-position of the benzoxazole moiety, this substituent was retained in the context of an examination to identify substitutes for the benzoxazole scaffold. An initial study focused on the benzofuranyl moiety with the 4-phenylbutyl and amide groups disposed as shown in compounds **3** and **4**. Moreover, we sought to extend studies evaluating the replacement of the amide side chains in **3** and **4**. To this end, we examined application of the aminopyrrolidine heterocycle that we have previously demonstrated to confer substantial activity to melatoninergic ligands.[16] In the present paper, we report the identification of a novel series of potent, orally active agonists of melatonin receptors based on the benzofuran nucleus and exemplified by structures **3**–**5**.



Scheme 1. Reagents and conditions: (a) MCPBA, $NaHCO_3$, CH_2Cl_2, rt, 80%; (b) Ac_2O, Py, rt, 98%; (c) DDQ, dioxane, reflux, 85%; (d) $Ph_3P(CH_2)_3PhBr$, *n*-BuLi, THF, 0–25 °C, 85%; (e) 10% Pd/C, H_2, EtOAc, 99%; (f) $LiAlH_4$, THF, rt, 96%; (g) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, 90%; (h) Ph_3PCHCO_2Et, THF, reflux, 99%; (i) CH_2N_2, $Pd(OAc)_2$, THF, 80%; (j) NaOH, MeOH, reflux, 99%; (k) $SOCl_2$, CH_2Cl_2, reflux; (l) NH_3, THF, −78–25 °C, 89% (two steps); (m) Red-Al, toluene, 0–25 °C, 88%; (n) R_1COCl, Et_3N, CH_2Cl_2 or R_2NCO, benzene.

The compounds discussed in this report contain a 2-(4-phenylbutyl)benzofuran nucleus and a general route to the first series of analogues, compounds **3a**–**f**, is described in Scheme 1. Epoxidation of 2-allylphenol **6**[17] with MCPBA was accompanied by a spontaneous intramolecular epoxide opening by the phenolic hydroxyl to afford dihydrobenzofuran **7** directly in 80% yield. An attempt to directly oxidize **7** to benzofuran **8** with DDQ failed, necessitating conversion of **7** to aldehyde **8** via a two step process that proceeded with 83% overall yield. This comprised acetylation of alcohol **7** with acetic anhydride followed by oxidation with excess DDQ in dioxane at reflux. Wittig olefination of **8** followed by hydrogenation in the presence of 10% Pd on charcoal as a catalyst gave compound **9**. The ester moiety of **9** was reduced with $LiAlH_4$ and the resultant alcohol oxidized under Swern conditions to produce aldehyde **10**. A second Wittig homologation with Ph_3PCHCO_2Et provided the *trans*-cinnamic ester **11** in 99% yield. Cyclopropanation of **11** with diazomethane using $Pd(OAc)_2$ as a catalyst gave rise to (±)-*trans*-cyclopropane **12** in 80% yield. The next series of reactions involved converting the ester functionality of **12** to the corresponding reverse amide or urea derivatives. In the event, ester **12** was treated with NaOH to afford the corresponding acid, which, in turn, was converted into the amide **13** using $SOCl_2$ and NH_3. Red-Al-mediated reduction of **13** provided the primary amine **14**, which was acylated with a series of acid chlorides or reacted with ethyl isocyanate to produce the amide and urea products **3a**–**f**, respectively. It should be noted that these compounds were prepared in racemic form and with the aforementioned *trans*-stereochemistry about the cyclopropane ring system.

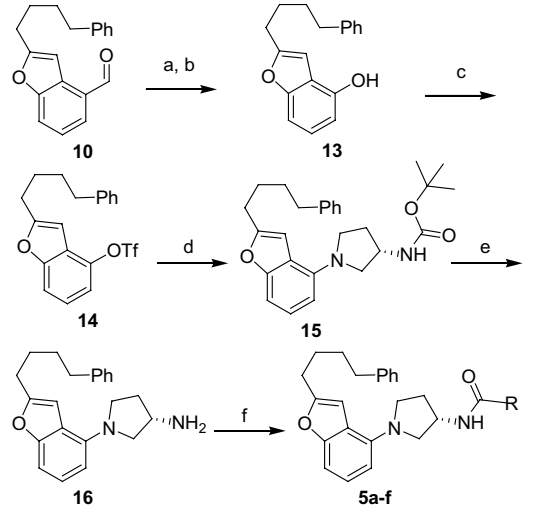
The benzofuran derivatives **4a**–**f** incorporating a simple alkyl side chain were synthesized as shown in Scheme 2. The aldehyde **10** was subjected to a Wittig reaction using (triphenylphosphoranylidene)acetonitrile to afford the unsaturated nitrile **11**, which was directly converted to the final amides **4a**–**d** by hydrogenation over Raney-nickel in the presence of the appropriate carboxylic acid anhydride. On the other hand, compounds **4e**–**f** were prepared through a two step sequence in which nitrile **11** was first reduced to the primary amine **12** and then acylated using cyclopropanecarbonyl chloride or reacted with ethyl isocyanate.



Scheme 2. Reagents and conditions: (a) Ph_3PCHCN, THF, reflux, 95%; (b) $(RCO)_2O$, H_2, Ra-Ni, THF; (c) $NaBH_4$, $CoCl_2{\cdot}H_2O$, MeOH, 90%; (d) R_1COCl, Et_3N, CH_2Cl_2 or R_2NCO, benzene.

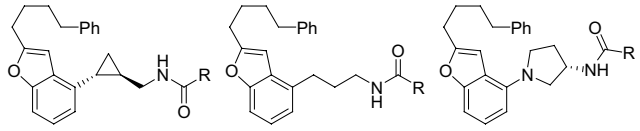
The synthetic route to the aminopyrrolidine derivatives **5a**–**f** is outlined in Scheme 3. The Baeyer–Villiger oxidation of aromatic aldehyde **10** followed by hydrolysis of the resulting aryl formate under acidic conditions provided phenol **13**. Exposure of **13** to triflic anhydride in the presence of pyridine provided the triflate **14** in 69% yield. Coupling of **14** with (*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 **15** in 90% yield. Deprotection of **15** with HCl in ethyl acetate gave amine **16** in 63% yield. The amide and urea products **5a**–**f** were prepared from **16** by treatment with a series of acid chlorides or reaction with ethyl isocyanate.

The K_i values of compounds **3**–**5** for human MT_1 and MT_2 melatonin receptor subtypes were determined in assays using 2-[^{125}I]-iodomelatonin according to the previously described assay method.[16,18] The chemical structures and K_i values of these compounds are reported in Table 1. As can be seen from Table 1, acetamide **3a** and



Scheme 3. Reagents and conditions: (a) MCPBA, $CHCl_3$; (b) HCl, MeOH, 22% (two steps); (c) Tf_2O, pyridine, 0–25 °C, 63%; (d) (*S*)-pyrrolidin-3-yl-carbamic acid *tert*-butyl ester, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 90%; (e) HCl/EtOAc, 69%; (f) R_1COCl, Et_3N, CH_2Cl_2 or R_2NCO, benzene.

Table 1. K_i of compounds **3a**–**f**, **4a**–**f**, and **5a**–**f** competing for the binding of 2-[^{125}I]-iodomelatonin to membrane preparations of NIH3T3 cells stably expressing human MT_1 or MT_2 melatonin receptor



Compd	R	MT_1 K_i (nM)	MT_2 K_i (nM)
Mel	—	0.3	0.7
3a	Me	2.6	10
3b	Et	2.1	2.8
3c	*n*-Pr	10	10
3d	*i*-Pr	23	54
3e	*c*-Pr	16	33
3f	NHEt	33	90
4a	Me	19	127
4b	Et	7.5	60
4c	*n*-Pr	16	82
4d	*i*-Pr	3.7	83
4e	*c*-Pr	6	95
4f	NHEt	21	52
5a	Me	22	14
5b	Et	2.4	6
5c	*n*-Pr	11	7.2
5d	*i*-Pr	105	162
5e	*c*-Pr	42	152
5f	NHEt	39	98

propionamide **3b** from the cyclopropyl series demonstrated high affinity for both MT_1 and MT_2 receptors. The binding affinity of these compounds is sensitive to the identity of the N-acyl group. Replacement of the methyl or ethyl group of the amide side chain by propyl (**3c**), *iso*-propyl (**3d**), or cyclopropyl (**3e**) resulted in a slight decrease in both MT_1 and MT_2 affinity. Replacing the terminal amide of **3a** with a simple ethyl urea provided compound **3f**, which showed a more marked reduction in binding affinity at both receptors. Replacement of the constrained cyclopropane moiety of right hand side chain of the compounds **3a**–**f** by a conformationally flexible alkyl side-chain provided compounds **4a**–**f**, which demonstrated high affinity for the MT_1 receptor and weaker MT_2 affinity. Further structural evolution of the cyclopropyl-bearing side chain in **3a**–**f** to the previously reported heterocyclic aminopyrrolidine gave compounds **5a**–**f**. From this set of derivatives, the propionamide **5b** proved to be the most active ligand for both MT_1 and MT_2 receptors, while acetamide **5a** and butyramide **5c** demonstrated good affinity for both MT_1 and MT_2 receptor subtypes. However, *iso*-propionamide **5d**, cyclopropylcarboxamide **5e**, and urea **5f** showed weak binding at both receptors.

These studies led to the identification of compounds **3a**, **3b**, and **5b** as the three most potent ligands for MT_1 and MT_2 receptors. While the propionamides **3b** and **5b** were more potent than acetamide **3a**, these two compounds were less attractive than **3a** since previous studies in this program had indicated that acetamides generally show better oral bioavailability than propionamides. Consequently, acetamide **3a** was chosen for further evaluation.

Table 2. Pharmacokinetic parameters of **3a**

PK parameters	Rat[a]
IV	
Dose (mg/kg)	1
$t_{1/2}$ (h)	3.8±1
Cl (mL/min/kg)	27.5±2.8
V_d (mL/kg)	557±76
PO	
Dose (mg/kg)	1
F (%)	90

[a] Compound dosed in rats as a solution in PEG-400.

It has been reported that melatonin has a marked ability to enhance α-adrenoceptor-mediated vasoconstriction of the rat tail artery.[13] Thus, the effect of compound **3a** on vascular smooth muscle was evaluated using the method previously described.[19] Compared to melatonin, compound **3a** showed reduced vasoconstrictive activity in assays conducted with rat caudal arteries (0.09 relative to melatonin).[20]

Compound **3a** was further tested for functional activity in NIH3T3 cells expressing melatonin MT_1 or MT_2 receptor using methodology previously described.[19] The agonist activity of the compound was assessed by comparing its ability to inhibit forskolin-stimulated cAMP accumulation with that of melatonin. Full agonist activity was confirmed for compound **3a** at both the human melatonin MT_1 and MT_2 receptor subtypes with EC_{50} s of 0.6 and 21 nM at the MT_1 and MT_2 receptors, respectively. The intrinsic activity relative to melatonin of **3a** was 0.97 and 0.79 at the MT_1 and MT_2 receptors, respectively.

Compound **3a** was further characterized in pharmacokinetic studies that are summarized in Table 2. The oral bioavailability in rats was 90%, significantly superior to the oral bioavailability of melatonin at the same dose (24%). The oral bioavailability of **3a** is presumably due to a combination of good absorption, predicted by the measured Caco-2 permeability (Pc 75 nm/s), and moderate clearance.

In conclusion, the benzoxazole scaffold that projects the 4-phenylbutyl and alkylamide groups was successfully replaced by an isosteric benzofuranyl moiety. This structural modification led to the discovery of a series of benzofuran derivatives as novel melatoninergic ligands and the subsequent identification of compound **3a** as an orally bioavailable agonist at MT_1 and MT_2 melatonin receptors with significantly lower vasoconstrictive activity in vitro in the rat tail artery and a longer biological half-life in rats than the natural ligand, melatonin.

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