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Design and synthesis of benzoxazole derivatives as novel melatoninergic ligands

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Abstract—A novel series of benzoxazole derivatives was synthesized and evaluated as melatoninergic ligands. The binding affinity of these compounds for human MT_1 and MT_2 receptors was determined using 2-[^{125}I]-iodomelatonin as the radioligand. The results of the SAR studies in this series led to the identification of compound **28**, which exhibited better MT_1 and MT_2 receptor affinities than melatonin itself. This work also established the benzoxazole nucleus as a melatoninergic pharmacophore, which served as an isosteric replacement to the previously established alkoxyaryl core.

Melatonin (*N*-acetyl-5-methoxy-tryptamine) (Fig. 1) is an indole-derived neurohormone present in all mammalian species that is synthesized and secreted primarily by the pineal gland in a circadian manner that closely follows the daily light/dark cycle.[1,2] It plays a major role in the regulation of circadian rhythms and the control of seasonal cycles.[3,4] Melatonin alleviates jet lag, regulates delayed sleep phase syndrome,[5] and induces sleep.[6] Moreover, melatonin has been reported to have antioxidant,[7] neuroprotective,[8] antiproliferative,[9] and immunomodulatory[10] properties. 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.[11] 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.[12]

The development of synthetic compounds capable of mimicking the effects of melatonin has progressed considerably during the past decade. These compounds are structurally diverse, and range from simple indole derivatives and its bioisosteres, to phenylalkyl amides and constrained melatoninergic agents.[13] Interestingly, a consistent structural motif found in almost all these melatonin receptor ligands is the presence of an amido group and an alkoxyaryl moiety. Hence, from the SAR available in the literature, these groups appear to be critical in determining the binding affinity and biological activity of these melatoninergic ligands. It should also be noted that as part of this body of work, melatonin receptor ligands have been identified which are selective for either the MT_2[14–21] or MT_1[21–22] receptor. Despite these findings, at the outset of this study there was surprisingly limited SAR established with respect to isosteric replacements of the alkoxyaryl and amido pharmacophoric elements. Herein we report the synthesis and biological activity of benzoxazole derivatives as novel and potent melatoninergic ligands.

A generic structure of the proposed melatonin pharmacophore is shown in Figure 1. The topology of the oxazole ring is such that the oxazole nitrogen may serve as a surrogate hydrogen bond acceptor[23] in place of the

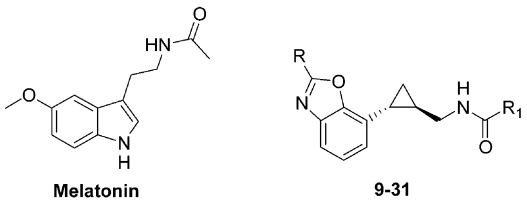

Figure 1.

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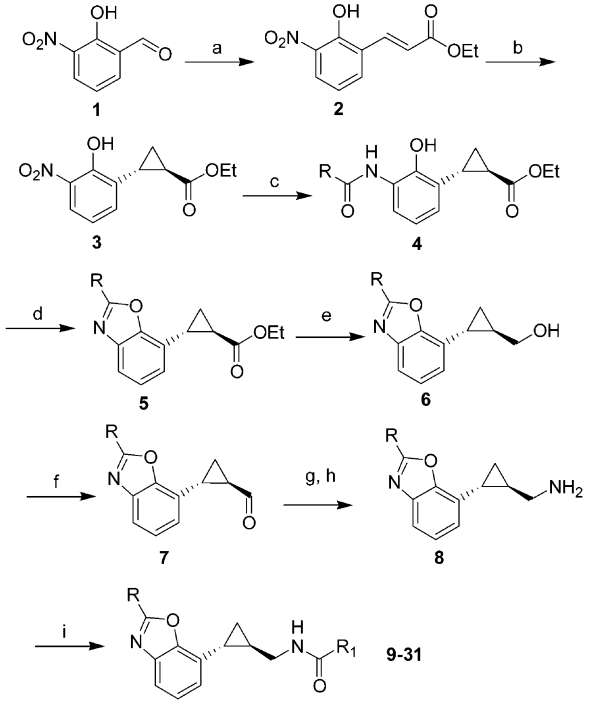
C5 methoxy group of melatonin. Moreover, the cyclopropyl bearing side chain in **9–31** was designed as a replacement for the C3 ethylamide side chain of melatonin.

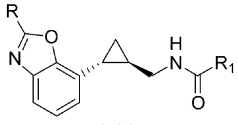
The general route to the melatonin analogues **9–31** is described in Scheme 1. Wittig reaction of commercially available 3-nitrosalicylaldehyde **1** with Ph_3PCHCO_2Et provided exclusively the (±)-*trans*-cinnamic ester **2** in 90% yield. Cyclopropanation of **2** with diazomethane using $Pd(OAc)_2$ as a catalyst gave rise to (±)-*trans*-cyclopropane **3** in 70% yield. The nitro moiety of **3** was reduced and acetylated by catalytic hydrogenation in the presence of an appropriate carboxylic acid anhydride or acid chloride to produce amide **4**. The requisite benzoxazole functionality, as found in **5**, was introduced by subjecting the hydroxy amide **4** to a solution of catalytic amount PPTS in xylene at reflux. The next series of reactions involved converting the ester functionality of **5** to the corresponding reverse amide or urea derivatives. In the event, ester **5** upon treatment with $LiAlH_4$ afforded the corresponding alcohol **6**. Swern oxidation of **6** to the corresponding aldehyde provided intermediate **7**, which was converted to the parent oxime and subsequently reduced to provide the primary amine **8**. Intermediate **8** was then acylated using a series of acid chlorides or reacted with isocyanates to produce the amide and urea products, respectively, **9–31**. It should be noted that these compounds were prepared in racemic form and with the aforementioned anti-stereochemistry about the cyclopropane ring system.

The K_i values of compounds **9–31** 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.[24] The chemical structures, K_i values and MT_1 and MT_2 selectivity ratios of these compounds are reported in Table 1. As is evident from the results, the oxazole nucleus conferred substantial activity to this series of melatoninergic ligands. Moreover, the SARs at both ends of the molecule are well defined. As seen in Table 1, compared to melatonin, 2-ethyl-benzoxazoles **9–14** exhibit excellent affinity for both human MT_1 and MT_2 melatonin receptors. The binding affinity and receptor subtype selectivity of these compounds are sensitive to the identity of the *N*-acyl group. Thus, while acetamide **9** displayed a similar MT_1 affinity to melatonin, this analogue showed a 3-fold decrease in MT_2 affinity. On the other hand, replacement of the methyl group of the amide side chain by an ethyl (**10**), propyl (**11**), *iso*-propyl (**12**), or cyclopropyl (**13**) resulted in a greater increase in MT_1 affinity than in MT_2 binding and therefore increased the overall selectivity for MT_1 receptors. From this set of compounds the cyclopropane carboxamide **13** proved to be the most active ligand for both MT_1 and MT_2 receptors with an 8- and 1.6-fold increased affinity when compared to melatonin at the respective receptors. However, replacing the terminal amide with a simple ethyl urea, as found in compound **14**, resulted in a 9- and 3-fold loss in activity at the MT_1 and MT_2 receptor subtypes, respectively.



Scheme 1. (a) Ph_3PCHCO_2Et, THF, reflux, 90%; (b) $Pd(OAc)_2$, CH_2N_2, ether, 0–25°C, 86%; (c) $(RCO)_2O$, 10% Pd/C, H_2, THF, rt; (d) PPTS, xylene, reflux; (e) $LiAlH_4$, THF; (f) $COCl_2$, DMSO, CH_2Cl_2, Et_3N; (g) $NH_2OH{\cdot}HCl$, NaOH, THF; (h) $NaBH_4$, $CoCl_2{\cdot}H_2O$, MeOH; (i) RCOCl, Et_3N, CH_2Cl_2 or RNCO, benzene.

Table 1. K_i of compounds **9–31** competing for the binding of 2-[^{125}I]-iodomelatonin to membrane preparations of NIH3T3 cells stably expressing human MT_1 or MT_2 melatonin receptors. Values represent mean from experiments performed in duplicate



9-31

Compd	R	R_1	MT_1 K_i (nM)	MT_2 K_i (nM)	Ratio MT_2/MT_1
Mel	—	—	0.4	0.3	0.7
9	Et	Me	0.35	0.88	2.5
10	Et	Et	0.05	0.44	8.8
11	Et	*n*Pr	0.05	0.33	6.6
12	Et	*i*Pr	0.2	0.75	3.8
13	Et	*c*Pr	0.05	0.19	3.8
14	Et	NHEt	3.1	2.2	0.7
15	*n*Pr	Me	0.96	0.13	0.1
16	*n*Pr	Et	0.22	0.08	0.4
17	*n*Pr	*n*Pr	0.94	0.04	0.04
18	*n*Pr	*i*Pr	4.9	0.28	0.06
19	*n*Pr	*c*Pr	0.18	0.14	0.8
20	*n*Pr	NHEt	3.5	1.0	0.3
21	*i*Pr	Me	5.1	1.2	0.2
22	*i*Pr	Et	4.9	0.32	0.07
23	*i*Pr	*n*Pr	1.8	0.8	0.4
24	*i*Pr	*i*Pr	1.3	2.5	1.9
25	*i*Pr	*c*Pr	0.6	2.2	3.7
26	*i*Pr	NHEt	11.8	12.6	1.1
27	$Ph(CH_2)_4$	Me	0.05	0.37	7.4
28	$Ph(CH_2)_4$	Et	0.05	0.07	1.4
29	$Ph(CH_2)_4$	*n*Pr	0.05	0.24	4.8
30	$Ph(CH_2)_4$	*i*Pr	0.13	1.9	14.6
31	$Ph(CH_2)_4$	*c*Pr	0.05	0.48	9.6

Modifications to the left hand aromatic moiety were somewhat limited but included the replacement of the ethyl substituent at the 2-position of the benzoxazole ring system with the homologous *n*-propyl group, compounds **15**–**20**. When compared to the aforementioned 2-ethyl-benzoxazoles, the majority of the 2-propyl-bearing analogues showed a simultaneous decrease in MT_1 affinity along with an increase in MT_2 affinity, albeit the latter was generally slight. As a consequence, 'reversed' selectivity was observed in this series, with analogues showing greater affinity to the MT_2 receptor subtype. For example, compound **11**, a 2-ethyl analogue, showed moderate MT_1 selectivity ($MT_2/MT_1 = 6.6$), while the corresponding 2-propyl compound **17** presented much better MT_2 selectivity ($MT_1/MT_2 = 24$). As observed with the 2-ethyl series, the amide cyclopropyl derivative **19** proved to be a highly potent analogue in the 2-propyl series, but once again at the expense of receptor selectivity.

2-*iso*-Propyl-benzoxazoles **21**–**26** were derived by substitution of the 2-ethyl group in the benzoxazole with an *iso*-propyl group. This structural modification has a greater effect on reducing MT_1 affinity compared to MT_2 binding. Thus, substitution of the ethyl group in compound **9** with an *iso*-propyl moiety (**21**) caused a 15-fold decrease in MT_1 affinity with negligible impact on MT_2 receptor binding. Changing the methyl group of the amide side chain of **21** to an ethyl (**22**), propyl (**23**) or *iso*-propyl (**24**) only slightly affected the potency at both receptor subtypes. Pursuing a similar substitution pattern with the cyclopropyl derivative **25** resulted in the achievement of subnanomolar MT_1 affinity that was accompanied by a 2-fold decrease in MT_2 binding. However, as previously observed, the ethylamino substituent, as in **26**, diminished binding to both receptors. In addition, these compounds showed MT_2/MT_1 selectivity ratios ranging between 1 to 4 and MT_1/MT_2 selectivity ratios varying between 2 to 15.

Of particular interest, a marked increase in binding affinity was achieved by replacing the 2-ethyl group of the benzoxazole **10** with a 4-phenyl-butyl group as in compound **28**. This modification imparted a 6-fold increase in MT_2 affinity while retaining MT_1 affinity comparable to the prototype **10**. Compound **28** was the most potent of this series with MT_1 and MT_2 affinities 8- and 4-fold more potent than melatonin, respectively. Replacement of the propionamide of **28** by acetamide (**27**), butyramide (**29**), or cyclopropylcarboxamide (**31**) caused a slight decrease in MT_2 activity but maintained MT_1 affinity. Pursuing a similar substitution with the *iso*-propyl derivative **30** resulted in a larger decrease in MT_2 potency than in MT_1 affinity, which, in turn, enhanced the MT_1 selectivity ($MT_2/MT_1 = 14.6$).

It has been reported that the methoxy group of melatonin and melatonergic ligands plays an important role in modulating melatonin receptor affinity. It has generally been assumed that the 5-OMe group of melatonin functions as a hydrogen bond acceptor for the atom - OH or NH of serine 115 of the MT_1 receptor.[25] The results of the current study support this hypothesis based on the fact that the oxazole nitrogen is also a hydrogen bond acceptor.[23]

In conclusion, benzoxazole derivatives[26] have been described as novel melatoninergic ligands that demonstrate high binding affinity to both the MT_1 and MT_2 receptors. SARs have been defined around both the terminal amide moiety and the C2 position of the benzoxazole ring. These findings establish the substituted benzoxazole ring system as a novel isostere for the alkoxyaryl moiety of the endogenous ligand and its close analogues.

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26. Selected spectral data. Compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, $J=7.9$ Hz, 1H), 7.16 (t, $J=7.8$ Hz, 1H), 6.86 (d, $J=7.6$ Hz, 1H), 5.99 (br, s, 1H), 3.51–3.42 (m, 1H), 3.26–3.17 (m, 1H), 2.88 (t, $J=7.6$ Hz, 2H), 2.22 (q, $J=7.6$ Hz, 2H), 2.11–2.03 (m, 1H), 1.97–1.84 (m, 2H), 1.58–1.47 (m, 1H), 1.25–1.13 (m, 4H), 1.08–0.97 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 166.8, 149.3, 141.0, 125.7, 124.0, 121.2, 116.7, 43.5, 30.5, 29.6, 21.3, 20.2, 17.1, 13.7, 13.1, 9.8; MS (ESI) 287 $(M+H)^+$. Compound **19**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, $J=7.9$ Hz, 1H), 7.14 (t, $J=7.8$ Hz, 1H), 6.84 (d, $J=7.6$ Hz, 1H), 6.45 (br, s, 1H), 3.51–3.42 (m, 1H), 3.26–3.17 (m, 1H), 2.86 (t, $J=7.4$ Hz, 2H), 2.09–2.01 (m, 1H), 1.94–1.82 (m, 2H), 1.59–1.48 (m, 1H), 1.42–1.33 (m, 1H), 1.21–1.14 (m, 1H), 1.05–0.92 (m, 6H), 0.70–0.64 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 166.8, 149.3, 141.0, 125.8, 124.0, 121.2, 116.6, 43.7, 30.4, 29.5, 21.3, 20.2, 17.1, 14.6, 13.3, 7.0; MS (ESI) 299 $(M+H)^+$. Compound **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, $J=7.8$ Hz, 1H), 7.30–7.26 (m, 2H), 7.20–7.16 (m, 4H), 6.89 (d, $J=7.6$ Hz, 1H), 5.86 (br, s, 1H), 3.52–3.43 (m, 1H), 3.28–3.19 (m, 1H), 2.95 (t, $J=7.3$ Hz, 2H), 2.70 (t, $J=7.6$ Hz, 2H), 2.22 (q, $J=7.6$ Hz, 2H), 2.10–2.03 (m, 1H), 1.99–1.90 (m, 2H), 1.83–1.73 (m, 2H), 1.59–1.49 (m, 1H), 1.25–1.14 (m, 4H), 1.05–0.98 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 166.7, 149.4, 141.9, 141.0, 128.3, 125.8, 124.1, 121.3, 116.7, 43.6, 35.4, 30.8, 29.6, 28.5, 26.4, 21.3, 17.1, 13.2, 9.8; MS (ESI) 377 $(M+H)^+$. Compound **29**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, $J=7.8$ Hz, 1H), 7.30–7.26 (m, 2H), 7.20–7.16 (m, 4H), 6.89 (d, $J=7.5$ Hz, 1H), 5.85 (br, s, 1H), 3.51–3.42 (m, 1H), 3.28–3.19 (m, 1H), 2.95 (t, $J=7.3$ Hz, 2H), 2.70 (t, $J=7.6$ Hz, 2H), 2.17 (t, $J=7.3$ Hz, 2H), 2.11–2.05 (m, 1H), 2.00–1.90 (m, 2H), 1.83–1.73 (m, 2H), 1.72–1.62 (m, 2H), 1.59–1.48 (m, 1H), 1.24–1.17 (m, 1H), 1.04–0.98 (m, 1H), 0.94 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 166.7, 149.4, 141.9, 141.0, 128.3, 125.8, 124.1, 121.3, 116.7, 43.5, 38.7, 35.4, 30.8, 28.5, 26.4, 21.3, 19.1, 17.1, 13.7, 13.2; MS (ESI) 391 $(M+H)^+$.