Bioorganic & Medicinal Chemistry Letters 20 (2010) 2582–2585

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of substituted *N*-[3-(3-methoxyphenyl)propyl] amides as highly potent MT_2-selective melatonin ligands

Yueqing Hu, Maurice K. C. Ho, King H. Chan, David C. New, Yung H. Wong *

Department of Biochemistry, the Biotechnology Research Institute, and the Molecular Neuroscience Centre, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

ARTICLE INFO

Article history:
Received 30 October 2009
Revised 27 January 2010
Accepted 22 February 2010
Available online 25 February 2010

Keywords:
Melatonin
Ligand
SAR

ABSTRACT

A series of substituted *N*-[3-(3-methoxyphenyl)propyl] amides were synthesized and their binding affinities towards human melatonin MT_1 and MT_2 receptors were evaluated. It was discovered that a benzyloxyl substituent incorporated at C6 position of the 3-methoxyphenyl ring dramatically enhanced the MT_2 binding affinity and at the same time decreased MT_1 binding affinity.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a vertebrate neurohormone secreted by the pineal gland during darkness.[1] It regulates the circadian rhythm and can be used to treat diseases associated with the desynchronization of biological rhythms, such as jet-lag, disturbed sleep-wake cycles and seasonal disorders.[2] Melatonin is also involved in a number of other physiological effects and has a variety of therapeutic potentials for the treatment of depression, cancer and neurodegenerative pathologies.[3a–c]

Melatonin exerts its physiological effects through the activation of specific receptors, including two G_i-coupled receptor subtypes MT_1 and MT_2[4a,b] that are widely expressed in different tissues.[5] Both receptors are expressed in the suprachiasmatic nucleus (SCN) which is the master control center of circadian rhythm. Studies in mice suggest that the firing of SCN neurons is suppressed by MT_1 thereby implicating MT_1 in sleep promotion.[6a–c] However, opposing results in meta-analyses of human studies have been reported for the efficacy of melatonin to alleviate sleep disturbance.[7a,b] A more recent study showed that MT_2 may promote sleep in rats.[8] While it has been shown that the phase advancement of rat SCN neuron firing is mediated by MT_2, discrepancies have been observed between different strains of mice.[9a,b] Subtype-selective ligands might help to clarify these inconsistencies.

Although melatonin is involved in a number of biological and physiological processes, its use in clinical applications is quite limited because of its short half-life (15–30 min)[10] and lack of subtype selectivity. Considerable interests have been devoted in the design and synthesis of novel melatonin receptor agonists and antagonists.[11a–c] It is hoped that the new analogues would not only be more metabolically stable, but also be more subtype selective. High affinity and subtype-selective analogues represent potential candidates for drug development as well as valuable experimental tools for the delineation of melatonin receptor pharmacology.[12a,b]

Our research goals were to design and synthesize novel compounds that exhibit potent binding affinity and good subtype selectivity at MT_1 and/or MT_2 receptors. A simplified form of melatonin, *N*-[3-(3-methoxyphenyl)propyl] propionamide was chosen as our starting compound. Despite its simple structure, it has good binding affinity (5.6 nM) towards melatonin receptors.[13] Surprisingly, there are only a few studies on substituted phenylalkyl amides analogues.[14a–c] Here, we report a series of benzyloxyl substituted phenylpropyl amides which showed high binding affinities toward MT_2 receptors.

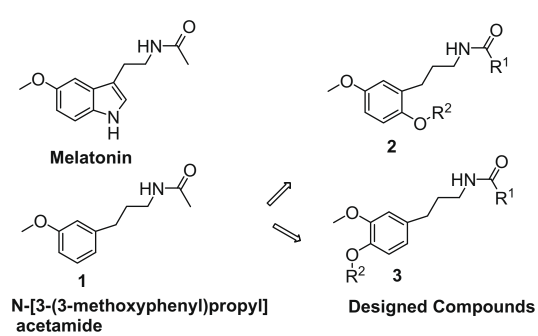


Figure 1. Structures of melatonin, *N*-[3-(3-methoxyphenyl)propyl] acetamide and the designed compounds.

* Corresponding author. Tel.: +852 2358 7328; fax: +852 2358 1552.
E-mail address: boyung@ust.hk (Y.H. Wong).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.02.084

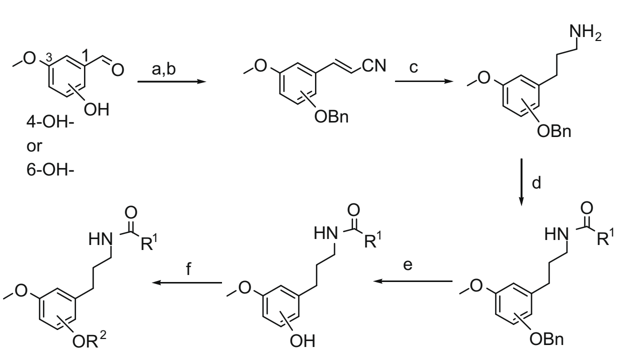
In terms of structure, *N*-[3-(3-methoxyphenyl)propyl] acetamide **1** is a truncated form of melatonin without the nitrogen-containing five member ring (Fig. 1). It has all the key components (aromatic ring, methoxy group, amido trimethylene chain), which are essential for potent binding to melatonin receptors. We decided to use *N*-[3-(3-methoxyphenyl)propyl] acetamide as a template for further optimization in order to discover novel, potent and subtype selective melatonin receptor ligands. A variety of different substituents were incorporated at the C6 (compound **2**) or C4 (compound **3**) positions of the 3-methoxyphenyl ring in order to modulate the binding affinity and selectivity. It was expected that an aromatic ring attached to the 3-methoxylphenyl ring through a linker might provide selectivity towards MT_2 subtype, as an extra phenyl group attached to the adjacent position of the amido ethyl chain was a common structure motif in a number of MT_2-selective ligands discovered so far.[12a]

The synthetic pathway towards the designed compounds of interest is shown in Scheme 1. The process began with commercially available 2-hydroxy-5-methoxybenzaldehyde or vanillin as starting materials. Alkylation of the hydroxyl group with benzyl bromide, followed by Horner–Emmons olefination with diethylcyanomethylphosphate of the aldehydes afforded the α,β-unsaturated nitriles as a mixture of *cis*- and *trans*-isomers in excellent yield. Reaction of the unsaturated nitriles with lithium aluminum hydride in refluxing ether reduced both the double bond and nitrile triple bond in one step to give the amines with modest yield (65%), which were converted to the desired amides by treatment with different acyl chlorides. To modify the C6 or C4 positions of the 3-methoxyphenyl ring, the benzyl group was removed under catalytic hydrogenation. The resulting free phenols were then alkylated with different halides to afford the desired products.[15]

Competitive binding characteristics of the compounds towards human MT_1 and MT_2 melatonin receptor subtypes stably expressed in Chinese hamster ovary (CHO) cells were determined by whole cell binding assays using 1 nM [^{3}H]melatonin as the probe.[16] The K_d of melatonin for MT_1 and MT_2 receptors was 0.296 nM and 0.429 nM, respectively, as determined by saturation binding assays (Supplementary Fig. 1). The K_i values of the compounds for MT_1 and MT_2 as well as their MT_1/MT_2 selectivity ratio are reported in Tables 1–3 and representative displacement curves are shown in Figure 2.

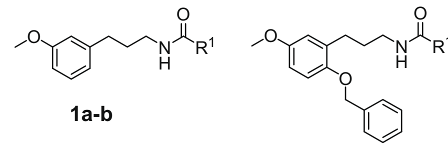
Carboxamides with different R^1 groups were evaluated first. Table 1 shows the melatonin receptor binding affinities for *N*-[3-(3-methoxyphenyl)propyl] amides with a benzyloxyl group substituted at the phenyl ring C6 position. For comparison, the binding data for melatonin and two analogues (**1a** and **1b**) that are unsubstituted at C6 position were also determined. Melatonin showed potent affinity towards both MT_1 and MT_2 receptors with little selectivity. As compared to melatonin, *N*-[3-(3-methoxyphenyl)propyl] acetamide **1a** and propionamide **1b** showed lower affinities toward both receptors with minor changes in selectivity. It was evident that an additional benzyloxy group at C6 position of the 3-methoxyphenyl ring dramatically enhanced the MT_2 affinity to sub-nM and sub-pM range while it decreased MT_1 affinity to over 200 nM. The different amides (with a different R^1 group) also modulated the MT_2 affinity. For compounds **2a**, **2b**, **2c**, and **2d**, the MT_2 binding affinity increased according to the following order of R^1 group: Ph << Me < *n*-Pr ~ Et, with R^1 equals ethyl group as the best. The K_i of compounds **2a–c** towards MT_2 were all in the low or sub-pM range, while their K_i towards MT_1 were over 200 nM (Fig. 2).

Next, the effects of a variety of R^2 groups were evaluated, while R^1 was kept as methyl group (Table 2). Compared to compound **2a** (K_i for MT_2 = 47 pM), compound **2e** without the benzyl group showed only modest binding affinity (K_i = 46.2 nM) towards MT_2. Considering compound **1a** without the extra hydroxyl group was reasonably active towards MT_1 (K_i = 23.3 nM) and MT_2 (K_i = 0.751 nM) binding, the hydroxyl group at C6 position of the



Scheme 1. Reagent and conditions: (a) BnBr, NaH, THF, rt, 100%; (b) NaH, $(EtO)_2PO(CH_2CN)$, THF, 95%; (c) $LiAlH_4$, Et_2O, reflux, ~65%; (d) R^1COCl, Et_3N, CH_2Cl_2, rt, 86–95%); (e) Pd/C, H_2 balloon, rt, 96%; (f) R^2Br or R^2Cl, K_2CO_3, DMF, rt, 100%.

Table 1
Binding affinity of the compounds **1a–1b**, **2a–2d** towards human MT_1 and MT_2 receptors expressed in CHO cells

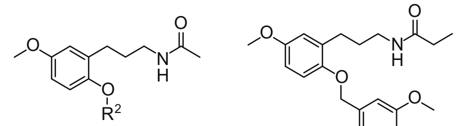


1a-b **2a-d**

	R^1	K_i (nM)		MT_1/MT_2
		MT_1	MT_2	
MT		0.296	0.429	0.69
1a	Me	23.3	0.751	31
1b	Et	85.4	7.07	12
2a[a]	Me	879	0.04705	1.87×10^4
2b[a]	Et	263	0.00055	4.73×10^5
2c[a]	*n*-Pr	1172	0.00103	1.14×10^6
2d	Ph	–	2300	–

[a] Spectral data for these compounds are given as Supplementary data.

Table 2
Binding affinity of the compounds **2a**, **2e–2q**

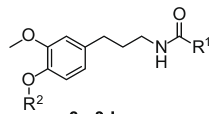


2e-p **2q**

	R^2	K_i (nM)		MT_1/MT_2
		MT_1	MT_2	
2a	Bn	879	0.047	1.87×10^4
2e	H	379	46.2	8.22
2f	Me	1280	26.1	49.1
2g	Et	183	1.59	116
2i	*n*-Pr	400	2.12	189
2j	Ph–$(CH_2)_2$–	2930	13.4	219
2k	Ph–$(CH_2)_3$–	–	72.2	–
2l	4-MeO–Bn	2700	27.9	97
2m[a]	3-MeO–Bn	441	0.0238	1.86×10^4
2n	4-Br-2-F–Bn	–	102	–
2o	3,5-Di-MeO-Bn	–	0.0326	–
2p	2-Py–CH_2–	1840	1.61	1150
2q[a]	3-MeO–Bn	705	0.00069	1.03×10^6

[a] Spectral data for these compounds are given as Supplementary data.

Table 3
Melatonin receptor binding of compounds **3a**–**3d**



3a-3d

	R^1	R^2	K_i (nM)		MT_1/MT_2
			MT_1	MT_2	
3a	Me	Bn	8980	69.3	130
3b	Et	Bn	2010	121	16.7
3c	Me	H	257	12.6	20.5
3d	Me	(3-MeO)Bn	1460	105	13.9

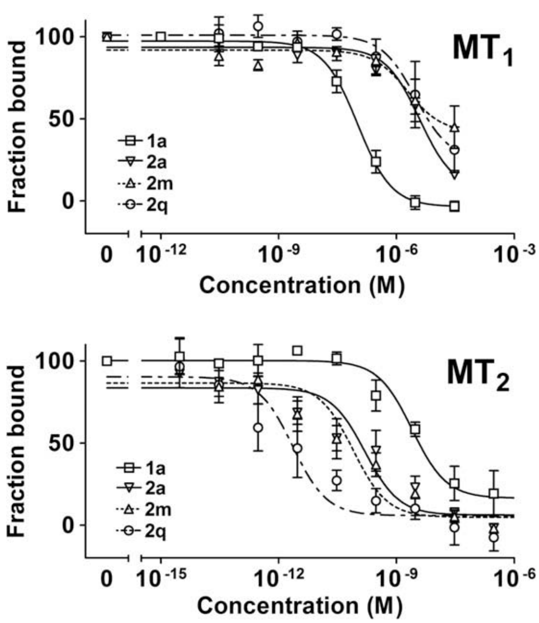


Figure 2. Competitive binding of selected compounds towards MT_1- or MT_2-expressing CHO cells. Data are mean ± SEM of four individual experiments done in duplicates. Correlation coefficients of the fitted dose response curve are in the range of 0.70–0.99 for MT_1, and 0.80–0.94 for MT_2.

3-methoxyphenyl ring was actually detrimental towards both MT_1 and MT_2 affinities. Alkylation of the hydroxyl group with a hydrophobic group, such as methyl, ethyl, propyl, phenethyl or phenylpropyl, restored its modest affinity towards MT_2, but not MT_1 receptor. The distance between the aromatic phenyl ring and the 3-methoxyphenyl core at C6 was also important, as compound **2j** with a three-atom chain as $-CH_2CH_2O-$, and compound **2k** with a four-atom chain $-CH_2CH_2CH_2O-$ did not exhibit potent binding activities towards MT_2. The optimal distance seemed to be a two-atom chain $-CH_2O-$. Alkylations with other aromatic ring substituted methyl group besides benzyl were also evaluated. It was discovered that a methoxyl substituent at C3 position on the benzyl ring was well tolerated towards MT_2 binding, as compounds **2m** and **2o** both exhibited similar K_i values comparing to compound **2a**. However, a methoxyl group at C4 position (compound **2l**) was not well tolerated. 3-Methoxylbenzyl was found to be the optimal group for R^2. It was evident that the aromatic 3-methoxyphenyl ring connected to the C6 position of the 3-methoxyphenyl core through $-CH_2O-$ chain conferred the profound binding affinity towards the MT_2 receptor.

After the optimal groups for R^1 and R^2 groups were identified, compound **2q** incorporating the optimal R^1 at the terminal end of the amide chain and R^2 groups at the C6 position of the 3-methoxyphenyl core (R^1 = Et, R^2 = 3-methoxybenzyl) was synthesized. As predicted, **2q** was extremely potent toward MT_2 receptor with sub-pM binding affinity, while its binding affinity toward MT_1 receptor was 705 nM.

Finally, a couple of alkyloxyl substitutions at C4 position were also evaluated. As shown in Table 3, it was obvious that the benzyloxyl group at C4 position was not as good as at C6 position for enhancing the binding affinities towards both MT_1 and MT_2 receptors.

In summary, we have identified a novel series of C6-benzyloxyl substituted *N*-[3-(3-methoxyphenyl)propyl] amides which showed potent binding affinities toward MT_2 receptor with high selectivity. In particular, several compounds (**2b**, **2c**, **2q**) exhibited pM to sub-pM affinity towards MT_2 and over 200 nM affinity towards MT_1. Preliminary results from FLIPR assays suggest that these compounds are potent MT_2-selective agonists as they stimulated intracellular Ca^{2+} release in CHO cells expressing the MT_2 receptor. The experimentally determined log *P* values of the test compounds are generally in the range of 2.66–3.34, which is comparable to melatonin (log *P* = 2.16) and that they are compatible with the Lipinski's Rule of Five. These easily synthesized compounds represent useful pharmacological tools to further investigate the biological functions of the MT_2 receptor. It remains to be determined if these compounds have better pharmacokinetic properties than melatonin to warrant their evaluation as drug candidates.

Acknowledgements

This study was supported in part by grants from the Hong Kong Jockey Club, the Innovation and Technology Fund (ITS/113/03), RGC (660108) and UGC (AoE/B-15/01) of Hong Kong.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.084.

References and notes

1. Reiter, R. J. *Endocrinol. Rev.* **1991**, *12*, 151.
2. Kennaway, D. J.; Wright, H. *Curr. Top. Med. Chem.* **2002**, *2*, 199.
3. (a) Blask, D. E.; Sauer, L. A.; Dauchy, R. T. *Curr. Top. Med. Chem.* **2002**, *2*, 113; (b) Dubocovich, M. L.; Markowska, M. *Endocrine* **2005**, *27*, 101; (c) Pandi-Perumal, S. R.; Trakht, I.; Srinivasan, V.; Spence, D. W.; Maestroni, G. J. M.; Zisapel, N.; Cardinali, D. P. *Prog. Neurobiol.* **2008**, *85*, 335.
4. (a) Dubocovich, M. L.; Rivera-Bermudez, M. A.; Gerdin, M. J.; Masana, M. I. *Front. Biosci.* **2003**, *8*, d1093; (b) New, D. C.; Tsim, S. T.; Wong, Y. H. *Neurosignals* **2003**, *12*, 59.
5. Ekmekcioglu, C. *Biomed. Pharmacother.* **2006**, *60*, 97.
6. (a) Liu, C.; Weaver, D. R.; Jin, X.; Shearman, L. P.; Pieschl, R. L.; Gribkoff, V. K.; Reppert, S. M. *Neuron* **1997**, *19*, 91; (b) Jin, X.; von Gall, C.; Pieschl, R. L.; Gribkoff, V. K.; Stehle, J. H.; Reppert, S. M.; Weaver, D. R. *Mol. Cell Biol.* **2003**, *23*, 1054; (c) Stehle, J.; Vanecek, J.; Vollrath, L. *J. Neural Transm.* **1989**, *78*, 173.
7. (a) Buscemi, N.; Vandermeer, B.; Hooton, N.; Pandya, R.; Tjosvold, L.; Hartling, L.; Vohra, S.; Klassen, T. P.; Baker, G. *BMJ* **2006**, *332*, 385; (b) Brzezinski, A.; Vangel, M. G.; Wurtman, R. J.; Norrie, G.; Zhdanova, I.; Ben-Shushan, A.; Ford, I. *Sleep Med. Rev.* **2005**, *9*, 41.
8. Fisher, S. P.; Sugden, D. *Neurosci. Lett.* **2009**, *457*, 93.
9. (a) Hunt, A. E.; Al-Ghoul, W. M.; Gillette, M. U.; Dubocovich, M. L. *Am. J. Physiol. Cell Physiol.* **2001**, *280*, C110; (b) Dubocovich, M. L.; Hudson, R. L.; Sumaya, I. C.; Masana, M. I.; Manna, E. *J. Pineal Res.* **2005**, *39*, 113.
10. Claustrat, B.; Brun, J.; Chazot, G. *Sleep Med. Rev.* **2005**, *9*, 11.
11. (a) Mor, M.; Plazzi, P. V.; Spadoni, G.; Tarzia, G. *Curr. Med. Chem.* **1999**, *6*, 501; (b) Zlotos, D. P. *Arch. Pharmacol. Chem. Life Sci.* **2005**, *338*, 229; (c) Garratt, P. J.; Tsotinis, A. *Min-Rev. Med. Chem.* **2007**, *7*, 1075.
12. (a) Boutin, J. A.; Audinot, V.; Ferry, G.; Delagrange, P. *Trends Pharmacol. Sci.* **2005**, *26*, 412; (b) Mody, S. M.; Hu, Y.; Ho, M. K. C.; Wong, Y. H. *Recent Pat CNS Drug Discovery* **2007**, *2*, 241.
13. Garratt, P. J.; Travard, S.; Vonhoff, S.; Tsotinis, A.; Sudgen, D. *J. Med. Chem.* **1996**, *39*, 1797.

14. (a) Takaki, K. S.; Karageorge, G. N.; Keavy, D. J.; Parker, M. F.; Watson, B. T. Eur. Pat Appl. EP 706994, 1996.; (b) Pegurier, C.; Curtet, S.; Nicolas, J. P.; Boutin, J. A.; Delagrange, P.; Renard, P.; Langlois, M. *Bioorg. Med. Chem.* **2000**, *8*, 163; (c) Sun, L.-Q.; Takaki, K.; Chen, J.; Bertenshaw, S.; Iben, L.; Mahle, C. D.; Ryan, E.; Wu, D.; Gao, Q.; Xu, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1345.
15. All of the compounds submitted for bioassays were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS, etc.
16. CHO cells stably expressing human MT_1 or MT_2 receptor have been described and characterized previously (New, D. C.; Wong, Y. H. *Assay Drug Dev. Technol.* **2004**, *2*, 269–280). Dissolution of test compounds and dilution methods were as described in the Supplementary data. Competitive binding assays were performed as described (Ho, M. K.; New, D. C.; Wong, Y. H. *Neurosignals* **2002**, *11*, 115–122.; Tian, Y.; New, D. C.; Yung, L. Y.; Allen, R. A.; Slocombe, P. M.; Twomey, B. M.; Lee, M. M.; Wong, Y. H. *Eur. J. Immunol.* **2004**, *34*, 785–795) with modifications for intact cells. Briefly, 1.5×10^5 cells were suspended in binding buffer (50 mM Tris, 2 mM $MgCl_2$, 1 mM EGTA, pH7.4) containing 1 nM [^{3}H]melatonin and increasing concentrations of a test compound. Assays were carried out at 4 °C for 60 min with occasional agitation and then terminated by rapid filtration through GF/C filters pre-soaked in 10 mM Tris, pH 7.4. Bound radioactivity was counted in Wallac 1450 Microbeta Jet scintillation counter. Competitive curves were fitted using a one-site competition nonlinear regression (GraphPad Prism 3.03). Data were means of 2–3 independent experiments performed in duplicates. Standard errors were typically within 10% of the mean value. Melatonin was employed as standard reference in every assay with reproducible K_i. K_i values were calculated using the Cheng–Prusoff equation.