Bioorganic & Medicinal Chemistry Letters 21 (2011) 1236–1242

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Design and synthesis of 4-arylpiperidinyl amide and *N*-arylpiperdin-3-yl-cyclopropane carboxamide derivatives as novel melatonin receptor ligands

Guiying Li [a,*], Hao Zhou [a], Yu Jiang [a], Holger Keim [a], Sidney W. Topiol [a], Suresh B. Poda [b], Yong Ren [b], Gamini Chandrasena [a], Darío Doller [a]

[a] Department of Chemical & Pharmacokinetic Sciences, Lundbeck Research USA, 215 College Rd., Paramus, NJ 07652, USA
[b] Department of Biological Research, Lundbeck Research USA, 215 College Rd., Paramus, NJ 07652, USA

ARTICLE INFO

*Article history:*
Received 21 October 2010
Revised 8 December 2010
Accepted 15 December 2010
Available online 19 December 2010

*Keywords:*
Melatonin
Melatonergic ligands

ABSTRACT

Two series of 4-arylpiperidinyl amide and *N*-arylpiperdin-3-yl-cyclopropane carboxamide derivatives exhibiting diverse functionality at rat $MT_1$ and $MT_2$ receptors are reported. Compounds **11f** and **18b** ($MT_1/MT_2$ agonist) have human microsomal intrinsic clearance comparable to ramelteon.



Melatonin (Fig. 1) is a pineal gland hormone, that is, secreted during darkness and plays an important role in the synchronization of circadian rhythms in both human and mammals.[1,2] It is synthesized from serotonin by the consecutive actions of two enzymes, 5-HT-*N*-aceyltransferase and hydroxyindole-*O*-methyltransferase.[3] In mammals, its biological actions are mediated via two high-affinity ($K_i$ values ca. 1 nM) G-protein coupled receptors, $MT_1$ and $MT_2$.[4–7] A third subtype, $Mel_{1c}$, is expressed in avian species but not in mammals.[4] In addition, a melatonin-sensitive form of the human enzyme Quinone Reductase 2, known as $MT_3$ with lower affinity for melatonin ($K_i$ values in the range of 10–60 nM), has been identified.[8] Melatonin has been suggested as a therapeutic agent for the treatment of delayed sleep phase syndrome,[9] jet lag,[10] shift work disturbances,[11] aging,[12] affective disorders associated with biological rhythm disturbances,[13,14] etc. However, its pharmacological use is limited due to its short biological half-life, poor bioavailability, and ubiquitous action.[15] Thus, during the past decade, considerable efforts have been devoted to developing melatonergic ligands which are characterized by a better pharmacokinetic profile, and/or subtype selectivity. A variety of structurally diverse melatonergic ligands, which range from simple indole derivatives and their bioisosteres to phenylalkyl amides and constrained derivatives, have been reported and elegantly summarized in several recent reviews.[16–20] More specifically, several melatonin ligands of existing chemical series related to our work, such as phenylalkyl amides **1**[21] and **2**,[22] *N*-(substituted-anilinoethyl)amide **3**,[23] and aminopyrrolidine derivatives **4** and **5**[24] are shown in Figure 1. Two dual $MT_1/MT_2$ agonists, agomelatine and ramelteon, are currently on the market, the former, also having weak 5-$HT_{2C}$ antagonism activity.[25,26] Upon embarking on our melatonin receptor modulation exploratory program, one of our main goals was to identify ligands with different subtype selectivity and with reasonable pharmacokinetic profile for evaluating the pathophysiological functions of $MT_1$ and $MT_2$ receptors in rat behavioral models. Hence, we developed binding and high throughput functional FLIPR assays against rat $MT_1$ and $MT_2$ receptors. We report herein the design, synthesis and SAR of two chemotypes, 4-arylpiperidinyl amides and *N*-arylpiperidin-3-yl-carboxamides exhibiting a diverse range of functional activities. In addition, we present a comparison of rat pharmacokinetic properties of select compounds with agomelatine and ramelteon.

The 4-arylpiperidinyl amide template (e.g., compound **11**) was derived from reported phenylalkyl amides **1** and **2** by conformational constraint (Fig. 2). The general synthetic route for analogs in this series is depicted in Scheme 1. Suzuki coupling of commercially available boronic acid **7** with an appropriate aryl iodide or bromide **6** produced compound **8**. Catalytic hydrogenation of compound **8** afforded compound **9**. Removal of the *N*-Boc protecting group from compound **9** was carried out using TFA, producing the TFA salt of amine **10**. Amidation of **10** with an appropriate acyl chloride $R^2COCl$ afforded amides **11a** to **11l**, **11p–t**, respectively. All reactions proceeded in good to excellent yield. De-methylation of compound **10** using $BBr_3$ in $CH_2Cl_2$ produced phenol **12** in 15%

* Corresponding author. Tel.: +1 201 350 0140.
*E-mail address:* guli@lundbeck.com (G. Li).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.12.068

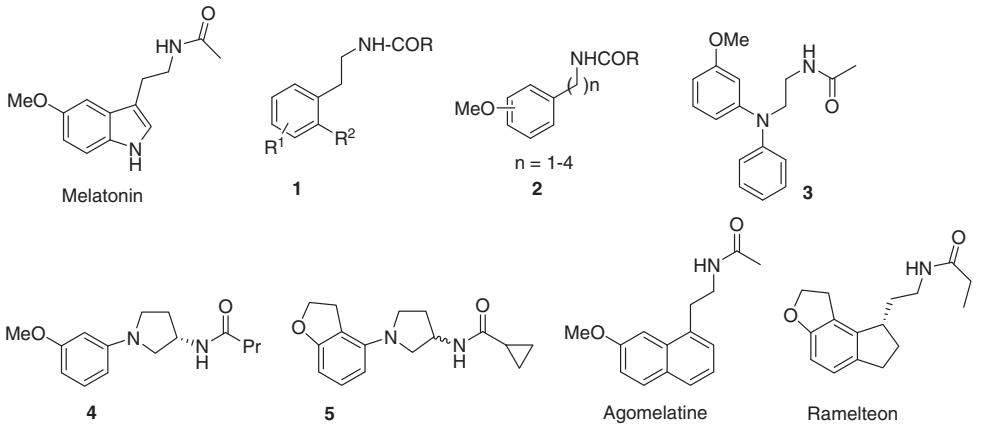



**Figure 1.** Melatonin receptor ligands.

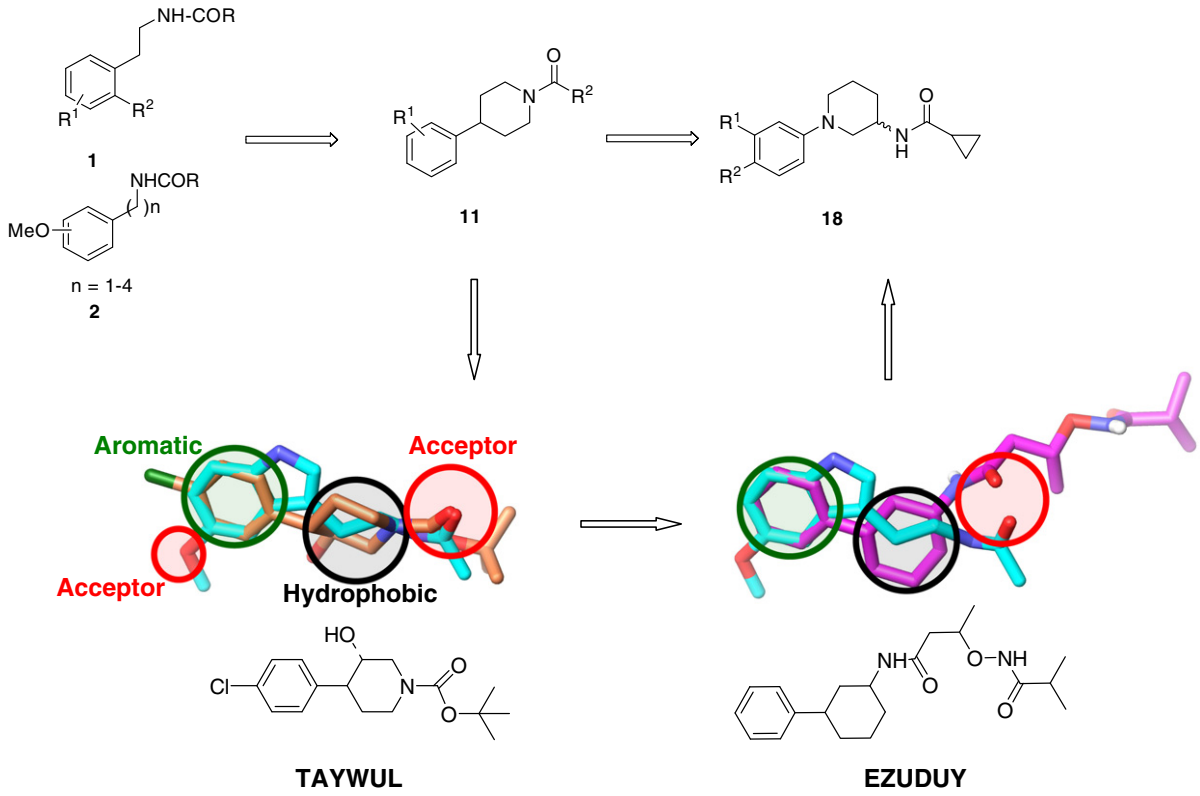
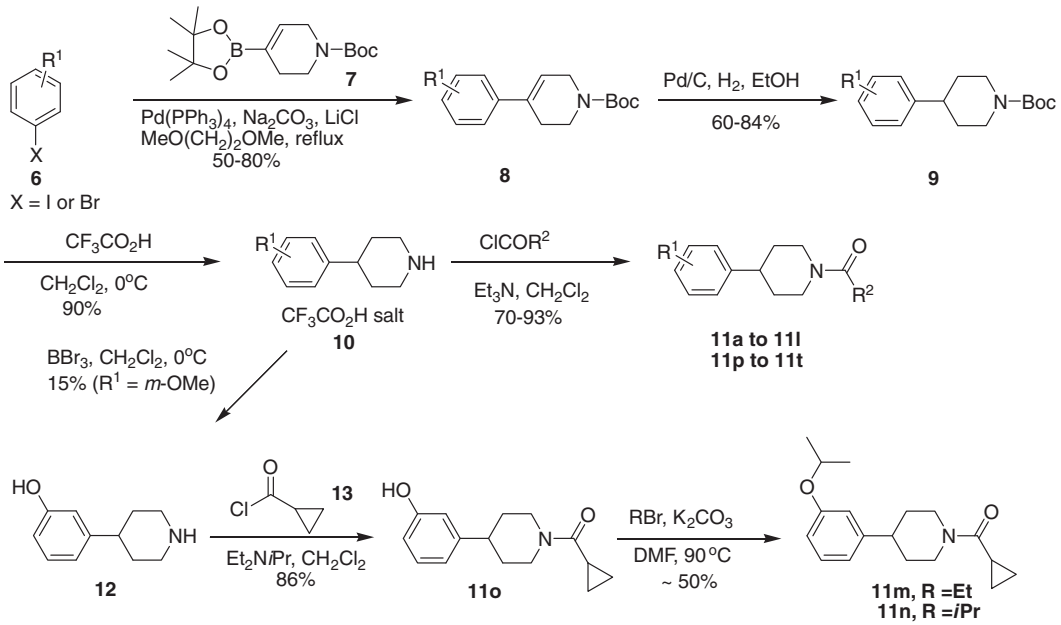



**Figure 2.** Design of novel melatonin ligands.

yield (unoptimized reaction conditions). Amidation of **12** with cyclopropylcarbonyl chloride **13** gave compound **11o** in 86% yield, which upon alkylation with ethyl bromide or isopropyl bromide in the presence of base $K_2CO_3$ in DMF at 90 °C afforded compound **11m** and **11n**, respectively, in about 50% yield. Experimental procedures, $^1$H NMR and/or LC–MS data are included as Supplementary data.

Amides **11a–t** were evaluated in vitro for their binding and functional activity at rat $MT_1$ and $MT_2$ receptors, and results are summarized in Table 1. The SAR of this template is narrower when compared to phenylalkylamides **1**[21] and **2**.[22] On the piperidinyl aryl ring, changing the position of the methoxy group from *m*- to *o*- or *p*-, or increasing methoxy to ethoxy resulted in a dramatic loss in potency. With respect to the amide, isopropyl and cyclopropyl are optimal. The most promising compound in this series is compound **11f**, which has four-fold receptor selectivity toward $MT_2$ receptor ($MT_1$ $K_i$/$MT_2$ $K_i$ = 4)

Using 3D pharmacophoric features coupled with X-ray crystallography structures we identified other targets as follows. Upon searching the Cambridge Crystallographic Database,[28] we found X-ray structures of both melatonin (CCDC ID: MELATN03) and compounds with the core of **11**. Using one of these structures (CCDC ID: TAYWUL) we found that an overlay as depicted in Figure 2 suggested a four-point pharmacophore as shown. Previous studies using pharmacophore and homology models on other rigidified analogs of melatonin[29,30] proposed that the side chain of melatonin and rigid analogs thereof are out-of-plane with respect to the indole core. For those analogs, X-ray structures of the compounds and their analogs were supportive of the models. The X-ray structure of melatonin adopts a nearly in-plane conformation of the side




**Scheme 1.** Synthesis of 4-arylpiperidinyl amides.

chain. For our series', for example, compound **11**, the melatonin X-ray structure yields a better overlap of the key features and was adopted herein as the template. We then considered a number of variations of **11** with different rings, topologies with respect to heteroatom placement, etc. We searched the CCDC for examples of each of the considered cores and found that in one such case, that is, the core shown as **18**, the related X-ray structure (CCDC ID: EZUDUY) allowed for a good overlap of three of the corresponding pharmacophore features with obvious access to introduction of the fourth via substitution at the *meta* position of the phenyl group. Thus, the right side of **11** was kept constant as cyclopropyl, and the core was modified to a 3-amino-piperdinyl (**18**, Fig. 2).

The general synthesis of *N*-arylpiperidin-3-yl-cyclopropane carboxamides (e.g., **18**) is depicted in Scheme 2. Amidation of commercially available **13** and **14** (racemate was used for the synthesis of **18a**; *S*-enantiomer was used for the synthesis of **18b**, **18d**, **18f**, **18g** and **18h**; *R*-enantiomer was used for the synthesis of **18c** and **18e**) produced compound **15**. Removal of the *N*-Boc protecting group of **15** yielded amine **16**. Reaction yields were excellent. *N*-arylation of **16** with commercially available aryl halide **17** afforded compounds **18a**–**g** in 10–50% yield. The reaction conditions were not optimized. De-methylation of compound **18b** with $BBr_3$, followed by alkylation with $Br(CH_2)_2OSi(Me)_2tBu$, and then treatment with TBAF, afforded **18h** in unoptimized 41% yield. Experimental procedures, $^1H$ NMR and LC–MS data are in Supplementary data.

The in vitro functional and binding activity of compounds **18a**–**h** were evaluated, and results are summarized in Table 2. It appears that chirality affects the affinity and the nature of the functional response. For example, the *S*-enantiomer 3-methoxy analog **18b** was a potent $MT_1/MT_2$ partial to full agonist, which is similar to the literature compound *S*-aminopyrrolidine amide **4** (human $MT_1$ $K_i$ = 3.7 nM, $MT_2$ $K_i$ = 2.6 nM);[24] while its *R*-enantiomer **18c** was a $MT_2$ antagonist with weak partial agonist activity toward the rat $MT_1$ receptor. The corresponding racemic compound **18a** showed full $MT_1/MT_2$ agonist activity in the FLIPR functional assay with about six-fold selectivity toward $MT_2$ receptor in the binding assay ($MT_1$ $K_i/MT_2$ $K_i$ = 5.6). Reduced electron density appears to have negative impact on binding affinity. For example, replacing –OMe with $–OCHF_2$ (**18f**) or $–OCF_3$ (**18g**) resulted in significantly loss of affinity to both $MT_1$ and $MT_2$ receptors. Both **18f** and **18g** were partial $MT_2$ agonists and weak $MT_1$ antagonists in the FLIPR functional assay. Chlorine on the *para*-position of the phenyl moiety was well tolerated. The *S*-enantiomer **18d** was a potent dual $MT_1/MT_2$ partial agonist, while its *R*-enantiomer **18e** was a $MT_1/MT_2$ full antagonist with about four-fold binding selectivity toward $MT_2$ receptor. The effect of chirality on receptor binding was also reported in the literature for bicyclic aminopyrrolidine amide **5** (The *S*-enantiomer of **5** was a non selective $MT_1/MT_2$ ligand: human $MT_1$ $K_i$ = 1.2 nM, human $MT_2$ $K_i$ = 2.8 nM; while the *R*-enantiomer of **5** had about nine-fold binding selectivity toward $MT_2$ receptor: human $MT_1$ $K_i$ = 3.9 nM, human $MT_2$ $K_i$ = 35 nM).[24] 5-HEAT, a melatonin analog with $–O(CH_2)_2OH$ replacing methoxy, has been reported to be a full agonist at human $MT_1$ and an antagonist/weak partial agonist at human $MT_2$ with lower affinity than melatonin.[31] In our case, the $–O(CH_2)_2OH$ analog **18h** was a full antagonist at both rat $MT_1$ and $MT_2$ receptors.

Next, we explored the hypothesis that core modification of *N*-arylpiperdin-3-yl-cyclopropane carboxamides could improve affinity and/or binding selectivity. Results are summarized in Figure 3. Replacement of the piperdin-3-yl moiety with pyridyl led to compound **19**, a very weak $MT_1$ partial antagonist and $MT_2$ partial agonist. Incorporation of a phenyl on the piperidin-3-yl moiety led to compound **20** (racemate), a $MT_1$ full/ $MT_2$ partial agonist with 11-fold binding selectivity toward $MT_2$ receptor. In comparison, the non-constrained *N*-phenyl substituted anilinoethyl amide **3** (also known as UCM765), a highly potent human $MT_2$-selective partial agonist ($MT_1$ $K_i$ = 4.2 nM, IAr = 0.79; $MT_2$ $K_i$ = 0.07 nM, IAr = 0.61),[23] was also an $MT_2$-selective partial agonist in our rat receptor assays ($rMT_1$ $K_i$ = 290 nM, FLIPR $EC_{50}$ = 78 nM, IA% = 81; $rMT_2$ $K_i$ = 8.7 nM, FLIPR $EC_{50}$ = 2.3 nM, IA% = 57). Replacement of the piperdin-3-yl moiety with pyrrolidin-2-yl-methyl was well tolerated. Compound **21** (racemate) was an $MT_1/MT_2$ full agonist with six-fold selectivity toward rat $MT_2$ receptor. Synthesis of compound **19** is illustrated in Scheme 3. Compounds **20** and **21** were made using the same route described in Scheme 2 for the synthesis of **18**, from cyclopropanecarboxylic acid (1,2,3,4-tetrahydro-quinolin-3-yl)-amide, which was made from the amidation of 3-aminoquinoline and cyclopropanecarbonyl chloride followed by hydrogenation, and *tert*-butyl 2-(aminomethyl)pyrrolidine-1-carboxylate, respec-

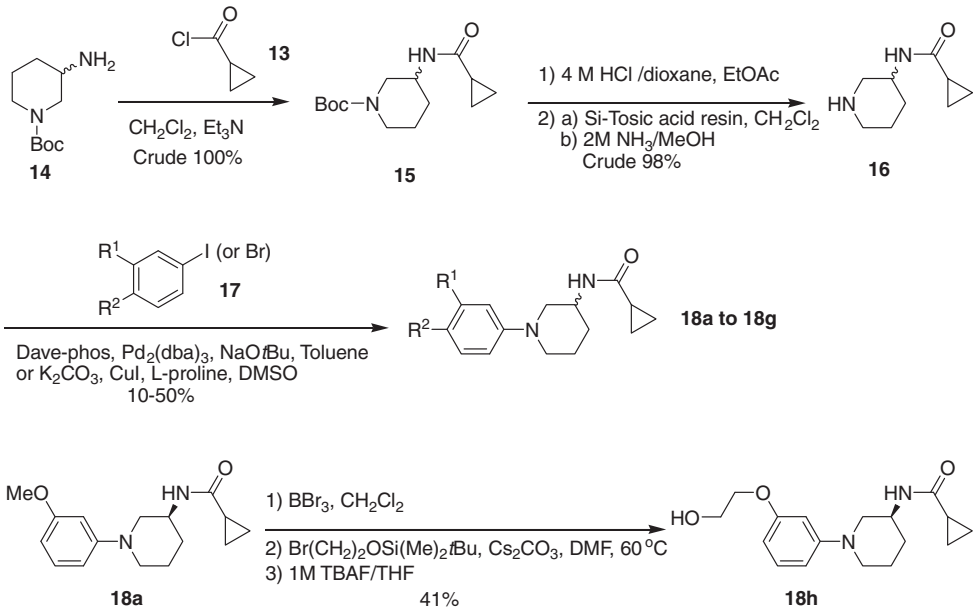
**Table 1**
Binding affinity and functional activity of 4-arylpiperidinyl amides[27]

| Compd | $R^1$ | $R^2$ | $EC_{50}$ (nM) (IA%) | | $K_i$ (nM) | |
|---|---|---|---|---|---|---|
| | | | $rMT_1$ | $rMT_2$ | $rMT_1$ | $rMT_2$ |
| Melatonin | | | 0.56 ± 0.1 (97 ± 1) | 1.1 ± 0.4 (97 ± 3) | 0.99 ± 0.01 | 0.58 ± 0.07 |
| Agomelatine | | | 2.1 ± 0.1 (94 ± 10) | 1.4 ± 0.2 (100 ± 2) | 1.2 ± 0.1 | 0.14 ± .0.01 |
| Ramelteon | | | 3.1 ± 0.6 (100 ± 4) | 0.89 ± 0.02 (95 ± 4) | 2.0 ± 0.1 | 0.03 ± 0.007 |
| **11a** | *m*-OMe | Me | >10,000 (6 ± 1) | >10,000 (19 ± 1) | n.d. | n.d. |
| **11b** | *m*-OMe | Et | 1300 ± 275 (31 ± 4) | 42 ± 9 (66 ± 14) | 810 ± 270 | 150 ± 37 |
| **11c** | *m*-OMe | *i*Pr | 98 ± 6 (87 ± 10) | 19 ± 9 (76 ± 3) | 160 ± 62 | 31 ± 3.6 |
| **11d** | *m*-OMe | 1-Ethylpropyl | 2300 ± 380 (32 ± 3) | 790 ± 62 (35 ± 2) | n.d. | n.d. |
| **11e** | *m*-OMe | *i*Bu | 1700 ± 300 (39 ± 1) | 1800 ± 200 (35 ± 2) | n.d. | n.d. |
| **11f** | *m*-OMe | Cyclopropyl | 65 ± 1 (41 ± 5) | 9.5 ± 2 (74 ± 4) | 40 ± 4 | 10 ± 0.8 |
| **11g** | *m*-OMe | (1*S*,2*S*)-2-Methyl-cyclopropyl | 610 ± 190 (19 ± 0.5) | 140 ± 16 (65 ± 2) | 150 ± 25 | 35 ± 4 |
| **11h** | *m*-OMe | 2,2-Dimethyl-cyclopropyl | 640 ± 158 (33 ± 3) | 147 ± 16 (30 ± 2) | n.d. | n.d. |
| **11i** | *m*-OMe | Cyclobutyl | 380 ± 29 (52 ± 0.1) | 200 ± 9 (62 ± 3) | 140 ± 42 | 67 ± 23 |
| **11j** | *m*-OMe | Cyclopentyl | 210 ± 20 (67 ± 0.5) | 110 ± 7 (74 ± 10) | n.d. | n.d. |
| **11k** | *m*-OMe | Cyclohexyl | 7500 ± 620 (42 ± 13) | 2800 ± 200 (36 ± 17) | n.d. | n.d. |
| **11l** | *m*-OMe | Ph | >10,000 (12 ± 1) | 4400 ± 140 (60 ± 5) | 650 ± 80 | 110 ± 7 |
| **11m** | *m*-OEt | Cyclopropyl | n.d. | n.d. | 5700 ± 370 | 4100 ± 34 |
| **11n** | *m*-O*i*Pr | Cyclopropyl | >10,000 (9 ± 1) | >10,000 (10 ± 2) | n.d. | n.d. |
| **11o** | *m*-OH | Cyclopropyl | >10,000 (96 ± 8) | 3600 ± 135 (23 ± 2) | n.d. | n.d. |
| **11p** | *m*-Me | Cyclopropyl | >10,000 (0.4 ± 0.1) | >10,000 (15 ± 1) | n.d. | n.d. |
| **11q** | *o*-OMe | Cyclopropyl | >10,000 (2 ± 0.1) | >10,000 (3 ± 1) | n.d. | n.d. |
| **11r** | *p*-OMe | Cyclopropyl | >10,000 (58 ± 3) | 5000 ± 100 (94 ± 9) | 1100 ± 127 | 870 ± 78 |
| **11s** | *p*-OMe | Me | 1700 ± 208 (34 ± 3) | 3400 ± 190 (140 ± 17) | 4700 ± 260 | 360 ± 60 |
| **11t** | *m*-OMe | OMe | >10,000 (9 ± 1) | 1000 ± 280 (54 ± 4) | 2000 ± 410 | 860 ± 400 |

tively; and using an Ullmann coupling for the *N*-arylation step. Experimental procedures, $^1$H NMR and LC–MS data are in Supplementary data.

The most promising compounds, **11f** (4-arylpiperidinyl amide series), **18b**, **18d**, and **18f** (*N*-arylpiperidine-3-yl-cyclopropane carboxamide series), were further evaluated for in vitro human and rat microsomal stability (Table 3). All showed high human microsomal intrinsic clearance ($CL_{INT}$), comparable to that of ramelteon but lower than agomelatine. Compound **11f** had comparable rat in vitro microsomal clearance to that of agomelatine and ramelteon, while compounds **18b**, **18d** and **18f** had higher rat in vitro microsomal clearance than agomelatine and ramelteon. Reducing the electron density of the oxygen in the methoxyphenyl moiety of **18b** with $-OCHF_2$ (**18f**) or blocking one of the potential metabolic sites with a chlorine atom on the phenyl ring (**18d**) resulted in no improvements of microsomal stability. Compounds **11f** and **18b** were tested in vivo in order to compare rat plasma and brain levels with agomelatine and ramelteon. When the compounds were dosed via intraperitoneal (IP) injection and 20% 2-hydroxypropyl-β-cyclodextrin (HP-βCD) was used as dosing vehicle, only ramelteon showed reasonable exposure (Table 3), while agomelatine had highly variable exposure (rat 1: [plasma] = 9.6 ng/mL, [brain] = 15 ng/mL; rat 2: [plasma] = 64 ng/mL, [brain] = 176 ng/mL). **11f** and **18b** had extremely low plasma and brain exposures(<5 ng/mL), which is presumably caused by high first pass metabolism, and perhaps solubility limited absorption. It was noted that agomelatine, **11f** and **18b** were dosed as suspensions rather than solutions in the vehicle used. Different vehicles were screened to make possible solution-dosing of these compounds, and their exposures were enhanced (Table 3). One hour after IP dosing, agomelatine, ramelteon and **11f** showed good ability to cross the blood–brain barrier; albeit compound **18b** less so. The brain-to-plasma ratios of compounds **11f** and agomelatine were similar, and higher than that of ramelteon. The brain-to-plasma ratio of compound **18b** was about 15% of agomelatine and 30% of ramelteon, respectively. The calculated physicochemical properties of these compounds (MW 243–310, *c*Log*P* 1.9–2.8, tPSA 30–42 Å$^2$, H-bond donor 0–1, H-bond acceptor 2) are all within the range targeted for CNS drugs, and they all showed good membrane permeability (19.8–25.0 × $10^{-6}$ cm/sec) in a PAMPA assay.[33] The observed low B/P ratio of **18b** may be caused by transporter efflux, which needs to be further investigated.

In summary, two novel series (4-arylpiperdinyl amide and *N*-arylpiperdin-3-yl-cyclopropane carboxamide derivatives) are reported




**Scheme 2.** Synthesis of *N*-arylpiperdin-3-yl-cyclopropane carboxamides.

**Table 2**
Binding affinity and functional activity of *N*-aryl piperidin-3-yl-cyclopropane carboxamides[27]

| Compd | Chirality | $R^1$ | $R^2$ | $EC_{50}$ (nM) (IA%) | | $IC_{50}$ (nM) (Inh. %) | | $K_i$ (nM) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $rMT_1$ | $rMT_2$ | $rMT_1$ | $rMT_2$ | $rMT_1$ | $rMT_2$ |
| **18a** | (±) | OMe | H | 9.7 ± 1.7 (85 ± 2.8) | 2.9 ± 0.6 (99 ± 0.8) | >10,000 | >10,000 | 15 ± 0.3 | 2.7 ± 0.1 |
| **18b** | S | OMe | H | 3.9 ± 0.6 (77 ± 3) | 6.2 ± 0.2 (74 ± 7) | n.d. | n.d. | 9.9 ± 0.9 | 2.7 ± 0.4 |
| **18c** | R | OMe | H | 3500 ± 1000 (30 ± 1) | 93 ± 9 (23 ± 10) | n.d. | 10 ± 1 (97 ± 2) | 1000 ± 27 | 95 ± 32 |
| **18d** | S | OMe | Cl | 0.74 ± 0.1 (73 ± 3) | 2.3 ± 0.5 (65 ± 4) | n.d. | n.d. | 1.2 ± 0.7 | 2.1 ± 0.6 |
| **18e** | R | OMe | Cl | >10,000 (19 ± 5) | >10,000 (23 ± 5) | 440 ± 59 (94 ± 3) | 24 ± 5 (98 ± 2) | 130 ± 24 | 31 ± 4 |
| **18f** | S | $OCHF_2$ | H | 390 ± 50 (21 ± 5) | 4.2 ± 0.7 (57 ± 5) | 870 ± 11 9 (96 ± 4) | n.d. | 410 ± 129 | 44 ± 6 |
| **18g** | S | $OCF_3$ | H | >10,000 (1 ± 0.3) | 12 ± 1 (53 ± 2) | 2100 ± 78 (95 ± 3) | n.d. | 190 ± 61 | 66 ± 11 |
| **18h** | S | $O(CH_2)_2OH$ | H | >10,000 (3 ± 0.5) | >10,000 (12 ± 1) | 930 ± 52 (91 ± 3) | 31 ± 5 (94 ± 4) | 610 ± 122 | 150 ± 97 |

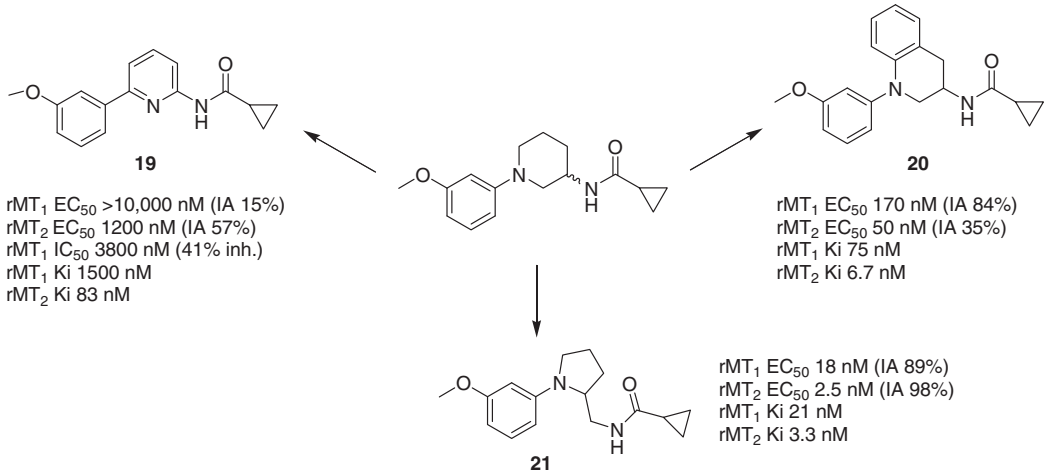
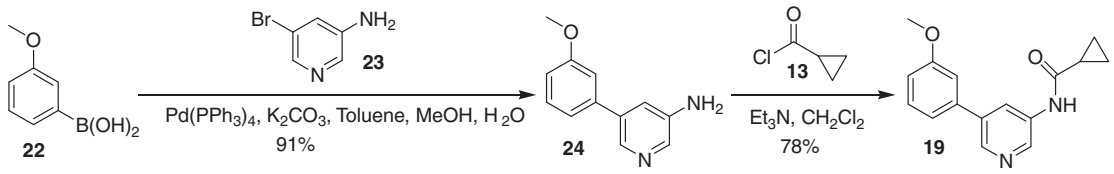



**Figure 3.** Core modifications of *N*-arylpiperdin-3-yl-cyclopropane carboxamide.




**Scheme 3.** Synthesis of compound **19**.

**Table 3**
In vitro microsomal $CL_{INT}$ and brain/plasma ratios of select compounds

| Compd | Microsomal $CL_{INT}$ (μL/min/mg)[32] | | Exposure in Rat (1 h, 10 mpk, IP) | | | |
|---|---|---|---|---|---|---|
| | Human | Rat | Brain (nM) | Plasma (nM) | B/P | Vehicle |
| Agomelatine | 361 | 144 | 1030 | 700 | 1.5 | 15% Solutol |
| Ramelteon | 47 | 60 | 390 | 580 | 0.7 | 20% HP-βCD |
| **11f** | 29 | 91 | 540 | 390 | 1.4 | 15% Solutol |
| **18b** | 28 | 616 | 180 | 730 | 0.2 | 10% DMSO, 2.5% PEG400 in 15% solutol |
| **18d** | 45 | 199 | — | — | — | |
| **18f** | 48 | 485 | — | — | — | |

as $MT_1/MT_2$ ligands. Design, syntheses, structure–property relationships and CNS disposition of select compounds are described.

## Acknowledgements

The authors acknowledge the contributions of colleagues in the DMPK and Analytical Chemistry Groups of the Chemical & Pharmacokinetic Sciences Department, Lundbeck Research USA, for in vitro and in vivo pharmacokinetic data, PAMPA results and HPLC purifications, and scientists in the Global HTS group of Biological Research Department, Lundbeck Research USA, for in vitro binding and functional data.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.068. These data include MOL files and InChiKeys of the most important compounds described in this article.

## References and notes

1. Reiter, R. J. *Endocr. Rev.* **1991**, *12*, 151.
2. Foulkes, N. S.; Borjigin, J.; Snyder, S. H.; Sassone-Corsi, P. *Trends Neurosci.* **1997**, *20*, 487.
3. Axerold, J. *Science* **1974**, *184*, 1341.
4. Reppert, S. M.; Weaver, D. R.; Godson, C. *Trends Pharmacol. Sci.* **1996**, *17*, 100.
5. Reppert, S. M.; Weaver, D. R.; Ebisawa, T. *Neuron* **1994**, *13*, 1177.
6. Reppert, S. M.; Godson, C.; Mahle, C. D.; Weaver, D. R.; Slaugenhaupt, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8734.
7. Dubocovich, M. L.; Cardinali, D. P.; Delagrange, P.; Krause, D. N.; Strosberg, A. D.; Sugden, D.; Yocca, F. D. *The IUPHAR Compendium of Receptor Characterization and Classification*; IUPHAR Media: London, 2000. p 271.
8. Nosjean, O.; Ferro, M.; Cogé, F.; Beauverger, P.; Henlin, J.-M.; Lefoulon, F.; Fauchère, J.-L.; Delagrange, P.; Canet, E.; Boutin, J. A. *J. Biol. Chem.* **2000**, *275*, 31311.
9. Oldani, A.; Ferini-Strambi, L.; Zucconi, M. .; Stankov, B.; Fraschini, F.; Smirne, S. *Neuroreport* **1994**, *6*, 132.
10. Nickelsen, T.; Lang, A.; Bergau, L. *Adv. Pineal Res.* **1991**, *5*, 303.
11. Sack, R. L.; Blood, M. L.; Lewy, A. J. *Sleep* **1992**, *15*, 434.
12. Reiter, R. J. *Exp. Gerontol.* **1995**, *30*, 199.
13. Rosenthal, N. E.; Sack, D. A.; Jacobsen, F. M.; James, S. P.; Parry, B. L.; Arendt, J.; Tamarkin, L.; Wehr, T. A. *J. Neural. Transm. Suppl.* **1986**, *21*, 257.
14. Wirz-Justice, A. *Int. Clin. Psychopharmacol.* **2006**, *21*, S11.
15. Le Bars, B.; Thivolle, P.; Vitte, P. A.; Bojkowski, C.; Chazot, G.; Arebdt, J.; Frackowiak, R. S. J.; Claustrat, B. *Nucl. Med. Biol.* **1991**, *18*, 357.
16. Zlotos, D. P. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 229.
17. Garratt, P. J.; Tsotinis, A. *Mini-Rev. Med. Chem.* **2007**, *7*, 1075.
18. Rivara, S.; Mor, M.; Bedini, A.; Spadoni, G.; Tarzia, G. *Curr. Top. Med. Chem.* **2008**, *8*, 954.
19. Mor, M.; Rivara, S.; Pala, D.; Bedini, A.; Spadoni, G.; Tarzia, G. *Expert Opin. Ther. Pat.* **2010**, *20*, 1059.
20. Hardeland, R. *Expert Opin. Investig. Drugs* **2010**, *19*, 747.
21. Langlois, M.; Brémont, B.; Shen, S.; Poncet, A.; Andrieux, J.; Sicsic, S.; Serraz, I.; Mathé-Allainmat, M.; Renard, P.; Delagrange, P. *J. Med. Chem.* **1995**, *38*, 2050.
22. Garratt, P. J.; Travard, S.; Vonhoff, S. *J. Med. Chem.* **1996**, *39*, 1797.
23. Rivara, S.; Lodola, A.; Mor, M.; Bedini, A.; Spadoni, G.; Lucini, V.; Pannacci, M.; Fraschini, F.; Scaglione, F.; Sanchez, R. O.; Gobbi, G.; Tarzia, G. *J. Med. Chem.* **2007**, *50*, 6618.
24. Sun, L.-Q.; Chen, J.; Mattson, R.; Epperson, J. R.; Deskus, J. A.; Li, W.; Takaki, K.; Hodges, D. B.; Iben, L.; Mahle, C. D.; Ortiz, A.; Molstad, D.; Ryan, E.; Yeleswaram, K.; Xu, C.; Luo, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4381.
25. Millan, M.; Brocco, M.; Gobert, A.; Dekeyne, A. *Psychopharmacology* **2004**, *177*, 448.
26. Kupfer, D. J. *Eur. Neuropsychopharmacol.* **2006**, *16*, S639.
27. Values were determined in triplicate and expressed as average ± SEM
(a) Functional assay: Melatonin functional assay
CHO-Gα16 cell line expressing rat $MT_1$ and rat $MT_2$ receptor were plated in 384-well poly-D-lysine-coated black FLIPR plates (Corning) 24 h before the assay. Cell plates were washed once with Hanks' balanced salt solution buffer (HBSS buffer) supplemented with 20 mM HEPES + 0.1% BSA pH 7.4 (Wisent Inc.) containing 2.5 mM Probenecid (Sigma). The plates were loaded with 50 μL HBSS containing the calcium-sensitive dye Fluo4-AM (Invitrogen) at 1.5 μM final concentration and pluronic acid (2% final concentration, Molecular Probes) and were incubated at 37 °C for 1 h in a humidified chamber (5% $CO_2$/95% air). Following the incubation step, cells were washed three times in HBSS buffer, leaving 30 μL of buffer in the plate after the last wash. Mobilization of intracellular $Ca^{2+}$ in response to different ligands was measured online using the FLIPR reader. For agonist assay, baseline fluorescence was measured for 15 s, 15 μL of test compound was added, and agonist response was monitored for 3 min. For antagonist assay, the test compound was added and incubated for 20 min at room temperature, 15 μL of agonist melatonin ($EC_{80}$) was then added, and fluorescence was read for an additional 3 min.
(b) [$^3$H]-Melatonin binding assay: Rat $MT_1$ and $MT_2$ membranes generated from CHO-Gα16 cell line were incubated with test compounds for 2 h at 37 °C in a buffer (Tris/HCl 50 mM, pH 7.4, 5 mM $MgCl_2$) in a final volume of 250 μL containing [$^3$H]-melatonin (0.5 nM final concentration, specific activity 80 Ci/mmol). Non-specific binding was defined with 10 μM melatonin. Reaction was stopped by rapid filtration through GF/B unifilters, followed by five successive washes with ice-cold buffer. Data were analysed by using the Activitybase XL fit program (IDBS, UK).The density of binding sites Bmax and the dissociation constant of the radioligand ($K_d$) values were calculated using non-linear regression model. For competition experiments, inhibition constants ($K_i$) were calculated according to the Cheng–Prussof equation: $K_i = IC_{50}/[1 + ([L]/K_d)]$, where $IC_{50}$ is the concentration resulting in 50% of maximum inhibition, and [L] is the concentration of [$^3$H] melatonin (Cheng, Y. C. and Prussoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099).
28. Searches were performed on The Cambridge Crystallographic Database from The Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK. See, e.g., 'The Cambridge Structural Database: a quarter of a million crystal structures and rising'; Allen, F. H. *Acta Crystallogr., Sect. B* **2002**, *58*, 380. Conquest was used to conduct the searches.

29. Rivara, S.; Diamantini, G.; Giacomo, B. D.; Lamba, D.; Gatti, G.; Lucini, V.; Pannacci, M.; Mor, M.; Spadoni, G.; Tarzia, G. *Bioorg. Med. Chem.* **2006**, *14*, 3383.
30. Davies, D. J.; Garratt, P. J.; Tocher, D. A.; Vonhoff, S.; Davies, J.; Teh, M.-T.; Sugden, D. *J. Med. Chem.* **1998**, *41*, 451.
31. Spadoni, G.; Bedini, A.; Guidi, T.; Tarzia, G.; Lucini, V.; Pannacci, M.; Fraschini, F. *ChemMedChem* **2006**, *1*, 1099.
32. (a) Conditions for measuring microsomal clearance: A 1 μM test compound was incubated in an assay mixture containing 0.5 mg/mL liver microsome proteins with a NADPH regenerating system. Each compound was incubated for 0, 5, 15, 30, 60 min, and the afforded supernatants from centrifugation of quenched incubations were analyzed by LC/MS to determine the remained drug concentrations for $T_{1/2}$ estimation. (b) Intrinsic clearance was calculated based on the following equations (Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber B. M.; Jones, B. C.; Macintyre, F.; Rance, D. J.; Wastall, P. *J. Pharmacol. Exp. Ther*. **1997**, *283*, 46.)

$$C = C_0 * e^{-kt};\ \mathrm{Ln}C = \mathrm{Ln}C_0 - kt;\ k = -\mathrm{slope};$$
$$C = (1/2)\ C_0 = T_{1/2} = 0.693/\mathrm{k}$$
$$\mathrm{CL_{INT}} = \frac{0.693}{T_{1/2}\ (\mathrm{min})} \times \frac{\text{incubation volume (mL)}}{\text{microsomal protein (mg)}}$$

33. The PAMPA assay was performed using conditions described in 'A Comparative Study of Artificial Membrane Permeability Assay for High Throughput Profiling of Drug Absorption Potential'. Zhu, C.; Jiang, L.; Chen, T.-M.; Hwang, K.-K. *Eur. J. Med. Chem.* **2002**, *37*, 399.