CONCISE ARTICLE

Novel difluoroacetamide analogues of agomelatine and melatonin: probing the melatonin receptors for MT$_1$ selectivity†

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Synthesis and pharmacological evaluation of novel agomelatine and melatonin analogues with structures combining the features generating MT$_1$ selectivity, namely the bulky hydrophobic ether moiety and the difluoroacetamide group, is reported. The dimeric agomelatine analogue linked by a three methylene spacer displayed the best affinity (K_i = 1.2 nM) and selectivity (7-fold) toward MT$_1$ receptors.

Introduction

The neurohormone melatonin (Fig. 1) exerts its diverse pharmacological actions mostly through activation of the two high affinity G-protein-coupled MT$_1$ and MT$_2$ receptors.[1–3] The distinct physiological role of MT$_1$ and MT$_2$ receptors has been only partly elucidated in animals studies. While MT$_1$-activation inhibits neuronal firing within the suprachiasmatic nucleus,[4] modulates visual function in retina,[5] and causes arterial vasoconstriction,[6] activation of MT$_2$ receptors induces vasodilation,[7] inhibits dopamine release in retina,[8] generates a phase shift in circadian rhythms,[9] and promotes nonrapid eye movement sleep.[10]

Although melatonin is a popular treatment of sleep problems caused by jet-lag, shift work, and delayed sleep phase syndrome, it shows poor pharmacokinetic properties, such as low oral bioavailability and short half-life. Currently, three synthetic melatonin analogues with improved pharmacokinetic profile, ramelteon, agomelatine, and tasimelteon are approved for the treatment of insomnia, major depression, and Non-24-Hour Sleep-Wake Disorder in blind people, respectively. The antidepressant effect of agomelatine (Fig. 1) is thought to be caused by the combination of its non-selective agonistic effect on MT$_1$ and MT$_2$ receptors and antagonistic action at 5-HT$_{2C}$ serotonin receptors.[11]

Melatonin displays equal subnanomolar affinity toward both MT$_1$ and MT$_2$ with binding constants between 0.15 and 1.00 nM depending on the cell lines used for receptor expression, and on the research laboratory. An accurate characterization of melatonin receptor-mediated functions requires MT$_1$ and MT$_2$-selective ligands. While many series of MT$_2$-selective agents have been reported, pronounced MT$_1$ selectivity is still a challenge with only few examples of MT$_1$-selective agents reported up to date.[3,12,13] Moreover, whereas for MT$_2$-selective agents K_i (MT$_1$)/K_i (MT$_2$) ratios > 1000 can be achieved, ligands preferentially binding to MT$_1$ reach maximally ~100-fold higher affinity for MT$_1$ than for MT$_2$ receptors. Structures of representative MT$_1$-selective ligands are compiled in Fig. 2.

Agomelatine is a non-selective melatonergic ligand displaying high-affinity at both MT$_1$ and MT$_2$ receptors ($K_i \approx$ 0.1 nM). Substantial MT$_1$ selectivity was achieved by introduction of two fluorine atoms into the N-acetyl group of agomelatine. The resulting difluoroacetamide 1 was reported to be 143-times more selective for MT$_1$ than for MT$_2$ receptors (MT$_1$: K_i = 0.03 nM, MT$_2$: K_i = 4.3 nM).[14] Preference for MT$_1$ receptors was also achieved by linking two agomelatine units via their ether oxygens by (CH$_2$)$_3$ and (CH$_2$)$_4$ spacers. The resulting dimeric ligands 2a (MT$_1$: K_i = 0.5 nM, MT$_2$: K_i = 112 nM) and 2b (MT$_1$: K_i = 0.6 nM, MT$_2$: K_i = 73.2 nM) display 224-fold and 120-fold selectivity toward MT$_1$, respectively.[15] However, binding data for compound 2a measured in our laboratory revealed much lower affinity (K_i = 112 nM) and just threefold selectivity for the MT$_1$-subtype.[16] Other

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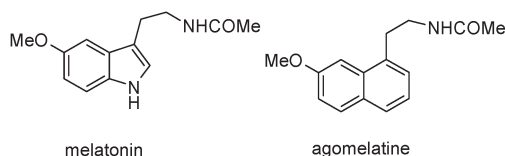
Fig. 1 Structures of melatonin and agomelatine.

1340 | Med. Chem. Commun., 2015, 6, 1340–1344

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1

2a n = 3
2b n = 4

agomelatine-biphenyl ligand

S24268

dimeric anilinoethylamide

phenylbutoxy anilinoethylamide

melatonin analogues

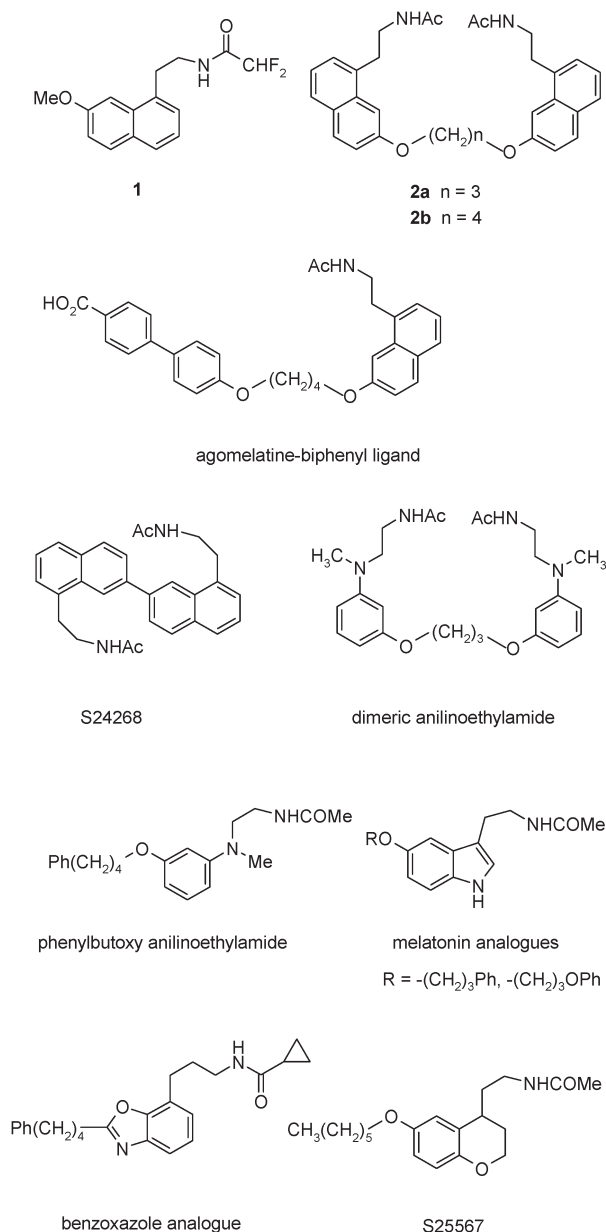R = -(CH₂)₃Ph, -(CH₂)₃OPh

benzoxazole analogue

S25567

Fig. 2 Structures of representative MT₁-selective ligands.

examples of agomelatine-derived MT$_1$-selective ligands are the homodimeric analogue S24268 (MT$_1$: K_i = 5.2 nM, MT$_2$: K_i = 246 nM),[17] and the agomelatine–biphenyl heterodimer (MT$_1$: K_i = 0.55 nM, MT$_2$: K_i = 51 nM).[18]

In the series of homodimeric (anilinoethyl) amides, for the most MT$_1$-selective ligand (MT$_1$: K_i = 20.4 nM, MT$_2$: K_i = 2089 nM), the head pharmacophores are separated by a (CH$_2$)$_3$ spacer.[19] The corresponding heteromeric analogue bearing a phenylbutoxy group showed (K_i (MT$_1$) = 1.17 nM and K_i (MT$_2$) = 91 nM).[20] Ph(CH$_2$)$_3$ and PhO(CH$_2$)$_3$ substituents are also present in the most MT$_1$-selective ligands from the series of melatonin analogues with the 5-OCH$_3$ group replaced by bulkier ethers.[16] Other compounds showing approximately 30-fold preference for MT$_1$ receptors are the

phenylbutyl substituted benzoxazole derivative[21] and the hexyloxy substituted chromane analogue S25567.[17]

A common structural feature conferring MT$_1$ selectivity is a bulky, hydrophobic ether replacing the methoxy group in a position equivalent to C5 of melatonin. The only exception is difluoroagomelatine **1** whose preferential binding to MT$_1$ receptors is attributed to the CH$_3$–CHF$_2$ exchange in the amide side chain. In this paper, we report the synthesis and pharmacological evaluation of novel agomelatine and melatonin analogues with structures combining the features responsible for MT$_1$ selectivity, namely the bulky hydrophobic ether moiety and the difluoroacetamide group. Moreover, the effect of introducing a third fluorine atom and of fluorine–chlorine exchange in compound **1** have been investigated.

Results and discussion

Chemistry

Agomelatine was synthesized according to our previously reported procedure.[22] 5-Methoxytryptamine and N-desacetylagomelatine **3** were prepared by amide hydrolysis of melatonin and agomelatine, respectively, using ethanolic KOH.[23] Trifluoroagomelatine **4** was prepared by acylation of **3** using trifluoroacetic anhydride in pyridine as previously reported.[24] Difluoroagomelatine **1** (ref. 14) and dichloroagomelatine **5** were obtained by acylation of **3** using methyl difluoroacetate, and methyl dichloroacetate, respectively (Scheme 1).

O-Desmethylagomelatine **6** was prepared by ether cleavage of the parent compound using BBr$_3$ as previously reported.[25] The phenylalkoxyagomelatine analogues **7–9** were synthesized by O-alkylation of **6** with appropriate arylalkylhalides using a standard procedure as shown in Scheme 2.

Our previously reported phenoxybutyl substituted melatonin analogue **10** (ref. 16) was subjected to amide hydrolysis and subsequent acylation using methyl difluoroacetate to give the difluoroacetamide **11** (Scheme 3.)
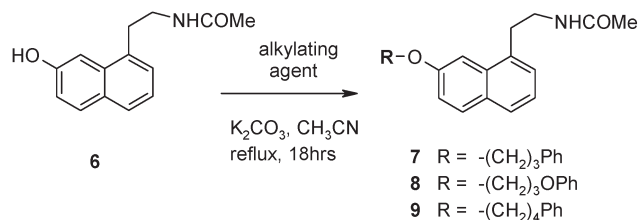
The dimeric difluoroacetamides **12a** and **12b** were prepared starting from the agomelatine dimers **2a** (ref. 16) and **2b** (ref. 15), respectively, as shown in Scheme 4.
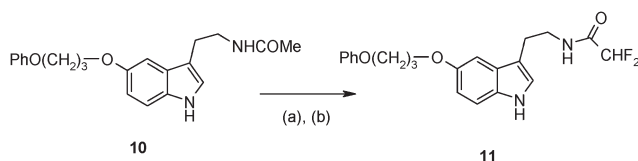
Pharmacology

The affinity of the target compounds for human MT$_1$ or MT$_2$ melatonin receptors expressed in CHO cells was measured by competition binding analysis using the radioligand, 2-[^{125}I]-iodomelatonin. Melatonin competition assays were run in
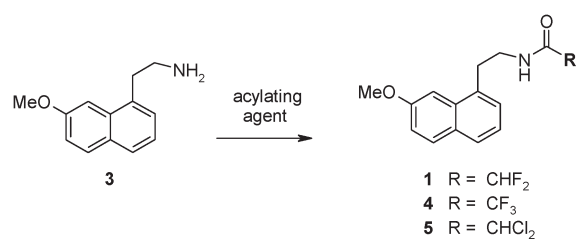
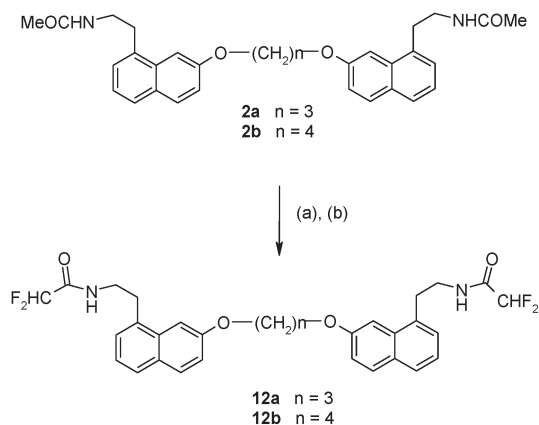**Scheme 1** Reagents and conditions 1: CHF$_2$COOCH$_3$, CF$_3$OH, reflux; 4: (CF$_3$CO)$_2$O, pyridine; 5: CHCl$_2$COOCH$_3$, reflux.

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Med. Chem. Commun., 2015, **6**, 1340–1344 | **1341**

Scheme 2 Reagents **7**: Cl(CH₂)₃Ph, **8**: Br(CH₂)₃OPh; **9**: Br(CH₂)₄Ph.

Scheme 3 Reagents and conditions: (a) KOH, EtOH, reflux, (b) CHF₂CO₂Me, CF₃OH, reflux.

Scheme 4 Reagents and conditions: (a) KOH, EtOH, reflux, (b) CHF₂CO₂Me, CF₃OH, reflux.

parallel and the affinity of melatonin for the MT₁ or MT₂ melatonin receptors was in the range of the reported literature. For the sake of comparison, the previously reported and structurally related MT₁-selective ligands **1**, **2a**, and **10** were included in our study. The results are compiled in Table 1.

Discussion

Development of MT₁-selective ligands remains a challenging task, and only few compounds displaying approximately 100-fold selectivity have been reported so far. MT₁-selective ligands bear a bulky arylalkyl substituent in a position topologically equivalent to the methoxy group of melatonin, except for the recently reported difluoroacatemide derivative of agomelatine **1**. Since binding constants for the same ligand may differ depending on the cell line used in the radioligand binding assay[17] and on the laboratory,[16,26] compound **1** has been included in this study as an MT₁-selective reference ligand. Notably, while **1** was reported to display K_i

Table 1 Binding affinity of the target compounds for the human MT₁ and MT₂ receptors expressed in CHO cells obtained in competition radioligand binding assays using 2-[¹²⁵-I]-iodomelatonin (pK_i values were calculated from IC₅₀ values obtained from competitive curves according to the method of Cheng and Prusoff and are the mean of at least three determinations performed in duplicate

	pK_i MT₁ ± SEM	pK_i MT₂ ± SEM
Melatonin	9.34 ± 0.10	9.02 ± 0.09
1	10.27 ± 0.52	9.07 ± 0.16
2a (ref. 16)	6.95 ± 0.03	6.45 ± 0.03
4	8.24 ± 0.06	8.75 ± 0.08
5	8.30 ± 0.14	8.75 ± 0.08
7	7.60 ± 0.10	7.67 ± 0.10
8	7.45 ± 0.01	7.36 ± 0.03
9	8.03 ± 0.37	8.05 ± 0.53
10 (ref. 16)	8.10 ± 0.08	7.06 ± 0.15
11	8.21 ± 0.13	8.11 ± 0.30
12a	8.91 ± 0.11	8.09 ± 0.05
12b	7.93 ± 0.03	7.84 ± 0.03

(MT₁) = 0.03 nM, and K_i (MT₂) = 4.3 nM,[14] we observed similar high-affinity at MT₁ (K_i = 0.054 nM) but considerably lower binding at MT₂ (K_i = 0.85 nM) resulting in reduced, 16-fold, preference towards MT₁ receptors.

Interestingly, the monofluorinated analogue is a nonselective MT₁/MT₂ ligand showing subnanomolar affinity similar to that of agomelatine.[14]

To explore the effect of an additional fluorine atom and of F–Cl exchange, trifluoroacetamide **4** and dichloroacetamide **5** have been elucidated. Both structure modifications led to reduced affinity for MT₁ (K_i ≈ 5 nM) and MT₂ (K_i ≈ 2–4 nM), and loss of MT₁ selectivity.

In the series of melatonin analogues with the 5-OCH₃ group replaced by bulkier ethers, the most MT₁-selective agents were substituted with a phenylpropyl or phenyloxypropyl group (compound **10**).[16] Since the majority of MT₁-selective ligands known to date are analogues of agomelatine (Fig. 1), the equally substituted agomelatine analogues **7** and **8**, as well as the phenylbutyl derivative **9** have been evaluated. Suprisingly, compounds **7–9** display just moderate affinity for MT₁ (K_i = 9–35 nM) and MT₂ (K_i = 9–44 nM) and no MT₁ selectivity. A direct comparison of the binding data between the PhO(CH₂)₃-substituted melatonin analogue **10** (MT₁: K_i = 7.9 nM, MT₂: K_i = 87 nM) and the identically substituted agomelatine derivative **8** (MT₁: K_i = 35 nM, MT₂: K_i = 44 nM) indicates that the effect of similar specific substitution in a position equivalent to the methoxy group of melatonin may be dependent on the core ring system, and, in this series, the indole nucleus is more suitable for generating MT₁ selectivity than naphthalene.

Finally, hoping for a synergistic effect, the structural features generating MT₁ selectivity, namely the phenyloxypropyl substitution and the difluoroacetamide group were combined in the melatonin analogue **11**. Unexpectedly, while MT₁-affinity (K_i = 6.2 nM) was very similar to that of the parent compound **10**, binding at MT₂ (K_i = 7.8 nM) was 10-fold higher leading to loss of MT₁ selectivity.

1342 | *Med. Chem. Commun.*, 2015, **6**, 1340–1344

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In our last attempt to achieve high MT_1-selectivity, the dimeric agomelatine analogues **2a–2b** were converted to the corresponding difluoroacetamides **12a–12b**. While the $(CH_2)_4$-linked dimer **12b** showed no selectivity toward MT_1 and moderate affinity at both subtypes (MT_1: K_i = 12 nM, MT_2: K_i = 15 nM), the dimeric ligand with the $(CH_2)_3$-linked spacer displayed low nanomolar and 7-fold higher affinity for MT_1 (K_i = 1.2 nM), than for MT_2 (K_i = 8.1 nM). These findings confirm that a three methylene linker confers the highest MT_1-selectivity in the series of dimeric melatonergic ligands. When compared to one of the most MT_1-selective ligands reported to date **2a**, compound **12a** is characterized by 90 times increased affinity and approximately doubled selectivity toward MT_1 receptors confirming that difluorosubstitution of the terminal acetamide group is favourable for ligand binding at MT_1 receptors. Compound **12a** could become a valuable pharmacological tool to examine distinct physiological functions of MT_1 and MT_2 receptors.

The findings indicate that the fragment merging approach to increase affinity and selectivity toward MT_1 receptors that was successful for the $(CH_2)_3$-linked dimeric agomelatines may not be generally applicable to all series of MT_1-selective melatonergic ligands.

Conclusions

Novel agomelatine and melatonin analogues with structures combining the features generating MT_1 selectivity, namely the bulky hydrophobic ether moiety and the difluoroacetamide group, were synthesized and pharmacologically evaluated. The dimeric agomelatine analogue linked by a three methylene spacer displayed the best affinity (K_i = 1.2 nM) and selectivity (7-fold) toward MT_1 receptors. The findings are important for the design of novel melatonergic ligands selectively targeting MT_1 receptors.

Acknowledgements

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