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Discovery of spiropiperidine-based potent and selective Orexin-2 receptor antagonists

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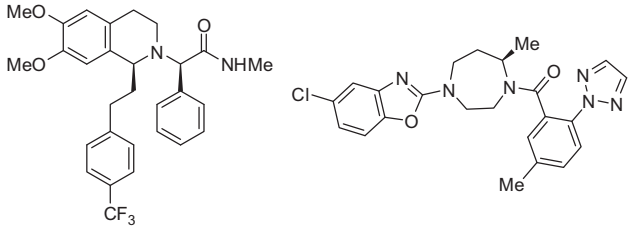
ABSTRACT

To generate novel human Orexin-2 Receptor (OX2R) antagonists, a spiropiperidine based scaffold was designed and a SAR study was carried out. Compound **4f** possessed the highest OX2R antagonistic activity with an IC_{50} value of 3 nM with 450-fold selectivity against Orexin-1 Receptor (OX1R).

The neuropeptides orexin-A and orexin-B are derived via proteolytic cleavage from a common precursor in hypothalamic neurons. These endogenous ligands bind two G protein–coupled receptors (GPCR) termed orexin receptors type 1 and type 2 (OX1R and OX2R).[1] OX1R is selective for orexin-A, whereas OX2R is a nonselective receptor for both orexin-A and orexin-B.[1] Anatomical studies have demonstrated that orexin neuronal projections reach throughout brain,[2–4] with dense concentration of orexin containing axon terminals in the brainstem, hypothalamus, and basal forebrain. In particular, OX1R mRNA is the most abundant in the locus coeruleus and OX2R mRNA in the tuberomamillary nucleus, whereas the dorsal raphe nucleus and the ventral tegmental area contain both OX1R and OX2R mRNA.[5] These regions are known to participate in the control of behavioral sleep and wakefulness. Activation of orexin neurons contributes to the promotion and maintenance of wakefulness, and conversely, relative inactivity of orexin neurons allows the onset of sleep.[6] Consequently, blocking orexin signaling with receptor antagonists should provide a mechanism for decreasing wakefulness and, thus, a novel therapeutic opportunity for the treatment of insomnia. In addition, orexin R antagonist might be expected to have other therapeutic potentials such as treatment of eating disorder,[7] Alzheimer's disease by reducing amyloid-β,[8] cognitive enhancer,[9] PTSD,[10] panic anxiety,[11] and addiction.[12]

Whilst numerous kinds of orexin receptor antagonists have been reported,[13] only two dual OX1R/OX2R receptor antagonists such as Almorexant and MK-4305 were entered in Phase-III,



Almorexant MK-4305

Figure 1. Almorexant and MK-4305

however, Almorexant was discontinued (Fig. 1).[14] Actelion has also reported that the Almorexant elicited somnolence without inducing cataplexy in rats, dogs, and humans,[14] and demonstrated superior efficacy in subjective and objective electrophysiological signs for treating insomnia compared to the currently available medications (e.g., GABA agonists).[14] In spite of fascinating profile of the dual orexin receptor antagonists, the intrinsic contributions of OX1R and OX2R in sleep regulation remain to be determined using pharmacological tools. Johnson & Johnson has reported that blockade of OX1R attenuates OX2R antagonist-induced sleep promotion in the rat.[15] Considering these results, our goal was to discover potent, selective OX2R antagonists for clinical development and their elucidation of pharmacological action.

Among various scaffolds of orexin receptor antagonists,[13] we regarded spiropiperidine **1** disclosed by a Merck research group as a promising CNS drug-like template because of low molecular weight (408.52), low PSA (74.8), moderate lipophilicity (*c* log *P*: 3.64), and only two rotatable bonds typical of a rigid structure

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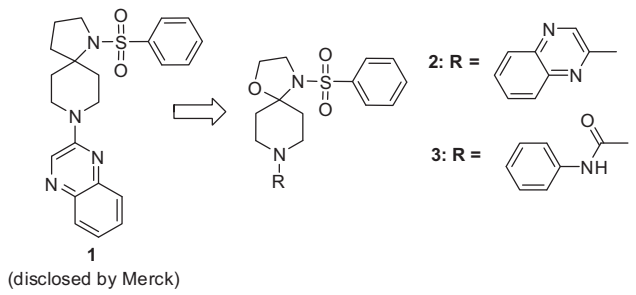


Figure 2. Spiropiperidine Compounds **2** and **3**.

(Fig. 2).[16,17] Inspired by this novel structure, we decided to modify the core of **1** to identify new chemical entities endowed with potency and selectivity against OX1R. In this article, we described scaffold morphing of compound **1** that led to a novel selective OX2R antagonist.

During the course of exploring a novel scaffold, we attempted to introduce an oxygen atom in the spiro-ring and truncation of pyrazine ring, to reduce lipophilicity. The designed compounds **2** and **3** were prepared according to Scheme 1 and 2. The synthesis of oxo-analogue **2** was achieved by four steps, namely S_NAr reaction, acetal deprotection, spiroketalization with 2-aminoethanol, and sulfonamidation. The yield of final spiroketalization and sulfonamide formation steps was relatively moderate as 20% because of low solubility of ketone **6**. In the case of the synthesis of following compound **3**, the yield of ketalization step was increased because these steps were undergone with soluble material **8** (Scheme 2).

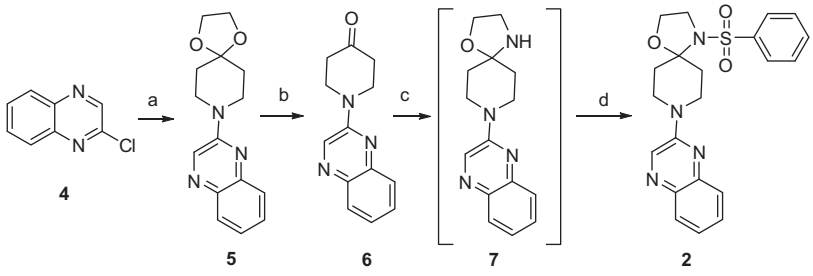
The synthesis of **3** began with spiroketalization of Cbz-protected piperidinone **8** with 2-aminoethanol giving intermediate **9**, which was directly sulfonamidated without purification to afford spiropiperidine **10**. Removal of Cbz group by hydrogenation gave a spiropiperidine **11** and subsequent acylation afforded the desired compound **3** (Scheme 2).

To measure potency, we utilized a FLIPR (Fluorometric Imaging Plate Reader) assay in which calcium flux is measured as a functional determinant of OX2 or OX1 antagonism. Spiropiperidine **2** showed acceptable OX2R antagonistic activity of 230 nM with sevenfold selectivity against OX1R, but the human metabolic stability (HLM) was poor (Table 1).[18] Phenyl urea **3** showed threefold decrease in potency compared with **2**, however, the selectivity against OX1R was slightly improved (8.9-fold). Additionally metabolic stability in human liver microsome was improved (Table 1). This improvement may be partially due to reduced lipophilicity (log *D*: 1.82 for **3** vs 3.07 for **2**).[19]

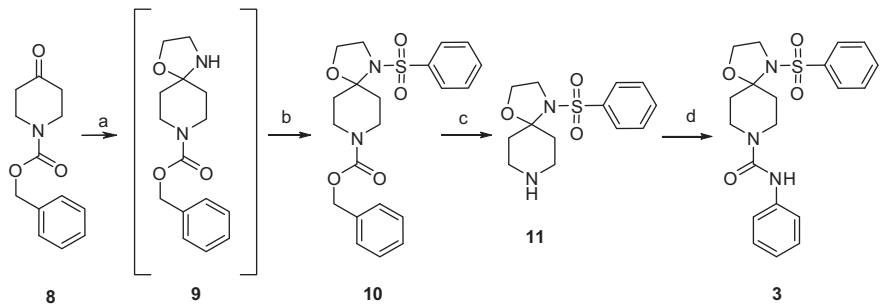
Both compounds **2** and **3** were evaluated by drug-like parameters such as ligand efficiency (LE), ligand-lipophilicity efficiency (LLE), and LELP.[20–23] LE of both compounds are comparable. However, LLE of **3** is better than that of **2** even though the potency of **3** was weaker. It was suggested that LELP is a more useful metric particularly in CNS drug and the value below 10 is recommended as acceptable to ensure maximum binding efficiency for molecular size and lipophilicity.[17d] LELP values for compounds **2** and **3** are 9.90, and 6.07, respectively. Judging from its acceptable profile of drug-like parameters and selectivity against OX1R, we selected **3** as a lead compound for further modification.

Although compound **3** showed 8.9-fold selectivity against OX1R, this compound was not stable under acidic conditions probably due to the aminal substructure. Additionally, the solubility of compound **3** was rather poor. In order to overcome those problems, we introduced a fluorine atom at the beta-position of the piperidine nitrogen, with the expectation that the strong electronegativity of fluorine would enhance the stability of oxazolidine in acidic conditions, and the collapse of the symmetrical structures would break the crystal packing and achieve better solubility.[24]

Monofluorinated analogues were prepared as shown below (Scheme 3). Formation of silyl enol ether, followed by fluorination

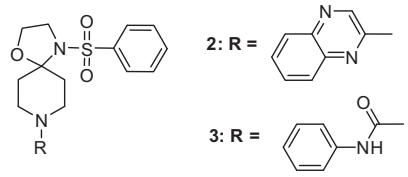


Scheme 1. Synthesis of oxo-analogue **2**. Reagents and conditions: (a) 1,4-dioxa-8-azaspiro[4.5]decane, NEt_3, EtOH, rt, 4 h, 87%; (b) *p*-TSA, acetone–water reflux, overnight, 12%; (c) 2-aminoethanol, toluene, *p*-TSA, reflux, 5 h; (d) $PhSOCl_2$,pyridine, rt, overnight, 20% over two steps.



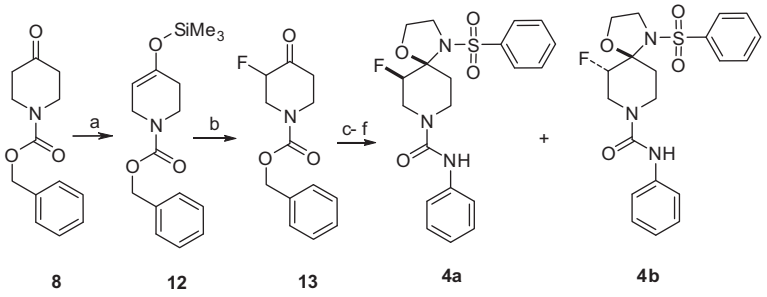
Scheme 2. Synthesis of phenyl urea **3**. Reagents and conditions: (a) 2-aminoethanol, toluene, *p*-TSA, reflux; (b) $PhSO_2Cl$,pyridine, rt, 92% over two steps for **11**; (c) 10% Pd/C, THF–water, rt, under H_2, quant.; d) PhNCO, NEt_3, THF, rt, 43%.

Table 1
OX2R/1R Antagonistic Activities, Selectivity Against OX1R, Human Metabolic Stabilities, LE, LLE, and LELP.

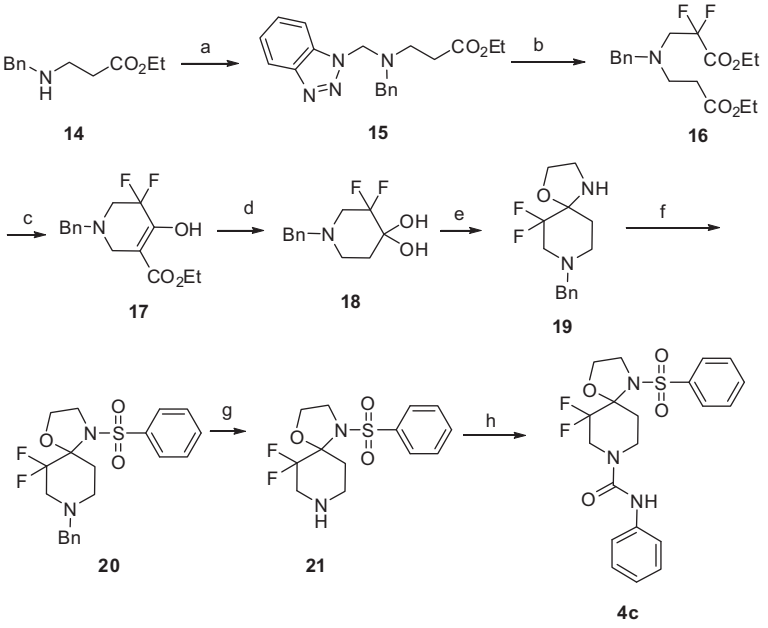


cmpd	OX-2[a] IC_{50} (nM)	OX-1[a] IC_{50} (nM)	Selectivity (OX-1/OX-2)	HLM[b] (mL/min/mg)	LE	LLE	LELP
2	230	1600	7.0	100	0.31	3.56	9.90
3	710	6300	8.9	43	0.30	4.33	6.07

[a] Antagonistic activity.
[b] HLM = metabolic stability by the incubation with human liver microsomes.



Scheme 3. Synthesis of Monofluorinated Compounds **4a** and **4b**. Reagents and conditions: (a) TMSCl, NEt_3, DMF, 80 °C, overnight, 48%; (b) Selectfluor, MeCN, rt, 2 h, 67%; (c) 2-aminoethanol, toluene, *p*-TSA, reflux; (d) $PhSO_2Cl$,pyridine, rt; (e) 10% Pd /C, THF–water, rt, under H_2; (f) PhNCO, NEt_3, THF, rt, 37% for **4a**, 19% for **4b**.

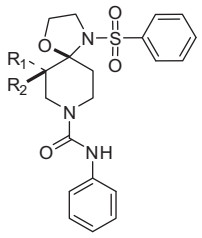


Scheme 4. Synthesis of Difluorinated Compounds **4c**. Reagents and conditions a) 1*H*-benzotriazole, formalin, MeOH, rt, 16 h; b) $BrCF_2CCO_2Et$, Zn, TMSCl, THF, rt, 2 h, 49%; c) LDA, THF, rt, 1 h, 38%; d) 6NHCl, 110 °C, 3 h, 46%; e) 2-aminoethanol, toluene, *p*-TSA, reflux; f) $PhSOCl_2$,pyridine, rt, 3 h, 39% over 2 steps; g) 20% $Pd(OH)_2$ / C, EtOH, under H_2, 78%; h) PhNCO, THF, rt, 15 min. 23%.

with Selectfluor provided monofluorinated piperidinone **13**.[25] Treatment of **13** in the same way as the preparation of **3** afforded desired compounds **4a** and **4b**. These monofluorinated compounds **4a** and **4b** were easily separated in 2:1 ratio by silica gel column chromatography. The stereochemistry of **4b** was confirmed by X-ray crystallographic analysis.

Difluorinated compound **4c** was prepared starting from material **14** (Scheme 4). Unsaturated ester **17** was prepared through three sequential steps, Mannich type reaction, Refortmatsky reaction and Dieckmann cyclization.[26] Treatment of compound **17** in 6 N HCl gave ketal **18**. Cyclization of the ketal **18** with aminoethanol furnished spiro-oxazolidine **19**, which was stable enough to be

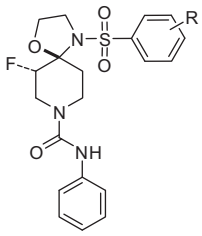
Table 2
OX2R/1R Antagonistic Activities, Selectivity Against OX1R, Human Metabolic Stabilities, Water Solubility, and Lipophilicity.



compd (R1, R2)	OX-2[a] IC_{50} (nM)	OX-1[a] IC_{50} (nM)	Selectivity (OX-1/OX-2)	HLM[b] (mL/min/mg)	JP1[c] (mg/mL)	JP2[c] (mg /mL)	log *D*
3 (H, H)	710	6300	8.9	43	<0.07	1.7	1.82
4a (H, F)	170	280	1.6	34	45	34	2.04
4b (F, H)	220	2500	11	2	8.5	12	1.50
4c (F, F)	1000	>10000	>10	84	21	18	1.93

[a] Antagonist activity.
[b] HLM = metabolic stability by the incubation with human liver microsomes.
[c] see Ref.[27]

Table 3
OX2R/1R Antagonistic Activities, Human Metabolic Stabilities, Water Solubility, and Lipophilicity.



cpds	R	OX-2[a] IC_{50} (nM)	OX-1[a] IC_{50} (nM)	Selectivity (OX-1/OX-2)	HLM[b] (mL/min/mg)	JP1[c] (mg /mL)	JP2[c] (mg /mL)	log *D*
4b	H	220	2500	11	2	8.5	12	1.50
4d	3-OMe	130	2300	18	64	2.1	2.4	1.75
4e	4-OMe	87	1600	18	4	0.49	0.58	1.63
4f	3,4-di(OMe)	3.3	1500	450	<1	5.9	4.5	1.35

[a] Antagonist activity.
[b] HLM = metabolic stability by the incubation with human liver microsomes.
[c] see Ref.[27]

isolated by purification through silicagel chromatography. This result suggested that fluorine atoms stabilized the aminal substructure. Subsequent four steps gave the desired compound **4c**.

The profiles of compounds **3** and **4a–4c** were shown in Table 2. Fluorinated compounds **4a–4f** described below were evaluated as racemic mixtures. Solubility in the stomach and intestine simulation fluid as the first fluid (JP1) and the second fluid (JP2) of the Japanese Pharmacopoeia was assessed.[27] All compounds **4a–4c** showed better solubility than **3**. This improvement may have resulted from a decrease in crystallinity because compounds **4b** and **4c** had lower melting point and **4a** is an amorphous solid.[28] JP2 is a buffer solution which mimics pH of intestine fluid. Therefore better solubility of compound **4a–4c** in JP2 would be advantageous for oral administration. Compound **3** was not found in JP1, however, **4a–4c** were detected in reasonable concentration in JP1. Since JP1 is an acidic solution, the finding suggests improved stability of compound **4a–4c** in acidic condition.

Monofluorinated compounds **4a** and **4b** showed improved antagonistic activity against OX2R compared to **3**. However, the activity of difluorinated compound **4c** was comparable with **3**. Compound **4a** diminished the selectivity against OX1R, while corresponding stereoisomer **4b** showed improved selectivity against OX1R (11-fold).

Lipophilicity (log *D*) of compound **4b** is lower than that of **3**, **4a**, and **4c**. The decreased lipophilicity may contribute to the better human metabolic stability of **4b** than the other compounds. Though it is not clear why only lipophilicity of **4b** is decreased, we speculated that the stereochemical environment of fluorine atom was likely responsible by enhancing the dipole moment.[29]

Finally, we investigated substituents of the phenylsulfonamide of **4b** in order to further improve potency against OX2R and selectivity against OX1R. Our investigation showed that introduction of methoxy group at 3- or 4- position (**4d, 4e**) could achieve good results for both issues (Table 3). These results led us to prepare 3,4-dimethoxy compound **4f**, with hope of a synergistic effect. As expected, OX2R antagonistic activity of **4f** reached single-digit nanomolar (3.3 nM) and the selectivity against OX1R was improved 40-fold over compound **4b**. While solubility was slightly decreased, human metabolic stability of **4f** was improved probably because 3,4- dimethoxy group is known as one of the best overall performing groups for improving HLM among disubstituted phenyl moieties.[30]

In summary, we have discovered a highly potent, novel, selective OX2R antagonist **4f**. It is noteworthy that the beneficial impacts of fluorine atom on spiropiperidine (**4b**) led to a compound with favorable drug-like properties such as potency, selectivity, HLM, solubility, stability, and lipophilicity. Multi-faceted optimiza-

tion starting from compound **2** resulted in compound **4f** with much improved drug like properties (LE: 0.31 (**2**) vs 0.35 (**4f**); LLE: 3.56 (**2**) vs 6.82 (**4f**); LELP: 9.90 (**2**) vs 3.86 (**4f**)).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.094.

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18. Metabolic stabilities were determined by the described method below.

Liver Metabolic Stability Assay Conditions

Condition	Method
Substrate Concentration	1 μM
Microsomal protein concentration	0.2 mg/mL
Co-enzyme	NADPH-regenerating systems
Temperature	37 °C
Quench Solvent	acetonitrile (1:1)
Incubation time points	0, 20 min
Detection	discrete analysis using LC/PDA
Calculations	CL (μL/min/mg) = 1000 × (1-R)/t/m t; time, 20 (min) m; microsomal protein, 0.2 (mg/mL) R; parent remaining
Criteria	Stable: <50 μL/min/mg Moderate: 50–100 μL/min/mg Unstable: >100 μL/min/mg

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