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Discovery of potent, selective, orally active benzoxazepine-based Orexin-2 receptor antagonists

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ABSTRACT

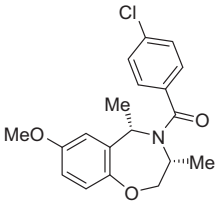
During our efforts to identify a series of potent, selective, orally active human Orexin-2 Receptor (OX2R) antagonists, we elucidated structure-activity relationship (SAR) on the 7-position of a benzoxazepine scaffold by utilizing Hammett σ_p and Hansch-Fujita π value as aromatic substituent constants. The attempts led to the discovery of compound **1m**, possessing good in vitro potency with over 100-fold selectivity against OX1R, good metabolic stability in human and rat liver microsome, good oral bioavailability in rats, and in vivo antagonistic activity in rats by oral administration.

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 binds only orexin-A, whereas OX2R binds both orexin–A and orexin-B.[1] Activation of orexin neurons contributes to the promotion and maintenance of wakefulness, and conversely, inactivation of orexin neurons leads to the onset of sleep.[2] 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.

Among numerous kinds of orexin receptor antagonists reported,[3a,3b] only two dual OX1R/OX2R receptor antagonists, Almorexant and MK-4305, have reportedly advanced into Phase–III development.[3b–e] In spite of the interesting profile of these dual orexin antagonists, the distinct contribution from either OX1R or OX2R in sleep regulation remains to be determined. In order to explore the potential of OX2R selective antagonism in the treatment of insomnia, we attempted to discover a series of novel, potent, and selective OX2R antagonists.

A high throughput screen generated a number of active chemotypes from which compound **1a** was selected as a hit (Fig. 1). 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. Compound **1a** showed potent OX2R antagonistic activity (74 nM), and good selectivity against OX1R (35-fold). However, **1a** suffered poor metabolic stability in human and rats liver microsome (HLM: 98 μL/min/mg and RLM: 224 μL/min/mg),[4] resulting in oral exposure below detection limit in rat cassette-dosing. We speculated that the metabolic vulnerability would come from metabolic liability of OMe group or an electron rich *p*-dimethoxylphenyl-like substructure, since it is well known that an electron rich aromatic ring is in general easily metabolized. This electron rich quinoid motif is also known as one of toxicophores triggering bioactivation,[5] which could lead to potential risk for drug-induced idiosyncratic toxicity in humans.[6] Thus, we focused on suitable replacement for OMe group at 7-position.

The representative synthesis of the analogues was outlined in Scheme 1 (the experimental procedure including the synthesis of intermediates **2** is reported in Supplementary data). Deprotection of Boc group of intermediate **2** gave spontaneously intramolecularly cyclized compound **3**. Reduction of **3** with $NaBH_4$ predominantly provided cis-isomer **4**, and acylation as the final step afforded the desired product **1**.



1a

OX2R IC_{50} : 74 nM
OX1R IC_{50} : 2600 nM
HLM: 98 μL/min/mg
RLM: 224 μL/min/mg
AUC_{po}*: < 1 ng / mL
F_{po}*: 0%

Figure 1. Hit Compound **1a**. *Male Sprague–Dewley rats (n = 3). Dose: iv infusion at 0.1 mg/kg; po at 1 mg/kg.

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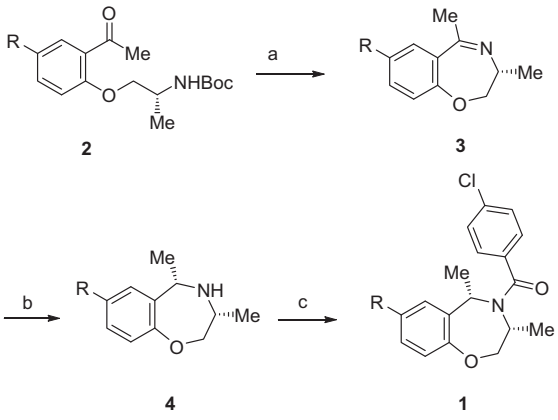
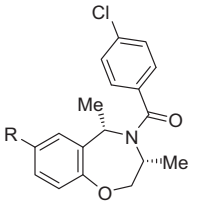


Scheme 1. Genaral Procedure of benzoxazepine synthesis. [a]Reagents and conditions: (a) TFA, DCM, rt, 30 min.; (b) $NaBH_4$, rt, 60 h; (c) 4-Chlorobenzoyl chloride, THF-1 N NaOH aq, rt, 1 h.

Table 1
OX2R Antagonistic Activities, and Human, Rat Metabolic Stabilities of OX2R Antagonists,[4] and Aromatic Substituent Constants[7]



Cmpds	R	OX-2[a] IC50 (nM)	HLM (μL/min/mg)	RLM[b] (uL/min/mg))	π^c	$\sigma_p{}^d$
1a	OMe	74	98	224	-0.02	-0.27
1b	H	6400	8	76	0	0
1c	Me	150	115	238	0.56	-0.17
1d	Et	160	146	206	1.02	-0.15
1e	SMe	6.4	146	227	0.61	0
1f	CN	8200	<1	<1	-0.57	0.66
1g	SO_2Me	4700	<1	<1	-1.63	0.72
1h	F	3000	15	17	0.14	0.06
1i	Cl	730	ND	11	0.71	0.23
1j	Br	430	<1	23	0.86	0.12
1k	CF_3	270	30	14	0.88	0.54
1l	OCF_3	140	7	<1	1.04	0.35
1m	$OCHF_2$	27	<1	5	0.31	0.18

[a] Antagonists activity.
[b] HLM = metabolic stability by the incubation with human liver microsomes; RLM = metabolic stability by the incubation with rat liver microsomes, c π value is lipophilic parameter prpposed by Hansch-Fuiita; σ_p value is Hammett electronic constant.

OX2R antagonistic activities, HLM, and RLM were summarized in Table 1. In order to recognize substitution effect on potency and metabolic stability, we annotate Hammet σ_p value as electron-donation or withdrawing nature and Hansch π value as lipophilicity (Table 1).[7] First of all, removal of OMe group showed significant drop in potency (**1b**), however, metabolic stability was improved, indicating that OMe group was liable to metabolic instability as we expected. Second, replacement of OMe with less electron-donating and lipophilic substituents such as Me and Et (**1c, 1d**) maintained potency, whereas replacement with SMe improved potency by 11-fold. However, the metabolic stability of all compounds was worse. This result indicates that those substituents like OMe are easily metabolized or their attenuating effect of electron-donation is not sufficient to improve metabolic stability.

In order to improve metabolic stability, we introduced less metabolically susceptible, polar, and electron-withdrawing groups such as CN and SO_2Me (**1f, 1g**), because increase in both polarity and electron deficiency of aromatic ring generally improves metabolic stability. As we expected, compounds **1f** and **1g** significantly improved metabolic stability to excellent levels, however, this improvement came at the cost of loss of potency.

At this point, we projected SAR to Craig plot consisting of Hansch π and Hammet σ_p as a classical quantitative SAR medicinal chemistry guideline.[8] From the standpoint of those physicochemical parameters, both CN and SO_2Me were plotted in $-\pi + \sigma_p$ region, which suggested their polar and electron-withdrawing nature may have caused the loss of potency. On the other hand, OMe, Me, Et, SMe groups were plotted in or around $+\pi - \sigma_p$ region and showed a good potency, which suggested that the increase in activity was not only electron-donation driven but also lipophilicity dependent. This observation prompted us to shift our interest to the third category, $+\pi + \sigma_p$ region, because lipophilic, electron-withdrawing and metabolically stable groups would increase potency without loss of metabolic stability. As we expected, the potency of compounds **1h–1l** with the increase of π value or lipophilicity (F < Cl < Br < CF_3 < OCF_3) was improved and all compounds showed good metabolic stability. The most potent compound **1l** with OCF_3 group showed acceptable potency at 140 nM.

To further improve potency, we focused on manipulation of the property of OCF_3. Considering that OCF_3 group is quite lipophilic and strong electronegative nature, we suspected that attenuation of electron-withdrawing nature of this group, or placing a group with a slightly stronger electron-donating property would afford more potent compound. On this concept, we designed $OCHF_2$ substituted compound **1m**, in which one fluorine atom is truncated and the electronegativity is reduced compared with **1l**.

The compound **1m** achieved good potency, 27 nM without reducing metabolic stability. Thus, we decided to select **1m** as a candidate for further evaluation. With respect to physicochemical properties, compound **1m** showed good water solubility as 16 μg/mL for JP2,[9] and lipophilicity (3.32 for log *D*), which is within CNS drug space.[10] This good water solubility and metabolic stability most likely attributed to good oral availability. Additionally **1m** exhibited >100-fold selectivity against OX1R with IC_{50} 3000 nM, suggesting the compound **1m** could be fairly regarded as a selective OX2R antagonist.

The representative compound **1m** was administered to rats by cassette-dosing (iv bolus at 0.1 mg/kg; po at 1 mg/kg). Oral exposure was significantly increased to an excellent level (658.1 ng/mL based on AUC_{po}) and oral availability (52%) was considered as very reasonable. Clearance was low (90 mL/h/kg), suggesting a potentially long acting profile (3.93 h based on MRT_{po}). In order to estimate CNS exposure potential, passive permeability and potential of P–gp substrate were evaluated using cell line expressing human MDR-1, which is considered to be the major efflux transporter at the blood brain barrier (BBB) and responsible for the efflux of a number of xenobiotic substances from the CNS. Compound **1m** showed good permeability and susceptibility of P-gp substrate (Passive permeability (MOCK): 226.6 nm/s; MDR1 directional transport ratio (B to A/A to B): 0.4).[11]

To determine whether a potent OX2R antagonist blocks the orexin signaling in vivo, we examined the effect of compound **1m** on the orexin-A peptide induced locomotion in rat.[13] Total activity (total beam breaks) was recorded in 1-min time bin for 120 min through use of a beam box. These experiments were performed with compound **1m** (10 mpk, 30 mpk) administered orally, 30 min before orexin-A i.c.v. injections. Central administration of orexin-A (1 nmol) significantly increased the total activity in rats. Responses from animals pretreated with compound **1m** were significantly attenuated in a dose-dependent manner (Fig. 2).

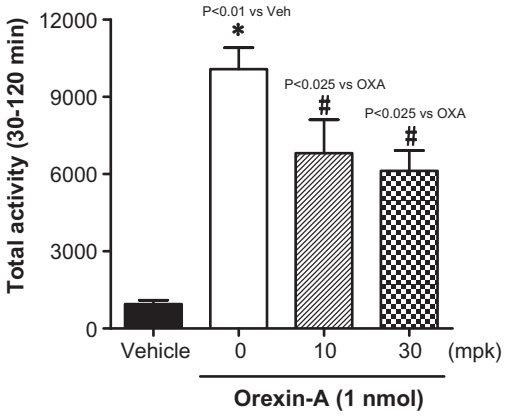


Figure 2. Effect of Compound **1m** on orexin-A induced locomotion. $^{*}p$ <0.01 versus Vehicle/Vehicle (Dunnetts test), $^{\#}p$ <0.025 versus Vehicle/OX-A (Williams test), n = 8 for each groups.

In summary, we have described SAR study of benzoxazepine scaffold leading to a novel, potent, selective, and orally available human OX2R antagonist. In attempts to improve potency and metabolic stability, we utilized Hammett σ_p and Hansch-Fujita π value as aromatic substituent constants. These efforts led to compound **1m**, which showed good in vitro potency (27 nM) with high LE as 0.40 and over 100–fold selectivity against OX1R, good metabolic stability in human and rat liver microsome, and good oral bioavailability in rats.[12] We further demonstrated the ability of compound **1m** to inhibit orexin-A mediated locomotion, when administered orally, in a dose dependent manner. Further in vivo evaluation will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.093.

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

Liver metabolic stability assay conditions

Condition	
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, 15, 30 min
Detection	5-in-One analysis using LC/MS/MS
Calculations	CL (μL/min/mg) = 1000*(1-exp(k*t))/ t/m t; time, 20 (min) m; microsomal protein, 0.2 (mg/mL) k; Rate constant (1/min) calculated from exponential fit of parent remaining vs. time curve, assuming first order kinetics
Criteria	Stable: <50 μL/min/mg, Moderate: 50–100 μL/min/mg, Unstable: >100 μL/min/mg

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