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Disubstituted piperidines as potent orexin (hypocretin) receptor antagonists

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ABSTRACT

A series of orexin receptor antagonists was synthesized based on a substituted piperidine scaffold. Through traditional medicinal chemistry structure–activity relationships (SAR), installation of various groups at the 3–6-positions of the piperidine led to modest enhancement in receptor selectivity. Compounds were profiled in vivo for plasma and brain levels in order to identify candidates suitable for efficacy in a model of drug addiction.

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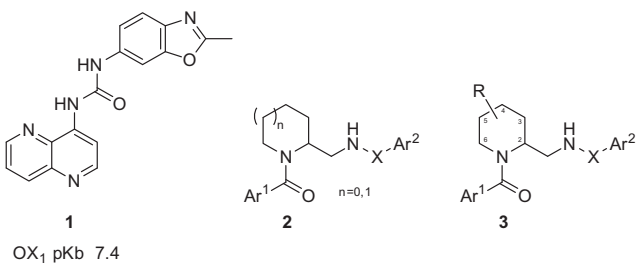
Orexins/hypocretins (Orexin-A and B, or hypocretin-1 and 2), simultaneously discovered by two groups in 1998, are a pair of hypothalamic neuropeptides with substantial amino acid identities.[1,2] Though produced by a small population of neurons in the posterior and lateral hypothalamus (LH), orexins exert multiple functions particularly in areas related to energy homeostasis, sleep, arousal and brain reward when binding to their respective G-protein coupled receptors OX_1 and OX_2 ($Hcrt_1$ and $Hcrt_2$ receptors, respectively). Due to the pharmacological potential from the modulation of these receptors, a significant effort has been poured into this area of research mostly in the area of insomnia.[3–9] The most advanced candidate Almorexant (a dual OX_1–OX_2 antagonist) from Actelion/GlaxoSmithkline(GSK) for the treatment of sleep disorders was dropped in late stage clinical development for safety concerns. Merck is also advancing a dual OX_1–OX_2 antagonist (Suvorexant) for sleep and is currently in PhIII.[10] Both of these drug candidates are dual OX_1–OX_2 antagonists with roughly equal potency on each receptor.

A growing body of evidence indicates that OX_1 receptors may play an important role in the behavioral adaptations associated with chronic drug exposure that may contribute to the development of addiction. Recently, compelling evidence has shown that activation of OX_1 in the brain plays a critical role in reward-seeking, drug relapse and addiction.[11] Chemical activation of LH orexin neurons reinstates extinguished morphine seeking behavior in rats, an effect blocked by the selective OX_1 receptor antagonist SB-334867.[11] Blockade of OX_1 transmission also decreases nicotine, and alcohol self-administration and attenuates cue-induced reinstatement of extinguished nicotine, alcohol and cocaine seeking, and attenuates stress-induced reinstatement of extinguished cocaine and alcohol seeking.[11–15] Injection of SB-334867 directly into the ventral tegmental area (VTA), a key brain area in drug addiction, attenuated the rewarding effects of morphine, as measured in a conditioned place preference (CPP) procedure and also mediated cue-induced cocaine seeking behavior.[16] These data suggest that orexin receptors, particularly those in the VTA, regulate the rewarding effects of drugs of abuse and support an important role for orexin transmission in drug-seeking and drug-taking behaviors. Thus, blockade of OX_1 receptors with OX_1 selective antagonists may provide a new mechanism and a promising therapeutic treatment for a variety of addiction related disorders.

The first OX_1 selective antagonist reported in the literature was SB-334867 (Fig. 1,1).[17,18] It has a reported OX_1 IC_{50} = 40 nM (Ca^{2+}) and is >100-fold selective for OX_1 versus OX_2. It was developed by GSK by modification of lead compounds from high throughput screening and is widely used in vitro and in vivo for OX_1 target



1
OX_1 pKb 7.4

2

3

Figure 1. Orexin antagonist scaffolds.

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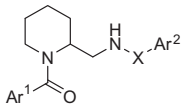
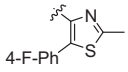
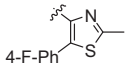
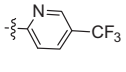
Table 1
2-Substitutedpiperidine (R=H) orexin receptor antagonists based on **2**



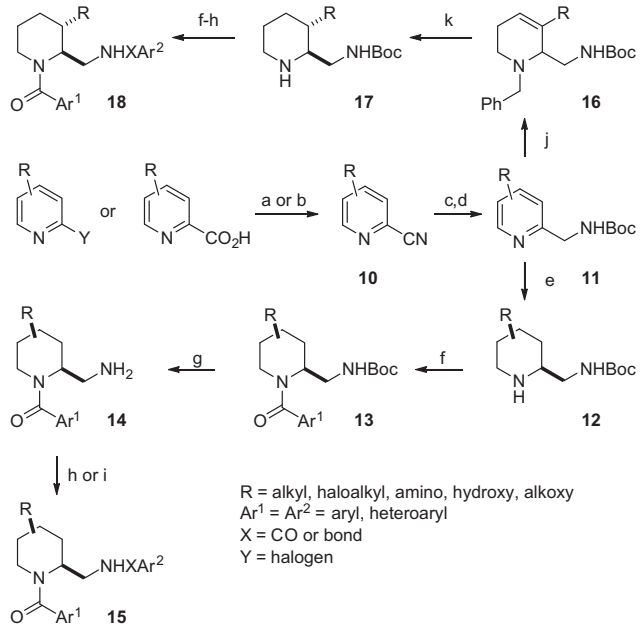
#	Ar^1	Ar^2	X	OX_1 IC_{50}[a] (nM)	OX_2 IC_{50}[a] (nM)
4			CO	3	2
5			CO	5	23
6			CO	4	4
7			CO	<1	<1
8			CO	6	8
9			—	13	8

[a] Values are means of at least three experiments.

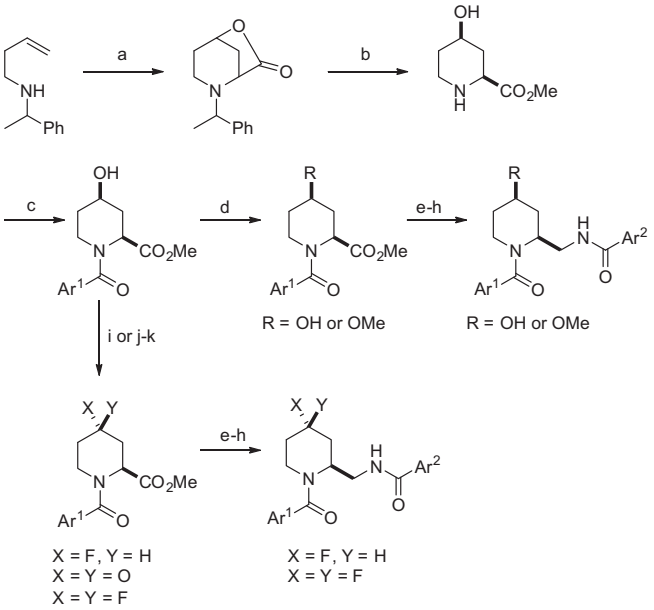
validation. However, the undesirable pharmacokinetic profile ($t_{1/2}$ = 0.4 h, 10% oral bioavailability) and potential for off-target activity at $5HT_{2B}$ and $5HT_{2C}$ hampered its progress beyond discovery phase.[18] Recently, another group further optimized this scaffold to dial out OX_2 completely, though no data is given with regards to off-target activity or pharmacokinetics.[19] Evaluation of both the primary and patent literature revealed that several orexin receptor antagonists have been developed based on a pyrrolidine or piperidine core with differentially substituted appendages at the N-1 and C-2 positions (Fig. 1,2).[6] When our research investigation began, there were scant reports of disubstituted piperidine antagonists (**3**). It wasn't clear if this was because ring substitution wasn't tolerated, or the chemistry simply hadn't yet advanced to this stage. We wondered if ring substitution could alter the chair topography of the piperidine ring, and subsequently affect selectivity for OX_1 versus OX_2. Recently, a patent application from Rottapharm S.P.A. published validating just such a strategy.[20] Herein we report the results of our investigation into substituted piperidines as orexin receptor antagonists.

To get a baseline and establish controls for comparison, we initially synthesized a variety of differentially substituted piperidines wherein we modified the N-1 acyl group and the substitution at C-2. These molecules have been reported mostly in the patent literature and contain little in vitro functional data.[21–24] Compounds were synthesized as described in the applications and screened in a functional cell-based assay using CHO cells stably expressing OX_1 (or OX_2 as a counterscreen) which is based on OX_A-stimulated intracellular calcium mobilization using a combination of calcium-sensitive dyes and a fluorescent imaging plate reader (FLIPR) (Table 1).[25]

These 2-substituted piperidines bearing a variety of heterocycles are potent dual OX_1–OX_2 receptor antagonists (Table 1). The most popular amides from the patent literature were chosen for N-1 substitution. The 2-biphenyl aryl amide and both phenyl-substituted thiazole isomers provided potent compounds (**4**–**9**). The 8-quinoline and 4-benzofuran amides, as well as the trifluoromethylpyridine anilines were chosen as C-2 substitutions. Compound **7** is the racemic version of SB649868, GSK's initial dual OX_1–OX_2 antagonist that advanced as far as PhII clinical trials before being pulled for preclinical toxicology findings.[26]



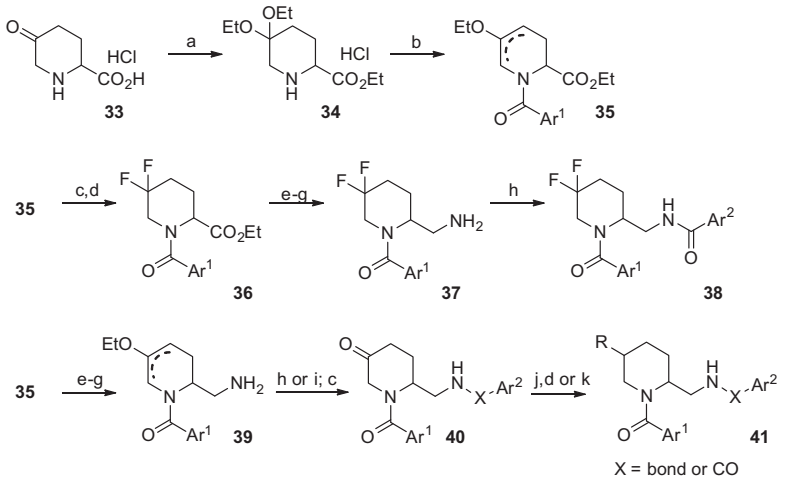
Scheme 1. Synthesis of 2,3- and 2,6-disubstitutedpiperidine orexin receptor antagonists. Reagents: (a) $ZnCN_2$, $Pd(PPh_3)_4$; (b) $SOCl_2$, NH_4OH, Tf_2O, DCM; (c) H_2, Pd/C, HOAc; (d) $(BOC)_2O$, Et_3N, DCM; (e) H_2, HOAc, Nishimura's catalyst; (f) Ar^1CO_2H, HATU, DIEA, DCM; (g) TFA, DCM; (h) Ar^2CO_2H, HATU, DIEA, DCM; (i) Ar^2Br, xantphos, Cs_2CO_3, DMAC; (j) (1) BnBr, CH_3CN; (2) $NaBH_4$, CH_3OH; (k) $Pd(OH)_2$, H_2, CH_3OH.



Scheme 2. Synthesis of 2,4-disubstitutedpiperidine orexin receptor antagonists. Reagents: (a) $OHCCO_2H$, CH_3CN, 4A sieves; (b) H_2, Pd/C, MeOH; (c) Ar^1CO_2H, HATU, DIEA, DCM; (d) For R = OMe, MeI, NaH, DMF; (e) $LiBH_4$, THF; (f) Phthalimide, DIAD, Ph_3P, THF; (g) N_2H_4, MeOH; (h) Ar^2CO_2H, HATU, DIEA, DMAC; (i) DAST, DCM; (j) Dess–Martin periodinane, DCM; (k) Deoxo–Fluor, DCM.

To probe the effects of additional substitution, we introduced substituents at positions 3–6 of the piperidine ring using the chemistry highlighted in Schemes 1–3.

We commenced the first round of SAR by introduction of substitution at the 3-position of the piperidine ring. This required access to 3-substituted 2-cyanopyridines, which were fairly easy to secure (**10**). Stepwise reduction and protection of the primary amine



Scheme 3. Synthesis of 2,5-disubstitutedpiperidines. Reagents: (a) $SOCl_2$, EtOH; (b) Ar^1CO_2H, HATU, DIEA, DMAC; (c) TFA, DCM; (d) Deoxo-Fluor, DCM; (e) $LiBH_4$, THF; (f) Phthalimide, DIAD, Ph_3P, THF; (g) N_2H_4, CH_3OH; (h) Ar^2CO_2H, HATU, DIEA, DMAC; (i) Ar^2Br, $Pd(OAc)_2$, xantphos, Cs_2CO_3; (j) $NaBH_4$, CH_3OH; (k) RNH_2, $NaBH(OAc)_3$, DCM.

at C-2 followed by saturation of the pyridine ring using Nishimura's catalyst afforded the 2,3-disubstituted piperidines (**12**). Compounds were almost exclusively 2,3-*cis*-oriented.[27–29] Standard manipulations afforded final products (Table 2). Synthesis of the 2,3-*trans* analogs was achieved from a common intermediate through a step-wise reduction of the pyridine ring after activation, followed by hydrogenation of the N-benzyl tetrahydropiperidine (**16**) to the deprotected piperidine (**17**). We were pleased to find that 3-substitution was indeed tolerated as most compounds exhibited good in vitro potency (Table 2).

A pair of bis-amide analogs showed almost 100-fold selectivity for OX_1 versus OX_2 (**19** and **23**) in the calcium flux assay, however, this trend was difficult to track as many analogs showed a more modest 10- to 20-fold selectivity. In the bis-amides, the bulkier CF_3 group provided no advantage over a standard methyl group (**25** vs **20**), nor did it appear that *cis* or *trans* methyl substitution made a difference (**27** vs **26**) in this bis-amide series. Interestingly, a 3-methyl substituted mono amide with an aminobenzoxazole in the side chain afforded a compound with an enhanced selectivity for OX_1 versus OX_2 (**28**). More analogs of this type are currently being synthesized to see if OX_2 activity can be completely abolished from this series.

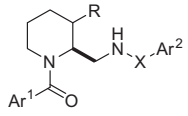
2,4-Disubstituted piperidine analogs were synthesized following standard literature chemistry as described in Scheme 2. Only a few analogs were made, as initial results weren't promising with regards to selectivity (Table 3). It's not clear how a simple fluorine substitution for hydrogen (**30** vs **4**) could result in an almost 50-fold drop in activity, but these results encouraged us to move on and to look at the C-5 and C-6 positions. In fact, all 2,4-disubstituted derivatives synthesized were less potent than their corresponding 2-substituted analogs.

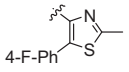
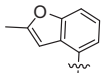
2,5-Disubstituted compounds could be made following chemistry as outlined in Scheme 1, but we also developed an alternate synthetic route, as we desired to expand the range of groups at this position on the ring. To that end, we synthesized 5-ketopipecolic acid (**33**) as a starting material as described in the literature.[30] Chemistry as outlined in Scheme 3 led to 2,5-disubstituted analogs (Table 4). Compounds resulting from reductive amination of the 5-ketopiperidine (**40**) gave mostly *trans*-2,5-disubstituted analogs resulting from axial delivery of hydride in the reduction step.[31]

Compounds in this series also tolerated substitution of the piperidine ring, and exhibited good in vitro activity (Table 4). There didn't appear to be much benefit, however, with regards to selectivity towards OX_1 versus OX_2. Some compounds showed a modest 5- to 10-fold selectivity for OX_1 (**43**, **44**, **46**), whereas others showed no selectivity at all.

Finally, the effect of installing a group at C-6 was examined (Table 5). Compounds were again synthesized following the general protocol as described in Scheme 1. Following saturation of

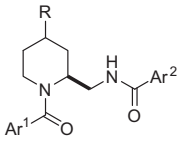
Table 2
2,3-Disubstitutedpiperidine orexin receptor antagonists

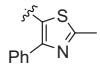
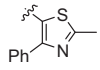


#	Ar^1	Ar^2	X	R	OX_1 IC_{50}[a] (nM)	OX_2 IC_{50}[a] (nM)
19			CO	Me (*cis*)	8	658
20			CO	Me (*cis*)	0.8	8.7
21			CO	Me (*cis*)	15	221
22			CO	CF_3 (*cis*)	9	40
23			CO	Me (*cis*)	145	>10,000
24			CO	Me (*cis*)	7	50
25			CO	CF_3 (*cis*)	10	40
26			CO	Me (*cis*)	2	58
27			CO	Me (*trans*)	2	82
28			—	Me (*cis*)	6	447

[a] Values are means of at least three experiments.

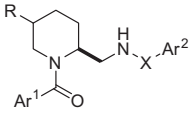
Table 3
2,4-Disubstitutedpiperidine orexin receptor antagonists

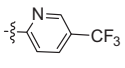


#	Ar^1	Ar^2	R	OX_1 IC_{50}[a] (nM)	OX_2 IC_{50}[a] (nM)
29			4-OH (*cis*)	40	62
30			4-F (*trans*)	146	541
31			4,4-diF	>2000	NT[b]
32			4-OMe (*cis*)	390	NT

[a] Values are means of at least three experiments.
[b] NT = not tested.

Table 4
2,5-Disubstitutedpiperidine orexin receptor antagonists



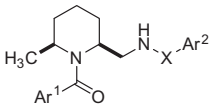
#	Ar^1	Ar^2	X	R	OX_1 IC_{50}[a] (nM)	OX_2 IC_{50}[a] (nM)
42			CO	F,F	2	2
43			CO	$NH(CH_2)_2OH$	27	137
44			CO	OH	2	13
45			CO	F	1	<1
46			CO	NH_2	121	2000
47			—	F	7	7

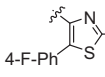
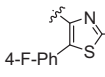
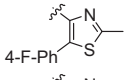
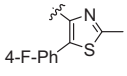
[a] Values are means of at least three experiments.

the pyridine ring, a single diastereomer was produced as indicated by ^1H NMR analysis (also single peak by reverse-phase analytical HPLC). It is presumed to be *cis* based on literature precedent.[27,32] No effort was made to synthesize the 2,6-*trans* isomer. A 6-methyl group was tolerated and imparted a modest 10-fold selectivity for OX_1 versus OX_2 (**48–51**). Synthesis of bulkier 6-substituted analogs is still on-going to see if more selectivity can be derived.

While chemical SAR has been on-going, in parallel, we have been routinely profiling compounds through drug metabolism in order to identify a compound suitable for in vivo use. We have been profiling compounds in microsomal stability, as well as looking for plasma and brain exposure in mice following ip dosing. Data is compiled in Table 6.

Table 5
2,6-Diubstitutedpiperidine orexin receptor antagonists



#	Ar^1	Ar^2	X	OX_1 IC_{50}[a] (nM)	OX_2 IC_{50}[a] (nM)
48			CO	0.8	8
49			CO	12	151
50			—	14	96
51			—	6	125

[a] Values are means of at least three experiments.

Table 6
In vitro and in vivo parameters of selected orexin antagonists

#	Microsome stability[a]	Mouse in vivo[b]		
	$t_{1/2}$	Plasma[c] (μm)	Brain (μm)	bp[d]
5	1/NT[e]/NT	1.5	0.06	4
7	1/1/1	33.7	18.7	55
9	NT/2/3	8.6	17.9	208
21	1/1/1	12.5	3	24
22	1/3/3	12.8	7.8	61
23	NT/NT/NT	20.8	10.1	49
28	1/1/1	1.6	0.9	56
45	1/7/1	4.6	0.5	11
46	7/15/52	27.6	1.4	5
49	4/6/5	0.3	0.1	33
50	1/3/2	12.4	14.3	115
51	1/1/1	3	1	33

[a] In mouse/rat/human liver microsomes.
[b] Dosed 50 mg/kg ip.
[c] Drug levels at l h timepoint.
[d] Brain penetration.
[e] NT = not tested.

The microsomal stability of most compounds examined in all three species (mouse, rat, human) was poor. Compounds were metabolized quite quickly. Even the racemate of GSK's PhII compound (**7**) was rapidly metabolized in microsomes. When compounds were dosed to mice (10 mg/kg, ip), there was little drug in plasma at $t = 1$ h, and even less in the brain.[33] Hence, the dose was increased to 50 mg/kg in an effort to increase plasma and brain levels that would support a compound's use in vivo. Data from these higher dosing experiments is shown in Table 6. For several analogs, plasma and brain levels at $t = 1$ h were quite high. In these cases, it is possible the metabolic mechanisms leading to low drug levels are being overwhelmed. However, this still does not explain the disconnect between microsomal stability and the fact that the enantiomer of **7** advanced into man. Nonetheless, these plasma and brain levels are likely sufficient to provide receptor coverage within the parameters of the in vivo study. Given the enhanced OX_1 selectivity of **23** and **28**, as well as their favorable drug plasma and brain levels at $t = 1$ h, these compounds may be useful in vivo candidates. Results of their in vivo efficacy in a model of drug addiction will be reported elsewhere.

In summary, we have described a series of orexin receptor antagonists based on substituted piperidines. SAR focusing on the

3-position revealed that it is possible to synthesize compounds with enhanced selectivity for the OX_1 receptor relative to OX_2. Substitution at other piperidine positions afforded potent compounds, with reduced or no selectivity for OX_1 versus OX_2. Further exploration of substitutions at the piperidine 3-position are still on-going. Evaluation of compounds such as **23** and **28** in an animal model of drug addiction will be reported in due course.

Acknowledgment

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25. *Measurement of $[Ca^{2+}]i$ using a FLIPR:* CHO-OX1or CHO-OX2 cells will be seeded into black-walled clear-base 384-well plates (Costar) at a density of 20,000 cells per well in F12-K medium supplemented with 10% FBS and selection antibiotic(500 μg/ml G418 or 15 μg/ml Blasticidin) and cultured overnight. The cells will be incubated with equal volume of calcium4 loading buffer (Molecular Devices, Inc.) containing 2.5 mM probenecid at 37 °C for 30 min, followed by putative OX1 receptor antagonists (dose-range 0.1 nM–10 μM)for another 30 min. The plates will then be placed into a FLIPR (Molecular Devices, Inc.) to monitor fluorescence ($\lambda_{excitation}$ 488 nm, $\lambda_{emission}$ 540 nm) before and after the addition of EC_{90} of [OXA].
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33. *Mouse brain penetration:* Compounds were dosed at 10 mg/kg intraperitoneally and after 2 h blood and brain were collected. Plasma was generated and the samples were frozen at −800 °C. The plasma and brain were mixed with acetonitrile (1:5 v/v or 1:5 w/v, respectively). The brain sample was sonicated with a probe tip sonicator to break up the tissue, and samples were analyzed for drug levels by LC–MS/MS. Plasma drug levels were determined against standards made in plasma and brain levels against standards made in blank brain matrix. All procedures were approved by the Scripps Florida IACUC.