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Proline bis-amides as potent dual orexin receptor antagonists

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Abstract—A series of OX_2R/OX_1R dual orexin antagonists was prepared based on a proline bis-amide identified as a screening lead. Through a combination of classical and library synthesis, potency enhancing replacements for both amide portions were discovered. N-methylation of the benzimidazole moiety within the lead structure significantly reduced P-gp susceptibility while increasing potency, giving rise to good brain penetration. A compound from this series has demonstrated in vivo central activity when dosed peripherally in a pharmacodynamic model of orexin activity.
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In 1998, two groups working independently discovered two neuropeptides; orexin A and orexin B.[1] Numerous studies have implicated a wide range of functions for orexin signaling in the CNS including regulating sleep–wake and feeding behaviors.[2] The two orexin peptides bind to their respective G-coupled protein receptors, OX_1R and OX_2R. While OX_1R exhibits about a 10- to 100-fold selectivity for orexin A over orexin B, OX_2R has an equal affinity for either peptide.[1a]

Orexin deficiencies have been implicated in narcolepsy.[3] Patients with narcolepsy often show low or undetectable levels of orexin A in the CSF and this decrease has been traced to a reduction of the neurons responsible for orexin production.[4] Orexins promote arousal and wakefulness and it is thought that low orexin levels in the brain are important in sleep initiation.[5] Several groups have developed orexin antagonist to explore the physiological role of the orexin receptors.[6] Inhibiting orexin signaling with receptor antagonists may provide a new mechanism for the treatment of insomnia. Actelion has recently reported that a dual OX_2R/OX_1R antagonist (ACT-078573) induced somnolence and increased electrophysiological markers for sleep in rats, dogs, and humans.[7] Herein, we describe a novel series of dual orexin antagonists based on a proline bis-amide scaffold and report their efficacy in a pharmacodynamic model of orexin induced hyperlocomotion.

The primary screens that we utilized were an in vitro binding assay measuring the ability of a compound to displace a high affinity radioligand bound to human orexin receptor (reported as K_i) and a cell based FLIPR assay (fluorometric imaging plate reader) which measures Ca^{2+} flux as a functional determinant of orexin binding.[8,9]

Recently, compound **1a** (Fig. 1), a benzimidazole containing proline bis-amide, was identified as a potent inhibitor of orexins from high throughput screening efforts. The original lead had unknown stereochemistry. Resynthesis of this lead in enantiomerically pure form identified the *S* stereochemistry as the more active enantiomer. The *S* enantiomer, **1b** (hOX_2R $K_i = 1$ nM, hOX_1R $K_i = 28$ nM), was a potent dual antagonist in

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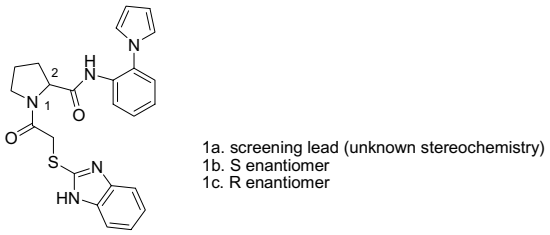
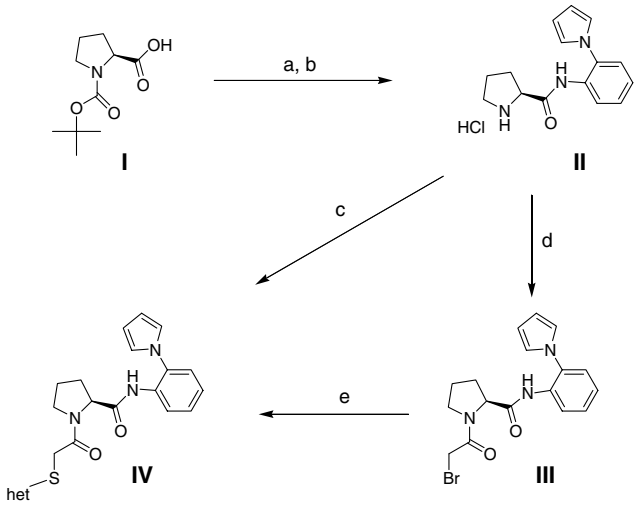


Figure 1. Proline bis-amide antagonists of orexin.

both the in vitro binding assay as well as the cell based FLIPR assay. The corresponding *R* isomer, **1c** (hOX_2R K_i = 131 nM, hOX_1R K_i = 2500 nM), was significantly less potent against both receptors. Fixing the L-proline moiety (*S* stereochemistry) as the central part of our molecule allowed us to explore the N-1 and C-2 amide portions independently.

Synthesis of these proline bis-amides began with a coupling of the appropriate aniline with Boc-L-proline (Scheme 1). Classical peptide coupling methodology (i.e. HOBt, mixed anhydride) was generally plagued with poor yields. An improved procedure using phosphorus oxychloride in pyridine at 0 °C was developed for the C-2 amide couplings.[10] Removal of the BOC group followed by standard peptide coupling conditions put the N-1 amide in place. In some cases where the N-1 amide chain contained a thioether, acylation of the proline with bromoacetyl bromide was followed by displacement of the α-bromide with a suitable heterocyclic thiol.

For substituted benzimidazoles where the thioether was replaced with a methylene unit, deviation from the first route began with compound **V** (Scheme 2). Amide coupling with 4-benzyloxybutyric acid was followed by catalytic hydrogenation to remove the benzyl group, giving the primary alcohol in quantitative yield. Oxidation via Dess–Martin periodinane afforded the aldehyde which was condensed with a 1,2-phenylenediamine to give the corresponding benzimidazole.[11] N-methylation of the benzimidazole was accomplished with iodomethane and cesium carbonate in DMF to afford compound **X**.



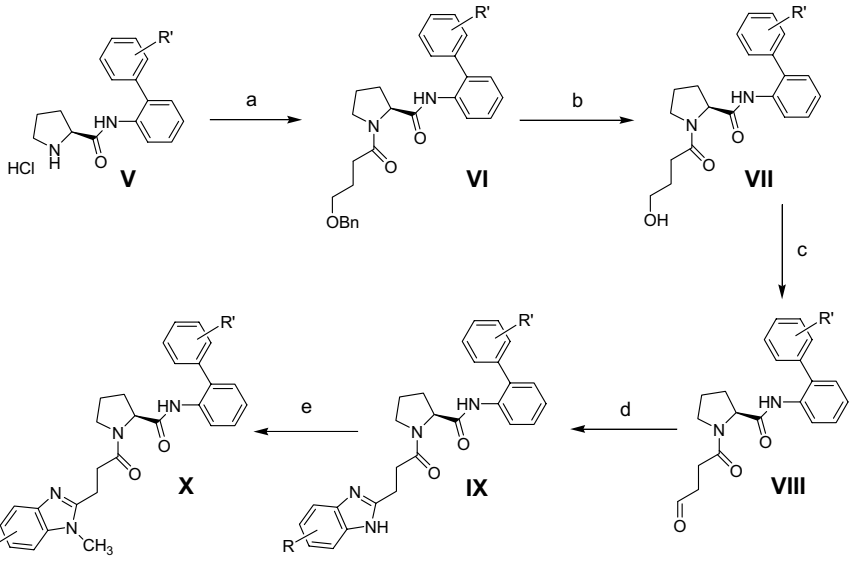
Scheme 1. General synthesis of proline bis-amides (route A). Reagents and conditions: (a) 1-(2-aminophenyl)pyrrole, $POCl_3$, pyridine, 0 °C, 62%; (b) EtOAc, HCl, 100%; (c) carboxylic acid, EDC, HOBt, Et_3N, DMF, 135 °C in microwave, 9–85%; (d) bromoacetyl bromide, Et_3N, CH_2Cl_2, 0 °C, 91%; (e) HS-het, Cs_2CO_3, DMF, 60 °C in microwave, 30–60%.

A large number of compounds were prepared in which the 2-pyrrolylphenyl carboxamide was replaced with various anilides (Table 1). Removal of the pyrrole group resulted in a significant loss of potency toward both orexin receptors (**2**). Replacement of the pyrrole with a phenyl group yielded a compound similar in hOX_2R binding, but significantly enhanced hOX_1R potency afforded a more balanced dual inhibitor (**3**). Moving the phenyl group to the *meta* (**4**) or *para* positions (**5**) was not well tolerated. Changing the 2-biphenyl to a 2-phenyl ether (**6**) resulted in a small loss of activity toward both receptors.

Compounds were prepared in which the sulfur was replaced with a carbon atom. These compounds were somewhat less potent than their respective sulfur containing counterparts but their SAR tracked similarly. Compound **7** is about 10-fold less potent in hOX_2R binding and half as potent in the FLIPR assay than its corresponding sulfur analogue (**3**). Additional C-1 amide replacements were evaluated with these carbon chain benzimidazoles. The 2-biphenylamides were the most promising compounds prepared in this series. Substitutions on the biphenyl such as *o*-methoxy (**8**) and *o*-fluoro (**9**) were also tolerated.

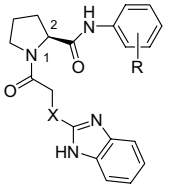
The ability of a compound to cross the blood–brain barrier and concentrate in the brain is an important consideration in developing centrally active drugs. A significant concern for CNS activity is a compound's susceptibility to P-gp transport. A P-gp efflux assay was used to determine if a compound is a substrate for P-gp.[12] It was determined that the benzimidazole N–H was a P-gp liability. Compounds **1b** (B to A/A to B ratio = 6.8) and **10** (B to A/A to B ratio = 13) were substrates for P-gp. Simply methylating **1b** and **10** gave compounds **11** (B to A/A to B ratio = 2) and **12** (B to A/A to B ratio = 2), respectively. Both of these are only moderate substrates for P-gp. An additional benefit of this methylation was a boost in potency, often of the order of 2- to 5-fold. Keeping the pyrrole fixed, a variety of benzimidazole replacements were evaluated (Table 2). The benzothiazole (**13**) was similar in hOX_2R potency to the unmethylated benzimidazole while the benzoxazole (**14**) was less potent against both orexin receptors.

Modification of the benzimidazole by substitution at the 5 position afforded still greater enhancement in potency. When a 5-methyl group is added to **1b** (**15**), it becomes equipotent to the *N*-methyl analogue **11**. Methylation of both positions (**16**) has an additive effect, yielding one of the most potent dual orexin antagonists to date. Moving the methyl to the 6 position (**17**) does not significantly change the orexin profile.[13]



Scheme 2. General synthesis of proline bis-amides (route B). Reagents and conditions: (a) 4-benzyloxybutyric acid, EDC, HOBt, Et_3N, DMF 42%; (b) Pearlman's catalyst, MeOH, hydrogen, 89%; (c) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2 100%; (d) substituted 1,2-phenylenediamines, $Mn(OAc)_3$, HOAc, 15–30%; (e) MeI, Cs_2CO_3, DMF, 100%.

Table 1. SAR of C-2 amide



Compound	R	X	hOX_2R binding (K_i, nM)[a]	hOX_2R FLIPR (IP, nM)[a]	hOX_1R binding (K_i, nM)[a]	hOX_1R FLIPR (IP, nM)[a]
1b	2-(1-Pyrrole)	S	1	6	28	240
2	H	S	560	4050	1400	20000
3	2-Ph	S	0.6	7	10	68
4	3-Ph	S	280	2640	500	20000
5	4-Ph	S	11	143	2300	1700
6	2-Phenoxy	S	3	16	69	450
7	2-Ph	C	6	20	40	240
8	2-(2-Methoxyphenyl)I	C	2	5	86	210
9	2-(2-Fluorophenyl)	C	3	4	27	68

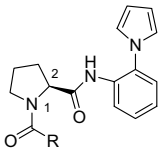
[a] Values are means of two or more independent experiments.

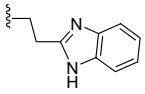
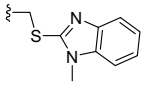
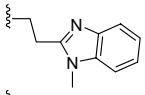
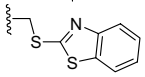
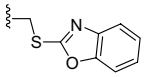
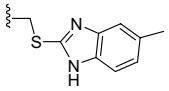
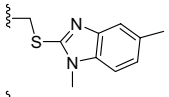
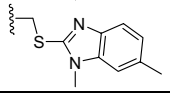
Combining advantageous substitutions of the C-2 and N-1 amides around the fixed proline constraint has an additive effect on orexin activity. Combining the biphenyl amide at C-2 with the *N*-methyl benzimidazole at N-1 (**18**), increased hOX_2R and hOX_1R potency 5- and 9-fold, respectively, compared to **1b**. Replacement of the sulfur in **18** with carbon (**19**) lowered the log *P* and improved rat oral bioavailability with little consequence on orexin binding. Both compounds are potent orexin antagonists and have acceptably low P-gp susceptibility. Compounds **18** and **19** represent optimized hOX_2R/hOX_1R antagonists (Table 3).

Brain/plasma levels were determined following intraperitoneal dosing (ip) in rats. Compounds **18** and **19** are brain penetrant, affording 2.4 and 13 μM levels, respectively, in the brain at 30 minutes after ip dosing (100 mpk).[14] Compounds **18** and **19** have water/octanol partition coefficients of 3.4 and 2.6, respectively, and neither compounds had any significant ancillary activity as determined through Panlabs (MDS Pharma).

In order to determine if a dual orexin antagonist could attenuate the actions of the ADL-orexin B peptide in vivo, a rat locomotion study was developed.[15] Movement parameters were measured through use of a beam box. After determining baseline activity for the rats, significant increases in activity were observed when rats received ICV injections of ADL-orexin B peptide.[16] The experiments were then performed with varying doses

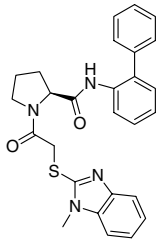
Table 2. SAR of N-1 amide

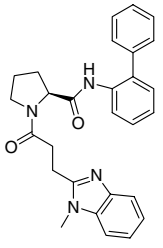


Compound	R	hOX_2R binding (K_i, nM)[a]	hOX_2R FLIPR (IP, nM)[a]	hOX_1R binding (K_i, nM)[a]	hOX_1R FLIPR (IP, nM)[a]
10		11	36	130	520[b]
11		0.3	2	9	22
12		2	3	49	480
13		2	11	39	545
14		8	28	120	1750
15		0.2	2	3	20
16		0.09	8	0.7	11
17		0.1	8	2	28

[a] Values are means of two or more independent experiments.
[b] Value is for rat OX_1R FLIPR.

Table 3. Optimized orexin inhibitors





Property	**18**	**19**
hOX_2R K_i (nM)[a]	0.2	0.8
hOX_1R K_i (nM)[a]	3	15
hOX_2R FLIPR (nM)[b]	4	5
hOX_1R FLIPR (nM)[a]	17	98
P-gp (B to A/A to B)	1.4	2
log P	3.4	2.6
Rat PK (Cl)[c]	3.7	35
Rat PK ($t_{1/2}$)	0.3 h	0.3 h
Rat PK (%F)	11	23
Brain/plasma/CSF (nM)[d]	2370/3500/43	13007/25705/860
Panlabs (IC_{50})[e]	3 hits < 10 μM	2 hits < 10 μM

[a] See Ref. 8 for and assay description and relevant reference therein.
[b] See Ref. 9 for an assay description.
[c] Measured in mL/min/kg.
[d] 100 mpk ip 30 min. See Ref. 14 for an assay description and relevant reference therein.
[e] Panlabs (MDS Phama): compound **18**: transporter, Monoamine: Protanoid, Thromboxane A_2 (TP); Thyrotropin releasing Hormone (TRH) **19**: Sodium Channel, Site 2; Thyrotropin Releasing Hormone (TRH).

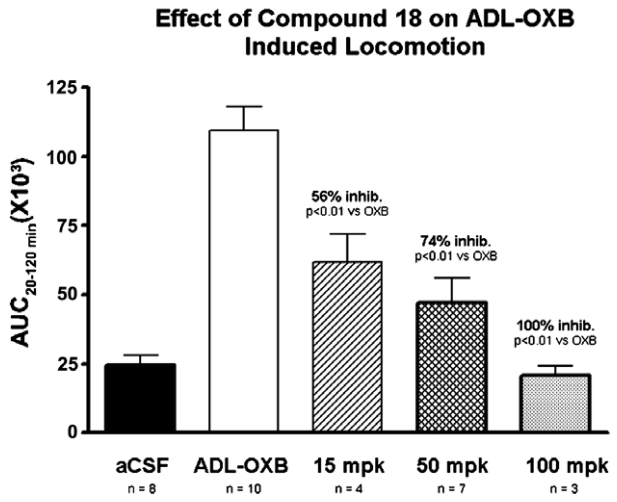


Figure 2. Induced Locomotion Inhibition.[17].

of compound **18** (15 mpk, 50 mpk, 100 mpk) administered intraperitoneally, 30 minutes before ADL-orexin B administration. Beam breaks were measured versus time and the AUCs over a 180 minute window were graphed (Fig. 2).[18]

A large increase in activity was observed in rats treated with ADL-orexin B alone. Pretreatment with compound **18** showed a clear, dose dependent inhibition of this excitation. At the 100 mpk dose locomotion was returned to baseline.

We have described a novel series of dual orexin antagonists based on a proline bis-amide scaffold. Starting with a screening lead, we were able to quickly identify antagonists with subnanomolar to low nanomolar potency for hOX_2R and hOX_1R. The series demonstrated excellent in vitro cell based activity, brain penetration, and low to moderate bioavailability in rats. We further demonstrated the ability of compound **18** to inhibit ADL-orexin B mediated locomotion, when dosed peripherally, in a dose dependent manner. Further improvements will be disclosed in due course.

Acknowledgments

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8. (a) *Radioligand binding assays for orexin receptors*. [^{3}H]-compound **11** was used to determine the OX_2R binding and [^{3}H]-compound **18** for OX_1R binding. Membranes were prepared from CHO/OX_2R and CHO/OX_1R cells. A dose-inhibition radioligand binding assay was performed by the Tecan robot by a standard protocol to test compounds synthesized by medicinal chemistry programs.[8b]; (b) Mosser, S. D.; Gaul, S. L.; Bednar, B.; Koblan, K. S.; Bednar, R. A. *JALA* **2003**, *8*, 54.
9. *FLIPR assay*. CHO-K1 cells expressing the orexin receptor (hOX_1R or hOX_2R) were seeded into 96-well plates and incubated with a cytoplasmic calcium indicator. After washing the cells, intercellular Ca^{2+} mobilization was monitored as a change in cell fluorescence intensity by FLIPR (Molecular Devices). Differing concentrations of orexin antagonists were added to the plates prior to addition of orexin A and antagonistic activity calculated.
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12. (a) P-gp is a *N*-glycosylated integral membrane protein with two ATP binding sites, which function as an energy-dependent drug efflux pump. P-gp efflux is determined by a transcellular transport study using human MDR1 cells and measuring the transport of a compound from basal to

apical and apical to basal cells then determining the ratio (B to A/A to B); (b) Hochman, J. H.; Pudvah, Q.; Yamazaki, M.; Tang, C.; Lin, J. H.; Prueksaritanont, T. *Pharm. Res.* **2004**, *21*, 1686; (c) Yamazaki, M.; Neway, W. E.; Ohe, T.; Chen, I.; Rowe, J. F.; Hochman, J. H.; Chiba, M.; Lin, J. H. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 723.

13. While the majority of the proline bis-amides described are dual inhibitors of orexin, it is noteworthy that considerable hOX_2R selectivity can be achieved in this series with certain modifications. When the upper amide contains the 1-(4-phenyl)anilide (compound **5**), a moderately potent hOX_2R antagonist with greater than 200-fold selectivity for hOX_2R versus hOX_1R binding is obtained.
14. (a) *Brain/plasma/CSF methods*. Male rats are dosed intraperitoneally (ip) with the test article or vehicle (control) and returned to their home cage for the test article incubation period, either 30 or 120 minutes. At the end of the incubation period, the rat is placed in an anesthesia induction chamber and anesthetized with isoflurane. Once anesthetized, the rat is placed on a breathing circuit (nose cone) and anesthesia is maintained. The head is shaved and the shaved area wiped with an alcohol pad. The rat is placed on an angled plexi-glass stand and the head is flexed over the top of the stand. The CSF is collected from the cisterna magna by needle puncture and gentle aspiration. The CSF is dispensed and immediately frozen on dry ice. The rat is then placed on its back and a blood sample is obtained from the same anesthetized rat via cardiac puncture. The blood is centrifuged and the plasma is removed via pipette, dispensed into an appropriate vial, and frozen on dry ice. After the blood is collected, the rat is euthanized and the brain is removed. The olfactory bulbs and hind brain are then removed and discarded. The CSF, plasma, and brain samples are stored at −70 °C until assayed by Analytical Chemistry for compound level; (b) Fujiki, N.; Yoshida, Y.; Ripley, B.; Honda, K.; Mignot, Emmanuel; Nishino, S. *NeuroReport* **2001**, *12*, 993.
15. *Locomotion assay*. Male ICV cannulated rats were individually housed, maintained on a 12/12 light/dark cycle with lights on at 6 AM, and had free access to food and water. Experiments were conducted between 8 AM and 2 PM each day using Med Associates Open Field activity chambers that employ infrared photobeam technology. Photobeam bars are mounted externally on the chamber and locomotor activity was recorded in the *xy*-plane (ambulations, fine movements, distance traveled) and *z*-plane (rearing) as the number of beams interrupted. Each rat was allowed to acclimate to the chamber for 90 minutes. The rat then received an intraperitoneal injection of either vehicle or test compound. Thirty minutes later, each rat received an ICV infusion of either vehicle (aCSF) or ADL-OXB ([Ala^{11}-D-Leu^{15}]-orexin-B) using a microdialysis pump delivering the peptide into the lateral ventricle. The ICV cannula was capped and the rat was then returned to the activity chamber where the locomotor activity was recorded for an additional 180 minutes. The data were tabulated in 10 minute bins. Mean responses and SEM were calculated and graphed using Sigmaplot graphing software. Area under the activity curve (AUC) was calculated and graphed using GraphPad Prism.
16. Asahi, S.; Egashira, S.; Matsuda, M.; Iwaasa, H.; Kanatani, A.; Ohkubo, M.; Ihara, M.; Morishima, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 111.
17. This graph represents effects on fine movement activity in ICV cannulated rats. Similar profiles were observed in the other three locomotion parameters measured as well (distance traveled, ambulatory movement, and vertical movement). Although locomotor activity was recorded for 180 minutes (as described in Ref. 15), the control response returns to baseline activity levels around 120 minutes. The AUC is calculated during this time frame. This is represented on the graph as what we considered to be the measurable response.
18. All animals used in these studies were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The Merck Research Laboratories Institutional Animal Care and Use Committee (IACUC) approved all studies described in this manuscript and experimental protocols were in accordance with all applicable guidelines regarding the care and use of animals. Animals were housed in an AALAC International approved facility with free access to food and water.