Synthesis of Di-Branched Heptasaccharide by One-Pot Glycosylation Using Seven Independent Building Blocks

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N-Acyl 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline: The First Orexin-2 Receptor Selective Non-peptidic Antagonist

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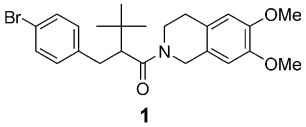
Abstract—The identification of potent and selective orexin-2 receptor (OX_2R) antagonists is described based on the modification of *N*-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogue **1**, recently discovered during high throughput screening (HTS). Substitution of an acyl group in **1** with *tert*-Leucine (*tert*-Leu), and introduction of a 4-pyridylmethyl substituent onto the amino function of *tert*-Leu improved compound potency, selectivity, and water solubility. Thus, compound **29** is a promising tool to investigate the role of orexin-2 receptors.

Orexin-A and -B are the novel neuropeptides that have been identified as ligands of orphan G protein coupled receptors (GPCR) from rat brain extracts.[1] Evidence continues to indicate that they are involved in the regulation of many neuronal functions, including feeding,[1] sleep/wake cycles,[2,3] neuroendocrine activity,[4,5] stress reactions,[6] and activation of the sympathetic nerve system.[7] The innervation of orexin neurons in the brain supports diversity in function of the orexins.[8] The physiological functions of orexins are evoked by two receptor subtypes, orexin-1R (OX_1R) and orexin-2R (OX_2R) which belong to a family of GPCR. Orexin-A is a 33-residue peptide with two intra-molecular disulfide bridges, which possessed potent agonistic activity toward both subtypes of orexin receptors. In contrast, orexin-B, which consists of 28-amino acids, possessed greater affinity for OX_2R than for OX_1R.[1] Moreover, the expression patterns of the two orexin receptors in the brain are different.[9] The orexin receptor subtypes are likely to be involved in different pharmacological functions. Recently, a small molecular OX_1R selective antagonist was developed as a potential tool to elucidate the functions of OX_1R.[10] Selective agonist of OX_2R, which can address the pharmacological functions of OX_2R, were recently identified;[11] however, OX_2R selective antagonists remain critical to understanding the physiological and pathophysiological roles of OX_2R. Therefore, the development of small molecular OX_2R selective antagonists for use as pharmacological tools is important for clarification of the functions of OX_2R. This communication describes the development of *N*-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogues as the first OX_2R selective non-peptidic antagonist and reports progress in optimizing this structural class, leading to compound **29**, which possesses potency, selectivity, and water solubility. As a part of our program to develop selective OX_2R antagonists, we discovered the non-selective orexin antagonist **1** (human OX_1R (hOX_1R) IC_{50}: 7 μM; human OX_2R (hOX_2R) IC_{50}: 2 μM) through HTS screening.[12]

Substitution and modification of the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline skeleton in **1** with other acyclic and cyclic amine moieties resulted in loss of potency against both hOX_1R and hOX_2R (data not shown). Thus, the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline skeleton seems to be an essential core for retaining biological activity. Based on these results, our attention focused on modification of the acyl portion of **1**.



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SAR of 3-Phenylpropanoyl Amides

To understand the structure–activity relationship (SAR) of **1**, initially we prepared *N*-(3-phenylpropanoyl) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogues. Removal of the bromine from the benzene ring of **1** resulted in a 30-fold increase in potency toward hOX_2R (**2**). Removal of the *tert*-butyl group from **2** afforded **3**, which did not possess antagonistic activity against either orexin receptor. The results of functional group substitutions for the *tert*-butyl group of **2** are listed in Table 1. A dimethylamino or benzylamino group at this position was not allowed (**5** and **6**), suggesting that neutral bulky substituents at this position are indispensable for antagonistic activity toward hOX_2R. Although replacement of the *tert*-butyl moiety with a benzoylamino group (**7**,**8**) led to significant reduction of activity toward hOX_2R compared to **2**, the (*S*)-isomer (**8**) showed relatively high potency than did its racemate (**7**). Introduction of neutral bulky substituent(s) on the benzoyl group in **8** improved potency toward hOX_2R; thus, the (*S*)-3,5-dichlorobenzoylamino analogue (**10**) brought about a 6-fold improvement in hOX_2R potency relative to **8**.

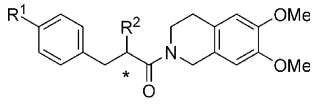
SAR of *N*-Substituted *tert*-Leu Amides

To improve water solubility and develop useful pharmacological tools, we modified the benzyl group in **1**. Introduction of hydrophilic substituents such as a dimethyl amino group on the benzene ring of **1** resulted in loss of potency toward both orexin receptors (data not shown). In some cases, substitution of the benzyl group with a substituted amino group afforded *tert*-Leu amide analogues and resulted in greater water solubility without loss of potency or selectivity (Table 2), especially in the case of the *N*-benzyl substituent (**15**). Reduction or elongation of the carbon chain between the nitrogen atom and phenyl group in **15** resulted in loss of potency (**12**, **13**, **18** and **19**). Substitution of a benzyl group in **15** for a cyclohexylmethyl group also resulted in complete loss of potency (**20**). Thus, aromatic functionality in the *N*-benzyl group seemed to be essential. Regarding to the stereochemistry of the *tert*-Leu portion, (*S*)-configuration is more preferable than (*R*)-configuration (**16** vs **17**).

None of the other *N*-benzyl amino acid analogues, such as (*N*-benzyl)phenylalanine **6**, *N*-benzyl-valine **21** or (*N*-benzyl)phenylglycine **22**, possessed greater potency toward hOX_2R than the *tert*-Leu analogue **15** (Table 3). Only the *N*-benzylvaline **21** analogue showed weak activity against hOX_2R (IC_{50}: 3300 nM).

Substitution of the benzyl group in **16** with other arylmethyl groups improved potency (Table 4). Replacement of the benzene ring in **16** with five-membered heteroaromatic rings generally improved hOX_2R potency. In particular, the 2-thienyl analogue **25** led to a

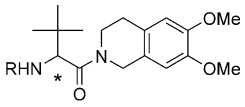
Table 1. Antagonistic activity of 1-(3-phenylpropanoyl)-tetrahydriisoquinoline analogues against hOX_1R and hOX_2R[a]



No	R^1	R^1	hOX_1R	hOX_2R
			(IC_{50}, nM)[b]	
1	Br	*tert*-Butyl	7000	2000
2	H	*tert*-Butyl	5500	56
3	H	H	> 10,000	> 10,000
4	H	Phenyl	> 10,000	395
5	H	Dimethylamino	> 10,000	> 10,000
6	H	Benzylamino[c]	> 10,000	> 10,000
7	H	Benzoylamino	> 10,000	900
8	H	Benzoylamino[c]	> 10,000	195
9	H	3,5-Dichlorobenzoylamino	5300	36
10	H	3,5-Dichlorobenzoylamino[c]	2300	30
11	H	3-Bromo-4-fluorobenzoylamino	5900	70

[a]All compounds were synthesized as racemic mixture otherwise noted.
[b]Values are the mean of more than two independent experiments performed in duplicate.
[c](*S*)-Isomer.

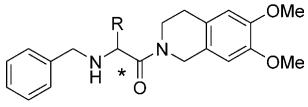
Table 2. Antagonistic activity of 1-(*tert*-leucyl)-tetrahydroisoquinoline analogues against hOX_1R and hOX_2R)[a]



No.	R	hOX_1R	hOX_2R
		(IC_{50}, nM)[b]	
12	H	> 10,000	> 10,000
13	Phenyl	> 10,000	2100
14	Benzoyl	> 10,000	> 10,000
15	Benzyl	> 10,000	910
16	Benzyl[c]	3850	130
17	Benzyl[d]	> 10,000	2900
18	2-Phenylethyl	> 10,000	> 10,000
19	3-Phenylpropyl	> 10,000	> 10,000
20	Cyclohexylmethyl	> 10,000	> 10,000

[a]All compounds were synthesized as racemic mixture otherwise noted.
[b]Values are the mean of more than two independent experiments performed in duplicate.
[c](*S*)-Isomer.
[d](*R*)-Isomer.

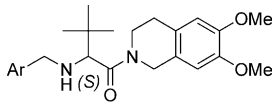
Table 3. Antagonistic activity of *N*-benzyl α-aminoacyl tetrahydroisoquinoline analogues against hOX_1R and hOX_2R[a]



No.	R	hOX_1R	hOX_2R
		(IC_{50}, nM)[b]	
6	Benzyl[c]	> 10,000	> 10,000
15	*tert*-Butyl	> 10,000	910
16	*tert*-Butyl[c]	3850	130
21	*i*-Propyl	> 10,000	3300
22	Phenyl[c]	> 10,000	> 10,000

[a]All compounds were synthesized as racemic mixture otherwise noted.
[b]Values are the mean of more than two independent experiments performed in duplicate.
[c](*S*)-Isomer.

Table 4. Antagonisitc activity of *N*-arylmethyl (*S*)-*tert*-leucyl tetrahydroisoquinoline analogues against hOX_1R and hOX_2R



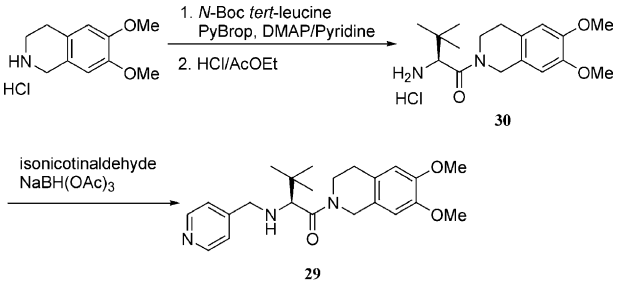
No.	Ar	hOX_1R	hOX_2R
		(IC_{50}, nM)[a]	
16	Phenyl	3850	130
23	2-(*N*-Methyl)pyrrolyl	3600	28
24	2-Thiazolyl	>10,000	59
25	2-Thienyl	1130	25
26	3-Thienyl	3670	35
27	2-Pyridyl	>10,000	1400
28	3-Pyridyl	>10,000	240
29	4-Pyridyl	>10,000	40

[a]Values are the mean of more than two independent experiments performed in duplicate.

5-fold improvement in the hOX_2R potency; however, its selectivity against hOX_2R over the hOX_1R was decreased compared to **16**. Nitrogen containing heteroaromatic analogues were generally showed high OX_1R/OX_2R selectivity. For the pyridine analogues, hOX_2R potency depended heavily on the position of the nitrogen. 4-Pyridyl methyl analogue **29** possessed the most potent activity for hOX_2R compared to other regio isomers (**27** and **28**). Furthermore, **29** showed over 250-fold selectivity for hOX_2R compared with hOX_1R (Table 4), as well as over 50 receptors, ion channels, and transporters (<30% inhibition at 10 μM), which includes G-protein coupled receptors associated with food intake including galanin and neuripeptide Y (data not shown). The high water solubility of **29** was another benefit (0.81 mg/mL at pH 7) to use pharmacological experiment. Full details of additional pharmacological testing of **29** will be described elsewhere.

Synthesis of Compound 29

Compound **29** was prepared according to Scheme 1. Commercially available 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrogen chloride was condensed with *N*-Boc *tert*-leucine using PyBrop[13] in pyridine in the presence of DMAP followed by treatment of HCl/AcOEt to yield the amine hydrogen chloride **30**. Conversion of **30** to the 4-pyridylmethyl analogue **29** was accomplished by the standard reductive amination procedure using isonicotinaldehyde and sodium triacetoxyborohydride.[14] Other compounds described here were synthesized by similar methods.



Scheme 1. Synthesis of compound **29**.

In summary, in this communication, the structure–activity relationship of the tetrahydroisoquinoline **1** toward hOX_2R was outlined. 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline was the essential core skeleton for hOX_1R and hOX_2R potency. *N*-Arylmethyl *tert*-leucyl 6,7-dimethoxy-1,2,3,4-tetrahydroiso-quinoline analogues generally showed high potency for the hOX_2R. Their selectivity could be enhanced by modifying the arylmethyl motif of the amino functionality. Nitrogen-containing hetero-aromatics were superior for improving both potency and selectivity toward hOX_2R. Finally, introduction of a 4-pyridylmethyl group on the amino function of the *tert*-Leu moiety yielded a potent hOX_2R antagonist **29** with high selectivity and high water solubility.

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12. Brief screening protocol: CHO-K1 cells stably expressing hOX_1R or hOX_2R were seeded into 96-well plates and incubated with a cytoplasmic calcium indicator, Fluo-3 AM. After the cells were washed four times, the intracellular Ca^{2+} mobilization evoked by 0.3 nM of orexins-A was monitored as a change in cell fluorescence intensity by FLIPR (Molecular Devices). Varying concentration of orexin antagonists were added to the plate 5 min prior to the addition of orexin-A. The antagonistic activities were calculated as IC_{50} values.
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14. Characterization of **29**: ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 and 1.02 (s each, 9H), 2.61–2.90 (m, 2H), 3.20–3.51 (m, 3H), 3.62–4.13 (m, 8H), 4.31–4.99 (m, 2H), 6.40, 6.60, 6.60 and 6.65 (s each, 2H), 7.09–7.32 (m, 2H), 8.40–8.56 (m, 2H).