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Novel substituted 4-phenyl-[1,3]dioxanes: potent and selective orexin receptor 2 (OX_2R) antagonists

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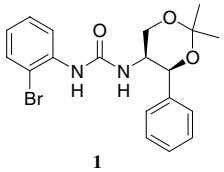
Abstract—Orexins, also termed hypocretins, consist of two neuropeptide agonists (orexin A and B) interacting with two known G-protein coupled receptors (OX_1R and OX_2R). In addition to other biological functions, the orexin-2 receptor is thought to be an important modulator of sleep and wakefulness. Herein we describe a series of novel, selective OX_2R antagonists consisting of substituted 4-phenyl-[1,3]dioxanes. One such antagonist is compound **9**, 1-(2,4-dibromo-phenyl)-3-((4*S*,5*S*)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea, which is bound by the OX_2R with a pK_i of 8.3, has a pK_b of 7.9, and is 600-fold selective for the OX_2R over the OX_1R.

1. Introduction

In 1998 two research groups, working independently and using different methods, discovered two neuropeptides, orexin A and orexin B.[1,2] The two neuropeptides, which are processed from the same gene and are produced by neurons in the lateral hypothalamus are also known as the hypocretins.[1,2] The orexin neuropeptides play a role in a variety of biological functions including the sleep/wake cycle, feeding behavior, regulation of gastric acid secretion, metabolic rate, and blood pressure.[3–10] Two receptors for orexin A and orexin B were also identified and they are known as orexin 1 (OX_1R) and orexin 2 (OX_2R).[2] While the orexin-2 receptor binds both neuropeptides with similar affinity, the orexin-1 receptor binds orexin A with slightly higher affinity than orexin B. Several reports of antagonists for the orexin-1 receptor have already appeared in the literature and suggest that this receptor is closely related to feeding, metabolism, and digestive function.[11–16] Recent data suggests that the orexin-2 receptor is associated with the sleep/wake cycle and that mutations to this receptor are related to canine narcolepsy.[5] Human narcolepsy appears to be linked to deficient orexin signaling, most likely related to immune ablation of orexinergic neurons in the lateral hypothalamus.[17,18] Herein we report on a novel series of nonpeptidic, small molecule antagonists for the orexin-2 receptor. These compounds are among the most potent and selective small molecule antagonists for this receptor to date and should prove useful for further elucidating the role of the OX_2R receptor.[19–22]

2. Chemistry

A high throughput screen (HTS) of our chemical library identified several hits including the novel urea-containing 4-phenyl-[1,3]dioxane, **1**, as an antagonist of the human orexin-2 receptor. Compound **1**, 1-(2-bromo-phenyl)-3-((4*S*,5*S*)-2,2-dimethyl-4-phenyl-[1,3]dioxan-5-yl)-urea, as a single enantiomer was also fairly selective for the



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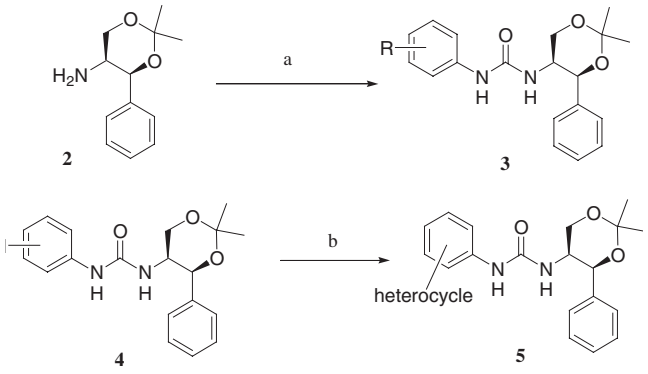
orexin-2 receptor (pK_i $OX_2R = 7.0$, $OX_1R = 5.7$).[23] Compound **1** and related analogs were readily synthesized by reacting commercially available isocyanates with amine **2** (Scheme 1).[24] In addition, Suzuki cross-coupling reactions[25,26] could be successfully performed on several of the resulting products, such as **4**, to provide compounds with a diverse range of functionality including heterocyclic substituents. In an effort to further increase receptor binding affinity, we initially chose to explore a variety of substituents with a range of different electronic properties at one or more positions on the phenyl ring arising from the isocyanate (Tables 1 and 2).[27]



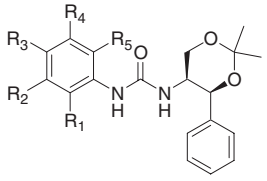
Scheme 1. Reagents and conditions: (a) isocyanate (R-PhNCO) and pentane, ~80–90%; (b) tetrakis(triphenylphosphine) palladium(0), ethylene glycol dimethyl ether, boronic acid, aqueous sodium carbonate ~50%.

We were also interested in identifying, which changes, if any, would be tolerated in the central urea portion of the molecule. Traditional urea replacement analogs such as those with amide, thiourea, and cyanoguanidine functionalities were synthesized. Thioureas were synthesized in the same manner as the urea analogs except that an isothiocyanate was used in place of an isocyanate in the reactions. Amides and carbamates were synthesized by reacting amine **2** with an acyl chloride or phenyl chloroformate in the presence of triethylamine. Cyanoguanidines were synthesized in two steps starting from diphenylcyanocarbonimidate and an appropriately substituted aniline (Scheme 2).

3. Biological results and discussion

Compounds **1** and **6**–**34** were evaluated for biological activity. Determination of receptor binding characteristics of the compounds was accomplished using adherent, cultured recombinant cells, and radioiodinated orexin A.[28] In addition, compound antagonism was demonstrated using a functional assay of intracellular Ca^{++} responses in cells expressing OX_2R.[29] Agonist binding to orexin receptors elevates the intracellular free calcium ion concentration through the activation of a Gq protein and the opening of voltage-activated plasma membrane calcium channels.[30] This effect was monitored using the fluorescent Ca^{++} indicator Fluo-3 AM (TefLabs, Austin, TX) and the Molecular Devices

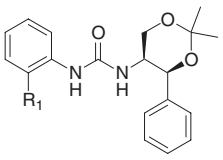
Table 1. Orexin receptor binding affinities of 4-phenyl-[1,3]dioxanes



Compound[a]	R_1	R_2	R_3	R_4	R_5	OX_2R[b]	OX_1R[b]	Selectivity[c]
1	Br	H	H	H	H	7.0 (6.8–7.1)	5.7 (5.3–6.1)	20
6	H	H	H	H	H	6.3 (6.1–6.4)	<5.0	>20
7	H	Br	H	H	H	6.0 (5.8–6.1)	<5.0	>9
8	H	H	Br	H	H	6.6 (6.3–7.0)	<5.0	>40
9	Br	H	Br	H	H	8.3 (8.0–8.6)	5.5 (5.3–5.8)	600
10	Cl	H	Cl	H	H	7.7 (7.6–7.9)	5.6 (5.4–5.8)	100
11	Cl	H	Br	H	H	8.2 (7.9–8.6)	<5.3	>900
12	Me	H	Br	H	H	7.4 (7.3–7.5)	<5.0	200
13	Cl	H	H	Me	H	7.2 (7.0–7.3)	<5.3	>80
14	Me	H	Me	H	H	6.9 (6.7–7.0)	<5.0	>70
15	Cl	H	H	Cl	H	7.2 (7.1–7.3)	<5.3	>80
16	Cl	H	H	H	Cl	<5.3	<5.0	>2
17	I	H	H	H	H	7.1 (7.0–7.3)	<5.3	>70
18	Cl	H	H	H	H	7.1 (7.0–7.2)	<5.0	>100
19	F	H	H	H	H	6.4 (6.1–6.6)	<5.0	>20
20	OMe	H	H	H	H	5.6 (5.3–5.9)	<5.0	>4
21	CF_3	H	H	H	H	7.0 (6.9–7.1)	<5.0	>100
22	*i*-Pr	H	H	H	H	5.6 (5.4–5.7)	<5.0	>4

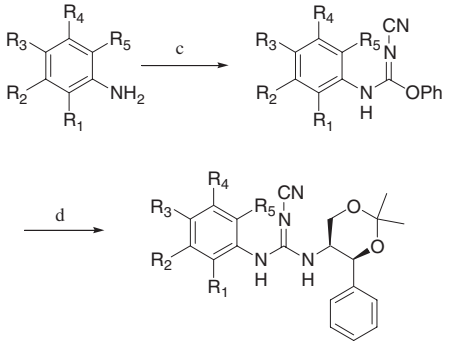
[a] See Ref. 27.
[b] pK_i with 95% confidence intervals in parenthesis.
[c] Selectivity = (OX_1R K_i/OX_2R K_i).

Table 2. Orexin receptor binding affinities of 4-phenyl-[1,3]dioxanes



Compound[a]	R_1	OX_2R[b]	OX_1R[b]	Selectivity[c]
23	2-Thiophene	7.3 (7.1–7.4)	6.0 (5.9–6.1)	20
24	3-Thiophene	7.1 (7.1–7.2)	5.7 (5.5–5.9)	30
25	Phenyl	7.0 (6.7–7.3)	6.3 (6.2–6.4)	4
26	2-Chlorophenyl	7.6 (7.5–7.7)	6.9 (6.7–7.0)	5
27	3-Pyridyl	6.5 (6.4–6.6)	<5.3	>20
28	3-Chlorophenyl	7.1 (7.0–7.2)	6.3 (6.2–6.4)	6

[a] See Ref. 27.
[b] pK_i with 95% confidence intervals in parenthesis.
[c] Selectivity = (OX_1R K_i/OX_2R K_i).



Scheme 2. Reagents and conditions: (c) diphenylcyanocarboimidate and acetonitrile ~22%; (d) amine **2** and acetonitrile ~23%.

(Sunnyvale, CA) FLIPR instrument. The pK_B was determined after Y.-C. Cheng and W. H. Prusoff[31] using the formula below:

$$pK_B = -\log(IC_{50}/(1 + (\text{agonist}/EC_{50})))$$

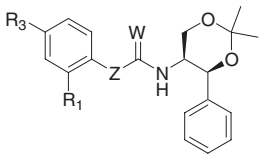
The presence of a halogen atom such as chlorine, bromine or iodine in the *ortho*-position (R_1) was very important for obtaining significant binding affinity to the orexin-2 receptor. By comparison, the unsubstituted analog, **6**, was significantly less active. After fully exploring numerous mono-substituted analogs, it was apparent that the most potent compounds were obtained with an electron-withdrawing or alkyl (preferably methyl) substituent on the phenyl ring. This same preference for electron-withdrawing and methyl substituents was also observed in analogs with multiple substitution patterns on the phenyl ring.

As shown in Table 1, the 2,4-disubstituted and 2,5-disubstituted positions were optimal for achieving activity as exemplified by **9**, **11**, and **13** with pK_i values of 8.3, 8.2, and 7.2, respectively, at the OX_2R receptor. In contrast, the 2,6-disubstitution pattern as illustrated by compound **16** was the most unfavorable and dramatically diminished binding affinity. Compounds **9** and **11** were also fairly selective for the orexin-2 receptor over the orexin-1 receptor with each possessing an OX_1R pK_i of $\leqslant 5.5$. In addition to halogen substituents, it should be noted that chlorophenyl substituents and heterocycles such as thiophenes were also well tolerated in the R_1 position of the phenyl ring (Table 2). The compounds in Table 2 indicate that there is probably room for variation in this region of the molecule unlike the central urea portion of the molecule, which is much more conserved. As can be observed in Table 3, even simple single atom substitutions resulted in a significant drop in binding affinity. Replacing the oxygen atom of **1** or **10** with a sulfur atom in **29** and **30**, respectively, yields a 3–10-fold drop in activity.

Altering the central urea to an amide (**31** and **33**) or carbamate (**32**) reduced the OX_2R affinity more than 10-fold and eliminated any OX_1R affinity for the compounds. Replacing the urea moiety with a cyanoguanidine, **34**, also resulted in a substantial drop in activity. These results were somewhat disappointing since many of the active compounds in this series possessed poor aqueous solubility. The urea moiety was regarded as the culprit for this poor aqueous solubility as is often the case and efforts are continuing to find a suitable replacement for this functionality.

In addition to testing orexin receptor binding, the 4-phenyl 1,3-dioxanes were tested for receptor specificity and antagonism. Receptor specificity was demonstrated for compounds **1**, **9**, **17**, and **26** in a CEREP ExpresS-Profile (CEREP, Redmond, WA) screen of 50 receptors, ion channels and transporters. These compounds applied at 10 μM failed to show >20% inhibition in any of the CEREP assays, with the exception of **9** (which displayed <40% inhibition in the K opiate, NPY1, and Neurokinin 3 receptor binding assays, but was otherwise inert). Antagonism of the OX_2R was verified using a functional assay. Examples of data from the functional assay are shown in Table 4 above. Active compounds were also tested for agonism in a manner similar to the pK_B assay (compound was tested without preincubation

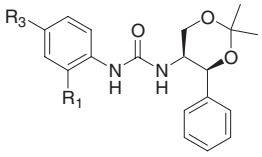
Table 3. Orexin receptor 2 binding affinities of 4-phenyl-[1,3]dioxanes



Compound[a]	R_1	R_3	W	Z	OX_2R[b]	OX_1R[b]	Selectivity[c]
29	Br	H	S	NH	6.4 (6.3–6.5)	<5.0	>30
30	Cl	Cl	S	NH	6.7 (6.6–6.8)	<5.0	>50
31	Cl	Cl	O	—	6.7 (6.4–7.1)	<5.0	>50
32	Cl	H	O	O	5.8 (5.6–5.9)	<5.0	>6
33	Br	H	O	CH_2	5.8 (5.7–6.0)	<5.0	>7
34	Br	Me	N-CN	NH	5.9 (5.6–6.2)	<5.0	>8

[a] See Ref. 27.
[b] pK_i with 95% confidence intervals in parenthesis.
[c] Selectivity = (OX_1R K_i/OX_2R K_i).

Table 4. Examples of antagonism of orexin receptor 2 by 4-phenyl-[1,3]dioxanes



Compound	R_1	R_3	OX_2R[a]
1	Br	H	6.3 (6.1–6.5)
18	Cl	H	6.0 (5.9–6.2)
17	I	H	6.5 (6.3–6.7)
9	Br	Br	7.9 (6.0–9.8)
10	Cl	Cl	7.0 (6.3–7.7)

[a] pK_B values with 95% confidence intervals in parenthesis.

or addition of the orexin agonist) and all active compounds were antagonists.

In conclusion, we have identified several potent and selective antagonists for the orexin-2 receptor. These antagonists may be useful in further elucidating the role played by the orexin-2 receptor and possibly in future treatments of sleep/wake disorders.

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23. This was confirmed via preparation and evaluation of the individual enantiomers. The 4*R*,5*R* enantiomer was inactive at both orexin-1 and orexin-2 receptors.
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27. All compounds in Tables 1–3 were characterized by ^{1}H NMR and MS data. A general procedure for the preparation of urea analogs is illustrated by the synthesis of compound **11**. To a stirred or shaken solution of 157 mg (0.76 mmol) of amine **2** in pentane (3.5 mL) was added dropwise a solution of 169 mg (0.73 mmol) of 4-bromo-2-chlorophenyl isocyanate in CH_2Cl_2 (0.7 mL). A white precipitate was observed within 2 h, collected, rinsed with pentane, and dried under vacuum to yield 280 mg (88%) of **11**. ^{1}H NMR (CD_3OD) 7.67 (d, $J = 9.0$ Hz, 1H), 7.46 (d, $J = 2.0$ Hz, 1H), 7.40 (m, 2H), 7.26 (m, 4H), 5.30 (d, $J = 2.0$ Hz, 1H), 4.39 (dd, $J = 12.0$ Hz, $J = 2.0$ Hz, 1H), 4.02 (m, 1H), 3.82 (dd, $J = 12.0$ Hz, $J = 2.0$ Hz, 1H), 1.62 (s, 3H), 1.58 (s, 3H). Elemental analysis calcd for $C_{19}H_{20}BrClN_2O_3$: C, 51.90; H, 4.58; N, 6.37. Found C, 52.24; H, 4.76; N, 6.33. MS $(M + Na)^+ = 461, 463$. When possible, pentane was used to dissolve the required isocyanate rather than CH_2Cl_2. In cases where the isocyanate was a liquid, it was added neat on small scale reactions such as this. However, the reaction is exothermic and caution should be used. In cases where a precipitate is not observed or the precipitate is not of sufficient purity, the reaction mixture was evaporated and purified by column chromatography (hexanes/ethyl acetate) to yield the desired product.
28. Radioligand binding assays were performed using clonal CHO-K1 cells expressing the orexin receptor grown on opaque, white 96-well tissue culture plates pretreated with poly-D-lysine (Bio-Coat plates, Becton Dickinson, Franklin Lakes, NJ). Binding reactions were performed in 0.1 mL of Dulbecco's phosphate buffered saline containing 0.1% w/v bovine serum albumin, 10^5 cpm ^{125}I-orexin A at 2200 Ci/mmol and test compounds or controls. After a 90–120 min incubation at room temperature, the assay was terminated by aspirating the liquid from the wells, washing the wells with iced binding buffer, aspirating the wells dry, adding 50 μL/well scintillation cocktail (MicroScint 40, Packard Biosciences, Meriden CT), and counting the plate(s) in a Packard TopCount scintillation counter. Data were analyzed using GraphPad Prizm software to calculate K_i values and 95% confidence intervals ($N = 3$ assays), which are shown in parentheses in the tables.
29. For screening purposes compounds were tested at a single dose (10 μM) to determine the percent inhibition of intracellular Ca^{++} signaling compared to a 100 nM orexin agonist stimulus. Antagonism was further evaluated by calculating pK_B values for compounds with a pK_i at the OX_2R of greater than 6.3. This was accomplished by determining the EC_{50} of orexin B and comparing the IC_{50} values determined from dilutions of antagonist compound(s), all on a single 96-well Viewplate of cells loaded with Fluo-3 AM. GraphPad Prism was used to calculate EC_{50} and IC_{50} values for each plate and to calculate 95% confidence intervals for the resulting pK_B values ($N = 3$ triplicate assays).
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