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Brief Article

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Design and Synthesis of Non-Peptide, Selective Orexin Receptor 2 Agonists

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ABSTRACT: Orexins are a family of neuropeptides that regulate sleep/wakefulness, acting on two G protein-coupled receptors, orexin receptors-1 (OX1R) and -2 (OX2R). Genetic and pharmacologic evidence suggests that orexin receptor agonists, especially OX2R agonist, will be useful for mechanistic therapy of the sleep disorder narcolepsy/cataplexy. We herein report the discovery of a potent (EC_{50} on OX2R = 0.023 μM) and OX2R-selective (OX1R/OX2R EC_{50} ratio = 70) agonist, 4'-methoxy-*N*,*N*-dimethyl-3'-[*N*-(3-{[2-(3-methylbenzamido)ethyl]amino}phenyl)sulfamoyl]-(1,1'-biphenyl)-3-carboxamide **26**.

Introduction

Orexins (orexin A and B, also known as hypocretin 1 and 2) are a pair of lateral hypothalamic neuropeptides originally identified as the endogenous ligands for two (then orphan) G protein-coupled receptors, orexin receptors-1 (OX1R) and -2 (OX2R).[1] They are derived from the single precursor peptide prepro-orexin (also termed prepro-hypocretin), of which the mRNA had also been identified by a differential cloning approach as a transcript highly enriched in the hypothalamus.[2,3] Orexin A, a C-terminally amidated 33-residue peptide with two intramolecular disulfide bridges and an N-terminal pyroglutamate residue, shows similar high potency for both OX1R and OX2R, whereas orexin B, a 28-amino-acid, C-terminally amidated linear peptide, exhibits a ≈10-fold selectivity for OX2R over OX1R.

An essential role of the orexin system in the regulation of sleep and wakefulness was initially demonstrated by the discoveries that OX2R-deficient dogs[4] and prepro-orexin knockout mice[5] both exhibit symptoms highly similar to the sleep disorder narcolepsy/cataplexy. Narcolepsy/cataplexy is a chronic neurological disorder characterized by non-rapid eye movement (REM) sleep-related symptoms such as excessive daytime sleepiness and "sleep attacks", as well as REM sleep-related signs including cataplexy (sudden bilateral loss of muscle tone often caused by a strong emotion), hypnagogic hallucination and sleep paralysis.[6] Whereas OX1R/OX2R double null mice exhibit a severe narcoleptic phenotype indistinguishable from that seen in prepro-orexin knockout mice, OX2R-null mice show a somewhat milder narcolepsy phenotype.[7] Moreover, OX1R-null mice exhibit no appreciable sleep/wakefulness-related phenotype,[8] suggesting that OX2R, rather than OX1R, plays a predominant role in sleep/wake regulation, and an intact OX2R-mediated signaling is sufficient to prevent the symptoms of narcolepsy/cataplexy. The importance of OX2R signaling in sleep/wakefulness regulation has been also demonstrated pharmacologically by the finding that OX2R-selective or OX1R/OX2R dual antagonists induce sleep, whereas OX1R-selective antagonists are largely devoid of effects on sleep.[9]

A vast majority (>90%) of human patients with narcolepsy/cataplexy has been shown to lack detectable levels of orexin peptides in their cerebrospinal fluid.[10,11] Orexin deficiency in narcolepsy patients is due to a highly selective loss of orexin-producing neurons in the lateral hypothalamus,[12,13] which is likely caused by an autoimmune mechanism.[14] These findings indicate that human narcolepsy/cataplexy is essentially an orexin-deficiency syndrome, and imply that a replacement of orexin signaling (especially OX2R signaling) can be a mechanistic therapy for the disorder. Indeed, genetic or pharmacologic replacement of orexin peptides effectively ameliorates the narcolepsy/cataplexy phenotype of a mouse model in which orexin neurons are genetically and postnatally ablated, providing a proof of concept for orexin replacement therapy.[15-17] However, since orexin peptides do not penetrate the blood-brain barrier efficiently,[18,19] it would be difficult to use the neuropeptides themselves as a therapeutic drug.

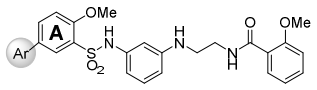
We expect that OX2R receptor agonists would be useful not only for mechanistic drug therapy of narcolepsy/cataplexy, but also for other pathologic conditions with excessive sleepiness, since it is well known that orexins exert potent wake-inducing effects upon central administration.[15,20,21] Although the role of endogenous orexin pathways is unclear[22] in narcolepsy without cataplexy (type-2 narcolepsy), OX2R agonists might be useful in this condition as well. In addition, OX2R agonists may potentially be useful in the prevention and treatment of obesity and metabolic syndrome, since it has recently been reported that genetic or pharmacologic enhancement of OX2R signaling has a net-negative effects on body weight homeostasis and prevents high fat diet-induced obesity in mice.[23] Indeed, it has also been reported that human patients with narcolepsy have higher body mass index and are more prone to metabolic syndrome than age-matched control individuals.[24,25]

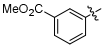
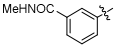
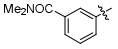
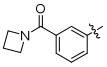
Motivated by these observations, we have initiated an effort to discover and optimize small-molecule, nonpeptide orexin receptor agonists. We have conducted a high-throughput screen of a library of ~250,000 drug-like compounds for OX2R agonist activity, and started a systematic medicinal chemistry effort based on the structural motifs of initial "hit" compounds from the screen. Here we report design and synthesis of novel potent, highly OX2R-selective agonists through such efforts.

Results and Discussion

We commenced with the screening of the universal chemical library.[26] We paid attention to the sulfonamide group of the resulting hit compound **1**, bearing two aromatic rings (A-ring and B-ring) connected to each other by a linker moiety at a certain length (Figure 1). We converted the compound **1** into the tertiary sulfonamide derivatives, termed type I compounds as shown in Figure 1. However, none of the obtained derivatives showed any activity, which led us to synthesize the secondary sulfonamides **2**–**6**, termed as type II compounds. As a result, the compounds **2**–**4** showed weak OX2R agonistic activity in NFAT-luciferase assay but were not effective in the calcium influx assay. These results suggested that the proton on the secondary sulfonamide group might play a role to afford agonistic activity. In addition, we found that the conversion of the 1,2-phenylenediamine moiety in the compound **4** into 1,3- and 1,4-phenylenediamine moieties (the compounds **5** and **6**) led to noticeable improvement in activities. When we changed the isopropyl group on the A-ring of more active derivative **5** into the phenyl group and removed the methyl group, the obtained compound **7** showed higher activity (NFAT-Luciferase activity: 41% (Efficacy at 10 μM), calcium influx: 48% (Efficacy at 10 μM)) as shown in Table 1. Finally, after examination of the position of the substituent R^1 on the aryl group (Ar) in the compound **7**, we found that substituent at the 3-position favored OX2R agonistic activity relative to the 2- or 4-positions. We then examined the introduction of other effective functional groups, shown as R^1, into the 3-position on the aryl group (Table 1, for experimental section, see supporting information). As a result, the efficacies for OX2R in both NFAT-Luciferase activity and calcium influx were further increased in many cases. The EC_{50} value of the compound **8** with the methoxy group was improved to 0.800 μM, meanwhile the introductions of the ester group or the fluorine led to decreased activity (the compounds **9** and **14**). Interestingly, the introduction of the amide groups remarkably increased both potency and efficacy, and the 3-pyridyl group also increased the activity (the compounds **10**–**12** and **15**).

Table 1. Structure activity relationships between the substituent Ar on the A-ring and calcium flux activity mediated by OX2R *in vitro* assay.

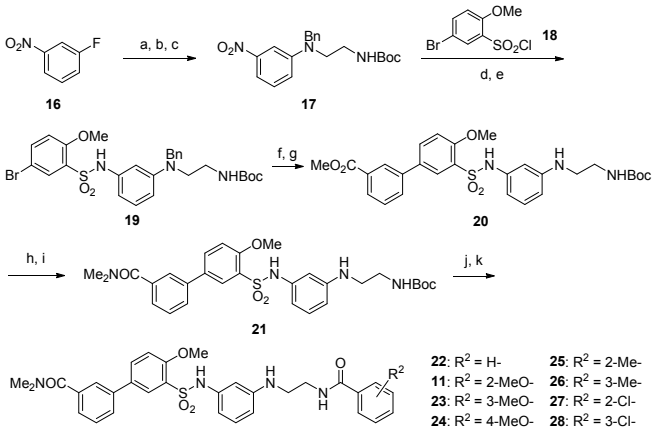


Compounds	Ar (R^1)	Calcium influx[a]	
		EC_{50} (μM)	E_{max} (%)
7		> 10	48[b]
8		0.800	54[c]
9		> 10	49[b]
10		0.182	98[c]
11		0.050	74[c]
12		0.235	85[b]
13		> 1.0	73[b]
14		> 10	47[b]
15		0.268	88[b]

[a]The value is the average of n = 5. E_{max} expressed as a percentage of OXA maximum. [b]Efficacy at 10 μM. [c]Efficacy at 1 μM.

Next, we focused on the compound **11** with the dimethyl amide group, which showed the highest activity in Table 1, and attempted to change the B-ring of **11**. The synthetic method of those compounds **11** and **22**–**28** is shown in Scheme 1. First, 3-fluoronitrobenzene **16** was easily transformed into the compound **17** through the three steps (i. treatment with ethylenediamine; ii. protection of the primary amine by a Boc group; iii. protection of the secondary nitrogen by a benzyl group). After the iron reduction of nitro group in **17**, the obtained aniline derivative was treated with sulfonyl chloride **18** to afford the sulfonamide **19**. Then, the Suzuki–Miyaura coupling of **19** with 3-methoxycarbonylphenyl boronic acid pinacol ester and the following removal of the benzyl group afforded the corresponding biaryl compound **20**. Hydrolysis of the methyl ester, followed by amidation with dimethylamine hydrochloride provided the compound **21**. Finally, the removal of Boc group, followed by amidation of **21** with a variety of benzoic acid derivatives using BOP reagent afforded the corresponding compounds **11** and **22**–**28** (for experimental section, see supporting information).

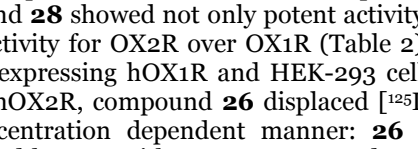
Scheme 1. Synthesis of the compounds 11 and 22–28.



Reagents and conditions: a) ethylenediamine, 100 °C; b) Boc_2O, Et_3N, CH_2Cl_2, rt, 79% in two steps; c) BnBr, TBAI, 50% aq. NaOH, CH_2Cl_2, rt, 78%; d) Fe powder, NH_4Cl, EtOH, H_2O, reflux, 99%; e) **18**, pyridine, CH_2Cl_2, rt, 92%; f) 3-methoxycarbonylphenylboronic acid pinacol ester, $PdCl_2$(dppf), Na_2CO_3, dioxane, H_2O, reflux, 87%; g) Pd/C, H_2, MeOH, rt, 99%; h) 1 M aq. NaOH, MeOH, 60 °C; i) Me_2NH·HCl, Et_3N, BOP rgt., CH_2Cl_2, rt, 73% in two steps; j) HCl-MeOH, 50 °C; k) $ArCO_2H$, Et_3N, BOP rgt., CH_2Cl_2, rt.

The introduction of the substituent R^2 on the B-ring afforded a substantial influence on both potency and efficacy as shown in Table 2. Removal of the methoxy group on the B-ring in **11** led to a significant loss in both potency and efficacy against OX2R (Compound **22**: EC_{50} = > 10 μM in OX2R). Changing the position of the methoxy group from the 2- to the 3-position on the B-ring slightly increased the potency and selectivity but not the efficacy (Compound **23**: EC_{50} = 0.033 μM, E_{max} = 54% in OX2R). On the other hand, substitution of the methoxy group at the 4-position led to a greater than 20-fold decrease in potency (Compound **24**: EC_{50} = 1.047 μM, E_{max} = 94% in OX2R). Introduction of a methyl group to the 2-position provided a decrease in potency (Compound **25**: EC_{50} = 0.800 μM, E_{max} = 85% in OX2R), while a methyl group at the 3-position increased both potency and efficacy (Compound **26**: EC_{50} = 0.023 μM, E_{max} = 98% in OX2R). Introduction of a chlorine to the 2- or 3-positions provided almost the same results as that of the methoxy group (Compounds **27** and **28**). The compounds **11**, **23**, **26**, **27**, and **28** showed not only potent activity, but also high selectivity for OX2R over OX1R (Table 2). In CHO cells overexpressing hOX1R and HEK-293 cells overexpressing hOX2R, compound **26** displaced [^{125}I]orexin-A in a concentration dependent manner: **26** bound to hOX2R and hOX1R with K_i = 0.14 μM and 0.77 μM, respectively.

Table 2. Structure activity relationships between the substituent R^2 on the B-ring and calcium flux activity mediated by OX2R *in vitro* assay.



Compounds	R^2	Calcium influx[a]				
		OX2R		OX1R		Selectivity
		EC_{50} (μM)	E_{max} (%)	EC_{50} (μM)	E_{max} (%)	OX1R EC_{50}/ OX2R EC_{50}
22	H	> 10	N.D.[b]	N.T.[c]		N.C.[d]
11	2-MeO	0.050	74	5.227	46	105
23	3-MeO	0.033	54	9.248	49	280
24	4-MeO	1.047	94	N.T.[c]		N.C.[d]
25	2-Me	0.800	85	N.T.[c]		N.C.[d]
26	3-Me	0.023	98	1.616	100	70
27	2-Cl	0.031	90	6.134	50	198
28	3-Cl	0.094	78	5.720	73	60

[a]The value is the average of n = 5. OXA EC_{50} (OX1R) = 1.5 nM. EC_{50} (OX2R) = 1.0 nM. E_{max} expressed as a percentage of OXA maximum. [b]N.D. = not detected. [c]N.T. = not tested. [d]N.C. = not calculated.

Next, we converted the sulfonamide group into the carboxyamide group in **26** to afford the compound **29** to examine the effect of the sulfonamide moiety to the OX2R agonistic activity. Surprisingly, the carboxyamide derivative **29** was inactive toward both OX1R and OX2R, which supports our aforementioned postulation that the sulfonamide moiety is one of the most important structural determinants for the OX2R agonistic activity (Figure 2).

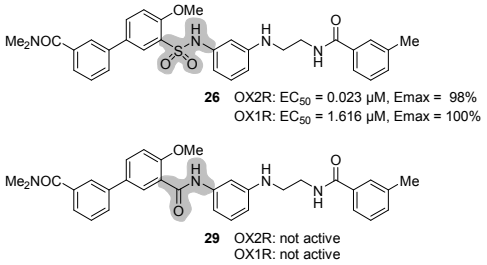


Figure 2. Structures of the sulfonylamide derivative **26** and the carbonylamide derivative **29**.

Recently, the X-ray crystal structure of the human OX2R bound to a dual orexin receptor antagonist, suvorexant[27,28] was solved at 2.5 Å.[29] Using this X-ray structure, the binding mode of **26** with OX2R was examined by molecular-docking calculations (see supporting information). The resulting binding mode of **26** (Figure 3) suggested that the dimethyl carbamoyl group on **26** was located deep in the ligand-binding site of OX2R, and its carbonyl group formed a hydrogen bond with N324 (transmebrane hexix 5, TM5). The compound **26** also used a nitrogen atom of the ethylenediamine moiety to form an additional hydrogen bond with the main-chain carbonyl group of C210 (TM3). The biphenyl group of **26** was located in the hydrophobic pocket consisting of V138 (TM3), F227 (TM5), Y317 (TM6), I320 (TM6), and V353 (TM7) to make hydrophobic interactions. The sulfonylamide group of **26** was in proximity to T111 (TM2), H350 (TM7), and Y354 (TM7) of OX2R (see supporting information's Figure 2). Interestingly, **26** directed two oxygens of sulfonylamide group to hydroxyl groups of T111 (TM2) and Y354 (TM7) and imidazole ring of H350 (TM7), respectively. The sulfonylamide group of **26** may form hydrogen bonds in the structural changes associated with receptor activation. This may explain that the carboxyamide derivative **29** was inactive, because the direction of oxygen was different between carboxyamide and sulfonylamide groups. T111 (TM2) of OX2R is mutated to Ser in OX1R. This mutation might be related to

high selectivity for OX2R of compounds in Table 2. The benzene groups in the middle and terminal portions of **26** also formed hydrophobic interactions with V114 (TM2), W120 (ECL1), and I130 (TM3) and with Y343 (TM7) and F346 (TM7). The neighborhood of the B-ring of the compound **26** was given in supporting information's Figure 3. This also may explain that compounds **23**, **26**, and **28** with a substituent group at the 3-position possessed potent activity, because we could see large space in the direction of 3-position to accommodate the substituent group. Figure 4 compares the binding modes of suvorexant and **26** with OX2R. As reported in the literature,[29] suvorexant adopted a π-stacked horseshoe-like conformation to direct its 7-membered ring to TM5 and TM6 of OX2R. This binding mode was believed to be responsible for the OX2R antagonist activity of suvorexant, as the 7-membered ring seems to inhibit inward movements of TM5 and TM6 relative to the rest of the TM bundle, which may be a general trigger for GPCR activation. In contrast, **26** binds to OX2R with an extended conformation, and we detected some spatial allowance between **26** and TM5 and TM6 of OX2R. Therefore, we could consider that the binding of **26** to OX2R may induce the inward movements of TM5 and TM6, which would allow **26** to have OX2R agonist activity.

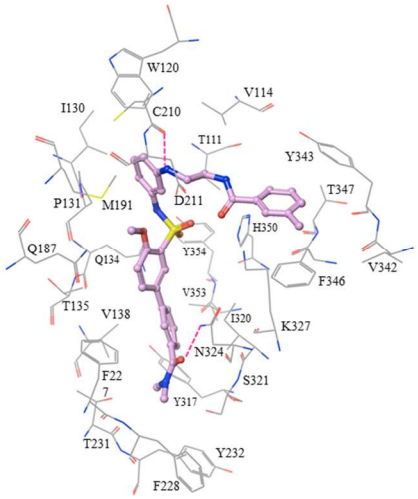


Figure 3. Binding mode of the compound **26** with the OX2R determined by our docking procedure. Hydrogen-bonding interactions are indicated by red dashed lines.

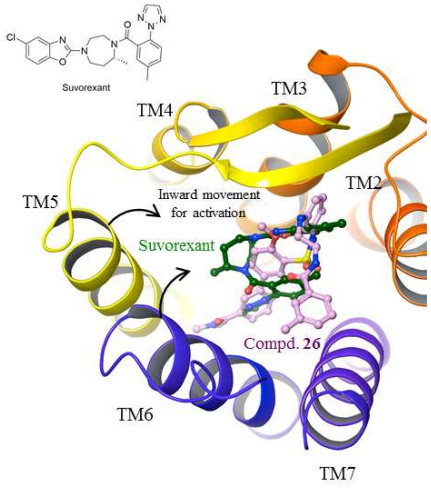


Figure 4. Superposition of suvorexant (green) and the compound **26** (purple) in the ligand-binding site of OX2R.

In Table 2, the most potent compound was 3-methyl derivative **26**. Meanwhile, the most selective compound for OX2R was 3-methoxy derivative **23**. However, the compounds **11** and **22**–**28** in Table 2 were barely soluble in water, which led to difficulty in confirming *in vivo* activity, although every compound showed satisfactory activities and potencies *in vitro*. Therefore, we introduced the 2-dimethylamino group on the B-ring to give more polar derivative **30** (Figure 5), and then converted **30** into dihydrochloride salt **31**, which could be dissolved in saline at 1.3 M. To evaluate the pharmacological effect of **31** *in vivo*, we administered it intracerebroventricularly in the light phase and observed the effect on sleep/wake states by recording EEG/EMG in C57BL/6J mice. **31** promoted wakefulness in a dose-dependent manner (Figure 6). 260 nmol of **31** increased wake time to 53 min, to a similar degree reported with 3.0 nmol orexin-A (58 min).[16] In contrast, **31** did not increase wakefulness in OX1R/OX2R double knockout (DKO) mice, demonstrating that the effect of **31** requires orexin receptors as expected. The intraperitorial injection of **31** afforded the similar effects. The details will be reported in separate papers.

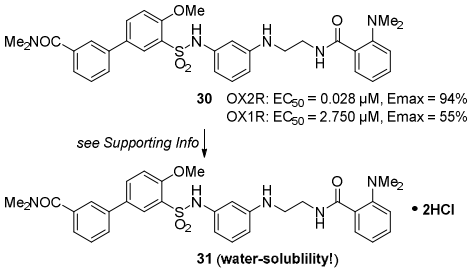


Figure 5. Structures of **30** and its water-soluble dihydrochloride salt **31**.

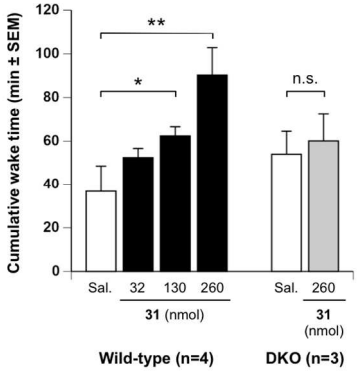


Figure 6. Effect of intracerebroventricular injection of **31** on sleep/wake states in wild-type and OX1R/OX2R DKO mice. Cumulative time spent in wake within 2 h after saline (sal.) or **31** administration at 6 h into the light phase (ZT6). *, $P < 0.05$; **, $P < 0.001$ saline vs. **31** in wild-type mice. n.s.: not significant.

Conclusions

We paid attention to the sulfonamide group of hit compound **1** and synthesized more than 1000 compounds. Those compounds were tested against a CHO cell line expressing the human orexin receptor using the NFAT-luciferase assay and the calcium influx assay. We found

the first lead compound **5**, then focused on the transformation of the A-ring and the B-ring in **5**. As a result, the first, potent and selective agonist **26** for OX2R was discovered. The conversion from the sulfonamide group in **26** to the carbonylamide group and the evaluation of the obtained **29** suggested that the sulfonamide moiety is one of the most important structural features for the OX2R agonistic activity. In addition, we also developed the dimethylamino derivative **30** and its dihydrochloride salt, which could be dissolved in saline at 1.3 M. The *in vivo* assay of the dihydrochloride salt showed a definite wake-promoting effect. These results would be helpful for further design of OXR agonists.

Experimental Section

Synthesis.

Methyl 3'-{*N*-[3-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)phenyl]sulfamoyl}-4'-methoxy-(1,1'-biphenyl)-3-carboxylate (20).

A mixture of **19** (2.36 g, 4.00 mmol), 3-methoxycarbonylphenylboronic acid pinacol ester (1.26 g, 4.80 mmol), 2.0 M aq. sodium carbonate (4.0 mL, 8.00 mol), and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (163 mg, 0.20 mmol) in dioxane (4.0 mL) was stirred under argon atmosphere at 100 °C for 18 h. After being diluted with ethyl acetate, the insoluble material was filtrated through a pad of Celite. The filtrate was concentrated *in vacuo*, and then the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 67/33 to 50/50) to give the desired coupling product (2.25 g, 87%) as a yellow amorphous powder. A mixture of the compound (2.25 g, 3.48 mmol) in methanol (20 mL) was stirred in the presence of catalytic 10% Pd/C at room temperature for 5 h under hydrogen pressure (balloon pressure). After filtration through a pad of Celite, the filtrate was concentrated *in vacuo*. The residure was purified by silica gel column chromatography (chloroform/methanol = 90/10) to give **20** (1.94 g, 99%) as a pale yellow amorphous powder.

***tert*-Butyl {2-[(3-{[3'-(dimthylcarbamoyl)-4-methoxy-(1,1'-biphenyl)]-3-sulfonamido}phenyl)amino]ethyl}carbamate (21).**

To a solution of **20** (1.94 g, 3.49 mmol) in methanol (30 mL) was added 1.0 M aq. sodium hydroxide (10.5 mL, 10.5 mmol) and the mixture was stirred at 60 °C for 3 h. After being neutralized by 1.0 M aq. hydrochloric acid, the mixture was extracted with chloroform/methanol (= 10/1), washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residual colorless amorphous powder was dissolved in dichloromethane (30 mL), and then triethylamine (1.59 mL, 11.4 mmol), dimethylamine hydrochloride (533 mg, 6.54 mmol), and BOP reagent (1.59 g, 3.60 mmol) were added. The mixture was stirred for 12 h at room temperature under argon atmosphere. The reaction mixture was quenched with saturated aq. sodium hydrogen carbonate, and extracted with chloroform. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5/95 to 0/100) to give the desired compound (1.45 g, 73% in two steps) as a colorless amorphous.

General Synthetic Procedure for the Compounds in Table 2.

A mixture of **21** (2.55 mmol) in 10% hydrogen chloride methanol solution (10 mL) was stirred at 50 °C for 2 h. The reaction mixture was concentrated *in vacuo*, and then the residue was purified by amine-silica gel column chromatography (ammonia-saturated chloroform/methanol = 100/0 to 95/5) to afford the desired primary amine. Then, a mixture of the primary amine (1.0 equiv), the benzoic acid derivative (1.0 equiv), trimethylamine (3.3 equiv), and BOP reagent (1.1 equiv) in dichloromethane (0.5 M) was stirred at room temperature for 12 h under argon atmosphere. The mixture was quenched with saturated aq. sodium hydrogen carbonate, and extracted with chloroform. The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate) to give the desired compound.

4'-Metoxy-*N*,*N*-dimethyl-3'-[*N*-(3-{[2-(3-methylbenzamido)ethyl]amino}phenyl)sulfamoyl]-(1,1'-biphenyl)-3-carboxamide (26).

Yield: 77% in two steps; colorless solid; MP 137–139 °C; IR (KBr) 3408, 2919, 1605, 1502, 1281, 1151 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.34 (3H, s), 3.02 (3H, brs), 3.13 (3H, brs), 3.19–3.22 (2H, m), 3.58–3.63 (2H, m), 4.04 (3H, s), 4.47 (1H, brs), 6.22 (1H, dd, *J* = 8.0, 2.0 Hz), 6.32 (1H, dd, *J* = 8.4, 2.0 Hz), 6.45 (1H, t, *J* = 2.0 Hz), 6.88 (1H, brs), 6.92 (1H, t, *J* = 8.0 Hz), 7.04 (1H, d, *J* = 8.4 Hz), 7.13–7.18 (1H, m), 7.21–7.34 (3H, m), 7.42 (1H, t, *J* = 8.0 Hz), 7.50–7.58 (4H, m), 7.68 (1H, dd, *J* = 8.4, 2.4 Hz), 8.12 (1H, d, *J* = 2.4 Hz); 13C NMR (100 MHz, $CDCl_3$) δ = 21.3, 35.4, 39.3, 39.6, 44.9, 56.6, 103.7, 109.2, 110.3, 112.7, 124.1, 125.6, 125.7, 126.5, 127.7, 127.8, 128.3, 128.8, 129.6, 129.8, 132.2, 132.7, 133.0, 134.1, 137.0, 137.7, 138.2, 139.4, 149.1, 155.9, 168.8, 171.3; HRMS-ESI: *m/z* $[M + Na]^+$ calcd for $C_{32}H_{34}N_4O_5SNa$: 609.2148; found: 609.2134. Purity was > 99% as assessed by HPLC (254 nm) and NMR.

ASSOCIATED CONTENT

Supporting Information.

General information and detailed experimental procedures, synthetic protocols, and chemical data. The Supporting Information is available free of charge via the Internet at http://pubs.acs.org."

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ABBREVIATIONS

OX1R, orexin receptor-1; OX2R, orexin receptor-2; REM, rapid eye movement; NFAT, nuclear factor of activated T-cells; Boc, *tert*-butoxycarbonyl; BOP, [(benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate; MP, melting point.

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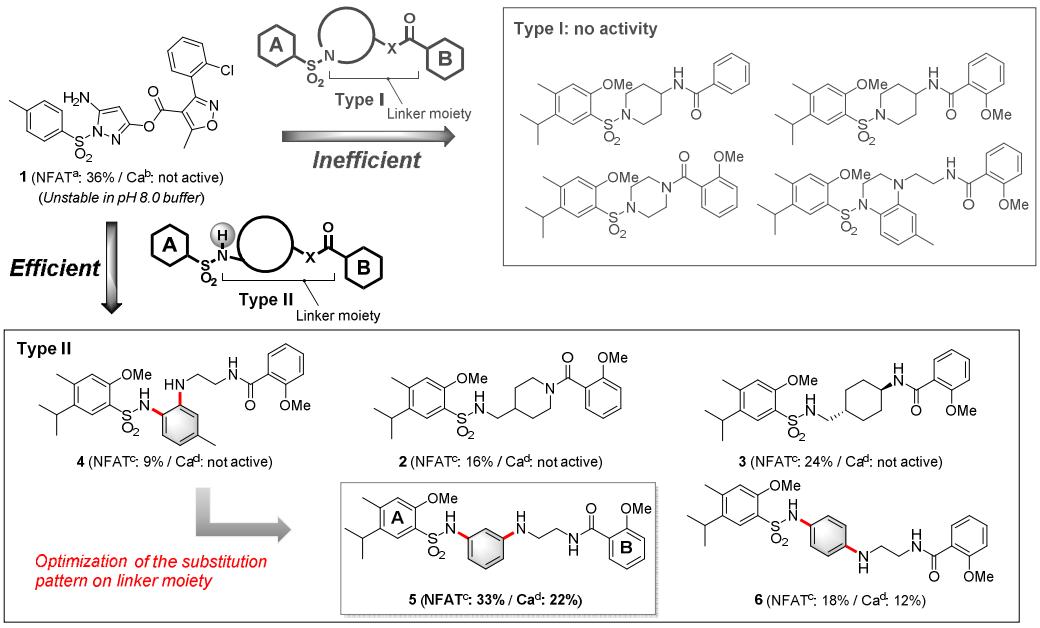

Figure 1. Drug design approach from the hit compound **1** to the lead compound **5**. [a]NFAT-Luciferase activity mediated by OX2R (Efficacy at 5 μM). [b]Ca^{2+} influx mediated by OX2R (Efficacy at 5 μM). [c]NFAT-Luciferase activity mediated by OX2R (Efficacy at 10 μM). [d]Ca^{2+} influx mediated by OX2R (Efficacy at 10 μM).

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