Title

Design and synthesis of novel orexin 2 receptor agonists based on naphthalene skeleton

Author List

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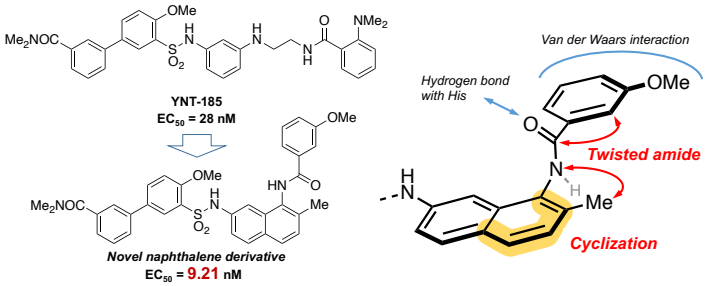
Graphical Abstract



Abstract

A novel series of naphthalene derivatives were designed and synthesized based on the strategy focusing on the restriction of the flexible bond rotation of OX_2R selective agonist YNT-185 (**1**) and their agonist activities against orexin receptors were evaluated. The 1,7-naphthalene derivatives showed superior agonist activity than 2,7-naphthalene derivatives, suggesting that the bent form of **1** would be favorable for the agonist activity. The conformational analysis of 1,7-naphthalene derivatives indicated that the twisting of the amide unit out from the naphthalene plane is important for the enhancement of activity. The introduction of a methyl group on the 2-position of 1,7-naphthalene ring effectively increased the activity, which led to the discovery of the potent OX_2R agonist **28c** (EC_{50} = 9.21 nM for OX_2R, 148 nM for OX_1R). The structure-activity relationship results were well supported by a comparison of the docking simulation results of the most potent derivative **28c** with an active state of agonist-bound OX_2R cryo-EM SPA structure. These results suggested important information for understanding the active conformation and orientation of pharmacophores in the orexin receptor agonists, which is expected as a chemotherapeutic agent for the treatment of narcolepsy.

Orexin A and B[1] (OXA, OXB; also known as hypocretin 1 and 2[2]) are hypothalamic neuropeptides that play an important role in the maintenance of wakefulness as well as the regulation of a variety of physiological events such as feeding behavior,[3] sleep–wake cycle,[3, 4] reward/addiction,[5] and energy homeostasis[6]. Orexins are derived from a single precursor peptide prepro-orexin and bind to the two subtypes of G-protein-coupled receptors (GPCRs), the orexin 1 receptor (OX_1R) and the orexin 2 receptor (OX_2R). Importantly, lack of prepro-orexin causes narcoleptic symptoms characterized by excessive daytime sleepiness, an abnormally short transition from wakefulness to rapid-eye-movement (REM) sleep, and cataplexy with sudden loss of bilateral skeletal muscle tone.[7] Orexin deficiency has also been found in a vast majority of human narcolepsy/cataplexy patients, which is caused by a highly selective loss of orexin-producing neurons in the lateral hypothalamus.[8] Genetic studies in mice revealed that OX_1R knockout mice exhibit no obvious sleep/wakefulness-related phenotype, but OX_2R knockout mice show a narcoleptic phenotype, suggesting that OX_2R plays a predominant role in maintenance of wakefulness, and the OX_2R-mediated signaling is sufficient to prevent the symptoms of narcolepsy/cataplexy.

Since the discovery of the crucial role of the orexin system on the sleep-wake cycle, many orexin receptor antagonists have been developed as indications for sleep disorder and dual orexin receptor antagonists (DORAs) such as suvorexant[9, 10] and lemborexant[11] have been launched for the treatment of insomnia so far.[12] However, the development of orexin receptor agonists has been slow due to less interest by the pharmaceutical industries and because only a few small molecule agonists with weak potency have been reported.[13] In 2015, we reported the first potent OX_2R selective agonist carrying diarylsulfonamide core, YNT-185 (**1**, Figure 1, EC_{50} = 28 nM for OX_2R, OX_1R/OX_2R = ca. 100),[14] and demonstrated the first proof-of-concept studies that the administration of an OX_2R-selective agonist promotes wakefulness in wild-type mice and attenuates narcoleptic symptoms in prepro-orexin knockout mice through the activation of OX_2R on histaminergic neurons in the mouse tuberomammillary nucleus (TMN).[15] We also demonstrated that **1** attenuates the morphine-induced sedative effects in rats.[16] Stimulated by our pioneering works, several types of OX_2R agonists have since been reported. The group of Takeda reported a series of 3-piperidyl sulfonamide derivatives as OX_2R selective agonists.[17] Among them, TAK-925 (**2**) with a 3-amino piperidine core showed potent OX_2R selective agonist activity (EC_{50} = 5.5 nM for OX_2R, OX_1R/OX_2R > 5,000) and this compound has recently entered phase I clinical trials for the treatments of narcolepsy and hypersomnia.[18] Based on the structure of diarylsulfonamide-type and 3-piperidyl sulfonamide-type agonists, several derivatives such as **3**–**8** have been reported (Figure 1).[19–27] Nonetheless, the structure-activity relationship information for expression of agonist activity has been insufficient.

During our drug discovery campaign targeting orexin receptors, we discovered the novel dual orexin receptor antagonist **A** (K_i = 5.23 nM for OX_1R, OX_1R/OX_2R = 6.6) and the OX_1R specific antagonist **B** (K_i = 2.17 nM for OX_1R) through the screening of the 1,3,5-trioxazatriquinanes bearing multiple effective residues (TriMER)-focused library.[28] In order to convert TriMER-type antagonists to agonists for OXR, we attempted to change the complex scaffold into a simple drug-like skeleton. The superimposition of the docking structures of **A** and **B** in OXRs is shown in Figure 2. Overall, these two molecules took quite similar binding forms: the lone electron pairs on the nitrogen in the convex and concave skeletons of the superimposed structures pointed in opposite directions and the side chains were oriented toward the same direction, which led us to speculate that a simple flat structure without a basic nitrogen such as a naphthalene would be sufficient for the basic skeleton.

The molecular shape of **1** is dramatically interconverted between the extended- and bent-forms depending on the orientation of the N1–C3 bond, suggesting that the constraint of the C2–N1 bond rotation would be an important indicator of the active conformation of **1** (Figure 3). Therefore, we designed and synthesized a new series of naphthalene-based derivatives that restrict the rotation of the C2–N1 bond in the flexible ethylene diamine moiety of YNT-185.

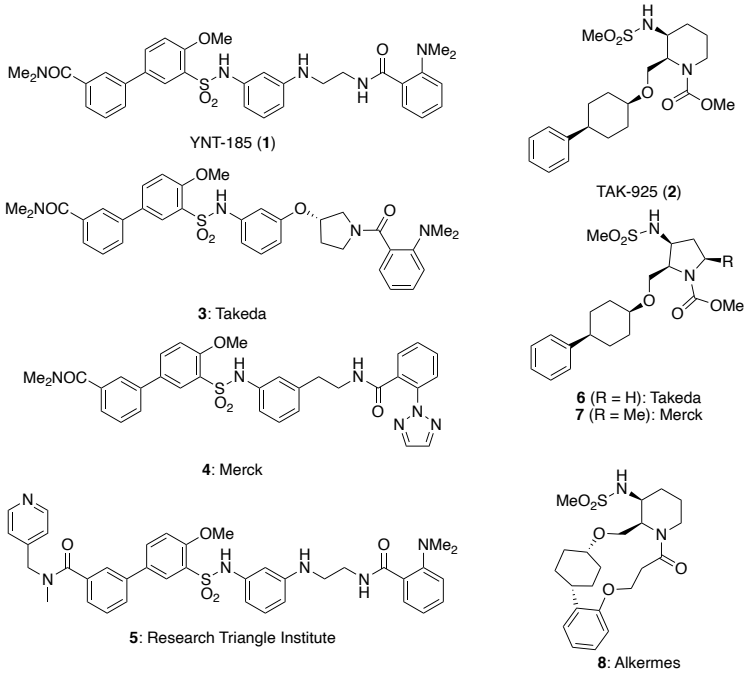


Figure 1. Structures of YNT-185 (**1**), TAK-925 (**2**) and derivatives of diarylsulfonamide-type (**3**–**5**) and 3-piperidyl sulfonamide-type (**6**–**8**) agonists

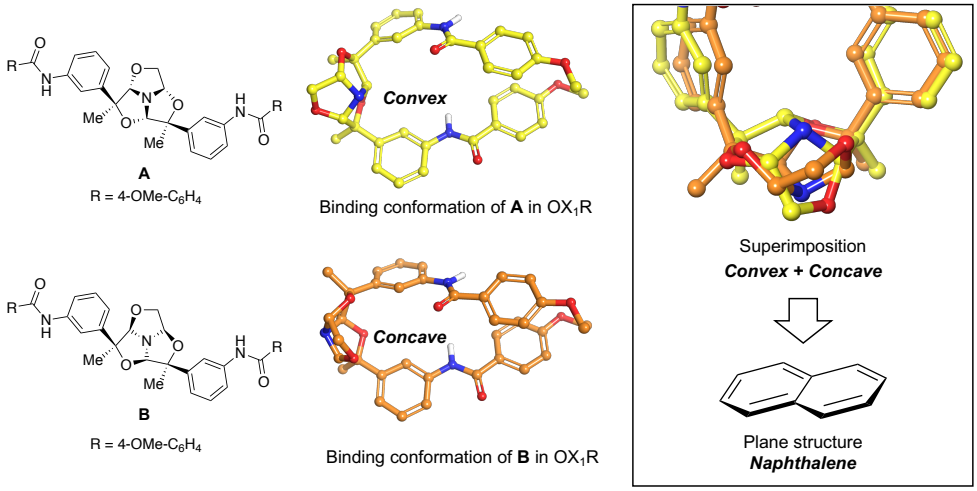


Figure 2. Structures and speculated binding conformations of TriMER-type orexin receptor antagonists **A** and **B**

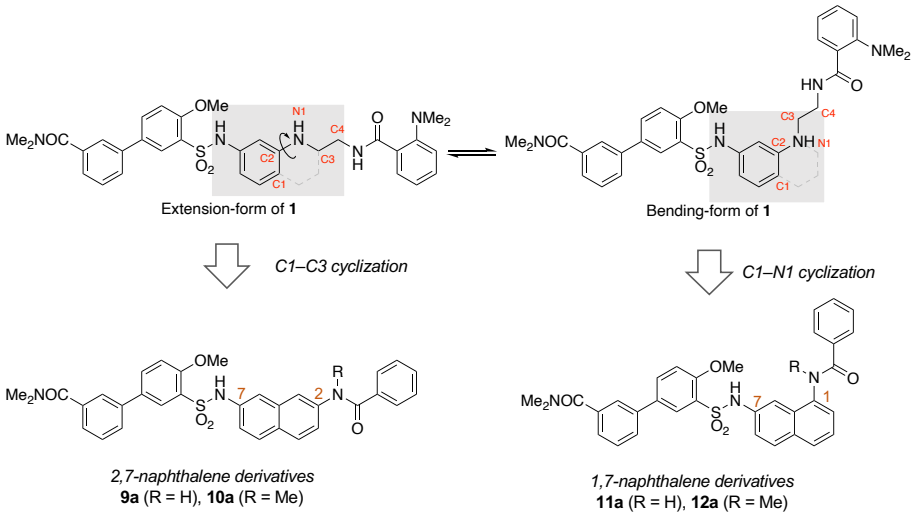
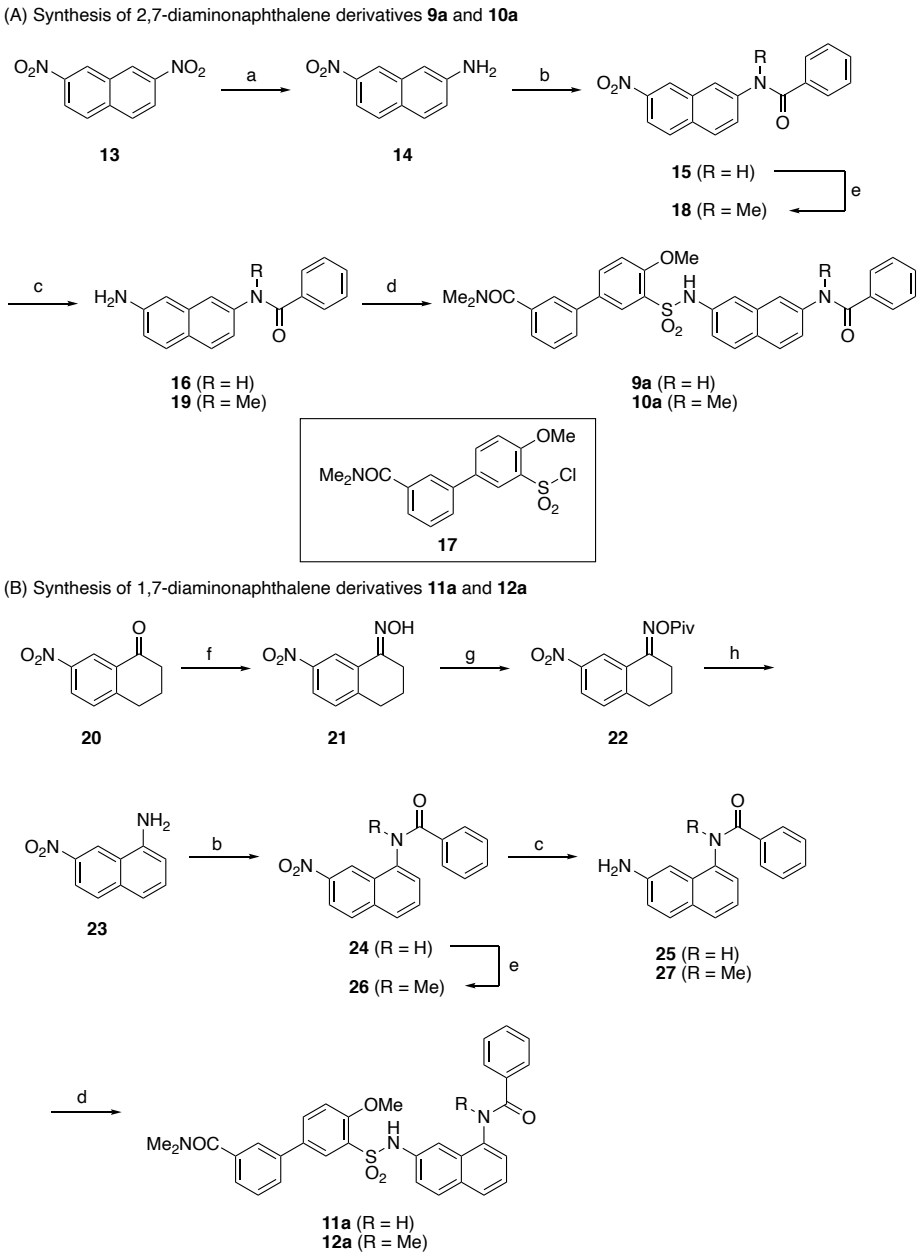


Figure 3. Molecular design of 2,7- and 1,7-naphthalene derivatives **9**–**12a** from YNT-185 (**1**)

First, we designed the 2,7- and 1,7-diaminonaphthalene derivatives **9a** and **11a** which mimic the extended- and bent-forms of YNT-185 (**1**), respectively, and the *N*-methyl amide derivatives **10a** and **12a**. The synthetic method of these compounds is shown in Scheme 1. The synthesis of 2,7-diaminonaphthalene derivative **9a** and **10a** began with the selective reduction of the unilateral nitro group of 2,7-dinitronaphthalene (**13**) with Na_2S[29] as a reductant (Scheme 1A). The amidation of the resulting amine **14** with BzCl afforded the common intermediate **15** for **9a** and **10a**. The Bechamp reduction followed by sulfonamidation with sulfonyl chloride **17** gave amine 2,7-diaminonaphthalene derivative **9a**. The *N*-methylation of **15** followed by the Bechamp reduction of the remaining nitro group gave amine **19**, which was converted to *N*-methyl amide derivative **12a** with **17**. Subsequently, the 1,7-diaminonaphthalene derivatives **11a** and **12a** were synthesized from 7-nitro-1-tetralone (**20**) (Scheme 1B). After the oxime formation followed by the esterification of **21** with pivaloyl chloride, the Pd-catalyzed Semmler-Wolff reaction[30] of **22** afforded 1-amino-7-nitro naphthalene (**23**). The amidation of **23** with BzCl afforded the common intermediate **24** for **11a** and **12a**. The resulting amide **24** was converted to the 1,7-diaminonaphthalene derivative **11a** and its *N*-methylated derivative **12a** through the same manner as **9a** and **10a**.

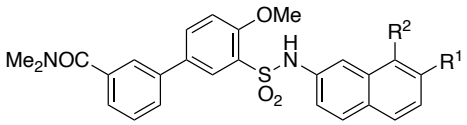


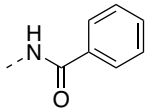
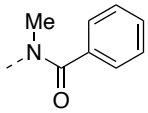
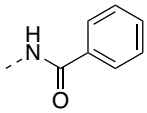
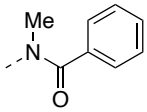
Scheme 1. Synthesis of naphthalene derivatives **9a**, **10a** (A) and **11a**, **12a** (B). (a) Na_2S, $NaHCO_3$, MeOH/H_2O (7:2), 60 ºC, **14**: 41%; (b) BzCl, Et_3N, CH_2Cl_2, rt, **15**: 94%, **24**: 80%; (c) Fe, NH_4Cl, EtOH/H_2O (5:1), 60 ºC, **16**: 31%, **19**: 97%, **25**: 79%, **27**: 100%; (d) 3'-(dimethylcarbamoyl)-4-methoxy-[1,1'-biphenyl]-3-sulfonyl chloride (**17**), CH_2Cl_2/pyridine, 0 °C to rt, **9a**: 87%, **10a**: 95%, **11a**: 100%, **12a**: 94%, (e) MeI, NaH, THF, 0 ºC to rt, **18**: 87%, **26**: 100%; (f) NH_2OH·HCl, NaOAc, MeOH, reflux, **21**: 98%; (g) pivaloyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, **22**: 100%; (h) $Pd(PPh_3)_4$, K_2CO_3, toluene, 95 °C, **23**: 72%.

With the naphthalene derivatives **9a**–**12a** in hand, we conducted cell-based calcium assays to evaluate the OXR agonist activities in a Chinese hamster ovary (CHO) cell line stably expressing OX_1R and OX_2R (Table 1). While the 2,7-diaminonaphthalene derivatives **9a** and **10a** mimicking the extended-form of **1** showed no agonist activity for either of the receptors (<5% efficacy *vs*. OXA at 10 μM), the 1,7-diaminonaphthalene derivatives **11a** and **12a** mimicking the bent-form of **1** showed moderate OX_2R agonist activity (**11a**: EC_{50} = 873 nM, **12a**: EC_{50} = 1,949 nM) with weak OX_1R agonist activity.

These results suggested that the bent form would be more favorable than the extended form of **1** to elicit the OX_2R agonist activity.

Table 1. Orexin receptor agonist activities of **9a**–**12a**



Compound	R^1	R^2	EC_{50} (nM) [E_{max} (%)[b]]		Selectivity (OX_1R/OX_2R)
			OX_1R[a]	OX_2R[a]	
9a		H	> 10,000 [ND[c]]	> 10,000 [ND[c]]	NC[d]
10a		H	> 10,000 [ND[c]]	> 10,000 [ND[c]]	NC[d]
11a	H		> 10,000 [31.8]	873 [112]	NC[d]
12a	H		>10,000 [50.8]	1,949 [119]	NC

[a]EC_{50} and E_{max} values are the means ± SEM of at least three independent experiments conducted in duplicate. [b]E_{max} expressed as a percentage of OXA maximum. [c]ND = not detected. [d]NC = not calculated.

In order to investigate the effect of *N*-methylation on the conformation in **11a** and **12a**, we carried out a conformational analysis for **11a** and **12a** within the +5.0 kcal/mol energy range (Figure 4).[31] The stable conformation of the secondary benzamide derivative **11a** had a *trans*-amide structure and the amide group was located in the same plane as the naphthalene ring. The C1–N1 bond was stable due to the resonance effect, and its bond rotation was observed at above + 3.0 kcal/mol. On the other hand, the stable conformation of the *N*-methyl benzamide derivative **12a** had a *cis*-amide structure, and the amide group located in the vertical direction with respect to the naphthalene plane by twisting the C1–N1 bond. The C1–N1 bond rotation barrier of the *N*-methyl *cis*-amide was lower than that of secondary *trans*-amide, yet it required > + 3.0 kcal/mol to occur the *cis-trans* isomerization. These data indicated that the orientation of the benzamide group would play an important role in the activation of orexin receptors.

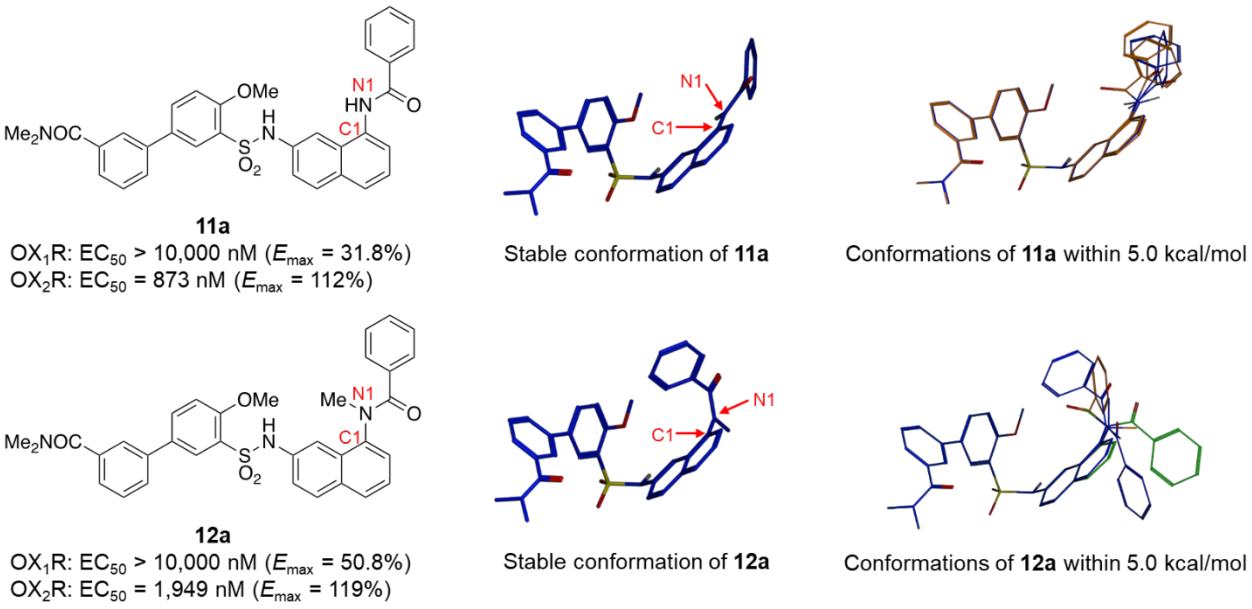
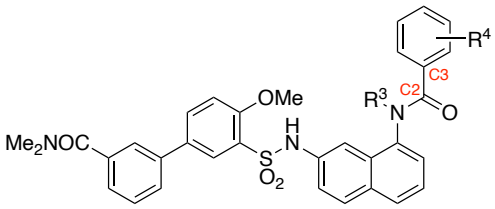


Figure 4. The stable conformations of **11a** and **12a** and superimposition of stable conformations within 5.0 kcal/mol from the lowest energy conformation. Blue: < + 1.0 kcal/mol, Green: 1–2 kcal/mol, Yellow: 2–3 kcal/mol, Orange: 3–5 kcal/mol.

In our previous study, the introduction of the methoxy group on the terminal benzamide was effectively involved in the agonist activity.[14] At that time, we synthesized the methoxy-substituted derivatives according to the same procedure as **11a** and **12a** (Supplemental Scheme S3) and evaluated the OXR agonist activities. While the *ortho*-methoxy derivative **12b** showed no activity for either receptor, the *meta*- and *para*-methoxy substitutions were effective for the improvement of agonist activity for each receptor (Table 2). The *meta*-methoxy derivative **12c** (EC_{50} = 22.7 nM for OX_2R, 232 nM for OX_1R) and *para*-methoxy derivative **12d** (EC_{50} = 51.1 nM for OX_2R, 654 nM for OX_1R) showed much potent agonist activities than **12a** for both receptors. The *ortho*-substituent forces the C2–C3 bond to twist between the amide and benzene ring more effectively than the *meta*- or *para*-substituents, which may cause the dramatic decrease of agonist activity. Notably, the secondary benzamide derivative with a *meta*-methoxy group **11c** (EC_{50} = 24.3 nM for OX_2R, 473 nM for OX_1R) showed a similar range of agonist activity compared to **12c**, suggesting that the *meta*-methoxy group would be an effective substituent to induce the agonist activity.

Table 2. Orexin receptor agonist activities of **12a**–**12d** and **11a**, **11c**



Compound	R^3	R^4	EC_{50} (nM) [E_{max} (%)[b]]		Selectivity (OX_1R/OX_2R)
			OX_1R[a]	OX_2R[a]	

12a	Me	H	>10,000 [50.8]	1,949 [119]	NC
12b	Me	2-OMe	> 10,000 [ND[c]]	> 10,000 [ND[c]]	NC[d]
12c	Me	3-OMe	232 [101]	22.7 [102]	10.2
12d	Me	4-OMe	654 [87.8]	51.1 [105]	12.8
11a	H	H	> 10,000 [31.8]	873 [112]	NC[d]
11c	H	3-OMe	473 [107]	24.3 [104]	19.5

[a]EC_{50} and E_{max} values are the means ± SEM of at least three independent experiments conducted in duplicate. [b]E_{max} expressed as a percentage of OXA maximum. [c]ND = not detected. [d]NC = not calculated.

With the expectation for the effective conformational control of the amide group toward the vertical direction from the naphthalene plane, we then synthesized 2-methyl naphthalene derivatives **28c** and **29c** in the same manner as **11c** and **12c** after the α-methylation of 7-nitro-1-tetralone **20** (Supplemental Scheme S4). The introduction of the 2-methyl group on the naphthalene moiety enhanced the agonist activity in the secondary amide derivative. Compound **28c** (EC_{50} = 9.21 nM for OX_2R, 148 nM for OX_1R) showed ca. 3 times higher activity for both receptors (Figure 5). However, the *N*-methyl tertiary amide derivative **29c** (EC_{50} = 31.1 nM for OX_2R, 260 nM for OX_1R) showed similar activity to **12c**. The results of conformational analysis using desmethoxy derivatives as a model structure showed that the stable structure of desmethoxy **28c** had a *trans*-amide structure and its C1–N1 bond was twisted from the naphthalene plane due to the structural repulsion with the 2-methyl group, as we predicted (Figure 5). The C1–N1 bond rotation of desmethoxy **28c** occurred frequently within + 3.0 kcal/mol, but the *trans-cis* isomerization was not observed in this energy range. On the other hand, the stable structure of desmethoxy **29c** was quite similar to that of **12a**, in which a *cis*-amide structure was located in a vertical direction from the naphthalene plane by twisting of the C1–N1 bond. These data suggested that the orientation of the aromatic ring by the *trans*-amide group as well as the orientation of the amide group by rotation of the C1–N1 bond is important for the activation of the orexin receptors.

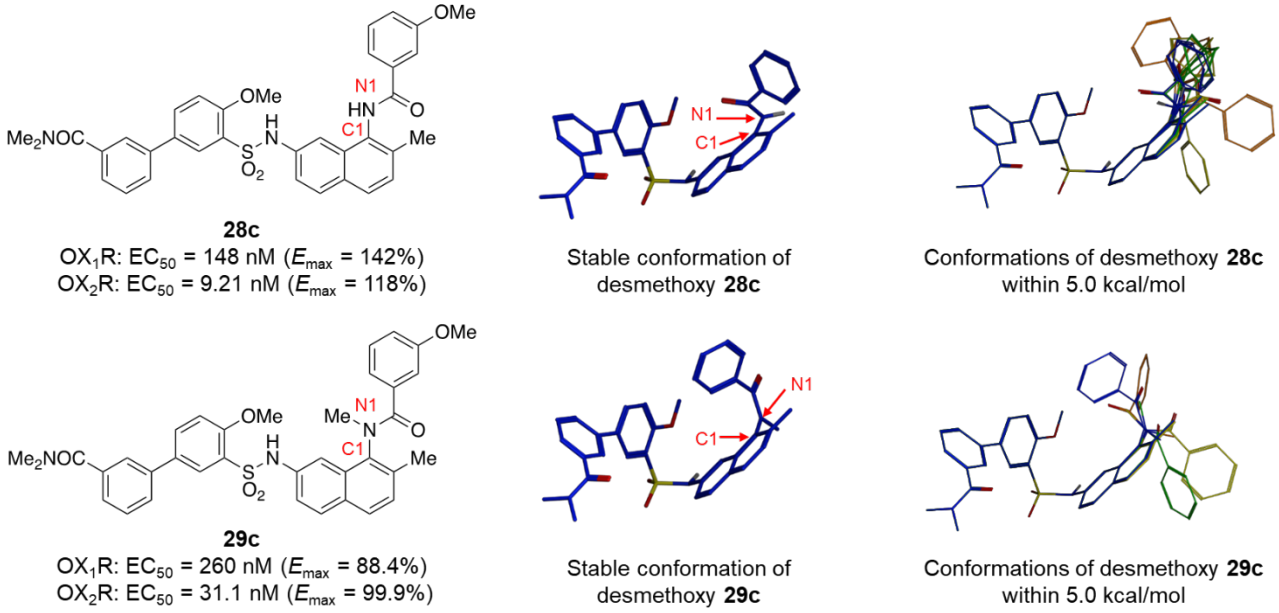


Figure 5. Structures of 2-methyl naphthalene derivatives **28c** and **29c**, the stable conformations of desmethoxy derivatives of **28c** and **29c**, and superimposition of stable conformations within 5.0 kcal/mol from the lowest energy conformation. Blue: < + 1.0 kcal/mol, Green: 1–2 kcal/mol, Yellow: 2–3 kcal/mol, Orange: 3–5 kcal/mol.

Quite recently, the cryogenic electron microscopy single-particle analysis (cryo-EM SPA) of the agonist-bound OX_2R with agonist **4** carrying the same diarylsulfonamide moiety as **1** (PDB ID: 7L1V) was reported.[26] We then conducted a docking study using the most potent naphthalene derivative **28c** to gain structural insight of our molecular conversion (Figure 6). The diarylsulfonamide moiety of **28c** forms a Donor-Acceptor double hydrogen bond with Q134 and the biphenyl moiety of **28c** is well overlapped with that of compound **4** (Figure 6a and 6b). The aniline part of **4** and the left naphthalene ring of **28c** are arranged in a similar manner, but the naphthalene ring is slightly twisted due to the rigidity and volume of the bicyclic system required to fit the binding pocket. The side chains of **4** extend in the bent direction (Figure 6c), which is consistent with the structure-activity relationship results in Table 1. Indeed, the 2-position of the naphthalene ring is close to the binding pocket wall (Figure 6d), which readily explains the low activity of the extended-form derivatives **9a** and **10a**. The *trans*-amide moiety of **28c** is twisted and its dihedral angle (C=C–N–CO) is 89.7º from the naphthalene plane, features which enable the hydrogen bond with H350 instead of the previously observed hydrogen bond between the terminal amide/triazole and H350 with **4**. The distance between the amide of **28c** and H350 (1.73 Å) is estimated to be shorter than that of **4** (2.29 Å), which indicates a tighter interaction for **28c** than with **4**. The terminal aromatic rings of each compound are located in a lipophilic pocket consisting of T135, V138, F227, T231, Y317, I320, and V353, and the anisole moiety is well overlapped with the triazole benzamide moiety of **4**. The dihedral angle (O=C–C=C) between the terminal aromatic ring and the carbonyl group of **28c** is twisted by –78.6º from the benzene plane to fit the lipophilic pocket. Although the twist of the carbonyl group on the benzamide was induced by the *ortho*-substituents, in our study, the introduction of *ortho*-substituents on the terminal aromatic ring was ineffective for the agonist activity. This outcome is due to the small space around the *ortho* position, which causes the steric

repulsion with the receptor. On the other hand, the spaces around the *para*- and *meta*-positions are rather open. Introduction of a substituent into these positions might heighten the van der Waals interaction. Although it is not clear why the OX_1R agonist activity of **28c** is more potent than that of **1** and **4**, the structural differences may be related to the composition of hydrophobic pockets. T135 (A127 in OX_1R) is located near the benzamide group, thereby forming a constricted lipophilic pocket. The substitution from threonine to alanine may alter the structure of the pocket, which may be acceptable for naphthalene derivatives with a more rigid and less conformationally variable amide structure. These data suggested that the restrictions of the flexible side chain and the amide bond rotation would improve the agonist activity of diarylsulfonamide-type agonists.

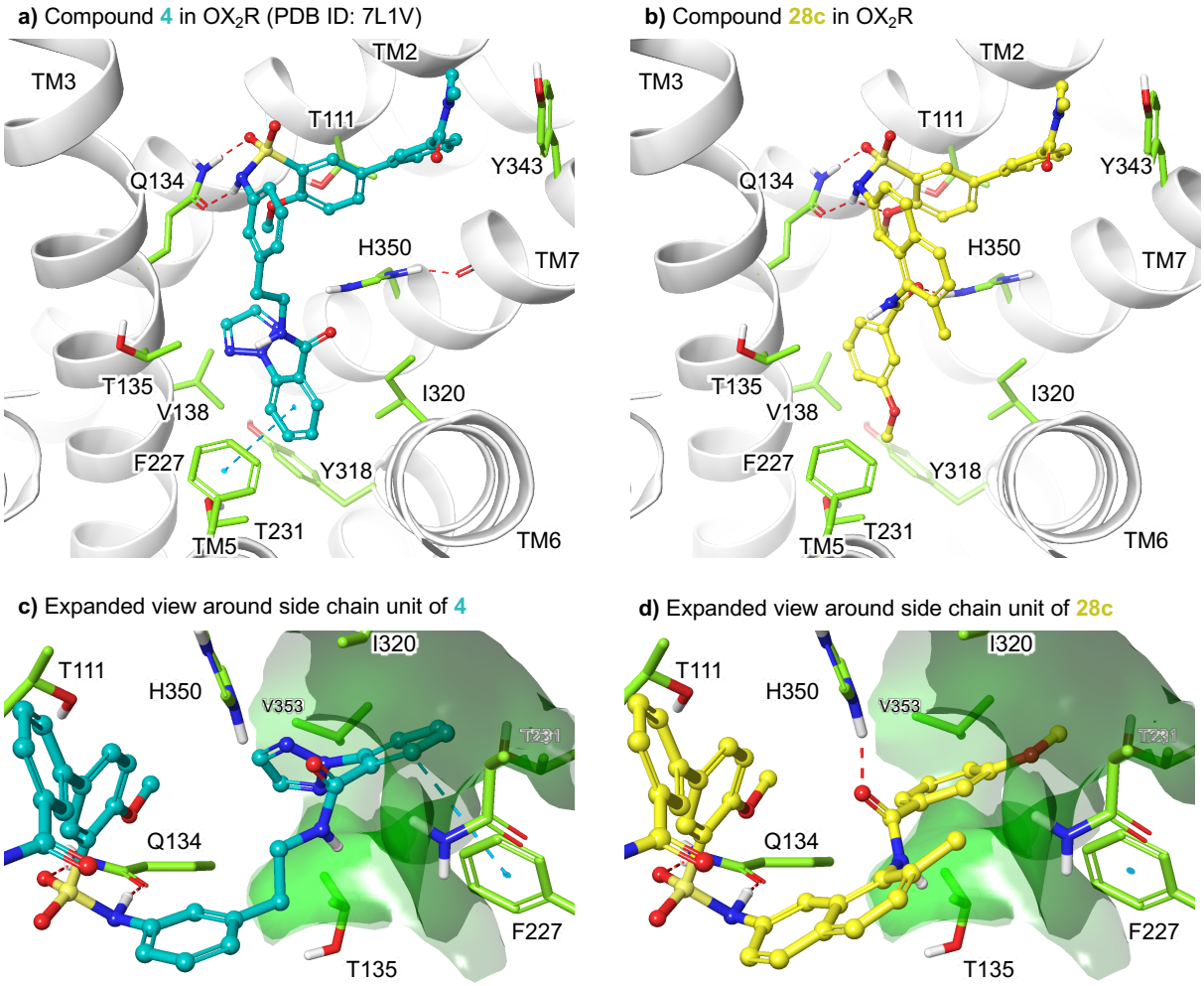


Figure 6. Binding modes of (a) compound **4** in complex with OX_2R (PDB ID: 7L1V) and (b) **28c** with OX_2R determined by our docking procedure. Hydrogen-bonding and CH-π-interactions are indicated by dashed lines. Expanded views around the side chain unit of compounds (c) **4** and (d) **28c**.

In conclusion, we have reported a novel series of potent naphthalene-type orexin receptor agonists based on the strategy focusing on the restriction of the flexible bond rotation of YNT-185 (**1**). The conformational control of a flexible side-

chain unit of **1** using the naphthalene ring revealed that the bent form is more favorable for agonist activity than the extended form. The conformational analysis also revealed that the twisting of the amide unit out from the naphthalene plane is important for the enhancement of activity, which was controlled predominantly by steric repulsion due to the introduction of a methyl group at the 2-position of the naphthalene ring. The geometric isomerism of the benzamide was preferably in the *trans* configuration. These structure-activity relationship results were well supported by comparison of the docking simulation results of the most potent derivative **28c** with an active state of **4**/OX_2R cryo-EM SPA structure. The estimated binding form of **28c** took a similar form to **4**, with the twisted amide group forming a tight hydrogen bond with H350 and the terminal aromatic ring occupying the lipophilic pocket. These results suggested important information for understanding the active conformation and orientation of pharmacophores in the diarylsulfonamide-type orexin receptor agonists.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/xxx.

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