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

Author List
Tsubasa Hino\textsuperscript{a} \textsuperscript{†}, Tsuyoshi Saitoh\textsuperscript{b} \textsuperscript{†}, Yasuyuki Nagumo\textsuperscript{b}, Naoshi Yamamoto\textsuperscript{b}, Noriki Kutsumura\textsuperscript{a} \textsuperscript{b}, Yoko Irukyama-Tomobe\textsuperscript{b}, Yukiko Ishikawa\textsuperscript{b}, Ryuji Tanimura\textsuperscript{a}, Masashi Yanagisawa\textsuperscript{a} \textsuperscript{d} \textsuperscript{e} and Hiroshi Nagase\textsuperscript{a} \textsuperscript{b} \textsuperscript{*}
\textsuperscript{a} Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki-305-8571, Japan
\textsuperscript{b} International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan
\textsuperscript{c} Pharmaceutical Research Laboratories, Toray Industries Inc., 10-1, Tebiro 6-choume, Kamakura, Kanagawa, 248-8555, Japan
\textsuperscript{d} R&D Center for Frontiers of Mirai in Policy and Technology (F-MIRAI), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki i305-8575, Japan
\textsuperscript{e} Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX75390, US
\textsuperscript{†}These authors contributed equally to this work.

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\textsubscript{2}R 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\textsubscript{2}R agonist 28c (EC\textsubscript{50} = 9.21 nM for OX\textsubscript{2}R, 148 nM for OX\textsubscript{1}R). 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\textsubscript{2}R 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\(_1\)R) and the orexin 2 receptor (OX\(_2\)R). 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\(_1\)R knockout mice exhibit no obvious sleep/wakefulness-related phenotype, but OX\(_2\)R knockout mice show a narcoleptic phenotype, suggesting that OX\(_2\)R plays a predominant role in maintenance of wakefulness, and the OX\(_2\)R-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 (DORA) such as suvorexant,\(^9,\)\(^10\) and lemborexan\(^11\) have been launched for the treatment of insomnia so far.\(^12\) However, the development of orexin receptor antagonists 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\(_2\)R selective agonist carrying diarylsulfonamide core, YNT-185 (1, Figure 1, EC\(_{50}\) = 28 nM for OX\(_2\)R, OX\(_1\)R/OX\(_2\)R = ca. 100),\(^14\) and demonstrated the first proof-of-concept studies that the administration of an OX\(_2\)R-selective agonist promotes wakefulness in wild-type mice and attenuates narcoleptic symptoms in prepro-orexin knockout mice through the activation of OX\(_2\)R on histaminergic neurons in the mouse tuberomammillary nucleus (TMN).\(^15\) We also demonstrated that it attenuates the morphine-induced sedative effects in rats.\(^16\) Stimulated by our pioneering works, several types of OX\(_2\)R agonists have since been reported. The group of Takeda reported a series of 3-piperidyl sulfonamide derivatives as OX\(_2\)R selective agonists.\(^17\) Among them, TAK-925 (2) with a 3-amino piperidine core showed potent OX\(_2\)R selective agonist activity (EC\(_{50}\) = 5.5 nM for OX\(_2\)R, OX\(_1\)R/OX\(_2\)R > 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\(_1\)R, OX\(_1\)R/OX\(_2\)R = 6.6) and the OX\(_1\)R specific antagonist B (\(K_i\) = 2.17 nM for OX\(_1\)R) through the screening of the 1,3,5-trioxaazatriquinanes 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
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$_2$S$_2$O$_4$ 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–12a. (A) Synthesis of 2,7-diaminonaphthalene derivatives 9a and 10a. (a) Na₂S, NaHCO₃, MeOH/H₂O (7:2), 60 °C, 14: 41%; (b) BzCl, Et₃N, CH₂Cl₂, rt, 15: 94%, 24: 80%; (c) Fe, NH₄Cl, EtOH/H₂O (5:1), 60 °C, 16: 31%, 19: 97%, 25: 79%, 27: 100%; (d) 3''-(dimethylcarbamoyl)-4-methoxy-[1,1''-biphenyl]-3-sulfonyl chloride (17), CH₂Cl₂/pyridine, 0 °C to rt, 9a: 87%, 10a: 95%, 11a: 100%, 12a: 94%, (e) MeI, NaH, THF, 0 °C to rt, 18: 87%, 26: 100%; (f) NH₂OH·HCl, NaOAc, MeOH, reflux, 21: 98%; (g) pivaloyl chloride, Et₃N, CH₂Cl₂, 0 °C to rt, 22: 100%; (h) Pd(PPh₃)₄, K₂CO₃, 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 OX₁R and OX₂R (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 OX₂R agonist activity (11a: EC₅₀ = 873 nM, 12a: EC₅₀ = 1,949 nM) with weak OX₁R agonist activity.
These results suggested that the bent form would be more favorable than the extended form of 1 to elicit the OX₂R agonist activity.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>EC₅₀ (nM)</th>
<th>Eₘ₅ (%)[b]</th>
<th>Selectivity (OX₁R/OX₂R)</th>
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<tbody>
<tr>
<td>9a</td>
<td>H</td>
<td>H</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>NC[4]</td>
</tr>
<tr>
<td>10a</td>
<td>Me</td>
<td>H</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>NC[4]</td>
</tr>
<tr>
<td>11a</td>
<td>H</td>
<td>N</td>
<td>&gt;10,000</td>
<td>31.8</td>
<td>873</td>
</tr>
<tr>
<td>12a</td>
<td>H</td>
<td>Me</td>
<td>&gt;10,000</td>
<td>50.8</td>
<td>1,949</td>
</tr>
</tbody>
</table>

EC₅₀ and Eₘ₅ values are the means ± SEM of at least three independent experiments conducted in duplicate. Eₘ₅ expressed as a percentage of OXA maximum. ND = not detected. 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).³ 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 (EC₅₀ = 22.7 nM for OX₂R, 232 nM for OX₁R) and para-methoxy derivative 12d (EC₅₀ = 51.1 nM for OX₂R, 654 nM for OX₁R) 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 (EC₅₀ = 24.3 nM for OX₂R, 473 nM for OX₁R) 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>R³</th>
<th>R⁴</th>
<th>EC₅₀ (nM) [Eₘ₅ (%)]</th>
<th>Selectivity (OX₁R/OX₂R)</th>
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<td></td>
<td></td>
<td>OX₂R³</td>
<td>OX₁R³</td>
</tr>
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<td>11a</td>
<td>Me₂NOC</td>
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<td></td>
<td></td>
</tr>
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<td>Me₂NOC</td>
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<table>
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<tr>
<th></th>
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<td>Me</td>
<td>H</td>
<td>&gt;10,000</td>
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<td>NC</td>
</tr>
<tr>
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<td>10,000</td>
<td>NC</td>
</tr>
<tr>
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<td>Me</td>
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<td>232</td>
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</tr>
<tr>
<td>11a</td>
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<td>873</td>
<td>NC</td>
</tr>
<tr>
<td>11c</td>
<td>H</td>
<td>3-OH</td>
<td>473</td>
<td>24.3</td>
<td>19.5</td>
</tr>
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</table>

*EC₅₀ and Eₘₐₓ values are the means ± SEM of at least three independent experiments conducted in duplicate. *EC₅₀ values are expressed as a percentage of OXA maximum. *ND = not detected. *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 (EC₅₀ = 9.21 nM for OX₂R, 148 nM for OX₁R) showed ca. 3 times higher activity for both receptors (Figure 5). However, the N-methyl tertiary amide derivative 29c (EC₅₀ = 31.1 nM for OX₂R, 260 nM for OX₁R) 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 OX2R 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 OX₁R 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 OX₁R) 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 OX₂R (PDB ID: 7L1V) and (b) 28c with OX₂R 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/OX2R 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.

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

References
31. The conformational analysis was conducted using Conformational Search function in MOE software package ver. 2018.0101 (Chemical Computing Group, Inc., Montreal, Canada) with LowModeMD method, MMFF94x force field, gas-phase electrostatics and no nonbonded interaction cutoff.