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Bioorganic & Medicinal Chemistry Letters
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Design and Synthesis of Propellane Derivatives with Selective Delta Opioid Receptor Agonism: Propellane Derivatives with a Quinoline Ring

Hiroshi Nagase,[a,b,*] Ryo Nakajima,[a,b,d] Naoshi Yamamoto,[a] Shigeto Hirayama,[b] Takashi Iwai,[b] Toru Nemoto,[b] Hiroaki Gouda,[c,e] Shuichi Hirono,[c] Hideaki Fujii[b]

[a] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan*
[b] *Laboratory of Medicinal Chemistry and* [c] *Laboratory of Physical Chemistry for Drug Design, School of Pharmacy, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[d] *Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan*
[e] *School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Opioid
DOR
Propellane structure
Binding mode
Binding free energy

ABSTRACT

Indolopropellane **2** was reported to show almost no binding affinity to the δ opioid receptor (DOR) in spite of the fact that **2** has both the propellane fundamental skeleton (message part) with binding ability to the opioid receptors and a possible DOR address structure (indole moiety). We developed the working hypothesis that almost no binding affinity of **2** to the DOR would be derived from its possibly stable bent conformer. To enable the propellane skeleton to adopt an extended conformation which would reasonably interact with the DOR, quinolinopropellanes **3a**–**d** were designed which had an additional pharmacophore, quinoline nitrogen. The calculated binding free energies of ligand-DOR complexes strongly supported our working hypothesis. The synthesized quinolinopropellane **3a** was a selective DOR full agonist, confirming our working hypothesis and the results of *in silico* investigation.

The opioid receptor, with which potent prescription analgesic morphine interacts, is one of the promising drug targets. Opioid receptors are classified into three major types, MOR (μ opioid receptor), DOR (δ opioid receptor), and KOR (κ opioid receptor). For several decades, medicinal chemists have focused on development of selective agonists and antagonists for each opioid receptor type. The message-address concept is a useful guideline for design of type selective opioid ligands.[1] For example, DOR antagonists such as NTI,[2] NTB,[3] BNTX,[4] and SB-205588,[5] DOR agonists such as TAN-67,[6] SB-219825,[5] NS-28,[7] and KNT-127,[8] KOR antagonists such as nor-BNI[9] and 5'-GNTI,[10] and the KOR agonist nalfurafine[11,12] were designed and synthesized according to this concept (Fig. 1). The message part plays an important role in exerting opioid functions. In contrast to the KOR ligands, which have the 4,5-epoxymorphinan functionality as the common message structure, the DOR ligands possess various message structures, including 4,5-epoxymorphinan, morphinan, and 4a-phenyldecahydroisoquinoline structures. Recently, we found propellane **1** (Fig. 2) as a novel message skeleton.[13] The propellane **1** bound to the MOR, DOR, and KOR with binding affinities (K_i) of 58.2 nM, 448 nM, and 17.4 nM, respectively. These results prompted us to develop novel ligands with the propellane skeleton. However, indolopropellane **2** (Fig. 3) was reported to show almost no affinity for opioid receptors[14] although **2** has not only a propellane skeleton as a message structure but also an indole moiety as a possible DOR address part like the selective DOR antagonist NTI.[1,2] To explain these observations, we developed the working hypothesis that **2** could adopt two different conformations, bent and extended (Fig. 3). The extended conformer, which resembles the stable conformation of NTI, could bind to the DOR whereas the bent conformer could not. Indeed the real binding conformation of NTI unveiled by the X-ray crystallographic analysis of the NTI-DOR complex[15] is an extended form (Fig. 4). The lack of binding of **2** to the DOR may be derived from the adoption of the bent conformer, which may be the more stable form.

This working hypothesis suggests that the introduction of an additional pharmacophore into the structure of **2**, which can interact with the DOR to stabilize the ligand-DOR complex, would enhance the binding affinity to the DOR. In the course of developing the selective DOR agonist TAN-67,[6] we assumed an interaction between the quinoline nitrogen and the DOR and that the interaction would trigger the precise conformational change of the DOR to exert the DOR agonist activity. On the basis of the above consideration, we designed quinolinopropellane **3a** (Fig. 3) as a DOR agonist which contains an additional possible pharmacophoric moiety like TAN-67. Herein, we report the conformational analyses of indolo- and quinolinopropellanes **2** and **3a** and the evaluation of the binding free energies of the ligands to the DOR. We also describe the synthesis of the designed quinolinopropellane derivatives **3a**–**d** and their *in vitro* profiles.

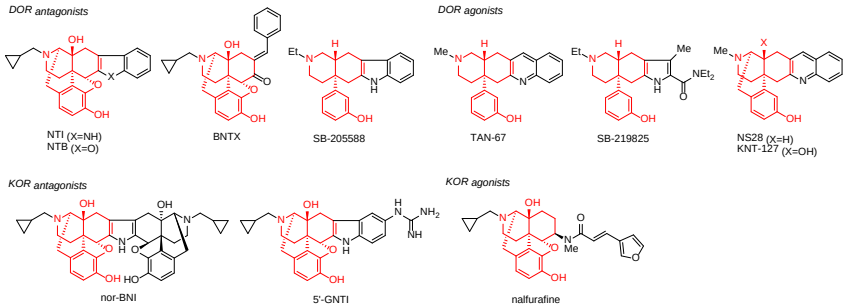


Figure 1. Structures of DOR antagonists, DOR agonists, KOR antagonists, KOR agonist. The message structures of these ligands are indicated in red.

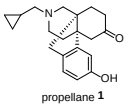


Figure 2. Structure of propellane **1**.

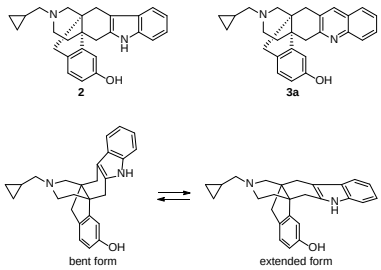
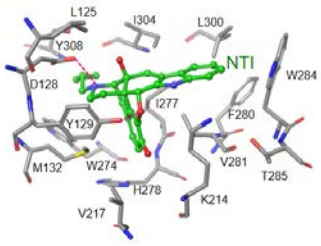


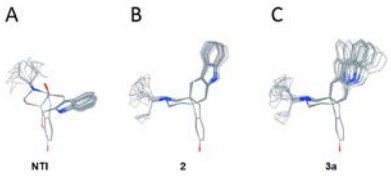
Figure 3. Structures of indolopropellane **2**, quinolinopropellane **3a**, and the bent and extended forms of **2**.

Figure 4. The binding mode of NTI observed in the X-ray structure of the NTI-DOR complex.

Figure 5. The superimpositions of the low-energy conformers of NTI, **2**, and



3a.



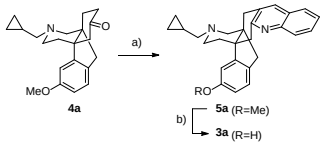
First, to investigate our proposal related to the bent and extended conformers of indolo- and quinolinopropellanes **2** and **3a**, we performed conformational analyses of NTI, **2**, and **3a** using Conformational Analyzer with Molecular Dynamics And Sampling (CAMDAS) 2.1 program.[16] When the low-energy conformers of NTI, **2**, and **3a** (those within 2.5 kcal/mol of the global minimum) were superimposed (Fig. 5), we found that the low-energy conformers of both **2** and **3a** adopted the bent form, while those of NTI had the extended form, as expected. The extended forms of **2** and **3a** roughly appeared at the energy difference of 3–5 kcal/mol from the global minimum.

Next, the binding modes of **2** and **3a** with the DOR and their binding free energies (ΔG_{bind} values) were examined by using a combination method of the molecular-docking calculation[17] and the molecular mechanics Generalized-Born surface area (MM-GBSA) free energy analysis[18,19]. The resulting binding modes of **2** and **3a** are displayed in Figure 6, and their calculated ΔG_{bind} values are given in Table 1. Indolopropellane **2** was found to bind with the DOR in its extended form (Fig. 6A). This result strongly supported our working hypothesis that the extremely low affinity of **2** to the DOR may be due to the fact that **2** could not bind to the DOR when the ligand was in the low-energy bent form. In other words, the binding of **2** to the DOR would require a considerable energy penalty to adopt the high-energy extended form, which is suited to bind to the DOR as shown in the crystal structure of the NTI-DOR complex[15] (Fig. 4). On the other hand, the binding mode of quinolinopropellane **3a** (Fig. 6B) suggested that the extended form of **3a** could also bind to the DOR[20]. Interestingly, we found that the lone electron pair on the nitrogen atom of the quinoline ring in **3a** could form a hydrogen bonding interaction with the NH_3^+ of the Lys[214] residue. A similar hydrogen bond was not observed in the **2**-DOR complex,

Table 1. Energy contributions (kcal/mol) to the binding free energy of **2** and **3a** to the DOR.

Contribution	**2**	**3a**	Difference[a]
ΔE_{int}[b]	3.19	2.80	0.39
ΔE_{VDW}[c]	–50.03	–48.59	–1.44
Δ_{Eelec}[d]	–11.93	–25.47	13.54
ΔG_{GB}[e]	11.06	13.99	–2.93
ΔG_{SA}[f]	–6.28	–8.15	1.87
ΔG_{bind}[g]	–53.99	–65.42	11.43

[a] Differences of energy contributions of **2** and **3a**.
[b] Internal contributions from bond, angle, dihedral terms.
[c] Nonbonded van der Waals.
[d] Nonbonded electrostatics.
[e] Electrostatic component to solvation.
[f] Nonpolar component to solvation.



[g] Total change of free energy in binding.

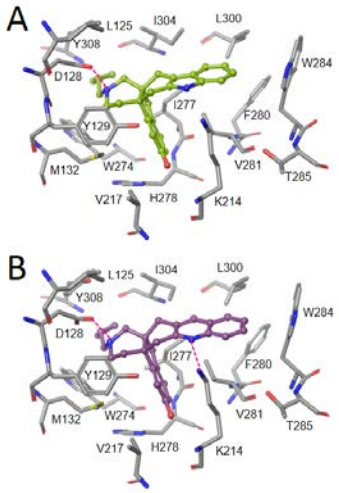
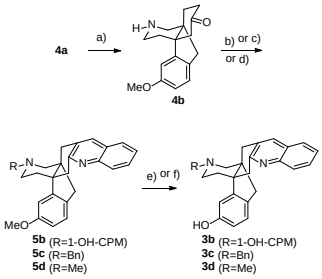


Figure 6. The binding modes of **2** (A) and **3a** (B) with the DOR determined by our docking procedure. Hydrogen-bonding interactions are indicated by red dashed lines.

because **2** possessed the indole ring which lacks a lone electron pair. Due to the additional hydrogen bonding interaction, the electrostatic interaction (ΔE_{elec}) of **3a** with the DOR was suggested to be much greater than that of **2** (Table 1). This situation inevitably led to a much better ΔG_{bind} value for **3a**. Taken together, the above observations suggested that the additional hydrogen bonding interaction in the **3a**-DOR complex might compensate for any energy penalty, allowing **3a** to adopt the high-energy extended form upon binding. The obtained binding mode of quinolinopropellane **3a** with the DOR included the hydrogen bonding with the Lys^{214} residue, whereas a corresponding interaction with the Lys^{214} residue was not observed in the crystal structure of the NTI (DOR antagonist)-DOR complex.[15] In the course of DOR agonist TAN-67 discovery, the hydrogen bonding with the DOR was proposed to be important in producing the DOR agonist activity.[6] Therefore, quinolinopropellane **3a** was expected to produce DOR agonism.

Scheme 1. Synthesis of quinolinopropellane **3a**. Reagents and conditions: (a) 2-Aminobenzaldehyde, $MeSO_3H$, EtOH, reflux; (b) HCl·Pyridine, 180 ˚C.

Scheme 2. Synthesis of quinolinopropellanes **3b**–**d**. Reagents and conditions: (a) (i) Troc-Cl, K_2CO_3, CH_2Cl_2, rt; (ii) Zn, AcOH, rt; (b) (i) 2-Aminobenzaldehyde, $MeSO_3H$, EtOH, reflux; (ii) 1-Acetoxy-1-cyclopropanecarboxylic acid, EDCI·HCl, DMAP, DMF, rt; (iii) $LiAlH_4$, H_2SO_4, THF, rt; (c) (i) 2-Aminobenzaldehyde, $MeSO_3H$, EtOH, reflux; (ii) BnBr, K_2CO_3, DMF, rt; (d) (i) MeI, Et_3N, CH_2Cl_2, rt; (ii) 2-Aminobenzaldehyde, $MeSO_3H$, EtOH, reflux; (e) BBr_3, CH_2Cl_2, 0 ˚C to rt; (f) HCl·Pyridine, 180 ˚C.



To confirm the *in silico* outcomes, we synthesized quinolinopropellanes **3a**–**d**. A quinolone moiety was constructed by the reaction of compound **4a** with 2-aminobenzaldehyde in the presence of methanesulfonic acid (Friedländer quinoline synthesis[21]), followed by demethylation of the *O*-Me group in **5a** to provide **3a** (Scheme 1). *Via* nor-compound **4b** obtained from **4a** by a reaction with Troc-Cl and subsequent treatment with Zn/AcOH, the conversion of *N*-substituents was carried out by two methods: 1) a Friedländer quinoline synthesis and followed by amidation with carboxylic acid and reduction of the obtained amide by alane,[22] and 2) a sequential Friedländer quinoline synthesis and alkylation (Scheme 2).

The binding affinities of the prepared quinolinopropellanes **3a**–**d** to the opioid receptors were evaluated by competitive binding assays (Table 2). As we expected, all the tested quinolinopropellanes **3a**–**d** exhibited high binding affinities and selectivities for the DOR. Quinolinopropellane **3a** with the *N*-cyclopropylmethyl group had the highest binding affinity for the DOR, while *N*-(1-hydroxycyclopropylmethyl) derivative **3b** showed the highest selectivity for the DOR, although its binding affinity for the DOR was slightly decreased compared with that of **3a**. Although a propellane **1** derivative with the *N*-methyl substituent was reported to be a strong binder to the MOR (K_i = 3.6 nM) with 122- and 71-fold greater selectivities over the DOR and KOR,[13] respectively, *N*-methylquinolinopropellane **3d** exerted low but evident selectivity for the DOR.

Table 2. Binding affinities of quinolinopropellanes **3a**–**d** to the opioid receptors[a]

Compound	R	K_i (nM)			Selectivity	
		MOR[b]	DOR[c]	KOR[d]	MOR/DOR	KOR/DOR
3a	CPM	112	0.941	84.6	119	89.9
3b	1-OH-CPM	415	1.10	879	378	801
3c	Bn	76.3	31.6	594	2.42	18.8
3d	Me	3.06	1.88	195	1.63	104

[a] Binding assays were carried out in duplicate.
[b] [3H] DAMGO was used.
[c] [3H] DPDPE was used.
[d] [3H] U-69,593 was used.
[e] CPM: cyclopropylmethyl

We next assessed the functional activities of a selected compound **3a**, which exhibited the highest binding affinity for the DOR, by [^{35}S]GTPγS binding assays. As we expected, **3a** exhibited DOR full agonist activity (EC_{50} (DOR) = 2.50 nM, E_{max} (DOR) = 88%, EC_{50} (MOR) = 197 nM, E_{max} (MOR) = 56%, EC_{50} (KOR) = 836 nM, E_{max} (KOR) = 57%). The functional DOR selectivities of **3a** were comparable to or higher than its binding DOR selectivities (EC_{50} ratio: MOR/DOR = 78.8, KOR/DOR = 334). The outcomes of *in vitro* evaluations strongly supported our

working hypothesis and the *in silico* experimental results. Moreover, these observations suggest that the hydrogen bonding interaction between a ligand and the Lys^{214} residue in the DOR plays a crucial role in not only obtaining strong binding ability but also exerting DOR agonist activity.

In conclusion, we have developed the working hypothesis that almost no binding affinity of indolopropellane **2** to the DOR would be derived from its possibly more stable bent conformer. To enable the propellane skeleton to adopt an extended conformation, which could reasonably be expected to interact with the DOR, quinolinopropellanes **3a**–**d** were designed which had an additional pharmacophore, the quinoline nitrogen. The calculated binding free energies of ligand-DOR complexes strongly supported our working hypothesis. The synthesized quinolinopropellane **3a** was a selective DOR full agonist, confirming our working hypothesis and the results of *in silico* investigation.

Acknowledgments

We acknowledge the financial supports from Shorai Foundation for Science, Uehara Memorial Foundation and Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST (AS2315040G) (to H.N., S.H., T.I., T.N., and H.F.). We also acknowledge the Institute of Instrumental Analysis of Kitasato University, School of Pharmacy for its facilities.

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