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Investigation of Beckett–Casy model 3: Synthesis of novel naltrexone derivatives with contracted and expanded D-rings and their pharmacology

Hiroshi Nagase [a,*], Satomi Imaide [a], Miyuki Tomatsu [a], Shigeto Hirayama [a], Toru Nemoto [a], Noriko Sato [a], Mayumi Nakajima [b], Kaoru Nakao [b], Hidenori Mochizuki [b], Hiroaki Gouda [a], Shuichi Hirono [a], Hideaki Fujii [a]

[a] School of Pharmacy, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan
[b] Pharmaceutical Research Laboratories, Toray Industries, Inc., 6-10-1, Tebiro, Kamakura 248-8555, Japan

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ABSTRACT

Novel naltrexone derivatives **7** and **8** with contracted and expanded D-rings were synthesized to investigate the importance of orientation of lone electron pair on the nitrogen for binding abilities to the opioid receptor. Compound **7** showed almost no binding affinity, whereas compound **8** was comparable to naltrexone (**6**) in binding affinity. Conformational analyses and NOE experiments in D_2O of compounds **6–8** suggested that the lone electron pairs of compounds **6** and **8** with respective six- and seven-membered D-rings would project in the pseudo-axial orientation, whereas compound **7** with five-membered D-ring would have the lone electron pair directing in pseudo-equatorial position. These results strongly supported the proposal that the axial orientation of the lone electron pair on nitrogen would provide sufficient binding abilities to the opioid receptor and that the 15–16 ethylene moiety in the morphine structure would play a role in fixation of the lone electron pair in the axial direction rather than interaction with the putative cavity in the Beckett–Casy model.

Three types of opioid receptors (μ, δ, and κ) are now well established not only by pharmacological studies but also by molecular biological studies.[1] The μ receptor type is believed to be involved in narcotic addiction, and therefore, δ and κ types are promising drug targets for analgesics without addiction. To obtain ideal analgesics free of addictive properties and other side effects derived from the μ receptor, we have focused our investigation on δ and κ receptor ligands to develop selective δ and κ agonists[2–6] and to discover other new reactions using naltrexone derivatives.[7–18] Recently, we have reported synthesis of 16,17-seco-naltrexone derivatives **1**[19] and 15–16 nornaltrexone derivatives **2–4** (Fig. 1),[20] and have discussed the importance of the putative cavity in the Beckett–Casy model[21–23] based on their binding affinities for the opioid receptor. This model proposes that the cavity would exist on the opioid receptor site and interact with the 15–16 ethylene moiety in the morphine structure (Fig. 2).[21–23] Although the investigation using 16,17-seco-naltrexone derivatives **1** seemed to support the existence of such a cavity structure, the intimate comparisons of the binding affinities of 15–16 nornaltrexone derivatives **2–4** and compound **5** suggested that the orientation of the lone electron pair on 17-nitrogen would influence the binding affinities of the compounds for the opioid receptor and that the 15–16 ethylene moiety in the morphine would play a role in fixation of the lone electron pair in the axial direction (see the structure of naltrexone (**6**) in Fig. 1) rather than interaction with the

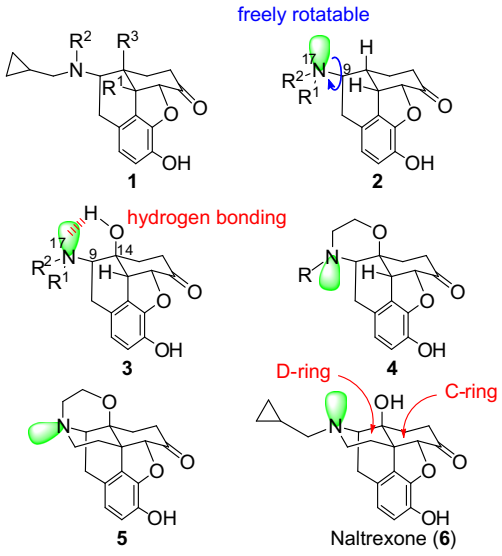

Figure 1. Structures of 16,17-seco-naltrexone derivatives **1**, 15–16 nornaltrexone derivatives **2–4**, compound **5**, and naltrexone (**6**). Lone electron pairs of compounds **2–6** are indicated.

* Corresponding author. Tel.: +81 3 5791 6372; fax: +81 3 3442 5707.
E-mail address: nagaseh@pharm.kitasato-u.ac.jp (H. Nagase).

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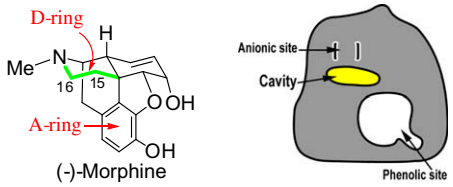


Figure 2. (−)-Morphine and the Beckett–Casy binding model. (−)-Morphine can bind the opioid receptor site by use of three pharmacophoric interactions; ionic, π–π (aromatic ring) interactions, and hydrogen bonding. Furthermore, the 15–16 bond (green line) projecting in front of and to the side of the line between the center of A-ring and the basic nitrogen in morphine is proposed to fit into the cavity moiety in this model.

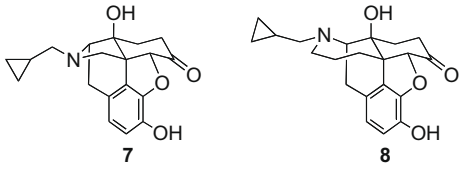


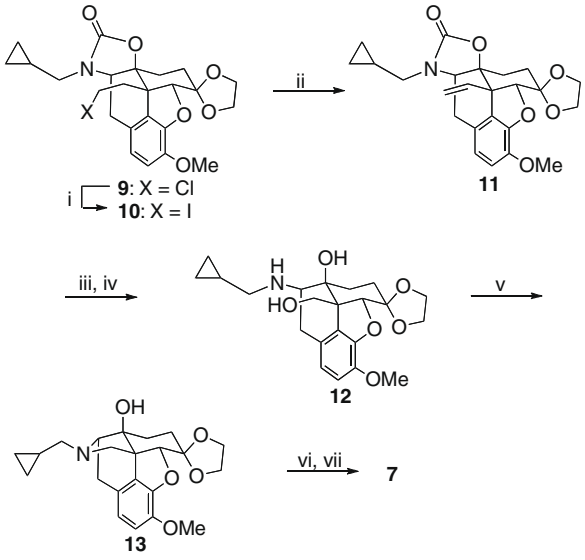
Figure 3. Structures of naltrexone derivatives **7** and **8** with contracted and expanded D-rings.

proposed cavity in the Beckett–Casy model.[21–23] However, as the compounds **4** and **5**, which gave the binding data supporting the proposal mentioned above, have an additional methylene structure between 17-nitrogen and 14-hydroxy group, a steric hindrance resulted from the methylene moiety may decrease in the binding abilities of these compounds. To clarify the importance of lone electron pair's orientation for binding ability, compounds which have lone electron pair projecting in various direction without steric hindrance would be required. The size of D-ring was predicted to influence the orientation of the lone electron pair, and especially a five-membered ring would restrict the lone electron pair's direction due to its rigid conformation. Therefore, we attempted to synthesize compounds **7** and **8** with contracted and expanded D-rings, respectively (Fig. 3). Herein, we report synthesis of compounds **7** and **8** with respective five- and seven-membered D-rings and their binding affinities for the opioid receptor, and discuss the importance of the lone electron pair's orientation on binding ability.

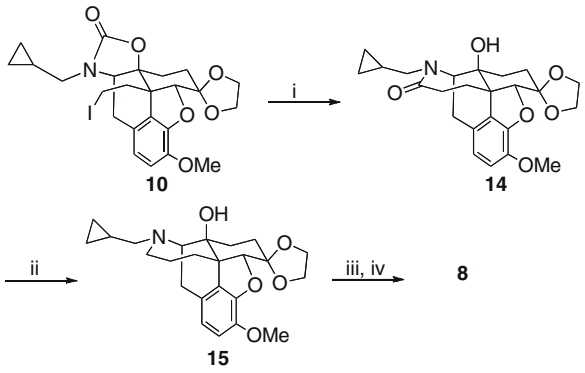
Synthesis of compound **7** with a contracted D-ring began from compound **9** prepared from naltrexone (**6**) by the 16–17 bond cleavage reaction[12] (Scheme 1). Halogen exchange reaction of compound **9** gave **10**, which was treated with *t*-BuOK to afford olefin **11**. The olefin moiety of **11** was converted into an alcohol by ozonolysis and subsequent $NaBH_4$ reduction, followed by hydrolysis to provide compound **12**. Treatment of **12** with PPh_3 and CCl_4 gave five-membered D-ring compound **13** via simultaneous cyclization. The objective compound **7** was obtained by appropriate deprotections of **13**.

Compound **8** with an expanded D-ring was also synthesized from 16–17 bond cleaved compound **10** (Scheme 2). Lithiation of **10** with *t*-BuLi afforded lactam **14** by intramolecular nucleophilic attack, and subsequent LAH reduction of **14** provided the seven-membered D-ring compound **15**. The objective compound **8** was obtained by appropriate deprotections of **15**.

Binding affinities of compounds **7**, **8**, and standard μ antagonist naltrexone (**6**) were evaluated by a competitive displacement binding assay. Compound **8** with a seven-membered D-ring showed affinity for the μ receptor comparable to naltrexone (**6**) (**8**: K_i (μ) = 0.41 nM, K_i (κ) = 2.09 nM, K_i (δ) = 10.35 nM; **6**: K_i (μ) = 0.27 nM, K_i (κ) = 0.70 nM, K_i (δ) = 12.27 nM), whereas compound **7** with a five-membered D-ring surprisingly exhibited



Scheme 1. Reagents and conditions: (i) NaI, DMF, 130 °C, 94%; (ii) *t*-BuOK, THF, 0 °C, 95%; (iii) O_3, CH_2Cl_2, −78 °C, then $NaBH_4$, MeOH, rt, 77%; (iv) 6 M NaOH aq, DMSO, 100 °C, 83%; (v) PPh_3, CCl_4, Et_3N, pyridine, 60 °C, 96%; (vi) 2 M HCl, MeOH, reflux, quant.; (vii) BBr_3, CH_2Cl_2, rt, 42%.



Scheme 2. Reagents and conditions: (i) *t*-BuLi, THF, −78 °C, 64%; (ii) LAH, THF, 50 °C, 81%; (iii) 2 M HCl, MeOH, reflux, quant.; (iv) BBr_3, CH_2Cl_2, rt, 27%.

almost no affinities for any opioid receptors (K_i > 1000 nM for μ, δ, and κ receptors).

Morphinan **16** and benzomorphan derivative **17** with five-membered D-rings and benzomorphan derivative **18** with a seven-membered D-ring were synthesized (Fig. 4) and their analgesic activities were reported.[24–26] Compound **16** had neither agonistic nor antagonistic activities,[27] while compound **17** showed antinociceptive effects but its potency was 36-fold weaker than that of morphine.[25] X-ray crystallographic analysis of compound **16**, carried out by Hardy, Ahmed, and colleagues, revealed that the D-ring of compound **16** was in the half-chair conformation, with the lone electron pair oriented toward the same side as the phenol ring, and the *N*-methyl group projecting toward the opposite side.[27,28] In comparisons of this result with reported X-ray crystallographic analyses of morphine and benzomorphans, these authors pointed out that the orientation of the lone electron pair was crucial to interaction with the opioid receptor. In contrast to **16**, compound **18** with a seven-membered D-ring exhibited antinociception as potent as morphine. X-ray crystallographic analysis of compound **18**, performed by Itai and co-workers, indicated that the D-ring[29] of compound **18** was in the quasi-chair conformation and that the lone electron pair projected toward the same side as

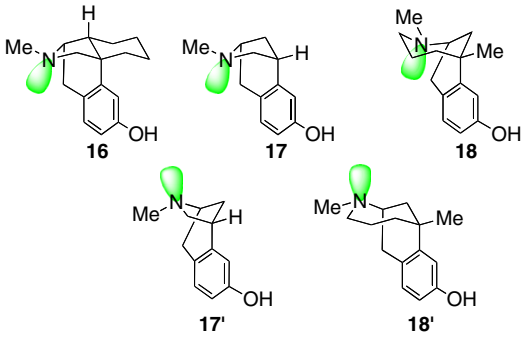


Figure 4. Structures of morphinan and benzomorphan derivatives **16** and **17** with contracted D-ring and benzomorphan derivative **18** with an expanded D-ring. Structures **17′** and **18′** indicate possible conformers with the alternative quasi-chair form in the D-rings.

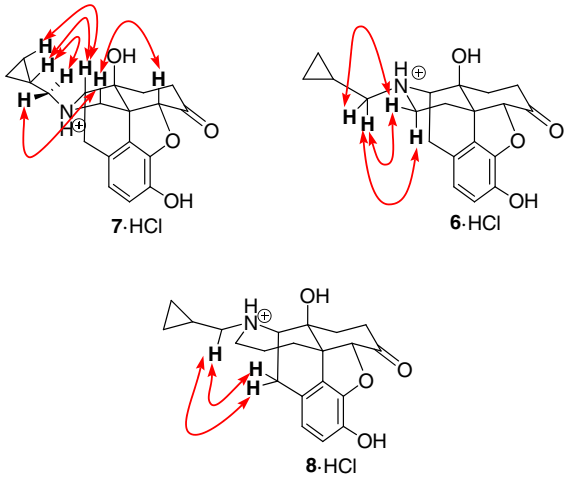


Figure 5. NOE correlations were observed between protons indicated by arrows.

the phenol while the *N*-methyl group projected to the opposite side.[30,31] However, the Itai and co-workers proposed that the D-ring could flip to adopt the alternative quasi-chair form **18′** (Fig. 4) in which the lone electron pair would take on the pseudo-axial orientation, that is, the opposite site to the phenol ring.[31] Taken together, it is estimated that the lone electron pair projecting in the pseudo-axial orientation, that is, the opposite side to the phenol ring, would induce a stronger interaction with the opioid receptor. The conformation of the five-membered ring in **16** would be too rigid to flip because of the presence of an additional structure, the C-ring. As a result, compound **16** was assumed to show neither agonistic nor antagonistic activities. Although the five-membered ring in **17** may have the same conformation as compound **16** as a stable conformation, it may not be so rigid that it can adopt another quasi-chair form due to the lack of the C-ring. Therefore, when a flip of the D-ring in **17** directed the lone electron pair in the pseudo-axial orientation (conformer **17′** in Fig. 4), **17′** was presumed to bind to the opioid receptor. On the other hand, the seven-membered ring in **18** is very flexible despite the presence of the additional structure, the C-ring, and it could easily adopt another quasi-chair form **18′** having its lone electron pair in the pseudo-axial direction, that is, the opposite site to the phenol ring. As a result, **18′** was estimated to bind to the opioid receptor to induce a strong antinociceptive effect.

Based on the above discussion, we attempted to analyze stable conformations of protonated compounds **7** and **8**, and naltrexone (**6**) using the Conformational Analyzer with Molecular Dynamics And Sampling (CAMDAS) 2.1 program.[32] Conformational analyses indicated the following results: in protonated compound **7** having a five-membered ring, a conformer with a lone electron pair in the pseudo-equatorial orientation was 1.935 kcal/mol more stable than a conformer with a lone electron pair in the pseudo-axial position; in protonated naltrexone (**6**) having a six-membered ring, a conformer with a lone electron pair in the axial direction was 3.196 kcal/mol more stable than a conformer with a lone electron pair in the equatorial orientation; and in protonated compound **8** having a seven-membered ring, a conformer with a lone electron pair in the pseudo-axial position was 1.197 kcal/mol more stable than a conformer with a lone electron pair in the pseudo-equatorial direction. These results supported the proposal that a conformer with a lone electron pair in the axial orientation is more favored to bind to the opioid receptor.

To further investigate actual conformations of compounds **6**–**8** in the solution state, we carried out NOE experiment in D_2O. The NOE correlations were observed between protons indicated by arrows, suggesting that hydrochlorides of compounds **6**–**8** in D_2O would adopt the conformation shown in Figure 5. These outcomes were in good agreement with the results obtained by conformational analyses using CAMDAS, and strongly support the proposal that the axial orientation of the lone electron pair on the nitrogen would provide sufficient binding abilities to the opioid receptor and that rather than interact with the putative cavity in the Beckett–Casy model, the 15–16 ethylene moiety in the morphine structure would play a role in fixation of the lone electron pair in the axial direction. This result suggests that the interaction between the 17-nitrogen and the receptor site should be not the non-directional ionic bond but the directional enforced ionic bond which was reinforced by the directional hydrogen bond.[33–35]

In conclusion, we synthesized novel naltrexone derivatives **7** and **8** with contracted and expanded D-rings to investigate the importance of orientation of a lone electron pair on the nitrogen for binding abilities to the opioid receptor. Compound **7** with a contracted D-ring showed almost no binding affinity, whereas compound **8** with an expanded D-ring was comparable to naltrexone (**6**) in binding affinity. Conformational analyses and NOE experiments in D_2O of compounds **6**–**8** suggested that the lone electron pairs of compounds **6** and **8** with respective six- and seven-membered D-rings would project in the pseudo-axial orientation, whereas compound **7** with a five-membered D-ring would have the lone electron pair directed in the pseudo-equatorial position. These results strongly support the proposal that the axial orientation of the lone electron pair on nitrogen would promote effective binding to the opioid receptor and that the role of the 15–16 ethylene moiety in the morphine structure is to fix the lone electron pair in the axial direction rather than to interact with the putative cavity in the Beckett–Casy model.

Acknowledgments

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