ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

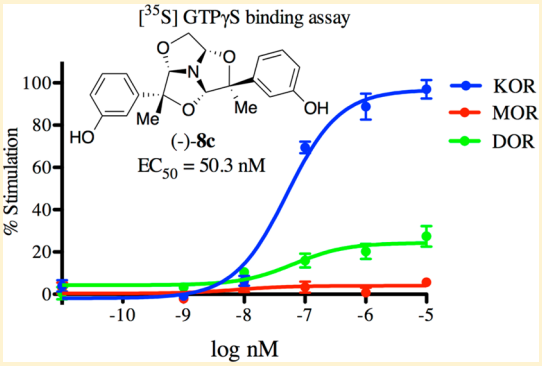
Synthesis and Pharmacology of a Novel κ Opioid Receptor (KOR) Agonist with a 1,3,5-Trioxazatriquinane Skeleton

Shigeto Hirayama, Naohisa Wada, Toru Nemoto, Takashi Iwai, Hideaki Fujii, and Hiroshi Nagase*,†

School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

Supporting Information

ABSTRACT: We designed and synthesized the 1,3,5-trioxazatriquinane derivatives with *m*-hydroxyphenyl groups. These compounds include the phenethylamine structure within them, which is a common structure observed in morphinan derivatives like morphine. Among the synthesized compounds, (−)-**8c** with two *m*-hydroxyphenyl groups selectively bound and exerted full agonist activity toward the κ opioid receptor (KOR). Subcutaneously administered (−)-**8c** exhibited significant antinociceptive effects via the KOR in a dose-dependent manner. These results suggest the emergence of a novel class of KOR agonist.



KEYWORDS: *Opioid, analgesics, phenethylamine structure*

Twin drugs consisting of two pharmacophore units in a single molecule have been described in numerous domains of medicinal chemistry. Symmetrical twin drugs with flexible linkers can simultaneously fit into symmetrical binding sites of a protein complex to afford increased activity or to increase selectivity. In contrast, nonsymmetrical twin drugs may bind to individual relevant binding sites to provide dual actions.[1] To obtain potential drugs with enhanced efficacy or improved safety, drug discovery campaigns have focused on a strategy designing multitarget drugs (DMLs)[2,3] from the viewpoint of polypharmacology.[4] In the opioid field, many twin drugs have been reported.[5] For example, Portoghese et al. designed nonsymmetrical twin drugs possessing selective agonists and/or antagonists for the opioid receptor type to investigate the heterodimer of the opioid receptor,[6,7] whereas Neumeyer et al. synthesized symmetrical and nonsymmetrical twin drugs for the purpose of seeking potent analgesics with fewer side effects.[8] Recently, we developed a novel synthetic method for attaching three identical or nonidentical pharmacophore units to a single scaffold, the 1,3,5-trioxazatriquinane skeleton, i.e., a "triplet".[9] These triplets exerted interesting pharmacological profiles. Subcutaneous administration of symmetrical triplet KNT-93 with three oxymorphone units or symmetrical twin drug KNT-123 with two oxymorphone units (Figure 1) showed profound antinociceptive effects mediated by the μ opioid receptor (MOR) in a dose-dependent manner.

The antinociception induced by KNT-93 and KNT-123 was 54- and 5-fold more potent, respectively, than that of morphine.[10,11] 4,5-Epoxymorphinan derivative SYK-134 (Figure 1) with the 1,3,5-trioxazatriquinane moiety, which was synthesized as an analog of KNT-93 and -123, showed agonistic activity selective to the KOR, while another derivative SYK-385 (Figure 1) was a selective MOR agonist.[12] It is interesting that SYK-385 exhibited the highest selectivity for the MOR over the KOR among the reported MOR selective nonpeptide ligands.[12] Although these results predicted that the 1,3,5-trioxazatriquinane derivatives would exert novel pharmacological profiles, all the above-mentioned derivatives contained large morphinan units.

We next focused on the 1,3,5-trioxazatriquinane derivatives with simple moieties. The derivatives **1** with aryl groups included the phenethylamine structure, which is a common structure observed not only in endogenous neuropeptides such as enkephalins, dopamine, and adrenaline but also in morphinan skeletons like morphine and naltrexone (Figure 2). Interestingly, when the 3D-alignment of a partial structure of compound **1** (R = *m*-OH) was superimposed onto that of naltrexone, the nitrogen atom, phenyl ring, and phenolic hydroxy group in both compounds were located in very similar positions to each other (Figure 3). This three point pharmacophore is well documented for ligands of opioid receptors.[13,14]

From these observations, we expected the compound **1** (R = *m*-OH) (Figure 2) to interact with the opioid receptor with adequate binding affinity. Herein, we report the synthesis of 1,3,5-trioxazatriquinane derivatives with one to three *m*-hydroxyphenyl groups (**1a-c** (R = *m*-OH), **8a-d**, and **11a,b**). The pharmacological properties of the synthesized derivatives were also evaluated.

Received: February 3, 2014
Accepted: June 26, 2014
Published: June 26, 2014

   dx.doi.org/10.1021/ml5000542 | ACS Med. Chem. Lett. 2014, 5, 868−872

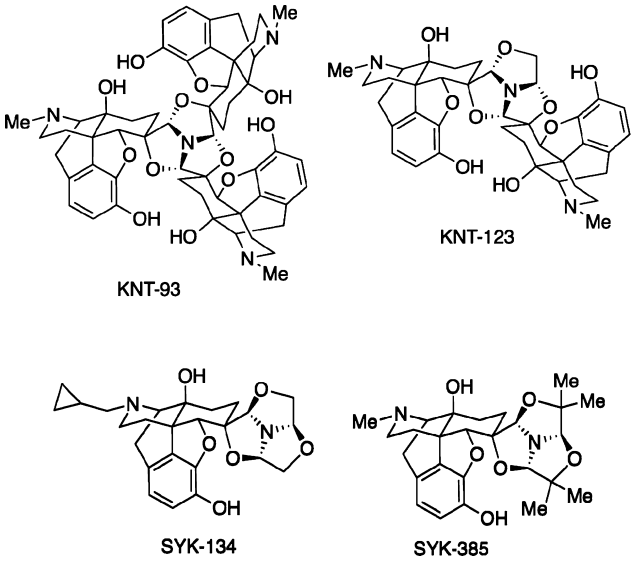


Figure 1. Structures of KNT-93, KNT-123, SYK-134, and SYK-385.

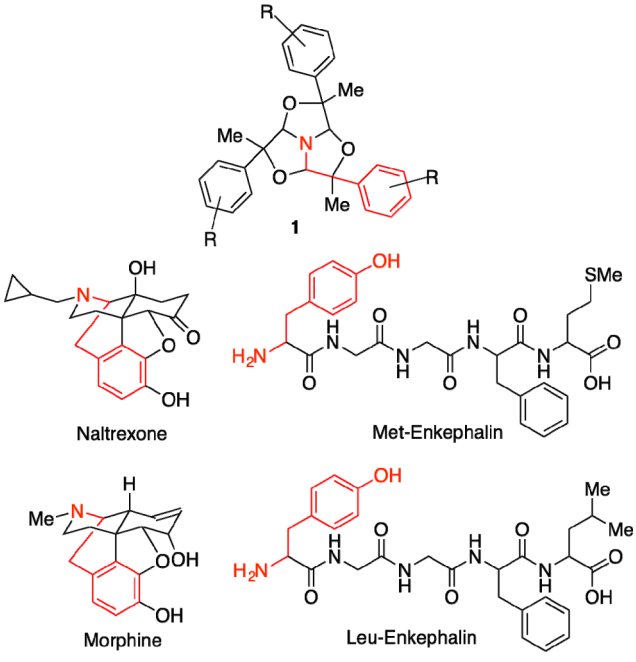


Figure 2. Structures of compounds containing the phenethylamine unit.

According to the previously reported method,[11,12] we prepared compounds **1a**–**c** (R = *m*-OH), **8a**–**d**, and **11a**,**b**. The synthesis of compounds **1a**–**c** (R = *m*-OH) commenced with *m*-methoxyacetophenone (**2**). α-Hydroxyaldehyde **3** and its hemiacetal dimer **4** derived from **2** were treated with ammonium chloride and sodium acetate to give oxazoline **5**. Oxazoline **5** reacted with the mixture of **3** and **4** in the presence of camphorsulfonic acid (CSA) to provide **6a**–**c** (Scheme 1). Compounds **7a**–**d** were prepared by the acidic condensation of **5** with glycolaldehyde dimer (Scheme 2). The key intermediate of the synthesis of **10a**,**b** was oxazoline **9** prepared from glycolaldehyde dimer and the mixture of **3** and **4** (Scheme 3). The *O*-methyl groups of thus prepared **6a**–**c**, **7a**–**d**, and **10a**,**b** were deprotected with boron tribromide or potassium thiolates to afford the corresponding compounds **1a**–**c**, **8a**–**d**, and **11a**,**b**, respectively. The treatment of **7c** with 130 mol % potassium 1-propanethiolate gave **8c**, **12a**, and **12b** (Scheme 4). The relative configurations of all synthesized compounds were determined by 2D-NMR experiments or X-ray crystallography (see the Supporting Information for details).

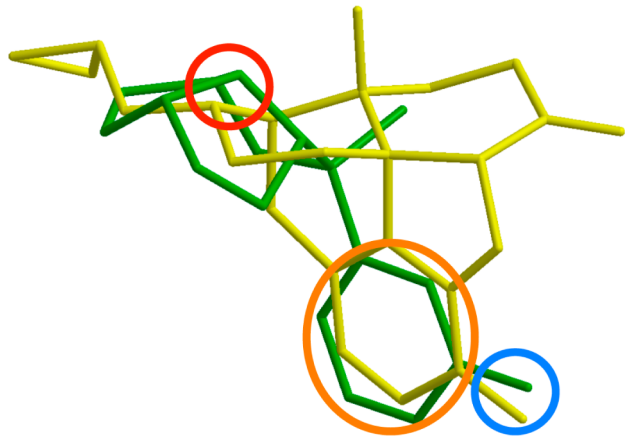


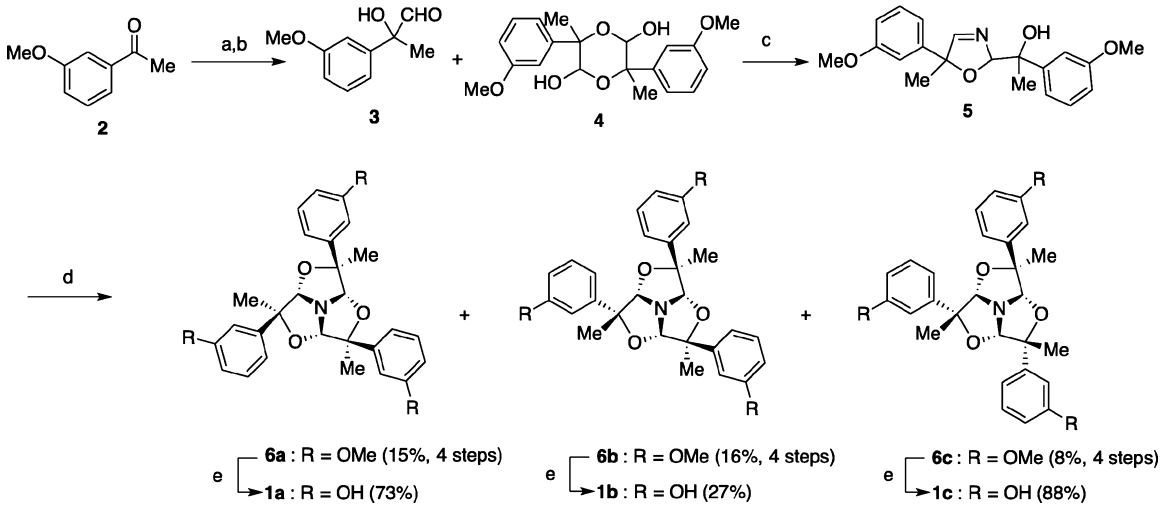
Figure 3. Superimposition of the 3D-alignment of naltrexone (yellow) on that of a 1,3,5-trioxaxatriquinane derivative with a *m*-hydroxyphenyl (green) group. The red circle indicates a nitrogen atom, the orange circle indicates a phenyl ring, and the blue circle indicates a phenolic hydroxy group. Two *m*-hydroxyphenyl groups were omitted from the 1,3,5-trioxazatriquinane derivative **1** (R = *m*-OH) for clarity.

The binding affinities of the prepared 1,3,5-trioxazatriquinanes for the opioid receptors were evaluated with competitive binding assays (Table 1). The assays were performed by a previously reported procedure.[15] Although most of the tested compounds did not bind to the opioid receptors, **1b**, **1c**, and **8a** exhibited submicromolar binding affinities to the MOR or to both the MOR and the KOR.

Surprisingly, compound **8c** strongly bound to the KOR with a K_i value of 6.09 nM. The affinity and selectivity of **8c** were almost the same as those of U-69,593 and U-50,488, known standard KOR agonists. (−)-**8c**[16] showed stronger binding affinity for the KOR with a K_i value of 4.63 nM, whereas its (+)-enantiomer **8c** hardly bound to the KOR. Very recently, Schmidhammer et al. reported phenethylamine derivatives with the *m*-hydroxy group as agonists selective for the KOR.[17] Taken together, this earlier report and our present results suggest that the phenethylamine moiety with the *m*-hydroxyphenyl group would be an important pharmacophore to interact with the opioid receptors. Compounds **1b** and **1c**, which included the structure of **8c**, hardly bound to the KOR. This outcome may result from the steric hindrance of the third aryl moiety. Although compounds **11a** and **11b** with a *m*-hydroxyphenyl group corresponded to partial structures of **8c**, these compounds exhibited no binding affinities, suggesting that both aryl moieties of **8c** would play an indispensable role in binding to the KOR. Both compounds **8b** and **8c** had two aryl groups with similar stereochemistry: one is in an *endo*-position, and the other is in an *exo*-position. However, compound **8c** strongly bound to the KOR, while **8b** did not. These observation indicated that the relationships between the spatial positions of these two aryl groups were important for binding to the KOR. Compound **7c**, which had the *m*-methoxyphenyl instead of *m*-hydroxyphenyl groups, did not bind to the KOR, suggesting that the hydroxy groups would be important structural determinants to achieve binding. Compound **12a** indicated a sufficient binding affinity for the KOR, but

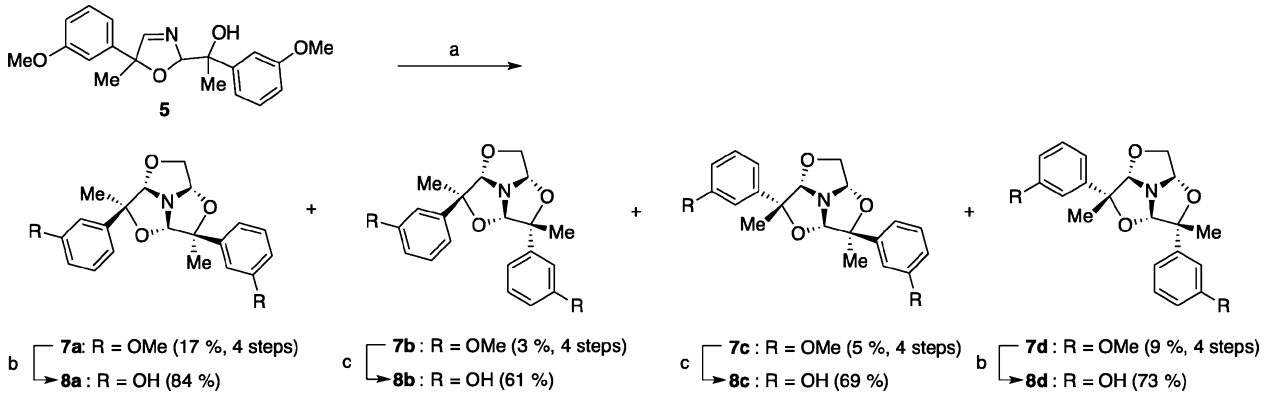
dx.doi.org/10.1021/ml5000542 | ACS Med. Chem. Lett. 2014, 5, 868–872

Scheme 1[a]



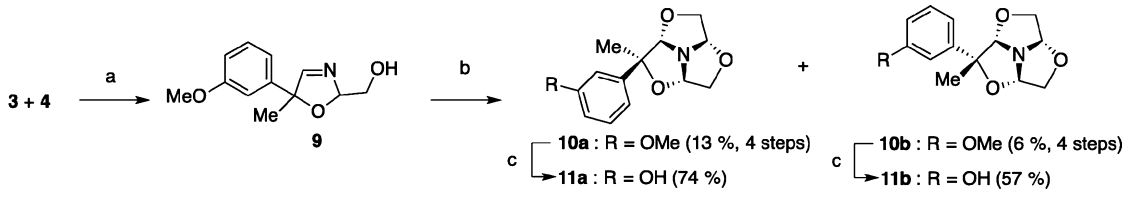
[a]Reagents and conditions: (a) TosMIC, K_2CO_3, MeOH, rt; (b) 2 M HCl aq, THF, rt; (c) NH_4Cl, AcONa, MeOH, reflux; (d) **3**, **4**, CSA, $CHCl_3$, reflux; (e) BBr_3, CH_2Cl_2, 0 °C to rt.

Scheme 2[a]



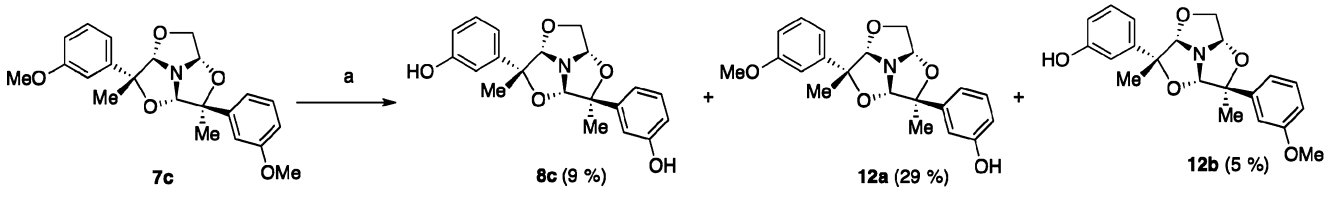
[a]Reagents and conditions: (a) glycolaldehyde dimer, CSA, $CHCl_3$, reflux; (b) 1-dodecanethiol, *t*-BuOK, DMF, 130 °C; (c) 1-propanethiol, *t*-BuOK, DMF, 130 °C.

Scheme 3[a]



[a]Reagents and conditions: (a) glycolaldehyde dimer, NH_4Cl, AcONa, MeOH, rt; (b) glycolaldehyde dimer, CSA, $CHCl_3$, reflux; (c) 1-dodecanethiol, *t*-BuOK, DMF, 130 °C.

Scheme 4[a]



[a]Reagents and conditions: (a) 1-propanethiol, *t*-BuOK, DMF, 130 °C.

dx.doi.org/10.1021/ml5000542 | *ACS Med. Chem. Lett.* 2014, 5, 868−872

Table 1. Binding Affinities of 1,3,5-Trioxazatriquinane Derivatives for Opioid Receptors[a]

	K_i (nM)		
	MOR[b]	DOR[c]	KOR[d]
U-69,593	>1000	>1000	1.37
U-50,488	>1000	>1000	1.67
1a	>1000	>1000	>1000
1b	652	>1000	667
1c	928	>1000	>1000
8a	735	>1000	793
8b	>1000	>1000	>1000
8c	>1000	>1000	6.09
(−)-**8c**	>1000	>1000	4.63
(+)-**8c**	293	651	>1000
8d	>1000	>1000	>1000
11a	>1000	>1000	>1000
11b	>1000	>1000	>1000
7c	>1000	>1000	>1000
12a	>1000	>1000	52.8
12b	>1000	>1000	>1000

[a]Binding assays were carried out in duplicate (KOR: cerebellum of guinea pig; MOR and DOR: whole brain without cerebellum of mouse). [b][^{3}H] DAMGO was used. [c][^{3}H] DPDPE was used. [d][^{3}H] U-69,593 was used.

Table 2. Agonist Activities of 1,3,5-Trioxazatriquinane Derivatives for Opioid Receptors[a]

	EC_{50} (Emax)		
	MOR	DOR	KOR
U-69,593	N.T.	N.T.	12.0 nM (100%)
8c	N.D.	N.D.	97.5 nM (98.2%)
(−)-**8c**	N.D.	83.7 nM (23.4%)	50.3 nM (96.8%)

[a][^{35}S] GTPγS binding assays were carried out in duplicate using human MOR, DOR, or KOR expressed in CHO cells. DAMGO, DPDPE, or U-69,593 was used as the standard MOR, DOR, or KOR agonist, respectively. N.T.: not tested; N.D.: not detected.

compound **12b** did not. The comparison of the binding affinities between **12a** and **12b** indicated the importance of the spatial location required for the *m*-hydroxyphenyl group. According to our initial working hypothesis (Figure 3), the phenolic hydroxy group on the *endo*-aryl group may play a crucial role in binding to the opioid receptor. However, it was not **12b** with the hydroxyl group but rather **12a** with the methoxy group in the *endo*-aryl group that showed sufficient binding to the KOR. This outcome indicated we should restructure our binding model of 1,3,5-trioxazatriquinane derivatives toward the opioid receptors. We are now investigating the binding mode of (−)-**8c** with the KOR using our three-dimensional pharmacophore model applicable to some KOR agonists.[18−20]

The functional activities of **8c** and its eutomer (−)-**8c** were assessed by the [^{35}S]GTPγS binding assays in human receptor transfected CHO cells. Procedures similar to those previously reported[27] were used. Both compounds showed selective full agonist activities for the KOR (Table 2). We next evaluated the analgesic effects of (−)-**8c** by the acetic acid writhing test in mice. Subcutaneous administration of (−)-**8c** dose-dependently exhibited significant antinociception (ED_{50}: 3.5 mg/kg, s.c., Figure 4a). The antinociceptive effects induced by (−)-**8c** were significantly reversed by the selective KOR antagonist nor-BNI

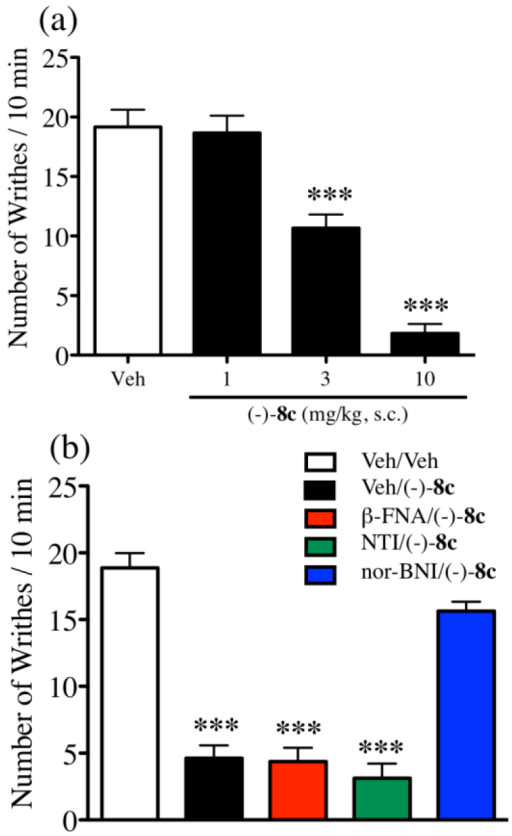


Figure 4. Analgesic effects induced by (−)-**8c** in the mouse acetic acid writhing test. Each mouse was injected intraperitoneally (i.p.) with 0.6% acetic acid at a dose of 10 mL/kg 15 min after (−)-**8c** administration (s.c.). After a 10 min delay, the animals were observed for an additional 10 min, during which the number of abdominal constrictions was counted. (a) Dose response effect; (b) Effects of pretreatment with MOR antagonist β-FNA, DOR antagonist NTI, or KOR antagonist nor-BNI on s.c. (−)-**8c**-induced antinociception in mice. Groups of mice were pretreated with β-FNA[21−25] (40 mg/kg), NTI (3 mg/kg), or nor-BNI[17,26−28] (20 mg/kg) at 24 h, 30 min, or 24 h, respectively, before s.c. administration of (−)-**8c** (7 mg/kg). Each group was comprised of 8 mice. Data are presented as group means ± SEM. Statistical significance of differences was assessed by repeated one-way ANOVA followed by the Bonferroni test. ***$p < 0.001$ vs veh or veh/veh.

but not by the selective MOR antagonist β-FNA or the selective DOR antagonist NTI (Figure 4b).

In summary, we have designed and synthesized the 1,3,5-trioxazatriquinane derivatives possessing *m*-hydroxyphenyl groups. Among the synthesized compounds, (−)-**8c** selectively bound and exerted full agonist activity for the KOR. Subcutaneous administration of (−)-**8c** exhibited significant antinociceptive effects via the KOR in a dose-dependent manner. These results suggest the emergence of a novel class of KOR agonist. We are now investigating the binding mode of (−)-**8c** with the KOR.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for the synthesis and characterization of the compounds, the *in vitro* activity assay, the *in vivo* mouse acetic acid writhing assay, and the spectral data of the reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/ml5000542 | ACS Med. Chem. Lett. 2014, 5, 868−872

AUTHOR INFORMATION

Corresponding Author

*E-mail: nagase.hiroshi.gt@u.tsukuba.ac.jp.

Present Address

[†]H.N.: International Institute for Integrative Sleep Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

CHO, Chinese hamster ovary; CSA, camphorsulfonic acid; DAMGO, [D-Ala^2, *N*-Me-Phe^4, Gly-ol^5]-enkephalin; DOR, δ opioid receptor; DPDPE, [D-Pen^2, D-Pen^5]-enkephalin; β-FNA, β-funaltrexamine; KOR, κ opioid receptor; MOR, μ opioid receptor; nor-BNI, nor-binaltorphimine; NTI, naltrindole; TosMIC, *p*-toluenesulfonylmethyl isocyanide

REFERENCES

(1) Contreras, J. M.; Sippl, W. Homo and Heterodimer Ligands: the Twin Drug Approach. In *The Practice of Medicinal Chemistry*, 3rd ed.; Wermuth, C. G., Ed.; Academic Press: London, 2008; Chapter 18.

(2) Morphy, R.; Rankovic, Z. Multi-target Drugs: Strategies and Challenges for Medicinal Chemistry. In *The Practice of Medicinal Chemistry*, 3rd ed.; Wermuth, C. G., Ed.; Academic Press: London, 2008; Chapter 27.

(3) *Designing Multi-Target Drugs*; Morphy, J. R., Harris, C. J., Eds.; The Royal Society of Chemistry: Cambridge, 2012.

(4) *Polypharmacology in Drug Discovery*; Peters, J. U., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2012.

(5) Fujii, H. Twin and triplet drugs in opioid research. *Top. Curr. Chem.* **2011**, *299*, 239−275 and references cited therein..

(6) Bhushan, R. G.; Sharma, S. K.; Xie, Z.; Daniels, D. J.; Portoghese, P. S. A bivalent ligand (KDN-21) reveals spinal δ and κ opioid receptors are organized as heterodimers that give rise to δ_1 and κ_2 phenotypes. selective targeting of δ-κ heterodimers. *J. Med. Chem.* **2004**, *47*, 2969−2972.

(7) Daniels, D. J.; Lenard, N. R.; Etienne, C. L.; Law, P. Y.; Roerig, S. C.; Portoghese, P. S. Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 19208−19213.

(8) Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. Synthesis and preliminary in vitro investigation of bivalent ligands containing homo- and heterodimeric pharmacophores at μ, δ, and κ opioid receptors. *J. Med. Chem.* **2006**, *49*, 256−262.

(9) Nagase, H.; Watanabe, A.; Harada, M.; Nakajima, M.; Hasebe, K.; Mochizuki, H.; Yoza, K.; Fujii, H. Novel synthesis of a 1,3,5-trioxazatriquinane skeleton using a nitorogen clamp. *Org. Lett.* **2009**, *11*, 539−542.

(10) Nagase, H.; Watanabe, A.; Nemoto, T.; Nakajima, M.; Hasebe, K.; Mochizuki, H.; Fujii, H. Synthesis of novel triplet drugs with 1,3,5-trioxazatriquinane skeletons and their pharmacologies. 1: Synthesis of triplet drugs with morphinan skeletons. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4023−4026.

(11) Nagase, H.; Koyano, K.; Wada, N.; Hirayama, S.; Watanabe, A.; Nemoto, T.; Nakajima, M.; Nakao, K.; Mochizuki, H.; Fujii, H. Synthesis of novel triplet drugs with 1,3,5-trioxazatriquinane skeletons and their pharmacologies. 2: Synthesis of novel triplet drugs with the epoxymethano structure (capped homotriplet). *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6198−6202.

(12) Wada, N.; Fujii, H.; Koyano, K.; Hirayama, S.; Iwai, T.; Nemoto, T.; Nagase, H. Synthesis of novel triplet drugs with 1,3,5-trioxazatriquinane skeletons and their pharmacologies. 3: Synthesis of novel triplet drugs with the bis(epoxymethano) or bis-(dimethylepoxymethano) structure (double-capped triplet). *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7551−7554.

(13) Beckett, A. H.; Casy, A. F. Synthetic analgesics: stereochemical considerations. *J. Pharm. Pharmacol.* **1954**, *6*, 986−1001.

(14) Beckett, A. H. Analgesics and their antagonists: some steric and chemical considerations. I. The dissociation constants of some tertiary amines and synthetic analgesics, the conformations of methadone-type compounds. *Pharm. Pharmacol.* **1956**, *8*, 848−859.

(15) Ida, Y.; Nemoto, T.; Hirayama, S.; Fujii, H.; Osa, Y.; Imai, M.; Nakamura, T.; Kanemasa, T.; Kato, A.; Nagase, H. Synthesis of quinolinomorphinan-4-ol derivatives as δ opioid receptor agonists. *Bioorg. Med. Chem.* **2012**, *20*, 949−961.

(16) The optical resolution of racemic **8c** and the determination of the absolute configuration of each enantiomer are described in detail in the Supporting Information.

(17) Spetea, M.; Berzetei-Gurske, I. P.; Guerrieri, E.; Schmidhammer, H. Discovery and pharmacological evaluation of a diphenethylamine derivative (HS665), a highly potent and selective κ opioid receptor agonist. *J. Med. Chem.* **2012**, *55*, 10302−10306.

(18) Yamaotsu, N.; Fujii, H.; Nagase, H.; Hirono, S. Identification of the three-dimensional pharmacophore of κ-opioid receptor agonists. *Bioorg. Med. Chem.* **2010**, *18*, 4446−4452.

(19) Yamaotsu, N.; Hirono, S. 3D-pharmacophore identification for κ-opioid agonists using ligand-based drug-design techniques. *Top. Curr. Chem.* **2011**, *299*, 277−307.

(20) According to our three-dimensional pharmacophore model of KOR agonists (see refs 18 and 19), the binding modes were classified into four types. (−)-**8c** may belong to the binding mode type IV, which differs from type II as indicated in Figure 3 and includes both aromatic and hydrogen-bonding accepting and/or donating interactions.

(21) We determined the dosage and administration time of β-FNA in accordance with refs 22−25.

(22) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. The irreversible narcotic antagonistic and reversible agonistic properties of the fumaramate methyl ester derivative of naltrexone. *Eur. J. Pharmacol.* **1981**, *70*, 445−451.

(23) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. Pharmacological profiles of β-funaltrexamine (β-FNA) and β-chlornaltrexamine (β-CAN) on the mouse vas deferens preparation. *Eur. J. Pharmacol.* **1982**, *80*, 377−384.

(24) Hayes, A. G.; Skingle, M.; Tyers, M. B. Effect of β-funaltrexamine on opioid side-effects produced by morphine and U-50,488H. *J. Pharm. Pharmacol.* **1985**, *37*, 841−843.

(25) Aceto, M. D.; Dewey, W. L.; Portoghese, P. S.; Takemori, A. E. Effects of β-funaltrexamine (β-FNA) on morphine dependence in rats and monkeys. *Eur. J. Pharmacol.* **1986**, *123*, 387−393.

(26) We determined dosage and administration time of nor-BNI in accordance with refs 17 and 27.

(27) Endoh, T.; Matsuura, H.; Tanaka, C.; Nagase, H. Nor-binaltorphimine: A potent and selective κ-opioid receptor antagonist with long-lasting activity *in vivo*. *Arch. Int. Pharmacodyn. Ther.* **1992**, *316*, 30−42.

(28) Nemoto, T.; Fujii, H.; Narita, M.; Miyoshi, K.; Nakamura, A.; Suzuki, T.; Nagase, H. Syntheses of 4,6′-epoxymorphinan derivatives and their pharmacologies. *Bioorg. Med. Chem.* **2008**, *16*, 4304−4312.