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

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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

win drugs consisting of two pharmacophore units in a I 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.

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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



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.

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).

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. (-)-8c¹⁶ showed stronger binding affinity for the KOR with a K, 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 mhydroxyphenyl 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 mhydroxyphenyl 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 Scheme 1^a



"Reagents and conditions: (a) TosMIC, K₂CO₃, MeOH, rt; (b) 2 M HCl aq, THF, rt; (c) NH₄Cl, AcONa, MeOH, reflux; (d) **3**, **4**, CSA, CHCl₃, reflux; (e) BBr₃, CH₂Cl₂, 0 °C to rt.



"Reagents and conditions: (a) glycolaldehyde dimer, CSA, CHCl₃, reflux; (b) 1-dodecanethiol, *t*-BuOK, DMF, 130 °C; (c) 1-propanethiol, *t*-BuOK, DMF, 130 °C.

Scheme 3^{*a*}



"Reagents and conditions: (a) glycolaldehyde dimer, NH₄Cl, AcONa, MeOH, rt; (b) glycolaldehyde dimer, CSA, CHCl₃, reflux; (c) 1-dodecanethiol, t-BuOK, DMF, 130 $^{\circ}$ C.

Scheme 4^{*a*}



^aReagents and conditions: (a) 1-propanethiol, t-BuOK, DMF, 130 °C.

Table 1. Bi	nding Affinities	of 1,3,5-Trioxa	zatriquinane
Derivatives	for Opioid Rec	ceptors ^a	

	$K_i (\mathrm{nM})$		
	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

^aBinding assays were carried out in duplicate (KOR: cerebellum of guinea pig; MOR and DOR: whole brain without cerebellum of mouse). $b^{5}_{3}H$] 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%)	

 $a[^{35}S]$ GTP γS 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 $[^{35}S]GTP\gamma S$ binding assays in human receptor transfected CHO cells. Procedures similar to those previously reported²⁷ 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 (ED₅₀: 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 -FNA²¹⁻²⁵ (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 \pm 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 1,3,5trioxazatriquinane 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.

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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-Ala², N-Me-Phe⁴, Gly-ol⁵]-enkephalin; DOR, δ opioid receptor; DPDPE, [D-Pen², D-Pen⁵]-enkephalin; β -FNA, β -funaltrexamine; KOR, κ opioid receptor; MOR, μ opioid receptor; nor-BNI, nor-binaltorphimine; NTI, naltrindole; TosMIC, *p*-toluenesulfonylmethyl isocyanide

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