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Letter

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Discovery of δ Opioid Receptor Full Inverse Agonists and Their Effects on Restraint Stress-induced Cognitive Impairment in Mice

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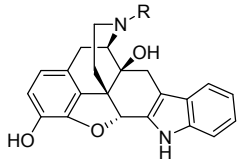
KEYWORDS: δ Opioid receptor, DOR, inverse agonist, constitutive activity, cognitive impairment

ABSTRACT: The cyclopropylmethyl group in classical δ opioid receptor (DOR) antagonist NTI, BNTX, and NTB was replaced with various electron-withdrawing groups to develop DOR inverse agonists. *N*-Benzyl NTB derivative SYK-657 was a potent DOR full inverse agonist and its potency was over 10-fold potent than that of a reference compound ICI-174,864. Intraperitoneal administration of SYK-657 induced the short-term memory improving effect in mice without abnormal behaviors.

G-Protein coupled receptors (GPCRs) are attractive and important drug targets, and over 25% of all approved drugs are known to provide their pharmaceutical effects through GPCRs.[1] The compounds interacting with GPCRs were classically divided into two classes: agonists with positive intrinsic activity and antagonists with no intrinsic activity. However, after discovering the constitutive activity of GPCRs, inverse agonists emerged and are characterized by having negative intrinsic activity. Many investigations on the constitutive activity of GPCRs have been undertaken and various inverse agonists have been reported.[2] The clinical relevance of inverse agonists is also being discussed.[2] Since the peptidic δ opioid receptor (DOR) inverse agonist ICI-174,864 was discovered by Costa and Herz,[3] which was a pioneering work in the inverse agonism field, several peptidic[4,5a] and non-peptidic[5] DOR inverse agonists have been reported. We also reported some 4,5-epoxymorphinan derivatives with DOR inverse agonistic properties (Figure 1).[6] The *N*-substituent in the selective DOR antagonist naltrindole (NTI)[7] was converted from the cyclopropylmethyl (CPM) group into the fluorinated ethyl group to afford an interesting change of the functional activity: conversion from an antagonist into an inverse agonist (Figure 1). It is well-known that the *N*-substituent in the morphinan derivatives dramatically affects their agonist/antagonist properties, but this is the first example that the transformation of the *N*-substituent in morphinan derivatives affords the inverse agonist. Although various DOR inverse



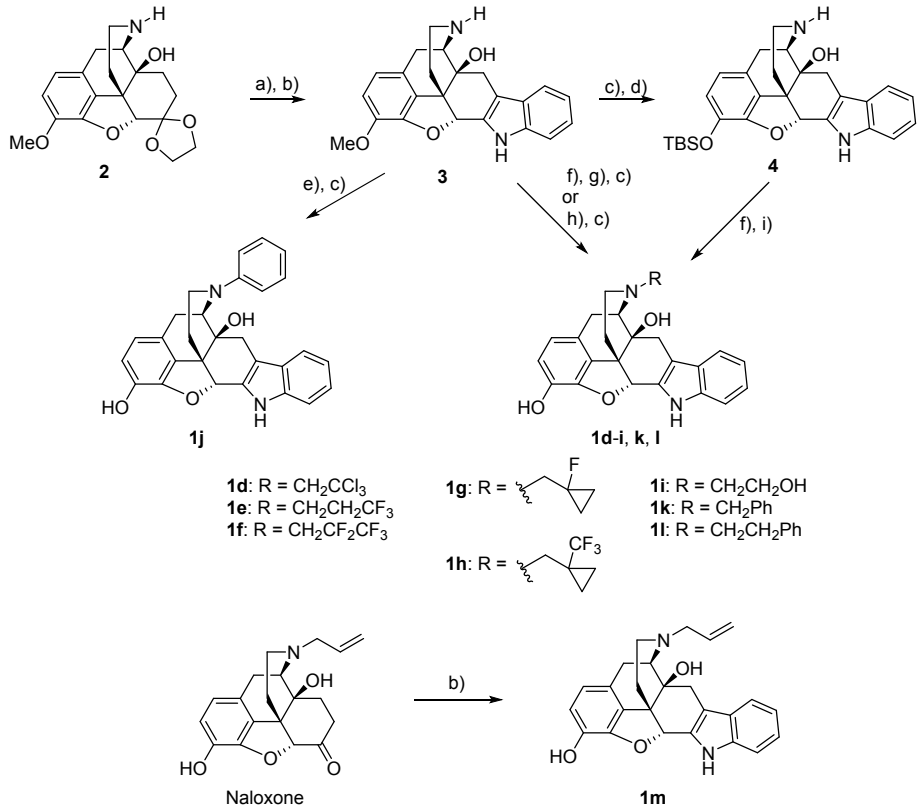
R = CPM (NTI): DOR antagonist
R = CH_2CF_3 (**1a**): DOR partial inverse agonist
R = CH_2CHF_2 (**1b**): DOR partial inverse agonist
R = CH_2CH_2F (**1c**): DOR partial inverse agonist

Figure 1. Structures of NTI and its derivatives with *N*-fluorinated ethyl group.

agonists have been reported,[4,5] there have been almost no investigation on *in vivo* pharmacological effects induced by a DOR inverse agonist. To the best of our knowledge, only Lilly's research group reported that the non-peptidic DOR inverse agonist LY255582 induced anorectic activity.[5d,8] However, the same research group later concluded that the anorexic effects produced by LY255582 resulted from a combination of μ (MOR), DOR, and κ opioid receptor (KOR) activities based on the examination of the binding of [^{3}H]LY255582 in mouse brain.[9] Therefore, the *in vivo* pharmacological effects by a DOR inverse agonist, and perhaps the physiological role in the constitutive activity of the DOR, remain unclear. To investigate the influence of the *N*-substituent on the DOR

inverse agonistic activity, we introduced various electron-withdrawing groups as the *N*-substituents in DOR antagonists with the morphinan skeleton like NTI because the fluorinated ethyl

Scheme 1. Synthesis of NTI derivatives[a]



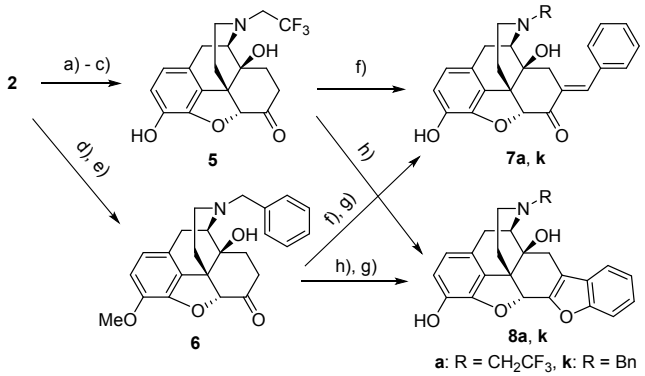
[a]Reagents and conditions: (a) 2 M HCl, MeOH, reflux; (b) $PhNHNH_2$·HCl, AcOH, reflux; (c) 1 M BBr_3, CH_2Cl_2, -10 °C; (d) TBSCl, imidazole, DMF, rt; (e) 2-(trimethylsilyl)phenyl trifluoromethanesulfonate, TBAF, THF, -40 °C; (f) trichloroacetic anhydride, CH_2Cl_2, rt, or R'CO_2H, EDCI·HCl, DMAP, CH_2Cl_2, rt; (g) BH_3·THF, THF, reflux; (h) RBr, K_2CO_3, DMF, rt; (i) BH_3·THF, THF, reflux, then 4 M HCl, rt. R = CH_2R'.

group is one of the representative electron-withdrawing groups. Previous reports suggested that the δ opioidergic neurons may be relevant to learning memory.[10,11] Therefore, using a DOR full inverse agonist obtained in this earlier research, we investigated *in vivo* pharmacological effects induced by a DOR inverse agonist to determine if such an agent could induce a short-term memory improving effect in mice. Herein, we report the synthesis of DOR antagonist derivatives bearing an electron-withdrawing group as the *N*-substituent to obtain some DOR full inverse agonists. The short-term memory improvement effect by a newly developed DOR full inverse agonist is also described.

RESULTS AND DISCUSSION

Chemical Synthesis NTI Derivatives with various *N*-substituents have been synthesized from noroxymorphone.[12] However, we chose the other synthetic routes delineated in scheme 1 because noroxymorphone is hardly available in our country. The synthesis of NTI derivatives **1d–l** with electron-withdrawing groups as the *N*-substituent commenced with compound **2**[13] (Scheme 1). The treatment of compound **2** with hydrochloric acid and subsequent Fischer indolization gave compound **3**. The objective compounds were prepared by two routes. In a route, the *N*-substituent was introduced by *N*-alkylation with the corresponding alkyl bromides or amidation with the corresponding carboxylic acid or acid anhydride

Scheme 2. Synthesis of BNTX and NTB derivatives[a]



[a]Reagents and conditions: (a) FTAA, CH_2Cl_2, rt; (b) BH_3·THF, THF, reflux; (c) 48% HBr, reflux; (d) BnBr, K_2CO_3, DMF, rt; (e) 2 M HCl, MeOH, reflux; (f) $PhCO_2H$, PhCHO, *i*-Pr_2EtN, Tol/DMF = 5/1, 140 °C; (g) 1 M BBr_3, CH_2Cl_2, -10 °C; (h) $PhONH_2$·HCl, $MeSO_3H$, EtOH, reflux.

following reduction of the intermediate amides with borane. Introduction of a phenyl group (compound **1j**) was achieved by using benzyne chemistry. Although Larock's reaction conditions[14] furnished 87% yield, our modified reaction conditions improved the yield (98%). The *O*-methyl group was deprotected with boron tribromide. In the alternative route,

Table 1. Binding Affinities and Selectivities of Synthesized Compounds **1a–m**, **7a**, **7k**, **8a**, **8k**, and Parent DOR Antagonists NTI, BNTX, and BNT for the Opioid Receptors[a] and Their Functional Activities for the DOR[b]

Compd	R	Opioid Receptor Binding					Functional Activity for DOR	
		Affinity K_i, nM (95% CI)			Selectivity			
		DOR	MOR	KOR	MOR/DOR	KOR/DOR	EC_{50} nM (95% CI)	E_{max} (%)
DPDPE[c]	–	N.T.[e]	N.T.[e]	N.T.[e]	N.T.[e]	N.T.[e]	4.66 (2.08-10.4)	100[c]
ICI-174,864[d]	–	422 (215-829)	N.T.[e]	N.T.[e]	N.T.[e].	N.T.[e]	114 (67.9-192)	-100[d]
NTI	CPM	0.46 (0.192-1.09)	30.7 (12.5-75.4)	14.7 (3.16-68.5)	66.7	32.0	N.D.[f] pA2 = 10.1 (9.39-10.8)	7.5[g]
1a[h]	CH_2CF_3	134 (75.4-239)	>1,000	>1,000	N.C.[i]	N.C.[i]	45.5 (17.5-118)	-44.8
1b[h]	CH_2CHF_2	15.5 (9.14-26.4)	>1,000	>1,000	N.C.[i]	N.C.[i]	17.2 (7.49-39.7)	-48.6
1c[h]	CH_2CH_2F	1.94 (1.29-2.93)	675 (373-1221)	397 (172-913)	348	204	220 (54.6- 885)	-38.1
1d	$CH_2CH_2CF_3$	40.2 (18.2-88.6)	1,050 (456-2,426)	1,490 (938-2,353)	26.1	37.1	40.3 (6.75- 241)	19.3
1e	$CH_2CF_2CF_3$	484 (154-1,517)	31,400 (4,380-224,905)	2,090 (1,137-3,845)	64.9	4.32	86.0 (3.33-2,222)	21.1
1f	CH_2CCl_3	40.4 (19.0-85.5)	1,850 (513-6,677)	1,340 (671-2,655)	45.8	33.2	N.D.[f] pA2 = 7.84 (7.14-8.53)	12.2[g]
1g	CH_2(1-F-*c*-C_3H_4)	1.11	460	34.8	414	31.4	8.67	22.9

		(0.697-1.76)	(233-910)	(23.5-51.4)			(0.295-255)	
1h	CH_2(1-(CF_3)-*c*-C_3H_4)	7.47 (4.69-11.9)	3,440 (1,356-8,724)	1,090 (744-1,594)	461	146	67.6 (9.43-484)	26.8
1i	CH_2CH_2OH	10.2 (6.08-17.2)	998 (606-1,644)	714 (464-1,098)	97.8	70.0	7.37 (0.424-128)	21.8
1j	Ph	25.5 (17.7-36.6)	1,260 (609-2,596)	825 (593-1,148)	49.4	32.4	N.D.[f] pA2 = 7.72 (7.07-8.38)	4.11[g]
1k	Bn	15.8 (7.23-34.7)	1,160 (605-2,213)	615 (443-853)	73.4	38.9	16.1 (7.31-35.4)	-56.5
1l	CH_2CH_2Ph	1.94 (1.50-2.50)	175 (114-267)	775 (637-943)	90.2	399	15.1 (2.67-85.0)	40.3
1m	allyl	0.61 (0.407-0.913)	182 (121-275)	181 (134-246)	299	297	101 (4.46-2,278)	16.2
BNTX	CPM	3.53 (2.38-5.23)	10.5 (6.61-16.8)	28.3 (22.5-35.7)	2.99	8.03	N.D.[f] pA2 = 9.47 (6.87-12.1)	-10.2[g]
7a	CH_2CF_3	1,680 (578-4,885)	1,750 (914-3,353)	7,450 (4,666-11,880)	1.04	4.43	94.7 (29.7-302)	-48.5
7k (SYK-656)	Bn	444 (263-751)	643 (459-901)	919 (582-1,452)	1.45	2.07	225 (132-383)	-103
NTB	CPM	0.603 (0.384-0.946)	31.0 (19.0-50.5)	30.4 (23.3-39.7)	51.5	50.4	N.D.[f] pA2 = 9.54 (8.68-10.4)	-1.6[g]
8a	CH_2CF_3	128 (82.5-199)	5,820 (3,449-9,825)	5,630 (3,664-8,638)	45.5	44.0	37.3 (9.38-148)	-59.7
8k (SYK-657)	Bn	177	3,930	1,740	22.2	9.82	10.6	-99.0

		(93.8-334)	(2,122-7,291)	(1,175-2,574)			(7.16-15.6)	

[a]Evaluated by ability of each compound to displace [^{3}H]DAMGO (MOR), [^{3}H]DPDPE (DOR), or [^{3}H]U-69,593 (KOR) binding to the CHO cells expressing human MOR, DOR, or KOR. The data represent means of three samples. [b]Membranes were incubated with [^{35}S]GTPγS and GDP with the compound. The δ human recombinant cell membrane (CHO) was used in this assay. The data represent means of four samples. [c]DPDPE (1 μM) was used as the standard DOR agonist. [d]ICI-174,864 (10 μM) was used as the standard DOR inverse agonist. [e]Not tested. [f]Not determined. [g]% Stimulation at 10 μM of the tested compound. [h]Reference 6. [i]Not calculated because K_i values for MOR or KOR were over 1,000 nM. 95% CI: 95% confidence interval.

the protecting group in the phenolic hydroxy group was changed from methyl into *t*-butyldimethylsilyl group. After introducing the *N*-substituent by a two step method (amidation and reduction), the silyl group was removed by acidic conditions. Allyl derivative **1m** was prepared from naloxone by Fisher indolization. We applied some *N*-substituents, which provided DOR inverse agonist activity mentioned below, to the other DOR antagonist 7-benzylidenenaltrexone (BNTX)[15] and naltriben (NTB)[16] having structures that differed from NTI. The synthesis of BNTX and NTB derivatives are shown in Scheme 2. 2,2,2-Trifluoroethyl and benzyl groups were introduced in compound **2** by previously reported methods[17] and subsequently treated with 48% hydrobromic acid and 2 M hydrochloric acid to give compounds **5** and **6**, respectively. According to the reported methods,[16,18] BNTX and NTB derivatives **7** and **8** were obtained from respective compounds **5** and **6**. Finally, all the objective compounds were converted to the hydrochloride or camphorsulfonate for pharmacological evaluation of the compounds.

Binding affinity and functional activity The binding affinities of the prepared compounds **1d–m**, **7a**, **7k**, **8a**, and **8k** were evaluated by competitive binding assays according to the previously reported methods[19] (Table 1). For the purpose of comparison, the results of the parent DOR antagonists NTI, BNTX, and NTB are also shown. All the NTI derivatives **1** except **1a** and **1e** strongly bound to the DOR with K_i values of double digit or lower nanomolar range and most derivatives showed selectivity for the DOR comparable to or higher than that of the parent compound NTI. These observations agreed with the previously reported results.[6] Compared to the NTI derivatives **1**, the binding affinities and selectivities for the DOR of BNTX derivatives **7** and NTB **8** variants were poorer.

We next assessed the functional activities of the compounds **1d–m**, **7a**, **7k**, **8a**, and **8k** for the DOR by [^{35}S]GTPγS binding assays according to the previously reported methods[19] (Table 1), and we compared the results to those obtained with the parent DOR antagonists NTI, BNTX, and NTB. The E_{max} values were calculated using the maximum response of reference compounds DPDPE and ICI-174,864 for the compounds with the positive and negative intrinsic activities, respectively. Concerning NTI derivatives **1**, only the *N*-benzyl derivative **1k** showed inverse agonistic activity with the E_{max} value of -56.5%, which was comparable to a little higher efficacy compared with those of *N*-trifluoroethyl and *N*-difluoroethyl derivatives **1a** and **1b**. Compounds **1f** and **1j** were DOR antagonists. Other derivatives, except for **1f**, **1j**, and **1l,** were agonists with almost no or low efficacy. *N*-Phenethyl derivative **1l** exhibited partial agonistic activity with a moderate efficacy (E_{max} = 40.3%), while **1l** was reported to be a full agonist in the electrically stimulated mouse vas deferens (MVD) assays.[20] Whereas *N*-allyl derivative **1m** was a partial agonist with a low efficacy (E_{max} = 16.2%), **1m** showed antagonist property in the MVD assays.[7b] These differences of the observed efficacies would stem from the distinct assay system. These results suggested that the replacement of CPM with some electron-withdrawing groups would convert the compound's property from an antagonist into an inverse agonist. The length between the nitrogen atom and fluorine atom or phenyl group may be an important structural determinant to exert inverse agonistic activities (**1a-c** vs. **1d**, **1k** vs. **1j** and **1l**). However, *N*-trifluoroethyl derivative **1a** was an inverse agonist, while *N*-trichloroethyl derivative **1f** was an antagonist. Although *N*-pentafluoropropyl derivative **1e** shared the same partial structure as *N*-trifluoroethyl derivative **1a**, **1e** was not an inverse agonist. Moreover, although the length between the fluorine and nitrogen atom in **1g** was the same as those in **1a-c**, which were inverse agonists, *N*-(1-fluorocyclopropyl)methyl derivative **1g** did not exert any inverse agonistic activity. These observations were interesting, but it is now difficult to rationally explain this phenomenon. The conformational distinction among the *N*-substituents might be also an important determinant. Among the tested substituents, only the fluorinated ethyl and benzyl groups afforded inverse agonist properties. Therefore, we applied these replacement of substituents to other DOR antagonists BNTX and NTB with chemotypes different from that of NTI. Fascinatingly, all the resulting compounds **7a**, **7k**, **8a**, and **8k** showed inverse agonism. *N*-Trifluoroethyl derivatives **7a** and **8a** were partial inverse agonists, while *N*-benzyl derivatives **7k** (SYK-656) and **8k** (SYK-657) were full inverse agonists. Particularly, SYK-657 (**8k**) was very potent (EC_{50} = 10.6 nM) and exhibited a greater than 10-fold more potent inverse agonistic activity than did the reference compound ICI-174,864. Unfortunately, a clear relationship between their inverse agonistic activities and binding affinities for the DOR was not observed.[21]

The Effects on Restraint Stress-Induced Cognitive Impairment With a potent DOR full inverse agonist (SYK-657) in hand, we sought to define the *in vivo* pharmacological effects induced by such an inverse agonist. It was previously reported that learning memory was impaired by DOR agonists.[10] In addition, preproenkephalin knockout mice, which cannot produce the endogenous DOR agonists enkephalins, were resistant to chronic stress, which induces memory impairment.[11] Thus, we evaluated the influence of SYK-657, which had almost no activities toward the MOR and KOR,[22] on repeated restraint stress-induced memory

impairment. Short-term memory performance was assessed by recording spontaneous alternation behavior in a Y-maze. The restraint stress caused cognitive impairment. Intraperitoneal administration of SYK-657 significantly increased % alteration, which meant that the short-term memory was improved. SYK-657 did not affect total arm entries, which are indices of movement (Figure 2). Moreover, no abnormal behaviors in mice injected with SYK-657 were observed in the open-field test (Figure S1, Supporting

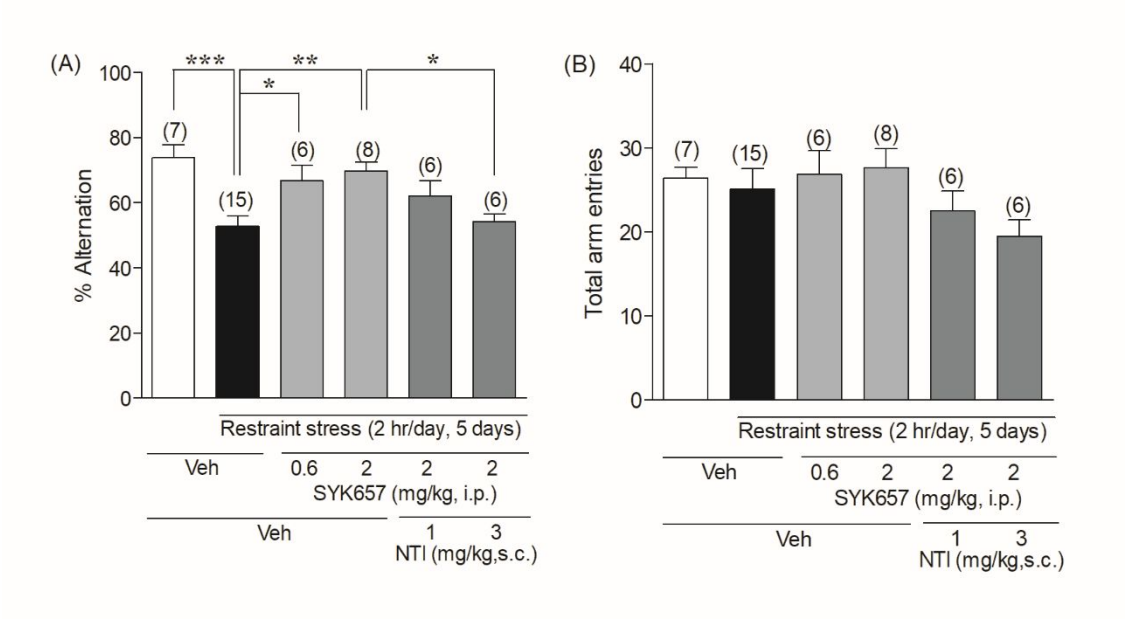


Figure 2. The effects of SYK-657 camphorsulfonate on restraint stress-induced cognitive impairment in mice.

The short-term memory performance was assessed using the Y-maze test. (A) The effects on % alteration. % Alteration was calculated by the following equation: % alternation = {(number of alternations)/(total number of arm entries - 2)}×100 (%). (B) The effects on the total entries. The numbers in parentheses show the sample size. *p<0.05, **p<0.01, ***p<0.001 (One-way analysis of variance followed by Bonferroni's multiple comparison test)

Information). The short-term memory improving effect by SYK-657 was reversed by the DOR antagonist NTI in a dose-dependent manner. NTI itself had no effects on the learning memory (Figure S2, Supporting Information). This result strongly indicated that the short-term memory improving effect by SYK-657 was mediated by the DOR. Although these outcomes suggested that the restraint stress might shift the receptor equilibrium between active and inactive forms toward the active form to provide higher constitutive activity of the DOR, further investigation would be required.

In conclusion, based on our previous results that *N*-fluorinated ethyl NTI derivatives **1a-c** showed DOR inverse agonistic activities, we designed and synthesized some NTI derivatives **1d-m** with various electron-withdrawing groups as *N*-substituents to find *N*-benzyl derivative **1k** as a DOR partial inverse agonist. Such transformation enabling the conversion of a compound's functional activity from an antagonist into an inverse agonist was applicable to other DOR antagonists BNTX and NTB having structures different from that of NTI. *N*-Trifluoroethyl derivatives **7a** and **8a** were DOR partial inverse agonists, while *N*-benzyl derivatives SYK-656 (**7k**) and SYK-657 (**8k**) showed DOR full inverse agonistic activities. The potency of SYK-657 was over 10-fold greater than that of a reference compound ICI-174,864. SYK-657 administered intraperitoneally induced a short-term memory improving effect in mice without abnormal behaviors. Its effect was blocked by DOR antagonist NTI. SYK-675 is expected to be a useful probe to explore pharmacology mediated by the DOR, especially constitutively active DOR.

METHODS

17-Benzyl-6,7-didehydro-4,5α-epoxy-benzofurano[2',3':6,7]morphinan-3,14β-diol (**8k**) camphorsulfonate (SYK-657 camphorsulfonate). Under Ar, to a solution of SYK-657-3*O*-Me ether (**S16**, Supprting Information)(107 mg, 0.23 mmol) in CH_2Cl_2 (4 mL) solution was added 1.0 M solution of BBr3 in CH_2Cl_2 (1.4 mL, 1.4 mmol) at -10 ºC and stirred at the same temperature for 1 hr. To the reaction mixture was added 25% ammonia solution and stirred for 30 min, and then extracted with $CHCl_3$. Combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. After removing the solvent *in vacuo*, the obtained crude product was purified by silica gel chromatography to give SYK-657 (**8k**) (53 mg, 52%) as a colorless oil;. IR (neat) cm^{-1}: 3303, 2924, 2832, 1615, 1453, 1218, 1117, 747. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.57 (br d, J = 11.2 Hz, 1H), 2.21 (ddd, J = 3.1, 11.8, 11.8 Hz, 1H), 2.33 (ddd, J = 4.6, 12.4, 12.4 Hz, 1H), 2.40 (d, J = 16.3 Hz, 1H), 2.46-2.55 (m, 1H), 2.61 (d, J = 16.3 Hz, 1H), 2.72 (dd, J = 6.3, 18.7 Hz, 1H), 3.00 (d, J = 6.3 Hz, 1H), 3.24 (d, J = 18.7 Hz, 1H), 3.68 (s, 2H), 4.79 (br s, 1H), 5.53 (s, 1H), 6.53 (d, J = 8.1 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1H), 7.16-7.21 (m, 1H), 7.22-7.36 (m, 4H), 7.38-7.47 (m, 3H), 7.54 (d, J = 8.3 Hz, 1H), 9.14 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.1, 28.7, 31.1, 43.2, 48.9, 59.1, 62.1, 72.9, 84.4, 111.5, 116.0, 117.3, 119.2, 129.7, 122.4, 124.8, 124.9, 127.5, 127.6, 128.6, 129.0, 130.0, 137.6, 139.1, 142.9, 147.6, 155.5. HR-MS (ESI): Calcd for $C_{29}H_{26}NO_4[M+H]^+$: 452.1862. Found: 452.1873.

To a solution of SYK-657 (**8k**) in MeOH was added (-)-10-camphorsulfonic acid at 0 ºC to give SYK-657 camphorsulfonate; mp (dec.): 241.8-243.2 ˚C. Anal. Calcd for $C_{29}H_{25}NO_4 \cdot CSA \cdot 0.8H_2O$: C, 67.09; H, 6.15; N, 2.01. Found: C, 67.05; H, 6.24; N, 2.00.

Evaluation of effects on restraint stress-induced cognitive impairment. All of the experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Kitasato University, and were carried out in accordance with the guidelines of the National Institutes of Health and the Japanese Pharmacological Society. ddY (5-week, 24-29 g) Male mice (Japan SLC Inc., Shizuoka, Japan) were used. The detailed procedures for habituation and Y-maze test were described in the Supporting Information. The mice were subjected to 5 days (day-1 to -5) of repeated restraint for 2 hr per day in 50 mL tubes with multiple air wholes. On day-6, short-term memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze, which is based on the tendency of rodents to enter an arm of the Y-maze that was not explored in the last two choices.[23] SYK-657 camphorsulfonate was suspended in a 0.5% Arabic gum solution, and administered 60 min before the Y-maze test. NTI hydrochloride was dissolved in phosphate-buffered saline, and administered 15 min before SYK-657 camphorsulfonate administration.

ASSOCIATED CONTENT

Supporting Information.
The Supporting Information is available free of charge on the ACS Publications website at at http://pubs.acs.org.
Synthetic procedures and characterization of all other compounds (PDF).

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Author Contributions

S.H., T.I., and E.H. contributed equally to this work. H.F. and K.I. oversaw and designed the chemistry. M.T. and T.I. oversaw and designed *in vivo* pharmacology. E.H., C.I., and T.N. synthesized compounds. S.H. performed *in vitro* assays. T.I. and M.N. executed *in vivo* pharmacological assays. S.H. and T.I. analyzed the data. H.F. and T.I. wrote the manuscript.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

BNTX, 7-benzylidenenaltrexone; CPM, cyclopropylmethyl; DOR, δ opioid receptor; GPCR, G-protein coupled receptors; KOR, κ opioid receptor; MOR, μ opioid receptor; MVD, mouse vas deferens; NTB, naltriben; NTI, naltrindole.

REFERENCES and NOTES

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(21) We used [^{3}H] DPDPE, the DOR agonist, as a labeled compound, which might have influenced the assay results. Agonists bind more strongly to the receptors in an active form, whereas inverse agonists indicate a stronger binding affinity against the receptors in an inactive form.

(22) SYK-657 showed almost no agonistic activities toward the MOR and DOR in [^{35}S]GTPγS binding assays (MOR: -3.8% at 10 μM, KOR: 7.5% at 10 μM). Moreover, as the binding affinities of SYK-657 were low against the MOR and KOR, SYK-657 would not also act as antagonists for the MOR and KOR.

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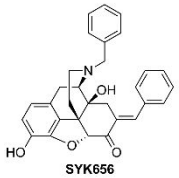
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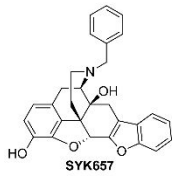
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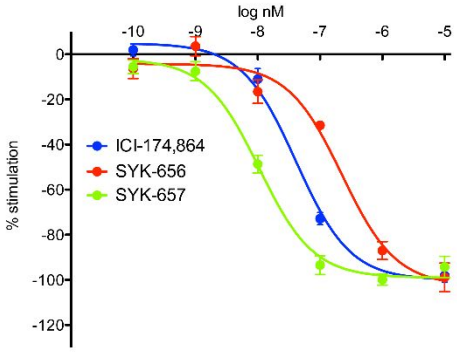
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