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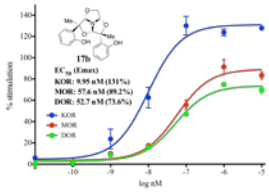

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Synthesis of a novel universal opioid receptor agonist with the 1,3,5-trioxazatriquinane skeleton and its pharmacologies

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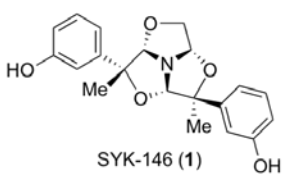
ABSTRACT

We designed and synthesized of 1,3,5-trioxazatriquinanes with *o*- or *p*-hydroxyphenyl rings as analogs of the κ opioid receptor agonist SYK-146 with *m*-hydroxyphenyl groups. Although almost all tested compounds did not bind to the opioid receptors, only **17b** with two *o*-hydroxyphenyl rings showed moderate or potent binding affinities and exhibited agonistic activities for three opioid receptor types. Because the basicity of the nitrogen atom in the 1,3,5-trioxazatriquinane structure was predicted to be very low due to the electron withdrawing effect of three oxygen atoms, **17b** was a novel non-morphinan and nonpeptidic opioid universal agonist lacking a basic nitrogen atom.

The opioid receptors are categorized into three types (μ, δ, and κ) and each type displays its various pharmacological effects. The κ opioid receptor (KOR) agonists are believed to be applicable to analgesia, diuresis, treatment of drug dependence and uremic pruritus.[1] Since the discovery of the first nonpeptidic KOR agonist U-50,488 in the early 1980s,[2] KOR agonists have been intensively sought by many medicinal chemists and were revealed to exert undesirable side effects including sedation[3] and psychotomimetic effects[4, 5] at effective doses. The nonpeptidic KOR agonists have been classified into seven types: 1) benzomorphan derivatives including bremazocine, 2) morphinan derivatives including nalfurafine, 3) arylacetamide derivatives including U-50,488 and U-69,593, 4) diazabicyclononanone derivatives including HZ2, 5) bicyclic guanidine derivatives including TPI 614-1, 6) benzodiazepine derivatives including tifluadom, and 7) neoclerodane diterpene derivatives including salvinorin A.[6] The KOR agonist nalfurafine hydrochloride (TRK- 820) was launched in Japan as an antipruritic for dialysis patients and this drug exhibited neither aversive nor addictive effects at effective doses.[1] Nalfurafine is characterized by the structural feature of a phenethylamine unit, a common structure observed in endogenous opioid peptides such as enkephalins, dynorphins, and β-endorphin. Recently, a diphenethylamine derivative HS665 was reported as a selective KOR agonist by Schmidhammer *et al*.[7] We also reported that a 1,3,5-trioxazatriquinane derivative SYK-146 (**1**) with *m*-hydroxyphenyl groups, which also included the phenethylamine moiety, was a selective KOR agonist and that its eutomer (-)-SYK-146 exhibited dose-dependent antinociceptive effects in the mouse acetic acid writhing test.[8] The structure-activity relationship (SAR) investigation of SYK-146 derivatives with *m*-hydroxyphenyl groups suggested that the hydroxy groups on the phenyl rings would be the significant structural motif for exerting the KOR agonistic activity. Therefore, we planned to investigate the effect of the position of the hydroxy groups on the KOR agonistic activity. Herein, we report the synthesis of 1,3,5-trioxazatriquinane derivatives with *o*- or *p*-hydroxyphenyl groups and their *in vitro* pharmacological properties.

According to the previously reported method,[8] we prepared 1,3,5-trioxazatriquinanes **13a-c** and **17a-d** with *o*-hydroxyphenyl groups. The synthesis of **13a-c** commenced with *o*-methoxyacetophenone (**2**). α-Hydroxyaldehyde **5** and its



SYK-146 (**1**)

Figure 1. Structure of SYK-146.

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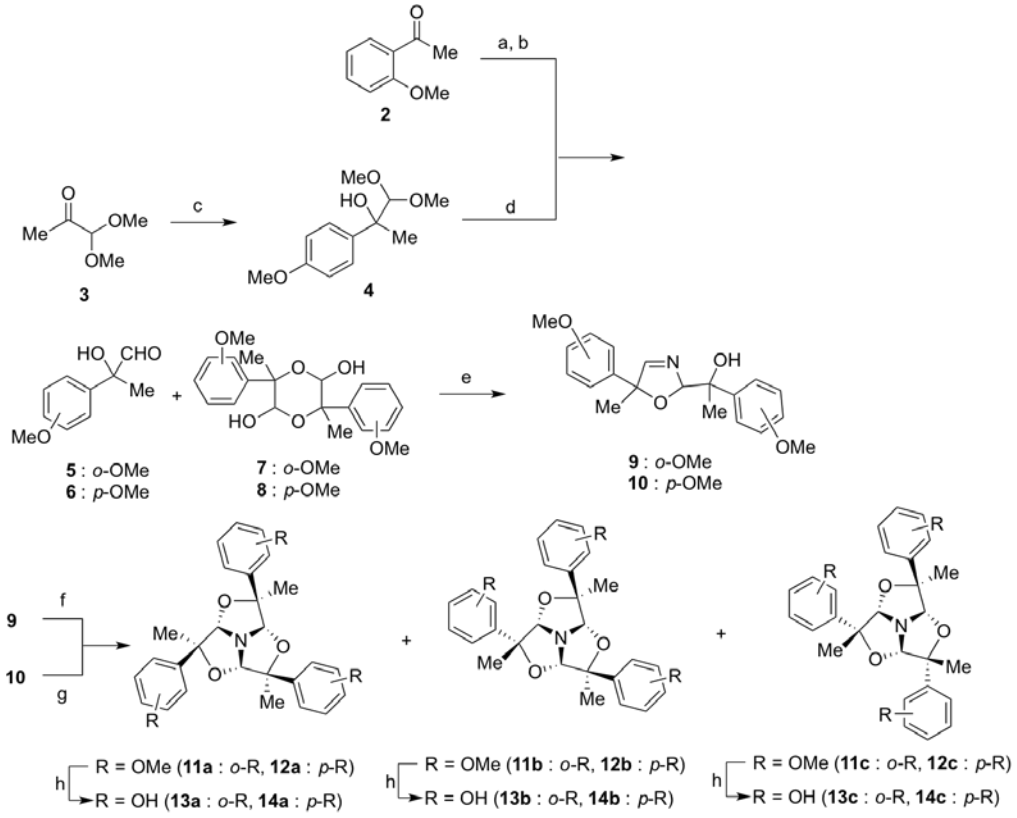
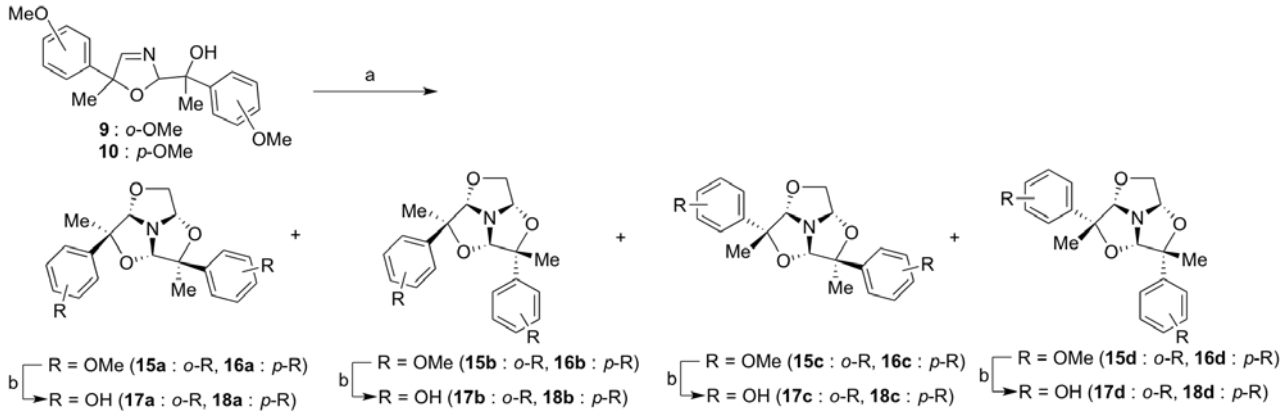
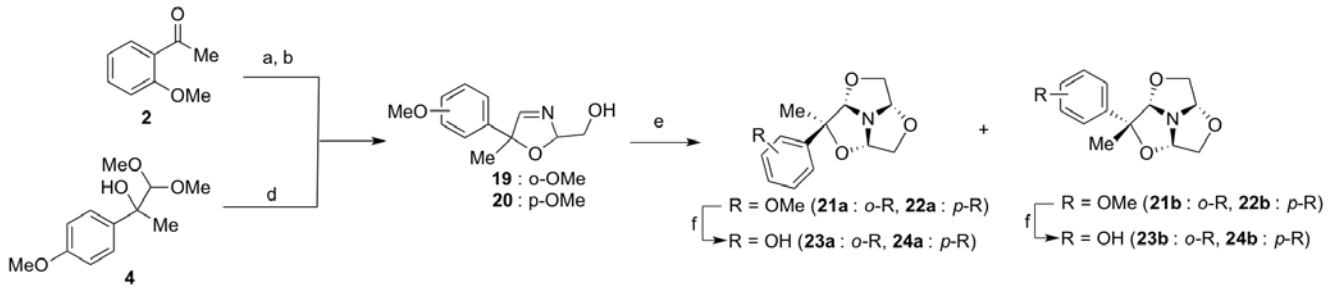


Scheme 1. Reagents and conditions; (a) TosMIC, K_2CO_3, MeOH, rt; (b) 2 M HCl aq, THF, rt; (c) *p*-Methoxyphenyl magnesium bromide, THF, 0 °C to rt; (d) AcOH, H_2O, reflux; (e) NH_4Cl, AcONa, MeOH, reflux; (f) **5**, **7**, CSA, $ClCH_2CH_2Cl$, reflux; (g) **6**, **8**, CSA, $CHCl_3$, rt, 2 weeks, **11a**: 17%, **11b**: 19%, **11c**: 7% from **2**, **12a**: 7%, **12b**: 10%, **12c**: 8% from **3**; (h) 1-Propanethiol, *t*-BuOK, DMF, 130°C, **13a**: 71% from **11a**, **13b**: 71% from **11b**, **13c**: 55% from **11c**, **14a**: 73% from **12a**, **14b**: 73% from **12b**, **14c**: 67% from **12c**.



Scheme 2. Reagents and conditions; (a) glycolaldehyde dimer, CSA, $CHCl_3$, reflux **15a**: 20%, **15b**: 6%, **15c**: 12%, **15d**: 7% from **2**, **16a**: 16%, **16b**: 13%, **16c**: 14%, **16d**: 5% from **4**; (b) 1-propanethiol, *t*-BuOK, DMF, 130 °C, **17a**: quant from **15a**, **17b**: quant from **15b**, **17c**: quant from **15c**, **17d**: quant from **15d**, **18a**: 87% from **16a**, **18b**: 91% from **16b**, **18c**: quant from **16c**, **18d**: 60% from **16d**.

hemiacetal dimer **7** derived from **2** were treated with ammonium chloride and sodium acetate to give oxazoline **9**. Oxazoline **9** reacted with the mixture of **5** and **7** in the presence of camphorsulfonic acid (CSA) to provide **11a-c** (Scheme 1). An attempt to synthesize **6** and **8** with *p*-methoxyphenyl groups by the same synthetic method of **5** and **7** furnished a recovery of starting materials. Therefore, **6** and **8** were prepared by a reaction of pyruvic aldehyde dimethyl acetal (**3**) with *p*-methoxyphenylmagnesium bromide and subsequent hydrolysis of acetal **4**. The resulting mixture of **6** and **8** was treated with ammonium chloride and sodium acetate in MeOH under reflux to afford oxazoline **10**. Oxazoline **10** was treated with the mixture of **6** and **8** in the presence of CSA to provide **12a-c** (Scheme 1). Derivatives **15a-d** or **16a-d** having two identical aryl units were prepared by the acidic condensation of **9** or **10** with glycolaldehyde dimer, respectively (Scheme 2). The key intermediates of the synthesis of 1,3,5-trioxazatriquinane derivatives **21a**, **b** and **22a**, **b** were the respective oxazolines **19** and **20**. Ketone **2** was treated with *p*-toluenesulfonylmethyl isocyanide (TosMIC) at rt in the presence of potassium carbonate, followed by hydrolysis with 2 M HCl, subsequent addition of hexamethyldisilazane (HMDS) for neutralization, and then treatment with ammonium chloride and sodium acetate in MeOH at rt to give oxazoline **19**. Oxazoline **20** was prepared from acetal **4** by sequential treatments similar to those of **19**. 1,3,5-Trioxazatriquinanes with a methoxyphenyl group **21a**, **b** or **22a**, **b** were prepared by the acidic condensation of **19** or **20** with glycolaldehyde dimer, respectively (Scheme 3).



Scheme 3. Reagents and conditions; (a) TosMIC, K_2CO_3, MeOH, rt; (b) 2 M HCl aq, MeOH, rt, then HMDS, glycolaldehyde dimer, NH_4Cl, AcONa, MeOH, rt; (c) *p*-Methoxyphenyl Grignard reagent, THF, 0 °C to rt; (d) AcOH, H_2O, reflux, then HMDS, glycolaldehyde dimer, NH_4Cl, AcONa, MeOH, rt; (e) glycolaldehyde dimer, CSA, $CHCl_3$, reflux, **21a**: 34%, **21b**: 20% from **2**, **22a**: 27%, **22b**: 16% from **3**; (f)1-propanethiol, *t*-BuOK, DMF, 130 °C, **23a**: 84% from **21a**, **23b**: 76% from **21b**, **24a**: 84% from **22a**, **24b**: 76% from **22b**.

Table 1. Binding affinities of 1,3,5-trioxazatriquinane derivatives for opioid receptors.[a]

	K_i (nM)		
	MOR[b]	DOR[c]	KOR[d]
DAMGO	0.83	N.T.[e]	N.T.[e]
DPDPE	N.T.[e]	0.90	N.T.[e]
U-69,593	> 1000	> 1000	1.37
SYK-146 (**1**)[f]	> 1000	> 1000	6.09
13a	> 1000	> 1000	> 1000
13b	> 1000	> 1000	> 1000
13c	> 1000	> 1000	> 1000
14a	> 1000	> 1000	> 1000
14b	> 1000	> 1000	> 1000
14c	> 1000	> 1000	> 1000
17a	> 1000	> 1000	> 1000
17b	20.5	88.8	9.17
17c	> 1000	> 1000	> 1000
17d	> 1000	> 1000	> 1000
18a	> 1000	> 1000	> 1000
18b	> 1000	> 1000	> 1000
18c	> 1000	> 1000	> 1000
18d	> 1000	> 1000	> 1000
23a	> 1000	> 1000	> 1000
23b	> 1000	> 1000	> 1000
24a	> 1000	> 1000	> 1000
24b	> 1000	> 1000	> 1000

[a] Binding assays were carried out in duplicate (MOR and DOR: whole brain without cerebellum of mouse, KOR: cerebellum of guinea pig).

[b] [^{3}H]DAMGO was used.

[c] [^{3}H]DPDPE was used.

[d] [^{3}H]U-69,593 was used.

[e] N.T.: not tested

[f] Ref. 8

Table 2. Agonist activities of 1,3,5-trioxazatriquinane derivatives for opioid receptors.[a]

	EC_{50} (E_{max})		
	MOR	DOR	KOR
DAMGO	7.57 nM (100%)	N.T.[b]	N.T.[b]
DPDPE	N.T.[b]	9.29 nM (100%)	N.T.[b]
U-69,593	N.T.[b]	N.T.[b]	8.03 nM (100%)
SYK-146 (**1**)[d]	N.D.[c]	N.D.[c]	97.5 nM (98.2%)
17b	57.6 nM (89.2%)	52.7 nM (73.6%)	9.95 nM (131%)

[a] [^{35}S] GTPγS binding assays were carried out in duplicate using human MOR, DOR, or KOR expressed CHO cells. DAMGO, DPDPE, or U-69,593 was used as the standard MOR, DOR, or KOR agonist, respectively.

[b] N.T.: not tested

[c] N.D.: not detected

[d] Ref. 8

The *O*-methyl groups of thus prepared 1,3,5-trioxazatriquinanes **11a-c**, **12a-c**, **15a-d**, **16a-d**, **21a**, **b** and **22a**, **b**[9] were removed by a treatment with a potassium thiolate to afford the corresponding compounds **13a-c**, **14a-c**, **17a-d**, **18a-d**, **23a**, **b** and **24a**, **b**, respectively.

The binding affinities of the synthesized 1,3,5-trioxazatriquinanes for the opioid receptors were evaluated with competitive binding assays (Table 1). Assays were performed by a previously reported procedure.[8] Although almost all tested compounds showed no binding affinities to the opioid receptors, **17b** alone, with two *o*-hydroxyphenyl groups, bound to the opioid receptors. This tendency was observed in the series of 1,3,5-trioxazatriquinanes with *m*-hydroxyphenyl groups: mono or trisubstituted 1,3,5-trioxazatriquinane derivatives did not bind to the opioid receptors due to the absence of a pharmacophore unit (the second hydroxyphenyl group) or the steric hindrance of the third hydroxyphenyl group. Among the disubstituted 1,3,5-trioxazatriquinanes, only one of the derivatives demonstrated remarkable binding to the opioid receptors. Contrary to **1**, which had a selective and strong binding affinity to the KOR, **17b** with

two *o*-hydroxyphenyl groups exhibited moderate binding affinities to the μ opioid receptor (MOR) and the δ opioid receptor (DOR) with K_i values of 20.5 and 88.8 nM, respectively. Furthermore, **17b** also strongly bound to the KOR with a K_i value of 9.17 nM. It is noteworthy that the configuration of **17b** was different from that of **1**. The functional activities of **17b** were assessed by [^{35}S] GTPγS binding assays with human opioid receptor-transfected CHO cells. Previously reported procedures[8] were used. Compound **17b** showed universal agonistic activities to the opioid receptors (Table 2). Although many ligands have been reported to bind nonselectively to the opioid receptor types,[10] most of them were universal antagonists or agonist/antagonists.[11] To the best of our knowledge, a few ligands have shown universal agonistic activities, for example etorphine, (-)-ethylketocyclazocine.[10] It is interesting that **17b** exhibited a more potent and efficacious agonistic activity for the KOR than did **1**. Until the discovery of salvinorin A as a KOR agonist,[12, 13] the basic nitrogen was believed to be the essential structural determinant for binding to the opioid receptor. The salvinorin A derivative herkinorin was reported to be an MOR agonist possessing no basic nitrogen atom.[13-15] **17b** may also be an opioid agonist lacking the basic nitrogen because the basicity of the nitrogen in 1,3,5-trioxazatriquinanes was predicted to be low due to the electron withdrawing effect of the three oxygen atoms. Indeed, the *pK*a value of **17b** was calculated to be 3.42 by ADMET Predictor.[16,17] **17b** was a potent KOR agonist comparable to salvinorin A (EC_{50} value: 40 nM, E_{max}: 120%) and a more potent MOR agonist than herkinorin (EC_{50} value: 500 nM, E_{max}: 130%).[14] Future investigations of the binding mode of **17b** to the opioid receptor types would shed light on the role and necessity of the basic nitrogen in the opioid agonists. Our results also indicated that the substitution position of the hydroxyl group would remarkably affect the *in vitro* pharmacological profiles of 1,3,5-trioxazatriquinanes. We are now exploring the binding mode of **17b**.

In summary, we have designed and synthesized the 1,3,5-trioxazatriquinane derivatives with *o*- or *p*-hydroxyphenyl groups. Among the synthesized compounds, **17b** exhibited moderate binding affinities to the MOR and the DOR, and strong binding affinity to the KOR. Furthermore, 1**7b** showed universal agonistic activities to the three opioid receptor types. These results suggest that **17b** is a novel non-morphinan and nonpeptidic opioid universal agonist lacking a basic nitrogen atom.

References and notes

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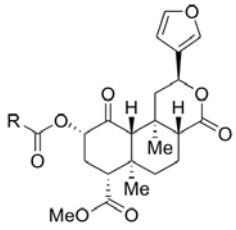
13. Structures of salvinorin A and herkinorin.



Salvinorin A: R = Me
Herkinorin: R = Ph

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16. We also calculated the *pK*a value of triethylamine to be 10.82. The *pK*a values were calculated by ADMET Predictor version 6.1.0001, Simulations Plus, Inc., Lancaster, CA, USA, 2012 (http://www.simulations-plus.com/).
17. This result suggested **1** was also the KOR agonist lacking a basic nitrogen atom.