Letter

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Discovery of SHR0687, a Highly Potent and Peripheral Nervous System-restricted KOR Agonist

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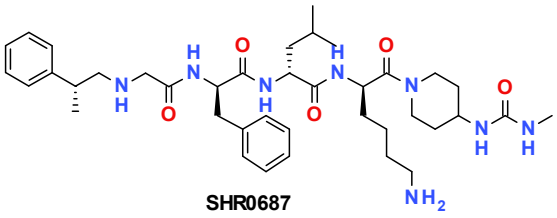
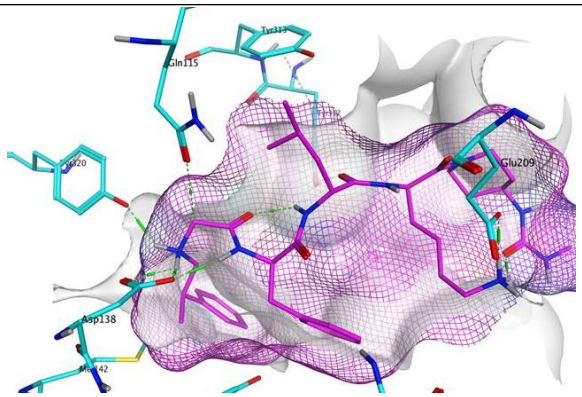


SHR0687

KOR EC_{50} = 0.53 pM
K*pu,u* = 0.006
Cl(mL/min/kg)= 10.1 (mouse), 10.5 (rat), 3.1 (dog)
Efficacious dose @ 0.1 mg/kg



ABSTRACT: Analgesics with no abuse liability are highly demanded in the market. KOR agonists have been proved to be strong analgesics without MOR agonist-related side effects, such as respiratory depression, tolerance, and dependence liability; however, activation of KOR in the central nervous system (CNS) may cause sedation and anxiety. It has been reported that peripheral KOR activation produces comparable bioactivity without CNS-related side effects. Herein, we designed and synthesized a novel tetrapeptide (SHR0687), which was shown to be a highly potent KOR agonist with excellent selectivity over other opioid receptors, such as MOR and DOR. In addition, SHR0687 displayed favorable PK profiles across species, as well as robust *in vivo* efficacy in a rat carrageenan-induced pain model. Notably, SHR0687 exhibited negligible BBB penetration, which was meaningful in minimizing CNS-related side effects.

KEYWORDS: Opioid receptors, kappa, analgesics, peripheral nervous system, BBB penetration, SHR0687.

Opioid receptors from family A of the G-protein-coupled receptors include three major subtypes (mu [MOR], kappa [KOR], and delta [DOR]), which are widely distributed in the brain, spinal cord, and digestive tract.[1,2,3] As well-known targets for pain treatment, opioid receptors are involved in the physiologic process of pain-related perception and modulation.[3] In the clinic setting, MOR agonists, such as morphine, are most widely used powerful analgesics.[4] However, activation of MOR often leads to serious side effects, such as respiratory depression, tolerance, and dependence liability.[5] DOR agonists are less anti-nociceptive with notable convulsions, which have limited therapeutic development.[6,3] As a potential alternative analgesic, KOR agonists have a strong anti-nociceptive effect with minimal respiratory depression and drug dependence.[7] Additionally, KOR agonists have been shown to be useful for treating pruritus, opiate dependence, and depression.[8]

Further research has verified that KOR agonists in the central nervous system (CNS) may cause sedation and anxiety,[9] whereas peripheral KOR activation produces comparable bioactivity without CNS-related side effects.[10] These inspiring results have spurred considerable interest of discovering novel KOR agonists with high region selectivity in the peripheral nervous system (PNS).

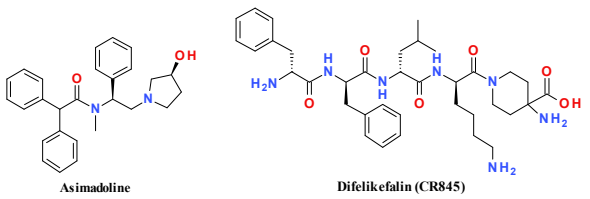
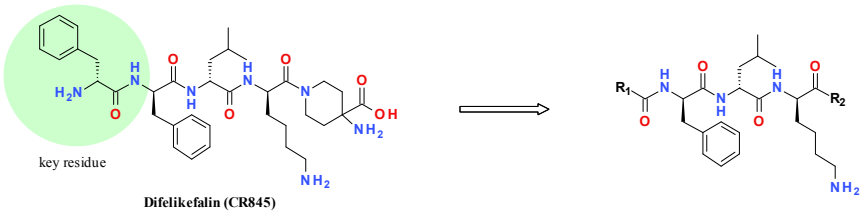


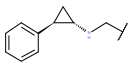
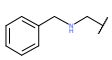
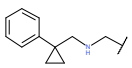
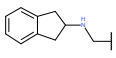
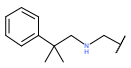
Figure 1. Structures of asimadoline and difelikefalin (CR845).

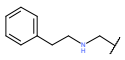
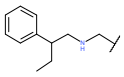
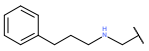
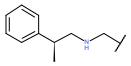
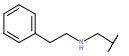
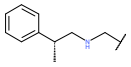
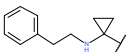
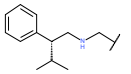
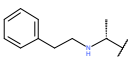
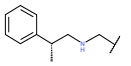
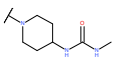
Recently, two peripherally selective KOR agonists were reported in clinical development at phase III. Asimadoline (Fig. **1**) has been shown to have reasonable efficacy for treatment of visceral pain.[11] Another peptide-based molecule, difelikefalin (CR845), is undergoing clinical trials for treating chronic and post-operative pain.[12,13] Considering the huge demand for analgesics without abuse liability, there has been considerable interest in developing diverse high-potency KOR agonists without CNS-related side effects. Our program focuses on the structure-activity relation (SAR) exploration of peptide oligomers as KOR agonists with high topologic polar surface areas (TPSAs), which presumably have limited ability to passively cross the brain-blood barrier (BBB).

Based on previous publications, the *N*-terminal D-phenylamine (Phe) functional group of CR-845 is a key pharmacophore and this area is very sensitive for the binding activity of KOR.[14-16] Herein, we focused on exploring alternative novel unnatural amino acids to mimic the key Phe group (Table **1**). The initial idea was to introduce cyclized phenylamine tetrahydroisoquinoline **1**, but unfortunately the potency decreased completely. Then, the open-chained benzyl amine **2** was shown to have no KOR activity. Subsequently, a cyclization ring afforded 2,3-dihydro-1H-inden-2-amine **3**, which was still inactive (EC_{50} > 1000 pM). Interestingly, the phenethyl amine **4** exhibited high potency by extension of one more carbon chain from compound **2**. Further extending the chain to afford **5** with an EC_{50}= 45 pM satisfied KOR potency. We preferred to select compound **4** considering it had a less flexible side chain that might be better for physicochemical properties. As shown in Table **1,** the R_2 group with either morphine or amino acid displayed the same level of potency (compounds **6** and **4**). Inspired by these results, additional compounds with mono and bis substitutions between the amide group and phenylpropyl amine linker (**7** and **8**) were designed. Unfortunately, the potency dropped completely. It was speculated that the steric effect close to the amide most likely affected the binding affinity. As a result, a new strategy was adjusted to the other side of the *N* atom close to the phenyl group. Inserting cyclopropane into the benzyl amine afforded compound **9** to maintain the potency compared with **6**. Thus, more effort was focused on the study of substitution groups (**10-15**). It was found that di-substituted group, such as cyclopropane or di-methyl (**10** and **11**) were well-tolerated and the potency was comparable to compound **6**. Unexpectedly, introducing the racemic ethyl substitution improved potency (compound **12**). This result indicated that the less steric group was more favorable for potency. Subsequently, two mono methyl with ***R*** and ***S*** configuration compounds were investigated. Interestingly, the ***R*** group **14** was more potent than the ***S*** configuration **13**. Next, the ***R*** configuration isopropyl **15** was shown to decrease potency compared with **14**. This result further confirmed the impact of steric effects on potency. Finally, the tail group R_2 was optimized and it was found that the methyl urea group yielded compound **16** to exhibit the highest potency in this series.

Table 1. Human KOR agonist in vitro activity [a]



Cpds ID	R1	R2	KOR EC_{50} (pM)	Cpds ID	R1	R2	KOR EC_{50} (pM)
1			*>1000*	*9*			*80*
2			*>1000*	*10*			*57*
3			*>1000*	*11*			*78*

4			*48*	*12*			*2.9*
5			*45*	*13*			*15.5*
6			*54*	*14*			*1.4*
7			*>1000*	*15*			*9.5*
8			*>1000*	*16*			*0.53*

[a] EC_{50} values are mean of two or more runs with CR845 as positive control for each batch (CR845 KOR EC_{50} = 2.62 ± 0.75 pM, n = 5).

This optimized pharmacophore residue was also supported by computational docking studies. Based on the overlay of the agonist-bound KOR protein structure (PDB code: 6B73) and the 3D-bound ligand conformation of the tetrapeptide agonist (DIPP-NH2) in DOR co-Xray crystal structure (PDB code: 4RWA), we modeled the bound conformation of compound **16** to KOR, as depicted in Figure **2**. The basic nitrogen in the phenethyl amine group makes strong salt bridge interaction with ASP138 and hydrogen bond with TYR320. The phenyl group of phenethyl amine is enclosed by three aromatic and hydrophobic side chains of HIS291, TYR139, and ILE294. This pocket helped the gain of potency of ***R*** isomer **14** compare with compound **13** might due to a more favorable binding position. The peptidic backbone amide groups make hydrogen bonds with ASP138, GLN115, and TYR312, respectively. The lysine group of compound **16** is within the salt bridge distance to GLU209, while the tail urea motif is largely exposed towards the solvent. Additionally, the methyl urea group seemed to make two interactions in the binding pocket of KOR. One is intramolecular hydrogen bond with lysine NH, and the other is dipole interaction with Arg202. These two interactions possibly enhanced the potency.

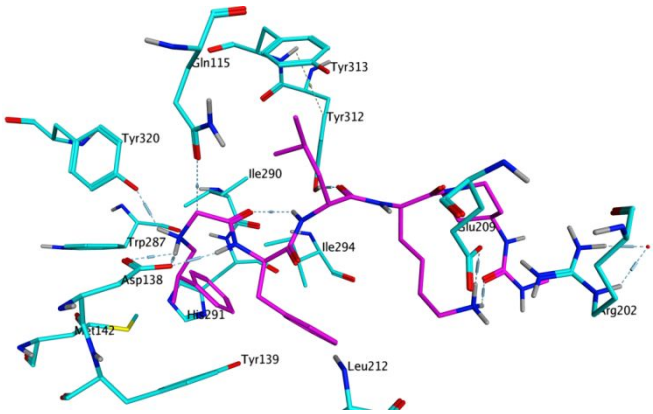


Figure 2. The modeled binding pose of compound **16** with agonist bound KOR protein structure.

As an interesting result, compound **16** (SHR0687) was selected for further *in vitro* and *in vivo* evaluations. As shown in Table **2**, this tetrapeptide displayed excellent selectivity over other opioid receptors, such as MOR and DOR, whose EC50 was > 50000 nM in cAMP accumulation assays.

Table 2. *In vitro* potency of SHR0687 [a]

Compound ID	SHR0687
MW / AlogP / PSA	720.9 /2.06 /186.8
hKOR EC_{50} (pM)	0.53
hMOR EC_{50} (nM)	>50000
hDOR EC_{50} (nM)	>50000

[a] The positive control for hMOR and hDOR test was Morphine (hMOR EC_{50} = 79.12 ± 9.91 nM, n = 3; hDOR EC_{50} = 203.97 ± 33.51 nM, n = 3).

In addition, SHR0687 was stable in *in vitro* liver microsomes across species, such as rats, mice, dogs, and humans (Table **3**), which correlated well with the *in vivo* PK results (Table **5**). Moreover, the cytochrome P450 inhibition of SHR0687 was investigated to assess the potential likelihood of drug−drug interactions (DDIs) with major CYP isoforms, including CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. SHR0687 was found with no obvious CYP450 inhibition with all CYP isoform IC50 values >30 μM. The hERG IC50 of SHR0687 was > 30 μM, indicating a higher cardiac safety margin.

Table 3. LMS, CYP, and hERG data of SHR0687

Compound ID	SHR0687
LMS $t_{1/2}$ (min)[a]	40 (h); >268 (r); 115 (m); 216 (d)
hCYPs IC_{50} (μM) 1A2/2C9/2D6/3A4(m)[a]/3A4(t)[c]2C19	All >30
hERG IC_{50} (patchliner, μM)	>30

[a]h = humans, r = rats, m = mice, d = dogs; [b]m: midazolam; [c]t: testosterone (FDA recommended 3A4 substrates)

Considering that our goal was discovery of a KOR agonist with good PNS selectivity and avoiding CNS side effects, we investigated the brain permeability of SHR0687 in a rat BBB model using the $K_{pu,u}$ value for evaluation of CNS penetration.[17,18] As summarized in Table **4**, the $K_{pu,u}$ value of SHR0687 was 0.006, suggesting that the BBB penetration capacity of SHR0687 was very limited.

Table 4. BBB penetration data of SHR0687 on SD rat (iv, 1 mg/kg)

Cmpd	fu_rat plasma	fu_brain tissue	plasma (ng/ml)	Brain tissue (ng/g)	$K_{pu,u}$
SHR0687	21.1%	2.58%	1144	57.2	0.006*

*T-test (*P=0.007*)

As summarized in Table **5**, SHR0687 has a favorable PK profile across species with low clearance and good *in vivo* exposure (AUC=1656 ng/mL*h on mice; AUC=1553 ng/mL*h on rats; AUC=2619 ng/mL*h on dogs). The adequate exposure was helpful for the following rat *in vivo* pain model study at a relatively lower dosage.

Table 5. Intravenous PK data of SHR0687 [a,b]

Species	Mouse	Rat	Dog
AUC_{0-t} (ng/mL*h)	1656	1553	2619
$t_{1/2}$(h)	0.34	4.78	4.45
CL (mL/min/kg)	10.1	10.5	3.1
Vz (mL/Kg)	297	4515	1236

[a]CL is clearance; t1/2 is the half-life of the compound exposure in plasma; AUC is the area under the curve. [b]The dosage for mouse and rat was 1 mg/kg and for dog was 0.5 mg/kg.

Furthermore, SHR0687 was investigated in a rat carrageenan-induced pain model to evaluate the therapeutic effect.[19] In agreement with the high *in vitro* potency, SHR0687 displayed remarkable efficacious characteristics, even at a 0.03 mg/kg dosage. A clear dose-dependent effect was observed from 0.03-0.3 mg/kg (28.9%-66.7%; Fig. **3**). It is worth mentioning that SHR0687 exhibited comparable or slightly better efficacy at the same dosage (66.7% vs. 61.9% @ 0.3 mg/kg) with CR-845 employed as a control group in the same study.

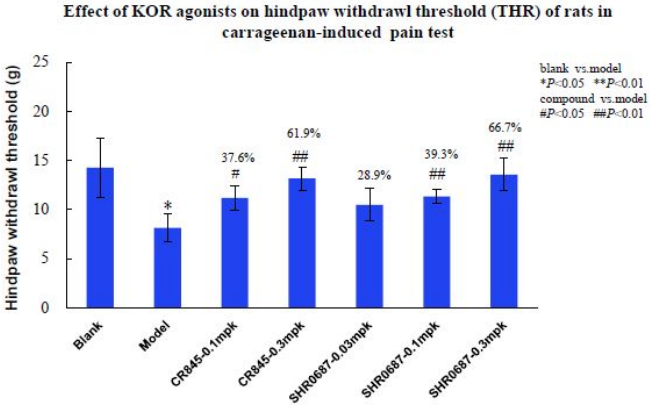
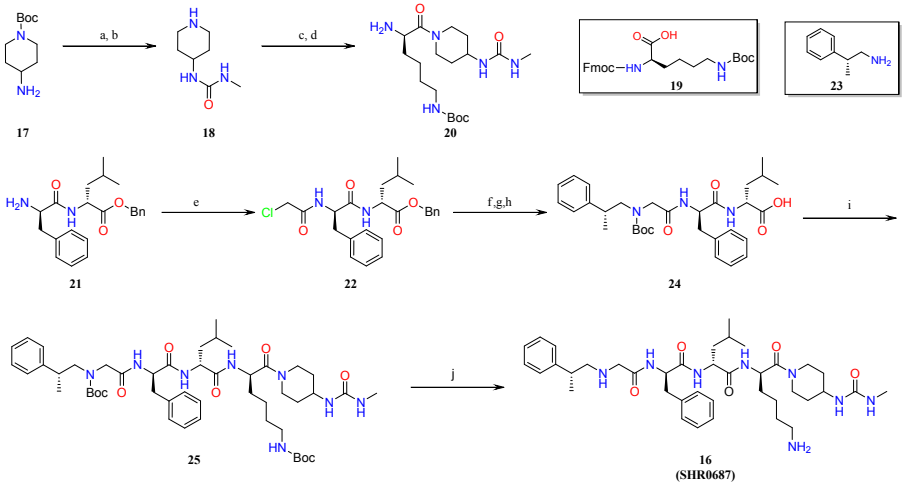


Figure 3. Efficacy study of SHR0687 on a rat carrageenan-induced pain model. N=8 rats in each group. Statistical comparisons were performed using the Excel software t-test. The data between the model and control groups were analyzed and compared to determine whether there was a significant statistical significance. *P <0.05 indicates that there is a significant difference between the model and control groups, ** P <0.01 indicates that there is a highly significant difference between the model and control groups, #P<0.05 indicates that there is a significant difference between the model and control groups, ##P <0.01 indicates that there is a highly significant difference between the model and the administration groups.

The synthesis of SHR0687 was straightforward, as shown in scheme **1**. Our strategy was to synthesize *N* and *C* terminal scaffolds in parallel, then couple two pieces at the late stage. The *N* terminal scaffold of methyl urea intermediate **20** was prepared in four steps. Initially, commercially available primary amine **17** was treated with methylcarbamic chloride, followed by deprotection of Boc group to afford urea substrate **18**. Then, **18** was coupled with carboxylic acid **19,** followed by removal of the Fmoc protecting group to achieve N terminal substrate **20** in a decent yield. The C terminal scaffold synthesis began with known intermediate **21** (see supporting information). Treating intermediate **21** with chloroacetyl chloride yielded compound **22**, which was coupled by (*R*)-2-phenylpropan-1-amine **23,** then followed by Boc protection. Subsequently the benzyl ether group was removed via hydrogenation afforded carboxylic acid **24** in an overall yield of 82% for four steps. The last two steps were coupling intermediates **24** and **20,** followed by removal of the Boc group, with successful production of compound **16** (SHR0687).

Scheme 1. Synthetic route of SHR0687



Reagents and conditions: (a) methylcarbamic chloride, DIPEA, DCM, r.t. 2h (b) HCl/Dioxane, DCM, r.t. 2h (c) **19**, HATU, Et_3N, DMF, r.t. 4h (d) Et_3N, DMF, r.t. 12h, 32% four steps (e) 2-chloroacetyl chloride, Et_3N, DCM, r.t. 12h (f) **23**, KI, K_2CO_3, DMF, 60° C 12h (g) $(Boc)_2O$, Et_3N, DCM, r.t. 12h (h) Pd/C, H_2, MeOH, r.t. 12h, 82% four steps (i) **20**, HATU, Et_3N, DMF, r.t. 12h (j) HCl/Dioxane, DCM, r.t. 12h, 50% two steps.

In summary, SHR0687 exhibited a highly potent KOR agonist with excellent selectivity over other opioid receptors, such as MOR and DOR. This novel tetrapeptide displayed favorable PK profiles across species, as well as robust *in vivo* efficacy in a rat carrageenan-induced pain model. Notably, SHR0687 showed minimal BBB penetration with an extremely low K*pu,u* value, thus indicating marginal CNS-related side effects for the potential therapeutic treatment of pain.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, analytical data, *in vitro* assay protocols, *in vivo* pharmacokinetic studies, and *in vivo* efficacy model details are available free of charge on the ACS publications website.

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ABBREVIATIONS

MOR, mu-opioid receptor; KOR, kappa-opioid receptor; DOR, delta-opioid receptor; PNS, peripheral nervous system; CNS, central-nervous system; BBB, blood-brain barrier; DCM, dichloromethane; hERG, human ether-a-go-go related gene; CYP, cytochrome P450 enzymes; PK, pharmacokinetics; HATU,2-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate; Boc, *t*-Butyloxy carbonyl; DIPEA, *N*,*N*-Diisopropylethylamine.

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