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Kappa-opioid receptor-selective dicarboxylic ester-derived salvinorin A ligands

Prabhakar R. Polepally [a], Kate White [b], Eyal Vardy [b], Bryan L. Roth [b], Daneel Ferreira [a], Jordan K. Zjawiony [a,*]

[a] Department of Pharmacognosy, and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677-1848, USA
[b] Department of Pharmacology, School of Medicine and Division of Chemical Biology and Medicinal Chemistry, School of Pharmacy, NIMH Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, NC 27599, USA

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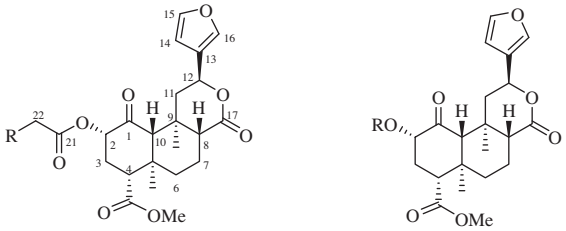
ABSTRACT

Salvinorin A, the active ingredient of the hallucinogenic plant *Salvia divinorum* is the most potent known naturally occurring hallucinogen and is a selective κ-opioid receptor agonist. To better understand the ligand–receptor interactions, a series of dicarboxylic ester-type of salvinorin A derivatives were synthesized and evaluated for their binding affinity at κ-, δ- and μ-opioid receptors. Most of the analogues show high affinity to the κ-opioid receptor. Methyl malonyl derivative **4** shows the highest binding affinity (K_i = 2 nM), analogues **5**, **7**, and **14** exhibit significant affinity for the κ-receptor (K_i = 21, 36 and 39 nM).

The neoclerodane diterpenoid salvinorin A (**1**) isolated from the leaves of hallucinogenic sage *Salvia divinorum*, is a potent and selective κ-opioid receptor (KOR) agonist.[1,2] Since its discovery, a large number of analogues have been prepared by semi-synthesis to probe the pharmacophore and mode of binding.[3] Some of these analogues present interesting pharmacological profiles from full KOR agonist to partial δ-opioid receptor (DOR) or μ-opioid receptor (MOR) agonist and antagonists. The current objective is to utilize the knowledge about salvinorin A–KOR interactions to rationally design salvinorin A derivatives with different pharmacological profiles and therapeutic potential. In the course of our work on the molecular mechanism of interaction of salvinorin A with KOR, we reported irreversible binding of 22-thiocyanatosalvinorin A (**2**) (Fig. 1) with the sulfhydryl group of Cys-315 at the κ-opioid receptor.[4,5] Our previous work on the KOR model and analysis of the mode of binding of **2** suggest that Cys-315 may be a good anchoring amino acid at the binding site in KOR.[4,6] The presence of ester substituents at C-22 may enhance electrophilicity of this center and thereby leads to stronger binding with the thiol group of Cys-315 of KOR.

Herein, we report the synthesis of series of new dicarboxylic ester salvinorin A derivatives and their binding affinity to κ-, δ- and μ-opioid receptors.

Salvinorin A (**1**) was isolated from dried leaves of *Salvia divinorum* and purified as previously reported.[7] It was hydrolyzed to salvinorin B (**3**), which served as the starting material for the preparation of the C(2)-modified dicarboxylic ester analogue library **4–16** (Schemes 1 and 2). Compounds **4–10**, **14**, and **15** were prepared[8] in yields of 57–69% by reacting **3** with the corresponding acid halides in the presence of Et_3N in DCM (Schemes 1 and 2). Analogues **11–13** and **16** were synthesized[9] in yields of 40–51% via the reaction of **3** with appropriate anhydrides using DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as base (Schemes 1 and 2). The physical data (^{1}H, 13C NMR and HR-ESIMS) were consistent with the proposed structures.



R = H, Salvinorin A (**1**)
R = SCN, 22-Thiocyanato salvinorin A (**2**)

R = H, Salvinorin B (**3**)

Figure 1. The structures of salvinorin A, 22-thiocyanatosalvinorin A, and salvinorin B.

* Corresponding author.
E-mail address: jordan@olemiss.edu (J.K. Zjawiony).

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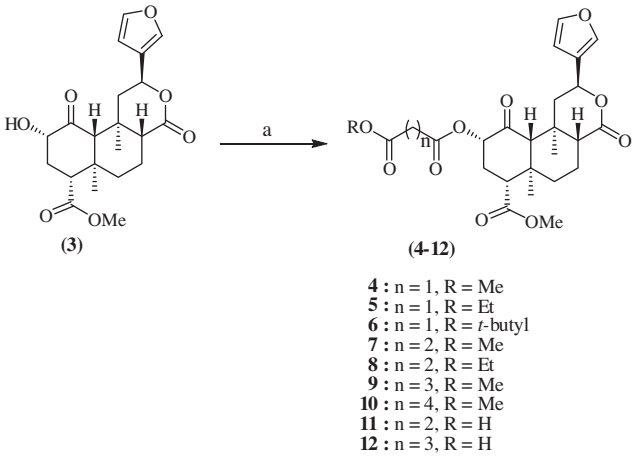


Scheme 1. Synthesis of dicarboxylic ester-type salvinorin A analogues **4**–**12**. Reagents and conditions: (a) appropriate acid chloride, Et_3N, dry DCM, N_2, rt, 2–3 h, or appropriate acid anhydride, DBU, dry DCM, N_2, rt, 6–8 h.

The κ-, δ- and μ-opioid receptor binding affinities of synthesized compounds **4–16** were evaluated at the NIMH-sponsored Psychoactive Drug Screening Program (PDSP), University of North Carolina at Chapel Hill using radioligand binding assays conducted as previously described,[1,5] and the data are collated in Tables 1 and 2. For comparison purposes, opioid binding affinity data for compounds **1–3** and positive controls for MOR and DOR are included in Table 1. Most of the dicarboxylic ester derivatives show appreciable binding affinity to KOR, and no affinity to MOR and DOR. The methyl malonyl derivative **4** had higher affinity than **1** at KOR (K_i = 2.0 vs 6.2 nM) (Table 1). The ethyl malonyl and methyl succinyl ligands **5** and **7**, also have significant affinities to KOR, these being respectively three and sixfold reduced compared to **1** (K_i = 21.0 and 36, respectively, vs 6.2 nM). The KOR affinities of analogues **6**, **8**, and **9** were decreased 24-, 70- and 49-fold as compared to **1** (K_i = 148, 437 and 302, respectively, vs 6.2 nM). Similarly, compounds **10**, **12**, and **15** show reduced affinity (K_i = 575, 263, and 427 nM, respectively) at KOR. Interestingly,

Table 1
Binding affinities of C(2)-dicarboxylic ester-derived salvinorin A analogues (**4**–**16**) at MOR, DOR, and KOR

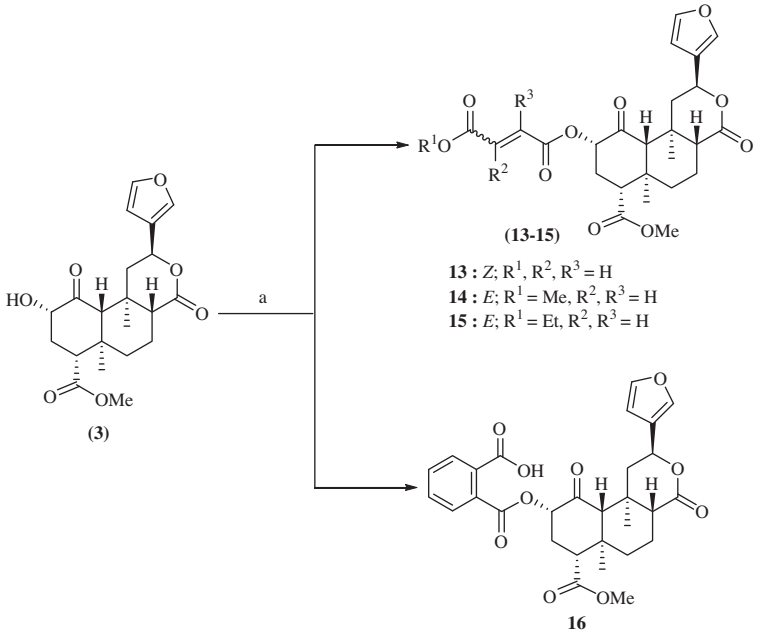
Compound	Affinities[a]	K_i ± SD (nM)		Selectivity	
	MOR[b]	DOR[c]	KOR[d]	MOR/KOR	DOR/KOR
1	>10,000	>10,000	6.2 ± 2.2		
Naltrindole	ND	0.9 ± 0.3	ND		
DAMGO	0.9 ± 0.2	ND	ND		
2[e]	>10,000	>10,000	0.6 ± 0.2		
3	>10,000	>10,000	>10,000		
4	>10,000	4320 ± 680	2.0 ± 0.3	>5000	2160
5	>10,000	>10,000	21.0 ± 5.6	>476	>476
6	>10,000	>10,000	148 ± 32	>67	>67
7	>10,000	>10,000	36 ± 8	>277	>277
8	>10,000	>10,000	437 ± 129	>23	>23
9	5012 ± 1400	>10,000	302 ± 94	16	>33
10	>10,000	>10,000	575 ± 183	>17	>17
11	>10,000	>10,000	4070 ± 1300	>2	>2
12	4570±1300	>10,000	263 ± 86	17	>38
13	>10,000	>10,000	>10,000	1	1
14	711 ± 134	>10,000	39 ± 11	18	>256
15	724 ± 137	>10,000	427 ± 134	2	>23
16	>10,000	>10,000	2291 ± 492	>4	>4

ND—not determined.
[a] K_i determined against [^{3}H]U69,553 ligand.
[b] See Ref. [4], K_i of **2** is 0.6 versus 1.9 nM of **1** for the KOR.
[c] Data are mean of three experiments performed in duplicate.
[d] The binding affinity constant (K_i) determined against [^{3}H]DAMGO ligand.
[e] K_i determined against [^{3}H]DADLE ligand.

Table 2
Kappa-opioid receptor functional assay: Efficacies of $G\alpha_i$ activation in live HEK293 cells using a cAMP biosensor Glosensor-22F (Promega)

Compound	EC_{50} ± SD (nM)[a]
1	5 ± 3
4	137 ± 15
5	52 ± 23
7	855 ± 130
14	70 ± 25

[a] Data are mean of four experiments.



Scheme 2. Synthetic route for the designed ligands **13**–**16**. Reagents and conditions: (a) appropriate acid chloride, Et_3N, dry DCM, N_2, rt, 3 h, or appropriate acid anhydride, DBU, dry DCM, N_2, rt, 6–8 h.

the methyl fumaryl ligand **14** had a sixfold reduced but still considerable affinity for the κ-opioid receptor compared to **1** (K_i = 39 vs 6.2 nM). Analogues **11** and **16** exhibited less affinity for the κ-receptor (K_i = 4070 and 2291 nM, respectively), and derivative **13** has no affinity for any of the opioid receptors.

To characterize the relative efficacy of these dicarboxylic ester ligands, compounds **4**, **5**, **7**, and **14** together with salvinorin A (**1**) were selected for a functional assay. Table 2 shows the EC_{50} values of these ligands in stimulating $G\alpha_i$ signaling mediated by the κ-opioid receptor.

In summary, a series of new dicarboxylic ester-type analogues of salvinorin A were synthesized in an effort to explore the effects of C-2 substitution towards selectivity at κ-, δ-, and μ-opioid receptors. Most of the analogues showed high binding affinity to the κ-opioid receptor with little affinity for δ- and μ-opioid receptors. Compound **4** possessing a C-2 methyl malonyl group exhibited increased selectivity for KOR compared to the parent salvinorin A (**1**).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.111.

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8. *Procedure for the synthesis of analogues **4**–**10**, **14** and **15**:* Compound **3** (15 mg, 0.05 mmol) and a catalytic amount of triethylamine were dissolved in DCM (3 mL). An appropriate acid chloride (0.125 mmol) was added, and the reaction mixture was stirred for 2–3 h at rt. After TLC indicated completion of the reaction, the mixture was quenched with water and the organic layer separated. The organic phase was washed with dilute aqueous HCl (0.01 mol/L, 2 mL) followed by saturated $NaHCO_3$ (2 mL). The organic layer was dried over anhydrous Na_2SO_4, evaporated, and the residue was purified by column chromatography (SiO_2; eluent:*n*-hexane/EtOAc) to obtain the target product. In some cases purification was done by preparative HPLC (C_{18} column, MeCN–water).
9. *Method for the synthesis of derivatives **11**–**13** and **16**:* To a solution of compound **3** (15 mg, 0.05 mmol) in DCM (3 mL), a catalytic amount of DBU and an appropriate acid anhydride (0.15 mmol) were added. The mixture was stirred at room temperature for 6–8 h. After TLC indicated completion of the reaction, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (SiO_2; eluent:*n*-hexane/EtOAc) and HPLC (C_{18} column, MeCN–water) to yield the target product.