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Synthesis and in vitro pharmacological studies of new C(2) modified salvinorin A analogues

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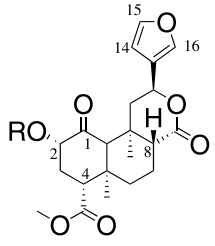
Abstract—Salvinorin A is the most potent naturally occurring opioid agonist yet discovered with high selectivity and affinity for κ-opioid receptor. To explore its structure and activity relationships, a series of salvinorin A derivatives modified at the C(2) position were prepared and studied. These salvinorin A derivatives were screened for binding and functional activities at the human κ-opioid receptor. Compound **4**, containing a methoxymethyl group at the 2-position, was a full κ-agonist with an EC_{50} value at 0.6 nM, which is about 7 times more potent than salvinorin A.

Activation of the κ-opioid receptor triggers many effects, including analgesia, dysphoria, antipruritic effect, corticosteroid elevations, diuresis, immunomodulation, and decreases in pilocarpine-induced seizures and associated mossy fiber sprouting and hilar neuron loss.[1] The κ-opioid receptors also participate in the expression of chronic morphine-induced withdrawal syndromes and mediate the aversive effects of Δ-9-tetrahydrocannabinol.[2,3] Synthetic arylacetamides, including U50488H, U69593, spiradoline, enadoline, ICI-204448, and asimadoline, have been demonstrated to be selective κ-opioid receptor agonists.[4,5] Interestingly, κ-agonist U69593 produces depressive-like effects and κ-antagonists, such as norBNI (nor-binaltorphimine) and ANTI (5′-acetamidinoethylnaltrindole), produce antidepressant-like effects in animal models.[6,7] Furthermore, κ-agonists appear to affect the mood in humans.[8,9] Other κ-active compounds, such as TRK 820 and HZ2, were reported to be useful as analgesics, water diuretics, and antipruritic drugs.[10–13]

Recently, salvinorin A has been identified as a selective κ-agonist.[14–17] Salvinorin A (**1**) was isolated from the dry leaves of *Salvia divinorum*, a 'magic mint' that has been in use for several hundreds of years mainly because of its psychoactive (hallucinogenic) effects during the divination rites of the Mazatec people of Mexico (Fig. 1).[18] Salvinorin A, a non-nitrogenous neoclerodane diterpenoid, was isolated and identified to be the key ingredient producing these psychoactive effects.[19] As part of our ongoing research on the development of κ-active ligands, we initiated the synthesis of C(2) modified salvi-



1: Salvinorin A, R= Ac
3: Salvinorin B, R= H

Figure 1.

Keywords: κ Opioid-receptor; Salvinorin A; Diterpenoid; Agonist; Binding activity.
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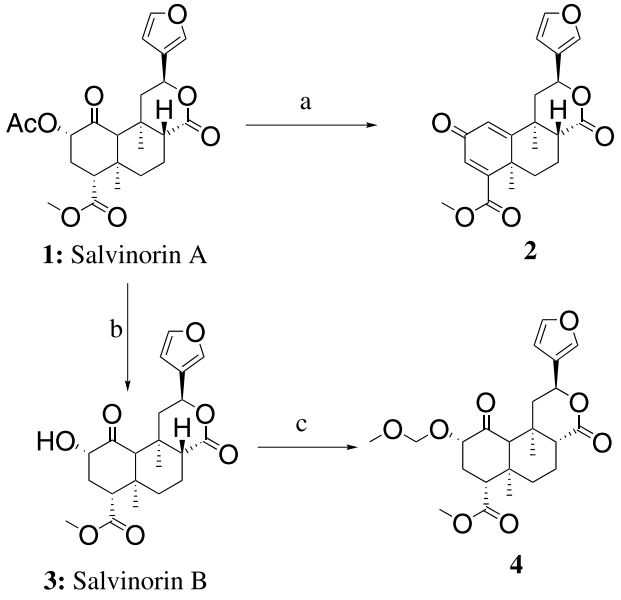
norin A analogues (Fig. 1). Our own findings, as well as those reported by several other laboratories, suggest that C(2) is a sensitive and critical site for binding the κ-receptor, with very limited tolerance in regard to size and electronegativity of the substituent group.[16,20]

Salvinorin A (**1**) was first converted to salvinorin B (**3**) by using potassium carbonate as the base at 0 °C in methanol (Scheme 1).[21] The C(8)-epimer (*epi*-**3**) was also isolated in this process, which has the same R_f value as **1** ($R_f = 0.61$, in 50% EtOAc/hexane). Various inorganic bases and organic bases have been tested and afforded no improvement in the yield of **3**. When $Ba(OH)_2$ was employed as the base in methanol, an unexpected oxidative-eliminated product **2** was isolated and identified. ^{1}H NMR shows two additional peaks at 6.92 and 6.98 ppm, in addition to furan protons at C(14), C(15), and C(16). ^{13}C NMR shows a corresponding peak pattern in which the C(1) signal had disappeared. Structural assignment of **2** was also confirmed by extensive H-H COSY and HMQC studies. Compound **4** was synthesized by reacting **3** with chloromethyl methyl ether in the presence of Hünig's Base and 4-(dimethyl-amino)pyridine (DMAP) (Scheme 1).

Although previous modifications at the C(2) position and corresponding binding studies have generated numerous C(2)-analogues, only the 2-propionate and formate derivatives, however, showed submicromolar affinity,[16,22] equivalent to salvinorin A, for the human κ-opioid receptor (hKOR). Several reports show that salvinorin B (**3**) is inactive.[16,23] A molecular modeling study reveals that residue **Y313** in the 7th transmembrane helix of the hKOR might interact with the carbonyl group at C(2) via H-bonding. To further explore the SAR at the C(2)-position and synthesize potential agonists and antagonists of hKOR, we have synthesized a series of C(2)-esters and carbonates, as shown in Table 1.



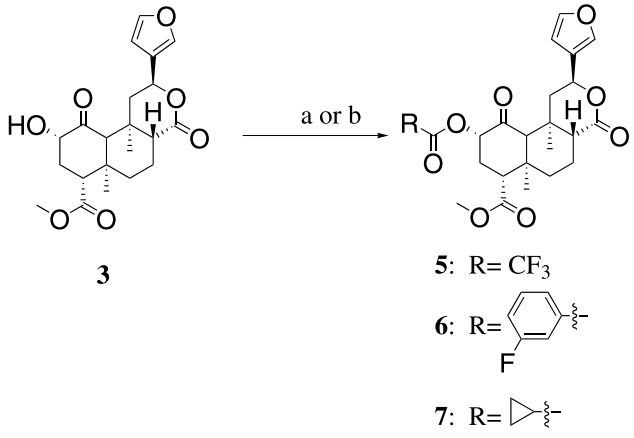
Scheme 1. Reagents and conditions: (a) $Ba(OH)_2$, MeOH, rt (75%); (b) K_2CO_3, MeOH, 0 °C, (70%); (c) MOM-Cl, ^{i}Pr$_2$NEt, DMAP, CH_2Cl_2, rt (72%).

Esters **5**, **6**, and **7** were prepared according to standard acylation procedures (Scheme 2). By reacting salvinorin B (**3**) with trifluoroacetic anhydride in the presence of pyridine at room temperature, **5** was obtained in good yield. Fluoride atoms have been used extensively in drug discovery on account of their unique electronic proper-

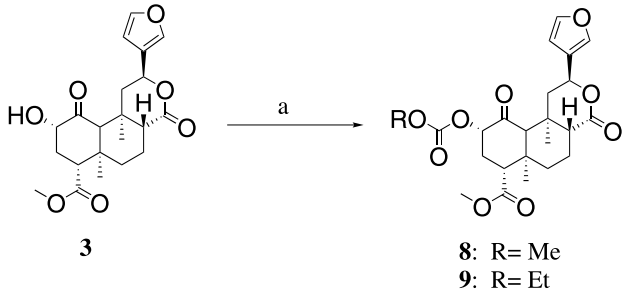
Table 1. Affinities (K_i), potencies (EC_{50}), and efficacies of C(2)-substituted salvinorins **1–9** at the κ-opioid receptor

Compound	K_i[a,b] (nM)	EC_{50}[b,c] (nM)	Efficacy[d]
2	>1000	—[e]	—
3	111 ± 12	492 ± 75	97
epi-**3**	43 ± 5	193 ± 4	102
Esters			
1	1.3 ± 0.5	4.5 ± 1.2	106
epi-**1**	77 ± 4	307 ± 92	94
5	>1000	—[e]	—
epi-**5**	>1000	—[e]	—
6	>1000	—[e]	—
7	>1000	—[e]	—
Carbonates			
8	>1000	—[e]	—
9	>1000	—[e]	—
Ethers			
4	0.4 ± 0.02	0.6 ± 0.2	98
epi-**4**	30 ± 3	92 ± 31	100
U50,488H	1.4 ± 0.3	4.5 ± 1.2	100

[a] K_i values of salvinorin A (**1**) and its analogues in inhibiting [^{3}H]diprenorphine binding to the human κ-receptor.
[b] Each value represents the mean of at least three independent experiments performed in duplicate.
[c] EC_{50} values in activating the human κ-receptor to enhance [^{35}S]GTPγS binding.
[d] Efficacy determined as the percentage of maximal response produced by U50,488H.
[e] Not determined.



Scheme 2. Reagents and conditions: (a) Compound **5**—$(CF_3CO)_2O$, Pyr., DMAP, CH_2Cl_2, rt (62%); (b) RCOCl, DMAP, CH_2Cl_2, rt.



Scheme 3. Reagents and conditions: (a) Chloroformate, DMAP, CH_2Cl_2, rt.

ties and steric similarity to hydrogen atoms. In addition, compounds **6** and **7** were synthesized conveniently by making **3** react with *m*-fluorobenzoyl chloride and cyclopropanecarbonyl chloride, respectively.

Incorporation of carbonates at the C(2)-position was carried out according to Scheme 3. The following scheme illustrates a one step synthesis of carbonate derivatives from **3**. Treatment of salvinorin B (**3**) with methyl or ethyl chloroformate in the presence of DMAP gave compounds **8** and **9** in good yield.

As we have mentioned before, the C(8)-position can be easily isomerized under basic conditions to give corresponding epimers in an almost 1:1 ratio. It is conceivable that the C(8)–C(9) bond underwent a base-promoted cleavage.[24] C(8)-Epimers of compounds **1**, **3**, **4**, and **5** were also synthesized by similar approaches and subjected to binding studies. All spectral data (^{1}H NMR, ^{13}C NMR, and high-resolution mass) obtained were consistent with the structures proposed. The absolute configurations of both epimers were assigned based on NMR and literature data.

The compounds **1–9** and their corresponding epimers were examined for binding to the κ-opioid receptor by competitive inhibition of [^{3}H]diprenorphine binding to membranes that were prepared from Chinese hamster ovary (CHO) cells stably transfected with hKOR.[17] At 1 μM, **3**, *epi*-**3**, **1**, *epi*-**1**, **4**, and *epi*-**4** showed >50% inhibition and their K_i values were determined from competitive inhibition of [^{3}H]diprenorphine binding by a series of concentrations of each compound (Table 1). The compounds were then assessed for their abilities to enhance [^{35}S]GTPγS binding to the membranes of CHO-hKOR cells, and EC_{50} and E_{max} values, indicators of potencies and efficacies, respectively, were calculated from the dose–response curves (Table 1). The selective κ agonist, U50,488H, served as the reference compound with a relative efficacy of 100.

In previous reports,[16] the bulky substituents other than acetate (**1**) and propionate derivatives showed no binding affinities to hKOR and salvinorin B was inactive. However, our studies show that salvinorin B (**3**) exhibited moderate affinity and potency for the κ-receptor (K_i = 111 ± 12 nM, EC_{50} = 492 ± 75 nM, and efficacy = 97%), which is about 80-fold lower than salvinorin A and U50,488H. The differences may have resulted from the different radiolabeled ligands used in the two studies, [^{3}H]bremazocine in the study of Chavkin and co-workers[16] and [^{3}H]diprenorphine in ours. Rusovici et al.[25] have demonstrated that bremazocine and U69,593 bind to low- and high-affinity states of the human KOR expressed in CHO cells. In theory, [^{3}H]diprenorphine, an antagonist, binds to both the states. Under our binding conditions, U50,488H showed a K_i value of 1.4 nM in competitive inhibition of [^{3}H]diprenorphine binding, consistent with the notion that [^{3}H]diprenorphine binds to at least the high-affinity state (For more details, see Lee, D.Y.W. et al., submitted for publication.) Interestingly, *epi*-**3** showed a higher affinity for hKOR (K_i = 43 ± 5 nM) than **3**. On the contrary, *epi*-**1** was far less potent (K_i = 77 ± 4 nM) than salvinorin A (**1**) (K_i = 1.3 ± 0.5 nM).

Compounds **6** and **7** showed no binding affinities to hKOR, which was consistent with the earlier observations of Chavkin and co-workers.[16] Similar SAR patterns were observed in the carbonate series (**8**, **9**: K_i > 1000 nM). However, compounds **5** and *epi*-**5** did not show any appreciable affinities to hKOR, despite the fact that their size was similar to that of **1**. It suggests that the electronic effect may also play an important role in binding to the κ-receptor. It was not totally unexpected that compound **2** was inactive because of the dramatic change compared to the acetoxy group of salvinorin A. Surprisingly, compound **4** with a methoxymethyl moiety at C(2) showed a potency (EC_{50} = 0.6 ± 0.2 nM) approximately 7 times greater than salvinorin A (**1**) (EC_{50} = 4.5 ± 1.2 nM). Interestingly, replacement of the carbonyl group of the carbonate **8** with a methylene (**4**) actually enhances the affinity and potency. A short straight chain with two oxygen atoms appears to fit the binding pocket of KOR better than an acetyl group, and the carbonyl group at C(2) may not be necessary as a H-bond acceptor.

The compounds *epi*-**1**, **3**, *epi*-**3**, **4**, and *epi*-**4** showed high to moderate binding affinities to the hKOR, but at 10 μM, *epi*-**1**, **3**, *epi*-**3**, and *epi*-**4** inhibited [^{3}H]diprenorphine binding to the μ or δ opioid receptor stably expressed in CHO cells by <50% and compound **4** inhibited the same by <70% (data not shown). Thus, these compounds, like such as salvinorin A (**1**), exhibit selectivity for the KOR over μ and δ opioid receptors.

In summary, the SAR information collected so far suggests that the size and shape of the substituents at the 2-position are both important factors for binding to the κ-receptor. A carbonyl functional group at C(2) may not be necessary, and compound **4** with a short ether linker showed a greater binding affinity to the κ-receptor. In addition, electronic factors are also important and should be explored further.[26,27]

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26. *Experimental procedure* (*epi*-**3**) K_2CO_3 (200 mg) was added to a solution of salvinorin A (200 mg) in MeOH (100 mL) at room temperature and the mixture was stirred for 20 min. The reaction mixture was then diluted with 50 mL water, and the pH was adjusted to 7.0 and extracted with EtOAc (2×50 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford a white solid. The crude product was purified further by column (1:1, EtOAc: hexane) to give the pure *epi*-**3** (Yield: 91 mg, 50.5%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.41 (d, J = 1.5 Hz, 1H), 6.37 (s, 1H), 5.31 (dd, J = 4.5, 11.4 Hz, 1H), 4.01 (t, 1H), 3.69 (s, 3H), 3.60 (d, J = 3.3 Hz, 1H), 2.72 (dd, J = 3, 13.5 Hz, 1H), 2.47–2.38 (m, 3H), 2.23 (s, 1H), 2.18 (d, J = 1.8 Hz, 1H), 2.07–1.82 (m, 3H), 1.64 (s, 3H), 1.56–41 (m, 2H), 1.06 (s, 3H); ^{13}C NMR (70.5 MHz, $CDCl_3$): δ 209.2, 173.4, 172.1, 143.6, 139.6, 123.5, 108.4, 74.4, 70.0, 63.6, 52.3, 51.6, 48.2, 45.2, 42.6, 34.5, 34.2, 33.8, 24.6, 17.5, 15.3.
27. *NMR data for compound* **2** ^{1}H NMR ($CDCl_3$, 300 MHz): 1.68 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 2.0 (m, 2 H), 2.25 (m, 1H), 2.50 (m, 1H), 3.00 (m, 1H), 3.10 (m, 1H), 3.83 (s, 3H, $COOCH_3$), 5.40 (dd, J = 2.4 Hz, 7.5 Hz, 1H), 6.40 (d, J = 0.7 Hz, 1H), 6.92 (s, 1H), 6.98 (s, 1H), 7.40 (m, 1H), 7.48 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.91, 24.98, 28.35, 30.20, 36.80, 37.70, 42.28, 44.90, 52.59, 70.87, 108.42, 124.57, 128.19, 139.61, 139.93, 143.64, 145.64, 157.50, 165.37, 173.10, 180.74.