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Synthesis and in vitro pharmacological studies of C(4) modified salvinorin A analogues

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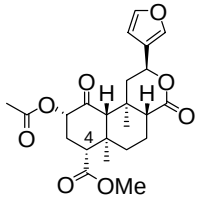
Abstract—Salvinorin A is the most potent naturally occurring opioid agonist with a high selectivity and affinity for κ-opioid receptor. To explore its structure–activity relationships, modifications at the C(4) position have been studied and a series of salvinorin A derivatives were prepared. These C(4)-modified salvinorin A analogues were screened for binding and functional activities at the human κ-opioid receptor and several potent new agonists have been identified.

Opioid receptors belong to a class of seven transmembrane spanning (7TM) G-protein-coupled receptors (GPCRs).[1] It is well known that GPCRs mediate many of the actions of neurotransmitters and hormones. Three decades of pharmacological studies have identified three major subtypes of opioid receptors, μ, δ, and κ, along with other less well-characterized subtypes.[2] Many of these receptors appear to be involved in determining psychological states. Specifically, it has been postulated that κ agonists produce prodepressant-like effects in behavioral models of depression in rats, whereas antagonists produce antidepressant-like effects.[3]

Many classes of ligands are known to act on μ, δ, and κ-opioid receptors (KORs), yet few ligands selectively bind to the κ receptor. Salvinorin A (Fig. 1), a neoclerodane diterpene isolated from a Mexican mint *Salvia divinorum*,[4] is one of the most potent naturally occurring opioid agonists with a high selectivity and affinity for KORs.[5] More interestingly, it represents the only known non-nitrogenous and terpenoid KOR selective agonist. The effective dose of salvinorin A is 200–1000 μg in humans, which is at the same scale as other synthetic hallucinogens such as lysergic acid diethylamide and 4-bromo-2,5-dimethoxy-phenylisopropylamine.[6] Salvinorin A represents a promising lead for the development of more potent and selective clinically useful KOR agonists and antagonists.[6a,7]



Salvinorin A

Figure 1. Selective κ-opioid receptor agonist.

A molecular modeling study reveals that residues **Y312**, **Y313**, and **Y139** on the KOR might interact with the carbonyl groups at C(2), C(4), and C(17) via H-bonding.[5] Several reports show salvinorin B, the major metabolite and a C(2) deacetyl compound, to be inactive.[8a] Interestingly, previous modifications at C(2) position and corresponding binding studies have generated numerous C(2) analogues, but only the 2-propio-

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nate derivative showed submicromolar affinity, equivalent to salvinorin A, for human KORs.[8b] Other steric hindered ester derivatives such as pivalate, 1-naphthoate, and carbonates were all inactive.

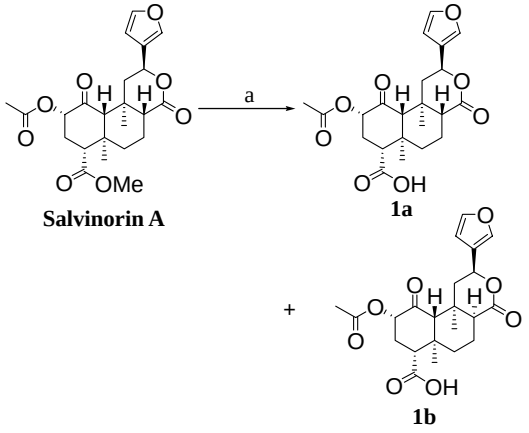
To further explore the structure–activity relationship (SAR) at C(2), we have synthesized a series of C(2) esters and carbonates and found that the methoxymethyl analogue showed approximately 7 times greater binding affinity than salvinorin A (**1**).[9,10] Thus, a short straight chain with two oxygen atoms appears to fit the binding site better than an acetyl group, and the carbonyl group at C(2) may not be necessary as a H-bond acceptor. In summary, C(2) is a sensitive and crucial site for binding to the κ receptor with very limited structure tolerance in terms of size and electronegativity of the substituent group. As little is known regarding the SAR at C(4), we started to investigate modifications at C(4). Our initial approach was to keep the C(4) carbonyl functional group intact and we synthesized a series of ester and amide derivatives.

The starting material, salvinorin A, was isolated from dry *Salvia divinorum* leaves and purified using a modified procedure.[8a,11] Obviously, C(4) methyl ester must be cleaved to couple with other building blocks. In the literature, the C(4) carboxylic acid **1** was prepared by cleaving both the ester groups at C(2) and C(4) simultaneously, followed by re-acetylation at C(2).[12] To cleave the C(4) ester selectively, we have tried many hydrolysis conditions and found that lithium iodide in pyridine could fulfill the task and gave the acid **1a** and **1b** as a 1:1 mixture in good yield (Scheme 1).[13] 1D and 2D NMR experiments reveal that epimerization occurred at the C(8) position. The configuration of 8-*epi*-**1** (**1b**) is determined by coupling between H-8 and H-7. Coupling constants of H-8 and H-7 showed *trans* and *gauche* pattern in **1a**, while only *gauche* pattern in **1b**. In addition, the H-12 multiplet (δ 5.50 ppm, dd) in **1a** showed the expected coupling to both protons of H-11 (COSY), while only the H-12 doublet (δ 5.30 ppm) showed in **1b**. A small portion of starting materials and corresponding C(8) epimer were also isolated from this transformation.[4b] The acid mixture was separable by flash column chromatography; however, it is easier to separate the further derivatized C(4) esters because the carboxylic acid functional group tends to dominate the polarity on silica gel and make the isolation of individual acids very difficult.

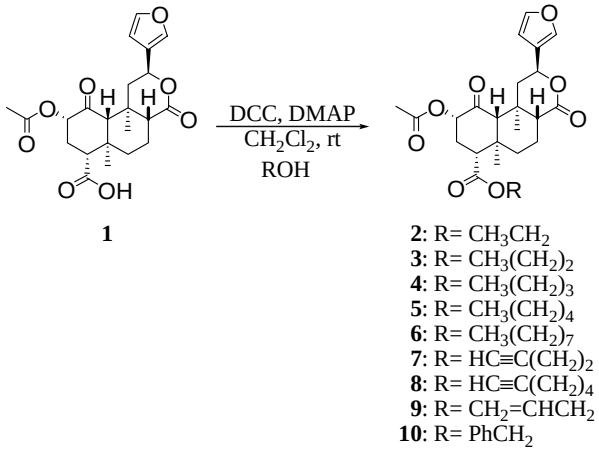
The C(4) esters were synthesized according to standard published procedures. A mixture of **1a** and **1b** was treated with dicyclohexylcarbo-diimide (DCC) and 4-(dimethyl-amino)pyridine (DMAP) as the catalyst in the presence of various alcohols (Scheme 2). To further examine the role of unsaturated functional groups at the C(4) position, several terminal alkynes and alkenes were linked at the C(4) site. Esters **2a**–**10a** as well as epimers **2b**–**10b** were obtained from the corresponding alcohols. In all the cases, the ester epimers were successfully separated by chromatography.

Nitrogen-containing building blocks often play important roles in drug design and provide enhanced interaction between pharmacophore and receptor sites. We attached a series of N-containing units to C(4) position. Amides **11** and **12** were prepared by reacting acid **1** with benzyl and phenylethyl amine, respectively (Scheme 3). Compound **13** was synthesized as an intermediate for the isostere of salvinorin acid.[14] As shown in compound **14**, a terminal hydroxyl functional group was introduced to improve solubility.

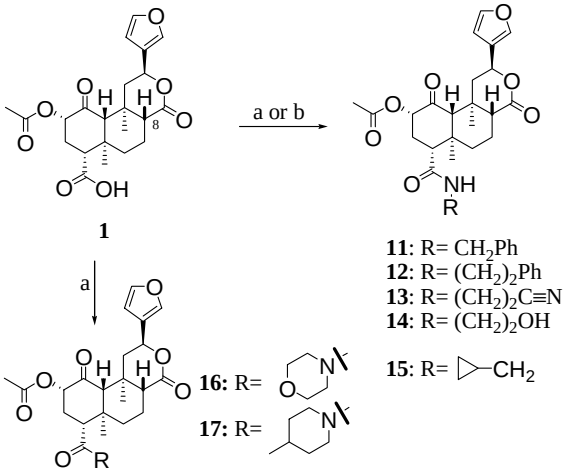
To further examine the steric effect, several bulky cyclic units were linked to the C(4) carbonyl group as shown in Scheme 3 (**15**, **16**, and **17**). In most of the cases, 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimide hydrochloride (EDCI) in DMF was used as the coupling reagent and 1-hydroxybenzotriazole hydrate (HOBt) served as the catalyst and base.[15] Since amino acid residues can often interact with the active site of receptors and play a pivotal role via H-bond and charge effects, L-(+)-alanine analogues of both acid epimers **18a** and **18b** were synthesized. In addition, derivatives of other amino acids, such as glycine, L-(+)-serine, L-(−)-proline, and L-(+)-histidine, were also synthesized by a similar approach (Scheme 4).



Scheme 1. Synthesis of salvinorin A acids. Reagents and conditions: (a) LiI pyridine, reflux 36 h, **1a** (39%) and **1b** (33%).



Scheme 2. Synthesis of C(4) esters.

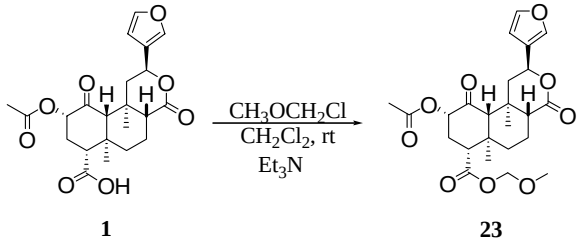


Scheme 3. Synthesis of C(4) amides. Reagents and conditions: (a) EDCI, HOBt, DMF, rt; (b) EDCI, DMAP, DMF, rt; (c) EDCI, HOBt, Et_3N, DMF, rt.

Our previous studies indicated that a methoxymethyl moiety at C(2) can enhance the binding of salvinorin derivatives to the κ receptor.[10] MOM-protected C(4) acids **23a** and **23b** were also synthesized by standard methods for both the natural and unnatural isomers (Scheme 5).

All spectra data (^1H NMR, ^{13}C NMR, and high-resolution mass) obtained were consistent with the structures provided. The absolute configurations of both epimers were assigned according to their NMR data.

The target compounds were examined for their ability to bind the human KOR (hKOR) by competitive inhibition of [^3H]diprenorphine binding to the receptor in membranes prepared from Chinese hamster ovary cells stably transfected with hKOR (CHO-hKOR).[16] Compounds were evaluated first at 1 μM in radioligand binding assays. For those producing more than 50% inhibition, dose–response curves were generated and K_i values were determined. In addition, the potencies (EC_{50}) and efficacies of compounds at the κ receptor

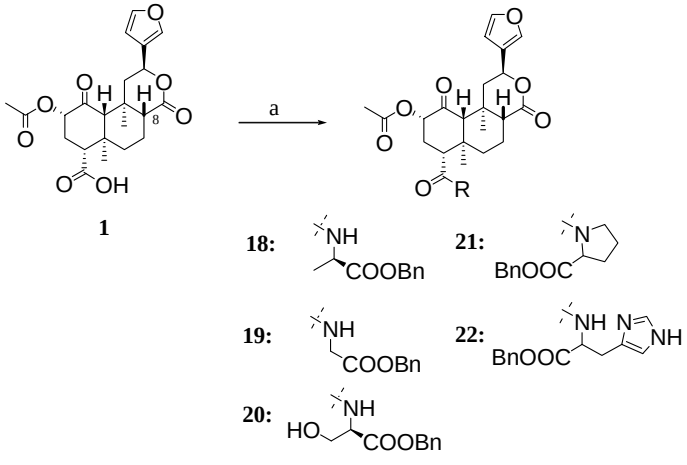


Scheme 5. Synthesis of MOM ester.

were determined by their abilities to enhance [^{35}S]GTPγS binding to membranes of CHO-hKOR cells. The selective κ full agonist, U50,488H, served as the reference standard with a relative efficacy of 100. The in vitro pharmacological data for C(4) derivatives are listed in Table 1.

Except **1a/1b**, **15a/15b**, and **23a/23b**, C(8) epimers show much lower binding affinity than the corresponding natural isomers, indicating the requirement of C(8)-H being *trans* to C(12)–H in most derivatives for optimized activity at the hKOR. **1a** is inactive at the hKOR, indicating the importance of the methyl ester at C(4). Among the ester derivatives, only **2a**, **8a**, **23a**, and **23b** had K_i values <1 μM, demonstrating that a longer chain at the C(4) position decreases the binding affinity. None of the C(4) amide derivatives exhibited significant binding affinity for the hKOR. Only compound **15b** shows moderate binding affinity compared to the reference standard. Three of the α-amino acid derivatives (**18a**, **19a**, and **21a**) had K_i values <1 μM. These compounds were identified as full agonists at the hKOR. In general, longer side chains or bulky substituents at C(4) decrease binding affinity.

In summary, a series of C(4) derivatives of salvinorin A were synthesized. The C(4) carboxylic acid was prepared by selective cleavage of the C(4) ester in the presence of C(2) acetyl group by using LiI in pyridine. These C(4) analogues of salvinorin A were characterized by radioligand binding assays at cloned human κ-opioid receptor. Several potent agonists have been identified and further



Scheme 4. Synthesis of C(4) amino acid derivatives. Reagents and conditions: (a) EDCI, HOBt, Et_3N, DMF, rt.

Table 1. Affinities (K_i), potencies (EC_{50}), and efficacies of C(4)-modified salvinorin A analogues at human κ-opioid receptor

Compound	K_i[a,b] (nM)	EC_{50}[b,c] (nM)	Efficacy[d]
Acids			
1a[f]	>1000	—[e]	—
1b[f]	48.6 ± 4.4	74.1 ± 2.2	94
Esters			
Sal. A	1.3 ± 0.5	4.5 ± 1.2	106
epi-Sal. A	77 ± 4	307 ± 92	94
2a[f]	28.5 ± 0.9	94.4 ± 4.1	110
2b[f]	>1000	—	—
3	>1000	—	—
4	>1000	—	—
5	>1000	—	—
6	>1000	—	—
7	>1000	—	—
8a[f]	201 ± 26	223.5 ± 3.7	104
8b[f]	>1000	—	—
9	>1000	—	—
10	>1000	—	—
23a[f]	99.6 ± 15.9	58.2 ± 5.7	105
23b[f]	110 ± 15	191 ± 5	102
Amides			
11	>1000	—	—
12	>1000	—	—
13	>1000	—	—
14	>1000	—	—
15a[f]	>1000	—	—
15b[f]	475 ± 41	840 ± 34	95
16	>1000	—	—
17	>1000	—	—
Amino acids			
18a[f]	26.9 ± 1.8	46.7 ± 7.3	95
18b[f]	>1000	—	—
19a[f]	470 ± 92	227 ± 15	105
19b[f]	>1000	—	—
20	>1000	—	—
21a[f]	210 ± 32	348 ± 26	100
21b[f]	>1000	—	—
22	>1000	—	—
U50,488H	1.4 ± 0.3	3.4 ± 0.7	100

[a] K_i values of salvinorin A and analogues in inhibiting [^{3}H]diprenorphine binding to the human κ-opioid receptor.
[b] Each value represents the mean values of at least three independent experiments performed in duplicate.
[c] EC_{50} values in activating the human κ-opioid receptor to enhance [^{35}S]GTPγS binding.
[d] Efficacy determined as the % of maximal response relative to that of U50,488H.
[e] Not determined.
[f] **a** refers to compounds with the natural configuration at C(8), whereas **b** refers to compounds with the unnatural configuration at C(8).

SAR studies to find more potent agonists and antagonists are being pursued.

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