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

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Abstract—Salvinorin A, a compound isolated from the plant *Salvia divinorum*, is a potent and highly selective agonist for the κ opioid receptor. For exploration of its structure and activity relationships, further modifications, such as reduction 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 new full agonists have been identified.

Activation of the κ-opioid receptor (KOR) produces many effects including analgesia, dysphoria, anti-pruritic effect, corticosteroid elevations, water diuresis, and immunomodulation.[1] The KOR also participates in the expression of chronic morphine-induced withdrawal syndromes and mediate the aversive effects of Δ-9-tetrahydrocannabinol.[2,3] Synthetic arylacetamides, including U50,488H, U69,593, spiradoline, enadoline, ICI-204,448 and asimadoline, have been demonstrated to be selective KOR agonists.[4,5] Interestingly, the κ-agonist U69,593 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 mood in humans.[8] Nalfurafine (TRK-820), a potent κ-agonist, is used as an anti-pruritic drug.[9–11]

Recently, salvinorin A, a neoclerodane diterpene isolated from a mint, *Salvia divinorum* native to Oaxaca, Mexico, was identified as one of the most potent naturally occurring opioid agonists with a high selectivity and affinity for KOR.[12–16] Salvinorin A represents a promising lead for the development of more potent and selective KOR agonists and antagonists.[17] However, it was short acting. We initiated a comprehensive drug synthesis and screening program in an attempt to find new KOR agonists or antagonists with higher chemical stability.[18–21] As part of our ongoing investigation, a series of salvinorin A analogues have been synthesized and tested. So far, several potent human KOR (hKOR) full agonists have been found. For instance, the 2-MOM salvinorin-A analog is seven times more potent than salvinorin A.[20] According to our findings as well as those reported by several other laboratories, a limited understanding of SAR has been established which suggests that C(2) and C(4) positions are sensitive and critical sites for binding to KOR with very limited tolerance in terms of size and electronegativity of the substituent group.

As reported previously, salvinorin A can be demethylated to form compound **1a** in pyridine media, accompanied with C-8 epimerization (Scheme 1).[19,22] The analogues with natural configurations at C(2) and C(8) usually show higher affinities and efficacies at hKOR. To further explore the SAR at the C(4) position, the corresponding C(4) alcohol **2** was obtained with 50% yield by using borane–THF complex as the reducing reagent.[22a] The corresponding C(8) epimer of compound **2** can be synthesized following the same

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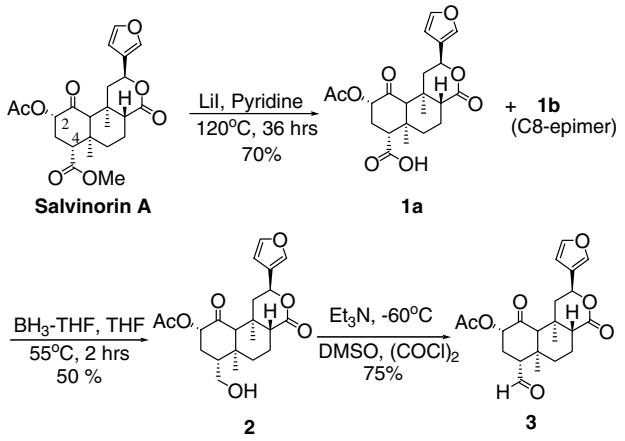
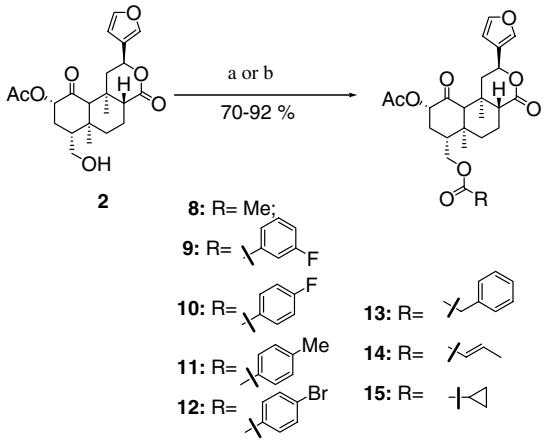


Scheme 1. Synthesis of compounds **2** and **3**.



Scheme 3. Synthesis of C(4) ester derivatives **8–15**. Reagent and conditions: (a) acid, DCC, DMAP, CH_2Cl_2, rt; (b) acyl chloride, DMAP, CH_2Cl_2, rt.

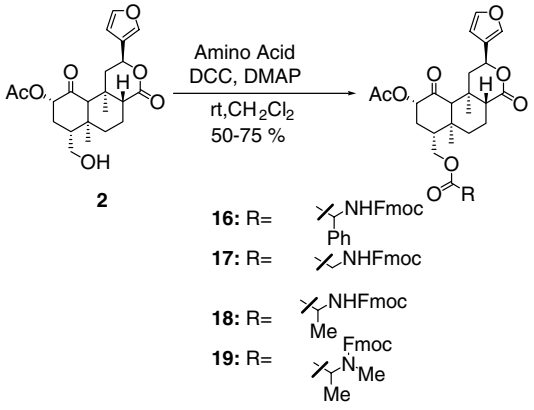
procedure. Compound **2** was then converted to aldehyde **3** by Swern Oxidation in 15 min at −60 °C.[23] Based on Cache® molecular modeling, the C(4) position was more tolerant in terms of the space occupied by its attached groups. Our previous approach was to keep the C(4) carbonyl intact and we synthesized a series of corresponding ester and amide derivatives.[19]

To continue to explore the SAR at the C(4) position, compound C(4)-alcohol **2** was chosen as the key intermediate. Although removal of the C(4) adjacent carbonyl might affect the affinity and selectivity at hKOR, these C(4) ether type derivatives can provide definite answers to the influence of a C(4) adjacent carbonyl group. Following the published standard procedure,[24] compound **2** was treated with silver oxide at 40 °C with various alkyl halides (X = Br or I) (Scheme 2). Both simple alkyl and allylic type ethers were synthesized with moderate to good yield.

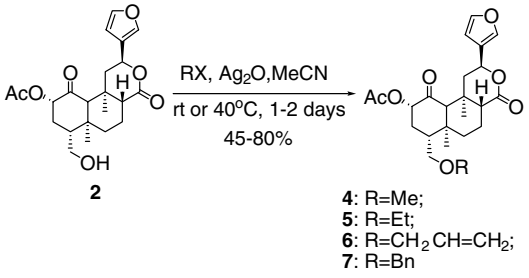
Since carbonyl functional groups play a key role in H-bonding to amino acid residues of macromolecules, the C(4) ester derivatives will allow us to investigate the SAR of a carbonyl group which is located two atoms away from the C(4) position. Compound **2** was treated with dicyclohexylcarbo-diimide (DCC) and 4-(dimethyl-amino)pyridine (DMAP) in the presence of various acids or acid chlorides (Scheme 3). To examine the role of different substituent patterns, several 3- or 4-substituted benzoic acyl chlorides were coupled to the C(4) primary alcohol. Simple unsaturated (**14** and **15**) and propyl acyl chloride were also used.

Nitrogen containing building blocks often play important roles in drug design and provide enhanced interaction between pharmacophore and receptor sites. In a previous report, C(4) amide derivatives showed enhanced interaction and resulted in potent full agonists at hKOR.[19] Similarly, a number of Fmoc-protected amino acid derivatives **16–18** (Scheme 4) and methylated nitrogen compound **19** were synthesized.

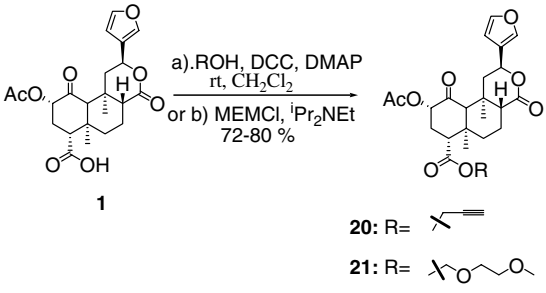
Additional ester derivatives from acid **1** were synthesized and subjected to binding studies (Scheme 5). An ester **20** with a short alkynyl chain was also synthesized.[25]



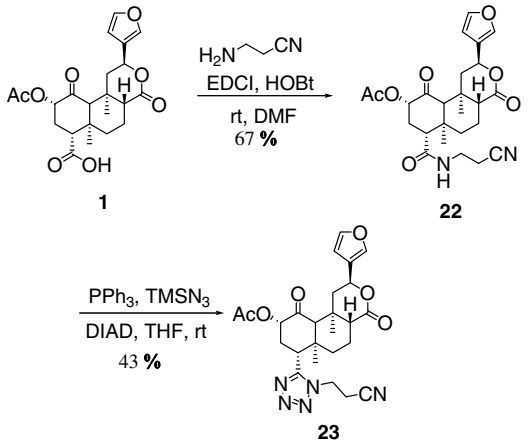
Scheme 4. Synthesis of C(4) amino acid derivatives.



Scheme 2. Synthesis of C(4) ether derivatives **4–7**.



Scheme 5. Synthesis of C(4) ester derivatives **20** and **21**.



Scheme 6. Synthesis of the isostere (**23**) of salvinorin acid.

As reported previously, a methoxymethyl protected salvinorin B at the C(2) position has very high affinity and efficacy at hKOR[18] suggesting that a short straight chain with several oxygen atoms appears to better fit the binding site. The C(4) position could share a similar trend for ligand–receptor interaction. Therefore, compound **21** with MEM side chain was synthesized by using MEMCl, $^{i}Pr_2NEt$ in methylene chloride at room temperature.[26]

Compound **23** was synthesized according to published procedure (Scheme 6).[27] Compound **1** was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimide hydrochloride (EDCI) and amine in DMF, and 1-hydroxybenzotriazole hydrate (HOBt) as the catalyst and base.[28] The resulting compound **22** was then further converted to compound **23** via a series of rearrangement reactions. This compound introduced tetrazole, a new type of N-containing cycle to salvinorin, which is a dramatic change in terms of steric and electronic factors.

Binding affinities of the target compounds to the hKOR were determined by competitive inhibition of [^{3}H]diprenorphine binding to the receptor in membranes of Chinese hamster ovary cells stably transfected with the hKOR (CHO-hKOR).[29] Compounds were evaluated first at 1 μM in radioligand binding assays. For those producing more than 50% inhibition of [^{3}H]diprenorphine binding, competitive inhibition binding curves

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

Compound	4-Substituents	K_i[a,b] (nM)	EC_{50}[b,c] (nM)	Efficacy[d]
Sal-A	C(O)OMe	1.3 ± 0.5	4.5 ± 1.2	106
1a[f]	C(O)OH	>1000	—[e]	—
1b	C(O)OH	48.6 ± 4.4	74.1 ± 2.2	94
2	CH_2OH	43.4 ± 4.93	20.7 ± 1.0	107
3	C(O)H	>1000	—[e]	—
23	Tetrazole	>1000	—	—
Ethers	CH_2O–R			
4	Me	>1000	—	—
5	Et	>1000	—	—
6	$CH_2CH{=}CH_2$	>1000	—	—
7	Bn	>1000	—	—
Esters	$CH_2OC(O)$–R			
8	Me	>1000	—	—
9	*m*-F–Ph	>1000	—	—
10	*p*-F–Ph	>1000	—	—
11	*p*-Me–Ph	>1000	—	—
12	*p*-Br–Ph	>1000	—	—
13	CH_2Ph	>1000	—	—
14	$CH{=}CHCH_3$	>1000	—	—
15	Cyclopropyl C(O)O–R	221 ± 19.1	358 ± 72	92
20	$CH_2C{\equiv}CH$	>1000	—	—
21a[f]	$CH_2OCH_2CH_2OMe$	613 ± 54.1	210 ± 47	98
21b	$CH_2OCH_2CH_2OMe$	>1000	—	—
Amino acids	CH_2–R			
16	Fmoc-Phe	>1000	—	—
17	Fmoc-Gly	>1000	—	—
18	Fmoc-Ala	>1000	—	—
19	Fmoc-*N*-Me-Ala	>1000	—	—
U50,488H		1.4 (±0.3)	3.4 (±0.7)	100

[a] K_i values of salvinorin A and analogs in inhibiting [^{3}H]diprenorphine binding to the human κ-opioid receptor.
[b] Each value represents the mean ± SEM 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 of U50,488H.
[e] Not determined.
[f] **a** refers to the compounds with the natural configuration at C(8) whereas **b** refers to the compounds with the unnatural configuration at C(8).

were generated and K_i values were determined. In addition, the potencies (EC_{50}) and efficacies of the compounds at the κ-receptor 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 compound with its relative efficacy defined as 100. The in vitro pharmacological data for C(4) derivatives are listed in Table 1.

In previous reports, three of the α-amino acid derivatives bearing a carbonyl functional group at C(4) position had K_i values <1 μM.[19] After reversing the connection of carbonyl to the salvinorin skeleton, only one derivative **15** with cyclopropyl side chain exhibited moderate affinity and potency for the κ-receptor ($K_i = 221 \pm 19$ nM, $EC_{50} = 358 \pm 72$ nM), which are 100-fold lower than salvinorin A and U50,488H. The normal ester derivative **21a** also showed moderate affinity and potency for hKOR, which indicates that a polyoxygenated side chain improves affinity compared to a saturated carbon side chain. Interestingly, full reduction of salvinorin acid **1** to alcohol **2** increased the potency and exhibited enhanced efficacy ($EC_{50} = 20.7 \pm 1$ nM with efficacy 107%). However, the efforts to mask the primary alcohol were unsuccessful, because none of the ether derivatives showed sub-micromolar affinities. This also supports the observation that a carbonyl functional group adjacent to C(4) is essential for binding to hKOR.

In summary, a series of new C(4) derivatives of salvinorin A were synthesized. The C(4) primary alcohol and corresponding aldehyde were prepared by selective reduction and oxidation of the C(4) functional group in the presence of C(2) acetyl group. These C(4) analogues of salvinorin A were characterized by radioligand binding assays on hKOR in vitro. Several full agonists with moderate affinities and potencies have been identified which provide a better understanding of the SAR of salvinorin A analogues.[30]

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30. *Experimental procedure (**3**).* A 10 mL schlenk tube containing a magnetic stirring bar was equipped with a low-temperature thermometer under argon atmosphere. The tube was charged with a solution of oxalyl chloride (75 μL, 0.15 mmol) in 1 mL methylene chloride. The solution was cooled to −78 °C in a dry ice–acetone cooling bath. DMSO (24 mg, 0.3 mmol) was added to above solution dropwise via syringe over 5 min. The reaction mixture was warmed up to −60 °C and stirred for 20 min. The alcohol **2** (20 mg, 50 μmol) were dissolved in dry methylene chloride (1 mL) under argon in a flask and was added to reaction mixture dropwise via syringe in 30 min. The reaction was allowed to stir another 30 min, and then slowly warm up to −50 °C. Diisopropylethylamine (0.1 mL) was added to above reaction mixture at

−78 °C, then warmed up to 0 °C. Carefully add 2 mL 1 N HCl aq solution to quench the reaction, then stir another 5 min. The solution was dilute and extracted into CH_2Cl_2 (3× 20 mL). Combining organic phase and washed with satd $NaHCO_3$ soln. and brine. Standard drying and FCC (10–25% EtOAc/hexane gradient) monitored by TLC gave **2** as a white solid (15 mg, 75%); mp 162–164 °C.

NMR data for compound ***3***.^{1}H NMR ($CDCl_3$): 9.91 (1H, s), 7.45 (1H, d, J = 0.9 Hz), 7.39 (1H, t, J = 1.8 Hz), 6.39 (1H, br s), 5.28 (1H, d, J = 11.7 Hz), 5.11 (1H, dd, J = 12.6, 7.0 Hz), 2.78 (1H, dd, J = 13.0, 3.3 Hz), 2.51 (1H, d, J = 3.9 Hz), 2.40–2.30 (3H, m), 2.26 (1H, m), 2.09 (1H, 1H, d, J = 13.2 Hz), 2.17 (3H, s), 2.03–1.96 (1H, m), 1.64 (3H, s), 1.55 (1H, dd, J = 9.0, Hz), 1.30–1.23 (2H, m), 1.01 (3H, s); ^{13}C NMR ($CDCl_3$): 202.0, 199.8, 173.7, 169.9, 144.1, 140.0, 123.3, 108.5, 75.8, 70.3, 64.1, 59.2, 48.3, 45.7, 42.9, 35.0, 34.2, 28.6, 24.7, 20.9, 18.0, 16.9.