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Synthesis and in vitro pharmacological evaluation of salvinorin A analogues modified at C(2)

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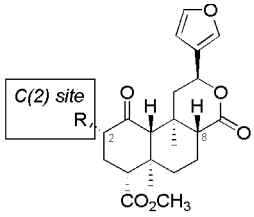
Abstract—Salvinorin A is the only known non-nitrogenous and specific κ-opioid agonist. A series of salvinorin A derivatives were prepared and tested for in vitro activity at the κ-opioid receptor. Unsubstituted carbamate **9** was a potent κ-agonist (EC_{50} = 6.2 nM) and should be more stable than salvinorin A toward metabolic transformations. Compound **10**, containing an *N*-methyl carbamate at C(2), showed partial agonist activity with 81% efficacy when compared with the full agonist U50,488H. No antagonist ligands were observed.

κ-Opioid receptor agonists appear to affect mood in human subjects,[1,2] and recent studies indicate that molecules that selectively bind to κ-opioid receptors have behavioral effects in tests that may reflect mood states in rodents.[3,4] In particular, Carlezon and co-workers demonstrated that the selective κ-agonist U69,593 produces depressive-like effects in behavioral models often used in rats to study depression. In contrast, the selective κ-antagonists norBNI and ANTI produce antidepressant-like effects in these models.[3,4] We are interested in designing and testing novel agents that selectively bind to κ-opioid receptors in order to explore whether selective κ-ligands may be useful to treat symptoms of depression and bipolar disorder.

Classes of compounds known to bind to the κ-receptor include morphinans, benzomorphans, 4-phenyl-piperidines, and arylacetamides. The structure–activity relationships (SARs) for these compounds have been well characterized.[5] Roth and co-workers recently found that the neoclerodane diterpene, salvinorin A (**1**), was a selective and potent agonist at the κ-receptor.[6] Salvinorin A makes an attractive lead compound for several reasons. It is an active κ-agonist (K_i = 1.3 nM) that seems to cross the blood–brain barrier.[7] A recent study suggests that salvinorin A has low toxicity in rodents;[8,9] similarly, both salvinorin A and its parent plant, *Salvia divinorum*, seem to be non-toxic[10] and to induce mood effects in humans.[9,11]



1: salvinorin A, R = CH_3CO_2
2: salvinorin B, R = HO

Little is known regarding the SAR of salvinorin A toward the κ-receptor. In Hall's open field tests conducted when salvinorin A was first isolated and characterized, the C(2) acetate appeared to be important for activity: salvinorin A is behaviorally active, while the deacetylated

Keywords: Salvinorin; Kappa-opioid receptor; Structure–activity relationship; Agonist; Partial agonist.
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salvinorin B is not.[12] Another recent report indicates that the inactive salvinorin B is the major metabolite of salvinorin A,[13] which might explain salvinorin A's short duration of action. Chavkin et al. made a series of esters and carbonates at the C(2) site.[14] The binding data suggest that the size of the C(2) substituent has a significant effect on binding to the κ-receptor, with smaller substituents increasing binding affinity. We further explored the role the C(2) position plays in binding to κ-receptors. We prepared a series of compounds to determine the effect of various functional groups in vitro with two goals: first, to identify candidate compounds with increased stability over salvinorin A in vivo, and second, to investigate if it is possible to change the biological profile of a salvinorin A-based ligand from full agonist at κ-receptors to partial agonist or antagonist. We introduced various ester (**3**–**8**, Fig. 1), carbamate (**9**–**11**), amine (**12**–**14**), and ether (**15**–**20**) substituents at this site and evaluated their in vitro κ-receptor activities.

Salvinorin A (**1**) was isolated from dry *S. divinorum* leaves and purified using a modification of procedures described previously.[12,15] Compound **1** was then converted to salvinorin B (**2**), which served as the starting material for the preparation of the C(2)-modified salvinorins **3**–**20**. Methanolysis of **1** to **2** was best accomplished using a modification of the published procedure.[16] During the course of the reaction, formation of a minor side product was consistently observed. Careful analysis of the 1D and 2D NMR data indicated that epimerization occurred at C(8), which is consistent with earlier observations reported by Koreeda et al.[17] While we did not eliminate the formation of the C(8) epimer, it could be separated from **2** by chromatography or crystallization.

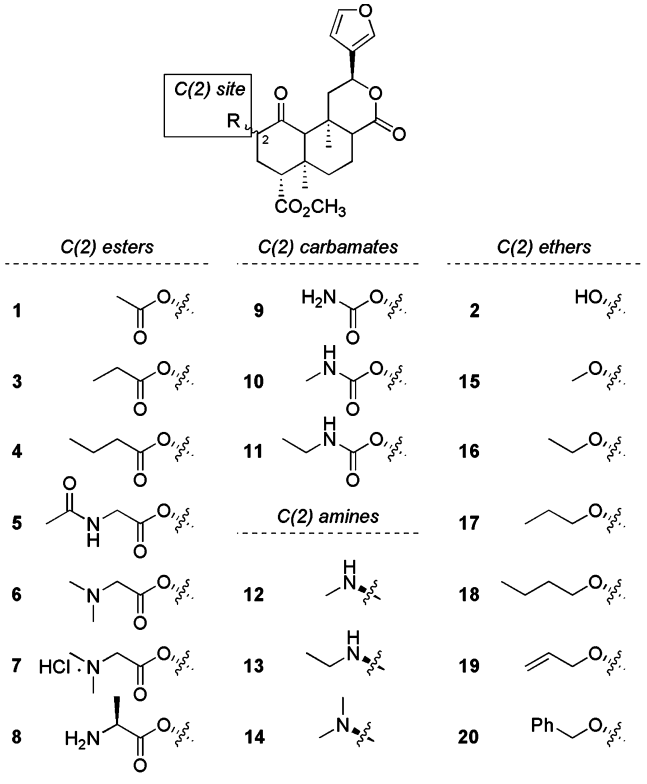
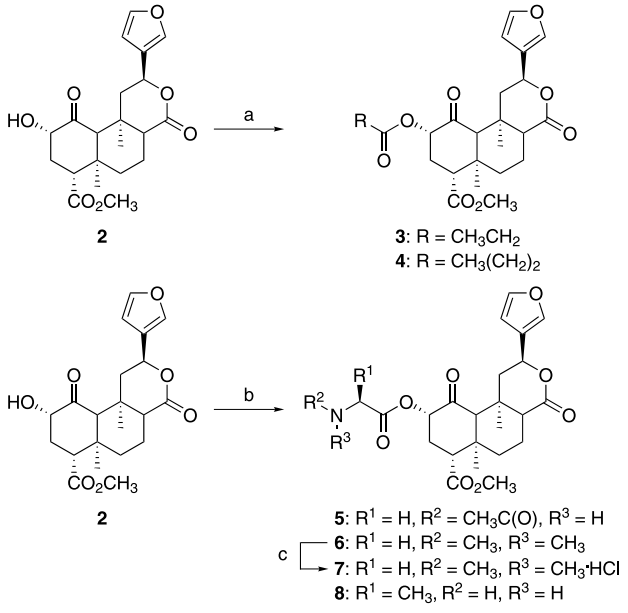


Figure 1. Salvinorin derivatives selected for study.

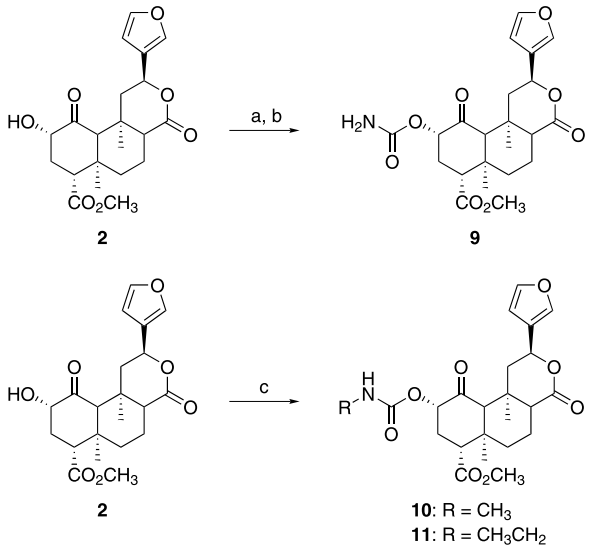
The C(2) esters (**3**–**8**) were synthesized using standard acylation procedures (Scheme 1). Salvinorin B (**2**) was treated with propionyl or butyryl chloride in the presence of pyridine to give **3** and **4**. In order to test the roles charge and hydrogen bond donors may play in binding, several small amino acids were added at C(2) as well. Esters **5** and **6** were formed from **2** and *N*-acetylglycine or *N*,*N*-dimethylglycine, respectively, using dicyclohexylcarbodiimide (DCC) as the coupling agent, and 4-(dimethylamino)pyridine (DMAP) as a catalyst. Ester **6** was then precipitated from CH_2Cl_2 as the hydrochloride salt **7**. Ester **8** was prepared in two steps: **2** was treated with 9′-fluorenylmethoxycarbonyl-*N*-(L)-alanine in the presence of DMAP and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), then was deprotected with piperidine. The glycine and sarcosine analogues were synthesized using a similar sequence, but the free amines were unstable.

Carbamates **10** and **11** were prepared by reacting **2** with methyl isocyanate and ethyl isocyanate, respectively (Scheme 2). For the preparation of **9**, we used the carbamoylation procedure reported by Kocovsky:[18] **2** was first treated with trichloroacetyl isocyanate followed by in situ hydrolysis of the trichloroacetyl moiety to give **9** as a stable white solid in 91% yield from **2**.

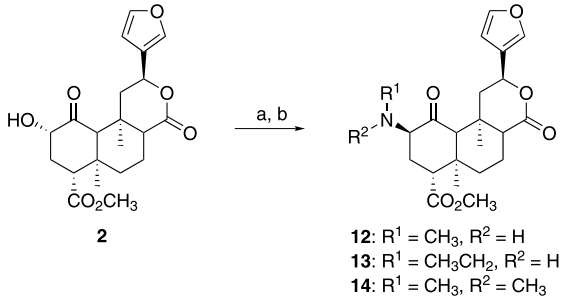
Scheme 3 describes the two-step synthesis of secondary and tertiary amines **12**–**14** from **2**. Accordingly, treatment of **2** with trifluoromethanesulfonic anhydride provided an activated triflate intermediate, which was displaced by methylamine, ethylamine, or dimethylamine to form **12**, **13**, and **14**, respectively. The structure



Scheme 1. Syntheses of C(2) esters. Reagents and conditions: (a) appropriate acyl chlorides, pyridine, 36–61%; (b) appropriate amino acids, DCC or EDCI, DMAP, CH_2Cl_2 (followed by deprotection with piperidine for the preparation of **8**), 51–91%; (c) HCl, Et_2O, 58%.



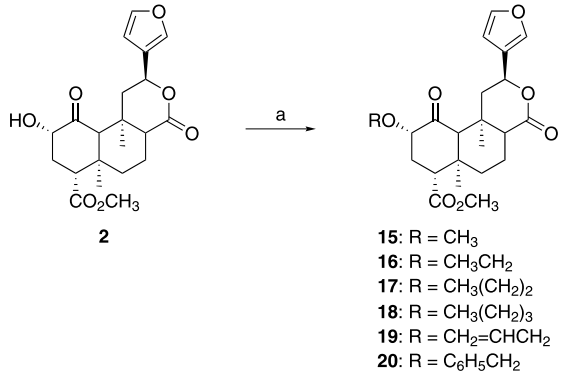
Scheme 2. Syntheses of C(2) carbamates. Reagents and conditions: (a) $CCl_3C(O)NCO$, CH_2Cl_2; (b) Al_2O_3, 91%, over two steps; (c) appropriate isocyanates, DMAP, pyridine, 53–68%.



Scheme 3. Syntheses of C(2) amines. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; (b) R^1R^2NH, THF, 26–29%, over two steps.

of **12** was determined by 1D NOESY experiments, which confirmed that the nucleophilic substitution proceeded with inversion of configuration at C(2).

Incorporation of ethers at C(2) was accomplished according to Scheme 4. Treatment of **2** with the appropriate alkyl halides in the presence of Ag_2O provided compounds **15**–**20** in good yields. Our attempts to prepare branched alkyl ethers failed under these reaction conditions.



Scheme 4. Syntheses of C(2) ethers. Reagents and conditions: (a) appropriate alkyl iodide (or bromide for **20**), Ag_2O, CH_3CN, 36–77%.

Spectral data (^1H NMR, ^{13}C NMR, high-resolution mass) consistent with the proposed structures were obtained for all the compounds prepared in this study.

The binding affinities of **1**–**20** were determined by competitive inhibition of [^3H]-diprenorphine binding to membranes prepared from Chinese hamster ovary cells (CHO-hKOR) stably transfected with the human κ-opioid receptor (hKOR).[19] The potencies and efficacies of **1**–**20** on hKOR were determined by their abilities to regulate [^{35}S]GTPγS binding to membranes of CHO-hKOR cells.[20] The selective full κ-agonist, U50,488H, was used as a reference compound. The in vitro pharmacological data for **1**–**20** are listed in Table 1.

When the seven C(2) esters (**1**, **3**–**8**) were evaluated in in vitro studies, we observed a decrease in affinity as we increased the size of the alkyl ester substituent. These data are consistent with the earlier observations of Chavkin et al.[14] Accordingly, C(2) *n*-propyl (**3**) and C(2) *n*-butyl (**4**) esters showed similar affinity to the κ-receptor in binding (K_i values (nM): **3**, 7.2 ± 0.5; **4**, 4.9 ± 0.6) and efficacy (EC_{50} values (nM): **3**, 20.4 ± 3.4; **4**, 9.9 ± 0.6), with an average 4-fold lower affinity than salvinorin A (K_i = 1.3 ± 0.5 nM; EC_{50} = 4.5 ± 1.2 nM). Introduction of various small amino acids at C(2) (**5**–**8**) led to a complete loss of binding activity (K_i >10 μM), with the nature of the amino acid having no effect on the in vitro binding properties at κ-receptors.

In the C(2) carbamate substituted compounds (**9**–**11**), we observed an important effect on in vitro pharmacological properties as we varied the C(2) moiety. Unsubstituted carbamate **9** was a potent full agonist (K_i = 3.2 ± 0.2 nM; EC_{50} = 6.2 ± 1.4 nM, 99% efficacy). Its K_i and EC_{50} were comparable with salvinorin A and U50,488H (K_i = 1.4 ± 0.3 nM; EC_{50} = 4.5 ± 1.2 nM, 100% efficacy). Introduction of an *N*-methylcarbamate substituent at C(2) led to decreased affinity for the κ-receptor: thus carbamate **10** had lower affinity than **9** and **1** in the κ-receptor binding assay (26- and 64-fold, respectively). Interestingly, **10** displayed partial agonist properties in the [^{35}S]GTPγS binding assay (81% efficacy when compared with U50,488H). Increasing the size of the C(2) carbamate substituent by one additional carbon led to a significant loss of in vitro activity. Thus, ethyl carbamate **11** did not show any appreciable potency at κ-receptors.

The size of the alkyl substituents in the amine series (**12**–**14**) was also critical for activity. Introduction of a methylamine at C(2) gave **12**, which was devoid of binding activity (K_i >10 μM). However, increasing the size of the C(2) secondary amine (**13**) or converting it to a tertiary amine (**14**) significantly increased the affinity for κ-receptors (>100-fold).

Similar SAR patterns were observed in the ether series (**15**–**20**). Methyl ether **15** and salvinorin B (**2**) showed

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

Compound	C(2) substituent	K_i, nM[a,b]	EC_{50}, nM[b,c]	Efficacy[d]
1	CH_3CO_2	1.3 ± 0.5	4.5 ± 1.2	99
3	$CH_3CH_2CO_2$	7.2 ± 0.5	20.4 ± 3.4	94
4	$CH_3(CH_2)_2CO_2$	4.9 ± 0.6	9.9 ± 0.6	97
5	$CH_3C(O)N(H)CH_2CO_2$	>10,000	—[e]	—[e]
6	$(CH_3)_2NCH_2CO_2$	>10,000	—[e]	—[e]
7	$HCl(CH_3)_2NCH_2CO_2$	>10,000	—[e]	—[e]
8	(*S*)-$H_2NCH(CH_3)CO_2$	>10,000	—[e]	—[e]
9	H_2NCO_2	3.2 ± 0.2	6.2 ± 1.4	99
10	$CH_3N(H)CO_2$	83.0 ± 8.5	201 ± 10	81
11	$CH_3CH_2N(H)CO_2$	462 ± 20	>1000	—[e]
12[f]	$CH_3N(H)$	>10,000	—[e]	—[e]
13[f]	$CH_3CH_2N(H)$	28.9 ± 1.0	68.9 ± 5.3	111
14[f]	$(CH_3)_2N$	90.9 ± 2.5	343 ± 12	105
2	HO	155 ± 23	371 ± 49	98
15	CH_3O	220 ± 12	389 ± 76	98
16	CH_3CH_2O	7.9 ± 0.3	18.6 ± 2.6	103
17	$CH_3(CH_2)_2O$	28.7 ± 3.0	67.4 ± 9.9	100
18	$CH_3(CH_2)_3O$	35.8 ± 5.1	104 ± 17	105
19	$CH_2{=}CHCH_2O$	60.1 ± 9.1	145 ± 33	106
20	$PhCH_2O$	75.7 ± 5.9	161 ± 14	102
U50,488H		1.4 ± 0.3	4.5 ± 1.2	100

[a] K_i values in inhibiting [^{3}H]diprenorphine binding to hKOR.
[b] Each value represents the mean of at least three independent experiments performed in duplicate.
[c] EC_{50} values in activating the hKOR to enhance [^{35}S]GTPγS binding.
[d] Efficacy determined as the % of maximal response to U50,488H.
[e] Not determined.
[f] 1D NOESY experiment showed (*R*) configuration at C(2).

very low affinities and potencies at the κ-receptor. Increasing the size of the C(2) ether substituent by one carbon led to a pronounced increase in affinities and potencies at κ-receptors. Ethyl ether **16** was the most potent κ-agonist in the ether series (K_i = 7.9 ± 0.3 nM; EC_{50} = 18.6 ± 2.6 nM, 103% efficacy). Further homologation of the carbon side chain led to compounds with weaker activities (**17**, **18**). Introduction of unsaturation at C(2) had little effect on κ-agonist activity: allyl ether **19** and its isostere, **17**, had comparable K_i and EC_{50} values. The bulkier benzyl ether **20** displayed modest in vitro activity and potency.

These studies suggest that the size of the substituent at the C(2) position is critical for activity at the κ-receptor, with three-atom branched chains giving the highest affinities and potencies (i.e., compounds **1** and **9**). Larger substituents gradually lost affinity, which is consistent with the findings of Chavkin et al.[14] Smaller substituents (**2**, **12**, and **15**) were inactive. The addition of a hydrogen bond acceptor improved binding 2–7-fold (**1** vs **16**, **3** vs **17**, and **4** vs **18**), while the addition of a hydrogen bond donor decreased activity (e.g., **9** vs **1** and **12** vs **15**). Finally, configuration at C(2) may not be a critical factor for binding, though more compounds are needed to confirm this. In summary, unsubstituted carbamate **9** was almost as potent as salvinorin A and should be more stable toward metabolic deacetylation. This derivative suggests potential modifications to salvinorin A that may increase biological stability and maintain high activity. Also of particular interest was the observation that methyl carbamate **10** was a partial κ-agonist in the [^{35}S]GTPγS binding assay. We will determine the selectivities of these κ-ligands toward μ- and δ-opioid receptors before they can be considered as candidates for evaluation in behavioral models used to study mood. Such compounds might stabilize the activity of neural systems that regulate mood, and thus be useful clinically if they are tolerated by humans.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.bmcl.2005.03.113.

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