Letters

Studies toward the Pharmacophore of Salvinorin A, a Potent κ Opioid Receptor Agonist

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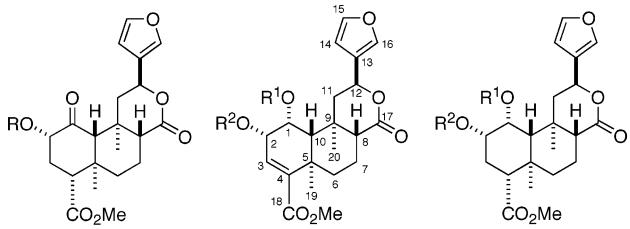
Abstract: Salvinorin A (**1**), from the sage *Salvia divinorum,* is a potent and selective κ opioid receptor (KOR) agonist. We screened other salvinorins and derivatives for binding affinity and functional activity at opioid receptors. Our results suggest that the methyl ester and furan ring are required for activity but that the lactone and ketone functionalities are not. Other salvinorins showed negligible binding affinity at the KOR. None of the compounds bound to μ or δ opioid receptors.

Salvinorin A (**1**)[1] was isolated from *Salvia divinorum*, a traditional medicine of the Mazatec Indians of Oaxaca, Mexico. Infusions of the leaves induce visions, and **1** is a potent hallucinogen in humans.[2] **1** is a selective agonist at the κ opioid receptor (KOR),[3] with comparable potency and efficacy to the synthetic KOR agonists U50488 and U69593.[4] KOR ligands appear to have therapeutic potential against a range of conditions, including pain,[5] nausea,[6] depression,[7] and HIV infection.[8] **1** is the only non-nitrogenous KOR agonist known, with no structural similarity to other such compounds.[3] Given this, **1** represents a valuable lead for the development of more potent and selective KOR ligands. At present, little is known of the compound's structure−activity relationships. The deacetyl compound salvinorin B (**2**), also isolated from *S. divinorum*,[9] is inactive at the KOR, while substitution of more hindered esters at the 2-position reduces or abolishes activity.[4] To probe the importance of other functional groups in **1**, we set out to test related compounds from this plant, as well as semisynthetic derivatives of **1**, for binding affinity and functional activity at cloned opioid receptors.

Salvinorins A (**1**), D (**4**), and E (**5**) were isolated as described previously.[10] Acetylation of **4** gave salvinorin C (**6**).[11] The known 1α-hydroxy derivative **8**[9,12a] and diacetate **9**[11] were prepared by published procedures.

A number of new derivatives of **1** were also synthesized, and the structures were verified by NMR analysis, including DEPT, COSY, HMQC, HMBC, and nOe experiments. As a preliminary step, **1** itself was reexamined using these techniques. This confirmed the original ^{1}H and ^{13}C assignments (based on decoupling experiments)[9] in all cases except the overlapping multiplets of H-6 and H-7. The HMQC spectrum showed cross-peaks from C-6 (δ 38.1 ppm) to multiplets at δ 1.57 and 1.78 ppm and from C-7 (δ 18.1 ppm) to δ 1.63 and 2.15 ppm (see the Supporting Information for the revised assignments).



	R
1	Ac
2	H
3	CHO

	R^1	R^2
4	Ac	H
5	H	Ac
6	Ac	Ac

	R^1	R^2
7	H	H
8	H	Ac
9	Ac	Ac

Until recently, no satisfactory procedure for deacetylation of **1** had been published. Brown reported that KCN in refluxing MeOH/THF gave **2** in quantitative yield,[13a] but in our hands 8-*epi*-**2** was the major product. Additionally, the epimers were not resolved on silica using Brown's solvent system (3% MeOH/CH_2Cl_2). These problems have recently been overcome by Prisinzano and co-workers.[14] Using their conditions (a suspension of **1** and Na_2CO_3 in MeOH), we detected only a trace of 8-*epi*-**2**, which was removed by trituration in MeOH, giving **2** in 76% yield without chromatography. Koreeda and co-workers have on several occasions proposed a complex mechanism for this epimerization, involving cleavage of the C-8/9 bond.[9,13b,15] We see no reason to look beyond enolate formation, followed by protonation from the opposite face.

8-*epi*-Salvinorin A (8-*epi*-**1**), previously reported without experimental detail or characterization data,[16] was formed by treatment of **1** with $NaHCO_3$ in dry DMPU or DMF at 150 °C (addition of water caused deacetylation). In commercial DMPU (98% purity) the $NaHCO_3$ could be omitted, presumably because of a basic impurity; heating in distilled DMPU alone gave no reaction. The configuration of 8-*epi*-**1** was apparent from the lack of a diaxial coupling constant for H-8 (dd, 5.0 and 2.2 Hz compared to 11.6 and 3.1 Hz in **1**). Also, irradiation of H-12 gave a strong nOe enhancement of H-8. The corresponding experiment on **1** gave an enhancement of H-20 but not H-8.

The formate (**3**) was prepared from **2** using formic acid/acetic anhydride mixture.[17] NMR assignments were inferred from the near-identical spectra of **1**. Tetrahydrosalvinorin A (**10**) was synthesized by hydrogenation of **1**, using rhodium catalyst to minimize the hydrogenolysis of the lactone caused by palladium.[9] For characterization, one epimer was separated by HPLC. By ^{1}H NMR, H-12 showed a new coupling to H-13, but its coupling constants to H-11 were scarcely affected, suggesting little change of conformation in the lactone.

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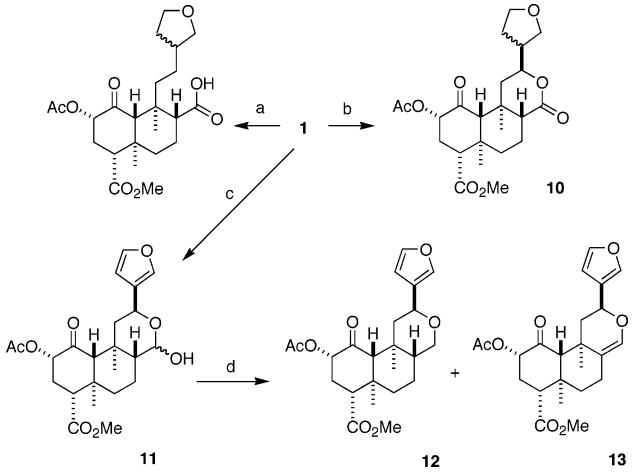
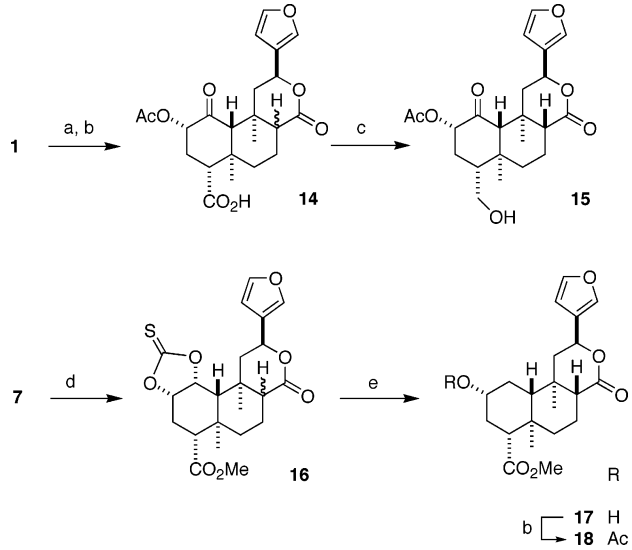
Scheme 1[a]



[a] Reagents: (a) H_2 (1 atm), Pd/C (5%), MeOH, room temp; (b) H_2 (4 atm), Rh/C (5%), CH_2Cl_2/MeOH, room temp; (c) DIBALH, THF, −78 °C; (d) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C.

17-Deoxysalvinorin A (**12**) was synthesized by deoxygenation of known lactol **11**[13c] using Et_3SiH and $BF_3{\cdot}Et_2O$.[18] By ^1H NMR, the H-17 oxymethylene appeared as a doublet, δ 3.58 ppm, coupling to H-8 (COSY cross-peak). As with **1**, irradiation of H-12 gave a strong nOe enhancement of H-20 rather than H-8, confirming the configuration at C-8. The enol ether (**13**) was also formed as byproduct. An attempted alternative route to **12**, using Amberlyst 15 resin,[19] instead gave **13** in 76% yield. Indeed, **11** proved extremely prone to elimination; storage overnight in $CDCl_3$ at −20 °C gave 52% yield of **13**, presumably catalyzed by DCl. The quaternary C-8 peak (δ 117.0 ppm) of **13** showed HMBC correlations to H-6, -7, and -17. A long-range coupling (1.8 Hz) was evident between one of the H-7 protons and H-17. Formation of the 18-hydroxy derivative **15** proved challenging. We decided to proceed via borane reduction of the acid **14**; given the base sensitivity of **1**, the acid would be derived by nucleophilic cleavage of the methyl ester. Of many procedures tried, only lithium ethanethiolate (LiSEt) in DMPU proved satisfactory. This was based on a published procedure (LiSMe in HMPA),[20] modified to avoid the use of methanethiol (intense odor) and HMPA (carcinogen). Ester cleavage was accompanied by deacetylation; standard acetylation conditions gave the acid **14** in good yield as an inseparable mixture of 8-epimers (methylation of a small portion with CH_2N_2 in Et_2O/CH_2Cl_2 at 0 °C gave a mixture of **1** and 8-*epi*-**1** by TLC and ^1H NMR). The acid mixture was then treated with $BH_3{\cdot}THF$ at 55 °C, giving the desired alcohol **15** in low yield (23%), along with its 8-epimer. The COSY spectrum of **15** showed a cross-peak between the complex H-4 multiplet (δ 1.89 ppm) and the diastereotopic H-18 oxymethylene (δ 3.94 and 3.49 ppm). The chemical shifts and coupling constants for H-8 and H-12 were near-identical to those of **1**, and irradiation of H-12 gave a strong nOe enhancement of H-20 rather than H-8, confirming the configuration at C-8. Deoxygenation of the ketone (to give **18**) was also challenging. The two-step route via hydride reduction of a tosylhydrazone was attempted, but **1** proved unreactive toward tosylhydrazide under a range of conditions, including sonication and microwave irradiation. We therefore decided to reduce the ketone

Scheme 2[a]



[a] Reagents: (a) LiSEt, DMPU, 55 °C; (b) Ac_2O, DMAP, pyridine, room temp; (c) BH_3, THF, 55 °C; (d) thiocarbonyldiimidazole, DMF, 90 °C; (e) Bu_3SnH, AIBN, toluene, 80 °C.

prior to deoxygenation. The standard approaches to hydroxyl deoxygenation, involving hydride reduction of sulfonate derivatives or radical reduction of thiocarbonyl derivatives,[21] would require derivatization of the 1α-hydroxyl group, which is extremely hindered and unreactive. For example, Valdés and co-workers found that acetylation of diol **7** gave **8** rather than diacetate **9**[11] but overcame this obstacle by installing an ortho ester between the two hydroxyl groups. We took an analogous approach to radical deoxygenation, employing the cyclic thionocarbonate **16**. This was formed in high yield by the reaction of **7** with thiocarbonyldiimidazole in DMF. Since these conditions again caused epimerization, and the epimers were inseparable on silica gel, separation of the epimers of starting material **7** was superfluous. Under standard radical reduction conditions (Bu_3SnH and AIBN in toluene at 80 °C),[22] **16** then gave the separable deoxy compounds **17** and 8-*epi*-**17** in 47% combined yield, along with a byproduct (presumably the 1-hydroxy regioisomer) that was not characterized due to contamination by tin compounds. Acetylation of **17** gave 1-deoxysalvinorin A (**18**). The H-2 multiplet (δ 4.74 ppm) showed the expected couplings to H-1 and -3 (COSY), including two diaxial couplings (11 Hz).

The target compounds were screened in radioligand binding assays at cloned opioid receptors. None of the compounds showed significant binding to μ or δ opioid receptors at 1 μM; binding affinities at the KOR are listed in Table 1. Those with submicromolar affinity were also screened for functional activity using a calcium flux assay (see Supporting Information for details of all assays). Since hindered esters at the 2-position interfere with binding to the KOR,[4] we suspected that the less-hindered formate **3** might prove more potent, but in fact both affinity and potency were lower; the acetoxy group is therefore the optimal alkyl chain length. Salvinorin C (**6**) showed negligible binding affinity compared to **1**. Consistent with this, in recent human testing **6** showed no psychoactivity.[23] Previously, however, a mixture of **1** and **6** showed significantly greater sedative activity in mice than pure **1**.[11] It is

Table 1. Binding Affinities[a] and Functional Activities[b] at Cloned KORs

compd	$K_i \pm$ SEM (nM)	$EC_{50} \pm$ SEM (nM)	$E_{max} \pm$ SEM (%)
1	4 ± 1	46 ± 8	100 ± 19
8-*epi*-**1**	163 ± 50	244 ± 102	78 ± 17
3	18 ± 2	315 ± 35	108 ± 11
4	>10000		
5	>10000[c]		
6	1022 ± 262		
8	1125 ± 365		
9	>10000		
10	156 ± 18	126 ± 36	108 ± 14
11	59 ± 11	78 ± 21	107 ± 5
12	6 ± 1	223 ± 60	103 ± 13
13	6 ± 2	624 ± 200	116 ± 10
15	347 ± 53	>10000	
18	18 ± 2	141 ± 43	122 ± 27

[a] Binding against [^{3}H]U69,593 in cloned rat KORs expressed in HEK 293 cells. [b] Intracellular Ca^{2+} mobilization. [c] Against [^{3}H]diprenorphine.

possible that these compounds undergo some in vivo interaction not apparent in our assay; however, the mouse assay used appears to have been confounded.[12b] Salvinorins D (**4**) and E (**5**) were inactive in our binding assay. Since the remaining terpenoids isolated from *S. divinorum*[10,24] lack the 2-ester essential to binding, it appears that **1** is the sole KOR agonist present in the plant. Another implication of these results, along with the very low affinities of **8** and **9**, is that reduction of the ketone to an α-hydroxy or -acetoxy group dramatically reduces or abolishes binding affinity. This was surprising; molecular modeling indicated that this modification would not affect binding.[3] Although the very low affinities of **5**, **6**, **8**, and **9** might appear to suggest that the ketone is part of the pharmacophore, the relatively high affinity and potency of 1-deoxy compound **18** show that it is not. This suggests that a 1α-hydroxy/acetoxy group interacts unfavorably with the KOR. Curiously, saturating the 3,4-double bond in **5** raises binding affinity (compare **8**) but has the opposite effect in **6** (compare **9**).

The substantially reduced affinities of 8-*epi*-**1** and tetrahydro derivative **10** suggest that the furan ring is part of the pharmacophore, as predicted.[3] However, the reduction in potency in the functional assay was far smaller, particularly in **10**. These compounds represent relatively minor modifications of **1**; more drastic modifications, less amenable to a semisynthetic approach, would be required to definitively resolve the role of the furan ring. By contrast, the predicted role of the methyl ester is strongly confirmed by the results for 18-hydroxy derivative **15**; the compound appears to be an antagonist, since it binds but does not activate the receptor. Contrary to predictions, the high affinities of **11**−**13**, all full agonists, show that the lactone carbonyl is not essential for binding or activity. However, **12** and especially **13** showed reduced potency in the functional assay. One possible explanation for inaccuracies in the initial model is differences in binding orientation between **1** and its derivatives; for instance, the 1-acetoxy group in **6** may serve as a poor substitute for the 2-acetoxy group in **1**.

In conclusion, our results indicate that **1** is the only compound from *S. divinorum* with significant affinity at the KOR. Other salvinorins show negligible affinity, and other compounds reported from the plant lack the features required for binding. In addition, we have tested at least one modification to every functional group in **1** and identified those not essential for binding to the KOR: namely, the lactone and ketone. This information should prove valuable in the development of selective KOR ligands, simplifying the design of synthetic analogues of **1**. Compound **15** suggests that still further simplification may be possible, particularly in the development of KOR antagonists. It is also noteworthy that none of the target compounds bound to μ or δ opioid receptors. This suggests that, as a lead compound, **1** will withstand considerable modification without loss of selectivity. Finally, given the lack of structural similarity to other opioids, these results should be of value in modeling opioid receptors.

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Supporting Information Available: Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra, and IUPAC/NIST Chemical Identifiers (INChIs) of the target compounds (the spectra of **4** and **5** were published previously).[10] This material is available free of charge via the Internet at http://pubs.acs.org.

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