Synthetic studies of neoclerodane diterpenes from *Salvia divinorum*: Exploration of the 1-position

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Abstract—Modification of the C-1 ketone of salvinorin A (**2a**) produces analogues with opioid antagonist properties. Of particular significance is the finding that 1-deoxo-1,10-dehydrosalvinorin A (**11a**) is a moderately potent antagonist at all three opioid receptor subtypes, and that herkinorin (**2b**), a μ agonist, is converted to a weak antagonist by removal of the C-1 ketone (**3b** and **11b**). These observations suggest that the ketone of **2b** is a key structural feature responsible for μ agonist activity.
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The opium poppy, *Papaver somniferum*, has been used for centuries for the relief of pain and to induce sleep. Among the most important constituents in opium are the alkaloids morphine (**1a**) and codeine (**1b**) (Fig. 1). Many of the opiate agonists and antagonists derived from these alkaloids are essential for the effective practice of modern medicine. However, new agents are needed with fewer side effects and developing these agents can allow the exploration of the mechanisms of action that induce tolerance and dependence.[1,2]

Morphine and related opiates exert their major pharmacological effects by interacting with opioid receptors (μ, δ, and κ).[3] The search for potent agonists and antagonists selective for each of these opioid receptors has engaged the interest of medicinal chemists for many years because of their potential as therapeutic agents and pharmacological tools.[3]

As a consequence of target drug design and synthetic efforts, we have achieved a better understanding of opioid receptors. Moreover, these efforts have opened new avenues for chemical investigation. Salvinorin A (**2a**), a
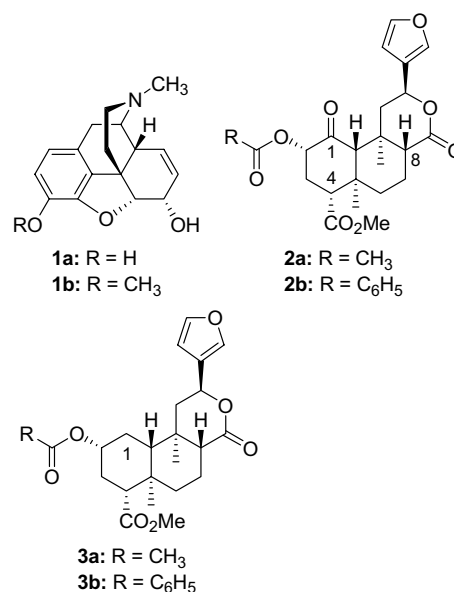
Figure 1. Structures of morphine (**1a**), codeine (**1b**), salvinorin A (**2a**), herkinorin (**2b**), 1-deoxosalvinorin A (**3a**), and 1-deoxoherkinorin (**3b**).

neoclerodane diterpene, is a potent and selective κ opioid receptor (κOR) agonist,[4] and represents a novel scaffold for the development of opioid ligands with potentially reduced side effects.[5] Several recent reports

have begun to characterize the structure–activity relationships of **2a** at κ opioid receptors.[6–8] Our own efforts have identified analogues of **2a**, such as **2b**, with affinity for κORs as well as μORs and δORs.[9–14]

As part of a program to develop novel agents to treat drug dependence, we have prepared several C-1 modified analogues of **2a**. These compounds were prepared to better elucidate the pharmacophore of **2a** at opioid receptors. In particular, we were interested in better understanding the role of the C-1 ketone. In a previous report, **3a** was prepared from **2a** in four steps.[6,11] To facilitate the preparation of additional analogues, a new synthetic route to **3a** and related analogues was undertaken.

Salvinorin A (**2a**), isolated from *Salvia divinorum*,[15] was reduced with an aqueous solution of NaBH₄ in THF to afford 1α-hydroxysalvinorin A (**4a**)[16] in 77% yield (Scheme 1). There was no evidence of C-8 epimerization, and the major byproducts from this reaction were reduction at both C-17 and C-1 (9%). Similar conditions using **2b** afforded 1α-hydroxyherkinorin (**4b**) in 45% yield. Treatment of **4a** with methanesulfonic anhydride and DMAP in CH₃CN afforded 1α-mesyloxysalvinorin A (**5**) in 99% crude yield. Basic hydrolysis of mesylate **5** in MeOH/

CH₂Cl₂ at −10 °C gave crystalline 1α-mesyloxysalvinorin B (**6**) in 70% overall yield from **4a**. Attempts to directly reduce **5** to **3a** or to displace the mesyloxy group in **5** with a halide followed by reduction were unsuccessful. Similarly, efforts to convert **4a** into **3a** using Barton deoxygenation were unsuccessful. To circumvent this problem, **6** was reacted with DMAP in DMSO at 170 °C to afford a mixture of 1-deoxo-1,10-dehydrosalvinorin B (**7**) in 76% yield and 2-keto-1-deoxosalvinorin A (**8**) (22%). Oxidation of the allylic alcohol with MnO₂ in toluene gave α,β-unsaturated ketone **9** in 78% yield. Reduction of **8** with NaBH₄ in acetonitrile gave 1-deoxosalvinorin B (**10**)[6] in 41% yield. Although reduction of **8** proceeded in high yield, it was complicated by a small amount of a byproduct, which ran just ahead of **10** on TLC. Two recrystallizations from ethyl acetate/*n*-hexanes removed this material, which is believed to be the 2β-epimer based on the finding that oxidation of the mixture produced pure **8**. Alternatively, **10** could be prepared from **6** in 47% overall yield using a sequence of elimination, followed by Jones' oxidation of the crude mixture of **7** and **8**, followed by reduction of the crude oxidation product using a new selective reducing agent derived from NaBH₄ and DMAP. Treatment of **10** with acetic anhydride or benzoyl chloride and a catalytic amount of DMAP afforded 1-deoxosalvinorin A (**3a**)[6] and 1-deoxoherkinorin (**3b**), respectively. The reaction of
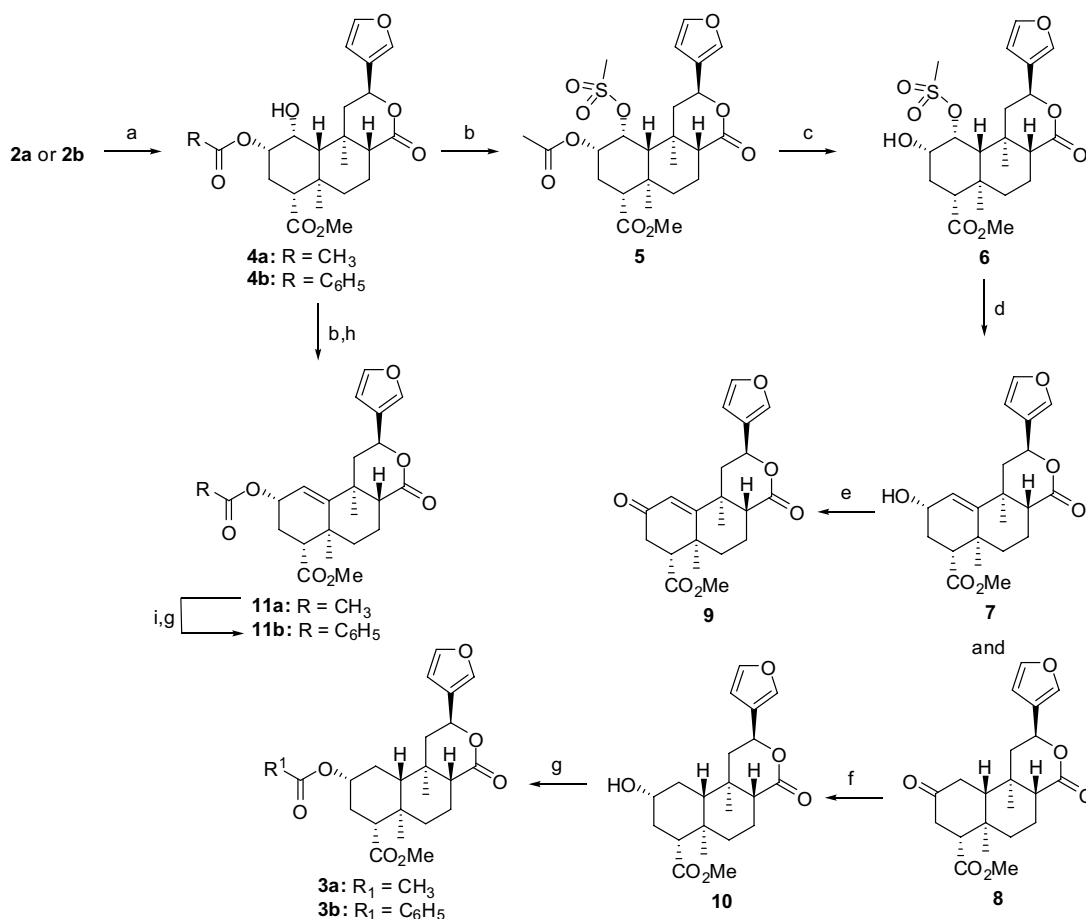
Scheme 1. Reagents and conditions: (a) NaBH₄, THF/H₂O; (b) (CH₃SO₂)₂O, DMAP, CH₃CN; (c) NaOH, MeOH, CH₂Cl₂; (d) DMAP, DMSO; (e) MnO₂, toluene; (f) NaBH₄, CH₃CN; (g) acetic anhydride or benzoyl chloride, DMAP, CH₂Cl₂; (h) trimethylphenylammonium chloride, CH₃CN; (i) Na₂CO₃, MeOH.

Table 1. Comparison of agonist activities

Compound	[³⁵S]GTP-γ-S		G$_{\alpha16}$-hκOR calcium flux	
	κ EC$_{50}$ ± SE (nM)	κ E$_{max}$[a] ± SE	EC$_{50}$ ± SE (nM)	E$_{max}$[a] ± SE
(−)-U69,593	330 ± 70	100 ± 7	2.25 ± 0.82	100 ± 12
2a	45 ± 10[b]	108 ± 4[b]	1.24 ± 0.56	118 ± 3
3a	280 ± 60	49 ± 5	3.34 ± 0.11	93 ± 3
5	2700 ± 400	95 ± 12	NT[c]	NT[c]

[a] E_{max} is relative to the maximum stimulation of binding observed with U69,593 run in parallel with the test compounds.
[b] Values from Ref. 10.
[c] Not tested, no experiments were done to determine value.

4a with methanesulfonic anhydride followed by heating with trimethylphenylammonium chloride in CH$_3$CN produced **11a** in 76% yield. Finally, treatment of **7** (obtained from hydrolysis of **11a**) with benzoyl chloride using similar conditions for **3b**, gave 1-deoxo-1,10-dehydroherkinorin **11b**. Efforts to reduce **11a** directly to **3a** resulted in hydrogenolysis of the allylic acetate as the major product.

Compounds **3a**, **3b**, **4a**, **4b**, **5**, **9**, **11a**, and **11b** were evaluated for opioid receptor activity as described previously (Tables 1 and 2).[10] Removal of the 1-ketone group in **2a** (**3a**) decreased agonist activity 6-fold at κORs (EC$_{50}$ = 280 nM vs. EC$_{50}$ = 45 nM). This result is in general agreement with previous work which showed 3-fold loss in activity.[6] However, in our hands **3a** was found to have lower efficacy than previously reported (E_{max} = 50% relative to U69,593 vs. E_{max} = 122% relative to **2a**). It should be noted that **2a** has higher efficacy than U69,593.[10] In addition, we found **3a** to also have significant antagonist activity at μORs (K_e = 170 nM) and δORs (K_e = 100 nM). One potential reason for this discrepancy is the different functional assays used to characterize the compounds: [³⁵S]GTP-γ-S binding[17] versus the mobilization of internal calcium.[18,19] To further probe this possibility, we evaluated **3a** in our own calcium mobilization assay.[10] In this assay, **3a** was also 3-fold less potent (EC$_{50}$ = 3.34 nM vs. EC$_{50}$ = 1.24 nM) than **2a**, but it remained less efficacious (E_{max} = 93% vs. E_{max} = 118%). However, the efficacy of **3a** increased relative to that measured in the [³⁵S]GTPγS binding assay such that it was nearly a full agonist relative to U69,593 (E_{max} = 100%). This increase in efficacy was not accompanied by a change in relative potency compared to our standard compound, U69,593 (EC$_{50}$ = 2.25 nM), in keeping with our [³⁵S]GTPγS assay data.

Table 2. Inhibition of agonist stimulated [³⁵S]GTP-γ-S binding by compounds in cloned human opioid receptors

Compound	K_e ± SD (nM)		
	μ, DAMGO	δ, DPDPE	κ, U69,593
3a	170 ± 20	100 ± 3	NT[a]
3b	4700 ± 910	8780 ± 2130	580 ± 30
4a	2300 ± 80	1790 ± 510	240 ± 5
4b	1830 ± 930	2900 ± 150	1050 ± 600
9	700 ± 220	3500 ± 1700	460 ± 70
11a	200 ± 30	400 ± 90	570 ± 140
11b	1740 ± 230	2300 ± 20	580 ± 210

[a] Not tested, no experiments were done to determine value; the K_e data represent means ± SD from 2 independent determinations.

There are several potential explanations for the differences in **3a** efficacy in our assays. The effects of agonists, and in particular partial agonists, can vary considerably depending on the number of cell surface-expressed receptors, leading to a greater proportion of receptors in an active receptor conformation recognized by the agonist and, thus, increased efficacy.[20–22] The type of G-protein to which a GPCR is coupled can also affect efficacy.[23–25] In keeping with these observations, it is not surprising that the partial agonist **3a** displays assay-specific efficacies. Despite the differences in efficacy between our two assays, it is clear, **3a** has agonist properties and we undertook further studies to determine the role of structure in conferring this activity.

Replacement of the acetyl group in **3a** with a benzoyl group (**3b**) resulted in an antagonist at μ, δ, and κ receptors. Benzoate **3b** had highest activity at κORs (K_e = 580 nM). This change also decreased antagonist activity 28-fold at μORs (K_e = 4700 nM vs. K_e = 170 nM) and greater than 88-fold at δORs (K_e = 8780 nM vs. K_e = 100 nM). This finding is curious given our previous investigations that showed that introducing an aromatic moiety increased activity at μORs compared to κORs.[11,13] This would suggest that the C-1 deoxo analogues may be interacting at μORs in a non-identical manner compared to C-1 keto analogues. Reduction of the 1-ketone in **2a** to the corresponding α-alcohol (**4a**) changed the efficacy at κORs from a full agonist (**2a**: E_{max} = 108%) to an antagonist (**4a**: K_e = 240 nM). Alcohol **4a** was 3-fold less selective over μORs (K_e = 2300 nM) and 2-fold less selective for δORs (K_e = 1800 nM). Addition of a benzene ring to **4a** (**4b**) decreased activity 2-fold at κORs (K_e = 450 nM vs. K_e = 240 nM), whereas the introduction of a mesylate group (**5**) resulted in a loss of antagonist activity at κ receptors (EC$_{50}$ = 2700 nM).

Finally, introduction of a 1,10-alkene functionality was explored. This modification was chosen for several reasons. First, it would probe the role of sp^2 hybridization at C-1 and second it would also examine the effect of stereochemistry at the C-10 position on the activity of **3a**. The presence of the 1,10-alkene (**11a**) resulted in a switch of efficacy from partial agonist (**3a**: κ EC$_{50}$ = 340 nM, E_{max} = 49% relative to U69,593) to antagonist (K_e = 570 nM). Furthermore, **11a** had higher antagonist activity at μORs (K_e = 200 nM) and δORs (K_e = 400 nM). When compared to **3a**, **11a** had similar antagonist activity at μORs (K_e = 200 nM vs. K_e = 170 nM) and reduced

activity at δORs ($K_e = 400$ nM vs. $K_e = 100$ nM). Replacement of the acetyl of **11a** with a benzoyl group (**11b**) decreased activity 9-fold at μORs ($K_e = 1740$ nM vs. $K_e = 200$ nM) and 6-fold at δORs ($K_e = 2310$ nM vs. $K_e = 400$ nM), but had little effect on κORs ($K_e = 720$ nM vs. $K_e = 570$ nM). This also suggests that the 1,10-dehydro analogues are not interacting in a similar manner to analogues which contain a C-1 keto group. To further confirm this observation, we evaluated ketone **9**. If these analogues were interacting in an identical manner to their C-1 ketone analogues, removal of the acetyl group would have a deleterious effect on activity. However, **9** was found to have similar antagonist activity as **11a** at κORs ($K_e = 460$ nM vs. $K_e = 570$ nM) but reduced activity at μORs ($K_e = 700$ nM vs. $K_e = 200$ nM) and δORs ($K_e = 3500$ nM vs. $K_e = 400$ nM). This finding also suggests that the 1,10-alkene analogues may be a productive scaffold for the development of selective opioid antagonists. This, however, needs validation through further synthesis and testing.

The results presented here indicate that the interaction of neoclerodane diterpenes with opioid receptors may be more complicated than first thought. Previous work in the opioid field has highlighted the importance of the message-address concept in the recognition of ligands by opioid receptors.[26] This work was originally applied to the recognition elements of peptide hormones such as dynorphin,[27] but later work illustrated its utility in the design of selective non-peptide opioid antagonists.[28] Based on our previous finding that the introduction of a benzene ring increases activity at the μOR,[11] we synthesized **3b** and **11b**. It was thought that perhaps the benzene ring of **2b** was functioning as an address moiety to μORs. However, this proved not to be the case. It is therefore possible that once the recognition elements of neoclerodane diterpenes with opioid receptors are better understood, more selective agents for μORs and δORs can be better designed.

In summary, modification of the C-1 ketone of **2a** has been found to alter its potent κ opioid agonist activity to produce, in almost every case, compounds that are antagonists at all three opioid receptor subtypes. The major exception is **3a**, which is a partial agonist at κ receptors but is, however, a moderately potent antagonist at μ and δ receptors. These studies suggest that the C-1 ketone of **2a** imparts agonist activity and that analogues lacking this structural feature bind to opioid receptors in a different manner, generally producing antagonist responses. Last, one should keep in mind that partial agonists have the potential to have different efficacies in different tissues or brain regions depending on the number of target receptors expressed and their effector coupling, and caution must be used in comparison of data from different in vitro functional assays.

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