Published in final edited form as:

J Med Chem. 2017 May 11; 60(9): 3866–3878. doi:10.1021/acs.jmedchem.7b00148.

Addressing Structural Flexibility at the A-Ring on Salvinorin A: Discovery of a Potent Kappa Opioid Agonist with Enhanced Metabolic Stability

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Abstract

Previous structure-activity studies on the neoclerodane diterpenoid salvinorin A have demonstrated the importance of the acetoxy functionality on the A-ring in its activity as a kappa opioid receptor agonist. Few studies have focused on understanding the role of conformation in these interactions. Herein we describe the synthesis and evaluation of both flexible and conformationally restricted compounds derived from salvinorin A. One such compound, spirobutyrolactone **14**, was synthesized in a single step from salvinorin B and had similar potency and selectivity to salvinorin A ($EC_{50} = 0.6 \pm 0.2$ nM at κ ; $>10,000$ nM at μ and δ). Microsomal stability studies demonstrated that **14** was more metabolically resistant than salvinorin A. Evaluation of analgesic and anti-inflammatory properties revealed similar *in vivo* effects between **14** and salvinorin A. To our knowledge, this study represents the first example of bioisosteric replacement of an acetate group by a spirobutyrolactone to produce a metabolically resistant derivative.

Graphical abstract

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Supporting Information. Data includes ¹H NMR, ¹³C NMR spectra, HPLC chromatograms for **4**, **14**, **20-25**, and supplementary figures and tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Chemical derivatization and modification of central nervous system (CNS)-active natural products have provided valuable, sometimes serendipitous, scientific and medicinal discoveries in the past that have furthered our understanding of the mind and body (Figure 1).¹ Notable examples include Albert Hoffmann's discovery of LSD (**1**) from his semisynthesis work on lysergic acid (**2**), the development of the Food and Drug Administration (FDA) approved antiemetic synthetic cannabinoid Nabilone (**3**) from THC (**4**), the antitussive medication dextromethorphan (**5**), which contains an enantiomeric morphine (**6**) core, and finally, the phenyltropane WIN 35,428 (**7**) has facilitated imaging studies of the dopamine transporter and was the result of synthetic efforts aimed at enhancing the metabolic stability of cocaine (**8**).² Over the last decade, the natural product salvinorin A (**9**) has emerged as a valuable non-nitrogenous scaffold for designing compounds primarily targeting the kappa opioid receptor (KOR), and more recently, the salvinorin A scaffold has also been used to target the mu-opioid receptor (MOR).³ The progress of organic chemistry plays a pivotal role in such discoveries, as the major challenge in natural product semisynthesis is the discovery and optimization of chemical procedures able to elicit selective transformations in the presence of the complex chemical functionality provided by nature.⁴

In the case of salvinorin A, chemical liabilities include: a furan ring prone to oxidative degradation, an epimerizable stereocenter at C8, and/or a hydrolyzable ester, lactone, and acetate group (Figure 2). Combined, these groups narrow the available pool of viable chemical transformations, and it is often challenging to predict the success of a reaction without prior experimental rigor. Limited only by these inherent liabilities of completing chemical transformation on a complex natural product, extensive modification of the salvinorin A scaffold has produced hundreds of biologically active compounds to date.⁵ Previously obtained data have demonstrated that manipulation of functionality on the A-ring can elicit significant changes in activity at opioid receptors (Figure 3).⁶ Structure-activity relationship (SAR) studies and computational models suggest that a key receptor interaction, perhaps a hydrogen-bonding situation, arising from substitution at the C2 position on salvinorin A may exist.^{3d} Supporting this hypothesis, replacement of the C2 acetoxy group with hydrogen as in **11** has produced a compound that is completely inactive at the KOR (*vide infra*). Alternatively, the introduction of unsaturation to the A-ring and conversion of the acetate to a benzoate as in Kurkinorin (**10**) surprisingly led to a completely selective agonist at the MOR with no KOR activity.⁷

The KOR is an intriguing target in the CNS and chemical probes have played a critical role in understanding its purpose in the mind. In humans, activation of these receptors by other known agonists besides salvinorin A (**9**), such as enadoline (**12**, Figure 4), have also been shown to elicit hallucinations, dissociation, and feelings of dysphoria.⁸ Furthermore, the endogenous KOR peptide agonist dynorphin is detected in elevated levels under conditions of stress in mammals.⁹ Collectively, these evidence suggest that the KOR may contribute to an evolved mechanism responsible for the mediation of damaging or aversive behaviors.¹⁰ Specifically, the ability of KOR agonists to suppress mesolimbic dopamine release within reward circuitry is thought to contribute to their dysphoric effects.¹¹ As such, the use of

exogenous KOR agonists in a therapeutic setting to modulate dopaminergic signaling may help mitigate drug-seeking behaviors observed in people suffering from addiction and represents a potentially viable mechanism by which to approach this unresolved problem in society.¹²

KOR agonists have also been shown to possess antinociceptive effects and modulate inflammation.¹³ The lack of rewarding effects of KOR agonists suggests that they may hold potential for development as pain medications with reduced abuse potential.¹⁴ Additionally, it has been hypothesized that the dysphoric effects of KOR agonism may be the result of different KOR-mediated pathways than the analgesic effects; therefore, it may be possible to decouple the undesirable hallucinogenic/dysphoric properties of agonism at the KOR by the development of functionally selective ligands.¹⁵ Through the development of chemical transformations that are tolerated by the sensitive functionalities in **9**, new KOR agonists can be developed to overcome some of the shortcomings of **9** itself for the potential use as drug abuse or pain therapies. The work outlined below has demonstrated a variety of such chemical transformations capable of providing access to both conformationally flexible and rigid compounds derived from **9**; biological activities of these novel compounds were subsequently established.

Results

Chemistry

Salvinorin B (**13**) is readily attainable without the need for chromatography by simple hydrolysis of **9** and serves as a useful launching point for chemical derivatization.¹⁶ The transformations outlined below were discovered by screening conditions able to take advantage of the C1-C2 α -hydroxy ketone functionality present in **13**. In this case, the α -hydroxy ketone was regarded as a single functional group rather than a discrete alcohol or ketone. Our previous work has suggested that neither of these positions behave with typical reactivity. For example, oxime formation at the C1 ketone, an otherwise facile reaction, has never been accomplished on salvinorin A/B in our hands. Given the nature of this functionality at the C1-C2 positions, we hypothesized that it could be taken advantage of to elicit selective transformations at the A-ring of **9**.

Formation of the rigid spirobutyrolactone ligand was made possible using chemistry recently described by Krische by utilization of ruthenium-catalyzed hydrohydroxyalkylation of methyl acrylate with **13** (Scheme 1).¹⁷ Preliminary experiments produced a cluster of products with polarity similar to **9** as indicated by thin layer chromatography (Figure S1). The cluster was fractionated and five logical intermediates were identified and partially isolated by performing preparative HPLC-UV-MS (Figure S2). Enriched isolates containing >70 % single compound were initially screened for *in vitro* KOR functional activity, evaluating forskolin-induced cAMP accumulation. Only the fraction that contained a product with a mass corresponding to **14** demonstrated high activity with an EC₅₀ value below 10 nM (Table S1). This compound was subsequently rigorously purified, structurally characterized, and reevaluated to confirm precise KOR activity.

To our surprise, the major isolated spirobutyrolactone product **14** demonstrated reversal of the stereochemistry about the carbon oxygen bond at C2 relative to starting material **13**, which was unambiguously revealed by single crystal X-ray diffraction (Scheme 1). The HPLC-UV-MS analysis of reaction mixtures suggested that other isomeric products or epimers, normally indicated by close retention time and identical mass, were detectable in minimal amounts. As shown in the chromatogram of crude reaction mixture (Figure 5), peak 7 (t_R 1.77 min) in green provided compound **14**. A minor isomer was also apparent at peak 9 (t_R 1.79 min). An enriched isolate of minor peak 9 demonstrated about 20-fold lower activity in KORs, as measured in the forskolin-induced cAMP accumulation assay, compared to pure **14** at peak 7 (Table S1). Though we were unable to rigorously purify and characterize this peak 9 isomer, a C2 epimer, C8 epimer, or isomeric spirobutyrolactone at C1 relative to **14** are all reasonable hypothetical products.

According to Krische's proposed catalytic mechanism, the reaction proceeds through the pseudosymmetric oxidized diketone transition state **15**, and a concurrent loss of stereochemical information at C2 occurs (Scheme 2). The regioselectivity would then presumably be controlled by the ruthenium-catalyzed C-C coupling of methyl acrylate to form intermediate **16**. The HPLC-UV-MS data of crude reaction mixtures also provided masses for the possible side-products **17-19**, which all appeared as discrete chromatographic peaks with only trace indication of epimers or regioisomers present. Collectively, the fact that isomeric signals (i.e. those with different retention time and the same mass spectrum) relative to major chromatographic peaks were either undetectable or were present with less than 10 % of the area relative to the major isomers suggested that the reaction proceeded with good stereo- and regio-specificity at the C2 carbon on **13** to produce **14**.

Initial experiments paralleled Krische's method and were conducted utilizing conventional oil-bath heating for extended reaction times, typically requiring 8-14h for complete disappearance of starting material. Under these conditions, thermal decomposition of **13** was evident, leading to increasingly difficult isolation and purification of products. We found that microwave irradiation could be used alternatively for heating, which resulted in a significant reduction of reaction time (3-4 h) and minimized unwanted side reactions. Additionally, in order to achieve dissolution of **13** in *meta*-xylene, the solvent volume had to be increased by a factor of 10 compared to the original report.¹⁷ Consequently, catalysts and ligands were also scaled accordingly to maintain suitable concentrations. With the optimized procedure, spiro lactone **14** was typically isolated in 20 - 30% yield following purification.

In contrast to the state-of-the art described above, oxidative cleavage of cyclic α -hydroxy ketones by lead tetraacetate was first described over 7 decades ago,¹⁸ yet no previous report exists describing its use on the salvinorin A scaffold to produce aldehydes. Entry into the flexible scaffold was made possible by this approach on **13** under methanolic conditions to isolate the three compounds shown in Scheme 3 as major products. Over multiple reactions, isolation of aldehyde **20** was found to be variable with competing formation of several other oxidized compounds. The reaction was found to be sensitive to the presence of moisture. Stirring the reaction mixture in the presence of molecular sieves under strictly anhydrous conditions tended to favor aldehyde formation. At best, over 200 mg of crystalline aldehyde **20** was isolated in 48 % yield, providing the material used for the X-ray crystal structure

shown in Scheme 3. When the reaction was run without molecular sieves in an open flask, the formation of acid **21** tended to predominate. Independent of moisture present, varying amounts of the unanticipated α -keto enol **22** was also isolated, again with single crystal x-ray diffraction providing the unambiguous identity of this compound.

Subsequent manipulations of aldehyde **20** were mostly straightforward (Scheme 4). Borohydride reduction afforded the acyclic C2 primary alcohol **23**. Subsequent acetylation provided compound **24**, a conformationally flexible derivative of salvinorin A (**9**). An attempt was made to oxidize the aldehyde directly to the corresponding methyl ester **26** by potassium peroxymonosulfate (OXONE) in methanol by a known procedure,¹⁹ however high-resolution mass spectrometry (HRMS) and proton nuclear magnetic resonance (¹HNMR) data unambiguously confirmed the major product to be dimethylacetal **25**.

The desacetoxy-salvinorin A derivative (**11**) is a desirable compound for testing the effects of the deletion of substitution at C2 and is also a valuable intermediate for semisynthesis. Our previously published synthetic method to produce **11** suffers from low yields, typically less than 28% desired product, and epimerization at C8 accounting for another 13% of the reaction mix.²⁰ In an effort to develop a more efficient method for obtaining **11**, a method that worked for a similar transformation on another terpene natural product, curcubitacin B, was determined to be effective (Scheme 5).²¹ This method proceeds through a previously described thiocarbonylimidazole intermediate (**27**) and uses much more mild conditions for the Barton-McCombie deoxygenation reaction to afford **11** in high yields (96% for this step) with no C8 epimerization observed. In addition to increasing the yield of the intermediate formation to 88% by allowing the reaction to run overnight, **11** can now be reached in 2 steps from **13** in 85% yield. Now that **11** can be accessed in reliable, high-yielding steps, it can be used as an intermediate for other substitutions at the C2 position, potentially affording a compound without the C-O bond to impart metabolic stability, a moiety that has not been installed on **9** to date.

Pharmacological Evaluation

Using the previously mentioned and described²² functional KOR assay, the compounds were evaluated and EC₅₀ values at the KOR were determined (Table 1). With an EC₅₀ value of 600 pM, **14** proved to be of similar potency to salvinorin A. This result was surprising given the 450-fold difference in activity of **9** compared to previously obtained data on compound **29**, its C2 epimer. Given the typical requirement of a hydrogen bond acceptor at C2, we hypothesized that directed hydrogen bonding might explain the high activity of compound **14**.

The KOR activity of the more flexible salvinorin A derivatives (**20**, **21**, **23-25**) demonstrated an apparent correlation with the hydrogen-bonding character of the substitution at the flexible alkyl arm (Figure 6). Both the alcohol **23** and acid **21** were inactive with EC₅₀ values >10,000 nM whereas compounds **20**, **24**, and **25** possessing H-bond acceptors at the alkyl arm terminus possessed EC₅₀ values in the 10-30 nM range.

Comparing the activity of **13**, a C2 H-bond donor, to **9**, a C2 H-bond acceptor, demonstrates only a 10-fold reduction in activity at the KOR. The reduced sensitivity to H-bond reversal

in **9** and **13** compared to **24** and **23** suggests that the flexible alkyl arm of **24** may be able to access alternative receptor interactions. Considering this, we hope to direct future studies towards studying the functional selectivity of flexible agonists such as **24** compared to more rigid derivatives such as **14**.

Compared to **13**, the reversal of the H-bond character in **22** at the C1 and C2 positions as well as alteration of the A-ring geometry were possible explanations for this change in activity. Removal of all groups at the C2 position as in **11** resulted in complete loss of KOR activity as well.

Additionally, all compounds possessing potency less than 100 nM at KORs were evaluated for DOR and MOR activity. As expected, all compounds maintained complete KOR selectivity like the parent compound **9**, with no activity seen up to 10 μ M at either the DOR or MOR.

Overall, these data support the hypothesis that the electronics and geometry of the A-ring on **9** are critical for activity at the KOR. Increasing the flexibility of the A-ring diminishes interaction with the KOR slightly, provided an H-bond acceptor is present, and significantly reduces activity if an H-bond donor is used. Conformational restriction of the acetate moiety into the rigid spirobutyrolactone as in **14** does maintain KOR potency. Removal of C2 substitution (**11**) or altering the H-bonding character of the C1-C2 positions (**22**) from that of **9** diminished activity at the KOR.

Ligand-based Alignment Studies

In an effort to provide a model able to potentially illustrate how the conformational constraint of **14** maintains similar KOR activity to **9** despite the relative reversal of C2 stereochemistry about the carbon-oxygen, especially in light of the greatly diminished activity associated with the epimer of **9**, **29**, several ligand-based alignment studies were performed on the three compounds. The only known structure of the KOR is bound to the antagonist JDTic, and thus the receptor is in an inactive conformation. Studies of agonists docked at the inactive receptor are difficult to interpret and must be validated via mutagenesis or further experimental studies; therefore, we chose to study the conformations of these compounds in the absence of the receptor crystal structure. Using the software Forge (v 10.4.2, Cresset, Litlington, Cambridgeshire, UK; <http://www.cresset-group.com/forge/>), compounds can be evaluated based upon their molecular fields.²³ Cresset's XED force field technology was used to generate the most energetically favorable conformation of **9** and the corresponding field points and electrostatic surfaces were generated.²⁴

Conformations of compounds **14** and **29** were aligned to **9** and these conformations were then scored based on the similarity of the field points (Figure 7, see Figure S3 for individual field points and electrostatic surfaces). Compound **14** shares a higher field similarity with **9**, with a score of 0.898, while compound **29** scored at 0.853. The alignments of **9** and **14** indicate that their similar activities may be due to the fact that both molecules (shown in light pink and light blue, respectively, Figure 7) direct the hydrogen bond accepting carbonyl group towards the same region, or field space, whereas the less active epimer **29** (shown in yellow in Figure 7) prefers an orientation that directs the carbonyl in the opposite direction.

Based on the activity data and *in silico* models, we hypothesize that receptor-bound **9** may preferentially direct the acetoxy carbonyl in the direction depicted in Figure 7. This data will be interesting to validate in the event that an agonist-bound crystal structure of the KOR is made available.

Microsomal Stability Studies

One of the biggest challenges to overcome in developing analogues of **9** with *in vivo* activity is its short half-life, which can be primarily attributed to esterase cleavage at the C2 acetate.²⁵ Therefore, we were particularly interested in discovering metabolically stable bioisosteric replacements for this labile acetate group. Esterase metabolism of **9** results in the formation of **13**, which has poor bioavailability and decreased KOR activity.

We hypothesized that in addition to providing conformational rigidity, the spirobutyrolactone group in **14** could potentially address this metabolic liability. This hypothesis was tested using rat liver microsomes in either the presence or the absence of the CYP450 cofactor NADPH. Metabolism in the presence of NADPH indicates a CYP450-mediated oxidative process and in the absence of NADPH a non-CYP450-mediated process. **9** is a known substrate for CYP450 enzymes as well as other metabolic enzymes such as carboxylesterases.²⁵⁻²⁶ The installation of the spirobutyrolactone moiety was intended to reduce metabolism by esterases, and the observed stability after 2.5 h does in fact show that **14** is more stable than **9**. Not only does the spiro lactone moiety impart resistance to non-CYP450-mediated metabolism, presumably esterases, but the CYP450-mediated oxidation is also reduced (Figure 8).

In vivo Antinociceptive Effects

We utilized the known analgesic properties of KOR agonists^{13a} to investigate the effects of **14** in comparison to **9**. We utilized the warm-water tail-withdrawal assay to induce a spinal cord reflex in mice to evaluate the duration of the analgesic effects (Figure 9a). Both **9** and **14** showed a rapid onset of action with significant analgesic effects seen at 5 and 10 min, respectively, and lasting until 30 min at a dose of 2 mg/kg (i.p.) (compared to vehicle-treated controls). To further explore the potency of **14** *in vivo* we assessed tail-withdrawal latencies following cumulative dosing and show that **14** has similar potency ($ED_{50}=0.7592$ (mg/kg), 95% CI [0.1508, 3.822]) compared to **9** ($ED_{50}=1.28$ (mg/kg), 95% CI [0.583, 2.82]) and a non-significant reduction in efficacy (**14**, $E_{max}=62.4 \pm 18\%$)(**9**, $E_{max}=100 \pm 14\%$) (Figure 9b).

To further explore the effects of **14** on pain responses we used the 2% intradermal formalin model to evaluate acute nociceptive and inflammatory pain responses (Figure 9c). Two-way repeated measures ANOVA revealed a significant effect of time ($p<0.0001$) and dose ($p=0.0002$). Vehicle/formalin treated mice show both phase one, nociceptive and phase two, inflammatory responses, which were significantly attenuated by administration of **9** and **14**. This attenuation in pain is prevented in mice pretreated with the selective KOR antagonist nor-BNI showing the analgesic effects are KOR mediated.

Area under the curve analyses (AUC) of phase one and phase two pain show **14** significantly reduces phase 2 pain ($p=0.0035$) but attenuation of phase one pain is not significantly different from vehicle (Figure 9d-e). Measurements of paw edema show both **9** and **14** significantly reduce the formation of edema induced by intradermal formalin administration (**9** $p<0.0001$, **14** $p<0.0001$) (Figure 9f). Taken together this data shows that both **9** and **14** have similar abilities to attenuate thermal spinal cord reflexes and inflammatory pain responses in mice. Responses in the dose-response tail-withdrawal assay, however, do show lower efficacy in thermally induced spinal reflexes. This result may be due to reduced CNS penetrance, or **14** may have partial KOR agonist effect *in vivo*. Given the interest in partial KOR agonists for modulating addiction,²⁷ further investigation is warranted and ongoing. Additionally, in these studies the *in vivo* duration of action of **14** and **9** are similar despite their significant differences in the *in vitro* microsomal studies. These results highlight the complexity of a whole animal system when compared to microsomes as well as possible differences between rat and mouse metabolic pathways.²⁸ Additionally, the i.p. injection method has been reported to cause saturation of first-pass hepatic metabolism, thereby negating the microsomal differences seen *in vitro*.²⁹

Conclusion

Several chemical strategies have been utilized to take advantage of the α -hydroxy ketone functionality in salvinorin B to produce derivatives rich in chemical diversity and pharmacology. The pharmacological data collected further confirmed the hypothesis that the molecular features of the A-ring in **9** contribute significantly to its activity at KORs. From these studies, a particularly promising compound emerged containing a rigid spirobutyrolactone functionality. Computational models of this molecule suggested that its activity may arise from a directed H-bond acceptor. Metabolic studies in liver microsomes demonstrated that the spirobutyrolactone was highly resistant to esterase metabolism and somewhat resistant to oxidation. *In vivo*, the spirobutyrolactone derivative attenuated nociceptive and inflammatory pain in mice. We hope to direct future studies towards studying additional pharmacological properties such as the functional selectivity of flexible salvinorin A derivatives compared to more rigid compounds such as the spirobutyrolactone.

Experimental Section

General Procedures

Salvinorin A was isolated from the leaves of *Salvia divinorum* as previously described.²⁰ All other chemical reagents were purchased from commercial suppliers and used without further purification. All solvents were obtained from a solvent purification system in which solvent was passed through two columns of activated alumina under argon. Reactions performed in standard glassware were performed under an atmosphere of argon using glassware dried overnight in an oven at 120 °C and cooled under a stream of argon. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm Analtech GHLF silica gel plates and visualized using a UV Lamp (254 nm) and vanillin solution. Flash column chromatography was performed on silica gel (4-63 mm) from Sorbent Technologies. ¹H and ¹³C NMR were recorded a 500 MHz Bruker AVIII spectrometer equipped with a

cryogenically-cooled carbon observe probe using tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. High-resolution mass spectrum (HRMS) was performed on a LCT Premier (Micromass Ltd., Manchester UK) time of flight mass spectrometer with an electrospray ion source in either positive or negative mode. Melting points were measured with a Thomas Capillary Melting Point Apparatus and are uncorrected. HPLC was carried out on an Agilent 1100 series HPLC system with diode array detection at 209 nm on an Agilent Eclipse XDB-C18 column (250 $\times$ 10 mm, 5 mm). Compounds were identified as 95% pure by HPLC before all *in vitro* and *in vivo* analyses unless otherwise noted.

Methyl (2S,4aR,6aR,7R,10aS,10bR)-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-2H-benzo[f]isochromene-7-carboxylate (11)

In a two-neck flask fitted with a reflux condenser, thiocarbonylimidazole-SVB **27**, (50 mg, 0.1 mmol) was dissolved in dry toluene (5 mL). Diphenylsilane (0.15 mL, 8 eq.) was added and the reaction was heated to reflux. Every 15 min., 0.2 mL of a solution of Luperox LP in toluene (60 mg, 1.5 eq., per mL), was injected into the side arm of the flask until reaction complete by TLC (45 min at 50 mg scale.) Once all starting material had been used, the reaction was allowed to cool to room temperature and the solvent was removed under reduced pressure. Purification of the reaction residue via flash column chromatography (elution 30 $\rightarrow$ 50% EtOAc in pentane) afforded pure **4** as a white solid in 96% yield. Compound characterization matches previously reported data.²⁰

Methyl (2S,4aR,6aR,7R,9R,10aS,10bR)-2-(furan-3-yl)-6a,10b-dimethyl-4,5',10-trioxododecahydro-2H,3'H,4H-spiro[benzo[f]isochromene-9,2'-furan]-7-carboxylate (14)

A flame-dried microwave vial was charged with a stir bar, **13**¹⁶ (117 mg, 0.3 mmol), Ru₃(CO)₁₂ (38 mg, 0.060 mmol), 1,3-bis(diphenylphosphino)propane (74 mg, 0.18 mmol) and benzoic acid (37 mg, 0.30 mmol). A septum cap was crimped in place and the vial was flushed with argon. Anhydrous m-xylene (3 mL) was added and the resulting deep red mixture was sonicated until the solids were homogenized. Methyl acrylate (500 μ L, 5.5 mmol) was added through the septum via syringe. The reaction was subjected to microwave irradiation such that 135 $^{\circ}$ C was maintained for 3.5 h whereby complete consumption of salvinorin B starting material was observed. The volatile components were removed under reduced pressure. The residue was resolved to two chromatographically distinct fractions using a small plug of silica and 1:1 EtOAc/hexanes. The less polar cluster of products contained 45 mg of white solid which proved to be spirobutyrolactone in about 80% purity and the substance was further purified by preparative RPHPLC (60% acetonitrile / water isocratic) to collect **14** (37 mg, 28 %) as a white solid with purity >95%. X-ray quality crystals were prepared by allowing HPLC fractions (acetonitrile/water) to sit undisturbed at room temperature for two weeks; R_f = 0.3 (30% EtOAc in hexanes); mp: 215–217 $^{\circ}$ C; ¹H NMR (500 MHz, Chloroform-d) δ 7.47 - 7.40 (m, 2H), 6.40 (dd, J = 1.9, 0.9 Hz, 1H), 5.56 (dd, J = 11.7, 5.1 Hz, 1H), 3.74 (s, 3H), 3.07 (dd, J = 13.1, 3.8 Hz, 1H), 2.97 (ddd, J = 13.2, 9.2, 5.9 Hz, 1H), 2.82 (s, 1H), 2.69 - 2.53 (m, 2H), 2.43 - 2.29 (m, 2H), 2.26 - 2.12 (m, 3H), 1.84 - 1.74 (m, 2H), 1.70 - 1.63 (m, 1H), 1.47 (s, 3H), 1.28 (s, 2H), 1.10 (s, 3H); ¹³C NMR (126 MHz, Chloroform-d) δ 203.01, 174.87, 172.09, 171.16, 143.82, 139.54, 125.06, 108.44, 87.11, 71.88, 61.84, 51.98, 51.67, 51.24, 43.01, 42.63, 38.39, 37.70, 35.30, 28.24,

26.48, 18.06, 16.32, 15.10; HRMS (ES+) calc. for C₂₄H₂₉O₈ [M+H]⁺: 445.1857, Found: 445.1822.

Methyl (2S,4aR,6aS,7R,10bS)-2-(furan-3-yl)-10-hydroxy-6a,10b-dimethyl-4,9-dioxo-1,4,4a,5,6,6a,7,8,9,10b-decahydro-2H-benzo[f]isochromene-7-carboxylate; methyl (3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-6-((R)-1-methoxy-1,4-dioxobutan-2-yl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromene-5-carboxylate; (R)-3-((3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-5-(methoxycarbonyl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromen-6-yl)-4-methoxy-4-oxobutanoic acid (22; 20; 21)

To an ice cold stirring suspension of salvinorin B (140 mg, 0.36 mmol) in methanol (3 mL), benzene (12 mL), and 4Å molecular sieves (~200 mg), lead tetraacetate (213 mg, 0.48 mmol) was added in four portions over 30 minutes producing a red solution that slowly faded to transparent pale yellow as the reaction mixture came to room temperature and stirred for 4h. The reaction was quenched by addition of 1M Na₂S₂O₃ (15 mL) and sat. NaHCO₃ (15 mL). The resulting mixture stirred for 30 minutes and was then extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine (50 mL), dried (Na₂SO₄) and concentrated. The resulting solid was purified by flash column chromatography:

Methyl (3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-6-((R)-1-methoxy-1,4-dioxobutan-2-yl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromene-5-carboxylate (20)

(5% EtOAc in CH₂Cl₂) to afford aldehyde (**20**) as a white solid (46 mg, 30% yield); X-ray quality crystals were prepared by recrystallizing the compound from methanol containing a trace amount of benzene; *R*_f = 0.3 (5% EtOAc in CH₂Cl₂); mp: 127–129 °C; ¹H NMR (500 MHz, Chloroform-*d*) δ 9.72 (s, 1H), 7.48 - 7.39 (m, 2H), 6.42 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.47 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 2.99 (ddd, *J* = 18.1, 11.4, 1.0 Hz, 1H), 2.81 (dd, *J* = 11.3, 2.4 Hz, 1H), 2.59 (dd, *J* = 18.0, 2.3 Hz, 1H), 2.28 (s, 1H), 2.19 - 2.10 (m, 2H), 2.03 (dd, *J* = 13.7, 5.5 Hz, 1H), 1.90 - 1.82 (m, 1H), 1.74 - 1.65 (m, 2H), 1.38 (s, 3H), 1.31 (s, 3H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 199.57, 173.76, 171.72, 170.92, 143.87, 139.60, 108.47, 99.96, 71.64, 58.57, 51.73, 51.39, 50.51, 49.21, 44.69, 42.20, 38.51, 36.97, 35.08, 19.01, 18.27, 15.58; HRMS (ES+) calc. for C₂₂H₂₉O₈ [M+H]⁺: 421.1857, Found: 421.1832.

(R)-3-((3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-5-(methoxycarbonyl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromen-6-yl)-4-methoxy-4-oxobutanoic acid (21)

(5% → 50% EtOAc in CH₂Cl₂ with 1% HOAc) to afford acid (**21**) as a white solid (28 mg, 18% yield); *R*_f = 0.6 (80% EtOAc in CH₂Cl₂ with 1% HOAc); mp: 183 - 185 °C; ¹H NMR (500 MHz, Chloroform-*d*) δ 7.49 - 7.41 (m, 2H), 6.44 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.53 (dd, *J* = 11.6, 5.3 Hz, 1H), 3.73 (s, 3H), 3.68 (s, 3H), 2.94 - 2.80 (m, 2H), 2.58 (dd, *J* = 14.7, 0.9 Hz, 1H), 2.34 (dd, *J* = 13.7, 5.3 Hz, 1H), 2.32 (s, 1H), 2.22 - 2.09 (m, 2H), 1.93 - 1.84 (m, 1H), 1.77 - 1.57 (m, 3H), 1.40 (s, 3H), 1.39 (s, 3H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 174.46, 174.02, 172.51, 170.94, 143.86, 139.51, 125.20, 108.45, 71.84, 58.33, 52.07, 52.03, 51.84, 50.64, 44.67, 38.64, 36.95, 34.64, 31.99, 19.48, 18.22, 15.73; HRMS (ES+) calc. for C₂₂H₂₈O₉ [M+H]⁺: 437.1806, Found: 437.1793.

Methyl (2S,4aR,6aS,7R,10bS)-2-(furan-3-yl)-10-hydroxy-6a,10b-dimethyl-4,9-dioxo-1,4,4a,5,6,6a,7,8,9,10b-decahydro-2H-benzo[f]isochromene-7-carboxylate (22)

(elution 0 → 5% EtOAc in CH₂Cl₂) to afford enol **22** as a white solid (31 mg, 22 % yield); Allowing chromatographic fractions (CH₂Cl₂/EtOAc) to sit undisturbed for 1 week formed X-ray quality crystals; *R_f* = 0.8 (5% EtOAc in CH₂Cl₂); mp: = 215-216 °C (dec); ¹H NMR (500 MHz, Chloroform-*d*) δ 7.49 - 7.42 (m, 2H), 6.79 (s, 1H), 6.45 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.59 (ddd, *J* = 10.5, 6.4, 1.1 Hz, 1H), 3.75 (s, 3H), 3.75 - 3.73 (m, 1H), 3.19 - 3.08 (m, 1H), 3.11 - 3.01 (m, 1H), 2.74 (dd, *J* = 17.3, 3.0 Hz, 1H), 2.46 (dd, *J* = 12.4, 3.0 Hz, 1H), 2.22 (dq, *J* = 14.2, 3.4 Hz, 1H), 2.16 - 2.03 (m, 1H), 1.80 (tdd, *J* = 14.0, 12.3, 3.0 Hz, 1H), 1.71 (dt, *J* = 13.6, 3.2 Hz, 1H), 1.46 (s, 3H), 1.43 (s, 3H), 1.27 (s, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 192.99, 171.84, 171.70, 143.82, 139.36, 139.25, 126.03, 108.62, 71.54, 52.00, 51.74, 50.57, 41.53, 38.64, 37.94, 37.24, 34.82, 29.72, 24.44, 19.02, 17.77; HRMS (ES+) calc. for C₂₁H₂₄O₇: [M+H]⁺: 389.1606 Found: 389.1595.

Methyl (3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-6-((R)-4-hydroxy-1-methoxy-1-oxobutan-2-yl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromene-5-carboxylate (23)

To a solution of aldehyde **20** (20 mg, 0.05 mmol) in methanol (1 mL) 0°C was added NaBH₄ (2 mg, 0.05 mmol) in one portion. The reaction mixture came to room temperature over 15 minutes and was monitored for completion by TLC (about 30 minutes) and was subsequently quenched by dropwise addition of saturated aqueous NH₄Cl (1 mL). The resulting suspension was diluted with several drops of water until solids dissolved and was extracted with CH₂Cl₂ (3 × 5 mL). The combined extracts were dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography (elution 30 → 40 % EtOAc in pentanes) to afford alcohol (**23**) as a white solid (20 mg, 95 % yield); *R_f* = 0.3 (40% EtOAc in pentanes); mp: 129–130 °C; ¹H NMR (500 MHz, Chloroform-*d*) δ 7.51 – 7.43 (m, 2H), 6.44 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.49 (dd, *J* = 11.6, 5.3 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.69 – 3.60 (m, 1H), 3.60 – 3.50 (m, 1H), 2.42 (dd, *J* = 11.8, 2.4 Hz, 1H), 2.31 (s, 1H), 2.19 – 2.11 (m, 2H), 2.07 – 1.96 (m, 1H), 1.98 – 1.87 (m, 1H), 1.89 – 1.65 (m, 4H), 1.40 (s, 3H), 1.35 (s, 3H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 175.18, 171.92, 171.16, 143.84, 139.62, 125.24, 108.53, 71.66, 61.40, 58.70, 53.03, 51.36, 51.24, 50.60, 44.67, 38.84, 36.95, 34.45, 30.62, 18.82, 18.34, 15.53; HRMS (ES+) calc. for C₂₂H₃₁O₈ [M+H]⁺: 423.2014, Found: 423.1977.

Methyl (3S,4aS,5S,6S,8aR)-6-((R)-4-acetoxy-1-methoxy-1-oxobutan-2-yl)-3-(furan-3-yl)-4a,6-dimethyl-1-oxooctahydro-1H-isochromene-5-carboxylate (24)

To a solution of **23** (20 mg, 0.05 mmol) in CH₂Cl₂ (1 mL) was added DMAP (about 1 mg) and Ac₂O (7 μL, 0.07 mmol). The resulting solution stirred overnight then was applied directly to a flash silica column (elution 0 → 5% EtOAc in CH₂Cl₂) to afford acetate (**24**) as a white solid (16 mg, 70 % yield); *R_f* = 0.4 (5 % EtOAc in CH₂Cl₂); mp: 123–125 °C; ¹H NMR (500 MHz, Chloroform-*d*) δ 7.40 – 7.33 (m, 2H), 6.35 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.40 (dd, *J* = 11.6, 5.3 Hz, 1H), 3.98 – 3.81 (m, 2H), 3.61 (s, 3H), 3.60 (s, 3H), 2.25 (dd, *J* = 11.7, 2.1 Hz, 1H), 2.19 (s, 1H), 2.12 – 2.01 (m, 2H), 1.96 (s, 3H), 1.96 – 1.86 (m, 2H), 1.82 – 1.75 (m, 1H), 1.77 – 1.67 (m, 2H), 1.68 – 1.59 (m, 1H), 1.45 (dt, *J* = 12.7, 2.8 Hz, 1H), 1.30 (s, 3H), 1.25 (s, 3H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 174.57, 171.90, 171.07, 170.82,

143.86, 139.62, 125.21, 108.51, 71.65, 62.93, 58.81, 53.33, 51.42, 51.30, 50.57, 44.64, 38.92, 36.97, 34.59, 26.90, 20.88, 18.52, 18.32, 15.49; HRMS (ES+) calc. for C₂₄H₃₃O₉ [M+H]⁺: 465.2119, Found: 465.2094.

Methyl (3S,4aS,5S,6S,8aR)-3-(furan-3-yl)-4a,6-dimethyl-1-oxo-6-((R)-1,4,4-trimethoxy-1-oxobutan-2-yl)octahydro-1H-isochromene-5-carboxylate (25)

To a solution of aldehyde **20** (20 mg, 0.05 mmol) in methanol (1 mL) was added oxone (8 mg, 0.05 mmol). The resulting suspension stirred overnight. The methanol was removed under reduced pressure and the resulting residue was purified by flash column chromatography (elution 30 % EtOAc in pentanes) to afford dimethyl acetal (**25**) as a white solid (15 mg, 65 % yield); Formation of the desired methyl ester was not observed; *R_f* = 0.2 (30% EtOAc in pentanes); mp: 138–140 °C; ¹H NMR (500 MHz, Chloroform-*d*) δ 7.49 – 7.42 (m, 2H), 6.44 (dd, *J* = 2.0, 0.9 Hz, 1H), 5.48 (dd, *J* = 11.6, 5.3 Hz, 1H), 4.24 (t, *J* = 5.5 Hz, 1H), 3.69 (d, *J* = 16.3 Hz, 6H), 3.30 (d, *J* = 4.8 Hz, 6H), 2.37 (dd, *J* = 11.8, 2.0 Hz, 1H), 2.25 (s, 1H), 2.18 – 1.96 (m, 4H), 1.91 – 1.82 (m, 1H), 1.80 – 1.65 (m, 3H), 1.53 (dt, *J* = 12.6, 2.8 Hz, 1H), 1.39 (s, 3H), 1.33 (s, 3H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 174.76, 171.82, 171.14, 143.84, 139.64, 125.23, 108.53, 103.78, 71.64, 58.53, 53.72, 52.81, 52.21, 51.27, 51.25, 50.57, 44.66, 38.89, 36.96, 34.77, 31.06, 18.75, 18.33, 15.53; HRMS (ES+) calc. for C₂₄H₃₅O₉ [M+H]⁺: 467.2276, Found: 467.2266.

***In vitro* pharmacology**

Cell lines and cell culture—The HitHunter™ Chinese hamster ovary cells (CHO-K1) stably expressing the human κ-opioid receptor (OPRK1, catalog # 95-0088C2), the human μ-opioid receptor (OPRM1, catalog # 95-0107C2), or the human δ-opioid receptor (OPRD1, catalog # 95-0108C2) were purchased from DiscoverX Corp. (Fremont, CA) and maintained in F-12 media with 10% fetal bovine serum (Life Technologies, Grand Island, NY), 1% penicillin/streptomycin/ L-glutamine (Life Technologies), and 800 μg/mL Geneticin (Mirus Bio, Madison, WI). Cells were grown at 37 °C and 5% CO₂ in a humidified incubator.

Forskolin-induced cAMP accumulation—Assays proceeded and were processed as previously described.²²

Data analysis—Data were analyzed using nonlinear regression analysis in GraphPad Prism v7.02 software (GraphPad, La Jolla, CA) to generate sigmoidal dose-response curves. cAMP accumulation data were normalized to vehicle and forskolin only control values. All compounds were run in parallel assays in triplicate in 2 individual experiments. EC₅₀ and E_{max} values are reported as the means ± S.E.M. and represent the average of each individual experiment following nonlinear regression analysis.

Ligand-based Alignment Studies

Ligand-based alignment studies were performed using Forge (v 10.4.2, Cresset, Litlington, Cambridgeshire, UK; <http://www.cresset-group.com/forge/>) as used previously.^{23a, 24} All settings were left as default, unless noted otherwise. All compounds were imported in “use input protonation state,” as none of the compounds contain ionizable moieties. Briefly, the 2-dimensional structure of **9** was imported and the preferred conformation was found using

the “very accurate and slow” method in the conformation hunt mode, with a maximum of 1000 conformations evaluated. The final conformation of **9** used had a conformation energy of 65.56 kcal/mol. Using the “alignment” tool, this conformation of **9** was set to the “reference” role and the 2-dimensional structures of **14** and **29** were imported in the “prediction set.” The conformation hunt and alignment were set to “very accurate and slow” and “substructure,” respectively. The “substructure” setting weights shape similarity and alignment with the reference equally and holds the common core of the “prediction set” compounds in place while searching for the best conformation of the rest of the molecule that varies from the common core. The final conformation energies of **14** and **29** were 74.63 and 66.35 kcal/mol, respectively.

Microsomal Stability Analysis

Materials—Pooled IGS Sprague-Dawley rat liver microsomes (male, 20mg/mL) were purchased from Sekisui XenoTech, LLC (Kansas City, KS). Nicotinamide adenine dinucleotide phosphate tetrasodium salt hydrate (reduced form, NADPH, Fisher Scientific), tolbutamide, dextromethorphan hydrobomide, and (±)-verapamil hydrochloride (Sigma Aldrich, St. Louis, MO), MeCN, (HPLC grade, Fisher Scientific)

In Vitro Metabolic Stability in Liver Microsomes—Metabolism studies were carried out as described previously,³⁰ with minor changes. Briefly, in 96-well plates, solutions contained 50 mM potassium phosphate buffer solution with 2 mM MgCl₂, at pH 7.4, and 0.25 mg/mL rat liver microsomes 0.25 mg/mL. The 0-minute time points were performed on ice, with the exception of compound **14** t = 0 samples which were prewarmed to prevent compound from crashing out of solution. The assay plate was pre-warmed to 37°C in an incubator for 15-20 minutes before adding 20 µL of test compound (125 µM, final compound concentration of 10 µM) and 10 µL of NADPH in buffer (final concentration 1mM). At the end of each time point, 150 µL of reaction mix was removed and mixed in a separate 96-well plate containing 150 µL of quench solution per well (1 µM tolbutamide in MeCN with 1% formic acid). For the test samples in the absence of NADPH, the same procedure as above was conducted with the addition of 10 uL buffer instead of the NADPH solution. The plate was centrifuged at 1500 rpm for 10 min to precipitate any protein before quantifying remaining compound using LCMS, using water and acetonitrile with 0.1% formic acid, from 40% to 80% acetonitrile over 3.4 minutes and held at 80% acetonitrile for 0.4 minutes. The flow rate was 0.25ml/min and the injection volume was 15uL. Each well was sampled twice. Verapamil and dextromethorphan were included as controls in the stability assays, and evaluated as described above. Both compounds are known substrates for CYP-450s, and both were significantly metabolized in the presence of NADPH compared to in the absence of NADPH, p <0.0001 for both sets of data.

Data analysis—Analysis of the metabolism data proceeded as described previously³⁰ using GraphPad Prism v7.02 software (GraphPad, La Jolla, CA).

Animal studies

All experimental work was performed on adult male mice (B6-SJL) weighing 25-35g. Experiments were approved by the Victoria University of Wellington (VUW) Animal Ethics

Committee and were conducted in accordance with their animal care guidelines. All experiments, breeding and housing of animals occurred in the VUW Small Animal Facility (New Zealand) under a controlled 12:12 hr light:dark cycle. All animals had *ad libitum* access to food and water except during experimental procedures. Four days prior to conducting experiments, animals were habituated to the experimenter and the experimental room for 60 min daily. On the test day animals were habituated for 60 min before beginning the procedure. All experimental procedures were conducted during the light cycle and in the presence of background white noise.

Preparation of Drugs—All KOR agonists used for *in vivo* studies were dissolved in a 2:1:7 vehicle containing DMSO: Tween-80: saline. KOR agonists and vehicle controls were administered via intraperitoneal (i.p.) injection for the time-course tail-withdrawal assay and the intradermal formalin assay and by subcutaneous (s.c.) injection for the dose response tail-withdrawal assay. Nor-binaltorphimine (nor-BNI) (Tocris Bioscience), a KOR antagonist, and saline controls were administered (10 mg/kg, s.c.) 24 hours prior to KOR agonist injections.

Warm-Water Tail-Withdrawal Assay—The warm-water tail-withdrawal assay was performed in mice following the protocol outlined in Simonson et al. (2015). Animals were habituated to restraint stress over 4 daily trials, 5 min each in Plexiglas restrainers (internal diameter 24 mm). On test day, withdrawal latencies were obtained by immersing one third of the tail into a water bath at a temperature of 50°C (± 0.5) and time until tail-withdrawal recorded. A maximal latency time of 10 s was used to prevent potential tissue damage. Baseline latencies were determined from the average of 3 withdrawal latencies. Measurements were then taken at 5, 10, 15, 30, 45, 60, 90 and 120 min following either KOR agonist or vehicle. The following formula was then used to calculate the Maximum Possible Effect (MPE): $MPE (\%) = 100 \times (\text{test latency} - \text{control latency}) / (10 - \text{mean control latency})$.

Dose-response tail-withdrawal assays were also performed using a within animal design to reduce animal numbers, as previously described (Bohn et al. 2000). Briefly, following baseline latency calculations, mice were given s.c. injections every 30 min at increasing concentrations to create the following cumulative doses: 0.3, 0.6, 1.0, 2.5, 5.0, 7.5 and 10 mg/kg and tail-withdrawal latencies recorded 30 min following each dose.

Intradermal Formalin Assay—Test chambers consisted of a glass base and a mirror at a 45° angle beneath the box allowing a clear view of the hind paws (Lamb et al. 2012). Mice were habituated to the test-box for 15 min, removed to measure paw size (height, width and depth of the right hind paw) using digital calipers followed by either vehicle or KOR agonist administration and returned to the chamber for 10 min. At 10 min the mice were given a intradermal (i.d.) injection of 2% formalin in a volume of 20 μ l to the planar surface of the right hind footpad, before being returned to the chamber and behaviors recorded using a digital video camera for 60 min. This time interval was chosen because analogues of 9 are known to have fast onsets, and specifically in the tail withdrawal assay described above, the E_{max} was seen at 10-15 minutes. Pain behavior was scored according to the weight bearing method outlined by Dubuisson and Dennis (1977). Normal behavior was given a score of 0,

partial weight bearing = 1, paw raised = 2 and paw bitten, licked or shaken = 3. Pain scores were assessed every 5 s of the 60 min recordings by an analyst blinded to the treatment groups. The average score of each 5 min time period is reported for 60 min. At 60 min, the paw measurements were repeated to determine extent of paw swelling.

Data analysis—All statistical tests were performed using GraphPad Prism software v6.07 (La Jolla, CA, USA). One-way ANOVAs were used to compare data from multiple treatment groups. Two-way ANOVAs were used to test for significant effects of two factors. For the dose response experiment, non-linear regression analysis was used to calculate the potency (ED_{50}) and the efficacy (E_{max}), with data normalized to salvinorin A a full KOR agonist. A p value of 0.05 was used to define significance. When significant differences were found for one- and two-way ANOVAs Bonferroni post-hoc tests were used. All values presented are means $\pm$ the standard error of the mean (S.E.M.).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: This work was supported by DA018151, GM111385 (to TEP), GM008545 (to RSC), AFPE Pre-doctoral Fellowship in Pharmaceutical Sciences (to RSC), and the Health Research Council of New Zealand (to BMK).

Support for the NMR instrumentation was provided by NIH Shared Instrumentation Grant #S10RR024664 and NSF Major Research Instrumentation Grant #0320648.

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Abbreviations

DOR	delta opioid receptor
E_{max}	maximum efficacy
KOR	kappa opioid receptor
MOR	mu opioid receptor
MPE	maximal possible effect
SEM	standard error of the mean

Figure 1.
CNS-active drug discoveries made possible by natural products.

Figure 2.
Chemical liabilities in salvinorin A.

Figure 3. Pharmacological diversity of modification at the A-ring

Enadoline (12)

Figure 4.
Enadoline, another kappa opioid agonist known to produce hallucinations, dissociation, and feelings of dysphoria in humans.

Figure 5.
HPLC-UV-MS trace of reaction products with similar polarity to **9**. Peaks 7 and 9 (green) contain products with high-resolution mass $[M + H]^+ = 445.186$ for isomers corresponding to compound **14**.

R = H-bond acceptor - **active**

R = H-bond donor - **inactive**

Figure 6. General trend regarding H-bonding character of alkyl arm terminus in relation to KOR activity

Figure 7. Ligand-based alignment. A) Alignment of **9** (light pink), **14** (light blue), and **29** (yellow), oxygen atoms in red, via substructure alignment using Forge (v 10.4.2, Cresset, Litlington, Cambridgeshire, UK; <http://www.cresset-group.com/forge/>). B) **9** and **14** alignment with field points shown; C) **9** and **29** alignment with field points shown. Field points of **9** shown as icosahedra and field points of **14** and **29** shown as spheres. The size of the points indicates relative strength of interaction. Blue and red field points indicate negative and positive electrostatic potential, respectively. Gold and yellow points indicate regions favoring hydrophobic and van der Waals interactions, respectively.

Figure 8.

Rat liver microsomal stability study. Compounds were incubated in rat liver microsomes in either the presence (stripes) or absence (no shading) of NADPH and quantified via LCMS over time. Data is presented as percent remaining at 150 minutes. **14** is less metabolized than **9** in both the presence of NADPH ($***p<0.0005$) and the absence ($**p<0.005$). The known CYP450 substrates verapamil and dextromethorphan were included as controls in the stability assays, and both were significantly metabolized in the presence of NADPH and not metabolized in its absence ($****p<0.0001$). Analysis via two-tailed, unpaired t-test.

Figure 9.

Antinociceptive and anti-inflammatory effects. a) Time-course analysis of analgesia in the 50°C warm-water tail withdrawal latency in mice following i.p. administration of **9** or **14**. The maximal possible effect (%MPE $\pm$ SEM) at each time point was calculated as a percentage based on pre-treatment control latencies. Two-way repeated measures ANOVA, followed by Bonferroni multiple comparisons test showed a significant effect of treatment for **9** at 5-30 min and **14** at 10-30 min compared to vehicle (n=8-9 per group). b) Dose-response effects in the tail-withdrawal assay and non-linear regression analysis was used to calculate ED₅₀ values (mg/kg) of **9** = 1.285, 95% CI [0.585, 2.81] and **14** = 0.759, 95% CI [0.151, 2.822] and the Emax of **14** = 62.4% (compared to **9**) (n=8 per group). c) Time-course analysis of pain behavior following intradermal formalin, showing significant attenuation of phase one pain at 5 and 10 min with **9**, and 10 min with **14**. A significant reduction in phase two with **9** (between 20-50 min) and **14** (20-45 min) compared to vehicle/formalin treated controls (n=7 per group). The effect of **14** was prevented with pre-treatment with the KOR antagonist nor-BNI (10 mg/kg, s.c.) (n=4). Two-way repeated measures ANOVA, followed

by Bonferroni multiple comparisons tests. d) Area under the curve (AUC) analysis of phase one (0-15 min) and e) phase two (15-60 min) pain revealed a significant reduction in both phases of pain following treatment with **9** (phase one $p=0.0011$, phase two $p=0.0008$). Treatment with **14** reduced phase two pain ($p=0.0035$), which was reversed by pre-treatment with nor-BNI (10 mg/kg, s.c.) ($p=0.0159$). f) Footpad edema at 60 min following 2% intradermal formalin shows a significant reduction in edema in mice pre-treated with **9** ($p<0.0001$) and **14** ($p<0.0001$) compared to vehicle/formalin treated controls. One-way ANOVA followed by Bonferroni multiple comparison tests ($n=7$ per group). a-f) * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, drug (green for **9**, purple for **14**) compared to vehicle. # $p<0.05$, ## $p<0.01$, **14** compared to **14**+nor-BNI. Data shown as mean $\pm$ SEM.

Scheme 1.

Ruthenium-catalyzed hydrohydroxyalkylation of methyl acrylate with **13** and x-ray crystal structure of major isolated product **14**.

Scheme 2.

A plausible mechanism for the formation **14** with proposed side-products identified by HPLC-UV-MS data.

Scheme 3.

Isolated products by the action of lead tetraacetate in methanolic benzene on salvinorin B with single crystal x-ray diffraction data on **20** and **22**.

Scheme 4.
Synthetic manipulations of aldehyde **20**.

Scheme 5. Revised strategy to desacetoxy-salvinorin A (11)

Table 1

KOR pharmacology. Inhibition of forskolin-induced cAMP accumulation.

#	Structure	κ -opioid receptor Potency $EC_{50} \pm SEM^{a,b}$ (nM)	Calculated Values:		
			SLogP ^f	PSA ($\text{\AA}^2$) ^f	Ligand Efficiency ^g
9		0.21 ± 0.06 <i>de</i>	2.40	109.1	0.43
11		>10000 ^c	2.90	82.8	0.25

#	Structure	κ -opioid receptor Potency $EC_{50} \pm SEM^{a,b}$ (nM)	Calculated Values:		
			SL $LogP^f$	PSA ($\text{\AA}^2$) ^f	Ligand Efficiency ^g
13		2.4 ± 0.8 <i>de</i>	2.20	103.0	0.42
14		0.6 ± 0.2 <i>de</i>	2.60	109.1	0.39
20		30 ± 10 <i>de</i>	2.30	109.1	0.34

#	Structure	κ -opioid receptor Potency $EC_{50} \pm SEM^{a,b}$ (nM)	Calculated Values:			Ligand Efficiency ^g
			SL _{LogP} ^f	PSA ($\text{\AA}^2$) ^f		
21		>10000 ^c	2.20	132.2		0.22
22		>10000 ^c	2.70	103.0		0.24
23		>10000 ^c	2.50	112.3		0.23

#	Structure	κ -opioid receptor Potency $EC_{50} \pm SEM^{a,b}$ (nM)	Calculated Values:		
			SL _{LogP} ^f	PSA ($\text{\AA}^2$) ^f	Ligand Efficiency ^g

24		13 ± 3 ^{d,e}	2.60	118.3	0.33
25		11.7 ± 0.4 ^{d,e}	2.70	110.5	0.33
29		110 ± 20 ²⁰	2.40	109.1	0.31

^aMean $\pm$ SEM (standard error of the mean); n = 2 individual experiments run in triplicate.

^b κ OR E_{max} = 100% unless noted otherwise.

^c κ OR E_{max} = 0% up to 10 μ M.

^dDOR E_{max} = 0% up to 10 μ M.

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MOR E_{max} = 0% up to 10 μM.

Properties calculated using Forge 10.4.2 Revision: 24876, Copyright 2006-2015, Cresset BioMolecular Discovery, Ltd.

$LE = -1.37x \log \left(\frac{\text{potency (M)}}{\text{Heavy Atom Count}} \right)$

g_{Ligand} Efficiency calculated using the following equation: