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Modular Approach to *pseudo*-Neoclerodanes as Designer Kappa Opioid Ligands

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Abstract

Informed by previous semisynthetic work on salvinorin A, a modular total synthesis has been developed capable of producing novel compounds targeting the kappa opioid receptor. The strategy has permitted the deliberate simplification and introduction of functionality about the target molecule to provide access to molecular features on salvinorin A otherwise unattainable by semisynthesis. Using this approach, a potent *pseudo*-neoclerodane kappa opioid receptor ligand (**2**) has been realized.

Graphical abstract

Salvinorin A (**1**) is an intriguing neoclerodane natural product with an extensive ethnobotanical history. In traditional preparations, crude salvinorin A extract from *Salvia divinorum* has been used by Mazatec shamans to induce altered states of consciousness to explore the origins of maladies.¹ In a similar sense, salvinorin A has recently gained considerable interest among the scientific community for its utility in the exploration of the central nervous system, owing to its uniqueness as a non-nitrogenous, potent and selective kappa-opioid receptor (KOR) agonist.²

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Supporting Information

Data includes experimental procedures, ¹H NMR spectra and ¹³C NMR spectra of compounds **2**, **8–19**, GC chiral analysis of **11**, and HPLC purity analysis and X-ray crystallographic data for **2** (PDF).

Salvinorin A provides a valuable chemical probe for the study of opioid receptors and semisynthesis work has yielded several notable discoveries.^{1d} For example, recent data has unexpectedly demonstrated that subtle changes in salvinorin A's structure can completely reverse its selectivity for the KOR in favor of the mu-opioid receptor (MOR).³ Such novel MOR ligands are especially relevant today, given the prevalence of opioid use disorders and the need for novel medications devoid of the detrimental aspects of morphine-derived opiates.⁴

Strategic manipulation of salvinorin A's pharmacokinetic properties has also been a useful approach to derivative compounds. Given that the C2 acetate moiety has been implicated in its short half-life due to rapid metabolism by esterases,⁵ derivatives with modifications in this position have been studied for their KOR activity.^{1d, 6} The methoxymethyl derivative **3** is one derivative that possesses both higher activity at KOR and a more metabolically stable C2 substituent.⁷

Other semisynthesis work has provided valuable probes that have contributed towards understanding salvinorin A's mode of binding within the KOR and has, in part, identified key molecular features in salvinorin A that are necessary for activity.^{3a} This data has been especially valuable, given that the active-state crystal structure of the KOR has not yet been reported in the literature and that general consensus has not been established on salvinorin A's precise pose within the receptor.⁸ The ability to systematically explore structure activity relationships of compounds inspired by salvinorin A is therefore of significant interest.

Figure 1 illustrates several previously described semisynthetic derivatives and their corresponding relative activities. These semisynthetic derivatives are representative of those used to generate the hypothesized 3-point binding model responsible for salvinorin A's activity at the KOR. The C2 acetate in **1** or the methoxymethyl group in **3** is required for activity,⁷ further exemplified by **4**, which is completely inactive.⁹ Semisynthetic manipulations at the C4 carbomethoxy on **1** have been surprisingly difficult and limited semisynthetic data at this position exists. However, some analogues with C4-modifications have been evaluated for their KOR activity, including carboxylic acid **5**. Their relative activities suggest that reversal of the H-bond character at this position significantly reduces activity,¹⁰ implying that an H-bond accepting group attached to C4 may be required for activity. Reduction-elimination of the C17 lactone carbonyl oxygen has yielded **6**, with only a slight loss in activity.¹¹ Additional unpublished work by our group has demonstrated that substitutions to the C17 lactol derivative of **1** are also well tolerated. Taken collectively, the eastern portion of the molecule has tolerated slight distortions in ring geometry as well as steric bulk, suggesting no significant interactions with the KOR. Though small substitutions at the C12 furan have been tolerated,¹² large non-aromatic groups, as in **7**, are detrimental to activity.¹³ Finally, our lab has previously reported activity data on modified analogues at the C1 ketone. Overall, any changes to the C1 ketone have reduced activity to varying degrees, suggesting that the C1 ketone imparts a supplemental, though not crucial interaction with the KOR. The C1 ketone was therefore included in the hypothetical 3-point binding model as a supplement to the critical interaction arising from the adjacent C2 substitution.¹⁴

Informed by generalization of the above semisynthetic data, we propose that salvinorin A's high activity and selectivity at the KOR arises predominantly from the proper orientation of the C1 ketone, C2 acetate, C4 carbomethoxy, and from a size-limited aromatic interaction from the C12 furan. Features not anticipated to contribute significantly to receptor interaction are the C5 and C9 methyl groups as well as the eastern portion of the molecule near the C17 lactone. To collectively address this hypothesis, we envisaged designing a robust total synthesis approach to *pseudo*-neoclerodanes inspired by salvinorin A that would allow systematic access to each of these positions.

While several total synthesis efforts of salvinorin A and other neoclerodane diterpenes have been accomplished,¹⁵ none have attempted to explore the KOR activity of derivative compounds, likely in part due to the dense structural complexity of these natural products. Of the known methods, we were particularly inspired by the seminal work of Evans,^{15a} which utilized a diastereoselective transannular cascade reaction on a bisenone macrolide to assemble the tricyclic core representative of **1**.¹⁶ We considered this to be the superior approach to derivative compounds, as other total synthesis efforts have relied on the early formation of the A-ring system by building off of preformed scaffolds such as Wieland-Miescher ketone,^{15d} thus potentially impeding access to manipulations capable of exploring structural modifications at the A-ring. Using Evans's precedent as a template,^{15a} combined with the design rationale discussed above, a synthetic strategy to **2** was designed to incorporate elements of modularity, high diastereoselectivity, and acceptable efficiency to reach target compounds in a reasonable amount of time (Figure 2).

Key simplifications and divergences from Evans's total synthesis procedure include the early introduction of the C2 stereocenter derived from commercially available enantiopure glycidol, organocatalytic asymmetric hydroxymethylation to provide the C4 substitution, the use of diketene acetone-adduct phosphonate as a precursor to the β -keto ester functionality, and a Nozaki-Hiyama-Kishi (NHK) macrocyclization reaction to form the macrolide. Additionally, the dual usefulness of the C2 methoxymethyl as both a design feature and suitable protecting group, omission of the methyl groups at C5 and C9, and the inclusion of the eastern enol further simplified the reaction scheme allowing the overall step count to be reduced to 9-steps from known starting materials (Scheme 1).

Molecular complexity was rapidly achieved by Boeckman's organocatalytic hydroxymethylation protocol¹⁷ on known aldehyde **9**¹⁸ followed immediately by Horner-Wadsworth-Emmons olefination using phosphonate **10** derived from diketene acetone adduct, also first described by Boeckman¹⁹ years earlier, to obtain grams of **14** in good yield and diastereoselectivity as the E-olefin exclusively. The resulting primary alcohol **14**, representing a valuable point of divergence for future studies, was readily methylated using trimethyloxonium tetrafluoroborate to give the desired H-bond accepting C4 methyl ether **15**. The remaining carbon skeleton was completed by capturing alcohol **13** in hot xylene with the β -acylketene intermediate generated from **15** to give a β -ketoester that was immediately reduced using Luche conditions to provide the corresponding allylic alcohol **16** as a mixture of inconsequential epimers. Attempts to proceed with the unmodified labile β -ketoester were unfruitful, necessitating the reduction and protection of the allylic alcohol as the tert-butyltrimethylsilyl ether **17**. Additionally, efforts to form the macrolide by

intramolecular acylketene capture were unsuccessful, necessitating the alternative NHK route described below. The vinyl iodide module **13** was produced by a one-pot hydrozirconation-iodination-deprotection from the triethylsilyl ether **12** of known homopropargyl alcohol **11**.^{15a} Given the availability of many asymmetric homopropargyl alcohols²⁰ analogous to **11**, intermediate **15** also represents a valuable point of divergence by implementing analogues of vinyl iodide module **13** to achieve various C12 substitutions in final compounds.²¹

Following protection of the allylic alcohol **16**, tethered aldehyde **18** was revealed in two steps using DDQ followed by Dess-Martin periodinane. Aldehyde **18** was then smoothly cyclized using the classic NHK conditions²² to give an inconsequential mixture of 4 diastereomers that were immediately deprotected with TBAF to give diol **19**. Catalytic variants of the NHK process,²³ utilizing stoichiometric manganese, have also been explored to produce **19** with mixed success, with classical conditions proving to be the most reliable. Global oxidation of **19** provided the desired bisenone macrolide **8**, which contained an unanticipated and unresolvable minor diastereomer.

The diastereoselectivity of the final TBAF-mediated transannular cascade reaction was controlled by the configurations at the C2, C4, and C12 stereocenters, which provided reinforcement of the pseudo-chair transition state of **8**, thus providing an additional 3 stereocenters in a single step to yield target compound **2**. The resulting 2:1 mixture of diastereomers was easily separated chromatographically to provide pure **2** as a single diastereomer, which was unambiguously confirmed by single crystal X-ray diffraction, as well as an uncharacterized minor diastereomer. It is likely that the lower diastereoselectivity observed in this reaction arose from the unknown minor diastereomer in **8** and not the transannular reaction itself. Efforts are underway to identify the source of epimerization in the earlier intermediates.

Utilizing the previously described functional KOR assay,^{12b} *pseudo*-neoclerodane **2** was evaluated and its EC₅₀ value was determined in comparison to **1** and the endogenous KOR peptidic agonist dynorphin A (Table 1). Demonstrating a low nanomolar EC₅₀ value at the KOR, **2** was within 2 orders of magnitude of the activity of **1**, suggesting that compound **2** has, in part, validated the proposed hypothetical 3-point binding model at the kappa opioid receptor. **2** has specifically demonstrated that KOR activity of **1** (and **3**) can be maintained with the deletion of the C5 and C9 methyl groups, substitution of the C4 carbomethoxy for a methyl ether and with the added steric bulk and distortion of ring geometry added by the enol on the eastern portion of the molecule. A follow-up study is underway to address each of these positions systematically to further probe their role in the activity of **1**.

In summary, compound **2** was rationally designed by careful analysis and generalization of previous semisynthesis data on salvinorin A and represents the first kappa opioid agonist in its class, illustrating a significant structural divergence from its inspiration, salvinorin A, while still retaining high activity at the KOR. With this information, efforts are now underway to systematically explore structure-activity relationships of novel compounds in order to design valuable research tools and therapeutic medications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Salvinorin A (1) and representative semi-synthetic derivatives (3–7) in support of 3-point bind-ing model hypothesis. Values indicate fold-change in activity relative to 1 in the assay readout evaluated.

Figure 2. Disconnect strategy and retrosynthesis to 14-membered bisenone macrolide **8** that is capable of undergoing a diastereoselective transannular cascade reaction to **2**.

Synthetic route to *pseudo*-neoclerodane **2** with unambiguous characterization by single crystal X-ray diffraction.

Table 1

KOR activity: Inhibition of forskolin-induced cAMP accumulation. Summary of pseudo-neoclerodane 2 compared to 1 and dynorphin A.

#	κ -opioid receptor potency EC ₅₀ ± SEM ^{a,b} (nM)
1	0.08 ± 0.02
2	9 ± 2
dynorphin A	0.41 ± 0.07

^aMean ± SEM (standard error of the mean); n = 3 individual experiments run in triplicate.

^bKOR E_{max} = 100%