Synthesis and biological evaluation of C-2 halogenated analogs of salvinorin A

David Y. W. Lee [a],*, Lu Yang [a], Wei Xu [b], Gang Deng [a], Lin Guo [a], Lee-Yuan Liu-Chen [b]

[a] Bio-Organic and Natural Products Laboratory, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA
[b] Department of Pharmacology and Center for Substance Abuse Research, School of Medicine, Temple University, 3420 N. Broad Street, Philadelphia, PA 19140, USA

## ABSTRACT

Salvinorin A (**1**), the main active ingredient of *Salvia divinorum*, is a potent and selective κ opioid receptor (KOPR) agonist. Based on the SAR, its C-2 position is one of the key binding sites and has very little space tolerance (3–4 carbons atoms) and limited to only lipophilic groups. In our attempt to prepare PET brain imaging agent for mapping KOPR, a series of C-2 halogenated analogs have been synthesized and screened for binding affinity at κ (KOPR), μ (MOPR), and δ (DOPR). These C-2 halogenated analogs with sequential changes of atomic radius and electron density serve as excellent molecular probes for further investigating the binding pocket at C-2, particularly on the effects of α verses β configuration at C-2 position. The results of KOPR binding and functional studies reveal β isomer in general binds better than α isomer with the exception of iodinated analogs and none of the C-2 halogenated analogs shows any improvement of KOPR binding affinity. Interestingly, functional assay has characterized that **6b** is a partial agonist with E_{max} of 46% of the kappa receptor full agonist U50,488H at 250 nM (K_i). We have also observed that the affinity to the kappa receptor increases with atomic radius (I > Br > Cl > F) which is in good agreement with halogen bonding interactions reported in the literature.

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Salvia divinorum, a Mexican psychoactive plant, has been used in traditional spiritual practices for centuries.[1–4] Salvinorin A (**1**), a neoclerodane diterpenoid isolated from *S. divinorum*,[5,6] is identified to be a potent and selective kappa (κ) opioid receptor (KOR) agonist.[7,8] Smoking of *S. divinorum* leaves fortified with salvinorin A extract has been the most efficient method for its hallucinogenic effects in human. Ingestion of *S. divinorum* in humans causes hallucinations or delusional episodes that mimic psychosis.[9,10] It is increasingly used as a recreational drug in North America and Europe. Salvinorin A is the principal active ingredient responsible for the observed hallucinogen; however, its action in human brain remains unclear. Structurally, **1** represents the first non-alkaloid opioid receptor type-selective drug. The unique structural and biological features of **1** make it an attractive candidate for exploring the opioid pharmacology and a potential template for development of novel F-18 (PET) or I-123 (SPECT) substituted imaging agent for its sites of action in the brain.

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3 R= (structure)
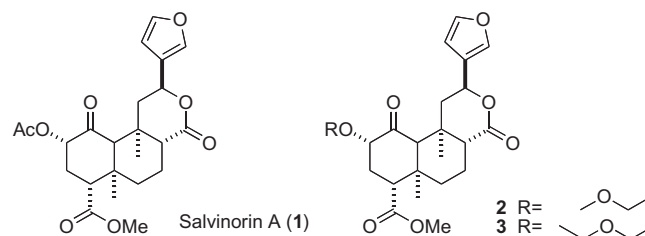
Prior structure–activity relationship studies of **1** by our group and others have led to the synthesis of a significant number of C-2 analogs. Among them, methoxymethyl ether (**2**)[11] and ethoxymethyl ether (**3**)[12] display higher binding affinity and potency than **1** at KOPR. Compound **2** also exhibits longer duration of action than **1** in vivo.[13] It has been demonstrated that the C-2 position of **1** is one of its key binding sites and highly sensitive to size and electronegativity of the substituent. In general, C-2 pocket has very little space tolerance (3–4 carbons atoms) and limited to only lipophilic groups. The synthesis of C-2 halogenated analogs would provide a series of interesting molecules for further investigation of the binding pocket at C-2, particularly with regard to the orientation of the C-2 α verses β isomers. In addition, F-18 and I-123 are usually the most popular radionuclides of choice for PET and SPECT studies, respectively, it would be necessary to evaluate the binding data

with non-radioactive compounds. Since the synthesis of C-2 fluoro- and iodo-analogs of **1** and their corresponding binding data have not yet been reported and the halogen bonding in biomolecular systems are becoming popular for rational drug design,[14] our study would be useful for providing such information particularly in view of the limited options of introducing halogens to the salvinorin A structure.

Salvinorin A (**1**) is isolated from *S. divinorum* and then converted to salvinorin B (**2a**) and 2-*epi*-salvinorin B (**2b**), as previously described.[15] The reaction of **2a** and **2b** with Deoxo-Fluor reagent[16] affords the C-2 fluoro analogs **3b** and its C-2 epimer **3a** (Scheme 1). Initial attempts to synthesize the C-2 chloro analogs **4a** and **4b** by treatment of **2a** and **2b** with TCT and DMF at room temperature[17] lead to the C-2 formate **4c**,[18] which may have formed due to presence of H_2O in the solvent. However, an elevated temperature at 35 °C gives exclusively the chlorinated products.

Addition of NaBr in the synthesis of **4b** provides a mixture of **4b** and the bromo-analog **5b** with a ratio of 3:2 (Scheme 2). Efforts to increase the yield of **5b** by adding phase transfer catalyst 15-crown-5 or separate **5b** from **4b** with preparative HPLC are unsuccessful. Alternatively, PBr$_3$ is exploited in brominating of **2a** and an unexpected rearrangement product **5c** is formed. To our knowledge, the opening of the lactone ring of **1** under basic conditions has been reported by several groups,[19] however, acid catalyzed

ring opening has not yet been addressed. Compound **5c** is also obtained when **2a** was treated with PPh$_3$ and bromine in the presence of a catalytic amount of imidazole.[20] Unfortunately, the previously reported procedure[21] in converting **2a** to **5b** is not reproducible in our hands and the synthesis of **5a** and **5b** is eventually achieved by adopting a two-step-route as shown in Scheme 2.[15]

Reaction of **2a** with I$_2$, PPh$_3$ and imidazole in toluene yields predominantly **6b** with small amount of C-2 epimer **6a** and deoxygenation product **6c**.[20] Surprisingly, when **2b** is treated with the identical conditions, **6b** is also the major product, which could be explained by a free-radical substitution mechanism. As shown in Scheme 3, when heated in toluene, the C-2 epimers **2a** and **2b** may proceed to form the common free-radical intermediate **6d** with the loss of configuration on C-2. Since the axial attack of the iodo radical at C-2 is favored by both stereoelectronic effects and torsional effects,[22,23] the C-2-β epimer **6b** is preferentially formed as expected. The C-2-α epimer **6a** is more favorable under S_N2 conditions evidenced by treatment of **6b** with lithium iodide in DMF leads to an exclusive conversion to **6a**.

A total of 8 halogenated salvinorin A analogs is synthesized and evaluated for binding to the μ, δ, and κ-opioid receptor by competitive inhibition of [³H]diprenorphine (∼0.3 nM) binding to membranes prepared from Chinese hamster ovary (CHO) cell lines stably transfected with rat μ, mouse δ, or human κ opioid receptor.
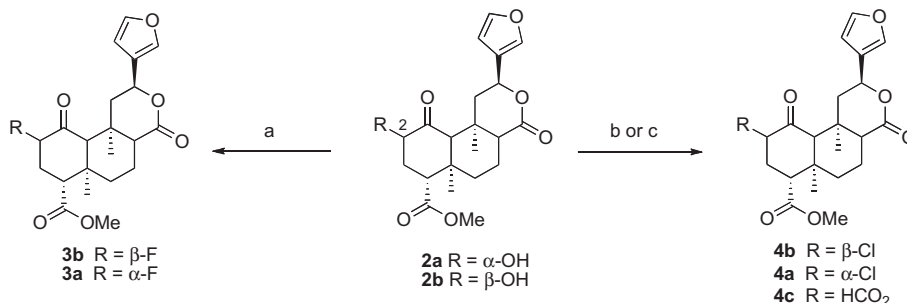
Scheme 1. Reagents and conditions: (a) Deoxo-Fluor reagent, CH$_2$Cl$_2$, −78 °C, 2 h, rt, 2 h, **3b** (74%), **3a** (80%); (b) TCT, DMF/CH$_2$Cl$_2$, 35 °C, 12 h, **4b** (90%), **4a** (81%); (c) TCT, DMF/CH$_2$Cl$_2$, rt, 2 h; yield varies according to reaction time.
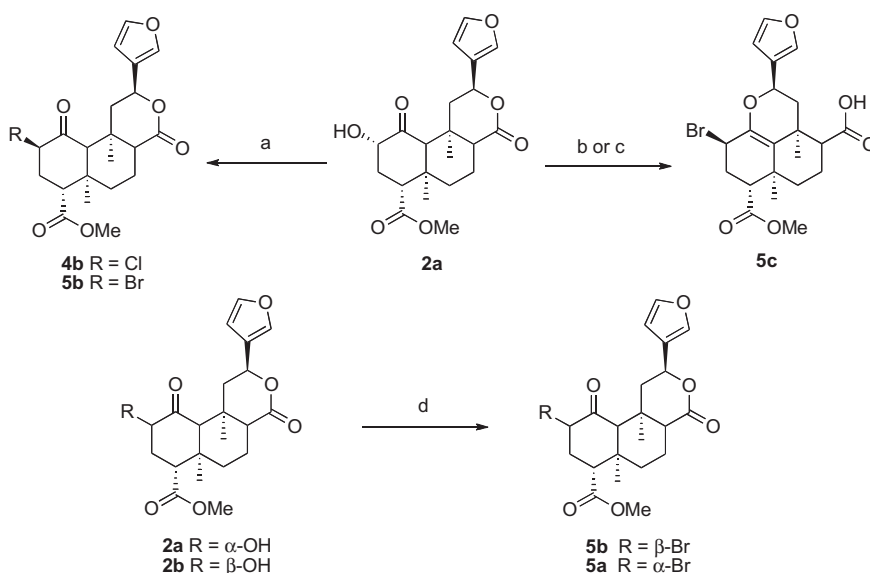
Scheme 2. Reagents and conditions: (a) TCT, DMF/CH$_2$Cl$_2$, NaBr, overnight, 92%, **4b:5b** = 3:2; (b) PBr$_3$, C$_6$H$_6$, rt, 86%; (c) PPh$_3$, Br$_2$, imidazole, toluene, 48%; (d) (i) (CF$_3$SO$_2$)$_2$O, pyridine, CH$_2$Cl$_2$, 0 °C; (ii) NaBr, DMF, rt, 75% in two-steps.
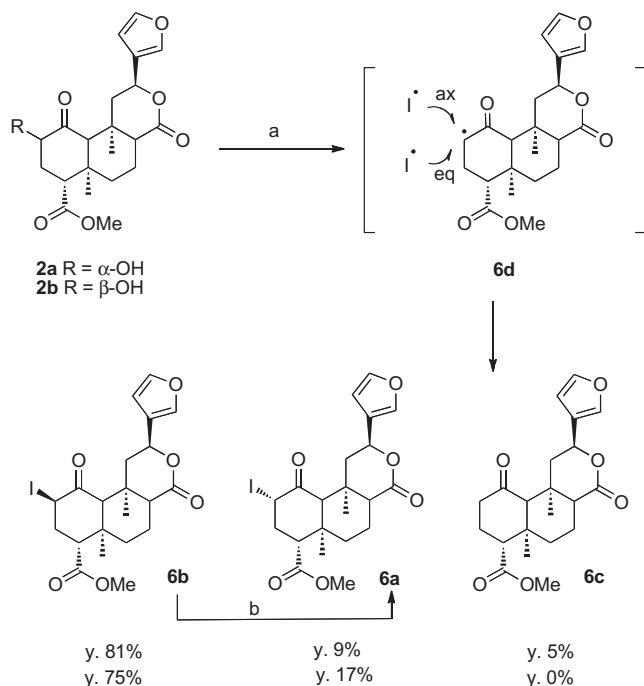
Scheme 3. Reagents and conditions: (a) I_2, PPh_3, imidazole, Toluene, 80 °C, 1 h, 92–95%; (b) LiI, DMF, rt, overnight, 95%.

Table 1
K_i values and EC_{50} and E_{max} to kappa opioid receptor

Sample	K_i (nM) ($n = 3$)	EC_{50} (nM) ($n = 3$ and 4)	E_{max} (% of U50,488H)
3a	>1000	—	—
4a	>1000	—	—
5a	422 ± 44	1966 ± 94	87
6a	198 ± 43	648 ± 152	92
3b	197 ± 14	539 ± 50	77
4b	167 ± 6.5	673 ± 111	112
5b	42.3 ± 3.3	282 ± 8.5	90
6b	245 ± 14	239 ± 23	46
6c	51.6 ± 0.9	266 ± 40	124
4c	24.3 ± 0.9	83.3 ± 10.9	103
5c	>1000		—
1		7.5 ± 1.7	106
U50,488H	1.2 ± 0.3	6.3 ± 0.7	100

The μ, δ, and κ-opioid receptor agonist, etorphine, is used as the reference compound in the binding assays. At 3 μM, most of the compounds have exhibited >50% inhibition of [3H]diprenorphine binding to the kappa, but not to mu or delta, opioid receptor, indicating significant affinity for only the kappa opioid receptor. K_i values of these compounds for the kappa opioid receptor are determined. As shown in Table 1, β isomer in general binds better than α isomer with the exception of iodinated analogs and none of the C-2 halogenated analogs has shown any improvement of binding activity than the parent compound (**1**). It also appears that affinity to the kappa receptor increases with atomic radius (I > Br > Cl > F). Our results are in good agreement with halogen bonding interaction detected within small model complexes.[14] Subsequent kappa receptor functional assay has been carried out to determine the potencies and efficacies of these compounds and U50,488H is used as the reference compound. [35S]GTPγS binding is used as the functional measure of activation of the kappa opioid receptor. Dose–response curves of these compounds are generated and EC_{50} and E_{max} values have been determined (Table 1). One noteworthy finding is that C-2 iodo analog **6b** is a partial agonist with E_{max} of 46% of U50,488H.

In view of the fact that halogen bonding in biomolecular systems are becoming popular for rational drug design and the synthesis of C-2 fluoro- and iodo-analogs of **1** and their corresponding binding data have not yet been reported, our study would be useful for providing such information particularly with a limited options of introducing halogens to the salvinorin A structure. A total of 8 C-2-halogenated salvinorin A analogs have been synthesized and evaluated for the MOPR, DOPR, and KOPR affinities by competitive inhibition of [3H]diprenorphine (~0.3 nM) binding to membranes prepared from Chinese hamster ovary (CHO) cell lines stably transfected with rat MOPR, FLAG-mouse DOPR, and human KOPR. As summarized in Figure 1 and Table 1, C-2-β isomer in general binds better than α isomer with the exception of iodinated analogs and none of the C-2 halogenated analogs showed any improvement of binding affinity than the parent compound **1**. It also appears that affinity to the kappa receptor increases with atomic radius (I > Br > Cl > F). These results agree with the characteristics reported within small model halogen bonded systems. Subsequent kappa receptor functional assay (Fig. 1) showed that **6b** is a partial agonist with E_{max} of 46% of U-50,488 H at 250 nM (K_i). It is conceivable that with further improvement of affinity, **6b** could be developed as a potential kappa antagonist.
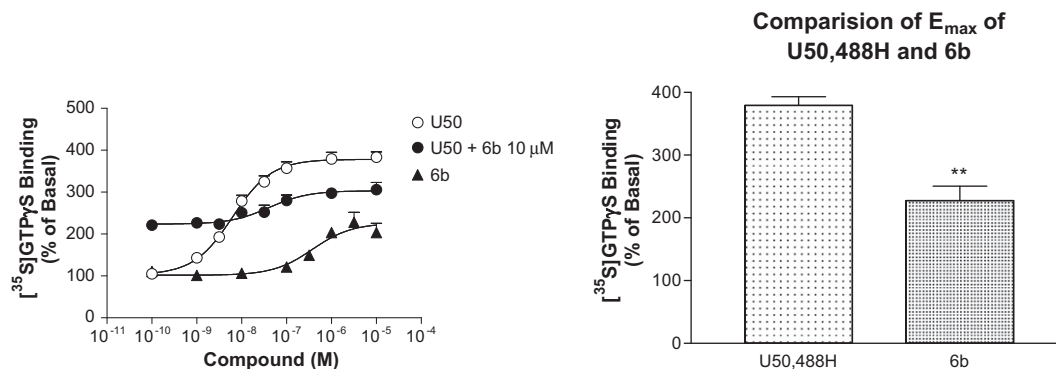
Acknowledgements

Figure 1.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.001.

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