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Synthesis and Biological Evaluation of 2-Alkyl-2-Methoxymethyl-Salvinorin Ethers as Selective *κ*-Opioid Receptor Agonists

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Abstract: The synthesis of a new series of C-2-alkyl-2-methoxymethyl-salvinorin ethers and their binding affinities at κ-, μ-, and δ-opioid receptors are presented. We have developed a synthesis that enables installation of alkyl-substituents at C-2 while maintaining the integrity of the C-2 methoxymethyl ether and retaining κ-opioid receptor binding activity. Among these new compounds, 2-methyl-2-methoxymethyl-salvinorin ether (9a) is a potent full agonist at the κ receptor and shows comparable potency in Ki and EC50 with salvinorin A and U50488H. These C2-alkylated analogs have been identified as full κ agonists.

Keywords: κ-opioid, receptor, affinity, agonist

Introduction

The *κ*-opioid receptor (KOR) is one of the three types of opioid receptors which belong to the seven-transmembrane receptor family; the other two are *δ* (DOR) and *μ* (MOR).[1] Activation of endogenous KOR results in analgesia, dysphoria, anti-pruritis, and water diuresis, among other effects.[2] Many selective KOR agonists have been synthesized, including HZ-2,[3] U50,488H,[4] U69,593,[5] U62,066,[2] and enadoline,[6] as shown in Figure 1. Structurally, each of these compounds contains at least one nitrogen atom. Salvinorin A (**1**), a neoclerodane diterpene isolated from *Salvia divinorum*, is the first known natural product acting on KOR selectively.[7] Notably, it does not contain a basic nitrogen atom in the structure. Salvinorin A is a potent and selective KOR agonist. It binds to KOR with a higher affinity (K_i=1.3 nM) than U50,488H (*K*i=2.7 nM), a prototypic highly selective KOR agonist, but does not show significant affinities to DOR, MOR, and nociceptin/orphanin FQ receptors.[8] It has also been reported that salvinorin A possesses higher selectivity for KOR than U50,488H and U69,593.[9] However, the duration of action of salvinorin A *in vivo* is short, which is likely due to the hydrolysis of the acetate by esterase to produce Salvinorin B (**2**), a compound with a comparatively much lower affinity to KOR. We have previously reported that 2-methoxymethyl salvinorin B (**3**)[10] and corresponding 2-ethoxymethyl-salvinorin B[11] both have greater potency (x2) than salvinorin A and much longer durations of action *in vivo*.

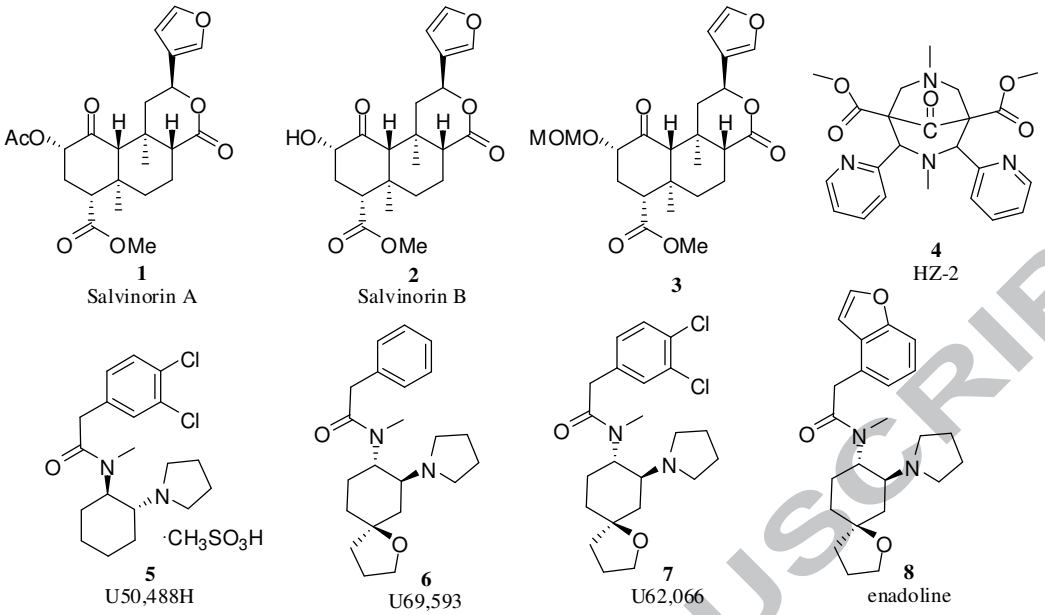


Figure 1. Structures of KOR agonists

To date, all previous modifications at the C-2 position of salvinorin A have been limited to either replacing the C-2 acetate with various ester or ether derivatives, or with halogens.[11] Alkylation at C-2 has not been reported because of the difficulties encountered in introducing an alkyl group at this position, which is adjacent to a carbonyl group and ester. In our attempt to fully explore the effect of 3D-alignment at C-2 position to structure-activity relationship (SAR) of salvinorin A, a series of 2-alkyl-2-methoxymethyl-salvinorin ethers were synthesized and evaluated for affinity, potency, and efficacy as KOR ligands. As shown in Table 1, 2-methyl-2-methoxymethyl-salvinorin ether (**9a**) was found to be a potent full agonist at the KOR, and shows a comparable potency in K_i and EC_{50} to salvinorin A and U50488-H. In addition, **9a**, which lacks a labile ester moiety, is much more stable than salvinorin A. This report opens up a synthetic strategy for introducing a series of stable C2-β-alkylated derivative of salvinorin A for pharmacological evaluation.

Table 1

Affinities (K_i) at the κ-opioid receptors

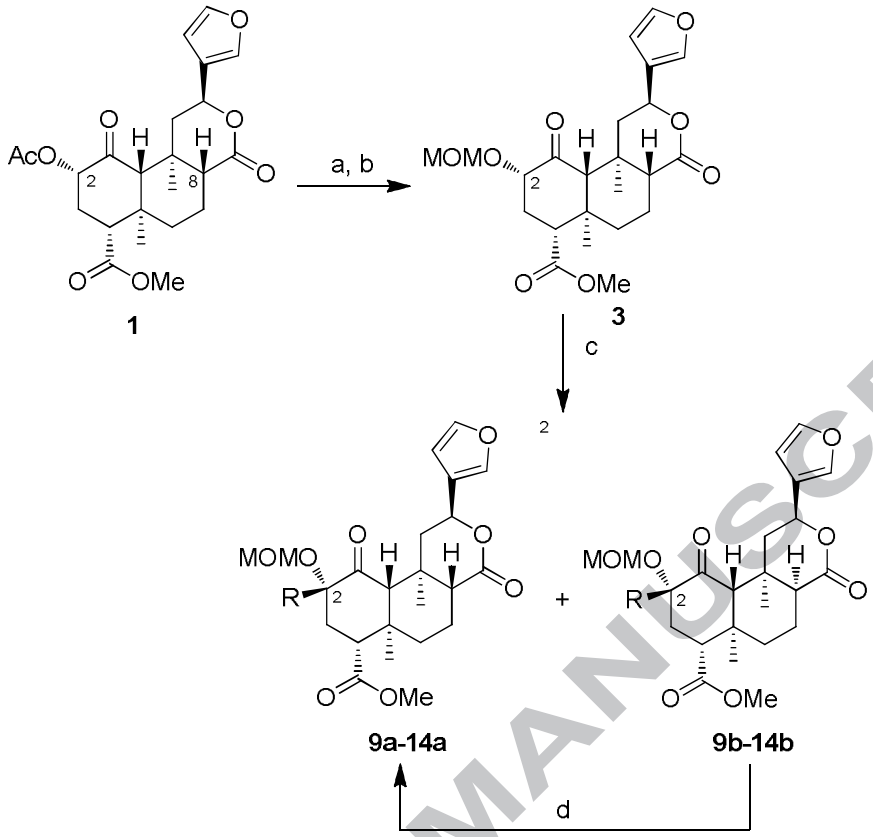
Compound	R	K_i ± SEM (nM) [a,b]		
		κ	δ	μ
9a	$-CH_3$	4.7±0.3	>1,000	>1,000
9b	$-CH_3$	20.5±6.5	>1,000	>1,000
10a	$-CH_2CH_3$	301±24	>1,000	>1,000
10b	$-CH_2CH_3$	210±22	>1,000	>1,000
11a	$-CH_2CH_2CH_3$	>1,000	>1,000	>1,000
11b	$-CH_2CH_2CH_3$	>1,000	>1,000	>1,000
12a	$-CH_2CH{=}CH_2$	48±6	>1,000	>1,000
12b	$-CH_2CH{=}CH_2$	250±51	>1,000	>1,000
13a	$-CH_2(CH_2)_3CH_2I$	>1,000	>1,000	>1,000

13b	$-CH_2(CH_2)_3CH_2I$	>1,000	>1,000	>1,000
14a	$-CH_2CH_2OCH_2CH_2I$	108±19	>1,000	>1,000
14b	$-CH_2CH_2OCH_2CH_2I$	316±70	>1,000	>1,000
Etorphine		0.5±0.05	2.9±0.6	0.14±0.02
1 (Salvinorin A)		1.3±0.5	>1,000	>1,000
3		0.73±0.06	>1,000	>1,000

[a] Inhibition of [^{3}H]diprenorphine binding hKOR in membranes of CHO-hKOR cells;
[b] Mean ± s.e.m. of at least three independent experiments performed in duplicate.

Chemistry

Salvinorin A (**1**) was isolated from dry *Salvia divinorum* leaves by following the chromatographic procedures worked out in our laboratory.[8] As shown in Scheme 1, deacetylation of **1** using a reported procedure provided the Salvinorin B (**2**) and the C8-epimer almost in 1:1 ratio.[12] It would be ideal to be able to control the epimerization and shift the ratio to the desirable natural isomer.[13] In order to improve the ratio of C8-epimerization and increase the overall yield of the intermediate compound **2**, an efficient and mild deacetylation method (H_2O_2/$NaHCO_3$) for compounds with an adjacent α carbonyl group[14] similar to the structure of **1** was employed. Unfortunately, a significant amount of the starting material (~40%) was recovered even under various concentrations and ratios of reagents or prolonged reaction time. However, this problem was overcome by simply adding the sodium-selective complexing agent, 15-crown-5, and prolonged vigorous stirring (36 h) to afford a nearly quantitative yield of salvinorin B. Notably, no C8-epimer product was detected following this deacetylation procedure, affording salvinorin B exclusively in good yield.



Scheme 1. Reagents and conditions: a) $NaHCO_3$, H_2O_2, 15-Crown-5, THF:MeCN=1:1, rt; b) MOMCl, DIPEA, DMF, rt; c) NaH, Alkyl halide , THF, -50 ^oC; d) K_2CO_3, MeOH, rt.

With a sufficient amount of intermediate **2** in hand, derivatization of the C2-hydroxyl as the methoxymethyl ether proceeded smoothly employing previously reported methods.[15] The next step involved alkylation at the C-2 position with various groups. With a bulky group introduced at C-2, we were concerned about the reactivity of C-2 under standard alkylation conditions. Therefore, we chose iodomethane as the model substrate to react with the enolate anion generated by various bases including LDA, triphenylmethyllithium, sodium hydride, lithium bis(trimethylsilyl)amide, lithium 2,2,6,6-tetramethylpiperidide and lithium hexamethyldisilazide. Unsurprisingly, employment of most of these bases resulted in either the C8-epimerization of 2-methoxymethyl-salvinorin B, or the decomposition of the starting material even at -78^oC. Interestingly, only NaH gave the desired 2-methyl-2-methoxymethyl-salvinorin ether product without C8-epimerization (Scheme 1, Table 1). The configuration of the methyl group at C-2 position was confirmed to be oriented in the β position by 2D NOESY. This may due to the fact that axial methyl groups at C-5 and C-9 along with the C-5 carboxyl methyl ester group block the attack of iodomethane from the α face. Encouraged by this result, we successfully employed a series of alkylating agents with various carbon chain lengths, affording yields from 30% to 70%. However, the C8-epimer of the 2-alkyl-2-methoxymethyl-salvinorin ether always accompanied the product, and even comprised the sole product in one case (**10b**). Nevertheless, epimerization of the undesired C8-epimer to its natural isomer was easily achieved through use of K_2CO_3 in methanol at room temperature. All spectral data (^{1}H-NMR, ^{13}C-NMR and high-resolution mass)

obtained were consistent with the structures proposed.

Results and discussion

All these compounds were evaluated for binding affinities to the KOR, DOR, and MOR by competitive inhibition of [^{3}H] diprenorphine binding to membranes prepared from Chinese hamster ovary cells (CHO) stably transfected with either rat MOR, mouse DOR, or human KOR (hKOR). Inhibition of [^{3}H] diprenorphine binding was first performed with 3 μM of each compound, and those exhibiting >50% inhibition were further evaluated for determination of K_i values.[16] The potencies and efficacies on hKOR were determined by their abilities to enhance [^{35}S]GTPγS binding to the membranes of CHO-hKOR cells. The selective full κ agonist U50,488H was used as the reference compound with a relative efficacy of 100. Data are shown in Table 1 and Table 2.

Table 2

Affinities (K_i), potencies (EC_{50}) and efficacies at the κ-opioid receptor

Compound	R	K_i (nM)[a,b]	EC_{50} (nM)[b,c]	E_{max}[d]
1	-	1.3±0.5	4.5±1.2	110±7
3	H	0.73±0.06	0.6±0.2	115±15
9a	$-CH_3$	4.7±0.3	19.2±2.0	123±16
9b	$-CH_3$	20.5±6.5	80.9±8.7	103 ± 16
10a	$-CH_2CH_3$	301±24	ND[e]	83 at 1 μM
10b	$-CH_2CH_3$	210±22	ND[e]	81 at 1 μM
11a	$-CH_2CH_2CH_3$	>1,000	NT[f]	NT[f]
11b	$-CH_2CH_2CH_3$	>1,000	NT[f]	NT[f]
12a	$-CH_2CH{=}CH_2$	48±6	ND[e]	99 at 1 μM
12b	$-CH_2CH{=}CH_2$	250±51	ND[e]	84 at 1 μM
13a	$-CH_2(CH_2)_3CH_2I$	>1,000	NT[f]	NT[e]
13b	$-CH_2(CH_2)_3CH_2I$	>1,000	NT[f]	NT[e]
14a	$-CH_2CH_2OCH_2CH_2I$	108±19	ND[e]	98 at 1 μM
14b	$-CH_2CH_2OCH_2CH_2I$	316±70	ND[e]	85 at 1 μM
U50,488H	-	2.7±0.2	10.6±1.8	100

[a] Inhibition of [^{3}H]diprenorphine binding hKOR in membranes of CHO-hKOR cells;

[b] Mean ± s.e.m. of at least three independent experiments performed in duplicate;

[c] Enhancement of [^{35}S]GTPγS binding to membranes of CHO-hKOR cells;

[d] E_{max} values were determined as the percentage of maximal response to U50,488H;

[e] ND: not determined because the response did not reach a plateau or it was tested at only 1 μM

[f] NT: not tested due to low affinity.

Most of the newly synthesized C-2 alkyl analogs have submicromolar affinity and potency. The compounds with K_i values < 1 μM exhibited agonist activity, but no antagonist activity was detected. When the aliphatic 2-alkyl derivatives (**9a**-**11a**, **9b**-**11b**) were evaluated, we found that by increasing the size of alkyl groups, affinities to the KOR were decreased accordingly. Similar results at C-2 alpha position were reported by Chavkin et al.[17] and Béguin et al.[18] For instance, the

affinity of **9a** was about 6-fold lower (K_i value = 4.7±0.3 nM) than lead compound **3** (K_i value = 0.73±0.06 nM), which lacks an alkyl substituent. Subsequently, compound, **9a** was found to be a potent full agonist at the KOR (123% efficacy relative to the full agonist U50,488H) and showed a 2- to 5-fold higher K_i and EC_{50} than **1** (K_i values (nM): **9a**, 4.7±0.3; **1**, 1.3±0.5; EC_{50} values (nM): **9a**, 19.2±2.0; **1**, 4.5±1.2) and than U50,488H (K_i values (nM): 2.7±0.2; EC_{50} values (nM): 10.6±1.8). This suggests that **9a**, if labeled with ^{14}C-methyl, may serve as a molecular probe for mapping the KOR in the brain. Introduction of an ethyl group (**10a** and **10b**) at the C-2 position led to decreased affinity (about 70-fold lower than **9a**). Extending the aliphatic alkyl group to *n*-propyl at C-2(**11a** and **11b**) resulted in the loss of binding activity (K_i >1.0 μM). When the unsaturated allyl group was introduced at C-2(**12a**, **12b**), a pronounced increase in affinity (K_i values (nM): 48±6 and 250±51, respectively) to the KOR was observed compared to its corresponding saturated analog (**11a**, **11b**, K_i values (nM): >1,000 for both), and both of them showed agonist activities (E_{max} values of 99% and 84%, respectively, compared to U50,488H). These data are different from the C-2 allyl ether reported by Béguin et al,[17] which showed a 2-fold decrease in affinity and potency compared to its isostere. When a 5-iodopentyl group was introduced, the affinity to KOR was abolished (K_i values (nM) >1,000). Interestingly, affinity to KOR was somewhat recovered when the linear 2'-iodoethoxyethyl group (**14a**, **14b**, K_i values (nM): 108±19, 316±70, respectively) was introduced at C-2.

Most of the C2-alkyl analogs with the natural configuration (8β-H) show better affinity and potency than its corresponding epimer (8α-H), except **10a, 10b**, in which the natural configuration **10a** (K_i values (nM): 301±24) shows 1.5-fold lower affinity than its unnatural epimer (**10b**, K_i values (nM): 210±22). This result was also reported in our previous publication where salvinorin B showed 3-fold lower affinity than its epimer.

In summary, the present results suggest that the affinity and potency at KOR is closely related to the size of the alkyl substituent at the C-2 position. Introduction of alkyl groups at C-2 led to decrease in binding affinity and potency compared to lead compound **3,** especially with a bulky alkyl group. This may due to the fact that the 3D-alignment at C-2 affected interaction with Tyr313.[19] In addition, an ether functionality at C2 (**14a, 14b**) was found to enhance affinity to the KOR compared to alkyl substituents lacking an ethereal oxygen (**13a, 13b**). Since the 2-methyl substituted derivative **9a** shows only 2- to 5-fold lower affinity than salvinorin A and U50,488H, its ^{14}C-labeled **9a** would be an excellent molecular probe for mapping the KOR in the brain, a highly interesting approach which is being investigated.

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References and notes

1 Dhawan, B.N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P.B.; Portoghese, P.S.; Hamon, M. *Pharmacol Rev*. **1996**, *48*, 567.

2 Liu-Chen LY. *Life Sci*. **2004**; 75, 511.

3 Siener, T; Cambareri, A; Kuhl, U; Englberger, W; Haurand, M; Kögel, B; Holzgrabe, U. *J Med Chem*. **2000**, *43*, 3746.

4 Von Voigtlander, P.F.; Lewis, R.A. *Prog. Neuropsychopharmacol. Biol. Psych*. **1982**, *6*, 467.

5 La Regina, A.; Petrillo, P.; Sbacchi, M.; Tavani, A. *Life Sciences* **1988**, *42*, 293.

6 Walsh, S.L.; Strain, E.C.; Abreu, M.E.; Bigelow, G.E. *Psychopharmacology (Berl.)* **2001**, *157*, 151.

7 Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P and Rothman RB. Proc Natl Acad Sci U S A . **2002**, 99, 11934.

8 Lee DY, He M, Liu-Chen LY, Wang Y, Li JG, Xu W, Ma Z, Carlezon WA Jr, Cohen B. *Bioorg Med Chem Lett.* **2006**; 16, 5498.

9 Baker, L.E.; Panos, J.J.; Killinger, B.A.; Peet, M.M.; Bell, L.M.; Haliw, L.A.; Walker, S.L. *Psychopharmacology (Berl).* **2009**, *203*, 203.

10 Wang, Y.; Chen, Y.; Xu, W.; Lee, D.Y.; Ma, Z.; Rawls, S.M.; Cowan, A.; Liu-Chen, L.Y. *J. Pharmacol. Exp. Ther*. **2008**, *324*, 1073.

11 Lee, D.Y.; Karnati, V.V.; He, M.; Liu-Chen, L.Y.; Kondaveti, L.; Ma, Z.; Wang, Y.; Chen, Y.; Beguin, C.; Carlezon, W.A. Jr; Cohen, B. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3744.

12 Tidgewell, K.; Harding, W.W.; Schmidt, M.; Holden, K.G.; Murry, D.J.; Prisinzano, T.E. *Bioorg Med Chem Lett.* **2004**, *14*, 5099.

13 Scheerer, J.R.; Lawrence, J.F.; Wang, G.C.; Evans, D.A. *J Am Chem Soc*. **2007**, *129*, 8968.

14 Zheng, Q.Y.; Darbie, L.G.; Cheng, X.; Murray, C.K. *Tetrahedron Lett*. **1995**, *36*, 2001.

15 Béguin C, Richards MR, Wang Y, Chen Y, Liu-Chen LY, Ma Z, Lee DY, Carlezon WA Jr, Cohen BM. *Bioorg Med Chem Lett*. **2005**; 15, 2761.

16 Huang, P.; Kehner, G. B.; Cowan, A.; Liu-Chen, L.-Y. J. *Pharmacol Exp Ther*. **2001**, 297, 688.

17 Chavkin C, Sud S, Jin W, Stewart J, Zjawiony JK, Siebert DJ, Toth BA, Hufeisen SJ, Roth BL. *J Pharmacol Exp Ther.* ***2004***, 308, 1197.

18 Béguin C, Richards MR, Li JG, Wang Y, Xu W, Liu-Chen LY, Carlezon WA Jr, Cohen BM. *Bioorg Med Chem Lett*. **2006**, 16, 4679.

19 McGovern DL, Mosier PD, Roth BL, Westkaemper RB. *J Mol Graph Model*. **2010**, 28, 612.

Graphic abstract