Potential Drug Abuse Therapeutics Derived from the Hallucinogenic Natural Product Salvinorin A

Katherine M. Prevatt-Smith[a], **Kimberly M. Lovell**[a], **Denise S. Simpson**[a], **Victor W. Day**[b], **Justin T. Douglas**[c], **Peter Bosch**[e], **Christina M. Dersch**[d], **Richard B. Rothman**[d], **Bronwyn Kivell**[e], and **Thomas E. Prisinzano**[a,*]

[a]Department of Medicinal Chemistry, University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045 [b]Small Molecule X-Ray Crystallography Lab, University of Kansas, 1251 Wescoe Hall Drive, Malott 6044, Lawrence, KS 66045 [c]Nuclear Magnetic Resonance Laboratory, University of Kansas, 1251 Wescoe Hall Drive, Malott 3002, Lawrence, KS 66045 [d]Clinical Psychopharmacology Section, National Institute on Drug Abuse, National Institutes of Health, 333 Cassell Drive, Triad Building, Suite 4400, Baltimore, MD 21224 [e]School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

Abstract

Previous structure-activity relationship studies of salvinorin A have shown that modification of the acetate functionality off the C-2 position to a methoxy methyl or methoxy ethyl ether moiety leads to increased potency at KOP receptors. However, the reason for this increase remains unclear. Here we report our efforts towards the synthesis and evaluation of C-2 constrained analogs of salvinorin A. These analogs were evaluated at opioid receptors in radioligand binding experiments as well as in the GTP-γ-S functional assay. One compound, **5**, was found to have affinity and potency at κ opioid (KOP) receptors comparable to salvinorin A. In further studies, **5** was found to attenuate cocaine-induced drug seeking behavior in rats comparably to salvinorin A. This finding represents the first example of a salvinorin A analog that has demonstrated anti-addictive capabilities.

Introduction

The neoclerodane diterpene salvinorin A is the active component of *Salvia divinorum* L. Epling&Jativa (Lamiaceae), a widely available psychoactive plant.[1] *S. divinorum* is a member of the sage family and is indigenous to Oaxaca, Mexico.[1, 2] For centuries it has been used by the Mazatec Indians in divination ceremonies as well as to treat headaches, rheumatism, and *panzón de barrego*, a semi-magical disease.[1] Salvinorin A was isolated from *S. divinorum* and determined to be a potent hallucinogen when vaporized and inhaled, with an active dose in humans of 4.5 – 21 μg/kg.[3] This rivals the potency of classical hallucinogens such as lysergic acid diethylamide (LSD) and 4-bromo-2,5-dimethoxyamphetamine (DOB).[4] However, unlike the classical hallucinogens, salvinorin A does not have activity at serotonin $5HT_{2A}$ receptors.[4–6] Instead, it was found to be a potent and selective κ opioid (KOP) receptor agonist.[4] KOP agonists have received recent research focus because they may have applications as drug abuse therapeutics.[7]

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[*]Corresponding author. Fax: 785 864 5326; Tel: 785 864 3276; prisinza@ku.edu.

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Recently it has been reported that converting the acetate group at the C-2 position of salvinorin A to a methoxy- or ethoxymethyl ether produces ligands of greater affinity and potency at KOP receptors than salvinorin A.[8, 9] However, the structural basis that underlies these changes in affinity and potency is unknown. We hypothesize that the ether moieties at C-2 are relatively flexible and can adopt different conformations when interacting with KOP receptors; for example, an eclipsed or an extended conformation as shown in Figure 1. Thus, we applied the concept of conformational constraint in order to design ligands in which the flexible portions are pre-organized in the hopes that such ligands would aid in the elucidation of the structural basis for the observed changes in affinity and potency. Here, we report our synthetic efforts towards this end, as well as the biological evaluation of C-2 constrained analogs of salvinorin A.

Chemistry

Compounds **3** and **4** were synthesized according to Scheme 1. Salvinorin A (**1**) was extracted from commercially available dried *S. divinorum* leaves and converted to salvinorin B (**2**) as previously described.[10] Treatment of **2** with either chloromethyl methyl ether or chloromethyl ethyl ether in the presence of *N,N*-diisopropylethylamine (DIPEA) afforded the previously reported ethers **3** and **4**.[8]

Compounds **5**, **6**, and **7** were synthesized according to Scheme 2. A solution of **2** in CH_2Cl_2 was treated with 3,4-dihydro-2*H*-pyran or 2,3-dihydrofuran in the presence of a catalytic amount of pyridinium *p*-toluene sulfonate (PPTS) to afford the previously reported tetrahydropyran (**5** and **6**) and novel tetrahydrofuran (**7**) C-2 constrained derivatives.[8] Under these conditions, diastereomers were formed and, in the case of tetrahydropyrans **5** and **6**, were separated from each other through flash column chromatography, and characterized by NMR, and X-ray crystallography (Figures 2 and 3). In the case of the diastereomers of **7**, they were separated from each other through flash column chromatography and characterized by NMR.

NMR spectroscopy and X-Ray crystallography were used to assign the stereochemistry of the new stereocenter in compounds **5** and **6**. In carbohydrate chemistry, anomeric $^1J_{C,H}$ values are useful for the assignment of configuration at that position since pyranoses with an axial proton have a $^1J_{C,H}$ value that is approximately 10 Hz lower than the corresponding value in compounds with an equatorial anomeric proton.[11, 12] Using this method, we determined that the anomeric $^1J_{C,H}$ values for compounds **5** and **6** are 168.55 Hz and 159.36 Hz, respectively, which are consistent with previous reports of substituted pyranoses. Given these data, we propose that **5** contains an equatorial, β-H (*R*) and **6** contains an axial, α-H (*S*) (Figure 2).[13] Compound **5**, the less polar diastereomer, was crystallized and we determined its structure using X-ray crystallography, confirming the orientation of the hydrogen predicted by our NMR analysis (Figure 3). In this structure, the tetrahydropyran moiety forms an α-glycosidic bond with the salvinorin A core in an axial position and the anomeric hydrogen in an equatorial, β position. Subsequently, compound **6** must be the other diastereomer.

Compounds **8** and **9** were synthesized according to Scheme 2. In the presence of a catalytic amount of PPTS, a suspension of **2** in ethyl vinyl ether was refluxed for 2 h, affording a diastereomeric mixture of previously reported ethers **8**.[8] These diastereomers were separated from each other by flash column chromatography, and the less polar diastereomer (**8a**) was isolated. The more polar diastereomer (**8b**) appeared to degrade during purification and could not be completely purified (purity = 81.938 %) through trituration, flash column chromatography, or HPLC. The impurity was not determined, although there was no amount of **8a** present as detectable by HPLC. Both **8a** and **8b** were characterized by NMR. In order

to produce novel ether **9**, ethyl vinyl ether was added to a solution of Br_2 in CH_2Cl_2, followed by the addition of DIPEA and finally **2**. These conditions afforded a mixture of diastereomers which were subsequently separated from each other by HPLC and characterized by NMR.

Compounds **10**, **11**, and **12** were synthesized according to Scheme 2. Following a literature procedure, a mixture of acetic ahydride and acetic acid was added to a mixture of **2** in pyridine at 0 °C.[14] The mixture was allowed to warm to room temperature and stirred for 30 min, affording formate **10**. Known carbonate **11**[9, 15] was produced by treating a solution of **2** in CH_2Cl_2 with ethyl chloroformate in the presence of DMAP and TEA at room temperature for 24 h. Following a literature procedure, carbamate **12** was produced by treating a mixture of **2** in pyridine with ethyl isocyanate at 35 °C overnight.[16]

Finally, compound **14** was synthesized according to Scheme 3. This compound was inspired by a previously published analog (**15**) that incorporated the piperidylamide in place of the furan ring of **1**, and demonstrated retained affinity for the KOP receptor.[17] Through the synthesis and evaluation of compound **14**, we sought to combine both the piperidyl amide and methoxy methyl ether features into one molecule that would have high KOP receptor affinity compared to **1**. Following a procedure developed in our laboratory, the furan ring of **3** was oxidatively degraded to carboxylic acid **13** using catalytic $RuCl_3 \cdot 3H_2O$ in the presence of $NaIO_4$.[18] With **13** in hand, standard peptide coupling conditions (as employed before in our laboratory) were used to generate amide **14**.[17]

Biology

Compounds **3–12**, and **14** were evaluated for affinity at human opioid receptors using methodology previously described (Table 1).[19, 20] For previously reported ethers **3** and **4**, the results of the radioligand binding assay are in agreement with published data.[8] In past literature reports, the tetrahydropyran diastereomers **5** and **6** were not completely separated from each other before biological evaluation. For a mixture of predominantly one of the diastereomers, absolute configuration unknown, the reported binding affinity and potency were $K_i = 4.0 \pm 0.4$ nM and $EC_{50} = 2.8 \pm 0.3$ nM.[8] We were able to separate and isolate each diastereomer, and the observed binding affinities for **5** and **6** in our hands reveal a eutomer (**5**) and a distomer (**6**), indicating a preference for the hydrogen of the new stereocenter to be in the β position or *R* configuration. Interestingly, the related tetrahydrofuran derivatives **7a** and **7b** have modest binding affinities at KOP receptors that are very similar to each other and show no preference for stereochemistry. One possible explanation is that by reducing the ring size by one carbon, the orientation of the new stereocenter of **7a** and **7b** in the receptor has been altered (relative to **5** and **6**) such that neither diastereomer has a particularly unfavorable interaction with the receptor.

In similar fashion to the tetrahydropyran derivatives, ethers **8a** and **8b** are previously reported, but the diastereomers were not completely separated from each other before biological evaluation.[8] The published data show both diastereomers as having very similar binding affinities ($K_i = 11 \pm 1$ nM and $K_i = 6.6 \pm 0.3$ nM) and potencies (10 ± 1 nM and 5.7 ± 0.7 nM). We were able to separate the diastereomers and isolate the less polar one (**8a**). By HPLC, the more polar diastereomer (**8b**) appeared to degrade during purification and was not completely purified (purity = 81.938 %), although it is important to note that there was no amount of **8a** present. In our hands ethers **8a** and **8b** also reveal a eutomer (**8a**) and distomer (**8b**), indicating a preference for the configuration of the new stereocenter, and reflecting our observations of tetrahydropyrans **5** and **6**. In our NMR characterization of **8a** and **8b**, we observed $^1J_{C,H}$ values at the new stereocenter that followed the accepted trend of pyranoses as previously described, although the difference was less pronounced (data not

shown). Given this observation and the pattern in the radioligand binding data in which the less polar diastereomers **5** and **8a** had the highest affinity, we propose that the configuration of the new stereocenter in **8a** is very likely the same as that of **5**. However, this needs to be confirmed through X-ray crystallography. The related brominated ethers **9a** and **9b** had poor binding affinities at the KOP receptor and show no preference for stereochemistry. One explanation for this observation is that the bromine atom was too bulky and/or too lipophilic such that it precluded receptor binding altogether.

Formate **10** produced radioligand binding affinity data that was well in agreement with published reports.[14, 22] However, in our hands compounds **11** and **12** behaved differently than previously reported. In two previously published radioligand binding studies, carbonate **11** showed no appreciable affinity at the KOP receptor vs. [^{3}H]bremazocine[15] or [^{3}H]diprenorphine.[9] Our data show that carbonate **11** displaces [^{3}H]U69,593 at the KOP receptor at $K_i = 171 \pm 7$ nM. Additionally, carbamate **12** was reported to have a KOP receptor binding affinity (vs. [^{3}H]diprenorphine) and potency of $K_i = 462 \pm 20$ nM and $EC_{50} = >1000$ nM.[16] We found that while carbamate **12** has no appreciable affinity at the other opioid receptors, it does displace [^{3}H]DAMGO at the μ opioid(MOP) receptor at $K_i = 1540 \pm 140$ nM. While this particular K_i is unimpressive, it does agree with previously published reports in which alkyl carbamates at the C-2 position of salvinorin A were found to lose KOP affinity and gain MOP affinity relative to the parent compound.

Compound **14** is reflective of a furan ring analog of **1** that was previously produced in our lab (Scheme 3). Amide **15** had binding affinity at KOP receptors of $K_i = 140 \pm 10$ nM, and potency and efficacy of $ED_{50} = 5110 \pm 1800$ nM, $E_{max} = 85 \pm 13$.[17] Our hypothesis was that through the combination of the amide in place of the furan ring (from **15**), and the methoxy methyl ether in place of the C-2 acetate (from **3**), we would be able to observe improved (or at least retained) binding affinity relative to the parent analogs. Through the addition of the methoxy methyl ether off the C-2 position, the KOP receptor binding affinity of **14** was improved over that of **15** by approximately 2-fold to $K_i = 59 \pm 4$ nM. However, the binding affinity of **14** is roughly 31-fold worse compared to **3**. Still, the improvement in receptor binding affinity from compound **14** over compound **15** is of similar magnitude (roughly 2–4 fold) to the improvement in receptor binding affinity from compound **3** over compound **1**.

Compounds **3**, **4**, **5**, **7**, **8a**, and **14** were also evaluated for functional activity at KOP receptors using a [^{35}S]GTP-γ-S functional assay (Table 2).[19, 20] Compounds **3**, **4**, **5**, and **8a** were found to have potencies comparable to or better than **1** as agonists. Compounds **7a** and **7b** were observed to be approximately 30-and 17-fold less potent than **1** as agonists, respectively. Finally, compound **14** was 48-fold less potent as an agonist than **1**, which is actually an approximately 2.5-fold improvement over the previously published furan analog **15** (approximately 125-fold less potent than **1** as an agonist).[17] However, **14** was also roughly 322-fold less potent than **3** as an agonist. Thus, the incorporation of the methoxy methyl ether at C-2 improved the binding affinity and potency of compound **14** over parent compound **15** by approximately 2-fold and 2.5 fold, respectively. These improvements are of similar magnitude to the improvements in the binding affinity and potency (approximately 4-fold and 7-fold, respectively) of compound **3** over compound **1**. However, the combination of furan substitution with C-2 acetate substitution in compound **14** ultimately greatly decreased the potency of the compound relative to ether **3** as well as compound **1**.

Finally, among the compounds reported, compound **5** had KOP receptor binding affinity and potency comparable to **1**. Compound **5** also lacks a hydrolyzable ester at C-2 and other analogs of **1** with this particular feature have been found to have a greater duration of action than **1**.[23] Therefore, compound **5** met our criteria for evaluation *in vivo*. Using methodology

previously reported, compound **5** was evaluated for its effect on cocaine-induced drug-seeking in rats at doses of 0.3 mg/kg and 1 mg/kg (Figure 4).[24]

Briefly, rats were trained on a fixed ratio 5 schedule of reinforcement for cocaine self-administration where a single cocaine infusion was received (0.5 mg/kg/infusion, paired with a light cue) for every 5 correct active lever responses. The first phase involved daily 2 h sessions until responding showed <20% variation across three consecutive days (cocaine baseline). In phase 2, cocaine was replaced with heparinised saline and daily 2 h sessions continued in the same operant chambers until active lever responses were <20 for a session (typically 2–3 days). When the extinction criteria had been achieved, reinstatement of cocaine-induced cocaine-seeking was tested the following day. Animals received IP injections of vehicle (75% DMSO), compound **5** (0.3 mg/kg or 1 mg/mg), or compound **1** (0.3 mg/kg) 5–10 min prior to a priming injection of cocaine (IP, 20 mg/kg). Immediately following the cocaine injection, animals were placed into the operant chamber and responding was recorded for a further 2 h. Results show a statistically significant reduction in responding between vehicle and the 1 mg/kg dose of **5**. In fact, this dose appeared to attenuate responding comparably to 0.3 mg/kg salvinorin A (**1**), our positive control, which we have previously shown to attenuate cocaine-primed reinstatement of drug seeking at a dose of 0.3 mg/kg or greater.[24]

Conclusions

In summary, we have evaluated C-2 modified analogs of **1** for opioid receptor affinity and efficacy. We have isolated and confirmed the binding affinities and potencies of two agonists, compounds **5** and **8a**. In comparison to their diastereomers (**6** and **8b**), these analogs show preferential interactions with the KOP receptor. Since our NMR-based assignment of the absolute configuration of compound **5** was confirmed through X-ray crystallography, and given our observation that the less polar diastereomers appear to have the highest binding affinities, we propose that the new stereocenter in ether **8a** shares the same absolute configuration as that of tetrahydropyran **5**. This will need to be further investigated through X-ray crystallography. Interestingly, we did not observe a stereochemical influence in the other related diastereomeric compounds (**7a**, **7b**, **9a**, and **9b**). This may be accounted for in steric considerations: the smaller ring size of **7a** and **7b** has changed their orientation in the receptor such that neither diastereomer interacts particularly unfavourably; and **9a** and **9b** are too large and/or lipophilic to allow for receptor interaction at all. Additionally, we have shown that contrary to previous reports, compound **11** does show affinity for the KOP receptor in radioligand binding assays. We have also demonstrated that compound **12**, in agreement with previous reports, has very little affinity at KOP receptors, but does have some affinity for the MOP receptor, in agreement with past radioligand binding studies of similar compounds. We also found that compound **14**, which is reflective of a previously published furan ring analog (**15**), demonstrated improved binding affinity over the parent compound upon the incorporation of the methoxy methyl ether moiety off C-2. However, the potency of **14** relative to compounds **3** and **1** was greatly diminished. Finally through further evaluation, it was found that compound **5** attenuated cocaine-induced drug seeking behavior comparably to salvinorin A (**1**). This finding is consistent with other reported KOP receptor agonists, and represents the first example of a salvinorin A analog that has demonstrated anti-addictive capabilities. The identification of differences in chirality and receptor tolerances for these ring constrained analogs should be of great value for the design of new compounds and or pharmacophore models of the binding site of **1**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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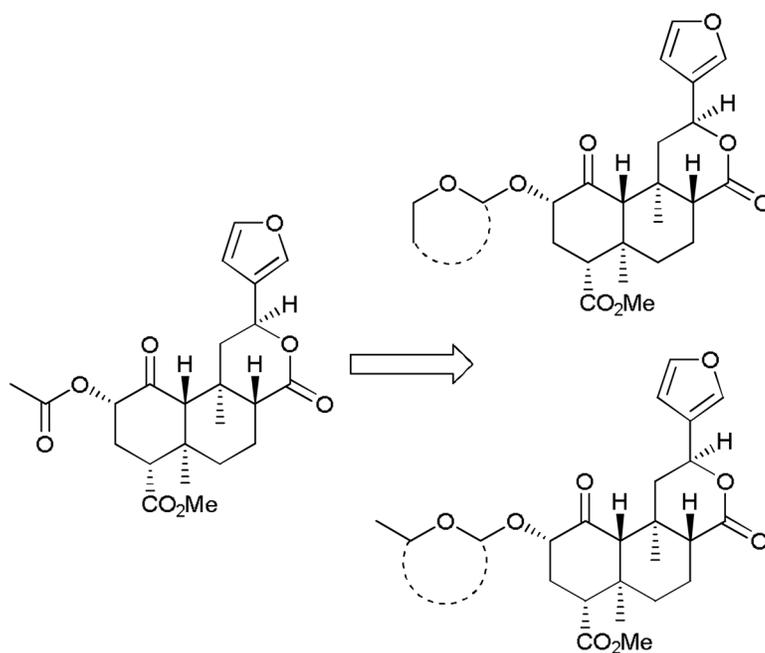
Figure 1.
Eclipsed (upper) or extended (lower) conformations of the ethoxymethyl ether at C-2 compared to salvinorin A.
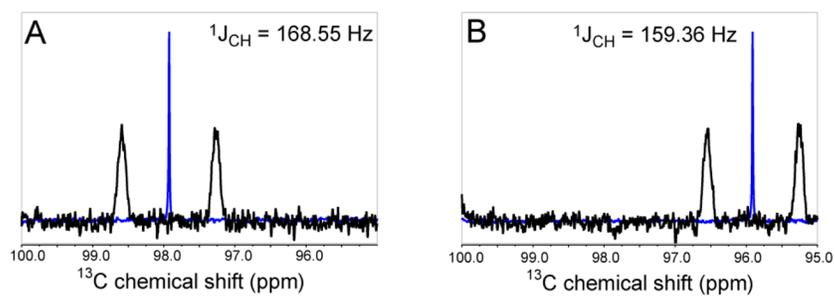
Figure 2.
1D ^{13}C NMR spectrum with composite pulse ^{1}H-decoupling (blue) and gated decoupling to show $J_{C,H}$ (black) for the anomeric carbon of **5**(A) and **6** (B).
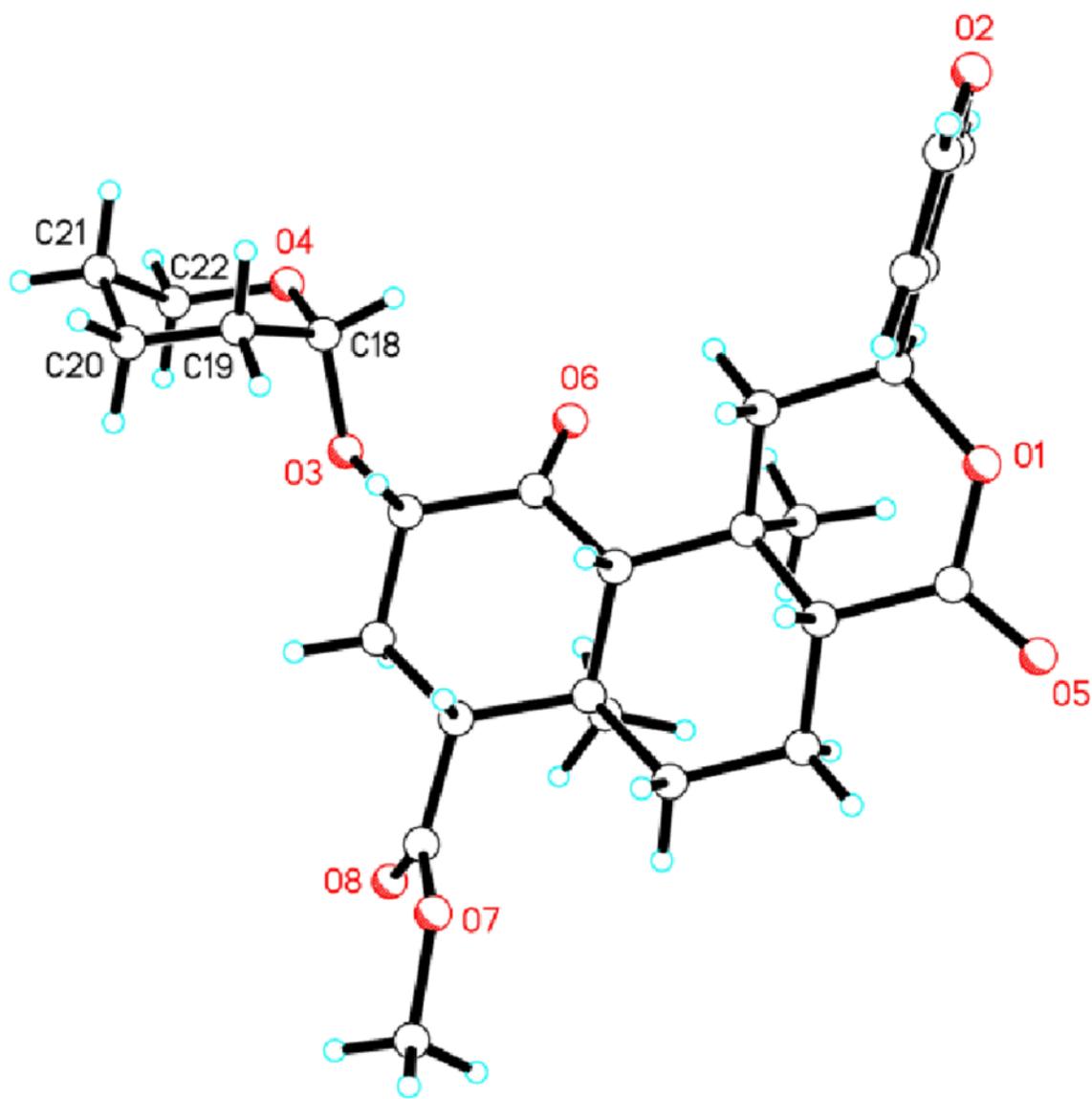
Figure 3.
X-ray crystallographic structure of **5**.
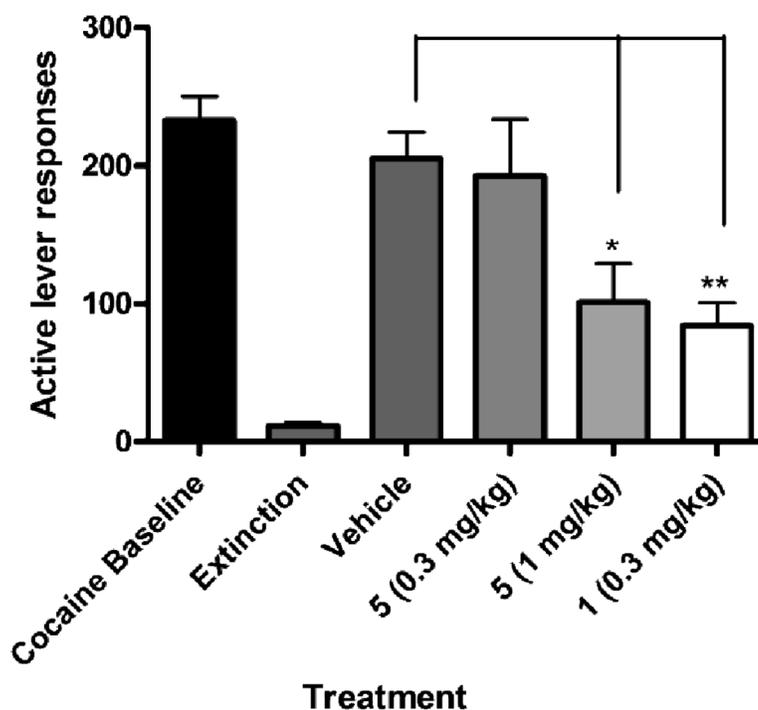
Figure 4.
The effect of **5** on cocaine-induced drug-seeking. In the first phase (cocaine baseline), the active lever delivered an infusion of cocaine. Recorded active lever responses consisted of access to cocaine (0.5 mg/kg/inf) for a period of 2 h in operant chambers. At the beginning of the second phase (extinction), cocaine was replaced with sterile heparinized saline and responses were recorded for 2 h, until active lever responses were <20 for a 2 h daily session. At the beginning of the third phase, animals received an I.P. injection of vehicle (75% DMSO, N = 7), **5** (0.3 mg/kg, N = 3), **5** (1 mg/kg, N = 6), or **1** (0.3 mg/kg, N = 7) 5–10 min prior to a priming injection of cocaine (20 mg/kg). Responding in phase 3 was measured for 2 h (± SEM).
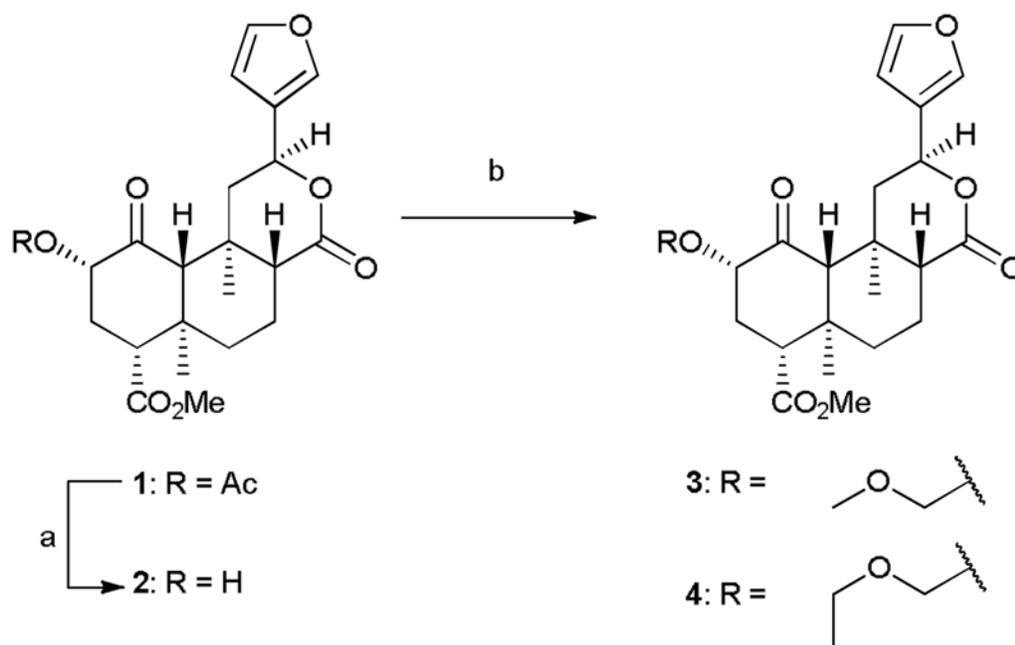
Scheme 1.
Reagents and conditions: (a) Na_2CO_3, MeOH; (b) appropriate chloromethyl ether, DIPEA, CH_2Cl_2.
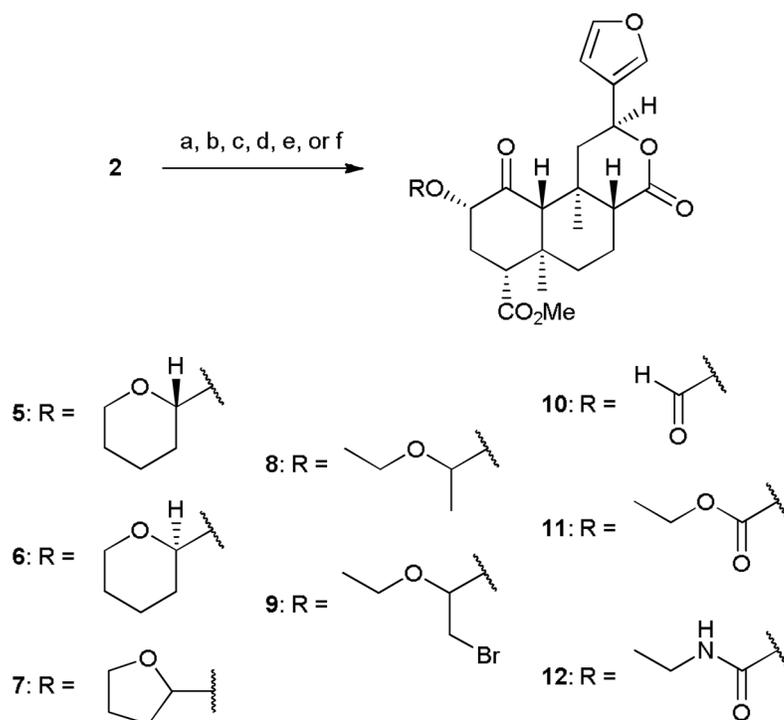
Scheme 2.
Reagents and conditions: (a) 3,4-dihydro-2*H*-pyran or 2,3-dihydrofuran, PPTS, CH$_2$Cl$_2$; (b) PPTS, ethyl vinyl ether, reflux; (c) Br$_2$, ethyl vinyl ether, DIPEA, CH$_2$Cl$_2$; (d) Ac$_2$O/ HCO$_2$H, pyridine; (e) ethyl chloroformate, DMAP, TEA, CH$_2$Cl$_2$; (f) ethyl isocyanate, DMAP, pyridine.
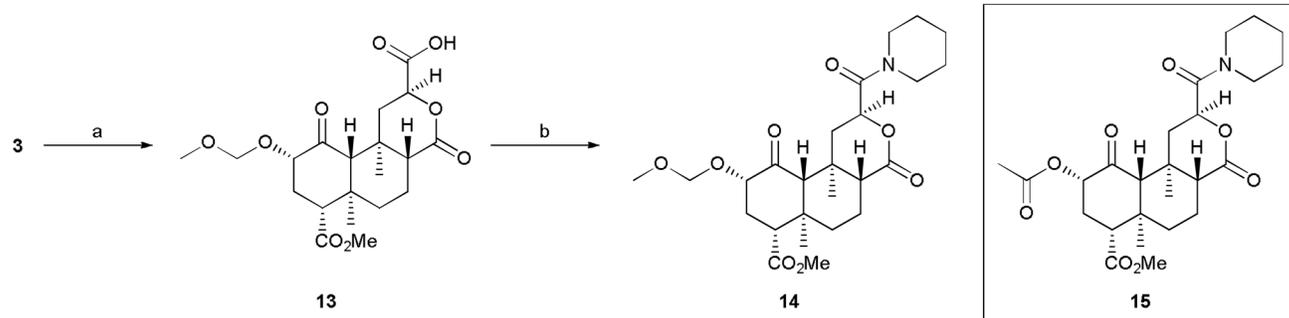
Scheme 3.
(a) RuCl$_3$·3H$_2$O, NaIO$_4$, CCl$_4$/CH$_3$CN/H$_2$O; (b) piperidine, EDCI, HOBt, TEA, CH$_2$Cl$_2$.

Table 1

Opioid receptor binding affinity for compounds **3–12**, and **14**.

Cmpd	$K_i \pm SD$ nM[a]		
	[³H]DAMGO (μ)	[³H]DADLE (δ)	[³H]U69,593 (κ)
1[b]	$EC_{50} = 2860 \pm 980$ $E_{max} = 75 \pm 8$ %	>10,000	7.4 ± 0.7
3	390 ± 20	2,840 ± 180	1.9 ± 0.2
4	41 ± 3	1,017 ± 99	3.13 ± 0.40
5	>10,000	>10,000	6.21 ± 0.40
6	>10,000	>10,000	300 ± 23
7a	>10,000	>10,000	75 ± 3
7b	>10,000	>10,000	81 ± 6
8a	777 ± 40	>5,000	6.7 ± 0.32
8b	>5,800	>5,000	1,233 ± 33
9a	>3,000	>5,000	5,261 ± 680
9b	3,523 ± 389	>5,000	7,438 ± 764
10[c]	ND	ND	41 ± 1
11[c]	ND	ND	171 ± 7
12[c]	1,540 ± 140	ND	ND
14	>3,000	>3,000	59 ± 4

[a]Receptor binding was performed in CHO cells which express the human MOP, DOP, or KOP receptors. All results are N = 3.

[b]Data from reference 21.

[c]ND indicates that K_i was found to be >10,000 nM in a range finding study.

Table 2

$[^{35}S]GTP\text{-}\gamma\text{-}S$ functional assay for compounds **3**, **4**, **5**, **7,8a**, and **14.**

Cmpd	$ED_{50} \pm SD^a$ (κ) nM	$E_{max} \pm SD^b$ (κ)
1[c]	40 ±10	120 ± 2
3	6 ± 1	118 ± 2
4	0.65 ± 0.17	127 ± 5
5	60 ± 6	109 ± 3
7a	1220 ± 230	112 ± 8
7b	690 ± 80	103 ± 4
8a	150 ± 14	101 ± 3
14	1934 ± 239	113 ± 5

[a]ED_{50} = Effective dose for 50% maximal response.

[b]Emax is % at which compound stimulates $[^{35}S]GTP\text{-}\gamma\text{-}S$binding compared to (−)-U50,488 (500 nM) at KOP receptors.

[c]Data from reference 21.