Synthetic Studies of Neoclerodane Diterpenes from Salvia divinorum: Role of the Furan in Affinity for Opioid Receptors


Abstract
Further synthetic modification of the furan ring of salvinorin A (1), the major active component of Salvia divinorum, has resulted in novel neoclerodane diterpenes with opioid receptor affinity and activity. A computational study has predicted 1 to be a reproductive toxicant in mammals and is suggestive that use of 1 may be associated with adverse effects. We report in this study that piperidine 21 and thiomorpholine 23 have been identified as selective partial agonists at kappa opioid receptors. This indicates that additional structural modifications of 1 may provide ligands with good selectivity for opioid receptors but with reduced potential for toxicity.

Introduction
The neoclerodane diterpene, salvinorin A (1), is the main active component of the hallucinogenic mint plant Salvia divinorum.1, 2 Lately, salvinorin A-containing products have become increasingly available and self-administration of these products has been reported.3–6 Historically, S. divinorum has been used medicinally by the Mazatecs of Oaxaca, Mexico for several conditions, but its mode of action was only recently discovered.7 Studies indicate that 1 has high affinity and activity at κ opioid (KOP) receptors, and has no affinity for the molecular targets of other hallucinogenic substances such 5-HT2, C B1/CB2, NMDA, or muscarinic receptors.8 Furthermore, 1 appears to have unique properties as a ligand at KOP receptors, including ultra-high efficacy in particular transduction systems, and a reduced propensity to cause receptor desensitization.9, 10

A growing number of experimental studies have explored the effects of 1 in animals.11–21 For example, 1 was found to substitute for KOP agonist U69,593 but did not substitute for DOM in DOM-trained non-human primates.19, 20 This suggests that the hallucinogenic experience elicited by 1 is qualitatively different than that of classical hallucinogens such as DOM. In rodents, 1 has been found to decrease striatal dopamine overflow and block the locomotor effects of cocaine.16, 18 These findings provide further evidence for the potential utility of 1 and related analogues as stimulant abuse therapeutics.22

It is relatively rare for natural products, such as 1, to have sufficiently attractive ADME/Tox (Absorption, Disposition, Metabolism, Excretion, and Toxicity) properties to be marketable,
despite their excellent potency and selectivity. ADME/Tox has become integrated into the drug discovery process and is a tremendous asset in guiding selection and optimization.\textsuperscript{23, 24} Thus, the ability to improve these properties by semi- or total synthetic chemistry is important in drug seeking campaigns.

Generally, furan-containing natural products, such as \textbf{1}, are of limited medicinal value. This results from their potential for toxicity after bioactivation.\textsuperscript{25–27} For instance, previous work has shown that teucrin A (\textbf{2}), a neoclerodane present in germander (\textit{Teucrium chamaedrys} L.; Lamiaceae), has the ability to produce hepatotoxicity.\textsuperscript{28} This results from the formation of an enedial formed from the metabolism of the furan ring by cytochrome P450 enzymes (CYP450s).\textsuperscript{29, 30} The resulting enedial is then capable of reacting with peptides to form stable conjugates.\textsuperscript{29} Using a proteomic analysis, the major targets of \textbf{2} were found to be mitochondrial and ER-associated proteins and enzymes involved in small molecule metabolism and cell maintenance.\textsuperscript{30} Aflatoxin B\textsubscript{1} (\textbf{3}), a difurano-containing natural product produced by \textit{Aspergillus} species, is a known hepatic carcinogen. Bioactivation of aflatoxin B\textsubscript{1} by CYP450s is thought to involve a similar dial intermediate to the one formed from \textbf{2}.\textsuperscript{31} As a furan-containing natural product, \textbf{1} also has the potential to form reactive metabolites resulting from bioactivation by CYP450s. This hampers its further development as a potential stimulant abuse therapeutic.\textsuperscript{32}

One approach to circumventing the potential toxicity of a furan-containing natural product is to explore different structural replacements for the furan ring. Ideally, these modifications should have low to no potential for toxicity but retain affinity and activity at opioid receptors. Previously, we found that modification of the furan ring of \textbf{1} resulted in neoclerodanes that possessed reduced affinity and efficacy at opioid receptors.\textsuperscript{33–35} These previous findings, as well as a recent report,\textsuperscript{36} suggested that additional structural modifications of \textbf{1} may lead to analogues with higher potency and potential utility as drug abuse medications. Here, we report our efforts to identify furan modified neoclerodanes with affinity and activity at KOP receptors.

\section*{Results and Discussion}

Initially, a computational toxicological study (MC4PC, MultiCASE, Inc.) was performed on \textbf{1} and three structurally similar compounds (columbin,\textsuperscript{37} diosbulbin G,\textsuperscript{38} and salvinorin B\textsuperscript{2}) identified using the Derwent World Index and Prous Integrity databases. The results of this investigation found that at pre-clinical endpoints \textbf{1} is predicted to be a reproductive toxicant in mammals (rabbits, rats, and mice). This suggests that \textbf{1} may have adverse effects associated with its use. However, there are few experimentally determined reports on the toxicological effects of \textbf{1}. A limited study in mice found that \textbf{1} produced no histological differences in control animals in the liver, spleen, kidney bone marrow, or brain tissue.\textsuperscript{39} However, more detailed studies on the potential toxicological effects of \textbf{1} are warranted.

Given the strong likelihood that the potential adverse effects of \textbf{1} are due to the presence of the furan ring, we sought to find other structural motifs that could potentially mimic the furan at its binding site. While a model has been proposed for the binding of \textbf{1} to the KOP,\textsuperscript{40, 41} the binding pocket of \textbf{1} will not be known definitively until the structure of a KOP – \textbf{1} complex is solved. Given the lack of these published studies, other indirect methods are needed to more fully elucidate the nature of binding of \textbf{1} at the KOP receptor. Our approach was to systematically change the structure of the position corresponding to the furan ring and probe its effects on opioid receptor affinity and activity.

We initially targeted amide and ester derivatives due to their relative ease of synthesis, potential for reduced toxicity, and, likely improved water solubility. Our design strategy
consisted of two parts: (1) the carbonyl of the amide or ester might participate in a hydrogen bonding interaction similar to the furanyl oxygen; and (2) enhanced affinity and/or activity might result from the addition of a suitable group off the amide or ester bond. Our results are described herein.

**Chemistry**

The synthesis of analogues 5 – 30 and 34 – 37 is outlined in Schemes 1 and 2. Salvorin A (1) was extracted from commercially available *S. divinorum* leaves as described previously.

The treatment of 1 with NaIO₄ and a catalytic amount of RuCl₃ afforded key intermediate 4. The coupling of acid 4 with the appropriate aniline or alkylamine using EDCI in CH₂Cl₂ afforded analogues 5 – 30 in 18 – 75 % yield.

The treatment of acid 4 with CDMT and N-methylmorpholine followed by ethanethiol afforded thioester 31 in 88% yield. The reduction of 31 with triethylsilane and Pd/C gave aldehyde 32 in 82% yield. Asymmetric allylation of aldehyde 32 was accomplished in 50% yield with B-allyl-(10S)-(trimethylsilyl)-9-borabicyclo[3.3.2]decene (prepared in situ from 9-[1(R,2R)-pseudoephedrinyl]-(10S)-(trimethylsilyl)-9-borabicyclo[3.3.2]decane and allylmagnesium bromide. To determine the absolute stereochemistry of alcohol 33a, mosher ester 33b was prepared in 82% yield using oxalyl chloride and (+)-α-methoxy-α-trifluoromethylphenylacetic acid. NMR analysis of 33b and its (S)-epimer made from the S-acid, was not definitive as to the configuration of the stereocenter. Therefore, X-ray analysis of 33b was conducted and established the correct relative stereochemistry; the absolute configuration was then assigned using the known stereochemistry for much of the molecule.

Acetylation of 33a with acryloyl chloride under basic conditions gave the corresponding ester which was then treated with 2nd generation Grubbs catalyst to afford 5,6-dihydro-2H-pyran-2-one 34 in 23% yield for the two steps. The reaction of 4 with either (2-hydroxybenzyl)triphenyl- or (2-thiobenzyl)triphenyl-phosphonium bromide afforded the benzofuran (35) and benzothiophene (36), respectively. Finally, coupling of the acid 4 with N-hydroxybenzene-carboximidamide using EDCI followed by cyclization afforded 4-phenyl-1,3,5-oxadiazole 37.

**Biology**

Compounds 1, 5 – 30, and 34 – 37 were then evaluated for affinity at human opioid receptors using methodology previously described (Table 1). As mentioned earlier, we and others have found previously that modification of the furan ring of 1 resulted in altered affinity and activity at opioid receptors. These findings suggested that further modification to the furan ring may result in analogues with improved potency and potential as drug abuse medications. To further elucidate the structure-activity relationships of neoclerodane diterpenes at opioid receptors, we synthesized an additional series of furan modified analogues.

Our initial modification was to replace the furan ring in 1 with an anilido group (5). It was envisioned that the benzene ring would participate in a hydrophobic interaction similar to the furan ring and the amide group could participate in a hydrogen bonding interaction. This modification was not well tolerated and affinity was lost at opioid receptors (Kᵢ > 10,000 nM). The reasons for this loss in affinity were not readily apparent. One potential reason for this loss in affinity was the flexibility of the aromatic ring relative to the amide bond. To address this issue, we synthesized indoline 6 as a more conformationally restricted analogue of 5. This change, however, did not enhance affinity suggesting that either the conformation was not optimal or that alkylation of the aniline portion was not tolerated. Another potential
reason for the lack of affinity of 5 is the presence of the aromatic ring. Thus, we prepared cyclohexane 7 and cyclopentane 8. Replacement of the phenyl ring in 5 with these saturated systems resulted in a greater than 10-fold and 5-fold increase in affinity at KOP receptors, respectively. The replacement of the phenyl ring with a 3-pyridine (9) was not tolerated.

Recent molecular modeling and site-directed mutagenesis studies have indicated that the oxygen in the furan ring present in 1 forms a critical hydrogen bond with Y119 at the KOP receptor.\textsuperscript{40, 41, 54, 55} This suggested to us that the inclusion of a hydrogen bond acceptor group to anilide 5 may enhance affinity at KOP receptors. Unfortunately, this design strategy met with little success. Generally, the addition of a methoxy group to anilide 5 (10 – 16) did not increase affinity for opioid receptors. Interestingly, 12 and 13 were found to have weak affinity for MOP and DOP receptors and no affinity for KOPs. To further explore the role of electronics in the binding of these compounds to opioid receptors, we prepared brominated analogues 17 – 19. Surprisingly, 17 – 19 possessed affinity at MOP receptors. This suggests that the furan ring may also have a role in determining the selectivity for opioid receptors. However, other modifications will need to be explored to further define the role of the furan ring in opioid receptor selectivity. In addition, these findings suggested that these compounds, while similar in structure to 1, may be interacting with opioid receptors in a different manner.

We also explored additional structural modifications of the furan. The replacement of the furan ring in 1 with a 5,6-dihydro-2H-pyran-2-one (34) was found to greatly reduce affinity for KOPs. This molecule was prepared as a potential neoclerodane based affinity label. However, its low affinity for KOPs greatly hampers its potential utility in this capacity. Other structural modifications may lead to more useful pharmacological tools to better characterize the interactions of neoclerodanes with opioid receptors. Replacement of the furan with a 2-benzofuran (35) greatly decreases affinity for KOPs compared to 1. However, this modification had little effect on MOP affinity but enhanced affinity for DOP receptors. Interestingly, bioisosteric replacement of the 2-benzofuran (35) with a 2-benzothiophene (36) resulted in similar affinity for MOPs and DOPs but led to a loss in affinity for KOPs (\(K_i > 10,000\) nM). Similar results were seen when the 2-benzofuran was replaced with a 4-phenyl-1,3,5-oxadiazole (37). This is in agreement with recent findings of Beguin et al.\textsuperscript{36} However, 37 was found to have affinity for MOP receptors (\(K_i = 1,610\) nM).

Given the increased affinity of 7 and 8 for KOPs relative to 5, we explored additional nonaromatic substitutions. First the cyclohexylamine moiety of 7 was contracted into a piperidine ring (21). This change resulted in a 9-fold increase in affinity for KOP receptors relative to 7 (\(K_i = 140\) nM vs. \(K_i = 1,220\) nM). Replacement of the piperidine ring with a pyrrolidine ring (22) resulted in a 9-fold loss in affinity (\(K_i = 1,210\) M vs. \(K_i = 140\) nM). Given this interesting result, we decided to further probe the role of piperidine ring in 21. Replacement of piperidine ring with either a morpholine ring (20) or a thiomorpholine ring (23) was well tolerated but did not further enhance affinity for KOP receptors. Addition of a 4-bromo group to the piperidine ring (24) resulted in a 9-fold loss in affinity. This suggests that substitution in this position is not well tolerated. However, additional modifications need to be explored to further substantiate this finding.

Previously, we showed that the tetrahydrofuran analogue of 1 retained high affinity for KOP receptors.\textsuperscript{34} Based on this finding, we prepared several additional tetrahydrofurans. Unfortunately, this strategy also met with little success. The insertion of an amide linkage (25 – 27) resulted in a loss in affinity at KOP receptors. Surprisingly, 25 was found to have low affinity DOP receptors (\(K_i = 3,090\) nM). This is interesting because this is the first report of a neoclerodane with some selectivity for DOPs. Its modest affinity augers for the identification of other neoclerodanes with enhanced affinity for DOPs. The insertion of an
ester linkage (28 – 30) was better tolerated but did not result in high affinity agents. The highest affinity compound in this series identified was bicycle 28 ($K_i = 610$ nM).

Compounds 21 and 23 were then evaluated for functional activity at KOP receptors using a $[^{35}]$GTP-γ-S assay (Table 2). Despite their affinity in the 100 nM range, 21 and 23 were found to be approximately 100-fold less active than 1 as agonists. However, the replacement of the furan ring with a piperidino or thiomorpholinocarbonyl group was found to reduce efficacy at KOP receptors. This suggests that additional structural modifications may lead to analogues with enhanced activity and reduced efficacy.

**Conclusions**

In summary, we have evaluated 30 furan ring modified analogues of 1 for opioid receptor affinity. This manuscript reports the first salvinorin A analogue with some selectivity for DOP receptors (25). This suggests the likelihood of identifying other diterpenes with this characteristic from natural sources. Piperidine 21 and thiomorpholine 23 were found to be selective partial agonists at KOPs. However, they are less potent than 1. Furthermore, this is the first report to discuss the potential toxicological concerns with the use of 1. Additional structural modifications of 1 are currently being explored and will be reported in due course.

**Experimental section**

Unless otherwise indicated, all reagents were purchased from commercial suppliers and are used without further purification. All melting points were determined on a Thomas–Hoover capillary melting apparatus and are uncorrected. NMR spectra were recorded on either a Bruker Avance-300 spectrometer, Bruker DRX-400 with qnp probe and/or a Bruker AV-500 with cryoprobe using δ values in ppm (TMS as internal standard) and J (Hz) assignments of 1H resonance coupling. High resolution mass spectrometry data was collected on either a LCT Premier (Waters Corp., Milford, MA) time of flight mass spectrometer or an Agilent 6890N gas chromatograph in conjunction with a Quatro Micro GC mass spectrometer (Micromass Ltd, Manchester UK). Thin-layer chromatography (TLC) was performed on 0.25 mm plates Analtech GHFL silica gel plates using EtOAc/n-hexanes, 1:1 as the solvent system. Spots on TLC visualized with phosphomolybdic acid in ethanol. Column chromatography was performed with Silica Gel (32–63 μ particle size) from Bodman Industries (Atlanta, GA). Analytical HPLC was carried out on an Agilent 1100 Series Capillary HPLC system with diode array detection at 254.8 nm on an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) with isotropic elution in 60% CH3CN/40% H2O at a flow rate of 5.0 mL/min unless otherwise noted.

**General Procedure A**

A solution of 4 (1 equiv), appropriate amine or alcohol (1.5 equiv), EDCI (2.5 equiv), HOBT (2.5 equiv) and Et3N (10 equiv) in CH2Cl2 was stirred at room temperature overnight. The mixture was then washed with saturated aqueous NaHCO3 (3 × 15 mL), H2O (3 × 15 mL), and brine (15 mL) and dried over anhydrous Na2SO4. The solvent was evaporated in vacuo and the resulting residue purified by flash column chromatography on silica gel using mixtures of EtOAc-hexanes.

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(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(piperidine-1-carbonyl)dodecahydro-1H-benzo[f]isochromene-7-carboxylate (21)

Compound 21 was synthesized from 4 using procedure A and piperidine to afford 0.0870 g (75.0%) as a white solid, mp 188 – 191 °C. 1H NMR (400 MHz, CDCl3) δ 5.27 – 5.09 (m, 2H), 4.18 – 4.05 (m, 1H), 3.88 (dd, J = 14.4, 4.5 Hz, 1H), 3.76 – 3.58 (m, 5H), 2.83 – 2.52 (m, 4H), 2.43 – 2.23 (m, 5H), 2.17 (s, 3H), 2.08 (d, J = 14.2 Hz, 1H), 1.90 (dd, J = 13.4, 7.7 Hz, 1H), 1.66 (ddd, J = 31.6, 25.8, 12.0 Hz, 4H), 1.37 (s, 3H), 1.07 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 202.43, 171.81, 171.14, 169.96, 167.43, 75.15, 71.59, 64.66, 52.12, 49.29, 48.72, 45.52, 42.16 (2C), 37.88, 35.24 (2C), 30.89, 28.21, 27.47, 20.76, 18.33, 17.17, 16.20. HRMS (m/z): [M+Na] calcd for C24H33NO8Na, 496.2055; found, 496.2047. HPLC tR = 8.80 min; purity = 97.04%.

(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(thiomorpholine-4-carbonyl)dodecahydro-1H-benzo[f]isochromene-7-carboxylate (23)

Compound 23 was synthesized from 4 using procedure A and thiomorpholine to afford 0.0430 g (23.8%) as a white solid, mp 102 – 105 °C. 1H NMR (400 MHz, CDCl3) δ 5.09 (m, 2H), 4.18 – 4.05 (m, 1H), 3.88 (dd, J = 14.4, 4.5 Hz, 1H), 3.76 – 3.58 (m, 5H), 2.83 – 2.52 (m, 4H), 2.43 – 2.23 (m, 5H), 2.17 (s, 3H), 2.08 (d, J = 14.2 Hz, 1H), 1.90 (dd, J = 13.4, 7.7 Hz, 1H), 1.66 (ddd, J = 31.6, 25.8, 12.0 Hz, 4H), 1.37 (s, 3H), 1.07 (s, 3H). 13C NMR (101 MHz, CDCl3) δ 202.40, 171.81, 171.14, 169.96, 167.43, 75.15, 71.59, 64.66, 52.12, 49.29, 48.72, 45.52, 42.16 (2C), 37.88, 35.24 (2C), 30.89, 28.21, 27.47, 20.76, 18.33, 17.17, 16.20. HRMS (m/z): [M+H]+ calcd for C24H33NO8S, 496.2055; found, 496.1985. HPLC tR = 12.47 min; purity = 98.40%.

(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(ethylthiocarbonyl)-6a,10b-dimethyl-4,10-dioxo-dodecahydro-1H-benzo[f]isochromene-7-carboxylate (31)

A solution of 4 (1.16 g, 2.83 mmol), CDMT (1.49 g, 8.49 mmol), and N-methylmorpholine (1.86 mL, 16.96 mmol) in anhydrous THF (25 mL) was stirred at room temperature for 1 h under an argon atmosphere. Ethanethiol (1.25 mL, 16.96 mmol) was then added and reaction was stirred at room temperature for an additional 48 hours. H2O (25 mL) was added and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined EtOAc portion was washed with saturated NaHCO3 (3 × 20 mL), 2N HCl (3 × 20 mL), and saturated NaCl (3 × 20 mL) and dried (Na2SO4). The solvent was removed under reduced pressure and the resulting residue purified by flash column chromatography on silica gel using a mixture of EtOAc/n-hexanes to afford 1.13 g (88.0% yield) of 31 as a white solid, mp 187 – 191 °C. 1H NMR (500 MHz, CDCl3) δ 5.18 – 5.11 (m, 1H), 4.97 (dd, J = 7.2, 9.7 Hz, 1H), 3.72 (s, 3H), 2.90 (q, J = 7.4 Hz, 2H), 2.73 (dd, J = 5.4, 11.4 Hz, 1H), 2.62 (dd, J = 7.2, 13.7 Hz, 1H), 2.35 – 2.26 (m, 2H), 2.18 (s, 3H), 2.17 – 2.10 (m, 2H), 2.10 – 2.02 (m, 1H), 1.81 – 1.73 (m, 1H), 1.70 – 1.62 (m, 1H), 1.62 – 1.51 (m, 2H), 1.37 (s, 3H), 1.27 (td, J = 2.3, 7.3 Hz, 3H), 1.08 (s, 3H). 13C NMR (126 MHz, CDCl3) δ 201.81, 199.44, 171.72, 170.08, 170.04, 80.41, 75.01, 64.42, 53.54, 52.24, 50.57, 42.13, 39.54, 37.96, 35.59, 30.85, 23.23, 20.81, 18.33, 16.36, 16.09, 14.54. HRMS (m/z): [M+Na] calcd for C22H30O8SNa, 477.1559; found, 477.1536. HPLC tR = 26.93 min; purity = 98.31%.
(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-formyl-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (32)

A mixture of 31 (0.700 g, 1.54 mmol), triethylsilane (0.737 mL, 4.62 mmol), and 10% palladium on carbon (32.7 mg) in CH$_2$Cl$_2$ (25 mL) was stirred at room temperature overnight under an argon atmosphere. Upon completion, the reaction mixture was filtered through a pad of Celite and the solvent was removed under reduced pressure. A mixture of EtOAc/n-hexanes (1:25) (40 mL) was added to the residue. The resulting solid was collected by filtration and dried to afford 0.498 mg (81.9% yield) of 32 as a white solid, mp 194 – 197 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 9.68 (s, 1H), 5.16 (dd, $J$ = 8.6, 11.6 Hz, 1H), 4.89 (dd, $J$ = 7.2, 9.8 Hz, 1H), 3.73 (s, 3H), 2.75 (dd, $J$ = 5.2, 11.6 Hz, 1H), 2.52 (dd, $J$ = 7.1, 13.7 Hz, 1H), 2.36 – 2.24 (m, 2H), 2.18 (s, 3H), 2.16 – 2.07 (m, 1H), 1.96 (dd, $J$ = 3.1, 11.8 Hz, 1H), 1.78 (dt, $J$ = 3.1, 13.4 Hz, 1H), 1.72 – 1.50 (m, 4H), 1.39 (s, 3H), 1.09 (s, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 201.91, 197.98, 171.69, 170.36, 107.71, 75.05, 64.50, 53.52, 52.24, 50.98, 42.16, 37.93, 36.64, 35.39, 30.82, 20.80, 18.35, 16.41, 16.37; HRMS (m/z): [M +H]$^+$ calcd for C$_2$H$_7$O$_7$Na, 395.1706; found, 395.1712. HPLC $r_t$ = 7.561 min; purity = 95.63%.

(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-((S)-1-hydroxybut-3-enyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (33a)

A solution of 9-[[1R,2R]-pseudoehedrinyl]-[10S)-trimethylsilyl]-9-borabicyclo[3.3.2]decane (0.546 g, 1.47 mmol) in anhydrous Et$_2$O (20 mL) at −78 °C was treated with allylmagnesium bromide (1.47 mL, 1.47 mmol). The mixture was stirred at room temperature for 1 h and then cooled to −78 °C. A solution of 32 (0.580 g, 1.47 mmol) in dry THF (3 mL) was added in a dropwise manner and the solution was warmed to 10 °C overnight. The solvent was removed under reduced pressure and the residue was redissolved in acetonitrile (10 mL) and (1R,2R)-(−)-pseudoehephrine (0.243 g) was added. The resulting mixture was heated at reflux overnight. The solvent was removed under reduced pressure and CH$_2$Cl$_2$ (5 mL) added and the mixture was cooled to 0 °C in an ice bath. The mixture was filtered to remove the white precipitate. The filtrate was evaporated to dryness and the residue was purified by column chromatography (eluent: 30 – 50% EtOAc/n-hexanes) to afford 0.289 g (50% brsm) of 33a as a clear oil; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.57 (dq, $J$ = 7.1, 10.1 Hz, 1H), 5.08 (dd, $J$ = 6.8, 13.8 Hz, 3H), 4.36 (ddd, $J$ = 9.1, 12.0, 21.0 Hz, 1H), 3.65 (s, 3H), 3.48 – 3.37 (m, 1H), 2.71 (dd, $J$ = 5.8, 11.0 Hz, 1H), 2.52 (s, 1H), 2.33 (dd, $J$ = 7.9, 14.8 Hz, 2H), 2.23 (t, $J$ = 7.1 Hz, 2H), 2.17 (s, 1H), 2.15 – 2.05 (m, 4H), 2.00 (t, $J$ = 10.0 Hz, 2H), 1.70 (d, $J$ = 7.8 Hz, 1H), 1.54 (ddd, $J$ = 10.5, 19.3 Hz, 3H), 1.28 (s, 3H), 1.00 (s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 202.37, 171.91, 171.77, 170.06, 133.99, 118.55, 78.65, 75.21, 72.57, 64.05, 53.53, 52.04, 50.80, 42.16, 38.53, 38.18, 37.80, 34.96, 30.89, 20.70, 18.30, 16.32, 15.25; HRMS (m/z): [M+Na] calcd for C$_{23}$H$_{32}$O$_8$Na, 459.1995, found, 459.1824.

(2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-6a,10b-dimethyl-4,10-dioxo-2-((S)-1-((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyloxy)but-3-enyl)dodecahydro-1H-benzo[f]isochromene-7-carboxylate (33b)

33b was prepared in 82% from 33a and (R)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid using previously described methods.$^{39}$ $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.65 – 7.52 (m, 2H), 7.52 – 7.39 (m, 3H), 5.79 – 5.59 (m, 1H), 5.21 – 5.02 (m, 2H), 5.01 – 4.88 (m, 1H), 4.75 (td, $J$ = 6.9, 0.9 Hz, 1H), 4.55 – 4.44 (m, 1H), 3.73 – 3.65 (m, 3H), 3.54 (t, $J$ = 5.4 Hz, 3H), 2.64 – 2.48 (m, 3H), 2.31 – 2.15 (m, 2H), 2.15 – 2.06 (m, 4H), 2.01 – 1.85 (m, 2H), 1.62 – 1.51 (m, 1H), 1.36 (ddt, $J$ = 11.5, 6.7, 5.7 Hz, 1H), 1.30 – 1.08 (m, 6H), 0.88 (s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 201.98, 171.74, 171.01, 170.09, 166.03, 133.66, 131.97, 129.83 (2C), 128.81 (2C), 127.41, 120.14, 83.78, 76.60, 75.00, 74.80, 63.73, 56.37, 53.19, 52.19, 49.80, 41.99, 37.91, 37.75, 34.76, 34.62, 30.85, 29.86, 20.73, 18.26, 16.16, 14.99.

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A solution of 33a (0.099 g, 0.227 mmol), acryloyl chloride (0.55 mL, 6.80 mmol), NEt3 (0.95 mL, 6.80 mmol), and a catalytic amount of DMAP in CH2Cl2 (10 mL) was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography to afford 0.045 g of the corresponding acrylate which was used with out further purification. A solution of the acrylate and 2nd generation Grubb’s catalyst (10 mol%) in CH2Cl2 (10 mL) was heated at reflux overnight under an argon atmosphere. The solvent was removed under reduced pressure and the residue was purified by column chromatography (eluents: 50% EtOAc/n-hexanes) to afford 0.0245 g (57.6%) of residue was purified by column chromatography to afford 0.0245 g (57.6%) of 34 as a white solid, mp > 250 °C; 1H NMR (300 MHz, CDC13) δ 7.05 – 6.87 (m, 1H), 6.03 (dd, J = 9.8, 2.4 Hz, 1H), 5.22 – 5.04 (m, 1H), 4.55 (dd, J = 11.3, 6.2 Hz, 1H), 4.32 (dd, J = 13.0, 3.3 Hz, 1H), 3.72 (s, 3H), 3.09 – 2.91 (m, 1H), 2.77 (dd, J = 11.1, 5.6 Hz, 1H), 2.38 – 2.08 (m, 9H), 1.95 (t, J = 12.2 Hz, 1H), 1.84 – 1.50 (m, 4H), 1.35 (s, 3H), 1.06 (s, 3H). 13C NMR (75 MHz, DMSO) δ 202.55, 171.81, 171.05, 170.01, 163.54, 145.84, 120.99, 77.60, 75.93, 75.36, 63.96, 53.59, 52.13, 50.56, 42.30, 38.11, 37.12, 35.05, 30.95, 26.01, 20.73, 18.31, 16.32, 15.24. HRMS (m/z): [M+Na] calcd for C24H30O9Na, 485.1787; found, 485.1769. HPLC tR = 19.918 – min; purity = 100%.

A solution of 4 (0.200 g, 0.487 mmol), 2-chloro-4,6-dimethoxytriazine (0.086 g, 0.487 mmol), and NEt3 (0.07 mL, 0.487 mmol) in CH2Cl2 (4 mL) was stirred at room temperature for 1 h. (2-Hydroxybenzyl)triphenyolphosphonium bromide (0.220 g, 0.487 mmol), NEt3 (0.21 mL, 1.46 mmol) and toluene (10 mL) were added and the resulting mixture was heated at reflux overnight. Removal of the solvent under reduced pressure gave a crude residue that was purified by flash column chromatography (eluents: 25% EtOAc/n-hexanes) to yield 0.0610 g (26%) of 35 as a white solid, mp 192 – 196 °C (dec); 1H NMR (300 MHz, CDC13) δ 7.55 (d, J = 7.4 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.35 – 7.17 (m, 2H), 6.71 (s, 1H), 5.66 (dd, J = 11.3, 5.7 Hz, 1H), 5.15 (t, J = 10.0 Hz, 1H), 3.73 (s, 3H), 2.85 – 2.71 (m, 1H), 2.57 (dd, J = 13.4, 5.7 Hz, 1H), 2.38 – 2.09 (m, 8H), 1.99 (dd, J = 24.3, 11.8 Hz, 1H), 1.86 – 1.55 (m, 3H), 1.49 (s, 3H), 1.10 (s, 3H). 13C NMR (75 MHz, CDCl3) δ 202.15, 171.76, 170.85, 170.12, 155.35, 153.94, 127.74, 125.19, 123.27, 121.67, 111.59, 105.42, 75.25, 72.52, 64.31, 53.77, 52.20, 51.25, 42.32, 40.60, 38.32, 35.64, 30.97, 20.76, 18.39, 16.53, 15.40. HRMS (m/z): [M+H]+ calcd for C27H30O6, 482.1941; found, 482.1945. HPLC tR = 10.606 min; purity = 98.10%.

A solution of 4 (0.200 g, 0.487 mmol), 2-chloro-4,6-dimethoxytriazine (0.086 g, 0.487 mmol), and NEt3 (0.07 mL, 0.487 mmol) in CH2Cl2 (4 mL) was stirred at room temperature for 1 h. 2-(Mercaptobenzyl)triphenyolphosphonium bromide (0.227 g, 0.487 mmol), and N-methylmorpholine (0.16 mL, 1.46 mmol) and toluene (10 mL) were added and the resulting mixture was heated at reflux overnight. Removal of the solvent under reduced pressure gave a crude residue that was purified by flash column chromatography (eluents: 20% EtOAc/n-hexanes) to yield 0.0846 g (35%) of 36 as a white solid, mp 223 – 225 °C; 1H NMR (500 MHz, CDCl3) δ 7.83 – 7.78 (m, 1H), 7.74 – 7.70 (m, 1H), 7.37 – 7.30 (m, 2H), 7.23 (s, 1H), 5.92 – 5.79 (m, 1H), 5.19 – 5.09 (m, 1H), 3.73 (s, 3H), 2.83 – 2.64 (m, 2H), 2.35 – 2.27 (m, 2H), 2.22 – 2.11 (m, 6H), 1.84 – 1.74 (m, 2H), 1.72 – 1.58 (m, 2H), 1.50 (s, 3H), 1.13 (s, 3H). 13C NMR (126 MHz, CDCl3) δ 201.99, 171.54, 170.60, 169.97, 143.65, 139.34, 139.04, 124.71, 124.55, 123.84, 122.43, 121.40, 75.13, 75.00, 64.04, 53.52, 52.01, 51.27,
HRMS (m/z): [M+H]+ calcd for C₂₇H₃₁O₇S, 499.1791; found, 499.1777. HPLC tR = 12.324 min; purity = 100%.

X-Ray Crystallographic Study of 33b

Colorless crystals of C₃₃H₃₉F₃O₁₀ are, at 100(2) K, monoclinic, space group P2₁-C₂ (No. 4) with a = 6.6372(4) Å, b = 18.637(1) Å, c = 12.6435(8) Å, β = 93.235(1)°, V = 1561.5(2) Å³ and Z = 2 molecules (dcalcd = 1.388 g/cm³; μa(MoKα) = 0.113 mm⁻¹). A full hemisphere of diffracted intensities (1850 10-second frames with an ω scan width of 0.30°) was measured for a single-domain specimen using graphite-monochromated MoKα radiation (λ = 0.71073 Å; fine-focus sealed x-ray tube) on a Bruker SMART APEX CCD Single Crystal Diffraction System. A total of 18602 integrated reflection intensities having 2θ(MoKα)<61.00° were produced using the Bruker program SAINT; 9099 of these were unique and gave Rint = 0.048 with a coverage which was 98.6% complete. The data were corrected empirically for variable absorption effects using equivalent reflections. The Bruker software package SHELXTL was used to solve (direct methods) and refine (weighted full-matrix least-squares) the structure with F₀² data.

The three ethylenic hydrogens were located from a difference Fourier and included in the structural model as independent isotropic atoms. The remaining hydrogen atoms were included in the structural model as idealized atoms (assuming sp²- or sp³-hybridization of the carbon atoms and C-H bond lengths of 0.95 – 1.00 Å) with isotropic thermal parameters fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameter of the carbon atom to which they are covalently bonded. The final structural model incorporated anisotropic thermal parameters for all nonhydrogen atoms and isotropic thermal parameters for all hydrogen atoms. A total of 432 parameters were refined using 1 restraint and 9099 data. Final agreement factors at convergence are: R₁(unweighted, based on F) = 0.045 for 8245 independent absorption-corrected “observed” reflections having 2θ(MoKα)<61.00° and I>2σ(I); R₁(unweighted, based on F) = 0.049 and wR²(weighted, based on F²) = 0.109 for all 9099 independent absorption-corrected reflections having 2θ(MoKα)<61.00°. The final difference map had maxima and minima of 0.51 and −0.22 e⁻/Å³, respectively. The Flack parameter [0.0(4)] did not allow the absolute configuration to be established directly from the X-ray analysis and this was assigned using the known stereochemistry for much of the molecule. Atomic coordinates for compound 33b have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 723548). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2, 1EZ, UK [fax, +44(0)-1223-336033; e-mail, deposit@ccdc.cam.ac.uk].
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank (Dr. Todd Williams, Mr. Robert Drake, Mr. Larry Sieb) of the KU mass spectrometry Laboratory for their efforts in acquiring the ESI spectra. LCT Premier was purchased with support from NIH SIG S10 RR019398. This work was supported by Grant Numbers R01DA018151 and R01DA018151S1 (to TEP) from the National Institute on Drug Abuse (NIDA). Portions of this work were also supported by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, DHHS. In silico computational toxicology testing was completed with the support of NIDA’s Addiction Treatment Discovery Program through the use of Interagency Agreement Y1-DA6003. The content is the sole responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.

Notes and references

Figure 1.
Results from the X-ray analysis on 33b drawn from the experimentally determined coordinates.
Scheme 1.
Reagents and conditions: (a) NaIO₄, RuCl₃•3H₂O, CH₃CN/CCl₄/H₂O; (b) Appropriate amine or alcohol, EDCI, HOBT, Et₃N, CH₂Cl₂
Scheme 2.
Reagents and conditions: (a) CDMT, NMM, EtSH, CH₂Cl₂, 88%; (b) Pd/C, Et₃SiH, CH₂Cl₂, 82%; (c) 9-(1R, 2R-pseudoephedrinyl)-(10S)-(trimethylsilyl)-9-borabicyclo[3.3.2]decane, allylMgBr, Et₂O; (d) 1. (R)-(+)−α-Methoxy-α-trifluoromethylphenylacetic acid, oxalyl chloride, benzene; 2. DMAP, NEt₃, CH₂Cl₂, 82%; (e) acryloyl chloride, DMAP, Et₃N, CH₂Cl₂, 40%; (f) Grubbs II, CH₂Cl₂, 58%; (g) Appropriate Wittig reagent; (h) 1. EDCI, C₆H₅C(NH₂)=NOH, CH₂Cl₂; 2. Toluene, heat
Table 1
Opioid receptor binding affinity for compounds 1, 5 – 30, and 34 – 37.\textsuperscript{53}

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>$[^{3}H]$DAMGO (MOP)</th>
<th>$[^{3}H]$DADLE (DOP)</th>
<th>$[^{3}H]$U69,593 (KOP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1370 ± 130</td>
<td>&gt;10,000</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>5</td>
<td>&gt;2,800</td>
<td>&gt;4,700</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>6</td>
<td>&gt;2,800</td>
<td>&gt;4,700</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>7</td>
<td>&gt;2,800</td>
<td>&gt;4,700</td>
<td>1,930 ± 220</td>
</tr>
<tr>
<td>8</td>
<td>&gt;2,800</td>
<td>&gt;4,700</td>
<td>4,060 ± 280</td>
</tr>
<tr>
<td>9</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
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<td>11</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>12</td>
<td>2,490 ± 80</td>
<td>3,690 ± 330</td>
<td>&gt;10,000</td>
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<tr>
<td>13</td>
<td>2,150 ± 140</td>
<td>3,200 ± 390</td>
<td>8,290 ± 670</td>
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<tr>
<td>14</td>
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<td>&gt;10,000</td>
<td>&gt;10,000</td>
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<tr>
<td>15</td>
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<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>17</td>
<td>1,630 ± 100</td>
<td>&gt;10,000</td>
<td>7,580 ± 720</td>
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<tr>
<td>18</td>
<td>1,610 ± 80</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
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<tr>
<td>19</td>
<td>1,570 ± 90</td>
<td>2,600 ± 220</td>
<td>7,280 ± 400</td>
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<td>34</td>
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<td>8,060 ± 720</td>
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<tr>
<td>35</td>
<td>1,900 ± 90</td>
<td>3,380 ± 240</td>
<td>2,340 ± 120</td>
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<tr>
<td>36</td>
<td>1,510 ± 100</td>
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<td>37</td>
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<td>&gt;10,000</td>
<td>1,210 ± 80</td>
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<tr>
<td>23</td>
<td>980 ± 70</td>
<td>&gt;3,000</td>
<td>160 ± 10</td>
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<tr>
<td>24</td>
<td>&gt;3,000</td>
<td>&gt;3,000</td>
<td>1,250 ± 130</td>
</tr>
<tr>
<td>25</td>
<td>&gt;10,000</td>
<td>3,090 ± 220</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>26</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>27</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
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<tr>
<td>28</td>
<td>&gt;10,000</td>
<td>3,360 ± 340</td>
<td>610 ± 30</td>
</tr>
<tr>
<td>29</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>2,980 ± 240</td>
</tr>
<tr>
<td>30</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>2,190 ± 300</td>
</tr>
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Receptor binding was performed in CHO cells which express the human MOP, DOP or KOP receptors. All results are n = 3.
Table 2

Opioid receptor activity for 1, 21, and 23.

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>KOP ED$_{50}^{a}$</th>
<th>KOP E$_{\text{max}}^{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41 ± 6</td>
<td>124 ± 6</td>
</tr>
<tr>
<td>21</td>
<td>5110 ± 1800</td>
<td>85 ± 13</td>
</tr>
<tr>
<td>23</td>
<td>3780 ± 970</td>
<td>57 ± 6</td>
</tr>
<tr>
<td>U50,488H</td>
<td>27 ± 6</td>
<td>100</td>
</tr>
</tbody>
</table>

$^{a}$ED$_{50}$ = Effective dose for 50% maximal response;

$^{b}$E$_{\text{max}}$ is % which compound stimulates binding compared to (-)-U50,488 (500 nM) at KOP receptors.