

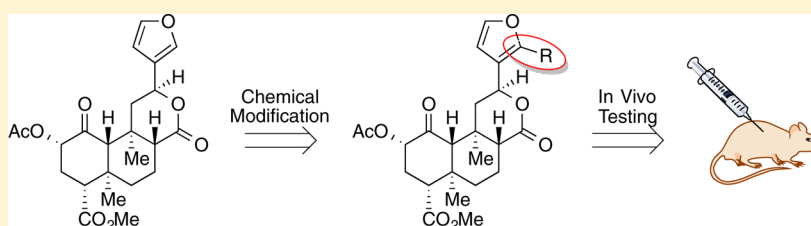
Synthesis and κ -Opioid Receptor Activity of Furan-Substituted Salvinorin A Analogues

Andrew P. Riley,[†] Chad E. Groer,[‡] David Young,[§] Amy W. Ewald,[§] Bronwyn M. Kivell,[§] and Thomas E. Prisinzano^{*,†,‡}

[†]Department of Chemistry and [‡]Department of Medicinal Chemistry, School of Pharmacy, The University of Kansas, Lawrence, Kansas 66045, United States

[§]School of Biological Sciences, Centre for Biodiscovery, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand

S Supporting Information



ABSTRACT: The neoclerodane diterpene salvinorin A, found in the leaves of *Salvia divinorum*, is a potent κ -opioid receptor agonist, making it an attractive scaffold for development into a treatment for substance abuse. Although several successful semisynthetic studies have been performed to elucidate structure–activity relationships, the lack of analogues with substitutions to the furan ring of salvinorin A has prevented a thorough understanding of its role in binding to the κ -opioid receptor. Herein we report the synthesis of several salvinorin A derivatives with modified furan rings. Evaluation of these compounds in a functional assay indicated that sterically less demanding substitutions are preferred, suggesting the furan ring is bound in a congested portion of the binding pocket. The most potent of the analogues successfully reduced drug-seeking behavior in an animal model of drug-relapse without producing the sedation observed with other κ -opioid agonists.

INTRODUCTION

κ -Opioid receptors (KOR) and their endogenous ligands, the dynorphins, are widely expressed in the central nervous system (CNS),^{1–3} and modulation of this system may prove useful in several therapeutic areas, including pain, drug abuse, and depressive disorders.^{4–9} KOR agonists can block pain perception^{10,11} and the rewarding effects of psychostimulants,^{12–15} while antagonists at this receptor may prove useful in preventing drug abuse relapse, depression, or anxiety.^{7–9,16,17} Although many KOR ligands have been studied for these purposes, their development as therapeutic agents is hindered by unfavorable side effects. For example, KOR agonists are known to produce dysphoria,^{17–19} sedation,²⁰ and depression,⁴ and classical antagonists are hindered by unusually long pharmacokinetic profiles.^{21–23}

In order to develop superior KOR-directed therapies, a better understanding of how ligand structure influences KOR function is needed. It is becoming increasingly clear that structurally unique ligands acting at the same receptor can preferentially activate different signaling pathways, which in turn dictate behavioral profiles.^{24,25} This phenomenon, known as functional selectivity, may be a means to harness the beneficial effects of KOR ligands and reduce the unwanted side effects associated with traditional KOR ligands.^{10,26–28}

Natural products are a robust source of unique structural scaffolds. In fact, the study of psychoactive natural products has had a significant impact on our understanding of CNS function.^{8,9,29,30} For example, studies of the alkaloid morphine from *Papaver somniferum* and Δ^9 -tetrahydrocannabinol (THC) from *Cannabis sativa* led to the identification of the endogenous opioid and endocannabinoid systems, respectively.^{31–33} Furthermore, well over half of currently approved drugs are natural products or derivatives of natural products.^{34–36} Therefore, continued investigation of natural products promises to yield additional biological probes and novel therapies.

Salvinorin A (1, Figure 1) is a natural product neoclerodane diterpene and is the principal active component of *Salvia divinorum* Epling and Jativa (Lamiaceae).^{37,38} *S. divinorum* is a mint plant native to southern Mexico that has been used in traditional medicine for treatment of diarrhea, headache, and rheumatism and for its powerful hallucinogenic effects during spiritual practices.³⁹ More recently, *S. divinorum* has been gaining popularity as a recreational hallucinogen⁴⁰ and thus is receiving attention from regulatory agencies. Interestingly, salvinorin A does not interact with serotonin 5-HT_{2A} receptors,^{41,42} which mediate the effects of other hallucinogens,

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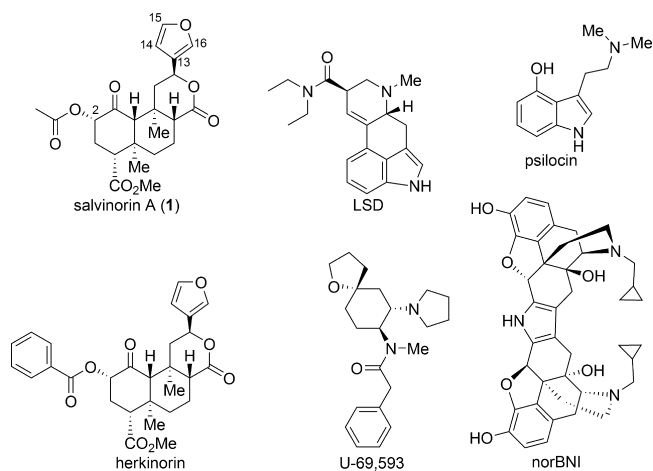


Figure 1. Salvinorin A is a structurally unique hallucinogen and KOR ligand. While salvinorin A, LSD, and psilocin are all hallucinogens, the latter two act through serotonin 5-HT_{2A} receptors. In contrast, salvinorin A is a potent and selective KOR agonist but is structurally dissimilar to other KOR ligands, such as U69,593 and nor-BNI. The closely related salvinorin A analogue herkinorin is a potent MOR agonist.

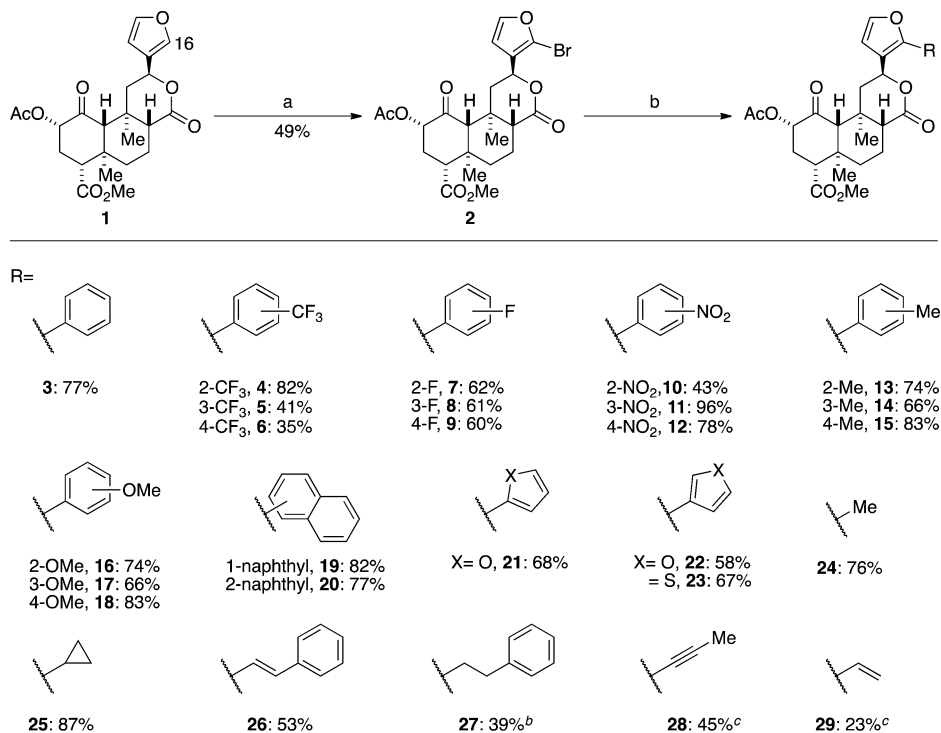
such as LSD and psilocin (Figure 1). In fact, **1** is a potent and selective KOR agonist,^{42,43} but it is structurally dissimilar to other opioids, including classical KOR ligands,⁴⁴ such as (+)-(5 α ,7 α ,8 β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69,593) and nor-binaltorphimine (nor-BNI) (Figure 1). The most striking feature of **1** is the lack of a basic nitrogen, which was once thought to be required for opioid receptor binding.⁴⁵ This has led to the

proposal of several binding models including one based on the recently solved KOR crystal structure.^{42,46–49}

The unique structure and binding of **1** to the KOR may lead to the unique pharmacology of this ligand. In vitro evaluation of **1** showed that while it was a potent activator of KOR-mediated G protein signaling, it promotes much less internalization of this receptor than other KOR agonists.⁴³ Additionally, a μ -opioid receptor (MOR) selective derivative of **1**, herkinorin, was also shown to activate G protein coupling, while not promoting receptor internalization or β -arrestin recruitment,⁵⁰ two cellular events that usually accompany G protein activation. The unique structure and pharmacology of **1** suggests that this compound may be useful in developing novel KOR ligands with reduced potential for unwanted side effects. To this end, we began an extensive structure–activity relationship (SAR) campaign to better understand how specific structural features of **1** determine opioid receptor activity.

Salvinorin A contains many opportunities for structural modification, and several of these have been explored extensively.⁵¹ Briefly, alteration of the C-1 ketone, C-4 carbomethoxy group, or C-17 lactone reduces affinity and efficacy at the KOR. Similarly, hydrolysis of the C-2 acetate eliminates activity. However, analogues that replace the acetyl moiety with functional groups that mimic the carbonyl oxygen retain activity. Interestingly, aromatic esters at this position shift selectivity toward MOR activity. Despite the proposed importance of the furan in binding models, considerably less work has been done to understand the SAR at this position. The majority of the studies that have taken place have focused on replacement of the furan ring with other heterocycles, only some of which appear to be well-tolerated. Substitution of the

Scheme 1. Bromination and Suzuki–Miyaura Reaction of **1**^a



^aReagents and conditions: (a) NBS (1.4 equiv), Br₂ (cat.), CH₂Cl₂; (b) RB(OH)₂ (2.0 equiv), Pd₂dba₃ (0.04 equiv), SPhos (0.16 equiv), K₃PO₄ (3.0 equiv), PhMe, 60 °C. ^b13% of **1** was recovered from the reaction mixture. ^cAlternate reaction conditions used: RBF₃K (1.1 equiv), Pd(dppf)·CH₂Cl₂ (0.09 equiv), Cs₂CO₃ (3.0 equiv) THF/H₂O (20:1), 65 °C.

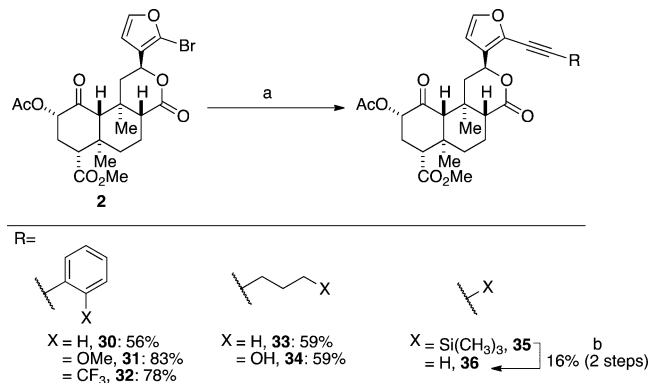
furan ring itself may provide better insight into how **1** interacts with the KOR.⁵² Furthermore, introduction of steric bulk or electron-withdrawing groups on the furan ring may hinder the action of CYP-450 enzymes responsible for the oxidative metabolism of furan rings, thus improving metabolic stability.⁵³

In the present study, analogues of **1** were generated by functionalizing the furan ring, and their activities at the KOR were measured to determine how the substituents may be effecting KOR binding and activity. This analysis allowed for the identification of several potent analogues that were further evaluated in animal models of substance abuse.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the analogues began by utilizing our recent method for selectively modifying furan-containing natural products.⁵⁴ Selective bromination at the C-16 position of **1** through a combination of NBS and catalytic Br₂ produced **2**. In addition to serving as a useful probe, **2** allowed for a variety of aryl, heteroaryl, alkenyl, and alkyl groups to be introduced in moderate to good yield using Suzuki–Miyaura couplings (Scheme 1). Alkynyl groups were also introduced using Sonogashira couplings (Scheme 2). In addition to alkyl- and aryl-substituted alkynes, the terminal alkyne **36** was accessed after fluoride-mediated removal of the trimethylsilyl-protecting group from **35**.

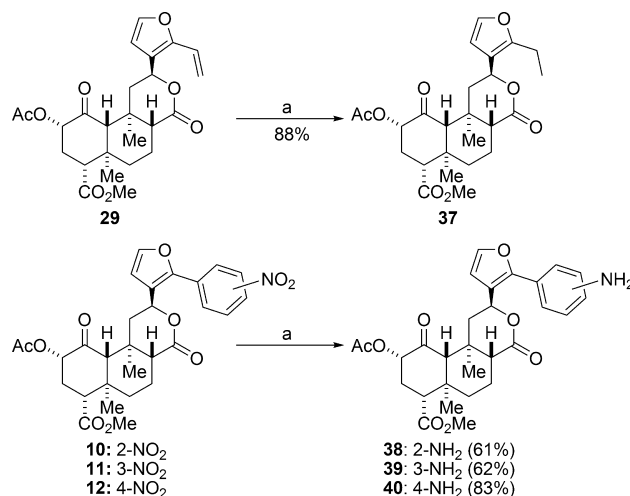
Scheme 2. Synthesis of Alkynyl-Substituted Analogues via Sonogashira Couplings^a



^aReagents and conditions: (a) appropriate terminal alkyne (2.0 equiv), PdCl₂(PPh₃)₂ (0.05 equiv), CuI (0.1 equiv), THF/Et₃N (1:1), 80 °C; (b) TBAF (2.0 equiv), CH₂Cl₂.

Despite the range of functional groups tolerated by these general conditions, some probe molecules could not be accessed using these reactions. The gaseous nature of propyne prevented the use of Sonogashira couplings to synthesize **28** (Scheme 1). Instead, a Suzuki–Miyaura coupling utilizing potassium propynyltrifluoroborate produced the desired probe in acceptable yield.⁵⁵ Despite aqueous Cs₂CO₃ being used in the reaction, the C-2 acetate remained intact, an interesting observation considering it is easily cleaved with methanolic Na₂CO₃.⁵⁶ Similar reaction conditions were used to synthesize **29**, a compound previously prepared via a Stille coupling.⁵⁷ Selective reduction of **29** to **37** using Pd/C was accomplished without over-reduction of the furan ring (Scheme 3). Additionally, these conditions successfully reduced the aromatic nitro compounds **10–12** to the corresponding anilines **38–40**.

Scheme 3. Selective Reduction of **29**, **10**, **11**, and **12**^a

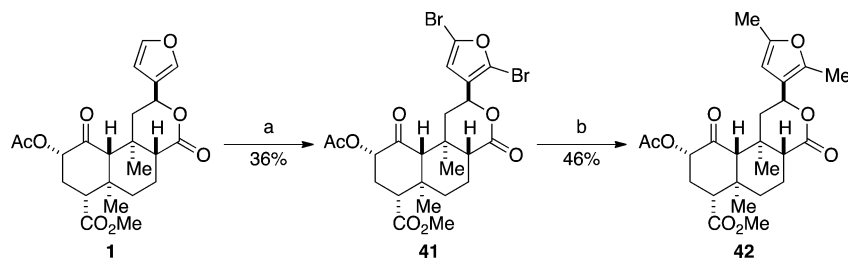


^aReagents and conditions: (a) H₂, Pd/C (10%), MeOH/THF (3:2).

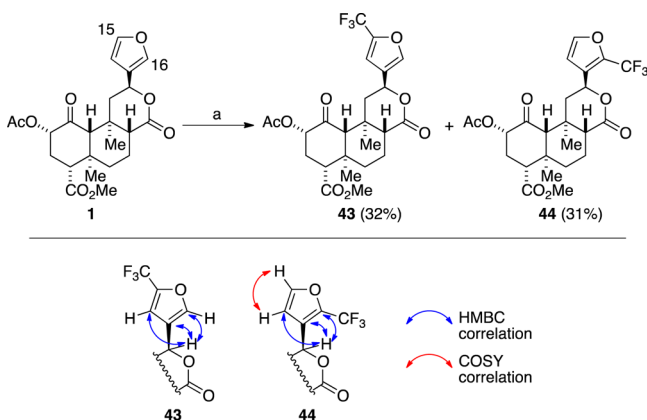
Disubstituted probes were also prepared by first treating **1** with stoichiometric amounts of Br₂ and NBS to produce the dibrominated derivative **41** (Scheme 4). **41** was then dimethylated with the same Suzuki–Miyaura conditions developed for the coupling of **2**, to produce **42**.

In the final attempt to alter the electronic and steric characteristics of the furan ring of **1**, trifluoromethyl groups were introduced at either C-15 or C-16 using a photoredox protocol developed by MacMillan (Scheme 5).⁵⁸ The products **43** and **44** were easily separable on silica gel in approximately a 1:1 ratio. Although we expected that the position of the trifluoromethyl groups could be assigned upon the basis of ¹H coupling constants, both pairs of furanyl protons appeared as two singlets. Thus, the position of the trifluoromethyl groups were assigned upon the basis of a combination of 2D-NMR and C–F coupling constants (Scheme 5). The HMBC spectra of **43** and **44** show a correlation of the proton on C-12 and carbons C-13, C14, and C-16. A correlation between H-14 and H-15 was observed in **44**, but no such correlation was observed in **43**. With these assignments made, the regiochemistries were made by measuring the C–F coupling constants. **43** is particularly interesting because only one other example of a C-15 monosubstituted derivative has been reported.⁵⁹

During our initial evaluation of these compounds, it became apparent that sterically encumbering substitutions to the furan ring resulted in a decrease in potency (vide infra). Specifically, the vinyl- and ethynyl-substituted derivatives appeared to be among the most potent compounds. To test whether the electronic properties may also have an effect, we synthesized the C-16 aldehyde and nitrile derivatives, which are similar in shape and size to the vinyl and ethynyl substitutions, respectively. Treating **1** with paraformaldehyde in warm acetic acid led to a complex mixture.⁵⁹ In addition to the mono- and dihydroxymethylated products (**45**, **46**) observed by Munro et al., a mixture of acetylated products (**47**, **48**) was also isolated from the reaction mixture (Scheme 6). Unfortunately, the selectivity or yields of this reaction could not be improved by extended or shortened reaction times, variation of the solvent employed, addition of Lewis acid catalysts including BF₃·OEt₂ and LiCl, use of aqueous formaldehyde, or slow addition of the paraformaldehyde. The monohydroxylated **45** was, however, successfully oxidized to **49** via a Swern reaction. Compound **49**

Scheme 4. Synthesis of Disubstituted Probes^a

^aReagents and conditions: (a) NBS (1.0 equiv), Br₂ (1.0 equiv), CH₂Cl₂; (b) MeB(OH)₂ (4.0 equiv), Pd₂dba₃ (0.04 equiv), SPhos (0.16 equiv), K₃PO₄ (3.0 equiv), PhMe, 60 °C.

Scheme 5. Trifluoromethylation of 1 Using Photoredox Catalysis and Relevant NMR Correlations^a

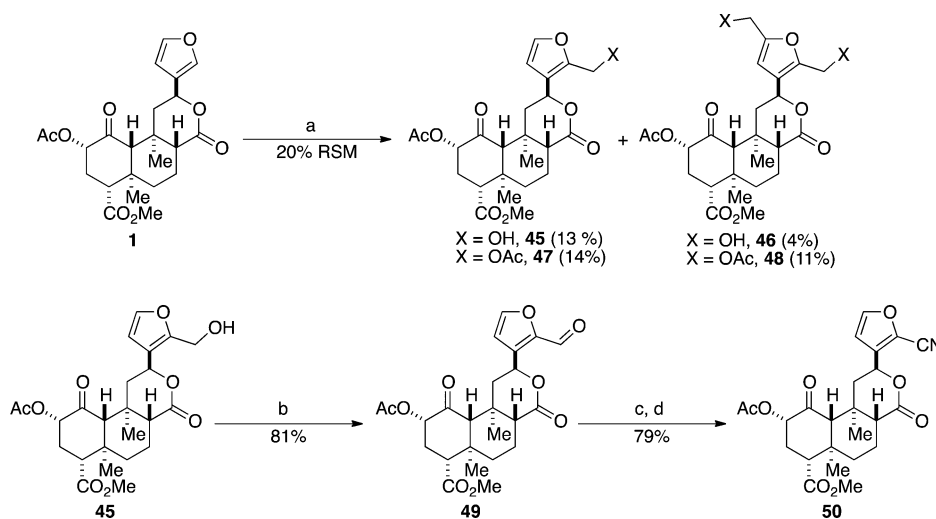
^aReagents and conditions: (a) Ru(phen)₃Cl₂·H₂O (0.02 equiv), CF₃SO₂Cl₂ (4.0 equiv), KH₂PO₄ (3.0 equiv), *hν*, MeCN.

was then converted to **50** by tosyl chloride-promoted dehydration of the corresponding oxime.

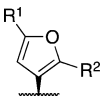
In Vitro KOR Activity. To determine how the substitutions at C-15 and C-16 of **1** affected KOR activity, the derivatives were tested for inhibition of forskolin-induced cAMP accumulation in CHO cells expressing the KOR (Table 1).

While most derivatives retained full efficacy for KOR activity, a reduction in potency was observed for most furan-substituted derivatives of **1**. Several of the probes with sterically less demanding C-16 substitutions, however, retained potency similar to that of **1**. Brominating the furan ring produced no significant change in the potency (Table 1; EC₅₀ = 0.030 ± 0.004 nM for **1** vs 0.040 ± 0.010 nM for **2**). However, conversion of the bromine atom to a methyl group via a Suzuki–Miyaura coupling led to a 11-fold reduction in potency (EC₅₀ = 0.41 ± 0.15 nM for **24**). In contrast, inclusion of an ethynyl group at C-16 produced a probe slightly more potent than **1** (EC₅₀ = 0.019 ± 0.004 nM), making **2** and **36** two furan-modified analogues with potencies similar to that of **1**. The difference in potency between **24** and **36** suggests that the C-16 position of **1** is oriented in a sterically congested region of the binding pocket.

To further probe this effect, substitutions were made at the end of the alkyne. The addition of a methyl or *n*-propyl group (**28** and **33**, respectively) reduced the potency to 26.0 ± 10.0 and 9250 ± 260 nM, respectively. A 10-fold increase in potency was observed by including an alcohol at the terminal position of the *n*-propyl group; however, it appears as though nonterminal alkynes extend too far into the binding pocket and result in decreases in potency. A similar decrease in potency was observed when aryl-substituted alkynes were appended (**30**–

Scheme 6. Synthesis of Hydroxymethyl-, Formyl-, and Cyano-Substituted Analogues^a

^aReagents and conditions: (a) (CH₂O)_{*n*} (5.43 equiv), AcOH, 75 °C; (b) DMSO (27 equiv), (COCl)₂ (14 equiv), Et₃N (5.2 equiv), CH₂Cl₂, -78 °C to rt; (c) NH₂OH·HCl, pyr (9.3 equiv), MeOH, 65 °C; (d) TsCl (1.1 equiv), DIPEA (2.6 equiv), CH₂Cl₂.

Table 1. C-15,16 Substituted Salvinorin A Derivatives—KOR Potency for Inhibition of cAMP Accumulation in CHO Cells


compd	R ₁	R ₂	EC ₅₀ ± SEM ^{a,b} (nM)
U69,593			0.80 ± 0.40
dynorphin A			0.41 ± 0.07
1	H	H	0.030 ± 0.004 ^d
2	H	Br	0.040 ± 0.010 ^d
3	H	Ph	1.3 ± 0.4
4	H	2-CF ₃ C ₆ H ₄	8.4 ± 2.3
5	H	3-CF ₃ C ₆ H ₄	27.0 ± 6.0
6	H	4-CF ₃ C ₆ H ₄	1020 ± 250
7	H	2-FC ₆ H ₄	1.2 ± 0.1
8	H	3-FC ₆ H ₄	12.0 ± 3.0
9	H	4-FC ₆ H ₄	54.0 ± 7.0
10	H	2-NO ₂ C ₆ H ₄	360 ± 60
11	H	3-NO ₂ C ₆ H ₄	450 ± 100
12	H	4-NO ₂ C ₆ H ₄	170 ± 50
13	H	2-MeC ₆ H ₄	5.4 ± 1.1
14	H	3-MeC ₆ H ₄	3.4 ± 1.1
15	H	4-MeC ₆ H ₄	2.3 ± 0.4
16	H	2-MeOC ₆ H ₄	10.0 ± 2.0
17	H	3-MeOC ₆ H ₄	8.6 ± 2.6
18	H	4-MeOC ₆ H ₄	16.0 ± 5.0
19	H	1-naphthyl	37.0 ± 9.0
20	H	2-naphthyl	25.0 ± 7.0
21	H	2-furyl	6.7 ± 1.6
22	H	3-furyl	6.5 ± 1.3
23	H	3-thienyl	4.3 ± 0.5
24	H	Me	0.41 ± 0.15 ^d
25	H	cyclopropyl	1.00 ± 0.30
26	H	PhC(H)=C(H)	20.0 ± 2.0
27	H	PhCH ₂ CH ₂	38.0 ± 5.0
28	H	MeC≡C	26.0 ± 10.0
29	H	H ₂ C=C(H)	0.96 ± 0.24
30	H	C ₆ H ₅ C≡C	100 ± 5
31	H	2-MeOC ₆ H ₄ C≡C	6100 ± 2000
32	H	2-CF ₃ C ₆ H ₄ C≡C	430 ± 50
33	H	CH ₃ (CH ₂) ₂ C≡C	9250 ± 260
34	H	HO(CH ₂) ₃ C≡C	890 ± 30
36	H	HC≡C	0.019 ± 0.004 ^d
37	H	Et	2.9 ± 0.6
38	H	2-NH ₂ C ₆ H ₄	430 ± 60
39	H	3-NH ₂ C ₆ H ₄	630 ± 160
40	H	4-NH ₂ C ₆ H ₄	620 ± 140 ^c
41	Br	Br	240 ± 50
42	Me	Me	250 ± 50
43	CF ₃	H	3.1 ± 0.3
44	H	CF ₃	31.0 ± 11.0
45	H	HOCH ₂	3.3 ± 0.2
49	H	CHO	7.3 ± 2.5
50	H	CN	9.4 ± 2.2

^aMean ± standard error of the mean; $n \geq 3$. ^bKOR $E_{\max} = 100\%$, unless otherwise indicated. ^c $E_{\max} = 65\%$. ^dEC₅₀ ≥ 10 000 nM for MOR.

32), although electronic composition of the aryl moiety seems to have an effect.

To observe the effects of hybridization, **36** was also compared to the known vinyl-substituted **29** and its reduction product **37**. Reduction of the triple bond decreased the potency to 0.96 ± 0.24 nM for **29** and 2.9 ± 0.6 nM for **37**. We believe the dependence on hybridization can be explained by noting that reductions will add to the steric bulk of the substitution and thus reduce the ability to interact with the receptor. This same trend was *not* observed when a phenyl group was present at the end of the alkyne. In this case, reduction of the alkyne reduces the extension of the phenyl group into the binding pocket, and thus, an increase in potency was observed. However, further reduction to the phenethyl results in a reduction of potency.

Clearly, the steric properties of the substitutions have a significant effect on the activity of the molecule. To determine if electronic properties of the substitution also play a role, small, electron-withdrawing groups were added to the furan ring. The aldehyde in **49** and nitrile in **50** are of similar shape and size to the alkene and alkyne in **29** and **36**, respectively, but they remove electron density from the furan ring. In both cases, this resulted in a significant decrease in potency, with **50** being more than 500 times less potent than **36**, and **49** being approximately 7 times less potent than **29**. It is interesting to note that **49** and **50** are nearly equipotent. This suggests that either the electronic properties of the substitution are more important than steric effects or that the difference in activity between **29** and **37** may be the result of their terminal hydrogens and not the geometries of the substitutions.

With these results in mind, it is not surprising that the addition of a phenyl group (**3**) at C-16 decreases potency relative to that of **1**. Nevertheless, **3** is still a potent agonist at the KOR with an EC₅₀ = 1.3 ± 0.4 nM. With the wide range of commercially available aryl boronic acids, substitution to the phenyl ring could be easily introduced and the resulting compounds compared to **3** to probe for additional ligand–receptor interactions (Table 1). Thus, fluoro- and trifluoromethyl-groups were used to probe for a halogen bond interaction. Methoxy- and amino-substituted phenyl-groups as well as furanyl- and thienyl-substitutions (Table 1) were included to explore hydrogen bond acceptors and donors. Given the relative frequency of the “methyl effect” tolyl-substituted derivatives were examined.⁶⁰ The π -system was also extended using the naphthyl-substituted analogues. Despite the wide range of functionality that was introduced, no substitution produced an increase in potency relative to that of **3**. Instead, steric properties of the substitution dictate the differences in potency, with bulkier substituents generally producing less potent compounds. The lone trend that could be observed was in the substitution pattern of the fluoro (**7–9**) and trifluoromethyl (**4–6**) phenyl substitutions. In these derivatives, the ortho-substituted phenyl rings were more potent than the meta-substituted phenyl rings, which were more potent than the para-substituted phenyl rings. However, no dependence on substitution pattern was observed in the methyl-, methoxy-, nitro-, or amino-substituted analogues. In addition to probing for specific interactions, the effect of electron-withdrawing and electron-donating groups was also tested. Once again, no obvious trend could be inferred, and steric parameters of the substitution appeared to control the potency of the probe molecule.

Finally, substitution at the C-15 position was investigated (Table 1). Because bromination selectively occurs at the C-16 position, however, selective derivitization was not as straightfor-

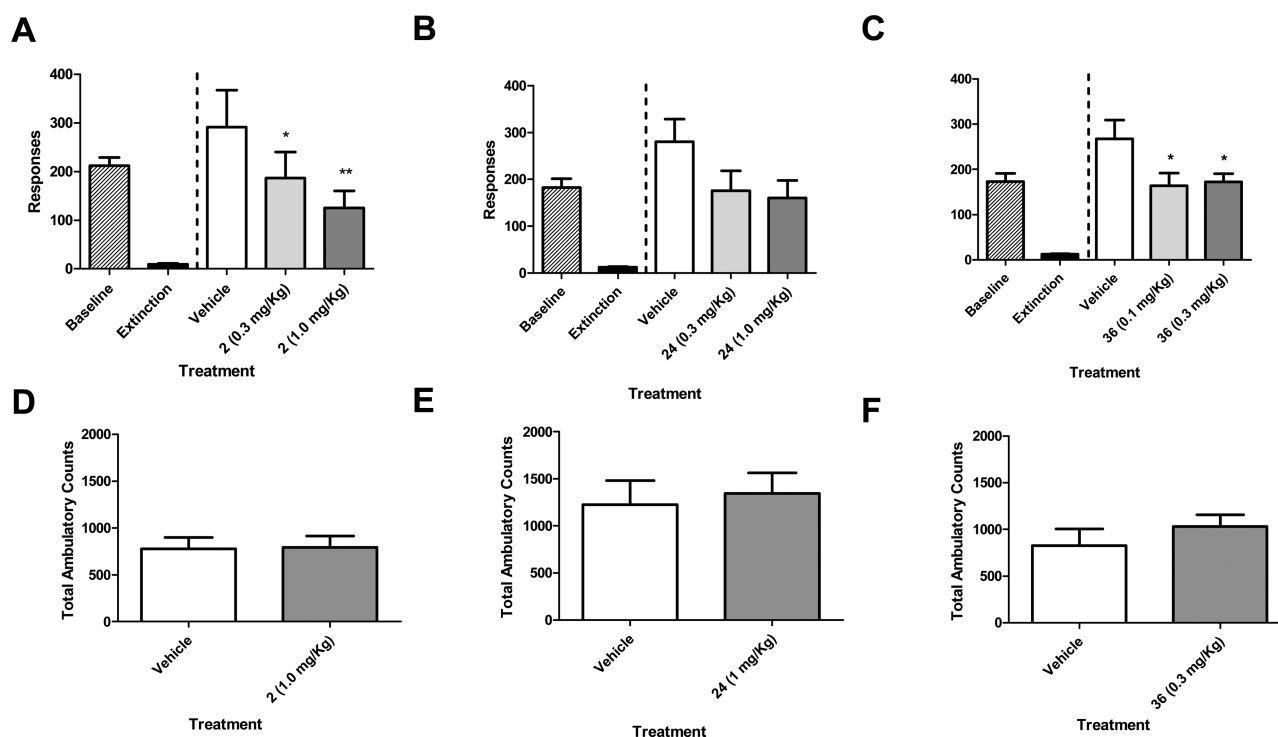


Figure 2. Active lever responses for cocaine self-administration during baseline and extinction (pretest) and during reinstatement following a priming injection of cocaine (20 mg/kg) for **2** (A), **24** (B), and **36** (C). Repeated measures ANOVA followed by Dunnett's multiple comparison test (* $P < 0.05$; ** $P < 0.01$) ($n = 5$ or 6). Effect of **2** (D) ($P = 0.9326$), **24** (E) ($P = 0.7289$), and **36** (F) ($P = 0.3659$) on spontaneous locomotor activity in the rat shown as total ambulatory counts over 60 min. Student t test ($n = 6$ or 7).

ward. Fortunately, employing Nagib and MacMillian's photo-redox system nonselectively appends trifluoromethyl groups at either the C-15 or C-16 positions on the furan ring of **1**, thus offering us a point of comparison. The potency of both **43** and **44** was lower than that of **1**; however, the size and electron-withdrawing nature of the trifluoromethyl group prevents us from determining if this is an electronic or steric effect. Comparing the potency of **43** and **44** does indicate a 10-fold difference in favor of the C-15-substituted regioisomer. This difference does suggest that steric effects are at least partially responsible for the decrease in potency, as both regioisomers are isoelectronic. Furthermore, it appears that groups appended to C-15 are oriented in a less sterically encumbered position of the binding pocket. Alternatively, rotation about the bond between C-12 and C-13 due to the added trifluoromethyl group may cause the furan ring to adopt a different mode of binding.

To better assess the effect of substituting the C-15 position of **1**, the dibrominated **41** and dimethylated **42** were synthesized and compared to their monosubstituted derivatives. The additional C-15 bromine decreased potency from 0.040 ± 0.010 nM for **2** to 240 ± 50 nM for **41**. The additional C-15 methyl group decreased potency from 0.41 ± 0.15 nM for **24** to 250 ± 50 nM for **42**. Thus, compounds **43** and **44** demonstrate that substitution at C-15 is more tolerated than a C-16 monosubstitution; however, disubstitution leads to a significant decrease in potency. This suggests that substitutions at both C-15 and C-16 may prevent the furan ring from adopting a favorable conformation within the KOR binding pocket.

In Vivo KOR Activity. Although the majority of the substitutions made to the furan ring of **1** resulted in a loss of activity at KOR, compounds **2**, **24**, and **36** were identified as subnanomolar agonists with potencies similar to that of **1**. Before assessing these compounds in vivo, their selectivities for

the KOR were determined. Although **1** and its derivatives typically display only moderate activity at the δ -opioid receptor (DOR), some modifications lead to significantly increased activity at the MOR.⁶¹ However, these compounds were further characterized as highly selective ligands, with no activity at the MOR (Table 1). To explore how these modified natural products would affect an in vivo system, we evaluated them for their ability to attenuate cocaine-priming-induced reinstatement of drug seeking in male Sprague–Dawley rats. In this model of relapse, stably responding cocaine-self-administering rats on a fixed ratio 5 (FR5) schedule of reinforcement underwent extinction until responses were less than 20% of baseline before being given a priming injection of cocaine and lever press responses recorded. Pretreatment with known KOR agonists such as U69,593 and *trans*(-)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50,488) have previously been shown to attenuate cocaine-priming-induced drug-seeking behavior.^{14,62} Both **2** and **36** significantly reduced the number of responses to the previously active lever relative to vehicle, suggesting that they attenuate cocaine-seeking behavior (Figure 2a). Consistent with our in vitro data, a larger dose of **2** (0.3 mg/kg for **2**; 0.1 mg/kg for **36**) was required to produce these results. Furthermore, a general trend toward decreasing responses was apparent for increasing doses of **24**; however, even at the highest dose (1.0 mg/kg), these results were not statistically different from those of vehicle.

Although these results clearly demonstrate that analogues of **1** containing small substitutions to the furan ring attenuate drug-seeking behavior, KOR agonists, including **1**, are known to cause sedation and/or motor incoordination.⁶³ Prior studies have shown that **1** reduces drug-seeking behavior at a dose that does not affect spontaneous locomotor activity.⁶² To determine if the observed decrease in responding was the result of

sedation or motor incoordination, the effects of acute treatments of **2** (1.0 mg/kg), **24** (1.0 mg/kg), and **36** (0.3 mg/kg) on spontaneous locomotor activity were examined. At doses that were able to reduce cocaine-seeking behavior, no significant difference from vehicle-treated animals was observed. Additionally, no significant changes in inactive lever pressing during the self-administration, extinction, and reinstatement periods were observed for animals treated with **2** (1.0 mg/kg), **24** (1.0 mg/kg), and **36** (0.3 mg/kg) (Supplementary Figure 1, Supporting Information). Therefore, decreased drug-prime responses are specific to anticocaine effects and are not the result of sedation. Taken together, these *in vivo* data suggest that **2**, **24**, and **36** are promising compounds to be investigated for drug abuse therapies. Studies aimed at investigating the effects of novel KOR agonists on drug self-administration and the rewarding effects of drugs of abuse as well as other potential side effects are currently being performed and will be reported in due course.

CONCLUSIONS

Utilizing recently developed methods for selectively substituting the furan ring of **1**, 47 novel KOR agonists were synthesized. Evaluation of this collection using a functional inhibition of cAMP assay demonstrated that only small substitutions are well-tolerated, indicating that the furan ring of **1** binds in a sterically congested portion of the KOR binding pocket. The most potent compounds (**2**, **24**, and **36**) were shown to successfully attenuate the drug-induced reinstatement of cocaine in an animal model of drug abuse without causing sedation.

EXPERIMENTAL SECTION

General Methods. All reagents were purchased from commercial sources and were used without further purification, unless noted otherwise. All glassware was dried in an oven at 120 °C overnight and cooled under a stream of argon prior to use. Melting points were determined on a Thomas-Hoover capillary melting apparatus. NMR spectra were recorded on 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 were collected on a LCT Premier (Waters Corp., Milford, MA) time-of-flight mass spectrometer. Column chromatography was performed with silica gel (40–63 μm particle size) from Sorbent Technologies (Atlanta, GA). The purity of compounds was determined to be >95% by analytical HPLC using an Agilent 1100 Series capillary HPLC system with diode array detection at 254 nm on an Agilent Eclipse XDB-C18 column (250 \times 10 mm, 5 μm) using isocratic elution with 60% $\text{CH}_3\text{CN}/40\%$ H_2O unless otherwise specified. Compounds with purity <95% were repurified by semipreparative HPLC using the same instrumentation above. Salvinorin A (**1**) was isolated from *S. divinorum* as previously described.⁶⁴ Compounds **2–9**, **16–27**, **30–36**, **41**, **45**, and **46** were prepared according to literature precedent,^{54,59} and their identities were confirmed by comparison of their ^1H and ^{13}C NMR and HRMS spectra and melting point.

General Procedure A: Suzuki–Miyaura Coupling. A conical vial was charged with **2** (60 mg, 1.0 equiv), Pd_2dba_3 (4.3 mg, 0.04 equiv), SPhos (7.7 mg, 0.16 equiv), K_3PO_4 (74.7 mg, 3.0 equiv), and the appropriate boronic acid (2.0 equiv). The vial was sealed with a Biotage Reseal cap and flushed with argon for 5 min. Toluene (1.6 mL) was added through the septum, and the reaction was stirred at room temperature for an additional 5 min and then heated to 60 °C for 16 h. The reaction was then cooled to room temperature, diluted with EtOAc, and filtered through a thin pad of Celite and silica gel, with rinsing with 3 \times 15 mL EtOAc. Solvent was removed in vacuo and the residue purified by flash column chromatography (EtOAc/pentane).

General Procedure B: Suzuki–Miyaura Coupling Utilizing a Potassium Trifluoroborate Salt. A conical vial was charged with **2** (50.0 mg, 1.0 equiv), appropriate potassium trifluoroborate salt (1.10 equiv), Cs_2CO_3 (95.6 mg, 3.0 equiv), and $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$ (7.2 mg, 0.09 equiv). The vial was fitted with a Biotage Reseal cap and flushed with argon for 5 min before the addition of THF (1.0 mL) and argon-sparged H_2O (0.05 mL). The reaction was heated to 65 °C for 16 h and then cooled to room temperature. The layers were separated, and the organic layer was filtered through a thin pad of silica, with rinsing with EtOAc. Solvent was removed in vacuo and residue purified by flash column chromatography (EtOAc/pentane).

General Procedure C: Reduction. A solution of the appropriate nitrophenyl/olefin in MeOH/THF (5 mL, 3:2) was treated with Pd/C (10% w/w, 0.10 equiv). The suspension was placed under an atmosphere of H_2 by applying a vacuum and backfilling with H_2 (3 \times). After 5 h, the reaction was filtered through a thin pad of Celite and the solvent removed in vacuo. The resulting residue was purified by flash column chromatography (EtOAc/pentane).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-2-(2-(2-nitrophenyl)furan-3-yl)-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (10**).** This compound was synthesized using general procedure A to yield a pale yellow solid (27.9 mg, 43%). Mp: 196–198 °C. ^1H NMR (500 MHz, CDCl_3): δ 8.46 (t, J = 1.90 Hz, 1H), 8.21 (ddd, J = 0.99, 2.27, 8.24 Hz, 1H), 7.91 (ddd, J = 1.03, 1.61, 7.77 Hz, 1H), 7.64 (t, J = 8.00 Hz, 1H), 7.52 (d, J = 1.96 Hz, 1H), 6.54 (d, J = 1.94 Hz, 1H), 5.71 (dd, J = 4.86, 12.07 Hz, 1H), 5.13 (m, 1H), 3.74 (s, 3H), 2.77 (m, 1H), 2.45 (dd, J = 4.88, 13.66 Hz, 1H), 2.30 (m, 2H), 2.18 (m, 3H), 2.14 (s, 3H), 1.82 (m, 1H), 1.68 (m, 3H), 1.48 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.81, 171.51, 170.85, 170.01, 148.69, 148.62, 143.15, 132.25, 131.63, 129.97, 122.81, 121.62, 121.49, 110.86, 75.00, 71.50, 64.00, 53.61, 52.04, 51.74, 43.00, 42.12, 38.17, 35.60, 30.72, 20.56, 18.15, 16.50, 15.15. HRMS: $M + \text{Na}^+$ 576.1846 (calcd), 576.1855 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-2-(2-(3-nitrophenyl)furan-3-yl)-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (11**).** This compound was synthesized using general procedure A to yield a pale yellow solid (62.4 mg, 96%). Mp: 119–123 °C. ^1H NMR (500 MHz, CDCl_3): δ 8.46 (t, J = 1.89 Hz, 1H), 8.21 (ddd, J = 0.97, 2.26, 8.22 Hz, 1H), 7.91 (ddd, J = 1.05, 1.56, 7.77 Hz, 1H), 7.64 (t, J = 8.02 Hz, 1H), 7.52 (d, J = 1.93 Hz, 1H), 6.54 (d, J = 1.93 Hz, 1H), 5.70 (dd, J = 4.86, 12.07 Hz, 1H), 5.14 (m, 1H), 3.74 (s, 3H), 2.77 (m, 1H), 2.45 (dd, J = 4.87, 13.66 Hz, 1H), 2.30 (m, 2H), 2.19 (m, 3H), 2.14 (s, 3H), 1.82 (m, 1H), 1.70 (m, 3H), 1.48 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.83, 171.51, 170.87, 170.01, 148.68, 148.61, 143.14, 132.24, 131.62, 129.97, 122.80, 121.61, 121.49, 110.86, 75.00, 71.50, 63.97, 53.59, 52.04, 51.72, 42.98, 42.11, 38.16, 35.59, 30.71, 20.56, 18.15, 16.50, 15.15. HRMS: $M + \text{Na}^+$ 576.1846 (calcd), 576.1843 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-2-(2-(4-nitrophenyl)furan-3-yl)-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (12**).** This compound was synthesized using general procedure A to yield a pale yellow solid (50.7 mg, 78%). Mp: 184–188 °C. ^1H NMR (500 MHz, CDCl_3): δ 8.32 (d, J = 9.00 Hz, 2H), 7.75 (d, J = 9.00 Hz, 2H), 7.54 (d, J = 1.86 Hz, 1H), 6.57 (d, J = 1.92 Hz, 1H), 5.70 (dd, J = 4.89, 12.04 Hz, 1H), 5.13 (m, 1H), 3.74 (s, 3H), 2.77 (m, 1H), 2.45 (dd, J = 4.92, 13.64 Hz, 1H), 2.31 (m, 2H), 2.22 (m, 2H), 2.16 (m, 1H), 2.14 (s, 3H), 1.83 (m, 1H), 1.68 (m, 3H), 1.47 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.87, 171.48, 170.81, 170.01, 148.99, 147.14, 143.69, 135.89, 127.18, 124.29, 122.56, 111.03, 75.00, 71.34, 63.96, 53.60, 52.05, 51.72, 42.91, 42.11, 38.14, 35.56, 30.70, 20.55, 18.15, 16.50, 15.14. HRMS: $M + \text{Na}^+$ 576.1846 (calcd), 576.1860 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(2-(*o*-tolyl)furan-3-yl)dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (13**).** This compound was synthesized using general procedure A to yield a white solid (45.4 mg, 74%). Mp: 166–167 °C (dec). ^1H NMR (500 MHz, CDCl_3) δ 7.46 (d, J = 1.95 Hz, 1H), 7.33 (m, 1H), 7.29 (dd, J = 1.62, 5.81 Hz, 2H),

7.26 (m, 1H), 6.47 (d, $J = 1.99$ Hz, 1H), 5.38 (dd, $J = 5.17, 11.90$ Hz, 1H), 5.13 (dd, $J = 8.84, 11.26$ Hz, 1H), 3.73 (s, 3H), 2.76 (dd, $J = 5.51, 11.36$ Hz, 1H), 2.32 (m, 3H), 2.29 (s, 3H), 2.17 (m, 2H), 2.15 (s, 3H), 2.06 (m, 1H), 1.79 (dd, $J = 2.93, 10.00$ Hz, 1H), 1.64 (m, 3H), 1.34 (s, 3H), 1.09 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.94, 171.58, 171.28, 169.97, 152.35, 142.17, 138.36, 130.76, 130.47, 129.33, 129.05, 125.74, 120.36, 109.29, 75.00, 72.07, 64.05, 53.59, 52.01, 51.47, 43.53, 42.11, 38.20, 35.50, 30.75, 20.59, 20.30, 18.16, 16.39, 14.95. HRMS: $M + \text{Na}^+$ 545.2151 (calcd), 545.2157 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(2-(*m*-tolyl)furan-3-yl)dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (14). This compound was synthesized using general procedure A to yield a white solid (40.5 mg, 66%). Mp: 172–175 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.43 (d, $J = 1.87$ Hz, 1H), 7.37 (m, 1H), 7.34 (d, $J = 4.11$ Hz, 2H), 7.19 (m, 1H), 6.47 (d, $J = 1.96$ Hz, 1H), 5.70 (dd, $J = 4.95, 12.03$ Hz, 1H), 5.14 (m, 1H), 3.74 (s, 3H), 2.77 (m, 1H), 2.41 (dd, $J = 5.12, 13.50$ Hz, 1H), 2.41 (s, 3H), 2.30 (m, 2H), 2.17 (m, 3H), 2.14 (s, 3H), 1.81 (m, 1H), 1.66 (m, 3H), 1.44 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.86, 171.58, 171.25, 169.94, 151.79, 142.05, 138.54, 129.97, 129.28, 128.73, 127.75, 124.28, 119.16, 110.14, 74.98, 72.07, 64.04, 53.61, 52.01, 51.67, 43.36, 42.12, 38.23, 35.55, 30.77, 21.51, 20.57, 18.19, 16.46, 15.05. HRMS: $M + \text{Na}^+$ 545.2151 (calcd), 545.2157 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(2-(*p*-tolyl)furan-3-yl)dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (15). This compound was synthesized using general procedure A to yield a white solid (50.9 mg, 83%). Mp: 204–206 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.43 (dd, $J = 4.99, 10.86$ Hz, 3H), 7.26 (d, $J = 6.26$ Hz, 2H), 6.47 (d, $J = 1.96$ Hz, 1H), 5.67 (dd, $J = 4.98, 12.02$ Hz, 1H), 5.14 (m, 1H), 3.74 (s, 3H), 2.77 (dd, $J = 5.67, 11.17$ Hz, 1H), 2.41 (m, 1H), 2.40 (s, 3H), 2.30 (m, 2H), 2.19 (m, 3H), 2.14 (s, 3H), 1.81 (m, 1H), 1.66 (m, 3H), 1.44 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.89, 171.58, 171.28, 169.93, 151.99, 141.88, 138.47, 129.53, 127.24, 127.07, 118.65, 110.05, 74.99, 72.08, 64.05, 53.61, 52.01, 51.63, 43.31, 42.12, 38.22, 35.54, 30.76, 21.34, 20.56, 18.19, 16.45, 15.04. HRMS: $M + \text{Na}^+$ 545.2151 (calcd), 545.2142 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(2-(prop-1-yn-1-yl)furan-3-yl)dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (28). This compound was synthesized using general procedure B to yield a white solid (20.7 mg, 45%). Mp: 210–212 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.26 (d, $J = 2.0$ Hz, 1H), 6.35 (d, $J = 2.0$ Hz, 1H), 5.57 (dd, $J = 11.7, 5.3$ Hz, 1H), 5.13 (m, 1H), 3.73 (s, 3H), 2.76 (m, 1H), 2.48 (dd, $J = 13.6, 5.4$ Hz, 1H), 2.31 (m, 1H), 2.30 (s, 1H), 2.18 (m, 2H), 2.16 (s, 3H), 2.11 (s, 3H), 2.10 (m, 1H), 1.80 (m, 1H), 1.62 (m, 3H), 1.47 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.98, 171.56, 171.11, 169.90, 142.89, 127.10, 109.16, 94.61, 75.02, 72.02, 68.53, 64.01, 53.60, 52.01, 51.50, 43.22, 42.12, 38.21, 35.53, 30.75, 20.56, 18.16, 16.39, 15.07, 4.67. HRMS: $M + \text{Na}^+$ 493.1838 (calcd), 493.1828 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-ethylfuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (37). This compound was synthesized from 29 (23.0 mg, 0.0502 mmol) using general procedure C to yield 37 (20.3 mg, 88%) as a white solid. Mp: 160–162 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.26 (d, $J = 1.96$ Hz, 1H), 6.25 (d, $J = 1.96$ Hz, 1H), 5.47 (dd, $J = 5.10, 11.97$ Hz, 1H), 5.13 (m, 1H), 3.73 (s, 3H), 2.77 (m, 1H), 2.63 (ddd, $J = 4.66, 7.57, 15.09$ Hz, 2H), 2.32 (m, 3H), 2.17 (m, 2H), 2.16 (s, 3H), 2.09 (dd, $J = 2.98, 11.41$ Hz, 1H), 1.80 (m, 1H), 1.61 (m, 3H), 1.46 (s, 3H), 1.22 (t, $J = 7.56$ Hz, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 202.05, 171.58, 171.34, 169.94, 154.47, 140.96, 117.41, 108.51, 75.04, 71.89, 64.01, 53.58, 52.00, 51.50, 43.83, 42.14, 38.20, 35.50, 30.76, 20.57, 19.69, 18.16, 16.41, 15.04, 12.94. HRMS: $M + \text{Na}^+$ 483.1995 (calcd), 483.1973 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-(2-aminophenyl)furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (38). This compound was synthesized from 10 (34.9 mg, 0.0630 mmol) using general

procedure C to yield 38 (20.1 mg, 61%) as a white solid. Mp: 200–202 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.48 (d, $J = 1.87$ Hz, 1H), 7.20 (m, 2H), 6.80 (td, $J = 1.05, 7.53$ Hz, 1H), 6.76 (d, $J = 7.99$ Hz, 1H), 6.49 (d, $J = 1.99$ Hz, 1H), 5.54 (dd, $J = 5.15, 11.84$ Hz, 1H), 5.12 (dd, $J = 9.67, 10.47$ Hz, 1H), 4.07 (s, 2H), 3.73 (s, 3H), 2.75 (m, 1H), 2.36 (dd, $J = 5.18, 13.62$ Hz, 1H), 2.29 (s, 1H), 2.29 (m, 1H), 2.15 (m, 2H), 2.14 (s, 3H), 2.08 (dd, $J = 2.86, 11.31$ Hz, 1H), 1.79 (dd, $J = 2.97, 10.10$ Hz, 1H), 1.62 (m, 3H), 1.37 (s, 3H), 1.09 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.85, 171.57, 171.29, 169.96, 150.43, 145.32, 142.41, 130.84, 130.44, 120.69, 118.17, 116.08, 114.58, 109.87, 75.00, 72.00, 64.03, 53.59, 52.00, 51.47, 43.28, 42.11, 38.21, 35.52, 30.74, 20.58, 18.16, 16.39, 15.03. HRMS: $M + \text{Na}^+$ 546.2104 (calcd), 546.2097 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-(3-aminophenyl)furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (39). This compound was synthesized from 11 (30.3 mg, 0.0547 mmol) using general procedure C to yield 39 (17.8 mg, 62%) as a solid. Mp: 123–127 °C. ^1H NMR (500 MHz, CDCl_3): δ 7.41 (d, $J = 1.90$ Hz, 1H), 7.23 (t, $J = 7.83$ Hz, 1H), 6.92 (m, 1H), 6.88 (m, 1H), 6.69 (ddd, $J = 0.88, 2.36, 8.02$ Hz, 1H), 6.46 (d, $J = 1.95$ Hz, 1H), 5.70 (dd, $J = 5.00, 12.01$ Hz, 1H), 5.14 (m, 1H), 3.73 (s, 3H), 2.77 (m, 1H), 2.40 (dd, $J = 5.02, 13.66$ Hz, 1H), 2.29 (m, 2H), 2.21 (m, 2H), 2.14 (s, 3H), 2.12 (m, 1H), 1.80 (m, 1H), 1.65 (m, 3H), 1.44 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.90, 171.59, 171.33, 169.94, 151.91, 146.77, 141.92, 130.98, 129.79, 119.12, 117.48, 115.26, 113.49, 110.15, 74.98, 72.05, 64.01, 53.58, 52.00, 51.62, 43.28, 42.11, 38.20, 35.53, 30.76, 20.57, 18.18, 16.45, 15.09. HRMS: $M + \text{Na}^+$ 546.2104 (calcd), 546.2089 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-(4-aminophenyl)furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (40). This compound was synthesized from 12 (63.2 mg, 0.114 mmol) using general procedure C to yield 40 (49.6 mg, 83%) as a white solid. Mp: 222–223 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 7.36 (m, 3H), 6.74 (d, $J = 8.65$ Hz, 2H), 6.43 (d, $J = 1.98$ Hz, 1H), 5.62 (dd, $J = 5.00, 11.99$ Hz, 1H), 5.14 (m, 1H), 3.83 (s, 2H), 3.73 (s, 3H), 2.77 (m, 1H), 2.39 (dd, $J = 5.01, 13.66$ Hz, 1H), 2.29 (m, 2H), 2.21 (m, 2H), 2.14 (s, 3H), 2.12 (m, 1H), 1.80 (m, 1H), 1.64 (m, 3H), 1.43 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 201.92, 171.60, 171.40, 169.92, 152.62, 146.79, 141.22, 128.58, 120.46, 117.24, 115.08, 109.90, 74.99, 72.31, 64.06, 53.59, 52.00, 51.59, 43.25, 42.12, 38.22, 35.52, 30.77, 20.57, 18.19, 16.44, 15.05. HRMS: $M + \text{Na}^+$ 546.2104 (calcd), 546.2112 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2,5-dimethylfuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[*f*]isochromene-7-carboxylate (42). This compound was synthesized from 41 (50.0 mg, 0.0847 mmol) using general procedure A modified to use 4.0 equiv of $\text{MeB}(\text{OH})_2$ to yield 42 (18.0 mg, 46%) as a white solid. Mp: 125–128 °C (dec). ^1H NMR (500 MHz, CDCl_3): δ 5.84 (s, 1H), 5.39 (dd, $J = 5.12, 11.93$ Hz, 1H), 5.12 (m, 1H), 3.73 (s, 3H), 2.76 (m, 1H), 2.30 (m, 2H), 2.22 (s, 3H), 2.20 (s, 3H), 2.17 (m, 3H), 2.16 (s, 3H), 2.06 (dd, $J = 3.04, 11.52$ Hz, 1H), 1.80 (m, 1H), 1.61 (m, 3H), 1.45 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 202.07, 171.59, 171.43, 169.97, 150.47, 147.51, 118.72, 104.36, 75.08, 72.28, 64.06, 53.63, 52.01, 51.49, 43.65, 42.16, 38.24, 35.46, 30.76, 20.57, 18.16, 16.39, 15.02, 13.38, 11.77. HRMS: $M + \text{Na}^+$ 483.1995 (calcd), 483.1987 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(5-(trifluoromethyl)furan-3-yl)-dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (43) and (2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(2-(trifluoromethyl)furan-3-yl)-dodecahydro-1H-benzof[*f*]isochromene-7-carboxylate (44). An oven-dried glass tube was charged with 1 (200 mg, 1.0 equiv), K_2HPO_4 (238 mg, 3.0 equiv), and dichlorotris(1,10-phenanthroline)-ruthenium(II) hydrate (6.6 mg, 0.02 equiv) and fitted with a Teflon screwcap. The reaction vessel was flushed with argon. Dry, degassed acetonitrile (4 mL) was added. After stirring for 10 min, triflyl chloride (0.10 mL, 2.0 equiv) was added and the reaction vessel was placed next to a fluorescent light bulb. Two additional portions of 0.05 mL of

triflyl chloride were added after 3 and 24 h. After 48 h, the reaction was quenched by the addition of H₂O (10 mL) and extracted with Et₂O (3 × 10 mL) and CH₂Cl₂ (3 × 10 mL). The combined organic layers were dried over Na₂SO₄ and decanted, and the solvent was removed in vacuo. The resulting residue was purified by flash column chromatography (30–45% EtOAc/pentane) to yield **44** (70.6 mg, 31%) and **43** (74.8 mg, 32%) as white solids. **43**: Mp: 104–106 °C. ¹H NMR (500 MHz, CDCl₃): δ 7.51 (s, 1H), 6.80 (s, 1H), 5.52 (dd, *J* = 4.98, 11.87 Hz, 1H), 5.14 (m, 1H), 3.73 (s, 3H), 2.75 (m, 1H), 2.52 (dd, *J* = 5.10, 13.39 Hz, 1H), 2.30 (s, 1H), 2.30 (dd, *J* = 6.28, 14.49 Hz, 1H), 2.17 (s, 3H), 2.16 (m, 2H), 2.08 (dd, *J* = 2.87, 11.62 Hz, 1H), 1.80 (m, 1H), 1.60 (m, 3H), 1.46 (s, 3H), 1.12 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 202.02, 171.48, 170.63, 170.00, 143.02 (q, *J* = 43.05 Hz), 141.41, 126.42, 118.68 (q, *J* = 267.33 Hz), 110.18 (q, *J* = 2.69 Hz), 75.01, 71.28, 63.88, 53.56, 52.02, 51.47, 43.18, 42.07, 38.08, 35.48, 30.72, 20.57, 18.09, 16.43, 15.15. HRMS: M + Na⁺ 523.1556 (calcd), 523.1570 (found). **44**: Mp: 147–150 °C. ¹H NMR (500 MHz, CDCl₃): δ 7.48 (s, 1H), 6.48 (s, 1H), 5.73 (dd, *J* = 5.03, 12.04 Hz, 1H), 5.12 (m, 1H), 3.73 (s, 3H), 2.76 (m, 1H), 2.41 (dd, *J* = 5.07, 13.56 Hz, 1H), 2.29 (s, 1H), 2.29 (dd, *J* = 6.37, 14.59 Hz, 1H), 2.16 (s, 3H), 2.15 (m, 3H), 1.80 (m, 1H), 1.60 (m, 3H), 1.46 (s, 3H), 1.12 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 201.78, 171.53, 170.62, 169.94, 144.58, 137.20 (q, *J* = 41.81 Hz), 126.83 (q, *J* = 1.97 Hz), 119.33 (q, *J* = 268.22 Hz), 110.21, 74.94, 70.45, 63.75, 53.52, 52.01, 51.45, 43.76, 42.05, 38.06, 35.63, 30.74, 20.58, 18.11, 16.46, 15.01. HRMS: M + Na⁺ 523.1556 (calcd), 523.1539 (found).

Reaction of 1 with Paraformaldehyde under Acidic Conditions.⁵⁹ A mixture of **1** (305 mg, 1.0 equiv) and paraformaldehyde (115 mg, 5.43 equiv) was dissolved in glacial acetic acid (5 mL) under an atmosphere of argon. The reaction was slowly warmed to 75 °C. After 20 h, reaction was cooled to room temperature and the solvent removed in vacuo. The resulting residue was purified by flash column chromatography (40 → 100% EtOAc/pentane) to yield **1** (60.9 mg, 20%), **47** (51.4 mg, 14%), and **48** (45.2 mg, 11%), as well as the previously described **45** (42.2 mg, 13%) and **46** (14.7 mg, 4%).⁵⁸ **47**: Mp: 81–85 °C. ¹H NMR (500 MHz, CDCl₃): δ 7.38 (d, *J* = 1.88 Hz, 1H), 6.33 (d, *J* = 1.89 Hz, 1H), 5.65 (dd, *J* = 5.01, 11.99 Hz, 1H), 5.10 (dd, *J* = 19.97, 28.28 Hz, 1H), 5.08 (d, *J* = 9.32 Hz, 2H), 3.73 (s, 3H), 2.76 (m, 1H), 2.37 (dd, *J* = 5.02, 13.53 Hz, 1H), 2.29 (dd, *J* = 6.36, 13.55 Hz, 2H), 2.16 (m, 3H), 2.15 (s, 3H), 2.08 (s, 3H), 1.80 (m, 1H), 1.62 (m, 3H), 1.48 (s, 3H), 1.12 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 201.85, 171.56, 171.09, 170.57, 169.95, 146.17, 143.25, 123.77, 109.19, 75.03, 71.61, 63.91, 56.08, 53.57, 52.00, 51.42, 43.73, 42.09, 38.17, 35.52, 30.72, 20.78, 20.57, 18.12, 16.45, 15.06. HRMS: M + Na⁺ 527.1893 (calcd), 527.1867 (found). **48**: Mp: 78–81 °C. ¹H NMR (500 MHz, CDCl₃): δ 6.36 (s, 1H), 5.62 (dd, *J* = 5.02, 12.00 Hz, 1H), 5.06 (d, *J* = 5.29 Hz, 2H), 5.05 (m, 1H), 4.99 (d, *J* = 4.53 Hz, 2H), 3.73 (s, 3H), 2.76 (dd, *J* = 7.38, 9.44 Hz, 1H), 2.36 (dd, *J* = 5.07, 13.59 Hz, 1H), 2.30 (dd, *J* = 6.87, 13.52 Hz, 2H), 2.16 (m, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 1.80 (m, 1H), 1.59 (m, 3H), 1.47 (s, 3H), 1.12 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 201.81, 171.55, 170.94, 170.57, 170.53, 169.99, 150.34, 146.62, 124.73, 110.05, 75.07, 71.40, 63.92, 57.71, 56.01, 53.62, 52.01, 51.44, 43.66, 42.12, 38.20, 35.52, 30.71, 20.87, 20.79, 20.57, 18.11, 16.43, 15.03. HRMS: M + Na⁺ 599.2105 (calcd), 599.2110 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-formylfuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[isochromene-7-carboxylate (49). A solution DMSO (0.08 mL, 27 equiv) in CH₂Cl₂ (0.5 mL) was added to a solution of oxalyl chloride (0.05 mL, 14 equiv) in CH₂Cl₂ (2 mL) at –78 °C. After 40 min, a solution of **45** (19.0 mg, 1.0 equiv) in CH₂Cl₂ (0.4 mL) was added slowly. After an additional 30 min, Et₃N (0.03 mL, 5.2 equiv) was added dropwise and the reaction slowly warmed to room temperature and stirred for 3 h. Reaction was quenched with the addition of H₂O and extracted with CH₂Cl₂ (3 × 10 mL). Combined organic layers were washed sequentially with 2 N HCl, saturated NaHCO₃, and brine and then dried over Na₂SO₄. Solvent was removed in vacuo and the resulting residue purified by flash column chromatography (55% EtOAc/pentane) to yield **49** (15.3 mg, 81%) as a solid. Mp: 215–220 °C (dec). ¹H NMR (500 MHz, CDCl₃): δ 9.83

(s, 1H), 7.59 (d, *J* = 1.70 Hz, 1H), 6.58 (d, *J* = 1.63 Hz, 1H), 5.97 (dd, *J* = 5.13, 11.99 Hz, 1H), 5.09 (m, 1H), 3.73 (s, 3H), 2.74 (m, 1H), 2.52 (dd, *J* = 5.15, 13.34 Hz, 1H), 2.29 (s, 1H), 2.29 (m, 2H), 2.22 (m, 1H), 2.15 (s, 1H), 2.15 (s, 3H), 1.81 (m, 1H), 1.62 (m, 3H), 1.50 (s, 3H), 1.13 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 201.65, 179.50, 171.54, 170.78, 169.98, 147.88, 147.17, 134.31, 112.10, 74.98, 71.65, 63.85, 53.59, 52.01, 51.31, 42.79, 42.09, 38.14, 35.71, 30.73, 20.59, 18.09, 16.45, 15.06. HRMS: M + Na⁺ 483.1631 (calcd), 483.1623 (found).

(2S,4aR,6aR,7R,9S,10aS,10bR)-Methyl 9-Acetoxy-2-(2-cyano-furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzof[isochromene-7-carboxylate (50). To a solution of **49** (30.5 mg, 1.0 equiv) in MeOH (5 mL) was added NH₂OH·HCl (18.4 mg, 4.0 equiv) and pyridine (0.05 mL, 9.3 equiv). The resulting solution was heated to reflux for 5 h and then cooled to room temperature. Solvent was removed in vacuo, the resulting residue was extracted with CH₂Cl₂ (20 mL) and H₂O (10 mL), and the layers were separated. The aqueous layer was extracted with CH₂Cl₂ (2 × 20 mL). The combined organic layers were washed sequentially with 2 N HCl and brine and then dried over Na₂SO₄. The solvent was removed in vacuo, and the resulting residue was placed under an atmosphere of argon and dissolved in dry CH₂Cl₂ (5 mL). To this solution were added TsCl (13.9 mg, 1.1 equiv) and DIPEA (0.03 mL, 2.6 equiv). The reaction was stirred at room temperature overnight before being diluted with CH₂Cl₂ (10 mL) and 6 N HCl (10 mL). The layers were separated, and the aqueous layer was extracted with CH₂Cl₂ (3 × 10 mL). Combined organic layers were washed sequentially with saturated NaHCO₃ and brine and then dried over Na₂SO₄. The solvent was removed in vacuo and the resulting residue was purified by flash column chromatography (45 → 50% EtOAc/pentane) to give **50** (23.8 mg, 79%) as a solid. Mp: 108–114 °C. ¹H NMR (500 MHz, CDCl₃): δ 7.53 (d, *J* = 1.86 Hz, 1H), 6.50 (d, *J* = 1.88 Hz, 1H), 5.61 (dd, *J* = 5.17, 12.04 Hz, 1H), 5.12 (m, 1H), 3.73 (s, 3H), 2.75 (m, 1H), 2.51 (dd, *J* = 5.18, 13.52 Hz, 1H), 2.31 (s, 1H), 2.31 (m, 1H), 2.17 (m, 3H), 2.17 (s, 3H), 1.81 (m, 1H), 1.63 (m, 3H), 1.48 (s, 3H), 1.13 (s, 3H). ¹³C NMR (126 MHz, CDCl₃): δ 201.83, 171.46, 170.01, 169.99, 147.51, 137.13, 123.63, 110.51, 110.45, 75.00, 70.98, 63.72, 53.53, 52.04, 51.50, 43.29, 42.09, 37.99, 35.71, 30.69, 20.57, 18.06, 16.43, 14.99. HRMS: M + Na⁺ 480.1634 (calcd), 480.1626 (found).

Cell Lines and Cell Culture. Chinese hamster ovary cells (CHO-K1) stably expressing the human κ-opioid receptor (accession # AF498922) (KOR-CHO) or the human μ-opioid receptor (accession # NM_009143) (MOR-CHO) were purchased from DiscoverRx Corp. (Fremont, CA) and maintained in F-12 media with 10% fetal bovine serum (Life Technologies, Grand Island, NY), 1% penicillin/streptomycin/L-glutamine (Life Technologies), and 800 μg/mL Geneticin (Life Technologies). Cells were grown at 37 °C and 5% CO₂ in a humidified incubator.

Opioid Receptor Agonist Activity. On day 1, ~80% confluent KOR-CHO or MOR-CHO cells were detached from culture plates using nonenzymatic cell dissociation buffer (Life Technologies) and counted using a hemocytometer. Cells were plated at 10 000 cells/well in 20 μL of Cell Plating Reagent 2 (DiscoverRx) in 384-well tissue culture plates and incubated at 37 °C overnight. On day 2, stock solutions of all compounds were generated by dissolution in 100% DMSO (Alfa Aesar, Ward Hill, MA) to 10 mM. Stock solutions were used to make 10 serial dilutions in 100% DMSO at 100× final compound concentrations, and 100× compound concentrations were diluted in assay buffer [Hank's Buffered Salt Solution (HBSS, Life Technologies) with 10 mM HEPES (Life Technologies)] containing forskolin (DiscoverRx) to yield 5× compound concentrations, 100 μM forskolin, and 5% DMSO in assay buffer. The DiscoverRx HitHunter cAMP Assay was used according to manufacturer's instructions. Briefly, media was removed from cells, and cells were washed with 10 μL of assay buffer. Assay buffer containing antibody reagent (20 μL/well) was added to cells. A 5 μL portion of 5× compound/forskolin solution were added to cells (final concentrations were 1× compound, 20 μM forskolin, and 1% DMSO). Cells were incubated at 37 °C and 5% CO₂ for 30 min, followed by incubation with detection reagents according to manufacturer's instructions at room temperature

protected from light overnight. On day 3, luminescence was quantified using a Synergy 2 plate reader with Gen5 software (BioTek, Winooski, VT). Data were normalized to vehicle and forskolin only control values and analyzed using nonlinear regression with GraphPad Prism 5.0.

Rat Reinstatement Assay. Animals were housed in polycarbonate cages in a temperature and humidity (55% relative humidity, 19–21 °C) controlled animal facility with a 12/12 h cycle with lights on at 0700 h. Food and water were available ad libitum except during experimental sessions. All experimental procedures were reviewed and approved by the Animal Ethics Committee of Victoria University of Wellington. Self-administration protocols were followed as previously described.^{16,62} Briefly, male Sprague–Dawley rats weighing between 300 and 350 g were trained to intravenously self-administer a sterile solution of cocaine HCl (0.5 mg/kg per infusion) in a saline (NaCl, 0.9%) and heparin (3 units/mL) solution (saline-Hep) via an indwelling jugular cannula in operant chambers (Med Associates ENV-001). Rats self-administered cocaine in daily 2 h sessions. Delivery of a cocaine infusion was paired with a light cue above the active lever. Following stable responding on a fixed-ratio 5 (FR5) schedule of reinforcement (<20% variation in lever presses across 3 days), the light stimulus was removed and the cocaine replaced with saline-Hep. Cocaine-seeking behavior was considered extinguished when right-hand (active) lever responses had fallen below 20 presses in a single session, typically between 2 and 3 days. Reinstatement testing consisted of a cocaine prime injection (20 mg/kg ip in saline) immediately prior to being placed into the operant box. Rats self-administered saline-Hep solution during the reinstatement test. All KOR compounds were dissolved in a vehicle of dimethyl sulfoxide (DMSO), polysorbate 80 (Tween 80), and distilled water at a ratio of 2:1:7, respectively, and injected ip 10 min prior to the cocaine prime, at doses of 0.1 mg/kg (36), 0.3 mg/kg (36, 24 and 2), or 1.0 mg/kg (24 and 2). All experiments were performed in the light phase of a 12-h light–dark cycle.

Rat Locomotor Activity. Spontaneous locomotor activity was performed as previously described.⁶⁵ Briefly, open field chambers (Med Associates ENV-520) equipped with photocells were used to measure horizontal and vertical locomotion in male Sprague–Dawley rats (280–430 g). Testing was conducted in the dark between 0900 and 1700 h in the presence of white noise. On the test day, following a 30 min habituation phase, rats were injected with either vehicle (DMSO:Tween 80:distilled water at a ratio of 2:1:7, ip) or KOR agonist and ambulation counts measured for 60 min.

Statistics. One-way repeated measures ANOVA was performed on reinstatement tests followed by Dunnett's multiple comparison test to compare vehicle and treatment groups. Total locomotor activity was analyzed using Student's *t* test. Results were considered significant when $P \leq 0.05$. Statistical tests were performed using Prism GraphPad 5.0.

■ ASSOCIATED CONTENT

● Supporting Information

Supporting data includes ¹H NMR and ¹³C NMR spectra of 10–15, 28, 37–40, 42–44, and 47–50; COSY, HSQC, and HMBC spectra for 43 and 44; HPLC chromatograms for 1–34, 36–45, 49, and 50; and Supplementary Figure 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone (785) 864-3267. Fax: (785) 864-5326. E-mail: prisinza@ku.edu.

Author Contributions

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

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

KOR, κ -opioid receptor; DOR, δ -opioid receptor; MOR, μ -opioid receptor; FR5, fixed ratio 5; ANOVA, analysis of variance.

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