JOURNAL OF NATURAL PRODUCTS

ARTICLE

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Opioid Receptor Probes Derived from Cycloaddition of the Hallucinogen Natural Product Salvinorin A

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Supporting Information

ABSTRACT: As part of our continuing efforts toward more fully understanding the structure−activity relationships of the neoclerodane diterpene salvinorin A, we report the synthesis and biological characterization of unique cycloadducts through [4+2] Diels−Alder cycloaddition. Microwave-assisted methods were developed and successfully employed, aiding in functionalizing the chemically sensitive salvinorin A scaffold. This demonstrates the first reported results for both cycloaddition of the furan ring and functionalization via microwave-assisted methodology of the salvinorin A skeleton. The cycloadducts yielded herein introduce electron-withdrawing substituents and bulky aromatic groups into the C-12 position. Kappa opioid (KOP) receptor space was explored through aromatization of the bent oxanorbornadiene system possessed by the cycloadducts to a planar phenyl ring system. Although dimethyl- and diethylcarboxylate analogues **5** and **6** retain some affinity and selectivity for KOP receptors and are full agonists, their aromatized counterparts **13** and **14** have reduced affinity for KOP receptors. The methods developed herein signify a novel approach toward rapidly probing the structure−activity relationships of furan-containing natural products.

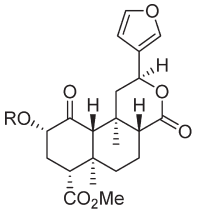
Salvia divinorum Epling & Játiva (Lamiaceae) is a hallucinogenic mint species found in Oaxaca, Mexico, and recently in parts of California. Traditionally, *S. divinorum* has been used by the natives of Oaxaca for their divination ceremonies, along with the treatment of headaches, rheumatism, and abdominal swelling.[1] The main active component of *S. divinorum* has been identified as the neoclerodane diterpene salvinorin A (**1**).[2,3] While many neoclerodane diterpenes have been found to possess biological activity, **1** was found to be a potent hallucinogen in humans with an active dose between 200 and 500 μg.[4] Furthermore, **1** was found to be a full agonist at κ-opioid (KOP) receptors despite having no structural similarity to other known KOP receptor ligands, such as the benzomorphinan cyclazocine or the arylacetamide U50,488.[5] The potential for abuse has led to the regulation of *S. divinorum* and **1** in several U.S. states and a growing number of countries abroad. However, the unique characteristics of **1** have also helped spark significant scientific interest as well. Even though **1** is a potent hallucinogen, it does not have any affinity for the 5-HT$_{2A}$ receptor,[5] which is the target receptor for classical hallucinogens such as LSD, mescaline, and psilocin. Experiments with **1** in rats have demonstrated that **1** can block the locomotor effects of cocaine[6,7] along with having opioid-mediated antinociceptive properties,[8,9] providing evidence for the potential utility of **1** and related analogues as stimulant abuse therapeutics and analgesics. On the basis of the potential therapeutic applications, we and others have begun to investigate the structure−activity relationships (SAR) of **1**.[10−12]

To date, the majority of analogues of **1** prepared and evaluated have explored the role of the C-2 acetoxy group. These investigations have found that the appropriate substituent in this position may alter selectivity for opioid receptors (**2**), as well as increase potency and extend duration of action *in vivo* (**3** and **4**).[13−15] By comparison, the role of the furan ring is less understood. Additional studies are necessary due to the implication of the furan ring in binding at KOP receptors by several modeling studies,[16,17] as well as the potential for furan-containing natural products to be hepatotoxic upon bioactivation by various CYP450 enzymes.[18−23] In an effort to circumvent potential hepatotoxicity and provide further insight into the SAR of **1**, furan-modified analogues were sought.

In the development of additional SAR for the furan ring present in **1**, two approaches were considered. The first approach considered was the incorporation of conformational constraint. This strategy is often a fruitful technique and has been used previously for the interactions of natural products at receptors.[24] In our case this approach is synthetically challenging and would

Received: October 30, 2010
Published: February 21, 2011

dx.doi.org/10.1021/np1007872 | *J. Nat. Prod.* 2011, 74, 718–726



1: R = Ac
2: R = Bz
3: R = MOM
4: R = EOM

require the development of a new or modification of an existing synthesis of **1**;[25,26] thus this approach was not undertaken. The alternative approach followed was to incorporate additional steric bulk directly to the natural product. This approach has been used successfully with opium alkaloids[27] and is particularly informative in cases where the X-ray crystal structure of a given molecular target, such as the KOP receptor, is unavailable. Furthermore, this approach was expected to provide details in a more timely manner.

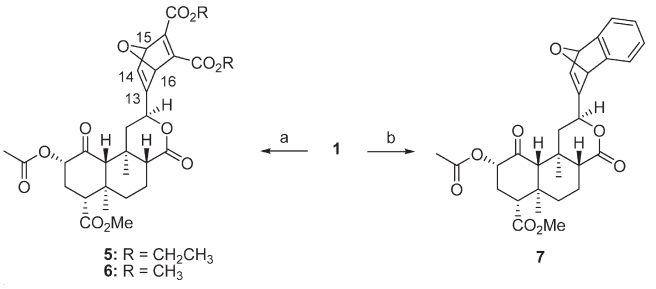
Furan rings have demonstrated themselves as versatile building blocks for the construction of molecules in semi- and total synthesis.[28] One of the more popular reactions that furan rings undergo is the Diels–Alder reaction where furan rings act as the 4π diene. Thus, we sought to explore the binding requirements of the KOP receptor through the incorporation of steric bulk into **1** through the utilization of Diels–Alder reactions. Our results are presented below.

RESULTS AND DISCUSSION

Synthesis. Initially, maleic anhydride and maleimide were reacted with **1** in an attempt to form the corresponding adducts, as both dienophiles have been found in the literature to react readily with furan rings.[28] However, after employing several different solvents (THF, ether, CH_2Cl_2, toluene) no reaction with the dienophiles maleic anhydride and maleimide was noted. Several other well-known dienophiles including benzoquinone, dimethyl maleate, dimethyl fumarate, diethyl maleate, diethyl fumarate, methyl vinyl ketone, di-*tert*-butyl diazo-dicarboxylate, diethyl diazo-dicarboxylate, and dibenzyl diazo dicarboxylate were explored in a variety of reaction conditions. However, none proved successful in generating the desired cycloadducts.

Failure of the cycloaddition reaction to proceed may be attributed to reduced reactivity of the furan ring in **1**. Although a 1999 publication reported a noncatalyzed Diels–Alder cyclization between maleic anhydride and the furan-containing labdane diterpenoid hedychenone,[29] a subsequent detailed study[30] described the necessity for Lewis acid catalysis using $BF_3 \cdot OEt_2$ and high temperatures, conditions that we anticipated would surely result in prominent degradation of **1**.[31] Attempts using $AlCl_3$ and $TiCl_4$ to further activate the dienophiles still failed to produce cyclization, with degradation seeming to dominate. One possible explanation for this may be the presence of multiple oxygen moieties in **1** competing for coordination with the Lewis acid, resulting in degradation. It was thought that while $HfCl_4$ is not as reactive as other Lewis acids,[32] it may be less likely to coordinate with the oxygens of **1**, thus circumventing the issues seen with $AlCl_3$; however, $HfCl_4$ also failed to catalyze cycloadduct formation. The reason that **1** failed to cyclize under the Lewis acid catalyzed conditions required to induce cyclization in hedychenone could very well be due to the considerable difference in oxygen functionality between these highly structurally dissimilar secondary metabolites.

Scheme 1[a]



5: R = CH_2CH_3
6: R = CH_3

7

[a] Reagents and conditions: (a) Appropriate alkyne, toluene, reflux; (b) 2-(trimethylsilyl)phenyl trifluoromethane sulfonate, CsF, CH_3CN, room temperature.

With the failure of the alkene-type dienophiles, alkynes were explored, as they have been reported to successfully form cycloadducts with furan rings.[33] Additionally, an advantage to using alkynes in the Diels–Alder reaction is the inability to form additional stereoisomers with respect to the substituents on the existing dienophile (*exo* vs *endo*). The reaction of diethyl acetylenedicarboxylate with **1** at reflux over the course of two days produced cycloadduct **5** as the major product (Scheme 1). While Diels–Alder reactions have been employed for the total synthesis of neoclerodane diterpenes, to our knowledge, this is the first example of a neoclerodane diterpene undergoing chemical modification via the Diels–Alder reaction.

Investigation of the NMR spectra illustrated that the ^1H proton signals, which correspond to C-15 (dd at δ_H 7.39) and H-16 (multiplet at δ_H 7.41) and ^{13}C signals for C-15 (δ_C 143.7) and C-16 (δ_C 139.4) of **1**, were no longer present in cycloadduct **5** (Scheme 1). Signals for the ethoxy groups were readily apparent from the six-proton multiplet at δ_H 1.32 and four-proton multiplet at δ_H 4.28. Additionally, two new signals representing bridgehead protons were found at δ_H 5.66 (dd) and 5.60 (doublet) along with their corresponding ^{13}C shifts at δ_C 85.55 and 85.46. The remaining signals in the ^{13}C spectra were found to be indicative of two carbonyls and four sp^2 carbons, which fit the expected shifts of the newly introduced α,β-unsaturated esters via the Diels–Alder mechanism. These data are consistent with formation of a new oxanorbornadiene ring system in **5**. The regiochemistry of the oxygen bridge is dependent on the facial orientation between the alkyne and the furan ring of **1** (Figure 1). The production of diastereomers is possible, and while HPLC analysis shows a single peak, we are unable to determine the relative configuration at this time. However, additional efforts toward this goal are underway and will be reported in due course.

When reacted under similar conditions, dimethyl acetylene dicarboxylate and **1** also successfully form cycloadduct **6**. There remained a caveat with the formation of cycloadducts **5** and **6**, specifically that prolonged exposure to heat would cause the cycloadduct to undergo the retro Diels–Alder reaction. This was proven by heating the pure compound **5** in fresh toluene, which yielded a distribution of **5** and **1** upon isolation. Interestingly, retro Diels–Alder reactions did not result in the salvinorin-alkyne and 3,4-substituted furan ring, as might have been predicted; rather, the retro [4+2] resulted in a return of **1** and

dx.doi.org/10.1021/np1007872 |*J. Nat. Prod.* 2011, 74, 718–726

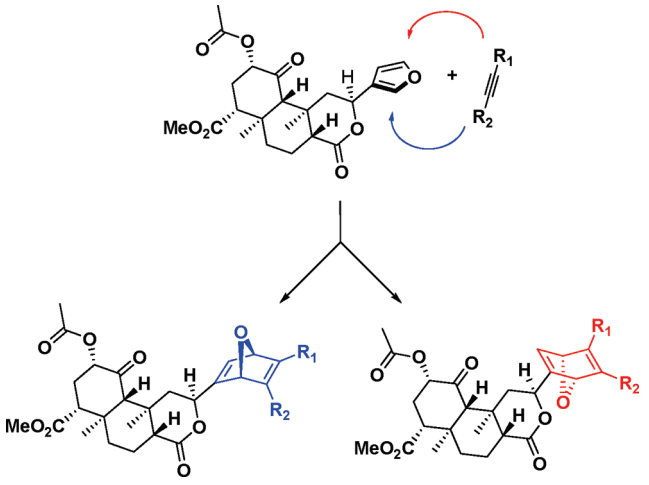


Figure 1. Formation of oxanorbornadiene analogues.

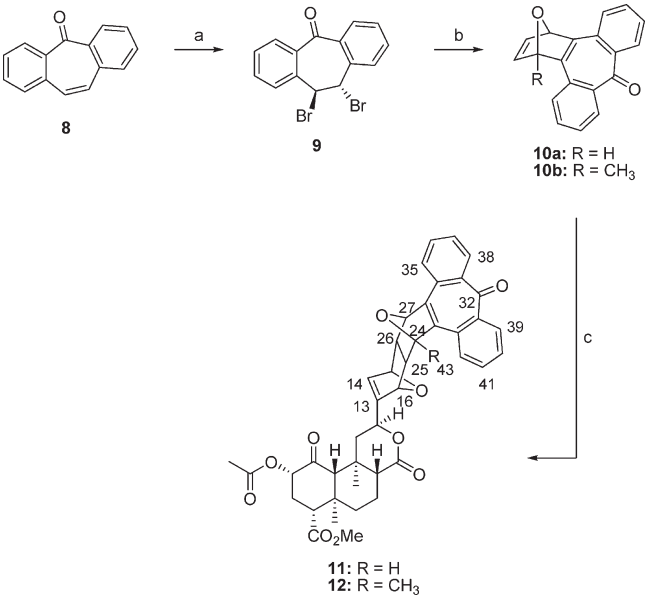
Table 1. Microwave Reaction Conditions for the Synthesis of Cycloadducts 5 and 6

time (min)	absorbance	temp (°C)	solvent	product	yield %
30	normal	50	dioxane	**5** or **6**	21
60	normal	50	dioxane	**5** or **6**	23
30	low	100	dioxane	**5** or **6**	62
60	low	100	dioxane	**5** or **6**	64
30	normal	100	dioxane	**5** or **6**	70
60	normal	100	dioxane	**5** or **6**	70
30	normal	50	toluene	**5** or **6**	24/15
60	normal	50	toluene	**5** or **6**	30
30	low	100	toluene	**5** or **6**	55
60	low	100	toluene	**5** or **6**	55
30	normal	100	toluene	**5** or **6**	67
60	normal	100	toluene	**5** or **6**	67

the starting alkyne. With the prolonged times needed for cycloadduct formation to occur, it was thought that this phenomenon may be affecting overall yields. In an effort to avoid this retro reaction and to optimize Diels–Alder reactions with **1** and alkynes, microwave irradiation was utilized. Several solvents were screened for reaction in the microwave based on their ability to solubilize reagents and polarity, including toluene, xylene, benzene, and dioxane. While various times and absorbance levels were explored, optimal conditions were found to involve either the use of dry and degassed toluene or dioxane at 100 °C for 30 min (Table 1).

With optimal microwave conditions found, several other alkynes were reacted with **1** in an attempt to form cycloadducts including methyl propiolate, methyl 2-butynoate, methyl phenyl propiolate, and acetylene dicarboxylate. Additionally, the alkene-type dienophiles (maleic anhydride, maleimide, diazo dicarboxylates, benzoquinone, dimethyl maleate, dimethyl fumarate, diethyl maleate, diethyl fumarate) were reacted with **1** under the defined microwave conditions; unfortunately, no cycloadducts incorporating the desired moieties were observed. Failure of the alkynes to form cycloadducts may be due to the presence of only one electron-withdrawing group. Diethyl and dimethyl acetylenedicarboxylate have their sp carbons flanked by electron-withdrawing groups, making the alkyne dienophile increasingly electron poor, which may contribute to its ability to overcome

Scheme 2[a]



[a] Reagents and conditions: (a) Br_2, CH_2Cl_2; (b) KO*t*-Bu, THF, furan or 2-methyl furan; (c) **1**, toluene, reflux.

the reduced activity of the furan ring of **1**, allowing cycloadduct formation to occur.

To further explore the reactivity of the furan ring in **1**, its reaction with benzyne was investigated. There are several studies that have demonstrated that benzyne can be effectively trapped by furan rings to form the corresponding cycloadduct.[34−36] Initial attempts to form the benzyne *in situ* using anthranilic acid and isoamyl nitrite and trap it with the furan of **1** were unsuccessful. However, it was found that when **1** was treated with benzyne generated from 2-(trimethylsilyl)phenyl trifluoromethanesulfonate and cesium fluoride in acetonitrile at room temperature, cycloadduct **7** was formed in 24% overall yield (Scheme 1).[35] The structure was confirmed through HRESIMS along with NMR experiments including ^{1}H NMR, ^{13}C NMR, and ^{135}DEPT. As with compounds **5** and **6**, the relative configuration of the newly formed oxygen bridge in **7** has not been determined.

While the furan of **1** can participate in Diels–Alder reactions, it seems to react optimally only with very reactive/electron-poor dienophiles, which allows for proper orbital overlap. Potentially the linearity of the alkynes is allowing for the required p-orbital overlap to overcome the activation energy required for reaction, whereas the alkenes would be hindered from the proper overlap due to the positioning over the salvinorin core for the favored *endo* conformation to form. To further investigate this phenomenon, we investigated the reaction of **1** with 3,6-epoxy-3,6-dihydrotribenzocycloheptatrienone (**10a**).[37,38] Trienone **10a** and similar compounds have been shown to readily react in Diels–Alder reaction with furans with the trienone serving as the dienophile (Scheme 2). The added advantage of selecting these compounds is that they exhibit mild blue light-emitting effects and are direct precursors to a recently described class of fluorescent ligands.[39] In addition to serving as dienophiles that would add steric bulk to **1**, they may also provide new avenues toward fluorescent neoclerodanes, potentially helping elucidate how **1** interacts at opioid receptors.

dx.doi.org/10.1021/np1007872 |*J. Nat. Prod.* 2011, 74, 718–726

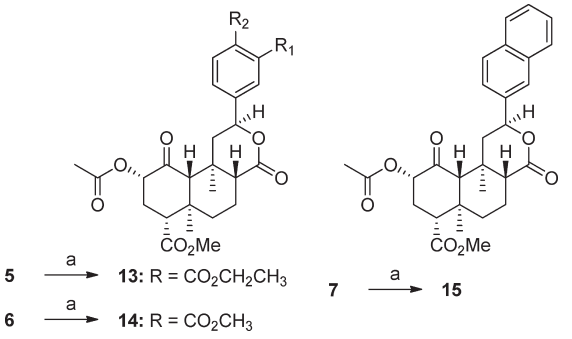
The reaction of dibenzosuberenone (**8**) with Br_2 in CH_2Cl_2 at 0 °C yielded the dibromo intermediate (**9**), which was treated with KO*t*-Bu and either furan or 2-methylfuran in THF to produce compounds **10a** and **10b** (Scheme 2). Reaction of **1** with **10a** under standard reflux conditions yielded cycloadduct **11** as a mixture of C-15/C-16 *exo* and *endo* isomers. The suggestion of a mixture of *endo* and *exo* isomers was corroborated by the presence of a doubling of signals in both the ^{1}H NMR and ^{13}C NMR spectra as well as the corresponding mass of cycloadduct **11** observed by HRESIMS. While cycloadduct **11** was obtained as a mixture of *exo* and *endo* products, cycloadduct **12** was obtained as a single compound as seen from the lack of signal doubling in the ^{1}H NMR and ^{13}C NMR spectra. HRESIMS analysis corresponded to a molecular formula that matched the predicted formula of **12**.

To determine the location of the methyl group and if the cycloadduct was *endo* or *exo* regarding its orientation, extensive NMR experiments were conducted. The first step was to determine whether C-43 was proximal or distal (*cis* or *trans*) to the core of **1**. HMBC experiments were able to show ^{1}H and ^{13}C couplings to trace the carbon framework of **12**, showing that C-43 is in fact proximal or *cis* to the salvinorin core (Figure S4). This also helped to establish the carbon framework of **12**, which was found to be in agreement with the proposed product of the Diels–Alder reaction. With regard to the assignment of *endo* or *exo* configuration, it was anticipated that steric effects would force cycloaddition to occur through an *endo,exo* approach. As described previously,[40] an *endo,exo* approach eliminates unfavorable steric interactions between dienophile and diene, resulting in formation of a single product. This would cause **10b** to approach from the face of the furan opposite the tricyclic core of **1**, as depicted by the red arrow in Figure 1, causing the methyl group to be oriented as shown in Scheme 2. Although we were unable to verify this with a crystal structure, analysis of the $^3J_{H,H}$ coupling values (for details, see the Supporting Information) seen in the ^{1}H NMR spectrum of **12** supports the hypothesis that the final product exhibits an *endo,exo* configuration. The results seen with the reactions of **10a** and **10b** with **1** appear to be in agreement with those described previously,[40] wherein the inclusion of alkyl substituents at the bridgehead carbons caused a shift in *endo/exo* preference (as seen in **12**), with nonsubstituted reagents showing no selectivity (as seen in **11**).

The synthesis of cycloadducts **5**–**7** not only provided analogues that introduced steric bulk at the furan position of **1**, but also provided useful synthetic intermediates. Treatment of compound **5** with $Fe_2(CO)_9$ caused a reductive elimination of water and consequent aromatization to produce compound **13** (Scheme 3).[41] Analysis of HRESIMS data verified the expected mass of the new compound, and examination of ^{1}H and ^{13}C spectra verified that the oxygen bridge present in **5** was indeed absent. Furthermore, new aromatic signals were observed in both the ^{1}H and ^{13}C spectra that were in good agreement with the proposed new structure. This reaction also proved successful in transforming compounds **6** and **7** to their corresponding aromatic rings at the C-12 position, **14** and **15**, respectively. These compounds are significant from a synthetic standpoint, as they are the first reported examples of non-heterocyclic aromatic rings directly attached to the core of neoclerodane diterpenes.

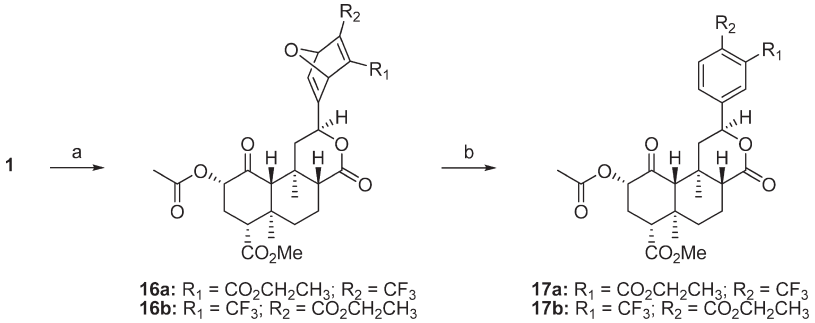
Finally, we sought to explore the reactivity of the furan ring with a nonsymmetrical alkyne. The reaction of ethyl 4,4,4-trifluoro-2-butynoate with **1** at 95 °C gave a mixture of **16a** and **16b** (Scheme 4) in a 91:9 ratio, as determined by analytical HPLC. While our initial predictions assumed a mixture of regioisomers would form, this hypothesis was realized upon careful examination of multiple NMR spectra, including HSQC, DEPT135, and COSY NMR. Particularly, there was a doubling of signals realized for the sp^2-hybridized methine at C-14, which appeared as a doublet of doublets instead of an anticipated doublet. These signals were coupled with two carbon signals at δ_C 84.42 and 85.01, which corresponds to the western bridgehead carbon (C-15). Additionally, simultaneous $^3J_{CF}$ coupling was seen with both bridgehead carbons C-15 and C-16 in the fluorine-coupled carbon spectrum, which suggests that the CF_3 group was attached to both C-26 and C-27 at the same time. This could be explained by the sample containing a mixture of regioisomers **16a** and **16b**. Our attempts to separate **16a** and **16b** on a preparative scale

Scheme 3[a]



[a] Reagents and conditions: (a) $Fe_2(CO)_9$, toluene, reflux.

Scheme 4[a]



[a] Reagents and conditions: (a) ethyl 4,4,4-trifluoro-2-butynoate, toluene, reflux; (b) $Fe_2(CO)_9$, toluene, reflux.

dx.doi.org/10.1021/np1007872 |*J. Nat. Prod.* 2011, 74, 718–726

using column chromatography under various solvent conditions were unsuccessful, meaning that the unambiguous determination of **16a** and **16b** was not possible. The preference to form cycloadduct **16a** over **16b** can potentially be explained by the difference in electron-withdrawing groups that flank the alkyne. We propose that the stronger electron-withdrawing CF_3 group promotes a more electron-rich end to the alkyne that pairs to C-15 due to the inductive push of electron density to C-16 from the core of **1**.

Given our success with converting oxanorbornadienes to the corresponding phenyl rings, we decided to convert the mixture of **16a** and **16b** to their corresponding phenyl derivatives. It was envisioned that a mixture of the phenyl derivatives would be more easily separated than the mixture of oxanorbornadienes. As expected, the treatment of the mixture of **16a** and **16b** with $Fe_2(CO)_9$ in toluene gave the corresponding phenyl rings. To our delight, **17a** and **17b** (Scheme 4) were isolated in 62% and 4% yield, respectively. The structure of **17a** was elucidated in a fashion similar to **16a** and **16b** by first identifying the three aromatic protons at δ 7.72, 7.69, and 7.50, which all correlate to aromatic carbons in the HSQC. Additionally the two protons at δ 7.72 and 7.50 are correlated to each other in the COSY spectrum, which would represent C-14 and C-15 (Figure 2). Further analysis of the COSY spectrum showed that the proton signal at δ 7.69 was not correlated to any other protons, indicating that it was attached to the C-16 position. Examination of the HMBC spectrum showed that the proton signal at δ 7.72 was correlated to C-25, C-27, and C-13, while the proton signal at δ 7.69 was correlated to C-28, C-26, and C-12. This would suggest that the COSY uncoupled proton signal from δ 7.69 is attached to C-16 and the COSY coupled proton signal from δ 7.72 is attached to C-15. The structure of **17b** was elucidated in a similar manner to **17a**. The success of our methodology suggests that this approach may be applicable to other furan ring containing natural products in efforts to rapidly explore their SAR.

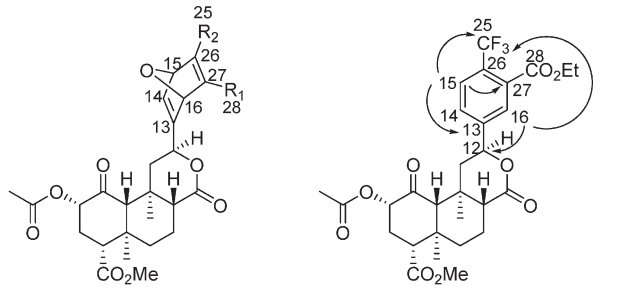


Figure 2. Partial numbering of compounds **16a**, **16b**, and **17a** with selected HMBC correlations.

Biological Results. The synthesized compounds were evaluated for affinity and efficacy at opioid receptors.[42] It was the thought that the cycloadducts prepared would give greater insight into the position of the oxygen atom and its ability to participate in hydrogen bonding. Additionally, these compounds would provide some measure of the amount of steric bulk that was tolerated around the furan ring. Cycloadduct **6** had decreased but still appreciable affinity for KOP receptors in comparison to **1** (K_i = 60 nM vs K_i = 7.4 nM) (Table 2). The diethyl analogue **6** (**5**) saw a decrease in affinity at KOP receptors when compared to its methyl counterpart, **6** (K_i = 120 nM vs K_i = 60 nM). While affinity for KOP receptors was diminished in **5**, affinity for delta opioid (DOP) receptors increased over 4-fold in comparison to **1** (K_i = 2,260 nM vs K_i > 10 000 nM). As mentioned above, we were unable to assign the relative configuration of the oxygen of the oxanorbornadiene in **5** and **6**. It is therefore possible that one regioisomer of the oxanorbornadiene is preferred for interacting with opioid receptors. However, additional synthesis and biological testing will be required to test this hypothesis.

Cycloadduct **7** also explored the role of steric bulk in activity at opioid receptors along with increasing the lipophilic/hydrophobic characteristics of **1** through the introduction of a benzene ring instead of esters, allowing us to investigate how these characteristics factor into activity at opioid receptors. Cycloadduct **7** was found to have decreased affinity at KOP receptors (K_i = 790 nM) in comparison to **1**. This decreased affinity may be the result of **7** not having the ability to form hydrogen bonds like its ester-containing cycloadduct counterparts and/or the regiochemistry of the oxanorbornadiene is not optimal. The installation of substituents that may partake in hydrogen bonding on the benzene ring of **7** may improve affinity at opioid receptors and warrants further investigation.

Table 2. Binding Affinities of Cycloadduct Analogues[a]

	$K_i \pm$ SD (nM)				
compound	MOP	DOP	KOP	MOP/KOP	DOP/KOP
1	EC_{50} = 2860 ± 980[b] E_{max} = 75 ± 8%	>10 000	7.4 ± 0.7	N.D.[c]	>1351
5	>3200	2260 ± 280	120 ± 10	>25	19
6	>4800	>5000	60 ± 10	>80	>83
7	>2500	>10 000	790 ± 200	>3	>13
11	>2700	>5200	>13 000	>0.2	>17
12	>1700	>5000	290 ± 20	>6	>17
13	>3000	>5000	228 ± 12	>13	>22
14	>3000	>5000	286 ± 19	>8	>17
15	>3000	>5000	>8000	>0.4	>0.6
16a/16b	1670 ± 150	>5000	1970 ± 80	0.85	>2

[a] [^{3}H]DAMGO was used for MOP, [^{3}H]DADLE for DOP, and [^{3}H]U69,593 for KOP receptors. [b] A more complete analysis provided this value, which corrects our previously reported value.[12] [c] Not determined.

dx.doi.org/10.1021/np1007872 |J. Nat. Prod. 2011, 74, 718–726

Table 3. [^{35}S]GTP-γ-S Activity Assay of Cycloadducts

compound	KOP ED_{50}[a] ± SD, nM	KOP E_{max}[b] ± SD, nM
1	40 ± 10	120 ± 2
5	2150 ± 500	90 ± 10
6	980 ± 200	100 ± 10

[a] ED_{50} = effective dose for 50% maximal response. [b] E_{max} is % at which compound stimulates in comparison to (−)-U50,488 (500 nM) at KOP receptors.

Further probing how steric bulk affects the interactions of **1** at opioid receptors, the incorporation of large appendages to the core structure of **1** resulted in the synthesis of **11** and **12**. Given that the core structures of **11** and **12** had fluorescent properties, it was the hope that these analogues of **1** could be used as potential visualizing agents for opioid receptors, along with exploring the effect of increased steric bulk. Unfortunately, cycloadduct **11** had no affinity at KOP receptors, as it had a K_i of >13 000 nM and no appreciable affinity for mu opioid (MOP) or DOP receptors as well. Interestingly, the introduction of a methyl group into the bridgehead of **11** (**12**) increased affinity for KOP receptors 44-fold (K_i = 290 nM vs K_i > 13 000 nM). The rationale behind this observed result is not immediately apparent but may be attributed to **11** being tested as a mixture of *endo* and *exo* isomers, while **12** was tested as a single isomer. Further studies are being explored to evaluate this hypothesis.

The phenyl ring analogues were also evaluated to explore steric bulk and the effects of non-heterocyclic aromatics at the C-12 position. The napthyl derivative of **7** (**15**) had no affinity at opioid receptors (K_i > 10 000 nM), indicating that the lack of any groups that may hydrogen bond is detrimental for affinity at opioid receptors. This possible explanation seems to be corroborated by phenyl derivatives of **5** (**13**) and **6** (**14**), as they retained some affinity for KOP receptors though less than **1** (**14**: K_i = 228 nM; **15**: K_i = 286 nM vs K_i = 7.4 nM).

Along with the bridgehead oxygen, it was thought that the carbonyls of the additional esters might be hydrogen bonding at opioid receptors. Investigation of this hypothesis was possible with **16**, where one of the ethyl esters of **5** was replaced with a CF_3 group. Introduction of the CF_3 group led to a 16-fold decrease in affinity at KOP receptors in comparison to **5** (K_i = 1970 nM vs 120 nM). This suggested that an additional carbonyl in this position assists in binding; however, a clear explanation has yet to be determined, which is in part due to **16** being tested as a mixture of regioisomers. Evaluation of each individual regioisomer will be necessary to further investigate the impact of the carbonyl moiety as well as the position of the CF_3. This will aid in the investigation of our initial hypothesis pertaining to the hydrogen-bonding capabilities of the esters and their role in binding at opioid receptors.

With cycloadducts **5** and **6** having relatively high affinity for KOP receptors ($K_i \leq$ 150 nM), they were evaluated for efficacy at opioid receptors in the [^{35}S]GTP-γ-S assay. Cycloadduct **6** was found to be a full agonist at KOP receptors compared to (−)-U50,488 (E_{max} = 100 ± 10) (Table 3). Cycloadduct **5** was also found to have high efficacy in this assay (E_{max} = 90); however both compounds were less potent than **1** (**6** ED_{50} = 980 nM vs **5** ED_{50} = 2150 vs ED_{50} = 40 nM). These compounds show that extension of the cycloadduct esters has more of an effect on potency than overall efficacy, and both compounds further illustrate that the furan ring **1** of is not essential for binding or efficacy at opioid receptors and some steric bulk is tolerated at this position.

In conclusion, a series of Diels−Alder cycloadduct analogues of salvinorin A were synthesized in an effort to explore the effects of steric bulk and the position of the oxygen ring on binding and activity at opioid receptors. Microwave conditions were optimized to further enhance the amenability of the furan ring in salvinorin A to serve as a 4π diene in the [4+2] Diels−Alder reaction. This work signifies the first reported example of the Diels−Alder reaction to modify a neoclerodane diterpene. Furthermore, several of the cycloadduct analogues were themselves useful as synthetic intermediates, as they were able to undergo reductive elimination to produce their phenyl ring counterparts. *In vitro* evaluation found that steric bulk around the furan ring of salvinorin A seems to be tolerated for binding at the KOP receptor, as cycloadducts **5**, **6**, and **12** maintained appreciable affinity. Although all compounds explored had reduced affinity relative to salvinorin A, this indicates that the furan ring is not a stringent requirement for KOP receptor affinity, as previously believed. Additionally, cycloadducts **5** and **6** were found to be full agonists at KOP receptors as compared to the known KOP agonist U50,488. Furthermore, phenyl derivatives **13** and **14** maintained modest affinity at KOP receptors, which further indicates that the furan ring is not essential for affinity at opioid receptors. Further investigation into the role of steric bulk at the furan ring will yield valuable information into the nature of how **1** interacts at opioid receptors. This information can then be used to aid in the development of opioid therapeutics with enhanced pharmacological properties.

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on a Thomas-Hoover capillary melting apparatus. NMR spectra were recorded on either a Bruker Advance-300 spectrometer, a Bruker DRX-400 with qnp probe, or a Bruker AV-500 with cryoprobe using δ values in ppm (TMS as internal standard) and J (Hz) assignments of ^{1}H resonance coupling. High-resolution mass spectrometry data were collected on either a LCT Premier (Waters Corp.) time-of-flight mass spectrometer or an Agilent 6890 N gas chromatograph in conjunction with a Quattro Micro GC mass spectrometer (Micromass Ltd.). Thin-layer chromatography (TLC) was performed on 0.25 mm Analtech GHLF silica gel plates using EtOAc/*n*-hexanes, in 1:1 ratio, as the solvent system unless otherwise noted. Spots on TLC were visualized by UV (254 or 365 nm), phosphomolybdic acid in EtOH, or vanillin/H_2SO_4 in EtOH. Column chromatography was performed with silica gel (32−63 μm particle size) from MP Biomedicals. 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 mm) with isocratic elution in 60% CH_3CN/40% H_2O at a flow rate of 5.0 mL/min unless otherwise noted. The systematic name for salvinorin A (**1**) is (2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate. Salvinorin A was isolated from *S. divinorum* as previously described.[43]

Diethyl 5-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (5). A solution of **1** (200 mg, 0.462 mmol), diethyl acetylene dicarboxylate (85 mg, 0.500 mmol), and toluene (20 mL) was allowed to stir at room temperature and gradually heated to reflux over 45 min. The solution was heated at reflux for two days. The solvent was removed under reduced pressure, and the resulting residue was purified by column chromatography

dx.doi.org/10.1021/np1007872 |*J. Nat. Prod.* 2011, 74, 718–726

(eluent: EtOAc/*n*-hexanes, 2:3) to afford 262 mg of **5** (70%) as a white powder: mp 84 − 86 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (1H, ddd, J = 1.9, 3.6 Hz), 5.66 (1H, t, J = 1.8 Hz), 5.60 (1H, d, J = 1.6 Hz), 5.39 (1H, dd, J = 3.8, 11.7 Hz), 5.29 (1H, d, J = 7.7 Hz), 5.22−5.08 (2H, m), 4.33−4.23 (4H, m), 3.73 (3H, s), 2.78−2.72 (1H, m), 2.43 (1H, d, J = 5.6 Hz), 2.41 (1H, d, J = 5.5 Hz), 2.31 (2H, dd, J = 3.2, 11.0 Hz), 2.17 (4H, d, J = 3.1 Hz), 1.80−1.75 (1H, m), 1.50 (2H, dd, J = 11.3, 23.9 Hz), 1.40 (3H, d, J = 1.6 Hz), 1.32 (6H, ddd, J = 2.8, 6.3, 10.1 Hz), 1.10 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 171.6, 170.7, 169.9, 163.1, 157.8, 152.8, 151.9, 138.0, 136.4, 85.6, 85.3, 74.9, 73.9, 64.1, 61.8, 61.6, 53.5, 52.0, 51.2, 42.0, 41.2, 40.3, 38.0, 35.4, 30.8, 20.6, 18.1, 16.3, 15.3, 14.1; HRESIMS m/z 625.2203 [M + Na] (calcd for $C_{31}H_{38}O_{12}Na$, 625.2261); HPLC t_R = 6.802 min; purity = 96%.

Dimethyl 5-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (6). *Method A.* Compound **6** was prepared from **1** and dimethyl acetylene dicarboxylate using a similar procedure to that for **5** to afford 152 mg (70%) as a white solid.

Method B. A solution of **1** (100 mg, 0.23 mmol), dimethyl acetylene dicarboxylate (35 mg, 0.25 mmol), and toluene (25 mL) was placed in a sealed 25 mL quartz tube and irradiated in a microwave reactor (Biotage Initiator) at 100 °C for 30 min with normal absorbance levels. Solvent was removed under reduced pressure, and the resulting residue was purified using column chromatography (EtOAc/*n*-hexanes, 2:3) to afford 69 mg (63%) as a white solid: mp 107−110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, d, J = 12.2 Hz), 5.71 (1H, d, J = 1.6 Hz), 5.67 (1H, s), 5.63 (1H, d, J = 1.6 Hz), 5.40−5.35 (1H, m), 5.32−5.26 (1H, m), 3.85 (3H, s), 3.82 (3H, d, J = 1.4 Hz), 3.73 (3H, s), 2.74 (1H, s), 2.42 (1H, s), 2.31 (2H, d, J = 7.4 Hz), 2.17 (4H, d, J = 3.7 Hz), 2.05 (2H, s), 1.80−1.75 (1H, m), 1.47 (2H, s), 1.40 (3H, s), 1.10 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.8, 171.5, 170.6, 170.0, 162.8, 157.8, 137.9, 136.5, 84.3, 84.2, 84.1, 73.6, 72.5, 71.7, 62.7, 62.7, 52.2, 51.4, 51.2, 50.8, 49.7, 40.7, 39.8, 36.7, 34.1, 19.4, 16.8, 15.1, 14.0; HRESIMS m/z 597.1921 [M + Na] (calcd for $C_{29}H_{34}O_{12}Na$, 597.1948); HPLC t_R = 4.757 min; purity = 97%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(7-benzooxabicyclo[2.2.1]hepta-2,5-dien-2-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (7). To a solution of **1** (150 mg, 0.35 mmol), 2-(trimethylsilyl)-phenyl trifluoromethanesulfonate (310.6 mg, 1.04 mmol), and CH_3CN (20 mL) was added CsF (319 mg, 2.1 mmol), and the solution was allowed to stir at room temperature overnight. Upon completion, the reaction was diluted with H_2O (25 mL) and ether (25 mL). The aqueous layer was extracted with ether and dried (Na_2SO_4). The compound was purified by column chromatography (eluent: EtOAc/*n*-hexanes, 1:1) to afford 42.4 mg of **7** (24%) as a white powder: mp 242 − 245 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.31 (1H, m), 7.25−7.22 (1H, m), 7.01 (2H, dd, J = 3.0, 5.1 Hz), 6.69 (1H, t, J = 2.0 Hz), 5.72 (2H, d, J = 11.9 Hz), 5.17−5.09 (2H, m), 3.72 (3H, s), 2.73−2.68 (1H, m), 2.37−2.33 (1H, m), 2.31−2.26 (2H, m), 2.17 (3H, s), 2.11 (1H, s), 1.95 (1H, d, J = 11.9 Hz), 1.76 (1H, d, J = 13.4 Hz), 1.66−1.58 (1H, m), 1.53−1.45 (2H, m), 1.37 (3H, s), 1.31 (1H, s), 1.07 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.7, 169.1, 168.5, 167.6, 153.9, 146.2, 145.7, 134.1, 123.1, 122.9, 118.3, 117.8, 80.5, 79.8, 72.7, 71.4, 61.8, 51.1, 49.6, 48.5, 39.8, 37.6, 35.6, 32.8, 28.3, 18.2, 15.6, 13.9, 13.2; HRESIMS m/z 531.1961 [M + Na] (calcd for $C_{29}H_{32}O_8Na$, 531.1995); HPLC t_R = 8.260 min; purity = 98%.

3,6-Epoxy-3-methyl-3,6-dihydrotribenzocycloheptatrienone (10b). A solution of dibenzosuberenone (500 mg, 2.4 mmol) and Br_2 (774 mg, 4.8 mmol) in CH_2Cl_2 (100 mL) was allowed to stir at −30 °C for 2 h, during which time a precipitate was formed. The precipitate was collected by filtration and placed in a sealed tube containing THF (100 mL). The resulting suspension was cooled to 0 °C and then treated with KO*t*-Bu (815 mg, 7.3 mmol) and 2-methylfuran (200 mg, 2.4 mmol). TLC indicated that the reaction was complete after approximately 3 h. The solvent was removed under reduced pressure, and CH_3OH was added to the residue, resulting in an off-white precipitate. The precipitate was filtered to afford 416 mg of **8b** (60%) as an off-white powder: mp 130−132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (2H, ddd, J = 1.1, 4.6, 7.9 Hz), 7.84−7.76 (2H, m), 7.73−7.63 (3H, m), 7.60−7.53 (2H, m), 7.43 (1H, s), 6.02 (1H, d, J = 1.8 Hz), 2.22 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.0, 149.8, 148.4, 144.1, 142.0, 138.1, 137.0, 130.3, 130.2, 130.2, 129.4, 128.3, 128.0, 127.5, 127.1, 121.8, 121.6, 92.5, 82.5, 27.7; HRESIMS m/z 287.1055 [M + H] (calcd for $C_{20}H_{15}O_2$, 287.1072); HPLC t_R = 32.15 min; purity = 98%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(8,8a,9,12,12a,13-hexahydro-8,13:9,12-diepoxy-5*H*-dibenzo[3,4:6,7]-cyclohept[1,2]naphthanen-5-on-10-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (11). Compound **11** was synthesized as described for **5** from **1** using **8a**[38] to afford 43.2 mg of the *exo* and *endo* isomers of **11** (32%) as a white powder: mp 211−214 °C; ^{1}H NMR (500 NMR, $CDCl_3$) δ 8.12 (5H, ddd, J = 1.2, 6.8, 12.3 Hz), 7.74−7.64 (6H, m), 7.55 (4H, d, J = 7.5 Hz), 7.51−7.44 (3H, m), 7.40 (3H, d, J = 7.9 Hz), 6.18 (1H, d, J = 2.1 Hz), 6.11 (2H, t, J = 2.0 Hz), 5.32 (2H, s), 5.23 − 5.21 (3H, m), 5.14 (2H, s), 3.73 (5H, s), 3.69 (3H, s), 3.06−2.97 (5H, m), 2.82−2.57 (7H, m), 2.31 (5H, dd, J = 7.5, 14.8 Hz), 2.20 (4H, d, J = 2.5 Hz), 2.18 (4H, s), 2.14−2.08 (3H, m), 2.05−2.00 (2H, m), 1.45 (3H, s), 1.43 (4H, s), 1.11 (2H, s), 1.10 (2H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.9, 199.6, 191.7, 191.6, 169.20, 169.19, 168.9, 168.7, 167.7, 145.9, 145.6, 142.72, 142.69, 142.2, 136.2 (2C), 136.04, 136.01, 129.7 (2C), 129.6, 129.44, 129.38, 128.24, 128.22, 128.1, 128.0, 127.7, 127.6(2C), 126.72, 126.70, 126.65, 126.6, 125.6, 125.2, 122.7, 122.6, 122.3, 122.2, 78.5, 78.3, 78.1, 77.7, 77.2, 76.9, 76.5, 74.8, 74.6, 74.4, 72.7, 72.64, 72.60, 70.8, 61.9, 61.6, 51.1, 51.0, 49.61, 49.56, 48.70, 48.69, 47.9, 47.8, 47.4, 47.2, 39.7, 39.6, 39.0, 38.3, 35.7, 35.5, 33.0, 32.7, 28.4, 18.3, 18.2, 15.8, 15.7, 14.0, 13.9, 13.3, 12.9; HRESIMS m/z 727.2549 [M + Na] (calcd for $C_{42}H_{40}O_{10}Na$, 727.2519); HPLC t_R = 5.657 min; purity = >99%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(8,8a,9,12,12a,13-hexahydro-8-methyl-8,13:9,12-diepoxy-5*H*-dibenzo[3,4:6,7]cyclohept[1,2]naphthanen-5-on-10-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (12). Compound **12** was synthesized as described for **5** from **1** using **9b** to afford 14.2 mg of **12** (26%) as a white powder, mp 198−200 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (2H, d, J = 7.8 Hz), 7.65 (1H, dt, J = 1.4, 7.5 Hz), 7.59 (1H, m), 7.52 (3H, d, J = 8.7 Hz), 7.43 (1H, d, J = 7.32 Hz), 6.17 (2H, t, J = 1.9 Hz), 5.21 (3H, m), 5.05 (2H, s), 3.73 (3H, s), 3.17 (3H, m), 2.76 (2H, dd, J = 10.8, 6.1 Hz), 2.63 (1H, dd, 13.3, 5.8 Hz), 2.31 (2H, m), 2.18 (3H, s), 1.81 (1H, s), 1.70 (3H, s), 1.59 (1H, m), 1.42 (3H, s), 1.26 (2H, s), 1.11 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.4, 194.9, 171.6, 170.7, 169.5, 148.0, 147.2, 146.2, 138.8, 131.7, 130.8, 129.8, 129.6, 129.3, 129.1, 129.0, 128.5, 128.4, 124.7, 123.9, 88.8, 79.5, 79.3, 78.5, 77.6, 75.01, 74.98, 64.4, 53.5, 53.0, 52.5, 52.0, 51.0, 42.1, 41.5, 38.1, 35.4, 30.8, 20.6, 18.2, 17.5, 16.3, 15.7; HRESIMS m/z 741.2693 [M + Na] (calcd for $C_{43}H_{42}O_{10}Na$, 741.2676); HPLC t_R = 10.938 min; purity = 99%.

Diethyl 4-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)phthalate (13). A solution of **5** (100 mg, 0.17 mmol), $Fe_2(CO)_9$ (150 mg, 0.41 mmol), and toluene (15 mL) was allowed to stir at 60 °C for 20 min. Once the solution turned black, it was gradually heated to reflux and allowed to stir for 2 h. The solution was filtered through a pad of Celite, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (eluent: 50% EtOAc/50% *n*-hexanes) to afford 55 mg of **13** (70%) as a white powder: mp 110−112 °C; ^{1}H NMR (500 NMR MHz, $CDCl_3$) δ 7.73 (1H, d, J = 8.0 Hz), 7.62 (1H, d, J = 1.8 Hz), 7.43 (1H, dd,

J = 1.7, 8.1 Hz), 5.60 (1H, dd, J = 5.1, 11.9 Hz), 5.12−5.06 (1H, m), 4.40−4.33 (4H, m), 3.73 (3H, s), 2.73 (1H, dd, J = 6.3, 10.5 Hz), 2.55 (1H, dd, J = 5.1, 13.6 Hz), 2.33−2.26 (2H, m), 2.19 (1H, s), 2.16 (3H, s), 2.14 (1H, s), 2.12 (1H, d, J = 3.1 Hz), 1.81 (1H, d, J = 13.2 Hz), 1.72−1.62 (1H, m), 1.58 (1H, s), 1.50 (3H, d, J = 8.0 Hz), 1.47−1.42 (1H, m), 1.37 (6H, q, J = 7.1 Hz), 1.13 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.7, 171.2, 170.6, 169.5, 167.1, 166.7, 143.3, 132.8, 131.4, 129.2, 127.3, 125.3, 77.5, 74.6, 63.5, 61.5, 61.4, 53.2, 51.7, 51.3, 44.8, 41.8, 37.8, 35.5, 30.4, 22.4, 20.2, 17.8, 16.1, 13.80, 13.78; HRESIMS m/z 609.2332 [M + Na] (calcd for $C_{31}H_{38}O_{11}Na$, 609.2314); HPLC t_R = 11.978 min; purity = >99%.

Dimethyl 4-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)phthalate (14). Compound **14** was synthesized as described for **13** from **6** to afford 55.2 mg of **14** (77%) as a white powder: mp 116−119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (1H, d, J = 8.0 Hz), 7.64 (1H, s), 7.45 (1H, d, J = 7.9 Hz), 5.60 (1H, d, J = 6.9 Hz), 5.13−5.06 (1H, m), 3.91 (3H, s), 3.91 (3H, s), 3.73 (3H, s), 2.72 (1H, d, J = 6.3 Hz), 2.55 (1H, d, J = 8.5 Hz), 2.31 (2H, d, J = 9.8 Hz), 2.22 (1H, s), 2.19 (1H, s), 2.16 (3H, s), 2.12 (1H, s), 1.81 (1H, d, J = 13.2 Hz), 1.66 (1H, s), 1.59 (2H, s), 1.51 (3H, s), 1.13 (3H, s); ^{13}C NMR δ 200.1, 169.6, 169.0, 168.0, 165.8, 165.5, 141.9, 130.9, 129.5, 127.6, 126.0, 123.8, 75.9, 73.1, 62.0, 51.7, 50.9, 50.8, 50.1, 49.7, 43.2, 40.2, 36.2, 33.9, 28.8, 18.7, 16.2, 14.5, 13.3; HRESIMS m/z 581.1994 [M + Na] (calcd for $C_{29}H_{34}O_{11}Na$, 581.1999); HPLC t_R = 4.103 min; purity = 96%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-6a,10b-dimethyl-2-(naphthalen-2-yl)-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (15). Compound **15** was synthesized as described for **15** from 7 to afford 41 mg of **15** (70%) as a white powder: mp 240−242 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (3H, dd, J = 7.2, 12.6 Hz), 7.75 (1H, s), 7.51−7.46 (2H, m), 7.38 (1H, d, J = 1.8 Hz), 5.72 (1H, dd, J = 5.2, 11.8 Hz), 5.11−5.06 (1H, m), 3.73 (3H, s), 2.76−2.70 (1H, m), 2.62 (1H, dd, J = 5.3, 13.6 Hz), 2.30 (2H, dd, J = 5.8, 13.1 Hz), 2.22 (3H, s), 2.16 (2H, d, J = 4.2 Hz), 2.15 (3H, s), 1.81 (1H, s), 1.63 (1H, s), 1.55 (3H, s), 1.14 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.2, 169.7, 169.6, 168.1, 135.6, 131.3, 131.2, 126.8, 126.2, 125.9, 124.6, 124.5, 122.6, 121.3, 74.9, 73.2, 62.2, 51.8, 50.2, 49.8, 43.6, 40.3, 36.4, 34.0, 28.9, 27.9, 18.7, 16.4, 14.6; HRESIMS m/z 515.1949 [M + Na] (calcd for $C_{29}H_{32}O_7Na$, 515.1945); HPLC t_R = 9.175 min; purity = >99%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(5-(ethoxycarbonyl)-6-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (16a) and (2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(6-(ethoxycarbonyl)-5-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (16b). Compounds **16a** and **16b** were synthesized as described for **5** from **1** using ethyl 4,4,4-trifluoro-2-butynoate to afford 402 mg of **16a** and **16b** (48%) as a white powder: mp 119−121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, dd, J = 1.9, 3.9 Hz), 5.76 (1H, s), 5.64 (1H, dt, J = 1.8, 5.5 Hz), 5.61 (1H, s), 5.39 (1H, dd, J = 4.7, 11.0 Hz), 5.33−5.27 (1H, m), 5.15 (1H, dd, J = 11.0, 19.9 Hz), 4.35−4.23 (3H, m), 3.73 (4H, s), 2.79−2.72 (1H, m), 2.43 (1H, td, J = 5.4, 13.3 Hz), 2.31 (2H, dd, J = 4.3, 10.6 Hz), 2.17 (5H, d, J = 2.5 Hz), 2.13 (1H, s), 2.09 (1H, d, J = 8.7 Hz), 2.03 (1H, d, J = 12.3 Hz), 1.78 (1H, d, J = 10.1 Hz), 1.61 (5H, dd, J = 12.4, 21.2 Hz), 1.54−1.46 (2H, m), 1.41 (4H, s), 1.37−1.29 (4H, m), 1.11 (4H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 171.54, 171.53, 170.7, 170.4, 170.0, 169.9, 162.0, 161.5, 158.5, 157.9, 151.3 (q, $^3J_{CF}$ = 4.66), 151.0 (q, $^2J_{CF}$ = 35.57), 150.8 (q, $^3J_{CF}$ = 4.87), 137.4, 135.8, 121.5 (q, $^1J_{CF}$ = 269.57), 121.4 (q, $^1J_{CF}$ = 269.63), 85.7 (d, $^3J_{CF}$ = 2.67), 84.5 (d, $^3J_{CF}$ = 2.52), 75.0, 74.9, 73.9, 72.7, 64.0, 64.0, 62.2, 62.1, 53.50, 53.47, 52.0, 51.2, 51.0, 42.04, 41.99, 41.1, 40.4, 38.02, 37.96, 35.5, 35.3, 30.8, 30.7, 20.61, 20.58, 18.12, 18.08, 16.4, 15.3, 15.1, 13.9; HRESIMS m/z 621.1986 [M + Na] (calcd for $C_{29}H_{33}F_3O_{10}Na$, 621.1924); analytical HPLC **16a** t_R = 12.858 min; ratio = 91%; **16b** t_R = 11.759 min; ratio = 9%.

(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(3-(ethoxycarbonyl)-4-(trifluoromethyl)phenyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (17a) and (2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(4-(ethoxycarbonyl)-3-(trifluoromethyl)phenyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (17b). A solution of **16a** and **16b** (200 mg, 0.33 mmol), $Fe_2(CO)_9$ (150 mg, 0.41 mmol), and toluene (15 mL) was allowed to stir at 60 °C for 20 min. Once the solution turned black, it was gradually heated to reflux and allowed to stir for 2 h. The solution was filtered through a pad of Celite, and solvent was removed under reduced pressure. The residue was purified by column chromatography (gradient eluent: 10% EtOAc/90% *n*-hexanes to 30% EtOAc/70% *n*-hexanes) to afford 121 mg of **17a** (62%) and 8.2 mg of **17b** (4.2%).

17a: white powder; mp 110−112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (1H, d, J = 8.2 Hz), 7.69 (1H, s), 7.50 (1H, d, J = 8.0 Hz), 5.62 (1H, dd, J = 5.0, 12.0 Hz), 5.13 − 5.06 (1H, m), 4.40 (2H, q, J = 6.9 Hz), 3.73 (3H, s), 2.75−2.71 (1H, m), 2.56 (1H, dd, J = 5.1, 13.6 Hz), 2.30 (2H, dd, J = 7.8, 13.5 Hz), 2.24−2.18 (1H, m), 2.16 (3H, s), 2.13 (2H, d, J = 8.3 Hz), 1.82 (1H, d, J = 13.2 Hz), 1.66 (2H, dd, J = 14.2, 26.0 Hz), 1.52 (3H, s), 1.49−1.43 (1H, m), 1.39 (3H, t, J = 7.2 Hz), 1.13 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 171.5, 170.8, 169.9, 166.5, 144.4, 132.2 (q, $^3J_{CF}$ = 1.80), 128.4 (q, $^2J_{CF}$ = 32.99), 127.8, 127.2 (q, $^3J_{CF}$ = 5.20), 127.0, 123.2 (q, $^1J_{CF}$ = 273.41), 77.6, 75.0, 63.8, 62.3, 53.6, 52.0, 51.6, 45.1, 42.1, 38.1, 35.8, 30.7, 20.6, 18.1, 16.4, 15.2, 13.9; HRESIMS m/z 605.1959 [M + Na] (calcd for $C_{29}H_{33}F_3O_9$, 605.1974); HPLC t_R = 16.767 min; purity = >99%.

17b: white powder; mp 100−102 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (1H, d, J = 8.0 Hz), 7.66 (1H, s), 7.51 (1H, d, J = 8.0 Hz), 5.62 (1H, dd, J = 5.0, 12.0 Hz), 5.12−5.07 (1H, m), 4.39 (2H, q, J = 7.1 Hz), 3.73 (3H, s), 2.78−2.71 (1H, m), 2.55 (1H, dd, J = 5.1, 13.6 Hz), 2.30 (2H, dd, J = 7.7, 13.5 Hz), 2.21 (1H, d, J = 13.9 Hz), 2.17 (3H, d, J = 5.8 Hz), 1.82 (1H, d, J = 13.2 Hz), 1.69 (1H, d, J = 14.4 Hz), 1.60 (3H, s), 1.52 (3H, s), 1.46 (1H, t, J = 12.8 Hz), 1.38 (3H, t, J = 7.1 Hz), 1.14 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.0, 171.5, 170.8, 169.9, 166.4, 143.7, 131.3 (q, $^3J_{CF}$ = 1.83), 130.8, 129.4 (q, $^2J_{CF}$ = 32.66), 128.6, 123.7 (q, $^3J_{CF}$ = 5.38), 123.1 (q, $^1J_{CF}$ = 273.83), 77.7, 75.0, 63.8, 62.2, 53.6, 52.1, 51.7, 45.1, 42.1, 38.1, 35.9, 30.7, 20.6, 18.1, 16.5, 15.2, 13.9; HRESIMS m/z 605.1979 [M + Na] (calcd for $C_{29}H_{33}F_3O_9$, 605.1974); HPLC t_R = 16.705 min; purity = 90%.

■ ASSOCIATED CONTENT

Supporting Information. NMR (^{1}H, ^{13}C) and HPLC analysis of compounds **5**−**7** and **11**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors would like to thank the National Institute on Drug Abuse (DA018151) for financial support of ongoing research. Portions of this work were supported by the Intramural Research Program, National Institute on Drug Abuse, NIH, DHHS. 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.

DEDICATION

Dedicated to Professor James M. Cook on the occasion of his 65th birthday.

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