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Synthetic Studies of Neoclerodane Diterpenes from *Salvia divinorum*: Preparation and Opioid Receptor Activity of Salvinicin Analogues

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Further modification of salvinorin A (**1a**), the major active component of *Salvia divinorum*, has resulted in the synthesis of novel neoclerodane diterpenes with opioid receptor affinity and activity. We report in this study that oxadiazole **11a** and salvidivin A (**12a**), a photooxygenation product of **1a**, have been identified as the first neoclerodane diterpenes with κ antagonist activity. This indicates that additional structural modifications of **1a** may lead to analogues with higher potency and utility as drug abuse medications.

Introduction

Salvia divinorum (Epling & Játiva-M, *Lamiaceae*) has been used for centuries in ceremonial rituals by the Mazatec Indians of Oaxaca, Mexico.[1] Taken in large doses (20−60 pairs of fresh leaves) an infusion of *S. divinorum* is reported to induce oral, aural, or visionary hallucinatory experiences.[2] In subhallucinogenic doses (four or five fresh leaves) an infusion is used medicinally to cure anemia, headache, rheumatism, and the disease panzón de barrego (swollen abdomen).[2] Chewing fresh leaves or smoking dried leaves will also produce hallucinogenic-like experiences.[1,3−5] *S. divinorum* leaves, tinctures, and extracts can be readily purchased on the Internet, and young adults have begun to experiment with this readily available, hallucinogenic substance.[6−8] Currently, the plant is unregulated in the United States but is regulated in a number of European countries and in Australia. Owing to its growing popularity as a recreational drug, the DEA has recently placed *S. divinorum* on the list of "drugs of concern".

The interesting pharmacological activity of *S. divinorum* has led to several phytochemical investigations of the plant.[9−15] Among the constituents identified is the neoclerodane diterpene salvinorin A (**1a**, Figure 1).[9−11] Salvinorin A (**1a**) possesses potent hallucinogenic activity; a smoked dose of 200−500 μg produces profound hallucinations lasting up to an hour, thus rivaling the potency of the synthetic hallucinogen LSD.[4] **1a** was found to be a potent and selective κ-opioid receptor agonist.[16] A number of recent reports have further documented the in vivo pharmacological effects of **1a**.[17] It has been shown that **1a** has depressive-like effects in a forced swim test, disrupts climbing behavior on an inverted screen task, and has rewarding effects that are different from its motor activity.[18−20] Other reports have focused on the antinociceptive activity of **1a**.[21−24] These studies revealed that **1a** produces a dose-dependent antinociceptive effect that was mediated by the κ opioid receptor (κOR) and is short in duration. Together, these studies confer a high degree of interest in **1a** and we and others, have begun to further study its pharmacological actions.

As part of our program to study the phytochemical constituents of *S. divinorum*, we recently identified two congeners of **1a**, salvinicins A (**2a**) and B (**3a**).[14] These compounds were isolated in low yield from an acetone extract of *S. divinorum* and possess a rare dimethoxydihydroxytetrahydrofuran ring. Salvinicin A (**2a**) was found to be a partial agonist at the κ opioid receptor, while salvinicin B (**3a**) possessed μ opioid antagonist activity.[14] It was intriguing to observe from these results that the stereochemistry of the dimethoxy groups of the tetrahydrofuran ring was important for selectivity and activity among the opioid receptors. The molecular basis for these differences was not readily clear. Thus, we set out to explore the structure−activity relationships of **2a** and **3a** at opioid receptors. Herein, we report the semisynthesis and biological activity of furan-modified analogues of **1a**.

Chemistry

Diterpene **1a** was extracted from commercially available dried *S. divinorum* leaves as described previously.[25] The treatment of **1a** with *N*-bromosuccinimide in acetonitrile afforded bromofuran **4**.[26] Alternatively, the reaction of **1a** with Br_2 in MeOH at low temperature afforded the dimethoxy alkene derivative (**5**) as a mixture of cis and trans isomers (Scheme 1).[26] Reduction of **5** with rhodium on carbon afforded trans dimethoxytetrahydrofuran isomers **6**.[27] Hydrogenation of **1a** using rhodium on carbon afforded a 1:1 mixture of **7** and acid **8**.[28] Further purification of (13*R*)-**7** using HPLC gave a mixture of a major peak and several minor components that were not obtained in sufficient quantities for identification. Analysis of the ^{1}H NMR, ^{13}C NMR, HMBC, and HMQC data of (13*R*)-**7** led to the assignment of the gross structure. The relative stereochemistry at C13 was derived from the NOESY data. The NOESY spectrum of the major peak of (13*R*)-**7** showed cross-peaks between H12, H13, and CH_3-20. These correlations suggested that these protons were close in space. The absolute stereochemistry at C13 was determined to be *R* from the single-crystal X-ray data (Figure 2).

The treatment of **1a** with $NaIO_4$ and a catalytic amount of $RuCl_3$ afforded the corresponding C13 acid.[27] The coupling of the C13 acid with selenophenol followed by decarbonylation with Bu_3SnH and AIBN afforded **9** (Scheme 2).[27] Additional

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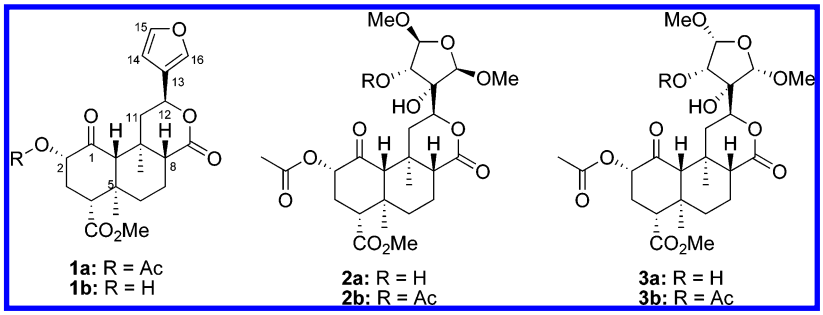
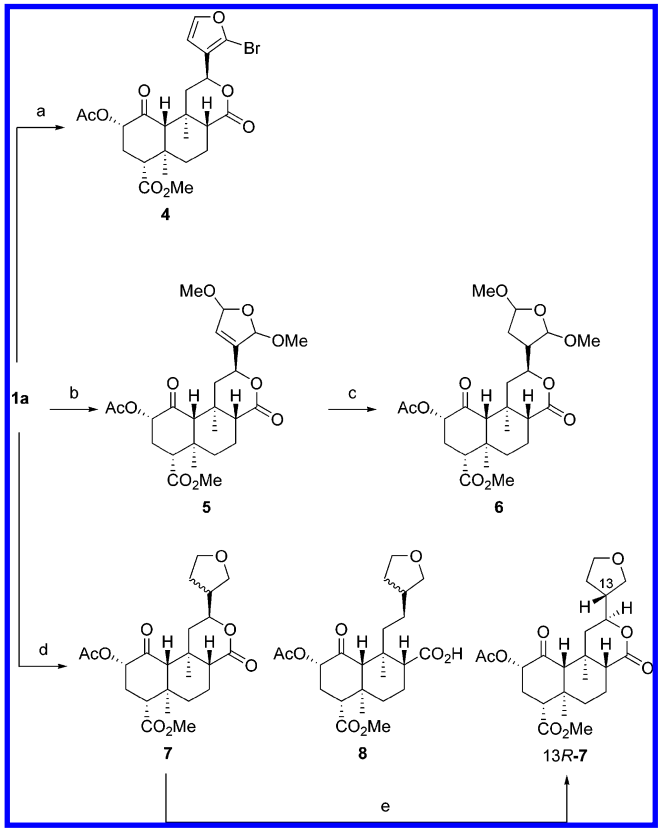


Figure 1. Structures of salvinorin A (**1a**), salvinorin B (**1b**), salvinicin A (**2a**), and salvinicin B (**3a**).

Scheme 1[a]



[a] Reagents and conditions: (a) NBS, CH_3CN; (b) Br_2, MeOH, CH_2Cl_2, −30 °C; (c) H_2, Rh/C, MeOH; (d) H_2, Rh/C, CH_2Cl_2/MeOH; (e) HPLC (40% CH_3CN/60% H_2O).

coupling of the C13 acid with either aminoethanol or acetamide oxime using EDCI followed by cyclization afforded **10** and a mixture of **11a** and **11b**, respectively. Photooxidation of **1a** in the presence of sunlight afforded γ-hydroxybutenolides **12a** and **12b**.[29] While our studies were in progress, salvidivin A (**12a**) and salvidivin B (**12b**) were reported from a methanol extract of commercially available dried *S. divinorum* leaves.[30] This synthesis is therefore the first reported preparation of salvidivins A and B from **1a**.

Given the success of the purification of **7** using HPLC, we sought to separate the major stereoisomers of **5** (Scheme 3). The mixture of cis and trans isomers of **5** was purified by HPLC to give compounds **13a** and **13b**. The gross structures of **13a** and **13b** were assigned based on ^{1}H NMR, ^{13}C NMR, HMBC, COSY, and HMQC data (Table 1). For **13b**, the NOESY spectrum showed cross-peaks between H15 and H16, H15 and H12, and H16 and H12. This indicated that H15 and H16 were on the same face of the molecule as H12. H15 and H16 were therefore assigned as α relative to H12. For **13a**, the relative stereochemistry of C14 to C16 was assigned by careful analysis

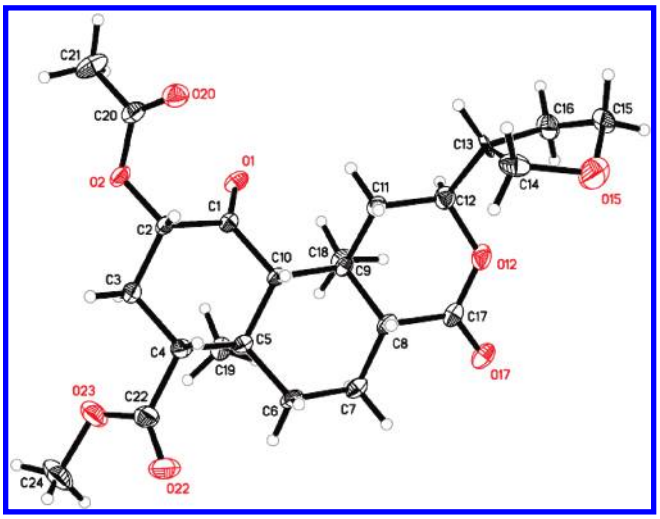


Figure 2. X-ray crystallographic structure of (13*R*)-**7**.

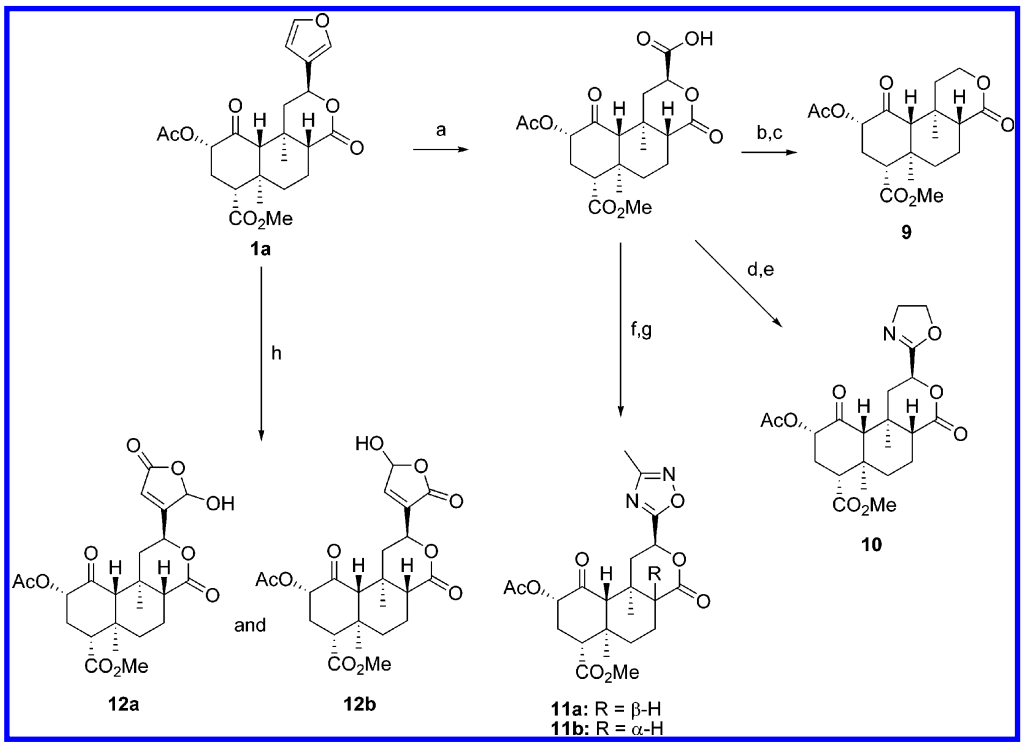
of the NOESY data and by comparison to the NOESY spectrum of **13b**. In the NOESY spectrum of **13a**, no cross-peaks were seen between H15 and H16. Cross-peaks were evident between H12 and H15. While the absence of NOE's between H15 and H16 is not conclusive evidence for stereochemical assignments, the presence of NOE's between H15 and H16 of **13b**, as well as the presence of H12/H15 NOE's in **13a**, led us to propose the *trans*-dimethoxy structure of **13a**.

Finally, the 15,16-dimethoxytetrahydrofuran isomers **6** were separated by HPLC to yield as the major products the *trans*-15,16-dimethoxytetrahydrofurans **14a** and **14b** (Scheme 4). The structures of **14a** and **14b** were assigned on the basis of ^{1}H NMR, ^{13}C NMR, HMBC, COSY, and HMQC data (Table 2). For **14a**, NOESY correlations were observed between H12, H13, and H15. No cross-peaks were seen between H15 and H16. Cross-peaks were evident between the CH_3O-15 and H16 and between CH_3O-16 and H15. Thus, by analogy to the **13** series of analogues, the relative stereochemistry as shown for **14a** is proposed. For **14b**, there were no NOE correlations between H15 and H16. Cross-peaks were observed between H12, H16, H13, and CH_3-20, and H10, CH_3O-15, and H15. These data indicated that the relative stereochemistry of **14b** is as shown.

Biological Results

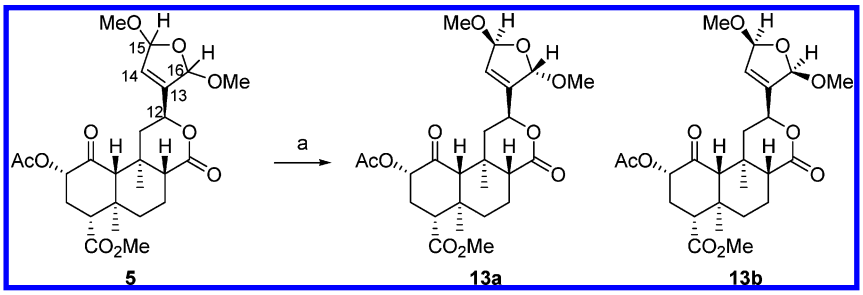
Compounds **1b** and **2**−**14** were then evaluated for affinity at human opioid receptors using methodology previously described (Table 3).[31] In an earlier study, **1a** was found to have high affinity (K_i = 1.9 nM) and selectivity for κ receptors over δ receptors.[21] Salvinorin B (**1b**) was found to have 147-fold lower affinity at κORs compared to **1a** (K_i = 280 nM vs K_i = 1.9 nM) and negligible affinity at μ and δ receptors (K_i > 10 000 nM). To facilitate direct comparison with **1a**, salvinicins A (**2a**) and B (**3a**) were reevaluated for opioid receptor affinity.

Scheme 2[a]



[a] Reagents and conditions: (a) $RuCl_3 \cdot 3H_2O$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$; (b) $PhOPOCl_2$, C_6H_5SeH, NEt_3, THF; (c) Bu_3SnH, AIBN, toluene; (d) EDCI, HOBt, ethanolamine, NEt_3, CH_2Cl_2; (e) Deoxo-Fluor, CH_2Cl_2, −30 °C; (g) EDCI, $CH_3C(NH_2)$═NOH, CH_2Cl_2; (g) toluene, heat; (h) photoxidation in the presence of sunlight.

Scheme 3[a]



[a] Reagents and conditions: (a) HPLC [40% CH_3CN/60% H_2O (0.1% TFA)].

Diterpenes **2a** and **3a** were found to have greatly reduced affinity for κORs compared to **1a** (K_i = 390 nM and K_i = 7020 nM vs K_i = 1.9 nM) and negligible affinity for μ or δ receptors (K_i > 10 000 nM). Our efforts then shifted to further probe the effects of the furan ring of **1a**−**3a**.

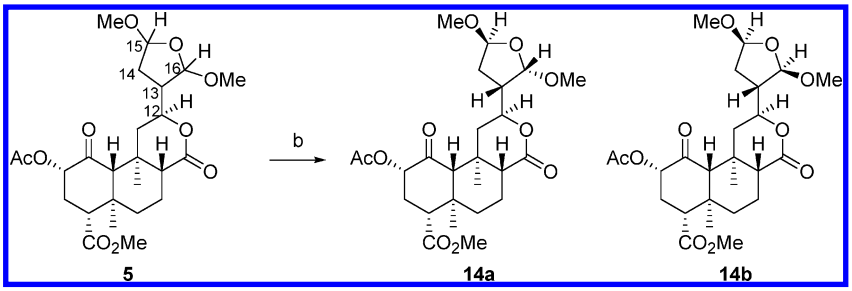
Selective acetylation of the C14 hydroxyl group of **2a** and **3a** afforded **2b** and **3b**, respectively. C14 acetylation was not tolerated, as significant affinity at μ, δ, and κ receptors was lost (K_i > 10 000 nM). However, bromination of the furan ring of **1a** was well-tolerated, as compound **4** retained high affinity for κORs (K_i = 3.0 nM vs K_i = 1.9 nM) and selectivity over μ and δ receptors (μ/κ = 483 and δ/κ = 2540, respectively). Removal of the C13 and C14 hydroxyl groups and introduction of an alkene between C13 and C14 of **2a** and **3a** affords dimethoxydihydrofuran **5**. Initially, **5** was evaluated as a mixture of stereoisomers, based on the ease of synthesis.[26] Alkene **5** was found to have negligible affinity for μ and δ receptors (K_i >10 000 nM) but to have similar affinity at κORs compared to **2a** (K_i = 440 nM vs K_i = 390 nM). This indicated that the C13 and C14 hydroxyl groups in **2a** and **3a** are not required for high affinity binding. To further probe this finding, the C13 alkene in **5** was reduced to afford **6**.[27] Interestingly, this modification resulted in an 11-fold increase in affinity at κORs (K_i = 440 nM vs K_i = 40 nM). To our surprise, this modification also increased affinity approximately 2-fold at μORs (K_i = 4810 nM vs K_i > 10 000 nM).

Removal of the methoxy groups in **6** affords tetrahydrofuran **7**.[28] This change resulted in an approximately 3-fold increase in affinity at κORs compared to **6** (K_i = 14 nM vs K_i = 40 nM). A previous report showed that **7** had 39-fold less affinity for κORs compared to **1a**.[28] In our hands, **7** had 7-fold lower affinity for κORs compared to **1a** (K_i = 14 nM vs K_i = 1.9 nM). One reason for this discrepancy could be the use of [^{125}I]IOXY rather than [^{3}H]U69,593 used by the other research group as the radioligand. In synthesizing **7**, a major side product is acid **8**. This compound also gives some insight as to whether the intact lactone ring is needed for high affinity binding at κORs. Diterpene **8** had 65-fold less affinity for κORs compared to **7** (K_i = 910 nM vs K_i = 14 nM), suggesting that the lactone ring is necessary for high affinity binding at κORs. Diterpene (13*R*)-**7** retained high affinity for κORs compared to **1a** (K_i = 3.7 nM vs K_i = 1.9 nM). This result indicates that an aromatic ring is not necessary for high affinity at κORs. However, complete removal of the furan ring (**9**)[27] resulted in over a 1700-

Table 1. NMR Spectroscopic Data (600 MHz, $CDCl_3$) for **13a** and **13b**

	13a		**13b**	
position	δ_C, mult	δ_H, mult (*J* in Hz)	δ_C, mult	δ_H, mult (*J* in Hz)
1	202.12, C		202.17, C	
2	75.01, CH	5.16, m	74.98, CH	5.18, m
3	30.79, CH_2	2.30, m	30.80, CH_2	2.32, m
4	53.60, CH	2.72, dd (4.7, 12.1)	53.60, CH	2.73,dd (4.6, 12.1)
5	42.08, C		42.07, C	
6	38.12, CH_2	1.55, m	38.13, CH_2	1.56, m
		1.80, dd (3.1, 11.8)		1.80, dd (3.1, 11.8)
7	18.15, CH_2	1.62, m	18.13, CH_2	1.62, m
		2.12, m		2.12, m
8	51.28, CH	2.02, dd (3.2, 11.8)	51.32, CH	2.02, dd (3.2, 11.8)
9	35.28, C		35.31, C	
10	64.12, CH	2.15, s	64.07, CH	2.18, s
11	40.46, CH_2	1.55, m	40.78, CH_2	1.55, m
		2.47, dd (5.5, 13.4)		2.50, dd (5.4, 13.4)
12	71.64, CH	5.14, m	72.01, CH	5.16, m
13	143.87, C		143.81, C	
14	127.26, CH	5.91, d (1.2)	126.55, CH	5.92, d (1.0)
15	107.26, CH	5.88, d (3.8)	106.56, CH	5.60, d (6.1)
16	107.97, CH	5.85, d (1.2)	106.26, CH	5.92, d, (1.0)
17	170.57, C		170.69, C	
18	171.54, C		171.55, C	
19	16.37, CH_3	1.10, s	16.38, CH_3	1.12, s
20	15.32, CH_3	1.40, s	15.28, CH_3	1.40, s
$OCOCH_3$ (2)	169.92, C		169.88, C	
$OCOCH_3$ (4)	20.60, CH_3	2.18, s	20.60, CH_3	2.20, s
$COOCH_3$ (4)	52.00, CH_3	3.72, s	52.00, CH_3	3.75, s
OCH_3 (15)	55.14, CH_3	3.45, s	55.31, CH_3	3.45, s
OCH_3 (16)	54.37, CH_3	3.38, s	54.39, CH_3	3.40, s

Scheme 4[a]



[a] Reagents and conditions: (a) HPLC (35% CH_3CN/65% H_2O).

fold loss in affinity at κORs compared to **1a** (K_i = 3400 nM vs K_i = 1.9 nM). Replacement of the 3-substituted furan ring with a 2-oxazoline ring (**10**) resulted in over a 150-fold loss in affinity (K_i = 300 nM vs K_i = 1.9 nM). Substitution of the furan ring with a 4-methyl-1,3,5-oxadiazoline ring (**11a**) resulted in a 29-fold loss in affinity compared to **1a** (K_i = 56 nM vs K_i = 1.9 nM). Inversion of the C8 stereochemistry of **11a** (**11b**) reduced affinity 18-fold for κORs compared to **11a** (K_i = 990 nM vs K_i = 56 nM).

Chromatographic separation of **5** affords **13a** and **13b**. Trans-isomer **13a** had similar affinity for κORs compared to **5** (K_i = 420 nM vs K_i = 440 nM). However, **13a** had approximately 3-fold higher affinity for μORs than **5** (K_i = 3190 nM vs K_i >10 000 nM). Inversion of the C16 stereochemistry of **13a** (**13b**) increased affinity 2-fold for κORs (K_i = 180 nM vs K_i = 440 nM) but had little effect on affinity for μORs (K_i = 3740 nM vs K_i = 3190 nM). The *trans*-2,5-dimethoxy analogues of (13*R*)-**7**, **14a** and **14b**, showed high affinity for κORs (**14a**, K_i = 25 nM and **14b**, K_i = 125 nM). However, **14a** had 7-fold lower affinity for κORs compared to (13*R*)-**7** (K_i = 25 nM vs K_i = 3.7 nM).

To further explore these developments, **4**, (13*R*)-**7**, **11a**, **12a**, **13a**, **13b**, **14a**, and **14b** were evaluated for activity at opioid receptors using the [^{35}S]GTP-γ-S binding assay (Table 4).[21,26] Bromo analogue **4** was found to be a full agonist at κORs (E_{max} = 104% relative to the full agonist U69,593) equipotent with **1a** (EC_{50} = 50 nM vs EC_{50} = 45 nM). This suggests that **4**, if labeled with ^{76}Br, might have potential utility as a κOR selective PET imaging agent. Tetrahydrofuran (13*R*)-**7** was 17-fold less potent at κORs than **1a** (EC_{50} = 750 nM vs EC_{50} = 45 nM) and is a partial agonist (E_{max} = 74% relative to U69,-593). This is in contrast to previous findings that showed that **7** was 3-fold less active than **1a** (EC_{50} = 126 nM vs EC_{50} = 46 nM).[28] One potential reason for this discrepancy is that the formerly evaluated tetrahydrofuran analogue was tested as a mixture of C13 epimers. Our results from evaluation of the 13*R* epimer imply that the 13*S* epimer may have higher activity. This, however, needs validation through synthesis and testing.

Given the activity seen with (13*R*)-**7**, we then evaluated **11a**, **11b**, and **12a** for activity at opioid receptors. Oxadiazole **11a** was found to have antagonist activity at μORs (K_e = 430 nM) and κORs (K_e = 360 nM). This is the first report of a neoclerodane diterpene with κ antagonist activity and identifies it as a novel scaffold for κOR antagonists. The C8 epimer of **11a** (**11b**) had 4-fold lower activity at μORs (K_e = 1530 vs K_e = 430 nM) and 16-fold lower activity at κORs (K_e = 5680 and K_e = 360 nM) compared to **11a**. Salvidivin A (**12a**), a photooxidation product of **1a**, had similar antagonist activity at

Table 2. NMR Spectroscopic Data (600 MHz, $CDCl_3$) for **14a** and **14b**

	14a		**14b**	
position	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)
1	202.02, C		202.21, C	
2	75.02, CH	5.15, m	74.96, CH	5.15, m
3	30.81, CH_2	2.30, m	30.86, CH_2	2.28, m
4	53.59, CH	2.74, dd (5.1, 11.5)	53.60, CH	2.74, m
5	42.11, C		42.12, C	
6	38.17, CH_2	1.55, m	38.19, CH_2	1.78, d (13.2)
		1.78, dd (3.0, 11.8)		1.55, m
7	18.17, CH_2	1.58, m	18.19, CH_2	1.62, m
		2.11, m		2.11, m
8	51.36, CH	1.93, m	51.33, CH	1.93, dd (3.4, 11.8)
9	35.11, C		35.15, C	
10	64.09, CH	2.12, s	64.24, CH	2.10, s
11	41.52, CH_2	1.25, t (12.7)	41.27, CH_2	1.12, t (12.4)
		2.32, m		2.39, dd (5.3, 13.2)
12	76.59, CH	4.56, m	76.95, CH	4.55, m
13	50.53, CH	2.18, m	50.98, CH	2.18, m
14	32.59, CH_2	2.30, m	32.17, CH_2	2.23, m
		1.94, m		1.57, m
15	105.32, CH	4.90, d (3.6)	105.02, CH	5.05, dd, (1.9, 5.3)
16	104.99, CH	5.10, m	106.34, CH	5.23, d (0.8)
17	171.07, C		171.01, C	
18	171.57, C		171.58, C	
19	16.32, CH_3	1.09, s	16.31, CH_3	1.10, s
20	15.11, CH_3	1.35, s	15.17, CH_3	1.37, s
O*C*OCH$_3$ (2)	169.88, C		169.92, C	
OCO*CH*$_3$ (4)	20.61, CH_3	2.17, s	20.68, CH_3	2.18, s
COO*CH*$_3$ (4)	51.98, CH_3	3.74, s	51.97, CH_3	3.72, s
O*CH*$_3$ (15)	56.07, CH_3	3.40, s	55.49, CH_3	3.38, s
O*CH*$_3$ (16)	55.67, CH_3	3.38, s	55.08, CH_3	3.32, s

Table 3. Binding Affinities of Salvinorin A Analogues at Opioid Receptors Using [^{125}I]IOXY as Radioligand[33−35]

	$K_i \pm$ SD, nM			selectivity	
compd	μ	δ	κ	μ/κ	δ/κ
1a[a]	>1000[b]	5790 ± 980	1.9 ± 0.2	>526	3050
1b	>10 000	>10 000	280 ± 20	>35	>35
2a	>10 000	>10 000	390 ± 30	>26	>26
2b	>10 000	>10 000	>10 000	ND[c]	ND
3a	>10 000	>10 000	7020 ± 750	1.4	1.4
3b	>10 000	>10 000	>10,000	ND	ND
4	1450 ± 60	7620 ± 180	3.0 ± 0.2	483	2540
5	>10 000	>10 000	440 ± 30	>20	>20
6	4810 ± 420	>10 000	40 ± 1	120	>250
7	7240 ± 480	>10 000	14 ± 1	517	714
(13*R*)-**7**	9790 ± 1090	>10 000	3.7 ± 0.2	2646	>2700
8	>10 000	>10 000	910 ± 40	>10	>10
9	>10 000	>10 000	3400 ± 150	>3	>3
10	>10 000	>10 000	300 ± 20	>30	>30
11a	>10 000	>10 000	56 ± 3	>170	>170
11b	>10 000	>10 000	990 ± 60	>10	>10
13a	3190 ± 230	>10 000	420 ± 20	8	>23
13b	3740 ± 240	>10 000	180 ± 20	21	>55
14a	>10 000	>10 000	25 ± 1	>400	>400
14b	>10 000	>10 000	125 ± 4	>80	>80

[a] Data from ref 21. [b] Partial inhibitor. [c] Not determined

μORs (K_e = 760 vs K_e = 430 nM) and κORs (K_e = 440 nM and K_e = 360 nM) compared to **11a**.

Dihydrofurans **13a** and **13b** were then evaluated for functional inhibition of opioid receptors. Compounds **13a** and **13b** were found to be weak nonselective antagonists at μ, δ, and κ receptors. However, **13b** had 3-fold higher activity at δ receptors (K_e = 1280 nM vs K_e = 3280 nM) and 2-fold higher activity at κ receptors than the trans isomer **13a** (K_e = 2370 nM vs K_e = 9700 nM). This indicates that the *S* configuration of the C16 methoxy group is favored for antagonist activity.

Finally, *trans*-dimethoxytetrahydrofurans **14a** and **14b** were evaluated in the functional assays. Reduction of the alkene in **13a** (**14a**) increases antagonist potency at μORs 2-fold (K_e = 1370 nM vs K_e = 2320 nM) and decreases potency approximately 3-fold at δORs (K_e >10 000 nM vs K_e = 3280 nM). Interestingly, this modification also changed the activity of **14a** at κORs from an antagonist into a full agonist (EC_{50} = 2350 nM, E_{max} = 95% relative to U69,593). Furthermore, **14a** is 3-fold less potent than **7** at κORs (EC_{50} = 2350 nM vs EC_{50} = 750 nM) but it appeared to be more efficacious (E_{max} = 95% vs E_{max} = 81% relative to U69,593). These data indicate that the addition of the methoxy groups decreases antagonist activity at μ and δORs but imparts an agonist conformation at κ receptors. Last, **14b** was found to have no activity (agonist or antagonist) at opioid receptors. Collectively, these findings indicate that additional structural modifications to the furan ring of **1a** may lead to antagonists with higher potency. Furthermore, the highly lipophilic nature

Table 4. Results from [^{35}S]GTP-γ-S Functional Assay Carried out in Stably Transfected CHO Cells Containing DNA for Human μ, δ, and κ Receptors[26] [a]

	$K_e \pm$ SD, nM			κ	
compd	μ	δ	κ	$EC_{50} \pm$ SE, nM	E_{max}[b] $\pm$ SE
1a	ND[c]	ND	ND	45 ± 10	108 ± 4
4	ND	ND	ND	50 ± 10	104 ± 4
(13*R*)-**7**	inactive	inactive	ND	750 ± 60	81 ± 7
11a	430 ± 80	inactive	360 ± 140	ND	ND
11b	1530 ± 740	4230 ± 2200	5680 ± 2280	ND	ND
12a	760 ± 320	2830 ± 320	440 ± 140	ND	ND
13a	2320 ± 1650	3280 ± 1640	9700 ± 1000	ND	ND
13b	2930 ± 1000	1280 ± 270	2370 ± 1240	ND	ND
14a	1370 ± 400	>10,000	ND	2350 ± 870	95 ± 5
14b	inactive	inactive	inactive	ND	ND
(−)-U69,593	ND	ND	ND	207 ± 34	100 ± 15

[a] The K_e data represent the mean ± SD from at least two independent determinations and the EC_{50} and E_{max} data represent the mean ± SE from at least three independent experiments. E_{max} values were compared using one-way ANOVA (Prism v4, GraphPad Inc., San Diego, CA). None of the E_{max} values were significantly different from that of U69,593 at $p < 0.05$ (F1,14 = 1.651; Dunnett's test). [b] E_{max} is the percent stimulation of [^{35}S]GTP-γ-S binding relative to the maximum stimulation of binding observed with U69,593 run in parallel with the test compounds. [c] Not determined.

of **2a** and its analogues raises the possibility that interactions with the cell membrane may influence the binding of these compounds to the opioid receptors.[32]

Conclusion

In summary, we have evaluated several furan ring modified analogues of **1a** for opioid receptor affinity and activity. This paper reports the first synthetic preparation of salvidivins A (**12a**) and B (**12b**). Bromo analogue **4** has been identified as a potent κOR agonist, and oxadiazole **11a** and salvidivin A (**12a**) have been identified as the first neoclerodane diterpenes with κ antagonist activity. Among the salvinicin analogues, it appears that the *S* configuration of the C16 methoxy group is favored for antagonist activity. Synthetic routes to additional analogues of **1a** are currently being explored and will be reported in due course.

Experimental Section

Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz on a Bruker Avance-300 spectrometer or on a Bruker AMX-600 spectrometer using $CDCl_3$ as solvent, δ values in ppm (TMS as internal standard), and *J* (Hz) assignments of ^{1}H resonance coupling. HMBC and HMQC data were collected on the AMX-600 spectrometer. Thin-layer chromatography (TLC) was performed on 0.25 mm Analtech GHLF silica gel plates. Spots on TLC were visualized with vanillin/H_2SO_4 in EtOH. Silica gel (32−63 μm particle size) from Bodman Industries (Atlanta, GA) was used for column chromatography. HPLC was carried out on an Agilent 1100 Series capillary HPLC system with diode array detector. Peaks were detected at 209, 214, and 254 nm. MPLC was performed on a RT Scientific PurChrom 150-GCS system equipped with a silica gel column (1.1 cm × 30 cm). The purity of the compounds was determined on an analytical scale by HPLC on the same instrument described above. An Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm) was utilized for these analyses.

Purification of (13*R*)-7. The crude product from the Rh/C hydrogenation of **1a** (**7**)[28] (108 mg) was further purified by HPLC (40% CH_3CN/60% H_2O) on a Phenomenex Luna C18 column (10 mm × 250 mm, 5 μm) at a flow rate of 3.5 mL/min to afford (13*R*)-**7** (77 mg). Crystallization from 40% CH_3CN/60% H_2O afforded a white solid: mp 179−182 °C; HPLC t_R = 16.7 min; purity = 99.6%.

Synthesis of Salvidivin A (12a) and Salvidivin B (12b). A solution of **1a** (365.5 mg, 0.845 mmol) in a mixture of CH_2Cl_2/MeOH (1:1) (5 mL) was stirred at room temperature for 7 days in the presence of sunlight. Removal of the solvent under reduced pressure afforded a crude material which was separated by repeated silica gel MPLC (eluent: 3% MeOH/CH_2Cl_2). Unreacted **1a** (207.0 mg) was recovered as a white solid, mp 236−239 °C (lit.[9,10] mp 240−242 °C). Fractions containing UV-active spots by TLC analysis were collected and evaporated to dryness. Crystallization from *n*-hexane/CH_2Cl_2 (4:1) gave 11.5 mg (6.7%) of **12a**, mp 212−215 °C (lit.[30] mp 217−222 °C), and 31.1 mg (18.2%) of **12b**, mp 230−234 °C (lit.[30] mp 216−221 °C) as white solids. The ^{1}H and ^{13}C spectra of **12a** and **12b** in $CDCl_3$/CD_3OD (1:1) were identical to that previously reported.[30]

Chromatographic Separation of 13a and 13b. Compound **5**[26] (100 mg) was further separated by HPLC [40% CH_3CN/60% H_2O (0.1% trifluoroacetic acid in H_2O)] on a Phenomenex Luna C18 column (10 mm × 250 mm, 5 μm) at a flow rate of 3 mL/min to afford **13a** (30 mg) and **13b** (26 mg).

(2*S*,4a*S*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-(Acetyloxy)-2-(2*S*,5*S*-dimethoxy-2,5-dihydrofuran-3-yl)dodecahydro-6a,10b-dimethyl-4,10-dioxo-2*H*-naphtho[2,1-*c*]pyran-7-carboxylic acid methyl ester (13a): ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; HREIMS *m*/*z* 501.2312 (calcd for $C_{25}H_{34}O_{10}Li$, 501.2325); HPLC t_R = 31.6 min; purity = 99.4%.

(2*S*,4a*S*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-(Acetyloxy)-2-(2*R*,5*S*-dimethoxy-2,5-dihydrofuran-3-yl)dodecahydro-6a,10b-dimethyl-4,10-dioxo-2*H*-naphtho[2,1-*c*]pyran-7-carboxylic acid methyl ester (13b): ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; HREIMS *m*/*z* 501.2312 (calcd for $C_{25}H_{34}O_{10}Li$, 501.2325); HPLC t_R = 33.4 min; purity = 99.4%.

Chromatographic Separation of 14a and 14b. Compound **6**[27] (124 mg) was further separated by HPLC (35% CH_3CN/65% H_2O) on a Phenomenex Luna C18 column (10 mm × 250 mm, 5 μm) at a flow rate of 4 mL/min to afford **14a** (40 mg) and **14b** (23 mg).

(2*S*,4a*S*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-(Acetyloxy)-2-(2*S*,5*S*-dimethoxy-2,3*R*,4,5-tetrahydrofuran-3-yl)dodecahydro-6a,10b-dimethyl-4,10-dioxo-2*H*-naphtho[2,1-*c*]pyran-7-carboxylic acid methyl ester (14a): ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2; HREIMS *m*/*z* 503.2469 (calcd for $C_{25}H_{36}O_{10}Li$, 503.2463); HPLC t_R = 24.8 min; purity = 98%.

(2*S*,4a*S*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-(Acetyloxy)-2-(2*R*,5*R*-dimethoxy-2,3*R*,4,5-tetrahydrofuran-3-yl)dodecahydro-6a,10b-dimethyl-4,10-dioxo-2*H*-naphtho[2,1-*c*]pyran-7-carboxylic acid methyl ester (14b): ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2; HREIMS *m*/*z* 503.2469 (calcd for $C_{25}H_{36}O_{10}Li$, 503.2463); HPLC t_R = 27.9 min; purity = 98%.

X-ray Crystal Structure of (13*R*)-7. Single-crystal X-ray diffraction data on (13*R*)-**7** were collected at 103 K using Mo Kα radiation and a Bruker APEX II CCD area detector. A 0.085 × 0.092 × 0.575 mm^3 crystal was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was mounted on a MiTeGen MicroMesh mount (Mitegen LLC, Ithaca, NY) and transferred immediately to the cold stream (103 K) on the diffractometer. The orthorhombic crystal was a nonmerohedral twin in space group

$P2_12_12_1$ with unit cell dimensions $a = 6.3428(7)$ Å, $b = 10.563(2)$ Å, $c = 31.962(6)$ Å. Corrections were applied for Lorentz, polarization, and absorption effects using TWINABS v1.05 (Bruker AXS Inc., Madison, WI). Data selected for use in the final refinement were nonoverlapped reflections from the major component of the twin and were 99.6% complete to 29.70° θ (approximately 0.73 Å) with an average redundancy greater than 7 prior to merging. The structure was solved by direct methods and refined by full-matrix least-squares on F^2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.14, 2000, Bruker AXS Inc., Madison, WI). Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model (coordinate shifts of C applied to H atoms) with C−H distance set at 0.98 Å. The absolute configuration was set on the basis of previously determined structures. Atomic coordinates for compound **7** have been deposited with the Cambridge Crystallographic Data Centre (deposition number 638730). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax, +44(0)-1223−336033; e-mail, deposit@ccdc.cam.ac.uk].

In Vitro Pharmacology. Cell culture, [^{35}S]GTP-γ-S binding assay, and [^{125}I]IOXY binding assays proceeded as described elsewhere.[26,33] Briefly, recombinant CHO cells (hMOR-CHO, hDOR-CHO, and hKOR-CHO) were produced by stable transfection with the respective human opioid receptor cDNA and provided by Dr. Larry Toll (SRI International, CA). All drug dilution curves are made up with buffer containing 1 mg/mL BSA. [^{125}I]IOXY (6β-iodo-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan) (SA = 2200 Ci/mmol) was used to label μ, δ, and κ binding sites.[33] These assays took place in 50 mM Tris-HCl, pH 7.4, with a protease inhibitor cocktail [bacitracin (100 μg/mL), bestatin (10 μg/mL), leupeptin (4 μg/mL) and chymostatin (2 μg/mL)], in a final assay volume of 1.0 mL. Nonspecific binding was determined using 10 μM naloxone. Salvinorin A related compounds were made up in 100% DMSO + 10 mM 2-mercaptoethanol to produce a 10 mM solution. Aliquots (50 μL) of this stock solution were pipetted into multiple microfuge tubes and stored frozen at −80 °C. On the day of the assay, an appropriate number of tubes were thawed and used to make up dilution curves. Any leftover compound was discarded.

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Supporting Information Available: CIF file of X-ray data for compound (13*R*)-**7** and purities of compounds (13*R*)-**7**, **13a**, **13b**, **14a**, and **14b** from HPLC analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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