Review

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Chem. Rev., **2008**, 108 (5), 1732-1743 • DOI: 10.1021/cr0782269 • Publication Date (Web): 14 May 2008

Salvinorin A Analogs as Probes in Opioid Pharmacology

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Received October 4, 2007

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1. Introduction

The opium poppy, *Papaver somniferum*, is one of several plants that have profoundly affected human history.[1] It has provided an unmatched medicine for the relief of pain for centuries.[2] More than 30 alkaloids have been identified in opium, and among the most relevant are morphine (**1a**) and codeine (**1b**) (Figure 1).[3] These alkaloids are important for modern medicine as an analgesic and a cough suppressant, respectively. These and related opiates exert their pharmacological effects by interacting with opioid receptors.[4]

Research over the past 30 years has given great insight into the pharmacology, biochemistry, and biology of opioid receptors.[5–7] Opioid receptors are members of the G-protein-coupled receptor (GPCR) superfamily of receptors and are divided into three types, μ (MOP), δ (DOP), and κ (KOP). In addition, there is a fourth member of the family, the nociception (NOP) receptor, which has low affinity for traditional opioids but has structural similarity with opioid receptors. Various pharmacological studies suggest the existence of additional opioid receptor subtypes.[8–10] Recent data suggests that these subtypes may arise from the formation of receptor heterodimers.[11] Each opioid receptor type plays a role in antinociception, as well as other biological responses.[12] In addition to being important targets for medications used to treat pain, opioid receptors are potential targets for obesity,[13] depression,[14,15] and alcoholism.[16]

In 2002, opioid receptors were implicated in the actions of the psychoactive mint *Salvia divinorum*.[17] The main active constituent isolated from the leaves of *S. divinorum* is the neoclerodane diterpene salvinorin A (**2a**).[18,19] Diterpene **2a** has been shown to produce short and very intense hallucinations in humans with a potency similar to lysergic acid diethyl amide (LSD) and 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB).[20,21] This activity was interesting given its lack of structural similarity to other psychotomimetic substances including LSD (**3**), phencyclidine (PCP, **4**), or Δ^9-tetrahydrocannabinol (Δ^9-THC, **5**). It came as little surprise that this molecule had a different mechanism of action given its lack of structural similarity to these substances.[20] It was striking, however, that **2a** was found to be a potent and selective agonist for κ opioid receptors over a battery of other receptors, including the 5-hydroxytryptamine (2a) (5-HT_{2A}) receptor, which mediates the psychotomimetic effects of LSD, *N*-methyl-D-aspartate acid (NMDA) receptors, the target of PCP like agents, and cannabinoid receptors, which mediate the psychotomimetic effects of cannabinoids.[17]

As a neoclerodane diterpene, **2a** is a truly unique opioid receptor ligand. Structurally it has little in common with other nonpeptidic opioid receptor ligands, such as **1a**, metopon (**6**),[22] bremazocine (**7**),[23] sufentanil (**8**),[24] SNC 80 (**9**),[25] U50,488 (**10**),[26] or etonitazene (**11**)[27] (Figure 2).[28,29] One common motif among these opioids is a basic nitrogen. Until the discovery of **2a**, it had been assumed that the presence of a positively charged nitrogen atom in opioid compounds represented an absolute requirement for their interaction with opioid receptors.[30] A general assumption was that the cationic amino charge of the opioid ligand would form a salt bridge with the side chain carboxyl group of an aspartate residue located in transmembrane (TM) III of the opioid receptor.[31–33]

Pharmacological and behavioral studies provide evidence that **2a** acts as a KOP agonist. Diterpene **2a**, for example, produces antinociception in mice that is blocked by a KOP antagonist.[34,35] Importantly, the antinociceptive and hypothermic effects of **2a** are not observed in KOP knockout mice.[36] The KOP agonist U69,593 produces depressive-like behaviors in animal models, including increased mobility in the forced swim test, decreased extracellular dopamine in

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10.1021/cr0782269 CCC: $71.00
Published on Web 05/14/2008

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Richard B. Rothman received an M.D. and a Ph.D. degree in pharmacology from the University of Virginia, Charlottesville, VA, in 1982. After postdoctoral studies at the National Institute of Mental Health (NIMH) (1982−1984), Dr. Rothman served a residency in psychiatry at St Elizabeth's Hospital, Washington, DC (1984−1987) and later a 2-year fellowship in psychiatry at the NIMH. In 1991, Dr. Rothman joined the Intramural Research Program of the National Institute on Drug Abuse (NIDA-IRP) in 1991. He has coauthored more than 340 original articles focused mostly on the pharmacology of opioids and stimulants and is an inventor on several U.S. patents. Dr. Rothman is board certified in psychiatry and currently serves as Chief of the Clinical Psychopharmacolgy Section, Chemical Biology Research Branch.

the nucleus accumbens,[37] and increases in threshold for intracranial self-stimulation.[38] A recent study showed that **2a** produced the same effects as U69,593 in these models, confirming that **2a** acts as a KOP agonist in vivo.[39] Other notable findings include the finding that, as expected for a KOP agonist, **2a** produced an aversive response in the conditioned place preference assay,[40] blocked the locomotor-stimulant effects of cocaine,[41] and did not exert DOM-like effects in nonhuman primates.[42]

This review describes the chemistry and structure–activity relationships of the growing number of nonpeptidic agonists and antagonists derived from **2a**. It provides a review of the

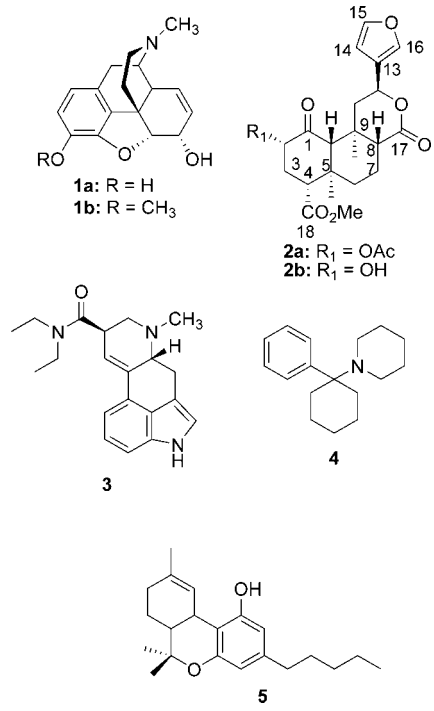

Figure 1. Structures of morphine (**1a**), codeine (**1b**), salvinorin A (**2a**), salvinorin B (**2b**), LSD (**3**), PCP (**4**), and Δ^9-THC (**5**).

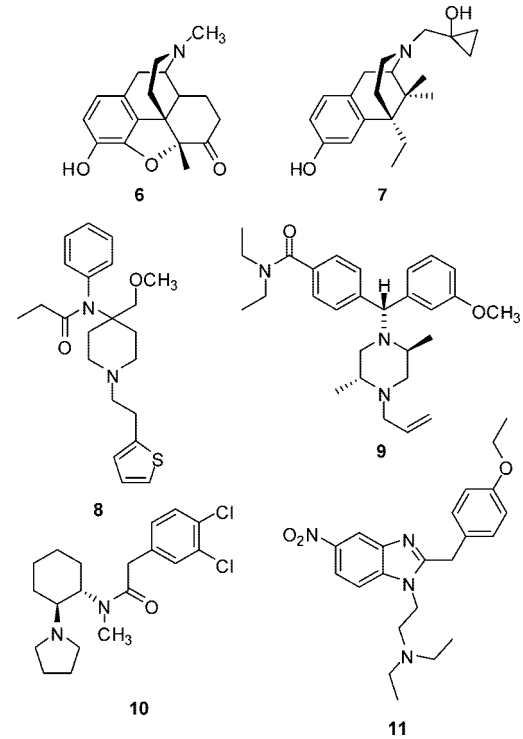

Figure 2. Structures of metopon (**6**), bremazocine (**7**), sufentanil (**8**), SNC 80 (**9**), U50,488H (**10**), and etonitazene (**11**).

scientific literature up to the middle of 2007 without attempting to describe the detailed pharmacology of **2a**. For this information, the reader is directed to several recent reviews on the pharmacology of **2a**.[43–45]

2. Phytochemistry

S. divinorum is a relatively rare plant, and few chemical studies have characterized its components. The first compounds isolated from *S. divinorum* were the neoclerodane

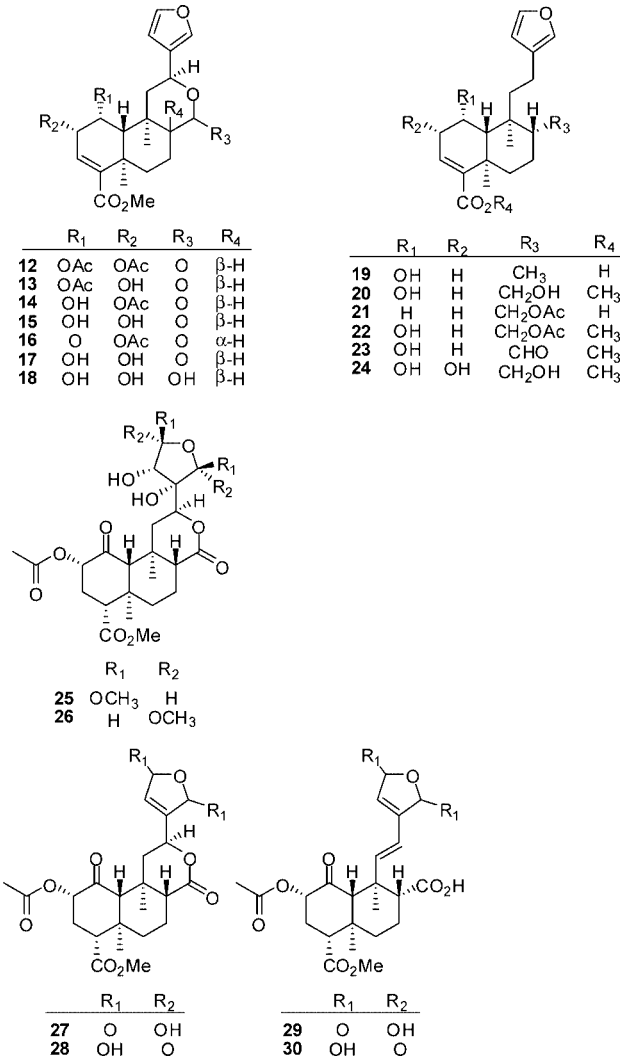

Figure 3. Naturally occurring analogs of salvinorin A.

diterpenes salvinorin A (**2a**) and salvinorin B (**2b**).[18,46] Prior to this report, Valdés was working on the isolation and characterization of the psychoactive substance from *S. divinorum*.[47] Infusions of the plant had been shown to possess psychotropic activity,[48] but the component responsible for this activity and its mechanism of action were not known.[49] Having ascertained the active component to be a terpenoid, efforts were initiated by Valdés to identify the molecular target of this compound.[47] These efforts were largely unsuccessful. A manuscript describing the isolation of the psychotropic terpenoid divinorin A and its congener divinorin B was then submitted to the *Journal of Organic Chemistry*.[19] Comparison of the structures of divinorin A with **2a** isolated by Ortega et al.[18] found these compounds to be identical. Therefore, divinorin A and B are now called salvinorin A and B, respectively.

2.1. Chemical Constituents

Additional work by Valdés et al. on *S. divinorum* isolated a novel neoclerodane diterpene, salvinorin C (**12**) (Figure 3).[50] Further phytochemical investigations have isolated salvinorins D−I (**13**−**18**),[51−54] divinatorins A−F (**19**–**24**),[52,53,55] salvinicins A and B (**25** and **26**),[56] and salvidivins A−D (**27**–**30**).[53] Eight additional constituents have been characterized from this plant: nepetoidin B, dehydrovomifoliol, harwickiic acid, isololiolide, methyl caffeate, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxybenzaldehyde, and loliolide.[53,57] To date, no alkaloids have been reported in *S. divinorum*.

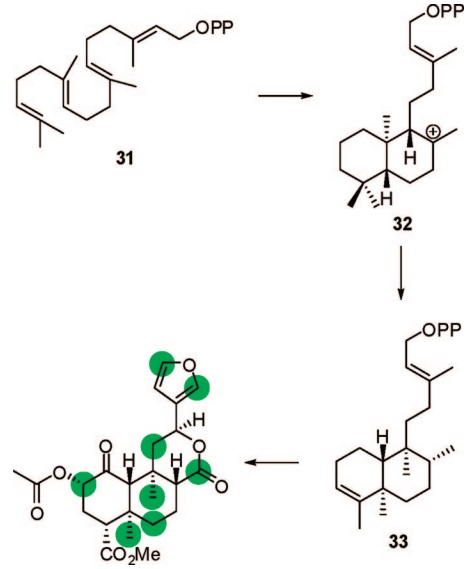

Figure 4. Proposed biosynthetic pathway for salvinorin A. Green labels indicate incorporation pattern of [1-^{13}C]-glucose isotopically labeled IPP derived from the DOXP pathway. Reprinted from Phytochemistry, 68, Lukasz Kutrzeba, Franck E. Dayan, J'Lynn Howell, Ju Feng, José-Luis Giner, and Jordan K. Zjawiony, Biosynthesis of salvinorin A proceeds via the deoxyxylulose phosphate pathway, pages 1872–1881, Copyright 2007, with permission from Elsevier.

2.2. Biosynthesis

Recently, the biosynthetic route of **2a** was studied using the incorporation of [1-^{13}C]glucose, [CH_3-^{13}C]methionine, and [1-^{13}C;3,4-$^{2}H_2$]-1-deoxy-D-xylulose into its structure.[58] Neoclerodane **2a**, like other terpenoids, results from the assembly of isopentenylpyrophosphate and dimethylallyl pyrophosphate to form geranylgeranyl pyrophosphate (**31**) (Figure 4).[59] Cyclization of **31** affords the labdanyl cation (**32**), which after several methyl shifts yields clerodane pyrophosphate (**33**). Further oxidation, acetylation, and methylation of **33** constructs **2a**. Despite low incorporation, feeding experiments with [1-^{13}C;3,4-$^{2}H_2$]-1-deoxy-D-xylulose (DOX) yielded a pattern of incorporation consistent with the deoxyxylulose phosphate pathway.[58] Furthermore, enrichment of the C-23 methoxy group suggests the participation of an *S*-adenosyl-L-methionine dependent type III *O*-methyltransferase. However, the enzymes responsible for the oxygenation of **33** remain uncharacterized.

3. Structure–Activity Relationships of Salvinorin A Analogs

Over the last 150 years, opioid receptor ligands have remained an active area in central nervous system (CNS) drug discovery. Opioid agonists are used clinically for the management of cancer pain, chronic pain, and cough and to treat diaherra.[28] Opioid antagonists, such as naloxone and naltrexone, are used to treat opioid overdose, narcotic addiction, and alcohol dependence.[60] Preclinical studies suggest that opioid agonists may have utility in treating stimulant dependence.[61] Additional studies indicate that opioid antagonists may have utility in treating mood disorders,[39,62] opioid abuse,[63] stress-induced reinstatement of cocaine-seeking behavior,[64] opioid-induced constipation,[65]

and gambling addiction.[66] Thus, **2a**, based on its novel structure, is a valuable lead in the development of opioid agents to treat all of these conditions.

Diterpene **2a** possesses a rigid tricyclic core with seven chiral centers. This structural architecture opens various avenues for chemical investigation. Presently, there are a growing number of investigations into the activity and selectivity of **2a** for κ opioid receptors. Interestingly, although **2a** has low affinity for the MOP,[5] **2a** has been shown to be an allosteric modulator of the MOP.[67] To date, structure–activity relationships of **2a** have focused on several main areas: (1) the 2-position acetoxy group, (2) the 4-position carbomethoxy group, (3) the 17-position carbonyl, and (4) the furan ring (Table 1). In addition, several attempts toward an asymmetric synthesis have been reported.

3.1. Other Salvinorins

Many naturally occurring analogues of **2a** have been evaluated for affinity at κ opioid receptors.[52,68,69] Generally, these compounds were found to have no affinity at KOPs (K_i > 10 000 nM).[52,68,69] However, there are a few exceptions. Salvinorin C (**12**) was found to have 250-fold lower affinity compared with **2a** (K_i = 1022 nM vs K_i = 4 nM).[68] Divinatorins D (**22**) and E (**23**) also had reduced affinity at KOPs compared with **2a** (K_i = 230 nM and K_i = 418 nM, respectively, vs K_i = 1.0 nM).[52] More recently, salvinicin A (**25**) was identified as having affinity for KOPs (K_i = 390 nM), but salvidivin A (**27**) was identified as the first naturally occurring neoclerodane with κ antagonist activity (K_e = 440 nM).[69] These findings suggest the possibility of additional naturally occurring analogues with opioid receptor affinity and activity.

3.2. Role of the 2-Position Acetyl Group

The most extensively studied substituent of **2a** has been the C-2 acetoxy group. This is likely due to the ease of preparation from **2b** and the early observation of **2b** having little psychotropic activity.[20] Modifications have been made in order to study (1) C-2 ester modifications, (2) preparation of carbamates or carbonates, (3) conversion to ethers or amines, (4) bioisosteric replacement to amides or thioesters, and (5) conversion to sulfonate esters.

3.2.1. Ester Modifications

Initially the role of the 2-acetyl group of **2a** on affinity and selectivity for κ opioid receptors was investigated.[70] Structural modification of this position was found to vary activity from full agonism to partial agonism for inhibition of forskolin-stimulated cAMP production. In particular, **2a** was found to be a full agonist, while propionate **34** and heptanoate **35** were found to be partial agonists in this assay (Figure 5).[70] Surprisingly, **2a** was found to be more efficacious than the selective κ agonist U50,488 and similar in efficacy to the naturally occurring peptide ligand for κ receptors, dynorphin A. However, salvinorin B (**2b**), as well as, **36**–**42** were found to have no affinity at μ, δ, or κ receptors.[70] Finally, the C-8 epimer of **2a** was found to have 41-fold lower affinity for κ receptors compared with **2a** (K_i = 163 nM vs K_i = 4 nM).[68]

Replacement of the 2-acetyl group with a formate (**43**) decreased affinity and activity approximately 5-fold at KOPs compared with **2a**.[68] However, the introduction of a butyl group (**44**) was found to decrease affinity and activity approximately 2-fold.[71] Additional studies found that **44** also had affinity at MOPs.[72] Further extension of the carbon chain (**45** and **46**) decreased affinity for KOPs but had little effect on MOP binding.[72] Introduction of an acetamido (**47**) or dimethylamino (**48**) to **2a** abolished affinity at KOPs (K_i > 10 000 nM).[71] Addition of an amino group to **34** (**49**) was also not tolerated. More recently, the addition of a *tert*-butoxycarbonylamino group (**50**) reduced affinity 47-fold at KOPs compared with **2a** (K_i = 90 nM vs K_i = 1.9 nM).[72]

The effect of branching and size of the 2-position has also been examined.[73] Insertion of a methyl group to **34** (**51**) decreased affinity 10-fold at KOPs. Introduction of an alkene (**52**) decreased affinity 3-fold for KOPs but increased affinity 11-fold at MOPs. Replacement of the 2-methylacroyl group with a methyl glyoxyl group (**53**) decreased affinity 11-fold at KOPs. Introduction of a benzoyl group (**54**) resulted in 47-fold loss of affinity at KOPs compared with **2a**. This change, however, increased affinity 25-fold at MOPs compared with **2a**. Functional studies showed **54** to be a full agonist at MOPs and KOPs. This was the first example of a non-nitrogenous μ agonist.[73]

The pharmacological properties of **54**, termed herkinorin, were examined in detail.[74,75] Benzoate **54** did not promote recruitment of β-arrestin-2 to the MOP and internalization of the MOPs, even in cells that overexpress G-protein-coupled receptor kinase.[75] In contrast, morphine, which does not normally recruit β-arrestin-2 and does not promote MOP internalization in HEK cells that express the MOP, will do so in the presence of G-protein-coupled receptor kinase overexpression. Thus, **54** provided a striking example of biased agonism.[76] Recent work suggests that the ability of a μ agonist to produce MOP internalization contributes to its ability to produce tolerance and dependence. The availability of **54** provided a useful tool to test this hypothesis using CHO cells that express the MOP.[74] The results showed that both noninternalizing (**54**) and internalizing (DAMGO) μ agonists produced tolerance, receptor desensitization, and up-regulation of the cAMP system. The major difference between the two types of μ agonists was that chronic **54** induced the formation of constitutively active MOPs to a profound degree.

Given the unique characteristics of **54**, additional studies were undertaken to more fully understand its preference for MOPs over KOPs.[72] Replacement of the benzoyl group with a nicotinoyl group (**55**) decreased affinity 6-fold at MOPs and 20-fold at KOPs. Introduction of a one carbon spacer between the carbonyl and phenyl ring (**56**) decreased affinity at MOPs and KOPs. The addition of a second methylene unit (**57**) increased affinity at MOPs and KOPs compared with **56**. Introduction of a bromo group in the 2-position (**58**) or 3-position (**59**) of the benzene ring had no effect on KOP affinity but decreased affinity for MOPs 9-fold compared with **54**. The presence of a 4-bromo group (**38**) decreased affinity for KOPs 8-fold compared with **54** (K_i = 740 nM vs K_i = 90 nM). In contrast to a previous report, which indicated that **38** had no affinity for MOPs,[70] this modification was found to retain high affinity for MOPs (K_i = 10 nM vs K_i = 12 nM).[72] Finally, the bioisosteric replacement of the benzene ring with a 2-thiophene (**60**) reduced affinity for KOPs 3-fold but had no effect on affinity for MOPs or DOPs.

More recently, the effects of additional modification to **54** were explored.[77] The addition of a 2-methoxy group (**61**)

Table 1. Opioid Receptor Affinity of Salvinorin A Analogs

compd	MOP $K_i \pm$ SD	DOP $K_i \pm$ SD	KOP $K_i \pm$ SD	refs	compd	MOP $K_i \pm$ SD	DOP $K_i \pm$ SD	KOP $K_i \pm$ SD	refs
2a	>10000 nM	>10000 nM	18.74 ± 3.38 nM	17,70	**82**	[a]	[a]	60.1 ± 9.1 nM	71
2b	>10000 nM	>10000 nM	>10000 nM	70	**83**	[a]	[a]	75.7 ± 5.9 nM	71
12	>1 μM	>1 μM	1022 ± 262 nM	68	**84**	>10000 nM	>10000 nM	1610 ± 120 nM	73
13	>1 μM	>1 μM	>10000 nM	68	**85**	[a]	[a]	0.4 ± 0.02 nM	86
14	>1 μM	>1 μM	>10000 nM	68	**86**	[a]	[a]	328 ± 40 nM	79
15	[a]	[a]	418 ± 117 nM	52	**87**	[a]	[a]	65.2 ± 24.6 nM	79
16	[a]	[a]	[a]		**88**	[a]	[a]	17.6 ± 3.1 nM	79
17	[a]	[a]	[a]			111 ± 49 nM	>10 μM	4.5 ± 2.0 nM	110
18	[a]	[a]	[a]		**89**	[a]	[a]	168 ± 10 nM	79
19	[a]	[a]	[a]		**90**	[a]	[a]	2.3 ± 0.6 nM	79
20	[a]	[a]	[a]		**91**	[a]	[a]	149 ± 1 nM	79
21	[a]	[a]	>10000 nM	52		4180 ± 310 nM	>10000 nM	30 ± 2 nM	77
22	[a]	[a]	230 ± 21 nM	52	**92**	[a]	[a]	374 ± 19 nM	79
23	[a]	[a]	>10000 nM	52	**93**	[a]	[a]	3.2 ± 0.1 nM	79
24	[a]	[a]	[a]			135 ± 4 nM	1690 ± 285 nM	0.37 ± 0.30 nM	110
25	>10000 nM	>10000 nM	390 ± 30 nM	69	**94**	[a]	[a]	1.6 ± 0.1 nM	79
26	>10000 nM	>10000 nM	7020 ± 750 nM	69		15 ± 3 nM	366 ± 38 nM	0.11 ± 0.10 nM	110
27	[a]	[a]	[a]		**95**	[a]	[a]	27.6 ± 1.8 nM	79
28	[a]	[a]	[a]		**96**	[a]	[a]	38.1 ± 1.9 nM	79
29	[a]	[a]	[a]		**97**	3.1 ± 0.4 nM	810 ± 30 nM	7430 ± 880 nM	77
30	[a]	[a]	[a]		**98**	[a]	[a]	38.1 ± 1.9 nM	81
34	>10000 nM	>10000 nM	32.63 ± 15.7 nM	70		4370 ± 310 nM	3990 ± 290 nM	5.7 ± 0.4 nM	77
35	>10000 nM	>10000 nM	3199 ± 961.2 nM	70	**99**	[a]	[a]	54.5 ± 25.7 nM	81
36	>10000 nM	>10000 nM	>10000 nM	70	**100**	290 ± 70 nM	1930 ± 70 nM	1410 ± 80 nM	77
37	>10000 nM	>10000 nM	>10000 nM	70	**101**	6820 ± 660 nM	>10000 nM	2.3 ± 0.1 nM	73
38	>10000 nM	>10000 nM	>10000 nM	70	**102**	>10000 nM	>10000 nM	60 ± 6 nM	72
	10 ± 1 nM	1410 ± 80 nM	740 ± 40 nM	72	**103**	220 ± 20 nM	3720 ± 400 nM	50 ± 5 nM	72
39	>10000 nM	>10000 nM	>10000 nM	70	**104**	>10 μM	>10 μM	2.9 ± 0.4 μM	84
40	>10000 nM	>10000 nM	>10000 nM	70	**105**	>1 μM	>1 μM	1125 ± 365 nM	68
41	>10000 nM	>10000 nM	>10000 nM	70	**106**	>1 μM	>1 μM	18 ± 2 nM	68
42	>10000 nM	>10000 nM	>10000 nM	70	**107**	[a]	[a]	[a]	
	>10000 nM	>10000 nM	410 ± 40 nM	77	**108**	[a]	[a]	[a]	
43	>1 μM	>1 μM	18 ± 2 nM	68	**109**	[a]	[a]	[a]	
44	520 ± 50 nM	4030 ± 250 nM	4 ± 1 nM	72	**110**	[a]	[a]	[a]	
45	310 ± 50 nM	3970 ± 270 nM	15 ± 2 nM	72	**111**	[a]	[a]	[a]	
46	520 ± 80 nM	4240 ± 290 nM	70 ± 4 nM	72	**112**	[a]	[a]	[a]	
47	[a]	[a]	>10000 nM	71	**113**	>10 μM	>10 μM	>10 μM	84
48	[a]	[a]	>10000 nM	71	**114**	[a]	[a]	1000 ± 269 nM	79
49	[a]	[a]	>10000 nM	71	**115**	[a]	[a]	>10000 nM	79
50	5660 ± 250 nM	>10000 nM	90 ± 10 nM	72	**116**	[a]	[a]	>10000 nM	79
51	2980 ± 110 nM	>10000 nM	19 ± 2 nM	73	**117**	[a]	[a]	>10000 nM	79
52	260 ± 6 nM	8880 ± 390 nM	42 ± 1 nM	73	**118**	[a]	[a]	>1000 nM	86
53	>10000 nM	>10000 nM	430 ± 10 nM	73	**119**	[a]	[a]	28.5 ± 0.9 nM	86
54	12 ± 1 nM	1170 ± 60 nM	90 ± 2 nM	73	**120**	[a]	[a]	>1000 nM	86
55	73 ± 2 nM	4820 ± 300 nM	1930 ± 50 nM	73	**121**	[a]	[a]	201 ± 26 nM	86
56	1090 ± 250 nM	>10000 nM	290 ± 40 nM	72	**122**	[a]	[a]	99.6 ± 15.9 nM	86
57	280 ± 40 nM	9330 ± 1010 nM	180 ± 10 nM	72	**123**	[a]	[a]	613 ± 54.1 nM	87
58	110 ± 10 nM	>10000 nM	90 ± 7 nM	72	**124**	[a]	[a]	>10000 nM	79
59	110 ± 10 nM	>10000 nM	70 ± 7 nM	72	**125**	[a]	[a]	>10000 nM	79
60	10 ± 2 nM	1380 ± 130 nM	260 ± 20 nM	72	**126**	[a]	[a]	>10000 nM	79
61	1640 ± 90 nM	>10000 nM	230 ± 20 nM	77	**127**	[a]	[a]	26.9 ± 1.8 nM	86
62	7550 ± 970 nM	>10000 nM	900 ± 50 nM	77	**128**	[a]	[a]	470 ± 92 nM	86
63	30 ± 2 nM	1140 ± 60 nM	550 ± 30 nM	77	**129**	[a]	[a]	210 ± 32 nM	86
64	>10000 nM	>10000 nM	800 ± 50 nM	77	**130**	>1 μM	>1 μM	59 ± 11 nM	68
65	70 ± 4 nM	1860 ± 140 nM	540 ± 40 nM	77	**131**	3670 ± 230 nM	>10000 nM	7 ± 1 nM	
66	260 ± 210 nM	>10000 nM	570 ± 40 nM	77	**132**	2620 ± 150 nM	>10000 nM	10 ± 1 nM	
67	180 ± 20 nM	>10000 nM	5490 ± 640 nM	77	**133**	>1 μM	>1 μM	6 ± 1 nM	68
68	10 ± 1 nM	580 ± 30 nM	70 ± 2 nM	77	**134**	>1 μM	>1 μM	6 ± 2 nM	68
69	10 ± 1 nM	690 ± 30 nM	80 ± 3 nM	77	**135**	7240 ± 480 nM	>10000 nM	14 ± 1 nM	69
70	1030 ± 80 nM	>10000 nM	2010 ± 110 nM	77	***S*-135**	9790 ± 1090 nM	>10000 nM	3.7 ± 0.2 nM	69
71	[a]	[a]	3.2 ± 0.2 nM	71	**136**	1450 ± 60 nM	7620 ± 180 nM	3.0 ± 0.2 nM	69
72	[a]	[a]	83.0 ± 8.5 nM	71	**137**	>10000 nM	>10000 nM	300 ± 20 nM	69
73	[a]	[a]	462 ± 20 nM	71	**138**	>10000 nM	>10000 nM	8530 ± 550 nM	89
74	640 ± 30 nM	6460 ± 390 nM	120 ± 4 nM	73	**139**	>10000 nM	>10000 nM	840 ± 90 nM	89
75	16 ± 1 nM	230 ± 10 nM	93 ± 3 nM	73	**140**	>10000 nM	>10000 nM	410 ± 30 nM	89
76	[a]	[a]	>10000 nM	86	**141**	>10000 nM	>10000 nM	1620 ± 110 nM	89
77	[a]	[a]	>10000 nM	86	**142**	>10000 nM	>10000 nM	56 ± 3 nM	69
78	[a]	[a]	220 ± 12 nM	71	**143**	>10000 nM	>10000 nM	25 ± 1 nM	69
79	[a]	[a]	7.9 ± 0.3 nM	71	**144**	>10000 nM	>10000 nM	125 ± 1 nM	69
80	[a]	[a]	28.7 ± 3.0 nM	71	**145**	3190 ± 230 nM	>10000 nM	420 ± 20 nM	69
81	[a]	[a]	35.8 ± 5.1 nM	71	**146**	>10000 nM	>10000 nM	125 ± 4 nM	69

[a] Not determined.

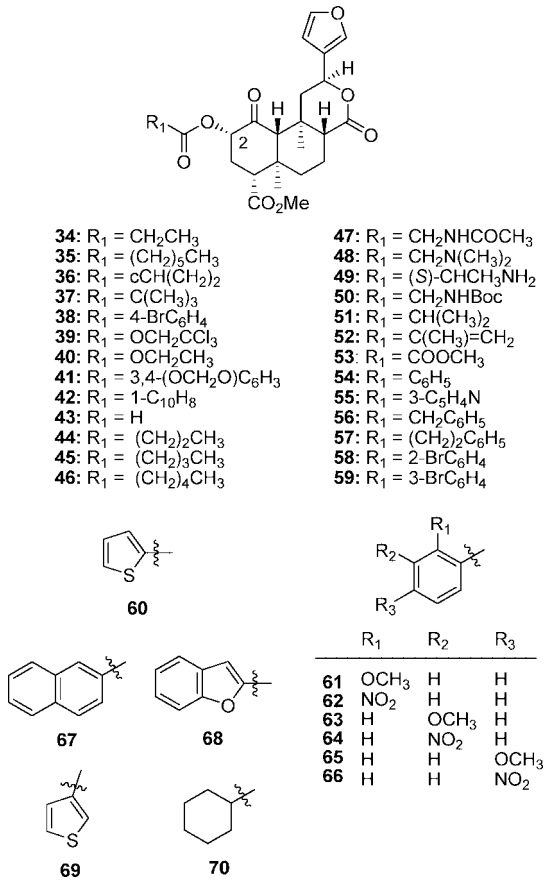

Figure 5. C-2 ester analogs of salvinorin A.

or a 2-nitro group (**62**) decreased affinity for MOPs compared with **54**. These results suggest that factors such as sterics are likely involved in the binding of 2-position analogues. However, this awaits further investigation. Introduction of a methoxy group in the 3-position of the benzene ring (**63**) also decreased affinity for MOPs and KOPs compared with **54**. This modification, however, improved selectivity for MOPs over KOPs compared with **54**. Substitution of a 3-nitro group (**64**) abolished affinity at MOPs (K_i > 10 000) and decreased affinity approximately 10-fold at KOPs compared with **54**. The presence of 4-methoxy group (**65**) leads to an approximately 6-fold decrease in affinity and similar selectivity for MOPs compared with **54**. The addition of a 4-nitro group (**66**) decreased affinity over 20-fold for MOPs and over 6-fold for KOPs compared with **54**. These results indicate that factors other than electronics are likely involved in the binding of 4-position analogues to MOPs.

Replacement of the benzoyl group in **54** with a 1-naphthoyl group (**42**)[70] decreased affinity roughly 1000-fold at MOPs, whereas substitution of a 2-naphthoyl group (**67**) reduced affinity at MOPs approximately 10-fold compared with **54**. Introduction of a 2-benzofuran (**68**) or a 3-thiophene (**69**) was well tolerated as **68** and **69** showed equal affinity for MOPs compared with **54**. Reduction of the benzene ring to a cyclohexane ring (**70**) reduced affinity for MOPs and KOPs compared with **54**.

The effect of C-2 position stereochemistry on the affinity and activity of **2a** has been probed.[78,79] Inversion of the C-2 substituent of **2a** decreased activity at KOPs 9-fold.[78] A similar trend was seen when the C-2 position was inverted in **34** and **44**.[79]

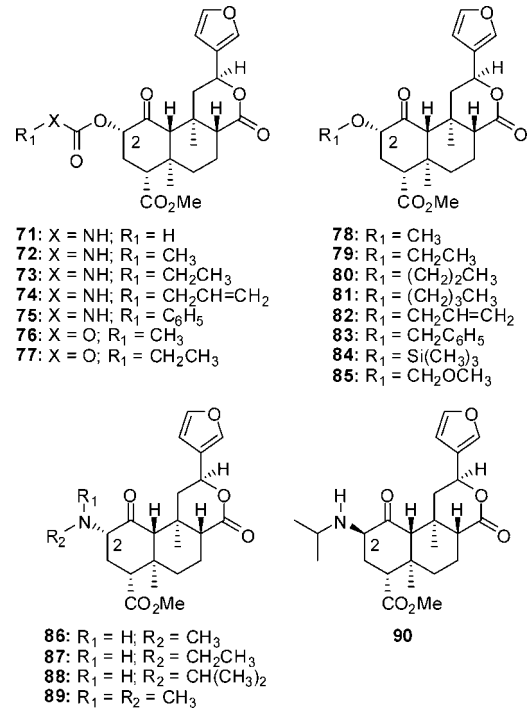

Figure 6. C-2 carbamate, carbonate, ether, and amine analogs of salvinorin A.

3.2.2. Carbamate and Carbonate Analogs

Replacement of the acetyl group in **2a** with a carbamoyl group (**71**) was well tolerated at KOPs (Figure 6).[71] Addition of methyl group (**72**) decreased affinity and activity. Extension of the methyl group to an ethyl group (**73**) was not well tolerated, and affinity and activity were further decreased. Exchange of the acetyl group in **2a** with an allyl carbamoyl group (**74**) decreased affinity 63-fold at KOPs.[73] Interestingly, this change resulted in moderate affinity at MOPs. Substitution of a phenylcarbamoyl group (**75**) for the allyl carbamoyl group in **74** had little effect at KOPs but increased affinity for MOPs and DOPs.[73] Conversion of **72** and **73** to the corresponding carbonates, **76** and **77**, respectively, was poorly tolerated at KOPs and affinity was abolished (K_i > 1000 nM).[80]

3.2.3. Ether and Amine Analogs

The conversion of **2a** to various ethers has been studied.[71,79,80] The addition of a methyl group to **2b** (**78**) has little effect on affinity or efficacy at KOPs.[71] Extending the chain to ethyl (**79**) increases affinity and activity 20-fold compared with **78**. Further extension of the chain (**80** and **81**) decreased affinity and activity compared with **79**. Allyl ether **82** and benzyl ether **83** were found to have similar activity at KOPs but were less potent than **79**.[71] Trimethylsilyl ether **84** was found to have reduced affinity compared with **2a**.[73] Finally, introduction of a methoxymethyl group (**85**) was found to increase affinity and activity at KOPs compared with **2a**.[80] This is the most potent salvinorin A derived κ agonist described to date.

The conversion of the methoxy group in **78** to a methylamino group (**86**) had little effect on affinity but decreased activity at KOPs.[79] Extension of the chain to an ethylamino group (**87**) increased affinity and activity compared with **86**. Substitution of an isopropylamino group (**88**) increased activity at KOPs compared with **87**. Addition of an *N*-methyl

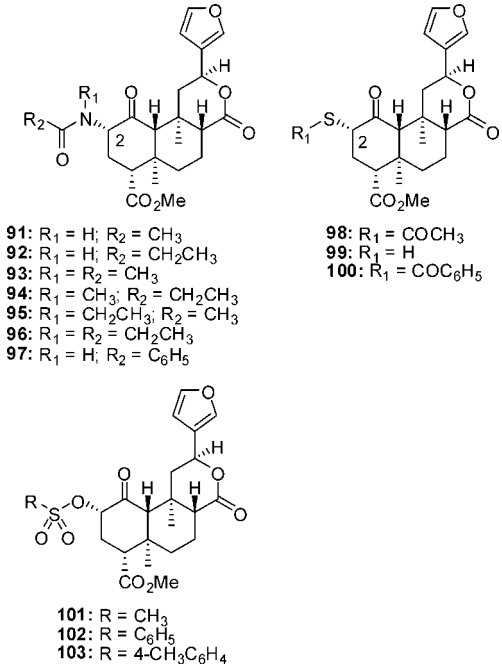

Figure 7. C-2 bioisosteric replacements of salvinorin A.

group to **86** (**89**) also increased activity at KOPs. Generally, inversion of C-2 stereochemistry of these analogues was found to increase activity at KOPs. The most potent analogue (**90**) was found to be roughly equipotent with **2a** (EC_{50} = 7.2 nM vs EC_{50} = 4.5 nM).[79]

3.2.4. Amides and Thioesters

Bioisosteric replacement of the acetoxy group in the 2-position of **2a** with acetamido and thioacetoxy has also been investigated.[71,77,79,81–83] The substitution of an acetamido group (**91**) for the acetoxy group in **2a** decreases affinity and activity at KOPs (Figure 7).[79] Extension of the carbon chain to a propionamido group (**92**) decreased affinity and activity at KOPs. The addition of an *N*-methyl group to **91** (**93**) increased affinity and activity at KOPs.[79] A similar effect was seen when an *N*-methyl group was added to **92** (**94**) resulting in a derivative more potent than **2a** (EC_{50} = 0.75 nM vs EC_{50} = 4.5 nM). Introduction of an *N*-ethyl group to **91** (**95**) and **92** (**96**) increased activity at KOPs, but these analogues were less potent than **94** and **95**. Generally, inversion of the C-2 stereochemistry in **91–96** decreased affinity and activity.[79] Conversion of the benzoyloxy group in **54** to a benzamido group (**97**) was found to increase affinity and selectivity for MOPs (K_i = 3.4 nM vs K_i = 12 nM).[77] Amide **97** is the most potent μ agonist derived from **2a** (EC_{50} = 360 nM) described to date.

The substitution of a thioacetoxy group (**98**) for the acetoxy group in **2a** decreased affinity and activity at KOPs (EC_{50} = 4.77 nM vs EC_{50} = 2.82 nM).[77,81] Removal of the acetyl group in **98** (**99**) decreased affinity and activity at KOPs. Similarly, inversion of the C-2 stereochemistry in **87** and **88** decreased activity at KOPs. As shown in the amide and ester series, introduction of a benzene ring to **98** (**100**) increased affinity for KOPs. However, **100** had reduced affinity compared with **54** and **97**.[77]

3.2.5. Sulfonyl Esters

An additional modification studied was the bioisosteric replacement of the acetyl group with a sulfonate ester.[73]

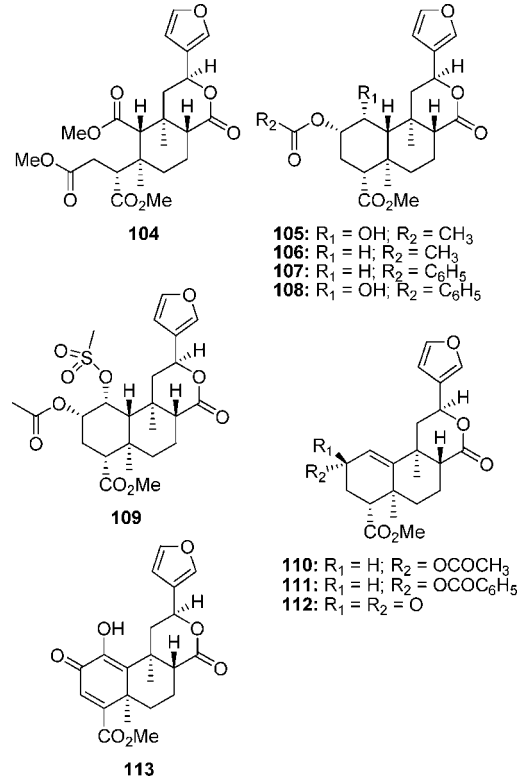

Figure 8. Ring A modified salvinorin A analogs.

Substitution of a mesylate group (**101**) was well tolerated as this change had little effect on binding to KOPs. Mesylate **101** was also found to be slightly more potent than **2a** as an agonist at KOPs (EC_{50} = 30 nM vs EC_{50} = 40 nM).[73] Replacement of the mesylate in **101** with a benzenesulfonate (**102**) reduced affinity at KOPs compared with **101**. This is in good agreement with previous SAR studies in the ester series where the replacement of a methyl group with a phenyl group (**54**) decreased affinity for KOPs.[73] Surprisingly, **102** had no affinity for MOPs (K_i > 10 000 nM). An analogous replacement of methyl by phenyl (**54**) showed an increase in MOP affinity.[73] Introduction of a 4-methyl group to **102** (**103**) had no effect on KOP affinity (K_i = 50 nM vs K_i = 60 nM) and increased affinity for DOPs (K_i = 3720 nM vs K_i > 10 000 nM) compared with **102**. This change, however, increased affinity for MOPs compared with **102** (K_i = 220 nM vs K_i > 10 000 nM). These changes, however, are not parallel to the ester series suggesting that the sulfonate esters are not binding in an identical manner at either MOPs and KOPs.

3.3. Other A Ring Changes

There have been several other changes to the A ring studied.[80,84,85] Ring-opened analogue **104** was found to have weak affinity at KOPs (K_i = 2.9 μM) (Figure 8).[84] Reduction of the C-1 ketone to an α-alcohol (**105**) reduced affinity over 250-fold compared with **2a** (K_i = 1125 nM vs K_i = 4 nM).[68] This modification also changed the efficacy at KOPs from a full agonist (**2a**, E_{max} = 108%) to an antagonist (**105**, K_e = 240 nM).[85] Removal of the ketone (**106**) resulted in a 5-fold loss of affinity compared with **2a** (K_i = 18 nM vs K_i = 4 nM).[68] Additional testing found that **106** was 3-fold less potent but more efficacious as a KOP agonist than **2a**. A more recent study found that **106** was approximately as potent but less efficacious than **2a**.[85] In this study, **106** was

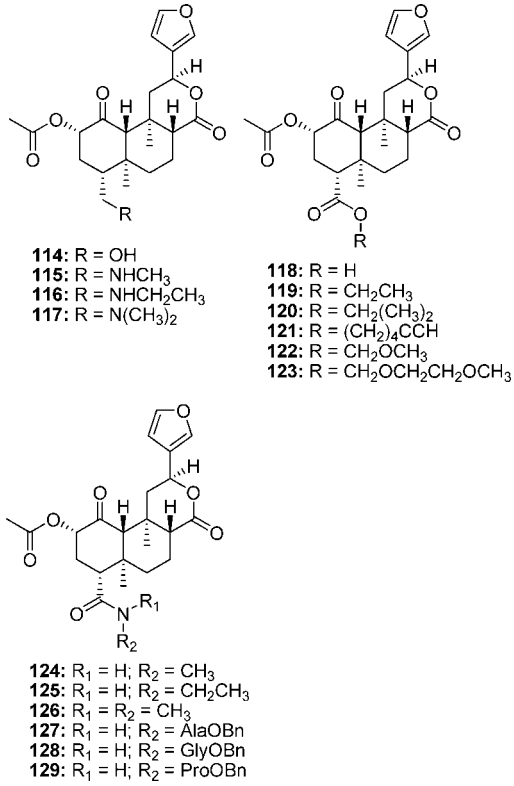

Figure 9. C-4 modified analogs of salvinorin A.

found to also have antagonist activity at MOPs and DOPs. Replacement of the acetyl group in **106** with a benzoyl group (**107**) resulted in an antagonist at μ, δ, and κ receptors. This finding suggests that the C-1 deoxo analogues may be interacting at μORs in a nonidentical manner compared with C-1 keto analogues. Addition of a benzene ring to **105** (**108**) decreased activity 2-fold at KOPs (K_e = 450 nM vs K_e = 240 nM), whereas, the introduction of a mesylate group (**109**) resulted in a loss of antagonist activity at κ receptors.

The presence of the 1,10-alkene has also been investigated.[85] Introduction of a 1,10-alkene to **106** (**110**) resulted in a switch of efficacy from partial agonist to antagonist at KOPs. Furthermore, **110** had higher antagonist activity at MOPs and DOPs than at KOPs. When compared with **106**, **110** had similar antagonist activity at MOPs and reduced activity at DOPs. Replacement of the acetyl of **110** with benzoyl group (**111**) decreased activity 9-fold at MOPs and 6-fold at DOPs but had little effect on KOPs. This also suggests that the 1,10-dehydro analogues are not interacting in a similar manner to analogues that contain a C-1 keto group. Several additional α,β-unsaturated ketones have been investigated. Ketone **112** was found to have similar antagonist activity as **110** at KOPs but reduced activity at MOPs and DOPs, whereas, **113** was found to have no affinity for KOPs (K_i > 10 μM).[80,84,85]

3.4. Role of the 4-Position Carbomethoxy Group

Several reports have investigated the role of the 4-position carbomethoxy group.[68,79,86,87] Reduction of the carbomethoxy group to the primary alcohol (**114**) reduced affinity 89-fold at KOPs (Figure 9).[68] Bioisosteric replacement of the alcohol with a primary amine (**115**) was not tolerated as affinity for KOPs was lost (K_i > 10 000 nM).[79] *N*-Alkylation of **115** (**116** and **117**) did not lead to an enhancement of affinity.

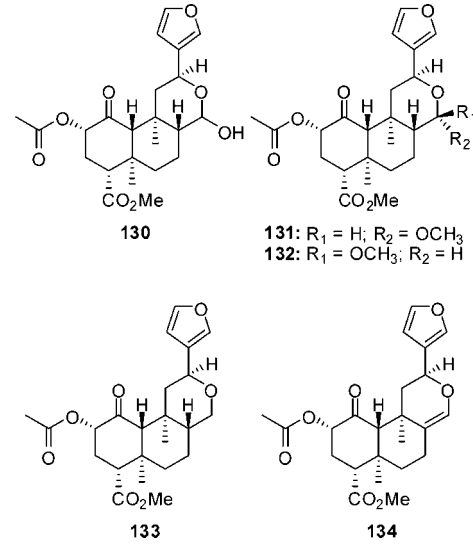

Figure 10. C-17 modified analogs of salvinorin A.

Similarly, epimerization of the C-8 position of **115**–**117** did not lead to an increase in affinity for KOPs.[79]

Hydrolysis of the methyl ester in **2a** to the corresponding acid (**118**) resulted in a loss of affinity at KOPs (K_i > 1000 nM).[86] Extension of the carbon chain in **2a** to an ethyl group (**119**) also resulted in a loss of affinity and activity at KOPs. Further extension of the alkyl chain, such as **120**, was not tolerated.[86] However, incorporation of an alkyne (**121**) did result in modest affinity at KOPs (K_i = 201 nM). Addition of a methoxymethyl (MOM) group to **118** (**122**) resulted in a loss in affinity and activity at KOPs. Interestingly, the C-8 epimer of **122** had similar affinity for KOPs but was 3-fold less potent as an agonist.[86] Replacement of the MOM group with a MEM group (**123**) decreased affinity and activity at KOPs.[87]

Conversion of the 4-position methyl ester into various amides has also been accomplished.[79,86,87] Bioisosteric replacement of the methyl ester with a methyl amide (**124**) resulted in over a 500-fold loss of affinity at KOPs.[79] Extension of the carbon chain (**125**) or addition of an *N*-methyl group (**126**) were not tolerated as affinity at KOPs was lost (K_i > 10 000 nM). Substitution of other alkyl chains was also not tolerated.[86] However, several amino acid derivatives (**127**–**129**) were found with affinity and activity less than **2a**.[86] The most potent of these analogues was alanine derivative **127** (EC_{50} = 46.7 nM).

3.5. Role of the 17-Position Carbonyl

Reduction of the lactone carbonyl to a lactol (**130**) was found to reduce affinity 14-fold and activity 2-fold at KOPs (Figure 10).[68] Addition of a methyl group to **130** creates **131** and **132**. This change was well tolerated, and C-17 stereochemistry was found to have little effect on binding as **131** and **132** had similar affinities at μ, δ, and κ receptors (**131**, μ K_i = 3670 ± 230 nM; δ K_i > 10 000 nM; κ K_i = 7 ± 1 nM vs **132**, μ K_i = 2620 ± 150 nM; δ K_i > 10 000 nM; κ K_i = 10 ± 1 nM).[88] Removal of the carbonyl (**133**) was found to have little effect on affinity at KOPs compared with **2a** (K_i = 6 nM vs K_i = 4 nM), but activity was reduced 5-fold.[68] Similarly, introduction of C-8−C-17 alkene (**134**) had little effect on binding, but activity was reduced 14-fold compared with **2a** (EC_{50} = 624 nM vs EC_{50} = 46 nM).[68]

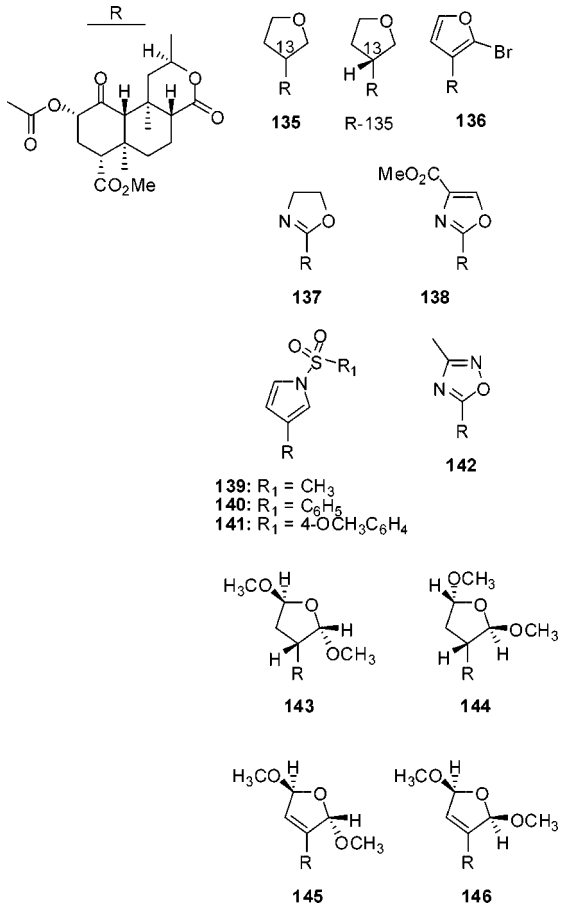

Figure 11. C-12 modified analogs of salvinorin A.

3.6. Role of the Furan Ring

Additional work has focused on the role of the furan ring.[68,69,89] Reduction of the furan ring to a mixture of C-13 epimers (**135**) reduced affinity for κ receptors compared with **2a** (K_i = 156 nM vs K_i = 4 nM) (Figure 11).[68] Additional testing found **135** to possess high affinity at KOPs (K_i = 14 nM).[69] The *R* epimer (*R*-**135**) was found to have similar affinity for KOPs as **2a** but was 17-fold less active than **2a**. Bromination of the furan ring is well tolerated as **136** retained high affinity and activity at KOPs. Replacement of the furan ring with a 2-oxazoline ring (**137**) or a 4-carbomethoxyoxazole (**138**) decreased affinity for KOPs.[69,89] A series of *N*-sulfonylpyrroles (**139**–**141**) were also found to have reduced affinity for KOPs compared with **2a**.[89] Interestingly, these modifications resulted in partial agonism at KOPs. Substitution of the furan ring with a 4-methyl-1,3,5-oxadiazole (**142**) resulted in a 29-fold loss in affinity at KOPs compared with **2a**. However, this change resulted in antagonist activity at MOPs and KOPs.[69] The addition of 2,5-dimethoxy groups to *R*-**135** (**143** and **144**) was also probed and found to decrease affinity at KOPs. Incorporation of a C-13−C-14 alkene to **143** (**145**) and **144** (**146**) did not enhance affinity at KOP.

3.7. Total Synthesis Efforts

The first studies toward the total synthesis of **2a** were reported by Lingham et al.[90] The proposed synthetic route dissected **2a** into cyclohexanone **147** and lactone **148** (Figure 12). It was envisioned that Michael addition followed by olefin metathesis and hydrogenation would afford **2a**. This

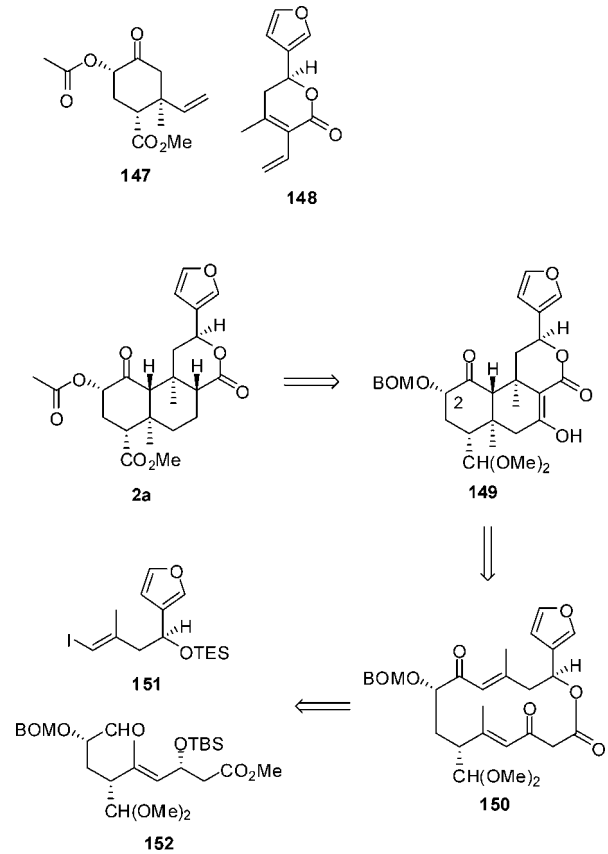

Figure 12. Synthetic targets and intermediates used in synthesis efforts of salvinorin A. Adapted from ref 91.

work, however, did not report a completed synthesis but rather an enantioselective route to ring A and a model route to ring C.[90]

More recently, an asymmetric synthesis of **2a** in 33 steps was reported.[91] The construction of the tricyclic core of **2a** (**149**) was accomplished by a transannular Michael reaction cascade of macrocycle **150**. Bisenone **150** was prepared in a convergent assembly from vinyl iodide **151** and aldehyde **152**. However, this synthetic route produces predominantly the C-8 epimer of **2a** but epimerization studies produced **2a** identical to natural material. This synthesis demonstrates the utility of a transannular reaction cascade in constructing polycyclic structures and offers a new method of preparing analogues of **2a** with modified C-12 functionality.[91]

4. Studies on the Mode of Binding of Salvinorin A

A number of models have been proposed to explain the selectivity and binding of opioid receptor ligands. Current thinking is that KOP-selective opiate antagonists recognize three elements within the KOP: (1) a highly conserved aspartate in TM III; (2) an aromatic pocket formed by TMs V, VI, and VII; (3) a KOP specific selectivity site in TM VI.[92–97] This model, however, does not readily apply to other classes of KOP ligands, such as **10**. However, several models of κ agonist binding have been reported.[98–103] It has been proposed that the Asp138 carboxylate in TM III forms a salt bridge with the protonated nitrogen of arylacetamides and benzomorphans, a hydrophobic pocket consisting of Tyr312, Leu224, Leu295, and Ala298 side chains hosts the phenyl ring of arylacetamides such as **10**, and His291 makes a hydrogen bond with the phenolic group of the benzomor-

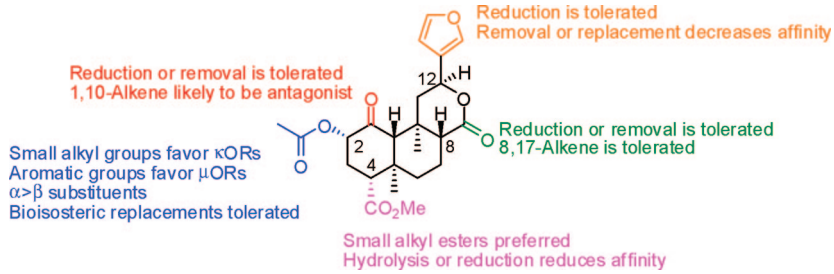

Figure 13. Summary of SAR for salvinorin A analogs.

phans such as **7**.[98] Another model was developed to study the mode of binding of dynorphin A(1–8).[102] This model found that amino acid residues in EL2, TM III, TM IV, and TM V determine the selectivity of peptide agonists for KOPs.

There have been several studies directed at elucidating the mode of binding of **2a** at the KOP.[17,83,104,105] Initially, molecular modeling studies suggested four interactions that might explain the binding of **2a**: (1) Gln115 forming a hydrogen bond with the furan oxygen; (2) Tyr139 interacting with the C-17 carbonyl; (3) Tyr312 interacting with the 4-carbomethoxy group; (4) Tyr313 interacting with the C-2 acetyl group.[17] This model, however, was revised based on structure–activity relationship studies. A more recent model[83] proposed three points of interaction: (1) the furan oxygen interacting with Tyr119 and Tyr320; (2) the 4-carbomethoxy group interacting with Glu297 and Ile294; (3) the 2-acetyl group interacting in a hydrophobic manner with Tyr313. This model was developed out of site-directed mutagenesis studies using thiol **99**. For the Tyr313 mutant, the affinity of **99** was enhanced compared with **2a** suggesting that this residue in helix 7 is close to the C-2 position of **2a**.[83] Collectively, the mutagenesis studies imply that **2a** utilizes unique residues within the commonly shared binding pocket of the KOP.

An additional model has been proposed by Kane et al. through chimeras and single-point mutations.[104] This work proposes that **2a** recognizes the KOP through a unique binding epitope involving interactions in TM II, TM IV, and EL-2.[104] In the qualitative binding-site model, **2a** vertically spans residues Tyr119, Tyr320, Gln115, Tyr313, and Tyr312. As in the model proposed by Yan et al., **2a** is in close proximity to EL-2. Overall, this model begins to elucidate how **2a** binds using hydrophobic interactions rather than salt-link interactions at the KOP.

Recently, an approach utilizing chimeras, site-directed mutagenesis, and the substituted cysteine accessibility method was used to investigate the selectivity of **2a** for KOPs.[105] It was found that helix 2 is required for the binding of **2a** to the KOP and two residues, Val108 and Val118, are responsible for the selectivity for the KOP. These two residues result in a differential pattern of amino acids that have access to the binding pocket. This finding supports **2a** having a novel mode of binding that imparts subtype selectivity for GPCR ligands.

5. Summary and Outlook

The body of knowledge of the chemistry, pharmacology, and neurobiology of opioid receptors continues to grow rapidly. Despite these advances, many aspects of the three-dimensional structure of μ, δ, and κ opioid receptors remain elusive. *Salvia divinorum* and its major active component **2a** have the potential to identify novel opioid receptor probes that will aid in addressing these aspects, as well as opening additional areas for chemical investigation. Opioid agonists based on **2a** have the potential to treat pain, cough, diarrhea, stimulant dependence, and mood disorders. Antagonists derived from **2a** have potential utility in treating a number of conditions including drug dependence, depression, opioid-induced constipation, and obesity. Thus, analogues of **2a** may prove to be excellent research tools and give greater insight into opioid receptor mediated phenomena.

Medicinal chemistry efforts have begun to explore the structure–activity relationship studies of **2a**. To date, these have mainly focused on its high affinity and selectivity for the KOP. Future work is likely to identify additional analogues of **2a** with altered selectivity for MOPs and DOPs, as well as novel allosteric modulators of opioid receptors.[67] The SAR known at this time is summarized in Figure 13. At the C-1 position, (1) reduction or removal of the carbonyl is tolerated, and (2) introduction of a 1,10-alkene increases the likelihood of antagonist activity. At the C-2 position, (1) small alkyl esters favor binding to KOPs, whereas aromatic esters favor binding to MOPs, (2) replacement of the acetoxy with ethers and amines is tolerated, (3) generally α-substituents are preferred over the corresponding β-substituents, and (4) bioisosteric replacement of the acetoxy group with amide, thioester, and sulfonate esters is tolerated. At the C-4 position, (1) small alkyl chains are preferential for binding to KOPs, (2) hydrolysis or reduction of the carbomethoxy group leads to reduced affinity at KOPs, and (3) generally conversion to an amide is not tolerated. At the C-17 position, (1) reduction or removal of the carbonyl is tolerated, and (2) introduction of an 8,17-alkene is also tolerated. Finally at C-12, the furan ring may be reduced or replaced, but this leads to a reduction in affinity. Several models suggest that the affinity and selectivity of **2a** for KOPs is the result of interactions with unique residues compared with other KOP ligands.

In 1929, the eminent pharmacologist Reid Hunt articulated a guiding premise for the development of a program at the NIH to address drug abuse problems. He stated that, "A thorough study of the morphine molecule might show a possibility of separating the analgesic from the habit forming property . . . work along these lines would involve cooperation between the highest type of organic chemists and pharmacologists."[106–108] Studies under this directive lead to the identification of metopon (**6**).[22] Early preliminary pharmacological studies of **6** in animals and humans showed this compound to retain morphine-like analgesia but cause fewer undesirable side effects than morphine. These observations provided the initial "proof of principle" that permeates contemporary opioid research.[108] Continued research into chemistry and pharmacology of opioid receptor ligands, such as **2a** or other natural products, may yet yield the holy grail of opioids.[109]

6. Acknowledgments

The authors thank the National Institute on Drug Abuse, NIH, and the Universities of Iowa and Kansas for financial support of ongoing research programs. 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.

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