

LOOKING AHEAD

SELECTIVE NATURAL κ OPIOID AND CANNABINOID RECEPTOR AGONISTS WITH A POTENTIAL ROLE IN THE TREATMENT OF GASTROINTESTINAL DYSFUNCTION

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Salvia divinorum (Epling and Jativa-M.), also termed "diviner's sage", "magic mint", "hojas de la Pastora", "xka (ska) Pastora" (the leaves of the shepherdess), or "hierba María" (the Virgin Mary's herb), is a plant traditionally used by the Mazatec Indians of Mexico in their divination rites. The major active ingredient, neoclerodane diterpenoid salvinorin A (Fig. 1) is a potent and selective κ opioid receptor (KOR) agonist.¹ Other neoclerodane diterpenoids obtained from *S. divinorum* include salvinorin B (Fig. 1), salvinorins C-I,²⁻⁵ divinorins A-F,⁴⁻⁶ salvinicins A-B⁷ and salvidivins A-D.⁵

In traditional medicine, extracts from *S. divinorum* are used in the treatment of diarrhea. Recent studies on extracts from *S. divinorum* and pure salvinorin A showed potent effects on gastrointestinal (GI) motility in vitro and in vivo, supporting a future role in the treatment of human GI disorders. This report aims to summarize the pharmacology of salvinorin A and give an overview on the present knowledge of the effects of salvinorin A on GI function.

PHARMACOLOGY OF SALVINORIN A

Salvinorin A and κ opioid receptors

Biological actions of opiates and opioid peptides are mediated by opioid receptors, namely μ (MOR), δ (DOR) and KOR (for review,

see ⁸). Opioid receptors are coupled to G_i/G_o proteins and affect numerous cellular mechanisms, which include inhibition of adenylyl cyclase, enhancement of K^+ conductance, decrease in Ca^{2+} conductance through inhibition of N-, P-, Q- and R-type voltage-activated Ca^{2+} channels, and activation of p42/p44 mitogen-activated protein kinases (for review, see ⁹). All of these mechanisms are known to be involved in the regulation of GI function.

Activation of KOR in vivo mediates pain perception and antinociception,^{10,11} mood,¹² hypolocomotor activity¹³ and responses to drugs of abuse, such as expression of chronic morphine-induced withdrawal syndromes.^{13,14} KORs, together with MORs, also play an important role in the GI tract. For example, activation of KOR inhibits cholinergic and noncholinergic excitatory trans-

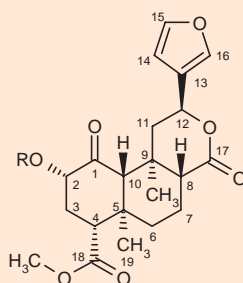
Analogues of salvinorin A, an active ingredient in *Salvia divinorum*, provide an attractive target as future therapeutics in human gastrointestinal dysfunctions.

SUMMARY

Salvinorin A is the major active ingredient of *Salvia divinorum*, a plant used by the Mazatec Indians of Mexico for spiritual and medical purposes. Different preparations from *S. divinorum* are also used in traditional healing practices to treat gastrointestinal disorders, including diarrhea. The recent extensive research on salvinorin A and other neoclerodane diterpenoids derived from *S. divinorum* resulted in a large number of reports describing their isolation, synthesis and structure-activity relationship. This review summarizes the present knowledge on salvinorin A and its analogues, with a focus on the effects on gastrointestinal tissues. We furthermore discuss structural changes in salvinorin A that may facilitate future use of its derivatives in human disease.

mission within myenteric plexus and thus decreases smooth muscle contractility,^{15,16} This KOR-induced decrease in muscle contractility in vitro translates into a decrease of intestinal transit in vivo.¹⁶

Salvinorin A was found to be a potent KOR agonist (EC_{50} of 1.05 nM for the inhibition of adenylyl cyclase activity in HEK-293 cells expressing human KOR), with high affinity and



Salvinorin A, R = Ac
Salvinorin B, R = H

Figure 1. Structures of salvinorin A and salvinorin B isolated from *Salvia divinorum*.

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selectivity at both cloned and physiological (guinea pig brain) binding sites (Table I).¹ The agonist efficacy of salvinorin A was not significantly different from that of dynorphin A(1-17), an endogenous KOR peptide ligand¹⁷ (Fig. 2) and other KOR-selective agonists.¹⁸ Salvinorin A had negligible affinity at other known receptors for psychoactive compounds, e.g., MOR, DOR, NOP, cannabinoid CB₁ and CB₂, muscarinic and nicotinic acid, as well as ionotropic and metabotropic glutamate receptors.¹¹⁷

Salvinorin B, which may be formed from salvinorin A via an esterase-mediated hydrolysis, had modest ($K_i = 65.9 \pm 8.6$ nM) to low ($> 10,000$ nM) affinity and low potency at KOR.^{4,17} Interestingly, the salvinorin-A-related natural compound salvidivin A has been identified as the first neoclerodane diterpenoid with KOR antagonist activity.²⁰

The antinociceptive profile of salvinorin A was characterized in chemo (*p*-phenylquinone writhing, acetic acid abdominal constriction) and thermal (tail flick, hot plate) nociceptive assays (Table II). The studies revealed that both central (intracerebroventricular, intrathecal) and peripheral (intraperitoneal, subcutaneous) administration of salvinorin A produced a dose-dependent nor-binaltorphimine (nor-BNI, KOR antagonist)-reversible

Table I. Opioid receptor binding data for salvinorin A, salvinorin B and selective mu (MOR), delta (DOR) and kappa (KOR) opioid receptor ligands^a

	K_i (nM)			³⁵ S]-GTPγS EC ₅₀ (nM)		
	MOR	DOR	KOR	MOR	DOR	KOR
Salvinorin A	> 1000	> 1000	7.90 ± 0.80 ^b 18.7 ± 3.40 ^c	> 1000	> 1000	4.6 ± 1.2
Salvinorin B	> 1000	> 1000	> 1000	> 1000	> 1000	> 1000
Dynorphin A	8.10 ± 0.20	5.80 ± 0.80	1.70 ± 0.10	65 ± 34	72 ± 12	5.65 ± 2.08
U-50488	294 ± 49	> 1000	0.20 ± 0.01	> 1000	> 1000	9.31 ± 2.54
U-69593	692 ± 97	> 1000	0.70 ± 0.05	> 1000	> 1000	26.1 ± 10.7

^aData from ¹⁷⁻¹⁹

^bData from ¹⁷

^cData from ¹⁸

antinociception.^{18,24-27} Other *in vivo* effects of salvinorin A, mediated by KOR, included hypothermia,²⁴ sedation and motor incoordination³¹ (Table II). Carlezon et al. reported that salvinorin A possessed prodepressant-like effects in the rat forced swimming test and the intracranial self-stimulation assay.²⁸ The duration of the effects of salvinorin A *in vivo* was short, regardless of species, behavioral endpoint or administration route.³² A pharmacokinetic study in nonhuman primates

showed that the elimination $t_{1/2}$ of salvinorin A in serum was less than 1 h.³³

μ Opioid receptor-mediated effects of salvinorin A

The results of receptor binding studies demonstrated that salvinorin A does not possess any significant affinity at the MOR.¹ Interestingly, functional binding assays indicated that salvinorin A may have some partial agonist activity at the MOR. However, in

Table II. Biological actions of salvinorin A (*in vivo*)

Phenomenon	Observed effect	Receptors involved	Species	Site of administration	Ref.
Gastrointestinal motility	Decreased	KOR, CB ₁ , CB ₂ (healthy tissue) KOR (inflamed tissue)	Mouse	i.p.	21-23, 85
Pain	Antinociception	KOR	Mouse	i.c.v. i.t. s.c. i.p.	24 25 26 27
Depression	Prodepressive	KOR	Rat	i.p.	28
Drug addiction, reward	Conditioned place preference rewarding effects	KOR, CB ₁ KOR, CB ₁	Zebrafish Rat	i.c.v., s.c.	29 30
Locomotor activity	Increased (0.1 and 0.2 μg/kg) Decreased (5 and 10 μg/kg)	KOR, CB ₁	Zebrafish		29
Sedation, ataxia	Sedation, motor incoordination	KOR	Mouse	i.p.	31
Thermoregulation	Hypothermia	KOR	Mouse	i.c.v.	24

KOR, κ opioid receptor; CB₁, cannabinoid receptor 1; i.c.v., intracerebroventricular; i.t., intrathecal.

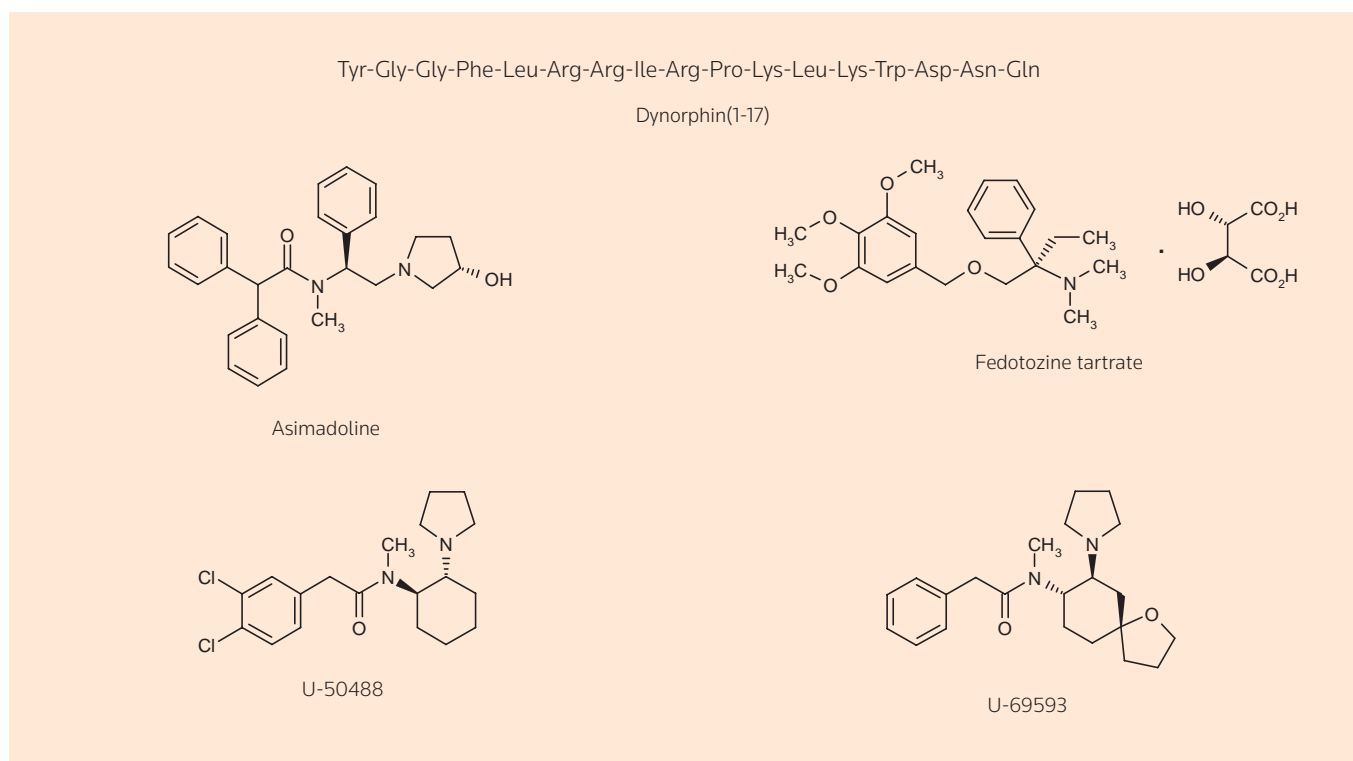


Figure 2. Structures of κ opioid receptor agonists.

KOR knock-out mice, salvinorin A did not induce antinociception, suggesting that the potency of salvinorin A at the MOR is probably too low to produce detectable effects.²⁴ Recently published data suggested that salvinorin A and some of its analogues are negative allosteric modulators of MOR binding in Chinese hamster ovary cells and rat brain membranes, but the physiological relevance of this observation has yet to be determined.^{26,34,35}

Notably, salvinorin-A-related salvinicin B exhibited antagonist activity at the MOR with a $K_i > 1.9 \mu\text{M}$ and was the first neoclerodane diterpenoid with antagonist effects at this opioid receptor.⁷ However, others could not confirm these observations and salvinicin B was found to have negligible affinity at the MOR and the DOR ($K_i > 10,000 \text{ nM}$).²⁰

Salvinorin A and cannabinoid receptors

The potential for abuse of salvinorin A has been recently evaluated in various animal models. Butelman et al.³⁶ demonstrated that salvinorin A produced discriminative stimulus effects in rhesus monkeys, similar to those obtained with the KOR agonist

U-69593, while Willmore-Fordham et al.³⁷ observed analogous activity in rats. The direct stimulation of cannabinoid receptors CB_1 and CB_2 was not observed; however, some rewarding effects were noticed in animal models.²⁹ When given at very low dose, salvinorin A was able to induce place preference in rats and rewarding effects in the zebrafish model.²⁹ This rewarding activity was significantly blocked by the pretreatment with either the KOR antagonist nor-BNI or the CB_1 receptor antagonist rimonabant, suggesting that both KOR and CB_1 receptors are involved.

Kinetic studies of salvinorin A

Kinetic studies of salvinorin A were recently summarized by Wang et al.,³² who described the duration of the effects of salvinorin A in vivo as short regardless of species, behavioral endpoint or administration route. The following is an overview on the studies that examined in vivo duration of the effects of salvinorin A.

In mice, the antinociceptive effect of salvinorin A disappeared within 20 min after intraperitoneal injection in the acetic acid abdominal constriction test.^{18,27} In the mouse

tail flick test, the antinociceptive effect was undetectable 20 min after intrathecal injection or 30 to 45 min after intracerebroventricular injection.^{24,25} Intravenous administration of salvinorin A increased prolactin levels in rhesus monkeys, but the duration was only 30 min, shorter than that of U-69593 (90 min) at the same dose.³⁸ A pharmacokinetic study in nonhuman primates showed that the elimination $t_{1/2}$ of salvinorin A in serum was less than 1 h, a finding which is in agreement with the duration of effects observed in animal studies.³³

In humans, the hallucinogenic effect of salvinorin A was brief when inhaled, reaching full effect in about 30 s, lasting 5–10 min, and subsiding in 20–30 min. However, a smoked dose of 200–500 μg of salvinorin A produced profound hallucinations lasting up to 1 h.³⁹

Recently, Hooker et al.⁴⁰ synthesized [^{11}C]-labeled salvinorin A and used positron emission tomography (PET) to measure its pharmacokinetics and distribution in the brain and peripheral organs of female primates. These PET studies indicated extremely rapid brain uptake reaching a peak accounting for 3.3% of the total administered dose within

40 s and clearing with a half-life of 8 min. [^{11}C]-Salvinorin A was distributed throughout the brain with the highest concentration in the cerebellum and a notable concentration in the visual cortex, probably accounting for its physiological effects when smoked. Peripheral organ kinetics suggested at least two modes of metabolism and excretion occur via the renal and biliary systems.⁴⁰ The exceptionally rapid uptake and brief duration of salvinorin A in the brain matched the time course of visual hallucinations for *S. divinorum* when smoked.

Effects of salvinorin A in humans

The Mazatec Indians have been using *S. divinorum* in their traditional spiritual practices for ritual divination and curing.⁴¹ In those practices, fresh leaves are either masticated and swallowed or crushed to extract the juices; another way of ingestion is by smoking dry leaves.⁴² Since salvinorin A is rapidly degraded in the GI tract, absorption through oral mucosa seems to be the most effective way of administration of salvinorin A and its natural derivatives.

S. divinorum preparations containing sub-hallucinogenic doses of salvinorin A (< 3 $\mu\text{g}/\text{kg}$ salvinorin A) have been used by the Mazatec Indians to help cure anemia, headache, rheumatism, depression and a disease named "panzon de barrego" (which translates best into swollen abdomen).⁴¹

High doses of salvinorin A (3–7.5 $\mu\text{g}/\text{kg}$) induce intense, short-lasting (up to 1 h), aural or visionary hallucinatory experiences, accompanied by changes in depth perception or increase in sensual and esthetic appreciation.^{42,43} The hallucinogenic potency of salvinorin A (200–500 μg) is comparable to that of classical hallucinogens, such as lysergic acid diethylamide (LSD, effective human dose 20–250 μg) or 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB, effective human dose 500–1000 μg).^{41,42,44} However, salvinorin A has no activity at the serotonin 5-HT_{2A} receptor, the presumed molecular target for LSD and DOB, and therefore it remains unclear by which pathway salvinorin A directly or indirectly induces central effects.⁴⁵

EFFECTS OF SALVINORIN A IN GASTROINTESTINAL TRACT

A significant number of reports on medicinal or recreational use of salvinorin A have been published, but only some refer to effects

besides hallucinogenic ones in a systematic manner. A few reports characterize the effects of salvinorin A on the GI tract.

Capasso et al. evaluated the effect of a standardized extract from the leaves of *S. divinorum* and salvinorin A on cholinergic transmission in guinea pig ileum.²³ *S. divinorum* and salvinorin A reduced electrically evoked neurogenic contractions of intestinal smooth muscle without modifying the contractions elicited by exogenous acetylcholine, suggesting a prejunctional neuronal site of action for salvinorin A. The effect on cholinergic transmission was most likely mediated through activation of KORs, since it was counteracted by the specific KOR antagonist nor-BNI and the universal opioid receptor antagonist naloxone.

Recently, the same group evaluated the effects of *S. divinorum* and salvinorin A on motility in mice in vivo, both in physiological states and during croton-oil-induced intestinal inflammation.²² In physiological states, salvinorin A (only at higher doses, 3 mg/kg and 10 mg/kg) inhibited motility through a non-KOR-mediated mechanism. The potency of salvinorin A significantly increased during intestinal inflammation while this was not observed for the KOR-selective agonist U-50488. The results demonstrated that salvinorin A may have different effects and targets in the inflamed and non-inflamed gut.

This observation was confirmed in a subsequent study, which reported the involvement of CB₁ receptors in the inhibitory effect of salvinorin A on GI motility.²¹ Previous studies in the central nervous system (CNS) have already suggested that KORs and CB receptors undergo some crosstalk. For instance, the rewarding effects induced by salvinorin A administration in zebrafish and rats were significantly reversed by either nor-BNI or the CB₁ antagonist rimobant.^{29,30} Another report additionally pointed out a common mechanism for Δ^9 -tetrahydrocannabinol (THC), a nonselective CB receptor agonist, and KORs in mediating antinociception.⁴⁶ However, binding experiments showed only very weak affinity of salvinorin A for CB receptors and no inhibitory effect on endocannabinoid hydrolysis and cellular uptake.²¹

Presently, we can only speculate on potential effects of acutely or chronically administered salvinorin A on the human GI tract.

These effects could be produced either via the CNS or peripheral sites (Fig. 3).

KORs are widely distributed in the human CNS, but they are localized distinctly from other opioid receptors.^{47,48} Since intracerebroventricular administration of KOR agonists does not influence GI motility,^{49,50} it could be suggested that low doses of salvinorin A would not produce any effect on the GI tract via central KORs. However, salvinorin A might activate central CB₁ receptors which could influence gastric motility and lower esophageal sphincter function,⁵¹ as well as gut homeostasis via alterations in the hypothalamic–pituitary–adrenal axis.⁵²

Like other KOR agonists, salvinorin A may influence energy levels and body weight. It was shown that dynorphin A(1-17) induced ingestive behavior in satiated rats via KOR₁ receptors.⁵³ Since salvinorin A also acts via KOR₁ receptors,²⁴ weight gain after administration of salvinorin A may be expected. This effect could be potentiated by salvinorin-A-mediated activation of CB₁ receptors in the brain, which increases appetite and is crucial in control of food intake and body weight.⁵⁴ Overall, salvinorin A could be a future appetite-stimulating agent, but this potential has yet to be detailed.

The peripheral site of action for salvinorin A is the enteric nervous system (ENS), where neurons of myenteric plexus ganglia strongly express KORs,^{55,56} and visceral sensory neurons, where KORs are responsible for antinociception to colonic distension.⁵⁷ Since salvinorin-A-induced effects may also involve CB receptors, as suggested by Capasso et al.,²¹ it is important to note that CB receptors are substantially expressed in the ENS.^{58,59} One study showed colocalization of KORs and CB receptors in cultured myenteric neurons, supporting the notion of a KOR–CB crosstalk.⁶⁰

Since salvinorin A displays high selectivity at KORs over MORs and DORs,¹ it is unlikely that it could produce significant effects on the GI tract via other types of opioid receptors.

SALVINORIN A AS A POTENTIAL DRUG FOR GASTROINTESTINAL DISEASES

Due to its unique pharmacological activity, salvinorin A might become an important template for the design and synthesis of future therapeutic agents, which act not

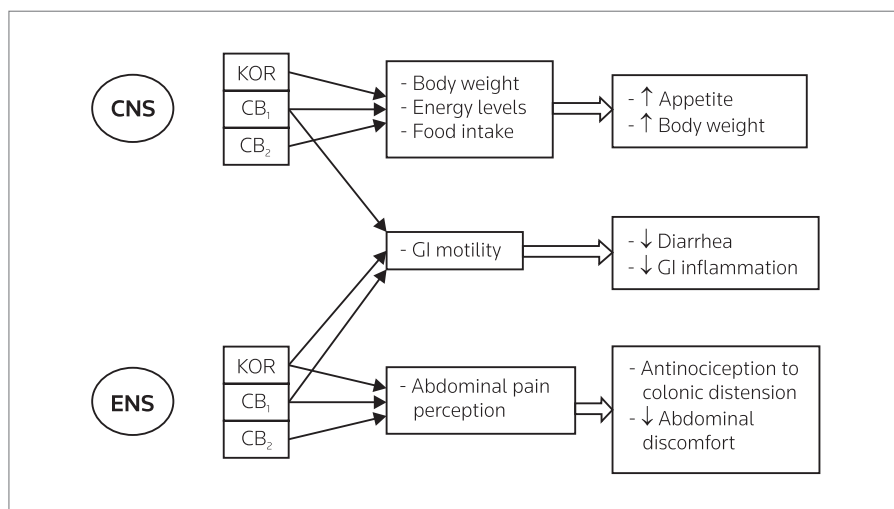


Figure 3. Possible involvement of central and peripheral κ opioid (KOR) and cannabinoid (CB) receptors in the effects of salvinorin A on the gastrointestinal (GI) tract. CNS, central nervous system; ENS, enteric nervous system (myenteric plexus ganglia and visceral sensory neurons).

only at the KOR, but also at CB binding sites, thus making use of the beneficial properties of two classes of receptors. The problem of the central side effects that presently limits a broad pharmacological use of compounds like salvinorin A can be addressed by targeted drug development of salvinorin A derivatives, which cannot cross the blood–brain barrier. Table III provides a summary of all potential GI indications as discussed below.

A peripherally restricted salvinorin-A-based drug would have a dual effect on GI motility and function. Such a drug could activate peripheral KORs, which have been shown on sensory afferent fibers to reduce visceral nociception.⁵⁷ Since KORs and CB₁ receptors are present in the ENS, salvinorin-A-derived compounds might also exert antidiarrheal properties by slowing GI transit through depression of cholinergic neurotransmission, as shown for salvinorin-A in animal studies.^{21–23} Due to these beneficial effects on GI motility and abdominal pain perception, diarrhea-predominant irritable bowel syndrome (IBS) may be a good target for potential salvinorin-A-related drugs. IBS is a combination of symptoms, including abdominal discomfort and pain and alterations in the pattern of defecation, as defined in the Rome III criteria. It can be subdivided as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C) and an alternating or mixed IBS subgroup (IBS-A).

Alternative approaches in reducing visceral pain have already integrated the use of KOR agonists. These attempts used KOR agonists without central opioid effects, as suggested for salvinorin-A-derived compounds.^{57,61} Among them, asimadoline and fedotozine (Fig. 2), two very potent and selective KOR agonists, were shown to act on peripheral nerve endings of sensory vagal and nonvagal afferent pathways, where they increased thresholds of perception in patients during colonic distension without modifying colonic compliance and small bowel transit.^{62–67} Asimadoline had very low distribution to the brain,⁶⁸ did not elicit opioid-like dependence in humans⁶⁵ and displayed a good safety profile in healthy volunteers.⁶⁵ In clinical trials, asimadoline favorably affected symptoms of IBS and produced significant improvement on pain or discomfort in IBS-A and IBS-D patients, who had at least moderate pain at baseline.⁶⁹

The occurrence of GI motor disturbances, associated with a delay in gastric emptying and small intestine transit is a further aspect of IBS. Fedotozine was found to block the colonic distension-induced delay on gastric motility and emptying.⁷⁰ In functional dyspepsia, however, a disorder based on hypersensitivity to mechanical stimuli in the stomach, asimadoline did not significantly alter symptoms of postprandial fullness and satiation.⁷¹

ADL-10-0101 is another peripherally restricted KOR agonist that has been shown to reduce abdominal pain associated with pancreatitis in humans.⁷² Clinical studies of ADL-10-0101 are under way.⁷³

Even though the role of asimadoline as a therapeutic agent was recently questioned (lack of dose–response relationship and no significant change in severity of abdominal pain compared to placebo after on-demand administration),^{65,69} most of the clinical studies on peripherally active KOR indicate the potential of these drugs in the treatment of IBS and suggest that salvinorin-A-derived compounds with limited central activity warrant further investigation and should be employed in clinical trials.

It is well known that CB₁ receptor activation protects against 2,4-dinitrobenzene sulfonic acid (DNBS)- and dextran sulfate sodium (DSS)-induced experimental colitis in mice.⁷⁴ Peripherally restricted salvinorin A derivatives targeting the KOR and the CB systems should produce relaxant, antinociceptive and anti-inflammatory activities in the gut without the typical psychoactive side effects of salvinorin A. Because GI inflammation upregulates CB₁ receptor expression,⁷⁴ low doses of such a derivative may already be efficient in ameliorating symptoms associated with colitis, as lower doses of salvinorin A were more effective in slowing GI transit in inflamed than in healthy mice.²¹

STRUCTURAL MODIFICATIONS OF SALVINORIN A TOWARDS A FUTURE HUMAN USE

The main goals of the structure–activity relationship studies of salvinorin A are 1) to obtain peripherally restricted analogues with high KOR potency and selectivity, and increased in vivo stability, and 2) converting salvinorin A into a selective antagonist at the KOR. In the following we summarize the most important findings, which might lead to the design of future salvinorin-A-based therapeutics.

Position 1

The presence of a C-1 ketone group seems important, but not crucial for agonist activity at the KOR. In the most recent study, the removal of the C-1 ketone group decreased the agonist activity at the KOR 6-fold ($EC_{50} = 280$ nM vs. $EC_{50} = 45$ nM) and increased antagonist activity at the MOR

Table III. Potential indications of salvinorin-A-derived compounds

	Antidiarrheal	Anticipated effect Alleviation of abdominal discomfort and pain	Anti-inflammatory
Inflammatory bowel disease	+		+
Irritable bowel syndrome	+	+	
Diseases with increased GI transit (diarrhea)	+		
Nonspecific abdominal pain		+	

GI, gastrointestinal.

($K_e = 170$ nM) and DOR ($K_e = 100$ nM) (Fig. 4a).⁷⁵ Reduction of the C-1 ketone to produce the corresponding α -alcohol changed the efficacy at KOR from a full agonist to an antagonist ($E_{max} = 108\%$ vs. $K_e = 240$ nM, respectively) (Fig. 4b). Similarly, the introduction of a 1,10-alkene functionality resulted in a switch of efficacy from partial agonist to antagonist of the KOR ($K_e = 570$ nM) (Fig. 4c).

Position 2

C-2 is a sensitive and crucial site for binding to the KOR with very limited structure tolerance in terms of size and electronegativity of the substituent group. Beguin et al.⁷⁶ suggested that the three-atom branched chains give the highest affinities and potencies. They also demonstrated that the natural configuration at C-2 provides better molecular complementarity with the KOR for C-2 esters, ethers and amides, but the trend is reversed when charged substituents are introduced.⁷⁷

The group of Zjawiony developed and biologically evaluated several salvinorin A analogues substituted at C-2, including bioisosteres⁷⁸ and thio-derivatives, using binding and functional assays.⁷⁹ Of interest, the C-2 thioacetate isostere (Fig. 5a) dis-

played affinity at the KOR ($K_i = 8$ nM vs. $K_i = 5$ nM) comparable to salvinorin A.

One of the most potent C-2 analogues synthesized so far is a methoxymethyl (MOM) derivative, displaying greater KOR binding affinity than salvinorin A and U-50488 (Fig. 5b).⁸⁰ MOM-salvinorin A was more potent than U-50488 and salvinorin A in hKOR-mediated G protein activation and in promoting internalization and downregulation of the hKOR in vitro.³² In vivo studies demonstrate that it produced significant antinociceptive and hypothermic effects. Also of note, the 2-fluoroethoxymethyl ether showed potency and affinity approximately equal to those of salvinorin A and is the first potent, selective fluorinated ligand at the KOR to be reported (Fig. 5c).⁸¹

Surprisingly, the introduction of an aromatic moiety in position 2 increased affinity at the MOR.²⁶ This salvinorin A derivative, designated herkinorin, represents the first neoclerodane diterpenoid that binds selectively to the MOR. Herkinorin displayed 25-fold higher affinity and potency at the MOR compared to salvinorin A ($K_i = 12$ nM vs. $K_i > 1000$ nM) and a 5-fold higher affinity at the DOR ($K_i = 1170$ nM vs. $K_i = 5790$ nM) (Fig. 5d). The

in vitro studies demonstrated that herkinorin activated the MOR and induced signaling through a single mitogen-activated protein kinase pathway, without stimulating receptor- β -arrestin-2 interactions and receptor internalization.⁸² Such compounds may be therapeutically useful and may represent promising candidates in the development of opiate analgesics that promote pain relief with limited adverse effects, including antinociceptive tolerance, constipation and respiratory suppression. In the in vivo assays, peripheral injections of herkinorin produced localized analgesia, blocked by the opioid antagonist naloxone. However, systemic injections did not produce any significant antinociceptive effect, which might be due to poor solubility of the compound and susceptibility to metabolic degradation.

One analogue of herkinorin was shown to display a decreased affinity at the KOR and significantly increased affinity and activity at the MOR, compared to the parent compound⁸¹ (Fig. 5e). Therefore, it has been identified as the most potent neoclerodane MOR agonist described to date. However, unlike other MOR ligands derived from herkinorin, it induced robust β -arrestin translocation to the plasma membrane and MOR internalization in HEK-293 cells. These effects were similar to those produced by DAMGO and other classic MOR agonists.

Positions 4 and 18

The predicted role of the methyl ester at C-4 as one of the pharmacophores of salvinorin A was initially confirmed by Munro et al.⁸³ in that the 4-hydroxymethyl analogue of salvinorin A (Fig. 6b) appeared to be a KOR antagonist, which labeled, but did not activate the receptor. However, the study of Lee et al.⁸⁴ produced contradictory results in which full

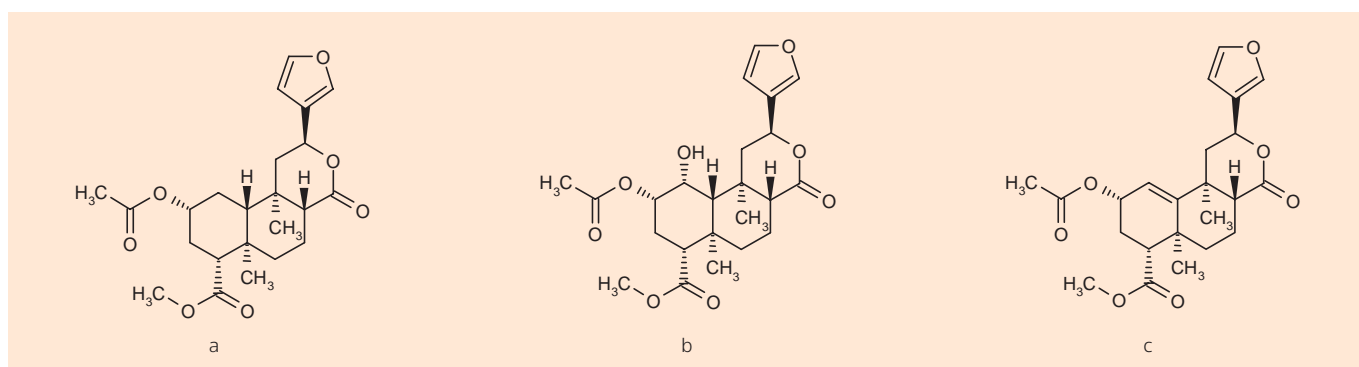


Figure 4. Salvinorin A analogues modified in position 1.

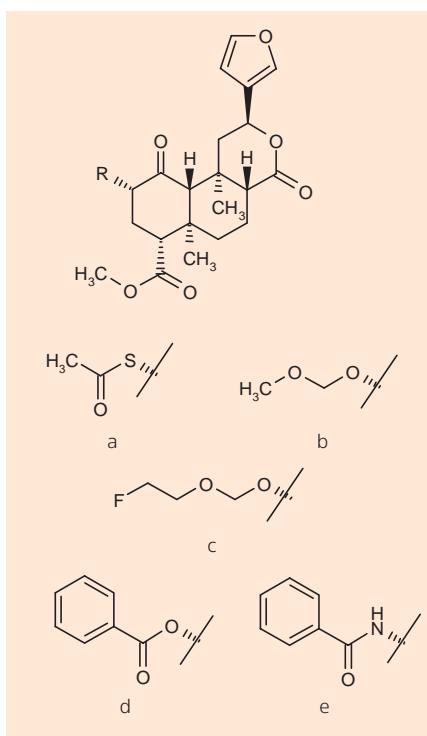


Figure 5. Salvinorin A analogues modified in position 2.

reduction of acid (Fig. 6a) to alcohol (Fig. 6b) increased potency and efficacy at the KOR ($EC_{50} = 20.7 \pm 1$ nM, with efficacy of 107%).

In an effort to convert salvinorin A into an antagonist, Beguin et al.⁷⁷ synthesized a number of C-18-modified analogues, using C-4 acid as a template. All molecular changes at C-18 induced a significant loss of activity, indicating that the introduction of hydrogen bond donors or charged residues and/or increasing the size of the substituent at C-18 prevents the molecule from binding to a tight lipophilic pocket of the KOR.

Position 12/Furan ring

The removal of the furan ring resulted in a greater than 1700-fold loss in affinity at the KOR compared to salvinorin A ($K_i = 3400$ nM vs. $K_i = 1.9$ nM), indicating its crucial role in binding at this receptor²⁰ (Fig. 7a). Replacement of the furan ring with 4-methyl-1,3,5-oxadiazoline resulted in > 29-fold loss of affinity compared to salvinorin A (Fig. 7b).²⁰ However, this analogue was the first neoclerodane diterpenoid with antagonistic effect at the MOR ($K_e = 430$ nM) and the KOR ($K_e = 360$ nM).

SUMMARY AND FUTURE PERSPECTIVES

Salvinorins and their derivatives are selective and powerful agonists of the KOR and CB receptors in the GI tract. Due to their distinct actions they may be used in the future in a broad range of pathologies—ranging from drug abuse and dependence, to cancer imaging and treatment, and GI disorders. To warrant the future applicability of salvinorin A analogues in the treatment of GI tract diseases, highly selective compounds with suppressed hallucinogenic activity and a clearly defined central/peripheral site of action need to be developed. So far, the most promising salvinorin-A-derived drug candidate is MOM-salvinorin A, a KOR-selective agonist resistant to enzymatic degradation and therefore with long-lasting activity. However, better understanding of salvinorin A metabolism and kinetics, its interaction with the KOR, as well as further structure–activity studies are still required.

Future studies need to address, whether — based on the structure of salvinorin A— it is

possible to develop drugs with a higher receptor selectivity, better tissue penetration, oral bioavailability and peripherally restricted activity, as well as a more favorable side effect profile. Additionally, basic scientific studies then need to specifically assess these drugs' effects on altered motility, intestinal inflammation and abdominal pain in respective disease models. The drugs that prove to be promising in these models may then proceed to clinical trials.

The opioid and endocannabinoid systems are complex; the KOR and CB receptors and their endogenous ligands are widely distributed throughout the body and affect a wide variety of processes. Knowledge on the localization and the role these receptors, possible interactions and activated intracellular pathways will provide the basis for the development of therapies involving salvinorin-A-based drugs. Furthermore, better understanding of the interaction between the CNS and ENS and their effect on GI function in humans is needed.

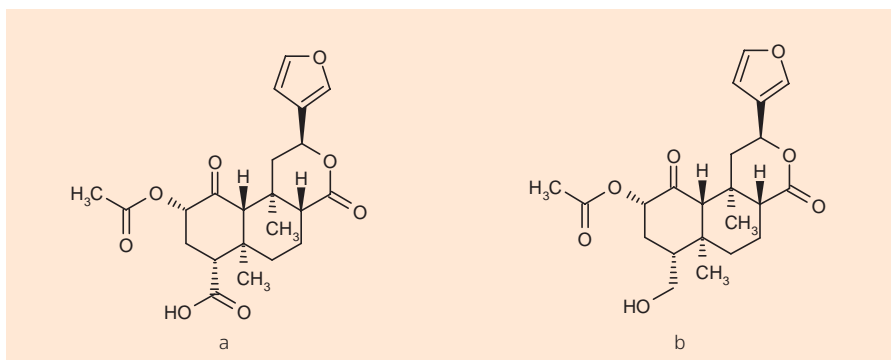


Figure 6. Salvinorin A analogues modified in position 4.

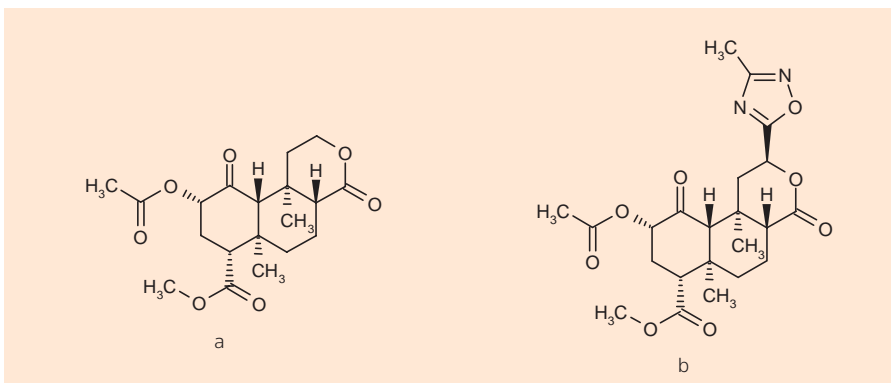


Figure 7. Salvinorin A analogues modified in position 12/furan ring.

In conclusion, salvinorin A analogues provide an attractive target as future therapeutics in human GI dysfunctions.

During the editorial process of this review paper, our research group published an article demonstrating that salvinorin A slows colonic motility and inhibits neurogenic ion transport in mice via three different receptors: the KOR, CB₁ and CB₂. Interestingly, the inhibitory effect of salvinorin A on smooth muscle contractions in vitro was mediated by the KOR and CB receptors, while its in vivo effect was mainly mediated by the KOR. These differences are most probably related to the pharmacokinetics and/or pharmacodynamics of salvinorin A.⁸⁵

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DISCLOSURE

The authors have no conflicts of interest to declare.

REFERENCES

- Roth, B.L., Baner, K., Westkaemper, R., et al. Salvinorin A: A potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc Natl Acad Sci U S A* 2002, 99(18): 11934-9.
- Valdes, L.J., III, Chang, H.M., Visger, D.C. and Koreeda, M. Salvinorin C, a new neoclerodane diterpene from a bioactive fraction of the hallucinogenic Mexican mint *Salvia divinorum*. *Org Lett* 2001, 3(24): 3935-7.
- Munro, T.A. and Rizzacasa, M.A. Salvinorins D-F, new neoclerodane diterpenoids from *Salvia divinorum*, and an improved method for the isolation of salvinorin A. *J Nat Prod* 2003, 66(5): 703-5.
- Lee, D.Y., Ma, Z., Liu-Chen, L.Y., Wang, Y., Chen, Y., Carlezon, W.A., Jr. and Cohen, B. New neoclerodane diterpenoids isolated from the leaves of *Salvia divinorum* and their binding affinities for human kappa opioid receptors. *Bioorg Med Chem* 2005, 13(19): 5635-9.
- Shirota, O., Nagamatsu, K. and Sekita, S. Neo-clerodane diterpenes from the hallucinogenic sage *Salvia divinorum*. *J Nat Prod* 2006, 69(12): 1782-6.
- A.K., Munro, T.A., Rizzacasa, M.A. and Robins-Browne, R.M. Divinatorins A-C, new neoclerodane diterpenoids from the controlled sage *Salvia divinorum*. *J Nat Prod* 2003, 66(9): 1242-4.
- Harding, W.W., Tidgewell, K., Schmidt, M. et al. Salvinicins A and B, new neoclerodane diterpenes from *Salvia divinorum*. *Org Lett* 2005, 7(14): 3017-20.
- Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H. and Watson, S.J. Anatomy of CNS opioid receptors. *Trends Neurosci* 1988, 11(7): 308-14.
- Law, P.Y., Wong, Y.H. and Loh, H.H. Molecular mechanisms and regulation of opioid receptor signaling. *Annu Rev Pharmacol Toxicol* 2000, 40: 389-430.
- Vonvoigtlander, P.F., Lahti, R.A. and Ludens, J.H. U-50,488: A selective and structurally novel non-Mu (kappa) opioid agonist. *J Pharmacol Exp Ther* 1983, 224(1): 7-12.
- Dykstra, L.A., Gmerek, D.E., Winger, G. and Woods, J.H. Kappa opioids in rhesus monkeys. I. Diuresis, sedation, analgesia and discriminative stimulus effects. *J Pharmacol Exp Ther* 1987, 242(2): 413-20.
- Pfeiffer, A., Brantl, V., Herz, A. and Emrich, H.M. Psychotomimesis mediated by kappa opiate receptors. *Science* 1986, 233(4765): 774-6.
- Simonin, F., Valverde, O., Smadja, C. et al. Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal. *EMBO J* 1998, 17(4): 886-97.
- Shippenberg, T.S. and Rea, W. Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* 1997, 57(3): 449-55.
- Chamouard, P., Klein, A., Martin, E., Adloff, M. and Angel, F. Regulatory role of enteric kappa opioid receptors in human colonic motility. *Life Sci* 1993, 53(14): 1149-56.
- De Schepper, H.U., Cremonini, F., Park, M.I. and Camilleri, M. Opioids and the gut: Pharmacology and current clinical experience. *Neurogastroenterol Motil* 2004, 16(4): 383-94.
- Chavkin, C., Sud, S., Jin, W. et al. Salvinorin A, an active component of the hallucinogenic sage *salvia divinorum* is a highly efficacious kappa-opioid receptor agonist: Structural and functional considerations. *J Pharmacol Exp Ther* 2004, 308(3): 1197-203.
- Wang, Y., Tang, K., Inan, S. et al. Comparison of pharmacological activities of three distinct kappa ligands (Salvinorin A, TRK-820 and 3FLB) on kappa opioid receptors in vitro and their antipruritic and antinociceptive activities in vivo. *J Pharmacol Exp Ther* 2005, 312(1): 220-30.
- Toll, L., Berzetei-Gurske, I.P., Polgar, W.E. et al. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res Monogr* 1998, 178: 440-66.
- Simpson, D.S., Katavic, P.L., Lozama, A. et al. Synthetic studies of neoclerodane diterpenes from *Salvia divinorum*: preparation and opioid receptor activity of salvinicins analogues. *J Med Chem* 2007, 50(15): 3596-603.
- Capasso, R., Borrelli, F., Cascio, M.G. et al. Inhibitory effect of salvinorin A, from *Salvia divinorum*, on ileitis-induced hypermotility: Cross-talk between kappa-opioid and cannabinoid CB1 receptors. *Br J Pharmacol* 2008, 155(5): 681-9.
- Capasso, R., Borrelli, F., Zjawiony, J. et al. The hallucinogenic herb *Salvia divinorum* and its active ingredient salvinorin A reduce inflammation-induced hypermotility in mice. *Neurogastroenterol Motil* 2008, 20(2): 142-8.
- Capasso, R., Borrelli, F., Capasso, F., Siebert, D.J., Stewart, D.J., Zjawiony, J.K. and Izzo, A.A. The hallucinogenic herb *Salvia divinorum* and its active ingredient salvinorin A inhibit enteric cholinergic transmission in the guinea-pig ileum. *Neurogastroenterol Motil* 2006, 18(1): 69-75.
- Ansonoff, M.A., Zhang, J., Czyzyk, T. et al. Antinociceptive and hypothermic effects of Salvinorin A are abolished in a novel strain of kappa-opioid receptor-1 knockout mice. *J Pharmacol Exp Ther* 2006, 318(2): 641-8.
- John, T.F., French, L.G. and Erlichman, J.S. The antinociceptive effect of salvinorin A in mice. *Eur J Pharmacol* 2006, 545(2-3): 129-33.
- Harding, W.W., Tidgewell, K., Byrd, N. et al. Neoclerodane diterpenes as a novel scaffold for mu opioid receptor ligands. *J Med Chem* 2005, 48(15): 4765-71.
- McCurdy, C.R., Sufka, K.J., Smith, G.H., Warnick, J.E. and Nieto, M.J. Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist. *Pharmacol Biochem Behav* 2006, 83(1): 109-13.
- Carlezon, W.A., Jr., Beguin, C., Dinieri, J.A. et al. Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J Pharmacol Exp Ther* 2006, 316(1): 440-7.
- Braida, D., Limonta, V., Pegorini, S., Zani, A., Guerini-Rocco, C., Gori, E. and Sala, M. Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid

- receptor involvement. *Psychopharmacology (Berl)* 2007, 190(4): 441-8.
30. Braidia, D., Limonta, V., Capurro, V. et al. *Involvement of kappa-opioid and endocannabinoid system on Salvinorin A-induced reward.* *Biol Psychiatry* 2008, 63(3): 286-92.
 31. Fantegrossi, W.E., Kugle, K.M., Valdes, L.J., III, Koreeda, M. and Woods, J.H. *Kappa-opioid receptor-mediated effects of the plant-derived hallucinogen, salvinorin A, on inverted screen performance in the mouse.* *Behav Pharmacol* 2005, 16(8): 627-33.
 32. Wang, Y., Chen, Y., Xu, W. et al. *2-Methoxymethyl-salvinorin B is a potent kappa opioid receptor agonist with longer lasting action in vivo than salvinorin A.* *J Pharmacol Exp Ther* 2008, 324(3): 1073-83.
 33. Schmidt, M.D., Schmidt, M.S., Butelman, E.R. et al. *Pharmacokinetics of the plant-derived kappa-opioid hallucinogen salvinorin A in nonhuman primates.* *Synapse* 2005, 58(3): 208-10.
 34. Rothman, R.B., Murphy, D.L., Xu, H. et al. *Salvinorin A: Allosteric interactions at the mu-opioid receptor.* *J Pharmacol Exp Ther* 2007, 320(2): 801-10.
 35. Ehler, F.J. *Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods.* *Mol Pharmacol* 1988, 33(2): 187-94.
 36. Butelman, E.R., Harris, T.J. and Kreek, M.J. *The plant-derived hallucinogen, salvinorin A, produces kappa-opioid agonist-like discriminative effects in rhesus monkeys.* *Psychopharmacology (Berl)* 2004, 172(2): 220-4.
 37. Willmore-Fordham, C.B., Krall, D.M., McCurdy, C.R. and Kinder, D.H. *The hallucinogen derived from Salvia divinorum, salvinorin A, has kappa-opioid agonist discriminative stimulus effects in rats.* *Neuropharmacology* 2007, 53(4): 481-6.
 38. Butelman, E.R., Mandau, M., Tidgewell, K., Prinsinzano, T.E., Yuferov, V. and Kreek, M.J. *Effects of salvinorin A, a kappa-opioid hallucinogen, on a neuroendocrine biomarker assay in nonhuman primates with high kappa-receptor homology to humans.* *J Pharmacol Exp Ther* 2007, 320(1): 300-6.
 39. Siebert, D.J. *Localization of salvinorin A and related compounds in glandular trichomes of the psychoactive sage, Salvia divinorum.* *Ann Bot (Lond)* 2004, 93(6): 763-71.
 40. Hooker, J.M., Xu, Y., Schiffer, W., Shea, C., Carter, P. and Fowler, J.S. *Pharmacokinetics of the potent hallucinogen, salvinorin A in primates parallels the rapid onset and short duration of effects in humans.* *Neuroimage* 2008, 41(3): 1044-50.
 41. Valdes, L.J., Hatfield, G.M., Koreeda, M. and Paul, A.G. *Studies of Salvia divinorum (Lamiaceae), an hallucinogenic mint from the Sierra Mazateca in Oaxaca, Central Mexico.* *Economic Botany* 1987, 41(2): 283-91.
 42. Siebert, D.J. *Salvia divinorum and salvinorin A: New pharmacologic findings.* *J Ethnopharmacol* 1994, 43(1): 53-6.
 43. Gonzalez, D., Riba, J., Bouso, J.C., Gomez-Jarabo, G. and Barbanoj, M.J. *Pattern of use and subjective effects of Salvia divinorum among recreational users.* *Drug Alcohol Depend* 2006, 85(2): 157-62.
 44. Sheffler, D.J. and Roth, B.L. *Salvinorin A: The "magic mint" hallucinogen finds a molecular target in the kappa opioid receptor.* *Trends Pharmacol Sci* 2003, 24(3): 107-9.
 45. Glennon, R.A., Titeler, M. and McKenney, J.D. *Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents.* *Life Sci* 1984, 35(25): 2505-11.
 46. Smith, P.B., Welch, S.P. and Martin, B.R. *Interactions between delta 9-tetrahydrocannabinol and kappa opioids in mice.* *J Pharmacol Exp Ther* 1994, 268(3): 1381-7.
 47. Gray, A.C., Coupar, I.M. and White, P.J. *Comparison of opioid receptor distributions in the rat central nervous system.* *Life Sci* 2006, 79(7): 674-85.
 48. Peckys, D. and Landwehrmeyer, G.B. *Expression of mu, kappa, and delta opioid receptor messenger RNA in the human CNS: a 33P in situ hybridization study.* *Neuroscience* 1999, 88(4): 1093-135.
 49. Porreca, F., Cowan, A., Raffa, R.B. and Tallarida, R.J. *Ketazocines and morphine: effects on gastrointestinal transit after central and peripheral administration.* *Life Sci* 1983, 32(15): 1785-90.
 50. Ward, S.J. and Takemori, A.E. *Relative involvement of receptor subtypes in opioid-induced inhibition of gastrointestinal transit in mice.* *J Pharmacol Exp Ther* 1983, 224(2): 359-63.
 51. Storr, M.A. and Sharkey, K.A. *The endocannabinoid system and gut-brain signalling.* *Curr Opin Pharmacol* 2007, 7(6): 575-82.
 52. Pagotto, U., Marsicano, G., Cota, D., Lutz, B. and Pasquali, R. *The emerging role of the endocannabinoid system in endocrine regulation and energy balance.* *Endocr Rev* 2006, 27(1): 73-100.
 53. Silva, R.M., Grossman, H.C., Hadjimarkou, M.M., Rossi, G.C., Pasternak, G.W. and Bodnar, R.J. *Dynorphin A(1-17)-induced feeding: pharmacological characterization using selective opioid antagonists and antisense probes in rats.* *J Pharmacol Exp Ther* 2002, 301(2): 513-8.
 54. Di Marzo, V. and Matias, I. *Endocannabinoid control of food intake and energy balance.* *Nat Neurosci* 2005, 8(5): 585-9.
 55. Fickel, J., Bagnol, D., Watson, S.J. and Akil, H. *Opioid receptor expression in the rat gastrointestinal tract: a quantitative study with comparison to the brain.* *Brain Res Mol Brain Res* 1997, 46(1-2): 1-8.
 56. Sternini, C., Patierno, S., Selmer, I.S. and Kirchgessner, A. *The opioid system in the gastrointestinal tract.* *Neurogastroenterol Motil* 2004, 16(Suppl. 2): 3-16.
 57. Sengupta, J.N., Su, X. and Gebhart, G.F. *Kappa, but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon.* *Gastroenterology* 1996, 111(4): 968-80.
 58. Duncan, M., Mouihate, A., Mackie, K. et al. *Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats.* *Am J Physiol Gastrointest Liver Physiol* 2008, 295(1): G78-87.
 59. Coutts, A.A., Irving, A.J., Mackie, K., Pertwee, R.G. and Anavi-Goffer, S. *Localisation of cannabinoid CB1 receptor immunoreactivity in the guinea pig and rat myenteric plexus.* *J Comp Neurol* 2002, 448(4): 410-22.
 60. Kulkarni-Narla, A. and Brown, D.R. *Opioid, cannabinoid and vanilloid receptor localization on porcine cultured myenteric neurons.* *Neurosci Lett* 2001, 308(3): 153-6.
 61. Burton, M.B. and Gebhart, G.F. *Effects of kappa-opioid receptor agonists on responses to colorectal distension in rats with and without acute colonic inflammation.* *J Pharmacol Exp Ther* 1998, 285(2): 707-15.
 62. Corazzari, E. *Role of opioid ligands in the irritable bowel syndrome.* *Can J Gastroenterol* 1999, 13(Suppl. A): 71A-5A.
 63. Delvaux, M., Louvel, D., Lagier, E., Scherrer, B., Abitbol, J.L. and Frexinos, J. *The kappa agonist fedotozine relieves hypersensitivity to colonic distention in patients with irritable bowel syndrome.* *Gastroenterology* 1999, 116(1): 38-45.
 64. Camilleri, M. *Novel pharmacology: Asimadoline, a kappa-opioid agonist, and visceral sensation.* *Neurogastroenterol Motil* 2008, 20(9): 971-9.
 65. Szarka, L.A., Camilleri, M., Burton, D. et al. *Efficacy of on-demand asimadoline, a peripheral kappa-opioid agonist, in females with irritable bowel syndrome.* *Clin Gastroenterol Hepatol* 2007, 5(11): 1268-75.
 66. Delgado-Aros, S., Chial, H.J., Camilleri, M. et al. *Effects of a kappa-opioid agonist, asimadoline, on satiation and GI motor and sensory functions in humans.* *Am J Physiol*

- Gastrointest Liver Physiol 2003, 284(4): G558-66.
67. Delgado-Aros, S., Chial, H.J., Cremonini, F., Ferber, I., McKinzie, S., Burton, D.D. and Camilleri, M. *Effects of asimadoline, a kappa-opioid agonist, on satiation and postprandial symptoms in health.* Aliment Pharmacol Ther 2003, 18(5): 507-14.
 68. Barber, A., Bartoszyk, G.D., Bender, H.M. et al. *A pharmacological profile of the novel, peripherally-selective kappa-opioid receptor agonist, EMD 61753.* Br J Pharmacol 1994, 113(4): 1317-27.
 69. Mangel, A.W., Bornstein, J.D., Hamm, L.R., Buda, J., Wang, J., Irish, W. and Urso, D. *Clinical trial: Asimadoline in the treatment of patients with irritable bowel syndrome.* Aliment Pharmacol Ther 2008, 28(2): 239-49.
 70. Gue, M., Junien, J.L. and Bueno, L. *The kappa agonist fedotozine modulates colonic distention-induced inhibition of gastric motility and emptying in dogs.* Gastroenterology 1994, 107(5): 1327-34.
 71. Talley, N.J., Choung, R.S., Camilleri, M., Dierkhising, R.A. and Zinsmeister, A.R. *Asimadoline, a kappa-opioid agonist, and satiation in functional dyspepsia.* Aliment Pharmacol Ther 2008, 27(11): 1122-31.
 72. Eisenach, J.C., Carpenter, R. and Curry, R. *Analgesia from a peripherally active kappa-opioid receptor agonist in patients with chronic pancreatitis.* Pain 2003, 101(1-2): 89-95.
 73. Lembo, A. *Peripheral opioids for functional GI disease: A reappraisal.* Dig Dis 2006, 24(1-2): 91-8.
 74. Massa, F., Marsicano, G., Hermann, H. et al. *The endogenous cannabinoid system protects against colonic inflammation.* J Clin Invest 2004, 113(8): 1202-9.
 75. Holden, K.G., Tidgewell, K., Marquam, A., Rothman, R.B., Navarro, H. and Prisinzano, T.E. *Synthetic studies of neoclerodane diterpenes from Salvia divinorum: Exploration of the 1-position.* Bioorg Med Chem Lett 2007, 17(22): 6111-5.
 76. Beguin, C., Richards, M.R., Wang, Y. et al. *Synthesis and in vitro pharmacological evaluation of salvinorin A analogues modified at C(2).* Bioorg Med Chem Lett 2005, 15(11): 2761-5.
 77. Beguin, C., Richards, M.R., Li, J.G. et al. *Synthesis and in vitro evaluation of salvinorin A analogues: Effect of configuration at C(2) and substitution at C(18).* Bioorg Med Chem Lett 2006, 16(17): 4679-85.
 78. Stewart, D.J., Fahmy, H., Roth, B.L., Yan, F. and Zjawiony, J.K. *Bioisosteric modification of salvinorin A, a potent and selective kappa-opioid receptor agonist.* Arzneimittelforschung 2006, 56(4): 269-75.
 79. Bikbulatov, R.V., Yan, F., Roth, B.L. and Zjawiony, J.K. *Convenient synthesis and in vitro pharmacological activity of 2-thioanalogs of salvinorins A and B.* Bioorg Med Chem Lett 2007, 17(8): 2229-32.
 80. Lee, D.Y., Karnati, V.V., He, M. et al. *Synthesis and in vitro pharmacological studies of new C(2) modified salvinorin A analogues.* Bioorg Med Chem Lett 2005, 15(16): 3744-7.
 81. Tidgewell, K., Groer, C.E., Harding, W.W. et al. *Herkinorin analogues with differential beta-arrestin-2 interactions.* J Med Chem 2008, 51(8): 2421-31.
 82. Groer, C.E., Tidgewell, K., Moyer, R.A., Harding, W.W., Rothman, R.B., Prisinzano, T.E. and Bohn L.M. *An opioid agonist that does not induce micro-opioid receptor-arrestin interactions or receptor internalization.* Mol Pharmacol 2007, 71(2): 549-57.
 83. Munro, T.A., Rizzacasa, M.A., Roth, B.L., Toth, B.A. and Yan, F. *Studies toward the pharmacophore of salvinorin A, a potent kappa opioid receptor agonist.* J Med Chem 2005, 48(2): 345-8.
 84. Lee, D.Y., He, M., Liu-Chen, L.Y. et al. *Synthesis and in vitro pharmacological studies of new C(4)-modified salvinorin A analogues.* Bioorg Med Chem Lett 2006, 16(21): 5498-502.
 85. Fichna, J., Schicho, R., Andrews, C.N. et al. *Salvinorin A inhibits colonic transit and neurogenic ion transport in mice by activating kappa-opioid and cannabinoid receptors.* Neurogastroenterol Motil 2009, Advance publication.

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