LOOKING AHEAD

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

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

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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.

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] divinatorins A-F,[4-6] salvinicins A-B[7] and salvidivins A-D.[5]

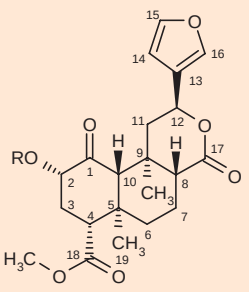
In traditional medicine, extracts from *S. divinorum* are used in the treatment of diarrhea. Recent studies on extracts from *S. divinorum* and pure salvinorin A showed potent effects on gastrointestinal (GI) motility in vitro and in vivo, supporting a future role in the treatment of human GI disorders. 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 activity[13] 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 transmission 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, R = Ac
Salvinorin B, R = H

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

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

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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 ligand[17] (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 CB_1 and CB_2, muscarinic and nicotinic acid, as well as ionotropic and metabotropic glutamate receptors.[1,17]

Salvinorin B, which may be formed from salvinorin A via an esterase-mediated hydrolysis, had modest (K_i = 65.9 ± 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 termal (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 antinociception.[18,24-27] Other in vivo effects of salvinorin A, mediated by KOR, included hypothermia,[24] sedation and motor incoordination[31] (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]

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

[a]Data from [17-19]
[b]Data from [17]
[c]Data from [18]

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; CB_1, cannabinoid receptor 1; i.c.v., intracerebroventricular; i.t., intrathecal.

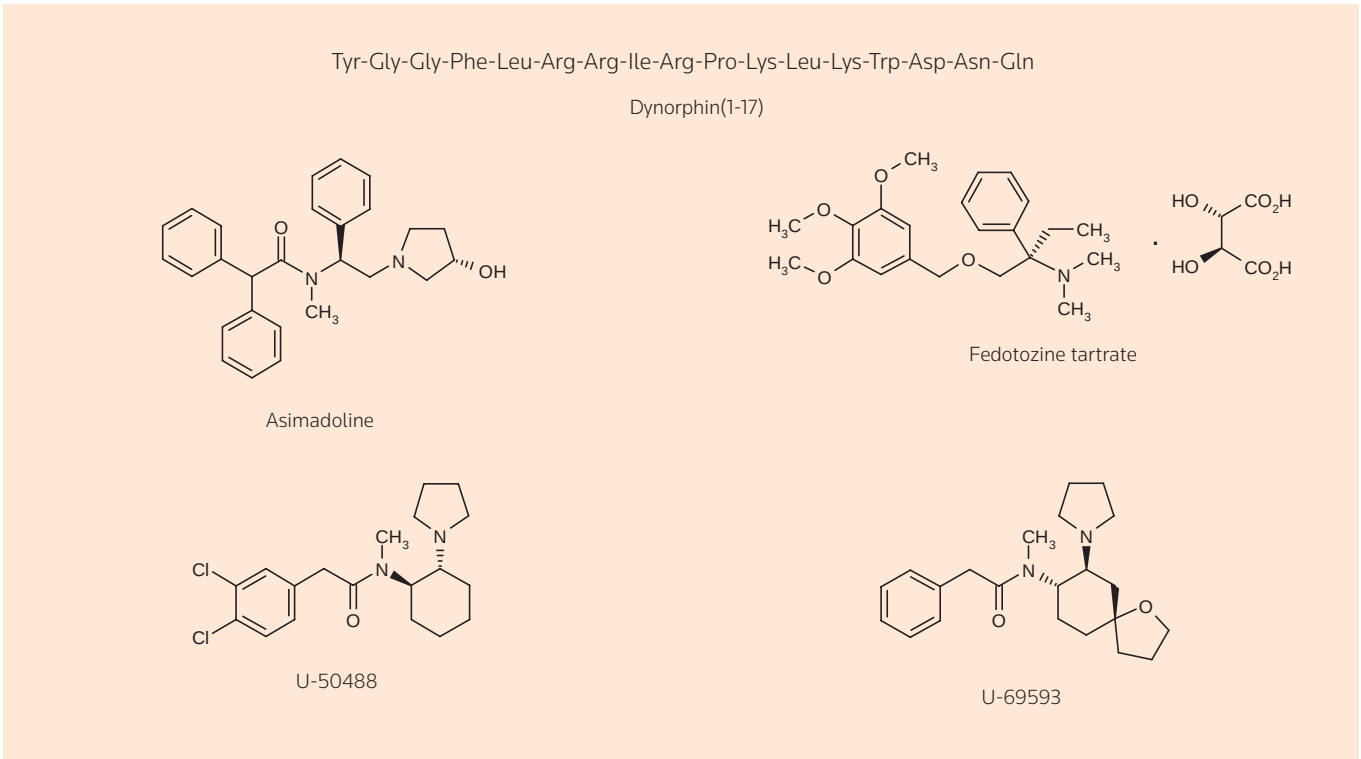

Figure 2. Structures of κ opioid receptor agonists.

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 μM 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 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 subhallucinogenic doses of salvinorin A (< 3 µg/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 µg/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 CB$_1$ 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 CB$_1$ antagonist rimonabant.[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 CB$_1$ 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 KOR$_1$ receptors.[53] Since salvinorin A also acts via KOR$_1$ receptors,[24] weight gain after administration of salvinorin A may be expected. This effect could be potentiated by salvinorin-A-mediated activation of CB$_1$ 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

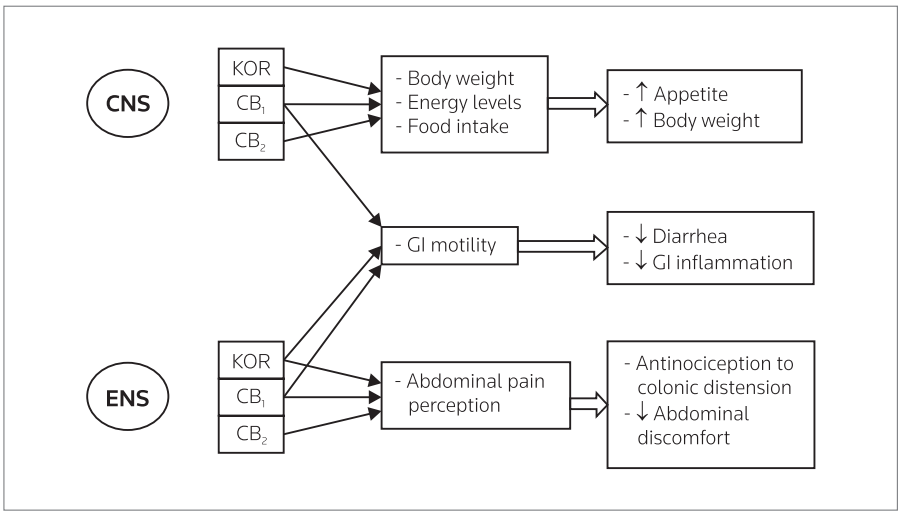

Figure 3. Possible involvement of central and peripheral κ opioid (KOR) and cannabinoid (CB) receptors in the effects of salvinorin A on the gastrointestinal (GI) tract. CNS, central nervous system; ENS, enteric nervous system (myenteric plexus ganglia and visceral sensory neurons).

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 CB_1 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 humans[65] 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 CB_1 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 CB_1 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*

	Anticipated effect		
	Antidiarrheal	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 bioisosteres[78] and thio-derivatives, using binding and functional assays.[79] Of interest, the C-2 thioacetate isostere (Fig. 5a) displayed 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 compound[81] (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

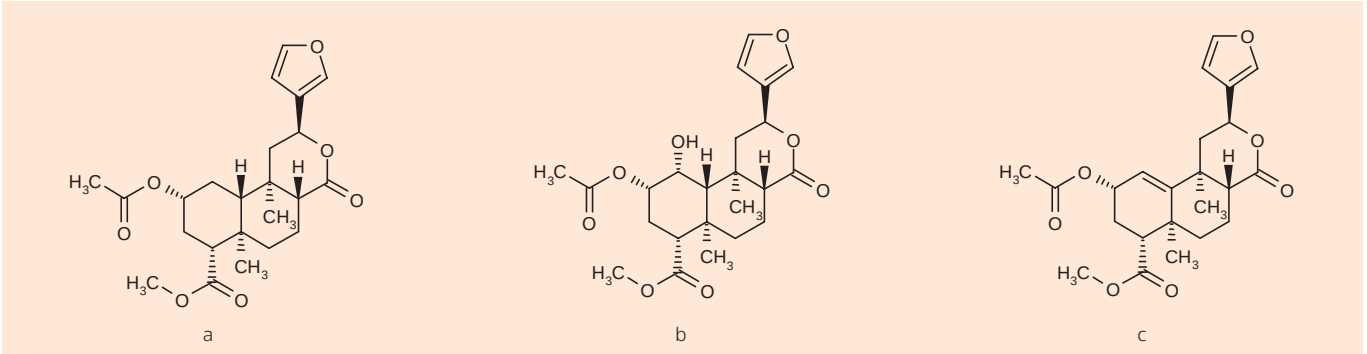

Figure 4. Salvinorin A analogues modified in position 1.

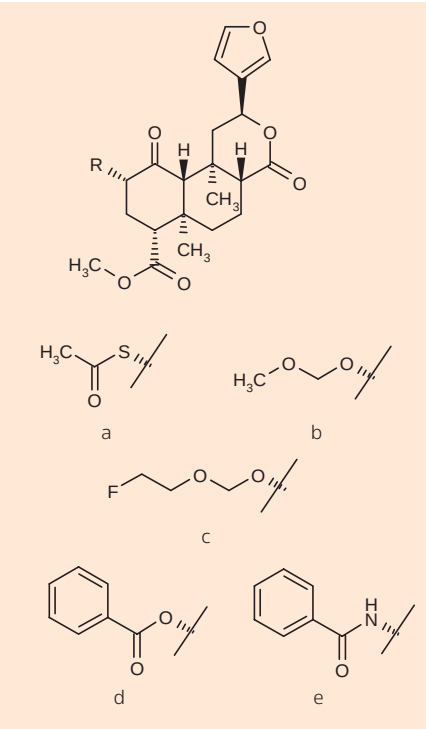

Figure 5. Salvinorin A analogues modified in position 2.

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.

reduction of acid (Fig. 6a) to alcohol (Fig. 6b) increased potency and efficacy at the KOR (EC_{50} = 20.7 ± 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 receptor[20] (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).

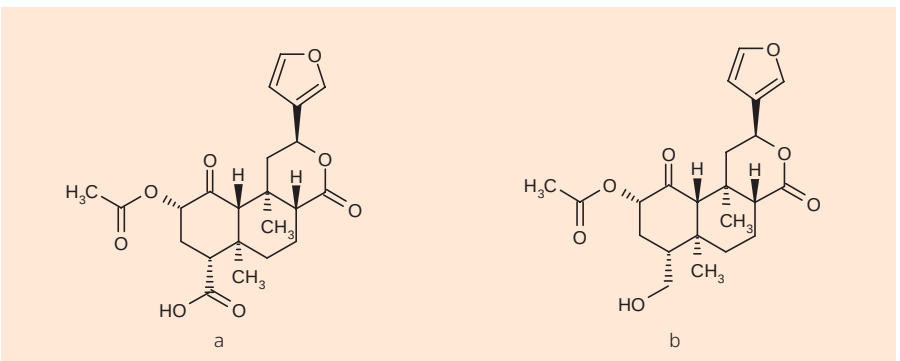

Figure 6. Salvinorin A analogues modified in position 4.

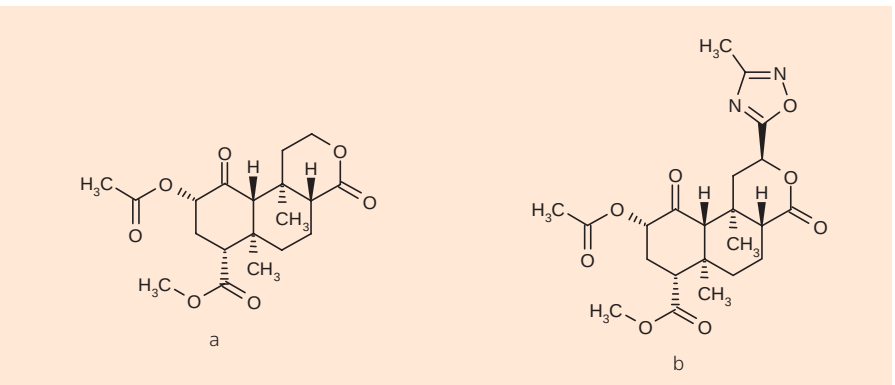

Figure 7. Salvinorin A analogues modified in position 12/furan ring.

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, CB_1 and CB_2. 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.

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