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N-Methylacetamide Analog of Salvinorin A: A Highly Potent and Selective κ-Opioid Receptor Agonist with Oral Efficacy[S]

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ABSTRACT

Several preclinical studies indicate that selective κ-opioid receptor (KOR) antagonists have antidepressant-like effects, whereas KOR agonists have opposite effects, suggesting that each might be useful in the treatment of mood abnormalities. Salvinorin A (salvA) is a valuable KOR agonist for further study due to its high potency and receptor selectivity. However, it has short lasting effects in vivo and limited oral bioavailability, probably due to acetate metabolism. We compared the in vitro receptor binding selectivity of salvA and four analogs containing an ethyl ether (EE), isopropylamine (IPA), *N*-methylacetamide (NMA), or *N*-methylpropionamide (NMP) at C-2. All compounds showed high binding affinity for the KOR (K_i = 0.11–6.3 nM), although only salvA, EE, and NMA exhibited KOR selectivity. In a liver microsomal assay, salvA was least stable, whereas NMA and IPA displayed slower metabolic transformations. Intraperitoneal (i.p.) administration of salvA, NMA, and NMP dose-dependently elevated brain reward thresholds in the intracranial self-administration (ICSS) test, consistent with prodepressive-like KOR agonist effects. NMA and NMP were equipotent to salvA but displayed longer lasting effects (6- and 10-fold, respectively). A dose of salvA with prominent effects in the ICSS test after i.p. administration (2.0 mg/kg) was inactive after oral administration, whereas the same oral dose of NMA elevated ICSS thresholds. These studies suggest that, although salvA and NMA are similar in potency and selectivity as KOR agonists in vitro, NMA has improved stability and longer lasting actions that might make it more useful for studies of KOR agonist effects in animals and humans.

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Brain κ-opioid receptors (KORs) have been implicated in many functions, including regulation of mood (Mague et al., 2003; Todtenkopf et al., 2004; Cohen and Murphy, 2007), responses to drugs of abuse (Shippenberg and Rea, 1997), sense of space and time (Pfeiffer et al., 1986; Walsh et al., 2001; Dortch-Carnes and Potter, 2005), and pain perception (Barber and Gottschlich, 1997; Dortch-Carnes and Potter, 2005). Therefore, selective KOR ligands may enable a more detailed understanding of the molecular mechanisms that

ABBREVIATIONS: KOR, κ-opioid receptor; MOR, μ-opioid receptor; DOR, δ-opioid receptor; 3FLB, diethyl-2,4-di-[3-fluorophenyl]-3,7-dimethyl-3,7-diazabicyclo[3.3.1]nonane-9-one-1,5-dicarboxylate; 5HT, serotonin; DMSO, dimethyl sulfoxide; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; EE, salvinorin B ethyl ether; ICSS, intracranial self-stimulation; IPA, *N*-(2-propyl)-2-amido salvinorin B (using IUPAC functional replacement nomenclature); MPA, mobile phase A; MPB, mobile phase B; NMA, *N*-acetyl-*N*-methyl-2-amido salvinorin B (using IUPAC functional replacement nomenclature); NMP, *N*-propionyl-*N*-methyl-2-amido salvinorin B (using IUPAC functional replacement nomenclature); salvA, salvinorin A; SAR, structure-activity relationship; TRK-820, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride; U50,488, (1*S*,2*S*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide hydrochloride; U69,593, (+)-(5*R*,7*S*,8*S*)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzenacetamide; h, human; r, rat; D, dopamine; H, histamine; M, muscarinic; CI-977, [5*R*-(5α,7α,8β)]-*N*-methyl-*N*-[7-(1-pyrrolidinyl)1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide; ICI-199441, 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-phenyl-2-(1-pyrrolidinyl)ethyl]acetamide; PD-117302, (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzothiophene-4-acetamide.

regulate complex brain function and possess the potential for clinical applications.

A small number of synthetic KOR agonists with high selectivity for the KOR have been developed. The most specific of these, U50,488 and U69,593 and their congeners spiradoline and enadoline, have limited efficacy when administered orally (Endoh et al., 1999). Salvinorin A (salvA) is a natural substance that is the major component of *Salvia divinorum*, a plant used in shamanistic rituals in Mexico. Recent work has established that salvA is a potent and highly selective KOR agonist (Roth et al., 2002; Butelman et al., 2004; Vortherms and Roth, 2006). SalvA appears to bind to KOR via both conserved and nonconserved amino acid residues (Yan et al., 2005; Vortherms et al., 2007), contributing to its extraordinary selectivity. In vitro, salvA has properties that are unique among KOR agonists. For example, in a specific assay designed to monitor the KOR-mediated activation of potassium (Kir3 channel) currents, salvA was more efficacious than U50,488, U69,593, or dynorphin A, the endogenous peptide neurotransmitter for the KOR (Chavkin et al., 2004). SalvA also caused less human KOR (hKOR) internalization and down-regulation than TRK-820 or 3FLB (Wang et al., 2005). It is not known whether these properties are maintained in vivo or whether they contribute to unique behavioral and cognitive effects of salvA.

Most in vivo studies of salvA have focused on its effects on pain (Wang et al., 2005; Ansonoff et al., 2006; John et al., 2006; McCurdy et al., 2006) and behaviors that reflect motivation (Zhang et al., 2005; Carlezon et al., 2006). One recent report suggested that salvA might reduce cocaine self-administration (Prisinzano et al., 2005), whereas another indicated that salvA induced rewarding effects at low doses and aversive effects at high doses in place-conditioning tests involving zebrafish (Braida et al., 2007). SalvA has dose-dependent analgesic effects in the mouse tail-flick, hot-plate, and acetic acid abdominal constriction tests after i.p., intracerebroventricular, or intrathecal administration (Ansonoff et al., 2006; John et al., 2006; McCurdy et al., 2006). The time course of salvA effects was consistently brief across these analgesia studies: it produced maximal antinociceptive effects 10 to 15 min postadministration, and these effects had waned by 30 to 60 min. However, the behavioral and neurochemical effects of the drug appear somewhat more sustained in other tests (Zhang et al., 2005; Carlezon et al., 2006).

As part of an effort to identify novel treatments for affective disorders, we have characterized the effects of selective KOR ligands in animal models often used to study mood (Mague et al., 2003; Todtenkopf et al., 2004; Carlezon et al., 2006). SalvA increased immobility behavior in the forced swim test and elevated intracranial self-stimulation (ICSS) thresholds (Carlezon et al., 2006), putative indicators of reduced motivation, and depressive-like effects. These observations raised the possibility that selective KOR agonists might relieve symptoms of mania, a disorder characterized by dysregulated motivation.

Although these preclinical studies suggested potential therapeutic applications for salvA, they also confirmed its relatively short duration of behavioral effects. A longer lasting salvA-like agent that is orally available may offer some advantages in the study and treatment of mood and other disorders. The relatively short duration of action of salvA and its low activity when taken orally (Siebert, 1994) is reportedly due to rapid hydrolysis of salvA acetate to form the weak KOR agonist salvinorin B (Schmidt et al., 2005a,b). We modified the functionality of the C-2 substituent of salvA with the goal of increasing metabolic stability. Structure-activity relationship (SAR) studies led to the identification of chemical alterations compatible with maintaining KOR recognition. Using an in vitro guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate assay, we found that the salvinorin analogs (Fig. 1) featuring ethyl ether (EE), isopropylamine (IPA), *N*-methylacetamide (NMA), or *N*-methylpropionamide (NMP) moieties at C-2 were potent KOR agonists (Béguin et al., 2005, 2006). To characterize the pharmacodynamic and pharmacokinetic properties of these new derivatives, we examined their effects in assays that quantify receptor binding selectivity, microsomal stability, and effects on motivated behavior.

Materials and Methods

Materials. Dried *S. divinorum* leaves were purchased from Bouncing Bear Botanicals (Lawrence, KS). SalvA (Fig. 1, **1**) was extracted, isolated, and purified using published methods (Munro and Rizzacasa, 2003; Lee et al., 2005). The C-2-modified salvinorin analogs EE (Fig. 1, **2**) (Béguin et al., 2005) and IPA (Fig. 1, **3**) (Béguin

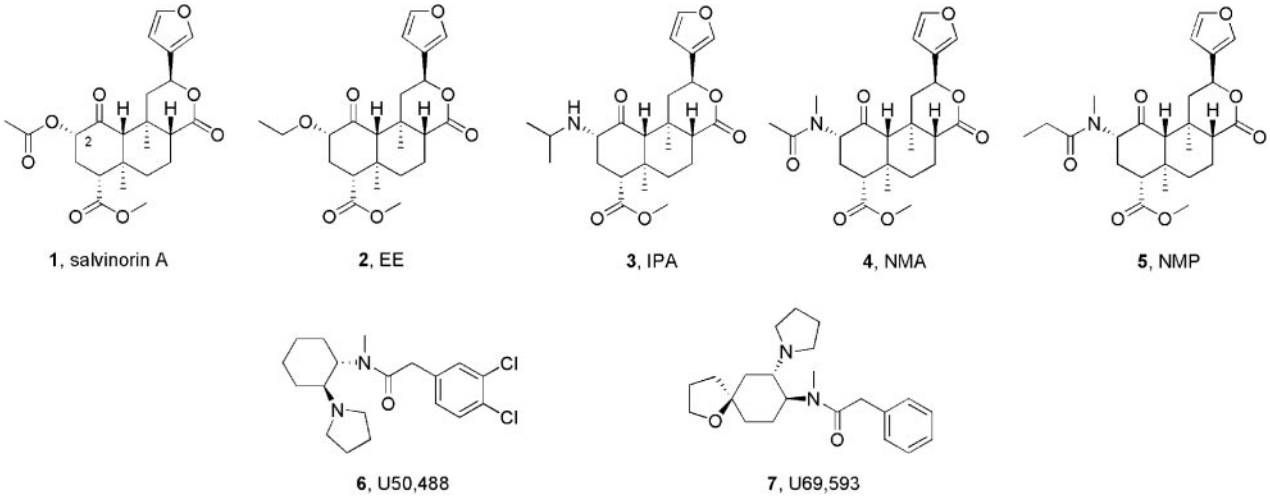

Fig. 1. Chemical structures of salvA, C-2-modified analogs (EE, IPA, NMA, and NMP), (1*S*, 2*S*) U50,488, and U69,593.

et al., 2006) were synthesized from salvA as previously described. NMA (Fig. 1, **3**) and NMP (Fig. 1, **4**) were prepared as described previously (Béguin et al., 2006), omitting purification of the intermediate 2-epi-salvinorin B to improve yields. The final products were purified by column chromatography (SiO_2, NMA: 0–10% MeOH/Et_2O; NMP: 5% MeOH/45% CH_2Cl_2/50% hexanes) to give NMA in 49% yield and NMP in 51% yield over five steps from salvinorin B. ^{1}H NMR spectroscopy showed only trace impurities (residual solvents that cannot account for the in vitro and in vivo properties of the compound). Solutions of each test compound in 75% dimethyl sulfoxide (DMSO)-25% distilled water were administered by i.p. injection or oral gavage in a volume of 1 ml/kg. Human and rat liver microsomes were purchased from BD Biosciences (San Jose, CA). (1*S*,2*S*)-U50,488, U69,593, nicotinamide adenine dinucleotide phosphate (reduced, NADPH), potassium phosphate, and magnesium chloride were purchased from Sigma-Aldrich (St Louis, MO).

In Vitro Radioligand Binding Studies. Radioligand-binding assays at human cloned G protein-coupled receptors, ion channels, and transporters were performed as described in the literature (Rothman et al., 2000; Shapiro et al., 2003) by using the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). Specifically, KOR radioligand-binding assays were performed using cloned rat KOR (rKOR) and [^{3}H]U69,593 as the radioligand. δ-Opioid receptor (DOR) binding affinities were determined using cloned human DOR (hDOR) and [^{3}H]Tyr-D-Ala-Gly-Phe-D-Leu as the radioligand. Finally, the affinity of the test compounds for μ-opioid receptors (MOR) were obtained using cloned human MOR (hMOR) and [^{3}H]Tyr-D-Ala-Gly-*N*-methyl-Phe-Gly-ol as the radioligand. Detailed on-line protocols are available for all assays at the NIMH-PDSP website (http://pdsp.med.unc.edu). Initial screening assays were performed in quadruplicate using test compound (10 μM), and the percentage inhibition of specific binding was determined. Where the test compound (10 μM) inhibited >50% of specific binding, K_i determinations were performed by using six concentrations of unlabeled ligand spanning a 10,000-fold dose range. K_i values were calculated by using GraphPad Prism (GraphPad Software, Inc., San Diego, CA) and represent the mean ± S.E.M. of quadruplicate determinations.

In Vitro Metabolism Experiments and Cl_{int} Calculations. The in vitro intrinsic clearances (CL_{int}) of the test compounds were determined in incubations with human and rat liver microsomes. Control compounds verapamil, dextromethorphan, and diclofenac were included as indicators of microsomal CYP450 activity, specifically CYP3A4, CYP2D6 and CYP2C9 in human liver microsomes. The 400-μl incubations contained 0.25 mg/ml of microsomal protein, 1 mM NADPH, 2 mM $MgCl_2$, and 50 mM potassium phosphate buffer (pH 7.4). To begin the reactions, test compounds were added to the prewarmed (37°C) incubation mixtures at the final concentration of 1 μM. At 0, 10, 20, 30, and 40 min following the addition of the test compound, aliquots of incubation mixture (35 μl) were collected into an equal volume of acetonitrile and internal standard (1 μM tolbutamide). For the controls without NADPH, compounds were incubated as above excluding NADPH, and samples were collected at 0, 20, and 40 min. The samples were centrifuged at 3000*g* for 15 min and analyzed on a liquid chromatography-tandem mass spectrometry system consisting of two Shimadzu LC-10AD high-performance liquid chromatography pumps and a DGU-14A degasser (Shimadzu, Columbia, MD), a CTC PAL autoinjector (Leap Technologies, Carrboro, NC) and a API3000 liquid chromatography/mass spectrometry-mass spectrometry system. Chromatography was conducted on a Sprite Armor C18 (20 × 2.1 mm, 10 μm) analytical column (Analytical Sales and Products, Pompton Plains, NJ) with a 0.5-μm poly-ether-ether-ketone guard filter using the following mobile phase gradient program: MPA = H_20 with 0.1% formic acid; MPB = acetonitrile with 0.1% formic acid; 0 min = 98% MPA, 2% MPB; 0.3 min = 98% MPA, 2% MPB; 0.7 min = 5% MPA, 95% MPB; 1.3 min = 5% MPA, 95% MPB; 1.4 min = 98% MPA, 2% MPB; 1.7 min = end of run; approximately 2 min between sample injections. For each compound, peak areas for the 10-, 20-, 30-, and 40-min samples were converted to the natural log of the percentage remaining relative to the 0-min samples (Obach, 1999). The resulting slope (k) of these values relative to time was used to calculate in vitro $T_{1/2}$ where $T_{1/2} = -0.693/k$. CL_{int} was calculated using the following equation: $CL_{int} = (0.693/T_{1/2}) \times (1/0.25)$.

Rats. A total of 22 male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were used in these studies. Rats were housed individually and weighed 350 to 400 g at the time of stereotaxic surgery. All rats were maintained on a 12-h light/12-h dark cycle (7:00 AM–7:00 PM) with free access to food and water except during testing. Experiments were conducted in accordance with the Institute of Laboratory Animal Resources (1996), as well as McLean Hospital and National Institute on Drug Abuse-Intramural Research Program (NIDA-IRP) policies.

Intracranial Self-Stimulation. ICSS experiments were conducted as described previously (Carlezon et al., 2006). Each of the 22 rats was anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.; Henry Schein, Port Washington, NY) and given atropine sulfate s.c. (0.25 mg/kg; Sigma-Aldrich) to minimize bronchial secretions. Each rat was then implanted with a monopolar, stainless steel electrode (0.25 mm diameter; Plastics One, Roanoke, VA) aimed at the left medial forebrain bundle and at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from the midsagittal suture, and 7.8 mm below the dura) (Paxinos and Watson, 1986). The electrodes were coated with polyamide insulation except at the flattened tip. Skull screws (one of which served as the ground) and the electrode were secured to the skull with dental acrylic.

After 1 week of recovery, the rats were trained on a continuous reinforcement schedule (FR1) to respond to brain stimulation (Todtenkopf et al., 2004; Carlezon et al., 2006). Each lever press earned a 0.5-s train of square-wave cathodal pulses (0.1-ms pulse duration) at a set frequency of 141 Hz. Responses during the 0.5-s stimulation period did not earn additional stimulation. The stimulation current (100–250 μA) was adjusted gradually to the lowest value that would sustain a reliable rate of responding (at least 30 rewards per min). Once an appropriate current was found for each rat, it was held constant for the remainder of the experiment.

Each rat was then adapted to brief tests with each of a descending series of 15 stimulation frequencies (126–25 Hz). Each series comprised 1-min test trials at each frequency. For each frequency tested, there was an initial 5-s "priming" phase, during which noncontingent stimulation was given, followed by a 50-s test phase, during which the number of responses was counted. After the test phase, there was a 5-s time out period, during which no stimulation was available. The stimulation frequency was then lowered by approximately 10% (0.05 log_{10} units), and another trial was started. After responding had been evaluated at each of the 15 frequencies, the procedure was repeated, such that each rat was given six such series per day (90 min of training). During the training procedure, the stimulation current was adjusted for each rat so that only the highest six to eight frequencies would sustain responding. To characterize the functions relating response strength to reward magnitude, a least-squares line of best fit was plotted across the frequencies that sustained responding at 20, 30, 40, 50, and 60% of the maximal rate using customized analysis software. ICSS threshold was defined as the frequency at which the line intersected the *x*-axis [theta-0 (θ_0); see Carlezon et al., 2006]. Drug testing started when mean ICSS thresholds varied by less than 10% over three consecutive sessions.

We performed three consecutive experiments to determine a) the dose-response curves and time course (0–90 min) after i.p. administered salvA, NMA, and NMP; b) the time course (3–24 h) of effects of NMA and NMP in the ICSS test after i.p. injection; and c) the ICSS effect of orally administered salvA and NMA. For each experiment, three rate-frequency functions ("curves") were determined for each rat immediately before drug treatment. The first curve served as a warm-up period and was discarded because it tended to be unreli-

able. The second and third curves were averaged to obtain the baseline parameters for threshold and maximal response rates.

For the dose-response curves and 0 to 90-min time course experiment, each rat then received an i.p. injection of drug or vehicle, and six more 15-min rate-frequency curves were obtained (90 min of testing). Eight rats received the same daily treatments in a standardized order: saline, vehicle (75% DMSO), and salvA at 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 mg/kg i.p. Six rats received the same daily treatments in a standardized order: saline, vehicle (75% DMSO), and NMA at 0.25, 0.5, 1.0, and 2.0 mg/kg i.p. Eight rats received the same daily treatments in a standardized order: saline, vehicle (75% DMSO), and NMP at 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/kg i.p. To test whether drug treatment induces tolerance or desensitization, the doses were given in ascending and then descending order, such that each rat received vehicle and each dose of the drug twice. To determine whether there were differences between the first and second test with each treatment, the effects of saline, vehicle (75% DMSO), salvA, NMA, and NMP on ICSS thresholds and maximal response rates over the test period were evaluated in separate two-way analyses of variance (drug dose $\times$ test number) with repeated measures. Data from the first and last tests with saline were used. Because there was no significant effect of dose order, as is typical of ICSS (see Carlezon et al., 2001), the first and second tests at each dose were then combined into single means, and the drug effects on thresholds and maximal rates were evaluated with separate one-way analyses of variances. Significant effects were analyzed further using post-hoc Dunnett's tests. Because the strongest effects of salvA ICSS thresholds occurred within the first 15 min postadministration, dose-response curve thresholds were determined using the first 15-min rate-frequency curve. The ED_{50} values were determined using GraphPad Prism.

When determining the time course of drug actions, the effects from the first 90 min of testing were obtained from the dose-response curves data set. For the determination of the ICSS effects at the other time points (3, 10, and 24 h), each rat ($n = 8$) received an i.p. injection of drug (NMA, 2 mg/kg, or NMP, 1 mg/kg) or vehicle (75% DMSO) immediately after the warm up and the two baseline rate-frequency curves. One 15-min rate-frequency curve was obtained immediately after injection to confirm the expected drug effect. Three sets of three rate-frequency curves (three 45-min sessions) then were collected 2.5, 9.5, and 23.5 h postinjection. The first and last rate-frequency curve of each set was discarded, and the second one was used to obtain the thresholds at 3-, 10-, and 24-h time points. As always, the average of the second and third 15-min rate-frequency functions obtained immediately before drug administration served as baseline thresholds.

For the studies of oral drug administration, a small number of rats ($n = 4$) received vehicle or 2 mg/kg drug (salvA or NMA) by gavage, and six 15-min rate-frequency curves were recorded (90 min of testing). For the time course and oral administration experiments, the effects of the test compound on ICSS thresholds and maximal response rates were evaluated in separate two-way analyses of variance with repeated measures. At each time point, significant effects were further analyzed by post-hoc Dunnett's tests.

Rats were drug-free for at least 1 week between each experiment. In addition, on alternate days, rats were tested after i.p. injections of saline to ensure that they had recovered from prior treatment and to minimize the possibility of conditioned drug effects.

Histology. Each rat was overdosed with pentobarbital (130 mg/kg i.p.) and perfused with 4% paraformaldehyde. The fixed brains were sliced in 40-μm sections for cresyl violet staining to confirm electrode placements.

Results

In vitro radioligand binding studies (salvA, EE, IPA, NMA, and NMP) were screened against 39 receptors and transporters ($5HT_{1a}$, $5HT_{1b}$, $5HT_{1d}$, $5HT_{1e}$, $5HT_{2a}$, $5HT_{2b}$, $5HT_{2c}$, $5HT_3$, $5HT_{5a}$, $5HT_6$, $5HT_7$, α_{1a}, α_{1b}, α_{2a}, α_{2b}, α_{2c}, β_1, D_1, D_2, D_3, D_4, D_5, dopamine transporter, DOR, H_1, H_2, H_3, H_4, KOR, M_1, M_2, M_3, M_4, M_5, MOR, norepinephrine transporter, serotonin transporter, and σ_1,σ_2) using the NIMH-PDSP comprehensive radioligand binding assay protocol. The most potent isomer of U50,488 [(1*S*,2*S*) isomer] and U69,593 were tested under the same conditions for comparison. The binding affinities for the receptors, at which at least one of the test compounds (10 μM) showed >50% displacement of radioligand, are summarized in Table 1. SalvA, EE, IPA, NMA, and NMP did not bind to any of the nonopioid receptors. Consistent with previous findings (Roth et al., 2002), salvA was a potent and selective rKOR agonist. As expected from previous studies (Béguin et al., 2005, 2006), the four C-2 analogs (EE, IPA, NMA, and NMP) showed high rKOR binding affinity (K_i = 0.11–6.3 nM). The C-2 substituent did not have a strong effect on the interaction of the molecule with the hDOR; only NMP showed moderate affinity for the hDOR (K_i = 366 nM). Furthermore, with the exception of EE (K_i = 6,938 nM), modifications at C-2 led to an increase in hMOR affinity: the *N*-containing salvinorin analogs showed weak to high affinity for the hMOR (K_i = 15–135 nM). Under the same experimental conditions, the synthetic KOR agonists (1*S*,2*S*)-U50,488 and U69,593 displayed high rKOR/hDOR and rKOR/hMOR selectivity (K_i ratios >1,000). However the selectivity of

TABLE 1

Binding affinities (K_i) of salvA, C-2 modified analogs, U50,488 and U69,593

Data represent mean ± S.E.M. of computer-derived estimates of K_i and pK_i values for $n > 3$ separate experiments. Each compound was screened against 39 receptors and transporters. We included in the table only the receptors at which any test ligand (at 10 μM) showed >50% inhibition of radioligand binding. SalvA, EE, NMA, NMP, U50,488, and U69,593 are highly selective for the KOR over DOR and MOR.

Compound	K_i					K_i Ratio	
	α_{2C}[a]	σ_2[a]	DOR[a,b]	KOR[c]	MOR[a,d]	DOR/KOR	MOR/KOR
			nM				
1, salvA	–[e]	–[e]	6404 ± 1080	0.28 ± 0.22	4879 ± 5055	>10,000	>10,000
2, EE	–[e]	–[e]	–[e]	6.3 ± 3.6	6938 ± 2216	>529	1101
3, IPA	–[e]	–[e]	–[e]	4.5 ± 2.0	111 ± 49	>740	25
4, NMA	–[e]	–[e]	1690 ± 285	0.37 ± 0.30	135 ± 4	4568	365
5, NMP	–[e]	–[e]	366 ± 38	0.11 ± 0.10	15 ± 3	3327	136
U50,488	118 ± 20	401 ± 33	1772 ± 249	0.42 ± 0.22	1095 ± 186	4219	2607
U69,593	–[e]	–[e]	–[e]	2.5 ± 0.3	5286 ± 2117	>1333	2114

[a] Using cloned human receptors expressed in human embryonic kidney-293 cells.
[b] Using [^{3}H]Tyr-D-Ala-Gly-Phe-D-Leu as the radioligand.
[c] Using cloned rat opioid receptors and and [^{3}H]U69,593 as the radioligand.
[d] Using [^{3}H]Tyr-D-Ala-Gly-*N*-methyl-Phe-Gly-ol as the radioligand.
[e] Test ligand (10 μM) caused <50% inhibition of radioligand binding.

(1*S*,2*S*)-U50,488 for the KOR is diminished by its affinity for the α_{2c} (K_i = 118 nM) and σ_2 (K_i = 401 nM) receptors.

SalvA, EE, IPA, NMA, and NMP were also screened against the mDOR and rMOR at Temple University (see supplemental data). The mDOR and rMOR K_i values were compared with the previously reported hKOR K_i values (Béguin et al., 2005, 2006) to estimate receptor selectivity. In general, the binding data obtained for EE, IPA, NMA, and NMP were comparable to the ones obtained using the PDSP resource and suggested similar KOR selectivity profiles.

In Vitro Metabolism Experiments. Each test compound was incubated in the presence of human or rat liver microsomes with or without the P450 cofactor NADPH. Table 2 shows the in vitro CL_{int} values; a lower CL_{int} indicates greater in vitro stability. SalvA was the least stable substrate in the presence of NADPH (rat CL_{int} = 226 $\mu l \cdot min^{-1} \cdot mg^{-1}$, human CL_{int} = 233 $\mu l \cdot min^{-1} \cdot mg^{-1}$). In comparison, C-2-modified salvinorin analogs EE, IPA, NMA, and NMP were more stable in both species (rat CL_{int} = 24–140 $\mu l \cdot min^{-1} \cdot mg^{-1}$, human CL_{int} = 5–17 $\mu l \cdot min^{-1} \cdot mg^{-1}$). SalvA also displayed significant NADPH-independent metabolism (rat CL_{int} = 109 $\mu l \cdot min^{-1} \cdot mg^{-1}$, human CL_{int} = 168 $\mu l \cdot min^{-1} \cdot mg^{-1}$), whereas the other four compounds did not. This may be indicative of esterase-dependent clearance of salvA in the liver and, by implication, blood in vivo and in vitro. Overall, the rank order of metabolic stability in rat liver microsomes was NMA > IPA > NMP > EE > salvA, and in human liver microsomes NMA ~ IPA > EE > NMP > salvA. Verapamil, dextromethorphan, and diclofenac were used as control substances to verify assay performance.

Intracranial Self-Stimulation. In pilot ICSS studies designed to identify dose ranges of the salvA derivatives, EE had no effect on ICSS thresholds at doses up to 8.0 mg/kg i.p. (data not shown). Given the lack of solubility of EE at higher doses in 75% DMSO and its lower in vitro binding affinity, compared to salvA, IPA, NMA, and NMP, we did not further evaluate EE in the ICSS test. IPA, NMA, and NMP showed elevations of ICSS thresholds at 1, 0.5, and 0.25 mg/kg, respectively (data not shown). We did not characterize the dose-response curve of IPA because of its reduced in vitro KOR selectivity (Table 1, MOR/KOR K_i ratio = 25) compared to salvA, NMA, and NMP. In a newly trained group of rats, salvA, NMA, and NMP dose-dependently increased ICSS thresholds (Fig. 2, a, c, and e), and the effect depended on treatment (salvA: $F_{7.63} = 9.14$, $P < 0.01$; NMA: $F_{5.35} = 7.26$, $P < 0.01$; salvA: $F_{6.55} = 7.25$, $P < 0.01$). The dose-response curve data (Fig. 2, a, c, and e) suggest that salvA, NMA, and NMP were equipotent. There was a trend for decreases in ICSS maximal rates at the highest doses tested (Fig. 2, b, d, and f) but none reached significance. Vehicle (75% DMSO) had negligible effects on thresholds and maximal response rates. Figure 3 shows representative response-frequency functions for vehicle and NMA (2 mg/kg i.p.).

Using doses that produced maximal elevations of ICSS thresholds (Fig. 2, ~140%), we next examined the effects of vehicle, NMA (2 mg/kg i.p.), and NMP (1 mg/kg i.p.) at 3, 10, and 24 h postinjection. There was a significant interaction between time and drug treatments (Fig. 4, $F_{16.136} = 4.61$, $P < 0.01$). The data suggested that salvA showed maximal elevation of ICSS thresholds within the first 15 min postinjection ($P < 0.01$, Dunnett's test) and that the effect decreased to nonsignificant levels 30 min postinjection. In comparison, the effects of NMA peaked within the first 15 min postinjection ($P < 0.01$) and remained stable until 1.5 h postinjection ($P < 0.01$) before returning to baseline. Similar to NMA, NMP displayed long-lasting effects, with significant effects still observed 3 h postinjection ($P < 0.01$).

These experiments suggested that IPA and NMP showed reduced in vitro KOR selectivity and that EE lacked in vivo efficacy. Therefore, NMA was the most attractive candidate for preliminary oral efficacy studies. To compare the ICSS effects of NMA and salvA after oral administration, rats (n = 4) were administered vehicle (75% DMSO), NMA, or salvA by oral gavage. There was a significant interaction between time and drug treatments (Fig. 5, $F_{10.45} = 2.05$, $P < 0.05$). When rats were administered vehicle (75% DMSO p.o.) or salvA (2 mg/kg p.o.), their ICSS thresholds remained unchanged. In comparison, NMA (2 mg/kg p.o.) caused a significant elevation of ICSS thresholds (Fig. 5, $P < 0.01$). Vehicle (75% DMSO) seemed to decrease response rates, but there was no significant difference between vehicle and each drug treatments (Fig. 5).

TABLE 2

Intrinsic clearances (CL_{int}) of salvA, C-2-modified analogs, verapamil, dextromethorphan, and diclofenac

Data represent the intrinsic clearances obtained by incubating each test compound with rat or human liver microsomes, in the presence or absence of the P450 cofactor NADPH. Verapamil, dextromethorphan, and diclofenac served as standard compounds. The order of in vitro metabolic stability in rat microsomes was NMA > IPA > NMP > EE > salvA. The order of in vitro metabolic stability in human microsomes was NMA ~ IPA > EE > NMP > salvA.

Compound	CL_{int}			
	Rat		Human	
	+ NADPH	− NADPH	+ NADPH	− NADPH
	($\mu l \cdot min^{-1} \cdot mg^{-1}$) ± S.D.			
1, SalvA	226 ± 3	109 ± 20	233 ± 4	168 ± 5
2, EE	140 ± 5	<5	9.70 ± 6.18	<5
3, IPA	41.8 ± 6.7	<5	<5	<5
4, NMA	24.2 ± 4.3	<5	<5	<5
5, NMP	77.2 ± 5.1	<5	17.2 ± 4.3	<5
Verapamil	273 ± 10	<5	166 ± 10	<5
Dextromethorphan	247 ± 5	<5	32 ± 6	<5
Diclofenac	254 ± 4	<5	362 ± 22	<5

Discussion

We have previously reported the SAR of C-2-modified salvinorin derivatives and found that three to four atom long substituents led to high KOR agonist potency (Béguin et al., 2005, 2006). In particular, four C-2-modified salvinorin analogs designed to have increased metabolic stability (EE, IPA, NMA, and NMP) were identified as potent KOR agonists. In this study, we further characterized these compounds by first determining their KOR binding selectivity. SalvA, EE, IPA, NMA, and NMP were screened against common central nervous system targets (39 receptors and transporters) using the PDSP screening facility (Table 1). As previously observed using hKOR (see supplemental data) (Béguin et al., 2005, 2006), EE, IPA, NMA, and NMP exhibited high binding affinity for the rKOR (Table 1, K_i = 0.11–6.3 nM). The rank order of KOR selectivity was salvA > NMA ~ EE > NMP > IPA. For comparison, we tested the standard KOR agonists U50,488 and U69,593 against the same 39 receptors or transporters. The synthetic KOR agonist U50,488 can exist in four diastereoisomeric forms. The racemic mixture of the two

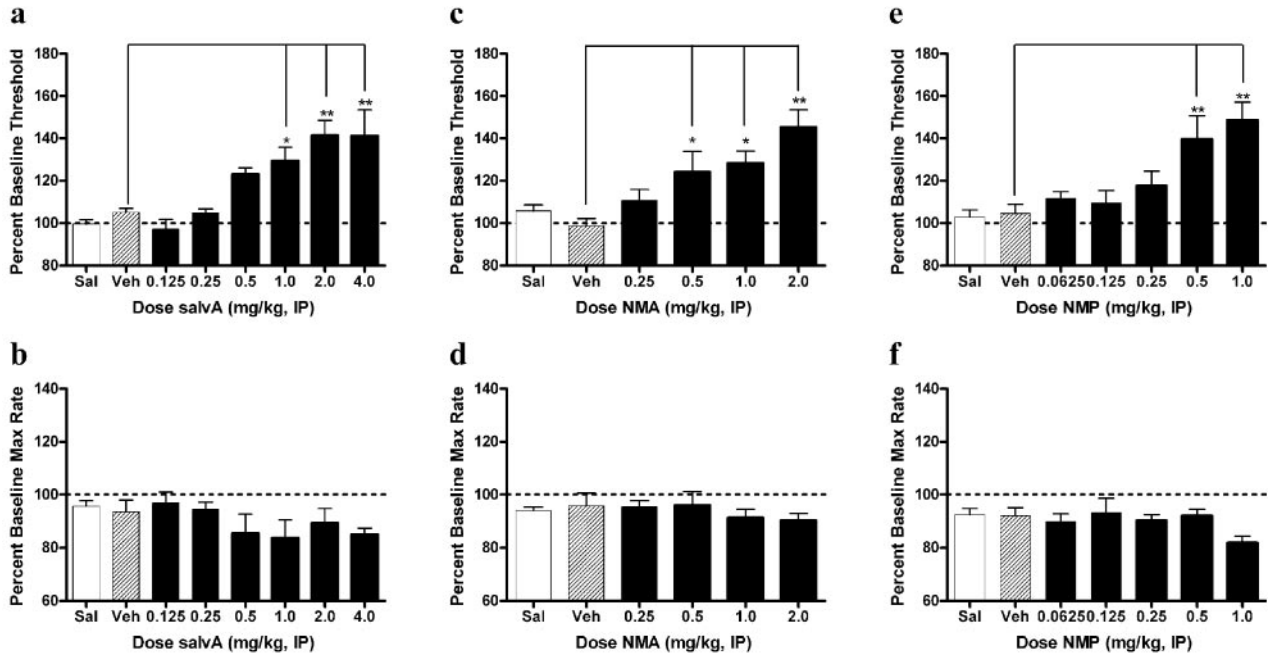

Fig. 2. Effect of salvA, NMA, and NMP on ICSS after i.p. administration. a, vehicle (75% DMSO) had no significant effect on ICSS thresholds (mean ± S.E.M.) over the 15-min test session. SalvA dose-dependently increased ICSS thresholds. *, $P < 0.05$; **, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group. b, in comparison with vehicle, none of the doses of salvA affected response capabilities (maximal rates). c, NMA dose-dependently increased ICSS thresholds during the 15-min session. *, $P < 0.05$; **, $P < 0.01$, compared with vehicle, Dunnett test, six rats per group. d, in comparison with vehicle, none of the doses of NMA affected response capabilities (maximal rates). e, NMP dose-dependently increased ICSS thresholds during the 15-min session. **, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group. f, in comparison with vehicle, none of the doses of NMP affected response capabilities (maximal rates).

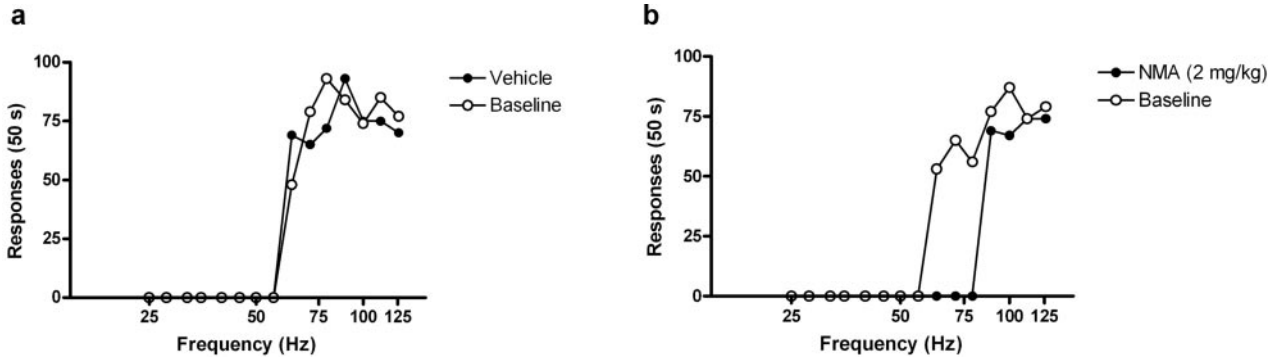

Fig. 3. a, vehicle had no effect on rate-frequency curves compared to baseline. b, NMA (2 mg/kg i.p.) caused rightward shifts in rate-frequency functions compared to baseline. Data are from representative rats.

trans-isomers is most commonly used in pharmacological studies. Previous investigations have shown that the *trans*-isomer (1*S*,2*S*)-U50,488 was the most potent isomer with subnanomolar K_i value at the KOR (Rothman et al., 1989). Consistent with this findings, (1*S*,2*S*)-U50,488 showed high rKOR binding affinity (Table 1). As previously reported (Clark and Pasternak, 1988), it also had excellent KOR selectivity over DOR and MOR. However, its affinity for the α_{2C} and σ_2 receptors led to reduced KOR selectivity. In comparison, U69,593 was highly selective for the KOR in the PDSP-screening assay. Taken together, these data indicate that, from the subset of compounds selected in this study, U69,593, salvA, NMA, and EE are the most selective KOR ligands.

To determine whether C-2 chemical modifications led to increased metabolic stability, we incubated salvA, EE, IPA, NMA, and NMP with rat and human liver microsomes. The observed intrinsic clearances demonstrated that salvA was the least stable compound. As expected, our modifications at C-2 (EE, IPA, NMA, and NMP) led to increased resistance to metabolism, with NMA and IPA being the most stable derivatives in vitro. It was anticipated that the improved metabolic stability of EE, IPA, NMA, and NMP in vitro should translate to improved half-life and bioavailability in vivo.

We then determined the potencies of salvA, EE, IPA, NMA, and NMP in vivo. We used ICSS because it is sensitive to KOR agonist effects (Todtenkopf et al., 2004) and can provide information about the time course of drug actions. In pilot ICSS studies, the order of potency was NMP > NMA ~ salvA > IPA > EE. As predicted from in vitro results (using hKOR or rKOR binding assays), EE was the least potent analog and thus not further characterized in vivo. Likewise, the dose-response curve of IPA was not determined due to its reduced in vitro KOR selectivity. We had previously evaluated salvA (0.125–2 mg/kg i.p.) in the ICSS assay (Carlezon et al., 2006). Here we tested a more extensive dose range of

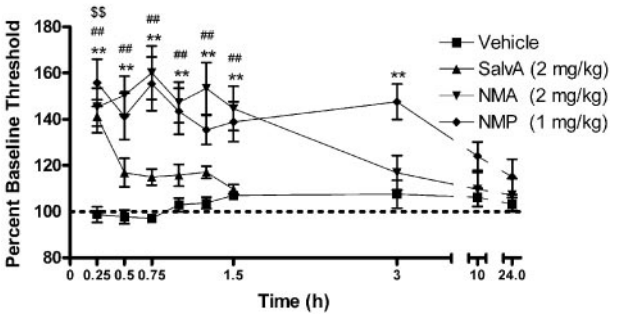

Fig. 4. Time course of effect of vehicle, salvA, and NMA on ICSS after i.p. administration. Vehicle (75% DMSO i.p.) had no significant effect on ICSS thresholds (mean ± S.E.M.) over 24 h. In comparison, salvA (2 mg/kg i.p.) increased ICSS thresholds 15 min postinjection. $$, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group. NMA (2 mg/kg i.p.) increased ICSS thresholds up to 1.5 h postinjection. ##, $P < 0.01$, compared with vehicle, Dunnett test, six rats per group. NMP (1 mg/kg i.p.) increased ICSS thresholds up to 3 h postinjection. **, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group.

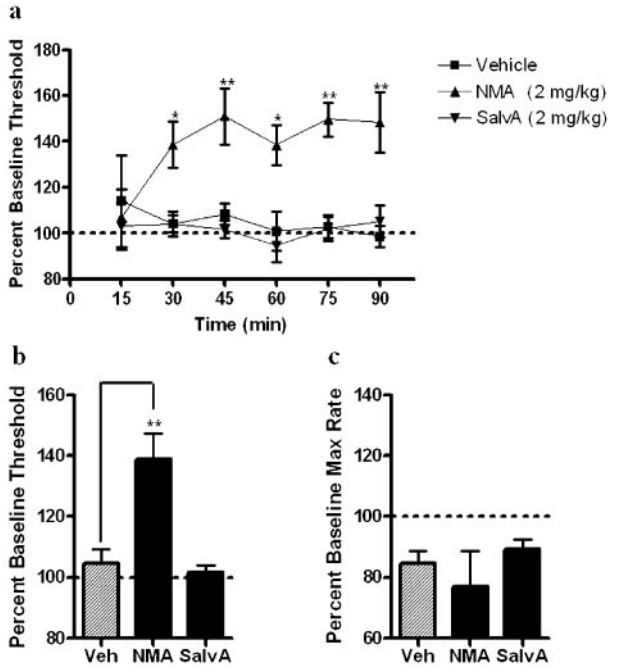

Fig. 5. Effect of vehicle, salvA, and NMA on ICSS after p.o. administration. a, vehicle (75% DMSO p.o.) and salvA (2 mg/kg p.o.) had no significant effect on ICSS thresholds (mean ± S.E.M.) over the 90-min test session. In comparison, NMA (2 mg/kg p.o.) increased ICSS thresholds. *, $P < 0.05$; **, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group. b, the average percent baseline thresholds over the 1.5-h test session showed similar effects. **, $P < 0.01$, compared with vehicle, Dunnett test, eight rats per group.

salvA to confirm its prodepressant-like effects and facilitate direct comparisons among the derivatives. We found that salvA, NMA, and NMP dose-dependently increased ICSS thresholds without significantly affecting maximal response rates, which can reflect treatment-induced alterations in motor capabilities. The effects were consistent with our previous findings using salvA (Carlezon et al., 2006) and U69,593 (Todtenkopf et al., 2004). In addition, salvA, NMA, and NMP were equipotent and produced similar maximal effects in this behavioral assay.

In vitro, NMP showed reduced KOR over MOR selectivity compared to salvA and NMA. However, the three test compounds induced comparable elevations in ICSS thresholds. It is conceivable that ICSS does not allow us to distinguish highly selective (salvA and NMA) from less selective (NMP) KOR agents, or the doses used were too low to detect any MOR-mediated effects. Alternatively, the prodepressant effects of KOR stimulation may mask the rewarding actions of MOR stimulation (Devine and Wise, 1994).

The purpose of our SAR study at C-2 was to design salvinorin analogs with increased in vivo stability. We compared the time courses of salvA, NMA, and NMP on ICSS. The time course of salvA effects on ICSS was consistent with its short-lived effects observed in antinociceptive assays (Ansonoff et al., 2006; John et al., 2006; McCurdy et al., 2006); it produced maximal threshold-elevating effects within the first 15 min postadministration, and these effects had waned by 30 to 60 min. NMA and NMP produced longer lasting prodepressant-like effects, 6- and 10-fold, respectively. These data suggest that the increase in duration of action of the C-2-modified salvinorin analogs may be due, at least in part, to their enhanced metabolic stability. Other C-2-modified salvinorin derivatives may also display longer lasting in vivo effects (L.-Y. Liu-Chen, personal communication).

A common characteristic of currently available selective KOR agonists is limited oral bioavailability. Using a mouse acetic acid-induced abdominal constriction test, Endoh et al. (1999) reported a 10-fold or higher difference between the ED_{50} obtained after p.o. and i.p. administrations of several KOR agonists (TRK-820, U50,488, CI-977, ICI-199441, and PD-117302), suggesting a limited oral absorption of these drugs. In our study, after oral administration, NMA (2 mg/kg) retained in vivo activity, whereas a similar dose of salvA was inactive. The slower onset of action of NMA after p.o. compared with i.p. administration was consistent with a slower rate of absorption. Vehicle (75% DMSO p.o.) had no effects on ICSS thresholds.

In conclusion, this study confirmed that salvA and U69,593 are potent and selective KOR ligands. We found that NMA had a similar highly selective in vitro pharmacological profile, whereas (1*S*,2*S*)-U50,488 had lesser KOR selectivity due to its moderate affinity for the adrenergic α_{2C} receptor. We confirmed that salvA had short lasting in vivo behavioral effects, most probably due to metabolic lability. In contrast, we found that NMA produced long-lasting prodepressant-like effects in the ICSS test, and preliminary data indicated that oral availability was improved by reducing metabolic lability. NMA may be a new pharmacological tool in experiments that require oral availability and long-lasting KOR activation. More exhaustive pharmacokinetics and toxicity studies are needed to determine whether NMA might have clinical applications.

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References

Ansonoff MA, Zhang J, Czyzyk T, Rothman RB, Stewart J, Xu H, Zjwiony J, Siebert DJ, Yang F, Roth BL, et al. (2006) Antinociceptive and hypothermic effects of salvinorin a are abolished in a novel strain of kappa-opioid receptor-1 knockout mice. *J Pharmacol Exp Ther* **318:**641–648.

Barber A and Gottschlich R (1997) Novel developments with selective, non-peptidic kappa-opioid receptor agonists. *Expert Opin Investig Drugs* **6:**1351–1368.

Béguin C, Richards MR, Wang Y, Chen Y, Liu-Chen L-Y, Ma Z, Lee DY, Carlezon WA Jr, and Cohen BM (2005) Synthesis and in vitro pharmacological evaluation of salvinorin A analogues modified at C(2). *Bioorg Med Chem Lett* **15:**2761–2765.

Béguin C, Richards MR, Li JG, Wang Y, Xu W, Liu-Chen L-Y, Carlezon WA Jr, and

Cohen BM (2006) Synthesis and in vitro evaluation of salvinorin A analogues: effect of configuration at C(2) and substitution at C(18). *Bioorg Med Chem Lett* **16:**4679–4685.
Braida D, Limonta V, Pegorini S, Zani A, Guerini-Rocco C, Gori E, and Sala M (2007) Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid receptor involvement. *Psychopharmacology (Berl)* **190:**441–448.
Butelman ER, Harris TJ, and Kreek MJ (2004) The plant-derived hallucinogen, salvinorin A, produces kappa-opioid agonist-like discriminative effects in rhesus monkeys. *Psychopharmacology (Berl)* **172:**220–224.
Carlezon WA Jr, Todtenkopf MS, McPhie DL, Pimentel P, Pliakas AM, Stellar JR, and Trzcinska M (2001) Repeated exposure to rewarding brain stimulation down-regulates GluR1 expression in the ventral tegmental area. *Neuropsychopharmacology* **25:**234–241.
Carlezon WA Jr, Béguin C, DiNieri JA, Baumann MH, Richards MR, Todtenkopf MS, Rothman RB, Ma Z, Lee DY, and Cohen BM (2006) Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J Pharmacol Exp Ther* **316:**440–447.
Chavkin C, Sud S, Jin W, Stewart J, Zjawiony JK, Siebert DJ, Toth BA, Hufeisen SJ, and Roth BL (2004) SalvA, an active component of the hallucinogenic sage *S. divinorum* is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther* **308:**1197–1203.
Clark JA and Pasternak GW (1988) U50,488: a kappa-selective agent with poor affinity for mu1 opiate binding sites. *Neuropharmacology* **27:**331–332.
Cohen BM and Murphy B (2007) The effects of pentazocine, a kappa agonist, in patients with mania. *Int J Neuropsychopharmacol*, in press.
Devine DP and Wise RA (1994) Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci* **14:**1978–1984.
Dortch-Carnes J and Potter DE (2005) Bremazocine: a kappa-opioid agonist with potent analgesic and other pharmacologic properties. *CNS Drug Rev* **11:**195–212.
Endoh T, Matsuura H, Tajima A, Izumimoto N, Tajima C, Suzuki T, Saitoh A, Suzuki T, Narita M, Tseng L, et al. (1999) Potent antinociceptive effects of TRK-820, a novel [kappa]-opioid receptor agonist. *Life Sci* **65:**1685–1694.
Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals* 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.
John TF, French LG, and Erlichman JS (2006) The antinociceptive effect of salvinorin A in mice. *Eur J Pharmacol* **545:**129–133.
Lee DY-W, Ma Z, Liu-Chen L-Y, Wang Y, Chen Y, Carlezon WA Jr, and Cohen BM (2005) Three new neoclerodane diterpenoids from the leaves of *S. divinorum*. *Bioorg Med Chem* **13:**5635–5639.
Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC Jr, Jones RM, Portoghese PS, and Carlezon WA Jr (2003) Antidepressant-like effects of kappa-opioid receptor antagonists in the forced swim test in rats. *J Pharmacol Exp Ther* **305:**323–330.
McCurdy CR, Sufka KJ, Smith GH, Warnick JE, and Nieto MJ (2006) Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist. *Pharmacol Biochem Behav* **83:**109–113.
Munro TA and Rizzacasa MA (2003) Salvinorins D-F, new neoclerodane diterpenoids from *Salvia divinorum*, and an improved method for the isolation of salvinorin A. *J Nat Prod* **66:**703–705.
Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27:**1350–1359.
Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd ed., Academic Press, San Diego, CA.
Pfeiffer A, Brantl V, Herz A, and Emrich HM (1986) Psychotomimesis mediated by kappa opiate receptors. *Science* **233:**774–776.
Prisinzano TE, Tidgewell K, and Harding WW (2005) Kappa opioids as potential treatments for stimulant dependence. *AAPS J* **7:**E592–E599.
Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P, and Rothman RB (2002) SalvA: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc Natl Acad Sci U S A* **99:**11934–11939.
Rothman RB, France CP, Bykov V, De Costa BR, Jacobson AE, Woods JH, and Rice KC (1989) Pharmacological activities of optically pure enantiomers of the kappa opioid agonist, U50,488, and its *cis*-diastereomer: evidence for three kappa receptor subtypes. *Eur J Pharmacol* **167:**345–353.
Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, and Roth BL (2000) Evidence for possible involvement of 5-HT2B receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* **102:**2836–2841.
Schmidt MD, Schmidt MS, Butelman ER, Harding WW, Tidgewell K, Murry DJ, Kreek MJ, and Prisinzano TE (2005a) Pharmacokinetics of the plant-derived kappa-opioid hallucinogen salvinorin A in nonhuman primates. *Synapse* **58:**208–210.
Schmidt MS, Prisinzano TE, Tidgewell K, Harding W, Butelman ER, Kreek MJ, and Murry DJ (2005b) Determination of Salvinorin A in body fluids by high performance liquid chromatography-atmospheric pressure chemical ionization. *J Chromatogr B Analyt Technol Biomed Life Sci* **818:**221–225.
Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, and Mailman R (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* **28:**1400–1411.
Shippenberg TS and Rea W (1997) Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* **57:**449–455.
Siebert DJ (1994) Savia divinorum and Salvinorin A: new pharmacologic findings. *J Ethnopharmacol* **43:**53–56.
Todtenkopf MS, Marcus JF, Portoghese PS, and Carlezon WA Jr (2004) Effects of kappa opioid ligands on intracranial self-stimulation in rats. *Psychopharmacology (Berl)* **172:**463–470.
Vortherms TA and Roth BL (2006) Salvinorin A: from natural product to human therapeutics. *Mol Interv* **6:**257–265.
Vortherms TA, Mosier PD, Westkaemper RB, and Roth BL (2007) Differential helical orientations among related G protein-coupled receptors provide a novel mechanism for selectivity. Studies with salvinorin A and the kappa-opioid receptor. *J Biol Chem* **282:**3146–3156.
Walsh SL, Strain EC, Abreu ME, and Bigelow GE (2001) Enadoline, a selective kappa opioid agonist: comparison with butorphanol and hydromorphone in humans. *Psychopharmacology (Berl)* **157:**151–162.
Wang Y, Tang K, Inan S, Siebert D, Holzgrabe U, Lee DY, Huang P, Li JG, Cowan A, and Liu-Chen L-Y (2005) 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* **312:**220–230.
Yan F, Mosier PD, Westkaemper RB, Stewart J, Zjawiony JK, Vortherms TA, Sheffler DJ, and Roth BL (2005) Identification of the molecular mechanisms by which the diterpenoid salvinorin A binds to kappa-opioid receptors. *Biochemistry* **44:**8643–8651.
Zhang Y, Butelman ER, Schlussman SD, Ho A, and Kreek MJ (2005) Effects of the plant-derived hallucinogen salvinorin A on basal dopamine levels in the caudate putamen and in a conditioned place aversion assay in mice: agonist actions at kappa opioid receptors. *Psychopharmacology (Berl)* **179:**551–558.

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