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Original Article

Effects of ATPM-ET, a novel κ agonist with partial μ activity, on morphine-induced physical dependence and behavior sensitization in mice

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Aim: To investigate the effects of ATPM-ET [(–)-3-N-Ethylaminothiazolo [5,4-b]-N-cyclopropylmethylmorphinan hydrochloride] on physical dependence and behavioral sensitization to morphine in mice.
Methods: The pharmacological profile of ATPM-ET was characterized using competitive binding and GTPγS binding assays. We then examined the antinociceptive effects of ATPM-ET in the hot plate test. Morphine dependence assay and behavioral sensitization assay were used to determine the effect of ATPM-ET on physical dependence and behavior sensitization to morphine in mice.
Results: The binding assay indicated that ATPM-ET ATPM-ET exhibited a high affinity to both κ- and μ-opioid receptors with K_i values of 0.15 nmol/L and 4.7 nmol/L, respectively, indicating it was a full κ-opioid receptor agonist and a partial μ-opioid receptor agonist. In the hot plate test, ATPM-ET produced a dose-dependent antinociceptive effect, with an ED_{50} value of 2.68 (2.34–3.07) mg/kg. Administration of ATPM-ET (1 and 2 mg/kg, sc) prior to naloxone (3.0 mg/kg, sc) injection significantly inhibited withdrawal jumping of mice. In addition, ATPM-ET (1 and 2 mg/kg, sc) also showed a trend toward decreasing morphine withdrawal-induced weight loss. ATPM-ET (1.5 and 3 mg/kg, sc) 15 min before the morphine challenge significantly inhibited the morphine-induced behavior sensitization ($P<0.05$).
Conclusion: ATPM-ET may have potential as a therapeutic agent for the treatment of drug abuse.

Keywords: behavioral sensitization; mu opioid receptors; morphine; kappa opioid receptors; physical dependence; addiction

Introduction

Opioid analgesic drugs, such as morphine, are widely used for the relief of severe pain. However, repeated use of morphine can induce tolerance and addiction (physical and psychological dependence)[1, 2]. Indeed, opioid addiction persists as a major public health problem[3–5]. Although much progress has been made, therapeutic strategies for the treatment of opioid addiction are still limited, largely due to the poor understanding of the mechanisms contributing to opioid addiction. Hence, it is necessary to further explore the neural mechanisms underlying opioid addiction and to develop new strategies for the treatment of opioid abuse.

Numerous studies have suggested that the μ-opioid receptor plays a major role in opioid addiction, whereas κ-opioid agonists may have therapeutic potential for opioid addiction. For example, previous studies in both nonhuman primates and rats have demonstrated that κ agonists functionally attenuate many of the behavioral effects of cocaine, including place preference[6–8], self-administration[9–12] and the reinstatement of extinguished drug-taking behavior in an animal model of relapse[12, 13]. However, these selective agonists produce many severe, undesirable side effects such as salivation, emesis, and sedation in nonhuman primates[10, 11], which may limit the clinical utility of κ agonists for the treatment of drug abuse.

Increasing evidence suggests that κ agonists with mixed activity at κ and μ receptors may be more promising candidates for drug abuse pharmacotherapy than highly selective κ agonists[14, 15]. Therefore, one potential treatment strategy for opioid addiction is to develop compounds with mixed activity at κ and μ receptors[16, 17]. A recent study from our group demonstrated that ATPM, a novel mixed κ agonist and μ agonist/
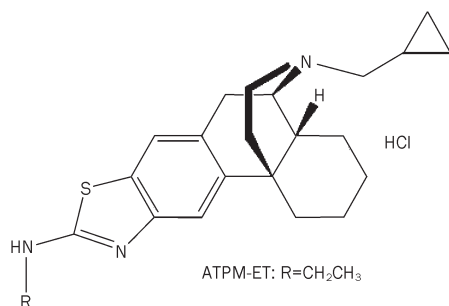
Figure 1. Chemical structure of ATPM-ET.

antagonist, can reduce heroin self-administration with relatively low levels of sedation[18]. The present study was undertaken to examine the effects of ATPM-ET, a derivate of ATPM, on morphine-induced physical dependence and behavioral sensitization (Figure 1).

Materials and methods

Cell culture and membrane preparation

CHO cells were transfected with κ-, μ-, or δ- opioid receptors using Lipofectamine (Invitrogen) according to the manufacturer's protocol. CHO cells stably expressing κ-, μ-, or δ-opioid receptors were maintained in F12 medium with 10% fetal calf serum and 0.25 mg/mL G418. Cells were incubated in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37 °C.

Cell membranes were prepared as described in our previous report[19]. Cells were detached by incubation with phosphate-buffered saline containing 1 mmol/L EDTA and centrifuged at 1000×g for 10 min (4 °C). The cell pellet was suspended in ice-cold homogenization buffer composed of 50 mmol/L HEPES, pH 7.4, 1 mmol/L $MgCl_2$, and 1 mmol/L EGTA. Cells were homogenized with 10 strokes using a glass Dounce homogenizer. After centrifugation at 40000×g for 10 min (4 °C), pellets were resuspended in homogenization buffer, homogenized, and centrifuged again. The final pellets were resuspended in 50 mmol/L Tris–HCl buffer, pH 7.4. Protein concentration was determined, and aliquots were stored at -80 °C.

Competitive binding assay

Membrane proteins were incubated with varying concentrations of ATPM-ET in a binding buffer composed of 50 mmol/L Tris-HCl, pH 7.5 at 25 °C. Incubation times of 60 min were used for the μ-selective peptide, [3H]DAMGO, and the κ-selective ligand, [3H]U69,593. A 3 h incubation was used with the δ-selective antagonist, [3H]naltrindole. The final concentrations of [3H]DAMGO, [3H]naltrindole, and [3H]U69,593 were 0.25, 0.2, and 1 nmol/L, respectively. Nonspecific binding was measured by the inclusion of 10 μmol/L naloxone. The binding was terminated by filtering the samples under reduced pressure through GF/B filters (Whatman). The bound radioactivity on each filter was determined by liquid scintillation counting (Beckman LS6500).

GTPγS binding assay

[35S]GTPγS binding was performed as described previously[19]. Briefly, membranes (15 μg/tube) were incubated with 0.1 nmol/L [35S]GTPγS in a binding buffer composed of 50 mmol/L Tris–HCl, pH 7.5, 1 mmol/L EDTA, 5 mmol/L $MgCl_2$, 100 mmol/L NaCl, and 40 μmol/L GDP at 30 °C for 1 h in the presence of increasing concentrations of ATPM-ET. Nonspecific binding was determined in the presence of nonradioactive GTPγS (10 μmol/L). Reactions were terminated by rapid filtration through GF/B filters (Whatman), and bound radioactivity was determined by liquid scintillation counting (Beckman LS6500).

Animals

Male Kunming mice (about 20 g) were obtained from the Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Mice were housed in groups of 10 and maintained on a 12 h light/dark cycle (lights on at 8:00 a.m.) in a temperature-controlled environment. Mice were given free access to food and water. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Hot plate test

Hot plate tests were performed using a plate temperature of 55 °C. Mice were placed on the heated smooth surface, and the jumping latency or the latency to begin licking or shaking the limbs was measured. Prior to drug administration, the nociceptive response of each mouse was measured twice. The average of these two responses was used as the pre-drug latency for each mouse. A cut-off time of 60 s was used to minimize tissue damage. Tests were performed 15 min after subcutaneous administration of drug. Antinociception was calculated using the following formula: % antinociception=100×(test latency – basal latency)/(60 – basal latency).

Physical dependence assay

Physical dependence assays were performed according to our previously published protocols[19]. Morphine was injected subcutaneously twice daily (8:30 and 16:30), and the dose was increased progressively from 20 to 100 mg/kg over a period of 5 days. Two hours following the final injection of morphine or ATPM-ET, withdrawal jumping was induced by subcutaneous injection of naloxone (3.0 mg/kg). Immediately following the naloxone injection, mice were placed on a circular platform (30 cm diameter and 70 cm height), and the jumping frequency of each mouse was recorded for 30 min. Body weight was measured initially and at 30 and 60 min after the naloxone injection. To study the effects of ATPM-ET and (−)U50,488H on morphine physical dependence, mice were treated with varying doses of ATPM-ET (0.1–2 mg/kg, subcutaneously) or (−)U50,488H (2 mg/kg, subcutaneously) 15 min prior to each morphine administration during a chronic morphine treatment paradigm. To evaluate the potential of ATPM-ET to induce physical dependence, animals were injected with ATPM-ET

twice daily with progressively increasing doses, ranging from 0.1 to 2 mg/kg, over a period of 5 days. Following this treatment regimen, naloxone-induced withdrawal jumping was measured as described above.

Behavioral sensitization assay

Locomotor activity was measured by a video tracking system (JLBehv-LAG, Shanghai, China). Each chamber of 26 cm×14 cm×15 cm (length×width×height) was equipped with a video camera above the center of the floor. The walls and floors were made of clear plastic that had been painted black. Total distance traveled was recorded in cm.

Mice were treated with morphine (10 mg/kg, subcutaneously) or saline for 7 consecutive days, and their activity was measured for 1 h after drug administration on days 1, 4, and 7. After a 7-d washout period, mice were challenged with morphine (10 mg/kg, subcutaneously), and their locomotor activity was measured for 1 h, as previously described[20].

Four groups of mice were injected with morphine (10 mg/kg, subcutaneously) for 7 consecutive days to induce behavioral sensitization, and one group of mice was injected with saline (10 mL/kg, subcutaneously). After 7 days of washout, mice were treated with either saline or ATPM-ET (0.75, 1.5, 3 mg/kg, subcutaneously) 15 min prior to morphine challenge (10 mg/kg, subcutaneously). The locomotor activity of the mice was then measured for 1 h.

Drugs

[^{35}S]GTPγS (1030 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ). (−)U50,488H, naloxone, GTPγS, and GDP were supplied by Sigma-Aldrich (St Louis, MO). Morphine was obtained from the National Institute of Forensic Science (Beijing, China). ATPM-ET was synthesized by Prof Ao ZHANG in our institute. ATPM-ET and U50,488H were dissolved in saline.

Statistical analysis

The data are expressed as means±SEM. Statistical analyses were performed using one-way ANOVAs and LSD *post hoc* tests. *P*-values less than 0.05 were considered statistically significant. When only two groups were compared, statistical significance was determined by an unpaired Student *t* test.

Results

Affinity, selectivity, and agonist activity of ATPM-ET

The binding affinity of ATPM-ET to μ-, δ-, and κ-opioid receptors was determined using competitive binding assays. As shown in Table 1, ATPM-ET exhibited a high affinity to both κ- and μ-opioid receptors with K_i values of 0.15 nmol/L and 4.7 nmol/L, respectively. The affinity to the κ-opioid receptor is 31-fold higher than the affinity to the μ-opioid receptor, indicting that ATPM-ET has a high selectivity for κ- over μ-opioid receptors. However, ATPM-ET exhibited a 287-fold lower affinity to the δ-opioid receptor (K_i of 43 nmol/L) than to the κ-opioid receptor.

To characterize the agonist activity of ATPM-ET, [^{35}S]GTPγS assays were performed with membranes prepared from CHO cells that had been stably transfected with either the κ- or the μ-opioid receptors. The maximal stimulatory response of (−)U50,488H and DAMGO was designated as the 100% effect for κ- and μ-opioid receptors, respectively. ATPM-ET produced a maximal stimulation (E_{max}) of 85% for the κ-opioid receptor and 36% for the μ-opioid receptor and exhibited an EC_{50} value of 1.7 nmol/L for the κ- and 61 nmol/L for the μ-opioid receptors (Table 1). The results of these [^{35}S]GTPγS binding assays indicate that ATPM-ET acts as a high-efficacy κ-opioid receptor agonist and a low-efficacy μ-opioid receptor agonist.

The antinociceptive effect of ATPM-ET

Activation of opioid receptors can produce antinociceptive effects against noxious stimuli, such as heat. Therefore, the antinociceptive effects of ATPM-ET in mice were studied using the hot plate test. As shown in Table 2, subcutaneous administration of ATPM-ET produced a dose-dependent antinociceptive effect, with an ED_{50} value of 2.68 (2.34–3.07) mg/kg in the hot plate test. Morphine and (−)U50,488H also resulted in a dose-dependent antinociceptive effect in the hot plate assay, with ED_{50} values of 4.42 (2.51–7.75) mg/kg and 6.95 (5.68–8.50) mg/kg, respectively.

The effect of ATPM-ET on morphine physical dependence

Withdrawal jumping is a widely used and highly reliable index of opiate withdrawal behavior. We evaluated the effects of ATPM-ET on morphine dependence by measuring withdrawal jumping and loss of body weight. An acute injection of

Table 1. Binding affinities for opioid receptors and efficacy in stimulating [^{35}S]GTPγS binding to membranes of ATPM-ET in CHO cells stably expressing opioid receptors. Data are expressed as the mean±SEM of independent experiments performed in triplicate.

| Compound | Receptor binding | | | | | GTPγS binding | | | |
| | K_i±SEM (nmol/L) | | | Selectivity | | κ | | μ | |
	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)	μ/κ	δ/κ	E_{max} (%)	EC_{50} (nmol/L)	E_{max} (%)	EC_{50} (nmol/L)
ATPM-ET	4.7±0.9	43±8	0.15±0.05	31	290	85±9	1.7±0.2	36±7	61±8

Table 2. ED$_{50}$ values and 95% confidence limits of the antinociception produced by subcutaneous injection of ATPM-ET, morphine and (−)50,488H in hot plate test. The antinociceptive ED$_{50}$ value of each drug was calculated from data obtained at 15 min after *sc* administration of each dose of compounds and for each dose at least 10 mice were used.

Compound	Hot plate test	
	ED$_{50}$ (mg/kg, *sc*)	95% Confidence limits
(−)U50,488H	6.95	(5.68–8.50)
Morphine	4.42	(2.51–7.75)
ATPM-ET	2.68	(2.34–3.07)

naloxone (3.0 mg/kg, *sc*) resulted in a robust increase in withdrawal jumping following repeated treatment of morphine for 5 consecutive days in mice compared to repeated treatment with saline (Figure 2A). Administration of ATPM-ET (1, 2 mg/kg, subcutaneously) prior to naloxone injection significantly inhibited withdrawal jumping. In addition, ATPM-ET (1, 2 mg/kg, subcutaneously) also showed a trend toward decreasing morphine withdrawal-induced weight loss (Figure 2B). In agreement with previous studies[21], administration of
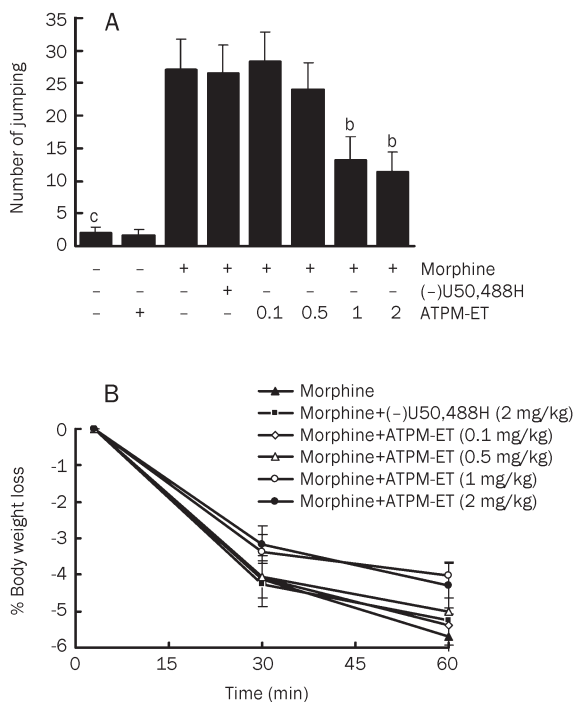

Figure 2. Effects of ATPM-ET and (−)U50,488H on naloxone-precipitated jumping and body weight loss in mice treated chronically with morphine. Mice were treated with progressively increasing doses of either ATPM-ET or morphine alone or treated with progressively increasing doses of morphine concomitantly with ATPM-ET (0.1–2 mg/kg, subcutaneously) or (-)U50,488H (2 mg/kg, subcutaneously) for 5 days. Morphine withdrawal jumping and body weight loss were precipitated by naloxone (3 mg/kg, subcutaneously). (A) The number of jumping events was measured for 30 min after naloxone injection. (B) Body weight loss was measured initially and 30 and 60 min after the naloxone injection. Data were presented as the mean±SEM from 10 animals. $^{b}P<0.05$, $^{c}P<0.01$ *vs* morphine alone.

(−)U50,488H (2 mg/kg, *sc*) did not inhibit naloxone-precipitated jumping or loss of body weight. Moreover, administration of ATPM-ET alone for 5 days did not induce morphine-like dependence after naloxone precipitation, which suggested that ATPM-ET has a lower abuse potential than morphine (Figure 2).

The effect of ATPM-ET on morphine-induced behavioral sensitization

It has been reported previously that behavioral sensitization is involved in opioid addiction and that κ-opioid agonists can attenuate many of the behavioral effects induced by opiates[21-23]. As a result, we decided to investigate the effects of ATPM-ET on morphine-induced behavioral sensitization.

First, we established the induction of morphine sensitization in mice as described previously[24]. Mice were treated with saline or morphine (10 mg/kg, sc) once per day for 7 consecutive days. Locomotor activity was recorded for 1 h following drug administration on days 1, 4, and 7. As shown in Figure 3, the initial injection of morphine (10 mg/kg, subcutaneously) elicited a significant increase in locomotor activity when compared to an injection of saline. Daily morphine injections led to a progressive increase in locomotor activity, with significantly elevated locomotion on days 4 and 7 compared to day 1. A two-factor repeated measures ANOVA shows significant effects of both drug ($F_{(1, 28)}=211.2$, $P<0.001$) and time of treatment ($F_{(2, 28)}=6.82$, $P<0.001$) as well as an interaction between the drug and the time of treatment ($F_{(2, 28)}=7.05$, $P<0.001$). In contrast, no enhancement of locomotor activity was observed in mice injected with saline for 7 days. These results indicate that morphine promotes the development of behavioral sensitization in mice, consistent with previous studies[24].

Next, we examined the effects of ATPM-ET on morphine-induced behavioral sensitization. After 7 days of washout, challenge with morphine (10 mg/kg, subcutaneously) induced a robust increase in locomotor activity in mice repeatedly treated with morphine (10 mg/kg) for 7 consecutive days compared to mice repeatedly injected with saline ($P<0.01$).
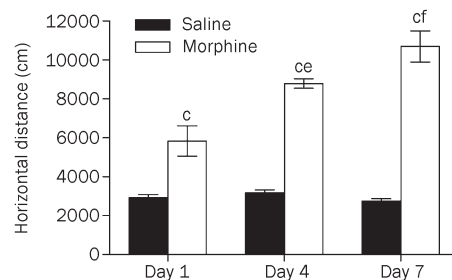

Figure 3. The induction of morphine sensitization in mice. Two groups were given morphine (10 mg/kg, subcutaneously) or saline (10 mL/kg, subcutaneously) for 7 consecutive days and their activity was measured for 1 h after each administration of drug (or saline) on days 1, 4, and 7. Data are shown as means±SEM. (*n*=10 per group). $^{b}P<0.05$, $^{c}P<0.01$ *vs* the corresponding saline group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* the first administration group.

One-way ANOVA revealed a significant effect of ATPM-ET on the expression of morphine sensitization ($F_{(4, 37)}$=6.42, $P<0.01$). LSD *post hoc* tests indicated that treatment with ATPM-ET (1.5, 3 mg/kg, subcutaneously) 15 min before the morphine challenge significantly inhibited morphine-induced behavior sensitization (Figure 4, $P<0.05$).
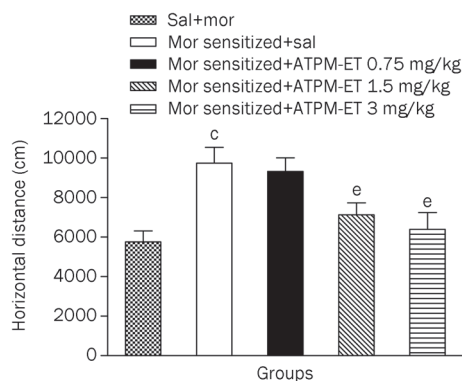

Figure 4. Effect of ATPM-ET on morphine-induced behavioral sensitization. Mice in the control group were given saline, and the other mice received morphine (10 mg/kg, subcutaneously) for 7 consecutive days. After a 7-d washout period, the mice were challenged with morphine (10 mg/kg, subcutaneously) 15 min after an injection of ATPM-ET (0.75, 1.5, 3 mg/kg, subcutaneously). The mice were then put into test chambers to measure their locomotor activity for 1 h. Data are presented as means±SEM (n=10 per group). $^c P<0.01$ *vs* saline group. $^e P<0.05$ *vs* morphine sensitized/saline group.

Discussion

A great deal of research has focused on κ-opioid receptor agonists with activity at the μ-opioid receptor as potential compounds to treat drug addiction[25, 26]. It has been reported that compounds with mixed κ and μ activity decrease cocaine self-administration[27–29]. These findings suggest that κ agonists with μ-receptor activity may be promising medications for drug addiction. To further confirm the effects of κ-opioid agonists with activity at the μ-opioid receptor on drug addiction, we investigated the effects of ATPM-ET, an N-ethyl substituted aminothiazolomorphinan, on morphine physical dependence and the expression of morphine-induced behavioral sensitization in mice.

Our current results demonstrate that ATPM-ET is a full κ agonist with partial μ agonist activity and that ATPM-ET displays a potent antinociceptive effect in mice in the hot plate test. The results from this study also show that the administration of ATPM-ET to chronic morphine-treated mice prior to naloxone precipitation significantly inhibits jumping and body weight loss induced by morphine withdrawal. However, consistent with previous studies[19, 21], the classic $κ_1$-opioid receptor agonist, (–)U50,488H, failed to inhibit withdrawal jumping and body weight loss induced by naloxone precipitation. Our findings support the hypothesis that κ agonists with partial μ agonist activity may have greater therapeutic benefits in the treatment of drug addiction.

Acute or repeated morphine administration in rodents can enhance locomotor activity and cause behavioral sensitization, a long-lasting behavioral alteration that has been proposed to play a prominent role in some of the motivational aspects of drug addiction, such as craving and drug-seeking[30]. Consistent with previous studies[31–33], we demonstrate that repeated administration of morphine can increase locomotor activity and induce behavioral sensitization. To further confirm that ATPM-ET may have utility for the treatment of drug abuse, we examined the effects of ATPM-ET on morphine-induced behavioral sensitization. The present study demonstrates that ATPM-ET dose-dependently inhibits the expression of behavioral sensitization induced by morphine treatment, which further supports that ATPM-ET may be a promising candidate agent for the treatment of drug abuse.

In conclusion, ATPM-ET is a new aminothiazolomorphinan analog with mixed κ agonist and μ agonist activities that produces dose-dependent antinociception in the hot plate test. More importantly, ATPM-ET can attenuate morphine-induced physical dependence and inhibit morphine-induced behavioral sensitization. Thus, ATPM-ET is a potentially promising candidate for the treatment of opioid addiction.

Author contribution

Jian-feng SUN designed and performed the research and wrote the paper; Yu-hua WANG, Gang LU, and Yun CHENG assisted in the research; Yi-min TAO, Xue-jun XU, and Jie CHEN helped with data analysis; Fu-ying LI, John L NEUMEYER, and Ao ZHANG provided the ATPM-ET; Zhiqiang CHI provided consultation; Jing-gen LIU designed the research and revised the paper.

References

1 Wells JL, Bartlett JL, Ananthan S, Bilsky EJ. *In vivo* pharmacological characterization of SoRI 9409, a nonpeptidic opioid mu-agonist/delta-antagonist that produces limited antinociceptive tolerance and attenuates morphine physical dependence. J Pharmacol Exp Ther 2001; 297: 597–605.

2 Sepulveda J, Ortega A, Zapata G, Contreras E. Acamprosate decreases the induction of tolerance and physical dependence in morphine-treated mice. Eur J Pharmacol 2002; 445: 87–91.

3 Fattore L, Spano MS, Deiana S, Melis V, Cossu G, Fadda P, *et al*. An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. Brain Res Rev 2007; 53: 1–16.

4 Lyvers M. Drug addiction as a physical disease: the role of physical dependence and other chronic drug-induced neurophysiological changes in compulsive drug self-administration. Exp Clin Psychopharmacol 1998; 6: 107–25.

5   Bell K, Salmon A. Pain, physical dependence and pseudoaddiction: redefining addiction for 'nice' people? Int J Drug Policy 2009; 20: 170–8.

6   Suzuki T, Shiozaki Y, Masukawa Y, Misawa M, Nagase H. The role of mu- and kappa-opioid receptors in cocaine-induced conditioned place preference. Jpn J Pharmacol 1992; 58: 435–42.

7   Crawford CA, McDougall SA, Bolanos CA, Hall S, Berger SP. The effects of the kappa agonist U-50,488 on cocaine-induced conditioned and unconditioned behaviors and Fos immunoreactivity. Psychopharmacology (Berl) 1995; 120: 392–9.

8   Shippenberg TS, LeFevour A, Heidbreder C. Kappa-opioid receptor agonists prevent sensitization to the conditioned rewarding effects of cocaine. J Pharmacol Exp Ther 1996; 276: 545–54.

9   Glick SD, Maisonneuve IM, Raucci J, Archer S. Kappa opioid inhibition of morphine and cocaine self-administration in rats. Brain Res 1995; 681: 147–52.

10 Negus SS, Mello NK, Portoghese PS, Lin CE. Effects of kappa opioids on cocaine self-administration by rhesus monkeys. J Pharmacol Exp Ther 1997; 282: 44–55.

11 Mello NK, Negus SS. Effects of kappa opioid agonists on cocaine- and food-maintained responding by rhesus monkeys. J Pharmacol Exp Ther 1998; 286: 812–24.

12 Schenk S, Partridge B, Shippenberg TS. U69593, a kappa-opioid agonist, decreases cocaine self-administration and decreases cocaine-produced drug-seeking. Psychopharmacology (Berl) 1999; 144: 339–46.

13 Schenk S, Partridge B, Shippenberg TS. Reinstatement of extinguished drug-taking behavior in rats: effect of the kappa-opioid receptor agonist, U69593. Psychopharmacology (Berl) 2000; 151: 85–90.

14 Mello NK, Negus SS. Interactions between kappa opioid agonists and cocaine. Preclinical studies. Ann N Y Acad Sci 2000; 909: 104–32.

15 Bowen CA, Negus SS, Zong R, Neumeyer JL, Bidlack JM, Mello NK. Effects of mixed-action kappa/mu opioids on cocaine self-administration and cocaine discrimination by rhesus monkeys. Neuropsychopharmacology 2003; 28: 1125–39.

16 Neumeyer JL, Gu XH, van Vliet LA, DeNunzio NJ, Rusovici DE, Cohen DJ, *et al*. Mixed kappa agonists and mu agonists/antagonists as potential pharmacotherapeutics for cocaine abuse: synthesis and opioid receptor binding affinity of N-substituted derivatives of morphinan. Bioorg Med Chem Lett 2001; 11: 2735–40.

17 Glick SD, Maisonneuve IM. Development of novel medications for drug addiction. The legacy of an African shrub. Ann N Y Acad Sci 2000; 909: 88–103.

18 Wang YJ, Tao YM, Li FY, Wang YH, Xu XJ, Chen J, *et al*. Pharmacological characterization of ATPM [(−)-3-aminothiazolo[5,4-b]-N-cyclopropylmethylmorphinan hydrochloride], a novel mixed kappa-agonist and mu-agonist/-antagonist that attenuates morphine antinociceptive tolerance and heroin self-administration behavior. J Pharmacol Exp Ther 2009; 329: 306–13.

19 Tao YM, Li QL, Zhang CF, Xu XJ, Chen J, Ju YW, *et al*. LPK-26, a novel kappa-opioid receptor agonist with potent antinociceptive effects and low dependence potential. Eur J Pharmacol 2008; 584: 306–11.

20 Li JX, Zhang Q, Liang JH. Valproate prevents the induction, but not the expression of morphine sensitization in mice. Behav Brain Res 2004; 152: 251–7.

21 Tsuji M, Takeda H, Matsumiya T, Nagase H, Yamazaki M, Narita M, *et al*. A novel kappa-opioid receptor agonist, TRK-820, blocks the development of physical dependence on morphine in mice. Life Sci 2000; 66: PL353–8.

22 Puig-Ramos A, Santiago GS, Segarra AC. U-69593, a kappa opioid receptor agonist, decreases cocaine-induced behavioral sensitization in female rats. Behav Neurosci 2008; 122: 151–60.

23 Collins SL, D'Addario C, Izenwasser S. Effects of kappa-opioid receptor agonists on long-term cocaine use and dopamine neurotransmission. Eur J Pharmacol 2001; 426: 25–34.

24 Li JX, Zhao WL, Liang JH. Effects of carbamazepine on morphine-induced behavioral sensitization in mice. Brain Res 2004; 1019: 77–83.

25 Zhang A, Xiong W, Hilbert JE, DeVita EK, Bidlack JM, Neumeyer JL. 2-Aminothiazole-derived opioids. Bioisosteric replacement of phenols. J Med Chem 2004; 47: 1886–8.

26 Neumeyer JL, Zhang A, Xiong W, Gu XH, Hilbert JE, Knapp BI, *et al*. Design and synthesis of novel dimeric morphinan ligands for kappa and micro opioid receptors. J Med Chem 2003; 46: 5162–70.

27 Stevenson GW, Wentland MP, Bidlack JM, Mello NK, Negus SS. Effects of the mixed-action kappa/mu opioid agonist 8-carboxamidocyclazocine on cocaine- and food-maintained responding in rhesus monkeys. Eur J Pharmacol 2004; 506: 133–41.

28 Neumeyer JL, Mello NK, Negus SS, Bidlack JM. Kappa opioid agonists as targets for pharmacotherapies in cocaine abuse. Pharm Acta Helv 2000; 74: 337–44.

29 Mello NK, Mendelson JH, Sholar MB, Jaszyna-Gasior M, Goletiani N, Siegel AJ. Effects of the mixed mu/kappa opioid nalbuphine on cocaine-induced changes in subjective and cardiovascular responses in men. Neuropsychopharmacology 2005; 30: 618–32.

30 Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993; 18: 247–91.

31 Hao Y, Yang JY, Wu CF, Wu MF. Pseudoginsenoside-F11 decreases morphine-induced behavioral sensitization and extracellular glutamate levels in the medial prefrontal cortex in mice. Pharmacol Biochem Behav 2007; 86: 660–6.

32 Kotlinska J, Bochenski M. Comparison of the effects of mGluR1 and mGluR5 antagonists on the expression of behavioral sensitization to the locomotor effect of morphine and the morphine withdrawal jumping in mice. Eur J Pharmacol 2007; 558: 113–8.

33 Zarrindast MR, Heidari-Darvishani A, Rezayof A, Fathi-Azarbaijani F, Jafari-Sabet M, Hajizadeh-Moghaddam A. Morphine-induced sensitization in mice: changes in locomotor activity by prior scheduled exposure to GABA$_A$ receptor agents. Behav Pharmacol 2007; 18: 303–10.