Pharmacological Characterization of ATPM [(–)-3-Amino-thiazolo[5,4-*b*]-*N*-cyclopropylmethylmorphinan hydrochloride], a Novel Mixed κ -Agonist and μ -Agonist/-Antagonist That Attenuates Morphine Antinociceptive Tolerance and Heroin Self-Administration Behavior

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ABSTRACT

ATPM [(–)-3-amino-thiazolo[5,4-*b*]-*N*-cyclopropylmethylmorphinan hydrochloride] was found to have mixed κ - and μ -opioid activity and identified to act as a full κ -agonist and a partial μ -agonist by in vitro binding assays. The present study was undertaken to characterize its in vivo effects on morphine antinociceptive tolerance in mice and heroin self-administration in rats. ATPM was demonstrated to yield more potent antinociceptive effects than (–)U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide). It was further found that the antinociceptive effects of ATPM were mediated by κ - and μ -, but not δ -opioid, receptors. In addition to its agonist profile on the μ -receptor, ATPM also acted as a μ -antagonist, as measured by its inhibition of morphine-induced antinociception. It is more important that ATPM had a greater ratio of the ED₅₀ value of sedation to that of antinociception than (–)U50,488 (11.8 versus

3.7), indicative of a less sedative effect than (–)U50,488H. In addition, ATPM showed less potential to develop antinociceptive tolerance relative to (–)U50,488H and morphine. Moreover, it dose-dependently inhibited morphine-induced antinociceptive tolerance. Furthermore, it was found that chronic treatment of rats for 8 consecutive days with ATPM (0.5 mg/kg s.c.) produced sustained decreases in heroin self-administration. (–)U50,488H (2 mg/kg s.c.) also produced similar inhibitory effect. Taken together, our findings demonstrated that ATPM, a novel mixed κ -agonist and μ -agonist/-antagonist, could inhibit morphine-induced antinociceptive tolerance, with less potential to develop tolerance and reduce heroin self-administration with less sedative effect. κ -Agonists with some μ -activity appear to offer some advantages over selective κ -agonists for the treatment of heroin abuse.

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Previous studies in both nonhuman primates and rats have demonstrated that κ -agonists functionally attenuate many behavioral effects of cocaine, including behavioral sensitization (Ukai et al., 1994; Crawford et al., 1995), place preference (Suzuki et al., 1992; Crawford et al., 1995; Shippenberg et al., 1996), and self-administration (Glick et al., 1995; Negus et al., 1997; Mello and Negus, 1998; Schenk et al., 1999). Administration of κ -agonists also attenuates the

ABBREVIATIONS: (–)U50,488H, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide; ATPM, (–)-3-amino-thiazolo[5,4-*b*]-*N*-cyclopropylmethylmorphinan hydrochloride; nor-BNI, nor-binaltorphimine; β -FNA, β -funaltrexamine; FR, fixed ratio; U69,593, (+)-(5 α ,7 α ,8 β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide; GTP γ S, guanosine-5'-O-(3-thio)triphosphate; PD117302, ($\pm$)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide; Mr2033, ($\pm$)-(1-*R*/S,5-*R*/S,2-*R*/S)-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan; MCL-101, 3-hydroxy-*N*-cyclobutylmethylmorphinan S-(+)-mandelate; Mr2034, (–)-(1*R*,5*R*,9*R*,2'*S*)-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan-D-tartrate; U69593, (5*a*,7*a*,8*b*)-(–)-*N*-methyl-*N*-(7-1-pyrrolidinyl)1-oxaspiro(4,5)dec-8-yl]benzeneacetamide.

reinstatement of extinguished drug-taking behavior in an animal model of relapse (Schenk et al., 1999, 2000). These inhibitory effects of κ -agonists on cocaine-induced abuse-related behaviors are achieved possibly by inhibiting the release of dopamine from dopaminergic neurons (Di Chiara and Imperato, 1988; Maisonneuve et al., 1994). In addition, selective κ -agonists such as dynorphin and (–)U50,488H also have been reported to suppress the development of antinociceptive tolerance to morphine (Yamamoto et al., 1988; Take-mori et al., 1992). Although highly selective κ -agonists do have utility for attenuating many cocaine-induced behaviors, these selective agonists produce many severe undesirable side effects such as salivation, emesis, and sedation in non-human primates (Negus et al., 1997; Mello and Negus, 1998), which may limit the clinical utility of κ -agonists for drug abuse treatment.

A growing body of evidence suggests that the compounds with mixed κ - and μ -opioid activity decreased cocaine self-administration more effectively and with fewer undesirable side effects than highly selective κ -agonists. For example, the nonselective κ -agonist ethylketocyclazocine, which possesses μ -opioid receptor-mediated effects in addition to its κ -agonist effects, was shown to suppress cocaine self-administration more potent and with fewer aversive effects, relative to the highly selective κ -agonist (–)U50,488H (Negus et al., 1997). Moreover, it has been shown that the selectivity of the ligand for κ - versus μ -opioid receptors also influences κ -opioid effects on cocaine self-administration. For example, by comparing the behavioral effects of three arylacetamide κ -agonists [enadoline, (–)spiradoline, and PD117302] and three morphinans (bremazocine, Mr2033, and cyclazocine), it was found that three of the morphinans were more effective in decreasing cocaine self-administration than three arylacetamide κ -agonists (Mello and Negus, 1998). This is because three morphinans display less selectivity for the κ -opioid but have relative high selectivity for μ -opioid receptors, in comparison with three arylacetamide κ -agonists (Davis et al., 1992; Butelman et al., 1993; Emmerson et al., 1994; France et al., 1994), although all of these compounds have high affinity for κ -opioid receptors as determined by *in vitro* receptor binding assays. In addition, it was further found that morphinans (MCL-101 and the benzomorphan Mr2034) decreased cocaine self-administration in rhesus monkeys in a more sustained manner and produced fewer side effects than the arylacetamide κ -agonist enadoline (Bowen et al., 2003). These findings suggest that κ -agonists with mixed activity at κ - and μ -receptors may be more promising candidate pharmacotherapies for drug abuse than highly selective κ -agonists (Mello and Negus, 2000).

In the continuing desire to produce more promising κ -agonists with activity at the μ -opioid receptor, we synthesized ATPM, an aminothiazole-derived morphinan (Zhang et al., 2004). With bioisosteric replacement of the phenolic fragment of its parent compound cyclorphan by the aminothiazole moiety, ATPM retains high affinity at the κ -receptor but with partial μ -agonist activity. The selectivity of ATPM for the κ -receptor is 30-fold higher than that for the μ -opioid receptor (Zhang et al., 2004). The present study was undertaken to evaluate the effects of ATPM on heroin self-administration in rats and morphine antinociceptive tolerance in mice, and the pharmacological properties of ATPM were also compared with highly selective κ -agonist (–)U50,488.

Materials and Methods

Drugs

(–)U50,488H, Nor-binaltorphimine, β -funaltrexamine, and naltrindole were purchased from Sigma-Aldrich (St. Louis, MO). Morphine hydrochloride was purchased from Qinghai Pharmaceutical General Factory (Qinghai, China).

Animals

Animals were Kunming strain mice weighing 20 to 24 g and male Sprague-Dawley rats weighing 280 to 330 g from the Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Animals were housed individually in a room maintained at $22 \pm 0.5^\circ\text{C}$ with an alternating 12-h light/dark cycle (lights on at 8:00 AM), with free access to food and water.

Antinociceptive Tests

Hot-Plate Test. Hot-plate tests were conducted according to our previously reported procedure (Tao et al., 2008). In brief, mice were placing on a 55°C heated surface, and the time to licking of the back paws or to an escape jump was recorded. Before drug administration, the nociceptive response of each mouse was measured three times. Mice not responding within 20 s were excluded from further studies. The cut-off time of 60 s for the temperature 55°C hot-plate test was used to minimize tissue damage. For drawing the dose-response curves, percentage analgesia was expressed as: $100 \times [(\text{test latency} - \text{predrug latency})/(\text{cut-off time} - \text{predrug latency})]$. The antinociceptive ED_{50} value of each compound was obtained as the dose that produced 50% analgesia.

Abdominal Constriction Test. Abdominal constriction tests were performed in mice according to the method described previously by us (Tao et al., 2008). In brief, an abdominal constriction was defined as a wave of contraction of the abdominal musculature followed by extension of the hind limbs. After receiving graded subcutaneous doses of opioid agonists for 15 min, an intraperitoneal injection of 0.6% acetic acid (10 ml/kg body weight) was administered to each mouse, and then the number of writhing signs displayed by each mouse was counted for 15 min. Percentage analgesia was expressed as: $100 \times (\text{number of mean control abdominal constriction} - \text{number of test abdominal constriction})/\text{number of mean control abdominal}$. The antinociceptive ED_{50} value of each compound was obtained as the dose that produced 50% analgesia.

Agonist Effects of ATPM. To further determine the *in vivo* opioid receptor profile of ATPM, mice were pretreated with a κ -selective (nor-BNI, 10 mg/kg s.c., –15 min) (Portoghesi et al., 1987), μ -selective (β -FNA, 20 mg/kg s.c., –24 h) (Kamei et al., 2000), and δ -selective (naltrindole, 3.0 mg/kg s.c., –30 min) (Saitoh et al., 2005) antagonists. Control mice received a vehicle injection (saline subcutaneously, –15 min, –24 h, or –30 min). Then, mice received ATPM administration. Antinociception was assessed after 15-min agonist injection.

Antagonist Effects of ATPM. Mice were injected with varying doses of morphine (2.5–10 mg/kg s.c.) after pretreatment with ATPM (0.5 mg/kg), given by subcutaneous administration. Control mice received a vehicle injection (saline subcutaneously). Antinociception was assessed 15 min after agonist injection.

Rotorod Test. This test was conducted by using a procedure described previously by Endoh et al. (1999). In brief, each mouse was individually trained to maintain their position on a Rotorod for 60 s or more using the Rotorod apparatus. The time duration for each mouse to fall off the Rotorod was recorded. Mice falling off the Rotorod within 60 s were excluded from further studies. If a mouse that had been injected with the test drugs fell off the Rotorod within 60 s, the time required to measure again.

Antinociceptive Tolerance Assays

Procedure for Development of Antinociceptive Tolerance. A 3-day consecutive administration regimen was used for induction

of antinociceptive tolerance of drugs, as reported by Suzuki et al. (2004) previously. Morphine (2.5–10 mg/kg), (–)U50,488H (2–8 mg/

Fig. 1. Chemical structure of ATPM.

kg), or ATPM (1–4 mg/kg) was administered twice on days 1 and 2 (9:00 AM and 4:00 PM) and once on day 3 (09:00). The tolerance to the antinociceptive effect was evaluated using hot-plate test (described above) on day 4. In brief, measurements were performed 30, 15, or 15 min after subcutaneous administration of morphine, (–)U50,488H, or ATPM, respectively. The dose of each test compound that produced approximately 80 to 90% antinociceptive efficacy in the vehicle-treated mice was chosen. Thus, the doses of morphine, (–)U50,488H, and ATPM were 10, 8, and 2 mg/kg s.c., respectively. Tolerant ED_{50} values were determined based on the repeated dose, resulting in a 50% reduction of the antinociceptive efficacy compared with the vehicle-pretreated mice.

Effect of ATPM on Morphine Antinociceptive Tolerance. Animals were treated concomitantly with ATPM (0.5–1.5 mg/kg s.c.) or (–)U50,488H (5 mg/kg s.c.) twice a day, immediately preceding the morphine injections during the induction period. Measurements were performed after the first and last treatments with morphine in the hot-plate test.

Self-Administration Behavior Studies

Surgery. The intravenous self-administration procedure has been described previously (Zhou et al., 2005). In brief, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and atropine sulfate. A permanent intravenous catheter was surgically implanted and secured to the right external jugular vein. To prevent infection, the rats were treated postsurgically with penicillin B for 5 days. All the animals were allowed to recover for at least 3 days.

Apparatus. The animals were transferred to stainless steel operant chambers (size, 30 × 30 × 30 cm) for the self-administration session, which were placed in a sound-attenuated, temperature-controlled room. Each chamber was equipped with two nose-poke operandum (ENV-114M; MED Associates, St. Albans, VT) in the right wall. There was a blue LED light inside active nose-poke hole. A house light (green, 28 V, 0.1 mA, ENV-215 M; MED Associates) was situated on the wall. Heroin solution was delivered through Tygon tubing, protected by a leash assembly (PHM-120; MED Associates) and suspended through the ceiling of the chamber from a plastic fluid swivel (PHM-115; MED Associates). The leash assembly was modified to fit a custom-made fluid connector fixed on the animal's jacket. The Tygon tubing was attached to a syringe pump (PHM-100; MED Associates) that delivered fluid at a speed of 1.2 ml/min using a 10-ml syringe. The

experimental events were controlled by an IBM-compatible PC using a MED Associates interface and running self-programmed software written in Borland Delphi 6.0 (OBSM version 4.0, operant behavioral schedule manager).

Heroin Self-Administration Training and Behavioral Testing Procedure. This procedure is performed according to the method described previously (Zhou et al., 2005), with slight modifications. During each daily 2-h self-administration training session, each session started with the illumination of the house light. The onset of the illumination of the house light served as discriminative stimulus signaling associated with heroin availability. Each active nose poke delivered a single infusion of heroin (60 µg/kg) under a fixed-ratio (FR) 1 schedule of reinforcement. Heroin was infused at a volume of 60 to 70 µl over a 3-s period, which was depending on the unit injection dose, the drug concentration, the pump speed, and the body weight of the rat. Each infusion was paired with 5-s illuminations of the blue light inside the active nose-poke hole and the noise of the infusion pump, serving as conditioned stimulus signaling paired with the heroin infusion. A timeout period was imposed for 20 s, during which the blue light inside the active nose-poke hole was extinguished and responding produced no programmed consequences but was still recorded. Responding in the inactive nose poke was recorded but had no programmed consequences. Each daily training session ended after 2 h or 25 heroin injections, and each rat was trained typically for 8 days on a continuous reinforcement (FR1) schedule until stable self-administration behavior was achieved. Responding was considered stable when animals were treated intraperitoneally daily 5 min before training with saline displayed accurate discrimination between the active and the inactive nose poke.

On day 9, the test session lasted for 2 h, and each animal was tested only once. During the test session, the connectors were attached to the swivels but not to the infusion lines. The rats were placed in the operant chamber and allowed to nose poke for 2 h without ATPM or (–)U50,488H pretreated in the absence of heroin, the blue nose-poke light, and the pump noise; however, the house lights were illuminated. Responses were recorded but had no consequence to show heroin-reinforced craving and drug-seeking behavior.

Drug Treatment. During the training session, 0.5 mg/kg ATPM, 2 mg/kg (–)U50,488H, or saline was injected intraperitoneally, and the rats were returned to the home cage. Five minutes later, they were transferred to the operant chambers, and the session was initiated. Each active nose poke delivered a single infusion of heroin (60 µg/kg). All the rats trained to self-administer heroin for 8 days on a continuous reinforcement (FR1) schedule.

Statistical Analysis. The data are presented as the mean ± S.E.M. A one-way repeated measures analysis of variance followed by Dunnett's test was used for the statistical evaluation ($p < 0.05$ and 0.01). The ED_{50} values, potency ratios, and 95% confidence limits were determined by Tallarida and Murray (1986). For each dose at least 10 mice were used.

TABLE 1

Affinity values (K_i) for the binding to opioid receptors and EC_{50} values and maximal effects in stimulating [35 S]GTPγS binding to membranes of ATPM in Chinese hamster ovary cells stably expressing opioid receptors

Membranes were incubated with varying concentrations of ATPM in the presence of 1 nM [3 H]U69,593, 0.25 nM [3 H][D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin, 0.2 nM [3 H]naltrindole, or 0.8 nM [35 S]GTPγS. Data are expressed as the mean ± S.E.M. for three independent experiments performed in triplicate (table from Zhang et al., 2004).

Agonist	Receptor Binding: K_i			[35 S]GTPγS Binding					
				Selectivity		κ		μ	
	[3 H]U69,593 (κ)	[3 H][D-Ala ² ,N-Me-Phe ⁴ ,Gly ⁵ -ol]-Enkephalin (μ)	[3 H]Naltrindole (δ)	κ/μ	κ/δ	EC_{50}	E_{max}	EC_{50}	E_{max}
<i>nM</i>									
ATPM	0.049 ± 0.005	1.5 ± 0.2	29 ± 2	30	590	2.4 ± 0.6	80 ± 6	73 ± 5	45 ± 4

Fig. 2. Dose-response curves for subcutaneous ATPM and (-)U50,488H in the hot-plate (a) and the abdominal constriction tests (b). Antinociception was assessed 15 min after agonist injection.

Results

In Vitro Studies: Affinity, Selectivity, and Efficacy of ATPM.

The binding of the novel aminothiazole-derived morphinan ATPM (Fig. 1) to all the opioid receptors was measured. ATPM had a K_i value of less than 0.05 nM for inhibiting the binding of [3 H]U69,593 to the κ -receptor in Chinese hamster ovary membranes stably transfected with the opioid receptors, which was 30- or 590-fold higher affinity for the μ - and δ -opioid receptors, respectively. In the [35 S]GTP γ S binding assay, ATPM produced an $E_{\max}$ value, percentage of maximal stimulation, of 80% for the κ -opioid receptor and 45% for the μ -opioid receptor. The EC_{50} values obtained for this compound were 2.4 and 73 nM for the κ - and μ -opioid receptors, respectively (Table 1) (Zhang et al., 2004).

In Vivo Studies

Antinociceptive Effects of ATPM against Heat and Chemical Nociception. Because ATPM has high affinity for both κ - and μ -opioid receptors, as measured in the receptor binding assays, the antinociceptive properties of this compound were characterized in the hot-plate and abdominal constriction tests. ATPM produced full dose-response curves with the ED_{50} values of 1.46 (1.26–1.67) mg/kg in the hot-plate test and 0.16 (0.11–0.24) mg/kg in the abdominal constriction test, respectively, which was approximately 3- or 5-fold more potent than that of (-)U50,488H in the hot-plate test or in the abdominal constriction test, respectively (Fig. 2; Table 2).

The duration of action for ATPM was shown in Fig. 3. The effect peaked at 15 min after injection in the abdominal constriction test and then gradually declined and returned to

the preinjection level 2 h after the injection. Likewise, (-)U50,488H displayed a similar time course on the production of antinociception.

Fig. 3. Time courses for the effects of ATPM and (-)U50,488H in producing antinociception. Mice were injected with either ATPM (0.4 mg/kg) or (-)U50,488H (1.5 mg/kg), given by subcutaneous administration. At varying times, antinociception was measured in the abdominal constriction test. Data are the mean $\pm$ S.E.M. from 10 mice/data point.

Fig. 4. Antinociceptive effects of ATPM were mediated by κ - and μ -, but not δ -opioid, receptors. The κ -opioid receptor antagonist nor-BNI, the μ -opioid receptor antagonist β -FNA, but not the δ -opioid receptor antagonist naltrindole, reduced ATPM-induced antinociception. Mice were pretreated with nor-BNI (10 mg/kg s.c.) for 15 min, β -FNA (20 mg/kg s.c.) for 24 h, and naltrindole (3.0 mg/kg s.c.) for 30 min and then injected with ATPM (0.25 mg/kg s.c.). Acetic acid solution was intraperitoneally injected 15 min after drug administration. Data are presented as the mean $\pm$ S.E.M. from 10 animals. **, $p < 0.01$; *, $p < 0.05$ in comparison with ATPM alone.

TABLE 2

ED_{50} values of the antinociception produced by subcutaneous injection of ATPM and (-)U50,488H evaluated with the hot-plate and the abdominal constriction tests

The antinociceptive ED_{50} value of each drug was calculated from data obtained at 15 min after drug administration. In parentheses are 95% confidence limits.

Agonist	Antinociception ED_{50}	
	Hot-Plate Test	Abdominal Constriction Test
	mg/kg s.c.	
ATPM	1.46 (1.26–1.67)	0.16 (0.11–0.24)
(-)U50,488H	4.42 (2.51–7.75)	0.89 (0.54–1.45)

Antinociceptive Effects of ATPM Were Mediated by κ - and μ -, but Not δ -Opioid, Receptors. Next, the selectivity of the agonist effect produced by ATPM in the abdominal constriction test was determined by the use of selective antagonists. As shown in Fig. 4, the κ -selective antagonist nor-BNI and the μ -selective antagonist β -FNA both reduced the antinociception induced by ATPM, but the δ -selective antagonist naltrindole had no effect on the antinociceptive properties of ATPM, supporting that ATPM is an agonist at both the κ - and μ -, but not δ -opioid, receptors.

Antagonist Properties of ATPM. ATPM is derived from the morphinan analog, cyclorphan, which has been shown to act as antagonist at the μ -opioid receptor (Neumeyer et al., 2000a,b, 2001), in addition to its agonist activity on the κ -receptors. To assess the antagonist activity of ATPM at the μ -receptor, morphine and ATPM were administered concomitantly, and antinociception was assessed 15 min after the injection. As shown in Fig. 5, a low-dose, 0.5 mg/kg ATPM antagonized morphine-induced antinociception. Morphine dose-dependently produced antinociception, with an ED_{50} value of 4.34 (3.76–5.03) mg/kg in the hot-plate test. However, in the presence of ATPM, the analgesic effect of morphine was decreased, with an ED_{50} value of 6.07 (5.27–6.99) mg/kg. There was a significant difference in potency, i.e., the 95% confidence limits (1.10–1.75) of the potency ratio (1.35) did not include one. These data demonstrated that ATPM acted as a μ -antagonist at low doses, in addition to its agonist activity at the μ -receptor.

ATPM with Less Sedative Side Effect Than (–)U50,488H. κ -Opioid agonists usually show a greater sedative effect, which is undesirable for therapeutic use. To determine the sedative effect of ATPM, inhibition of Rotorod performance (sedative activity) was determined by the ability of mice to maintain their position on an accelerating Rotorod. Both ATPM and (–)U50,488H caused a dose-related inhibition of Rotorod performance in mice. The sedative ED_{50} values for ATPM and (–)U50,488H were 1.94 (1.77–2.13) and 3.32 (2.65–3.94) mg/kg, respectively. The ratio of the ED_{50} value of sedative effect to that of antinociceptive effect (mouse acetic acid-induced abdominal constriction test) was found to be much greater for ATPM (ratio = 11.8) than that for (–)U50,488H (ratio = 3.7) (Table 3), suggesting that

TABLE 3
Sedative effects in mouse Rotorod test

Agonist	Sedative ED_{50} Values mg/kg s.c.	Sedative ED_{50} /Antinociception ED_{50} ^a
ATPM	1.94 (1.77–2.13)	11.8
(–)U50,488H	3.32 (2.65–3.94)	3.7

^a ED_{50} values obtained from mice abdominal constriction test.

ATPM has a less sedative adverse effect relative to (–)U50,488H.

ATPM with Less Potential to Develop Antinociceptive Tolerance Relative to Morphine and (–)U50,488H. Next, the potential of ATPM to develop antinociceptive tolerance was determined with the protocol reported by previous study (Suzuki et al., 2004). Mice were subcutaneously administered with various doses of ATPM (1, 2, and 4 mg/kg), (–)U50,488H (2, 4, and 8 mg/kg), or morphine (2.5, 5, and 10 mg/kg) twice daily for 3 consecutive days, respectively, and on day 4, a single dose of each compound was used to determine its tolerant ED_{50} value. To compare the tolerance-development potencies of each compound, the ratios of the ED_{50} value of tolerance to that of antinociception were calculated (Table 4). The larger ratio means less potential to develop tolerance. As shown in Fig. 6, after pretreatment with various doses of ATPM, (–)U50,488H, or morphine for 3 consecutive days, the analgesic effects of these compounds were decreased in a dose-dependent manner, indicative of the development of antinociceptive tolerance. Significant reduction of the analgesic effect was found upon treatment with more than 2 mg/kg ATPM, 4 mg/kg (–)U50,488H, and 5 mg/kg morphine. The ratio value was found to be greater for ATPM (1.99) than that for (–)U50,488H (1.54) and morphine (1.39), suggesting that ATPM has less potential to develop antinociceptive tolerance relative to morphine and (–)U50,488H.

Effect of ATPM on Morphine Antinociceptive Tolerance. Next, the effect of ATPM on the development of antinociceptive tolerance to morphine was examined in mice. As shown in Fig. 7a, injection of 10 mg/kg morphine s.c. to mice produced an antinociceptive effect in the hot-plate test. Acute coadministration of (–)U50,488H or high doses of ATPM with morphine produced no effect on the morphine-induced analgesia. Repeated administration of morphine (10 mg/kg s.c., twice daily) to mice for 5 consecutive days resulted in the reduction of morphine-induced antinociception, indicative of the development of antinociceptive tolerance. Coadministration of various doses of ATPM (0.5–1.5 mg/kg s.c.) with morphine dose-dependently suppressed the development of antinociceptive tolerance to morphine (Fig. 7b). (–)U50,488H (5 mg/kg s.c.) also reduced morphine antinociceptive tolerance, which was consistent with the result reported in the literature (Tsuji et al., 2000).

Effect of ATPM on Heroin Self-Administration. Because the characteristics of drug addiction included impaired ability to control drug taking and compulsive craving and drug-seeking behavior when the drug is unavailable, we evaluate the effects of ATPM and (–)U50,488H on heroin reinforcements in these aspects. As shown in Fig. 8a, during the training phase, animals were pretreated with ATPM (0.5 mg/kg), (–)U50,488H (2 mg/kg), or saline 5 min before the start of the daily training for 8 consecutive days. The rats

Fig. 5. Antagonism of morphine-induced antinociception by ATPM. Mice were injected with varying doses of morphine, alone or with ATPM, and after 15-min antinociception were measured in the mouse hot-plate test. Data are presented as the mean $\pm$ S.E.M. from 10 to 12 animals. *, $p < 0.05$ in comparison with morphine dose alone.

TABLE 4

The development of tolerance to antinociceptive effects of opioid agonists in the hot-plate test

Agonist	Tolerant ED ₅₀	Acute Antinociceptive ED ₅₀	Ratio (Tolerant ED ₅₀ /Acute Antinociceptive ED ₅₀)
		mg/kg s.c.	
ATPM	2.91 (2.41–3.52)	1.46 (1.26–1.67)	1.99
(-)-U50,488H	6.78 (5.77–8.08)	4.42 (2.51–7.75)	1.54
Morphine	6.22 (4.08–8.41)	4.34 (3.76–5.03)	1.39

Fig. 6. Development of tolerance to ATPM, (-)U50,488H, and morphine-induced antinociception in the mouse hot-plate test. Mice were administered various indicated doses of the agonist or vehicle for 3 days. On day 4, antinociceptive actions were measured as an injection of ATPM (2 mg/kg s.c.), (-)U50,488H (8 mg/kg s.c.), or morphine (10 mg/kg s.c.). Each value represents the mean $\pm$ S.E.M. of data obtained from 10 to 12 mice. *, $p < 0.05$; **, $p < 0.01$ versus repeated vehicle + each drug.

Fig. 7. Effects of ATPM and (-)U50,488H on morphine antinociceptive tolerance. **a**, acute effects of ATPM and (-)U50,488H on morphine-induced antinociception in mice. ATPM (0.5, 0.75, and 1.5 mg/kg s.c.) or (-)U50,488H (5 mg/kg s.c.) was coadministered with 10 mg/kg morphine; after subcutaneous administration of morphine for 30 min, the antinociceptive effects were measured in the hot-plate test. Each value represents mean $\pm$ S.E.M. of data obtained from 10 mice. *, $p < 0.05$ in comparison with the morphine group. **b**, occurrence of tolerance to morphine after coadministration with 10 mg/kg s.c. morphine and various doses of ATPM and (-)U50,488H twice daily for 5 days. The antinociceptive effects were measured after the last treatment with morphine in the hot-plate test. Each value represents mean $\pm$ S.E.M. of data obtained from 10 mice. ##, $p < 0.01$ in comparison with the acute morphine group; *, $p < 0.05$ in comparison with the chronic morphine group.

pretreated with chronic saline rapidly acquired heroin self-administration, which was manifested by an apparent increase in the number of infusions of heroin (limited to 25

injections per session). In contrast, when animals were pretreated with 0.5 mg/kg ATPM and 2 mg/kg (-)U50,488H, a sustained decrease in the number of infusions of heroin was

Fig. 8. Effects of ATPM and (-)U50,488H on heroin self-administration. a, acquisition of heroin self-administration. Animals were treated with ATPM (0.5 mg/kg) or (-)U50,488H (2 mg/kg) and then allowed to self-administer heroin for eight consecutive daily 2-h sessions. ATPM and (-)U50,488H decreased acquisition of heroin self-administration over the last 5 days. The number of animals per treatment group ranged between eight and 15. Data shown are the mean $\pm$ S.E.M. number of heroin infusion (limited to 25 injections per session). *, $p < 0.05$ in comparison with saline pretreatment on day 8. b, suppression of heroin-reinforced craving and drug-seeking behavior. On the test day, ATPM and (-)U50,488H decreased heroin-reinforced active nose-poke responses. Animals were allowed to respond for 2 h without ATPM or (-)U50,488H pretreated and heroin. Data shown are the mean $\pm$ S.E.M. number of the active nose-poke (reinforcement) responses. **, $p < 0.01$ in comparison with active nose poke responses of heroin self-administration (baseline).

observed. Figure 8b shows during the test session the total number of responses on the active nose poke and inactive nose poke in the absence of heroin under an FR1 schedule. Chronic administration of ATPM (0.5 mg/kg) and (-)U50,488H (2 mg/kg) resulted in the reduction of heroin-reinforced active responses from 20 ± 2.8 to 7.8 ± 1.5 and 8.2 ± 1.6 , respectively, which revealed that heroin-reinforced craving and drug-seeking behavior were decreased. No significant differences of inactive responding were found among all three groups. Thus, ATPM significantly attenuated the self-administration of heroin under an FR1 schedule of reinforcement. (-)U50,488H showed similar effects on heroin self-administration behavior as ATPM, which was consistent with the results reported in the literature (Kuzmin et al., 1997).

Discussion

The hypothesis that compounds with mixed κ - and μ -activity may have particular utility for the treatment of drug abuse (Archer et al., 1996) was supported by several previous studies (Negus et al., 1997; Bowen et al., 2003), which promoted continuing development of novel ligands that could bind the κ - and μ -receptors (Neumeyer et al., 2000a,b, 2001). ATPM is derived from the morphinan analog, cyclorphan that functions as a κ -agonist/ μ -antagonist (Neumeyer et al., 2000a,b, 2001), which was synthesized by bioisosteric replacement of the phenolic fragment of cyclorphan by the aminothiazole moiety (Zhang et al., 2004). Data obtained in vitro with ATPM demonstrated that this compound was a full κ -agonist with partial μ -agonist activity (Zhang et al., 2004). The purpose of the present study was to investigate the effects of ATPM on morphine antinociceptive tolerance and heroin self-administration behavior.

The results of the present study demonstrated that ATPM produced full dose-response curves and displayed more potent antinociceptive effects than (-)U50,488H in the abdominal constriction and hot-plate tests. The antinociception produced by ATPM was blocked by both κ - and μ -antagonists, indicating that the antinociceptive effects of ATPM are mediated by both κ - and μ -receptors, which is consistent with the results observed from other morphinan analogs whose antinociceptive effects were also confirmed to be induced by

the mixed κ - and μ -agonists (Mathews et al., 2005). In addition to its agonist profile on the μ -receptor, ATPM also acted as a μ -antagonist, as measured by its inhibition of morphine-induced antinociception at low doses, similar to its parental compound cyclorphan (Neumeyer et al., 2000a,b, 2001). This finding indicates that addition of an aminothiazole moiety in place of the phenolic fragment resulted in a compound that was a partial agonist, in addition to an antagonist at the μ -receptor.

The development of tolerance in response to chronic use of drugs is a characteristic of all the opioid analgesics and one of the major problems in clinical use. The present study clearly demonstrated that ATPM has less potential to develop antinociceptive tolerance relative to (-)U50,488H and morphine (Bhargava et al., 1989; He et al., 2002), moreover, it could inhibit the development of tolerance to the antinociceptive effect of morphine. The mechanisms underlying ATPM having less potential to develop antinociceptive tolerance currently are unclear. One of the possible explanations is associated with its mixed κ -/ μ -activity. It is not unreasonable to postulate that ATPM may exert a distinct effect on κ - or μ -opioid receptor trafficking because of its mixed κ -/ μ -activity. Cellular responses induced by ATPM are of great interest and need to be elucidated in future studies.

Several previous studies have demonstrated that mixed-action κ -/ μ -agonists can decrease cocaine-reinforced responding, with fewer side effects (Negus et al., 1997; Bowen et al., 2003). In the present study, we extend the findings by the results showing that chronic administration of ATPM (0.5 mg/kg) also decreased heroin-reinforced self-administration behavior in rats under an FR1 schedule. Furthermore, we also clearly demonstrated that ATPM did not produce appreciable sedation at the doses that produce antinociception, although it may also produce a sedative side effect at high doses. The dose of (-)U50,488H to produce sedative effects was similar to those to produce antinociception and suppression of heroin self-administration. Therefore, the findings suggest that ATPM has advantages over the highly selective κ -agonist (-)U50,488H, supporting that compounds with mixed activities at both κ - and μ -receptors have greater therapeutic benefits and fewer side effects than highly selec-

tive κ -agonists (Negus et al., 1997; Bowen et al., 2003). In addition, a previous study showed that the parental compound, cyclorphan of ATPM, a κ -agonist/ μ -antagonist, produced transient decreases in cocaine self-administration (Bowen et al., 2003). Although the present study demonstrated that ATPM, a mixed κ -agonist and μ -agonist/-antagonist, produced sustained decreases in heroin self-administration, which is consistent with previous studies of μ -agonist and -antagonist effects on cocaine self-administration (Mello et al., 1990; Negus and Mello, 2002; Bowen et al., 2003), suggesting that μ -agonist activity may be essential for sustained decreases in drug self-administration by κ -agonists with mixed activity at μ -receptors. Although it seems that κ -agonists with some μ -activity decreased cocaine or heroin self-administration more effectively and with fewer undesirable side effects than highly selective κ -agonists, the optimal κ/μ ratio to selectively reduce cocaine or heroin self-administration with minimal side effects remains to be determined.

In summary, ATPM, a mixed κ -agonist and μ -agonist/-antagonist, was found to be capable of suppressing heroin-reinforced self-administration behavior with less sedative side effect and to have less antinociceptive tolerance potential but suppress morphine antinociceptive tolerance. Taking all these findings together, κ -agonists with μ -agonist/-antagonist further strengthens the hypothesis that compounds with mixed κ - and μ -activity show promising therapeutic potential in the treatment of drug abuse.

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