PROGRESS REPORT FROM THE TESTING PROGRAM FOR STIMULANT AND DEPRESSANT DRUGS (2004)

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INTRODUCTION

A research group within the Drug Evaluation Committee has been involved in the evaluation of stimulant and depressant compounds for approximately 20 years. The group currently includes laboratories at The University of Texas Health Science Center at San Antonio (UTHSCSA; France, McMahon), the University of Michigan (UM; Fantegrossi, Woods), The University of Mississippi Medical Center (UMMC; Woolverton), and the State University of New York at Buffalo (SUNYB; Winter). As part of the Drug Evaluation Committee (Woods, Chair) of the College on Problems of Drug Dependence (CPDD), research is supported by both the CPDD and the National Institute on Drug Abuse (NIDA). One of the purposes of this group is to evaluate new compounds, generally classified as either stimulants or depressants, for their abuse liability and physical dependence potential. Compounds are received, coded and distributed by the Biologic Coordinator (Coop, University of Maryland School of Pharmacy at Baltimore) for blind testing in the various laboratories. Drugs are then evaluated for reinforcing effects in monkeys with histories of drug self-administration (UM), and for discriminative stimulus effects in monkeys that discriminate amphetamine (UMMC), midazolam (UTHSCSA), or flumazenil (UTHSCSA). This year, compounds were also tested for the capacity to induce the head-twitch response in mice (UM), and for LSD-like discriminative stimulus effects in rats (SUNYB) as well as binding to serotonin receptors in rat brain (SUNYB). This report includes the results of evaluation of CPDD 0066, CPDD 0067 and CPDD 0068. All studies were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committees at UTHSCSA, UM, UMMC, SUNYB and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

METHODS

Reinforcing Effects in Rhesus Monkeys (UM)

Subjects and Apparatus

Three adult rhesus monkeys (*Macaca mulatta*) experienced with self-administration of cocaine were surgically prepared with indwelling silicone rubber catheters using 10.0 mg/kg i.m. ketamine and 2.0 mg/kg i.m. xylazine as anesthetics. Catheters were implanted in either a jugular (internal or external), femoral, or brachial vein as necessary. Catheters passed s.c. to the mid-scapular region, exited the body, and continued through a hollow restraining arm to the outside rear of the cage. During these studies, each animal wore a Teflon mesh jacket (Lomir, Québec, Canada) connected to a flexible stainless steel spring arm attached to the rear of the cage. Animals were individually housed in 83.3 x 76.2 x 91.4 cm-deep stainless steel cages. A side-mounted panel was present in each cage, equipped with a row of three stimulus lamps (red-green-red) across the top, and two response levers (one mounted under each red light.) Animals were fed 10-12 Purina monkey chows twice per day along with fresh fruit and other preferred foods; water was available continuously. Environmental enrichment toys were provided on a regular rotating basis.

Procedure

Two experimental sessions were conducted each day: 1000 and 1600 hours. A red stimulus light signaled the onset of each session. In the presence of this light, the tenth lever press (fixed ratio [FR] 10) resulted in the operation of the infusion pump and delivery of 1 ml of saline or drug over 5 seconds. During the 5-second infusion, the red

stimulus light was extinguished, the center green light was illuminated, and further lever presses had no programmed consequence. For studies on CPDD 0066, immediately following each infusion, all stimulus lights were extinguished for a 10-second timeout during which lever presses had no programmed consequence. For studies on CPDD 0067 and CPDD 0068, this timeout was increased to 60 seconds. Session lengths for CPDD 0066 tests were approximately 120 minutes; CPDD 0067 and CPDD 0068 were examined in 60-minute test sessions.

Under baseline conditions, animals could respond for a dose of 0.01 mg/kg/injection of cocaine following the above outlined schedule requirements. To ensure that responding was maintained by drug, saline was substituted for cocaine every third or fourth session, usually for two consecutive sessions. CPDD 0066, CPDD 0067, and CPDD 0068 were studied two to three times per week, except on weekends. Drugs were studied in an ascending order of dose and saline was tested for at least three consecutive sessions prior to drug tests. Typically, each dose of each test compound was assessed at least twice in each animal.

Drugs

CPDD 0066, CPDD 0067, and CPDD 0068 were dissolved in sterile 0.9% saline. Test compounds were assessed over a dose range from 0.003 to 0.3 mg/kg/injection.

Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination, UMMC)

Subjects and Apparatus

Three adult rhesus monkeys that had received other drugs prior to these studies were individually housed in stainless-steel cages with water available continuously. Feeding consisted of 110-200 g of Teklad monkey chow approximately 3 hours after each session and monkeys received a chewable vitamin 3 days per week. During experimental sessions, each monkey was seated in a restraint chair and placed in a sound-attenuating cubicle that had two response levers, a white light above each lever, and a white house light mounted on the ceiling. Shoes were attached to the chairs and were fitted with brass plates through which electric shock could be delivered. Experimental events were programmed and recorded using an Apple Macintosh computer in an adjacent room.

Procedure

Monkeys had been trained in a discrete-trials paradigm to discriminate amphetamine (1.0 mg/kg) from saline (Woolverton et al., 1994). Each monkey was placed in the chair and moved to the test room where their feet were placed in shoes and held in place with a Velcro strap. Monkeys received an infusion of either saline (0.25 ml/kg) or the training drug, followed by 2 ml of flush i.g. via a nasogastric tube. Monkeys remained in the chair in the test room for 55 minutes then were placed in the experimental chambers. The session began with a 5-minute timeout, after which the house light and lever lights were illuminated (trial) and responding on the correct lever either prevented electric shock (8515 and Ou3) or delivered a 1-g banana-flavored food pellet (M163), and extinguished the lights. Responding on the incorrect lever reset the response requirement on the correct lever. The correct lever was determined by the pre-session infusion (drug or saline). If the response requirement (FR2, 8515; FR 5, M163, Ou3) was not satisfied on the correct lever within 10 seconds of the onset of the lights, then shock (250 millisecond duration, 5 mA intensity) was delivered (8515 and Ou3 only). If the response requirement was not satisfied within 4 seconds after this shock, then a second shock was delivered and the trial ended. For M163, if the response requirement was not satisfied within 10 seconds, the trial ended. Sessions terminated after two consecutive trials in which 2 shocks were received or food was not received. Trials were separated by a 30-second timeout, and sessions lasted for 30 trials or 20 minutes, whichever occurred first.

Training sessions were conducted five days a week according to the following two-week schedule: SDDSS, DSSDD, where S denotes sessions preceded by saline infusion and D denotes sessions preceded by drug infusion. Discrimination training continued until at least 80% of the responses in the first trial were on the correct lever and at least 90% of the total trials (27/30) were avoidance trials (8515 and Ou3 only) for seven out of eight consecutive sessions. Test sessions were conducted according to the following two-week schedule: SDTST, DSTDT, where T denotes test sessions. If the criteria for stimulus control were not satisfied during the training sessions, test sessions

were not conducted and the training sequence continued. Test sessions were identical to training sessions except that completion of the response requirement on either lever was reinforced.

Drugs

d-Amphetamine sulfate (Abbott Laboratories, N. Chicago, IL) was dissolved in sterile 0.9% saline to an infusion volume of 0.25 ml/kg. CPDD 0066 was prepared in sterile water. The dose of 1.0 mg/kg was administered in 0.1 ml/kg. Doses of 3.0 and 10 mg/kg were administered in the standard infusion volume of 0.25 ml/kg. A dose of 17 mg/kg was administered to one monkey in 1.0 ml/kg. CPDD 0068 was prepared in sterile water with doses administered in the standard infusion volume of 0.25 ml/kg. Doses of CPDD 0066 and CPDD 0068 were tested the day after a saline or a drug-training session. If in that test responding occurred predominately on the drug lever, the dose was tested again the day after the opposite training session.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations, UTHSCSA)

Subjects and Apparatus

Seven adult rhesus monkeys, weighing between 4.6 and 9.0 kg, were housed individually in stainless steel cages. Water was available continuously and monkeys received primate chow (Harlan Teklad, Madison, WI) daily as well as fresh fruit and peanuts several days per week.

Monkeys were seated in chairs that provided restraint at the neck. During experimental sessions, chairs were located in sound-attenuating, ventilated chambers that were equipped with two response levers, a food cup and stimulus lights. Chairs were equipped with shoes containing brass electrodes, to which brief (250 ms) electric shock could be delivered from an a.c. shock generator.

Procedure

Flumazenil Discrimination. Monkeys consumed 5.6 mg/kg of diazepam in fruit punch 3 hours prior to daily sessions in which they discriminated between s.c. injections of 0.1 mg/kg (two monkeys) or 1.78 mg/kg (one monkey) of flumazenil and vehicle while responding under an FR 5 schedule of food presentation (Gerak and France, 1999). Daily training sessions consisted of several discrete, 15-minute cycles. Each cycle comprised a 10-minute pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a 5-minute response period, during which the chamber was illuminated green and monkeys could receive a 300 mg banana-flavored food pellet by responding five times on the appropriate lever as determined by the s.c. injection administered during the first minute of the 10-minute timeout (e.g., left lever after vehicle, right lever after flumazenil). Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of flumazenil, CPDD 0066, CPDD 0067, or CPDD 0068 were administered during the first minute of the timeout and five consecutive responses on either lever resulted in the delivery of food. CPDD 0066, CPDD 0067, and CPDD 0068 were studied up to 2 hours after administration in tests comprising eight 15-minute cycles.

Midazolam Discrimination. Monkeys discriminated between s.c. injections of 0.32 mg/kg of midazolam and saline while responding under an FR 10 schedule of stimulus-shock termination (Lelas et al., 1999). Daily sessions comprised multiple, 15-minute cycles. Each cycle comprised a 10-minute pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated red and monkeys could postpone scheduled shock for 30 seconds by responding ten times on the appropriate lever as determined by the s.c. injection administered during the first minute of the 10-minute timeout (e.g., left lever after saline, right lever after midazolam). Failure to satisfy the response requirement within 15 seconds resulted in the delivery of a brief shock. The response period ended after 5 minutes or 4 shocks, whichever occurred first. Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of midazolam, CPDD 0066, CPDD 0067, or CPDD 0068 were administered during the first minute of the timeout and ten consecutive responses on either lever postponed the shock schedule. CPDD 0066, CPDD 0067, and CPDD 0068 were studied up to 2 hours after administration in tests comprising eight 15-minute cycles.

Drugs

Diazepam (Zenith Laboratories, Northvale, NJ) was suspended in 42-48 ml (depending on body weight) of fruit punch containing suspending Agent K to yield a dose of 5.6 mg/kg/daily drinking episode. Flumazenil (F. Hoffman LaRoche, LTD, Basel, Switzerland) was dissolved in a vehicle of 10% ethanol, 40% propylene glycol and 50% saline; midazolam hydrochloride (Roche Pharma, Inc., Manati PR) was purchased as a commercially-prepared solution. CPDD 0066, CPDD 0067 and CPDD 0068 were dissolved in sterile 0.9% saline and were studied up to doses of 1.0, 10.0, and 0.32 mg/kg s.c., respectively.

Discriminative Stimulus Effects in Rats (LSD discrimination, SUNYB)

Subjects and Apparatus

Male Fischer 344 rats were obtained at an age of approximately 6 weeks from Harlan Sprague-Dawley Inc. (Indianapolis, IN, U.S.A.), housed in pairs under a 12-hour light-dark cycle beginning at 0600 hours, and allowed free access to water in their home cages. All training and testing occurred during the light cycle. Caloric intake was controlled to maintain a mean body weight of 250 g. Subjects were fed standard rat chow following experimental sessions. Caloric control has been shown to lengthen the life span and decrease the incidence of a variety of pathologies in Fischer 344 rats (Keenan et al. 1994).

Small animal test chambers [MED Associates ENV-008] were used for all experiments and were housed in larger light-proof, sound-insulating boxes which contained a house light and an exhaust fan. Chambers contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water. Sessions were managed by a micro-computer using operant control software [MED-PC State Notation, Version IV].

Procedure

After learning to drink from the dipper, rats were trained to press first one, and then the other, of the two levers. The number of responses for each reinforcer was gradually increased from 1 to 10. During this time, the reinforced lever was alternated on a random basis. All subsequent training and testing sessions used an FR 10 schedule of reinforcement. Subjects were then trained to discriminate LSD (0.1 mg/kg i.p., 15 min pretreatment; Hirschhorn and Winter 1971). Following the administration of LSD, every tenth response on the LSD-appropriate lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced on an FR 10 schedule following the injection of saline. For half of the subjects, the left lever was designated as the drug-appropriate lever. During discrimination training, drug and saline were alternated on a daily basis. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, at least 83% of all responses prior to the delivery of the first reinforcer were on the appropriate lever, i.e., no more than 2 incorrect responses prior to completion of the FR10 on the correct lever.

After stimulus control with LSD was established, tests of generalization were conducted once per week in each animal. Tests were balanced between subjects trained on the previous day with saline and drug, respectively. During test sessions, no responses were reinforced and the session was terminated after the emission of 10 responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted prior to lever selection, that is, prior to the emission of 10 responses on either lever, by elapsed time. Data for any subject failing to emit 10 responses within the 10-minute test session were not considered in the calculation of the percent drug-appropriate responding but were included in the calculation of response rates.

Drugs

Lysergic acid diethylamide [(+)-LSD (+)-tartrate (2:1)] and [-]-2,5-dimethoxy-4-methylamphetamine (DOM) were generously provided by the National Institute on Drug Abuse (Rockville, MD). Doses of LSD and DOM were expressed as mg/kg of the salts; both drugs were dissolved in sterile 0.9% saline. A stock solution of pirenpirone (1

mg/ml) was prepared in a minimal volume of a 45 percent w/v aqueous solution of 2-hydroxy-propyl-β-cyclodextrin and solutions for i.p. injections were made by diluting the stock with sterile 0.9% saline.

Head-twitch Response in Mice (UM)

Subjects and Apparatus

Male NIH Swiss mice (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing approximately 20-30 g were housed 12 animals per 44.5 x 22.3 x 12.7 cm Plexiglas cage in a room that was maintained at 22 ± 2°C and 45-50% humidity under a 12-hour light/dark cycle. Animals were fed Lab Diet rodent chow (Laboratory Rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water *ad libitum* until immediately before testing. Neither food nor water was available during the tests. Animals were not used in experiments until at least two days after arrival in the laboratory. Each animal was used only once, and was sacrificed immediately after use.

Procedure

The drug elicited head-twitch response is a selective behavioral model for 5-HT_2 agonist activity in the rodent, and several previous studies have established that direct and indirect 5-HT agonists induce this effect (Peroutka *et al.* 1981; Colpaert and Janssen 1983; Green *et al.* 1983; Goodwin and Green 1985; Darmani *et al.* 1990a, 1990b, 1992). Further, 5-HT_2 receptor antagonists selectively block the head-twitch response (Lucki *et al.* 1984; Handley and Singh 1986) with a potency that is highly correlated with affinity for 5-HT_2 receptors (Peroutka *et al.* 1981; Ortmann *et al.* 1982).

On test days, mice were weighed, marked, and returned to the home cage. Individual animals were subsequently removed from the home cage, received saline i.p., and then placed into a 15.24 x 25.40 x 12.70 cm Plexiglas mouse cage. Ten minutes after the initial injection, mice received an injection of either saline or one of several doses of R(-)-DOM, CPDD-0066 or CPDD-0068 and were returned to the observation cage. Five minutes after this second injection, a camera mounted above the observation cage began recording behavior for 10 minutes. Videotapes were later scored for the head-twitch response (defined as a rapid rotational jerk of the head that is not contiguous with any grooming or scratching behaviors) by two observers who were blind to treatment.

Drugs

R(-)-DOM (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), CPDD 0066 and CPDD 0068 were dissolved in sterile 0.9% saline. All injections were i.p. at a volume of 1 ml/100 g.

Competition Binding in Rat Brain (SUNYB)

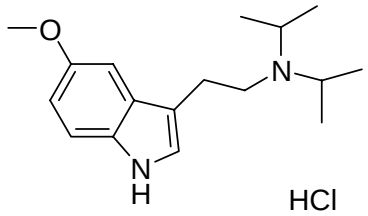
Receptor Binding

The frontal cortex (5-HT_{2A} receptors), hippocampus (5-HT_{1A} receptors), or brain stem (5-HT_{2C} receptors) from male CDF rats (Charles Rivers Laboratories) was homogenized (Dounce tissue grinder) in 50 mM Tris-HCl (pH 7.4). The homogenates were then centrifuged at 40,000 *g* for 15 minutes at 4°C. The resulting pellets were resuspended in the Tris buffer and stored at –80°C. On the day of the assays tissue samples were thawed and centrifuged at 40,000 *g* for 15 minutes at 4°C. The resulting pellets were resuspended in 30 ml warm 50 mM Tris-HCl (pH 7.4) and incubated for 10 minutes at 37°C to remove endogenous serotonin. Samples were again centrifuged at 40,000 *g* for 15 minutes at 4°C. Final resuspension of the pellets (frontal cortex: 6.7 mg/ml; hippocampus: 5 mg/ml; brain stem 13.3 mg/ml) was in Tris assay buffer (50 mM Tris-HCl, pH 7.4, containing 4 mM $MgCl_2$, 10μM pargyline and 0.1% ascorbate). For [^{3}H]8-OH-DPAT binding, assays were carried out for 30 minutes at 37°C in a final volume of 0.5 ml containing Tris assay buffer, 1 nM radioligand (129 Ci/mmole; Perkin-Elmer, Boston MA), appropriate drugs, and hippocampal membranes (2 mg wet weight/tube). For [^{3}H]ketanserin binding, assays were carried out for 30 minutes at 30°C in a final volume of 0.5 ml containing Tris assay buffer, 1.5 nM radioligand (88 Ci/mmole; Perkin-Elmer, Boston MA), 100 nM prazosin to prevent binding to α_1-adrenergic receptors, appropriate drugs, and frontal cortical membranes (2 mg wet weight/tube). For [^{3}H]mesulergine binding, assays were carried out for 45

minutes at 37°C in a final volume of 0.5 ml containing Tris assay buffer, 2 nM radioligand (77 Ci/mmole; Amersham Biosciences), 100 nM spiperone to prevent binding to 5-HT_{2A} and dopamine D_2 receptors, appropriate drugs, and membranes from the brain stem (4 mg wet weight/tube). Reactions were terminated by rapid vacuum filtration (Brandel harvester) through GF/B glass fiber filters presoaked in 0.1% polyethylenimine. Filters were washed twice with cold 50 mM Tris-HCl (pH 7.4) with the amount of bound radioactivity measured by scintillation spectrophotometry. Nonspecific binding was defined as the difference in the amount of radioligand binding in the absence and presence of either 10 μM 5-HT ([^{3}H]8-OH-DPAT binding), 20 μM 5-HT ([^{3}H]mesulergine binding) or 100 μM cinanserin ([^{3}H]ketanserin binding). Data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT).

RESULTS

CPDD 0066: 5-Methoxy-N,N-diisopropyltryptamine HCL



Reinforcing Effects in Rhesus Monkeys

A dose of 0.01 mg/kg/injection of cocaine maintained high rates of responding in all monkeys with an average of more than 100 injections of cocaine received per session. Up to five doses of CPDD 0066 were evaluated in three rhesus monkeys. Figure 1 shows the mean (± SEM) effects obtained with CPDD 0066. No animal self-administered this compound at rates greater than those engendered by saline.

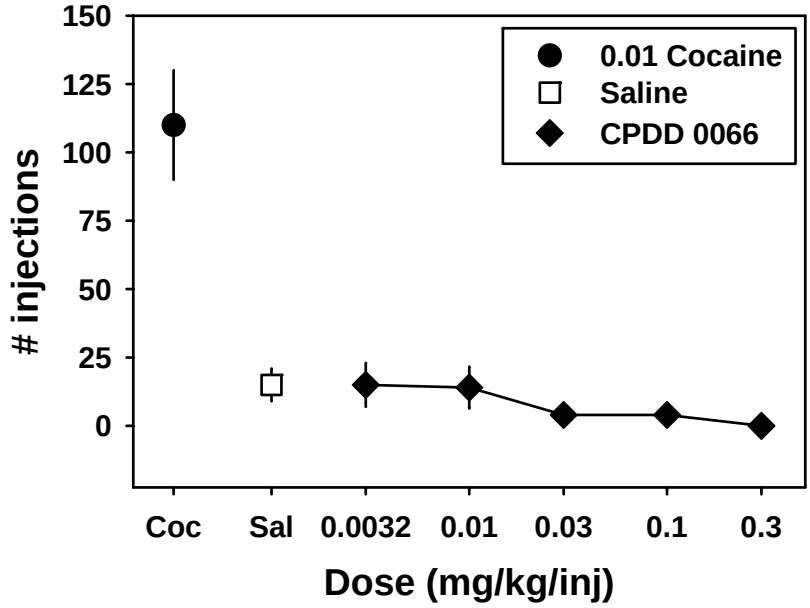


Figure 1. Self-administration studies with CPDD 0066.

Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination)

When administered 60 minutes before the session, CPDD 0066 generally lacked amphetamine-like discriminative stimulus effects up to a dose of 10 mg/kg (Table 1). Partial substitution at 3.0 mg/kg in monkey Ou3 was the result of averaging full substitution during the initial test session with no substitution when this dose was retested. This variable effect, and the lack of amphetamine-like responding at any dose in other monkeys, suggests that the full substitution seen in the initial test in Ou3 was a spurious result. CPDD 0066 was administered to an untrained monkey at a dose of 17 mg/kg. After 2-3 minutes the monkey began to seize. Seizures were controlled with diazepam and isoflurane and the monkey was conscious and sitting 4-5 hours later. Therefore, doses of CPDD 0066 larger than 10 mg/kg were not tested in trained monkeys.

Subject	TABLE 1 CPDD 0066 (D0SE) MG/KG AMPH	SALINE	1.0	3.0	10.0
8515	100 / 1.4	1.5 / 1.8	0 / 1.0	0 / 0.8	0 / 1.1
M163	100 / 1.8	5 / 1.4	0 / 2.3	0 / 2.5	0 / 1.7
Ou3	100 / 2.3	0 / 2.7	0 / 1.6	0 / 2.1	48 / 2.1

CPDD 0066 was administered via nasogastric tube 60 minutes prior to testing.

AMPH = amphetamine

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations)

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam and discriminating between 0.1 mg/kg (JI) or 0.178 mg/kg (JE) of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 2). Over the doses studied, flumazenil decreased response rate in JI and increased response rate in JE.

Subject	TABLE 2 Flumazenil Dose (mg/kg) Veh	0.01	0.032	0.1
JI	0 / 1.76	0 / 1.79	27 / 1.46	82 / 0.83
JE	0 / 0.46	0 / 0.38	74 / 0.90	88 / 1.04

Data represent percent drug-appropriate responding / response rate (responses / second)
Veh, vehicle

CPDD 0066 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the flumazenil discriminative stimulus (Table 3) up to doses (0.32 mg/kg in JE and 0.56 mg/kg in JI) that suppressed responding.

Data shown are an average of responding at 30 minutes after administration of CPDD 0066.

Subject	TABLE 3 CPDD 0066 Dose (mg/kg) Veh	0.1	0.32	0.56
JI	0 / 1.36	10 / 1.69	0 / 1.84	* / 0
JE	0 / 0.47	0 / 0.36	* / 0	NT

Data represent percent drug-appropriate responding / response rate (responses / second)
Veh, vehicle
*Discrimination data are not presented when response rate was <20% of control response rate
NT, not tested

At the largest doses (0.56 mg/kg in JI and 0.32 mg/kg in JE), the onset of action for CPDD 0066 to suppress responding was 15-30 minutes and the duration of action was 30-60 minutes (Table 4).

Subject (mg/kg of CPDD 0066)	TABLE 4 Min after CPDD 0066 15	30	45	60	75
JI (0.56)	14 / 1.60	* / 0	* / 0	65 / 1.53	0 / 1.80
JE (0.32)	* / 0	* / 0	* / 0	* / 0	0 / 0.76

See Table 3 for details

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug-associated lever with doses of 0.1 mg/kg and 0.32 mg/kg occasioning greater than 80% drug-lever responding in LI and GI, respectively (Table 5). Over the doses studied, midazolam increased response rate in LI and decreased response rate in GI. CPDD 0066 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the midazolam discriminative stimulus (Table 6) up to a dose (1.0 mg/kg) that markedly decreased responding and that produced pupil dilation and hyperventilation. Data shown are from 30 minutes after administration of CPDD 0066.

Subject	TABLE 5 Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
LI	0 /1.59	0 / 1.39	0 / 1.47	100 / 2.05	NT
GI	0 / 1.94	0 / 1.60	0 / 1.22	1 / 0.99	100 / 0.95

NT, not tested
See Table 3 for details

Subject	TABLE 6 CPDD 0066 Dose (mg/kg) Veh	0.32	0.56	1.0
LI	0 /1.34	0 / 1.58	0 / 1.43	* / 0
GI	0 / 1.98	0 / 2.62	0 / 1.70	* / 0.06

See Table 3 for details

At the largest dose (1.0 mg/kg), the onset of action for CPDD 0066 to suppress responding was 15 minutes and the duration of action was at least 30 min (Table 7).

Subject (mg/kg of CPDD 0066)	TABLE 7 Min after CPDD 0066 15	30	45	60	75
LI (1.0)	* / 0	* / 0	0 / 2.00	0 / 1.92	0 / 2.15
GI (1.0)	* / 0	* / 0.06	1 / 1.09	0 / 1.59	0 / 1.38

See Table 3 for details

Discriminative Stimulus Effects in Rats (LSD discrimination)

Up to a dose of 3 mg/kg, CPDD 0066 substituted partially for the LSD discriminative stimulus and also decreased rate of responding (Figure 2). Moreover, the partial LSD-like discriminative stimulus effects of 1 mg/kg of CPDD 0066 were completely attenuated by pretreatment with 0.16 mg/kg of pirenpirone.

Head-twitch Response in Mice

R(-)-DOM generated a biphasic dose-response function on the head-twitch response, consistent with previous studies in mice (Fantegrossi et al., 2004a). R(-)-DOM-induced head-twitches peaked at a mean of approximately 14 twitches in 10 minutes at a dose of 1.0 mg/kg (Table 8). CPDD 0066 induced a similar biphasic dose-response function for the head-twitch response, although this compound was less effective than DOM, eliciting a maximum of 8.5 twitches at a dose of 1.0 mg/kg.

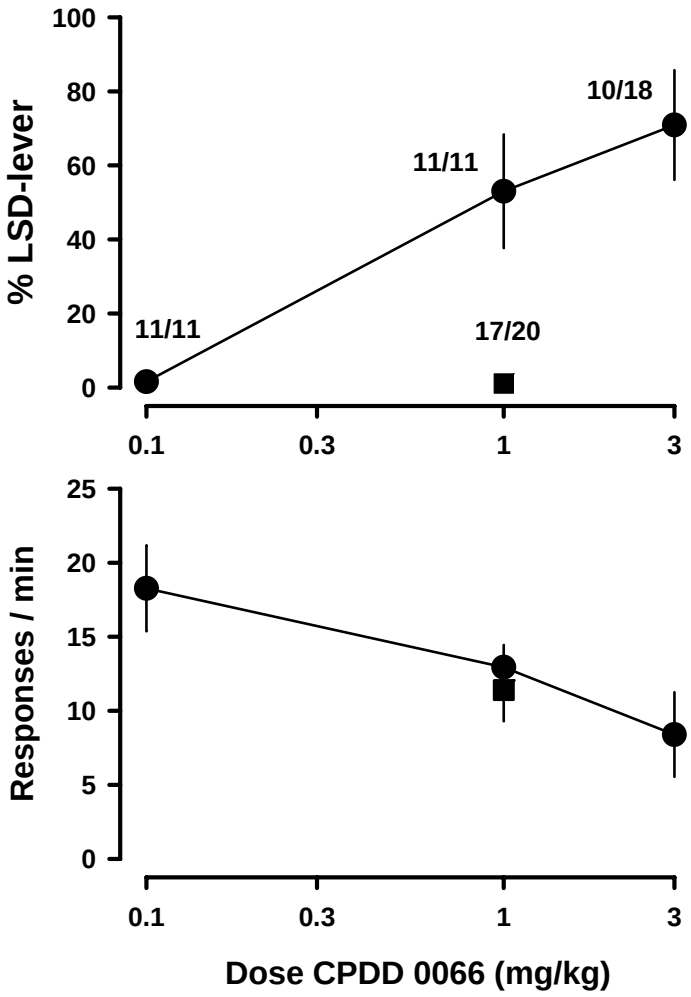


Figure 2. Discriminative stimulus (upper) and rate-altering (lower) effects of CPDD 0066 administered alone (circles) or 60 minutes after administration of 0.16 mg/kg of pirenpirone (squares) in rats discriminating between 0.1 mg/kg of LSD and saline. Ordinates: upper, average percentage of responses on the LSD-associated lever; lower, rate of lever pressing in responses/minute. Abscissa: dose in mg/kg body weight. CPDD 0066 was administered 15 minutes before testing. Other values (X/Y) indicate number of animals responding / number of animals studied.

Dose	Saline	DOM	CPDD 0066	CPDD 0068
0.0	**0.67 ± 0.33**	**-**		
0.3	**-**	**5.67 ± 0.76**	**1.67 ± 0.49**	**2.83 ± 0.60**
1.0	**-**	**14.17 ± 1.40**	**8.50 ± 1.38**	**15.60 ± 2.41**
3.0	**-**	**10.33 ± 3.77**	**6.33 ± 0.80**	**7.33 ± 0.95**
10.0	**-**	**7.17 ± 2.09**	**Not studied**	**Not studied**

Table 8. Head-twitch response for DOM, CPDD 0066 and CPDD 0068. Each value is the mean (± SEM) number of twitches per 10-minute observation period for different groups of mice.

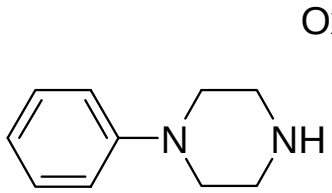
Competition Binding in Rat Brain

CPDD 0066 displaced binding in all three assays indicating binding affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors in rat brain (Table 9); CPDD 0066 had highest affinity for 5-HT_{1A} receptors.

CPDD #	pK_I [^{3}H]8-OH-DPAT	pK_I [^{3}H]ketanserin	pK_I [^{3}H] mesulergine
0066	7.44 ± 0.04	5.25 ± 0.04	5.77 ± 0.36
0067	6.51 ± 0.01	5.11 ± 0.06	5.57 ± 0.07
0068	5.93 ± 0.08	6.92 ± 0.17	7.41 ± 0.02

Table 9. Binding affinities at 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors. Affinities of the various compounds at [^{3}H]8-OH-DPAT binding sites in hippocampus [5-HT_{1A}], [^{3}H]ketanserin binding sites in frontal cortex [5-HT_{2A}], and [^{3}H] mesulergine binding sites in brain stem [5-HT_{2C}] were measured as described in the Methods section. Data are expressed as the mean (± SEM; n=3-5) negative log of the equilibrium dissociation constant (pK_I).

CPDD 0067: Phenylpiperazine Oxalate



Reinforcing Effects in Rhesus Monkeys

Four doses of CPDD 0067 were evaluated in three rhesus monkeys. Figure 3 shows the mean (± SEM) effects obtained with CPDD 0067. No animal self-administered this compound at rates greater than those engendered by saline.

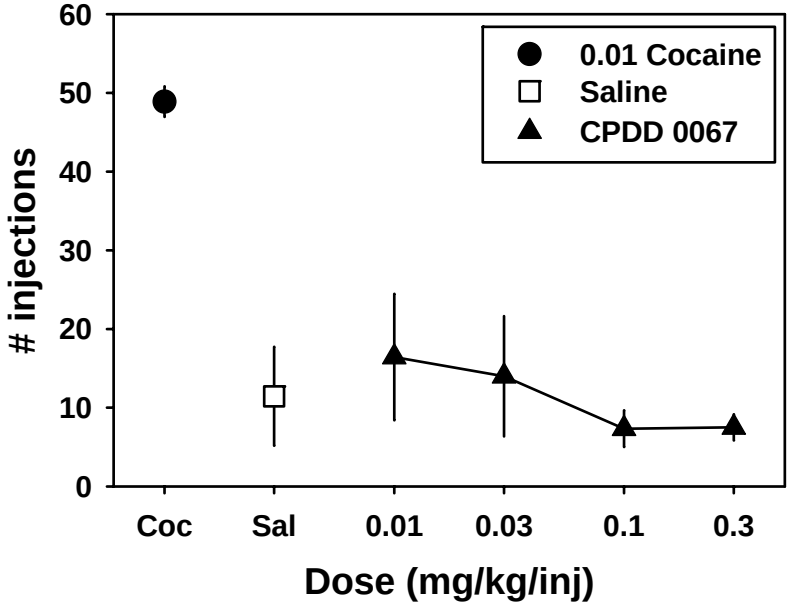


Figure 3. Self-administration studies with CPDD 0067.

No behavioral changes were noted following CPDD 0067 test sessions, although 2 of 3 animals failed to emit a single response in afternoon sessions following morning exposure to 0.1 and 0.3 mg/kg/injection of CPDD 0067.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations)

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam and discriminating between 0.1 mg/kg (JI) or 0.178 mg/kg (JE) of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug (flumazenil)-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in

each monkey (Table 10). Over the doses studied, flumazenil decreased response rate in JI and increased response rate in JE.

Subject	TABLE 10 Flumazenil Dose (mg/kg) Veh	0.01	0.032	0.1
JI	0 / 1.76	0 / 1.79	27 / 1.46	82 / 0.83
JE	0 / 0.46	0 / 0.38	74 / 0.90	88 / 1.04

Data represent percent drug-appropriate responding / response rate (responses / second)
Veh, vehicle

CPDD 0067 did not substitute for the flumazenil discriminative stimulus (Table 11) up to doses (1.0 mg/kg in JE and 3.2 mg/kg in JI) that suppressed responding. Data shown are an average of responding at 30 minutes after administration of CPDD 0067.

Subject	TABLE 11 CPDD 0067 (mg/kg) Veh	0.1	0.32	1.0	3.2
JI	0 /1.36	NT	0 / 1.59	0 / 0.85	* / 0
JE	0 / 0.45	0 / 0.70	0 / 0.13	* / 0	NT

Data represent percent drug-appropriate responding / response rate (responses / second)
*Discrimination data are not presented when response rate was <20% of control response rate
Veh, vehicle
NT, not tested

At the largest doses studied (3.2 mg/kg in JI and 1.0 mg/kg in JE), the onset of action for CPDD 0067 to suppress responding was 15 minutes and the duration of action was 90-120 minutes (Table 12).

Subject (mg/kg of CPDD 0067)	TABLE 12 Min after CPDD 0067 15	30	45	60	75	90	105	120
JI (3.2)	* / 0.10	* / 0	* / 0	0 / 0.54	4 / 0.19	2 / 0.16	0 / 0.43	0 / 0.49
JE (1.0)	* / 0	* / 0	* / 0	* / 0.05	0 / 0.20	0 / 0.55	0 / 0.63	0 / 0.75

See Table 11 for details

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug (midazolam)-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 13). Over the doses studied, midazolam increased response rate in RO and decreased response rate in GI. CPDD 0067 did not substitute for the midazolam discriminative stimulus (Table 14) up to a dose (10.0 mg/kg) that decreased responding. Data shown are from responding at 120 minutes after administration of CPDD 0067. At the largest dose (10.0 mg/kg) of CPDD 0067, the largest decrease in responding was observed in the last cycle of the 2-hr session (Table 15).

Subject	TABLE 13 Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
RO	0 /2.38	0 / 2.65	0 / 2.49	0 / 2.64	100 / 3.09
GI	0 / 1.94	0 / 1.60	0 / 1.22	1 / 0.99	100 / 0.95

See Table 11 for details

Subject	TABLE 14 CPDD 0067 Dose (mg/kg) Veh	3.2	5.6	10.0
RO	0 /2.24	0 / 1.72	0 / 2.37	0 / 1.26
GI	0 / 1.35	NT	0 / 1.89	0 / 0.70

See Table 11 for details

Subject (mg/kg of CPDD 0067)	TABLE 15 Min after CPDD 0067 15	30	45	60	75	90	105	120
RO (10.0)	0 / 2.05	0 / 1.69	0 / 1.76	0 / 1.47	0 / 1.47	0 / 1.33	0 / 1.35	0 / 1.26
GI (10.0)	0 / 1.34	0 / 1.34	0 / 1.80	0 / 1.60	0 / 1.30	0 / 1.99	0 / 1.72	0 / 0.70

See Table 11 for details

Discriminative Stimulus Effects in Rats (LSD discrimination)

Up to a dose that eliminated responding (3 mg/kg), CPDD 0067 failed to substitute for the LSD discriminative stimulus in rats (Figure 4). The largest dose of CPDD 0067 that did not eliminate responding (1.0 mg/kg) also was studied for its effects over a broader range of pretreatment times (Figure 5) and, under those conditions, failed to substitute for LSD.

Competition Binding in Rat Brain

Although CPDD 0067 displaced binding in all three assays, indicating some binding affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors in rat brain (Table 9), overall its affinity for all three receptors was comparatively low.

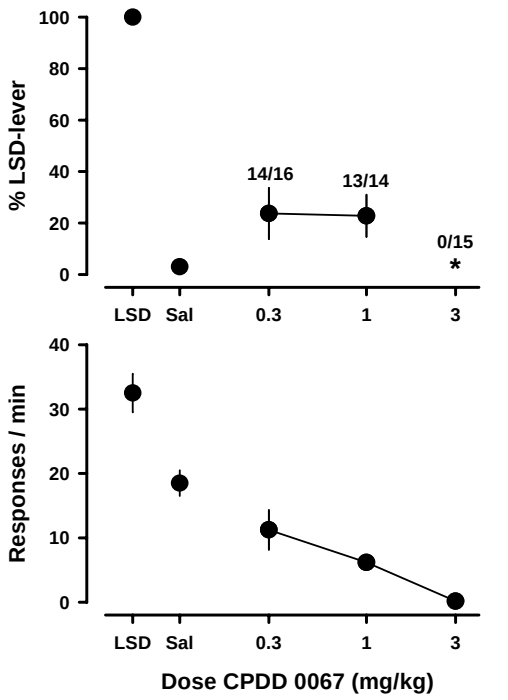


Figure 4. Discriminative stimulus effects of CPDD 0067 in rats discriminating between 0.1 mg/kg of LSD (triangles) and saline (inverted triangles). CPDD 0067 was administered 30 minutes before testing. See Figure 2 for other details.

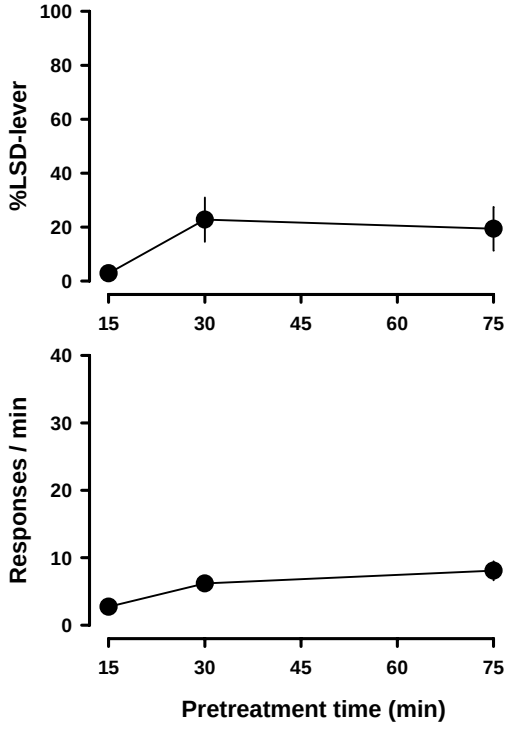
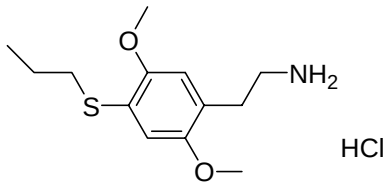


Figure 5. Time course of effects for 1.0 mg/kg of CPDD 0066 in rats discriminating LSD. See Figures 2 and 4 for other details.

CPDD 0068: 2,5-Dimethoxy-4-(n)-propyl-thiophenethylamine HCL



Reinforcing Effects in Rhesus Monkeys

Four doses of CPDD 0068 were evaluated in three rhesus monkeys. Figure 6 shows the mean (± SEM) effects obtained with CPDD 0068. No animal self-administered this compound at rates greater than those engendered by saline. Following sessions when large unit doses of CPDD 0068 were available, animals appeared sluggish and tended to display stereotyped jaw opening and head movements, especially after 0.01 and 0.03 mg/kg/injection. In addition, animals obtained fewer injections of cocaine in afternoon sessions following morning test sessions with CPDD 0068 as compared to sessions following morning sessions with cocaine (Figure 7).

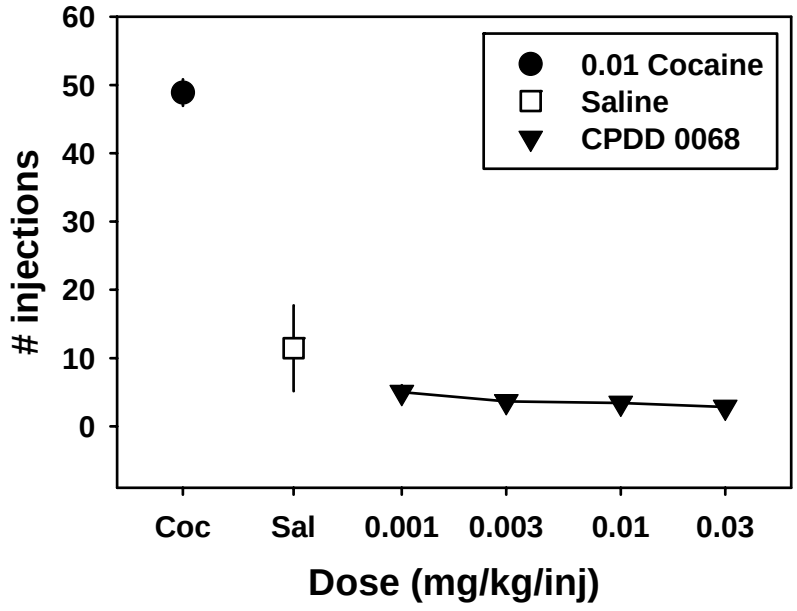


Figure 6. Self-administration studied with CPDD 0068

Discriminative Stimulus Effects in Rhesus Monkeys (amphetamine discrimination)

When administered 60 minutes before the session, CPDD 0068 lacked amphetamine-like discriminative stimulus effects and did not systematically alter response rate up to a dose of 3.0 mg/kg (Table 16). Following 3.0 mg/kg of CPDD 0068, monkeys were visibly affected (e.g., appeared more calm than usual and staring). All ate monkey chow offered after the session.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations)

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam and discriminating between 0.1 mg/kg of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug-associated lever with a dose of 0.1 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 17). Flumazenil dose-dependently decreased response rate.

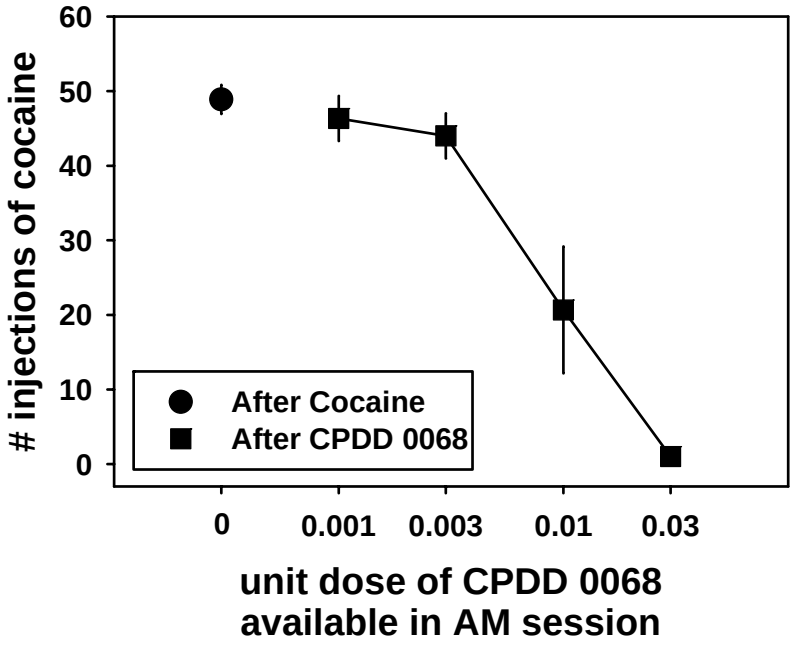


Figure 7. Cocaine self-administration in sessions following morning sessions with CPDD 0068.

Subject	TABLE 16 CPDD 0068 (D0SE) MG/KG AMPH	SALINE	0.3	1.0	3.0
8515	100 / 1.4	1.5 / 1.8	0 / 1.1	0 / 1.0	0 / 1.0
M163	100 / 1.8	5 / 1.4	0 / 2.0	0 / 2.3	0 / 1.9
Ou3	100 / 2.3	0 / 2.7	0 / 2.1	0 / 2.0	0 / 2.4

See Table 1 for other details.

Subject	TABLE 17 Flumazenil Dose (mg/kg) Veh	0.01	0.032	0.1
JI	0 / 1.41*	11 / 1.65	53 / 1.29	98 / 0.79
NA	0 / 1.00	13 / 0.95	51 / 0.40	84 / 0.17

*Data represent percent drug-appropriate responding / response rate (responses / second)
Veh = vehicle

CPDD 0068 did not substitute for the flumazenil discriminative stimulus (Table 18) up to a dose (0.32 mg/kg) that suppressed responding. Data shown are from 30 minutes after administration of CPDD 0068 (peak onset for rate-decreasing effects).

Subject	TABLE 18 CPDD 0068 Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
JI	9 / 1.59	NS	0 / 1.60	0 / 0.87	* / 0
NA	2 / 0.83	10 / 0.13	* / 0	NS	* / 0

*Discrimination data are not presented when food was not delivered
NS = not studied
See Table 17 for other details

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug-associated lever with a dose of 0.32 mg/kg occasioning greater than 80% drug-lever responding in each monkey (Table 19). The largest dose of midazolam (0.32 mg/kg) slightly decreased response rate.

CPDD 0068 did not substitute for the midazolam discriminative stimulus (Table 20) up to a dose (0.32 mg/kg) that significantly decreased response rate (GI) and that produced emesis and salivation. Data shown are from 30 minutes after administration of CPDD 0068 (peak onset for rate-decreasing effects).

Subject	TABLE 19 Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
SA	0 /3.25	0 / 3.01	0 / 2.69	33/ 1.68	100 / 1.63
GI	0 / 1.71	0 / 1.54	0 / 1.76	63/ 1.00	98 / 0.53

See Table 17 for other details

Subject	TABLE 20 CPDD 0068 Dose (mg/kg) Veh	0.032	0.1	0.32
SA	0 /2.77	0 / 2.89	0 / 2.99	0 / 2.82
GI	0 / 1.75	NS	0 / 0.74	0 / 0.14

See Tables 17 and 18 for other details

Discriminative Stimulus Effects in Rats (LSD discrimination)

Increasing doses of CPDD 0068 occasioned increased responding on the LSD-associated lever with an average of more than 70% responding on the LSD lever occurring at doses of 1 and 3 mg/kg (Figure 8). A dose of 3 mg/kg of CPDD 0068 markedly decreased rates of responding. Figure 9 shows a comparison of the time course of 0.3 mg/kg of DOM and 1 mg/kg of CPDD 0068. The most LSD-lever responding occurred with DOM 75 minutes after administration of CPDD 0068 45 minutes after administration.

Head-Twitch Response in Mice

CPDD 0068 induced the head-twitch response in mice (Table 8) at doses of 0.3-3.0 mg/kg. Similar to effects obtained with DOM, the dose-response function for CPDD 0068-induced head-twitching was biphasic, with a maximum of 15.6 twitches per 10 minutes occurring at a dose of 1.0 mg/kg.

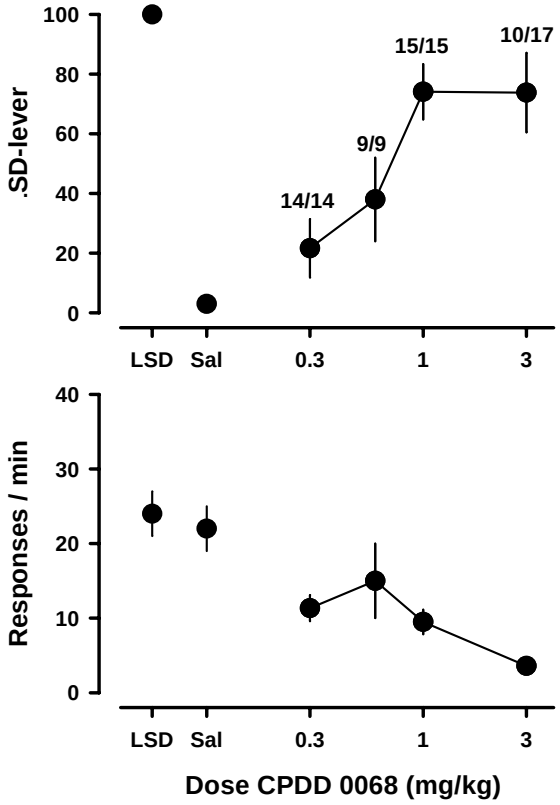


Figure 8. Discrimination stimulus effects of CPDD 0068 (circles) in rats discriminating between 0.1 mg/kg LSD (triangles) and saline (inverted triangles) 45 minutes prior to testing. See Figure2

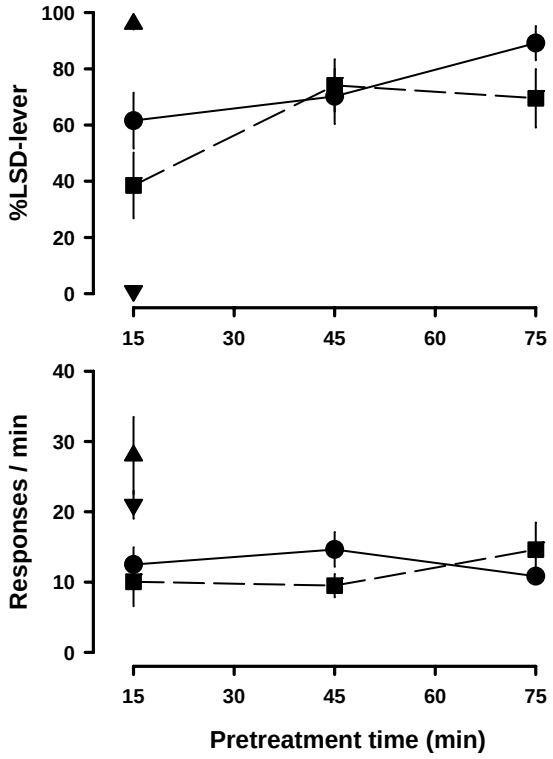
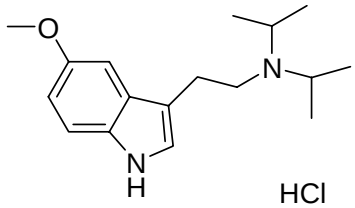


Figure 9. Time course of effects for 0.3 mg/kg of [-]-DOM (circles) and 1.0 mg/kg of CPDD 0068 (squares) in rats discriminating between 0.1 mg/kg of LSD (triangles) and saline (inverted triangles). See Figures 2 and 8 for other details.

Competition Binding in Rat Brain

CPDD 0068 displaced binding in all three assays indicating binding affinity for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors in rat brain (Table 9); however, CPDD 0068 had highest affinity for 5-HT_{2A}, and 5-HT_{2C} receptors.

CONCLUSIONS

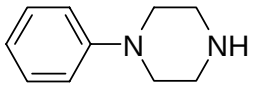


CPDD 0066

5-Methoxy-N,N-diisopropyltryptamine HCl

CPDD 0066 did not maintain self administration responding in monkeys and, up to doses that decreased rats of responding, did not substitute for amphetamine, midazolam or flumazenil in monkeys. However, like DOM, CPDD 0066 induced head-twitching in mice and substituted partially for an LSD discriminative stimulus in rats; like other tryptamines, CPDD 0066 had highest affinity for 5-HT_{1A} receptors. Moreover, the LSD-like effects of CPDD 0066 were attenuated by the 5-HT_{2A} receptor antagonist, pirenpirone. Collectively, these data are consistent with the view that this compound exerts LSD-like behavioral effects and, therefore, that its abuse could be due to LSD-like hallucinogenic activity at 5-HT receptors. The much greater potency of CPDD 0066 in altering responding in the midazolam and flumazenil discrimination procedures (s.c.), as compared to the amphetamine discrimination procedure (i.g.), indicates that the bioavailability of CPDD 0066 is greater after s.c. as compared to i.g. administration.

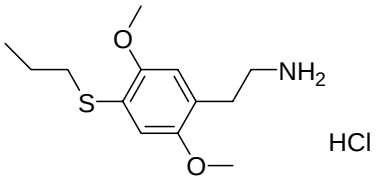
Oxalate



CPDD 0067

1-Phenylpiperazine Oxalate

CPDD 0067 was not self administered by rhesus monkeys and, up to doses that decreased rates of responding, did not substitute for midazolam or flumazenil in monkeys or for LSD in rats. CPDD 0067 had very low affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors and did not share behavioral actions with LSD. CPDD 0067 is structurally similar to benzylpiperazine (BZP; CPDD 0063); however, unlike BZP (Fantegrossi et al., 2004b) it was not self administered by monkeys. Ongoing studies are assessing whether this compound, like BZP (Fantegrossi et al., 2004b), shares discriminative stimulus effects with amphetamine in monkeys.



CPDD 0068

2,5-Dimethoxy-4-(n)-propyl-thiophenethylamine HCl

CPDD 0068, like CPDD 0066, has a profile of effects that is similar to well characterized hallucinogens (e.g., LSD, DOM). Specifically, CPDD 0068 wais not self administered by monkeys, did not substitute for amphetamine, and, up to doses that decrease rates of responding, did not substitute for midazolam or flumazenil. However, as might be expected of an analog of DOM, this compound had relatively high affinity for 5-HT_{2A} and 5-HT_{2C} receptors, it induced head-twitching in mice, and it substituted partially for an LSD discriminative stimulus in rats. Collectively, these data are consistent with the view that this compound exerts LSD-like behavioral effects and, therefore, that its abuse could be due to LSD-like hallucinogenic activity at 5-HT receptors. The much greater potency of CPDD 0068 in altering responding in the midazolam and flumazenil discrimination procedures (s.c.), as compared to the amphetamine discrimination procedure (i.g.), suggesting that the bioavailability of CPDD 0068 is greater after s.c. as compared to i.g. administration.

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