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Synthetic studies and pharmacological evaluations on the MDMA ('Ecstasy') antagonist nantenine

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ABSTRACT

The naturally occurring aporphine alkaloid nantenine, has been shown to antagonize behavioral and physiological effects of MDMA in mice. We have synthesized (±)-nantenine via an oxidative cyclization reaction with PIFA and evaluated its binding profile against a panel of CNS targets. To begin to understand the importance of the chiral center of nantenine with regards to its capacity to antagonize the effects of MDMA in vivo, (*R*)- and (*S*)-nantenine were prepared and evaluated in a food-reinforced operant task in rats. Pretreatment with either nantenine enantiomer (0.3 mg/kg ip) completely blocked the behavioral suppression induced upon administration of 3.0 mg/kg MDMA. (±)-Nantenine displayed high affinity and selectivity for the α_{1A} adrenergic receptor among several other receptors suggesting that this α_1 subtype may be significantly involved in the anti-MDMA effects of the enantiomers.

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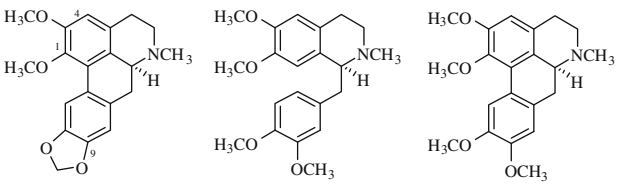
MDMA ('Ecstasy') is a synthetic amphetamine which produced both stimulant-like and hallucinogen-like effects in humans.[1] Abuse of this designer drug is particularly prevalent among young adults. Recent data shows that 6% and 4% of 12th grade students had used 'Ecstasy' in their lifetime and in the past year respectively.[2] MDMA causes acute adverse physiological effects including the development of hyperthermia.[3–6] Use of MDMA is also associated with memory and cognitive impairment as well as the development of dependence on the drug in some consumers.[6–13] At this time, there are no therapeutic agents which are specifically approved to treat the acute adverse effects of MDMA or MDMA dependence.

The aporphine alkaloid (*S*)-(+)-nantenine (**1**) ex *Nandina domestica* was shown to block and reverse a range of behavioral and physiological effects mediated by MDMA in mice.[14] At present, there is a paucity of knowledge concerning the receptor targets which may be involved in the reported antagonist effects of nantenine vs MDMA in vivo, although it would appear that 5-HT_{2A} and α_1 adrenergic receptors are involved.[14] Prior reports have established that (±)-nantenine is an antagonist at the 5-HT_{2A} receptor and α_1 adrenoceptors;[15–17] its pharmacological activity at other CNS receptors has not been ascertained. To date, (*R*)-(−)-nantenine has not been evaluated for its anti-MDMA effects in animal models. As part of our research endeavors to better understand the in vitro and in vivo pharmacological profile of nantenine, we have synthesized racemic nantenine and nantenine enantiomers and evaluated the CNS receptor binding profile and behavioral effects in rats trained to respond for food-reinforcement, respectively. Our efforts in this regard are reported herein.

A commonly used route to synthesize aporphines involves oxidative biaryl coupling of phenolic or phenol ether benzyltetrahydroisoquinoline intermediates in an essentially biomimetic process.[18] In the past, the oxidizing agents commonly used for this reaction include $VOCl_3$, VOF_3, $Pb(OAc)_4$ and $Tl(OCOCF_3)_3$.[19–24] These reagents are toxic and furthermore the yields obtained in these reactions are often quite low.

The reagent phenyliodine bis(trifluoroacetate) (PIFA) has emerged as an environmentally-friendly alternative for these biaryl cyclizations, providing aporphines in good yields. For example, Anakabe et al. have reported conversion of laudanosine (**2**, Fig. 1)



1 2 3

Figure 1. Structures of (+)-nantenine (**1**), laudanosine (**2**) and (+)-glaucine (**3**).

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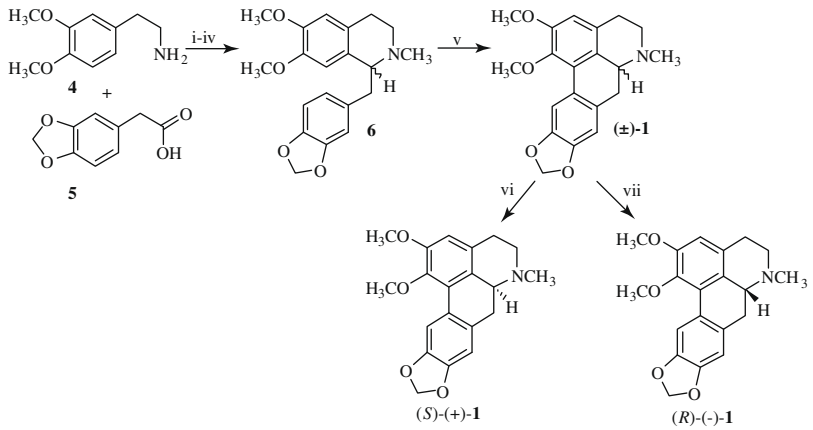


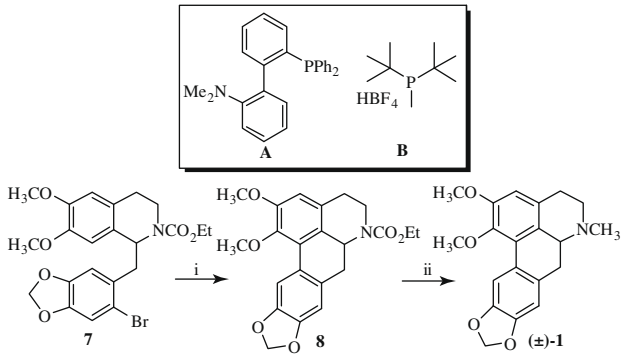
Scheme 1. Reagents and conditions: (i) CDI, THF, 99%; (ii) $POCl_3$, CH_2Cl_2, reflux, 87%; (iii) $NaBH_4$, MeOH, −78 °C, 77%; (iv) HCHO, $Na(OAc)_3BH$, DCM, 95%; (v) PIFA, $BF_3{\cdot}OEt_2$, HFIP, rt, 45 min, 16%; (vi) (−)-2,3-di-*p*-toluoyl-L-tartaric acid, EtOH then 10% aq NaOH, 30%, 96% ee; (vii) (+)-2,3-di-*p*-toluoyl-D-tartaric acid, EtOH then 10% aq NaOH, 32%, 97% ee.

to glaucine (**3**) in 75% yield using PIFA.[25] Others have recently reported the synthesis of (±)-nantenine in moderate (36%) yield with PIFA.[26] We initially engaged this method to prepare nantenine, with a secondary goal of optimizing reported conditions for the PIFA-mediated biaryl cyclization.

Following established protocols,[25] the tetrahydroisoquinoline **6** was prepared starting from amine **4** and acid **5** in a sequence involving amide coupling, Bischler–Napieralski cyclization, imine reduction and N-methylation as shown in Scheme 1. Cyclization of **6** with PIFA at −40 °C in DCM has been reported to give a moderate yield of **1**.[26] In our hands however, only a 12% yield was obtained under these conditions.

We found that variations in temperature (−78 °C, −20 °C and rt) did not improve this outcome in yield. We also substituted DCM with polar, non-nucleophilic solvents such as hexafluoroisopropanol (HFIP),[27,28] trifluoroethanol (TFE) and CH_3CN; only HFIP gave the required product albeit in low (16%) yield. No nantenine was detected when $RuO_2{\cdot}xH_2O$ or $Ce(OH)_4$ were used as oxidant (i.e., instead of PIFA). Our failed attempts at optimizing this reaction have since led us to opt for more efficient and versatile direct biaryl coupling procedures to prepare (±)-nantenine as well as several analogs (Scheme 2).[29,30]

The naturally occurring enantiomer of **1** (i.e., the (*S*)-(+)-enantiomer) has been shown to antagonize locomotor stimulant, head-twitch, lethality and hyperthermic effects of MDMA.[14] To gain some insight into the capacity of the (*R*)-(−)-enantiomer to function as an antagonist of MDMA's effects, we first resolved this compound from racemic **1** with (+)-2,3-di-*p*-toluoyl-D-tartaric acid.



Scheme 2. Reagents and conditions: (i) $Pd(OAc)_2$, solvent (DMA, DMF or DMSO), pivalic acid, K_2CO_3, ligand **A** or **B**, microwaves, 5 min, 15–88%; (ii) $LiAlH_4$, THF, 0 °C, 16 h, 90%.

(*R*)-(−)-Nantenine (96% ee as determined by chiral HPLC; $[\alpha]_D^{24}$ −117.0 (*c* 0.58, $CHCl_3$); stereochemistry determined by CD spectroscopy[31]) was then evaluated in a food-reinforced operant assay in rats trained under a fixed-ratio 20 (FR20) schedule in the presence of an illuminated stimulus light. This behavior was maintained by delivery of one 94 mg food pellet. Behavioral sessions were run daily and consisted of 4 discrete components; each component was terminated after rats earned 20 pellets, or after 20 min—whichever happened first. Each component was separated by a 10 min timeout where stimulus lights were extinguished and lever presses had no programmed consequences. The (*S*)-enantiomer [obtained by resolution of the racemate with (−)-2,3-di-*p*-toluoyl-L-tartaric acid; 97% ee by chiral HPLC; $[\alpha]_D^{24}$ +115.5 (*c* 0.51, $CHCl_3$)] was also evaluated in this assay for comparison. As a positive control, the 5-$HT_{2A/2C}$ antagonist ketanserin was also tested against the rate suppressant effects of MDMA.

Importantly, ketanserin has previously been shown to block a range of behavioral and physiological effects of MDMA in mice,[3]

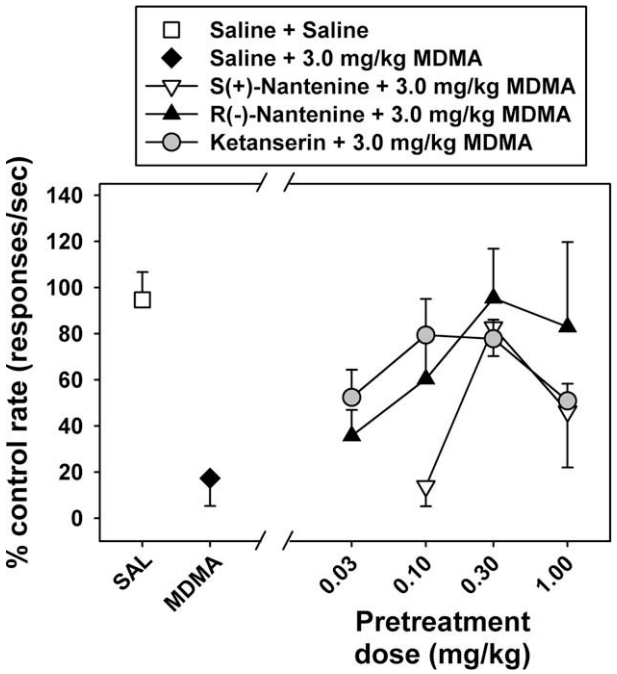


Figure 2. Rate suppressant assay with nantenine enantiomers and ketanserin.

Table 1
Affinity of (±)-nantenine (**1**) at CNS receptors

Receptor	K_i (nM)	Receptor	K_i (nM)	Receptor	K_i (nM)	Receptor	K_i (nM)
5-HT_{1B}	100 ± 3	α_{1A}-AR	2 ± 0.2	D1	895 ± 94	MOR	7265 ± 910
5-HT_{1D}	49 ± 5	α_{1B}-AR	1191 ± 145	D2	858 ± 86	H1	>10,000
5-HT_{1E}	>10,000	α_{1D}-AR	340 ± 34	D3	309 ± 50	H2	672 ± 23
5-HT_{2A}	832 ± 164	α_{2A}-AR	1288 ± 155	D4	262 ± 28	H3	>10,000
5-HT_{2B}	543 ± 53	α_{2B}-AR	252 ± 21	D5	2397 ± 410	H4	>10,000
5-HT_{2C}	1069 ± 131	α_{2C}-AR	181 ± 14	DAT	>10,000	M1	>10,000
5-HT_3	>10,000	β_1-AR	8565 ± 1084	SERT	244 ± 41	M2	>10,000
5-HT_{5A}	2224 ± 224	β_2-AR	>10,000	NET	>10,000	M3	>10,000
5-HT_6	257 ± 24	β_3-AR	>10,000	DOR	>10,000	M4	>10,000
5-HT_7	67 ± 7			KOR	>10,000	Sigma 2	>10,000

rats,[32] rhesus monkeys[33] and humans[34,35] which guided our selection of this compound as a standard against which to compare the effects of the nantenine enantiomers.

Rats were injected with various dose combinations of ketanserin, (*R*)- or (*S*)-nantenine, and 3.0 mg/kg racemic MDMA. Responding during the first behavioral component following drug administration, normalized to baseline data collected in sessions where no injections were administered, is presented in Figure 2. At a dose of 3.0 mg/kg (IP), MDMA profoundly suppresses operant behavior in this first component in all subjects, although responding gradually recovered throughout the remainder of the session. Since this rate of behavioral recovery in subsequent components differed among rats, we present only response data from the first behavioral component as an index of the apparent antagonist effects of the nantenine enantiomers against MDMA-induced behavioral suppression.

All pretreatments were administered 15 min prior to MDMA injection. Following two saline injections (separated by 15 min, open square), response rates were essentially unchanged from control. All rats responded at high rates and earned all 20 available food pellets. When 3.0 mg/kg MDMA was injected 15 min after an initial saline injection (filled diamond), response rates were reduced to approximately 20% of control levels, and rats earned 2 or fewer food pellets.

Injection of ketanserin 15 min before MDMA administration (filled circles) had biphasic effects on response rates. At a dose of 0.03 mg/kg ketanserin, MDMA continued to suppress operant behavior to approximately 50% of control levels. At higher doses (0.1 and 0.3 mg/kg), ketanserin blocked the rate suppressant effects of MDMA. At the highest ketanserin dose tested (1.0 mg/kg), ketanserin produced lethargy in all subjects during the pretreatment interval, and these effects likely explain the descending limb of the dose-effect function in Figure 2.

In comparison, injection of (*S*)-nantenine 15 min before MDMA administration (open inverted triangles) also had biphasic effects. The lowest tested dose (0.1 mg/kg) did not alter the effects of MDMA on operant behavior. However, 0.3 mg/kg (*S*)-nantenine completely blocked MDMA-induced behavioral suppression, and only 1 rat failed to earn all available food pellets at this dose. Higher doses likely had direct effects of their own, and administration of 10.0 mg/kg (*S*)-nantenine appeared to have sedative effects during the pretreatment period, with most rats failing to emit even a single response at this dose (not shown).

Like the other antagonists studied, administration of (*R*)-nantenine 15 min before MDMA injection (filled triangles) also had biphasic effects. The lowest dose tested (0.03 mg/kg) did not alter the effects of MDMA on operant behavior. At 0.1 mg/kg, (*R*)-nantenine partially blocked the rate suppressant effects of MDMA. The highest two doses tested (0.3 and 1.0 mg/kg) completely blocked the effects of MDMA on operant responding.

(±)-Nantenine, was evaluated for affinity at several human CNS receptors using the services of the NIH/NIMH Psychoactive Drug Screening Program. Experimental details for these assays may be found at the PDSP website (http://pdsp.med.unc.edu/). The data (Table 1) indicates that (±)-nantenine is a very selective α_{1A} ligand. Based on the observed selectivity it is plausible that this receptor plays a role in the anti-MDMA effects of the enantiomers. Another significant finding from this study is that opioid receptors (DOR, MOR, KOR), β-adrenergic receptors, or dopamine and norepinephrine transporters (DAT and NET) do not seem to be responsible for nantenine's anti-MDMA effects given the low affinity at these receptors. (However, the contribution of multi-receptor effects cannot be ruled out.) The involvement of α_1 adrenergic receptors in the effects of MDMA has become more prominent in the literature recently; α_1 antagonists have been reported to reverse or attenuate MDMA-induced hyperthermia and locomotor activity in rodent models.[36–39]

In conclusion, the PIFA-mediated oxidative biaryl coupling procedure for the synthesis of (±)-nantenine is low-yielding with a variety of conditions investigated. (±)-Nantenine was found to have high affinity and selectivity for the α_{1A} adrenoceptor, providing indications that this receptor plays a role in the observed anti-MDMA effects of the nantenine enantiomers. Both (*R*)- and (*S*)-nantenine (0.3 mg/kg ip) completely blocked behavioral suppression induced by MDMA. In the context of antagonism of MDMA's behavioral and physiological effects, the importance of the chiral center of nantenine towards its in vitro and in vivo pharmacological characteristics is deserving of further investigation. Such studies are ongoing in our laboratories and will be reported in due course.

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