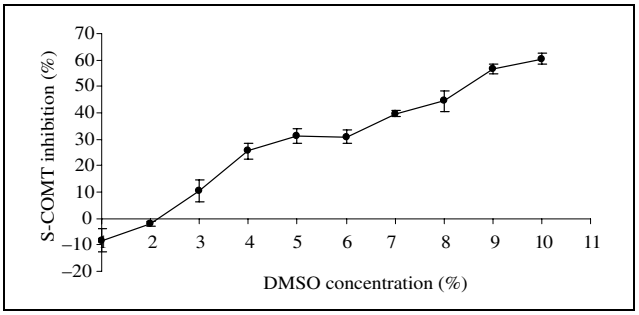


Fig.: DMSO tolerance of the S-COMT assay. The columns represent % inhibitions observed with increasing DMSO concentrations (mean ± SD, n = 3)

compounds. This seemed to interfere with the assay detection and is in fact seen as one of the limiting factor in high throughput screening concerning fluorescence-based assays (Comley 2003). Secondly, the assay was noted to tolerate a limited concentration of DMSO which is the universal solvent in dissolving library compounds. For example, the DMSO concentration of 5% resulted in 31% inhibition of S-COMT (Fig.). With very poorly soluble library compounds the limited DMSO tolerance of the assay may complicate the screening at high concentrations. In this study, the final DMSO concentration used was therefore kept at 1%.

In conclusion, this study shortly describes an approach by which the time and cost-efficiency of an *in vitro* screening process can be improved by a preceding *in silico* screening procedure. Here, the number of compounds screened *in vitro* was reduced from thousands to 49 using virtual screening, resulting in notable savings in time and money. After *in vitro* screening, two inhibitors were identified as reasonable drug candidates for further evaluation in cell-based assays and for chemical optimisation.

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## LE300 – New results on its ability to antagonize the discriminative stimulus effects of cocaine

**Dopamine/serotonin receptor antagonists, part XI**

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LE300, i.e. 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-benz[*d*]indolo[2,3-*g*]azecine, with nanomolar affinities to the $hD_1$ receptor family, suppresses *in vivo* spontanous locomotor activity and attenuates locomotor activity induced by cocaine. Therefore in this study, LE300 was investigated for its ability to antagonize cocaine's discriminative effects. LE300 was tested in doses from 0.5 to 10.0 mg/kg and partially antagonized the discriminative stimulus effects produced by 10 mg/kg of cocaine in rats. The partial antagonism (39% drug-appropriate responding) occurred following 5 mg/kg LE300. Response rate was decreased following 5 and 10 mg/kg, with the maximum effect (27% of cocaine control) following 10 mg/kg LE300.

The compound LE300 (Witt et al. 2000), which is 7-methyl-6,7,8,9,14,15-hexahydro-5*H*-benz[*d*]indolo[2,3-*g*]-azecine (Fig. 1), has been characterized both by radioligand binding assays and functional testings using cAMP and $[Ca^{2+}]$ as a very potent antagonist for the human dopamine receptors ($D_1$, $D_{2L}$, $D_{4.4}$ and $D_5$) with nanomolar affinities and a 10- to 20-fold selectivity for $D_1$ over $D_{2L}$ (Kassack et al. 2002): $K_i(D_1) = 1.9$ nM, $K_i(D_{2L}) = 44.7$ nM, $K_i(D_{4.4}) = 109$ nM, $K_i(D_5) = 7.5$ nM. LE300 served as a lead for numerous analogs which are highly potent and show selectivity towards the dopamine receptor subtypes, some of them show selectivity even within the $D_1$-subtype family (Wittig et al. 2004; Decker and Lehmann 2003). Moderate affinity at the $D_3$ receptor was determined ($K_i(D_3) = 86.9$ nM), nanomolar affinities were found at the 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors ($K_i$(5-$HT_{2A}$) = 11.9 nM; $K_i$(5-$HT_{2C}$) = 36.1 nM), micromolar affinity at the 5-$HT_{1A}$ receptor ($K_i$(5-$HT_{1A}$) = 1.2 μM) using binding assays (Decker et al. 2004). Functional studies indicate moderate antagonist activity at the 5-$HT_{2A}$ site ($K_e = 86.9$ nM; $pA_2 = 8.35$ nM). No activity was found at dopamine, serotonin and norepinephrine transporters. These results suggested the use of LE300 for cocaine addiction treatment (Piercy et al. 1992; Ciccocioppo et al. 2001). High activities were found *in vivo*: LE300 suppressed spontaneous locomotor activity with an $ID_{50}$ of 1.24 mg/kg and attenuated locomotor activity induced by 20 mg/kg cocaine with $AD_{50}$ of 1.50 mg/kg (Decker et al. 2004). LE300 was also tested for substitution for the dis-

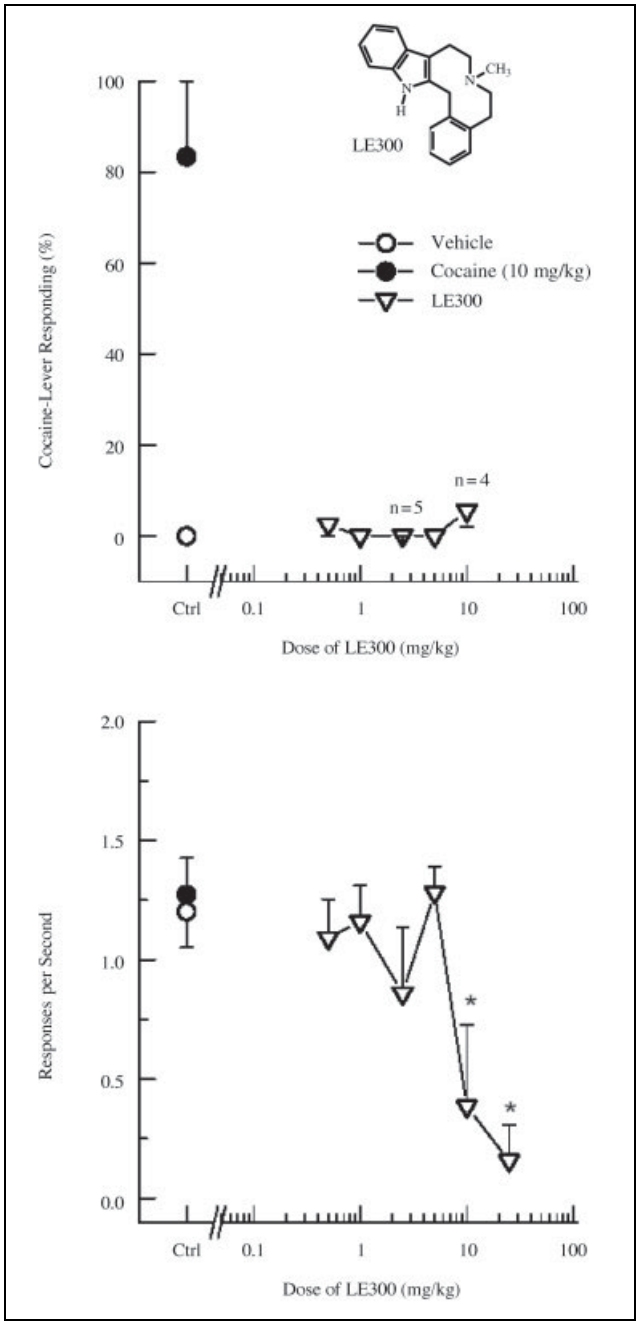


Fig. 1: *Total Session*: The upper panel shows the mean (±SEM) percentage of responses emitted on the cocaine-appropriate lever during the total session as a function of dose, for doses with three or more rats completing the first fixed ratio. The lower panel shows the mean response rate (±SEM) as a function of dose for all subjects tested. The sample size is equal to six at all data points except where noted. To the left of the axis break, control (Ctrl) data are shown for the vehicle (2% methylcellulose) and for the training dose of cocaine (10 mg/kg). Data for the antagonism study of LE300 for the training dose of cocaine are shown to the right of the axis break
* $p < 0.05$ compared with cocaine control

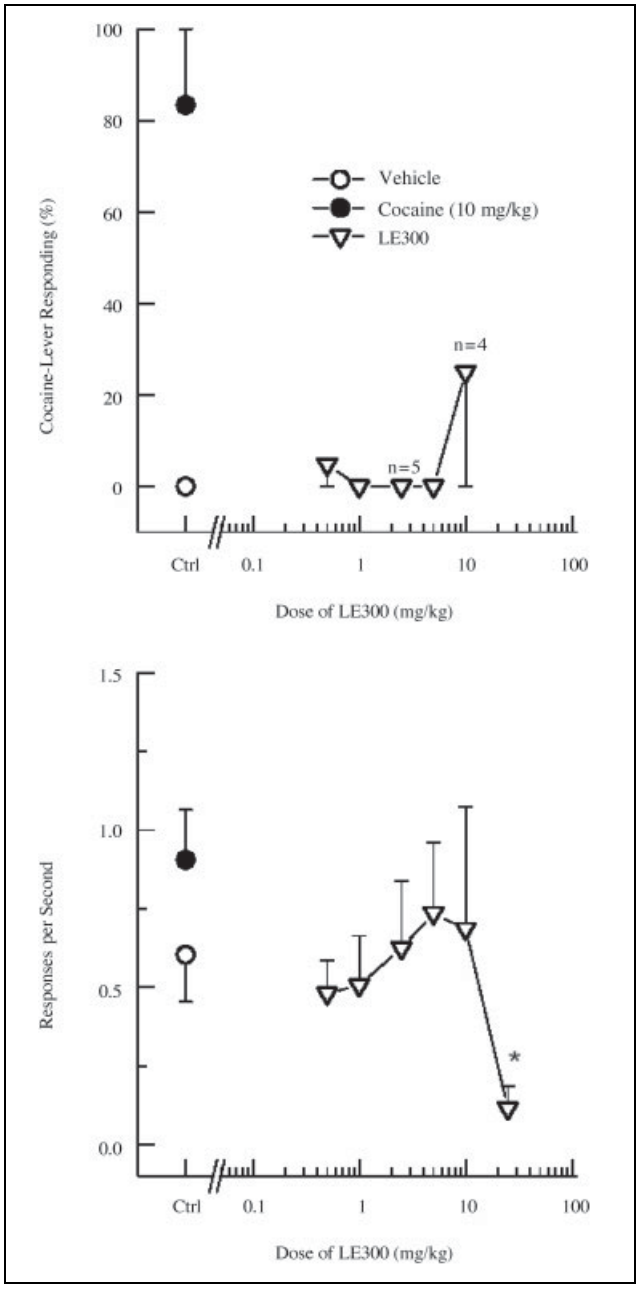


Fig. 2: *First Reinforcer:* The upper panel shows the mean (±SEM) percentage of responses emitted on the cocaine-appropriate lever for the first reinforcer as a function of dose, for doses with three or more rats completing the first fixed ratio. The lower panel shows the mean response rate (±SEM) as a function of dose for all subjects tested. The sample size is equal to six at all data points except where noted. To the left of the axis break, control (Ctrl) data are shown for the vehicle (2% methylcellulose) and for the training dose of cocaine (10 mg/kg). Data for the antagonism study of LE300 on the training dose of cocaine are shown to the right of the axis break
* $p < 0.05$ compared with cocaine control

criminative stimulus effects produced by cocaine; in this assay it did not generalize to cocaine (Decker et al. 2004), this means that LE300 does not produce cocaine-like effects in rats.

For further investigating these effects of LE300, it was tested for its ability to block the discriminative stimulus effects of cocaine (10 mg/kg) in rats.

*Total session*. LE300 partially antagonized the discriminative stimulus effects produced by 10 mg/kg cocaine. The partial antagonism (39% drug-appropriate responding) occurred following 5 mg/kg LE300. Response rate was decreased following 5 and 10 mg/kg, with the maximum effect (27% of cocaine control) following 10 mg/kg LE300. A one-way, repeated measures analysis of variance conducted on response rate for the total session indicated a significant overall effect $F(5.25) = 5.30$, $p = 0.002$; planned comparisons (a priori contrast) against the cocaine control indicated a significant difference for the 5 and 10 mg/kg doses, respectively (all $ps < 0.05$ denoted in Fig. 1 with an asterisk).

*First reinforcer.* When the first reinforcer measure was considered, LE300 failed to antagonize the discriminative stimulus effects produced by 10 mg/kg cocaine. For this measure, the minimum drug-appropriate responding was 65% at 5 mg/kg. The discrepancy between the total sessions and first reinforcer measures for the 5 mg/kg dose was the result of a shift from drug- to saline-appropriate responding by one rat after completion of the first fixed ratio. Response rate was decreased to 33% of cocaine control following 10 mg/kg of LE300. A one-way, repeated measures analysis of variance conducted on response rate for the first reinforcer failed to indicate a significant overall effect $F(5.25) = 1.83$, $p = 0.143$; planned comparisons (a priori contrast) against the cocaine control indicated a significant difference for the 10 mg/kg doses (all ps $< 0.05$ denoted in Fig. 2 with an asterisk).

*Other observations*. Four of six rats failed to complete the first fixed ratio when tested following 10 mg/kg LE300. No unusual effects were observed following any dose of LE300.

In principle, LE300 seems to be a suitable compound for the treatment of cocaine addiction, because it partially antagonizes the discriminative stimulus effects of cocaine. A drawback might be the fact that it also affects overall rates of responding. Out of this reason, promising analogs of LE300, which might show an improved profile, are currently evaluated pharmacologically for their activity.

## Experimental

Experiments were conducted according to the standard operating protocol for Cocaine Treatment Discovery Program (CTDP) drug discrimination testing in rats of NIH, National Institute on Drug Abuse, Bethesda, Maryland (USA).

Six male Sprague-Dawley rats were trained to discriminate cocaine (10 mg/kg) from saline using a two-lever choice methodology. Food was available as a reinforcer under a fixed ratio 10 schedule when responding occurred on the injection appropriate lever. All tests occurred in standard, commercially available chambers (Coulbourn Instruments), using 45 mg food pellets (Bioserve) as reinforcers.

Training sessions occurred in a double alternating fashion, and tests were conducted between pairs of identical training sessions (i.e., between either two saline or two cocaine training sessions). Tests occurred only if, in the two preceding training sessions, subjects met the criteria of emitting 85% of responses on the injection correct lever for both the first reinforcer (first fixed ratio) and the total session. Test sessions lasted for 20 min, or until twenty reinforcers had been obtained. Doses of the test compound for which fewer than three rats completed the first fixed ratio were not considered in the characterization of discriminative stimulus effects.

Intraperitoneal injections (1 ml/kg) of LE300, or its vehicle (2% methylcellulose), occurred 30 min prior to the start of the test session. Intraperitoneal injections of the training dose of cocaine occurred 10 min prior to the start of the test session. A starting dose for LE 300 of 1 mg/kg was determined based upon data previously determined (Decker et al. 2004), and a dose range of 0.5 to 10.0 mg/kg was examined. This range included doses that were inactive to those that had biological activity as evidenced by partial antagonism and a decrease in response rate to 27% of cocaine control.

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