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## **N-Substituted-3-alkoxy-derivatives of dextromethorphan are functional NMDA receptor antagonists in vivo: Evidence from an NMDA-induced seizure model in rats**

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### **Abstract**

Interest in developing minimal side-effect NMDA receptor antagonists for neurological and psychiatric disorders has been energized by the recent introduction of esketamine into clinical practice for treatment-resistant depression. Structural analogs of dextromethorphan bind with low affinity to the NMDA receptor ion channel, have functional effects in vivo, and generally display a lower propensity for side-effects than that of ketamine and other higher affinity antagonists. As such, the aim of the present study was to determine whether a series of N-substituted-3-alkoxy-substituted dextromethorphan analogs produce their effects in vivo through blockade of NMDA receptors. Compounds were studied against NMDA-induced seizures in rats. Compounds were administered intracerebroventricularly in order to mitigate confounds of drug metabolism that arise from systemic administration. Comparison of the anticonvulsant potencies to their affinities for NMDA,  $\sigma 1$ , and  $\sigma 2$  binding sites were made in order to help define the receptor population(s) governing NMDA antagonist efficacy. The potencies to block convulsions were positively associated with their affinities to bind to the NMDA receptor ion channel ( $[^3\text{H}]\text{-TCP}$  binding) ( $r = 0.71$ ,  $p < 0.05$ ) but not to  $\sigma 1$  receptors ( $[^3\text{H}]\text{-SKF 10047}$  binding) ( $r = -0.31$ ,  $p = 0.46$ ) or to  $\sigma 2$  receptors ( $[^3\text{H}]\text{-DTG}$  binding) ( $r = -0.38$ ,  $p = 0.36$ ). This is the first report demonstrating that these dextromethorphan analogs are functional NMDA receptor antagonists in

vivo. Given their potential therapeutic utility and favorable side-effect profiles, such low affinity NMDA receptor antagonists could be considered for further development in neurological (e.g., anticonvulsant) and psychiatric (e.g., antidepressant) disorders.

## Keywords

Dextromethorphan analogs; NMDA seizures; NMDA receptors; Sigma receptors

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N-Methyl-D-Aspartate (NMDA) receptors play important roles in the regulation of physiology and behavior. Their functional control in neurological disorders such as epilepsy and stroke, for example, has generated intense efforts to identify NMDA receptor antagonists that might safely modulate these disorders (Ghasemi and Schachter, 2011; Kalia et al., 2008; Wang and Reddy, 2017). Similar discovery efforts were launched for the identification of NMDA receptor antagonists that might function as novel anti-anxiety or antidepressant drugs (Ghasemi et al., 2017; Zanos et al., 2018). NMDA receptors have gained notoriety in the past decade due to their implicated role in major depressive disorder. The pioneering work of Trullas and Skolnick (1990) posited that NMDA receptor antagonism would be antidepressant based upon convergent data from a host of sources. Clinical validation of the Trullas and Skolnick hypothesis was born out ten years later by Berman and colleagues (2000) who reported rapid onset of antidepressant effects of the NMDA receptor antagonist ketamine in treatment-refractory patients. Subsequent systematic replication of this finding (Zarate et al., 2006) led to an explosion in the field of antidepressant drug discovery (Witkin, 2011; Witkin et al., 2018).

Although the efficacy of ketamine in patients for depression and pain (Hewitt, 2000) has been generally acknowledged, the use of ketamine as a therapeutic in these disease states and others such as epilepsy remains constrained by the side-effects and safety issues associated with ketamine. Ketamine produces concomitant motor-impairment and associated motor-excitation, psychotomimetic effects, abuse liability and brain lesions with prolonged use (Iadarola et al., 2015; Kim et al., 2016). Since it is known that blockade of the NMDA receptor ion pore not only drives efficacy but generates side-effects, solutions to the problem of identifying NMDA receptor antagonists without side-effects has focused on compounds binding in sites distinct from the ion channel (uncompetitive antagonists) (e.g., Willetts et al., 1990) and antagonists with low affinity for the binding domain within the ion channel. The rationale behind the later strategy is that low affinity ligands would control ion channel function in a dynamic fashion. With antagonist molecules moving on and off the NMDA receptor rapidly, it was hypothesized that NMDA receptor function could be dampened, but not completely blocked (Parsons et al., 1999a, b; Semenova et al., 1999). Therefore, low affinity channel blockers might create therapeutic advantage with reduced side effect burden. Proof of concept of this approach has been exemplified by a diversity of low affinity NMDA receptor antagonists that have NMDA receptor antagonist properties in vivo but do not fully recapitulate the side-effect profile of higher affinity drugs like phencyclidine, MK-801, or ketamine. For example, dextromethorphan does not produce full motor-impairing effects or the motor stimulation that originates with high affinity channel blockade (Ginski and Witkin, 1999; Zapata et al., 2003). Low affinity channel blockers are also predicted to

lack the psychotomimetic subjective effects that can be produced by high affinity ligands (Geter-Douglass and Witkin, 1999).

Indeed, dextromethorphan is approved for the treatment of pseudobulbar affect (Nguyen et al. 2016b), has been studied in Rett syndrome (Smith-Hicks et al., 2016), in opioid use disorder (Malek et al., 2013; Lee et al., 2015), and has shown efficacy against agitation in Alzheimer's disease (Cummings et al., 2015). Dextromethorphan has also been shown to produce antidepressant-like effects in rodent models that detect antidepressant drugs (Nguyen and Matsumoto, 2015; Nguyen et al., 2014, 2016a, 2017; Sakhaee et al., 2016) and efficacy in patients in a case report (Lauterbach et al., 2016). As an anticonvulsant, dextromethorphan has been studied in epileptic patients and shown efficacy (Wieser and Beck, 1998; Kimiskidis et al., 1999; Chien et al., 2012; see review by Nguyen et al., 2016).

A series of N-substituted-3-hydroxy and 3-alkoxy derivatives of dextromethorphan reported by Newman et al. (1992; 1996) have been characterized as binding with high affinity to  $\sigma 1$  receptors and as relatively low affinity at NMDA receptors, in radiolabeled binding experiments. Dextromethorphan analogs have also demonstrated efficacy against maximal electroshock-induced seizures (Newman et al., 1992, Tortella et al 1994) and against cocaine-induced seizures (Zapata et al., 2003). However, their mechanism of action in vivo has not been previously elucidated. Herein, we tested dextrophan, dextromethorphan, and selected dextromethorphan analogs (Newman et al., 1992, 1996; Zapata et al., 2003) (Fig. 1) in a model of NMDA-induced seizures that detects NMDA receptor antagonists and for which dextromethorphan is active (Ferkany et al., 1988). Variations of this model have been used as an in vivo model to identify NMDA receptor antagonists (Leander et al., 1988). Correlational analysis of the potencies of the compounds to block seizures compared to their binding affinities to NMDA receptors,  $\sigma 1$ , and  $\sigma 2$  receptors was utilized to derive insight into mechanistic processes.

## Methods

### Compounds.

Compounds were synthesized in house (A.H. Newman laboratory, NIDA, Baltimore, MD).

### [<sup>3</sup>H]SKF 10047, [<sup>3</sup>H]TCP, and [<sup>3</sup>H]DTG Binding.

Binding affinities were established by Newman et al. (1996) per methods described therein and are used here to compare potencies to the in vivo anticonvulsant activity of these compounds. In these studies, NMDA receptor affinity was obtained by displacement of [<sup>3</sup>H]TCP binding.  $\sigma 1$  affinity was determined by the displacement of [<sup>3</sup>H]SKF 10047 binding in the presence of MK-801.  $\sigma 2$  binding affinities were obtained by displacement of [<sup>3</sup>H]DTG binding in the presence of (+)-SKF-10047.

### NMDA-induced Seizures.

NMDA convulsions were induced in male Sprague-Dawley rats (200-250 g; Charles River) implanted with an i.c.v. cannula aimed at the right lateral ventricle under surgical anesthesia (70 mg/kg ketamine and 6 mg/kg xylazine, i.m.) Three to five days post-surgery the

animals were divided into groups (n=6 per group) for testing. As described previously (Tortella et al., 1994) a suprathreshold, non-lethal dose of 12.5 nM NMDA was administered i.c.v. to induce the NMDA receptor specific clonic "popcorn" convulsions. Two indices of convulsive activity were measured during a 120 sec observation period: the latency to onset to NMDA convulsions and the number of rats per group exhibiting convulsive behavior (% responding). In all cases, NMDA was injected i.c.v. as a bolus 15 min following the i.c.v. injection of either vehicle or the respective compound. All animals were housed and used in accordance with NIH guidelines for the care and use of experimental animals. In addition, the experiments were conducted under the overview and approval of an animal care and use committee and monitored by veterinary and animal care staff.

Dose-response curves were analyzed using the methods of Litchfield and Wilcoxon (1949) (Tallarida and Murray, 1987). Potencies of the compounds at each receptor were correlated with their potencies to block NMDA-induced seizures using the Pearson correlation coefficient; statistical significance was secondarily evaluated by linear regression (ANOVA). Comparisons of latencies to produce seizures were compared to one another with post-hoc Dunnett's test after statistically-significant ANOVA verification. All statistical evaluations were conducted with a priori value of  $p < 0.05$  being considered significant. Data were analyzed using GraphPad Prism software (ver. 5.02).

## Results

The in vitro affinities of the compounds studied are shown in Table 1. Potencies at NMDA receptors varied over two orders of magnitude from 460 nM for dextropran (DX) to >40  $\mu$ M for AHN 1080. Many of these compounds had higher affinity for  $\sigma$ 1 receptors than for either NMDA or  $\sigma$ 2 receptors including dextromethorphan (8 times higher affinity for  $\sigma$ 1 vs NMDA receptors).

Phencyclidine fully blocked NMDA-induced convulsions ( $ED_{50}=0.7 \mu$ g; 95% confidence limits: 0.5-1.0), as did dextropran (DX) and dextromethorphan (DM) (Table 1; Fig. 2). The analogs of dextromethorphan were anticonvulsant in this model with the exception of AHN 1047 (Table 1; Fig. 2). Latencies to produce convulsions were significantly different from vehicle control latencies ( $F_{8,63} = 30.4$ ,  $p < 0.0001$ ) for all compounds tested. However, AHN-1047 significantly differed in efficacy from dextromethorphan and the other analogues ( $p < 0.001$ ) except for AHN 1080 (Table 1; Fig. 2).

The potencies of these compounds to block NMDA-induced convulsions was positively associated with their affinities for the NMDA receptor ion channel ( $[^3H]$ -TCP binding) ( $r = 0.71$ ,  $p < 0.05$ ) (Fig. 3, top panel). Linear regression analysis showed significant deviation from zero ( $F_{1,6} = 6.26$ ,  $p < 0.05$ ). Elimination of the outlier compound, ANH 1047, did not significantly alter this positive statistical association. In contrast, no significant relationship was observed between anticonvulsant potencies and affinities for  $\sigma$ 1 receptors ( $[^3H]$ -SKF 10047 binding) ( $r = -0.31$ ,  $p > 0.05$ ) (Fig. 3, bottom panel). Linear regression did not detect a significant deviation from zero ( $F_{1,6} = 0.63$ ,  $p = 0.46$ ). Likewise, there was not a statistically-significant relationship between anticonvulsant potencies and  $[^3H]$ DTG binding ( $r = -0.38$ ,  $p > 0.05$ ). Linear regression confirmed this lack of relationship ( $F_{1,6} = 0.99$ ,  $p =$

0.36). However, this analysis for  $\sigma_2$  receptors is not precise as the values for binding were  $>1000$  or  $>10000$  nM for one half of the compounds tested.

## Discussion

A series of N-substituted-3-alkoxy-analogs of dextromethorphan were studied for their ability to attenuate NMDA-induced convulsions in rats. Compounds were given by i.c.v. administration so that the effects of the compounds per se could be evaluated with minimal contamination from their metabolites. For example, dextromethorphan is metabolized to the more potent NMDA receptor antagonist, dexrotrphan when given systemically (Taylor et al., 2016). All compounds blocked NMDA-induced convulsions (with AHN 1047 being an outlier) and antagonist  $ED_{50}$  values positively correlated with their potencies to bind to the NMDA receptor ion channel as measured by [ $^3$ H]-TCP binding.  $ED_{50}$  values did not significantly correlate with the potencies of the compounds to bind to either  $\sigma_1$  or  $\sigma_2$  binding sites.

The pharmacology of dextromethorphan is complex and identification of mechanisms of action have not been straightforward. Work using estimated free brain concentrations of dextromethorphan led to the conclusion that NMDA receptors might be one receptor target in humans (Taylor et al., 2016). In the present study, the findings with NMDA-induced convulsions provides the first in vivo evidence that these dextromethorphan analogs act in rats through the blockade of NMDA receptors. This relationship was not fully predicted. First, Newman et al. (1992, 1996) had shown that these compounds were generally more potent at  $\sigma_1$  receptors than at NMDA receptors. Secondly, compound potencies to block cocaine-induced clonic convulsions in mice were negatively associated with binding to the NMDA receptor ion channel ( $r=-0.79$ ,  $p<0.05$ ) (Zapata et al., 2003). That lower affinity compounds were more potent than higher affinity compounds, is opposite to the present positive association of potency to affinity found here despite prediction that cocaine convulsions and NMDA convulsions would be pharmacologically isomorphic since NMDA receptor antagonists also block convulsions induced by cocaine (Witkin and Tortella, 1991; Witkin et al., 1999).

Nevertheless, the NMDA antagonist activity of the dextromethorphan analogs in vitro suggested that NMDA receptor actions of these compounds might be achieved in vivo. Blockade of NMDA receptors produces antidepressant-like effects in rodent models (Trullas and Skolnick, 1990; Skolnick et al. 2009; Zanos et al., 2018). Some of these compounds, ketamine in particular, have likewise shown rapid-acting antidepressant effects in depressed patients and in patients that have been unresponsive to standard-of-care medicines (Witkin et al., 2018). Esketamine ((*S*)-ketamine) has recently been approved by the U.S. FDA for treatment of major depressive disorder (February, 2019). However, ketamine and high affinity NMDA receptor ligands have side-effect and safety issues that may limit their therapeutic utility. Therefore, safer alternatives are needed.

Dextromethorphan produces antidepressant-like effects in rodent models (Nguyen and Matsumoto, 2015; Nguyen et al., 2014, 2016a, 2017; Sakhaee et al., 2016) and efficacy in patients in a case report (Lauterbach et al., 2016; see Nguyen et al., 2016 for a

review). These effects have been attributed to blockade of NMDA receptors (Sakhaee et al., 2016) as well as to  $\sigma 1$  receptors (Nguyen et al., 2014). Based upon the functional activity of the structural analogs of dextromethorphan at NMDA receptors in vivo, it is predicted that they would also generate antidepressant-like phenotypes in rodents. Based upon the preclinical and clinical data, it is also predicted that these compounds would not produce side-effects in humans like those of higher affinity NMDA receptor antagonists like ketamine, phencyclidine, or MK-801. Preclinically, the dextromethorphan analogs did not produce complete motor-impairment or psychomotor stimulation like that of higher affinity ligands including dextrophan (Zapata et al., 2003). Clinical data with lower affinity NMDA receptor antagonists, like memantine, have also suggested that lower affinity ligands might circumvent the undesirable side-effect profiles of high affinity ion channel blockade (Parsons et al., 1999a). Dextromethorphan has demonstrated rapid response efficacy in a treatment-resistant patient (Lauterbach, 2016) and by retrospective analysis in bipolar II patients (Kelly & Lieberman, 2014).

In conclusion, we have identified an NMDA receptor antagonist mechanism of action of a series of N- and 3-alkoxy-substituted dextromethorphan analogs that may constitute a new inroad into the development of a pharmacologically safer way of transducing the medically beneficial effects of NMDA receptor antagonism. The safety and efficacy of dextromethorphan has already been exemplified by its approved use in combination with quinidine (Nuedexta) for the treatment of pseudobulbar affect in multiple sclerosis and amyotrophic lateral sclerosis (Pioro et al., 2010). Low affinity NMDA receptor antagonists, exemplified by this set of dextromethorphan analogues, may have as of yet unexploited therapeutic benefit in neurological and psychiatric disorders such as epilepsy and major depressive disorder. The 'opioid crisis' (Coussens et al., 2019; Stoica et al., 2019) is another area of potential impact for dextromethorphan analog-based NMDA receptor antagonists. Tolerance to the analgesic effects of opioids is one of reason for opioid dose escalation and the negative health consequences of opioid dose increases (e.g., dependence, respiratory depression; (Jaffe and Martin, 1985)). NMDA receptors provide a primary control mechanism for regulating tolerance development and dextromethorphan along with other NMDA receptor antagonists have been under consideration for this effect (Herman et al., 1995).

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## Non-standard Abbreviations

<b>DTG</b>	1,3-Di-o-tolylguanidine
<b>DM</b>	dextromethorphan

<b>DX</b>	dextrorphan
<b>SKF 10047</b>	N-allylnormetazocine
<b>PCP</b>	phencyclidine
<b>TCP</b>	tenocyclidine

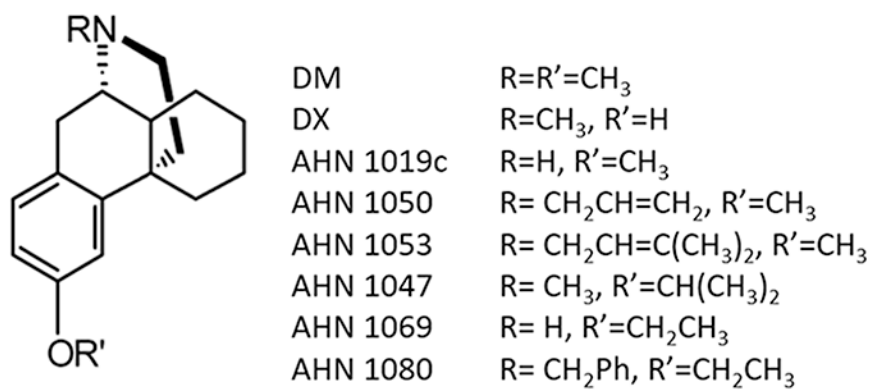
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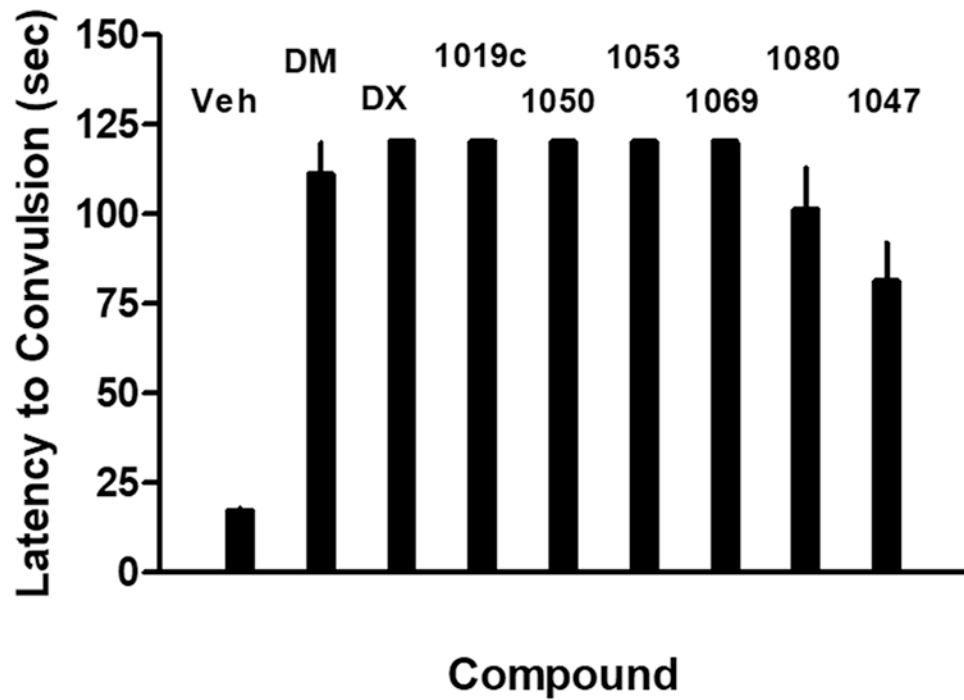
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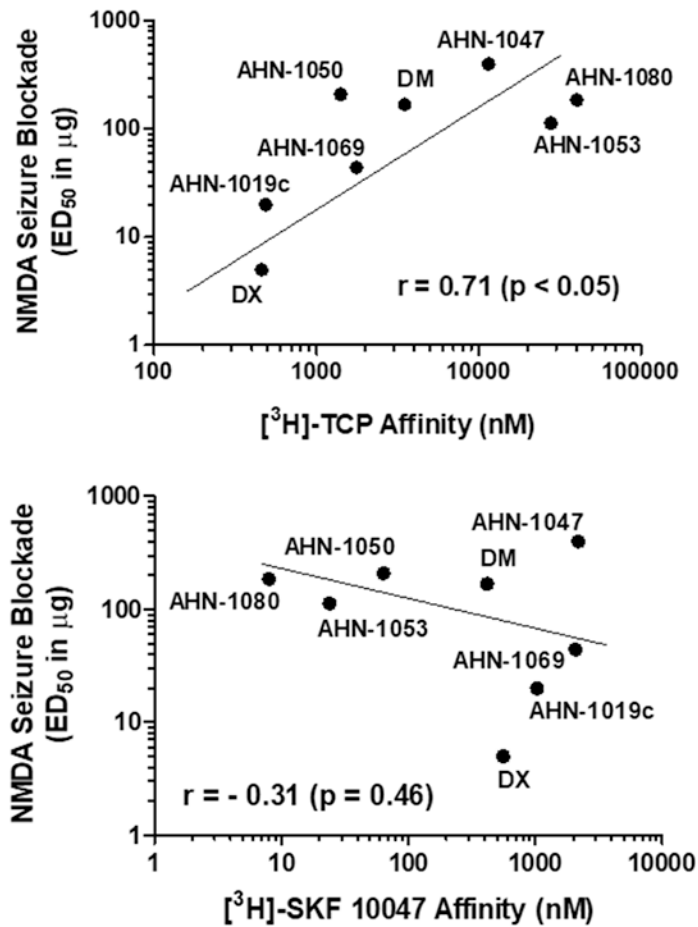
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**Figure 1.**  
Structures of the dextromethorphan analogs studied.



**Figure 2.** Latency to NMDA-induced convulsions in rats in the presence of vehicle or each of the dextromethorphan analogs studied. Each bar represents the mean + S.E.M. for 8 rats each. All compounds displayed higher latencies than vehicle ( $p < 0.001$ ). AHN 1047 differed significantly ( $p < 0.001$ ) from all other compounds except AHN 1080.



**Figure 3.** Relationship of NMDA ion channel (top panel) or  $\sigma$ 1 receptor (bottom panel) binding affinities to anticonvulsant potency. Each point represents the mean value for each of the dextromethorphan analogs studied as shown also in Table 1. Data for  $\sigma$ 2 binding are not plotted since half of the compounds studied had affinities designated as >1000 or > 10000 nM (Table 1).

**Table 1.**

Affinities of dextromethorphan (DM), dextrorphan (DX) and analogs at NMDA,  $\sigma$ 1, and  $\sigma$ 2 binding sites and their potencies to block NMDA-induced convulsions.

Compound	NMDA Receptor $K_i$ (nM)	$\sigma$ 1 Receptor $K_i$ (nM)	$\sigma$ 2 Receptor $K_i$ (nM)	NMDA-Induced Seizures $ED_{50}$ (95% confidence limit) ( $\mu$ g)
DX	460	559	1128	5 (2-11)
AHN 1019c	487	1034	>10,000	20 (11-36)
AHN 1050	1410	64	>1000	210 (171-258)
AHN 1069	1770	2078	>10,000	44 (25-76)
DM	3500	419	2639	168 (125-226)
AHN 1047	11,500	2181	>1000	>400
AHN 1053	27,800	24	948	113 (72-177)
AHN 1080	40,200	8	1050	186 (125-277)

Compounds are displayed in order of their NMDA receptor affinity. NMDA receptor affinity was obtained by displacement of [ $^3$ H]TCP binding.  $\sigma$ 1 receptor affinity was determined by the displacement of [ $^3$ H]SKF 10047 binding in the presence of MK-801.  $\sigma$ 2 receptor binding affinities were obtained by displacement of [ $^3$ H]DTG binding in the presence of (+)-SKF-10047. Binding data are from Newman, et al. (1996).