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# Structure–activity comparison of YZ-069, a novel σ ligand, and four analogs in receptor binding and behavioral studies

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**Abstract**

Earlier studies show that antagonism of σ receptors using high to moderate affinity compounds or antisense oligodeoxynucleotides targeting the $\sigma_1$ subtype significantly attenuates the behavioral effects of cocaine in mice. In this study, the novel σ receptor ligand YZ-069 [*N*-phenylpropyl-*N*′ -(3,4-dichlorophenethyl)piperazine] and four analogs (representing nitrophenyl and methoxyphenyl derivatives) were evaluated in receptor binding and behavioral studies to further delineate structural features that convey favorable anticocaine actions. In receptor binding studies, all of the compounds had low nanomolar affinities for $\sigma_1$ and $\sigma_2$ receptors but only micromolar affinities for monoamine transporters. Consistent with the favorable affinities of the compounds for σ receptors, they also significantly attenuated cocaine-induced convulsions in mice. The compounds with the 3,4-dichlorophenyl and methoxyphenyl substitutions provided better protection against cocaine-induced convulsions than the nitrophenyl derivative. This is consistent with the reduced lipophilicity of the nitro substitution, which would reduce its ability to cross the blood–brain barrier. The position of the substituent on the phenyl ring had no significant effect on binding affinity or behavioral protective actions. Together with earlier studies, the data suggest that favorable features of σ receptor ligands with anticocaine actions include high affinity for brain σ receptors, antagonistic actions at the receptor, and lipophilicity to facilitate crossing the blood–brain barrier.


*Keywords:* Cocaine; σ Receptor; Convulsions

## 1. Introduction

Significant densities of σ receptors are present in the brain and heart (Matsumoto et al., 2002; Novakova et al., 1995), where they may subserve some of the toxic effects of cocaine. The ability of cocaine to bind to σ receptors at concentrations that can be achieved in vivo (Sharkey et al., 1988; Spiehler and Reed, 1985) further supports the relevance of these interactions. Moreover, recent studies demonstrate that interfering with cocaine's access to σ receptors, using either pharmacological antagonists or antisense oligodeoxynucleotides, significantly reduces cocaine-induced behavioral toxicity (Matsumoto et al., 2001a,b,c, 2002; McCracken et al., 1999a; Skuza, 1999).

The idea that σ receptors are involved in cocaine's behavioral actions has gained increasing acceptance in recent years (Maurice et al., 2002) and these receptors appear to represent viable medication development targets for cocaine abuse (Matsumoto et al., 2003). There are two established σ receptor subtypes (i.e., $\sigma_1$ and $\sigma_2$), which can be distinguished based on their molecular sizes and drug selectivity patterns. The $\sigma_1$ subtype is thought to be a 25–29 kDa protein, while the $\sigma_2$ receptor is thought to be slightly smaller at 18–22 kDa and perhaps existing as a heterodimer (Hellewell and Bowen, 1990; Hellewell et al., 1994; Kavanaugh et al., 1988; Mei and Pasternak, 2001; Moebius et al., 1993a,b, 1996; Wilke et al., 1999). Cocaine interacts with both subtypes but has about a 10-fold better affinity for $\sigma_1$ receptors as compared with $\sigma_2$ receptors (Matsumoto et al., 2002). Both subtypes are found in the rodent brain (Bouchard and Quirion, 1997), but almost all of the σ receptors in the heart are of the $\sigma_1$ subtype (Novakova et al., 1995). Only the $\sigma_1$ receptor has thus far been cloned. It has been

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demonstrated to have high homology in the mouse brain, rat brain, guinea pig liver, and human placenta, with an amino acid sequence that is unlike any other mammalian protein (Hanner et al., 1996; Mei and Pasternak, 2001; Pan et al., 1998; Seth et al., 1997, 1998).

Recently, a large number of σ receptor ligands with anticocaine actions have been described. Many of these compounds belong to an aryl ethylenediamine synthetic series, representing analogs of the compound BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine; De Costa et al., 1992a,b; Zhang et al., 1996). However, the structural requirements of compounds that produce these anticocaine actions are still poorly understood. As part of an earlier structure–activity relationship study on this aryl ethylenediamine synthetic series, we described the ability of aryl monosubstituted analogs of BD1008 to attenuate cocaine-induced convulsions, lethality, and locomotor activity (Matsumoto et al., 2002). We examined the effects of nitro and methoxy aryl monosubstitutions on σ binding affinity and behavioral protection against cocaine. In addition, the influence of the position of the aryl monosubstitution was examined. The results of this earlier study revealed that compounds with high to moderate affinity for σ receptors attenuate cocaine-induced convulsions in mice (Matsumoto et al., 2002). Furthermore, the position of the substitution did not appear to affect binding or behavioral protection against cocaine-induced convulsions (Matsumoto et al., 2002). However, relative to their σ binding affinities, compounds with the nitro substitution required higher doses to produce behavioral protective effects than the methoxy-substituted compounds (Matsumoto et al., 2002). This difference was attributed to the reduced lipophilicity of the nitro substitution, which would be expected to result in poorer transport into the central nervous system and reduced ability to block the convulsions induced by cocaine.

The present study was thus undertaken to validate the structure–activity relationships that were observed in the aryl ethylenediamine synthetic series. YZ-069 [*N*-phenylpropyl-*N*′ -(3,4-dichlorophenethyl)piperazine] is a novel σ ligand from a *N*,*N*-disubstituted piperazine series that was synthesized as a continuation of the structure–activity characterization of the earlier aryl ethylenediamine series (Zhang et al., 1996, 1998). The structures of the compounds tested herein are depicted in Fig. 1. The effects of changing the 3,4-dichlorophenyl substituent on YZ-069 to a *m*-methoxyphenyl or *m*-nitrophenyl substitution, formingYZ-185 [*N*-phenylpropyl-*N*′ -(3-methoxyphenethyl)piperazine] and YZ-231 [*N*-phenylpropyl-*N*′ -(3-nitrophenethyl)piperazine], respectively, were investigated in receptor binding and behavioral studies to determine whether the nitro substituent would again convey a reduced behavioral protective action, relative to the other two substituents (3,4-dichlorophenyl and methoxyphenyl) in the piperazine series as it did in the aryl ethylenediamine series. Furthermore, the

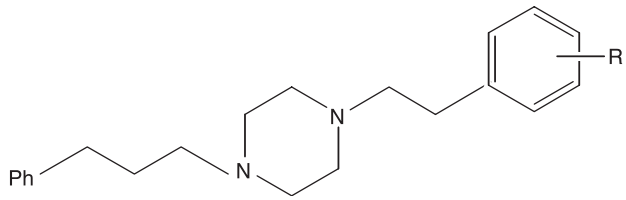


Fig. 1. Structures of the compounds. Compounds and corresponding substitutions at the *R* position: YZ-069 3,4-dichloro; YZ-067 *p*-$OCH_3$; YZ-184 *o*-$OCH_3$; YZ-185 *m*-$OCH_3$; YZ-231 *m*-$NO_2$.

effect of changing the position of the methoxy substitution on the phenyl ring was also investigated with a comparison of the effects of YZ-185 (*m*-substituted) with YZ-184 (*o*-substituted) and YZ-067 (*p*-substituted).

## 2. Methods

### 2.1. Animals

Male, Sprague–Dawley rats (150–200 g; Charles River, Boston, MA or Harlan, Indianapolis, IN) and frozen guinea pig brains (Pel-Freeze, Rogers, AR) were used for the receptor binding studies. Male, Swiss Webster mice (24–32 g; Harlan) were used for the behavioral studies and all in vivo evaluations were performed between 13:00 and 17:00 h. Before use, all animals were housed in groups with a 12:12 h light/dark cycle and ad libitum food and water. All procedures were performed as approved by the Institutional Animal Care and Use Committees where each experiment was conducted.

### 2.2. Drugs

YZ-069 and its analogs were synthesized as described previously (Zhang et al., 1998). Cocaine hydrochloride was obtained from Sigma (St. Louis, MO). Dextrallorphan was supplied by Dr. F.I. Carroll (Research Triangle Institute, Research Triangle Park, NC). The radioligands were purchased from NEN Life Sciences (Boston, MA). All other chemicals that were used for the receptor binding assays were obtained from standard commercial sources (Sigma-Aldrich, St. Louis, MO).

### 2.3. Receptor binding assays

The affinities of the ligands for σ receptors were determined in tissues enriched in each of the respective subtypes using methods described previously in detail (Bowen et al., 1993; Matsumoto et al., 1995; Zhang et al., 1998). Briefly, $\sigma_1$ receptors were labeled in homogenates from guinea pig brains minus cerebellum using 5 nM [$^3$H](+)-pentazocine. $\sigma_2$ Receptors were labeled in homogenates from rat livers with 3 nM [$^3$H]di-*o*-tolylguanidine ([$^3$H]DTG) in the presence of 1 μM dextrallorphan to mask $\sigma_1$ sites. Nonspecific

binding for both $\sigma_1$ and $\sigma_2$ assays was determined in the presence of 10 μM haloperidol. Liver homogenates were used for the $\sigma_2$ receptor assays because they are enriched in this subtype. Earlier studies demonstrated that $\sigma_2$ receptors in the liver and brain exhibit similar signature profiles for this subtype, with high affinity for haloperidol, and enantioselectivity for (−)-benzomorphans over the corresponding (+)-isomer (Bowen et al., 1993; Hellewell et al., 1994). Therefore, use of livers instead of brains to measure $\sigma_2$ binding is expected to yield a comparable pattern of results while taking advantage of the larger numbers of $\sigma_2$ receptors in the liver.

In addition, because cocaine has significant affinities for monoamine transporters, the affinities of the ligands for these sites were also determined using methods described previously in detail (Boja et al., 1994; Matsumoto et al., 2001a, 2002). Briefly, dopamine transporters were labeled in rat striata with 0.5 nM [$^3$H]WIN35,428; nonspecific binding was determined in the presence of 50 μM cocaine. Serotonin transporters were labeled in rat brainstem with 0.2 nM [$^3$H]paroxetine; nonspecific binding was determined in the presence of 1.5 μM imipramine. Norepinephrine transporters were labeled in rat cerebral cortex with 0.5 nM [$^3$H]nisoxetine; nonspecific binding was determined in the presence of 4 μM desipramine.

### 2.4. Cocaine-induced convulsions

Mice were injected intraperitoneally (i.p.) with one of the following treatments: saline ($n=10$), YZ-069 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg; $n=50$), YZ-231 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg; $n=55$), YZ-185 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg; $n=50$), YZ-184 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg; $n=50$), or YZ-067 (0.01, 0.05, 0.1, 0.5, and 1 mg/kg; $n=53$). After 15 min, the mice were challenged with a dose of cocaine (60 mg/kg i.p.) that reliably produces convulsions in 100% of our animals (Brackett et al., 2000; Matsumoto et al., 2001a,b,c, 2002; McCracken et al., 1999a). Following the injection with cocaine, each mouse

Table 1
Receptor binding affinities (in nM) for YZ-069 and analogs

| | $\sigma_1$ | $\sigma_2$ |
|---|---|---|
| YZ-069 | 2.2 ± 0.2 | 38.8 ± 1.6 |
| YZ-231 | 0.7 ± 0.1 | 9.4 ± 1.5 |
| YZ-185 | 1.4 ± 0.2 | 10.2 ± 0.5 |
| YZ-184 | 3.9 ± 0.6 | 7.5 ± 0.2 |
| YZ-067 | 1.3 ± 0.3 | 28.6 ± 1.9 |

| | Dopamine transporter | Serotonin transporter | Norepinephrine transporter |
|---|---|---|---|
| YZ-069 | 8196 ± 1005 | 2865 ± 484 | 15,245 ± 275 |
| YZ-231 | 1009 ± 64 | 247 ± 2 | 744 ± 52 |
| YZ-185 | 1408 ± 164 | 985 ± 126 | 3160 ± 611 |
| YZ-067 | 1466 ± 19 | 1726 ± 57 | 2279 ± 263 |

Values represent $K_i$ values (means ± S.E.M.s) from two or more assays, each performed in triplicate. The σ binding affinities have been reported previously by Zhang et al., (1998). YZ-184, the *o*-methoxyphenyl-substituted compound, was not tested against the monoamine transporters because of its limited quantities.

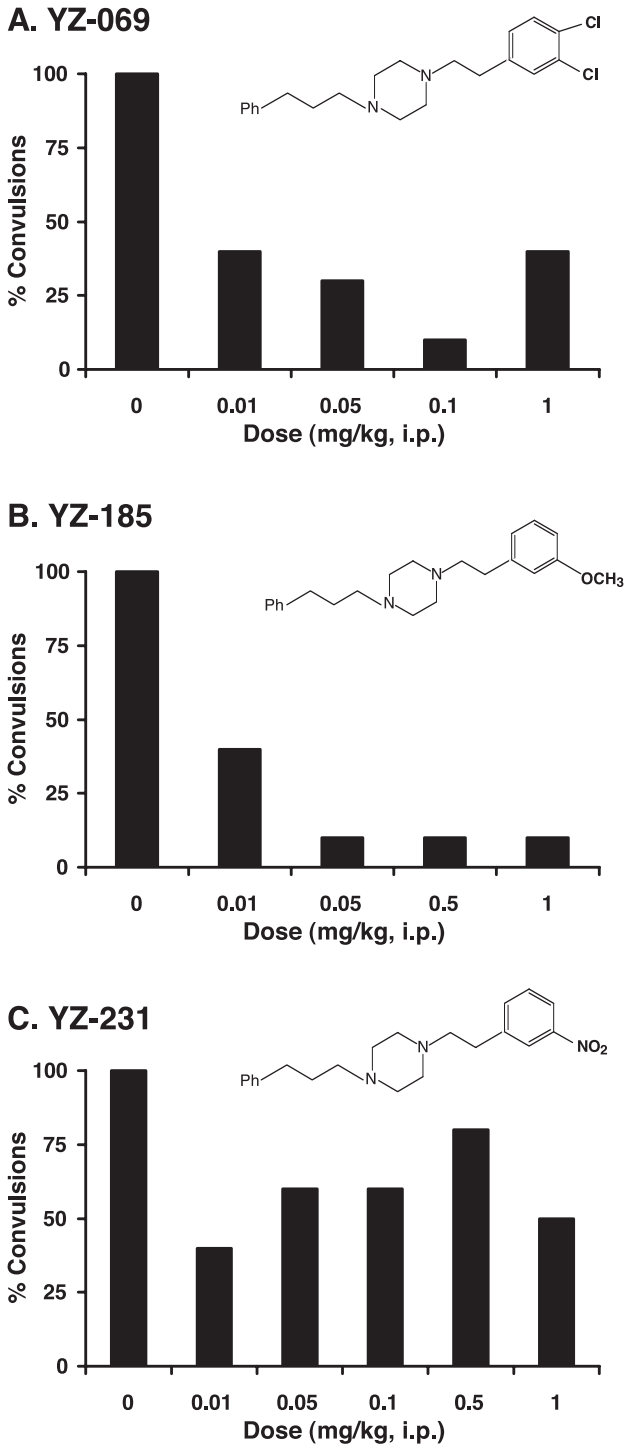


Fig. 2. Effect of phenyl substitution on cocaine-induced convulsions in mice. Mice were pretreated with (A) YZ-069, (B) YZ-185, or (C) YZ-231 (0–1 mg/kg i.p.) followed 15 min later with a convulsive dose of cocaine (60 mg/kg i.p.). Data represent the number of mice convulsing during the 30 min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's Exact Tests, $P<.05$).

was placed in a plastic observation chamber (56 × 38 cm) and the onset of convulsions during the 30 min testing session was recorded. Convulsions were operationally defined as the loss of righting reflexes for at least 5 s combined with the presence of clonic limb movements.

### 2.5. Data analysis

Data from the receptor binding studies were evaluated using GraphPad Prism (San Diego, CA) to calculate $IC_{50}$ values. Apparent $K_i$ values were then calculated using the Cheng–Prusoff equation and $K_d$ values that were determined in earlier saturation assays. Data from the behavioral studies were analyzed using Fisher's Exact Tests (InStat, San Diego, CA).

## 3. Results

### 3.1. Receptor binding assays

All of the compounds tested herein had high affinities for $\sigma_1$ and $\sigma_2$ receptors (Table 1). In contrast to the nanomolar affinities of the compounds for σ receptors, they had micromolar affinities for monoamine transporters (Table 1).

### 3.2. Effect of changing phenyl substituent on cocaine-induced convulsions

The effect of type of phenyl substituent was determined by comparing 3,4-dichloro (YZ-069), *m*-methoxy (YZ-185), and *m*-nitro (YZ-231) substituents. YZ-069, which has a 3,4-dichlorophenyl substitution, significantly attenuated cocaine-induced convulsions at low doses (Fig. 2). The magnitude of the protective effect of YZ-069 was statistically significant at doses of 0.01, 0.05, 0.1, and 1 mg/kg ($P < .05$).

Similar to YZ-069, the methoxyphenyl substitution of YZ-185 was associated with strong protective actions (Fig. 2). YZ-185 significantly antagonized cocaine-induced convulsions at doses of 0.01, 0.05, 0.5, and 1 mg/kg ($P < .05$).

In contrast, the nitrophenyl substitution of YZ-231 was associated with decreased protective actions against the convulsive effects of cocaine when compared with YZ-069 and YZ-185 (Fig. 2). The protective effect was barely statistically significant at the 0.01 mg/kg dose of YZ-231, and the drug failed to prevent cocaine-induced convulsions at the other tested doses.

### 3.3. Effect of changing position of substituent on cocaine-induced convulsions

All of the compounds with methoxyphenyl substitutions significantly attenuated cocaine-induced convulsions regardless of the position of the substituent on the phenyl ring (Fig. 3). Although there was no statistically significant difference among YZ-185 (*m*-position), YZ-184 (*o*-posi-

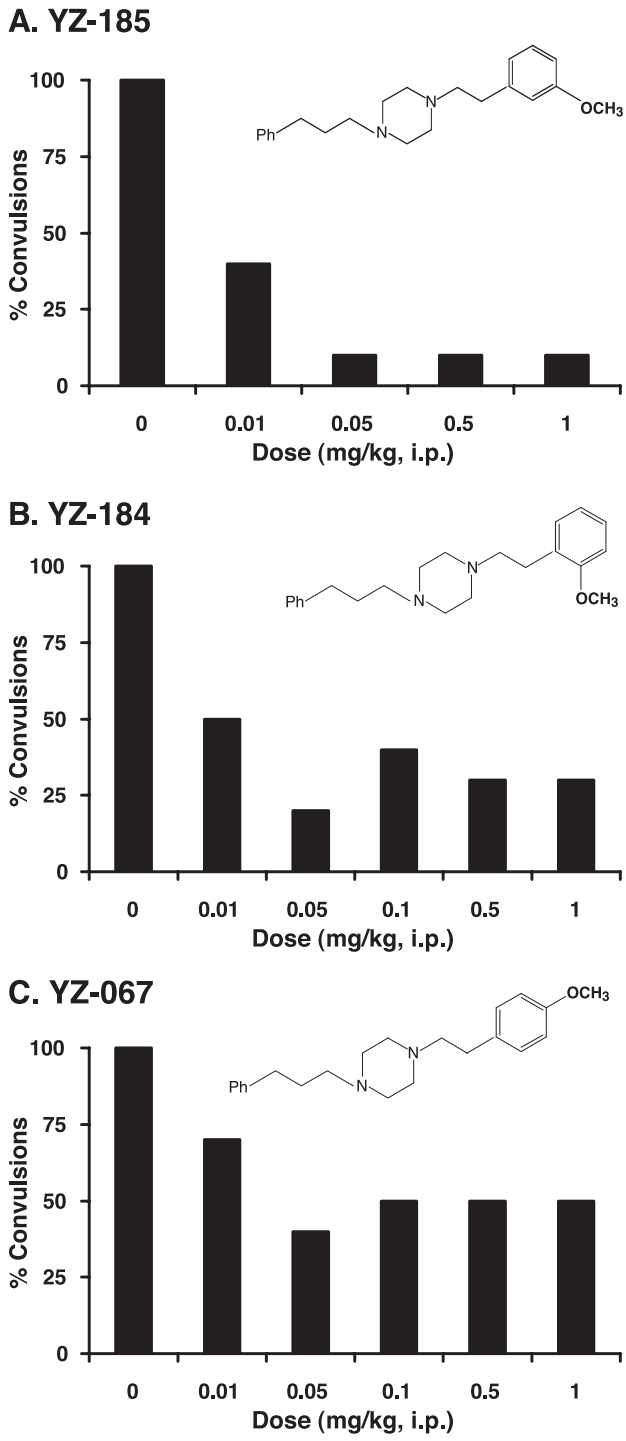


Fig. 3. Effect of position of methoxyphenyl substitution on cocaine-induced convulsions in mice. Mice were pretreated with (A) YZ-185, (B) YZ-184, or (C) YZ-067 (0–1 mg/kg i.p.) followed 15 min later with a convulsive dose of cocaine (60 mg/kg i.p.). Data represent the number of mice convulsing during the 30 min testing period/the total number of mice tested × 100%. Reductions of 50% or greater were statistically significant (Fisher's Exact Tests, $P < .05$).

tion), and YZ-067 (*p*-position), there was a trend for improved protective ability when the substituent was in the *m*-position.

## 4. Discussion

In the present study, the novel σ receptor ligand YZ-069 and several of its methoxyphenyl-substituted analogs (YZ-185, YZ-184, and YZ-067) significantly attenuated cocaine-induced convulsions in mice. Similar to earlier studies that have documented the ability of σ receptor antagonists and antisense oligodeoxynucleotides to prevent behavioral toxic and psychomotor stimulant effects of cocaine (Matsumoto et al., 2001a,b,c, 2002; McCracken et al., 1999a,b; Menkel et al., 1991; Ritz and George, 1997; Romieu et al., 2000; Skuza, 1999; Witkin et al., 1993), all of the compounds investigated herein have high affinity for σ receptors and much weaker affinity for monoamine transporters, suggesting that their interaction with cocaine occurs via σ receptors rather than monoamine transporters.

Regarding the involvement of the different σ receptor subtypes in the anticonvulsant actions produced by the novel ligands, the results obtained herein are consistent with earlier studies implicating the $\sigma_1$ subtype in these actions. Cocaine has been reported to have a 10-fold better affinity for $\sigma_1$ receptors as compared with $\sigma_2$ receptors (Matsumoto et al., 2001a). In addition, the ability of antisense oligodeoxynucleotides targeting $\sigma_1$ receptors to attenuate the convulsive effects of cocaine (Matsumoto et al., 2002) demonstrates that antagonism of this subtype alone is sufficient to prevent cocaine-induced convulsions. Almost all σ ligands possessing anticocaine actions have significant affinity for $\sigma_1$ receptors (Matsumoto et al., 2001a,b,c, 2002; McCracken et al., 1999a,b; Romieu et al., 2000; Skuza, 1999), including the compounds tested herein. The high affinity of YZ-069 and its analogs for $\sigma_1$ receptors thus provides a logical explanation for their anticocaine actions. The role of $\sigma_2$ receptors in the actions of cocaine is less definitive than with the $\sigma_1$ subtype. In earlier studies, the ability of rimcazole analogs to prevent cocaine-induced convulsions was significantly correlated with binding to $\sigma_2$ receptors (Matsumoto et al., 2001a), but the relationship to $\sigma_1$ receptors was stronger. The $\sigma_2$-preferring antagonist (±)-SM 21 has also been shown to significantly attenuate cocaine-induced convulsions in mice (Matsumoto and Mack, 2001), but again, the reductions were less robust than those exhibited by $\sigma_1$-preferring antagonists. Although the contribution of the anticonvulsive activity from $\sigma_2$ receptors is far from clear, it is conceivable that the novel compounds tested herein also produce some of their protective actions through $\sigma_2$ receptors, given their significant affinities for this subtype. Although additional studies are needed to fully characterize YZ-069 and its analogs, the data obtained thus far strongly suggest that they act at least in part through antagonism of σ receptors.

In terms of the structural features of σ ligands that convey favorable anticocaine actions, the results from the present aryl piperazine series were similar to those observed previously with the aryl ethylenediamines (Matsumoto et al., 2002). In the present study of aryl piperazines, the position of the substituent on the phenyl ring did not appear to have significant impact on the affinity or functionality of the compounds. This is similar to the pattern observed earlier in BD1008 analogs with aryl monosubstitutions where the position of the substitution also had little effect on σ binding affinity or magnitude of anticocaine actions (Matsumoto et al., 2002). It is also noteworthy that in the present study, YZ-231, the nitrophenyl-substituted analog, was much less efficacious in preventing cocaine-induced convulsions than the other compounds, although it had high affinity for σ receptors. A similar pattern was observed in BD1008 analogs with aryl monosubstitutions, where the nitro-substituted compounds (YZ-027, YZ-028, and YZ-029) were also less effective than their corresponding methoxy-substituted analogs (YZ-011, YZ-016, and YZ-018; Matsumoto et al., 2002). This pattern of results may be related to the reduced lipophilicity of the nitro substitution, which would be expected to reduce transport across the blood–brain barrier. It is further possible that the nitro derivatives may be partial agonists, which would account for their diminished antagonistic actions, as compared with what would be expected based on their affinity for σ receptors alone.

Due to the small quantities of the compounds, direct testing of locomotor behavior was not performed in this project, but it is unlikely that motor disturbances masked the actions of YZ-231 relative to the other compounds. Behavioral observation of the animals prior to receiving cocaine did not reveal obvious untoward side effects, such as motor malfunctions, with all groups of animals exhibiting comparable behaviors. Moreover, all σ receptor antagonists that we have reported on thus far, including the aryl nitro-substituted compounds from the BD1008 series (Matsumoto et al., 2002), produced no significant effects on locomotor behavior on their own as compared with control injections of saline (Matsumoto and Mack, 2001; Matsumoto et al., 2001b; McCracken et al., 1999a,b). Therefore, the weaker protective actions of YZ-231 is likely attributable to a factor other than motor malfunction.

YZ-231, in addition to its reduced lipophilicity and potential partial agonistic actions, was also the only compound in this series with significant affinity for serotonin transporters. Because selective serotonin reuptake inhibitors have been shown to facilitate cocaine-induced convulsions (O'Dell et al., 2000), the interaction of YZ-231 with serotonin transporters may have offset whatever protective effects are conveyed through σ receptors. Therefore, it appears that among the aryl piperazines examined herein as well as the aryl ethylenediamines evaluated earlier, 3,4-

dichlorophenyl and methoxyphenyl substitutions are preferred to nitrophenyl substitutions when designing σ receptor antagonists.

The data thus far demonstrate that YZ-069 and its analogs attenuate the convulsive effects of cocaine. Favorable features conveying anticocaine actions include high affinity for σ receptors, antagonism of σ receptors, and lipophilicity to facilitate crossing the blood–brain barrier. Together with earlier reports, the data suggest that effective compounds to mitigate the convulsive effects of cocaine can be designed by targeting σ receptors.

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