

Conversion of a Highly Selective Sigma-1 Receptor–Ligand to Sigma-2 Receptor Preferring Ligands with Anticocaine Activity

Christophe Mésangeau,[†] Sanju Narayanan,[†] Andrea M. Green,[†] Jamaluddin Shaikh,[‡] Nidhi Kaushal,[‡] Eddy Viard,[‡] Yan-Tong Xu,[‡] James A. Fishback,[‡] Jacques H. Poupaert,[§] Rae R. Matsumoto,[‡] and Christopher R. McCurdy^{*,†,‡}

Department of Medicinal Chemistry, Department of Pharmacology, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, and School of Pharmacy, Université Catholique de Louvain, Avenue Emmanuel Mounier 74, B-1200 Brussels, Belgium

Received October 30, 2007

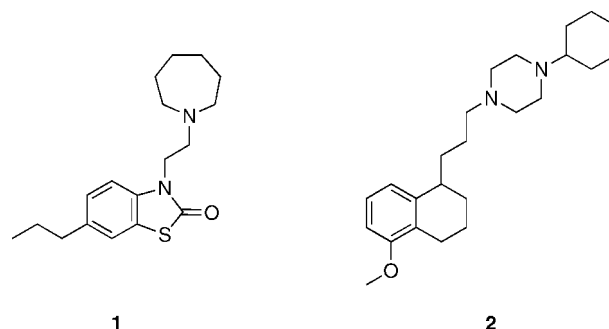
Cocaine's toxicity can be mitigated by blocking its interaction with sigma-1 receptors. The involvement of sigma-2 receptors remains unclear. To investigate their potential role, we have designed compounds through a convergent synthesis utilizing a highly selective sigma-1 ligand and elements of a selective sigma-2 ligand. Among the synthesized compounds was produced a subnanomolar sigma-2 ligand with an 11-fold preference over sigma-1 receptors. These compounds may be useful in developing effective pharmacotherapies for cocaine toxicity.

Introduction

Cocaine abuse is widespread and responsible for more serious intoxications and deaths than any other illicit drug.¹ Currently, there are no approved medications to treat cocaine abuse or addiction. For years, treatment approaches for cocaine abuse and addiction have targeted the dopaminergic system with limited success. Moreover, it is apparent that this strategy may only provide molecules that substitute for cocaine.² This has led researchers to shift the focus of medications development toward the involvement of other protein systems. Indeed, cocaine interacts with many proteins, and it is now well-established that cocaine interacts with sigma receptors at physiologically relevant concentrations.³ Earlier studies have indicated that reducing brain sigma-1 receptor levels with antisense oligonucleotides attenuates the convulsive and locomotor stimulant actions of cocaine.⁴ Synthetic small molecule antagonists for sigma receptors have also been shown to mitigate the actions of cocaine in animal models.^{3,4} From prior work, the role of the sigma-1 subtype has been clearly linked to the actions of cocaine. Although there has recently been literature indicating that the sigma-2 receptor may be involved in the actions of cocaine,⁵ its role has been less clear due to the lack of truly selective ligands for this subtype. To add to the complexity, the sigma-2 receptor has yet to be cloned. Furthermore, it is unclear if a compound with mixed affinity for both sigma-1 and sigma-2 receptors, with minimal to no affinity for other proteins, would be of benefit or utility in the treatment of cocaine toxicity or abuse.

Compound **1** (Chart 1) was previously reported⁶ to have high affinity and selectivity for sigma-1 receptors with respect to several other proteins and it was hypothesized that the 2(3*H*)-benzothiazolone moiety could be a useful template to improve the selectivity toward sigma receptors over other proteins. The recent report⁷ of compound **2** (Chart 1) provided a lead compound with high sigma-2 selectivity (40-fold over sigma-1 receptors), and along this line, we hypothesized that the

Chart 1. Structures of Lead Compounds



cyclohexylpiperazine moiety could be acting as a selectivity element for the sigma-2 receptor preference. With this in mind, we have designed and synthesized a series of compounds replacing the azepane ring in compound **1** with the putative selectivity element (N-cyclohexylpiperazine) for the sigma-2 receptor, exemplified by compound **2**, to obtain mixed affinity ligands or sigma-2 selective ligands. In addition to the cyclohexylpiperazine moiety affording sigma-2 affinity, it has been suggested that a four methylene spacer between the piperazine and the heterocyclic system is optimal for sigma-2 affinity over sigma-1.⁷ Therefore, it was warranted to examine the spacer length between the two portions by varying it from two to six carbons. We also wanted to investigate the effects of changing the 2-oxo position of the heterocycles to a sulfur to modulate the potential for hydrogen bonding interactions and determine their possible importance in receptor affinity and selectivity.

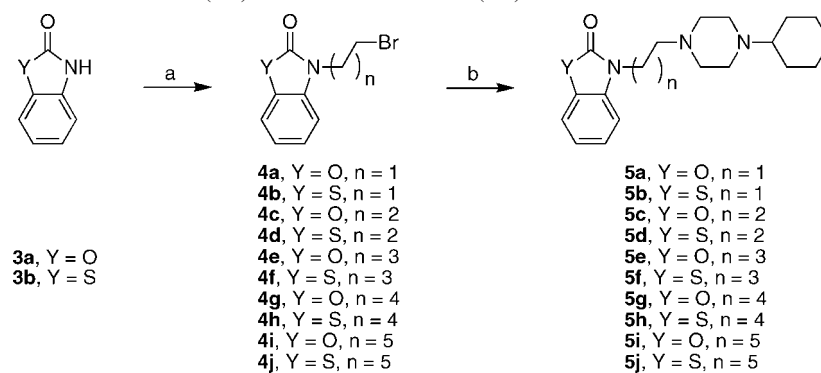
Chemistry. Our synthesis of the initial series of compounds was very straightforward as outlined in Scheme 1. Commercially available 2(3*H*)-benzoxazolone (**3a**) or 2(3*H*)-benzothiazolone (**3b**) were alkylated with various dibromoalkanes to give compounds with the desired spacer lengths (**4a–4j**). These were then coupled with the commercially available cyclohexylpiperazine to afford compounds **5a–5j**. Finally, hydrochloride salts were formed for biological testing. To investigate the effect of sulfur in the 2-position, compound **3a** was thionated with Lawesson's reagent to give **7** (Scheme 2). Initially, alkylation of the heterocycle with 1,4-dibromobutane was directed to the more reactive 2-thiocarbonyl. Subsequently, compound **7** (Scheme 2) was utilized to alkylate the cyclohexylpiperazine, resulting

* To whom correspondence should be addressed. Address: Department of Medicinal Chemistry, 419 Faser Hall, The University of Mississippi, University, MS 38677. Tel.: 662-915-5882. Fax: 662-915-5638. E-mail: cmccurdy@olemiss.edu.

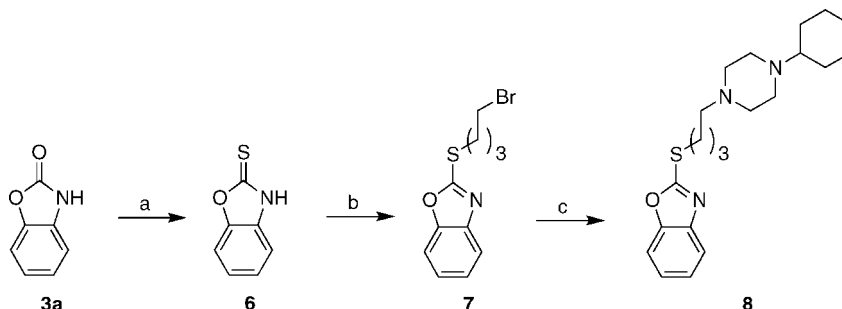
[†] Department of Medicinal Chemistry, The University of Mississippi.

[‡] Department of Pharmacology, The University of Mississippi.

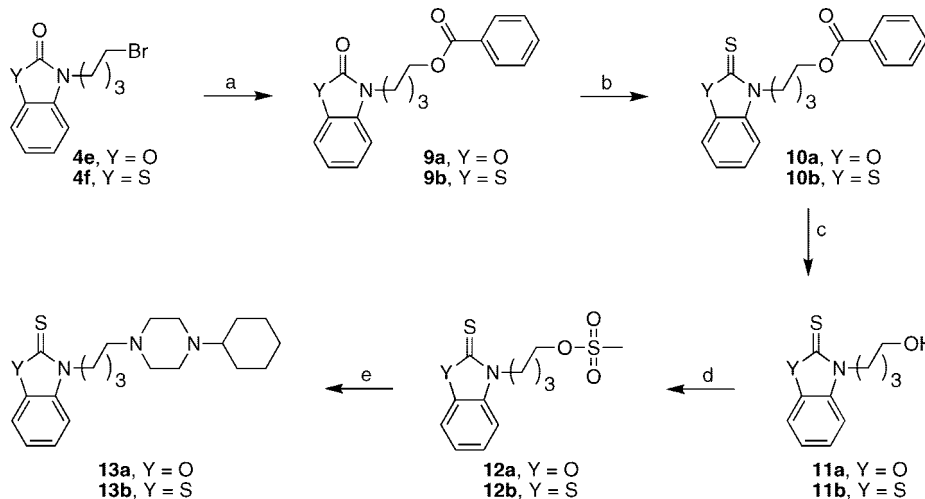
[§] Université Catholique de Louvain.

Scheme 1. Synthesis of Various Substituted 2(3*H*)-Benzoxazolones and 2(3*H*)-Benzothiazolones^a

^a Reagents and conditions: (a) dibromoalkane, K₂CO₃, DMF, 60° C, 2 h; (b) cyclohexylpiperazine, K₂CO₃, DMF, 60° C, 3 h.

Scheme 2. Synthesis of Compound **8**^a

^a Reagents and conditions: (a) Lawesson's reagent, toluene, reflux, 4 h; (b) 1,4-dibromobutane, K₂CO₃, DMF, 60° C, 2 h; (c) cyclohexylpiperazine, K₂CO₃, DMF, 60° C, 3 h.

Scheme 3. Synthesis of **13a** and **13b**^a

^a Reagents and conditions: (a) benzoic acid, K₂CO₃, 60° C, 1 h; (b) Lawesson's reagent, toluene, reflux, 24 h; (c) NaOH, H₂O, CH₃OH, reflux, 1 h; (d) CH₃SO₂Cl, Et₃N, -10° C, 1 h; (e) cyclohexylpiperazine, K₂CO₃, DMF, 60° C, 3 h.

in compound **8**. We felt it would be worthwhile to explore the attachment position of the heterocycle, and Scheme 3 outlines the synthesis of the desired N-alkylated compounds containing sulfur in the 2-position of the heterocycles. Compounds **4e** and **4f** were reacted with potassium benzoate to form the respective esters, **9a** and **9b**. These compounds were then treated with Lawesson's reagent to thionate the 2-oxo position, yielding **10a** and **10b**, respectively. Hydrolysis of the benzoate afforded the alcohols **11a** and **11b**, which were mesylated (**12a** and **12b**) and reacted with *N*-cyclohexylpiperazine to afford compounds **13a** and **13b** in high yields.

Biology. All final compounds were subjected to in vitro binding studies against sigma-1 and sigma-2 receptors in rat

brain homogenates using routine assay conditions.⁸ In addition, compound **13b** was subjected to selected nonsigma receptor binding assays in rat brain homogenates to determine the selectivity of this template over other proteins usually associated with sigma receptor-ligands. Because seizure is a common result of cocaine overdose and earlier sigma receptor antagonists attenuated the convulsive effects of cocaine in mice,⁴ we tested whether pretreatment with representative sigma receptor-ligands produced similar anticocaine actions.

Results and Discussion

As can be seen from Table 1, all compounds demonstrated high affinity for sigma-1 and sigma-2 receptors. Compounds

Table 1. Sigma Receptor Binding Affinities and Selectivity Ratios of **5a–5j**, **8**, **13a**, and **13b**^a

cmpd	σ -1 (K_i , nM)	σ -2 (K_i , nM)	σ -1/ σ -2
5a	6.90 ± 0.37	5.43 ± 0.78	1.27
5b	4.66 ± 0.74	2.25 ± 0.37	2.07
5c	5.22 ± 1.11	8.74 ± 2.30	0.60
5d	5.61 ± 0.74	3.05 ± 0.41	1.84
5e	11.26 ± 1.25	1.83 ± 0.17	6.15
5f	4.17 ± 0.62	0.39 ± 0.06	10.69
5g	10.55 ± 2.52	5.89 ± 1.31	1.79
5h	4.98 ± 0.42	2.44 ± 0.26	2.04
5i	4.60 ± 1.08	3.06 ± 0.45	1.50
5j	6.55 ± 0.25	1.49 ± 0.18	4.40
8	3.37 ± 0.28	3.77 ± 0.35	0.89
13a	4.90 ± 1.70	0.77 ± 0.06	6.36
13b	1.28 ± 0.38	0.55 ± 0.08	2.33

^a Affinities (K_i in nM) were determined in rat brain homogenates using standard assays conditions. Sigma-1 receptors were labeled with [³H](+)-pentazocine. Sigma-2 receptors were labeled with [³H]DTG in the presence of (+)-pentazocine to block sigma-1 receptors. Nonspecific binding was determined in the presence of haloperidol. The values in this table represent the mean ± SEM from replicate assays.

Table 2. Sigma Receptor Binding Affinities and Selectivity Ratios of Lead Compounds **1**, **2**, and Haloperidol^a

cmpd	σ -1 (K_i , nM)	σ -2 (K_i , nM)	σ -1/ σ -2
1	0.12 ± 0.0096	830.95 ± 72.35	0.00014
2	1.48 ± 0.40	1.11 ± 0.031	1.33
haloperidol	3.35 ± 0.80	80.60 ± 14.10	0.042

^a Affinities (K_i in nM) were determined in rat brain homogenates using standard assays conditions. Sigma-1 receptors were labeled with [³H](+)-pentazocine. Sigma-2 receptors were labeled with [³H]DTG in the presence of (+)-pentazocine to block sigma-1 receptors. Nonspecific binding was determined in the presence of haloperidol. The values in this table represent the mean ± SEM from replicate assays.

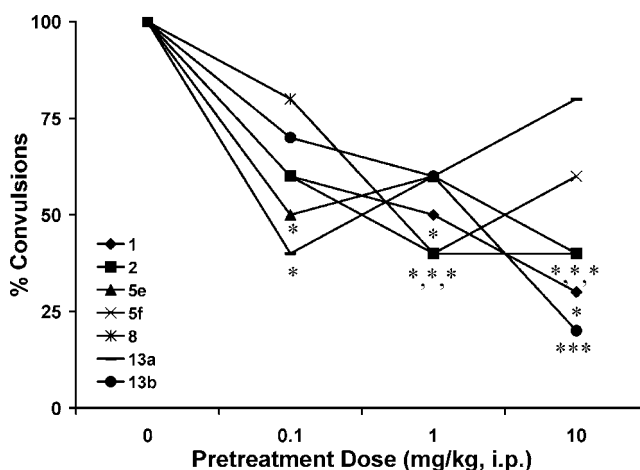
with a spacer length of four carbons (**5e**, **5f**, and **13a**) seemed to have the most preference for sigma-2 receptors. Furthermore, it can be noted that subnanomolar affinity for sigma-2 receptors resulted from at least one sulfur atom being present in the heterocycle. Because the binding mode of these analogs or the structure of the proteins is unknown, this data may indicate a presence of a hydrogen bonding element in the sigma-1 site that may either be absent or cause an unfavorable steric interaction in the sigma-2 site. In general, the 2(3*H*)-benzothiazoles had higher affinities for sigma-2 receptors than their respective 2(3*H*)-benzoxazole counterparts. Moreover, compounds **13a** and **13b**, where the 2-oxo was replaced with a sulfur, also resulted in compounds with subnanomolar affinity for sigma-2 receptors. It is interesting to note that compound **13b** where two sulfur atoms are present (in the 1 and 2 positions) resulted in high affinity, but no selectivity, for both the sigma-1 and sigma-2 receptors. Compound **8**, where the heterocycle is connected to the linker via the 2-thio position, was nonselective and showed equivalent affinity for both sigma-1 and sigma-2 receptors. This may indicate that the attachment position of the heterocycle may not be important for high affinity but potentially for selectivity.

The most selective compound for sigma-2 receptors, **5f**, was 11-fold selective over sigma-1. This was a surprising result as we envisioned these compounds having a similar selectivity profile to lead compound **2**. This result prompted us to evaluate compounds **1**, **2**, and haloperidol (as a control) under our receptor binding assay conditions, which differ slightly from those previously reported for each of them. This data is presented in Table 2. Haloperidol, a known sigma ligand, demonstrated binding affinities similar to those reported in the literature.⁹ Compound **1** retained high affinity and selectivity for sigma-1 receptors in our assays. Interestingly, compound **2** in our assay

Table 3. Nonsigma Protein Binding Affinities of Compound **13b**^a

	radioligand	nonspecific binding	K_i (nM)
Monoamine Transporters			
dopamine ^b	0.5 nM [³ H]WIN 35,428	50 μ M cocaine	1175 ± 10
serotonin ^c	0.2 nM [³ H]paroxetine	1.5 μ M imipramine	1402 ± 152
norepinephrine ^d	0.5 nM [³ H]nisoxetine	4 μ M desipramine	>10000
Other Receptors ^e			
opioid	0.5 nM [³ H]bremazocine	10 μ M levallorphan	>10000
NMDA	5 nM [³ H]TCP	10 μ M cyclozocine	>10000
dopamine D ₂	5 nM [³ H](–)-sulpiride	1 μ M haloperidol	1041 ± 9
5-HT ₂	2 nM [³ H]ketanserin	1 μ M mianserin	1326 ± 159

^a Affinities of **13b** for nonsigma binding sites were determined in rat brain homogenates. The values in this table represent the mean ± SEM from replicate assays. Values of >10000 nM signify that there was less than 30% displacement of the radioligand at that concentration. ^b Rat brain homogenates, striatum. ^c Rat brain homogenates, brainstem. ^d Rat brain homogenates, cortex. ^e Rat brain homogenates, whole brain.

**Figure 1.** Attenuation of cocaine-induced convulsions in Swiss Webster mice by select sigma ligands. * P < 0.05, *** P < 0.001. Asterisks separated by commas indicate significance for three different ligands at that datapoint.

exhibited high affinity for sigma-2 receptors but also had equally high affinity for sigma-1 receptors, revealing that it is not selective under our assay conditions.

The most interesting finding from this work is that our design indeed provided compounds with high mixed-affinity for sigma-1 and sigma-2 receptors, as well as compounds with a preference for sigma-2 receptors. Compound **13b** has the highest affinity values so far reported for both subtypes. We therefore examined **13b** in several nonsigma binding assays as shown in Table 3. Compound **13b** had essentially no affinity for other proteins of interest, indicating the validity of our design.

To determine the compound's abilities to attenuate the effects of cocaine-induced convulsions, we examined those compounds with high sigma-2 affinities (**2**, **5e**, **5f**, **8**, **13a**, and **13b**) in vivo along with compound **1** as a control. Indeed, as shown in Figure 1, compound **1** acts as a sigma-1 receptor antagonist and at least one dose of each of the compounds with sigma-2 affinity significantly attenuated cocaine-induced convulsions. Because attenuation of sigma-1 receptors alone is sufficient to mitigate cocaine-induced convulsions,⁴ it is difficult to distinguish the contribution of sigma-2 receptors in the mixed affinity ligands. Nevertheless, it is worth noting that other sigma-2 preferring compounds have been reported to attenuate cocaine-induced convulsions.⁵ However, until the development and testing of truly selective sigma-2 ligands, the role of sigma-2 receptors in cocaine-induced convulsions will remain suggestive, but unclear. The most efficacious compound was **13b**, which also

had the highest affinities for sigma receptors. These results are encouraging toward the development of new medications to treat cocaine toxicities, and further studies are underway to determine their ability to attenuate cocaine abuse.

Conclusion

We successfully converted a highly selective sigma-1 antagonist (**1**) into a series of compounds that have high, mixed-affinity for sigma-1 and sigma-2 receptors. We also have an interesting lead molecule (**5f**) for the development of highly selective sigma-2 compounds. Although the role of sigma-2 receptors in cocaine toxicity could not be clarified in this study, there may be a benefit to a high affinity, mixed sigma-1/sigma-2 ligand in the treatment of cocaine abuse. These data, taken together, suggest that further studies are warranted to evaluate their potential to treat cocaine abuse. Additionally, because many psychostimulants (e.g., methamphetamine and methylenedioxymethamphetamine) bind to sigma receptors, mixed affinity sigma-1/sigma-2 receptor-ligands represent a potential new class of drugs to treat psychostimulant abuse. Presently, we are investigating the utility of compound **13b** as a treatment of cocaine abuse in further in vivo assays to be reported in due time.

Experimental Section

Chemistry. Reagents and starting materials were obtained from commercial suppliers and were used without purification. Precoated silica gel GF Uniplates from Analtech were used for thin-layer chromatography (TLC). Column chromatography was performed on silica gel 60 (Sorbent Technologies). ^1H and ^{13}C NMR spectra were obtained on a Bruker APX400 at 400 and 100 MHz, respectively. The high resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-ToF Micro mass spectrometer with a lock spray source. The mass spectra (MS) were recorded on a Waters Acquity Ultra Performance LC with ZQ detector in ESI mode. Elemental analyses (C, H, N) were recorded on an elemental analyzer, Perkin-Elmer CHN/SO Series II Analyzer. Chemical names were generated using ChemDraw Ultra (CambridgeSoft, version 10.0).

General Procedure A. Synthesis of (bromoalkyl)benzo[d]oxazol-2(3H)-one and Derivatives. 3-(4-Bromobutyl)benzo[d]oxazol-2(3H)-one (**4e**). K_2CO_3 (9.2 g, 66.6 mmol) and 1,4-dibromobutane (21.0 mL, 177.6 mmol) were added, under stirring, to a solution of benzo[d]oxazol-2(3H)-one (3.0 g, 22.2 mmol) in anhydrous DMF (30 mL). The reaction mixture was heated at 60 °C for 3 h. After cooling, the mixture was poured into 100 mL of water and extracted with ethyl acetate (3 $\times$ 70 mL). The combined organic layers were washed with saturated aqueous NaCl and dried over magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl acetate (8:2) as the eluent to give 3.8 g (64%) of **4e** as a white solid. ^1H NMR (CDCl_3): δ 7.19–7.08 (m, 3H), 6.99–6.97 (m, 1H), 3.85 (t, J = 6.4 Hz, 2H), 3.44 (t, J = 6.0 Hz, 2H), 1.95–1.93 (m, 4H). MS m/z 272 ($\text{M}^+ + 2$), 270 (M^+).

General Procedure B. Synthesis of the Cyclohexylpiperazine Derivatives. 3-(4-(Cyclohexylpiperazin-1-yl)butyl)benzo[d]oxazol-2(3H)-one (**5e**). K_2CO_3 (0.46 g, 3.33 mmol) and 1-cyclohexylpiperazine (0.18 g, 1.11 mmol) were added, under stirring, to a solution of **4e** (0.3 g, 1.11 mmol) in anhydrous DMF (4 mL). The reaction mixture was heated at 60 °C for 3 h. After cooling, the mixture was poured into 20 mL of water and extracted with ethyl acetate (3 $\times$ 30 mL). The combined organic layers were washed with saturated aqueous NaCl and dried over magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using dichloromethane/methanol (9.5:0.5) as the eluent to give 0.27 g (69%) of **5e** as a white solid. ^1H NMR (CDCl_3): δ 7.21–6.98 (m, 4H), 3.84 (t, J = 6.7 Hz, 2H), 2.59–2.22 (m, 11H), 1.89–1.57 (m, 9H),

1.21–1.10 (m, 5H). HRMS calcd for $\text{C}_{21}\text{H}_{32}\text{N}_3\text{O}_2$ [$\text{M} + \text{H}$] $^+$, 358.2495; found, 358.2498. Anal. ($\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_2 \cdot \frac{1}{4}\text{H}_2\text{O}$) C, H, N.

Benzo[d]oxazole-2(3H)-thione (6). Lawesson's reagent (2.99 g, 7.4 mmol) was added, under stirring, to a solution of benzo[d]oxazol-2(3H)-one (1.5 g, 11.1 mmol) in anhydrous toluene (80 mL). The reaction mixture was heated under reflux for 4 h, cooled to room temperature, and purified by flash column chromatography (SiO_2) using petroleum ether/ethyl ether (8:2) as the eluent. The solid was then recrystallized from toluene/hexanes to give 1.2 g (72%) of **7** as a white solid. ^1H NMR ($\text{DMSO}-d_6$): δ 13.82 (s, 1H), 7.49–7.47 (m, 1H), 7.28–7.22 (m, 3H). MS m/z 150 ($\text{M}^+ - 1$).

4-(2-Oxobenzo[d]oxazol-3(2H)-yl)butyl Benzoate (9a). K_2CO_3 (2.35 g, 17 mmol) and benzoic acid (1.04 g, 8.51 mmol) were added, under stirring, to a solution of **4e** (1.77 g, 6.55 mmol) in anhydrous DMF (10 mL). The reaction mixture was heated at 60 °C for 2 h. After cooling, the mixture was poured into 100 mL of water, extracted with ethyl acetate (3 $\times$ 70 mL), and the combined organic layers were washed with a 0.5 N solution of KOH and with saturated aqueous NaCl. The solvent was dried over magnesium sulfate and removed in vacuo to give 2 g (98%) of **9a** as a white solid. ^1H NMR ($\text{DMSO}-d_6$): δ 7.92 (d, J = 7.2 Hz, 2H), 7.62 (t, J = 7.2 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 7.31 (d, J = 7.6 Hz, 2H), 7.19 (t, J = 7.6 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1H), 4.30 (t, J = 6.4 Hz, 2H), 3.88 (t, J = 6.4 Hz, 2H), 1.97–1.72 (m, 4H). MS m/z 334 ($\text{M}^+ + 23$).

4-(2-Oxobenzo[d]thiazol-3(2H)-yl)butyl Benzoate (9b). K_2CO_3 (11.18 g, 81 mmol) and benzoic acid (4.94 g, 40.5 mmol) were added, under stirring, to a solution of **4f** (8.91 g, 31.1 mmol) in anhydrous DMF (30 mL). The reaction mixture was heated at 60 °C for 1 h. After cooling, the mixture was poured into 100 mL of water, extracted with ethyl acetate (3 $\times$ 70 mL), and the combined organic layers were washed with a 0.5 N solution of KOH and with saturated aqueous NaCl. The solvent was dried over magnesium sulfate and removed in vacuo to give 10 g (98%) of **9b** as a white solid. ^1H NMR (CDCl_3): δ 8.01–7.99 (m, 2H), 7.54 (t, J = 7.2 Hz, 1H), 7.43–7.39 (m, 3H), 7.29 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 4.36 (t, J = 6.0 Hz, 2H), 4.02 (t, J = 6.8 Hz, 2H), 1.91–1.86 (m, 4H). MS m/z 350 ($\text{M}^+ + 23$).

4-(2-Thioxobenzo[d]oxazol-3(2H)-yl)butyl Benzoate (10a). Lawesson's reagent (1.9 g, 4.69 mmol) was added, under stirring, to a solution of **9a** (1.95 g, 6.26 mmol) in anhydrous toluene (80 mL). The reaction mixture was heated under reflux for 20 h. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl acetate (8:2) as the eluent to give 1.56 g (76%) of **10a** as a white solid. ^1H NMR ($\text{DMSO}-d_6$): δ 7.92–7.90 (m, 2H), 7.64–7.28 (m, 7H), 4.32–4.26 (m, 4H), 1.97–1.76 (m, 4H). MS m/z 350 ($\text{M}^+ + 23$).

4-(2-Thioxobenzo[d]thiazol-3(2H)-yl)butyl Benzoate (10b). Lawesson's reagent (1 g, 3 mmol) was added, under stirring, to a solution of **9b** (0.93 g, 2.3 mmol) in anhydrous toluene (50 mL). The reaction mixture was heated under reflux for 15 h. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl ether (8:2) as the eluent to give 0.66 g (63%) of **10b** as a colorless oil. ^1H NMR (CDCl_3): δ 8.03–8.00 (m, 2H), 7.57–7.02 (m, 7H), 4.51 (t, J = 6.8 Hz, 2H), 4.40 (t, J = 6 Hz, 2H), 2.03–1.90 (m, 4H). MS m/z 344 ($\text{M}^+ + 1$).

3-(4-Hydroxybutyl)benzo[d]oxazole-2(3H)-thione (11a). To a solution of **10a** (1.44 g, 4.39 mmol) in methanol (20 mL) was added a solution of sodium hydroxide (0.44 g, 10.98 mmol) in water (20 mL). The mixture was heated at 100 °C for 1 h, concentrated in vacuo, poured into 1 N HCl (20 mL), and extracted with ethyl acetate (3 $\times$ 20 mL). The combined organic layers were washed with a 10% solution of K_2CO_3 and brine and dried over magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl acetate (5:5) as the eluent to give 0.42 g (43%) of **11a** as a white solid. ^1H NMR (CDCl_3): δ 7.31–7.12 (m, 4H), 4.20 (t,

$J = 7.2$ Hz, 2H), 3.67 (t, $J = 6.4$ Hz, 2H), 2.28 (s, 1H), 1.94–1.89 (m, 2H), 1.66–1.60 (m, 2H). MS m/z 246 ($M^+ + 23$).

3-(4-Hydroxybutyl)benzo[d]thiazole-2(3H)-thione (11b). To a solution of **10b** (0.61 g, 1.77 mmol) in methanol (20 mL) was added a solution of sodium hydroxide (0.18 g, 4.44 mmol) in water (20 mL). The mixture was heated at 60 °C for 1 h, concentrated in vacuo, poured into 1 N HCl (20 mL), and extracted with ethyl acetate (3×20 mL). The combined organic layers were washed with saturated aqueous NaCl, dried over magnesium sulfate, and removed under reduced pressure. The residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl ether (8:2) as the eluent to give 0.30 g (71%) of **11b** as a white solid. ^1H NMR (CDCl_3): δ 7.46–7.23 (m, 4H), 4.44 (t, $J = 7.6$ Hz, 2H), 3.70 (t, $J = 6$ Hz, 2H), 2.41 (m, 1H), 1.71–1.66 (m, 2H), 1.91–1.86 (m, 2H). MS m/z 262 ($M^+ + 23$).

4-(2-Thioxobenzo[d]oxazol-3(2H)-yl)butyl Methanesulfonate (12a). A solution of methanesulfonyl chloride (0.16 mL, 2.01 mmol) in methylene chloride (5 mL) was slowly added to a solution of **11a** (0.3 g, 1.34 mmol) and triethylamine (0.56 mL, 4.03 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and the solvent was evaporated. The residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl acetate (6:4) as the eluent to give 7.4 g (97%) of **12a** as a white solid. ^1H NMR ($\text{DMSO}-d_6$): δ 7.56–7.53 (m, 2H), 7.39–7.29 (m, 2H), 4.26–4.23 (m, 4H), 3.15 (s, 3H), 1.91–1.73 (m, 4H). MS m/z 324 ($M^+ + 23$).

4-(2-Thioxobenzo[d]thiazol-3(2H)-yl)butyl Methanesulfonate (12b). A solution of methanesulfonyl chloride (2.75 mL, 35.4 mmol) in methylene chloride (20 mL) was slowly added to a solution of **11b** (5.65 g, 23.6 mmol) and triethylamine (8.3 mL, 59.1 mmol) in dichloromethane (120 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and poured into water. The two layers were separated and the organic layer was washed with water and dried over magnesium sulfate. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (SiO_2) using petroleum ether/ethyl acetate (6:4) as the eluent to give 7.4 g (98%) of **12b** as a white solid. ^1H NMR (CDCl_3): δ 7.49 (d, $J = 7.8$ Hz, 1H), 7.42 (t, $J = 7.5$ Hz, 1H), 7.30 (t, $J = 7.6$ Hz, 1H), 7.24 (d, $J = 8.2$ Hz, 1H), 4.48 (t, $J = 6.9$ Hz, 2H), 4.31 (t, $J = 6.0$ Hz, 2H), 3.02 (s, 3H), 2.00–1.90 (m, 4H). MS m/z 318 ($M^+ + 1$).

Radioligand Binding Assays. The assays were performed in rat brain homogenates using procedures previously published in detail.⁹ Sigma-1 receptors were labeled with 5 nM [^3H](+)-pentazocine. Sigma-2 receptors were labeled with 3 nM [^3H]DTG^a in the presence of 300 nM (+)-pentazocine to block sigma-1 receptors. Nonspecific binding was determined in the presence of 10 μM haloperidol. K_i values were calculated using the Cheng–Prusoff equation.¹⁰

Cocaine-Induced Convulsions. Male, Swiss Webster mice were pretreated (i.p.) with saline or test compound, then challenged 15 min later with a convulsive dose of cocaine (70 mg/kg, i.p.). Mice were observed for the next 30 min for convulsions, which were defined as a loss of righting reflexes for at least 5 s, combined with the presence of clonic limb movements or popcorn jumping. The data were analyzed using Fisher's exact tests.

^a Abbreviations: DTG, 1,3-di-*o*-tolylguanidine; i.p., intraperitoneal; NMDA, *N*-methyl *D*-aspartate; TCP, *N*-(1-[thienyl]cyclohexyl)piperidine; WIN 35,428, (–)-2-beta-carbomethoxy-3-beta-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate.

Acknowledgment. This project was supported by NIDA (DA013978, DA011979, DA023205) and NCRR (P20 RR021929). We appreciate the initial work from Prof. Daniel Lesieur's group that led to the discovery of compound **1**, and the technical assistance of Caroline Croom and Babuk Shariat-Madar in conducting some of the behavioral studies. This investigation was conducted in a facility constructed with support from a research facilities improvement program grant from NCRR (C06 RR14503).

Supporting Information Available: Detailed experimental data for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Drug Abuse Warning Network, 2005: National Estimates of Drug-Related Emergency Department Visits. DAWN Series D-29, DHHS Publication No. (SMA) 07-4256, Rockville, MD, 2007. (b) Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Drug Abuse Warning Network, 2003: Area Profiles of Drug-Related Mortality. DAWN Series D-27, DHHS Publication No. (SMA) 05-4023, Rockville, MD, March, 2005.
- (2) (a) Grabowski, J.; Shearer, J.; Merrill, J.; Negus, S. S. Agonist-like, replacement pharmacology for stimulant abuse and dependence. *Addict. Behav.* **2004**, *29*, 1439–1464. (b) Carroll, F. I.; Howell, L. L.; Kuhar, M. J. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. *J. Med. Chem.* **1999**, *42*, 2721–2736.
- (3) Matsumoto, R. R.; Liu, Y.; Lerner, M.; Howard, E. W.; Brackett, D. J. Sigma receptors: Potential medications development target for anti-cocaine agents. *Eur. J. Pharmacol.* **2003**, *469*, 1–12.
- (4) (a) Matsumoto, R. R.; McCracken, K. A.; Friedman, M. J.; Pouw, B.; De Costa, B. R.; Bowen, W. D. Conformationally restricted analogs of BD1008 and an antisense oligodeoxynucleotides targeting sigma-1 receptors produce anti-cocaine effects in mice. *Eur. J. Pharmacol.* **2001**, *419*, 163–174. (b) Matsumoto, R. R.; McCracken, K. A.; Pouw, B.; Zhang, Y.; Bowen, W. D. Involvement of sigma receptors in the behavioral effects of cocaine: evidence from novel ligands and antisense oligodeoxynucleotides. *Neuropharmacology* **2002**, *42*, 1043–1055.
- (5) Matsumoto, R. R.; Pouw, B.; Mack, A. L.; Daniels, A.; Coop, A. Effects of UMB24 and (+/–)-SM21, putative sigma2-preferring antagonists, on behavioral toxic and stimulant effects of cocaine in mice. *Pharmacol. Biochem. Behav.* **2007**, *86*, 86–91.
- (6) Yous, S.; Wallez, V.; Belloir, M.; Caignard, D. H.; McCurdy, C. R.; Poupaert, J. H. Novel 2(3H)-Benzothiazolones as highly potent and selective sigma-1 receptor ligands. *Med. Chem. Res.* **2005**, *14*, 158–168.
- (7) Berardi, F.; Ferorelli, S.; Abate, C.; Colabufo, N. A.; Contino, M.; Perrone, R.; Tortorella, V. 4-(Tetralin-1-yl)- and 4-(naphthalen-1-yl)alkyl derivatives of 1-cyclohexylpiperazine as sigma receptor ligands with agonist sigma-2 activity. *J. Med. Chem.* **2004**, *47*, 2308–2317.
- (8) Nguyen, E. C.; McCracken, K. A.; Liu, Y.; Pouw, B.; Matsumoto, R. R. Involvement of sigma receptors in the acute actions of methamphetamine: Receptor binding and behavioral studies. *Neuropharmacology* **2005**, *49*, 638–645.
- (9) (a) McCann, D. J.; Weissman, A. D.; Su, T. P. Sigma-1 and sigma-2 sites in rat brain: Comparison of regional, ontogenetic, and subcellular patterns. *Synapse* **1994**, *17*, 182–189. (b) Matsumoto, R. R.; Pouw, B. Correlation between neuroleptic binding to sigma-1 and sigma-2 receptors and acute dystonic reactions. *Eur. J. Pharmacol.* **2000**, *401*, 155–160.
- (10) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

JM701357M