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PII: S0223-5234(19)30029-7

DOI: https://doi.org/10.1016/j.ejmech.2019.01.019

Reference: EJMECH 11026

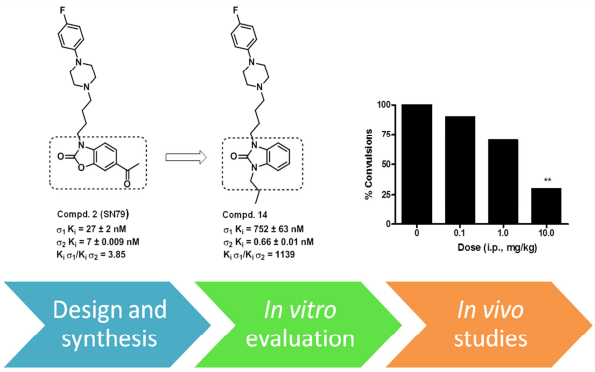
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 4 October 2018

Revised Date: 1 January 2019

Accepted Date: 8 January 2019

Please cite this article as: S. Intagliata, W.F. Alsharif, C. Mesangeau, N. Fazio, M. Seminerio, Y.-T. Xu, R.R. Matsumoto, C.R. McCurdy, Benzimidazolone-based selective σ_2 receptor ligands: Synthesis and pharmacological evaluation, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.01.019.



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Benzimidazolone-based selective σ_2 receptor ligands: synthesis and pharmacological evaluation

Sebastiano Intagliata [a], Walid F. Alsharif [b], Christophe Mesangeau [b], Nicola Fazio [b], Michael Seminerio [c], Yan-Tong Xu [c], Rae R. Matsumoto [c,d], Christopher R. McCurdy [a, b *]

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Abstract

Sigma receptors (σRs) are considered to be a significant and valid target for developing new medications to address several diseases. Their potential involvement in numerous central nervous system disorders, neuropathic pain, addiction, and cancer has been extensively reported. In particular, the σ_2R has been identified as potential target for the development of pharmaceutical agents intended to treat the negative effects associated with drugs of abuse. As a continuation of our previous efforts to develop new selective σ_2R ligands, a series of benzimidazolone derivatives were designed, synthesized, and characterized. The newly synthesized ligands were evaluated through *in vitro* radioligand binding assays to determine their affinity and selectivity towards both σ_1 and σ_2 receptors. Several derivatives displayed high affinity for the σ_2R (K_i = 0.66-68.5 nM) and varied from preferring to selective, compared to σ_1R (σ_1/σ_2 = 5.8-1139). Among them, compound 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-propyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**14**) displayed the ability to produce a dose-dependent reduction in the convulsive effects of cocaine in a rodent model after injecting 10

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mg/kg (i.p.). These preliminary results support the use of selective σ_2R ligands in the development of useful pharmacological tools or potential pharmacotherapies for cocaine toxicity.

Keywords: Sigma receptors, Sigma-2 receptors, Sigma-2 ligands, Benzimidazolone, Structure-affinity relationships, Cocaine-induced convulsions, Cocaine's toxicity.

1. Introduction

Sigma receptors (σRs) were originally classified as members of the opioid receptor family. This was based on the behavioral actions observed with the opiate benzomorphan, (±)-*N*-allyl-normetazocine, and its analogs [1]. The effects of (±)-*N*-allylnormetazocine were reported to be partially antagonized by the opioid antagonist naloxone, thus resulting in σRs being classified as a new opioid receptor subtype. Through subsequent studies using purified enantiomers, it was shown that the "psychotomimetic" effect produced by (-)-SKF-10047 was instigated through kappa opioid receptors and the "psychotomimetic" effect of (+)-SKF-10047 was not blocked by naltrexone, a potent analog of naloxone, and must be mediated through non-opioid receptors. (+)-SKF-10047 was eventually shown to interact with at least two distinct binding sites: 1) the phencyclidine (PCP) site on N-methyl-D-aspartate (NMDA) receptors, and it was due to this interaction that the term sigma-PCP was prevalent in the early 1980s, and 2) other binding sites, distinct from opioid and NMDA receptors, which today retains the designation of σRs [2, 3].

Later, several studies proved that σRs are unique proteins highly preserved across species, cell types, and organelles [4-7]. Additional studies identified two subtypes of the receptor, σ_1R and σ_2R. Although historical sigma ligands interacted with both σ_1R and σ_2R, the subtypes are distinguishable from each other by their amino acid sequences and pharmacological preferences [8-10]. The σ_1R was first cloned in 1996 from guinea pig liver and later from other sources, representing different species and tissues such as human placental choriocarcinoma cells [4], human brain [5], rat brain [6], and mouse brain [8]. The σ_1R is highly conserved across species with an amino acid sequence that is distinct from other known mammalian receptors [4]. The σ_2R has been more difficult to understand and clone. In 2011, the σ_2R was suggested to be part of the progesterone receptor membrane component 1 (PGRMC1) protein complex [11].

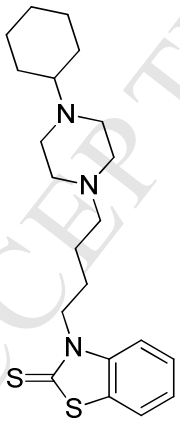
However, further investigations showed no correlation between PGRMC1 expression and σ_2R binding levels in knockout or over-expression studies [12]. More recently, the σ_2R was purified from calf liver tissue by affinity chromatography and, subsequently, the gene that codes for the σ_2R was identified as identical to Transmembrane Protein 97 (TMEM97), which is an endoplasmic reticulum-resident transmembrane protein that acts as a binding partner of the lysosomal cholesterol transporter NPC1. Indeed, this latest finding provided further evidence that the σ_2R and PGRMC1 are two distinct proteins [13]. Consensus in the field now accepts the TMEM97 protein as the σ_2R. Over-expression of σ_2R in proliferative cancer cells has been reported, suggesting that selective radiolabeled σ_2R ligands may be useful to pinpoint the malignant tissues in SPECT (single-photon emission computed tomography) and PET (positron emission tomography) imaging [14-16]. Moreover, literature-based reports have shown that σ_2R agonists are able to induce apoptosis and reduce cancer cell proliferation, thus, there is a rising interest in the development of σ_2R agents as therapeutics or adjuvants to treat cancer [17-19].

The involvement of σRs in several CNS disorders such as depression, schizophrenia, and dementia, as well as their potential role in other neurological and psychological disorders, drove much of the early interest in these receptors [20-24]. This interest was due to the majority of typical antipsychotics, such as haloperidol, having affinity for σRs. Also, agents that instigate psychotomimetic activity, such as (±)-*N*-allylnormetazocine and its analogs, shared affinity for σRs [25]. Moreover, evidence-based literature demonstrated that some σR antagonists were able to attenuate the effects of psychostimulant drugs such as cocaine [26-28]. Conversely, σR agonists aggravated some of these effects, including the convulsive effects of cocaine, resulting in a leftward shift in the cocaine convulsion dose–response curve [28, 29]. There still remains a need to develop new medications to attenuate the convulsions associated with an overdose of cocaine, which cannot be controlled with existing antiepileptic drugs. However, unlike the σ_1R, the involvement of the σ_2R in the toxicity of cocaine is still unclear, and improved, selective σ_2R ligands are needed [30].

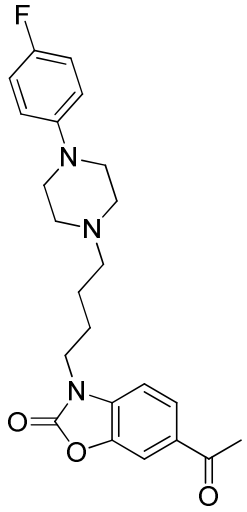
Previously, we reported a series of 1,3-benzothiazole-2(3*H*)-thione with high mixed affinity for σ_1Rs and σ_2Rs [31]. Among them, 3-[4-(4-cyclohexylpiperazin-1-yl)butyl]-2(3*H*)-benzothiazolethione compound **1** (CM156**,** Fig. 1) produced both acute and subchronic effects against cocaine in behavioral models in rodents [32]. Further studies showed the ability of **1** to mitigate the effects of methamphetamine in mice [33]. Furthermore, we developed 6-acetyl-3-{4-

[4-(4-fluorophenyl)piperazin-1-yl]butyl}-2(3*H*)-benzoxazolone compound **2** (SN79, Fig. 1) which reduced the toxic and stimulant effects of cocaine, and also caused sedation and incoordination with acute treatment [34]. More studies have suggested that pretreatment with compound **2** afforded protection against methamphetamine-induced hyperthermia, and striatal dopaminergic and serotonergic neurotoxicity in mice [35].

Within the frame of our efforts to develop novel selective σ_2R ligands [36], we have developed several σ ligands with high affinity for σ_2Rs and preference over σ_1Rs. Among them, the most selective σ_2R ligand was selected in order to study its effect against cocaine-induced convulsions in a preclinical rodent model, and to assess the selectivity requirement of both receptor subtypes. As a strategy in the current study, we replaced the 1,3-benzothiazole-2(3*H*)-thione, and the 1,3-benzoxazol-2(3*H*)-one of **1** and **2** with the bioisostere 1,3-dihydrobenzimidazol-2-one. In particular, the new versatile core allowed us to perform 1,3-bis-functionalization along with the introduction of an acetyl group in the 5-position of the heterocyclic ring. Similarly to **1** and **2**, the cyclic amine fragment of the new ligands were both 1-cyclohexylpiperazine and 1-(4-fluorophenyl)piperazine. Finally, based on our previous data, a four-carbon chain was selected as a linker as it results in greater σ_2R preference [31].



1 (CM156)
σ_1 K_i = 1.3 nM
σ_2 K_i = 0.6 nM
K_i σ_1/K_i σ_2 = 2.17



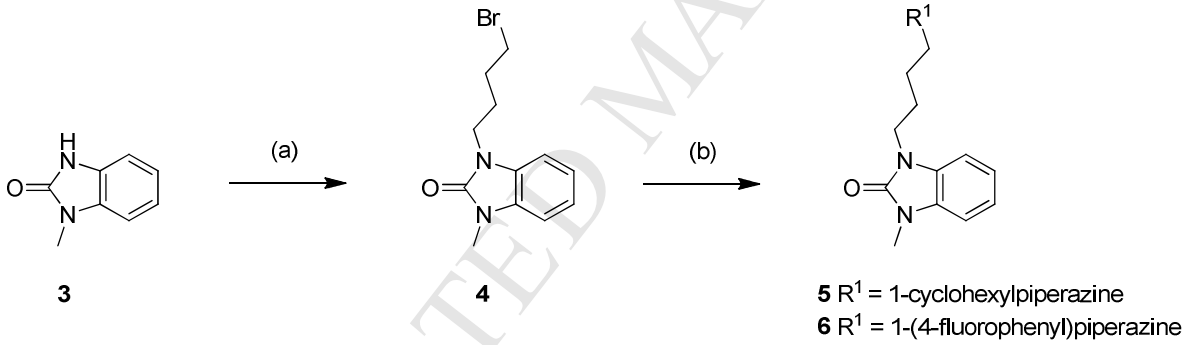
2 (SN79)
σ_1 K_i = 27 nM
σ_2 K_i = 7 nM
K_i σ_1/K_i σ_2 = 3.86

Fig. 1. Chemical structures and affinity values of compounds **1** and **2**.

2. Results and discussion

2.1. Chemistry

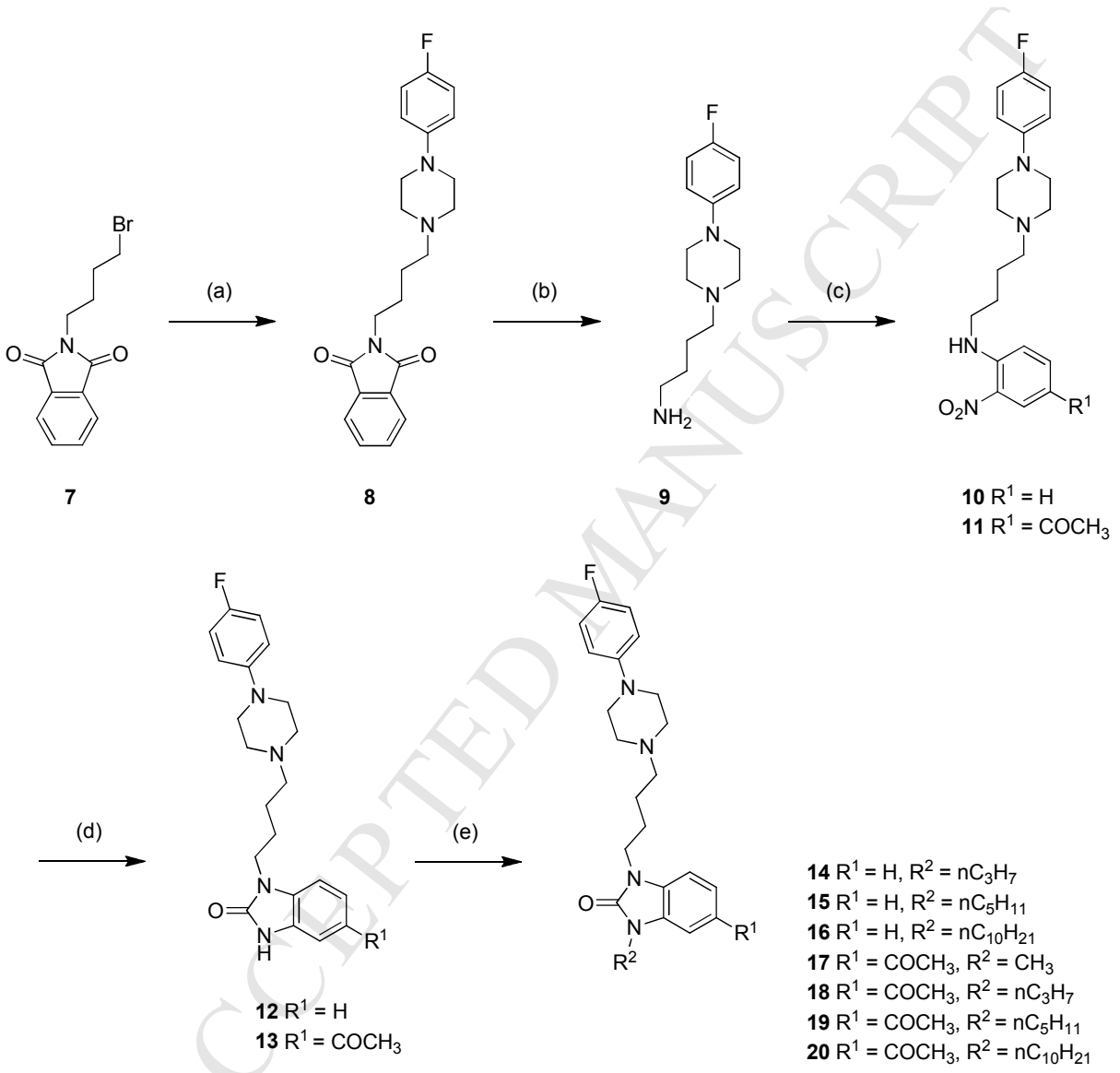
The synthesis of final compounds **5** and **6**, was very straightforward as outlined in Scheme 1. Treatment of commercially available compound **3** with 1,4-dibromobutane in the presence of potassium carbonate in DMF, gave the 4-bromobutyl derivative (**4**) which was then coupled with 1-cyclohexylpiperazine, or 1-(4-fluorophenyl)piperazine in the presence of potassium carbonate in DMF to afford target compounds **5** and **6,** respectively (Scheme 1).



Scheme 1. Reagents and conditions: (a) 1,4-dibromobutane, K_2CO_3, DMF, 60 °C, 70%; (b) cyclic amine, K_2CO_3, DMF, 60 °C, 56-62%.

The synthesis of final compounds **12-20** is depicted in Scheme 2. *N*-alkylation of commercially available 2-(4-bromobutyl)isoindole-1,3-dione (**7**) with 1-(4-fluorophenyl)piperazine provided compound **8**. The desired primary amine was then liberated from the phtalimide by hydrazinolysis to afford compound **9** which was subsequently reacted, through a nucleophilic aromatic substitution, with 1-fluoro-2-nitrobenzene to give **10** or, with 1-(4-chloro-3-nitrophenyl)ethanone to give **11**. The nitro groups of **10** and **11** were reduced in the presence of Pd/C and the corresponding 1,2-diamine derivatives which were reacted with 1,1′-

carbonyldiimidazole to give benzimidazolones **12** and **13**. The final compounds **14**-**20**, were obtained by *N*-alkylation with the appropriate alkyl halides.



Scheme 2. Reagents and conditions: (a) 1-(4-fluorophenyl)piperazine, K_2CO_3, DMF, 60 °C, 60%; (b) NH_2-NH_2, EtOH, reflux, 83%; (c) 1-fluoro-2-nitrobenzene or 1-(4-chloro-3-nitrophenyl)ethanone, DIPEA, DMF, 100-120 °C, 83-93%; (d) H_2 (40 psi), 10% Pd/C, EtOH then CDI, THF, 20 °C, 51-68%; (e) NaH, TBAI, alkyl halide, DMF, 20 °C, 33-80% or K_2CO_3, TBAI, alkyl halide, DMF, 60 °C, 84-89%.

2.2. Binding studies and extended non-σ binding profile

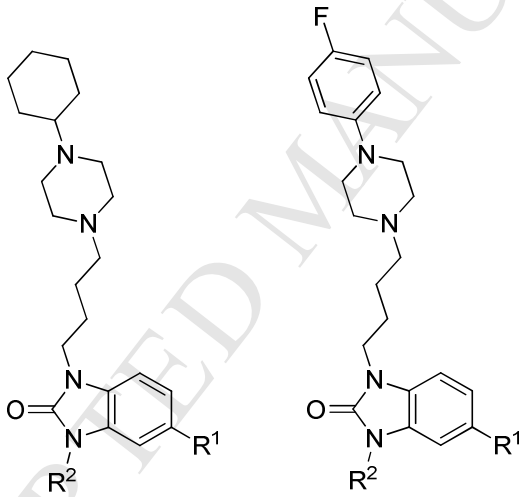
All final compounds were tested in radioligand binding studies at the σ_1R and σ_2R in rat brain membranes using well-established and previously described assay conditions [31]. The σ_1Rs were labeled with [^{3}H](+)-pentazocine. The σ_2Rs were labeled with [^{3}H]di-o-tolylguanidine (DTG) in the presence of (+)-pentazocine to block σ_1Rs. Nonspecific binding was determined in the presence of haloperidol. The binding affinities (K_i in nM), and selectivity ratios (K_i σ_1/ K_i σ_2) of the new compounds at σRs are summarized in Table 1.

Initially, we synthesized compounds **5** and **6** to investigate the effect of replacement of the heterocyclic scaffolds in **1** and **2** with a benzimidazolone moiety. This structural modification led to an increase of the σ_1/σ_2 selectivity ratio for both analogs (**5** *vs* **1**, **6** *vs* **2**). In particular, compound **6** resulted in 46-fold more selective preference than **2** for the σ_2R over the σ_1R. This result prompted us to maintain the 1-(4-fluorophenyl)piperazine ring as a fixed portion of the new series. Subsequently, the introduction of different alkyl substituents on the *N*-3 (such as nC_3H_7, nC_5H_{11}, and $nC_{10}H_{21}$), as well as an acetyl group at the 5-position of the benzimidazolone scaffold, gave compounds **12-20** which resulted in affinity values in the low or subnanomolar range for the σ_2R (K_i = 0.66-32.5 nM).

Based on the binding data, reported in Table 1, the main structure-affinity relationships (SARs) were as follows. In general, the new benzimidazolone derivatives retained high σ_2R affinity and showed increased selectivity over the σ_1R (selectivity 5.8-1139 fold). Replacement of the 1-cyclohexylpiperazine with 1-(4-fluorophenyl)piperazine, greatly improved the selectivity for the σ_2R subtype (**5** *vs* **6**). Functionalization at the *N*-3 of the benzimidazolone ring generally increased affinity for the σ_2R. Introduction of an acetyl group at the 5-position of the heterocyclic ring led to a loss of the σ_1/σ_2 selectivity. Thus, the presence of both a bulky substituent as R^2 and an acetyl group as R^1 was detrimental for σ_2R affinity. Regarding the set of compounds with no substituent on the benzo-fused ring (**6**, **12**, and **14**-**16**), the introduction of R^2 groups were ranked based on their affinity (K_i values) at σ_2R as follows: nC_3H_7 > CH_3 > nC_5H_{11} > $nC_{10}H_{21}$ > H. A different ranking was observed for the set of 5-acetyl-benzoimidazolone analogs **13**, and **17**-**20**: nC_3H_7 > nC_5H_{11} > CH_3 > H > $nC_{10}H_{21}$. Among both sets, *n*-propyl and *n*-pentyl analogs (**14**, **18**, and **15**, **19**) displayed better results in terms of affinity and selectivity for the σ_2R than other alkyl-substituted derivatives.

Based on these results, we selected compounds **14** and **15** which had the highest ratios of σ_1/σ_2 (>1000 fold and >500 fold, respectively) for further evaluation in extended non-σRs binding experiments (Table 2), using previously established and published methods [28]. Substantially, compound **14** did not exhibit significant affinities for the non-σ proteins tested, except for serotonin (5-HT_2) receptors (K_i = 54.70 nM, 5-HT_2/σ_2R = 83 fold preference for σ_2Rs). Compound **15** showed similar results at serotonin (5-HT_2) receptors (K_i = 249.6 nM, 5-HT_2/σ_2R = 58 fold selectivity for σ_2Rs), although possessed very low affinity for serotonin transporters (K_i = 445.9 nM, 5-HTT/σ_2R = 104 fold selectivity for σ_2Rs).

Table 1. σRs binding affinities and selectivity ratios of compounds **5**, **6**, **12-20,** and haloperidol.



5 **6**, **12-20**

Compd.	R^1	R^2	K_i (nM ± SEM)[a]		K_i σ_1/ K_i σ_2
			σ_1R	σ_2R	
5	H	CH_3	50.22 ± 7.59	2.57 ± 0.47	19.5
6	H	CH_3	366.23 ± 101	2.05 ± 0.33	178.6
12	H	H	144 ± 6.6	18.4 ± 1.4	7.8
13	$COCH_3$	H	166 ± 12	28.5 ± 8.6	5.8
14	H	nC_3H_7	752 ± 63	0.66 ± 0.01	1139
15	H	nC_5H_{11}	2274 ± 187	4.27 ± 0.29	532.5

16	H	$nC_{10}H_{21}$	724 ± 13.6	4.55 ± 0.21	159.1
17	$COCH_3$	CH_3	72.3 ± 2.6	7.6 ± 0.31	9.5
18	$COCH_3$	nC_3H_7	273.5 ± 1.33	1.81 ± 0.02	151.1
19	$COCH_3$	nC_5H_{11}	299 ± 32	2.15 ± 0.94	139
20	$COCH_3$	$nC_{10}H_{21}$	988 ± 53	32.5 ± 5.3	30.4
Haloperidol			3.35 ± 0.80	80.60 ± 14.10	0.042

[a]The values represent the mean ± SEM from triplicate assays.

Table 2. Binding profile of **14** and **15** for other non-σ proteins.

Compd.	K_i (nM ± SEM)[a]						
	Monoamine transporters			Other receptors			
	Dopamine	Serotonin	Norepinephrine	Dopamine (D_2)	Serotonin ($5\text{-}HT_2$)	NMDA	Opioid
14	>1000	>1000	>10000	>10000	54.70 ± 0.27	>10000	>10000
15	>1000	445.9 ± 3.2	>10000	>1000	249.6 ± 4.8	>10000	>10000

[a] Affinities (K_i values in nanomolar) were determined in rat brain homogenates. The values represent the mean ± SEM from triplicate assays. Values of >10000 nM represent less than 30% displacement of the radioligand at that concentration.

2.3. In vivo convulsion studies

Seizure is a common result of cocaine overdose and earlier σR antagonists attenuated the convulsive effects of cocaine in mice [27, 28]. Therefore, we tested whether pretreatment of mice with 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-propyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**14**), similarly attenuated cocaine-induced convulsions. Compound **14** was selected because it possessed a very good extended non-σ binding profile, thus acting as a relatively selective σ_2R ligand. As can be seen from Fig. 2, compound **14** produced a dose-dependent reduction in the convulsive effects of cocaine, with the protective effects being statistically significant at the 10 mg/kg pretreatment dose ($P<0.005$). These results suggest that

σ_2R-selective ligands can mitigate cocaine-induced convulsions, and support the hypothesis of an active role for this target in cocaine's toxicity.

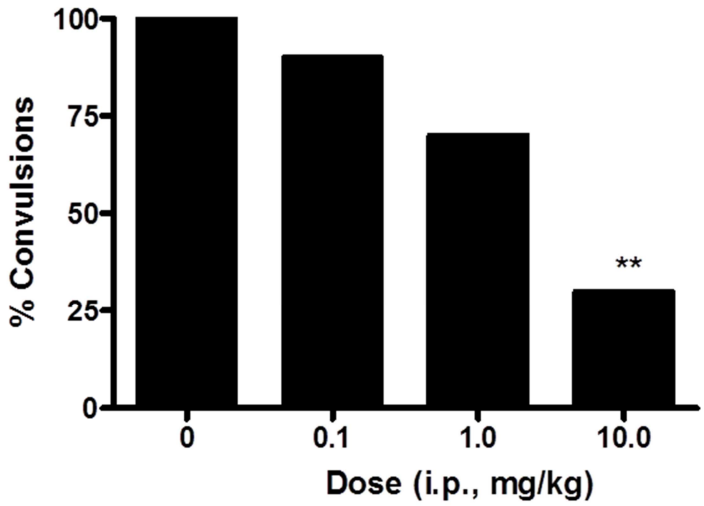


Fig. 2. In *vivo* cocaine-induced convulsion studies on compound **14**. ** P<0.005 *vs* vehicle response; *n* = 6-8 per group.

3. Conclusion

We successfully synthesized a series of benzimidazolone derivatives, and evaluated their binding affinity for σRs. In this work, a versatile 1,3-dihydrobenzimidazol-2-one scaffold was used, along with a 1-(4-fluorophenyl)piperazine as a cyclic amine moiety. Based on the SARs, both structural elements were identified as preferential for optimizing affinity and selectivity for the σ_2R. Derivatives **14**, and **15**, which gave the best results in terms of affinity for the σ_2R (K_i = 0.66 and 4.27 nM) and selectivity over the σ_1R (σ_1/σ_2 =1139 and 532), were selected for additional binding evaluation *versus* different non-σ sites. In particular, only moderate affinity for serotonin (5-HT_2) receptors (**14** and **15**, K_i = 54.70 and 249.60 nM respectively) and very low affinity for serotonin transporters (**15**, K_i = 445.9 nM) were observed. Noteworthy, *in vivo* convulsion studies in mice showed that compound **14** was able to dose-dependently attenuate cocaine-induced convulsions. Thus, the data reported in this paper are indicative of the potential utility of targeting σ_2Rs to mitigate the convulsive effects of cocaine. The synthesis of new

derivatives and further *in vivo* studies are currently under development and will be reported elsewhere.

4. Experimental protocols

4.1. 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). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker APX400 in $CDCl_3$ and DMSO-d_6 solution. Chemical shift (δ) values are given in parts per million (ppm) downfield to tetramethylsilane (TMS, δ = 0.00 ppm); coupling constants (*J* values) are given in hertz (Hz). For signal multiplicities, the following abbreviations are used as follows: s (singlet), d (doublet), dd (doublets of doublet), t (triplet), q (quartet), quint (quintet), br s (broad singlet) and m (multiplet). Except where otherwise noted, ^{1}H and ^{13}C NMR data for final compounds are given for materials in their salt form. 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 analysis (C, H, N) was recorded on an elemental analyzer, Perkin-Elmer CHN/SO Series II Analyzer.

4.1.1. 1-(4-bromobutyl)-3-methyl-1,3-dihydrobenzimidazol-2-one (**4**).

K_2CO_3 (0.56 g, 4.05 mmol) and 1,4-dibromobutane (1.12 mL, 9.45 mmol) were added, under mechanical stirring, to a solution of 3-methyl-1*H*-benzimidazol-2-one (**3**) (0.20 g, 1.35 mmol) in anhydrous DMF (8 mL). The reaction mixture was heated at 60 °C for 3 h. After cooling, the mixture was poured into 100 mL of water, extracted with ethyl acetate (3 × 40 mL), washed with saturated aqueous NaCl and dried. The solvent was removed *in vacuo*, and the residue was purified by chromatography on a silica gel column using petroleum ether/ethyl acetate (7:3) as the eluent to give **4** (0.27 g, 70%), as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06-7.03 (m, 2H), 6.96-6.91 (m, 2H), 3.88-3.86 (m, 2H), 3.41-3.38 (m, 2H), 3.63 (s, 3H), 1.89-1.85 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ154.41, 130.10, 129.17, 121.26, 121.23,

107.53, 107.50, 40.08, 33.13, 29.66, 27.15, 26.98. MS (ESI) *m/z* 305 $[M + Na]^+$ for ^{79}Br, *m/z* 307 $[M + Na]^+$ for ^{81}Br.

4.1.2. 1-[4-(4-cyclohexylpiperazin-1-yl)butyl]-3-methyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**5**).

K_2CO_3 (0.06 g, 0.44 mmol) and 1-cyclohexylpiperazine (0.04 g, 0.21 mmol) were added, under mechanical stirring, to a solution of **4** (0.05 g, 0.18 mmol) in anhydrous DMF (3 mL). The reaction mixture was heated at 60 °C for 2 h. After cooling, the mixture was poured into 20 mL of water, extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were washed with saturated aqueous NaCl and dried. The solvent was removed *in vacuo*, and the residue was chromatographed on a silica gel column using methylene chloride/methanol (95:5) as the eluent. The free base was converted into the hydrochloride salt by addition of HCl/dioxane to give **5** (0.05 g, 62%), as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 12.01-11.80 (m, 2H), 7.21-7.19 (m, 1H), 7.15-7.12 (m, 1H), 7.08-7.06 (m, 2H), 3.84-3.02 (m, 17H), 1.80-1.77 (m, 2H), 1.66-1.05 (m, 11H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.01, 129.80, 128.89, 121.33, 121.30, 108.09, 64.32, 55.37, 48.60, 45.39, 39.96, 27.13, 26.37, 25.28, 24.75, 24.55, 21.14. HRMS (ESI) calcd for $C_{22}H_{35}N_4O$ $[M + H]^+$ 371.2811, found 371.2828. Anal. calcd for $C_{22}H_{36}Cl_2N_4O.\ H_2O$: C, 57.26; H, 8.30; N, 12.14. Found: C, 57.46; H, 7.92; N, 12.11.

4.1.3. 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-methyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**6**).

This compound was prepared from **4** and 1-(4-fluorophenyl)piperazine as described for **5**. Yield 56%, as a white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 11.40 (br s, 1H), 7.30-7.00 (m, 9H), 3.88 (t, *J* = 6.8 Hz, 2H), 3.70-3.67 (m, 2H), 3.50-3.47 (m, 2H), 3.32 (s, 3H), 3.23-3.08 (m, 6H), 1.81-1.68 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.81 (d, *J* = 258.0 Hz), 149.09, 129.59, 128.69, 120.78, 120.75, 117.84 (d, *J* = 7.0 Hz), 115.25 (d, *J* = 22.0 Hz), 107.65, 107.63, 54.68, 50.33, 45.95, 39.64, 26.80, 25.16, 20.18. HRMS (ESI) calcd for $C_{22}H_{28}N_4OF$ $[M + H]^+$ 383.2247, found 383.2237. Anal. calcd for $C_{22}H_{29}Cl_2FN_4O$: C, 58.02; H, 6.42; N, 12.30. Found: C, 58.31; H, 6.14; N, 12.23.

4.1.4. 2-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-1H-isoindole-1,3(2H)-dione (**8**).

K_2CO_3 (8.45 g, 61.2 mmol) and 1-(4-fluorophenyl)piperazine (4.77 g, 26.50 mmol) were added, under mechanical stirring, to a solution of 2-(4-bromobutyl)isoindole-1,3-dione (**7**) (3.75 g, 20.00 mmol) in anhydrous DMF (30 mL). The reaction mixture was stirred at 60 °C for 1 h and at room temperature for 15 h. The reaction mixture was then poured into 40 mL of water, extracted with ethyl acetate (3 × 40 mL), and the combined organic layers were washed with saturated aqueous NaCl and dried. The solvent was removed *in vacuo*, and the residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol (10:0 to 9.5:0.5) as the eluent to give **8** (4.70 g, 60%), as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86-7.80 (m, 4H), 7.00 (t, J = 9.0 Hz, 2H), 6.90-6.87 (m, 2H), 3.58 (t, J = 7.0 Hz, 2H), 3.01-2.99 (m, 4H), 2.43-2.41 (m, 4H), 2.30 (t, J = 7.0 Hz, 2H), 1.63-1.57 (m, 2H), 1.48-1.42 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.88, 155.87 (d, J = 234.0 Hz), 147.89 (d, J = 1.8 Hz), 134.27, 131.56, 122.91, 116.91 (d, J = 7.5 Hz), 115.13 (d, J = 21.6 Hz), 57.14, 52.65, 48.92, 37.31, 25.90, 23.58.

4.1.5. 4-[4-(4-fluorophenyl)piperazin-1-yl]butan-1-amine (**9**).

A solution of **8** (4.25 g, 11.10 mmol), and hydrazine monohydrate (2.7 mL, 55.70 mmol) in ethanol (220 mL) was heated at reflux for 16 h. The reaction mixture was then cooled with an ice bath. The precipitate was eliminated by filtration, and the filtrate was evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol saturated with ammonia (10:0 to 8:2) as the eluent to give **9** (2.33 g, 83%), as a low melting point pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91-6.89 (m, 2H), 6.83-6.79 (m, 2H), 3.06 (t, J = 4.8 Hz, 4H), 2.67 (t, J = 6.8 Hz, 2H), 2.54 (t, J = 5.0 Hz, 4H), 2.34 (t, J = 7.0 Hz, 2H), 2.00 (br s, 2H), 1.55-1.40 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.24 (d, J = 237.1 Hz), 148.11 (d, J = 2.1 Hz), 117.88 (d, J = 7.5 Hz), 115.58 (d, J = 21.9 Hz), 58.52, 53.35, 50.23, 42.07, 31.57, 24.42.

4.1.6. N-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-2-nitroaniline (**10**).

A mixture of 1-fluoro-2-nitrobenzene (2.51 g, 17.80 mmol), compound **9** (2.24 g, 8.91 mmol), *N*,*N*-diisopropylethylamine (7.8 mL, 44.56 mmol) in DMF (30 mL) was heated at 120 °C for 2 h. After cooling, the mixture was poured into 50 mL of water, extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were washed with brine and dried. The solvent

was removed *in vacuo*, and the residue was purified by chromatography on a silica gel column using ethyl acetate/hexanes (6:4) as the eluent to give **10** (3.09 g, 93%), as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.14-8.11 (m, 1H), 8.04 (d, J = 8.6 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 7.01 (t, J = 8.8 Hz, 2H), 6.92-6.88 (m, 2H), 6.65 (t, J = 7.6 Hz, 1H), 3.36 (q, J = 6.3 Hz, 2H), 3.04-3.02 (m, 4H), 2.47-2.45 (m, 4H), 2.34 (t, J = 6.9 Hz, 2H), 1.68-1.61 (m, 2H), 1.57-1.50 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.90 (d, J = 234.0 Hz), 147.91 (d, J = 1.8 Hz), 145.21, 136.52, 130.79, 126.19, 116.93 (d, J = 7.5 Hz), 115.16 (d, J = 21.6 Hz), 115.00, 114.51, 57.09, 52.65, 48.96, 42.00, 26.07, 23.50.

4.1.7. 1-[4-({4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}amino)-3-nitrophenyl]ethan-1-one (**11**).

A mixture of 1-(4-chloro-3-nitrophenyl)ethanone (3.89 g, 19.50 mmol), compound **9** (2.45 g, 9.75 mmol), *N*,*N*-diisopropylethylamine (6.32 g, 48.74 mmol) in DMF (50 mL) was heated at 100 °C for 6 h. After cooling, the mixture was poured into 80 mL of water, extracted with ethyl acetate (3 × 80 mL), and the combined organic layers were washed with brine and dried. The solvent was removed *in vacuo*, and the residue was purified by chromatography on a silica gel column using a gradient of ethyl acetate and hexanes (6:4 to 10:0) as the eluent to give **11** (3.35 g, 83%), as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61-8.60 (m, 2H), 7.99 (d, J = 5.2 Hz, 1H), 7.16 (d, J = 9.1 Hz, 1H), 7.02 (t, J = 9.0 Hz, 2H), 6.93-6.90 (m, 2H), 3.45 (q, J = 2.5 Hz, 2H), 3.37 (s, 3H), 3.05 (t, J = 4.0 Hz, 4H), 2.48 (s, 4H), 2.37 (t, J = 6.9 Hz, 2H), 1.66 (quint, J = 7.0 Hz, 2H), 1.54 (quint, J = 6.9 Hz, 2H).

4.1.8. 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-1,3-dihydrobenzimidazol-2-one (**12**).

A solution of **10** (3.00 g, 8.06 mmol) in methanol (220 mL) was hydrogenated in the presence of 10% Pd/C (0.30 g) under pressure (40 psi) for 1 h. The catalyst was removed by filtration and the solvent was evaporated. The residue was dissolved in THF (80 mL), and 1,1′-carbonyldiimidazole (2.61 g, 16.10 mmol) was added. The reaction mixture was stirred under argon at room temperature. After 2 h, brine was added and the mixture was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with brine, dried and evaporated. The residue was purified by chromatography on a silica gel column using a gradient of methylene chloride and methanol (10:0 to 9.5:0.5) as the eluent to give **12** (2.03 g, 68%), as a white solid. A small portion was converted to the corresponding dihydrochloride salt for the

biological testing. ^{1}H NMR (free amine, 400 MHz, DMSO-d_6): δ 10.81 (s, 1H), 7.12-7.10 (m, 1H), 7.03-6.97 (m, 5H), 6.90-6.87 (m, 2H), 3.79 (t, *J* = 6.9 Hz, 2H), 3.01-2.99 (m, 4H), 2.44-2.42 (m, 4H), 2.30 (t, *J* = 7.0 Hz, 2H), 1.68-1.62 (m, 2H), 1.48-1.41 (m, 2H). ^{13}C NMR (free amine, 100 MHz, DMSO-d_6): δ 155.90 (d, *J* = 234.0 Hz), 154.73, 147.91 (d, *J* = 1.8 Hz), 130.19, 128.22, 120.62, 120.39, 116.93 (d, *J* = 7.5 Hz), 115.15 (d, *J* = 21.7 Hz), 108.64, 107.67, 57.04, 52.60, 48.93, 39.59, 25.61, 23.33. Anal. calcd for $C_{21}H_{27}Cl_2FN_4O.1/2\ H_2O$: C, 56.00; H, 6.27; N, 12.44. Found: C, 56.44; H, 6.02; N, 12.44.

4.1.9. 5-acetyl-1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-1,3-dihydrobenzimidazol-2-one (**13**).

This compound was prepared from **11** as described for **12**. Yield 51%, as a white solid. A small portion was converted to the corresponding dihydrochloride salt for the biological testing. ^{1}H NMR (free amine, 400 MHz, DMSO-d_6): δ 11.16 (br s, 1H), 7.71 (d, *J* = 6.5 Hz, 1H), 7.51 (s, 1H), 7.24 (d, *J* = 6.5 Hz, 1H), 7.00 (t, *J* = 7.0 Hz, 2H), 6.89-6.87 (m, 2H), 3.84 (t, *J* = 5.4 Hz, 2H), 2.99 (s, 4H), 2.53 (s, 3H), 2.41 (s, 4H), 2.30 (t, *J* = 5.6 Hz, 2H), 1.66 (quint, *J* = 5.3 Hz, 2H), 1.44 (quint, *J* = 5.4 Hz, 2H). ^{13}C NMR (free amine, 100 MHz, DMSO-d_6): δ 196.59, 155.93 (d, *J* = 237.3 Hz), 154.54, 147.93, 134.34, 130.18, 128.26, 122.59, 116.95 (d, *J* = 5.9 Hz), 115.19 (d, *J* = 17.3 Hz), 108.03, 107.22, 57.05, 52.64, 48.96, 40.01, 26.51, 25.66, 23.32. Anal. calcd for $C_{23}H_{29}Cl_2FN_4O_2.\ H_2O$: C, 55.09; H, 6.23; N, 11.17. Found: C, 55.25; H, 5.81; N, 10.67.

4.1.10. 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-propyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**14**).

Sodium hydride (60% in mineral oil, 0.02 g, 0.38 mmol) and tetrabutylammonium iodide (0.01 g, 0.03 mmol) were added to a solution of **12** (0.10 g, 0.27 mmol) in anhydrous DMF (4 mL) at room temperature under argon. After stirring for 15 min, iodopropane (0.03 mL, 0.32 mmol) in DMF (2 mL) was added. The reaction mixture was stirred for 4 h at room temperature, poured into brine (20 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were combined, washed with water, dried and evaporated. The residue was purified by chromatography on a silica gel column using ethyl acetate as the eluent. The free base was converted into the hydrochloride salt by the addition of HCl/dioxane to give **14** (0.10 g, 80%), as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (s, 1H), 7.23-7.22 (m, 1H), 7.19-7.18 (m,

1H), 7.11-7.01 (m, 6H), 6.39 (br s, 1H), 3.86 (t, *J* = 6.6 Hz, 2H), 3.79 (t, *J* = 6.9 Hz, 2H), 3.69 (d, *J* = 12.3 Hz, 2H), 3.48 (d, *J* = 11.3 Hz, 2H), 3.23-3.08 (m, 6H), 1.78-1.62 (m, 6H), 0.85 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.75 (d, *J* = 235.8 Hz), 153.50, 146.20, 129.09, 128.78, 120.89, 120.80, 118.03 (d, *J* = 7.7 Hz), 115.56 (d, *J* = 21.9 Hz), 107.96, 107.91, 54.76, 50.42, 46.10, 41.90, 39.69, 25.26, 21.22, 20.30, 11.08. Anal. calcd for $C_{24}H_{33}Cl_2FN_4O$: C, 59.63; H, 6.88; N, 11.59. Found: C, 59.38; H, 6.66; N, 11.10.

4.1.11. 1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-pentyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**15**).

This compound was prepared from **12** as described for **14**. Yield 68%, as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (s, 1H), 7.24-7.21 (m, 1H), 7.18-7.16 (m, 1H), 7.11-7.01 (m, 6H), 3.86 (t, *J* = 6.8 Hz, 2H), 3.81 (t, *J* = 7.0 Hz, 2H), 3.68 (d, *J* = 12.7 Hz, 2H), 3.49 (d, *J* = 11.2 Hz, 2H), 3.23-3.08 (m, 6H), 1.78-1.62 (m, 6H), 1.31-1.21 (m, 4H), 0.82 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.93 (d, *J* = 236.0 Hz), 153.61, 146.23, 129.11, 128.88, 121.11, 121.00, 118.23 (d, *J* = 7.6 Hz), 115.70 (d, *J* = 21.9 Hz), 108.09, 108.08, 54.92, 50.60, 46.32, 40.44, 39.81, 28.42, 27.63, 25.31, 21.86, 20.47, 13.97. Anal. calcd for $C_{26}H_{37}Cl_2FN_4O$: C, 61.05; H, 7.29; N, 10.95. Found: C, 60.74; H, 7.25; N, 10.65.

4.1.12. 1-decyl-3-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-1,3-dihydrobenzimidazol-2-one dihydrochloride (**16**).

This compound was prepared from **12** as described for **14**. Yield 33%, as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.15 (s, 1H), 7.24-7.22 (m, 1H), 7.19-7.16 (m, 1H), 7.09-7.01 (m, 6H), 5.85 (br s, 1H), 3.86 (t, *J* = 6.7 Hz, 2H), 3.81 (t, *J* = 7.0 Hz, 2H), 3.68 (d, *J* = 11.9 Hz, 2H), 3.48 (d, *J* = 11.2 Hz, 2H), 3.17-3.06 (m, 6H), 1.76-1.62 (m, 6H), 1.15-1.20 (m, 14H), 0.83 (t, *J* = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.61 (d, *J* = 235.7 Hz), 153.41, 146.37, 129.00, 128.77, 120.86, 120.76, 117.87 (d, *J* = 7.7 Hz), 115.48 (d, *J* = 21.9 Hz), 107.88, 107.87, 54.77, 50.49, 46.02, 40.30, 39.75, 31.23, 28.88, 28.87, 28.61, 28.59, 27.76, 26.09, 25.20, 22.03, 20.29, 13.91. Anal. calcd for $C_{31}H_{47}Cl_2FN_4O$: C, 64.01; H, 8.14; N, 9.63. Found: C, 63.94; H, 8.04; N, 9.59.

4.1.13. 5-acetyl-1-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)-3-methyl-1H-benzo[d]imidazol-2(3H)-one dihydrochloride (**17**)

A solution of **13** (0.11 g, 0.27 mmol), potassium carbonate (0.11 g, 0.80 mmol), tetrabutylammonium iodide (0.30 g, 0.08 mmol) in anhydrous DMF (10 mL) was heated at 60 °C under argon. After 30 min, a solution of iodomethane (16.7 µl, 0.27 mmol) in anhydrous DMF (8 mL) was added dropwise over 1 h. After 3 h, a solution of iodomethane (5 µl, 0.08 mmol) in anhydrous DMF (3 mL) was added dropwise and the reaction was stirred for another 3 h. The mixture was then poured into 80 mL of water, extracted with ethyl acetate (3 x 50 mL) and the solvent was evaporated. The residue was purified by chromatography on a silica gel column using a gradient of ethyl acetate and methanol saturated with ammonia (10:0 to 9:1) as the eluent. The free base was converted into the hydrochloride salt by addition of HCl/dioxane to give **17** (0.11 g, 89%), as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.17 (br s, 1H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.73 (s, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.11-6.99 (m, 4H), 5.20 (br s, 1H), 3.92 (t, *J* = 6.7 Hz, 2H), 3.69 (d, *J* = 11.7 Hz, 2H), 3.49 (d, *J* = 11.0 Hz, 2H), 3.40 (s, 3H), 3.18-3.06 (m, 6H), 2.58 (s, 3H), 1.74-1.71 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.80, 156.77 (d, *J* = 236.0 Hz), 154.01, 146.17, 132.92, 130.42, 129.76, 122.84, 118.05 (d, *J* = 8.5 Hz), 115.56 (d, *J* = 21.8 Hz), 107.42, 107.39, 54.82, 50.48, 46.15, 40.10, 27.14, 26.50, 20.29. Anal. calcd for $C_{24}H_{31}Cl_2FN_4O_2$. H_2O: C, 55.92; H, 6.45; N, 10.87. Found: C, 54.88; H, 6.05; N, 10.58.

4.1.14. 5-acetyl-1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-propyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**18**).

This compound was prepared from **13** as described for **17**. Yield 89%, as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (br s, 1H), 9.69 (br s, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.74 (s, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.11-7.04 (m, 4H), 3.93-3.84 (m, 4H), 3.69 (d, *J* = 12.1 Hz, 2H), 3.49 (d, *J* = 11.3 Hz, 2H), 3.22 (t, *J* = 12.1 Hz, 2H), 3.19-3.09 (m, 4H), 2.57 (s, 3H), 1.79-1.64 (m, 6H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.83, 156.79 (d, *J* = 235.7 Hz), 153.85, 146.12, 132.90, 130.47, 129.13, 122.78, 118.07 (d, *J* = 7.6 Hz), 115.57 (d, *J* = 21.9 Hz), 107.52, 107.44, 54.74, 50.41, 46.13, 42.04, 40.45, 26.69, 25.28, 21.23, 20.26, 11.06. Anal. calcd for $C_{26}H_{35}Cl_2FN_4O_2$. 1/2H_2O: C, 58.43; H, 6.79; N, 10.48. Found: C, 58.85; H, 6.46; N, 10.46.

4.1.15. 5-acetyl-1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-3-pentyl-1,3-dihydrobenzimidazol-2-one dihydrochloride (**19**).

This compound was prepared from **13** as described for **17**. Yield 84%, as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (br s, 1H), 8.75 (br s, 1H), 7.76 (d, *J* = 8.2 Hz, 1H), 7.71 (s, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.11-7.02 (m, 4H), 3.93-3.86 (m, 4H), 3.69 (d, *J* = 12.4 Hz, 2H), 3.51 (d, *J* = 11.4 Hz, 2H), 3.23 (t, *J* = 11.8 Hz, 2H), 3.20-3.09 (m, 4H), 2.57 (s, 3H), 1.79-1.62 (m, 6H), 1.31-1.22 (m, 4H), 0.81 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.82, 156.85 (d, *J* = 235.7 Hz), 153.79, 146.07, 132.93, 130.47, 129.08, 122.84, 118.13 (d, *J* = 7.7 Hz), 115.59 (d, *J* = 21.9 Hz), 107.53, 107.32, 54.74, 50.39, 46.19, 40.87, 40.45, 28.28, 27.53, 26.69, 25.28, 21.75, 20.27, 13.86. Anal. calcd for $C_{28}H_{39}Cl_2FN_4O_2.1/2\ H_2O$: C, 59.78; H, 7.17; N, 9.96. Found: C, 59.71; H, 7.04; N, 9.93.

4.1.16. 5-acetyl-3-decyl-1-{4-[4-(4-fluorophenyl)piperazin-1-yl]butyl}-1,3-dihydrobenzimidazol-2-one dihydrochloride (**20**).

This compound was prepared from **13** as described for **17**. Yield 87%, as a white solid. ^{1}H NMR (free amine, 400 MHz, $CDCl_3$): δ 7.71 (dd, *J* = 6.7, 1.5 Hz, 1H), 7.61 (d, *J* = 1.3 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.93-6.89 (m, 2H), 6.84-6.81 (m, 2H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.87 (t, *J* = 7.4 Hz, 2H), 3.09 (t, *J* = 5.0 Hz, 4H), 2.61 (t, *J* = 4.9 Hz, 4H), 2.59 (s, 3H), 2.45 (t, *J* = 7.4 Hz, 2H), 1.79 (quint, *J* = 7.3 Hz, 2H), 1.72 (quint, *J* = 7.2 Hz, 2H), 1.59 (quint, *J* = 7.8, 2H), 1.30-1.21 (m, 16H), 0.83 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (free amine, 100 MHz, $CDCl_3$): δ 197.22, 157.44 (d, *J* = 237.6 Hz), 154.64, 147.98, 133.67, 131.13, 129.91, 123.47, 118.08 (d, *J* = 8.0 Hz), 115.74 (d, *J* = 22.0 Hz), 107.32, 106.86, 57.73, 53.12, 50.01, 41.57, 41.23, 32.04, 29.70, 29.45, 29.43, 28.60, 27.00, 26.65, 26.41, 23.73, 22.83, 21.67, 14.28. Anal. calcd for $C_{33}H_{49}Cl_2FN_4O_2$: C, 63.55; H, 7.92; N, 8.98. Found: C, 63.49; H, 7.67; N, 8.81.

4.2. Competition binding assays for the σ_1R and σ_2R

Preparation of rat brain membranes and *in vitro* binding assays for the σ_1R and σ_2R were performed using methods published previously in detail [31]. In brief, homogenates of whole rat brain excluding the cerebellum (400-500 µg) were incubated with 5 nM [^{3}H](+)-pentazocine to label σ_1Rs, or 3 nM [^{3}H]-DTG in the presence of 300 nM (+)-pentazocine to label σ_2Rs. Non-

specific binding was determined in the presence of 10 μM haloperidol. Ten concentrations (0.1-1000 nM) of each test compound were incubated for 120 min at 25 ^{o}C in 50 mM Tris-HCl, pH 8.0 to measure their ability to displace each radioligand from its binding sites. The total reaction volume was 500 μl. All of the assays were terminated with the addition of ice-cold buffer and rapid vacuum filtration over glass fiber filters. Counts were extracted from the filters using Ecoscint (National Diagnostics, Manville, NJ) for at least 8 h prior to counting. K_i values were calculated using the Cheng-Prusoff equation [37] and K_d values determined in separate saturation assays.

4.3. Extended non-σ binding profile

The two compounds with the highest σ_1/σ_2 ratios (**14** and **15**) were tested for interactions with other binding sites known to interact with many historic σ compounds and psychostimulants: opioid receptors, PCP binding site on NMDA receptors, dopamine (D_2) receptors, serotonin (5-HT_2) receptors, monoamine transporters (dopamine, serotonin, norepinephrine). Well-established, previously published methods were used [28]. Briefly, dopamine transporters were labeled using 0.5 nM [^{3}H]WIN 35,428 in membranes prepared from rat striata; nonspecific binding was determined in the presence of 50 μM cocaine. Serotonin transporters were labeled using 0.2 nM [^{3}H]paroxetine in membranes prepared from rat brainstem; nonspecific binding was determined in the presence of 1.5 μM imipramine. Norepinephrine transporters were labeled using 0.5 nM [^{3}H]nisoxetine in membranes prepared from rat cerebral cortex; nonspecific binding was determined in the presence of 4 μM desipramine. All of the receptor assays were conducted using rat brain membranes minus cerebellum, similarly to those for *σ*Rs. Dopamine (D_2) receptors were labeled using 5 nM [^{3}H](-)-sulpiride and nonspecific binding was determined with 1 μM haloperidol. Serotonin (5-HT_2) receptors were labeled using 2 nM [^{3}H]ketanserin and nonspecific binding was determined with 1 μM mianserin. The PCP site on NMDA receptors were labeled using 5 nM [^{3}H]1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) and nonspecific binding was determined with 10 μM cyclazocine. Opioid receptors were labeled using 0.5 nM [^{3}H]bremazocine and nonspecific binding was determined with 10 μM levallorphan. All of the assays were terminated with the

addition of ice-cold buffer and vacuum filtration through glass fiber filters. Counts were extracted and K_i values determined as described for the σR assays.

4.4. In vivo convulsion studies

Male, Swiss Webster mice (21-30 g; Harlan, USA) were pretreated (i.p.) with either saline or compound **14** (0.1, 1 or 10 mg/kg), 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. Animals were housed 1-5 per cage with a 12:12H light/dark cycle (lights on 06:00 hours) and *ad libitum* food and water. They were allowed to climatize following their arrival for one week prior to being utilized in any experiment. All procedures were approved by the Institutional Animal Care and Use Committee at West Virginia University.

Acknowledgments

We appreciate the technical assistance of Matthew Robson, Colleen Beatty, and Bahar Noorbakhsh. This project was supported by grants from NIDA R01 DA013978 and R01 DA023205.

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Benzimidazolone-based selective σ_2 receptor ligands: synthesis and pharmacological evaluation

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Highlights

- A series of benzimidazolone derivatives as highly selective σ_2R ligands were prepared and characterized.
- Replacement of the heterocyclic scaffolds of the lead compounds with a benzimidazolone moiety increased the σ_2 selectivity.
- The cyclic amine fragment 1-(4-fluorophenyl)piperazine is preferred rather than 1-cyclohexylpiperazine for the σ_2 selectivity.
- Introduction of n-propyl and n-pentyl group at *N*-3 position of the benzimidazolone, resulted in better affinity and selectivity for the σ_2R.
- Selective σ_2R ligands might attenuate cocaine-induced convulsions.

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