Novel Highly Potent and Selective Sigma1 Receptor Antagonists Effectively Blocking the Binge Eating Episode in Female Rats

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ABSTRACT

In this paper the benzo-cracking approach was applied to the potent sigma1 (σ_1) receptor antagonist **1** to afford the less conformationally constrained 1,3-dioxane derivatives **2** and **3**. To evaluate the effect of the increase of the distance between the two hydrophobic structural elements that flank the basic function, the *cis* and *trans* diastereomers of **4** and **5** were also prepared and studied. Compounds **2** and **3** showed affinity values at σ_1 receptor significantly higher than that of the lead compound **1**. In particular, **3** displayed unprecedented selectivity over σ_2 receptor, the phencyclidine site of the NMDA receptor, and opioid receptor subtypes, as well as over dopamine transporter. Docking results supported the structure-activity relationship studies. Due to its interesting biological profile, derivative **3**, selected for an *in vivo* study in a validated preclinical model of binge eating, was able to counteract the overeating of palatable food only in binging-rats, without affecting palatable food intake in the control group and anxiety-like and depression-related behaviors in female rats. This result strengthened the involvement of σ_1 receptor in the compulsive-like eating behavior and supported σ_1 receptor as a promising target for the management of eating disorders.

Keywords: selective sigma1 ligands, binge eating episode, highly palatable food, open field test, forced swimming test.

INTRODUCTION

Sigma (σ) receptors are scarcely understood transmembrane proteins involved in a large number of cellular functions.[1] Initially, they were classified as subtypes of the opioid receptor family and, subsequently, it was hypothesized that they corresponded to the phencyclidine (PCP) binding site of the ionotropic *N*-methyl-D-aspartate (NMDA) receptor. At present, they are reported as a distinctive receptor family, comprised of two subtypes (σ_1 and σ_2 receptors).[1] Both subtypes have been cloned[2-5] and the crystal structures of σ_1 receptor complexed with known agonists and antagonists have recently been reported.[6, 7] σ_1 receptors work as molecular chaperones in the mitochondria-associated endoplasmic reticulum (ER) membrane and play a role in the cellular stress response and homeostasis.[8, 9]

Their wide distribution in the nervous system and their involvement in several physiological and pathological conditions make σ_1 receptors very promising targets for the management of numerous disorders. In particular, central σ_1 receptors are implicated in different neuropsychiatric and neurodegenerative diseases,[10-12] as well as in pain.[13] The observation that the σ_1 agonist ANAVEX™ (NCT02244541) and the σ_1 antagonist E-52862 (EudraCT number: 2012-000400-14) are being evaluated in clinical trials for the treatment of Alzheimer's disease and neuropathic pain, respectively, supports the validity of σ_1 receptors as clinical targets.[14] Moreover, experimental evidence has demonstrated that the blockade of σ_1 receptors can counteract the addictive effects elicited by psychostimulants[15, 16] and ethanol.[17-20] While several papers report the involvement of σ_1 receptors in drug abuse, very few studies suggest that this receptor system is implicated in binge eating behavior, despite many behavioral and brain mechanisms overlapping between food and drug addiction. In fact, compulsive fast overeating and strong craving, with a consequent withdrawal for hedonic food and impulsivity, are features correlated with binge eating behavior, similarly to the substance dependence.[21, 22] In a pioneering study, the σ_1 antagonist BD-1063 (Figure 1) proved to reduce binge-like eating and to block compulsive eating in palatable rats, suggesting that the σ_1 receptor system might play a role in binge-like eating following to neurobiological adaptations.[23]

Moreover, a relationship between food reinforced operant responding and σ_1 receptors has been recently highlighted. Indeed, the potent σ_1 antagonist PD144418 (Figure 1) demonstrated to decrease the motivational effort of a food-reinforced behavior maintaining food palatability.[24] Finally, in a recent study we demonstrated that the spipethiane analog 2-(1-benzylpiperidin-4-yl)thiochroman-4-one (Figure 1), behaving as a potent σ_1 receptor antagonist,[25] decreased the binge eating episode in female rats, supporting the involvement of σ_1 receptors in compulsive-like eating disorder.[26]

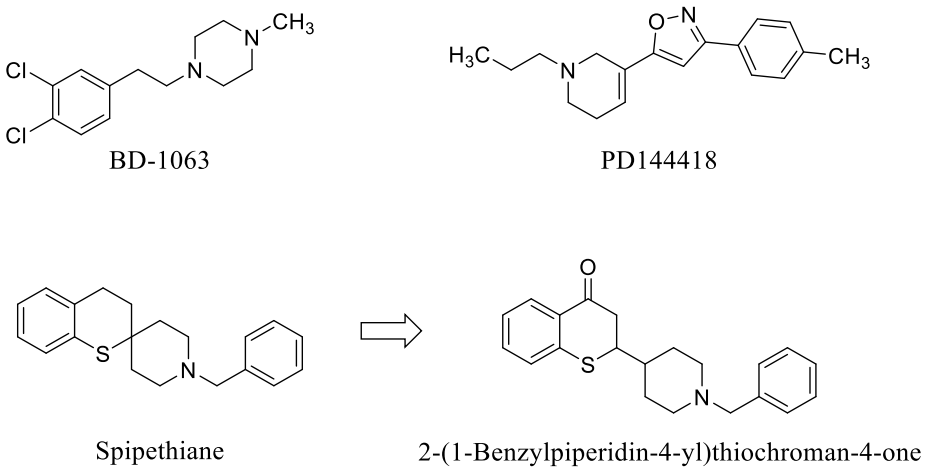


Figure 1. Structures of the σ_1 antagonists BD-1063, PD144418, spipethiane and 2-(1-benzylpiperidin-4-yl)thiochroman-4-one.

Among the analogs of spipethiane, another potent σ_1 receptor ligand (pK_i = 10.05), endowed with high σ_1/σ_2 selectivity (2515), is the 1,3-benzodioxane derivative **1** (Figure 2). Functional assays performed on MCF-7 and MCF-7/ADR highlighted the σ_1 antagonist profile of this compound.[25] With the aim to improve the σ_1 receptor affinity and selectivity over σ_2 subtype, the conformationally constrained 1,3-benzodioxane moiety of **1** was replaced by the more flexible 1,3-dioxane nucleus by benzo-cracking approach.[27] In particular, derivatives **2** and **3**, in which the phenyl substituent is linked to the positions 4 or 5 of the 1,3-dioxane ring, respectively, were prepared and studied (Figure 2). Moreover, to evaluate the effect of the distance between the two hydrophobic portions that flank the

basic function of **2** and **3**, the diastereomers **4a/b** and **5a/b** were also prepared and studied. In these novel derivatives, the *N*-benzylpiperidine moiety is spaced from the 1,3-dioxane ring (Figure 2), resulting in a further increase in the conformational flexibility of the molecule. The separation of the *cis* and *trans* diastereomers of **4** and **5** permitted us to evaluate the role played by the relative configuration on the σ_1 receptor affinity.

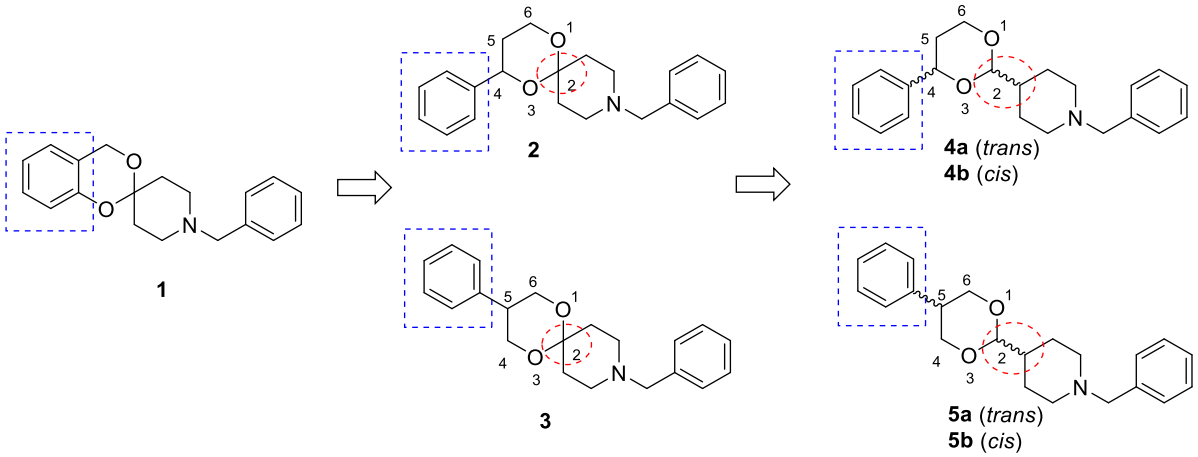
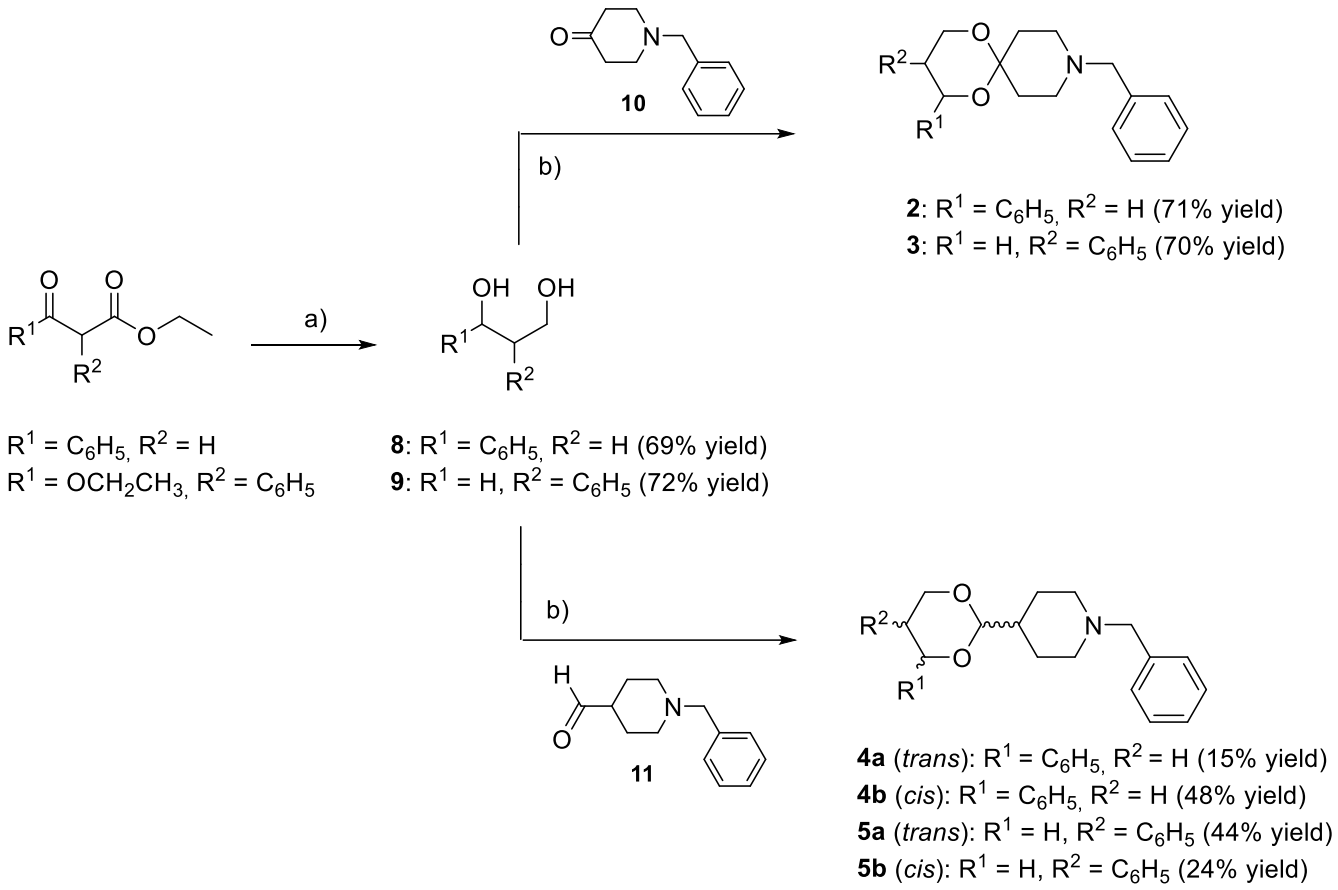


Figure 2. Structures of **2-5**, analogs of the potent σ_1 ligand **1**.

The novel derivatives **2-5** were tested by radioligand binding assays at σ_1 and σ_2 receptors. Moreover, to confirm the involvement of the σ_1 receptor system in binge-like eating disorder, the aim of this work was also the evaluation of the most interesting compound **3** in a female rat model of binge eating. Finally, the affinities of compounds **2** and **3** were also assessed at the PCP site of the NMDA receptor, opioid receptors and/or dopamine transporter (DAT), all of which playing a role in binge eating disorders,[28] considering that many σ_1 ligands also bind these targets with high affinity.

RESULTS AND DISCUSSION

Derivatives **2-5** were synthesized following the synthetic route reported in Scheme 1.

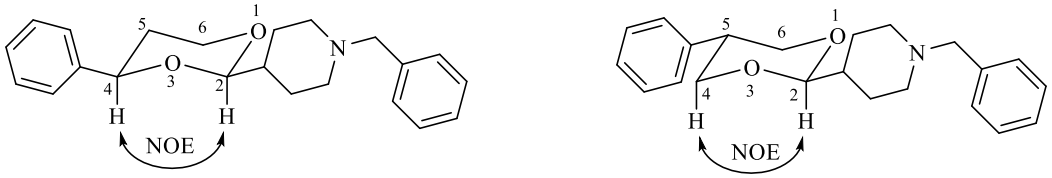


Scheme 1. a) $LiAlH_4$, Et_2O, r.t. for 2 h b) *p*-toluenesulfonic acid, toluene, reflux for 5 h.

The commercially available ethyl 3-oxo-3-phenylpropionate (**6**) and diethyl 2-phenylmalonate (**7**) were subjected to a reduction reaction with $LiAlH_4$ to the corresponding diols **8** and **9**, respectively. The condensation of **8** and **9** with the suitable *N*-benzylpiperidine carbonyl derivatives **10** and **11** in presence of *p*-toluenesulfonic acid afforded the desired derivatives **2** and **3** and the mixtures of the diastereomers **4a/b** and **5a/b**, respectively. The *cis* and *trans* diastereomers of **4** and **5** were separated by flash chromatography.

The stereochemical relationship between the *N*-benzylpiperidine moiety in the position 2 and the phenyl substituent in the positions 4 or 5 of **4a**/**b** and **5a**/**b**, respectively, was determined by ^{1}H NMR analysis (NOESY studies). In particular, an evident NOE was observed between the protons in the positions 2 and 4 (4.48 and 4.65 ppm, respectively) of **4b**, highlighting that both the piperidine and

phenyl rings in the positions 2 and 4, respectively, are equatorially oriented. Therefore, the stereochemical relationship between the substituents in the positions 2 and 4 is *cis* in **4b** and, consequently, *trans* in **4a** (Figure 3). Concerning **5a**, the axial proton in the position 4 (δ 3.78 ppm) showed two large coupling constants (J = 10.8 Hz and J = 11.3 Hz), one with the geminal equatorial proton and the other with the axial proton in the position 5. Consequently, the phenyl ring adopts an equatorial orientation. Moreover, a clear NOE was observed between the axial protons in the positions 2 and 4 at 4.36 and 3.78 ppm, respectively, evidencing that the *N*-benzylpiperidine moiety also adopts an equatorial orientation. Therefore, the relative configuration between the substituents in the positions 2 and 5 is *trans* in **5a** and, consequently, *cis* in **5b** (Figure 3).



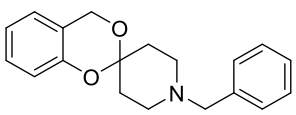
4b **5a**

Figure 3. Structure of compounds **4b** and **5a**.

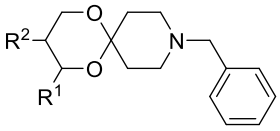
The affinities of compounds **2-5** for σ_1 and σ_2 receptors were assessed on guinea pig brain and rat liver membranes, respectively. [^{3}H]-(+)-pentazocine and [^{3}H]-di-*o*-tolylguanidine in presence of an excess of (+)-pentazocine were used as radioligands for σ_1 and σ_2 receptors, respectively.[29, 30] The pK_i values are reported in Table 1. The lead compound **1** was included for useful comparison.

Compounds **2** and **3** were also evaluated for their affinity for DAT, the PCP site of the NMDA receptor as well as μ, κ and δ opioid receptor subtypes. The assays were performed with rat striatal ([^{3}H]-WIN35,428), pig brain cortex ([^{3}H]-(+)-MK-801), guinea pig brain ([^{3}H]-DAMGO), guinea pig brain ([^{3}H]-U-69593) and rat brain ([^{3}H]-DPDPE) membranes for DAT, NMDA, μ-, κ- and δ-opioid receptors, respectively.[29, 31-33] The p*K*i values are shown in Table 1.

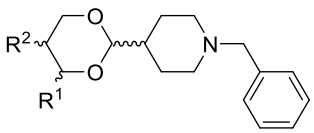
Table 1. Affinity values (pK_i)[a] of **1**-**5** at σ_1 and σ_2 receptors and of **2** and **3** at DAT, the PCP site of the NMDA receptor, μ, κ and δ opioid receptor subtypes.



1



2: $R^1 = C_6H_5$, $R^2 = H$
3: $R^1 = H$, $R^2 = C_6H_5$



4a (*trans*): $R^1 = C_6H_5$, $R^2 = H$
4b (*cis*): $R^1 = C_6H_5$, $R^2 = H$
5a (*trans*): $R^1 = H$, $R^2 = C_6H_5$
5b (*cis*): $R^1 = H$, $R^2 = C_6H_5$

Compd	pK_i						
	σ_1	**σ_2**	**DAT**	**NMDA**	**μ**	**κ**	**δ**
1	10.05±0.08	6.65±0.09	-	-	-	-	-
2	11.00±0.07	6.33±0.11	<5	<5	<5	<5	8.60±0.14
3	10.89±0.05	6.09±0.07	5.63±0.09	<5	<5	<5	5.82±0.08
4a	8.43±0.07	6.75±0.10	-	-	-	-	-
4b	9.62±0.15	7.42±0.08	-	-	-	-	-
5a	8.44±0.14	7.25±0.02	-	-	-	-	-
5b	8.31±0.06	6.60±0.10	-	-	-	-	-

[a]Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng-Prusoff equation.[34] The reported pK_i values are the mean ± S.E.M. of three to five independent experiments, each performed in triplicate, according to the methods described in the S.I.

The data reported in Table 1 reveal that the benzo-cracking approach performed on the 1,3-benzodioxane derivative **1** is favorable for the binding to σ_1 receptor, while causes a slight reduction in σ_2 receptor affinity, with a consequent increase in σ_1/σ_2 selectivity. In fact, both compounds **2** and **3** display very high affinity for σ_1 receptor and a remarkable σ_1/σ_2 selectivity. Several potent σ_1 ligands belonging to different chemical classes and highly selective over σ_2 receptor have been discovered.[35] Interestingly, **3** shows an impressive σ_1/σ_2 selectivity ratio (σ_1/σ_2 = 63096) and, to our knowledge, is the most selective σ_1 ligand reported so far. A significant reduction in affinity for σ_1 receptor and an increase in those for σ_2 are observed when the benzo-cracking approach is combined with the further increase in the distance between the two lipophilic moieties of **2** and **3** (compounds **4a/b** and **5a/b**, respectively). Consequently, the σ_1/σ_2 affinity ratios displayed by **4a/b** and **5a/b** are

significantly lower than those of **2** and **3**. Probably, the increase in the conformational freedom and in the distance between the two lipophilic portions is detrimental for the optimal interaction with σ_1 receptor. Stereochemistry appears to play a role in the binding to the σ_1 receptor when the phenyl ring is in the position 4 of the 1,3-dioxane nucleus, with the *cis* isomer **4b** showing an affinity value significantly higher than that of the *trans* diastereomer **4a**. On the contrary, the *trans* and *cis* 5-phenyl diastereomers **5a** and **5b** show similar affinity values.

From the results obtained with the off-targets it emerges that ligand **2** shows negligible affinity for DAT, NMDA, μ and κ opioid receptors, and high affinity for δ subtype (pK_i = 8.60), although it is 251-fold lower than that for σ_1. Interestingly, compound **3**, which also binds δ receptor with sub-micromolar affinity, displays a remarkable selectivity for σ_1 receptor over all the evaluated targets (σ_1/DAT = 181970, σ_1/NMDA > 776247, σ_1/μ > 776247, σ_1/κ > 776247, σ_1/δ = 117490). The binding profile of **3** is noticeable, given that many potent σ_1 ligands also bind to DAT, NMDA and/or opioid receptors with high affinity.[1, 36, 37]

To rationalize the affinity profiles of the proposed ligands at the σ_1 receptor, docking simulations were performed based on the resolved σ_1 structure (PDB Id: 5HK1) using the PLANTS software and following the same recently reported computational protocol.[26] As discussed below, the complex stability is evaluated by calculating the APBS score which is focused on the polar interactions.[38] Figure 4 compares the computed putative poses for **1** (Figure 4A) and **2** (Figure 4B) and reveals some differences which can justify the increase of affinity observed for the latter.

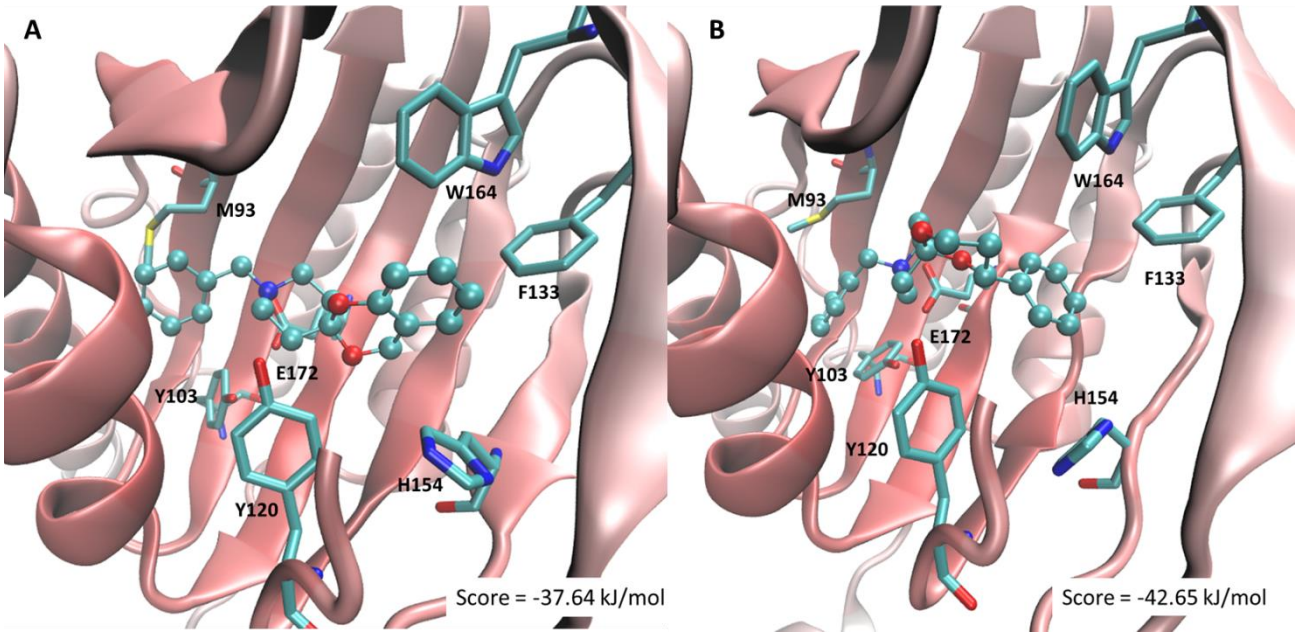


Figure 4. Main interactions stabilizing the putative complexes for **1** (A) e (*R*)-**2** (B) as computed using the resolved σ_1 receptor structure. The reported scores are calculated by using the APBS method.

In detail, Figure 4A highlights the key interactions stabilized by **1** which can be schematized as follows: (a) the ligand ammonium head stabilizes a clear ion-pair with Glu172 reinforced by a H-bond with Tyr103; (b) the benzyl moiety is inserted within a hydrophobic subpocket where it mostly contacts alkyl side-chains plus π–π stacking with Tyr103 and a π–Sulfur contact with Met93; (c) the benzodioxane system is accommodated within a subpocket lined by several aromatic residues while the O1 oxygen atom is engaged by a H-bond with Tyr120. The enantiomers of **2** afford very similar putative complexes and attention is here focused on the complex for (*R*)-**2** since it shows a slightly better APBS score compared to (*S*)-**2** (-42.56 vs. -41.38 kJ/mol). Specifically, Figure 4B emphasizes that (*R*)-**2** elicits an interaction pattern very similar to that already seen in Figure 4A, even though some key interactions appear to be enhanced when compared to those elicited by **1**. This positive effect can be seen in the contacts stabilized by: (a) the benzyl moiety which elicits an optimized π–π stacking with Tyr103; (b) the dioxane oxygen atoms which better approach Tyr120 and (c) the phenyl ring which is engaged by an extended set of π–π stacking interactions with Phe107, Phe133, His154

and Trp164. These reinforced contacts are reflected into better complex stability as encoded by the scores displayed in Figure 4A. As also confirmed by APBS score (-38.91 kJ/mol), compound **3** yields an in-between docking result, with the two aromatic rings being engaged by enhanced contacts, while the dioxane ring is unable to conveniently approach Tyr120, as seen in Figure 4B. Finally, the compounds **4** and **5** reveal computed poses rather similar to those observed for the previous ligands, even though the free dioxane ring assumes a rather different arrangement which hampers its interactions with Tyr120. The lack of this contact induces flipped poses of the most hindered ligands by which the dioxane ring approaches Tyr103.

Considering its intriguing σ_1 affinity and selectivity profile, compound **3** was selected for the *in vivo* study, using a validated preclinical animal model of binge eating, to further investigate the function of σ_1 receptor system on compulsive-like eating disorder. Female rats were used in relation to the higher prevalence of binge eating disorder and bulimia nervosa in women than in men.[39] In the binge eating model,[40-42] female rats were randomly separated into four groups: non restricted and not exposed to stress group (NR + NS); non restricted and exposed to stress group (NR + S); restricted and not exposed to stress group (R + NS); restricted and exposed to stress group (R + S). The association of three consecutive food restriction/refeeding periods and acute stress is able to trigger a strong increase of highly palatable food (HPF) consumption only in R + S rats in a short period of time (120 min). Stress is induced by placing a coffee cup containing HPF for 15 min, letting the animal see the cup and smell HPF odor, without the possibility to eat it. Thus, on the binge test day, NR + NS and R + NS had immediate access to HPF for 120 min, whereas NR + S and R + S had free access to it only after 15 min of stress. This stressful condition, although mild, has already shown to enhance the corticosterone blood level in stressed rats.[43-46] In line with our previous studies,[47, 48] the ANOVA in the vehicle groups revealed a marked interaction among the three factors (food restriction x stress x sessions time) [$F_{interaction}$ (3,72) = 4.8; $P < 0.01$]. Bonferroni post hoc test revealed a significant ($P < 0.01$) increase in HPF consumption in the first 15 min in the R + S group (binging group), compared to the other three groups. On the other hand, during the time of the other sessions

(15–30; 30-60; 60-120 min) no change in HPF intake was observed among all groups (Figure 5, left panel). At the end of the binge eating test (120 min) one-way ANOVA showed a two-way interaction (food restriction x stress) [$F_{interaction}$ (1,24) = 4.3; $P < 0.05$] and the post hoc analyses ($P < 0.01$) revealed that only R + S rats significantly enhanced HPF eating with respect to the other rats (Figure 5, right panel). Thus, the stress exposure induced binge-like behavior only in previously restricted rats, which consumed a large amount of HPF within 15 min and no compensatory changes during the remaining 15–120 min were detected.

Acute intraperitoneal (i.p.) injection of **3**, 30 min before giving access to HPF, selectively blocked the episode of binge eating in a dose-dependent manner in the R + S group, without affecting consumption in the other experimental groups during 120 min of observation (Figure 5B-E).

Specifically in R + S rats, ANOVA reported a significant effect of treatment at 0–15 min [$F(2,20) = 6.7$; $P < 0.05$] and in 0–120 min [$F(2,20) = 10.9$; $P < 0.01$]. Post hoc analyses indicated that both used dosages (3 or 7 mg/kg) significantly decreased HPF consumption in R + S at each time point as indicated in Figure 5E.

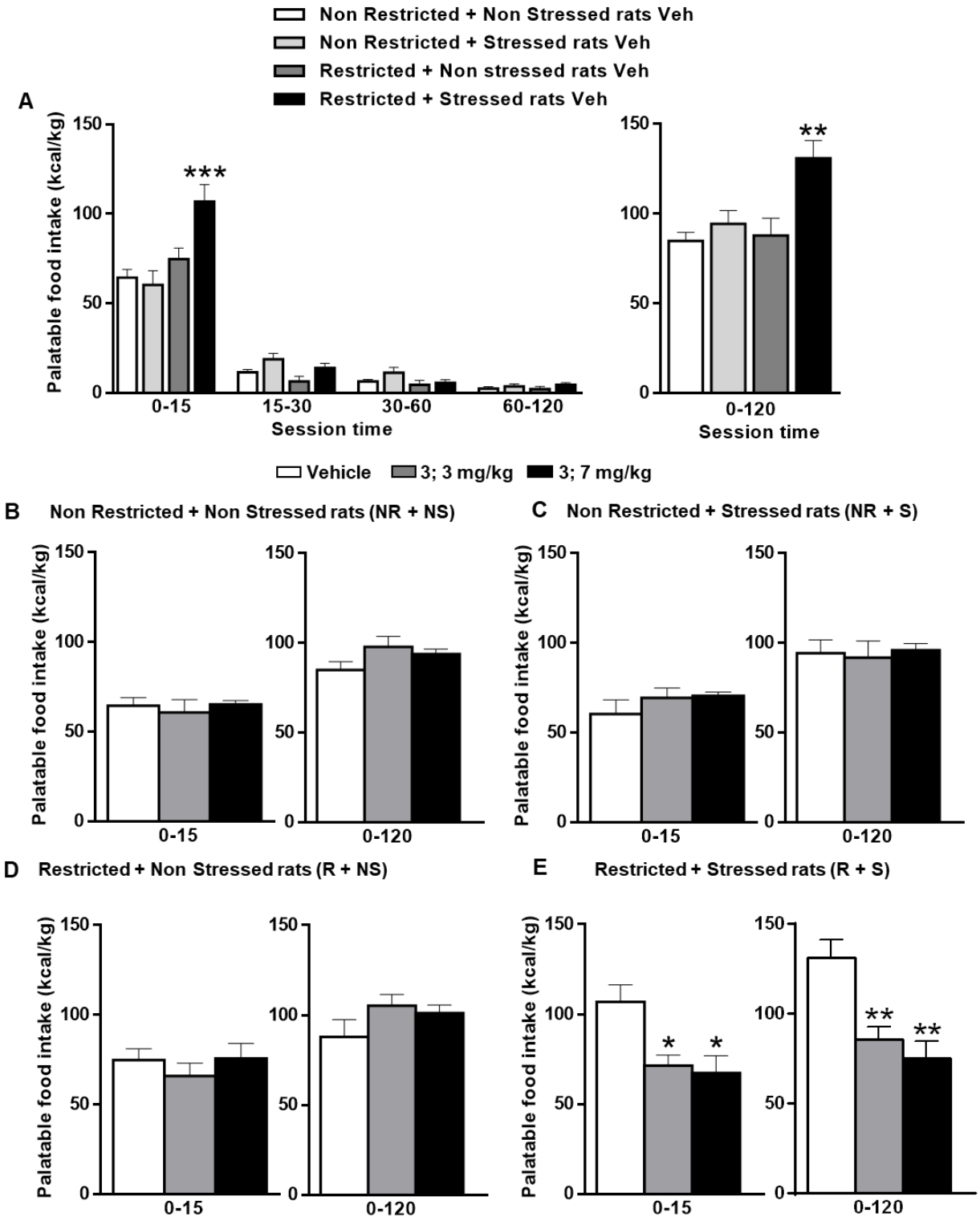


Figure 5. Administration of **3** blocked the episode of binge eating. **A**. HPF intake shown in kcal/kg at different sessions time (0-15, 15-30, 30-60, 60-120 min; left) and at 120 min (right) in the vehicle (veh) injected rats. **P < 0.01; ***P < 0.001 different from the other three groups. **B.** HPF eating (kcal/kg) after 15 min (left) or 120 min (right) to free access to cup containing chocolate paste in veh or treated rats: NR + NS (**B**, Non Restricted + Non Stressed), NR + S (**C**, Non Restricted + Stressed), R + NS (**D**, Restricted + Non Stressed), R + S (**E**, Restricted + Stressed) groups. *P < 0.05; ***P < 0.01 vs R + S veh. Data are expressed as mean ± SEM. N = 6-8 per group.

In addition, to assess if the systemic injection of **3** may influence different aspects of animal behavior in the control or binging group, the open field (OF) and forced swimming tests (FST) were performed. The OF test is a validated test, commonly used for evaluating locomotor activity and anxiety-like behavior in rodents in an unfamiliar environment,[49] while FST is a suitable tool for evaluating a depressed state.[50] The administration of **3** showed to not affect any measured behavioral parameters in these present tests. In fact, analyzing the locomotor activity in the entire OF arena, ANOVA showed a significant effect of restriction and stress conditions [$F_{restriction}$ (1,48) = 7.9; $P < 0.01$; F_{stress} (1,48) = 30.9; $P < 0.001$] and no effect of the treatment with **3** [$F_{treatment}$ (1,48) = 0.6; $P > 0.05$]. R + S veh and R + S **3** (7 mg/kg) showed the highest distance travelled compared to the other groups (Table 2).

Table 2. Behavioral parameters in female rats performing the open field and forced swimming tests

OPEN FIELD TEST								
Parameters	**NR + NS**		**NR + S**		**R + NS**		**R + S**	
	Veh	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**
Tot. dist. trav. (cm)	2305.3±357.3	2492.3±225.3	3602.9±269.3	3467.2±411.8	2626.2±242.5	3132.4±331.6	4335.9±419.3*	4461.5±346.3*
Tot. vert. counts	99±4	93.7±5	123.9±6.1	125.9±5.4	87±3.3	94.3±6.6	131.7±4.3	126.6±4.5
Jump counts	104.6±4.8	113±17.1	137.1±2.1	152±32.5	97.7±6.3	119.5±9.1	172±20.6	170.4±19
Stereot. counts	2367.1±116.9	2500.3±89.3	2477.4±161.4	2270.4±137.5	2030.5±280.8	2516.7±79.2	2305.1±79.7	2401±119.6
Cent. dist. trav. (cm)	101.2±25.6	112.7±7.1	140.3±30.1	148.1±13.1	84.3±17.1	96.4±6	179.9±24.8	177.5±37.5
Cent. zone entries	26.4±4.6	28.1±2.7	34.5±4.1	34±6.8	18.7±2.8	22.5±2.1	50.4±2.4	47.2±10
FORCED SWIMMING TEST								
Parameters	**NR + NS**		**NR + S**		**R + NS**		**R + S**	
	Veh	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**	**Veh**	**3 (7 mg/kg)**
Immobility time (s)	100.4±12.94	106.7±11.2	92.9±13.5	111.1±11.2	99.6±10.4	94.8±9.2	158.6±10*	163.5±22.5*

In the entire open field arena: Tot. dist. trav. (cm): total distance travelled; Tot. vert. counts: total vertical counts; Jump counts; Stereot. counts: stereotypic counts. In the central zone of the open field box: Cent. dist. trav. (cm): distance travelled in the center; Cent. zone entries: number of entrances in the central zone. Data are the mean ± SEM. *$p < 0.05$ vs the other groups. N = 6-8 per group.

Regarding the other parameters, jump and total vertical counts were significantly affected only by stress [F_{stress} (1,48) = 11.9; $P < 0.01$] and [F_{stress} (1,48) = 86.7; $P < 0.001$], respectively, but not by

restriction or treatment conditions. As shown in Table 2, the stress procedure appeared to increase the general arousal and this effect was confirmed by the significant gain in vehicle or treated stressed rats (NR + S and R + S) on distance travelled in the central zone [F_{stress} (1,48) = 19.7; P < 0.001] and on zone entries [F_{stress} (1,48) = 39.2; P < 0.001] into the central zone. In particular, the latest finding also suggested that stress does not influence anxiety-like behavior in stressed rats. Notably, the reduction of distance travelled or low numbers of entries into the central zone of the OF, marked an increased emotionality and anxiety in rodents.[51]

Finally, no difference in stereotypic counts was found among the groups [$F_{restriction}$ (1,48) = 0,7; P > 0.05; F_{stress} (1,48) = 0.02; P > 0.05; $F_{treatment}$ (1,48) = 1.6; P > 0.05].

In addition, using the FST, ANOVA revealed that the immobility time was significantly impacted by restriction [$F_{restriction}$ (1,49) = 8,2; P < 0.01], stress [F_{stress} (1,49) = 11.5; P < 0.01] and by the interaction between these two factors [$F_{interaction}$ (1,49) = 12.7, P < 0.01], while compound **3** [$F_{treatment}$ (1,48) = 1.6; P > 0.05] did not change the swimming/floating behavior.

Post hoc test exhibited a significantly longer immobility time in vehicle or treated R + S rats compared with the other groups, revealing that the cycle of food restriction plus stress may increase depression-like behaviors in female rats.

In summary the stressed rats, particularly R + S, showed an increase of spontaneous locomotor and exploratory activity, including the central zone of OF test and the vehicle binging rats revealed the longest immobility time in FST. In this context, **3** pretreatment did not impact the anxiety and depression-like behaviors in the control groups (NR + NS or NR + S or R + NS) and did not alter the emotional state detected in the binging rats.

CONCLUSIONS

The replacement of the conformationally constrained 1,3-benzodioxane structure of **1** with the more flexible 1,3-dioxane ring by benzo-cracking approach led to derivatives **2** and **3**, which show very high affinity for σ_1 receptor and a remarkable selectivity over σ_2 subtype. Docking studies rationalized

the affinity profiles of the proposed ligands on the σ_1 receptor and gave useful information about the binding mode of this class of compounds. Showing significant affinity also for δ opioid subtype, **2** might be considered a dual σ_1/δ receptor ligand. Interestingly, compound **3** displays an uncommon selectivity for σ_1 receptor over all the other evaluated targets. In *in vivo* studies, it was able to counteract the overeating of HPF only in binging-rats, without affecting HPF intake in the control group and anxiety-like and depression-related behaviors in female rats. These findings reinforce the potential use of σ_1 receptor antagonism to selectively block compulsive eating in binging rats, suggesting σ_1 receptor antagonists as promising candidates to treat binge episode, and are noteworthy, considering that, at present, the treatment approaches to manage pathological feeding behavior are limited.

METHODS

Chemistry

General

Instruments used for the synthesis and characterization of compounds **2-9** are reported in the Supporting Information.

9-Benzyl-2-phenyl-1,5-dioxa-9-azaspiro[5.5]undecane (2)

A mixture of **8** (1.7 g, 11.16 mmol), **10** (2.11 g, 11.16 mmol) and p-toluenesulfonic acid (0.85 g, 4.85) in toluene (50 mL) was heated at reflux for 5 h. After the mixture was cooled, water was added. The aqueous phase was basified with 2N NaOH and extracted three times with $CHCl_3$. The organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography. Eluting with cyclohexane/EtOAc (7:3) afforded an oil (71% yield). ^{1}H NMR ($CDCl_3$) δ 1.61-2.60 (m, 10H, C*H*$_2$ and piperidine), 3.51 (s, 2H, NC*H*$_2$Ar) 3.90 (m, 1H, C*H*$_2$O), 4.13 (m, 1H, C*H*$_2$O), 4.98 (dd, 1H, ArC*H*O), 7.21-7.42 (m, 10H, Ar*H*). ESI/MS: m/z 324.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from EtOH: mp 202-204 °C. Anal. Calcd for $C_{21}H_{25}NO_2 \cdot H_2C_2O_4$: C, 66.81%; H, 6.58%; N, 3.39%. Found: C, 67.05%; H, 6.42%; N, 3.50%.

9-Benzyl-3-phenyl-1,5-dioxa-9-azaspiro[5.5]undecane (3)

This compound was synthesized from **9** and **10** according to the procedure described for **2**: an oil was obtained (70% yield). ^{1}H NMR ($CDCl_3$) δ 1.82 (m, 2H, piperidine), 2.18 (m, 2H, piperidine), 2.50 (m, 4H, piperidine), 3.18 (m, 1H, C*H*Ar), 3.53 (s, 2H, NCH_2Ar) 3.99 (m, 4H, 2 x CH_2O), 7.21-7.39 (m, 10H, Ar*H*). ESI/MS: m/z 324.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from EtOH: mp 211-212 °C. Anal. Calcd for $C_{21}H_{25}NO_2 \cdot H_2C_2O_4$: C, 66.81%; H, 6.58%; N, 3.39%. Found: C, 66.59%; H, 6.40%; N, 3.19%.

1-Benzyl-4-(4-phenyl-1,3-dioxan-2-yl)piperidine (4)

This compound was synthesized from **8** and **11** according to the procedure described for **2**, to give a mixture of the diastereomers **4a** and **4b**, that were separated by flash chromatography, eluting with cyclohexane/EtOAc (95:5).

The isomer **4a** eluted first as an oil (15% yield). ^{1}H NMR ($CDCl_3$) δ 1.28-2.42 (m, 9H, piperidine), 2.94 (m, 2H, piperidine), 3.49 (s, 2H, NCH_2Ar), 3.92 (m, 1H, CH_2O), 4.16 (m, 1H, CH_2O), 4.42 (d, 1H, *J* = 6.5 Hz, OC*H*O), 5.19 (m, 1H, ArC*H*O), 7.20-7.42 (m, 10H, Ar*H*). ESI/MS: m/z 338.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from 2-PrOH: mp 101-102 °C. Anal. Calcd for $C_{22}H_{27}NO_2 \cdot H_2C_2O_4$: C, 67.43%; H, 6.84%; N, 3.28%. Found: C, 67.27%, H, 6.96%; N, 3.50%.

The second fraction was the isomer **4b** (48% yield). ^{1}H NMR ($CDCl_3$) δ 1.19-1.94 (m, 9H, piperidine), 2.92 (m, 2H, piperidine), 3.50 (s, 2H, NCH_2Ar), 3.89 (m, 1H, CH_2O), 4.20 (m, 1H, CH_2O), 4.48 (d, 1H, *J* = 5.6 Hz, OC*H*O), 4.65 (dd, 1H, *J* = 11.3, 2.3 Hz, ArC*H*O), 7.20-7.42 (m, 10H, Ar*H*). ESI/MS: m/z 338.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from EtOH: mp 161-162 °C. Anal. Calcd for $C_{22}H_{27}NO_2 \cdot H_2C_2O_4$: C, 67.43%; H, 6.84%; N, 3.28%. Found: C, 67.70%, H, 6.98%; N, 3.05%.

1-Benzyl-4-(5-phenyl-1,3-dioxan-2-yl)piperidine (5)

This compound was synthesized from **9** and **11** according to the procedure described for **2**, to give a mixture of the diastereomers **5a** and **5b**, that were separated by flash chromatography eluting with cyclohexane/EtOAc (95:5).

The isomer **5a** eluted first as an oil (44% yield). ^{1}H NMR ($CDCl_3$) δ 1.40-1.98 (m, 7H, piperidine), 2.88 (m, 2H, piperidine), 3.18 (m, 1H, C*H*Ar), 3.50 (s, 2H, NCH_2Ar) 3.78 (dd, 1H, *J* = 11.3, 10.8 Hz, CH_2O), 4.17 (dd, 1H, *J* = 11.3, 4.5 Hz, CH_2O), 4.36 (d, 1H, *J* = 4.9 Hz, OC*H*O), 7.12-7.38 (m, 10H, Ar*H*). ESI/MS: m/z 338.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from 2-PrOH: mp 158-160 °C. Anal. Calcd for $C_{22}H_{27}NO_2 \cdot H_2C_2O_4$: C, 67.43%; H, 6.84%; N, 3.28%. Found: C, 67.55%, H, 6.70%; N, 3.48%.

The second fraction was the isomer **5b** (24% yield). 1.42-1.97 (m, 7H, piperidine), 2.61 (m, 1H, C*H*Ar), 2.92 (m, 2H, piperidine), 3.50 (s, 2H, NCH_2Ar) 4.18 (m, 4H, 2 x CH_2O), 4.42 (d, 1H, *J* = 5.2 Hz, OC*H*O), 7.18-7.59 (m, 10H, Ar*H*). ESI/MS: m/z 338.2 $[M + H]^+$. The free base was transformed into the oxalate salt that was crystallized from 2-PrOH: mp 111-112 °C. Anal. Calcd for $C_{22}H_{27}NO_2 \cdot H_2C_2O_4$: C, 67.43%; H, 6.84%; N, 3.28%. Found: C, 67.61%, H, 6.97%; N, 3.41%.

1-Phenylpropane-1,3-diol (8)

A solution of **6** (Aldrich) (1 g, 4.23 mmol) in dry Et_2O (3 mL) was added dropwise to a suspension of $LiAlH_4$ (0.17 g, 4.5 mmol) in dry Et_2O (5 mL) at 0° C under a nitrogen atmosphere. The mixture was stirred for 2 h at room temperature, then it was poured onto ice and 2.5 M NaOH (12.65 mL) was added. After the precipitate was filtered off over Celite, the organic phase was dried (Na_2SO_4). The evaporation of the solvent afforded a residue that was purified by flash chromatography. Eluting with cycloexane/EtOAc (75:25) gave an oil (69% yield). ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 2H, CH_2), 3.24 (br s, 2H, exchangeable with D_2O, 2 x O*H*), 3.79 (m, 2H, CH_2O), 4.88 (dd, 1H, C*H*O), 7.25-7.36 (m, 5H, Ar*H*).

2-Phenylpropane-1,3-diol (9)

This compound was synthesized from **7** (Aldrich) according to the procedure described for **8**: a white solid was obtained (72% yield). M.p. 49-50 °C. ^{1}H NMR ($CDCl_3$) δ 1.95 (br s, 2H, exchangeable with D_2O, 2 x O*H*), 3.08 (m, 1H, C*H*Ar), 3.96-4.03 (m, 4H, 2 x CH_2O), 7.34-7.47 (m, 5H, Ar*H*).

Radioligand binding studies

The experimental details of the binding studies at σ_1, σ_2, NMDA, opioid receptors and DAT are reported in the Supporting Information.

***In vivo* studies**

Female Sprague-Dawley rats (Charles River, Italy), fifty two days old were submitted to the binge eating protocol as described in previous works[52] and in the Supporting information.

OF test was performed to evaluate locomotor activity, exploration, and anxiety-like behavior in rodents as described in previous studies.[53, 54] FST is a validated tool, previously described[50] to assess the depression-like behavior in rodents.

Compound **3** was dissolved in 5% solution of DMSO in distilled water and administered i.p. (2 ml / kg) at 3 or 7 mg / kg doses. On feeding test, **3** or the vehicle were injected 30 min before allowing access to HPF. For more detailed information see the Supporting Information.

All rats in the estrous phase were excluded from the results, since binge eating episodes did not occur during this stage in female rats in the same animal model.[55-57]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Instruments used for the synthesis and characterization of compounds **2-9**, experimental details of the binding studies at σ_1, σ_2, NMDA, opioid receptors and DAT and binge eating experimental procedure.

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F.D.B., G.G., P.P., A.P. and W.Q. designed, synthesized and characterized the new compounds. They wrote the associated chemical sections. A.B., B.W. and D.S. performed the binding experiments. G.V. performed the docking experiments. L.B., C.C., E.M.D.B. and M.V.M.D.B. performed the *in vivo* experiments and described the relative results and discussion. C.C., F.D.B., M.V.M.D.B. and W.Q. drafted the main text of the manuscript. All authors critically read and approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

PCP, phencyclidine; NMDA, *N*-methyl-D-aspartate; DAT, dopamine transporter; HPF, highly palatable food; i.p., intraperitoneal; veh, vehicle.

References

1. Smith, S. B. (2017) Introduction to Sigma Receptors: Their Role in Disease and as Therapeutic Targets, *Adv. Exp. Med. Biol. 964*, 1-4.

2. Hanner, M., Moebius, F. F., Flandorfer, A., Knaus, H. G., Striessnig, J., Kempner, E., and Glossmann, H. (1996) Purification, molecular cloning, and expression of the mammalian sigma1-binding site, *Proc. Natl. Acad. Sci. U. S. A. 93*, 8072-8077.

3. Pan, Y. X., Mei, J., Xu, J., Wan, B. L., Zuckerman, A., and Pasternak, G. W. (1998) Cloning and characterization of a mouse sigma1 receptor, *J. Neurochem. 70*, 2279-2285.

4. Alon, A., Schmidt, H. R., Wood, M. D., Sahn, J. J., Martin, S. F., and Kruse, A. C. (2017) Identification of the gene that codes for the sigma2 receptor, *Proc. Natl. Acad. Sci. U. S. A. 114*, 7160-7165.

5. Kekuda, R., Prasad, P. D., Fei, Y. J., Leibach, F. H., and Ganapathy, V. (1996) Cloning and functional expression of the human type 1 sigma receptor (hSigmaR1), *Biochem. Biophys. Res. Commun. 229*, 553-558.

6. Schmidt, H. R., Betz, R. M., Dror, R. O., and Kruse, A. C. (2018) Structural basis for sigma1 receptor ligand recognition, *Nat. Struct. Mol. Biol. 25*, 981-987.

7. Schmidt, H. R., Zheng, S., Gurpinar, E., Koehl, A., Manglik, A., and Kruse, A. C. (2016) Crystal structure of the human sigma1 receptor, *Nature 532*, 527-530.

8. Hayashi, T., and Su, T. P. (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival, *Cell 131*, 596-610.

9. Yano, H., Bonifazi, A., Xu, M., Guthrie, D. A., Schneck, S. N., Abramyan, A. M., Fant, A. D., Hong, W. C., Newman, A. H., and Shi, L. (2018) Pharmacological profiling of sigma 1 receptor ligands by novel receptor homomer assays, *Neuropharmacology 133*, 264-275.

10. Albayrak, Y., and Hashimoto, K. (2017) Sigma-1 Receptor Agonists and Their Clinical Implications in Neuropsychiatric Disorders, *Adv. Exp. Med. Biol. 964*, 153-161.

11. Behensky, A. A., Yasny, I. E., Shuster, A. M., Seredenin, S. B., Petrov, A. V., and Cuevas, J. (2013) Stimulation of sigma receptors with afobazole blocks activation of microglia and reduces toxicity caused by amyloid-beta25-35, *J. Pharmacol. Exp. Ther. 347*, 458-467.

12. Francardo, V., Bez, F., Wieloch, T., Nissbrandt, H., Ruscher, K., and Cenci, M. A. (2014) Pharmacological stimulation of sigma-1 receptors has neurorestorative effects in experimental parkinsonism, *Brain 137*, 1998-2014.

13. Merlos, M., Burgueno, J., Portillo-Salido, E., Plata-Salaman, C. R., and Vela, J. M. (2017) Pharmacological Modulation of the Sigma 1 Receptor and the Treatment of Pain, *Adv. Exp. Med. Biol. 964*, 85-107.

14. Vavers, E., Zvejniece, L., Maurice, T., and Dambrova, M. (2019) Allosteric Modulators of Sigma-1 Receptor: A Review, *Front. Pharmacol. 10*, 223.

15. Hiranita, T., Soto, P. L., Tanda, G., and Katz, J. L. (2010) Reinforcing effects of sigma-receptor agonists in rats trained to self-administer cocaine, *J. Pharmacol. Exp. Ther. 332*, 515-524.

16. Nguyen, E. C., McCracken, K. A., Liu, Y., Pouw, B., and Matsumoto, R. R. (2005) Involvement of sigma (sigma) receptors in the acute actions of methamphetamine: receptor binding and behavioral studies, *Neuropharmacology 49*, 638-645.

17. Blasio, A., Valenza, M., Iyer, M. R., Rice, K. C., Steardo, L., Hayashi, T., Cottone, P., and Sabino, V. (2015) Sigma-1 receptor mediates acquisition of alcohol drinking and seeking behavior in alcohol-preferring rats, *Behav. Brain. Res. 287*, 315-322.

18. Sabino, V., Cottone, P., Zhao, Y., Iyer, M. R., Steardo, L., Jr., Steardo, L., Rice, K. C., Conti, B., Koob, G. F., and Zorrilla, E. P. (2009) The sigma-receptor antagonist BD-1063 decreases ethanol intake and reinforcement in animal models of excessive drinking, *Neuropsychopharmacology 34*, 1482-1493.

19. Valenza, M., Blasio, A., DiLeo, A., Cottone, P., and Sabino, V. (2020) Sigma receptor-induced heavy drinking in rats: Modulation by the opioid receptor system, *Pharmacol. Biochem. Behav. 192*, 172914.

20. Hong, W. C., Yano, H., Hiranita, T., Chin, F. T., McCurdy, C. R., Su, T.-P., Amara, S. G., and Katz, J. L. (2017) The sigma-1 receptor modulates dopamine transporter conformation and cocaine

binding and may thereby potentiate cocaine self-administration in rats, *J. Biol. Chem. 292*, 11250-11261.

21. Avena, N. M., Rada, P., and Hoebel, B. G. (2008) Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake, *Neurosci. Biobehav. Rev. 32*, 20-39.

22. D'Addario, C., Micioni Di Bonaventura, M. V., Pucci, M., Romano, A., Gaetani, S., Ciccocioppo, R., Cifani, C., and Maccarrone, M. (2014) Endocannabinoid signaling and food addiction, *Neurosci. Biobehav. Rev. 47*, 203-224.

23. Cottone, P., Wang, X., Park, J. W., Valenza, M., Blasio, A., Kwak, J., Iyer, M. R., Steardo, L., Rice, K. C., Hayashi, T., and Sabino, V. (2012) Antagonism of sigma-1 receptors blocks compulsive-like eating, *Neuropsychopharmacology 37*, 2593-2604.

24. Tapia, M. A., Lee, J. R., Bathe, E. L., Rivera, L. L., Mason, K. L., Cessac, M. E., Bodeen, J. L., Miller, D. K., and Will, M. J. (2019) Sigma-1 receptor antagonist, PD144418, selectively reduces female motivation for food during negative energy balance, *Behav. Brain. Res. 373*, 112087.

25. Piergentili, A., Amantini, C., Del Bello, F., Giannella, M., Mattioli, L., Palmery, M., Perfumi, M., Pigini, M., Santoni, G., Tucci, P., Zotti, M., and Quaglia, W. (2010) Novel highly potent and selective sigma 1 receptor antagonists related to spipethiane, *J. Med. Chem. 53*, 1261-1269.

26. Del Bello, F., Micioni Di Bonaventura, M. V., Bonifazi, A., Wunsch, B., Schepmann, D., Giancola, J. B., Micioni Di Bonaventura, E., Vistoli, G., Giorgioni, G., Quaglia, W., Piergentili, A., and Cifani, C. (2019) Investigation of the Role of Chirality in the Interaction with sigma Receptors and Effect on Binge Eating Episode of a Potent sigma1 Antagonist Analogue of Spipethiane, *ACS Chem. Neurosci. 10*, 3391-3397.

27. Morice, C., and Wermuth, C. G. (2008) Chapter 9 - Ring Transformations. In *The Practice of Medicinal Chemistry (Fourth Edition)* (Wermuth, C. G., Aldous, D., Raboisson, P., and Rognan, D., Eds.), pp 243-266, Academic Press, San Diego.

28. Hutson, P. H., Balodis, I. M., and Potenza, M. N. (2018) Binge-eating disorder: Clinical and therapeutic advances, *Pharmacol. Ther. 182*, 15-27.

29. Bonifazi, A., Del Bello, F., Mammoli, V., Piergentili, A., Petrelli, R., Cimarelli, C., Pellei, M., Schepmann, D., Wunsch, B., Barocelli, E., Bertoni, S., Flammini, L., Amantini, C., Nabissi, M., Santoni, G., Vistoli, G., and Quaglia, W. (2015) Novel Potent N-Methyl-d-aspartate (NMDA) Receptor Antagonists or sigma1 Receptor Ligands Based on Properly Substituted 1,4-Dioxane Ring, *J. Med. Chem. 58*, 8601-8615.

30. Miyata, K., Schepmann, D., and Wunsch, B. (2014) Synthesis and sigma receptor affinity of regioisomeric spirocyclic furopyridines, *Eur. J. Med. Chem. 83*, 709-716.

31. Del Bello, F., Bonifazi, A., Giorgioni, G., Cifani, C., Micioni Di Bonaventura, M. V., Petrelli, R., Piergentili, A., Fontana, S., Mammoli, V., Yano, H., Matucci, R., Vistoli, G., and Quaglia, W. (2018) 1-[3-(4-Butylpiperidin-1-yl)propyl]-1,2,3,4-tetrahydroquinolin-2-one (77-LH-28-1) as a Model for the Rational Design of a Novel Class of Brain Penetrant Ligands with High Affinity and Selectivity for Dopamine D4 Receptor, *J. Med. Chem. 61*, 3712-3725.

32. Tangherlini, G., Kalinin, D. V., Schepmann, D., Che, T., Mykicki, N., Stander, S., Loser, K., and Wunsch, B. (2019) Development of Novel Quinoxaline-Based kappa-Opioid Receptor Agonists for the Treatment of Neuroinflammation, *J. Med. Chem. 62*, 893-907.

33. Battiti, F. O., Newman, A. H., and Bonifazi, A. (2020) Exception That Proves the Rule: Investigation of Privileged Stereochemistry in Designing Dopamine D3R Bitopic Agonists, *ACS Med. Chem. Lett., DOI: 10.1021/acsmedchemlett.9b00660*, in press.

34. Cheng, Y., and Prusoff, W. H. (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, *Biochem. Pharmacol. 22*, 3099-3108.

35. Arena, E., Dichiara, M., Floresta, G., Parenti, C., Marrazzo, A., Pittalà, V., Amata, E., and Prezzavento, O. (2018) Novel Sigma-1 receptor antagonists: from opioids to small molecules: what is new?, *Future Med. Chem. 10*, 231-256.

36. Cao, J., Kopajtic, T., Katz, J. L., and Newman, A. H. (2008) Dual DAT/sigma1 receptor ligands based on 3-(4-(3-(bis(4-fluorophenyl)amino)propyl)piperazin-1-yl)-1-phenylpropan-1-ol, *Bioorg. Med. Chem. Lett. 18*, 5238-5241.

37. Hiranita, T., Soto, P. L., Kohut, S. J., Kopajtic, T., Cao, J., Newman, A. H., Tanda, G., and Katz, J. L. (2011) Decreases in cocaine self-administration with dual inhibition of the dopamine transporter and sigma receptors, *J. Pharmacol. Exp. Ther. 339*, 662-677.

38. Ren, P., Chun, J., Thomas, D. G., Schnieders, M. J., Marucho, M., Zhang, J., and Baker, N. A. (2012) Biomolecular electrostatics and solvation: a computational perspective, *Q. Rev. Biophys. 45*, 427-491.

39. Hudson, J. I., Hiripi, E., Pope, H. G., Jr., and Kessler, R. C. (2007) The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication, *Biol. Psychiatry 61*, 348-358.

40. Cifani, C., Polidori, C., Melotto, S., Ciccocioppo, R., and Massi, M. (2009) A preclinical model of binge eating elicited by yo-yo dieting and stressful exposure to food: effect of sibutramine, fluoxetine, topiramate, and midazolam, *Psychopharmacology 204*, 113-125.

41. Romano, A., Micioni Di Bonaventura, M. V., Gallelli, C. A., Koczwara, J. B., Smeets, D., Giusepponi, M. E., De Ceglia, M., Friuli, M., Micioni Di Bonaventura, E., Scuderi, C., Vitalone, A., Tramutola, A., Altieri, F., Lutz, T. A., Giudetti, A. M., Cassano, T., Cifani, C., and Gaetani, S. (2020) Oleoylethanolamide decreases frustration stress-induced binge-like eating in female rats: a novel potential treatment for binge eating disorder, *Neuropsychopharmacology, DOI: 10.1038/s41386-020-0686-z.*

42. Micioni Di Bonaventura, M. V., Pucci, M., Giusepponi, M. E., Romano, A., Lambertucci, C., Volpini, R., Micioni Di Bonaventura, E., Gaetani, S., Maccarrone, M., D'Addario, C., and Cifani, C. (2019) Regulation of adenosine A2A receptor gene expression in a model of binge eating in the amygdaloid complex of female rats, *J. Psychopharmacol. 33*, 1550-1561.

43. Cifani, C., Di Bonaventura, M. V. M., Ciccocioppo, R., and Massi, M. (2013) Binge eating in female rats induced by yo-yo dieting and stress. In *Animal Models of Eating Disorders* pp 27-49, Springer.

44. Cifani, C., Micioni Di, B. M., Vitale, G., Ruggieri, V., Ciccocioppo, R., and Massi, M. (2010) Effect of salidroside, active principle of Rhodiola rosea extract, on binge eating, *Physiol. Behav. 101*, 555-562.

45. Micioni Di Bonaventura, M. V., Vitale, G., Massi, M., and Cifani, C. (2012) Effect of Hypericum perforatum Extract in an Experimental Model of Binge Eating in Female Rats, *J. Obes. 2012*, 956137.

46. Micioni Di Bonaventura, M. V., Micioni Di Bonaventura, E., Polidori, C., and Cifani, C. (2020) Preclinical Models of Stress and Environmental Influences on Binge Eating. In *Binge Eating: A Transdiagnostic Psychopathology* (Frank, G. K. W., and Berner, L. A., Eds.), pp 85-101, Springer International Publishing, Cham.

47. Micioni Di Bonaventura, M. V., Ubaldi, M., Liberati, S., Ciccocioppo, R., Massi, M., and Cifani, C. (2013) Caloric restriction increases the sensitivity to the hyperphagic effect of nociceptin/orphanin FQ limiting its ability to reduce binge eating in female rats, *Psychopharmacology 228*, 53-63.

48. Pucci, M., Micioni Di Bonaventura, M. V., Giusepponi, M. E., Romano, A., Filaferro, M., Maccarrone, M., Ciccocioppo, R., Cifani, C., and D'Addario, C. (2016) Epigenetic regulation of nociceptin/orphanin FQ and corticotropin-releasing factor system genes in frustration stress-induced binge-like palatable food consumption, *Addict. Biol. 21*, 1168-1185.

49. Ramos, A., and Mormède, P. (1998) Stress and emotionality: a multidimensional and genetic approach, *Neurosci. Biobehav. Rev. 22*, 33-57.

50. Porsolt, R. D., Anton, G., Blavet, N., and Jalfre, M. (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments, *Eur. J. Pharmacol. 47*, 379-391.

51. Royce, J. R. (1977) On the construct validity of open-field measures, *Psychol. Bull. 84*, 1098-1106.

52. Micioni Di Bonaventura, M. V., Ubaldi, M., Giusepponi, M. E., Rice, K. C., Massi, M., Ciccocioppo, R., and Cifani, C. (2017) Hypothalamic CRF1 receptor mechanisms are not sufficient to account for binge-like palatable food consumption in female rats, *Int. J. Eat. Disord. 50*, 1194-1204.

53. Micioni Di Bonaventura, M. V., Cifani, C., Lambertucci, C., Volpini, R., Cristalli, G., Froldi, R., and Massi, M. (2012) Effects of A(2)A adenosine receptor blockade or stimulation on alcohol intake in alcohol-preferring rats, *Psychopharmacology 219*, 945-957.

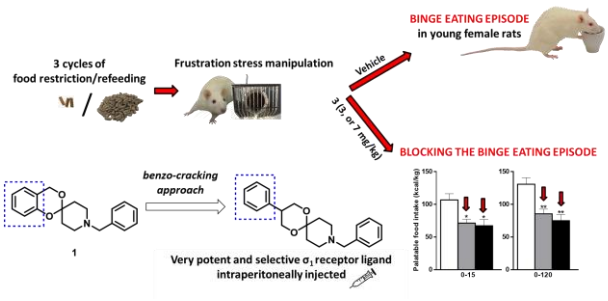
54. Micioni Di Bonaventura, M. V., Cifani, C., Lambertucci, C., Volpini, R., Cristalli, G., and Massi, M. (2012) A2A adenosine receptor agonists reduce both high-palatability and low-palatability food intake in female rats, *Behav. Pharmacol. 23*, 567-574.

55. Alboni, S., Micioni Di Bonaventura, M. V., Benatti, C., Giusepponi, M. E., Brunello, N., and Cifani, C. (2017) Hypothalamic expression of inflammatory mediators in an animal model of binge eating, *Behav. Brain. Res. 320*, 420-430.

56. Micioni Di Bonaventura, M. V., Lutz, T. A., Romano, A., Pucci, M., Geary, N., Asarian, L., and Cifani, C. (2017) Estrogenic suppression of binge-like eating elicited by cyclic food restriction and frustrative-nonreward stress in female rats, *Int. J. Eat. Disord. 50*, 624-635.

57. Pucci, M., Micioni Di Bonaventura, M. V., Zaplatic, E., Bellia, F., Maccarrone, M., Cifani, C., and D'Addario, C. (2019) Transcriptional regulation of the endocannabinoid system in a rat model of binge-eating behavior reveals a selective modulation of the hypothalamic fatty acid amide hydrolase gene, *Int. J. Eat. Disord. 52*, 51-60.

1 **Graphical Table of Contents**



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