Investigation of the Role of Chirality in the Interaction with σ Receptors and Effect on Binge Eating Episode of a Potent σ_1 Antagonist Analogue of Spipethiane

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ABSTRACT

The enantiomers of the potent σ_1 receptor antagonist (±)-**1** were synthesized and evaluated for their affinity at σ_1, σ_2 receptors and dopamine transporter (DAT). Analogously to (±)-**1**, both the enantiomers showed very high affinity for the σ_1 receptor and unprecedented selectivity over both the σ_2 receptor and DAT. The lack of enantioselectivity between (+)-**1** and (-)-**1** indicated that the center of chirality in 2-position of the benzothiochromane nucleus doesn't play a crucial role in the interaction with any of the studied targets. Docking studies confirmed that the configuration of the enantiomers has only marginal effects on the molecular interactions with the σ_1 receptor. In *in vivo* studies in a female rat model of binge eating, (±)-**1** dose-dependently decreased the binge eating episode elicited by a history of intermittent food restriction and stress, confirming and strengthening the important role played by the σ_1 receptor in bingeing-related eating disorders.

Keywords: selective σ_1 antagonists; chirality; docking studies, binge eating episode, palatable food

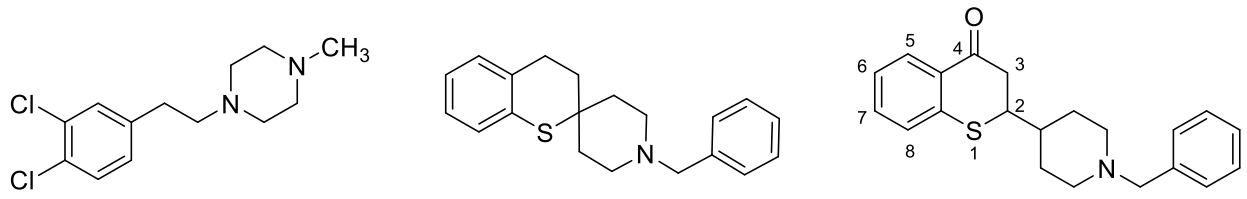
Sigma (σ) receptors are transmembrane proteins implicated in several cellular functions and are among the most enigmatic proteins from a biological point of view.[1] At present, they are considered to be a receptor family different from both the opioid receptor family and the phencyclidine site of the NMDA receptor,[2] and comprise σ_1 and σ_2 receptor subtypes, which are widespread expressed in the central nervous system (CNS) and in several peripheral tissues.[3] They are also localized in the immune and endocrine systems and are overexpressed in several tumour cell lines.[3]

While the σ_1 receptor has been cloned from various tissues of different species,[4-6] the σ_2 subtype has only recently been cloned and identified as transmembrane protein 97 (TMEM97).[7] The crystal structures of the human σ_1 receptor in complex with the potent σ_1 ligands 4-IBP and PD144418 provided the first detailed description of the σ_1 architecture, revealing a triangular trimer with only one transmembrane domain in each protomer.[8] The σ_1 receptor is predominantly found in the mitochondria associated membrane of the endoplasmic reticulum (ER) and in the nuclear and plasma membranes.[9] It has been reported to aid the inositol triphosphate receptor in the ER membrane in a chaperone-like mode, modulating proper Ca^{2+}-signalling. Moreover, it regulates glutamatergic, dopaminergic, and cholinergic neurotransmission as well as the opening of some cation (K^+, Na^+, Ca^{2+}) channels.[3]

Due to their wide localization throughout the human body and their involvement in many physiological functions, the σ_1 receptors are considered highly appealing targets for the treatment of several diseases. In particular, centrally active σ_1 receptor agonists might be useful in depression, cognition, delirium, akathisia and other hyperkinetic movement,[10] as well as in neurodegenerative diseases.[11,12] σ_1 receptor antagonists have been demonstrated to be efficacious in neuropathic pain, as well as in visceral, orofacial, and inflammatory pain.[13] Moreover, σ_1 antagonists can be used as anti-addictive compounds, blocking several effects induced by psychostimulant[14,15] and ethanol.[16,17] While a collection of evidence has demonstrated the role of the σ_1 receptor in drug abuse, just one study reports that it is involved in compulsive-like eating.[18] Moreover, very recently a correlation between σ_1 receptor and food reinforced operant responding has been discussed.[19]

Binge eating is a central trait of many eating disorders, such as bulimia nervosa and binge eating disorder (BED), consisting in compulsive, non-homeostatic consumption of abnormal amounts of highly palatable food within few hours, as described by the DSM-V.[20] BED is among the most frequent eating disorders, in which subjects assume an unusually large quantity of food, feeling unable to stop eating. Although it affects about 2-5% of adults and is more frequent in women than in men,[21] effective drugs are limited leading to the impetus for innovative treatments. The study by Cottone et al.[18] demonstrated that the σ_1 antagonist BD-1063 (Figure 1) (σ_1 pK_i = 8.05, σ_1/σ_2 selectivity ratio = 71)[22] reduced binge-like eating in a dose-dependent manner and blocked the increased eating rate in palatable rats. This result suggests that the σ_1 receptors might be implicated in neurobiological adaptations underlying compulsive-like eating.[18]

We have reported a series of spipethiane analogs showing high affinity for the σ_1 receptor and selectivity over the σ_2 subtype. Among these ligands, compound (±)-**1** (Figure 1) (σ_1 pK_i = 10.28) behaved as a very potent σ_1 receptor antagonist and showed the outstanding σ_1/σ_2 selectivity ratio of 29510.[23]



BD-1063 Spipethiane (±)-**1**

Figure 1. Chemical structures of BD-1063, spipethiane and (±)-**1**.

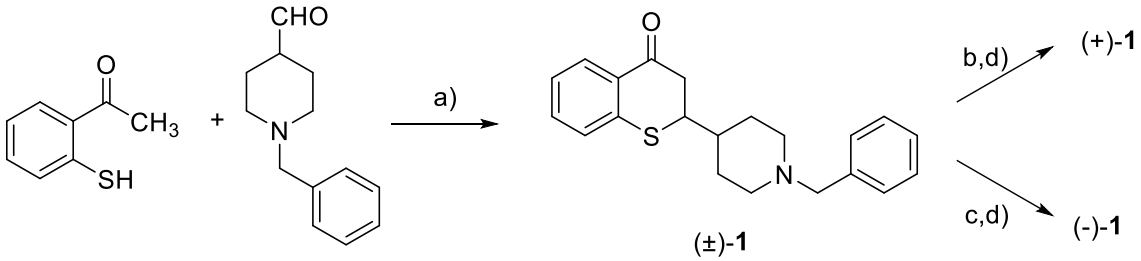
Since compound (±)-**1** shows both a markedly higher σ_1 affinity and σ_1/σ_2 selectivity with respect to BD-1063, to better clarify the role played by the σ_1 receptor in compulsive-like eating disorders, it was evaluated in a female rat model of binge eating.

Given that the biological profile of ligands might quantitatively and qualitatively be influenced by stereochemistry, the presence of a centre of chirality in (±)-**1** prompted us to preliminarily separate the two enantiomers (+)-**1** and (-)-**1** and evaluate their affinities for the σ_1 and σ_2 receptors.

Moreover, the affinities of (±)-**1** and its enantiomers were also evaluated at dopamine transporters (DAT), considering that DAT have been demonstrated to be involved in binge eating disorders[24] and that several σ_1 ligands also bind DAT.[22,25]

RESULTS AND DISCUSSION

Racemic compound (±)-**1** was prepared as reported in reference 23 (Scheme 1). The enantiomers (+)-**1** and (-)-**1** were separated by fractional crystallization of the salts of the racemic compound (±)-**1** with (+)-O,O′-di-*p*-toluoyl-D- and (-)-O,O′-di-*p*-toluoyl-L-tartaric acid, respectively (Scheme 1).



Scheme 1. a) pyrrolidine, MeOH;[23] b) (+)-O,O′-di-*p*-toluoyl-D-tartaric acid, crystallization from EtOH; c) (-)-O,O′-di-*p*-toluoyl-L-tartaric acid, crystallization from EtOH; d) 2N NaOH

The enantiomeric excess, determined by HPLC using DAICEL - Chiralpak AD-H (4.6 mm X 250 mm) as the chiral stationary phase and n-hexane/2-propanol 90/10 v/v as the mobile phase, was found to be >99% for (+)-**1** and >97% for (-)-**1** (see supporting information). ^{1}H NMR spectroscopy studies confirmed that the enantiomeric purity is >97% (detection limit) for both the enantiomers. Indeed, the ^{1}H NMR spectrum of the racemic compound (±)-**1** in the presence of the chiral solvating reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol shows two pairs of doublets at 3.50 ppm for the $PhCH_2N$

protons, whereas only two doublets were observed for (+)-**1** and (-)-**1** at 3.51 and 3.49 ppm, respectively.

The affinities of (±)-**1** and its enantiomers (+)-**1** and (-)-**1** were determined at the σ_1 receptor, using [^{3}H]-(+)-pentazocine as the radioligand, and at the σ_2 receptor, using [^{3}H]-di-o-tolylguanidine as the radioligand in the presence of an excess of (+)-pentazocine to ensure occupancy of σ_1 receptors. The experiments were performed on guinea pig brain and rat liver membrane preparations for the σ_1 and σ_2 receptors, respectively, according to previously reported procedures.[26,27] The DAT assay was performed with rat striatal membranes using the radioligand [^{3}H]-WIN35,428, analogously to previously reported procedures.[28,29]

The affinity values, expressed as pK_i values, are reported in Table 1 together with those of BD-1063 for useful comparison.[22]

Table 1. Affinity values, expressed as pK_i,[a] of (±)-**1**, its enantiomers (+)-**1** and (-)-**1** and BD-1063, at σ_1 and σ_2 Receptor and DAT.

Compound	pK_i				
	σ_1	σ_2	DAT	σ_1/σ_2	σ_1/DAT
(±)-**1**	10.85	6.53	5.80	20893	112202
(+)-**1**	10.92	6.87	5.77	11220	141253
(-)-**1**	10.66	6.39	5.67	18621	97724
BD-1063	8.05[b]	6.20[b]	5.10[b]	71	891

[a]Equilibrium dissociation constants (K_i) were derived from IC_{50} values using the Cheng-Prusoff equation.[30] The IC_{50} values were determined by displacement of [^{3}H]-(+)-pentazocine and [^{3}H]-DTG in the presence of 500 nM (+)-pentazocine from the σ_1 and σ_2 receptors, respectively, and by displacement of [^{3}H]WIN35,428 from DAT. Each experiment was performed in triplicate. pK_i and pIC_{50} values are means from three to five experiments, which agreed within ±20%. [b]Data from reference 22.

The data reported in Table 1 reveal that, similarly to the racemic compound (±)-**1**, both enantiomers (+)-**1** and (-)-**1** show very high affinity for the σ_1 receptor and impressive σ_1/σ_2 selectivity. Moreover, (±)-**1** and its enantiomers display sub-micromolar DAT affinity higher than 1 μM, and consequently an uncommon selectivity for the σ_1 receptor even over these transporters. This result is noteworthy, considering that most potent σ_1 ligands also bind DAT with high affinity.[22,25] Significant differences in the affinities towards the σ_1, σ_2 receptors and DAT between the enantiomers (+)-**1** and (-)-**1** were not observed, indicating that the configuration of the stereocenter in 2-position of the benzothiochromane system of (±)-**1** doesn't play a crucial role in the interaction with these molecular targets. To rationalize the lack of stereoselectivity between the enantiomers (+)-**1** and (-)-**1** at the σ_1 receptor, docking studies on the crystal structure of the human σ_1 receptor[8] were performed. In Figure 2 the best binding poses of the two enantiomers (+)-**1** and (-)-**1** at the σ_1 receptor are compared. The observation that the enantiomers reveal an almost identical arrangement supports that the configuration has only marginal effects on the interactions, since

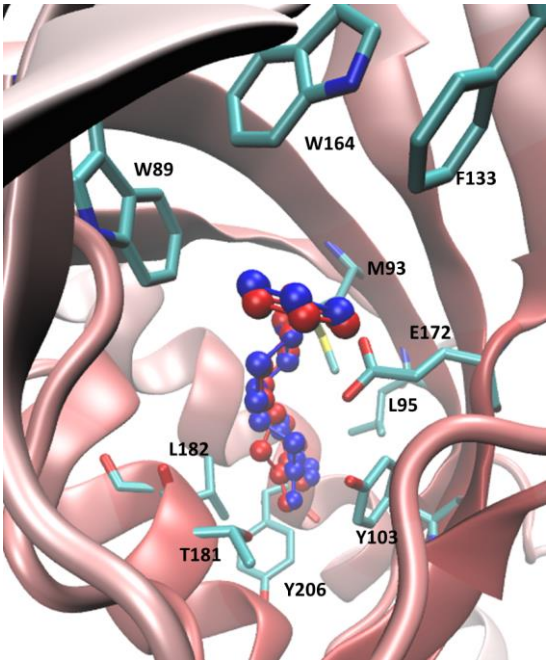


Figure 2. Superposition of the calculated binding poses of (*S*)-**1** (blue-colored ligand) and (*R*)-**1** (red-colored ligand).

Specifically, the two calculated, optimized complexes appear to be stabilized by the same interaction pattern, which can be summarized as follows: (1) the ammonium head of the ligand stabilizes a clear salt bridge with Glu172 reinforced by the H-bond with Tyr103, which in turn also approaches the thiochroman-4-one moiety; (2) the thiochroman-4-one system contacts a set of apolar side-chains (i.e. Met 93, Leu95, Leu105, Ile178, Leu182) and its carbonyl group elicits a H-bond with Thr181); (3) the phenyl ring is inserted in a subpocket completely lined by aromatic residues such as Trp89, Phe107, Phe133, His154 and Trp164. Clear similarity between the two generated complexes is further confirmed by the similar values obtained for some representative scoring functions such as the primary ChemPLP function (–123.49 kcal/mol (*S*) vs. –124.17 kcal/mol (*R*)), the electrostatic-based APBS score (–10.55 kcal/mol (*S*) vs. –10.39 kcal/mol (*R*)) and the empirical XScore (–10.99 kcal/mol (*S*) vs. –11.03 kcal/mol (*R*)). Overall, the presented docking results are in consensus with the observed lack of stereoselectivity between the two studied enantiomers, which are indeed able to stabilize almost identical interaction patterns with the σ_1 receptor.

Though the enantiomers (+)-**1** and (-)-**1** show similar affinities at σ_1/σ_2 and σ_1/DAT, they might manifest a different pharmacokinetic profile. The possible role of the configuration in determining the metabolic fate of the considered enantiomers was analyzed in silico by predicting the metabolic reactions they can undergo by a similarity-based approach involving the MetaQSAR database as described in reference 31. In detail and considering the molecules within MetaQSAR showing a Tanimoto similarity greater than 0.5, the following metabolic reactions can be figured out: 1) oxygenation of sulfide to sulfoxide and to sulfone; 2) hydroxylation on the carbon atom in alpha to both heteroatom and unsaturated systems; 3) hydroxylation on the phenyl ring (preferentially in para position); 4) reduction of the carbonyl group to alcohol. Among these putative reactions, (1) and (4) involve sites of metabolism close to the chiral center. In particular, the S-oxidation can show a certain degree of substrate stereoselectivity especially when catalyzed by FMO enzyme, whose cavity seems to be more sensitive to the substrate configuration compared to CYP450 enzymes as reported in

previous studies.[32] Focusing attention on metabolic reactions occurring close to the chiral center, one may suppose that differences in the metabolism of the two enantiomers might be eventually due to the S-oxidation reactions rather than the reduction of the carbonyl group considering that the involved aldehyde dehydrogenases are well-known for their product rather than substrate stereoselectivity.

Compared to BD-1063, the affinity values of (±)-**1**, (+)-**1** and (-)-**1** are significantly higher at the σ_1 receptor and only slightly higher at the σ_2 receptor and DAT. Consequently, marked increases in σ_1/σ_2 and σ_1/DAT selectivity ratios are observed for (±)-**1**, (+)-**1** and (-)-**1** with respect to BD-1063. Therefore, due to their interesting selectivity profiles, (±)-**1** and its enantiomers would allow us to confirm and strengthen the hypothesis that the σ_1 receptor system plays an important role in compulsive-like eating disorder. Since racemic compound (±)-**1** shows σ_1 affinity and selectivity profiles indistinguishable to those of both enantiomers, it was selected for the *in vivo* study, using a well-characterized animal model of binge eating. The episode of binge eating for palatable food was induced by combining stress with three consecutive cycles of food restriction/re-feeding[33] in female rats, which were used in consideration of the high prevalence of BED in adolescent and young adult females.[21]

As previously reported by us,[34] the body weight of rats was reduced in the 4 days of food restriction (in restricted rats), but during re-feeding period, the rats regained their body weight to values of controls (non-restricted rats) the last day of each cycle (values not displayed). At 15 min after palatable food access and at the end of the test (2 h time point), three-way ANOVA showed a significant interaction among the three factors (food restriction, stress and treatment) [15 min: $F_{(3,90)} = 3.16$, $p < 0.05$] [2 h: $F_{(3,90)} = 2.8$, $p < 0.05$]. As shown in Figure 3, post-hoc comparisons indicated that the palatable food intake of the vehicle restricted and stressed group was significantly higher than in the other vehicle groups (non-restricted and non-stressed; non-restricted and stressed; restricted and non-stressed) (15 min: $p < 0.01$; 2 h: $p < 0.05$).

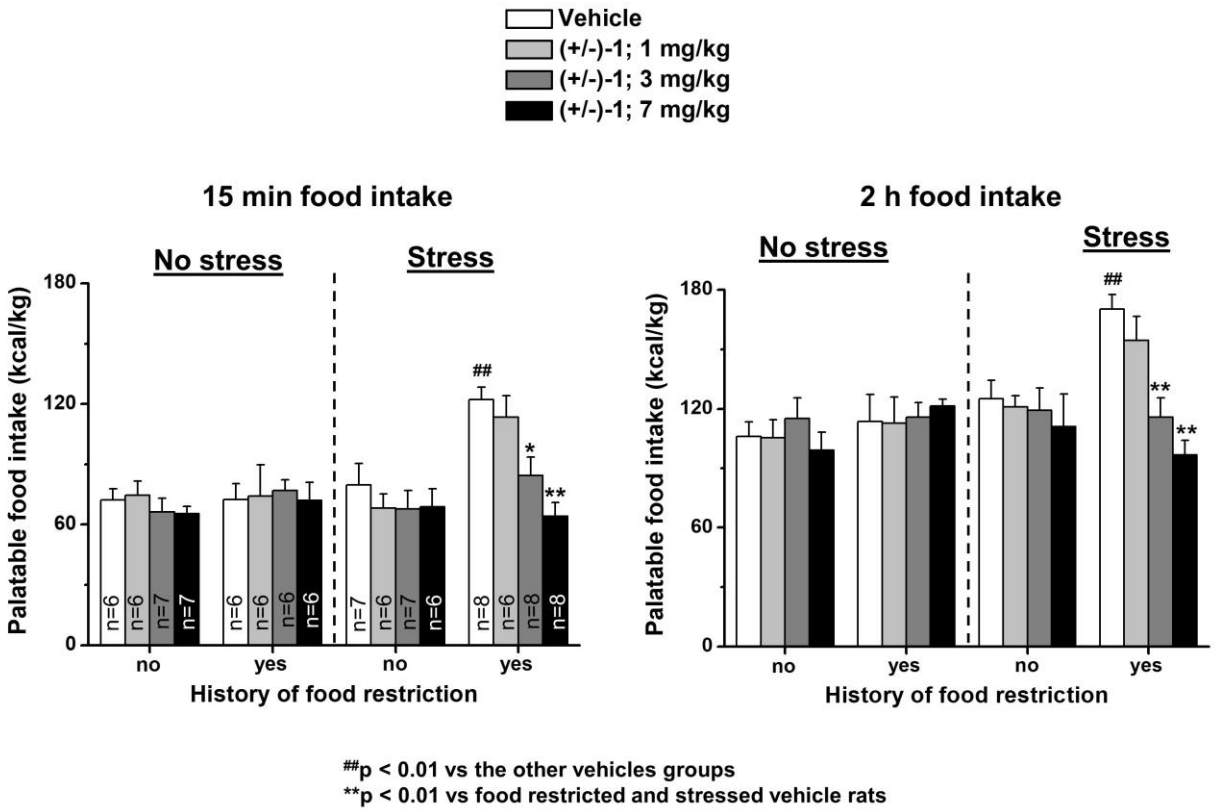


Figure 3. Systemic injections of compound (±)-**1** decreased the binge eating episode in rats with cycles of intermittent food restrictions and stress. Mean ± SEM (n = 6-8 for each group, as shown for each bar) palatable food intake (kcal/kg) in the first 15 min (left) and the total 2 h (right) test session. ##p < 0.01 vs the other vehicles groups; **p < 0.01 vs food restricted and stressed vehicle rats. At 15 min after palatable food access and at the end of the test (2 h time point), three-way ANOVA showed a significant interaction among the three factors (food restriction, stress and treatment) [15 min: $F_{(3,90)} = 3.16$, $p < 0.05$] [2 h: $F_{(3,90)} = 2.8$, $p < 0.05$].

Moreover, post-hoc comparisons showed a significant reduction in palatable food consumption in the binge eating group (restricted and stressed rats) treated with (±)-**1**, at 3 and 7 mg/kg at 15 min and 2 h time points in a dose-response relationship. These doses did not reduce palatable food intake in the other three groups. Compound (±)-**1** at the dose of 1 mg/kg was ineffective in all groups of rats.

Therefore, we found that systemic injections of (±)-**1** selectively blocked the episode of binge eating without a general inhibition of food intake, observed for serotonergic drugs, such as fluoxetine or sibutramine in the same animal model.[33] Considering the unprecedented selectivity of (±)-**1** for σ_1 over σ_2 and DAT, these results confirm and strengthen the important role played by the σ_1 receptor in the pharmacotherapy of bingeing-related eating disorders. Despite several works revealed the potential effect of σ_1 receptor antagonists to treat drug abuse, the precise mechanism of action remained unclear. The ability of the σ_1 antagonists to reduce the motivational effect of cocaine[14] methamphetamine[15] and alcohol[16,17] makes conceivable that the blockade of the σ_1 receptor may control the motivation for palatable food consumption under particular conditions, such as food restrictions and acute stress in our binge eating rat model, modulating the dopaminergic system.

METHODS

Chemistry

General

Instruments used for melting point determination, IR, NMR and mass spectra, elemental analyses and optical rotation are given in references 35 and 36.

Resolution of (2-(1-benzylpiperidin-4-yl)thiochroman-4-one) (±)-1 by fractional crystallization.

A solution of (+)-O,O'-di-*p*-toluoyl-D-tartaric acid (5.73 g, 14.8 mmol) in EtOAc (30 mL) was added to a solution of racemic compound (±)-**1**[23] (5 g, 14.8 mmol) in ethyl acetate (EtOAc) (60 mL) and the mixture was left at r.t. for 24 h. The white solid was crystallized five times from EtOH (2.24 g): mp 185-186 °C, $[\alpha]^{20}_D$ = +153.8 (c 1, CH_3OH). The salt was dissolved in water (50 mL) and the solution was cooled to 0 °C, made basic with 2 N NaOH and extracted with EtOAc (3 x 30 mL). Removal of dried solvent afforded (+)-**1** (1.18 g): mp 80-82 °C, $[\alpha]^{20}_D$ = +144.66 (c 1, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 1.38-2.02 (m, 7H), 2.80-3.12 (m, 4H), 3.35 (m, 1H), 3.50 (dd, 2H), 7.10-8.11 (m, 9H). The free base was converted into the oxalate salt, that was recrystallized from MeOH: mp 218-220 °C, $[\alpha]^{20}_D$

= +137.2 (c 1, CH_3OH). ESI/MS: m/z 338.2 $[M + H]^+$. Anal. Calcd for $C_{21}H_{23}NOS.C_2H_2O_4$: C, 64.62; H, 5.89; N, 3.28, S, 7.50. Found: C, 64.36; H, 6.23; N, 3.34, S, 7.42.

A solution of the amine recovered from the mother liquor (1.7 g, 5.04 mmol) in EtOAc (20 mL) was treated with a solution of (-)-O,O'-di-*p*-toluoyl-D-tartaric acid (1.95 g, 5.04 mmol) in EtOAc (10 mL) and left at r.t. for 24 h. The white solid was crystallized four times from EtOH (1.74 g): mp 182-183 °C, $[\alpha]^{20}_D$ = -148.54 (c 1, CH_3OH). The salt was dissolved in water (20 mL), and the solution was cooled to 0 °C, made basic with 2 N NaOH and extracted with EtOAc (3 x 10 mL). Removal of dried solvent afforded (-)-**1** (0.9 g): mp 81-82 °C, $[\alpha]^{20}_D$ = -143.57 (c 1, $CHCl_3$). The ^1H NMR spectrum was identical to those of (±)-**1**[23] and the enantiomer (+)-**1**. The free base was converted into the oxalate salt, that was recrystallized from MeOH: mp 219-220 °C, $[\alpha]^{20}_D$ = +137.0 (c 1, CH_3OH). ESI/MS: m/z 338.2 $[M + H]^+$. Anal. Calcd for $C_{21}H_{23}NOS.C_2H_2O_4$: C, 64.62; H, 5.89; N, 3.28, S, 7.50. Found: C, 64.38; H, 5.89; N, 3.34, S, 7.42.

Receptor binding studies

The experimental details of σ_1 and σ_2 receptors and DAT are given in references 26-29.

Computational methods

Docking simulations were based on the σ_1 receptor resolved structure in complex with PD144418 (PDB ID: 5HK1). The protein and ligand structures were prepared by applying standard protocols as previously described.[37] Docking simulations were performed by using PLANTS[38] and focusing the search within a 12 Å radius sphere around the bound ligand. For each enantiomer, 10 poses were generated and scored by the ChemPLP scoring function with the speed equal to 1. The obtained best poses were then minimized by fixing all atoms outside a 10 Å radius sphere around the bound ligands.

***In vivo* studies**

Material and methods

Subjects, diet composition and binge eating experimental procedure

One hundred and forty-four female Female Sprague–Dawley rats (Charles River, Italy) were used. The palatable food was prepared by mixing Nutella (Ferrero, Italy) chocolate cream (52%), ground food pellets (4RF18) (33%) and water (15%). The binge eating procedure was described in detail in our previous studies.[33,39-42]

Drug

Compound (±)-**1** was synthesized according to previously reported procedure[23] and dissolved in 5% dimethylsulfoxide in distilled water. It was administered intraperitoneally (2 mL/kg) at doses of 1, 3 or 7 mg/kg. On test day, compound (±)-**1** or the respective vehicle was administered 1 h before access to palatable food.

Experiment 1: Effect of compound (±)-1 in a model of binge eating

The purpose of this experiment was to evaluate the effects of compound (±)-**1** on binge eating behavior. One hundred and forty-four female rats, divided into 4 groups (n = 36 per group), were used. They were either exposed or not exposed to 3 consecutive 8-days cycles of food restriction/re-feeding, during which the access to the palatable food was provided to them for 2 h/day on days 5, 6, 13, and 14. On day 25, rats were either exposed or not exposed to 15 min of frustration stress before the 2 h palatable food binge eating test. On the test day, each group was separated in 4 subgroups and treated intraperitoneally with vehicle or (±)-**1** compound (1, 3 and 7 mg/kg), 30 min before the 2 h palatable food access. After testing, the estrous cycle phase was determined. During the estrous phase, stress-induced binge eating was not observed in our model.[43,44] Therefore, thirty-eight rats that were in this phase were excluded from the statistical analysis.

Statistical analysis

The data are reported as mean ± SEM. The results were analyzed with factorial ANOVAs (Systat Software 10.0) using the factors reported in Results. Bonferroni's post hoc tests were used to follow up on significant interactions or main effects ($p < 0.05$) from the factorial ANOVAs.

ASSOCIATED CONTENT

Supporting Information

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

Analytical HPLC method and Figure S1, reporting the HPLC chromatograms of (±)-**1** and its enantiomers.

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Author Contributions

[#]F.D.B. and M.V.M.D.B. contributed equally. F.D.B., G.G., W.Q. and A.P. designed the novel compounds and planned the procedures for their synthesis. They developed the chemical synthesis, characterized the novel compounds, wrote the relative chemical experimental parts and drafted the main text of the manuscript. A.B., B.W. and D.S. and performed binding experiments at σ_1 and σ_2 receptors. A.B. and J.B.G. performed binding experiments at DAT. G.V. carried out docking experiments. M.V.M.D.B., E.M.D.B., and C.C. performed in vivo assays and drafted the relative discussion. All authors critically discussed and approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The work was supported by the Italian Ministry of University and Research under grant PRIN-2015KP7T2Y to CC, by grant from the University of Camerino (Fondo di Ateneo per la Ricerca 2018), and by the Intramural Research Program of the National Institute on Drug Abuse (post-doctoral fellowship to A.B. and post-baccalaureate fellowship to J.B.G.).

ABBREVIATION USED

CNS, central nervous system; ER, endoplasmic reticulum; BED, binge eating disorder; DAT,

dopamine transporter; EtOAc, ethyl acetate.

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