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Opioid and sigma receptor studies. New developments in the design of selective sigma ligands*

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Abstract: New racemic and chiral methyl 2-{[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-1-phenylcyclopropanecarboxylate derivatives were synthesized in order to obtain sigma ligands with increased affinity and selectivity compared to (+)-MPCB and haloperidol. The *cis*-(±)-**7** racemic mixture showed a better binding affinity and selectivity than the (±)-**8** *trans* isomers. Between the two *cis* enantiomers, (+)-**7**, with configuration (1*R*,2*S*), showed a very high affinity and the best selectivity for σ_1. All compounds synthesized (**7–9**) showed a reduced or negligible affinity for opioid and dopaminergic D_1 and D_2 receptors. Nociceptive *in vivo* test confirms that (+)-**7** (namely **MR200**), such as non-selective antagonist haloperidol, increased the analgesic effect induced by the κ opioid selective ligand U50,488H and reversed the inhibiting effect of (+)-pentazocine on analgesia.

INTRODUCTION

Starting with the first studies reported by Martin and coworkers [1,2], many advances have been made in the field of sigma (σ) receptors. At present, this binding site represents a typical protein different from opioid, NMDA, dopaminergic, and other known neurotransmitter or hormone receptor families.

Pharmacological data based on ligand binding studies, anatomical distribution, and biochemical features distinguish at least two subtypes of σ receptors [3–5].

The σ_1 subtype is better characterized at the functional and structural level and shows a very high affinity for the *dextro* isomers of *cis*-normetazocine derivatives such as (+)-pentazocine, (+)-SKF-10,047, and (+)-cyclazocine. The (+)-pentazocine represents a typical selective agonist that was used as tritiated ligand to label σ_1 receptors. Other selective ligands are the putative antagonist NE-100 [6] and a new compound, namely AC915 [7] (Fig. 1).

Studies of anatomical distribution of σ_1 subtype showed that in the central nervous system (CNS) the regions with high levels are the areas involved in motor, sensory, and endocrine functions, and memory. In peripheral tissues, they are present in placenta, liver, immune cells, and gastrointestinal tract.

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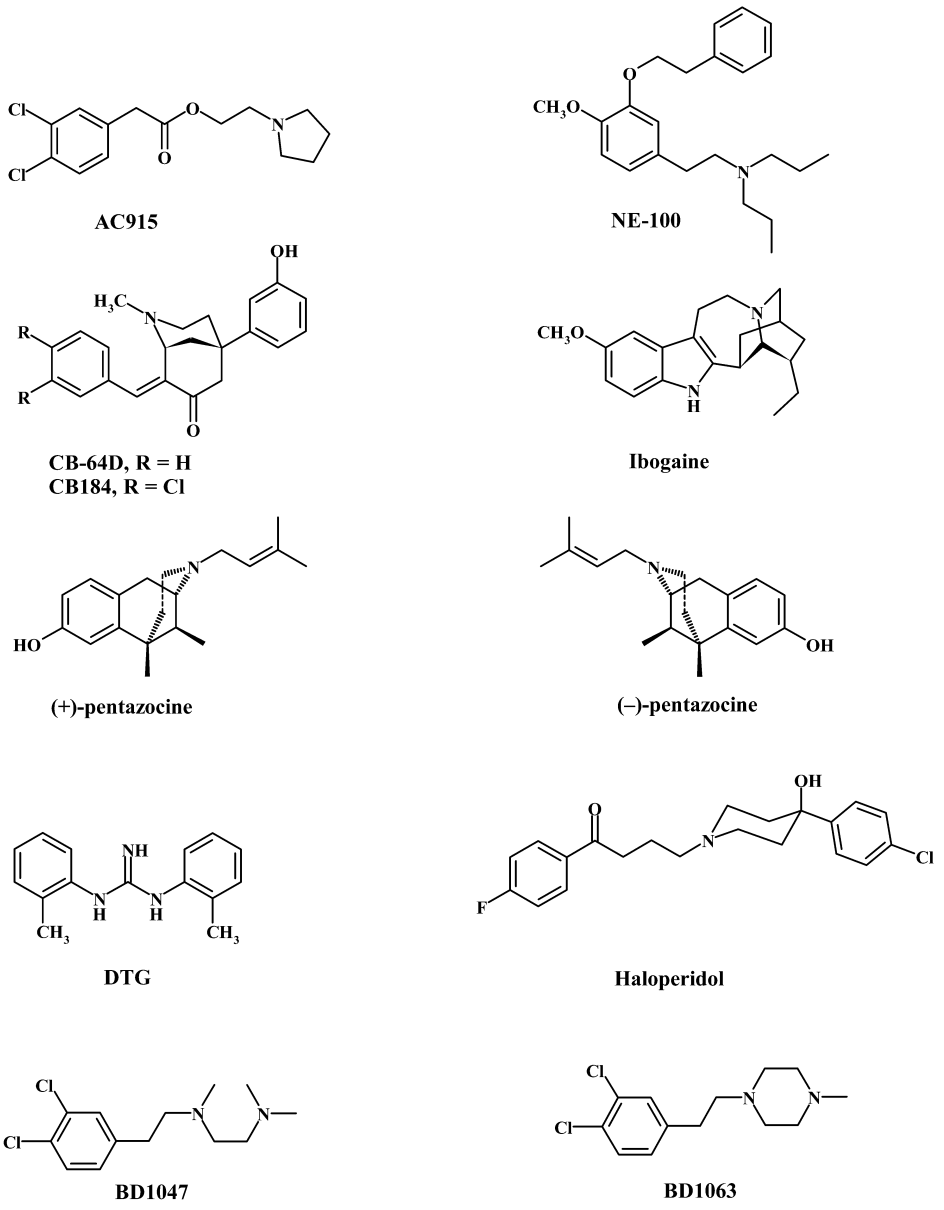


Fig. 1 Sigma ligands.

Recently, a protein of 223 amino acid corresponding to σ_1 receptors has been purified and cloned, first from guinea pig liver and subsequently from human, rat, and mouse tissues [8–10]. This protein shows no analogy with other mammalian proteins, but it has homology with a yeast protein (C_8-C_7 isomerase) involved in the ergosterol biosynthetic pathway [11].

At present, no clear data are reported about the endogenous ligand, but progesterone is believed to be one of them.

The high expression of σ_1 receptors in steroid-producing tissues and in CNS suggests their possible role in functions of neuroendocrine and central neuroactive steroid system.

Additional involvement of σ_1 receptors has been postulated in psychosis with modulation of synthesis and release of neurotransmitters such as acetylcholine and dopamine [12]. Moreover, they seem to be involved in modulation of the glutamatergic system with a neuroprotective effect and an improvement in learning and memory in animal models of amnesia [13,14].

Pasternak *et al.* showed that σ_1 receptors constitute a potent antiopioid system in which typical agonists such as (+)-pentazocine administered with analgesic opioid reduced notably the antinociceptive potency [15–17].

Conversely, the coadministration of analgesic opioid with putative σ_1 antagonist haloperidol increases opioid analgesia. The antiopioid modulator system involves all opioid receptors, but it seems that kappa opioid analgesia is particularly affected [18].

The molecular mechanism involved in all the above-reported effects is not completely clear, but new interesting data provide evidence that modulation of intracellular Ca^{++} level by σ_1 receptor/ankrin/IP_3 receptor-complex is a possible key of action (Fig. 2) [19,20].

Considering the σ_2 subtype, few biochemical data are available. The reasons might be the absence of cloned protein and the paucity of selective ligands with respect to σ_1 or other receptor systems. The (+)-pentazocine showed a very low affinity for σ_2 receptors. Conversely, analgesic (–)-pentazocine, antipsychotic haloperidol, guanidine DTG [*N*,*N*′-(o-tolyl)gunidine], possess high affinity for both σ subtypes [21]. Recently, selective σ_2 subtype compounds, such as alkaloid ibogaine and phenylmorphans CB-64D and CB-184 (Fig. 1), have been reported, but unfortunately these compounds showed also activity and affinity for NMDA (ibogaine) and μ opioid (CB-64D, CB-184) receptors [22,23].

Recent studies confirm that σ_2 subtypes have a different pattern of anatomical distributions in CNS and in peripheral tissues [24]. A very high concentration of σ_2 receptors in neuronal and non-neuronal tumor cell lines provides evidence of a possible role in cell proliferation and viability. In fact, putative σ_2 agonists, such as ibogaine and CB-64D, induce apoptotic death by a new mechanism involving the increase of intracellular Ca^{++} level by release from endoplasmic reticulum and subsequently from mitochondrial stores. These effects are inhibited by putative σ_2 antagonists BD1047 and BD1063 [25].

This physiological involvement of σ_2 receptors led to the consideration that potent and selective ligands could be diagnostic tools and drugs in anticancer therapy [26,27].

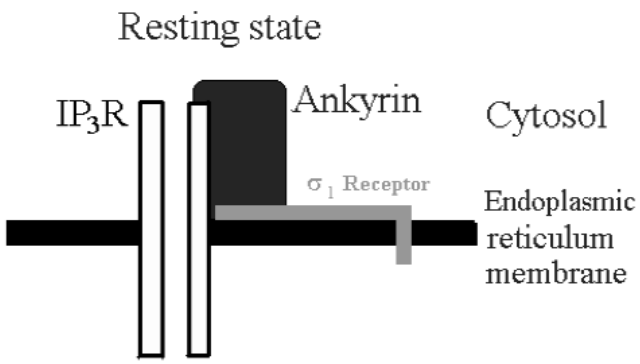


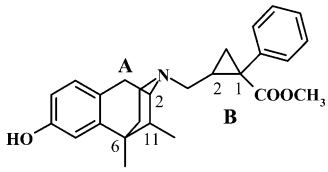
Fig. 2 Graphical representation of σ_1 receptor/ankrin/IP_3 receptor-complex, as reported by Su *et al*.

RATIONAL DRUG DESIGN

In previous studies, we addressed our interest in the design of selective analgesic κ opioid agonist with reduced side effects typical of classical opioid compounds [28]. Moreover, considering the recent advances in σ_1 receptors as modulating opioid analgesia, we focused attention on this field in order to obtain antagonist compounds capable of improving it and, if possible, reducing adverse reactions of opioid therapy.

On the bases of structure–activity relationships (SAR) and molecular modeling studies on selective peptidic and nonpeptidic ligands, we synthesized a new series of (+)- and (−)-*cis*-*N*-normetazocine derivatives in which the two diastereoisomers (+)- and (−)-**MPCB-a** (Table 1) showed a stereoselective interaction with σ_1 and κ opioid receptors, respectively [29]. Nevertheless, these very selective compounds showed only a moderate affinity, and thus, additional studies have been done in order to improve binding potency.

Table 1 Binding affinity to σ_1, κ, μ, and δ receptors (K_i, nM).



Compd.	A	B	σ_1	κ	μ	δ
(+)-MPCB-a	2*S*, 6*S*, 11*S*	1′*R*, 2′*S*	66.7	>1000	>1000	>1000
(+)-MPCB-b	2*S*, 6*S*, 11*S*	1′*S*, 2′*R*	1381	>1000	>1000	>1000
(−)-MPCB-a	2*R*, 6*R*, 11*R*	1′*R*, 2′*S*	>1000	240	>25 000	>25 000
(−)-MPCB-b	2*R*, 6*R*, 11*R*	1′*S*, 2′*S*	>1000	2640	>25 000	>25 000

In particular, modifications on aromatic ring of cyclopropylmethyl moiety provide a 4-chloro derivative, namely (−)-**CCB** with high affinity and specificity for κ opioid receptors (Ki = 0.4 nM for κ, >25 000 for μ and δ, >1000 for σ_1) [30].

Subsequently, studies on (+)-**MPCB-a** showed that substitution of (+)-*cis*-*N*-normetazocine nucleus with more simply amine-provided compounds with a comparable or improved affinity for σ receptors [31].

SAR studies reported by Largent *et al*. on butyrophenones [32] (typical dopamine antagonists with neuroleptic properties) showed that haloperidol, with 4-(4-chlorophenyl)piperidin-4-ol nucleus was the compound with the higher affinity for σ receptors compared to buspirone, spiperone, benperidol, and other butyrophenones having different amino nucleus.

As reported by Pasternak *et al*. [15–17], haloperidol is considered a σ_1 antagonist capable of increasing opioid analgesia by a mechanism involving σ_1 receptors, but not dopaminergic systems.

Thus, considering these data we decided to substitute the nucleus of *cis*-*N*-normetazocine with 4-(4-chlorophenyl)piperidin-4-ol (Fig. 3) of haloperidol in order to obtain compounds with an improved σ_1 affinity and selectivity compared to (+)-**MPCB-a** and haloperidol, respectively. In addition, we evaluated if it were possible to maintain σ_1 antagonist properties capable of increasing opioid analgesic effect like haloperidol.

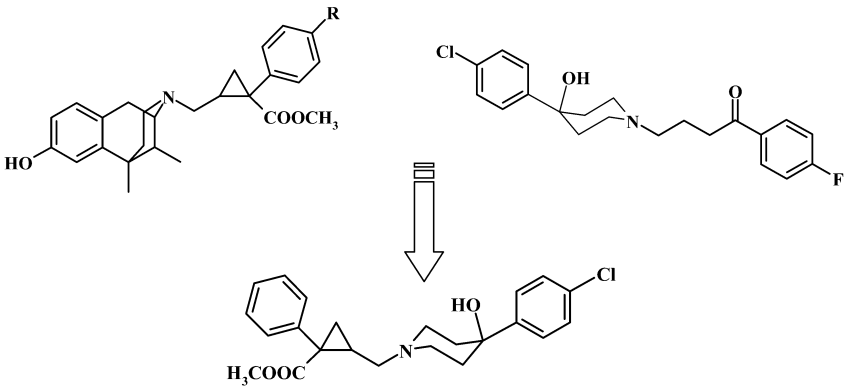


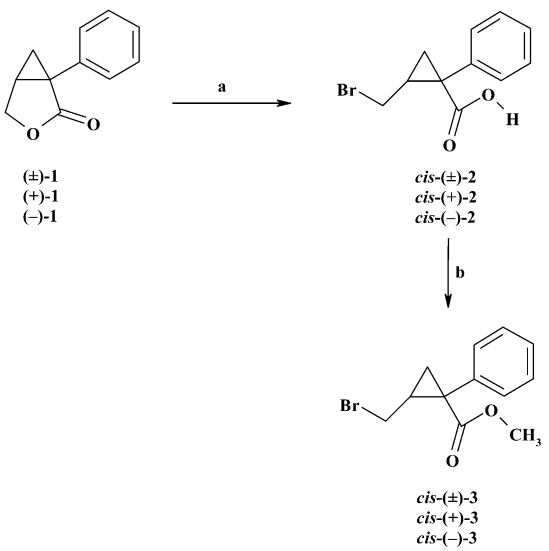
Fig. 3 Rational drug design.

CHEMISTRY

Compounds (±)-**1**, the respective enantiomer (+)- or (–)-**1**, were prepared as previously reported by Casadio *et al*. and Shuto *et al*. [33,34].

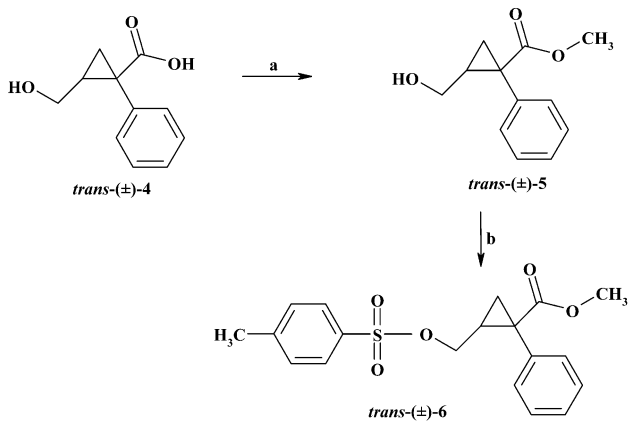
Treatment of the opportune lactone with HBr/CH_3COOH (33%) provided the racemic 2-(bromomethyl)-1-phenylcyclopropanecarboxylic acid *cis*-(±)-**2** and the enantiomers *cis*-(+)- and *cis*-(–)-**2**. Compounds (±)-**2**, (+)-**2** and (–)-**2**, were converted to methyl-2-(bromomethyl)-1-phenylcyclopropanecarboxylate, *cis*-(±)-**3**, *cis*-(+)-**3**, and *cis*-(–)-**3**, by subsequent esterification with $SOCl_2$ and 3N CH_3OH/HCl in benzene (Scheme 1).

Esterification of the *trans*-(±)-**4** carboxylic acid gave the corresponding methyl ester *trans*-(±)-**5**. The *trans*-methyl-2-({[(4-methylphenyl)sulfonyl]oxy}methyl)-1-phenylcyclopropanecarboxylate (±)-**6** was prepared by condensation with tosyl chloride (Scheme 2).

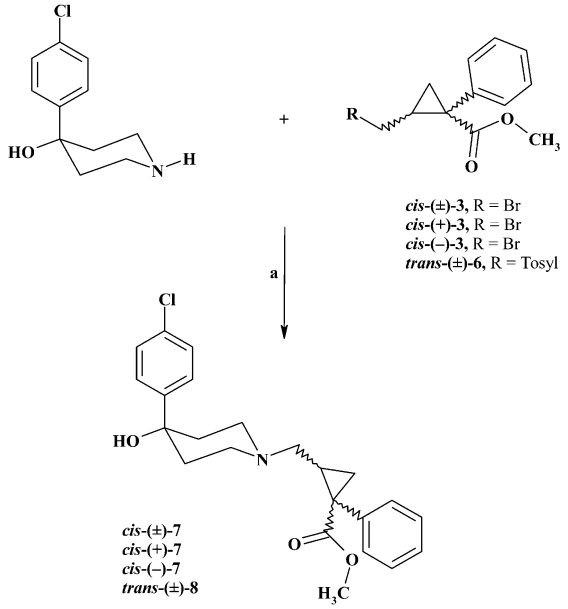


Scheme 1 a) HBr/CH_3COOH (33%), 80 °C, 2 h; b) C_6H_6, $SOCl_2$, 3N CH_3OH/HCl, 5 h.

The synthesis of compounds *cis*-(±)-**7**, *cis*-(+)-**7**, *cis*-(–)-**7**, and *trans*-(±)-**8** were performed by reaction of commercially available 4-(4-chlorophenyl)-4-hydroxypiperidine with the appropriate *cis* bromo or with the *trans* tosyl ester derivative (Scheme 3).



Scheme 2 a) C_6H_6, $SOCl_2$, 3N CH_3OH/HCl, 5 h; b) tosyl chloride, dichloromethane, pyridine.



Scheme 3 a) dimethylformamide, $NaHCO_3$, 70 °C, 8 h.

PHARMACOLOGY

Sigma$_1$ (σ_1) binding assays

σ_1 binding assays were carried out on guinea pig brain membranes prepared by the procedure of Matsumoto *et al.* [35]. The protein concentration of the suspension was evaluated by the method of

Lowry *et al*. [36] and generally ranged from 6.5 to 8.5 mg of protein/mL. Binding assays were performed as described by DeHaven *et al.* [37]. Each tube contained 500 µg of membrane protein, and was incubated with 3 nM [^{3}H]-(+)-pentazocine specific activity (sa) 45 *Ci*/mM; the value of the apparent dissociation constant (K_d) was 1.2 ± 0.3 nM, n = 3) in 50 mM Tris-HCl (pH 7.4). Test compounds were dissolved in dimethyl sulfoxide and then diluited in buffer for a total volume of 1 mL. Test compounds were added in concentration ranging from 10^{-5} to 10^{-11} M. Nonspecific binding was assessed by the addition of 10 µM of haloperidol. The reaction was performed for 150 min at 37 °C and terminated by filtering the solution through Whatman GF/B glass-fiber filters that were presoaked for 1 h in a 0.5% polyethylenimine solution. Filters were washed twice with 4 mL of ice-cold buffer.

Sigma$_2$ (σ_2) binding assays

σ_2 binding assays were carried out on guinea pig brain membranes prepared as described by Mach *et al.* [22]. The membranes were incubated with 3 nM [^{3}H]-DTG [1,3-di-(2-tolyl)-guanidine] (sa 31 *Ci*/mM; K_d = 9.9 ± 0.8 nM; n = 3) in the presence of 400 nM (+)-SKF10,047 to block σ_1 sites. Incubation was carried out in 50 mM TrisHCl (pH 8.0) for 120 min at room temperature, and assays were terminated by the addition of ice-cold 10 mM TrisHCl pH 8.0, followed by filtration through Whatman GF/B glass-fiber filters that were presoaked for 1 h in a 0.5% polyethylenimine solution. Filters were washed twice with 4 mL of ice-cold buffer. Nonspecific binding was evaluated in the presence of 5 µM DTG. Inhibition constants (Ki values) for test compounds were calculated using the EBDA/LIGAND program, purchased from Elsevier/Biosoft [38].

Dopaminergic D_1 and D_2 binding assays

Rats (weighing about 150 g) were killed by decapitation, and their brains were quickly removed. The striatum region was dissected and stored at –80 °C until used. The membranes were prepared as previously described [39,40], and the pellets obtained were resuspended in the incubation buffer (50 mM TrisHCl, pH 7.1, containing 10 µM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) just before the binding assay. [^{3}H]-SCH 23390 (sa 71.1 *Ci*/mM) binding to D_1 receptors was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (2 mg of tissue/sample), 0.25 mL of tritiated ligand (0.4 nM), and 10 µL of displacing agent or solvent. Nonspecific binding was evaluated in the presence of 10 mM of (–)-*cis*-flupentixol. [^{3}H]-spiperone (sa 19.0 *Ci*/mM) binding to D_2 receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (1 mg of tissue/sample), 0.5 mL of tritiated ligand (0.2 nM), and 20 µL of displacing agent or solvent. Nonspecific binding was evaluated in the presence of 100 µM of (–)-sulpiride. Incubations (15 min at 25 °C for D_1 at 37 for D_2) were stopped by rapid filtration under vacuum through Whatman GF/B glass-fiber filters using a Brandel apparatus (mod. M-48R) and washed 3 times with 4 mL of ice-cold buffer. The radioactivity trapped on the filters was counted in 4 mL of "Ultima Gold MV" (Packard) in a DSA 1409 (Wallac) liquid scintillation counter, with a counting efficiency of 50%. For inhibition experiments, the drugs were added to the binding mixture at 7–9 different concentrations. Inhibition curves were analyzed using the "Allfit" program [41] running on an IBM AT-PC. The Ki values were derived from the IC_{50} values using the Cheng and Prusoff equation [42].

Opioid binding assays

Total opioid receptor binding assays were performed using [^{3}H]-naloxone (sa 55.5 *Ci*/mmol; K_d = 6.6 ± 0.7 nM; n = 3), and rat brain membranes were prepared as previously reported [31]. Nonspecific binding was evaluated in the presence of 10 µM naloxone.

Nociceptive testing

Tail flick (TF) test was used in our study to determine changes in thermal withdrawal latency of the tail to noxious heat [15]. Briefly, the TF apparatus (Basile, Comerio, VA, Italy) consisted of a beam of radiant heat, provided by a 50-W projector lamp focused on the bottom of the tail. TF latency was automatically recorded by a photocell, located immediately below the tail, from onset of heating of the tail to withdrawal of the tail itself from the source of heat. Light intensity of the beam was controlled to provide a pre-drug latency between 3 and 4 s. A cut-off latency of 10 s was used to avoid tissue damage to the tail. The baseline latency corresponded to the average of the first three measurements.

Male Sprague Dawley rats (180 ± 10 g) were used in all experiments. Animals were divided randomly into separate groups of 6–10 rats each.

Data were expressed as the mean ±SEM of the values recorded in the animals of the same group. The statistical significance of differences between groups was assessed with a one-way of variance (ANOVA) followed by Dunnett's multiple comparisons. The criterion for statistical significance was $P < 0,05$ in all statistical evaluations.

RESULTS AND DISCUSSION

Binding affinity studies showed that replacement of *cis-N*-normetazocine with 4-(4-chlorophenyl) piperidin-4-ol nucleus provided compounds with higher affinity and selectivity for σ receptors compared to **MPCB** and haloperidol (Table 2). In particular, binding data showed a better binding profile of *cis*-(±)-**7** racemic mixture for σ receptors than the respective (±)-**8** *trans* isomers. Moreover, between the two *cis* enantiomers **7**, *dextro* isomer with configuration (1*R*,2*S*) showed a very high affinity, and the best selectivity for σ_1 compared to all synthesized compounds and haloperidol.

Considering the binding data for opioid and dopaminergic D_1 and D_2 receptors, except for *cis* enantiomer (−)-**7** with low affinity for D_2 receptors, all compounds (**7**–**8**) presented a very low or negligible affinity.

Thus, on the base of binding profile, compound **(+)-7 (MR200)** has been submitted to evaluation of *in vivo* studies in order to test if it improves analgesia of selective κ opioid ligands.

As reported in Fig. 4, (+)-pentazocine alone, at the dose utilized in this study (5 mg/kg/s.c.), had no effect on TF latency; the same result has been obtained with the injection of **MR200** (1 mg/kg/s.c.). As expected, subcutaneous administration of the κ opioid agonist U50,488H, at the dose of 4 mg/kg/s.c., caused a significant increase on TF latency. The peak of this analgesic effect (8.6 ± 0.34) was reached 30 min after the injection, then gradually declined and returned to the pre-injection level after 120 min (data not shown). The dose of the opioid was selected in order to avoid maximal effects.

As shown in Fig. 5, pretreatment with (+)-pentazocine (5 mg/kg/s.c.) inhibited the analgesic effect induced by subcutaneous injection of U50,488H (4 mg/kg/s.c.). The mean of the values registered at the TF test, reproposed the value of 4.53 ± 0.26 against the U50,488H analgesic peak of 8.6 ± 0.34.

Table 2 Binding affinity to σ_1, σ_2, opioid, D_1 and D_2 receptors (K_i, nM ± SEM).

Compd.	σ_1	σ_2	Opioid	D_1	D_2
(+)-MPCB-a	66.7 ± 2.2	3980 ± 42	>1000	>10 000	>10 000
(−)-MPCB-a	>1000	>10 000	378 ± 12	>25 000	>25 000
(±)-7 (MR 177)	2.43 ± 0.8	14.7 ± 2.7	>1000	>10 000	1018
(+)-7 (MR 200)	1.51 ± 0.6	21.9 ± 3.2	>1000	>10 000	3230
(−)-7 (MR 201)	5.6 ± 1.0	23.4 ± 3.5	>1000	>10 000	378 ± 49
(±)-8 (MR 204)	14.6 ± 1.5	61.1 ± 4.3	>1000	>10 000	5200 ± 241
Haloperidol	2.2 ± 0.5	16 ± 2.7	>1000	318 ± 59	2.1 ± 0.3

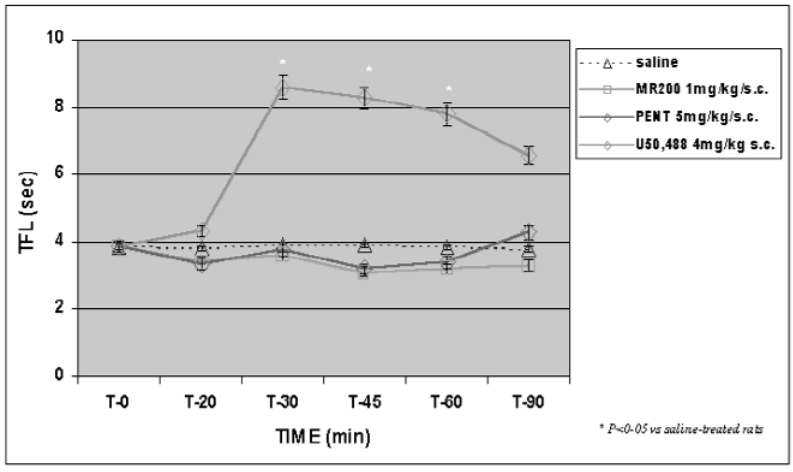


Fig. 4 Time-related effects of (+)-pentazocine (5 mg/kg/s.c.), **MR200** (1 mg/kg/s.c.), U50,488H (4mg/kg/s.c.) or saline.

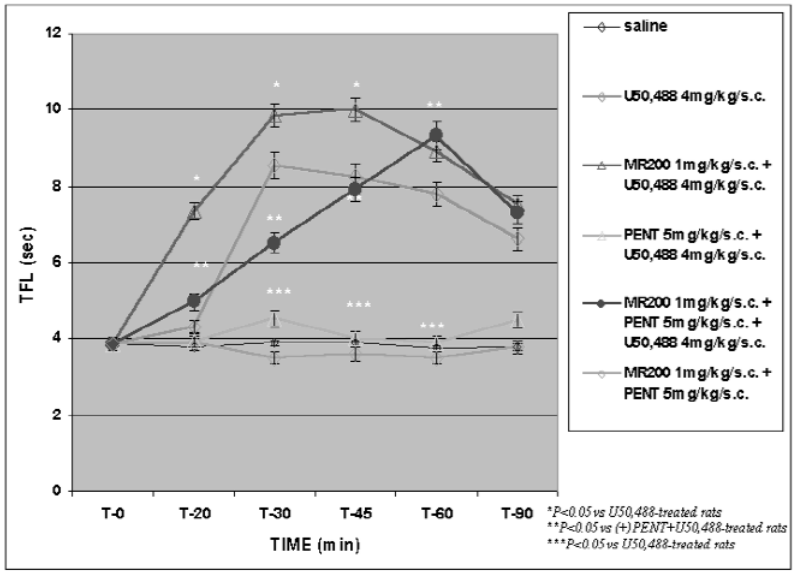


Fig. 5 Time-related effect of (+)-pentazocine (5 mg/Kg/s.c.) and **MR200** (1 mg/Kg/s.c.) on U50,488-induced antinociception.

On the contrary, pretreatment with **MR200** (1 mg/kg/s.c.) intensified U50,488H-induced analgesia. The antinociceptive peak was significantly enhanced from a value of 8.6 ± 0.34 to a value of 9.86 ± 0.39 registered at 30 min and to the cut-off time of 10 s at 45 min. A sequential treatment with **MR200**, (+)-pentazocine and U50,488H, respectively, was carried out to investigate the possible σ antagonistic activity of **MR200**. The results obtained showed that **MR200** was capable of reversing the (+)-pentazocine inhibition on U50,488H-induced antinociception, showing a significant analgesic profile.

However, the double treatment with **MR200** (1 mg/kg/s.c.) and (+)-pentazocine (5 mg/kg/s.c.) remarked the value of the baseline-latency.

In summary, neither **MR200** nor (+)-pentazocine alone modify TF latencies. In agreement with previous studies, (+)-pentazocine prevents the analgesic effect induced by U50,488H. The high affinity of (+)-pentazocine for σ_1 receptors confirms a possible functional antagonism of σ_1 receptors on opioid effects. Moreover, **MR200** as reported for nonselective σ_1 antagonist haloperidol, increases the analgesic effect induced by U50,488H and reverses the inhibiting effect of (+)-pentazocine on analgesia induced by the κ opioid.

CONCLUSIONS

In this preliminary study we reported our strategy to obtain new σ ligands with improved affinity and selectivity compared to (+)-**MPCB** and haloperidol. Moreover, we showed that selected σ ligand **MR200**, increasing κ opioid analgesia, represents a new speculative tool in the pathological pain control.

At present, we are evaluating the effect of **MR200** in the modulation of μ, δ, and ORL_1 analgesia, and, in particular, we are studying if it is possible to reduce the typical side effects of opioid therapy.

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