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Favourable involvement of α_{2A}-adrenoreceptor antagonism in the I_2-imidazoline binding sites-mediated morphine analgesia enhancement

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ABSTRACT

Aim of the present study was to obtain novel α_2-adrenoreceptor (α_2-AR) antagonists, possibly endowed with subtype-selectivity. Therefore, inspired by the non subtype-selective α_2-AR antagonist idazoxan, we designed 1,4-dioxane derivatives bearing an aromatic area in position 5 or 6 and the imidazoline nucleus in position 2. Among the novel molecules **1–6**, compound **2**, with a trans stereochemical relationship between 5-phenyl and 2-imidazoline groups, was able to antagonize the sole α_{2A}-subtype. Moreover, **2** showed an affinity at I_2-imidazoline binding sites (I_2-IBS) comparable to that at α_{2A}-AR. In in vivo studies **2** strongly increased morphine analgesia. This interesting behaviour appeared to be induced by the favourable involvement of α_{2A}-AR antagonism in the I_2-IBS-mediated morphine analgesia enhancement.

1. Introduction

α_2-Adrenoreceptors (α_2-ARs), belonging to the superfamily of G_i/G_o protein coupled receptors, have been classified into α_{2A}-, α_{2B}-, and α_{2C}-subtypes.[1] They are widely distributed throughout the peripheral and central nervous systems (PNS and CNS) and are considered attractive therapeutic targets for the treatment of a wide range of diseases.[2] Studies on mice lacking or overexpressing α_2-AR subtypes[3] indicated that the α_{2A}-subtype mediates hypotension, sedation and analgesia, the α_{2B}-subtype mediates hypertension, while the α_{2C}-subtype appears to be involved in many CNS processes.

The primary use of α_2-AR agonists has long been the treatment of hypertension, but the associated sedative effects have severely limited their prescription; in contrast, they still represent valuable tools for the relief of intractable cancer pain.[4] Besides being antidotes for reversing effects of agonist overdose, centrally acting α_2-AR antagonists have been postulated for the treatment of depression.[5] Moreover, α_2-AR antagonists may represent a rational strategy for the treatment of Alzheimer's and Parkinson's diseases.[6] Nevertheless, probably due to the inability to discriminate the α_2-AR subtypes, the commonly used α_2-AR antagonists idazoxan, phentholamine and yohimbine do not exhibit unequivocal pharmacological properties. Therefore, the therapeutic potential of α_2-AR subtype-selective antagonists might not have yet completely been explored.

The focus of our research over the years has been the development of ligands targeting α_2-AR subtypes and interesting α_{2C}- and α_{2A}/α_{2C}-agonists have been discovered.[7] In the present study, our aim was to obtain novel α_2-AR antagonists, possibly endowed with subtype-selectivity. Therefore, the quite planar 1,4-benzodioxane scaffold of the α_2-AR antagonist idazoxan[8] was replaced by the less conformationally constrained 1,4-dioxane nucleus substituted in position 5 or 6 with one phenyl group in both cis or trans stereochemical relationship with the imidazoline ring in position 2 (compounds **1–4**) (Chart 1). These modifications allowed us to evaluate the influence of different distances between the aromatic and basic moieties on the biological effects and the stereochemical requirements of the receptor interaction. Moreover, the effect of the enlargement of the aromatic area was examined by the insertion of two phenyl groups in position 5 or 6 (compounds **5** and **6**, respectively). This investigation was also prompted by our recent studies in which the 1,4-dioxane nucleus proved to be a suitable scaffold for potent muscarinic,[9] α_{1D}-adrenergic and 5-HT_{1A} serotoninergic receptor ligands.[10] Finally, the enantiomers of the

Abbreviations: AR, adrenoreceptor; PNS, peripheral nervous system; CNS, central nervous system; IBS, imidazoline binding sites; CHO, Chinese hamster ovary; (1*S*)-(+)-10-CSA, (1*S*)-(+)-10-camphorsulfonic acid; *m*-CPBA, *m*-chloroperbenzoic acid; MPE, Maximum Possible Effect.

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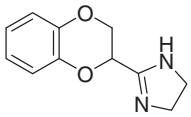
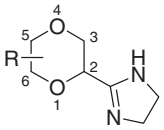


Idazoxan



1, 5-C_6H_5 cis
2, 5-C_6H_5 trans
3, 6-C_6H_5 cis
4, 6-C_6H_5 trans
5, 5-$(C_6H_5)_2$
6, 6-$(C_6H_5)_2$

Chart 1. Chemical structures of idazoxan and the compounds **1–6**.

most interesting compound **2** were prepared and studied. The affinity values and antagonist potencies of the novel compounds were evaluated on CHO cells expressing recombinant human α_2-AR subtypes, according to procedures previously reported.[7] Moreover, since some α_2-AR ligands are known to be able to interact also with α_1-ARs, 5-HT_{1A} receptor[8] and I_2-imidazoline binding sites (I_2-IBS),[11] the affinity values of **2** at these systems were assessed in agreement with procedures already reported.[10,12] Compound **2** was also evaluated in an algesiometric test.

2. Chemistry

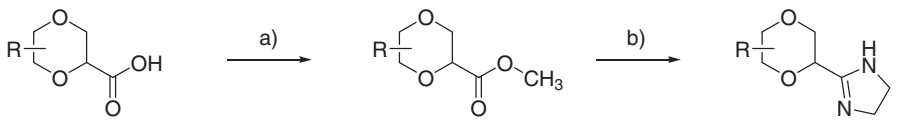
Compounds **1–6** were prepared by treatment of methyl esters **7–12**, obtained by the corresponding acids,[10] with ethylenediamine in the presence of $Al(CH_3)_3$ (Scheme 1). The two enantiomers (−)-**2** and (+)-**2** were obtained by fractional crystallization of the salts of (±)-**2** with hydrogen dibenzoyl-L- and hydrogen dibenzoyl-D-tartaric acid, respectively. The enantiomeric purity, determined by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol, was found to be >98% (detection limit) for both enantiomers. In fact, the ^{1}H NMR spectrum of racemic compound (±)-**2** showed two double doublets at δ 4.76 for the proton in position 5 of 1,4-dioxane nucleus, whereas only one double doublet was observed for (−)-**2** and (+)-**2** at δ 4.74 and δ 4.78, respectively. The (2*S*,5*R*) absolute configuration of the enantiomer (−)-**2** was determined by comparing the sign of its optical rotation with that of the (2*S*,5*R*) enantiomer obtained by stereoselective synthesis (Scheme 2). Compound (*R*)-(−)-**13**, obtained by treatment of methyl (*R*)-(−)-mandelate (commercially available) with allyl bromide in presence of Ag_2O, was reduced with $LiAlH_4$ to afford the intermediate alcohol (*R*)-(−)-**14.** The epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA) and subsequent cyclization with (1*S*)-(+)-10-camphorsulfonic acid [(1*S*)-(+)-10-CSA] in CH_2Cl_2 afforded a cis/trans diastereomeric mixture, from which (2*S*,5*R*)-(−)-**15** was obtained by flash chromatography. The oxidation of (2*S*,5*R*)-(−)-**15** with $KMnO_4$ to the corresponding acid (2*R*,5*R*)-(−)-**16**, followed by esterification with methanol, yielded the methyl-ester (2*R*,5*R*)-(−)-**8**, which was transformed into the corresponding imidazoline (2*S*,5*R*)-(−)-**2**.

The enantiomer (2*S*,5*R*)-(−)-**2**, obtained by stereoselective synthesis, showed an enantiomeric purity >98%, determined by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol. Since the ^{1}H NMR spectra of this enantiomer and of (−)-**2**, obtained by fractional crystallization, in the presence of the chiral shift reagent were identical, it reasonably follows that (−)-**2** has a (2*S*,5*R*) configuration.

3. Results and discussion

The affinity values (pK_i) and antagonist potencies (pK_b) of the novel compounds at α_2-AR subtypes are reported in Table 1 together with those of idazoxan[7] included for useful comparison. From these data it emerged that a general decrease of affinity and potency was observed for all the compounds. Nevertheless, noteworthy is the observation that **2**, bearing the phenyl group in position 5 in trans stereochemical relationship with the imidazoline ring, was able to antagonize the sole α_{2A}-subtype (pK_i = 6.30; pK_b = 6.40) and was devoid of agonist activity at the other α_2-AR subtypes (data not shown). The fact that the corresponding cis diastereomer **1** was endowed with negligible affinity and potency highlighted the important role played by stereochemistry in the receptor recognition. This observation was further strengthened by the study of the biological behaviour of the enantiomers of **2**. Indeed, only the levorotatory form (2*S*,5*R*)-(−)-**2** showed the same α_{2A}-affinity and antagonist potency of the racemic compound, whereas the enantiomer (2*R*,5*S*)-(+)-**2** was inactive up to 10 μM concentration. In addition, unlike idazoxan,[8] **2** showed no appreciable affinity at α_{1A}-ARs and 5-HT_{1A} receptor (pK_i <5).

The use of α_2-AR agonists (i.e. clonidine) as adjuvants with opioids continues to be the focus of clinical investigations. Nevertheless, Scheinin and co-workers reported that antinociceptive responses to partial opioid agonists were very markedly accentuated by targeted inactivation of the α_{2A}-AR gene in mice. The possible mechanisms included reversal of α_{2A}-AR-dependent desensitization of some components of the common intracellular signalling pathways of α_{2A}-ARs and μ-opioid receptors, and removal of conformational constraints imposed by hetero-oligomeric α_{2A}-ARs to μ-opioid receptor activation by partial agonists. Therefore, these authors hypothesized that selective α_{2A}-AR antagonists might enhance the antinociceptive effects of partial opioid agonists.[13] On the basis of this hypothesis and with the hope of improving the knowledge of the therapeutic potential of α_{2A}-AR antagonists, **2** has been evaluated in in vivo studies. Our aim was also encouraged by some recent observations indicating that α_2-AR antagonists,

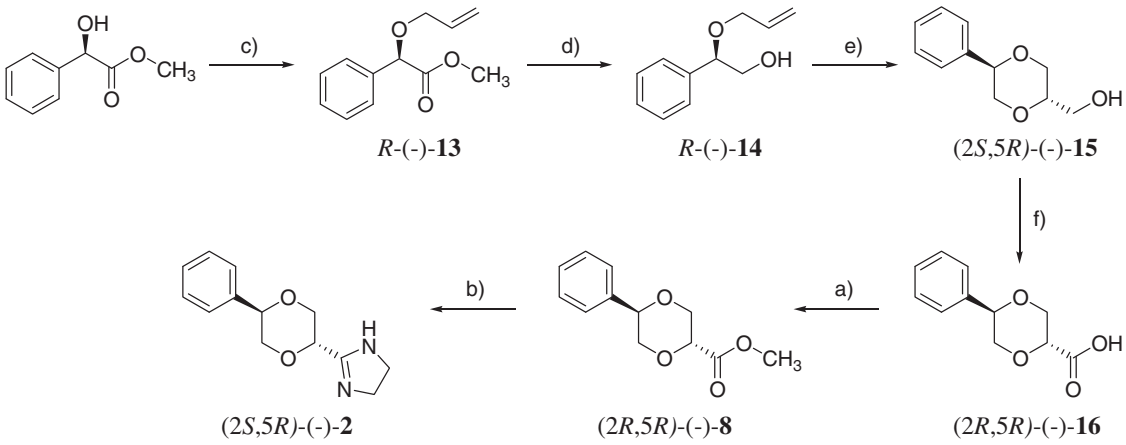


5-C_6H_5 cis
5-C_6H_5 trans
6-C_6H_5 cis
6-C_6H_5 trans
5-$(C_6H_5)_2$
6-$(C_6H_5)_2$

7, 5-C_6H_5 cis
8, 5-C_6H_5 trans
9, 6-C_6H_5 cis
10, 6-C_6H_5 trans
11, 5-$(C_6H_5)_2$
12, 6-$(C_6H_5)_2$

1, 5-C_6H_5 cis
2, 5-C_6H_5 trans
3, 6-C_6H_5 cis
4, 6-C_6H_5 trans
5, 5-$(C_6H_5)_2$
6, 6-$(C_6H_5)_2$

Scheme 1. Reagents: (a) CH_3OH/H_2SO_4; (b) $NH_2CH_2CH_2NH_2$, $Al(CH_3)_3$, toluene.



Scheme 2. Reagents: for (a) and (b) see Scheme 1; (c) allyl bromide, Ag_2O, Et_2O; (d) $LiAlH_4$, THF; (e) *m*-CPBA, CH_2Cl_2; (1*S*)-(+)-10-CSA, CH_2Cl_2; flash chromatography; (f) $KMnO_4$, KOH, H_2O.

Table 1
Affinity Constants (pK_i) [a] and Antagonist Potency (pK_b) [b] on Human α_2-AR Subtypes.

Compd	pK_i			pK_b		
	α_{2A}	α_{2B}	α_{2C}	α_{2A}	α_{2B}	α_{2C}
Idazoxan	8.15 ± 0.11	7.64 ± 0.09	7.75 ± 0.08	7.73 ± 0.09	7.16 ± 0.10	7.92 ± 0.08
1	<5	<5	<5	<5	5.50 ± 0.16	<5
2	6.30 ± 0.14	<5	<5	6.40 ± 0.11	<5	<5
(2*R*,5*S*)-(+)-**2**	5.10 ± 0.09	<5	<5	<5	<5	<5
(2*S*,5*R*)-(−)-**2**	6.40 ± 0.12	<5	<5	6.40 ± 0.16	<5	<5
3	5.27 ± 0.15	<5	<5	<5	5.18 ± 0.13	<5
4	5.20 ± 0.09	<5	<5	<5	<5	<5
5	<5	5.55 ± 0.09	5.13 ± 0.11	<5	<5	<5
6	5.42 ± 0.10	5.35 ± 0.13	5.21 ± 0.14	5.37 ± 0.10	<5	<5

[a] pK_i values were calculated from [^{3}H]RX 821002 on membrane preparations from CHO cells expressing individually each human α_2-AR subtype (α_{2A}, α_{2B}, α_{2C}).
[b] pK_b values were determined by applying the cytosensor microphysiometry system to the same cell models and they express the ability of the antagonist to shift the agonist (clonidine) concentration-effect curve. The data are expressed as means ± SEM of three to six separate experiments.

though endowed with moderate affinity (pK_i = 6.58) and potency (pK_b = 6.25), were anyhow able to evoke significant in vivo responses.[14] Therefore, **2** was administered to mice 15 min before morphine and was tested in the algesiometric paradigm mouse tail flick test. From in vivo dose–response studies (Fig. 1) it emerged that **2**, lacking antinociceptive properties alone, at low doses (1 or 2 mg/kg) caused a significant increase of morphine analgesia. The increased tail-flick latency was still observed 120 min after the administration. On the contrary, confirming previous data,[15] the known α_2-AR non subtype selective antagonists idazoxan or yohimbine,[7,16] used in this test as reference compounds, were devoid of this effect. These results, supporting Scheinin and co-worker's hypothesis,[13] suggested the favourable role played by the inhibition of the sole α_{2A}-AR subtype in morphine analgesia enhancement induced by **2**.

Also IBS, classified into I_1- and I_2-IBS, represent therapeutically interesting targets. While I_1-IBS participate in the regulation of cardiovascular function, I_2-IBS appear to be involved in Parkinson's disease, depression and modulation of morphine analgesia as well as tolerance and addiction to opioids.[17] We have previously demonstrated that ligands bearing a wholly carbon bridge between phenyl and imidazoline moieties induced selective I_2-IBS recognition. Indeed, phenyzoline, bearing an ethylene bridge, showed a pK_i value of 8.60 at I_2-IBS and an I_2-IBS/α_2-ARs selectivity ratio of 794.[18] Moreover, phenyzoline significantly enhanced morphine analgesia, while its ortho-phenyl derivative diphenyzoline, another selective I_2-IBS ligand, decreased morphine antinociceptive effect. Both the positive or negative morphine analgesia modulations

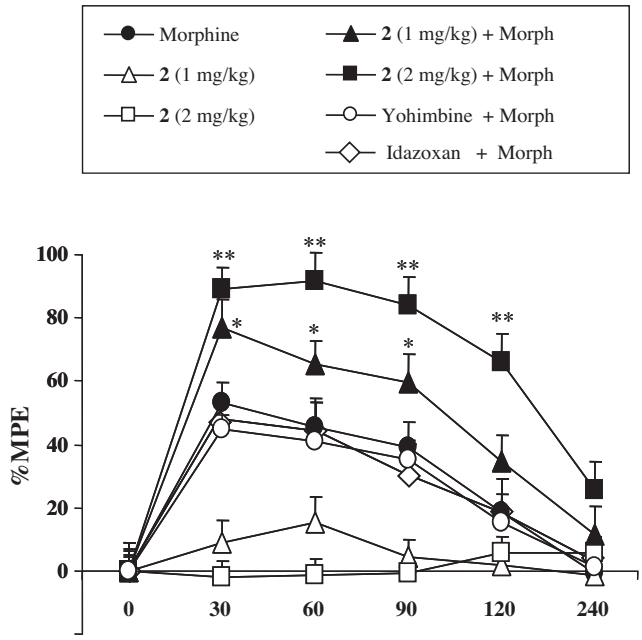


Figure 1. Effect of **2** (1 and 2 mg/kg, ip) pre-treatment on morphine (5 mg/kg, sc) analgesia in the tail-flick test. The reaction latencies were expressed as a percent of the Maximum Possible Effect (%MPE). Values are means ± SEM of 8–10 mice. $^*p<0.05$, $^{**}p<0.01$ compared to morphine group; where not indicated, the difference was not statistically significant.

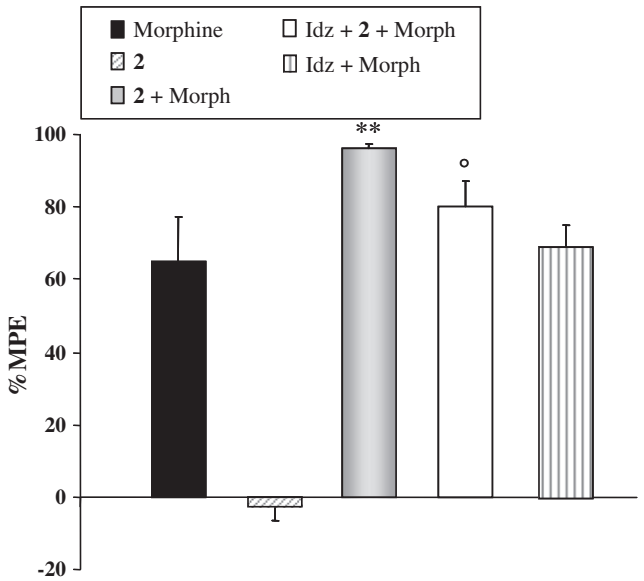


Figure 2. Effect of idazoxan (2.0 mg/kg, ip) on enhancement of morphine (5 mg/kg, sc) analgesia induced by **2** in the tail-flick test. The reaction latencies were expressed as a percent of the Maximum Possible Effect (%MPE). Values are means ± SEM of 8–10 mice. $^{**}p$ <0.01 compared to morphine group; $^{\circ}p$ <0.05 compared to **2**+morphine group.

proved to be mediated by I_2-IBS, because they were completely reversed by the mixed α_2-AR/I_2-IBS antagonist idazoxan. We also speculated that phenyzoline and diphenyzoline, interacting with I_2-IBS, might induce a perturbation of μ-opioid receptors to higher or lower, respectively, affinity state conformation for opioid ligands.[15]

From the aforementioned study[18] it also emerged that, instead, the presence of the oxygen atom in the bridge allowed ligand to interact with both α_2-ARs and I_2-IBS. Therefore, on the basis of these observations, the peculiar nature of 1,4-dioxane ring and its presence as benzocondensed fragment in idazoxan, compatible with the high I_2-IBS affinity of this ligand (pK_i = 8.37),[19] prompted us to assess the affinity values of **2** and its enantiomers at I_2-IBS. From the obtained data the racemic compound **2** showed an affinity value (pK_i = 6.35) comparable to that found for α_{2A}-AR. Also at this system (2*S*,5*R*)-(−)-**2** was the eutomer, its affinity value (pK_i = 6.52) being significantly higher than that of (2*R*,5*S*)-(+)-**2** (pK_i = 5.48). These results did not allow us to exclude the I_2-IBS contribute on morphine analgesia enhancement induced by **2**. Therefore, to verify the real I_2-IBS involvement, analogously to what made for phenyzoline,[15] we assessed the tail-flick latencies after pre-treatment of the mice with idazoxan. The observation that idazoxan partially contrasted morphine analgesia enhancement (Fig. 2) supported the involvement of I_2-IBS in the positive effect induced by **2**. Therefore, the biological profile of **2** was characterized by its ability both to selectively antagonize α_{2A}-AR subtype and to positively modulate I_2-IBS-mediated morphine analgesia.

In conclusion, considering that (i) yohimbine and idazoxan, non subtype selective α_2-AR antagonists, did not enhance morphine analgesia; (ii) phenyzoline, a selective I_2-IBS ligand positively modulating morphine analgesia, required significantly higher dose (10 mg/kg)[15] for producing an effect comparable to that of **2**, we think that α_{2A}-AR antagonism might favourably contribute to I_2-IBS-mediated morphine analgesia enhancement. Anyway, **2**, lacking sedative and hypotensive side effects due to its α_{2A}-AR antagonism, in combination with opioids, might be a novel promising tool therapeutically useful in pain management.

4. Experimental section

4.1. Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded on Perkin-Elmer 297 and Varian EM-390 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). The elemental compositions of the compounds were performed by the Microanalytical Laboratory of our department and agreed to within ±0.4% of the calculated value. When the elemental analysis was not included, crude compounds were used in the next step without further purification. Optical activity was measured at 20 °C with a Perkin–Elmer 241 polarimeter. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Chemical names were generated using ChemDraw Ultra (CambridgeSoft, version 9.0). The purity of the novel tested compounds was determined by combustion analysis and was ⩾95%.

4.1.1. *cis*-Methyl 5-Phenyl-1,4-dioxane-2-carboxylate (7)

A solution of cis-5-phenyl-1,4-dioxane-2-carboxylic acid (1.1 g, 5.23 mmol)[10] in MeOH (20 mL) and H_2SO_4 (1.5 mL) was heated to reflux for 5 h. Removal of the solvent under reduced pressure gave a residue, which was dissolved in water. The aqueous solution was basified with 2 N NaOH and extracted with Et_2O. Removal of dried solvent gave a residue which was purified by column chromatography, eluting with cyclohexane/EtOAc (95:5) to afford an oil: 89% yield. ^{1}H NMR ($CDCl_3$): δ 3.82 (dd, 1, cycle), 3.91 (s, 3, C*H*$_3$), 4.08 (dd, 2, cycle), 4.31 (d, 1, cycle), 4.42 (d, 1, cycle), 4.62 (dd, 1, cycle), 7.23–7.39 (m, 5, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.74; H, 6.60.

4.1.2. *trans*-Methyl 5-Phenyl-1,4-dioxane-2-carboxylate (8)

This was obtained following the procedure described for **7** starting from trans-5-phenyl-1,4-dioxane-2-carboxylic acid[10] to afford an oil: 87% yield. ^{1}H NMR ($CDCl_3$): δ 3.58 (dd, 1, cycle), 3.77 (dd, 1, cycle), 3.80 (s, 3, C*H*$_3$), 4.03 (dd, 1, cycle), 4.26 (dd, 1, cycle), 4.37 (dd, 1, cycle), 4.61 (dd, 1, cycle), 7.31–7.37 (m, 5, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.77; H, 6.22.

4.1.3. *cis*-Methyl 6-Phenyl-1,4-dioxane-2-carboxylate (9)

This was obtained following the procedure described for **7** starting from *cis*-6-phenyl-1,4-dioxane-2-carboxylic acid[10] to afford an oil: 87% yield. ^{1}H NMR ($CDCl_3$): δ 3.42 (dd, 1, cycle), 3.61 (dd, 1, cycle), 3.79 (s, 3, C*H*$_3$), 3.98 (m, 1, cycle), 4.18 (dd, 1, cycle), 4.51 (dd, 1, cycle), 4.75 (dd, 1, cycle), 7.24–7.42 (m, 5, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.99; H, 6.58.

4.1.4. *trans*-Methyl 6-Phenyl-1,4-dioxane-2-carboxylate (10)

This was obtained following the procedure described for **7** starting from *trans*-6-phenyl-1,4-dioxane-2-carboxylic acid[10] to afford an oil: 89% yield. ^{1}H NMR ($CDCl_3$): δ 3.51 (dd, 1, cycle), 3.82 (d, 1, cycle), 3.84 (s, 3, C*H*$_3$), 3.95 (dd, 1, cycle), 4.33 (d, 1, cycle), 4.42 (d, 1, cycle), 5.16 (dd, 1, cycle), 7.31–7.37 (m, 5, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.59; H, 6.10.

4.1.5. Methyl 5,5-diphenyl-1,4-dioxane-2-carboxylate (11)

This was obtained following the procedure described for **7** starting from 5,5-diphenyl-1,4-dioxane-2-carboxylic acid[10] to afford an oil: 86% yield. ^{1}H NMR ($CDCl_3$): δ 3.75 (d, 1, cycle), 3.78 (s, 3, C*H*$_3$), 3.96 (d, 1, cycle), 4.00 (d, 1, cycle), 4.40 (dd, 1, cycle), 4.62 (d, 1,

cycle), 7.19–7.52 (m, 10, Ar*H*). Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.70; H, 6.17.

4.1.6. Methyl 6,6-diphenyl-1,4-dioxane-2-carboxylate (12)

This was obtained following the procedure described for **7** starting from 6,6-diphenyl-1,4-dioxane-2-carboxylic acid[10] to afford an oil: 90% yield. ^{1}H NMR ($CDCl_3$): δ 3.61 (m, 2, cycle), 3.79 (s, 3, C*H*$_3$), 4.08 (dd, 1, cycle), 4.37 (dd, 1, cycle), 4.62 (d, 1, cycle), 7.21–7.59 (m, 10, Ar*H*). Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.19; H, 6.31.

4.1.7. cis-2-(5-Phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (1)

A solution of ethylenediamine (0.42 mL, 10.91 mmol) in dry toluene (10 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminum (5.4 mL, 10.91 mmol) in dry toluene (6 mL) at 0 °C in nitrogen atmosphere. The mixture was stirred at room temperature for 1 h then, after cooling to 0 °C, a solution of **7** (1.2 g, 5.4 mmol) in dry toluene (10 mL) was added dropwise. The reaction mixture was heated to 70 °C for 3 h, cooled to 0 °C, and quenched cautiously with MeOH (10 mL) followed by H_2O (0.3 mL). After addition of $CHCl_3$ (10 mL), the mixture was left at room temperature for 30 min to ensure the precipitation of the aluminum salts. The mixture was filtered and the organic layer was extracted with 2 N HCl. The aqueous layer was made basic with 10% NaOH and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated to give an oil, which was purified by flash chromatography eluting with ciclohexane/EtOAc/MeOH/33% NH_4OH (8:2:1:0.1) to afford a solid: 57% yield; mp 175–177 °C. ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 4, NC*H*$_2$C*H*$_2$N), 3.89 (m, 3, cycle), 4.00 (dd, 1, cycle), 4.38 (dd, 1, cycle), 4.69 (dd, 1, cycle), 5.78 (br, 1, N*H*, exchangeable with D_2O), 7.22–7.40 (m, 5, Ar*H*). The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 199–200 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}H_2C_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.76; H, 5.88; N, 8.78.

4.1.8. *trans*-2-(5-Phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (2)

This was obtained following the procedure described for **1** starting from **8** to afford a white solid: 63% yield; mp 178–180 °C. ^{1}H NMR ($CDCl_3$): δ 3.55 (dd, 1, cycle), 3.61 (s, 4, NC*H*$_2$C*H*$_2$N), 3.80 (dd, 1, cycle), 3.95 (dd, 1, cycle), 4.29 (dd, 1, cycle), 4.43 (dd, 1, cycle), 4.59 (dd, 1, cycle), 6.47 (br, 1, N*H*, exchangeable with D_2O), 7.25–7.38 (m, 5, Ar*H*). The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 202–204 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}H_2C_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.69; H, 5.49; N, 8.55.

4.1.9. *cis*-2-(6-Phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (3)

This was obtained following the procedure described for **1** starting from **9** to afford a white solid: 58% yield; mp 117–118 °C. ^{1}H NMR ($CDCl_3$): δ 3.48 (dd, 1, cycle), 3.62 (s, 4, NC*H*$_2$C*H*$_2$N), 3.63 (dd, 1, cycle), 3.85 (dd, 1, cycle), 4.18 (dd, 1, cycle), 4.61 (dd, 1, cycle), 4.76 (dd, 1, cycle), 5.66 (br, 1, N*H*, exchangeable with D_2O), 7.28–7.42 (m, 5, Ar*H*). The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 177–179 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}H_2C_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 60.08; H, 5.82; N, 8.59.

4.1.10. *trans*-2-(6-Phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (4)

This was obtained following the procedure described for **1** starting from **10** to afford a white solid: 57% yield; mp 98–100 °C. ^{1}H NMR ($CDCl_3$): δ 3.60–3.68 (m, 2, cycle), 3.72 (s, 4, NC*H*$_2$C*H*$_2$N), 3.90 (dd, 1, cycle), 4.39 (dd, 1, cycle), 4.48 (m, 1, cycle), 4.81 (dd, 1, cycle), 5.54 (br, 1, N*H*, exchangeable with D_2O), 7.24–7.41 (m, 5, Ar*H*). The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 171–172 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}H_2C_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.74; H, 5.42; N, 8.47.

4.1.11. 2-(5,5-Diphenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (5)

This was obtained following the procedure described for **1** starting from **11** to afford a white solid: 54% yield; mp 208–210 °C. ^{1}H NMR ($CDCl_3$): δ 3.58 (s, 4, NC*H*$_2$C*H*$_2$N), 3.60–3.80 (m, 2, cycle), 4.00 (dd, 1, cycle), 4.50 (dd, 1, cycle), 4.63 (d, 1, cycle), 5.98 (br, 1, N*H*, exchangeable with D_2O), 7.18–7.56 (m, 10, Ar*H*). The free base was transformed into the oxalate salt, which was crystallized from EtOH: mp 219–220 °C. Anal. Calcd for $C_{19}H_{20}N_2O_2{\cdot}H_2C_2O_4$: C, 63.31; H, 5.57; N, 7.03. Found: C, 63.12; H, 5.79; N, 7.25.

4.1.12. 2-(6,6-Diphenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (6)

This was obtained following the procedure described for **1** starting from **12** to afford an oil. ^{1}H NMR ($CDCl_3$): δ 3.60–3.76 (m, 6, NC*H*$_2$C*H*$_2$N and cycle), 4.12 (dd, 1, cycle), 4.40 (dd, 1, cycle), 4.61 (d, 1, cycle), 5.18 (br, 1, N*H*, exchangeable with D_2O), 7.20–7.58 (m, 10, Ar*H*). The free base was transformed into the oxalate salt and crystallized from EtOH: mp 207–208 °C. Anal. Calcd for $C_{19}H_{20}N_2O_2{\cdot}H_2C_2O_4$: C, 63.31; H, 5.57; N, 7.03. Found: C, 63.60; H, 5.44; N, 6.97.

4.1.13. Resolution of *trans*-2-(5-phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole (±)-2

Racemic **2** (1 g, 4.3 mmol) in 95% EtOH (30 mL) was treated with a solution of (−)-*O*,*O*′-dibenzoyl-L-tartaric acid (1.54 g 4.3 mmol) in 95% EtOH (35 mL) and left at room temperature for 30 h. The white crystals were crystallized twice from 95% EtOH: 1.2 g yield. The salt was dissolved in water (50 mL) and the ice-cooled solution was made basic with 2 N NaOH and extracted with ether (3 × 30 mL). Removal of dried solvent gave (2*S*,5*R*)-(−)-**2**: 0.4 g; $[\alpha]_D^{20}$ −11.09 (*c* 1, $CHCl_3$). The ^{1}H NMR spectrum was identical to that of the racemic compound **2**. A similar treatment of **2** with (+)-*O*,*O*′-dibenzoyl-D-tartaric acid gave the other enantiomer (2*R*,5*S*)-(+)-**2**: $[\alpha]_D^{20}$ +10.5 (*c* 1, $CHCl_3$). The ^{1}H NMR spectrum was identical to that of the racemic compound **2**.

4.1.14. (*R*)-(−)-Methyl 2-(Allyloxy)-2-phenylacetate [*R*-(−)-13]

Ag_2O (1.28 g, 5.58 mmol) was added portionwise, under vigorous stirring, to a solution of methyl (*R*)-(−)-mandelate (Aldrich) (1 g, 6.02 mmol) and allyl bromide (1 g, 8.32 mmol) in anhydrous diethyl ether (11.7 mL) at room temperature. The mixture was refluxed for 2 h, then it was cooled and filtered over Celite. The evaporation of dried (Na_2SO_4) organic layer gave a residue that was purified by flash chromatography eluting with cycloexane/EtOAc (95:5) to give an oil: 48% yield; $[\alpha]_D^{20}$ −112.41 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 3, COOC*H*$_3$), 4.08 (dd, 2, OC*H*$_2$), 4.98 (s, 1, C*H*O), 5.20–5.30 (m, 2, C*H*$_2$═CH), 5.90–6.00 (m, 1, C*H*═CH$_2$), 7.35–7.51 (m, 5, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.69; H, 6.69.

4.1.15. (*R*)-(−)-2-(Allyloxy)-2-phenylethanol [*R*-(−)-14]

A solution of *R*-(−)-**13** (0.4 g, 1.94 mmol) in anhydrous diethyl ether (2.43 mL) was added dropwise to a suspension of $LiAlH_4$ (0.1 g, 2.63 mmol) in anhydrous diethyl ether (3.04 mL) at 0 °C under a nitrogen atmosphere and vigorous stirring. The mixture was allowed to warm to room temperature; after 2 h it was poured onto crushed ice and 2.5 M sodium hydroxide (5.83 mL) was added. The precipitate was filtered off over Celite and washed with

diethyl ether (10 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography eluting with cycloexane/EtOAc (9:1) to give an oil: 65% yield; $[\alpha]_D^{20}$ −92.91 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.40 (br s, 1, O*H*, exchangeable with D_2O), 3.70 (d, 2, CH_2OH), 4.02 (dd, 2, OCH_2), 4.42 (dd, 1, C*H*O), 5.18 (m, 2, CH_2=CH), 5.84–6.01 (m, 1, C*H*=CH_2), 7.20–7.42 (m, 5, Ar*H*). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.39; H, 7.67.

4.1.16. (2*S*,5*R*)-(−)-(5-Phenyl-1,4-dioxan-2-yl)methanol [(2*S*,5*R*)-(−)-15]

m-Chloroperbenzoic acid (50%) (1.16 g, 3.36 mmol) was added to a solution of *R*-(−)-**14** (0.3 g, 1.68 mmol) in CH_2Cl_2 (12 mL). After 20 h at room temperature under stirring the reaction mixture was washed with 10% Na_2SO_3, 5% Na_2CO_3 and H_2O. Removal of dried solvents afforded a mixture of the two diastereomers as an oil. A solution of this mixture (2.94 g, 15.14 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (0.33 g, 14.21 mmol) in CH_2Cl_2 (13.4 mL) was refluxed for 8 h. The reaction mixture was then washed with $NaHCO_3$ saturated solution and dried over Na_2SO_4. Removal of the solvent afforded a residue, which was purified by column chromatography gradient eluent, eluting with cyclohexane/EtOAc (8:2 to 6:4). The trans diastereomer (2*S*,5*R*)-(−)-**15** eluted first as a solid: 34% yield; mp 79–81 °C; $[\alpha]_D^{20}$ −64.8 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.93 (t, 1, O*H*, exchangeable with D_2O), 3.52–4.04 (m, 7, OCH_2 and cycle), 4.58 (dd, 1, cycle), 7.28–7.40 (m, 5, Ar*H*). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.84; H, 7.07.

4.1.17. (2*S*,5*R*)-(−)-5-Phenyl-1,4-dioxane-2-carboxylic acid [(2*R*,5*R*)-(−)-16]

A solution of $KMnO_4$ (3.25 g, 20.6 mmol) in H_2O (15 mL) was added dropwise to a stirred mixture of (2*S*,5*R*)-(−)-**15** (2.16 g, 11.1 mmol) in 1 N KOH (15 mL) such that the temperature was maintained below 10 °C. After 18 h at room temperature the mixture was filtered over Celite, MeOH was added and the solvent was concentrated under vacuum. The resulting aqueous solution was acidified with 6 N H_2SO_4 and extracted with $CHCl_3$. After evaporation of the dried solvent, the residue was crystallized from EtOAc/petroleum ether: 40% yield; mp 165–167 °C; $[\alpha]_D^{20}$ −42.09 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.62 (dd, 1, cycle), 3.81 (dd, 1, cycle), 4.07 (dd, 1, cycle), 4.35 (dd, 1, cycle), 4.41 (dd, 1, cycle), 4.62 (dd, 1, cycle), 5.91 (br s, 1, COO*H*, exchangeable with D_2O), 7.31–7.41 (m, 5, Ar*H*). Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.34; H, 5.94.

4.1.18. (2*S*,5*R*)-(−)-Methyl 5-phenyl-1,4-dioxane-2-carboxylate [(2*R*,5*R*)-(−)-8]

This was obtained following the procedure described for **7** starting from (2*R*,5*R*)-(−)-**16** to afford an oil: 80% yield; $[\alpha]_D^{20}$ −76.26 (*c* 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound **8**. Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.90; H, 6.23.

4.1.19. (2*S*,5*R*)-(−)-2-(5-Phenyl-1,4-dioxan-2-yl)-4,5-dihydro-1*H*-imidazole [(2*S*,5*R*)-(−)-2]

This was obtained following the procedure described for **1** starting from (2*R*,5*R*)-(−)-**8** to afford a white solid: 57% yield. $[\alpha]_D^{20}$ −11.13 (*c* 1, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemic compound **2**. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}H_2C_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.77; H, 5.84; N, 8.81.

4.2. Pharmacology

4.2.1. In vivo assays

4.2.1.1. Animals. Male CD-1 mice (Harlan SRC, Milan, Italy), weighing 25–35 g, were used. Animals were kept in a room with a 12:12 h light/dark cycle (lights on at 9:00 a.m.), a temperature of 20–22 °C and a humidity of 45–55%. They were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). Ethical guidelines for the investigation of experimental pain in conscious animals were followed, and procedures were carried out according to EEC ethical regulations for animal research (EEC Council 86/609; D. Lgs. 27/01/1992, No. 116).

4.2.1.2. Nociceptive test. Nociception was evaluated by the radiant heat tail-flick test: briefly, it consists of the irradiation of the lower third of the tail with an I. R. source (Ugo Basile, Comerio, Italy). The basal pre-drug latency, ranged between 2–3 s, was calculated as the mean of two trials performed at 30 min interval. Then mice received **2** (1 and 2 mg/kg, ip) or related vehicle (distilled water), 15 min before morphine (5.0 mg/kg, s.c.) administration or saline. Yohimbine (1.250 mg/kg, ip) and idazoxan (2.0 mg/kg, ip) were used as reference compounds. The antinociceptive activity was evaluated 30, 60, 90, 120 and 240 min after morphine injection. A cut-off latency of 12 s was established to minimize tissue damage. As shown in Figure 1, **2** had no analgesic effect when given alone at all doses tested, but significantly increased the analgesic response of morphine at all times, as evidenced by increase reaction latencies in the tail-flick test expressed as % MPE. The analgesic effect of morphine (5 mg/kg, s.c.) was observed after 30 min, and then it decreased progressively. Pretreatment with **2** (1 and 2 mg/kg) significantly increased the antinociception produced by morphine until 90 and 120 min respectively. The ANOVA confirmed a statistically significant treatment effect [$F(6,54) = 17.928$; $p < 0.001$]. Both yohimbine and idazoxan did not affect morphine analgesia over the entire time course.

In order to evaluate the involvement of I_2-IBS on morphine analgesia, animals were pre-treated ip with idazoxan at the dose of 2.0 mg/kg, 15 min before **2** treatment. Figure 2 shows the effect of idazoxan on enhancement of morphine analgesia induced by **2**. Pretreatment with idazoxan slightly reduced the effect of **2** on morphine analgesia (−24%) [$F(4,36) = 38.574$; $p < 0.001$].

4.2.1.3. Statistical analysis. Antinociceptive effect was expressed as a percent of the Maximum Possible Effect (MPE) according to the following formula: %MPE = (measured latency−basal latency)/(cut-off time−basal latency) × 100%. Data are reported as means ± SEM. Statistical evaluation of data was carried out by analysis of variance (ANOVA) and sequential differences among means according to Student–Newman–Keuls test. Statistical significance was set at $p < 0.05$.

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