Novel Ligands Rationally Designed for Characterizing I_2–Imidazoline Binding Sites Nature and Functions[†]

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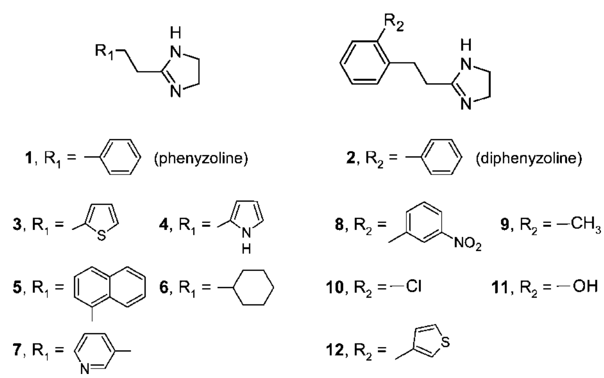
The study of two series of 2-aryl-ethylen-imidazolines **3**–**7** and **8**–**12** inspired by I_2–IBS ligands phenyzoline (**1**) and diphenyzoline (**2**), respectively, confirmed the interesting "positive" or "negative" morphine analgesia modulation displayed by their corresponding leads and demonstrated that these effects might be correlated with morphine tolerance and dependence, respectively. By comparative examination of rationally designed compounds, some analogies between binding site cavity of I_2–IBS proteins and α_{2C}-adrenoreceptor emerged.

Introduction

For over 15 years, the interest of our research group has been directed to the characterization of the imidazoline binding sites (IBS[a])[1] described for the first time by Bousquet in 1984. Although it has been possible to ascribe the IBS nature to distinct proteins in human and rat brain, the structures of these binding proteins have not yet been identified. Nevertheless, the IBS, classified into I_1–IBS and I_2–IBS, represent interesting therapeutic targets. While the I_1–IBS participate in the regulation of cardiovascular function,[1] the I_2–IBS appears to be involved in the Parkinsons's disease, depression, and modulation of analgesia as well as tolerance and addiction to opioids.[2] Recently, we examined the effect on morphine analgesia produced by **1** (phenyzoline)[3] (Chart 1), which might be considered as a particularly selective I_2–IBS ligand with respect to I_1–IBS and α_2-adrenoreceptors (α_2-ARs) (pK_i I_2 = 8.60; I_2/I_1 = 1479; I_2/α_2 = 794)[4] and by its ortho phenyl derivative **2** (diphenyzoline) (pK_i I_2 = 6.80; I_2/I_1 = 40; I_2/α_2 = 45), designed to induce modification of the biological profile of **1**.[3] The mouse tail-flick test showed that **1** and **2** significantly enhanced (60%) and decreased (−41%) morphine analgesia, respectively. The ability to decrease morphine analgesia had never been observed before in I_2–IBS ligands. Therefore, in the present study, to confirm the interesting "positive" or "negative" morphine analgesia modulatory effects observed for **1** and **2**, respectively, and to improve SAR knowledge for better I_2–IBS characterization, we designed two series of imidazoline molecules: **3**–**7** and **8**–**12**, based on the leads **1** and **2**, respectively (Chart 1). The already described compounds **3**,[5] **5**,[6] **9**,[7] and **11**[8] had never been studied from this point of view. The affinity values of **3**–**12** at I_1–IBS on rat kidney membranes, I_2–IBS, and α_2-ARs on rat whole brain membranes were determined. Morphine analgesia modulation was evaluated by the mouse tail-flick test.[3]

Chart 1. Imidazolines Structurally Related to Phenyzoline (**1**) and Diphenyzoline (**2**)



In addition, the lead **1** and imidazoline **9**, belonging to the first and second series, respectively, were examined for their ability to affect tolerance and dependence development induced by morphine.

Chemistry

The novel imidazolines **4**, **6**–**8**, **10**, and **12** were synthesized according to standard methods (Scheme 1): the imidazolines **4**, **6**, and **7** by catalytic hydrogenation over Pd/C of the corresponding vinyl precursors, and the imidazoline **8**, **10**, and **12** by condensation of suitable methyl ester or nitriles with ethylenediamine in different conditions. The new intermediates **13** and **15** were obtained starting from 3-(2-bromo-phenyl)-propionic acid or 3-(2-bromo-phenyl)-propionitrile[9] with the suitable commercially available arylboronic acid in the presence of tetrakis(triphenylphosphine)palladium(0). **14** was obtained by esterification of **13** with MeOH in presence of H_2SO_4.

Results and Discussion

We have previously demonstrated that the nature of the bridge between the aromatic portion and imidazoline nucleus played a crucial role for IBS or α_2-ARs selective recognition.[4] Therefore, the unsubstituted ethylenic bridge, which proved to be determinant in inducing high I_2–IBS selectivity with regard to I_1–IBS and α_2-ARs, was present in all the designed derivatives. No modification was performed on the imidazoline

[†] This article is dedicated to Dr. Francesco Gentili, who died prematurely at the age of 39.

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[a] Abbreviations: IBS, imidazoline binding sites; α_2-ARs, α_2-adrenoreceptors; DA, dopamine; DME, 1,2-dimethoxyethane; MPE, maximum possible effect.

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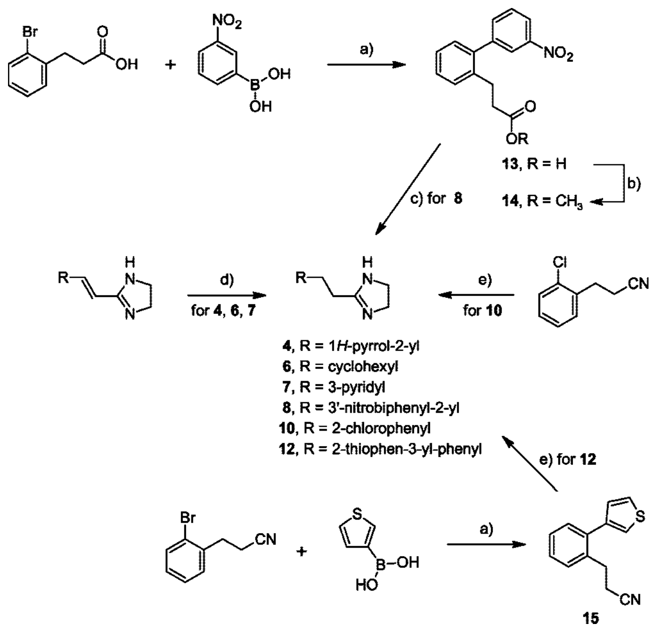
Scheme 1[a]



[a] Reagents: (a) Na_2CO_3, Pd[$(C_6H_5)_3P$]$_4$, DME; (b) MeOH, H_2SO_4; (c) $(CH_3)_3Al$, dry toluene, ethylenediamine, Δ; (d) H_2, Pd/C; (e) HCl_g, MeOH, ethylenediamine.

nucleus, as the previous observations indicated that the C- or N-substitution, in structurally related compounds, was detrimental for I_2−IBS affinity and selectivity.[10,11] Consequently, the derivatives of the two series (**3**−**7** and **8**−**12**) showed differences exclusively on the aromatic portion (Chart 1). In this portion, a 2D- and 3D-quantitative SAR study, performed on a series of imidazoline congeners (tracizoline derivatives), highlighted that good lipophilicity, extended also to the ortho position of the phenyl ring, was favorable but not decisive for significant I_2−IBS affinity.[7] Consequently, in the designed imidazoline molecules, the phenyl ring R_1 of **1** or the ortho pendant phenyl group R_2 of **2** were replaced by functions of different lipophilic character (compounds **3**−**7** and **8**−**12**, respectively). In particular, among the derivatives of **2** (diphenyzoline), as previously made for it,[3] the compounds **8**, **9**, and **12** were rationally designed to verify our hypothesis that I_2−IBS and α_2-ARs might present analogies in the nature and orientation of some critical binding functions.

The affinity and calculated logP values of **1**−**12**, reported in Table 1, indicated that the presence of portions endowed with good lipophilicity such as thiophen-2-yl (**3**), naphthalen-1-yl (**5**), cyclohexyl (**6**), *o*-tolyl (**9**), 2-chloro-phenyl (**10**), and 2-thiophen-3-yl-phenyl (**12**) induced in the ligands the highest I_2−IBS affinities. Lower I_2−IBS affinities were produced by the presence of polar portions such as 1*H*-pyrrol-2-yl (**4**), 3-pyridyl (**7**), and 2-hydroxy-phenyl (**11**). Although the 3′-nitrobiphenyl-2-yl (**8**) and biphenyl-2-yl (**2**) fractions displayed good lipophilicity, they negatively affected I_2−IBS affinity, probably owing unfavorable steric hindrance. The novel derivatives, except **8**, displayed significant I_2−IBS selectivity. The data obtained by the in vivo study corresponded to our expectations. Indeed, analogously to **1**, the compounds of the first series **3**−**7** exhibited no antinociceptive effect by themselves but increased morphine analgesia (34, 14, 16, 30, and 23%, respectively) (Figure 1). The compounds of the second series **8**−**12**, lacking in analgesic effect by themselves, analogously to **2**, reduced morphine analgesia (−29, −44, −34, −26, and −59%, respectively) (Figure 2). In the previous study,[3] the unambiguous involvement of I_2−IBS in the morphine analgesia modulatory effects of **1** and **2** has been demonstrated. In fact, these effects were not affected by treatment of animals with yohimbine (selective α_2-AR antagonist) or Efaroxan (I_1−IBS/α_2-AR antagonist) but were completely reversed by treatment with idazoxan (I_2−IBS/α_2-AR antagonist). In addition, **1** and **2** proved to be inactive at all three α_2-AR subtypes.[3] Therefore, I_2−IBS selectivity with regard to I_1−IBS and α_2-ARs, observed in **3**−**12**, and the correlation of the **3**−**7** series with **1** and **8**−**12** series with **2**, supported the involvement of I_2−IBS in the morphine analgesia modulatory effects of **3**−**12**. The above results, confirming what was observed for **1** and **2**, demonstrated that the introduction of substituents in the ortho position of the aromatic ring of **1** induced significant change of its morphine analgesia modulation.

In previous contributions from our laboratory, we demonstrated that the introduction of pendant groups in the ortho position of the aromatic ring of the α_2-AR ligand 2-(1-phenoxyethyl)-4,5-dihydro-1*H*-imidazole, structurally related to **1**, caused decisive biological profile modulation; in this case, from antagonist to agonist behavior was induced (biphenyline and analogues).[12,13] This observation allows us to suggest that α_2-AR and I_2−IBS ligands might interact with their corresponding binding sites in a similar fashion. In particular, in the above-mentioned biphenyline series, the interactions formed between the ortho phenyl or methyl (results not published) or 3-nitrophenyl or 3-thienyl pendant groups and the aromatic cluster present in transmembrane domain 6 of the α_2-AR binding cavity proved to be favorable to trigger high α_{2C}-subtype activation. Methyl or 3-nitrophenyl groups selectively activated the α_{2C}-subtype.[13] As above-reported, the same ortho phenyl, 3-nitrophenyl, methyl, and 3-thienyl pendant groups (compounds **2**, **8**, **9**, and **12**, respectively) induced also a change of the modulatory effect on morphine analgesia displayed by the unsubstituted precursor **1**. Therefore, we suggest that the I_2−IBS proteins amino acid residues domain interacting with the hydrophobic portions of the I_2−IBS ligands might share some degree of homology with the corresponding aromatic cluster involved in the α_{2C}-AR activation.[13] Moreover, if the pendant groups, interacting with hydrophobic residues, would lead to receptor activation, it might be reasonable, as previously reported,[3] to define the I_2−IBS ligands **8**−**12** as "putative agonists" and, consequently, the compounds **3**−**7** as "putative inverse agonists".

Among the many approaches investigated to overcome the undesired side effects of opiate drugs, the synergism with I_2−IBS mediated antinociceptive mechanisms has been reported.[2] Therefore, to find new possible therapeutic coadjuvants in the management of chronic pain with opiate drugs, we wished to evaluate the effects of the observed I_2−IBS mediated "positive" or "negative" morphine analgesia modulation on opioid tolerance and dependence. In this study, **1** and **9** were selected due to their very high I_2−IBS affinity and significant modulatory activity. Analogously to what previously made for **1** and **2**,[3] the selective involvement of I_2−IBS in the morphine analgesia modulatory effect of **9** has been confirmed (Supporting Information, Figure 5). Interestingly, both "positive" and "negative" modulation of the morphine analgesia was found to attenuate the development of side effects. In particular, in mice receiving morphine (twice daily for 5 days), the tolerance development determined the lack of morphine antinociceptive effect on day 5. In contrast, the pretreatment with **1** inhibited the tolerance expression phases, and the antinociceptive effect observed proved similar to that of the morphine alone treated

Table 1. Binding Affinities,[a] Selectivity Ratios,[b] and Calculated logP[c] of Compounds **1–12**

compd	I_1–IBSIC_{50} (nM)	I_2–IBSK_i (nM)	α_2-ARs K_i (nM)	I_1/I_2 selectivity ratio	α_2/I_2 selectivity ratio	calcd logP
1	3697 ± 230[d]	2.5 ± 0.49	1985 ± 200	1479	794	2.71
2	6340 ± 272[d]	158.5 ± 10.3	7132 ± 250	40	45	4.47
3	2877 ± 592	7.72 ± 1.64	819.9 ± 131.8	372.6	106.2	2.39
4	23890 ± 3829	254.3 ± 62.1	3540 ± 600	94	13.9	1.24
5	25812 ± 6017	1.16 ± 0.32	381.0 ± 27.1	22252	328.4	3.94
6	3569 ± 841	4.8 ± 0.49	2962 ± 683	743.5	617.1	3.64
7	211600 ± 2984	413.6 ± 66.1	64773 ± 8069	511.6	156.6	1.22
8	3998 ± 663	251.9 ± 60.1	1117.9 ± 436.3	16.0	4.4	4.00
9	7360 ± 898	1.68 ± 0.2	557.7 ± 77.1	4381	332.0	3.17
10	4255 ± 952	1.5 ± 0.9	295.4 ± 58.2	2836.6	196.9	3.31
11	7301 ± 1308	73.5 ± 12.9	925.9 ± 163.7	99.3	12.6	1.98
12	6891 ± 868	8.89 ± 2.74	292.7 ± 40.6	775.1	32.9	4.15

[a] Data for I_1–IBS affinity were determined on rat kidney membranes and values expressed in IC_{50} values (the concentration of ligand that inhibits 50% of specific binding). I_2–IBS and α_2–ARs binding was determined on rat brain membranes and values are expressed as K_i values. Data represent the mean ± SEM of 3–5 separate experiments performed in triplicate. [b] I_1/I_2 and α_2/I_2 are the ratios of I_1–IBS IC_{50} and α_2-ARs K_i, respectively, to I_2–IBS K_i. [c] Data from ACD/logP DB version. [d] K_i values performed on rat pheochromocytoma cells, PC 12.

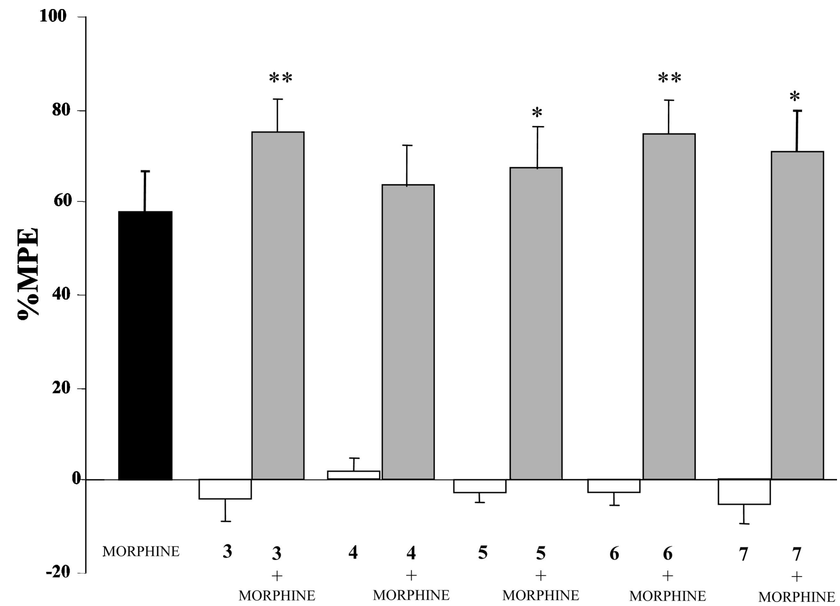


Figure 1. Effects of **3–7** (10 mg/kg, sc) on morphine (5.0 mg/kg, sc) analgesia in the tail flick test. The reaction latencies were expressed as a percent of the maximum possible effect (%MPE). To avoid tissue damage, a cutoff latency of 12–15 s was used. Each mouse was tested 1 and 0.5 h before vehicle or compound administration to determine baseline latency. Then mice were sc administered with compounds **3–7** or related vehicle. Morphine (5.0 mg/kg) or its vehicle were sc administered 30 min later. The antinociceptive activity was evaluated 30 min after morphine injection. Each column represents the mean ± SEM of 8–10 animals. Significant differences: *$p < 0.05$, **$p < 0.01$ compared to morphine treated group; where not indicated, the difference was not statistically significant.

group on day 1. Pretreatment with **9** proved ineffective (Figure 3). In mice treated with morphine (twice daily for 6 days to induce opioid dependence), on day 6, the naloxone injection induced severe signs of withdrawal syndrome, as manifested by jumping behavior. Interestingly, in mice coadministered with **9**, withdrawal signs were significantly reduced (−39%). The reduction induced by **1** proved less significant (Figure 4). It has been reported that BFI, currently considered a ligand of choice for the I_2–IBS study, was able to enhance morphine-induced analgesia[14] and displayed central dopamine (DA) releasing/deplete properties.[15] Therefore, to extend our study and discover novel I_2–IBS ligands potentially useful in the treatment of the DA system alterations on our most interesting I_2–IBS ligands, we intend to investigate the possible correlations between the ability to modulate the morphine analgesia with its side effects and influence on DA system functional relevance.

In conclusion, the aryl-ethylen-imidazoline molecules of the present study (i) extended the knowledge of the ligand structural characteristics, such as good lipophilicity and suitable steric hindrance, compatible with significant I_2–IBS affinity and selectivity; (ii) confirmed our previous observation that I_2–IBS ligands, depending on their structure, might behave as "putative inverse agonists" or "putative agonists"; (iii) demonstrated that the corresponding effects of morphine analgesia enhancement or decrease might be correlated with morphine tolerance or dependence, respectively. Finally, the comparative examination of rationally designed compounds pointed out some significant analogies between binding site cavity of I_2–IBS proteins and α_{2C}-AR subtype.

Experimental Section

2-(2-Cyclohexyl-ethyl)-4,5-dihydro-1*H*-imidazole (6). A solution of 2-((*E*)-2-cyclohexyl-vinyl-4,5-dihydro-1*H*-imidazole[7] (0.57 g, 3.2 mmol) in MeOH was hydrogenated for 3 h at rt under pressure (40 psi) using 10% Pd/C (0.6 g) as catalyst. Following catalyst removal and evaporation of the solvent, the residue was purified by flash chromatography eluting with $CHCl_3$/MeOH/33% NH_4OH (9:1:0.1) to give the free base (0.54 g, 94% yield), which was transformed into the oxalate salt and crystallized from EtOH: mp 130–131 °C. ^1H NMR (DMSO) δ 0.78–1.72 (m, 13H, C_6H_{11}–CH_2), 2.45 (m, 2H, CH_2), 3.84 (s, 4H, NCH_2CH_2N), 7.85 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{11}H_{20}N_2 \cdot H_2C_2O_4$) C, H, N.

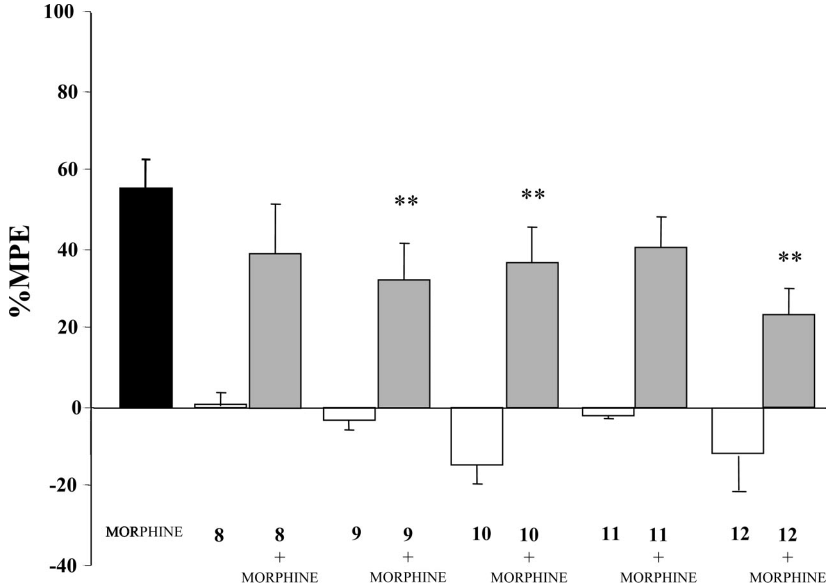


Figure 2. Effects of **8** (20 mg/kg) and **9−12** (10 mg/kg) on morphine (5.0 mg/kg, sc) analgesia in the tail-flick test. The reaction latencies were expressed as a percent of the maximum possible effect (%MPE). To avoid tissue damage, a cutoff latency of 12−15 s was used. Each mouse was tested 1 and 0.5 h before vehicle or compound administration to determine baseline latency. Then mice were sc administered with compounds **8−12** or related vehicle. Morphine (5.0 mg/kg) or its vehicle were sc administered 30 min later. The antinociceptive activity was evaluated 30 min after morphine injection. Each column represents the mean ± SEM of 8−10 animals. Significant differences: $^{**}p < 0.01$ compared to morphine treated group; where not indicated, the difference was not statistically significant.

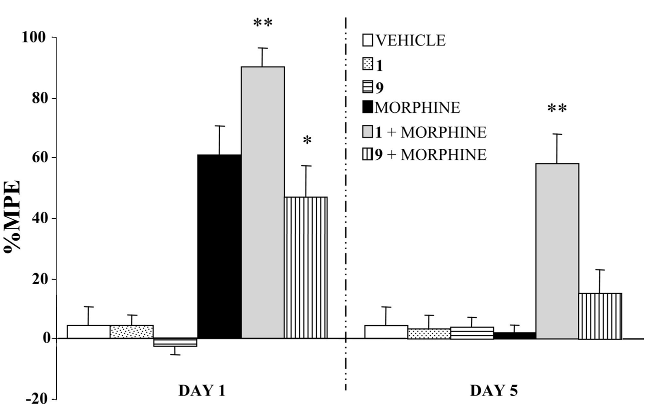


Figure 3. Effects of repeated coadministration of **1** and **9** (10 mg/kg) with morphine on the development of morphine tolerance to analgesia in mice. Morphine was sc injected twice daily for 5 days at the dose of 10 mg/kg (except for the day of the test when the dose of 5 mg/kg has been used). Compounds **1** and **9** were repeatedly administered 30 min before every morphine treatment. Morphine antinociceptive effect was assessed both on day 1 and on day 5. Significant differences: $^{*}p < 0.05$, $^{**}p < 0.01$ compared to morphine-treated mice; where not indicated, the difference was not statistically significant.

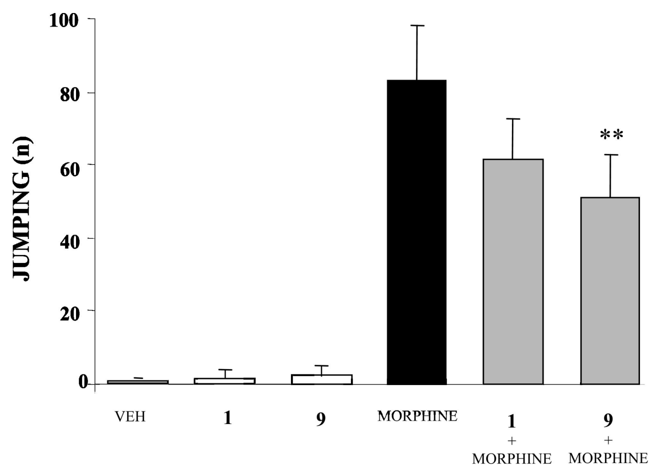


Figure 4. Effects of repeated coadministration of **1** and **9** (10 mg/kg, sc) with morphine (10 mg/kg, sc) on naloxone-precipitated withdrawal syndrome. I_2-imidazoline compounds were repeatedly administered, 30 min before every morphine treatment, twice daily for 5 days. On the sixth day, I_2−imidazoline compounds and naloxone (5 mg/kg, ip) were administered 30 min before and 2 h after morphine treatment, respectively. The development of dependence on morphine was determined by frequency of the precipitate withdrawal signs (expressed as jumping) for 15 min after naloxone injection. Significant differences: $^{**}p < 0.01$ compared to morphine-treated mice.

2-[2-(1*H*-Pyrrol-2-yl)-ethyl]-4,5-dihydro-1*H*-imidazole (4). This was prepared from 2-[(*E*)-2-(1*H*-pyrrol-2-yl)-vinyl]-4,5-dihydro-1*H*-imidazole[7] via the procedure described for **6**. The reaction mixture was purified by flash chromatography, and the free base (85% yield) was transformed into the hydrochloride salt, which was crystallized from EtOH: mp 146−148 °C. ^{1}H NMR (DMSO) δ 2.58 (t, 2H, $CH_2C{=}N$), 2.82 (t, 2H, CH_2), 3.62 (s, 4H, NCH_2CH_2N), 5.72−6.58 (m, 3H, ArH), 10.68 (br s, 2H, NH, exchangeable with D_2O). Anal. ($C_9H_{13}N_3 \cdot HCl$) C, H, N.

3-[2-(4,5-Dihydro-1*H*-imidazol-2-yl)-ethyl]-pyridine (7). This was prepared from 3-[(*E*)-2-(4,5-dihydro-1*H*-imidazol-2-yl)-vinyl]-pyridine[16] via the procedure described for **6**. The reaction mixture was purified by flash chromatography, and the free base (95% yield) was transformed into the oxalate salt, which was crystallized from EtOH: mp 140−141 °C. ^{1}H NMR (DMSO) δ 2.80 (t, 2H, $CH_2C{=}N$), 3.98 (t, 2H, CH_2), 3.88 (s, 4H, NCH_2CH_2N), 7.32−8.44 (m, 4H, ArH), 10.18 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{10}H_{13}N_3 \cdot H_2C_2O_4$) C, H, N.

2-[2-(3′-Nitro-biphenyl-2-yl)-ethyl]-4,5-dihydro-1*H*-imidazole (8). A solution of ethylenediamine (0.42 mL, 6.28 mmol) in dry toluene (6 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminum (3.2 mL, 6.28 mmol) in dry toluene (4 mL) at 0 °C in nitrogen atmosphere. After being stirred at rt for 1 h, the solution was cooled to 0 °C, and a solution of **14** (0.90 g; 3.14 mmol) in dry toluene (8 mL) was added dropwise. The reaction mixture was heated to 110 °C for 3 h, cooled to 0 °C, and quenched cautiously with MeOH (0.8 mL) followed by H_2O (0.2 mL). After addition of $CHCl_3$ (5 mL), the mixture was left for 30 min at rt. The mixture was filtered and the organic layer was extracted with

2N HCl. The aqueous layer was made basic with 10% NaOH and extracted with $CHCl_3$. Removal of dried solvent gave a residue that was purified by flash chromatography to give the free base (0.48 g, 52% yield). The oxalate salt was crystallized from EtOH: mp 180−182.2 °C. ^{1}H NMR (DMSO) δ 2.62 (t, 2H, $CH_2C{=}N$), 2.88 (t, 2H, CH_2), 3.82 (s, 4H, NCH_2CH_2N), 7.22−8.28 (m, 8H, ArH), 9.82 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{11}H_{13}N_3O_2 \cdot H_2C_2O_4 \cdot 0.5H_2O$) C, H, N.

2-[2-(2-Chloro-phenyl)-ethyl]-4,5-dihydro-1*H*-imidazole (10). HCl was bubbled through the stirred and cooled (0 °C) solution of 3-(2-chloro-phenyl)-propionitrile (0.89 g, 5.35 mmol) in MeOH (0.43 mL) and dry $CHCl_3$ (9.3 mL) for 45 min. After 12 h at 0 °C, the solvent was removed in vacuo to give an oil (0.61 g, 2.60 mmol) that was dissolved in absolute EtOH and added to a cooled (0 °C) and stirred solution of ethylenediamine (0.22 mL, 3.24 mmol) in absolute EtOH (12.5 mL). After 1 h, concentrated HCl (0.11 mL) was added to the reaction mixture, which was stored overnight in the refrigerator. The crude residue was then diluted with absolute EtOH (8.6 mL) and heated to 70 °C for 5 h. After cooling, the solid was collected and discarded and the filtrate was concentrated and filtered again. The filtrate, evaporated to dryness, gave a residue that was taken up in $CHCl_3$ (20 mL) and washed with 2N NaOH. Removal of the dried solvent gave a residue that was purified by flash chromatography to give the free base (0.40 g, 36% yield over all). The hydrochloride salt was crystallized from EtOH: mp 157.7−159 °C. ^{1}H NMR (DMSO) δ 2.82 (t, 2H, $CH_2C{=}N$), 3.12 (t, 2H, CH_2), 3.81 (s, 4H, NCH_2CH_2N), 7.30−7.52 (m, 4H, ArH), 10.18 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{11}H_{13}ClN_2 \cdot HCl \cdot 0.33H_2O$) C, H, N.

2-[2-(2-Thiophen-3-yl-phenyl)-ethyl]-4,5-dihydro-1*H*-imidazole (12). This was prepared from **15** via the procedure described for **10**. The purification by flash chromatography gave the free base (42% yield), which was transformed into the oxalate salt and crystallized from EtOH: mp 200.3−202.6 °C. ^{1}H NMR (DMSO) δ 2.68 (t, 2H, $CH_2C{=}N$), 2.98 (t, 2H, CH_2), 3.78 (s, 4H, NCH_2CH_2N), 7.20−7.68 (m, 7H, ArH), 9.66 (br s, 1H, NH, exchangeable with D_2O). Anal. ($C_{15}H_{16}N_2S \cdot H_2C_2O_4 \cdot 0.25H_2O$) C, H, N.

3-(3′-Nitro-biphenyl-2-yl)-propionic acid methyl ester (14). Na_2CO_3 (1.13 g, 10.7 mmol), H_2O (5.35 mL), and tetrakis-(triphenylphosphine)palladium(0) (0.255 g, 0.221 mmol) were added to a solution of 3-(2-bromo-phenyl)-propionic acid (1.00 g, 4.42 mmol) and 3-nitrophenylboronic acid (0.92 g, 5.52 mmol) in DME (8 mL). The mixture was heated at 90 °C for 14 h in the dark under nitrogen atmosphere. After cooling to rt the mixture was poured into AcOEt and ice, acidified and extracted with AcOEt. Removal of dried solvent gave the 3-(3′-nitro-biphenyl-2-yl)-propionic acid (**13**) (0.68 g, 2.51 mmol). ^{1}H NMR ($CDCl_3$) δ 2.52 (t, 2H, CH_2), 2.72 (t, 2H, CH_2−Ar), 7.22−8.30 (m, 8H, ArH), 11.04 (br s, 1H, COOH, exchangeable with D_2O).

13 was converted into the corresponding methyl ester by heating in CH_3OH in the presence of a catalytic amount of H_2SO_4. After purification by flash chromatography eluting with cyclohexane/AcOEt (95:5) compound **14** was obtained as an oil (0.71 g, 2.49 mmol; yield over all 56%). ^{1}H NMR ($CDCl_3$) δ 2.44 (t, 2H, CH_2), 2.92 (t, 2H, CH_2−Ar), 3.62 (s, 3H, OCH_3), 7.18−8.27 (m, 8H, ArH).

3-(2-Thiophen-3-yl-phenyl)-propionitrile (15). This was prepared from 3-(2-bromo-phenyl)-propionitrile[9] (0.93 g, 4.42 mmol) and 3-thiophenboronic acid (0.7 g, 5.52 mmol) via the procedure described for **13**. Compound **15** was obtained as an oil (60% yield). ^{1}H NMR ($CDCl_3$) δ 2.43 (t, 2H, CH_2), 3.04 (t, 2H, CH_2−Ar), 7.08−7.43 (m, 7H, ArH).

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Supporting Information Available: Chemical methodology, biological experiments and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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