Combined Interactions with I₁-, I₂-Imidazoline Binding Sites and α_2 -Adrenoceptors To Manage Opioid Addiction

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S Supporting Information

ABSTRACT: Tolerance and dependence associated with chronic opioid exposure result from molecular, cellular, and neural network adaptations. Such adaptations concern opioid and nonopioid systems, including α_2 -adrenoceptors (α_2 -ARs) and I₁- and I₂-imidazoline binding sites (IBS). Agmatine, one of the hypothesized endogenous ligands of IBS, targeting several systems including α_2 -ARs and IBS, proved to be able to regulate opioid-induced analgesia and to attenuate the development of tolerance and dependence. Interested in the complex pharmacological profile of agmatine and considering the nature of its targets, we evaluated two series of imidazolines, rationally designed to simultaneously interact with I₁-/I₂-IBS or I₁-/I₂-IBS/ α_2 -ARs. The compounds showing the highest affinities for I₁-/I₂-IBS or I₁-/I₂-IBS/ α_2 -ARs have been selected for their *in vivo* evaluation on opiate withdrawal syndrome. Interestingly, **9**, displaying I₁-/I₂-IBS/ α_2 -ARs interaction profile, appears more effective in reducing expression and acquisition of morphine dependence and, therefore, might be considered a promising tool in managing opioid addiction.

KEYWORDS: opioid addiction, imidazolines, α_2 -adrenoceptors, combined interactions, agmatine biological profile

Opioid addiction, involving tolerance, drug-seeking, and physical dependence, is defined as a chronic relapsing condition and represents a major health and social problem in most societies. Tolerance and dependence associated with chronic opioid exposure result from molecular, cellular, and neural network adaptations. Such adaptations concern opioid and nonopioid systems, including α_2 -adrenoceptors (α_2 -ARs) and imidazoline binding sites (IBS). Therefore, agents directed to these targets can also affect the pharmacological actions of opioids and represent useful tools in managing opioid addiction.¹

α_2 -ARs have been demonstrated to be extremely sensitive to opioid exposure and to play a key role in opiate withdrawal symptoms.² They are widely distributed both in the central nervous system (CNS) and in peripheral tissues.³ By pharmacological and molecular cloning techniques, α_2 -ARs have been classified into three subtypes, namely, α_{2A} -AR, mediating hypotension, sedation and analgesia, α_{2B} -AR, mediating vasoconstriction, and α_{2C} -AR, modulating many CNS processes and contributing to spinal α_2 -agonist mediated analgesia and adrenergic-opioid synergy.^{2–4}

IBS, discovered about 30 years ago by Bousquet et al.,⁵ are distributed in both central and peripheral nervous systems.⁶ At present they appear to be divided into three subtypes: I₁-IBS, preferentially recognized by [³H]-clonidine and related

compounds, I₂-IBS, recognized by [³H]-idazoxan, and I₃-IBS, atypical site constituting a potential target for the treatment of diabetes.^{6,7} I₁-IBS are involved in the regulation of cardiovascular function, in the modulation of ocular pressure, and in the secretion of renal sodium. I₂-IBS are involved in CNS pathologies such as Parkinson's disease, depression, tolerance and addiction to opioids, and food intake in rats.^{6,8}

For many years, the nonsubtype selective α_2 -AR agonist clonidine (Chart 1) has been the mainstay of nonopioid treatment for relief of withdrawal symptoms during detoxification.²

Since clonidine also interacts with I₁-IBS and is endowed with preferential I₁-IBS/ α_2 -AR selectivity (selectivity ratio = 4), a potential role of I₁-IBS signaling in addiction is hypothesized. It has also been reported that, as observed with moxonidine (I₁-IBS/ α_2 -AR selectivity ratio = 40), the coincident stimulation of α_2 -ARs and I₁-IBS prevents relapse of cocaine-seeking, indicating novel pharmacological approaches for addiction treatment.⁹

Moreover, a significant number of studies suggested that some substances such as 2-BFI (Chart 1), displaying high

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Chart 1. Chemical Structures of Clonidine, Moxonidine, 2-BFI, and Agmatine

affinity for I₂-IBS over α_2 -ARs, were able to regulate the opioid-induced analgesia,¹⁰ and to attenuate the development of tolerance and dependence.¹¹

Positive effects in managing opioid addiction are also provided by agmatine (Chart 1), which has been considered one of the endogenous ligands for IBS.¹² Several studies demonstrated that agmatine potentiates opioid analgesia and attenuates opioid tolerance and dependence mainly by interaction with IBS.¹³ Moreover, it attenuates ethanol withdrawal symptoms¹⁴ and displays anxiolytic, antidepressant, anticonvulsive, antiproliferative, and neuroprotective effects.¹⁵ Nevertheless, the efficacy of agmatine is weakened by the fact that, similarly to the other monoamine transmitter molecules, its fast peripheral metabolism reduces its penetration into the brain, limiting its use as a therapeutic agent. Moreover, only high and frequent doses of exogenous agmatine induced significant effects in animal models.¹⁶ Agmatine is one of the few neurotransmitters with multireceptorial affinities. Indeed, it interacts with serotonin 5-HT receptors, antagonizes NMDA receptors, inhibits nitric oxide synthase, and binds to α_2 -ARs and I₁-, I₂-IBS.^{12,15,16} The multiplicity of systems recognized by agmatine makes it difficult to identify its certain targets modulating opioid functions as well as other interesting aforementioned effects.

Some advantageous combined interactions also emerged by our studies regarding imidazolines able to interact with different systems, including IBS and α_2 -ARs, and built on the common scaffold I characterized by an aromatic area (Ar) linked to the position 2 of the imidazoline nucleus by a biatomic bridge (X) (Chart 2). Interestingly, our results highlighted that the peculiar chemical nature of X addressed the ligand to specific biological targets, whereas the *ortho*-phenyl substituents were responsible for its functional behavior.

In particular, the -OCH(CH₃)- bridge proved to be suitable for α_2 -AR and 5-HT_{1A} receptor (5-HT_{1A}-R) interactions,¹⁷ whereas the -O-CH₂- linker promoted the simultaneous α_2 -AR/I₂-IBS recognition (Chart 2).¹⁸ Indeed, we demonstrated that ligands such as allylphenylene (1), displaying effective α_{2C} -AR agonism/ α_{2A} -AR antagonism/5-HT_{1A}-R agonism, or compound 2, producing significant α_{2C} -AR agonism/ α_{2A} -AR antagonism/I₂-IBS interaction, behaved as multifunctional agents and were able, at the same dose, to reduce opioid withdrawal syndrome and associated depression.^{17–19} At low dose 1 also reduced hyperanxiety-like behavior after alcohol intoxication.²⁰

In contrast, the -CH=CH- bridge induced efficacious I₁- and I₂-IBS recognition, as demonstrated by trazolone (3) (pK_i: I₁-IBS 7.72, I₂-IBS 8.72, α_2 -ARs < 6),²¹ whereas the -NH-CH₂- chain might be tolerated by ligands addressed to I₁-, I₂-

Chart 2. Chemical Structures of Imidazolines 1–13 Sharing the Common Scaffold I

IBS and α_2 -ARs, as reported for 4 (pK_i: I₁-IBS 9.30, I₂-IBS 7.48, α_2 -ARs 7.14).^{22,23}

Our studies also indicated that the introduction of suitable decorations in the *ortho* position of the phenyl ring conferred to the ligand an interesting profile modulation from antagonism to agonism. Such a modulation was demonstrated both for selective α_2 -AR²⁴ and I₁-IBS ligands,²⁵ suggesting some analogies in the nature of critical binding sites for both systems, as hypothesized by Hieble and Ruffolo.²⁶ As aforementioned, the simultaneous stimulation of α_2 -ARs and I₁-IBS might be advantageous for addiction treatment.⁹

On the basis of these observations, interested in the complex pharmacological profile of agmatine, we focused our attention on some of its targets, namely, α_2 -ARs and I₁-, I₂-IBS. Therefore, we evaluated the *in vitro* IBS and α_2 -ARs profiles of two series of imidazolines rationally designed to simultaneously interact with I₁-/I₂-IBS or I₁-/I₂-IBS/ α_2 -ARs. Indeed, compounds 5–8, bearing the -CH=CH- bridge and being structurally related to 3, might interact with I₁- and I₂-IBS, whereas simultaneous I₁-/I₂-IBS/ α_2 -AR interactions might be expected for 9–13, structurally related to 4 and characterized by the -NH-CH₂- linker (Chart 2). Our aim was to discover compounds useful for exploring the biological effects modulated by these target interaction combinations and novel tools potentially useful in managing opioid addiction. Therefore, *in vivo* assays on the expression and acquisition of morphine dependence were performed with the most interesting compounds.

Imidazolines 7, 8, 11, and 12 have been synthesized for the first time in this study. To make relevant structure–activity relationships, compounds 5, 6²¹ and 9, 10, 13²⁷ have also been evaluated.

Compounds 7, 8, 11, and 12 were prepared according to the synthetic procedures reported in Scheme 1. In particular, the reaction of the aldehydes 14²⁸ and 15²⁹ with ethylenediamine in the presence of *N*-bromosuccinimide gave the imidazolines 7 and 8, respectively. The condensation of the commercially available anilines 16 and 17 with 2-(chloromethyl)-4,5-dihydro-1H-imidazole at 170 °C in diglyme afforded the imidazolines 11 and 12, respectively.

Scheme 1. Synthesis of Imidazolines 7, 8, 11, and 12^a

^aReagents and conditions: (a) ethylenediamine, dichloromethane, 1 h, then *N*-bromosuccinimide, overnight at room temperature; (b) diglime, 170 °C, 20 h.

The biological profiles of 5–13 were evaluated by radioligand competition binding assays at I₁-IBS, I₂-IBS, and α₂-ARs using [¹²⁵I]-*p*-iodoclonidine (on rat kidney membranes), [³H]-2-BFI (on rat brain membranes), and [³H]-RX821002 (on rat brain membranes), respectively, as radioligands, according to previous reported procedures.³⁰ Moreover, the functional profiles of ligands interacting with α₂-ARs (9–13) at α_{2A}, α_{2B}, and α_{2C}-AR subtypes have been assessed with Cytosensor microphysiometer on CHO cells expressing each individual human α₂-AR subtype.²⁴

The data reported in Table 1 are in agreement with our expectations. Indeed, compounds 5–8, bearing the -CH=CH- bridge, prove to be able to effectively produce

simultaneous I₁/I₂-IBS interactions. Similarly to compound 3,²¹ they are inactive at α₂-ARs. *ortho*-Phenyl substituents endowed with low steric bulk, such as -CH₃ or -Cl, appear to be more suitable for the binding at I₁- and I₂-IBS than bulkier substituents, such as allyl or phenyl groups, as demonstrated by the higher affinities showed by 5 and 6 with respect to 7 and 8. A preferential binding affinity for I₂- over I₁-IBS is also observed for all the compounds of this series.

On the contrary, compounds 9–13, bearing the -NH-CH₂- linker, show I₁/I₂-IBS/α₂-AR affinities. In particular, in the case of compound 13, a significant I₁-IBS selectivity vs I₂-IBS and α₂-AR is displayed. The *ortho*-methyl group proved to be particularly suitable for the combined I₁/I₂-IBS/α₂-AR interaction, as demonstrated by the affinity values of compound 9. In comparison to the first series of compounds (5–8), the imidazolines 9–13 display higher affinity for I₁-IBS and lower for I₂-IBS. The functional profile of the compounds bearing the -NH-CH₂- linker (9–13) at α₂-AR subtypes highlights the crucial role of the *ortho*-phenyl pendant. Indeed, while compound 13 significantly activates both α_{2C}-AR and α_{2A}-AR subtypes, compounds 9–12 show interesting α_{2C}-AR agonism/α_{2A}-AR antagonism. The behavior of 9–13 might be ascribed to the different conformations adopted by the ligands, similarly to what was previously observed for analogues bearing the -OCH(CH₃)- bridge.³¹

Because compounds 5 and 9 displayed the best biological profiles at I₁/I₂-IBS and I₁/I₂-IBS/α₂-ARs, respectively, they were selected for their *in vivo* evaluation on opiate withdrawal syndrome. Both of them are characterized by the presence of the *ortho*-methyl substituent in the phenyl ring that, in ligands selectively interacting with I₂-IBS, was demonstrated to be

Table 1. Binding Affinity (pK_i^a) at I₁- and I₂-IBS on Rat Brain Membranes; Binding Affinity (pK_i^a), Antagonist Potency (pK_b^b), Agonist Potency (pEC₅₀^b), and Intrinsic Activity (ia^b) at Human α₂-Adrenergic Receptors Subtypes on CHO Cells

Cpd	X	R	I ₁ -IBS	I ₂ -IBS	α ₂ -AR	α _{2A} -AR		α _{2B} -AR		α _{2C} -AR	
			pK _i	pK _i	pK _i	pEC ₅₀ (pK _b)	ia	pEC ₅₀	ia	pEC ₅₀	ia
3	-CH=CH-	-H	7.72±0.14 ^c	8.72±0.13 ^c	<6 ^c	-	-	-	-	-	-
4	-NHCH ₂ -	-H	9.30±0.10 ^d	7.48±0.16 ^d	7.14±0.09 ^d	-	-	-	-	-	-
5	-CH=CH-	-CH ₃	7.75±0.05	9.11±0.06 ^e	<6 ^e	-	-	-	-	-	-
6	-CH=CH-	-Cl	7.62±0.09	8.79±0.10 ^e	<6 ^e	-	-	-	-	-	-
7	-CH=CH-	-CH ₂ CH=CH ₂	7.16±0.07	8.30±0.17	<6	-	-	-	-	-	-
8	-CH=CH-		7.01±0.11	8.08±0.07	<6	-	-	-	-	-	-
9	-NHCH ₂ -	-CH ₃	8.70±0.12	8.06±0.08	7.73±0.08	(7.58±0.21)		5.50±0.07	0.40	7.66±0.12	0.80
10	-NHCH ₂ -	-Cl	8.12±0.11	7.90±0.13	7.59±0.09	(7.12±0.05)		6.11±0.06	0.45	6.65±0.10	0.85
11	-NHCH ₂ -	-CH ₂ CH=CH ₂	8.40±0.07	7.85±0.11	6.79±0.10	(6.77±0.14)		5.60±0.21	0.50	6.80±0.15	0.80
12	-NHCH ₂ -	-CH ₂ CH ₂ CH ₃	8.08±0.18	7.29±0.06	6.75±0.15	(6.71±0.12)		5.34±0.08	0.55	6.94±0.07	0.75
13	-NHCH ₂ -		8.85±0.06 ^e	6.10±0.08 ^e	6.75±0.13 ^e	7.20±0.08	0.85	6.02±0.11	0.50	6.83±0.12	0.80

^apK_i affinity values for I₁-IBS, I₂-IBS, and α₂-ARs were assessed by measuring the ability of the tested compounds to displace [¹²⁵I]-*p*-iodoclonidine (rat kidney membranes), [³H]-2-BFI (rat brain membranes), and [³H]-RX 821002 (rat brain membranes), respectively. ^bpK_b, pEC₅₀, and ia values were determined by applying the Cytosensor microphysiometry system to the CHO cells expressing individual each human α₂-AR subtype. Intrinsic activity of the tested compounds is expressed as the fraction of that of the full agonist (-)-noradrenaline taken as equal to 1. The data are expressed as mean ± SEM of 3–5 separate experiments. ^cReference 21. ^dReferences 22 and 23. ^eRe-examined in the experimental conditions of the present study.

compatible with a significant reduction of the morphine withdrawal signs.³⁰

Figures 1 and 2 show the effects of **5** and **9**, respectively, on naloxone-precipitated withdrawal syndrome behavior. Repeated

Figure 1. Effects of acute (A,B) or repeated (C,D) intraperitoneal (i.p.) administration of **5** (5, 10, mg/kg) on expression and acquisition of morphine dependence, respectively. Naloxone-precipitated withdrawal symptoms, both in control and morphine treated mice, are given as a measure of the frequency of jumping (A,C) and somatic signs (B,D) expressed as summary of rearing, forepaw tremors, and teeth chatter. Data represent mean ($\pm$ SEM) of 6–10 animals. Significant differences: ## p < 0.01, compared to vehicle; * p < 0.05, ** p < 0.01, compared to morphine group.

Figure 2. Effects of acute (A,B) or repeated (C,D) i.p. administration of **9** (5, 10, mg/kg) on expression and acquisition of morphine dependence, respectively. Naloxone-precipitated withdrawal symptoms, both in control and morphine treated mice, are given as a measure of the frequency of jumping (A,C) and somatic signs (B,D) expressed as summary of rearing, forepaw tremors, and teeth chatter. Data represent mean ($\pm$ SEM) of 6–9 animals. Significant differences: ## p < 0.01, compared to vehicle; ** p < 0.01, compared to morphine group.

administration of morphine produced physical dependence, as assessed by a summary of characteristic set of behavioral responses, including jumping, rearing, forepaw tremor and teeth chattering following the naloxone challenge, as compared to vehicle. Acute administration of **5** or **9**, 15 min prior to the naloxone injection, significantly decreased both the number of

jumping, a sign featuring the morphine withdrawal syndrome (Figures 1A and 2A), and the frequencies of other withdrawal manifestations, reported as the sum of the frequency of rearing, forepaw tremor, and teeth chattering (Figures 1B and 2B) in morphine-dependent mice.

In particular, treatment with **9** at the doses of 5 and 10 mg/kg almost completely nullifies the number of jumping (96% and 98%, respectively). Similarly, the reduction of the frequency of the other signs of withdrawal is 86% and 93% at 5 and 10 mg/kg, respectively.

The combined I_1 - and I_2 -IBS interactions produced by **5** appear less effective in reducing the expression of morphine dependence. Indeed, the reduction of the number of jumping is comparable to that produced by **9** only at 10 mg/kg (90%). Moreover, only half reduction of the other withdrawal signs is observed.

Figures 1 and 2 also show the effects of repeated coadministration of **5** or **9** with morphine on the naloxone-precipitated withdrawal symptoms. In particular, pretreatment of the mice with **5** or **9**, 15 min before each morphine injection, significantly attenuates both the development of jumping (Figures 1C and 2C) and the total signs of withdrawal (Figures 1D and 2D).

Also in this case **9** is the most effective compound, showing a reduction of about 80% of jumping and other withdrawal signs at 5 and 10 mg/kg. Analogous reduction of jumping was produced by **5** at the dose of 10 mg/kg, whereas no effect is observed at 5 mg/kg (Figure 1C). Moreover, significantly lower effects are also observed in the attenuation of the other signs of morphine withdrawal (34% and 46% at 5 and 10 mg/kg, respectively).

In conclusion, according to what was previously observed for compounds **3** and **4**, the $-\text{CH}=\text{CH}-$ and $-\text{NH}-\text{CH}_2-$ bridges of the imidazoline scaffold **I** reported in Chart 2 confirm to be suitable for ligands able to produce I_1 -/ I_2 -IBS or I_1 -/ I_2 -IBS/ α_2 -ARs interactions, respectively. Such combined interactions, rationally selected on the basis of some of the numerous targets of agmatine, were demonstrated to produce advantageous pharmacological responses. Indeed, **5** and **9**, showing the best biological profiles at I_1 -/ I_2 -IBS and I_1 -/ I_2 -IBS/ α_2 -ARs, respectively, significantly affected both expression and acquisition of morphine dependence. In particular, the combined I_1 -/ I_2 -IBS/ α_2 -ARs interaction appeared more favorable, as demonstrated by the higher efficacy displayed by **9** both in contrasting and preventing morphine withdrawal. Moreover, such a combination also appeared more favorable than that produced by **2** (I_2 -IBS/ α_2 -ARs), already evaluated in the same assays¹⁸ (see Table S2). Therefore, **9** might be considered a promising tool useful in managing opioid addiction, potentially lacking in sedative and hypotensive side effects, due to its α_{2A} -AR antagonist profile.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00290.

Synthetic procedures, experimental details of *in vivo* assays, and elemental analysis of the final compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to Prof. Maria Pigni, who died on February 8, 2016.

■ ABBREVIATIONS

5-HT_{1A}-R, 5-HT_{1A} receptor; α_2 -ARs, α_2 -adrenoceptors; CNS, central nervous system; IBS, imidazoline binding sites

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