Mini Review

The 2-substituted imidazoline ring linked to an aromatic moiety by a biatomic bridge: a bioversatile scaffold

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The first drug bearing the 2-substituted imidazoline ring as a structural motif was the α-adrenoreceptor (α-AR) agonist nafazoline [1], described in 1939 and still used as vasoconstrictor and nasal decongestant. Twenty-five years later Boehringer Ingelheim patented clonidine [2], a drug able to cause high sedation, reduction of blood pressure and cardiac rate, and inhibition of salivary secretion. It also showed analgesic properties useful in the treatment of neuropathic pain. Its central sympathetic inhibition was ascribed to the activation of the α_2-ARs in the medulla oblongata. However, in 1984 Bousquet hypothesized a complementary mechanism involving the I_1-imidazoline binding sites (I_1-IBS) [3]. Though IBS, also including I_2 and I_3 subtypes, are distinct from α_2-ARs, they similarly recognize ligands bearing the imidazoline ring. The discovery of the vasoconstrictor cirazoline (**1**) (Figure 1), emerged as an α_1-AR agonist/α_{2A}-AR antagonist/α_{2C}-AR agonist and efficacious I_2-IBS ligand [4], supported the versatility of the imidazoline nucleus as a structural motif of molecules with different biological profiles [5].

In the structure of cirazoline, as well as of many other active ligands directed to different biological systems, it is possible to recognize the bioversatile scaffold **I** reported in Figure 1, consisting of the 2-substituted imidazoline nucleus linked to an aromatic area (Ar) by a biatomic bridge (X-Y). Interestingly, the three portions forming such a scaffold display different functions. Pigini e coll. noticed that minor chemical modifications in the biatomic bridge determined the preferential recognition of one or more specific biological targets [6], whereas those in the aromatic moiety (in particular in the ortho position of the phenyl ring) were generally responsible for the functional behaviour of the ligand [7].

In particular, the $-OCH_2-$ chain is compatible with ligands directed to I_2-IBS and α_2-ARs. Indeed, several cirazoline analogues have been described as multitarget agents, producing efficacious α_{2C}-AR agonism/α_{2A}-AR antagonism/I_2-IBS interaction. *In vivo* experiments performed with compound **2** demonstrated that such a combination is useful for reducing opioid withdrawal syndrome and associated depression [8].

The introduction of a methyl group in the oxymethylene chain, leading to the $-OCH(CH_3)-$ bridge, proved to be particularly suitable for the α_2-ARs. Indeed, such a modification drastically reduced the I_2-IBS affinity. In particular, compound **3** behaved as a potent α_2-AR antagonist and showed high selectivity over IBS [6]. Interestingly, it has been observed that the insertion of an ortho phenyl substituent in the aromatic ring of **3** modulates its fuctional profile. Indeed, biphenyline (**4**) is characterized by an α_2-AR agonist profile. The eutomer *S*-(-)-**4**, tested in two algesiometric tests (rat hot-plate and tail-flick), displayed a potent and long lasting antinociceptive effect, clearly mediated by α_2-ARs, as it was completely blocked by the selective α_2-antagonist RX 821002 [9].

The results obtained with biphenyline stimulated the investigation of the effect produced by the insertion of different substituents in the ortho position of the aromatic ring of the antagonist **3**. In particular, substituents such as cyclopentyl or cyclohexyl groups, endowed with steric bulk comparable to that of the phenyl ring, led to compounds behaving similarly to **4** (agonists at both α_{2A}- and α_{2C}-AR subtypes). On the contrary, substituents such as allyl or cyclopropyl groups, with lower steric bulk (MR <20), induced significant modulation of the antagonist profile of **3** only at the α_{2C}-subtype. Indeed, they maintained the α_{2A}-antagonist behavior, but were potent α_{2C}-agonists [7]. This last *in vitro* biological profile proved to be responsible for the intriguing pharmacological behavior of the allyl derivative **5** (allyphenyline). Indeed, in *in vivo* studies in mice, allyphenyline enhanced morphine analgesia (due to its α_{2C}-agonism), was devoid of sedative effect (due to its α_{2A}-antagonism) [4], contrasted and prevented morphine tolerance and dependence at the very low dose of 0.05 mg/Kg [10]. Moreover, allyphenyline significantly reduced hyperanxiety-like behavior verified 6 days after alcohol intoxication and hyperlocomotor activity associated with alcohol withdrawal [11].

The evaluation of a large number of α_2-AR ligands, including the potent α_{2A}-AR antagonist/α_{2C}-AR agonist cyclomethyline (**6**), suggested that their α_{2A}-AR agonist or antagonist profile might be ascribed to different conformations adopted by the molecules. In particular, from a structural overlay investigation it emerged that a preferred extended conformation appeared to be associated with dual α_{2C}-AR agonism/α_{2A}-AR antagonism, whereas a folded conformation was associated with α_{2C}-/α_{2A}-AR agonism. This latter conformation is probably induced by the electron-rich ortho phenyl substituent, able to provide a stable π-cation like charge transfer complex with the imidazoline moiety [12].

Further studies performed with allyphenyline (**5**) and cyclomethyline (**6**) demonstrated that their efficacy against morphine dependence [10,13] proved to be even improved by their ability to reduce the depressed mood, partially due to the additional serotonin 5-HT_{1A} receptor activation. For this reason, at the same dose of 0.05 mg/kg **5** and **6** exerted a potent antidepressant-like effect in the mouse forced swimming test. Experiments carried out in the presence of the α_2-AR antagonist yohimbine and the serotonin 5-HT_{1A} receptor antagonist WAY100135 demonstrated that both α_{2C}-AR and 5-HT_{1A}
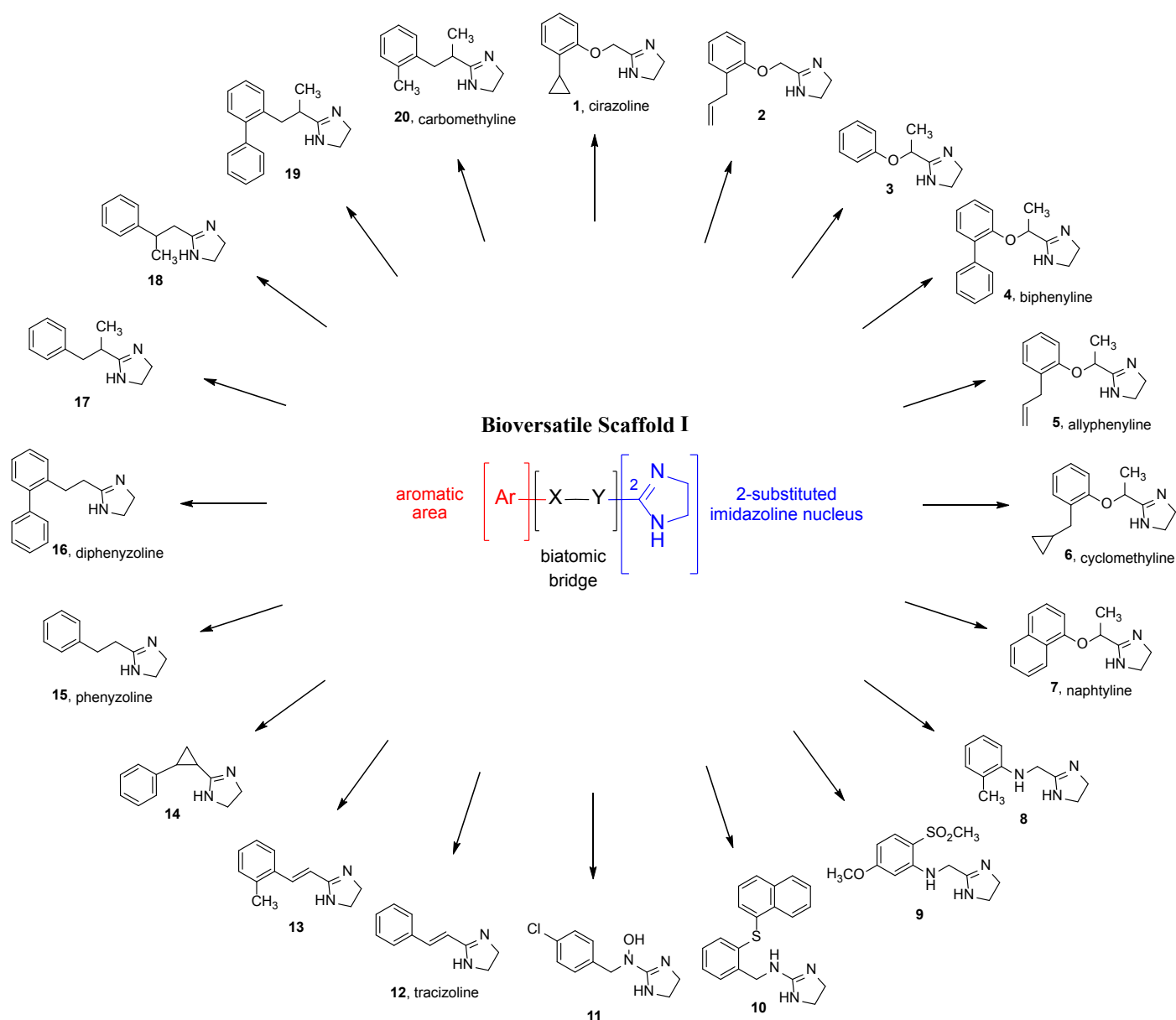
Figure 1. Chemical structures of compounds 1-20 sharing the common scaffold I.

receptor activation was involved in their antidepressant-like activity [13]. Moreover, the therapeutic potential of **5** and **6** was strongly enhanced by their favorable in vitro ADME profiles and limited activity on the hERG channel [13].

Recently, the analogue (*S*)-(+)-**7** [(*S*)-(+)-naphtyline] proved to exert an even more efficacious antidepressant-like effect in the mouse forced swimming test, being active at the very low dose of 0.01 mg/kg. This effect was also clearly mediated by the 5-HT$_{1A}$ receptor, as it was significantly reduced by the pretreatment with the antagonist WAY100635 [14].

The isosteric substitution of the oxygen in the bridge with an NH function, together with the insertion of suitable substituents in the ortho position of the aromatic moiety also led to biologically active compounds. Indeed, the -NH-CH$_2$- chain proved to be suitable for ligands interacting with I$_1$-, I$_2$-IBS and α$_2$-ARs. Among all the ligands,

8 proved to be effective in reducing expression and acquisition of morphine dependence in mice at the dose of 5 mg/kg and, therefore, might be considered a promising tool in managing opioid addiction [15].

The -NH-CH$_2$- bridge also demonstrated to be compatible with α$_1$-AR agonist activity, with compound **9** showing high selectivity for α$_{1a}$-AR over α$_{1b}$- e α$_{1d}$-AR (250 and 7000 fold, respectively) [16].

The reversed bridge -CH$_2$NH- is also present in several ligands, among which compound **10** showed significant *in vitro* antiplasmodial activity against both chloroquine-resistant P. falciparum W2 and atovaquone-resistant P. falciparum FCR3 strains [17].

The insertion of an hydroxy group at the nitrogen led to the -CH$_2$N(OH)- bridge, present in a series of *N*-(Imidazolidin-2-ylidene)-1-arylmethanamine oxides that bind to α-ARs and IBS. Particularly

interesting was the derivative **11**, which showed high affinity for I_2-IBS and good selectivity over I_1-IBS and α-ARs. Due to low lipophilicity, such a compound is not expected to cross the blood brain barrier and, therefore, might find application for the treatment of vascular hyperplasia [18].

Structure-activity relationship (SAR) studies also demonstrated that a wholly carbon bridge is responsible for effective selectivity for IBS over α-ARs. For example tracizoline (**12**), a ligand bearing a -CH=CH- bridge, is endowed with high affinity for both I_1- and I_2-IBS and good selectivity over both $α_1$- and $α_2$-ARs [6]. Such a biological profile is responsible for the ability of the tracizoline analogue **13** to contrast and prevent morphine dependence in mice at the dose of 5 mg/kg [15].

A selectivity between I_1- and I_2-IBS subtypes can be obtained by replacing the vinyl bridge with a cyclopropyl ring. In particular, the *trans* isomer of **14** showed higher affinity than the *cis* one for both I_1- and I_2-IBS, indicating that the distance between the phenyl ring and the imidazoline ring is a critical requirement for the affinity. The most important result was obtained by resolving the racemate of the *trans* isomer into the enantiomers (1*R*,2*R*)-(-)-**14** and (1*S*,2*S*)-(+)-**14**. At I_1-IBS the dextrorotatory enantiomer was more potent than the corresponding optical antipode. At I_2-IBS, enantioselective binding was also observed, but the eutomer was the levorotatory enantiomer. This was clearly a reversal of enantioselectivity with the (1*S*,2*S*)-(+)-**14** being 10 fold selective for I_1-IBS while the enantiomer (1*R*,2*R*)-(-)-**14** about 60-fold selective for I_2-IBS [19].

Further SAR studies demonstrated that the -CH_2CH_2- bridge was suitable for the I_2-IBS interaction. It is present in the ligand phenyzoline (**15**), showing very high selectivity for I_2-IBS over both $α_2$-ARs and I_1-IBS ($I_2/α_2$ = 794; I_2/I_1 = 1479) [6]. Analogously to what observed with $α_2$-ARs derivatives, the ortho-phenyl substitution also affected the functional profile of I_2-IBS ligands. Indeed, **15** and its ortho-phenyl derivative **16** (diphenyzoline) highlighted an interesting "positive" or "negative" morphine analgesia modulatory effect, respectively. In fact, **15** (s.c. 10 mg/kg) enhanced morphine analgesia, while **16** (s.c. 10 mg/kg) decreased it [20]. A subsequent study of two series of 2-aryl-ethylen-imidazolines, inspired by **15** and **16**, 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 [21].

The α- or β-methylation of **15** appeared extremely critical regarding the affinity and selectivity for I_1- or I_2-IBS interaction. Indeed, the α-methylation (compound **17**) favoured the I_1-IBS selectivity (I_1/I_2 = 186) and caused high stereospecificity of interaction (eudismic ratio (*S*)-(-)-**17**/(*R*)-(+)-**17** = 5888). Instead, even if at a lesser extent, the β-methylation (compound **18**) favored I_2-IBS selectivity (I_2/I_1 = 50) [6].

The insertion of a phenyl ring in the ortho position of **17**, leading to **19**, induced an interesting modulation of its biological profile from antagonism to agonism. Indeed, **19**, behaving as efficacious I_1-IBS agonist, produced significant hypotensive and bradicardic effects, prevented by the pre-treatment with **17**. Moreover, chirality appeared crucial in the activation of the I_1-IBS as the biological effects shown by **19** were produced only by the *S*-(+) enantiomer, while the distomer *R*-(-) was devoid of hemodynamic action [22]. Ten years later, the preparation of other ligands carrying selected substituents in the ortho position of the phenyl ring led to carbomethyline (**20**). This derivative and its (*S*)-(+) eutomer, showed a hypotensive effect higher than that evoked by **19** and selectively modulated by I_1-IBS [23]. At the same

time, this study confirmed that the introduction of ortho substituents with moderate steric hindrance and positive lipophilic contribution was able to induce antagonism to agonism modulation.

Altogether, the results described in this review strongly support the bioversatility of the imidazoline scaffold **I**, whose decorations can lead to selective or multitarget ligands. In the latter case, as observed for **5** and **6**, the biological profile can be potentially useful for the management of comorbidities. Therefore, the 2-substituted imidazoline ring linked to an aromatic moiety by a biatomic bridge might be considered a privileged structure and deserves the ironical definition of "biological picklock".

Acknowledgement

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