

# Imidazoline Antihypertensive Drugs: Selective I<sub>1</sub>-Imidazoline Receptors Activation

K. Nikolic & D. Agbaba

Faculty of Pharmacy, Institute of Pharmaceutical Chemistry, University of Belgrade, Vojvode Stepe, Belgrade, Serbia

## Keywords

$\alpha_2$ -Adrenergic receptors; Centrally acting antihypertensives; Clonidine; Hypertension; Imidazoline receptors; Rilmenidine.

## Correspondence

K. Nikolic, Faculty of Pharmacy, Institute of Pharmaceutical Chemistry, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia.

Tel: +381-63-84-30-677;

Fax: +381-11-3974-349;

E-mail: knikolic@pharmacy.bg.ac.rs

doi: 10.1111/j.1755-5922.2011.00269.x

## SUMMARY

Involvement of imidazoline receptors (IR) in the regulation of vasomotor tone as well as in the mechanism of action of some centrally acting antihypertensives has received tremendous attention. To date, pharmacological studies have allowed the characterization of three main imidazoline receptor classes, the I<sub>1</sub>-imidazoline receptor which is involved in central inhibition of sympathetic tone to lower blood pressure, the I<sub>2</sub>-imidazoline receptor which is an allosteric binding site of monoamine oxidase B (MAO-B), and the I<sub>3</sub>-imidazoline receptor which regulates insulin secretion from pancreatic  $\beta$ -cells. All three imidazoline receptors represent important targets for cardiovascular research. The hypotensive effect of clonidine-like centrally acting antihypertensives was attributed both to  $\alpha_2$ -adrenergic receptors and nonadrenergic I<sub>1</sub>-imidazoline receptors, whereas their sedative action involves activation of only  $\alpha_2$ -adrenergic receptors located in the locus coeruleus. Since more selective I<sub>1</sub>-imidazoline receptors ligands reduced incidence of typical side effects of other centrally acting antihypertensives, there is significant interest in developing new agents with higher selectivity and affinity for I<sub>1</sub>-imidazoline receptors. The selective imidazoline receptors agents are also more effective in regulation of body fat, neuroprotection, inflammation, cell proliferation, epilepsy, depression, stress, cell adhesion, and pain. New agonists and antagonists with high selectivity for imidazoline receptor subtypes have been recently developed. In the present review we provide a brief update to the field of imidazoline research, highlighting some of the chemical diversity and progress made in the theoretical studies of imidazoline receptor ligands.

## Introduction

Two decades ago Bousquet et al. [1] discovered that the clonidine-like centrally acting antihypertensives produced their pharmacological effect by interaction not only with the  $\alpha_2$ -adrenoceptors ( $\alpha_2$ -AR) but also with imidazoline receptors (imidazoline binding sites [IBSs]). The IBSs were pharmacologically distinct from the  $\alpha_2$ -AR because they were not activated by catecholamines [1]. They found a positive correlation between the hypotensive potency of imidazoline compounds and their affinity for imidazoline receptors but not for  $\alpha_2$ -adrenoceptors [2,3].

In the present study, we seek to distinguish the ligand selectivity profile of I<sub>1</sub>-IBS relative to other binding sites for imidazolines, demonstrate the specific activation of transmembrane signaling pathways by I<sub>1</sub>-imidazoline receptors and review the diverse and complex function of I<sub>1</sub>-imidazoline receptors ligands.

Specific imidazoline receptors, I<sub>1</sub>-imidazoline receptors (I<sub>1</sub>-IR), I<sub>2</sub>-imidazoline receptors (I<sub>2</sub>-IR), and I<sub>3</sub>-imidazoline receptors (I<sub>3</sub>-IR), have been characterized by extensive biochemical and

physiological studies [4,5] and highly selective novel imidazoline agents have been developed without  $\alpha_2$ -AR activity [6].

Imidazoline I<sub>1</sub>-receptors in the rostral ventrolateral medulla (RVLM) are important for the sympathoinhibitory action of clonidine-, rilmenidine-, and moxonidine-like antihypertensive drugs. The mechanism by which central antihypertensives lowers blood pressure is a result of activation of both  $\alpha_2$ -AR and I<sub>1</sub>-IRs in the RVLM [3,7]. The  $\alpha_2$ -AR agonists directly inhibit presympathetic RVLM neurons, while the I<sub>1</sub>-IR agonists increase the release of catecholamines in the RVLM. The catecholamines depress presympathetic RVLM neurons by activating  $\alpha_2$ -AR [3,7]. Compared with clonidine, newer centrally acting antihypertensive drugs such as rilmenidine and moxonidine are more selective for I<sub>1</sub>-imidazoline receptors than for  $\alpha_2$ -adrenergic receptors. Rilmenidine and moxonidine cause only few  $\alpha_2$ -adrenoceptor-mediated side effects because they possess better selectivity for I<sub>1</sub>-IRs.

In order to test the imidazoline hypothesis, the effects of clonidine on blood pressure and heart rate in  $\alpha_{2ABC}$ -deficient mice

were determined [8]. Clonidine, moxonidine, and rilmenidine failed to significantly decrease mean arterial pressure in  $\alpha_{2ABC}$ -deficient mice [8]. These findings have further confirmed that activation of  $\alpha_2$ -AR is required for hypotensive effect of the central antihypertensives.

The second-generation agents, such as rilmenidine and moxonidine, produce hypotension and sympathetic inhibition by an action principally on I<sub>1</sub>-imidazoline receptors rather than  $\alpha_2$ -adrenoceptors [3,7,9]. Many studies have investigated close interdependence and interaction of these two receptors at the cellular level.

Many efforts have been made to identify the proteins bearing the different imidazoline-binding sites. The I<sub>1</sub>-IR protein structures have not yet been solved to date. Only imidazoline receptor antisera-selected (IRAS-1) gene candidate for the I<sub>1</sub>-IR protein has been cloned [10–12].

Nischarin, a homolog of human IRAS [10], plays an important role in cell signaling and function [12]. New *in vivo* studies from Abdel Rahman's group presents evidence that nischarin is essential for the initiation of neuronal signaling triggered by I<sub>1</sub> receptor activation in the RVLM and subsequent hypotensive response [11]. This evidence is supported by the findings that knockdown of nischarin expression in the RVLM virtually abolishes the I<sub>1</sub>R (rilmenidine)-mediated enhancement of phosphorylated extracellular signal-regulated kinase (pERK)1/2 production in the RVLM and the associated hypotension [11]. Transfection studies with IRAS-1 cDNA, have revealed a modulatory role for imidazoline receptors on coincidentally activated receptors in the same cell, including the  $\alpha_{2A}$ -adrenergic and fibronectin receptors [13].

Since the IBS may only be formed when IRAS-1 is complexed to the fibronectin receptor or other partner proteins [13], characterization of I<sub>1</sub>-ligand—IRAS-1 complexes require special experimental procedure.

The I<sub>1</sub>-sites have been shown to be coupled to a G protein in synaptic plasma membranes of the bovine brainstem [14,15], in the human platelets [16], and in the rat pheochromocytoma cells (PC12) [17,18]. I<sub>1</sub> receptors are preferentially bound by 2-aminoimidazolines (<sup>3</sup>H]-clonidine), while show medium affinity for imidazolines (<sup>3</sup>H]-idazoxan), and low affinity for guanidines (amiloride) [19–21].

The I<sub>2</sub> receptors are allosteric binding site associated with the catalytic site of monoamine oxidase (MAO), but also on other non-MAO oxidative enzymes [22].

The I<sub>2</sub>-imidazoline-binding proteins were purified from rabbit kidney [23], adrenal chromaffin cells [24], and RINm5F pancreatic cells [25]. The sequence of these proteins presented some homology with that of the MAO enzyme [23–25].

The I<sub>2</sub> sites are located principally in the outer membrane of mitochondria of peripheral and central tissues [26,27], such as central nervous system (especially in glia cells [28]), blood platelets, liver, adipocytes, and kidney.

I<sub>2</sub> receptors are characterized by their high affinity for imidazolines and guanidines, and lower affinity for 2-aminoimidazolines [29]. Further pharmacological studies demonstrated existence of two I<sub>2</sub>-receptor subtypes, I<sub>2A</sub> and I<sub>2B</sub>, depending on their affinity for guanidine derivative-amiloride [26].

Some previous studies reported experimental data that pancreatic imidazoline receptors and sympathetic presynaptic imidazo-

line receptors did not fulfill the criteria for the definition of I<sub>1</sub> or I<sub>2</sub> receptors. Such sites were initially called non-I<sub>1</sub>, non-I<sub>2</sub> receptors [30,31] and later were defined as I<sub>3</sub> receptors [32,33]. The I<sub>3</sub> receptors in pancreatic  $\beta$ -cells are involved in regulation of insulin secretion in a manner, which is not typical of I<sub>1</sub>- or I<sub>2</sub>-receptor related phenomenon [30,32–35]. The imidazoline efaroxan is a selective agonist at the I<sub>3</sub> receptor and its imidazole analog (KU14R) is an antagonist [33]. However, attempts to characterize the associated IBSs have been unsuccessful because of the lack of specific radioligands.

The reduced incidence of side effects of more selective imidazoline receptor ligands together with difficulties in separating effects of an interaction with  $\alpha_2$ -AR from those of imidazoline receptors [34], were driving force to search for new selective imidazoline agents, to identify imidazoline receptors and their endogenous ligands, and to examine functional effects of stimulation of particular I<sub>1</sub>-, I<sub>2</sub>-, and I<sub>3</sub>-imidazoline receptors.

I<sub>1</sub>-imidazoline receptors ligands will be compared in function of their chemical structure, binding affinity, and selectivity for the I<sub>1</sub>-imidazoline receptors.

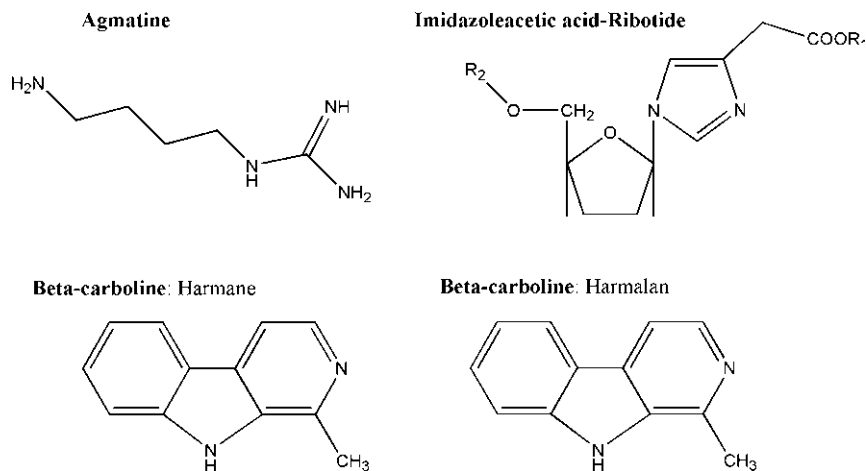
## Imidazoline Endogenous Ligands

The majority of the currently used hypotensive compounds are synthetic imidazoline derivatives. If the naturally occurring ligands could be identified, they may have an improved selectivity profile and hence could be the starting point for the development of a new range of antihypertensive agents. Therefore, elucidation of the structure of the endogenous ligand(s), called clonidine-displacing substances (CDS), for IBSs has been a major goal for many years. The CDS was originally identified in extracts of rat and bovine brain [36] but may also be present in peripheral tissues and in the circulation [37,38]. The CDS, such as agmatine, imidazole-4-acetic acid-ribotide, and  $\beta$ -carboline compounds (Figure 1), can bind with high affinity to imidazoline sites and to  $\alpha_2$ -adrenoceptors [39]. Agmatine possesses the common amidine motif and can accordingly displace specific radioligand binding from  $\alpha_2$ -adrenoceptors and IBSs [39].

Initially, agmatine [40] and imidazole-4-acetic acid ribotide [41] have been identified as endogenous ligands for the imidazoline receptors.

The agmatine is a polyamine formed by decarboxylation of L-arginine by arginine decarboxylase (ADC) in mammalian tissues [40]. Agmatine binds with a moderate affinity  $\alpha_2$ -adrenoceptors as well as to I<sub>1</sub>- and I<sub>2</sub>-binding sites [40]. This guanidine-aliphatic-amine seems to be either an endogenous antagonist or inverse agonist at imidazoline receptors, but also has major effects on NMDA receptors and neuronal nitric oxide synthase [42,43]. This putative neurotransmitter interacts with a variety of receptors and has been implicated in mediation of stress responses, analgesia, drug addiction and withdrawal, convulsions, and neuroprotection. [44]. Activation of imidazoline I<sub>2A</sub> receptors by agmatine in adrenal gland lowers plasma glucose in streptozotocin-induced diabetic rats (STZ rats) [45].

The imidazole-4-acetic acid-ribotide (IAA-RP) is the endogenous agonist for I<sub>1</sub>-IRs and I<sub>3</sub>-IRs in adrenal medulla and in pancreatic tissue [41].



**Figure 1** Chemical structures of endogenous ligands: Agmatine, Imidazoleacetic acid-Ribotide, Harmane, and Harmalan.

Also,  $\beta$ -carboline compounds, such as harmane [46,47] and harmalan [48], have been proposed as putative endogenous substrates of either I<sub>1</sub>- or I<sub>2</sub>-imidazoline receptors. These compounds have been shown to act as endogenous ligands at certain imidazoline receptors and have central effects on blood pressure [47]. The potency of harmane on blood pressure is similar to that of the clonidine [47].

The endogenous ligands were assessed for binding affinities at I<sub>1</sub>-IR, I<sub>2</sub>-IR, and  $\alpha_2$ -adrenoceptors on rat brain and kidney membranes [49,50] and binding affinities at I<sub>2</sub>-IR on human placenta and rat liver membranes [39].

## I<sub>1</sub>-Imidazoline Receptor Ligands

The I<sub>1</sub> subtype of imidazoline receptors has been thoroughly characterized by binding assays using radiolabeled clonidine analogs and its pharmacological selectivity assessed [7,51,52]. The coupling of I<sub>1</sub>-imidazoline receptors to G proteins has been suggested by the sensitivity of the imidazoline-specific binding to GTP or nonhydrolysable analogs in the canine prostate [53], in the chromaffin cells [16,51], and in the bovine brainstem [54]. Effects of imidazolines on classical second messenger systems of G protein-coupled receptors, either cAMP or inositol-phosphates and diacylglycerol (DAG), have been studied in various models, including rat adrenal glands, bovine chromaffin cells, and rat brain. Transduction pathways of the I<sub>1</sub>-imidazoline receptors in the PC12 cells have been associated with activation of a phosphatidylcholine-specific phospholipase C (PC-PLC) [17] and inhibition of an adenylate cyclase [18].

Benazoline and other I<sub>1</sub>-imidazoline receptor ligands are able to dose dependently decrease forskolin-stimulated cAMP content in cells expressing only I<sub>1</sub>-imidazoline receptors (PC12 cells and NG10815 cells) [18]. Interestingly, although benazoline and moxonidine were agonists for both PC-PLC and cAMP pathways, some agonists for the cAMP pathway (efaroxan) had antagonist effects on the PC-PLC pathway [17,55]. These findings have opened new fields of investigations aiming to determine the existence of a cross talk between the different transduction pathways and to identify

their respective contributions to the physiological roles of the I<sub>1</sub>-imidazoline receptors.

On the other hand, pharmacological studies have shown that I<sub>1</sub>-imidazoline receptors are involved in several functions such as regulation of the cardiovascular function [1,3], modulation of the ocular pressure [56], renal sodium excretion [57], and control of the catecholamine release from chromaffin cells [58].

The central hypotensive effect of the I<sub>1</sub>-imidazoline receptor ligands with mixed (I<sub>1</sub>/ $\alpha_2$ ) binding profiles has been in significant correlation with their affinity for I<sub>1</sub>-IRs [2,3]. Imidazoline antagonists such as idazoxan competitively antagonized the centrally induced hypotensive effect of the I<sub>1</sub>-IRs ligands, while yohimbine, a  $\alpha_2$ -ARs antagonist, blocks the hypotensive effect of the ligands but usually in a noncompetitive manner [59]. The hypotensive effects of more selective I<sub>1</sub>-imidazoline receptor ligands, such as rilmenidine and moxonidine, was clearly prevented by idazoxan, an imidazoline I<sub>1</sub>-IR antagonist, whereas it was very weakly antagonized by antagonist for the  $\alpha_2$ -adrenoceptors, such as yohimbine [60,61]. Furthermore, recent studies have found that hypotensive effect of the selective imidazoline agents was facilitated by the activation of  $\alpha_2$ -adrenoceptors using  $\alpha$ -methylnoradrenaline [62].

In more detail, the adrenergic cell provides excitatory signals to the preganglionic sympathetic neurons (PGSN) in the intermediolateral cell column of the spinal cord to alter sympathetic discharges to the peripheral tissues. Its activity can be inhibited by neurotransmitters that are released from the activation of I<sub>1</sub>-IRs located on the noradrenergic (NA) cell terminals, or from the stimulation of terminal  $\alpha_{2A}$ -adrenoceptors located on the interneuron containing  $\gamma$ -aminobutyric acid (GABA). The serotonergic (5-HT) innervation may enhance the excitatory signals from the adrenergic cell [59–62].

All these results can be summarized as follows:

- An action on medullary I<sub>1</sub>-imidazoline receptors alone is sufficient to inhibit vasomotor tone and therefore to reduce blood pressure.
- I<sub>1</sub>-imidazoline receptors are involved in the hypotensive effects of I<sub>1</sub>-IR ligands.

- The integrity of the  $\alpha_2$ -adrenoceptors included in the sympathetic centers and pathways appears to be required for the development of the hypotensive effects of at least  $\alpha_2$ -adrenergic agonists.
- A cooperative interaction between imidazoline receptors and  $\alpha_2$ -adrenoceptors seems to account for the marked and rapid hypotensive effect caused by I<sub>1</sub>-IR ligands [59–62].

Many different families of I<sub>1</sub>-imidazoline receptors ligands, such as guanidine derivatives, imidazoline compounds, endogenous amines, and carbolines have been synthesized and examined for I<sub>1</sub>-, I<sub>2</sub>-,  $\alpha_{2A}$ -,  $\alpha_{2B}$ -,  $\alpha_{2C}$ -binding affinities [7,15,18,38,49,51,63–65].

Compilation of agmatine structure and imidazoline ring leads to a new family of imidazoline/ $\alpha_2$ -adrenoceptor ligands, 4(5)-(2-aminoethyl)imidazoline derivatives. The guanidine moiety included into heterocyclic ring improves the affinities of the resultant fusion compounds in comparison to agmatine itself [64].

Previously used radioligands to characterize the I<sub>1</sub>-IR, such as [<sup>3</sup>H] clonidine and [<sup>125</sup>I] *p*-iodoclonidine [51,52], were able to bind with similar affinities to  $\alpha_2$ -ARs and to I<sub>1</sub>-IR [66]. Therefore adequate characterization of the I<sub>1</sub>-IR subtype was very difficult. Recently, new imidazoline analogs, such as *cis*-/*trans*-dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3H-pyrrol-2-yl)-amine (LNP-509) [67], 2-(2-chloro-4-iodo-phenylamino)-5-methyl-pyrroline (LNP 911) [63], (2-(5-azido-2-chloro-4-iodo-phenylamino)-5-methyl-pyrroline (LNP 906) [68], (+)-5-(2-bromophenoxy)-methyl-2-amino-4,5-dihydro-1,3-oxazole (S23515) [69], (+)-2-(2-fluoro-5-methylphenyl)-4,5-dihydro-1H-imidazole (S23757) [69], with high affinity and selectivity for the I<sub>1</sub>-IRs were synthesized. The LNP 509 and S23515 cause hypotension when injected alone into the brainstem [67,69]. The LNP 906 and S23757 clearly antagonized the decrease in forskolin-stimulated cAMP level induced by I<sub>1</sub>-IR agonists [68,69]. Finally, LNP 911 is not able to dose dependently decrease forskolin-stimulated cAMP content in cells expressing only I<sub>1</sub>-imidazoline receptors, but it behaves as an allosteric enhancer [63]. The LNP 911 was radiiodinated and its binding properties characterized in different membrane preparations. While the hypotensive actions of these highly selective agents, such as LNP 509 [67], S23515 [69], and S23757 [69], have been documented [67,69] no full description of their cardiovascular actions has appeared to date in the literature.

Because of diverse I<sub>1</sub>-IRs binding affinities of the ligands obtained with different radioligands (such as [<sup>125</sup>I] *p*-iodoclonidine ([<sup>125</sup>I] PIC) and [<sup>125</sup>I] LNP 911) and different membrane preparations (such as PC12 and human platelets, Table 1) [15,18,63], it is not possible to compare the ligands binding affinities data of various competition binding studies.

The correlation between the pKi of the high affinity I<sub>1</sub>-IR ligands (clonidine; lofexidine; BDF 6143; rilmenidine; *p*-iodoclonidine; LNP 911) on [<sup>125</sup>I] LNP 911 binding sites and the pKi of the same drugs on [<sup>125</sup>I] PIC binding sites [63], together with the fact that PIC and LNP 911 were able to displace the same amount of [<sup>125</sup>I] LNP 911 total binding [63] indicate that identical I<sub>1</sub>-binding sites are concerned.

Because moxonidine, efaroxan, and benzoline were described as high-affinity I<sub>1</sub>-IRs ligands in different models [17,18] but dis-

placed [<sup>125</sup>I] LNP 911 with rather low affinity, relationships between the ligands and [<sup>125</sup>I] LNP 911 were studied in details [63]. These analyses have found out that I<sub>1</sub>-IRs are submitted to a complex allosteric modulation by the use of LNP 911. Actually, the LNP 911 behaves as an allosteric enhancer and potentiates the effects obtained with I<sub>1</sub>-IR agonists [63].

The lack of a clearly identified molecular structure for the I<sub>1</sub>-IR [10–12] and modulatory role for imidazoline receptors on coincidentally activated  $\alpha_{2A}$ -adrenergic and fibronectin receptors in the same cell [13] are making virtual docking study very difficult. Furthermore, importance of I<sub>1</sub>-IR selective ligands being available for structural, functional, and pharmacological investigations on I<sub>1</sub>-IRs, are the factors that have encouraged scientists to undertake a modeling study addressing the development of pharmacophore and quantitative structure activity relationship (QSAR) models [70–80]. The modeling studies have indicated on the physicochemical interactions modulating I<sub>1</sub>-IR affinity and on the key molecular determinants of I<sub>1</sub>-IR/ $\alpha_2$ -AR and I<sub>1</sub>/I<sub>2</sub>-IR selectivity. The results from the modeling studies can be used for the rational design of new, more selective, and high affinity I<sub>1</sub>-IR ligands.

The developed QSAR models for I<sub>1</sub>-IR ligands (Figure 2) have indicated that an increase in lipophilicity ( $\log D_{pH7.4}$ ), molar refractivity, and dipole moment value, together with a decrease in N-charge in the heterocyclic moiety influence on better affinity for I<sub>1</sub> receptors [70,71]. Furthermore, highest occupied molecular orbital energy and lipophilicity (ClogP) of the ligands are important parameters for evaluation of I<sub>1</sub>/ $\alpha_2$ -selectivity [70].

The other 2D-QSAR studies [76–80] have selected the electrostatic and steric properties as the most relevant ones in explaining the modulation of both I<sub>1</sub> and I<sub>2</sub> affinity. In agreement with the 2D-QSAR study, the combination of the steric and electrostatic field (for affinity at I<sub>1</sub>-IR) and the steric field in combination with the lipophilic one (for affinity at I<sub>2</sub>-IR), were the most important molecular interaction fields (MIFs) parameters, identified by the 3D-QSAR CoMFA, GRID, and GOLPE approaches [80].

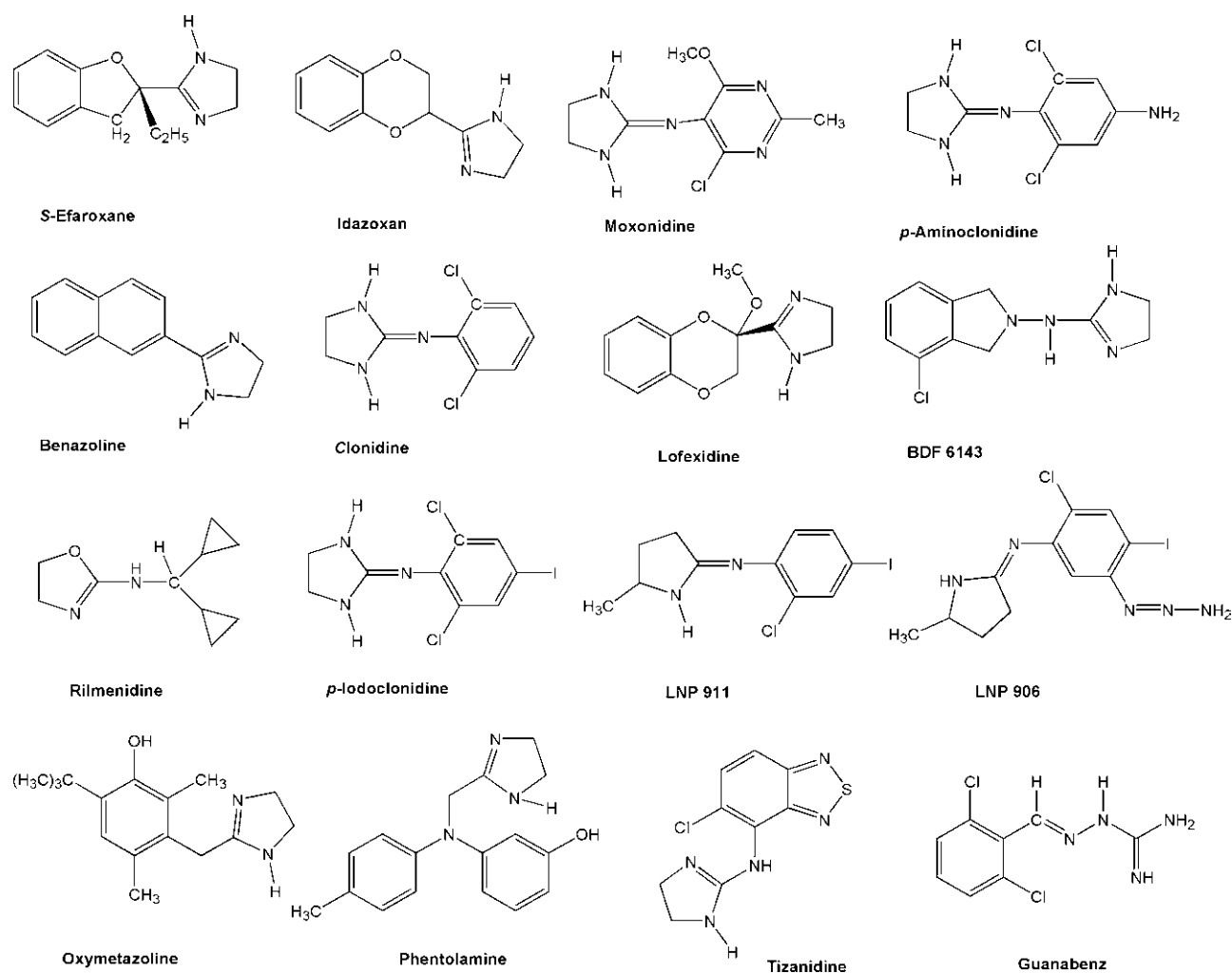
The pharmacophore hypotheses, developed for I<sub>1</sub>-IR ligands, indicated for the following five features: a hydrogen-bond donor group (HBD), two hydrophobic (HY1 and HY2) regions, an aromatic ring (AR), and a positive charge (PC) [80]. The pharmacophore for the I<sub>2</sub>-IR ligands was made up of only four features: two hydrophobic (HY1 and HY2) regions, an AR, and a PC [80]. 2D- and 3D-quantitative SAR study, performed on a series of imidazoline congeners, highlighted that good lipophilicity, extended also to the ortho position of the phenyl ring, was favorable but not decisive for significant I<sub>2</sub>-IR affinity [78,81]. Furthermore, the unsubstituted ethylenic bridge, between the aromatic portion and imidazoline nucleus of the 2-phenoxyethylimidazoline analogs, is proved to be determinant in inducing high I<sub>2</sub>-IR selectivity with regard to I<sub>1</sub>-IR and  $\alpha_2$ -ARs [65,81].

## Pharmacological Effects of I<sub>1</sub>-Imidazoline Agents

Hypertension is often observed as part of a more complex combination of diseases, such as obesity, hyperlipidemia, and hyperinsulinemia [82].

**Table 1** Inhibitory binding constants (K<sub>i</sub>) for ligands in competition binding studies with [<sup>125</sup>I] LNP 911 on PC12 cells [64], [<sup>125</sup>I] PIC on PC12 cells [18,64], and [<sup>125</sup>I] PIC at human platelet I<sub>1</sub>-IR [15]

Compound	K <sub>i</sub> (I <sub>1</sub> -IR) [nM] with [ <sup>125</sup> I]LNP 911 on PC12 cells [64]	K <sub>i</sub> (I <sub>1</sub> -IR) [nM] with [ <sup>125</sup> I]PIC on PC12 cells [18,64]	K <sub>i</sub> (I <sub>1</sub> -IR) [nM] with [ <sup>125</sup> I]PIC at human platelet I <sub>1</sub> -IR [15]
LNP 911	1.4 ± 1.7	0.3 [64]	
<i>p</i> -Iodoclonidine (PIC)	9.5 ± 6.3	0.8 [64]	4.8 ± 1.2
Rilmenidine	43.9 ± 0.5	20.0 [64]	59.2 ± 5.8
BDF 6143	62.3 ± 7.0	28 ± 6.0 [18]	232.0 ± 126.0
Lofexidine	507 ± 69	5.6 [64]	
Clonidine	693 ± 37	125 ± 75 [18]	55.0 ± 10.0
Benazoline	3,903 ± 437	1.3 [18]	
<i>p</i> -Aminoclonidine	4,492 ± 784		
Moxonidine	7,215 ± 461	34 ± 5 [18]	4.2 ± 3.2
Idazoxan	7,655 ± 876		1,255.0 ± 745.0
Efaroxan	8,567 ± 747	144 ± 170 [18]	52.4 ± 30.4

**Figure 2** Chemical structures of I<sub>1</sub>-IR ligands.

Rilmenidine and moxonidine induce fall in plasma catecholamines, rennin, and antidiuretic hormone [83–87], while clonidine, rilmenidine, and moxonidine effectively regress left ventricular hypertrophy [88–90] and reduce atrial natriuretic peptide levels [91]. Therefore, the drugs of the second generation were shown to increase renal blood flow, potassium excretion, natriuresis associated with inhibition of sodium reabsorption, and diuresis, whereas the sympathetic renal nerve activity was markedly decreased [92].

The  $\alpha_2$ -adrenoceptors in the pancreas reduced insulin secretion and increase glucagon release, while I<sub>1</sub>-IR activation had the opposite effect. Thus selective I<sub>1</sub>-IR agonists are capable of increasing the glucose-induced insulin secretion from pancreatic  $\beta$ -cells [93–96]. Furthermore, moxonidine improves the metabolic profile in patients with hypertension and diabetes mellitus or impaired glucose tolerance [97].

## References

- Bousquet P, Feldman J, Schwartz J. Central cardiovascular effects of alpha-adrenergic drugs: Differences between catecholamines and imidazolines. *J Pharmacol Exp Ther* 1984;**230**:232–236.
- Tibirica E, Feldman J, Bousquet P. Differences in the ability of yohimbine to antagonize the hypotensive effect of clonidine in normotensive and spontaneously hypertensive anesthetized rats. *J Pharmacol Exp Ther* 1988;**244**:1062–1066.
- Ernsberger P, Guiliano R, Willette RN, Reis DJ. Role of imidazole receptors in the vasodepressor response to clonidine analogs in the rostral ventrolateral medulla. *J Pharmacol Exp Ther* 1990;**253**:408–418.
- Regunathan S, Reis DJ. Imidazoline receptors and their endogenous ligands. *Ann Rev Pharmacol Toxicol* 1996;**36**:511–544.
- Ernsberger P, Haxhiu MA. The I<sub>1</sub>-imidazoline-binding site is a functional receptor mediating vasodepression via the ventral medulla. *Am J Physiol* 1997;**273**:R1572–R1579.
- Anastassiadou M, Danoun S, Crane L, et al. Synthesis and pharmacological evaluation of imidazoline sites I<sub>1</sub> and I<sub>2</sub> selective ligands. *Bioorg Med Chem* 2001;**9**:585–592.
- Chan CKS, Burke SL, Head GA. Contribution of imidazoline receptors and  $\alpha_2$ -adrenoceptors in the rostral ventrolateral medulla to sympathetic baroreflex inhibition by systemic rilmenidine. *J Hypertension* 2007;**25**:147–155.
- Knaus A, Zong X, Beetz N, Jahns R, Lohse MJ, Biel M, Hein L. Direct inhibition of cardiac HCN pacemaker channels by clonidine. *Circulation* 2007;**115**:872–880.
- Head GA, Chan CKS, Burke SL. Relationship between imidazoline and  $\alpha_2$ -adrenoceptors involved in the sympatho-inhibitory actions of centrally acting antihypertensive agents. *J Auton Nerv Syst* 1998;**72**:163–169.
- Piletz JE, Ivanov TR, Sharp JD, et al. Imidazoline receptor antisera-selected (IRAS) cDNA: Cloning and characterization. *DNA Cell Biol* 2000;**19**:319–329.
- Zhang J, Abdel-Rahman AA. Inhibition of nischarin expression attenuates rilmenidine-evoked hypertension and phosphorylated extracellular signal-regulated kinase 1/2 production in the rostral ventrolateral medulla of rats. *J Pharmacol Exp Ther* 2008;**324**:72–78.
- Lim KP, Hong W. Human nischarin/imidazoline receptor antisera-selected protein is targeted to the endosomes by a combined action of a PX domain and a coiled-coil region. *J Biol Chem* 2004;**279**:54770–54782.
- Alahari SK, Nasrallah H. A membrane proximal region of the integrin  $\alpha 5$  subunit is important for its interaction with nischarin. *Biochem J* 2004;**377**:449–457.
- Heemskerk FMJ, Dontenwill M, Grenney H, Vonthron C, Bousquet P. Evidence for the existence of imidazoline-specific binding sites in synaptosomal plasma membranes of the bovine brainstem. *J Neurochem* 1998;**71**:2193–2202.
- Piletz JE, Sletten K. Nonadrenergic imidazoline binding sites on human platelets. *J Pharmacol Exp Ther* 1993;**267**:1493–1502.
- Ernsberger P, Graves ME, Graff LM, et al. I<sub>1</sub> imidazoline receptors. Definition, characterisation, distribution and transmembrane signaling. *Ann N Y Acad Sci* 1995;**763**:22–42.
- Separovic D, Kester M, Ernsberger P. Coupling of I<sub>1</sub>-imidazoline receptors to diacylglyceride accumulation in PC12 rat pheochromocytoma cells. *Mol Pharmacol* 1996;**49**:668–675.
- Grenney H, Ronde P, Magnier C, et al. Coupling of I<sub>1</sub> imidazoline receptors to the cAMP pathway: Studies with a highly selective ligand, benazoline. *Mol Pharm* 2000;**57**:1142–1151.
- Heemskerk FMJ, Dontenwill M, Vonthron C, Bousquet P. [<sup>125</sup>I] Para-iodoclonidine reveals a new subtype of imidazoline specific sites in synaptosomal plasma membranes of the bovine brainstem. *J Neurochem* 1998;**71**:2193–2202.
- Meeley MP, Ernsberger PR, Granata AR, Reis DJ. An endogenous clonidine-displacing substance from bovine brain: Receptor binding and hypotensive actions in the ventrolateral medulla. *Life Sci* 1986;**38**:1119–1126.
- Bricca G, Dontenwill M, Molines A, Feldman J, Belcourt A, Bousquet P. Evidence for the existence of a homogenous population of imidazoline receptors in the human brainstem. *Eur J Pharmacol* 1988;**150**:401–402.
- Holt A, Wieland B, Baker GB. Allosteric modulation of semicarbazide-sensitive amine oxidase activities in vitro by imidazoline receptor ligands. *Br J Pharmacol* 2004;**143**:495–507.
- Limon I, Coupry I, Lanier SM, Parini A. Purification and characterization of mitochondrial imidazoline-guanidinium receptive site from rabbit kidney. *J Biol Chem* 1992;**267**:21645–21649.
- Wang H, Regunathan S, Meeley MP, Reis DJ. Isolation and characterization of imidazoline receptor protein from bovine adrenal chromaffin cells. *Mol Pharmacol* 1992;**42**:792–801.
- Remaury A, Paris H. The insulin-secreting cell line, RINm5F, expresses an  $\alpha_2$ -adrenoceptor and non adrenergic idazoxan binding sites. *J Pharm Exp Ther* 1991;**260**:417–426.
- Tesson F, Parini A. Identification of an imidazoline-guanidinium receptive site in mitochondria from rabbit cerebral cortex. *Eur J Pharmacol* 1991;**208**:81–83.
- Tesson F, Limon I, Parini A. Tissue-specific localization of mitochondrial imidazoline-guanidinium receptive sites. *Eur J Pharmacol* 1992;**219**:335–338.
- Martin-Gomez JJ, Ruiz J, Callado LF, Garibi JM, Aguinaco L, Barturen F, Javier Meana J. Increased density of I<sub>2</sub>-imidazoline receptors in human glioblastomas. *Neuroreport* 1996;**7**:1393–1396.
- Coupry I, Limon I, Tesson F, Lachaud V, Gargalidismoudanos C, Parini A. The imidazoline-guanidinium receptive site: A subtype of imidazoline receptors. *Therapie* 1992;**47**:519–524.
- Chan SL, Brown CA, Scarpello KE, Morgan NG. The imidazoline site involved in control of insulin secretion: Characteristics that distinguish

## Conclusion

The low incidence of the side effects, antiarrhythmic effects, and beneficial metabolic and renal effects of second-generation I<sub>1</sub>-IR ligands suggest that they may provide a very useful therapy, which may be further enhanced by the development of more selective I<sub>1</sub>-IR agents.

## Acknowledgment

This work was supported by the Ministry for Science, Technology and Environmental Protection of Serbia, contract No. 172033.

## Conflict of Interest

The authors have no conflict of interest

- it from I<sub>1</sub>- and I<sub>2</sub>-sites. *Br J Pharmacol* 1994;**112**:1065–1070.
31. Gothert M, Bruess M, Boenisch H, Molderings GJ. Presynaptic imidazoline receptors: New developments in characterization and classification. *Ann N Y Acad Sci* 1999;**881**:171–184.
  32. Morgan NG, Chan SL, Mourtada M, Monks LK, Ramsden CA. Imidazolines and pancreatic hormone secretion. *Ann NY Acad Sci* 1999;**881**:217–228.
  33. Clews J, Morgan NG, Ramsden CA. Preparation of the I<sub>2</sub> imidazoline receptor antagonist KU14R and related 2,3-dihydrobenzo[b]furan derivatives. *Synthesis* 2001;**10**:1546–1550.
  34. Eglen RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG, Dillon MP. 'Seeing through a glass darkly': Casting light on imidazoline 'I' sites. *Trends Pharmacol Sci* 1998;**19**:381–390.
  35. Efendic S, Efanov AM, Berggren PO, Zaitsev SV. Two generations of insulinotropic imidazoline compounds. *Diabetes* 2002;**5**(Suppl. 3):S448–S454.
  36. Atlas D, Burstein Y. Isolation of an endogenous clonidine-displacing substance from rat brain. *FEBS Lett* 1984;**170**:387–390.
  37. Hensley ML, Meeley MP, McCauley PM, Ernsberger P, Reis DJ. Clonidine-displacing substance is present in peripheral tissues of the rat. *Am J Hypertens* 1989;**2**:917–919.
  38. Meeley MP, Hensley ML, Ernsberger P, Felsen D, Ernsberger DJ. Evidence for a bioactive clonidine-displacing substance in peripheral tissues and serum. *Biochem Pharmacol* 1992;**44**:733–740.
  39. Atlas D. Molecular and physiological properties of clonidine-displacing substance. *Ann N Y Acad Sci* 1995;**763**:314–324.
  40. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine: An endogenous clonidine-displacing substance in the brain. *Science* 1994;**263**:966–969.
  41. Prell GD, Martinelli GP, Holstein GR, et al. Imidazoleacetic acid-ribotide: An endogenous ligand that stimulates imidazol(in)e receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 2004;**101**: 13677–13682.
  42. Halaris A, Piletz J. Agmatine: Metabolic pathway and spectrum of activity in brain. *CNS Drugs* 2007;**21**:885–900.
  43. Wu N, Su RB, Xu B, et al. IRAS, a candidate for I<sub>1</sub>-imidazoline receptor, mediates inhibitory effect of agmatine on cellular morphine dependence. *Biochem Pharmacol* 2005;**70**:1079–1087.
  44. Halaris A, Piletz JE. Relevance of imidazoline receptors and agmatine to psychiatry. A decade of progress. *Ann N Y Acad Sci* 2003;**1009**:1–20.
  45. Chang CH, Wu HT, Cheng KC, Lin HJ, Cheng JT. Increase of  $\beta$ -endorphin secretion by agmatine is induced by activation of imidazoline I<sub>2A</sub> receptors in adrenal gland of rats. *Neurosci Lett* 2010;**468**:297–299.
  46. Husbands SM, Glennon RA, Gorgerat S, et al. Beta-carboline binding to imidazoline receptors. *Drug Alcohol Depend* 2001;**64**:203–208.
  47. Musgrave IF, Badoer E. Harmaline produces hypotension following microinjection into the RVLM: Possible role of I<sub>1</sub>-imidazoline receptors. *Br J Pharmacol* 2000;**129**:1057–1059.
  48. Parker ChA, Anderson NJ, Robinson ESJ, et al. Harmaline and harmalan are bioactive components of classical clonidine-displacing substance. *Biochemistry* 2004;**43**:16385–16392.
  49. Parker CA, Hudson AL, Nutt DJ, et al. Extraction of active clonidine-displacing substance from bovine lung and comparison with clonidine-displacing substance extracted from other tissues. *Eur J Pharmacol* 1999;**378**:213–221.
  50. Lione LA, Nutt DJ, Hudson AL. Characterisation and localisation of [H-3]2-(2-benzofuranyl)-2-imidazoline binding in rat brain: A selective ligand for imidazoline I<sub>2</sub> receptors. *Eur J Pharmacol* 1998;**353**:123–135.
  51. Molderings GJ, Moura D, Fink K, Bonisch H, Gothert M. Binding of [<sup>3</sup>H]clonidine to I<sub>1</sub>-imidazoline sites in bovine adrenal medullary membranes. *Naunyn Schmiedeberg's Arch Pharmacol* 1993;**348**:70–76.
  52. Piletz JE, Andorn AC, Unnerstall JR, Halaris A. Binding of [<sup>3</sup>H]-p-aminoclonidine to  $\alpha_2$ -adrenoceptor states plus a non-adrenergic site on human platelet plasma membranes. *Biochem Pharmacol* 1991;**42**:569–584.
  53. Felsen D, Ernsberger P, Sutaria PM, et al. Identification, localization and functional analysis of imidazoline and alpha adrenergic receptors in canine prostate. *J Pharmacol Exp Ther* 1994;**268**:1063–1071.
  54. Ernsberger P, Shen IH. Membrane localization and guanine nucleotide sensitivity of medullary I<sub>1</sub> imidazoline binding sites. *Neurochem Int* 1997;**30**:17–23.
  55. Separovic D, Kester M, Haxhiu MA, Ernsberger P. Activation of phosphatidylcholine-selective phospholipase C by I<sub>1</sub>-imidazoline receptors in PC12 cells and rostral ventrolateral medulla. *Brain Res* 1997;**749**:335–339.
  56. Ogidigben MJ, Chu TC, Potter DE. Naphazoline-induced suppression of aqueous humor pressure and flow: Involvement of central and peripheral alpha<sub>2</sub>/I<sub>1</sub> receptors. *Exp Eye Res* 2001;**72**:331–339.
  57. Smyth DD, Penner SB. Peripheral and central imidazoline receptor-mediated natriuresis in the rat. *Ann N Y Acad Sci* 1999;**881**:344–357.
  58. Nguyen TT, De Lean A. Nonadrenergic modulation by clonidine of the cosecretion of catecholamines and enkephalins in adrenal chromaffin cells. *Can J Physiol Pharmacol* 1987;**65**:823–827.
  59. Bousquet P, Bruban V, Schann S, Greney H, Ehrhardt JD, Dontenwill M, Feldman J. Participation of imidazoline receptors and alpha<sub>2</sub>-adrenoceptors in the central hypotensive effects of imidazoline-like drugs. *Ann N Y Acad Sci* 1999;**881**:272–278.
  60. Urban R, Szabo B, Starke K. Involvement of alpha<sub>2</sub>-adrenoceptors in the cardiovascular effects of moxonidine. *Eur J Pharmacol* 1995;**282**:19–28.
  61. Feldman J, Tibiric E, Bricca G, Dontenwill M, Belcourt A, Bousquet P. Evidence for the involvement of imidazoline receptors in the central hypotensive effect of rilmenidine in the rabbit. *Br J Pharmacol* 1990;**100**:600–604.
  62. Bruban V, Estato V, Schann S, et al. Evidence for synergy between  $\alpha_2$ -adrenergic and nonadrenergic mechanisms in central blood pressure regulation. *Circulation* 2002;**105**:1116–1121.
  63. Greney H, Urošević D, Schann S, et al. [<sup>125</sup>I]2-(2-chloro-4-iodo-phenylamino)-5-methyl-pyrroline (LNP 911), a high-affinity radioligand selective for I<sub>1</sub> imidazoline receptors. *Mol Pharmacol* 2002;**62**:181–191.
  64. Treder AP, Andruszkiewicz R, Zgoda W, Ford C, Hudson AL. New analogues of agmatine with higher affinity to imidazoline receptors. *Bioorg Med Chem Lett* 2009;**19**:1009–1011.
  65. Gentili F, Bousquet P, Brasili L, et al. Imidazoline binding sites (IBS) profile modulation: Key role of the bridge in determining I<sub>1</sub>-IBS or I<sub>2</sub>-IBS selectivity within a series of 2-phenoxyethylimidazoline analogues. *J Med Chem* 2003;**46**:2169–2176.
  66. Ruffolo RR, Bondinell W, Hieble JP. Alpha- and beta-adrenoceptors: from the gene to the clinic. 2. Structure-activity relationships and therapeutic applications. *J Med Chem* 1995;**38**:3681–3716.
  67. Schann S, Bruban V, Pompermayer K, et al. Synthesis and biological evaluation of pyrrolic isosteres of rilmenidine. Discovery of cis-/trans-dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3H-pyrrol-2-yl)-amine (LNP 509), an I<sub>1</sub> imidazoline receptor selective ligand with hypotensive activity. *J Med Chem* 2001;**44**:1588–1593.
  68. Urošević D, Schann S, Ehrhardt JD, Bousquet P, Greney H. LNP 906, the first high-affinity photoaffinity ligand selective for I<sub>1</sub> imidazoline receptors. *Br J Pharmacol* 2004;**142**: 609–617.
  69. Bruban V, Feldman J, Greney H, et al. Respective contributions of  $\alpha$ -adrenergic and non-adrenergic mechanisms in the hypotensive effect of imidazoline-like drugs. *Br J Pharmacol* 2001;**133**:261–266.
  70. Nikolic K, Filipic S, Agbaba D. QSAR study of imidazoline antihypertensive drugs. *Bioorg Med Chem* 2008;**16**:7134–7140.
  71. Nikolic K, Filipic S, Agbaba D. QSAR study of selective I<sub>1</sub>-imidazoline receptor ligands. *SAR e<sup>t</sup> QSAR Environ Res* 2008;**20**:133–144.
  72. Filipic S, Nikolic K, Krizman M, Agbaba D. The quantitative structure: Retention relationship (QSRR) analysis of some centrally acting antihypertensives and diuretics. *QSAR Comb Sci* 2008;**27**:1036–1044.
  73. Eric S, Pavlovic M, Popovic, G, Agbaba D. Study of retention parameters obtained in RP-TLC system and their application on QSAR/QSPR of some alpha adrenergic and imidazoline receptor ligands. *J Chromatogr Sci* 2007;**45**:140–145.

74. Eric S, Solmajer T, Zupan J, Novic M, Oblak M, Agbaba D. Prediction of selectivity of  $\alpha$ 1-adrenergic antagonists by counterpropagation neural network (CP-ANN). *Farmaco* 2004;**59**:389–395.
75. Eric S, Solmajer T, Zupan J, Novic M, Oblak M, Agbaba D. Quantitative structure-activity relationships of  $\alpha$ 1 adrenergic antagonists. *J Mol Model* 2004;**10**:139–150.
76. Carrieri A, Brasili L, Leonetti F, Pignini M, Giannella M, Bousquet P, Carotti A. 2-D and 3-D modeling of imidazoline receptor ligands: Insights into pharmacophore. *Bioorg Med Chem* 1997;**5**:843–856.
77. Pignini M, Bousquet P, Carotti A, et al. Imidazoline receptors: Qualitative structure-activity relationships and discovery of trazizoline and benzazoline. Two ligands with high affinity and unprecedented selectivity. *Bioorg Med Chem* 1997;**5**:833–841.
78. Gentili F, Bousquet P, Carrieri A, et al. Rational design of the new antihypertensive I<sub>1</sub>-receptor ligand 2-(2-biphenyl-2-yl-1-methyl-ethyl)-4,5-dihydro-1H-imidazole. *Lett Drug Des Discov* 2005;**2**:571–578.
79. Pignini M, Bousquet P, Brasili L, et al. Binding of trazizolines to the imidazoline receptor. Role of lipophilicity in quantitative structure-activity relationship models. *Ann N Y Acad Sci* 1999;**881**:118–122.
80. Nicolotti O, Carotti A, Carrieri A, et al. Pharmacophore development and 3D-QSAR study of I<sub>1</sub> imidazoline binding site ligands. *Med Chem Res* 2004;**13**:170–189.
81. Gentili F, Cardinaletti C, Vesprini C, et al. Novel ligands rationally designed for characterizing I<sub>2</sub>-imidazoline binding sites nature and functions. *J Med Chem* 2008;**51**:5130–5134.
82. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction: Implications for the syndrome of insulin resistance. *J Clin Invest* 1996;**97**:2601–2610.
83. Genissel P, Bromet N, Fourtillan JB, Mignot A, Albin H. Pharmacokinetics of rilmenidine. *Am J Cardiol* 1988;**61**:47D–53D.
84. Valet P, Tran MA, Damase-Michel C, et al. Rilmenidine (S 3341) and the sympatho-adrenal system: Adrenoreceptors, plasma and adrenal catecholamines in dogs. *J Auton Pharmacol* 1988;**8**:319–326.
85. Trenk D, Wagner F, Jahnchen E, Planitz V. Pharmacokinetics of moxonidine after single and repeated daily doses in healthy volunteers. *J Clin Pharmacol* 1987;**27**:988–993.
86. Kirch W, Hutt HJ, Planitz V. Pharmacodynamic action and pharmacokinetics of moxonidine after single oral administration in hypertensive patients. *J Clin Pharmacol* 1990;**30**:1088–1095.
87. Mitrovic V, Patyna W, Huting J, Schlepfer M. Hemodynamic and neurohumoral effects of moxonidine in patients with essential hypertension. *Cardiovasc Drugs Ther* 1991;**5**:967–972.
88. Trimarco B, Rosiello G, Sarno D, Lorino G, Rubattu S, Deluca N, Volpe M. Effects of one-year treatment with rilmenidine on systemic hypertension- induced left ventricular hypertrophy in hypertensive patients. *Am J Cardiol* 1994;**74**:36A–42A.
89. Ollivier JP, Christen MO. I1-imidazoline-receptor agonists in the treatment of hypertension: An appraisal of clinical experience. *J Cardiovasc Pharmacol* 1994;**24**:S39–S48.
90. Sadowski Z. Regression of left ventricular hypertrophy in hypertensive patients after 1 year of treatment with rilmenidine: A double-blind, randomized, controlled (versus nifedipine) study. *J Hypertens Suppl* 1998;**16**:S55–S62.
91. Koldas L, Ayan F, Ikitimur B. Short term effects of rilmenidine on left ventricular hypertrophy and systolic and diastolic function in patients with essential hypertension: Comparison with an angiotensin converting enzyme inhibitor and a calcium antagonist. *Jpn Heart J* 2003;**44**:693–704.
92. Kline RL, Cechetto DF. Renal effects of rilmenidine in anaesthetized rats: importance of renal nerves. *J Pharmacol Exp Ther* 1993;**266**:1556–1562.
93. Chan SLF, Brown CA, Scarpello KE, Morgan NG. The imidazoline site involved in control of insulin secretion: Characteristics that distinguish it from I1 and I2 sites. *Br J Pharmacol* 1994;**112**:1065–1070.
94. Plant TD, Henquin JC. Phentolamine and yohimbine inhibit ATPsensitive K<sup>+</sup> channels in mouse pancreatic  $\beta$ -cells. *Br J Pharmacol* 1990;**101**:115–120.
95. Chan SLF, Dunne MJ, Stillings MR, Morgan NG. The  $\alpha$ <sub>2</sub>-adrenoceptor antagonist efaroxan modulates K<sup>+</sup> ATP channels in insulinsecreting cells. *Eur J Pharmacol* 1991;**204**:41–48.
96. Chan SLF, Brown CA, Scarpello KE, Morgan NG. Pancreatic  $\beta$ -cells express an imidazoline binding site that is distinct from I<sub>1</sub> and I<sub>2</sub> sites. *Ann N Y Acad Sci* 1995;**763**:153–156.
97. Fenton C, Keating GM, Lyseng-Williamson KA. Moxonidine: A review of its use in essential hypertension. *Drugs* 2006;**66**:477–496.