REVIEW

Cardiovascular Therapeutics

Imidazoline Antihypertensive Drugs: Selective I_1-Imidazoline Receptors Activation

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Keywords

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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_1-imidazoline receptor which is involved in central inhibition of sympathetic tone to lower blood pressure, the I_2-imidazoline receptor which is an allosteric binding site of monoamine oxidase B (MAO-B), and the I_3-imidazoline receptor which regulates insulin secretion from pancreatic β-cells. All three imidazoline receptors represent important targets for cardiovascular research. The hypotensive effect of clonidine-like centrally acting antihypertensives was attributed both to α_2-adrenergic receptors and nonadrenergic I_1-imidazoline receptors, whereas their sedative action involves activation of only α_2-adrenergic receptors located in the locus coeruleus. Since more selective I_1-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_1-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 α_2-adrenenoceptors (α_2-AR) but also with imidazoline receptors (imidazoline binding sites [IBSs]). The IBSs were pharmacologically distinct from the α_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 α_2-adrenoceptors [2,3].

In the present study, we seek to distinguish the ligand selectivity profile of I_1-IBS relative to other binding sites for imidazolines, demonstrate the specific activation of transmembrane signaling pathways by I_1-imidazoline receptors and review the diverse and complex function of I_1-imidazoline receptors ligands.

Specific imidazoline receptors, I_1-imidazoline receptors (I_1-IR), I_2-imidazoline receptors (I_2-IR), and I_3-imidazoline receptors (I_3-IR), have been characterized by extensive biochemical and physiological studies [4,5] and highly selective novel imidazoline agents have been developed without α_2-AR activity [6].

Imidazoline I_1-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 α_2-AR and I_1-IRs in the RVLM [3,7]. The α_2-AR agonists directly inhibit presympathetic RVLM neurons, while the I_1-IR agonists increase the release of catecholamines in the RVLM. The catecholamines depress presympathetic RVLM neurons by activating α_2-AR [3,7]. Compared with clonidine, newer centrally acting antihypertensive drugs such as rilmenidine and moxonidine are more selective for I_1-imidazoline receptors than for α_2-adrenergic receptors. Rilmenidine and moxonidine cause only few α_2-adrenoceptor-mediated side effects because they posses better selectivity for I_1-IRs.

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

were determined [8]. Clonidine, moxonidine, and rilmenidine failed to significantly decrease mean arterial pressure in α_{2ABC}-deficient mice [8]. These findings have further confirmed that activation of α_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_1-imidazoline receptors rather than α_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_1-IR protein structures have not yet been solved to date. Only imidazoline receptor antisera-selected (IRAS-1) gene candidate for the I_1-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_1 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_1R (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 coincidently activated receptors in the same cell, including the α_{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_1-ligand—IRAS-1 complexes require special experimental procedure.

The I_1-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_1 receptors are preferentially bound by 2-aminoimidazolines ([^{3}H]-clonidine), while show medium affinity for imidazolines ([^{3}H]-idazoxan), and low affinity for guanidines (amiloride) [19–21].

The I_2 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_2-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_2 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_2 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_2-receptor subtypes, I_{2A} and I_{2B}, depending on their affinity for guanidine derivative-amiloride [26].

Some previous studies reported experimental data that pancreatic imidazoline receptors and sympathetic presynaptic imidazoline receptors did not fulfill the criteria for the definition of I_1 or I_2 receptors. Such sites were initially called non-I_1, non-I_2 receptors [30,31] and later were defined as I_3 receptors [32,33]. The I_3 receptors in pancreatic β-cells are involved in regulation of insulin secretion in a manner, which is not typical of I_1- or I_2-receptor related phenomenon [30,32–35]. The imidazoline efaroxan is a selective agonist at the I_3 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 α_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_1-, I_2-, and I_3-imidazoline receptors.

I_1-imidazoline receptors ligands will be compared in function of their chemical structure, binding affinity, and selectivity for the I_1-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 β-carboline compounds (Figure 1), can bind with high affinity to imidazoline sites and to α_2-adrenoceptors [39]. Agmatine possesses the common amidine motif and can accordingly displace specific radioligand binding from α_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 α_2-adrenoceptors as well as to I_1- and I_2-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_{2A} 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_1-IRs and I_3-IRs in adrenal medulla and in pancreatic tissue [41].

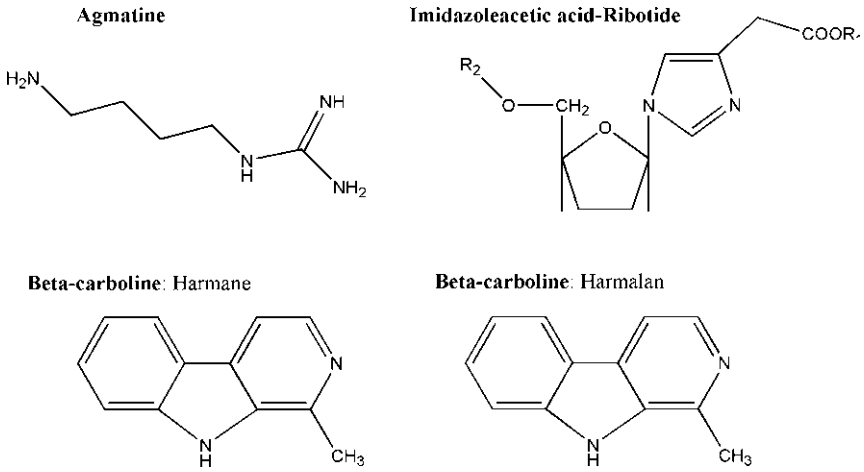

Figure 1 Chemical structures of endogenous ligands: Agmatine, Imidazoleacetic acid-Ribotide, Harmane, and Harmalan.

Also, β-carboline compounds, such as harmane [46,47] and harmalan [48], have been proposed as putative endogenous substrates of either I_1- or I_2-imidazoline receptors. These compounds have been shown that 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_1-IR, I_2-IR, and α_2-adrenoceptors on rat brain and kidney membranes [49,50] and binding affinities at I_2-IR on human placenta and rat liver membranes [39].

I_1-Imidazoline Receptor Ligands

The I_1 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_1-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_1-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_1-imidazoline receptor ligands are able to dose dependently decrease forskolin-stimulated cAMP content in cells expressing only I_1-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 field 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_1-imidazoline receptors.

On the other hand, pharmacological studies have shown that I_1-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_1-imidazoline receptor ligands with mixed (I_1/α_2) binding profiles has been in significant correlation with their affinity for I_1-IRs [2,3]. Imidazoline antagonists such as idazoxan competitively antagonized the centrally induced hypotensive effect of the I_1-IRs ligands, while yohimbine, a α_2-ARs antagonist, blocks the hypotensive effect of the ligands but usually in a noncompetitive manner [59]. The hypotensive effects of more selective I_1-imidazoline receptor ligands, such as rilmenidine and moxonidine, was clearly prevented by idazoxan, an imidazoline I_1-IR antagonist, whereas it was very weakly antagonized by antagonist for the α_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 α_2-adrenoceptors using α-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_1-IRs located on the noradrenergic (NA) cell terminals, or from the stimulation of terminal α_{2A}-adrenoceptors located on the interneuron containing γ-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_1-imidazoline receptors alone is sufficient to inhibit vasomotor tone and therefore to reduce blood pressure.
- I_1-imidazoline receptors are involved in the hypotensive effects of I_1-IR ligands.

- The integrity of the α_2-adrenoceptors included in the sympathetic centers and pathways appears to be required for the development of the hypotensive effects of at least α_2-adrenergic agonists.
- A cooperative interaction between imidazoline receptors and α_2-adrenoceptors seems to account for the marked and rapid hypotensive effect caused by I_1-IR ligands [59–62].

Many different families of I_1-imidazoline receptors ligands, such as guanidine derivatives, imidazoline compounds, endogenous amines, and carbolines have been synthesized and examined for I_1-, I_2-, α_{2A}-, α_{2B}-, α_{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/α_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_1-IR, such as [^{3}H] clonidine and [^{125}I] *p*-iodoclonidine [51,52], were able to bind with similar affinities to α_2-ARs and to I_1-IR [66]. Therefore adequate characterization of the I_1-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_1-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_1-IR agonists [68,69]. Finally, LNP 911 is not able to dose dependently decrease forskolin-stimulated cAMP content in cells expressing only I_1-imidazoline receptors, but it behaves as an allosteric enhancer [63]. The LNP 911 was radioiodinated 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_1-IRs binding affinities of the ligands obtained with different radioligands (such as [^{125}I] *p*-iodoclonidine ([^{125}I] PIC) and [^{125}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_1-IR ligands (clonidine; lofexidine; BDF 6143; rilmenidine; *p*-iodoclonidine; LNP 911) on [^{125}I] LNP 911 binding sites and the pKi of the same drugs on [^{125}I] PIC binding sites [63], together with the fact that PIC and LNP 911 were able to displace the same amount of [^{125}I] LNP 911 total binding [63] indicate that identical I_1-binding sites are concerned.

Because moxonidine, efaroxan, and benazoline were described as high-affinity I_1-IRs ligands in different models [17,18] but displaced [^{125}I] LNP 911 with rather low affinity, relationships between the ligands and [^{125}I] LNP 911 were studied in details [63]. These analyses have found out that I_1-IRs are submitted to a complex allosteric modulation by the use of LNP 911. Actually, the LNP 911 behaves as an allosteric enhancer and potentates the effects obtained with I_1-IR agonists [63].

The lack of a clearly identified molecular structure for the I_1-IR [10–12] and modulatory role for imidazoline receptors on coincidently activated α_{2A}-adrenergic and fibronectin receptors in the same cell [13] are making virtual docking study very difficult. Furthermore, importance of I_1-IR selective ligands being available for structural, functional, and pharmacological investigations on I_1-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_1-IR affinity and on the key molecular determinants of I_1-IR/α_2-AR and I_1/I_2-IR selectivity. The results from the modeling studies can be used for the rational design of new, more selective, and high affinity I_1-IR ligands.

The developed QSAR models for I_1-IR ligands (Figure 2) have indicated that an increase in lipophilicity ($logD_{pH7.4}$), molar refractivity, and dipole moment value, together with a decrease in N-charge in the heterocyclic moiety influence on better affinity for I_1 receptors [70,71]. Furthermore, highest occupied molecular orbital energy and lipophilicity (ClogP) of the ligands are important parameters for evaluation of I_1/α_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_1 and I_2 affinity. In agreement with the 2D-QSAR study, the combination of the steric and electrostatic field (for affinity at I_1-IR) and the steric field in combination with the lipophilic one (for affinity at I_2-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_1-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_2-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_2-IBS affinity [78,81]. Furthermore, the unsubstituted ethylenic bridge, between the aromatic portion and imidazoline nucleus of the 2-phenoxymethylimidazoline analogs, is proved to be determinant in inducing high I_2-IR selectivity with regard to I_1-IR and α_2-ARs [65,81].

Pharmacological Effects of I_1-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 (Ki) for ligands in competition binding studies with [^{125}I] LNP 911 on PC12 cells [64], [^{125}I] PIC on PC12 cells [18,64], and [^{125}I] PIC at human platelet I_1-IR [15]

Compound	K_i (I_1-IR) [nM] with [^{125}I]LNP 911 on PC12 cells [64]	K_i (I_1-IR) [nM] with [^{125}I]PIC on PC12 cells [18,64]	K_i(I_1-IR) [nM] with [^{125}I]PIC at human platelet I_1-IR [15]
LNP 911	1.4 ± 1.7	0.3 [64]	
p-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]	
p-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

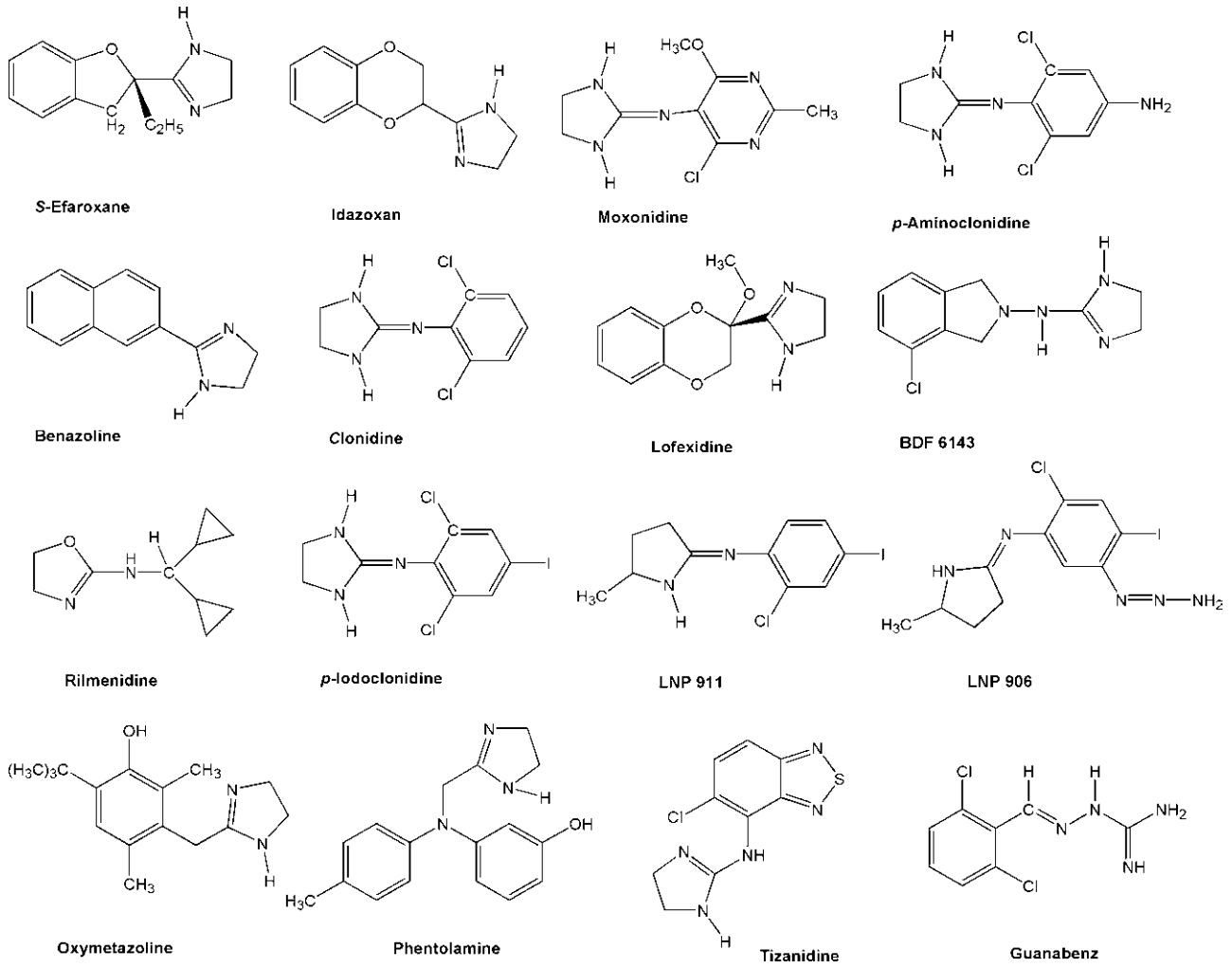

Figure 2 Chemical structures of I_1-IR ligands.

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 α_2-adrenoceptors in the pancreas reduced insulin secretion and increase glucagon release, while I_1-IR activation had the opposite effect. Thus selective I_1-IR agonists are capable of increasing the glucose-induced insulin secretion from pancreatic β-cells [93–96]. Furthermore, moxonidine improves the metabolic profile in patients with hypertension and diabetes mellitus or impaired glucose tolerance [97].

Conclusion

The low incidence of the side effects, antiarrhythmic effects, and beneficial metabolic and renal effects of second-generation I_1-IR ligands suggest that they may provide a very useful therapy, which may be further enhanced by the development of more selective I_1-IR agents.

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Conflict of Interest

The authors have no conflict of interest

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