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Pharmacophore Development and SAR Studies of Imidazoline Receptor Ligands

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Abstract: Relationship between biological responses and binding affinities at $I_1/I_2/I_3$ imidazoline receptors of compounds with imidazoline, pyrroline or oxazoline moieties was studied by 2D-QSAR, 3D-QSAR and quantitative pharmacophore development approaches. Since the I_1 imidazoline receptor is involved in central inhibition of sympathicus that produce hypotensive effect, the I_2 receptor is allosteric modulator of monoamine oxidase B (MAO-B) and the I_3 receptor regulates insulin secretion from pancreatic β-cells, design and synthesis of selective $I_1/I_2/I_3$ imidazoline ligands are very important for the development of new effective therapeutic agents. New agonists and antagonists with high selectivity for $I_1/I_2/I_3$ imidazoline receptor classes have been recently synthesized and examined. The present review will highlight the main chemical diversity and pharmacophore features of selective $I_1/I_2/I_3$ imidazoline receptor ligands.

Keywords: I1-imidazoline receptors, I2-imidazoline receptors, I3-imidazoline receptors.

INTRODUCTION

The imidazoline receptor research started with the discovery of hypotensive effect of the imidazoline derivative clonidine, which action was shown to be mediated by a central inhibition of sympathetic tone [1, 2]. The imidazoline receptor hypothesis was initiated with the evidence that the antihypertensive action of clonidine and other imidazoline derivatives was the result of their interaction with imidazoline receptors (IR) rather than with α_2-adrenoceptors (α_2-AR) in Rostral Ventrolateral Medulla (RVLM) [1, 3, 4].

Many studies have investigated close interaction and interdependence of I-IR and the α_2-AR at the cellular level [3, 5-8]. The imidazoline receptors were pharmacologically distinct from the α_2-AR because they were not activated by catecholamines [4]. The ability of the various imidazoline derivatives and related compounds to bind to the I-IR and the α_2-AR indicated that both receptor systems present considerable analogies in the orientation of their critical binding functions with different steric hindrance of the binding pockets. Therefore, the ligands with some modest structural modifications can display altered affinity and selectivity for the I-IR and the α_2-AR [9].

Extensive biochemical and physiological studies have determined three different subtypes of imidazoline receptors [10, 11]: I_1-imidazoline receptors (I_1-IR), I_2- imidazoline receptors (I_2-IR), and I_3-imidazoline receptors (I_3-IR).

The central hypotensive effect of the imidazoline derivatives, such as clonidine, rilmenidine and moxonidine, is the result of activation of both I_1-IR and α_2-AR in RVLM [5, 12-14]. Positive correlation between the hypotensive potency of imidazoline compounds and their affinity for I_1-IR but not for α_2-AR, was observed [5, 15]. It has been adequately demonstrated that some of their side effects, such as sedation and dry mouth, are mediated by α_2-adrenergic receptors in locus coeruleus [16, 17]. Newly developed centrally acting antihypertensives, such as rilmenidine and moxonidine, have expressed higher I_1-IR/α_2-AR selectivity and therefore cause lesser side effects than clonidine [5, 14, 18].

Moreover, it is demonstrated that the α_{2A}-AR are involved in the central hypotensive effect of clonidine-like drugs [19], while moxonidine was still able to reduce blood pressure in strain of transgenic mice with nonfunctional α_{2A}-AR [20]. All these results demonstrated that close interaction between I_1-IR and α_{2A}-AR might underlie the mechanism of the hypotensive effects of imidazoline ligands binding both to α_{2A}-adrenoceptors and I_1-IR [20].

Highly selective I_1-IR ligands, which only bind to I_1-IR and specifically avoiding α_2-AR, are potentially useful in the studies of the imidazoline receptor system, in the examination of functional effects of stimulation of particular I_1-, I_2-, and I_3-imidazoline receptors, and in the exploration of potential new therapeutic agents that rely exclusively on I_1-IR. The newly synthesized highly selective I_1-IR ligands, such as S23515 [21], S23757 [21], LNP509 [22], LNP 906 [23], and LNP911 [24], are good models for investigation of the I_1-imidazoline receptor systems.

The binding studies on the I_1-IR were performed with tritiated clonidine or iodinated paraiodoclonidine in the bovine brainstem membranes [25-27], human brainstem membranes [28], plasma membrane of bovine chromaffin cells [29], plasma membrane of rat pheochromocytoma cells (PC 12) [23, 24, 30-33], plasma membrane of human platelets [34], plasma membrane of canine prostate [35], and adrenal medullary plasma membranes [21, 22].

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Activation of phosphatidylcholine-sensitive phospholipase C (PC-PLC) [31, 36, 37] and inhibition of adenylcyclase [32] are two main signal pathways associated with I_1-IR activation. Therefore, particular I_1-IR ligands can act as agonists on both main pathways (agonists such as rilmenidine, benazoline and moxonidine), as agonist for the cAMP pathway and antagonists on the PC-PLC pathway (partial agonists such as efaroxan and BDF-6143) [31, 32, 36] or as antagonists for both main pathways (antagonists such as S23757 [21], LNP 906 [23], and LNP911 [24]). Since *in vivo* pharmacological effects of the I_1-IR agonists, I_1-IR-partial agonists and I_1-IR antagonists are very diverse, further studies need to define pharmacophores responsible for I_1-IR agonistic activity, select pharmacophores related to I_1-IR antagonistic activity and develop specific Quantitative Structure Activity Relationship (QSAR) models for prediction of I_1-IR agonistic and I_1-IR antagonistic activities.

Also, activation of the I_1-IR can induce transduction pathways such as: increase in phosphorylation of Mitogen-Activated Protein Kinases (MAPK1 and MAPK3) in RVLM neurons that produce hypotension [38]; activation of Protein Kinase C isoforms (PKC) [39]; activation of Extracellular Signal Regulated Kinase (ERK1 and ERK2) and c-jun Kinases [40, 41]; release of arachidonic acid and prostaglandin E2 [21, 41]; and increasing expression of phosphatase mitogen-activated protein kinase phosphatase 2 [40], and increasing expression of phenylethanolamine-N-methyl transferase [41].

The I_1-IR protein structures have not yet been solved to date, but the Imidazoline Receptor Antisera-Selected (IRAS) gene candidate for the I_1-IR protein has been cloned [42]. Transfection of human IRAS cDNA into rat pheochromocytoma cells (PC 12) and Chinese Hamster Ovary (CHO) cells resulted in expression of high-affinity I_1-receptor binding sites [42, 43]. The human IRAS protein, named nischarin, was also identified and cloned [42, 44, 45].

Recent findings indicate that the I_1-imidazoline receptors mediating effects of clonidine and moxonidine in PC12 and the transfected HEK293 cells belong to the S1P-receptor (sphingosine-1-phosphate) family [46]. Actually, the results obtained in PC12 cells suggest that the I1 imidazoline receptors represent a mixture of S1P1- and S1P3-receptors [46].

The expression level of nischarin is critical for retaining the binding of imidazoline ligands on I_1-IR and the initiation of I_1-IR associated cell-signaling pathways, such as activation of PC-PLC and ERK phosphorylation [45, 47-49]. Therefore, the nischarin may be the I_1-IR itself, functional subunit of I_1-IR, or membrane-associated mediator of the I_1-IR signaling [45, 47-49].

The nischarin also has an important role in cell signaling and function of the cytoskeleton regulated by Rho-family GTPase [42, 45]; initiation of cell-signaling cascades triggering to cell survival, growth, migration, and apoptosis [42, 45, 50]; suppression of Rac1-stimulated cell migration by interacting with PAK1 [50]; and inhibition of Rac1-stimulated NF-kB response element and cyclin D1 promoter activation [42, 45, 50].

Very recent study of breast cancer progression, has determined nischarine as a novel potent tumor suppressor [51].

Since the 3D-structure of the I_1-IR protein is not determined, virtual docking study on the I_1-IR is very difficult. Therefore, the Quantitative Structure Activity Relationship (QSAR) studies and the quantitative pharmacophore development approaches for I_1-IR ligands are very helpful tools in design and discovery of novel potent and selective I_1-IR ligands, as drug candidates with various pharmacological activities.

I_1-imidazoline receptors are preferentially bound by 2-aminoimidazolines ([^{3}H]-clonidine), whereas show medium affinity for imidazolines ([^{3}H]-idazoxan) and low affinity for guanidines (amiloride) [26, 52, 53].

I_2-imidazoline receptors (I_2-IR) are characterized by their high affinity for guanidines and imidazolines ([^{3}H]-idazoxan), and low affinity for 2-aminoimidazolines [54]. Existence of I_{2A} and I_{2B}-imidazoline receptor subtypes, depending on their affinity for amiloride, was determined by detailed studies [54]. The I_2-receptors are allosteric binding site associated with the catalytic site of monoamine oxidase (MAO) and other non-MAO oxidative enzymes [55]. Sequence of I_2 imidazoline-binding proteins expressed some homology with that of the MAO enzyme [56, 57].

Recent findings that brain creatine kinase (B-CK) possesses an I_2 imidazoline-binding site will greatly contribute to understanding of the effects of I_2 ligands in psychiatric disorders and to developing novel therapeutic drugs [58].

Although the I_2-IR has not been cloned, understanding of these receptors has been achieved by use of selective I_2-IR ligands. The I_2-imidazoline receptors have been associated with various neurobiological conditions, including depression [59, 60], hyperphagia [61, 62] and analgesia [63, 64]. The I_2-IRs are manly positioned in glia cells [65], blood platelets, liver, and adipocytes.

Induction of insulin secretion from pancreatic β-cells by imidazoline ligands are tentatively assigned to imidazoline I_3 imidazoline receptors [66, 67]. The precise mechanisms of I_3-IR modulated insulin secretion and glucose homeostasis remain unknown. The imidazoline efaroxan is defined as selective agonist at the I_3-IR and its imidazole analog (KU14R) is determined as an antagonist [68].

Pharmacological effects of imidazoline receptors activation have been described by several reviews [10, 13, 41, 60].

Design and synthesis of novel selective I_1-IR, I_2-IR, and I_3-IR agents will provide the discovery of new drugs with reduced incidence of side effects, comprehensive examination of effects associated with stimulation of particular I_1-, I_2-, and I_3-imidazoline receptors, and determination of the I-IR protein structures. Since new agonists and antagonists with high selectivity for $I_1/I_2/I_3$ imidazoline receptor classes have recently been synthesized and examined, the present review will highlight main chemical diversity and pharmacophore features of selective $I_1/I_2/I_3$ imidazoline receptor ligands,

classify them in function of their chemical structure and binding affinity and selectivity for the different I-IR subtypes.

ENDOGENOUS LIGANDS

The naturally occurring imidazoline receptors ligands may have an improved selectivity profile and therefore could be good molecular models for the development of new agents. Initially, agmatine [69] and imidazole-4-acetic acid ribotide (IAA-RP) [70] have been reported as endogenous ligands for the imidazoline receptors.

The agmatine binds with a moderate affinity on α_2-AR, I_1-IR and I_2-IR [71]. The agmatine acts as an endogenous antagonist or inverse agonist at imidazoline receptors [71]. The imidazole-4-acetic acid-ribotide (IAA-RP) is endogenous agonist for I_1-IR in adrenal medulla and I_3-IR in pancreatic tissue [70].

Also, harmane [72, 73] and harmalan [74] have been shown to act as endogenous ligands at I_1-IR and I_2-IR and have central effects on blood pressure similar to that of the clonidine [73].

I_1-IMIDAZOLINE RECEPTOR LIGANDS

The I_1 imidazoline receptors can be labeled by [^{3}H]-clonidine and [^{3}H]-idazoxan, while the I_2-IR can only be labeled by [^{3}H]-idazoxan. Since the non-adrenergic I_1-IR are involved in the hypotensive action of imidazoline drugs, the I_1-IR are assigned as therapeutic targets for antihypertensives.

Adequate characterization of the I_1-IR subtype is very difficult because of low selectivity of the I_1-IR radioligands, such as [^{3}H] clonidine and [^{125}I] *p*-iodoclonidine (PIC) [25, 29]. Discovery of new highly selective I_1-IR ligands, such as cis-/*trans*-dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3H-pyrrol-2-yl)-amine (LNP-509) [22], 2-(2-chloro-4-iodo-phenylamino)-5-methyl-pyrroline (LNP 911) [24], (2-(5-azido-2-chloro-4-iodo-phenylamino)-5-methyl-pyrroline (LNP 906) [23], (+)-5-(2-bromophenoxy)-methyl-2-amino-4,5-dihydro-1,3-oxazole (S23515) [21], (+)-2-(2-fluoro-5-methylphenyl)-4,5-dihydro-1H-imidazole (S23757) [21], allows the characterization of I_1-IR subtype even in cells expressing also α_2-AR or I_2-IR.

Since different I_1-IR binding affinities were obtained with [^{125}I] PIC) and [^{125}I] LNP 911 radioligands on PC 12 cells [24, 32] and human platelets [75] for the same compounds, results of the various competition binding studies couldn't be directly compared [76]. The correlation between the p*Ki* 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 p*Ki* of the same ligands on [^{125}I] PIC binding sites was observed [24].

Many different families of I_1-IR ligands, such as 2-aminoimidazolines, 2-aminooxazolines, aminopyrrolines, 2-arylimidazolines, 2-phenylimidazolines, 2-imidazolines, have been synthesized and examined for I_1-IR, I_2-IR, α_{2A}-AR, α_{2B}-AR, α_{2C}-AR binding affinities [21-24, 32, 33, 77-79] (Fig. (**1**) and Table **1**).

The reported binding affinities of the compounds relate to rabbit kidney preparations for I_2-IR and rat calf cerebral cortex for a α_2-AR.

Clonidine (Fig. (**1**), compound **16**) was the first identified I_1-IR ligand showing antihypertensive activity. Clonidine displayed higher affinity towards the I_1-IR and α_2-AR, than towards the I_2-IR (Table **1**). The *p*-iodoclonidine (Fig. (**1**), compound **29**) showed increased affinity and selectivity for I_1-IR in regards to the I_2-IR, but retained high potency of binding to α_2-AR (Table **1**). Further changes in the structure of clonidine yielded moxonidine and rilmenidine (Fig. (**1**), compounds 17 and 20), as potent and selective I_1-IR ligands in relation to the I_2-IR and t α_2-AR (Table **1**).

New family of aminopyrrolines analogs of rilmenidine (Fig. (**1**), compounds **1**, **2**, **6**, **8**, **10**, **11**, and **14**) was prepared and assayed at I_1-IR, I_2-IR and α_2-AR (Table **1**) [22]. The isosteric substitution of rilmenidine with a pyrrolinic ring eliminated the binding affinity to α_2-AR while I_1-IR affinity was little decreased.

The benazoline (Fig. (**1**), compound **27**) is very potent I-IR ligand able to activate both the I_1-IR and I_2-IR in a highly selective way towards α_1-AR and α_2-AR [32].

Inspired by benazoline structure, 2-phenylimidazolne derivatives were synthesized and assayed at I_1-IR, I_2-IR, α_1-AR and α_2-AR [77]. Several of these compounds were potent and selective I_1-IR ligands. For instance, the 2'-methoxyphenyl-2-imidazoline (Fig. (**1**), compound **28**) expressed very high I_1-IR affinity and selectivity toward I_2-IR, α_1-AR and α_2-AR (Table **1**) [77]. Thus, it was concluded that the I_1-imidazolne receptors bind preferably with 2-phenyl-imidazolines substituted with a methoxy or a methyl group in the 2' or 3' positions [77].

Significant increase in selectivity for I_1-IR towards I_2-IR was obtained by replacing the ethylene bridge of the phenyl-ethylene-imidazoline derivatives (Fig. (**1**), compound **5**) by a cyclopropane ring (Fig. (**1**), (1S,2S)-(+) isomer-compound **21**) [33]. Actually, the (1S,2S)-(+) isomer (Fig. (**1**), compound **21**) was more active at the I_1-IR, while the (1R,2R)-(-) isomer (Fig. (**1**), compound **12**) was more potent for the I_2-IR (Table **1**). Also, two enantiomers of the α-methyl derivative of the phenylethylen-2-imidazoline (Fig. (**1**), compounds **4** and **28**) were separated and examined. The (*S*)-(-) isomer (Fig. (**1**), compound **28**) expressed a high I_1-IR activity and I_1-IR/α_2-AR and I_1/I_2-IR selectivity, while the (*R*)-(+) enantiomer (Fig. (**1**), compound **4**) bound to the I-IR and α_2-AR with very low affinity (Table **1**). Observed stronger activity of one optical isomer of the compounds at one I-IR subtype indicated on some stereospecific requirements of the I_1-IR and I_2-IR [33].

Also, new family of I_1-IR ligands, 2-aminoimidazolines, was created by compilation of agmatine structure and imidazoline ring (Fig. (**2**)). The guanidine moiety, included into heterocyclic ring of the compounds, improves the I_1-IR affinities of the resultant new molecules [79].

Unsolved structure of the I_1-IR protein is making virtual docking study very difficult. Thus, QSAR studies and the quantitative pharmacophore development approaches for

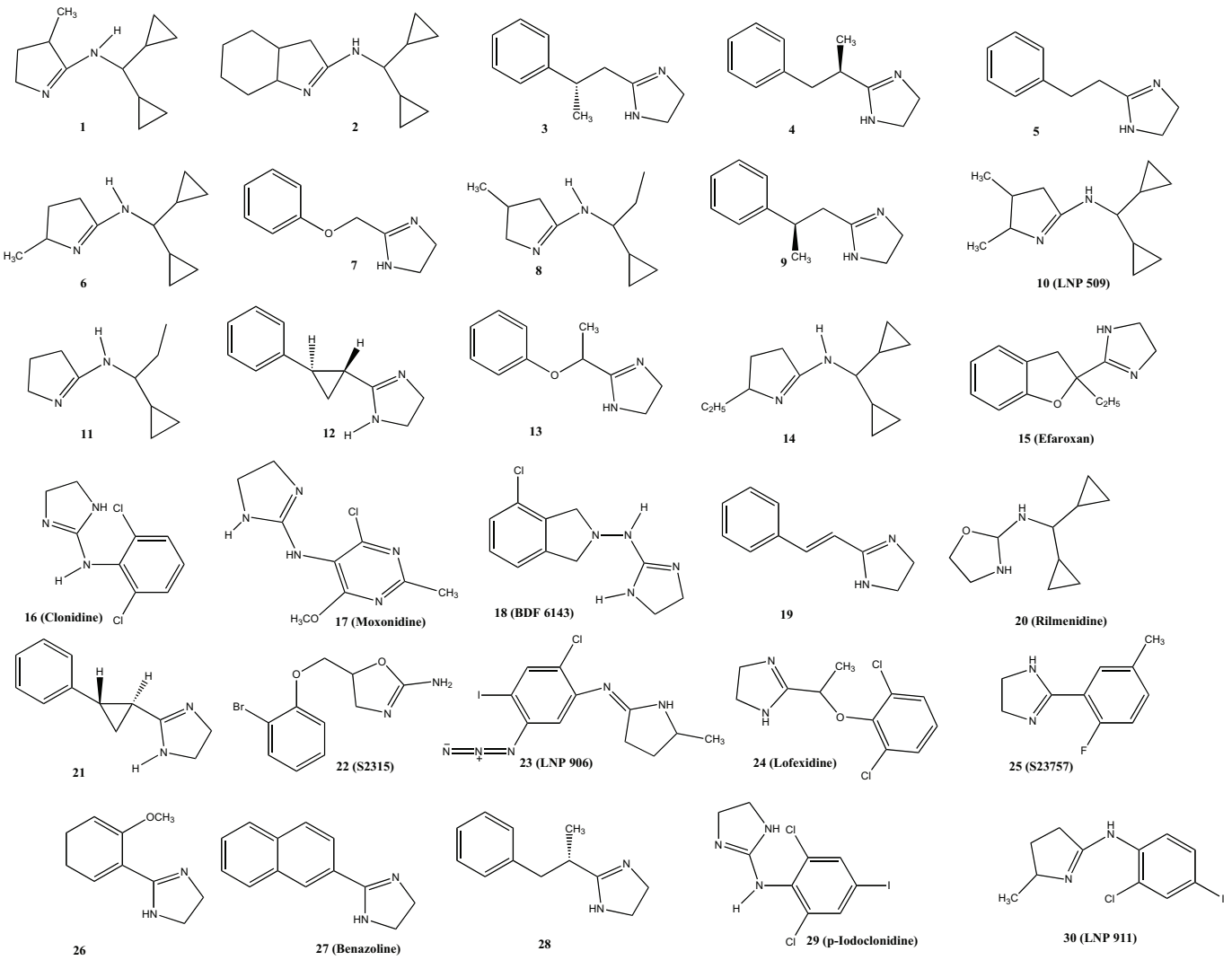
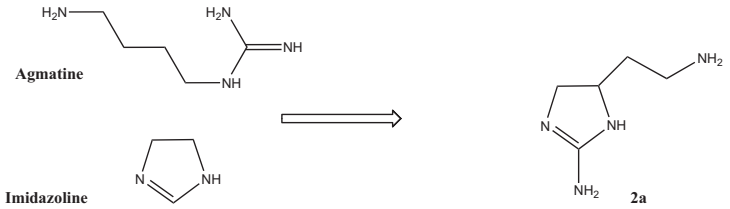


Fig. (1). Structural formulas of I_1-IR ligands.



Compounds	I_1 IC_{50} (nM)	I_2 Ki (nM)	α_2 Ki (nM)
2a (*S*)	477	54 950	11 770
Clonidine	366,2	364,5	8,7
Agmatine	36 532	416 700	31 700

Fig. (2). Chemical structure of compound formed by compilation of agmatine and imidazoline ring.

I_1-IR ligands are optimal tools in design and discovery of novel potent and selective I_1-IR ligands [78, 80-94]. The new ligands can be used for structural, functional and pharmacological investigations on I_1-IR and examined for various pharmacological activities.

Table 1. The I_1-IR, I_2-IR and α_2-AR Binding Affinities (p*Ki* = log(1/*Ki*)) of I_1-IR ligands.

Compounds	p*Ki* (I_1-IR)	I_1-IR Study: Radioligand Used, Membrane/Cell	p*Ki* (I_2-IR)	p*Ki* (α_2-AR)
1	4.00 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
2	4.00 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
3	5.14 [33]	[^{125}I] PIC, PC 12	7.00 [33]	5.80 [33]
4	5.20 [33]	[^{125}I] PIC, PC 12	4.90 [33]	5.40 [33]
5	5.43 [33]	[^{125}I] PIC, PC 12	8.60 [33]	5.70 [33]
6	5.80 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
7	6.15 [33]	[^{125}I] PIC, PC 12	9.05 [33]	7.28 [33]
8	6.19 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
9	6.23 [33]	[^{125}I] PIC, PC 12	5.60 [33]	5.90 [33]
10-LNP509	6.27 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
11	6.29 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
12	6.46 [33]	[^{125}I] PIC, PC 12	8.22 [33]	6.92 [33]
13	6.51 [33]	[^{125}I] PIC, PC 12	5.75 [33]	7.01 [33]
14	6.77 [22]	[^{3}H] clonidine, bovine chromaffin cells	<5 [22]	<5 [22]
15-Efaroxan	6.84 [32]	[^{125}I] PIC, PC 12	-	8.01 α_{2A}-AR, 8.00 α_{2B}-AR, 8.01 α_{2C}-AR [75]
16-Clonidine	6.90 [32]	[^{125}I] PIC, PC 12	6.02 [67]	8.06 α_{2A}-AR, 7.50 α_{2B}-AR, 8.03 α_{2C}-AR [75]
17-Moxonidine	7.47 [32]	[^{125}I] PIC, PC 12	<5 [32]	5.44 α_{2A}-AR, 5.59 α_{2B}-AR, 5.03 α_{2C}-AR [75]
18-BDF 6143	7.55 [32]	[^{125}I] PIC, PC 12	-	8.55 α_{2A}-AR, 8.31 α_{2B}-AR, 8.96 α_{2C}-AR [75]
19	7.72 [33]	[^{125}I] PIC, PC 12	8.72 [33]	4.85 [33]
20-Rilmenidine	7.90 [24]	[^{125}I] PIC, PC 12	<5 [22]	7.44 α_{2A}-AR, 7.37 α_{2B}-AR, 7.90 α_{2C}-AR [75]
21	7.93 [33]	[^{125}I] PIC, PC 12	6.91 [33]	6.62 [33]
22-S23515	8.19 [21]	[^{3}H] clonidine, adrenal medullary plasma membranes	<4 [21]	6.39 [21]
23-LNP 906	8.22 [23]	[^{125}I] PIC, PC 12	3.88 [23]	5.65 α_{2A}-AR, 5.43 α_{2B}-AR, 5.08 α_{2C}-AR [75]
24-Lofexidine	8.25 [24]	[^{125}I] PIC, PC 12	-	-
25-S23757	8.28 [21]	[^{3}H] clonidine, adrenal medullary plasma membranes	<4 [21]	8.21 [21]
26	8.53 [77]	[^{3}H] clonidine, adrenal medullary plasma membranes	5 [77]	<5 [77]
27-Benazoline	8.89 [32]	[^{125}I] PIC, PC 12	9.07 [92]	5.45 α_{2A}-AR [32]
28	8.97 [33]	[^{125}I] PIC, PC 12	6.84 [33]	5.30 [33]
29-*p*-Iodoclonidine (PIC)	9.10 [24]	[^{125}I] PIC, PC 12	<5 [24]	8.66 α_{2A}-AR, 8.13 α_{2B}-AR, 9.10 α_{2C}-AR [75]
30-LNP 911	9.75 [24]	[^{125}I] PIC, PC 12	4.79 [24]	<4 α_{2A}-AR [24]

The performed modeling studies have selected physicochemical features of the ligands essential for I_1-IR affinity and determined molecular descriptors for I_1-IR/α_2-AR and I_1/I_2-IR selectivity. The recently developed 2D-QSAR models for I_1-IR ligands have indicated that an increase in lipophilicity ($logD_{pH\ 7.4}$), 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 [80, 81], while lipophilicity (ClogP) and HOMO energy of the ligands are important descriptors of I_1-IR/α_2-AR selectivity [80]. The other 2D-QSAR studies [78, 92, 93] have indicated that the electrostatic and steric descriptors of the ligands have the strongest influence on the modulation of both I_1-IR and I_2-IR affinity.

Also the 3D-QSAR CoMFA, GRID and GOLPE approaches have selected the combination of the electrostatic and steric field as the most significant molecular interaction fields (MIFs) parameters for affinity at I_1-IR [94]. Finally,

the pharmacophore hypothesis of the I_1-IR ligands has selected two hydrophobic regions (HY1 and HY2), two hydrogen-bond donor groups (HBD), an aromatic ring (AR), and a positively charged moiety (PC) [94].

I_2-IMIDAZOLINE RECEPTOR LIGANDS

I_2-imidazoline receptors (I_2-IR) are characterized by their high affinity for guanidines and imidazolines ([^{3}H]-idazoxan), and low affinity for 2-aminoimidazolines [54]. Also, I_{2A} and I_{2B}-imidazoline receptor subtypes are defined depending on their affinity for amiloride [54]. The I_2-IR have first been located on the outer membranes of mitochondria and determined as allosteric binding site associated with the catalytic site of monoamine oxidase (MAO) and other non-MAO oxidative enzymes [54, 55]. However, it has been reported that there is no statistical association linking both MAO-B catalytic unit sites and I_2-IR densities in the human brain [95].

I_2-imidazoline receptors are involved in pathophysiology of various psychiatric disorders, such as: depression, Huntington's disease, Alzheimer's disease, and Parkinson's disease [60, 96].

Since no structural data for I_2-IR are available, the better understanding of the I_2-IR pharmacological functions is based on the discovery of selective I_2-IR ligands with very low I_1-IR and α_2-AR affinities.

Synthesized and examined I_2-IR ligands belong to: imidazoline, 2-aminoimidazoline, guanidine, and carboline derivatives. The reported binding affinities of the I_2-IR ligands relate to rabbit kidney preparations for I_2-IR, bovine adrenal gland in the case of I_1-IR, and rat calf cerebral cortex for a α_2-AR.

Idazoxan (Fig. (**3**), compound **30**) is α_2-AR antagonist with significant affinity for the I_1-IR, I_2-IR, and α_2-AR [97]. Minor structural modifications of idazoxan, such as introduction of a methoxy substituent on the carbon atom of the benzodioxane nucleus (RX 821002), are able to influence on a decrease of affinity for the I_2-IR in favor of the α_2-AR [98].

Various benzodioxane, benzodioxolane, and benzofuran analogs of idazoxan [99] (Fig. (**3**), compounds **31-37**) were assayed on I_2-IR and α_2-AR (Table **2**) [100]. Introduction of a double bond into the chromane cycle of the chromane analog of the idazoxan increase rigidity and planarity of the compounds and therefore enhance affinity and selectivity for I_2-IR (Fig. (**3**), compound **35**) [92]. The binding study of these compounds resulted in the discovery of potent and selective I_2-IR ligands such as benzofuran analogs of the idazoxan, 2-BFI and 7-chloro-2-BFI (Fig. (**3**), compounds **36** and **37**) [100].

However, it has been demonstrated that [^{3}H] 2-BFI binds, in membranes from human prefrontal cortex, to a second binding site different from the imidazoline I_2-IR receptors recognized by [^{3}H] idazoxan [101].

The N-substitution of the 2-BFI resulted in general decrease of the I_2-IR affinity associated with significant dopamine D_2-potency enhancement [102]. Since the I_2-IR are involved in psychiatric disorders, the designed chemical modifications of the 2-BFI were performed both in the aromatic region and to the imidazoline nitrogen without changing the core structure of the lead with goal to preserve a possible favorable synergism between I_2-IR and dopamine systems [102].

5-Isothiocyanato-2-BFI analog (Fig. (**3**), compound **38**-BU99006) is an irreversible I_2-IR ligand with high affinity and selectivity for I_2-IR in whole rat brain membranes [103].

Recently prepared bioisosteres of the idazoxan, such as benzoxazine derivatives and tricyclic analogs (Fig. (**3**), compounds **39-41**) were assayed on I_1-IR, I_2-IR, α_1-AR and α_2-AR [104]. The replacement of the 4-oxygen of idazoxan with a nitrogen atom (Fig. (**3**), compound **39**) resulted in similar binding affinity for I_2-IR and considerably increased I_2-IR/α_2-AR selectivity compared to the non-selective idazoxan (Table **2**) [104]. Mono substitution with methyl radical on the aromatic ring of the compound 38 (Fig. (**3**), compound **40**), giving the best activities on the I_1-IR, I_2-IR, and α_2-AR receptors and a moderate selectivity towards I_2-IR (Table **2**) [104].

In case of oxazinoindole and oxazinoquinoline tricyclic analogues, introduction of a double bond (Fig. (**3**), compound **41**) led to the most active and selective I_2-IR ligand (Table **2**), while increase of the size of the ring or decreasing the basic character of the oxazino nitrogen atom considerably decrease the I_2-IR activity [104].

2-Phenoxymethyl imidazoline derivative, cirazoline (Fig. (**3**), compound **42**), is a potent I_2-IR ligand, α_1-AR agonist and α_2-AR antagonist, with significant I_2-IR/α_1-AR selectivity [105, 106].

The bridge between imidazoline and the phenyl ring plays a key role in the modulation of the affinity and selectivity between I_2-IR and α-AR [92, 93]. Therefore, methylation at the β-position of the bridge (Fig. (**3**), compound **43**) significantly decreased the I_2-IR affinity and I_2-IR/α_2-AR selectivity of the cirazoline derivative (Fig. (**3**), compound **44**) [107]. Isosteric replacement of the oxygen atom of the bridge by a methylene group (Fig. (**3**), compound **45**) increased I_2-IR affinity, reduced α_2-AR activity and eliminated α_2-AR activity [92]. Hence, the compound **45** was selected as a new I_2-IR lead for further modifications.

Very recent study of two series of 2-aryl-ethylen-imidazolines (Fig. (**3**), compound **45**), confirmed the morphine analgesia modulation displayed by these compounds and demonstrated that these effects might be correlated with morphine tolerance and dependence [108]. For the examined aryl-ethylen-imidazoline molecules were selected good lipophilicity and suitable steric hindrance as factors with high influence on I_2-IR affinity and selectivity. Also, comparative examination of rationally designed aryl-ethylen-imidazolines pointed out some significant analogies between binding site cavity of I_2-IR proteins and α_{2C}-AR subtype [108].

The observed high I_2-IR affinity and I_2-IR/α_2-AR selectivity of the α-methylated analog of compound **45** (Fig. (**3**), compound **46**) and the high I_1-IR affinity and I_1-IR/α_2-AR

Table 2. The I_1-IR, I_2-IR and α_2-AR Binding Affinities (p*Ki* = log(1/*Ki*)) of I_2-IR ligands [77, 97-117].

Compounds	p*Ki* (I_2-IR)	p*Ki* (I_1-IR)	I_2-IR/ α_2-AR Selectivity	p*Ki* (α_2-AR)
31-Idazoxan	7.97	5.90	5.2	7.25
32	8.06	-	41.3	6.44
33	8.57	-	161.5	6.36
34	7.40	-	10	6.40
35	8.43	-	191	6.15
36-2BFI	8.89	-	2874	4.57
37	8.55	-	2192	6.18
38-BU99006	8.60	≈5.60	≈1000	≈5.60
39	7.29	-	>2000	≈3.99
40	8.70	7.66	35.3	7.15
41	8.45	7.88	102	6.44
42-Cirazoline	7.90	-	-	-
43	5.57	-	0.036	7.01
44	9.05	-	58.8	7.28
45	8.60	-	794	5.70
46	6.91	-	10	5.91 α_2-AR, 5.22 α_1-AR
47	8.74	5.37	7762	4.85
48-BU224	8.70	5.78	-	-
49-BU226	8.85	5.37	-	-
50	-	-	-	-
51	-	-	-	-
52	8.30	-	110	6.26
53	8.22 I_{2A}-IR, 8.70 I_{2B}-IR	-	-	-
54	7.47	-	6.3 α_{2A}-AR, 28 α_{2B}-AR, 57 α_{2C}-AR	6.67 α_{2A}-AR, 6.02 α_{2B}-AR, 5.71 α_{2C}-AR
55	9.37	-	23000	5.00
56	8.02	-	-	-
57	8.14	-	-	-
58	8.36	-	4226	4.73
59	7.75	-	>5649	<4
60-FTIMD	8.53	<5	>3000	<5

selectivity of the (S)-(-)-β-methylated analog of compound **45** (Fig. (**1**), compound **28**) indicated on the key role of the ethylene bridge in modulating I_1- and I_2-IR selectivity in these series of molecules [33]. The stereospecific requirement of the I_1-IR and I_2-IR is the remarkable feature of the binding sites.

The previous 2D-QSAR studies of the I_2-IR ligands [33, 91-93] have indicated on electrostatic and steric parameters of the compounds as descriptors with the strongest influence on the I_2-IR affinity.

Performed QSAR studies on 2-phenoxymethyl imidazoline analogs and related compounds [33, 78, 92, 93, 109] found out that good lipophilicity extended also to the *ortho* position of the phenyl ring facilitates I_2-IR affinity, the cyclopropyl moiety was not essential for either I_2-IR or α_1-AR affinity, and unsubstituted ethylenic bridge, between the aromatic moiety and imidazoline ring of the 2-phenoxymethylimidazolines, is determinant in inducing high I_2-IR selectivity with regard to I_1-IR and α_2-AR.

In agreement with the 2D-QSAR studies, the 3D-QSAR CoMFA, GRID and GOLPE approaches have selected the combination of lipophilic and steric field as most significant MIFs descriptors for affinity at I_2-IR [94]. Finally, the pharmacophore for the I_2-IR ligands contains two hydrophobic (HY1 and HY2) regions, an aromatic ring (AR) and a positively charge moiety (PC) [94]. The pharmacophore of the I_2-IR ligands is formed from four features (HY1, HY2, AR, PC), while the pharmacophore of the I_1-IR ligands contain five features (HY1, HY2, AR, PC, HBD). Since the pharmacophore of the I_2-IR ligands lacked the HBD feature, the HBD interactions are important determinant of the I_1-IR/I_2-IR selectivity [94].

Reduction of the conformational flexibility of compound **45** with a trans-styryl bridge in the tracizoline (Fig. (**3**), compound **47**) resulted in very potent and selective activity at the I_2-IR (Table **2**), while the more rigid molecule - benazoline (Fig. (**1**), compound **27**) expressed more potent activity at the I_2-IR and higher selectivity in relation to α-AR (pKi I_2 9.07; pKi α_2 4.40; pKi α_1 5.64) [92]. Furthermore, the relative position of the aromatic and imidazoline rings of the 2-trans-styryl-imidazolines plays a crucial role in the I_2-IR binding, whereas the aromatic region of the ligands need to be of limited size in order to display high affinity for I_2-IR [92].

Since the benazoline is very potent I_1-IR and I_2-IR ligand [110], new benazoline isosters able to discriminate between I_1-IR and I_2-IR (Fig. (**3**), compounds **48** and **49**) have been synthesized and examined. Substitution of the naphtyl ring by a quinoline (Fig. (**3**), compound **48**, BU224) and isoquinoline (Fig. (**3**), compound **49**, BU226), resulted in potent and selective I_2-IR ligands in relation to I_1-IR (Table **2**) [96].

The activities of these tracizoline congeners and cirazoline analogues were used to develop a 3D-QSAR models that selected the most relevant electrostatic, steric, and lipophilic interactions for high I_2-IR affinity [109] such as: favorable lipophilic/steric interactions around the 2-*ortho* and 3-*meta* positions of the phenyl ring; favorable electronic/ hydrophilic interactions on the 5-*meta* position; unfavorable high energy density above the *ortho*-region; unfavorable steric hindrance near and above the imidazoline ring and around distal part of planar polycyclic ligands [109].

Recently developed 3D-QSAR CoMFA on I_2-IR ligands, imidazoline derivatives, have defined set of steric and electronic favorable or unfavorable areas and proposed I_2-IR pharmacophore containing nonsubstituted imidazoline cycle (plane p1) and a 2.5 A° wide aromatic zone (plane p2) with imidazoline C2 dihedral angle of 142º [111].

Furthermore, aromatic guanidines, such as amiloride (Fig. (**3**), compound **50**) and guanabenz (Fig. (**3**), compound **51**) have been used for the characterization of I_{2A}-IR and I_{2B}-IR subtypes. The N,N-dimethyl-guanabenz derivatives were the most potent I_2-IR ligands, while the highest I_2-IR affinity and selectivity were observed for the 2-bromo N,N-dimethyl-guanabenz derivative (Fig. (**3**), compound **52**) [112].

Finally, the guanabenz-like molecule aganodine (Fig. (**3**), compound **53**), is very good ligand for both I_2-IR subtypes (pKi I_{2A} 8.22 and pKi I_{2B} 8.70) [113].

The 2-aminoimidazoline derivative such as brimonidine (Fig. (**3**), compound **54**) is displaying a high binding affinity to I_2-IR and low selectivity in relation to α_2-AR subtypes (Table **2**) [64, 114]. The 2-aminoimidazoline compound RS45041 (Fig. (**3**), compound **55**) is very selective I_2-IR ligand (Table **2**) [115].

Also, the 2-(4,5-dihydroimidazol-2-yl)-4-methyl- and 4-chloro-benzimidazole (Fig. (**3**), compound **56** and **57**) exhibited a high affinity at imidazoline I_2 receptors and high I_2/α_2 selectivity. Moreover, for this type of imidazoline derivatives has been proved that the I_2/α_2 selectivity ratio may depend upon pKa value [116].

Hybridization of 1,2,3,4-tetrahydro-β-carboline (Fig. (**3**), compound **58**) and 2-BFI (Fig. (**3**), compound **36**) resulted in fused imidazopyridoindole molecule (Fig. (**3**), compound **59**). The compound **59** showed similar I_2-IR affinity (pKi I_2 8.14) as the 1,2,3,4-tetrahydro-β-carboline (pKi I_2 8.02) and lower selectivity towards I_1-IR and α_2-AR, because of the embedded imidazoline structure [117].

The benazoline (Fig. (**1**), compound **27**) is very potent I-IR ligand able to activate both the I_1-IR and I_2-IR in a highly selective way toward α_1-AR and α_2-AR [32].

Following the benazoline structure, 2-phenylimidazolne derivatives were synthesized and assayed at I_1-IR, I_2-IR, α_1-AR and α_2-AR [77]. Several of these compounds were potent and selective I_2-IR ligands. For instance, the 3'-fluoro-4'methylphenyl-2-imidazoline (Fig. (**3**), compound **60**) expressed very high I_2-IR affinity and selectivity toward I_1-IR, α_1-AR and α_2-AR (Table **2**) [77]. Introduction of a fluoro group in *meta* position of the phenyl ring leads to an increase of affinity and selectivity for I_2-IR in relation to I_1-IR, α_1-AR and α_2-AR [77].

Two series of fentanyl-derived hybrid molecules bearing potent I_2–IR ligands (such as guanidine and BU224 moieties) linked with an aliphatic or aromatic spacer were synthesized and assayed at I_2–IR and μ-opoid receptors through competition binding studies on human postmortem brain membranes [118, 119]. The fentanyl-alkaneguanidine series exhibited remarkable affinities in the nanomolar range for both I_2–IR and μ-opoid receptors [118].

Also, 1,2,3,4-Tetrahydropyrazino[1,2-a]indoles are reported as a novel class of I_2–IR ligands. Specially, 8-methoxy-1,2,3,4- tetrahydropyrazino[1,2-a]indole has expressed high affinity and selectivity for I_2–IR (Ki=6.2 nM) relative to I_1–IR, α_2-AR [120].

It has recently been indicated that guanidinium derivatives, such as metformin (Fig. (**3**), compound **61**), might activate both I_{2A}- and I_{2B}-imidazoline receptor subtypes. While I_{2A}-IR link the increase of β-endorphin release the I_{2B}-imidazoline receptors influence on lowering of blood glucose in type-1 like diabetic rats [121].

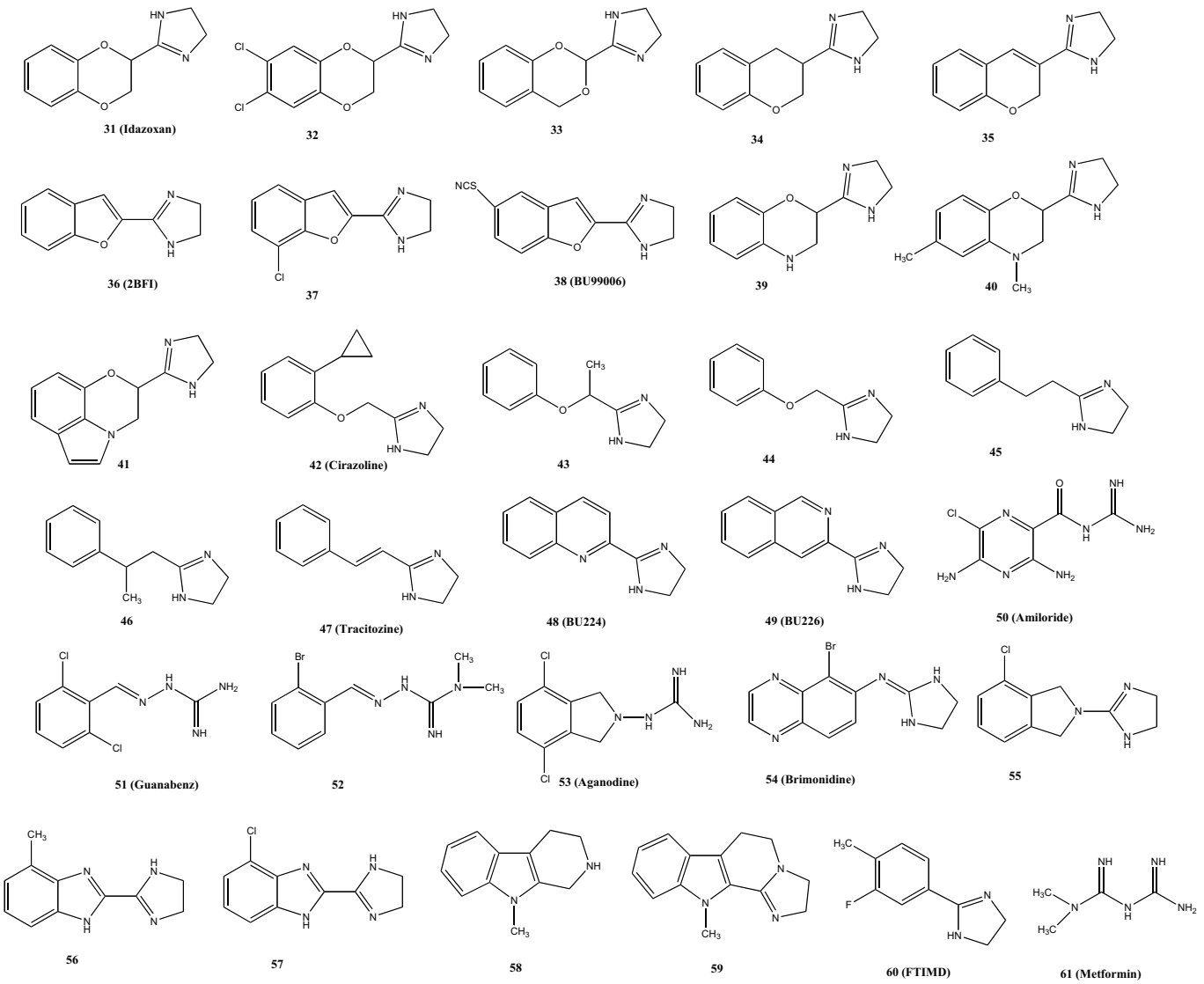


Figure 3.

Fig. (3). Structural formulas of I_2-IR ligands.

Important advancements in imidazoline I_2 receptor pharmacology are current findings that imidazoline I_2 receptor agonists exhibit anti-depressive effect [122], analgesic activity [123-125], and antipyretic effect [126]. The I_2 receptors are recently proposed as novel drug target for new analgesics [127]. These new results represent an important progress of imidazoline I_2 receptor pharmacology and provide new directions for future studies of the I_2 receptor ligands.

I_3-IMIDAZOLINE RECEPTOR LIGANDS

Induction of insulin secretion from pancreatic β-cells by imidazoline ligands is tentatively assigned to imidazoline I_3 imidazoline receptors [67, 68]. The precise mechanisms of I_3-IR modulated insulin secretion and glucose homeostasis remain unknown.

Pancreatic imidazoline receptors and sympathetic presynaptic imidazoline receptors have been experimentally determined as I_3-imidazoline receptors (I_3-IR) [69, 128-131]. The I_3-IR ligands can induce insulin secretion from pancreatic β-cells through activation of the I_3-IR, which is not mediated by I_1 or I_2 imidazoline receptors [67, 69, 128, 129, 132]. The insulotropic action of imidazolines mediated by I_3-IR has first been attributed exclusively to the closure of ATP-sensitive potassium channel (K_{ATP}) producing depolarization, calcium influx and release of insulin [133]. The pharmacological evidences suggest that I_3-IR binding site for imidazolines may lie within the ion conducting subunit of the K_{ATP} channel [134, 135].

However, attempts to characterize the I_3-IR have been unsuccessful because of the lack of specific radioligands.

Structural requirements for binding of the ligands to I_3-IR have not been fully defined. The efaroxan (Fig. (**1**), compound **15**) is defined as selective I_3-IR agonist whereas its imidazole analog KU14R (Fig. (**4**), compound **62**) is an I_3-IR antagonist [136].

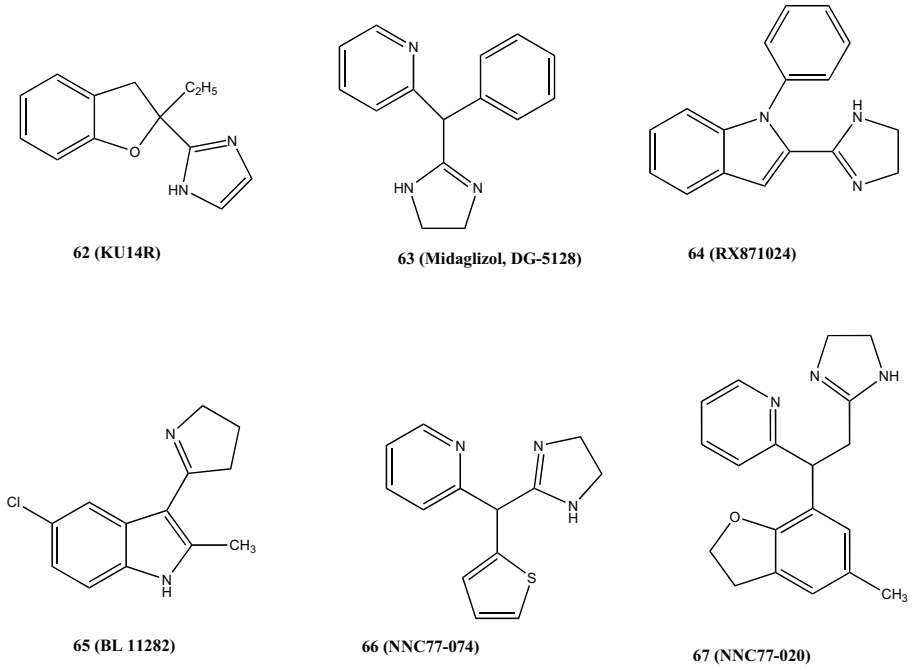


Fig. (4). Structural formulas of I_3-IR ligands.

One imidazoline compound, midaglizole (Fig. (**4**), compound **63**), is expressing antihyperglycaemic effects in animal models of diabetes and in patients with type-2 diabetes [137-139].

More recently, the imidazoline I_3-IR ligands are divided into classic group of agents (such as RX871024 (Fig. (**4**), compound **64**)), which produce insulotropic effect via the closure of K_{ATP} channel at both normal and elevated glucose levels [132], and agents of new generation (such as BL11282 (LY374284) (Fig. (**4**), compound **65**) [140], NNC77-0074 (Fig. (**4**), compound **66**) [141], NNC77-0020 (Fig. (**4**), compound **67**) [142]) which have only the glucose dependent insulotropic activity without affecting K_{ATP} channels [132, 140, 143]. The NNC77-0074 potently inhibited glucagon secretion from rat islets by modulation of exocytosis of the insulin- and glucagon-containing granules [141], while the NNC77-0020 modulated pancreatic hormone secretion by glucose-dependent stimulation of insulin and somatostatin secretion and inhibition of glucagon release [142].

Because of the dependency on glucose concentration, these novel I_3-IR ligands may significantly reduce the risk of hypoglycemic episodes and therefore may be used as leads for the development of novel anti-diabetic agents.

A recent binding study on insulin secreting HIT cells established a low and high affinity binding sites of [^{3}H]-clonidine and determined low-affinity binding site as the pore-forming subunit of the K_{ATP} channel [144]. The existence of diverse I_3-IR has also been proposed on the basis of pharmacological experiments with I_3-IR agonists efaroxan and harmane [145].

The imidazoline compounds, such as idazoxan (Fig. (**3**), compound **30**) and RX 821002 (2-methoxy-idazoxan), stimulate insulin release on freshly isolated islets by relieving the β-cell from the inhibitory effect of prebound endogenous catecholamines [146].

CONCLUSION

Progress in the imidazoline receptors research field is restricted by the lack of knowledge of the structure of IR that require more selective I_1-IR, I_2-IR, or I_3-IR ligands for such studies. Therefore, the development of new potent and selective I-IR ligands is based on QSAR studies of various families of I-IR ligands.

The pharmacological activity of I_1-IR ligands has been mainly related to hypertension. The low incidence of the side effects, antiarrhythmic activity, and beneficial metabolic and renal effects of more selective I_1-IR ligands suggest that they may provide a very useful therapy.

The pharmacological activity of I_2-IR is mainly linked to psychiatric disorders, analgesia, opiate withdrawal, Parkinson's disease and Alzheimer's diseases, while activation of the I_3-IR can induce insulin secretion from pancreatic β-cells.

Extensive research of imidazoline receptors indicated new applications of IR ligands, such as: prevention of cue-induced cocaine relapse by I_1-IR agonists [147], modulatory effects on the opoid induced analgesia by I_2-IR ligands [148, 149], and diagnosis of gliomas by use of the I_2-IR ligands [150].

The developed pharmacophore models for I_1-IR and I_2-IR ligands should be used for the high-throughput screening of the three-dimensional-multi-conformational databases for discovery of new leads targeting the I-IR. The QSAR and pharmacophore modeling studies for I_3-IR ligands are not performed because of small number of synthesized and assayed compounds.

CONFLICT OF INTEREST

None declared.

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