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Design and synthesis of novel imidazoline derivatives with potent antihyperglycemic activity in a rat model of type 2 diabetes

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Abstract—Imidazoline derivatives have been reported to show antihyperglycemic activity in vivo. In the present study, we first showed that there was no correlation between the in vivo antidiabetic activity and the in vitro affinities for the I_1/I_2 binding sites for several substituted aryl imidazolines. Among these compounds, 2-(α-cyclohexyl-benzyl)-4,5-dihydro-1*H*-imidazole **2** exhibited potent antihyperglycemic properties. It was then chosen as lead compound. Thirty-six new derivatives were synthesized by replacing the cyclohexyl/benzyl group by various cyclic systems or the imidazoline ring by isosteric heterocycles. These compounds were evaluated in vivo for their antihyperglycemic activity using an oral glucose tolerance test (OGTT) in a rat model of type-2 diabetes obtained by giving a single intravenous (iv) injection of a low dose of streptozotocin to rats (STZ rats) and in normal rats. Nine compounds with an imidazoline moiety, possibly substituted by a methyl group, had a potent effect on the glucose tolerance in normal or STZ-diabetic rats, after an oral (po) administration of the test compound at a dose of 30 or 10 mg kg^{-1}, without any hypoglycemia. Replacement of the imidazoline ring by isosteric heterocycles resulted in a total loss of activity.

1. Introduction

Type 2 diabetes mellitus or non-insulin-dependent diabetes mellitus (NIDDM) is a widespread syndrome characterised by fasting and post-prandial hyperglycemia affecting about 5% of the population in the industrialized nations.[1,2] At present, the treatment of type 2 diabetes mellitus is directed towards the reduction of the hyperglycemia by improving insulin secretion or reducing the insulin resistance of peripheral tissues. Currently available therapies for type 2 diabetes include the sub-cutaneous administration of insulin and the use of various oral agents: sulfonylureas,[3,4] biguanides[4–7] (metformin), α-glucosidase inhibitors,[8] benzoic acid derivatives,[9] thiazolidinediones[10,11] (pioglytazone, rosiglitazone). The pharmaceutical industries are constantly searching for new compounds with better tolerance and longer-lasting efficacy.[12] Among the new molecules tested, imidazoline derivatives[13–17] such as midaglizole[18] and efaroxan[19,20] have been reported to be α-antagonists[19,21] stimulating insulin secretion and thus showing antihyperglycemic activity in vivo. Further studies have shown that their activity was not correlated with their α_2-antagonistic properties[22–24] and suggested that the stimulatory effects of these compounds on insulin release were probably related to their imidazoline moiety. These compounds could interact with imidazoline preferring binding sites (IPBS), I_1 and I_2.

In a previous paper we described imidazoline-based structures that were potent and selective I_1 and/or I_2 ligands (reacting at nanomolar concentrations) and these compounds were devoid of affinities for both α_1 and α_2 adrenoreceptors.[25,26]

Keywords: Imidazoline; Imidazoline receptors; Type 2 diabetes mellitus; Glucose tolerance.
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✠ In memory of professor Marc Payard.

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The first aim of the present study was to evaluate the relationships between I_1 and/or I_2 affinities and antidiabetic activity for our compounds. We have selected several 2-phenyl imidazoline derivatives previously described[26] (compounds **Ia–q**, **IIa–c**) with two new compounds (**1** and **2**) and studied their antidiabetic activity. The molecules of series **I** are phenyl imidazolines substituted at the ortho, para or meta position. In series **II**, the imidazoline moiety is linked to heterocyclic or aromatic rings, with compounds **1** and **2** including a cycloalkyl group. Some of these imidazoline derivatives showed a high affinity and selectivity for imidazoline binding sites (I_1 and/or I_2). Compounds **In**, **IIa** were highly selective for the I_1 sites, compounds **Id**, **Ii**, **Ij**, **Im**, **Io**, **Ip** for the I_2 sites, while compound **II** was a rather poor ligand for the I sites and the other derivatives were less selective. Most of them did not bind to α-adrenergic receptors. These compounds were evaluated in vivo for their antidiabetic activity using an oral glucose tolerance test (OGTT) in a rat model of type-2 diabetes obtained by giving a single iv injection of a low dose (35 mg kg^{-1}) of streptozotocin to rats (=STZ rats).

As shown in Tables 1 and 2, there was no correlation between the antihyperglycemic properties in vivo and the affinities for I_1 and/or I_2 binding sites in vitro. It appears that the potency of the imidazoline derivatives for increasing insulin secretion, as has been previously described,[14,27] does not only result from their binding to the I_1 or I_2 binding sites of pancreatic islets. The hypoglycemic effects of these compounds are exerted via a mechanism of action which does not require binding to these receptors.

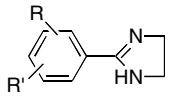
However, four compounds showed potent effects on glycemia. In these compounds, the imidazoline moiety is linked to a phenyl ring (**Ic** and **In**), a cycloalkyl group (compound **1**) or a cycloalkyl and an aromatic ring (compound **2**). In an effort to optimize these results, we chose compound **2** as the starting point for our research because it includes both a saturated and an aromatic ring.

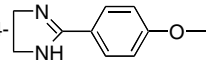
Starting with this lead compound we carried out various modifications (Fig. 1) such as: (i) replacement of the phenyl ring by a cycloalkyl group in order to determine the influence of the aromatic ring (compound **3**); (ii) incorporation of a methylene group in the cycloalkyl ring in order to bring the imidazoline moiety closer to the cycloalkyl/phenyl groups and to reduce the flexibility of the molecule (compounds **4–8**); (iii) modulation of the steric hindrance of the cycloalkyl group (compounds **6** and **8**); (iv) introduction of a chlorine atom on the phenyl ring, as halogens had been reported to lead to a significant increase in activity; (v) replacement of the aromatic ring of compound **4** by a methyl group (compound **9**). This methyl group can also be linked to the para position of the cyclohexyl group (compound **10**).

We have also synthesized compound **11** which is an analogue of compound **2** that is rigid because of the presence of the cyclopropyl bridge. It is also an analogue of cibenzoline[28,29] and midaglizole[21] which have been reported to be antihyperglycemic agents (Fig. 2).

Pharmacomodulation was also performed on the imidazoline moiety itself which was substituted by a methyl

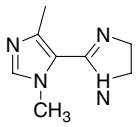
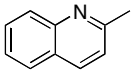
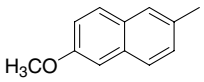
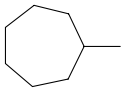
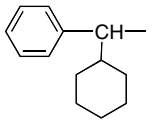
Table 1. pK_i values[a] in bovine brain (I_1), rabbit kidney (I_2) and calf cerebral cortex (α_1 and α_2), and antidiabetic activity[b] for compounds of series **I**



Compound	R	R′	pK_i				Activity (STZ rats)
			I_1	I_2	α_1	α_2	
Ia	3-Me	H	8.66	8.30	<5.00	<5.00	Inactive
Ib	4-Et	H	7.23	7.08	<5.00	<5.00	Inactive
Ic	4-Pr	H	6.22	7.16	<5.00	<5.00	Active
Id	4-*i*Pr	H	5.66	7.09	<5.00	<5.00	Inactive
Ie	4-*t*Bu	H	5.05	6.25	<5.00	<5.00	Inactive
If	4-Ph	H	6.69	8.00	6.00	<5.00	Inactive
Ig	4-PhO	H	6.34	7.34	5.11	5.26	Inactive
Ih	4-MeS	H	7.75	8.42	<5.00	<5.00	Inactive
Ii	2-F	H	<5.00	7.00	<5.00	<5.00	Inactive
Ij	4-CF_3	H	5.96	7.28	6.89	<5.00	Inactive
Ik	4-CF_3O	H	6.04	7.07	<5.00	<5.00	Inactive
Il	4-CH_3CONH	H	<5.00	<5.00	<5.00	<5.00	Inactive
Im	4- 	H	<5.00	6.00	<5.00	5.38	Inactive
In	2-Me	5-F	7.64	5.30	<5.00	<5.00	Active[c]
Io	4-Me	3-F	<5.00	8.53	<5.00	<5.00	Inactive
Ip	3-F	5-F	5.00	7.11	<5.00	<5.00	Inactive
Iq	2-Me	3-CN	7.64	8.30	<5.00	<5.00	Inactive

[a] Values are means of two experiments. The variability is less than 10%.
[b] Activity with po administration of 30 mg kg^{-1} of the test compound.
[c] Inactive with a po administration of 10 mg kg^{-1} of the test compound.

Table 2. pK_i values[a] in bovine brain (I_1), rabbit kidney (I_2) and calf cerebral cortex (α_1 and α_2), antidiabetic activity[b] for compounds of series **II**

Compound	R	pK_i				Activity (STZ rats)
		I_1	I_2	α_1	α_2	
IIa		6.39	<5.00	<5.00	<5.00	Inactive
IIb		7.34	8.82	5.82	5.74	Inactive
IIc		7.05	7.94	<5.00	5.46	Inactive
1		4.88	5.87	<5.00	5.64	Active
2		7.23	7.38	<5.00	<5.00	Active

[a] Values are means of two experiments. The variability is less than 10%.
[b] Activity with po administration of 30 mg kg^{-1} of the test compound.

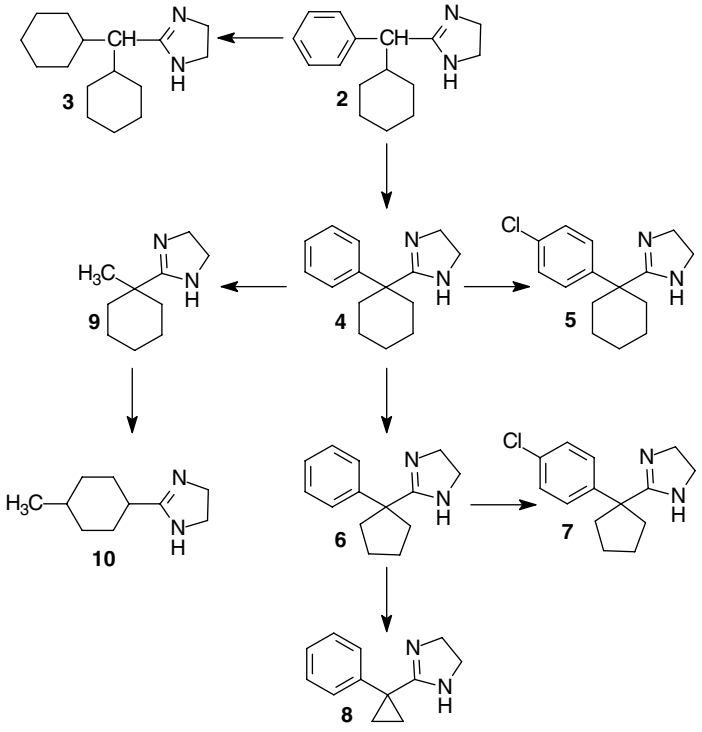
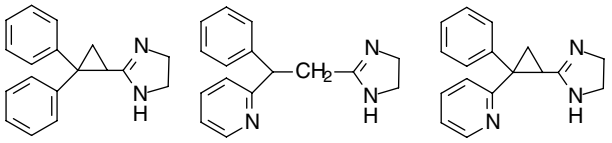


Figure 1. Imidazoline derivatives **2**–**10**.

group (series **a**), a phenyl group (series **b**) or replaced by isosteric groups such as oxazoline (series **c**), thiazoline (series **d**), tetrahydropyrimidine (series **e**), tetrazoles (series **f**–**h**) heterocycles with acidic properties, or amidines, open derivatives (series **i**). These modifications are shown in Schemes 2 and 3. All these compounds were evaluated in vivo for their antidiabetic activity using an oral glucose tolerance test (OGTT) on normal or STZ-diabetic rats.



Cibenzoline **Midaglizole** **11**

Figure 2. Imidazoline derivative **11**.

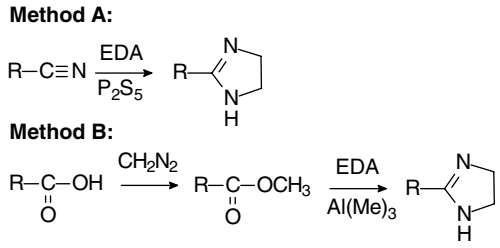
As the route of administration was by oral (po) ingestion, we used the rule of 5 established by Lipinski[30] to take into consideration the physicochemical properties of these compounds relevant to their intestinal absorption. This set of rules states that the majority of drug-like molecules that are active when administered orally are characterized by a $c\log P \leqslant 5$, a molecular weight $\leqslant 500$, a number of H-bond acceptors $\leqslant 10$, and a number of H-bond donors $\leqslant 5$. Molecules for which more than one of these conditions are not satisfied may have poor permeability. A qualitative structure–activity relationships study was then carried out and some structural elements governing biological activity were identified.

2. Chemistry

Imidazoline compounds were prepared as outlined in Scheme 1. Compounds **1**, **2**, **4** and **11** were synthesized by nucleophilic addition of ethylenediamine (EDA) to the corresponding nitriles in the presence of catalytic amounts of the sulfur reagent: P_2S_5[26,31] (Method A). The synthesis of compounds **3**, **5–10** was performed by the addition of EDA to the corresponding esters in the presence of trimethylaluminium ($Al(Me)_3$)[32,33] (Method B). Methyl esters **3′**, **5′–10′**, not commercially available, were prepared by the addition of diazomethane to the corresponding carboxylic acids.

The synthesis of the majority of the imidazoline analogues is described in Scheme 2. Compound **2a** was synthesized in two steps: the thioamide derivative was prepared by reaction of the nitrile and an aqueous solution of ammonium sulfide[34–36] followed by treatment with 1,2-diaminopropane (1,2-DAP). Compounds **4a**, **9a**, **2e**, **4e** and **9e** were obtained by addition of 1,2-DAP or 1,3-DAP to the corresponding nitrile in the presence of P_2S_5.

Δ-2-oxazolines **2c**, **4c** and **11c** were prepared by condensation of the corresponding nitriles with ethanolamine. It was necessary to use $CaCl_2$[37] or $ZnCl_2$[38] as catalysts and the best results were obtained with $ZnCl_2$. Condensation of the corresponding nitriles with cysteamine[39] produced the Δ-2-thiazolines **2d**, **4d** and **11d**.



Scheme 1. Synthesis of imidazoline derivatives **1–11**.

1*H*-tetrazoles **2f**, **4f** and **11f** were obtained by warming sodium azide in the presence of the corresponding nitriles.[40] Methylation of 1*H*-tetrazoles by diazomethane gave a mixture of 1*N*-methyl and 2*N*-methyl-tetrazoles **2g–h**, **4g–h** and **11g–h**. The identification of structural isomers of the methylated tetrazoles was performed according to literature data[41,42] on the basis of the ^1H and ^{13}C NMR chemical shifts of the methyl group and the carbon C-5. Chemical shifts $\delta N–CH_3$ (^1H) and $\delta N–CH_3$ (^{13}C) and δC_5 of 2*N*-methyl tetrazole were higher than those of 1*N*-methyl tetrazole. This study enabled us to assign the structure of 1*N*-methyl tetrazole to compounds **g** and the structure of 2*N*-methyl tetrazole to compounds **h**. These were confirmed by X-ray analysis of compound **11h** (Fig. 3, Table 3).

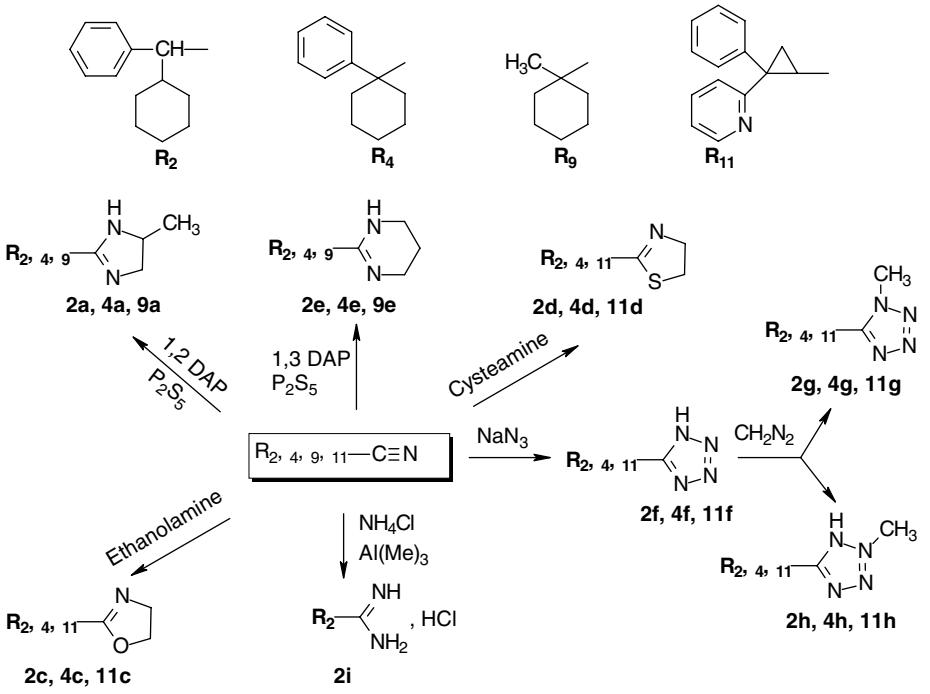
Carboxamidine hydrochloride **2i** was prepared from trimethyl aluminium ($Al(Me)_3$) and ammonium chloride added to the corresponding nitrile according to Garigipati.[43]

1*H*-Benzimidazole **2b**, **4b** and **9b** derivatives were obtained in two steps[44] from acid chloride by treatment with *o*-phenylendiamine to give a mixture of monoamides (**2b′**, **4b′** and **9b′**) and diamides (**2b″**, **4b″** and **9b″**) and cyclisation of monoamides **b′** (Scheme 3).

3. Biological activity

The antidiabetic properties of all the synthesized compounds were quantified, in vivo, by their ability to improve glucose tolerance during an OGTT performed on a normal (non-diabetic) rat or for active compounds, on a rat model of mild diabetes developed by Thibault.[45] Moderate diabetes is obtained by a single iv injection of a low dose (35 mg kg^{-1}) of streptozotocin (STZ). These diabetic rats exhibit moderate basal hyperglycemia, glucose intolerance and impairment of glucose-induced insulin secretion. Two weeks later measurements of the basal glycemia and an OGTT were performed, enabling the animals with moderate diabetes to be identified, that is a basal glycemia of between 7 and 10 mM.

OGTTs were carried out 1 h after a single po administration of 30, 10 or 3 mg kg^{-1} of each synthesized molecule and glucose tolerance was evaluated according to two parameters: G120 which is the blood glucose level at 120 min after the glucose administration and ΔG which represents the increase in blood glucose over the baseline integrated over a period of 120 min following the glucose load. To be considered as effective antidiabetic agents, compounds must induce a diminution of ΔG value. The activity was expressed by the ratio ΔG (control rats)/ΔG (treated rats). The score was recorded



Scheme 2. Synthesis of imidazoline analogues **2(a,c–i)**, **4(a,c–h)**, **9(a,c–e)**, **11(c,d,f–h)**.

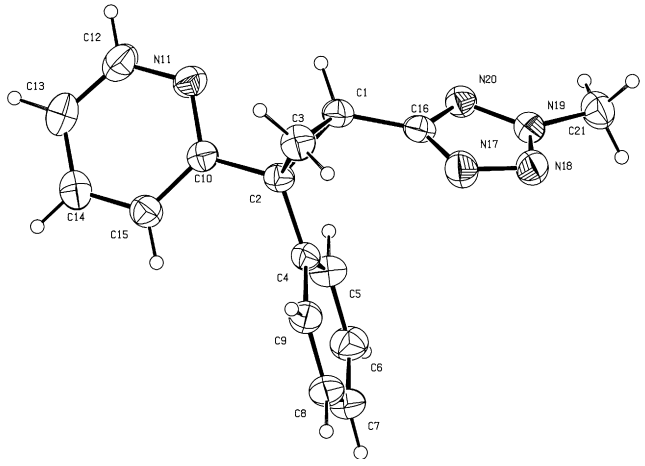


Figure 3. ORTEP view of **11h**.

as: + for a ratio value lower than 2, ++ between 2 and 10, and +++ higher than 10.

4. Results and discussion

4.1. Activity of imidazolines 1–11

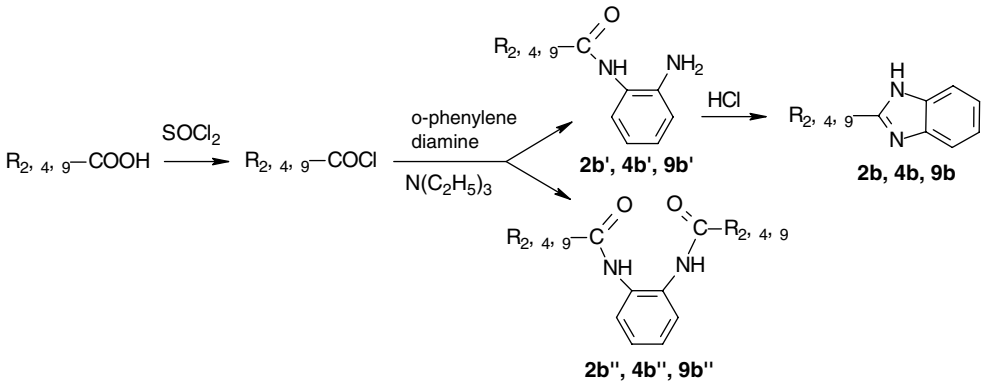
Initially, compounds **2–9** and **11** were tested on normal rats by oral administration at two different doses: 10 and 30 mg kg^{-1} po. These results are reported in Table 4. Then compounds **1**, **2**, **9**, **10** and **11** were tested on STZ rats (Table 5).

The biological effects observed, both in normal rats and STZ rats, were similar. And as the results are homogeneous it was possible to extrapolate the results from one series to another.

Table 3. Crystal data and structure refinement for **11h**

Empirical formula	C_{16} H_{15} N_5
Formula weight	277.33
Temperature	296(2) K
Wavelength	1.54180
Crystal system, space group	Orthorhombic, Pca21
Unit cell dimensions	a = 20.909(2) ; α = 90°
	b = 7.188(1) ; beta = 90°
	c = 9.598(1) ; gamma = 90°
Volume	1442.5(3) Å$'^3$
Z, calculated density	4, 1.277 mg/m^3
Absorption coefficient	0.641 mm^{-1}
F(000)	584
Crystal size	0.50 × 0.30 × 0.25 mm
Theta range for data collection	4.23–64.92°
Limiting indices	$0 \leqslant h \leqslant 24$, $0 \leqslant k \leqslant 8$, $0 \leqslant l \leqslant 11$
Reflections collected/unique	1303/1303 [R(int) = 0.0000]
Completeness to theta = 64.92	99.7 %
Max. and min. transmission	0.8561 and 0.7398
Refinement method	Full-matrix least-squares on F_2
Data/restraints/parameters	1303/1/191
Goodness-of-fit on F_2	1.116
Final R indices [$I > 2$sigma(I)]	$R1$ = 0.0435, $wR2$ = 0.1158
R indices (all data)	$R1$ = 0.0451, $wR2$ = 0.1177
Absolute structure parameter	0.6(7)
Extinction coefficient	0.057(5)
Largest diff. peak and hole	0.271 and −0.208 e Å$'^{-3}$

Compound **2** was the most potent in normal rats. ΔG (see footnotes of Table 4), which is an indication of glucose tolerance, decreased at the dose of 30 mg kg^{-1} (31.1 g L^{-1}/0.1 g L^{-1}) and at 10 mg kg^{-1} (20.5 g L^{-1}/11.3 g L^{-1}). The corresponding G120 values, 1.22 and 0.84 g L^{-1}, respectively, were close to the blood glucose values of normal rats. With this compound and also with the others, we did not observe any hypoglycemic



Scheme 3. Synthesis of benzimidazole derivatives **2b**, **4b**, **9b**.

Table 4. Variation of the glycemia parameters after po administration of imidazolines derivatives to normal rats

Compound	Dose (mg kg^{-1})	ΔG[a] (g L^{-1})	G120[b] (g L^{-1})	Activity[c]
2	30	31.1 (±3.3)/0.1 (±2.1)	1.22 (±0.03)/0.84 (±0.03)	+++
	10	20.5 (±2.5)/11.3 (±3.9)	1.22 (±0.03)/1.12 (±0.02)	+
3	30	36.4 (±3.4)/26.9 (±2.3)	1,15 (±0.06)/1.17 (±0.03)	–
4	30	17.6 (±1.3)/4.6 (±1.6)	1.23 (±0.01)/1.00 (±0.02)	++
	10	29.9 (±3.9)/11.9 (±2.1)	1.24 (±0.02)/1.20 (±0.03)	++
	3	31.5 (±2.4)/32.4 (±1.5)	1.20 (±0.03)/1.21 (±003)	–
5	10	33.3 (±4.1)/25.2 (±4.2)	1.25 (±0.04)/1.09 (±0.06)	+
	3	35.4 (±5.7)/32.2 (±2.7)	1.25 (±0.03)/1.21 (±0.04)	–
6	10	33.3 (±3.8)/17.1 (±2.5)	1.25 (±0.04)/1.16 (±0.04)	+
	3	17.7 (±3.2)/21.7 (±2.3)	1.13 (±0.03)/1,11 (±0.02)	–
7	30	17.7 (±1.3)/12.2 (±4.3)	1.23 (±0.01)/1.07 (±0.05)	+
8	10	33.3 (±3.7)/36.2 (±3.4)	1.25 (±0.04)/1.29 (±0.02)	–
9	30	30.3 (±2.5)/7.0 (±1.3)	1.20 (±0.05)/1.10 (±0.04)	++
	10	30.3 (±2.9)/13.0 (±1.2)	1.20 (±0.05)/1.18 (±0.02)	++
11	10	29.9 (±3.9)/21.9 (±2.6)	1.24 (±0.02)/1.21 (±0.03)	+

[a] ΔG, incremental glycemia values over baseline integrated over 120 min after compound administration, the two values refer to the variation in blood glucose of untreated normal rats and treated normal rats, respectively.
[b] G120 is the blood glucose value 120 min after glucose administration.
[c] The score is the ratio of untreated normal rats/treated rats, where + for a ratio value lower than 2, ++ between 2 and 10, and +++ higher than 10. Number of tested rats for each compound was between 4 and 8 (±SEM).

effects; the glycemia remained at a higher level than the basal value of 0.70 g L^{-1}. With the STZ diabetic rats, compound **2** had an interesting activity only at the dose of 30 mg kg^{-1} (Fig. 4a). Replacement of aromatic ring of compound **2** by a cycloalkyl group (compound **3**) resulted in a loss of activity probably due to a higher lipophilicity (Table 6). However, an increase in steric hindrance generated by the cycloalkyl group might also be an explanation.

While compound **4** exhibited an interesting activity (ΔG: 17.6 g L^{-1}/4.6 g L^{-1} at the dose of 30 mg kg^{-1} and 29.9 g L^{-1}/11.9 g L^{-1} at 10 mg kg^{-1}), it was nevertheless lower than compound **2**. Figure 5a shows the superimposition of compounds **2** and **4** (rigid fit): it can be seen that there is a great structural similarity between these two compounds. This reduction in the activity of **4** could result from the bringing together of the imidazoline cycle and the cyclohexyl/phenyl group by removal of the –CH– bridge. This also confers a strong rigidity to this molecule as well as constraints which may prevent it from docking in an optimal way into the imidazoline binding site.

In order to examine the role of the phenyl ring found on compound **4**, this latter was replaced by a methyl group (to give compound **9**). This replacement had no deleterious effects, and the results on normal rats were similar for both compounds at 30 and 10 mg kg^{-1} (Fig. 4b). Compound **10**, with a methyl group in the para position, exhibited poor activity. Thus the presence of a substituent at the α position of the imidazoline ring seems essential.

Table 5. Variation in blood glucose after po administration of imidazoline derivatives to STZ rats

Compound	Dose (mg kg^{-1})	ΔG[a] (g L^{-1})	G120[b] (g L^{-1})	Activity[c]
1	30	57.4 (±10.2)/41.8 (±2.2)	1.54 (±0.06)/1.52 (±0.08)	+
	10	50.6 (±3.6)/42.9 (±12.2)	1.37 (±0.07)/1.38 (±0.04)	–
2	30	54.2 (±9.7)/39.8 (±7.0)	1.34 (±0.05)/1.05 (±0.02)	+
	10	41.3 (±5.9)/23.4 (±4.3)	1.19 (±0.10)/1.05 (±0.02)	+
	3	40.5 (±5.0)/34.8 (±7.9)	1.27 (±0.06)/1.36 (±0.11)	–
9	30	67.9 (±8.7)/38.3 (±3.4)	1.50 (±0.09)/1.44 (±0.05)	+
	10	61.0 (±5.6)/53.5 (±4.2)	1.27 (±0.10)/1.33 (±0.06)	+
10	30	—	—	+
	10	55.6 (±1.5)/63.0 (±3.9)	1.39 (±0.07)/1.46 (±0.07)	–
11	30	40.5 (±7.8)/12.2 (±3.9)	1.18 (±0.07)/1.15 (±0.04)	++

[a] ΔG, incremental glycemia values over baseline integrated over 120 min after compound administration, the two values refer to the untreated STZ rats and treated STZ rats, respectively.
[b] G120 is the blood glucose value 120 min after glucose administration.
[c] The score is the ratio of untreated STZ rats/treated STZ rats, where + for a ratio value lower than 2, ++ between 2 and 10, and +++ higher than 10. Number of tested rats for each compound was between 4 and 8 (±SEM).

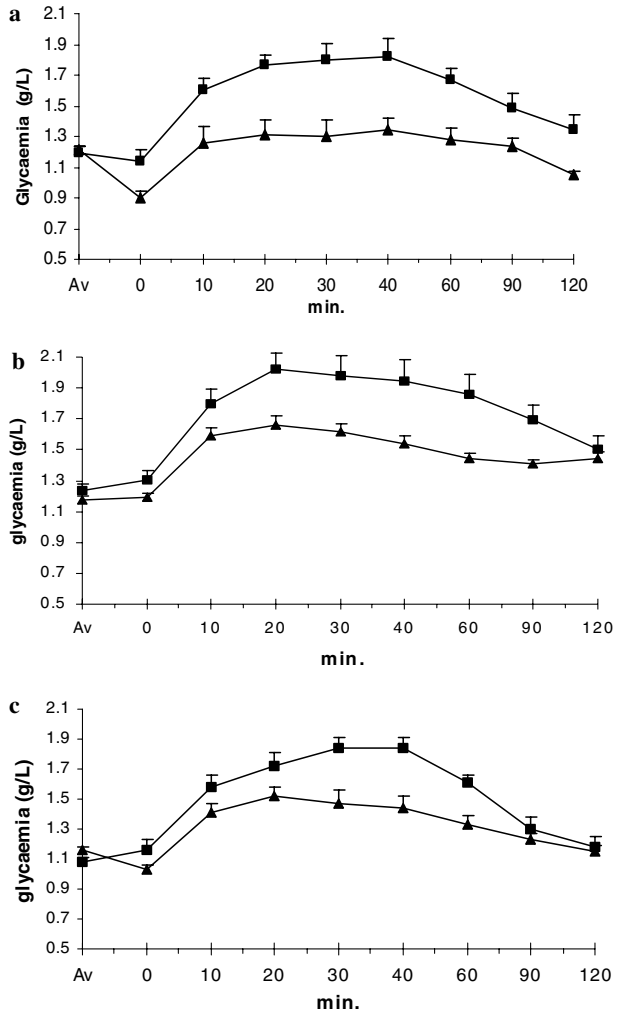


Figure 4. Effect of a single per os administration at 30 mg/kg of compounds **2** (graph **a**), **9** (graph **b**) and **11** (graph **c**) on glucose tolerance: (■) untreated STZ rat and (▲) treated STZ rat. $p < 0.05$. Values are means ± SEM ($n = 6$).

Table 6. pK_a and $c\log P$ values of compounds **1**–**11**

Compound	pK_a[a]	$c\log P$[b]
1	9.1	3.02
2	9.9	5.08
3	8.63	5.6
4	10.2	4.58
5	9.9	5.3
6	10.1	4.02
7	9.8	4.74
8	10.1	2.91
9	10.4	2.98
10	—	2.98
11	—	2.64

[a] The basicity of compounds was assessed by potentiometric titration in aqueous solution.
[b] Calculated from the Ponoma College Medicinal Chemistry program $c\log P$[48].

The reduction in the size of the cycloalkyl group was studied (compounds **4**, **6**, **8**). With compound **6**, carrying a cyclopentyl ring, the antidiabetic activity was preserved, whereas compound **8** with a cyclopropyl ring was inactive. Figures 5a, b and c show the superposition of molecules **4**, **6** and **8** with **2**. One can see that the cyclopentyl group (**6**) occupies the same zone of space as the cyclohexyl of **2**, in the same manner as **4**. Whereas in the case of **8**, the cyclopropyl ring cannot be pointed correctly and occupies a zone of the space which may make it incompatible with good anchoring in the imidazoline binding site. A slight hindrance of the cycloalkyl group seems favourable to good activity.

Compounds **5** and **7**, substituted by a chlorine atom in the para position of the aromatic ring, were found to be less active than the non-substituted compounds **4** and **6**. For the compound **4**, $\Delta G = 29.9$ g L^{-1}/11.9 g L^{-1} and for **5**, $\Delta G = 33.3$ g L^{-1}/25.2 g L^{-1} at a dose of 10 mg kg^{-1}. The phenyl group probably fills a tight area of the imidazoline binding site which does not accommodate the addition of bulky substituents.

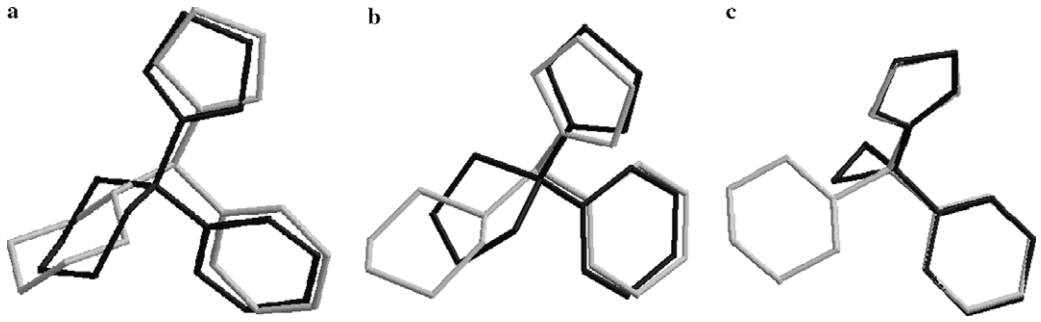


Figure 5. Superimposition of compound **2** (in grey) with (in black) (a) compound **4**; (b) compound **6**; (c) compound **8**.

The activity of the compound **11**, which exhibits structural analogies with midaglizole and cibenzoline, showed an interesting degree of blood glucose reduction: ΔG: 40.5 g L^{-1}/12.2 g L^{-1} at the dose of 30 mg kg^{-1} in STZ rats (Fig. 4c). This compound is the one which gave the best results in this test, though the effectiveness was less marked in normal rats.

It should be noted that all the studied compounds conform to the criteria of Lipinski. As pointed out above, the only real deviation was with the inactive compound **3** which has a *c*log*P* of 5.6 (Table 6). On the other hand, the molecular polar surface area which is acknowledged as another requirement for absorption[46,47] is in the same order as our other compounds (results not shown). We also measured the pK_a values of compounds **1**–**9** (Table 6). These values are in the same order and range from 8.63 (compound **3**) to 10.45 (compound **9**).

4.2. Effect of chemical modifications of the imidazoline group

Results of the chemical modifications of the imidazoline group are reported in Table 7. Substitution of the imidazoline ring by a methyl group in position 4 (compound **2a**) leads to a clear increase in activity. Indeed, this derivative was active at 3 mg kg^{-1} po. The aromatization of the cycle on '1*H*-benzimidazole' derivatives **2b**, **4b** and **9b** and '1*H*, 1*N* and 2*N*-tetrazoles' **2f–h**, **4f–h** and **11f–h** induces a loss of activity. The charges of these compounds, contrary to the imidazolines, are delocalized.

Table 7. Variation of the blood glucose levels after po administration of substituted imidazolines, benzimidazoles, tetrahydropyrimidines, oxazolines, thiazolines, tetrazoles and amidines to normal rats

Compound	Dose (mg kg^{-1})	ΔG[a] (g L^{-1})	G120[b] (g L^{-1})	Activity[c]
2a	10	17.9 (±1.7)/4.5 (±2.0)	1.22 (±0.04)/1.02 (±0.04)	++
2b	30	28.3 (±2.4)/32.2 (±1.5)	1.29 (±0.01)/1.34 (±0.06)	–
2c	10	22.3 (±2.5) /16.5(±3.2)	1.23 (±0.02) /1.18 (±0.05)	–
2d	30	22.9 (±3.10)/24.5 (±2.4)	1.10 (±0.03)/1.21 (±0.04)	–
2e	30	27.0 (±2.1)/24.7 (±1.7)	1.26 (±0.03)/1.20 (±0.03)	–
2f	30	38.7 (±3.3)/35.3 (±4.8)	1.30 (±0.04)/1.36 (±0.04)	–
2g	10	25.1 (±4.2)/21.6 (±2.3)	1.21 (±0.03)/1.21 (±0.02)	–
2h	10	17.9 (±3.3)/31.0 (±3.6)	1.22 (±0.04)/1.18 (±0.02)	–
2i	10	20.34 (±4.5)/22.8 (±3.8)	1.19 (±0.03)/1.08 (±0.04)	–
4a	ND[d]			
4b	10	35.1 (±4.6)/42.5 (±3.4)	1.11 (±0.05)/1.20 (±0.02)	–
4c	10	21.5 (±2.4)/23.0 (±1.9)	1.16 (±0.04)/1.26 (±0.03)	–
4d	10	35.1 (±5.6)/29.6 (±4.4)	1.11 (±0.05)/1.13 (±0.03)	–
4e	10	34.90 (±3.4)/29.3 (±1.5)	1.14 (±0.03)/1.15 (±0.02)	–
4f	10	25.8 (±3.9)/26.1 (±3.5)	1.28 (±0.03)/1.34 (±0.03)	–
4g	10	25.8 (±3.5)/26.3 (±3.0)	1.28 (±0.03)/1.29 (±0.03)	–
4h	30	35.6 (±4.6)/37.5 (±4.0)	1.28 (±0.03)/1.34 (±0.06)	–
9a	10	22.3 (±2.4)/19.27 (±3.5)	1.23 (±0.02)/1.24 (±0.03)	–
9b	10	20.4 (±4.3)/28.9 (±3.8)	1.19 (±0.03)/1.16 (±0.01)	–
9e	10	35.1 (±5.6)/36.5 (±2.5)	1.11 (±0.05)/1.25 (±0.06)	–
11c	10	21.5 (±2.4)/24.0 (±1.6)	1.16 (±0.04) /1.14 (±0.03)	–
11d	30	31.2 (±3.8)/25.5 (±3.2)	1.28 (±0.03)/1.30 (±0.05)	–
11f	10	25.1 (±2.3)/28.5 (±3.9)	1.21 (±0.03)/1.29 (±0.05)	–
11g	10	27.3 (±2.5)/32.0(±3.3)	1.13 (±0.05)/1.07 (±0.03)	–
11h	10	27.3 (±2.5)/32.7 (±4.8)	1.13 (±0.05)/1.24 (±0.05)	–

[a] ΔG, incremental glycemia values over baseline integrated over 120 min after compound administration, the two values refer to the variation in blood glucose of untreated normal rats and treated normal rats, respectively.

[b] G120 is the blood glucose value 120 min after glucose administration.

[c] The score is the ratio of untreated STZ rats/treated STZ rats, where + for a ratio value lower than 2, ++ between 2 and 10, and +++ higher than 10. Number of tested rats for each compound was between 4 and 8 (±SEM).

[d] Not determined.

Replacement of the imidazoline by a Δ-2-oxazoline **2c**, **4c** and **11c** or Δ-2-thiazoline **2d**, **4d**, **11d** did not produce better results. Replacement of one of the nitrogens by an oxygen or a sulphur abolishes the activity completely. The loss of activity could be due to the absence of prototropic rearrangement: the double bond being localized.

The enlarging of the cycle found on the '1,4,5,6-tetrahydropyrimidine' compounds **2e**, **4e** and **9e** involves a loss of activity although their charges, as imidazoline derivatives, are localized on the nitrogens. On the other hand, these compounds exhibit a more significant steric hindrance.

The opening of the cycle which gives the carboxamidine **2i** abolishes the activity. This compound, which is less bulky than the imidazoline analogue, exhibits a charge repartition different from those of the imidazolines, but has the possibility to form hydrogen bonds like imidazolines. The presence of the two carbon atoms at position 4 and 5 found on the imidazolines seems essential.

5. Conclusion

In conclusion, starting from a series of substituted aryl imidazolines, we established the lack of relationship between in vivo antidiabetic activity and in vitro affinities for the I_1/I_2 binding sites for several substituted aryl imidazolines. Among these compounds, 2-(α-cyclohexyl-benzyl)-4,5-dihydro-1*H*-imidazole **2** exhibited a potent antihyperglycemic activity and was chosen as lead compound. Eleven new imidazoline derivatives and 25 imidazoline analogues were synthesized and evaluated for their antidiabetic properties (blood glucose lowering) using glucose tolerance tests. Nine of them, with an imidazoline moiety, possibly substituted by a methyl group have a potent effect on the glucose tolerance in normal or STZ diabetic rats after a po administration of the test compound at a dose of 30 or 10 mg kg^{-1} without any hypoglycemic effect. All attempts to replace the imidazoline groups by benzimidazole, tetrahydropyrimidine, oxazoline, thiazoline, tetrazole, amidine resulted in a total loss of activity.

The structure–activity relationships that have been established show that an unsubstituted or methyl substituted imidazoline moiety is an obligatory requirement for potent antihyperglycemic properties. It was necessary to take into account the flexibility of the molecule which could interact with binding sites and these results indicate that electronic and steric factors seem to be strongly involved in the activity. Finally, it is noteworthy that the potent effects of these new compounds were observed after oral administration.

6. Experimental section

Melting points were determined with a DSC-50 Shimadzu apparatus. Infrared spectra were recorded on a Perkin-Elmer 983G spectrophotometer. NMR spectra were recorded using a Brucker AM 250 MHz spectrometer. For the ^1H and ^{13}C NMR data, chemical shifts are reported in parts per million (δ, ppm) downfield from $CHCl_3$ as an internal standard. NMR coupling constants (J values) are listed in hertz (Hz) and multiplicities are reported as s (singlet), d (doublet), t (triplet) or m (multiplet). The microanalyses (only for tested compounds) were performed in the Microanalytical Laboratory of ENSCT in Toulouse and the results obtained are within ± 0.4% of the theoretical values. Reactions were monitored by thin-layer chromatography (TLC) and product mixtures were purified by column chromatography using silica gel 60 F-254,70-200 Mesh. All yields are calculated for analytical pure materials.

6.1. Preparation of imidazoline derivatives 1, 2, 4 and 11 (Method A)

A stirred mixture of the appropriate nitrile (0.2 mol), ethylenediamine (15 mL, 0.22 mol) freshly distilled on KOH, and 0.15 g P_2S_5 was heated at 120 °C in an oil bath for 4 h. The reaction mixture was then cooled, poured into cold water and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude free base was collected and purified by recrystallisation from cyclohexane.

All these compounds gave the same IR absorption bands towards 3000 cm^{-1} (ν CH, CH_2, CH_3) and 3150 cm^{-1} (ν NH).

6.1.1. 2-Cycloheptyl-4,5-dihydro-1*H*-imidazole (1). Yield 17%, mp 131.2 °C, ^1H NMR ($CDCl_3$) δ 4.90 (s, 1H, NH), 3.87 (s, 4H, $2CH_2$ imidazoline), 2.98 (m, 1H, CH), 1.96–1.50 (m, 12H, H cycloheptyl), Anal. Calcd for $C_{10}H_{18}N_2$ (166.27): C, 72.24; H, 10.91; N, 16.85. Found: C, 72.22; H, 10.85; N, 17.01.

6.1.2. 2-(α-Cyclohexyl-benzyl)-4,5-dihydro-1*H*-imidazole (2). Yield 41%, mp 180.8 °C, ^1H NMR ($CDCl_3$) δ 7.30–7.21 (m, 5H, ArH), 4.25 (s, 1H, NH), 3.50 (s, 4H, $2CH_2$imidazoline), 3.14 (d, 1H, CH, J = 10.2 Hz), 2.01–0.85 (m, 11H, H cyclohexyl), Anal. Calcd for $C_{16}H_{22}N_2$ (242.36): C, 79.29; H, 9.15; N, 11.56. Found: C, 79.14; H, 9.34; N, 11.48.

6.1.3. 2-(1-Phenyl-cyclohex-1-yl)-4,5-dihydro-1*H*-imidazole (4). Yield 14% (method A), 34% (method B), mp 119 °C, ^1H NMR ($CDCl_3$) δ 7.44–7.18 (m, 5H, ArH), 3.72 (m, 5H, NH + $2CH_2$ imidazoline), 2.25–1.31 (m, 10H, H cyclohexyl), Anal. Calcd for $C_{15}H_{20}N_2$ (228.36): C, 78.90; H, 8.83; N, 12.27. Found: C, 79.11; H, 8.52; N, 11.97.

6.1.4. 2-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-4,5-dihydro-1*H*-imidazole (11). Yield 70%, mp 156.4 °C, ^1H NMR ($CDCl_3$) δ 8.49–8.47 (m, 1H, H_6 pyridine), 7.43–6.76 (m, 8H, H_3, H_4, H_5 pyridine + H phenyl), 3.38–3.14 (m, 5H, NH + $2CH_2$ imidazoline), 2.99–2.93 (m, 1H, CH cyclopropyl), 2.03–1.95 (m, 2H, CH_2

_cyclopropyl), Anal. Calcd for $C_{17}H_{17}N_3$ (263.34): C, 77.54; H, 6.51; N, 15.96. Found: C, 77.34; H, 6.73, N, 16.12.

6.2. Preparation of imidazoline derivatives 3, 5, 6, 7, 8, 9 and 10 (Method B)

6.2.1. Synthesis of ester derivatives 3′, 5′–10′. To an ethereal solution of diazomethane containing approximately 5 g (0.12 mol) of diazomethane, cooled at 0 °C, was added slowly 0.06 mol of appropriate carboxylic acid. After stirring at room temperature for 24 h, the solvent was evaporated and the crude product was used without purification. All these compounds are oils.

All these compounds gave the same IR absorption bands towards 3000 cm^{-1} (ν CH, CH_2, CH_3) and 1730 cm^{-1} (ν C=O).

6.2.1.1. Dicyclohexyl acetic acid, methyl ester (3′). Yield: 98%, ^{1}H NMR ($CDCl_3$) δ 3.59 (s, 3H, CH_3), 2.05–1.99 (t, 1H, CH–CO), 1.66-0.74 (m, 22H, H cyclohexyl).

6.2.1.2. 1-(4-Chlorophenyl)-1-cyclohexanecarboxylic acid, methyl ester (5′). Yield: 98%, ^{1}H NMR ($CDCl_3$) d 7.33–7.24 (m, 4H, ArH),3.62 (s, 3H, CH_3), 2.46–1.17 (m, 10H, H cyclohexyl).

6.2.1.3. 1-Phenyl-1-cyclopentanecarboxylic acid, methyl ester (6′). Yield: 98%, ^{1}H NMR ($CDCl_3$) δ 7.39–7.19 (m, 5H, ArH), 3.60 (s, 3H, CH_3), 2.70–1.66 (m, 8H, H cyclopentyl).

6.2.1.4. 1-(4-Chlorophenyl)-1-cyclopentane carboxylic acid, methyl ester (7′). Yield: 88%, ^{1}H NMR ($CDCl_3$) δ 7.10–7.04 (m, 4H, ArH), 3.64 (s, 3H, CH_3), 2.50–2.15 (m, 4H, H cyclopentyl), 1,75–1.54 (m, 4H, H cyclopentyl).

6.2.1.5. 1-Phenyl-1-cyclopropane carboxylic acid, methyl ester (8′). Yield: 92%, ^{1}H NMR ($CDCl_3$) δ 7.39–7.25 (m, 5H, ArH), 3.63 (s, 3H, CH_3), 1.65–1.19 (m, 4H, H cyclopropyl).

6.2.1.6. 1-Methyl-1-cyclohexane carboxylic acid, methyl ester (9′). Yield: 98%, ^{1}H NMR ($CDCl_3$) δ 3.66 (s, 3H, O-CH_3), 2.03–1.15 (m, 10H, H cyclohexyl), 1.13 (s; 3H, CH_3).

6.2.1.7. 4-Methyl-1-cyclohexane carboxylic acid, methyl ester (10′). Yield: 97%, ^{1}H NMR ($CDCl_3$) δ 3.64 (s, 3H, O-CH_3), 0.85 (m, 10H, H cyclohexyl and CH_3).

6.2.2. Synthesis of imidazoline derivatives 3, 5–10. A solution of ester (0.03 mol) in anhydrous toluene (100 mL) was added dropwise, at 0 °C, to a previously prepared solution of trimethylaluminium ((3.17 g, 0.044 mol) of a 2 M solution in toluene) and ethylenediamine (2.64 g, 0.44 mol) in anhydrous toluene (100 mL), so that the temperature did not exceed 10 °C. The solution was refluxed with stirring for 12 h. After cooling, the solution was treated dropwise with water (12 mL) diluted with MeOH (30 mL) and CH_2Cl_2 (30 mL) and the mixture heated again under reflux for 15 min. The $Al(OH)_3$ was then removed by filtration and the organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product obtained was extracted with CH_2Cl_2. The extracts were dried, the solvent removed under reduced pressure and the crude free base was collected and purified by recrystallisation from cyclohexane or hexane.

6.2.2.1. 2-(α-Dicyclohexylmethyl)-4,5-dihydro-1*H*-imidazole (3). Yield 20%, mp 155.5 °C, ^{1}H NMR ($CDCl_3$) δ 5.79 (s, 1H, NH), 3.32–3.25 (m, 2H, *CH_2*–NH), 2.82–2.78 (m, 2H, *CH_2*–N), 1.68-0.86 (m, 23H, H cyclohexyl), Anal. Calcd for $C_{16}H_{28}N_2$ (248.41): C, 77.36; H, 11.36; N, 11.28. Found: C, 77.45; H, 11.18; N, 10.99.

6.2.2.2. 2-[1-(4-Chlorophenyl)-cyclohex-1-yl]-4,5-dihydro-1*H*-imidazole (5). Yield 46%, mp 152 °C, ^{1}H NMR ($CDCl_3$) δ 7.35–7.25 (m, 4H, ArH), 3.42–2.65 (m, 5H, NH + $2CH_2$ imidazoline), 2.21–1.28 (m, 10H, H cyclohexyl), Anal. Calcd for $C_{15}H_{19}ClN_2$ (262.78): C, 68.56; H, 7.29; N, 10.66; Cl, 13.49. Found: C, 68.89; H, 7.14; N, 10.42.

6.2.2.3. 2-(1-Phenyl-cyclopent-1-yl)-4,5-dihydro-1*H*-imidazole (6). Yield 40%, mp 136.3 °C, ^{1}H NMR ($CDCl_3$) δ 7.36–7.15 (m, 5H, ArH), 3.93 (s, 1H, NH), 3.66–3.43 (m, 4H, $2CH_2$ imidazoline), 2.48–1.64 (m, 8H, H cyclopentyl), Anal. Calcd for $C_{14}H_{18}N_2$ (214.31): C, 78.46; H, 8.46; N, 13.07. Found: C, 78.62; H, 8.25; N, 13.14.

6.2.2.4. 2-[1-(4-Chlorophenyl)-cyclopent-1-yl]-4,5-dihydro-1*H*-imidazole (7). Yield 30%, mp 147 °C, ^{1}H NMR ($CDCl_3$) δ 7.32–7.28 (m, 4H, ArH), 3.89–3.31 (m, 5H, NH + $2CH_2$ imidazoline), 2.46–1.60 (m, 8H, H cyclopentyl), Anal. Calcd for $C_{14}H_{17}ClN_2$ (248.76): C, 67.60; H, 6.89; N, 11.26; Cl, 14.25. Found: C, 67.82; H, 6.78; N, 11.13.

6.2.2.5. 2-(1-Phenyl-cycloprop-1-yl)-4,5-dihydro-1*H*-imidazole (8). Yield 70%, mp 93 °C, ^{1}H NMR ($CDCl_3$) δ 7.37–7.18 (m, 5H, ArH), 4.12 (s, 1H, NH), 3.49 (s, 4H, $2CH_2$ imidazoline), 1.51–1.07 (m, 4H, H cyclopropyl), Anal. Calcd for $C_{12}H_{14}N_2$ (186.26): C, 77.38; H, 7.58; N, 15.04. Found: C, 77.56; H, 7.23; N, 14.87.

6.2.2.6. 2-(1-Methyl-cyclohex-1-yl)-4,5-dihydro-1*H*-imidazole (9). Yield 13%, mp 90.3 °C, ^{1}H NMR ($CDCl_3$) δ 4.27 (s, 1H, NH), 3.51 (s, 4H, $2CH_2$ imidazoline), 1.84–1.77 (m, 2H, 2Heq. cyclohexyl), 1.53–1.25 (m, 8H, $3CH_2$ + 2Hax. cyclohexyl), 1.10 (s, 3H, CH_3), Anal. Calcd for $C_{10}H_{18}N_2$ (166.27): C, 72.24; H, 10.91; N, 16.85. Found: C, 71.98; H, 11.12; N, 16.58.

6.2.2.7. 2-(Trans-4-methyl-cyclohexyl)-4,5-dihydro-1*H*-imidazole (10). Yield 15%, mp 156 °C, ^{1}H NMR ($CDCl_3$) δ 3.53 (m, 5H, NH + $2CH_2$ imidazoline), 2.07 (m, 1 H, H_1), 1.86 (m, 4H, $2CH_2$ cyclohexyl),

1.30 (m, 4H, $2CH_2$ cyclohexyl), 0.87 (m, 1H, *CH*–CH_3), 0.84 (d, 3H, CH_3), Anal. Calcd for $C_{10}H_{18}N_2$ (166.27): C, 72.24; H, 10.91; N, 16.85. Found: C, 71.92; H, 11.09; N, 17.03.

6.3. Preparation of compound 2a

The α-cyclohexyl phenyl thioacetamide intermediate was prepared as described in our previous publication[35] using the corresponding nitrile.

6.3.1. α-Cyclohexyl phenyl thioacetamide. Yield 11%, mp 170 °C, ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H, NH), 7.40–7.21 (m, 5H, ArH), 6.88 (s, 1H, NH), 3.44 (d, 1H, CH), 2.30–0.71 (m, 11H, cyclohexyl). IR ν (KBr, cm^{-1}): 3365, 3345 (NH_2), 3163, 2931, 2851 (CH_2, CH), 1610 (C=C), 1261 (C=S).

A stirred mixture of α-cyclohexyl phenyl thioacetamide (0.2 mol) and EDA in stoichiometric quantity, freshly distilled on KOH, was heated at 120 °C in an oil bath for 3 days. The reaction mixture was then cooled, poured into cold water and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$. The solvent was removed in vacuo. The crude free base was collected, chromatographed with EtOH as eluent and recrystallised from cyclohexane.

6.3.2. 2-(α-Cyclohexyl-benzyl)-4-methyl-4,5-dihydro-1*H*-imidazole (2a). Yield 11%, mp 151.9 °C, ^{1}H NMR ($CDCl_3$) δ 7.44–7.18 (m, 5H, ArH), 4.15 (m, 1H, *CH*–CH_3), 3.77–3.34 (m, 2H, CH_2 imidazoline), 3.13–3.09 (d, 1H, CH, J = 10,3 Hz), 2.16–0.69 (m, 15H, H cyclohexyl + NH + CH_3); IR ν (KBr, cm^{-1}): 3147 (NH), 3030, 2924, 2852 (CH, CH_2, CH_3), 1594 (C=N and C=C). Anal. Calcd for $C_{17}H_{24}N_2$ (256.39): C, 79.64; H, 9.43; N, 10.93. Found: C, 79.97; H, 9.24; N, 11.13.

6.4. Preparation of derivatives 4a and 9a

A mixture of corresponding nitrile (0.2 mol), 1,2-diaminopropane (DAP) (0.22 mol) and 0.15 g of P_2S_5 was stirred and refluxed. The reaction was monitored by thin-layer chromatography. The reaction mixture was then cooled, poured into water and extracted with CH_2Cl_2. The extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure and the crude product was recrystallised from cyclohexane.

6.4.1. 2-(1-Phenyl-cyclohex-1-yl)-4-methyl-4,5-dihydro-1*H*-imidazole (4a). Yield 13%, mp 130 °C, ^{1}H NMR ($CDCl_3$) δ 7.44–7.18 (m, 5H, ArH), 3.72–3.17 (m, 3H, *CH*–CH_3 + CH_2 imidazoline), 2.19–1.05 (m, 14H, NH + CH_3 + H cyclohexyl); IR ν (KBr, cm^{-1}): 3185 (NH), 3060, 2954, 2921, 2857 (CH, CH_2, CH_3), 1624, 1585, 1491 (C=N and C=C). Anal. Calcd for $C_{16}H_{22}N_2$ (242.36): C, 79.29; H, 9.15; N, 11.56. Found: C, 79.02; H, 9.07; N, 11.92.

6.4.2. 2-[(1-Methyl-cyclohex-1-yl)-4-methyl-4,5-dihydro-1*H*-imidazole (9a). Yield 13%, mp 76 °C, ^{1}H NMR ($CDCl_3$) δ 3.97–3.68 (m, 3H, *CH*–CH_3 + CH_2 imidazoline), 3.17 (s, 1H, NH), 1.87–1.26 (m, 13H, H cyclohexyl + CH_3), 1.12 (s, 3H, CH_3), IR ν (KBr, cm^{-1}): 3159 (NH), 2958, 2924, 2859 (CH_2, CH_3), 1588 (C=N). Anal. Calcd for $C_{11}H_{20}N_2$ (180.29): C, 73.28; H, 11.18; N, 15.54. Found: C, 73.52; H, 11.42; N, 15.21.

6.5. Preparation of 1*H*-benzimidazole derivatives 2b, 4b and 9b

6.5.1. Preparation of amide derivatives 2b′, 2b″, 4b′, 4b″, 9b′, 9b″. To a mixture of 1,2-phenylenediamine (0.02 mol) and triethylamine (0.04 mol) dissolved in anhydrous toluene was added dropwise the appropriate acid chloride diluted in toluene (20 mL). After stirring at room temperature for 18 h, the resulting mixture was evaporated under reduced pressure. The solid was washed with water (100 mL). The precipitate was collected by filtration and then purified by column chromatography with CH_2Cl_2 as eluent.

All amide compounds gave the same IR absorption bands towards 3300 cm^{-1} (ν NH), 3000 cm^{-1} (ν CH, CH_2), 1640 cm^{-1} (ν C=O).

6.5.1.1. *N*-(2′-aminophenyl)-2-cyclohexyl-2-phenyl-acetamide (2b′). Yield 30%, mp 202 °C, ^{1}H NMR ($CDCl_3$) δ 7.41–6.71 (m, 10H, NH + ArH), 3.52 (s, 2H, NH_2), 3.15–3.11 (d, 1H, CH, J = 10.12 Hz), 2.24–0.75 (m, 11H, H cyclohexyl).

6.5.1.2. 1,2-*N*,*N*′-phenyl-bis-(2-cyclohexyl-2-phenyl-acetamide) (2b″). Yield 10%, mp 111 °C, ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 2H, 2NH), 7.41–6.90 (m, 14H, ArH), 2.91–2.87 (d, 2H, CH, J = 10,36 Hz), 2.12–0.67 (m, 22H, H cyclohexyl).

6.5.1.3. *N*-(2′-aminophenyl)-1-phenyl-cyclohex-1-yl-carboxamide (4b′). Yield 22%, mp 146 °C, ^{1}H NMR ($CDCl_3$) δ 7.53–6.67 (m, 9H, ArH), 6.78 (s, 1H, NH); 3.45 (s, 2H, NH_2), 2.43–1.39 (m, 10H, H cyclohexyl).

6.5.1.4. 1,2-*N*,*N*′-phenyl-bis-(1-phenyl-cyclohex-1-yl-carboxamide) (4b″). Yield 18%, mp °C, ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 2H, 2NH), 7.38–6.98 (m, 14H, ArH), 2.28–1.40 (m, 20H, H cyclohexyl).

6.5.1.5. *N*-(2′-aminophenyl)-1-methyl-cyclohexane-1-carboxamide (9b′). Yield 19%, mp 140.8 °C, ^{1}H NMR ($CDCl_3$) δ 7.66–6.76 (m, 5H, NH + ArH), 3.79 (s, 2H, NH_2), 2.16–1.37 (m, 10H, H cyclohexyl), 1.29 (s, 3H, CH_3).

6.5.1.6. 1,2-*N*,*N*′-phenyl-bis-(1-methyl-cyclohexane-1-carboxamide) (9b″). Yield 27%, mp 142 °C, ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 2H, 2NH), 7.44–7.14 (m, 4H, ArH), 2.04–1.30 (m, 20H, H cyclohexyl), 1.25 (s, 6H, $2CH_3$).

6.5.2. Preparation of 1*H*-benzimidazole derivatives 2b, 4b and 9b. To 2 mmol of monoamide **b**′ dissolved in ethanol (10 mL) was added concentrated HCl (7 mL). The mixture was stirred and refluxed for 10 h. After cooling, the reaction mixture was neutralised with aqueous ammonia (15%), and the resulting solid filtered and crystallised from chloroform.

6.5.2.1. 2-(α-Cyclohexyl-benzyl)-1*H*-benzimidazole (2b). Yield 54%, mp 234.7 °C, ^{1}H NMR ($CDCl_3$) δ 9.01 (s, 1H, NH), 7.66–7.16 (m, 9H, ArH), 3.85–3.81 (d, 1H, CH, J = 10.13 Hz), 2.45–0.82 (m, 11H, H cyclohexyl), IR ν (KBr, cm^{-1}): 3220 (NH), 3077, 2930, 2849 (CH, CH_2), 1671, 1528, 1493 (C=N and C=C). Anal. Calcd for $C_{20}H_{22}N_2$ (290.41): C, 82.72; H, 7.63; N, 9.65. Found: C, 82.57; H, 7.35; N, 9.97.

6.5.2.2. 2-(1-Phenyl-cyclohex-1-yl)-1*H*-benzimidazole (4b). Yield 44%, mp 277.5 °C, ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H, NH), 7.80–7.16 (m, 9H, ArH), 2.61–1.39 (m, 10H, H cyclohexyl), IR ν (KBr, cm^{-1}): 3151 (NH), 3077, 3018, 2989, 2957, 2930 (CH, CH_2), 1619, 1601 (C=N and C=C). Anal. Calcd for $C_{19}H_{20}N_2$ (276.38): C, 82.57; H, 7.29; N, 10.14. Found: C, 82.86; H, 7.46; N, 9.97.

6.5.2.3. 2-(1-Methyl-cyclohex-1-yl)-1*H*-benzimidazole (9b). Yield 76%, mp 282 °C, ^{1}H NMR ($CDCl_3$) δ 9.06 (s, 1H, NH), 7.66–7.08 (m, 4H, ArH), 2.28–1.46 (m, 10H, H cyclohexyl), 1.37 (s, 3H, CH_3), IR ν (KBr, cm^{-1}): 3147 (NH), 3066, 2989, 2957, 2930 (CH, CH_2, CH_3), 1620, 1590 (C=N and C=C). Anal. Calcd for $C_{14}H_{18}N_2$ (214.31): C, 78.46; H, 8.46; N, 13.07. Found: C, 78.36; H, 8.12; N, 13.25.

6.6. Preparation of Δ-2-oxazoline derivatives 2c, 4c and 11c

To 25 mmol of the appropriate nitrile dissolved in 25 mL of ethanolamine (0.05 mol) was added anhydrous $CaCl_2$ (25 mmol, 2.8 g) or $ZnCl_2$ (25 mmol, 3.4 g). The reaction mixture was heated with stirring for 24 h at 120 °C. After cooling, the mixture was poured onto crushed ice (adjusting the pH to 7) and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and the solvent was removed in vacuo. To the resulting oil, NaOH 2 N (10 mL), cyclohexane (10 mL) were added and the mixture was stirred for 1 h on an ice bath. The solid that separated out was filtered off and washed with water. The crude product was purified by column chromatography using cyclohexane/CH_2Cl_2 (9:1) as eluent.

6.6.1. 2-(α-Cyclohexyl-benzyl)-Δ-2-oxazoline (2c). Yield 9%, mp 71.5 °C, ^{1}H NMR ($CDCl_3$) δ 7.35–7.16 (m, 5H, ArH), 4.22–4.18 (m, 2H, CH_2-O), 3.85–3.73 (m, 2H, CH_2–N), 3.30–3.26 (d, 1H, CH, J = 10.56 Hz), 2.00–1.01 (m, 11H, H cyclohexyl), IR ν (KBr, cm^{-1}): 3062, 3028, 2966, 2935, 2925 (CH, CH_2), 1661, 1599 (C=N and C=C). Anal. Calcd for $C_{16}H_{21}NO$ (243.34): C, 78.97; H, 8.70; N, 5.76. Found: C, 79.21; H, 8.45; N, 5.88.

6.6.2. 2-(1-Phenyl-cyclohex-1-yl)-Δ-2-oxazoline (4c). Yield 8% ($ZnCl_2$), mp 67.8 °C, ^{1}H NMR ($CDCl_3$) δ 7.44–7.17 (m, 5H, ArH), 4.17–4.09 (m, 2H, CH_2-O), 3.90–3.82 (m, 2H, CH_2–N), 2.56–1.53 (m, 10H, H cyclohexyl), IR ν (KBr, cm^{-1}): 3060, 2969, 2936, 2921 (CH, CH_2), 1646, 1591 (C=N and C=C). Anal. Calcd for $C_{15}H_{19}NO$ (229.32): C, 78.57; H, 8.35; N, 6.11. Found: C, 78.27; H, 8.46; N, 5.98.

6.6.3. 2-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-Δ-2-oxazoline (11c). Yield 8%, mp 230.8 °C, ^{1}H NMR ($CDCl_3$) δ 8.44-8.41 (m, 1H, H_6 pyridine), 7.50-6.84 (m, 8H, H_3, H_4, H_5 pyridine + H phenyl), 4.08-3.41 (m, 4H, $2CH_2$oxazoline), 3.29–3.23 (t, 1H, H cyclopropyl), 2.10–2.07 (d, 2H, H cyclopropyl). IR ν (KBr, cm^{-1}): 3050, 3009, 2980, 2917 (CH, CH_2), 1625, 1599 (C=N and C=C). Anal. Calcd for $C_{17}H_{16}N_2O$ (264.32): C, 77.25; H, 6.10; N, 10.60. Found: C, 76.97; H, 6.23; N, 10.72.

6.7. Preparation of Δ-2-thiazolines derivatives 2d, 4d and 11d

To 0.01 mol of the corresponding nitrile dissolved in EtOH (40 mL) was added cysteamine hydrochloride (1.14 g, 0.01 mol) in NaOH 1 N (20 mL). The solution was strirred and refluxed for 5 days. After cooling, the solvent was removed under reduced pressure. The crude product was chromatographed with CH_2Cl_2 as eluent.

6.7.1. 2-(α-Cyclohexyl-benzyl)-Δ-2-thiazoline (2d). Yield 33%, mp 60.3 °C, ^{1}H NMR ($CDCl_3$) δ 7.33–7.19 (m, 5H, ArH), 4.30–4.10 (m, 2H, CH_2–N), 3.50–3.46 (d, 1H, CH, J = 10.6 Hz), 3.21-3.13 (m, 2H, CH_2–S), 2.16–0.71 (m, 11H, H cyclohexyl), IR ν (KBr, cm^{-1}): 3061, 3025, 2921, 2849 (CH, CH_2), 1618, 1596 (C=N and C=C). Anal. Calcd for $C_{16}H_{21}NS$ (259.41): C, 74.08; H, 8.16; N, 5.40; S, 12.36. Found: C, 74.32; H, 8.26; N, 5.12.

6.7.2. 2-(1-Phenyl-cyclohex-1-yl)-Δ-2-thiazoline (4d). Yield 15%, mp 52.3 °C, ^{1}H NMR ($CDCl_3$) δ 7.95–7.17 (m, 5H, ArH), 4.34–4.18 (m, 1H, CH_2–N), 3.45–3.02 (m, 1H, CH_2–N), 2.35–1.26 (m, 12H, CH_2–S + H cyclohexyl), IR ν (KBr, cm^{-1}): 3086, 3028, 2996, 2972 (CH, CH_2), 1612, 1596 (C=N and C=C). Anal. Calcd for $C_{15}H_{19}NS$ (245.39): C, 73.42; H, 7.80; N, 5.71; S, 13.07. Found: C, 73.22; H, 7.53; N, 5.97.

6.7.3. 2-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-Δ-2-thiazoline (11d). Yield 31%, mp 91.9 °C, ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 1H, H_6 pyridine), 7.34–7.18 (m, 6H, H phenyl + H_5 pyridine), 7.03 (m, 1H, H_3 pyridine), 6.77 (m, 1H, H_4 pyridine), 4.07 (m, 1H, CH_2–N), 3.59 (m, 1H, CH_2–N), 2.68 (m, 1H, H cyclopropyl), 3.06 (m, 2H, CH_2–S), 2.22 (m, 1H, CH_2 cyclopropyl), 2.04 (m, 1H, CH_2 cyclopropyl), IR ν (KBr, cm^{-1}): 3077, 3021, 2978, 2950 (CH, CH_2), 1624, 1581 (C=N and C=C). Anal. Calcd for $C_{17}H_{16}N_2S$ (280.39): C, 72.82; H, 5.75; N, 9.99; S, 11.44. Found: C, 72.65; H, 5.89; N, 10.12.

6.8. Preparation of 1,4,5,6-tetrahydropyrimidine derivatives 2e, 4e and 9e

These derivatives were prepared as described for **2a**, **4a** and **9a**, using 1,3-DAP instead of 1,2-DAP.

6.8.1. 2-(α-Cyclohexyl-benzyl)-1,4,5,6-tetrahydropyrimidine (2e). Yield 65%, mp 185.4 °C, ^{1}H NMR ($CDCl_3$) δ 7.32–7.17 (m, 5H, ArH), 3.28–3.24 (m, 5H, NH + $2CH_2$ pyrimidine), 2.83–2.79 (d, 1H, CH, J = 10.7 Hz), 2.16–0.63 (m, 13H, H cyclohexyl + CH_2

pyrimidine), IR ν (KBr, cm^{-1}): 3201 (NH), 3024, 2924, 2850 (CH, CH_2), 1623 (C=N and C=C). Anal. Calcd for $C_{17}H_{24}N_2$ (256.39): C, 79.64; H, 9.43; N, 10.93. Found: C, 79.85; H, 9.28; N, 10.62.

6.8.2. 2-(1-Phenyl-cyclohex-1-yl)-1,4,5,6-tetrahydropyrimidine (4e). Yield 32%, mp 135 °C, ^1H NMR ($CDCl_3$) δ 7.44–7.17 (m, 5H, ArH), 3.98 (s, 1H, NH), 3.42–3.10 (m, 4H, $2CH_2$ pyrimidine), 2.18–1.34 (m, 12H, H cyclohexyl, CH_2 pyrimidine), IR ν (KBr, cm^{-1}): 3258 (NH), 3088, 3032, 2962, 2920 (CH, CH_2), 1630, 1610 (C=N and C=C). Anal. Calcd for $C_{16}H_{22}N_2$ (242.36): C, 79.29; H, 9.15; N, 11.56. Found: C, 79.54; H, 8.97; N, 11.42.

6.8.3. 2-(1-Methyl-cyclohex-1-yl)-1,4,5,6-tetrahydropyrimidine (9e). Yield 36%, mp 118 °C, ^1H NMR ($CDCl_3$) δ 4.50 (s, 1H, NH), 3.32 (m, 4H, $2CH_2$ pyrimidine), 1.81–1.23 (m, 12H, H cyclohexyl, CH_2 pyrimidine), 1.08 (s, 3H, CH_3), IR ν (KBr, cm^{-1}): 3152 (NH), 2934, 2859 (CH_2, CH_3), 1631, 1609, 1571 (C=N and C=C). Anal. Calcd for $C_{11}H_{20}N_2$ (180.29): C, 73.28; H, 11.18; N, 15.54. Found: C, 72.97; H, 10.89; N, 15.74.

6.9. Preparation of 1*H*-tetrazole derivatives 2f, 4f and 11f

To 100 mL THF precooled in an ice bath were added 0.025 mol of nitrile, 0.15 mol (9.75 g) of sodium azide, and 0.025 mol (3.3 g) of pulverised anhydrous $AlCl_3$. The mixture was then refluxed for 4 days with stirring. After cooling, 60 mL of HCl (15%) was added and the solution was extracted by CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was recrystallised from cyclohexane.

6.9.1. 5-(α-Cyclohexyl-benzyl)-1*H*-tetrazole (2f). Yield 82%, mp 206 °C, ^1H NMR ($CDCl_3$) δ 7.66–7.27 (m, 5H, ArH), 4.02–3.98 (d, 1H, CH, J = 9.85 Hz), 2.36–0.79 (m, 12H, NH + H cyclohexyl), IR ν (KBr, cm^{-1}): 3144 (NH), 3085, 2918, 2853 (CH, CH_2), 1557 (C=N and C=C). Anal. Calcd for $C_{14}H_{18}N_4$ (242.32): C, 69.39; H, 7.49; N, 23.12. Found: C, 69.70; H, 7.23; N, 23.02.

6.9.2. 2-(1-Phenyl-cyclohex-1-yl)-1*H*-tetrazole (4f). Yield 18%, mp 160.7 °C, ^1H NMR ($CDCl_3$) δ 7.41–7.17 (m, 5H, ArH), 2.58–1.41 (m, 11H, NH + H cyclohexyl), IR ν (KBr, cm^{-1}): 3091 (NH), 2944, 2855 (CH, CH_2), 1596 (C=N and C=C). Anal. Calcd for $C_{13}H_{16}N_4$ (228.29): C, 68.39; H, 7.06; N, 24.54. Found: C, 68.67; H, 6.82; N, 24.23.

6.9.3. 5-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-1*H*-tetrazole (11f). Yield 75%, mp 183.7 °C, ^1H NMR ($CDCl_3$) δ 8.59–8.57 (m, 1H, H_6 pyridine), 7.63–7.05 (m, 8H, H_3, H_4, H_5 pyridine + H phenyl), 3.63–3.58 (m, 1H, CH cyclopropyl), 2.48–2.44 (m, 1H, $CH_{2\beta}$cyclopropyl), 2.23–2.19 (m, 1H, $CH_{2\alpha}$cyclopropyl), 1.27 (s large, 1H, NH), IR ν (KBr, cm^{-1}): 3126 (NH), 3087, 3043, 3006 (CH, CH_2), 1587 (C=N and C=C). Anal. Calcd for $C_{15}H_{13}N_5$ (263.30): C, 68.42; H, 4.98; N, 26.60. Found: C, 68.72; H, 5.12; N, 26.07.

6.10. Preparation of 1*N* and 2*N*-methyl-tetrazoles 2g–h, 4g–h and 11g–h

To a solution of diazomethane (0.11 mol, 4.6 g) in diethyl ether (600 mL) prepared according to Boer's method[49] was added the tetrazole derivative (0.016 mol). The mixture was maintained at room temperature for a week. The solvent was evaporated and the residue was chromatographed with cyclohexane/CH_2Cl_2 (7:3) as eluent to give 1*N*-methyl tetrazoles (**g** or **h**) and 2*N*-methyl-tetrazoles (**g** or **h**).

6.10.1. 5-(α-Cyclohexyl-benzyl)-1*N*-methyl-tetrazole (2g). Yield 10%, mp 115.5 °C, ^1H NMR ($CDCl_3$) δ 7.31–7.27 (m, 5H, ArH), 3.86 (s, 3H, N–CH_3), 3.72–3.69 (d, 1H, CH, J = 10,4 Hz), 2.45–2.37 (m, 1H, H cyclohexyl), 1.77–0.85 (m, 10H, H cyclohexyl); ^{13}C NMR ($CDCl_3$) δ 156.99, 137.74, 129.29 (2C), 128.94 (2C), 128.03, 48.40, 42.19, 33.77, 32.50, 31.40, 26.61, 26.34, 26.32. IR ν (KBr, cm^{-1}): 3060, 3027, 2910, 2867 (CH, CH_2), 1598 (C=N, C=C and N=N). Anal. Calcd for $C_{15}H_{20}N_4$ (256.35): C, 70.28; H, 7.86; N, 21.86. Found: C, 70.03; H, 7.97; N, 22.10.

6.10.2. 5-(α-Cyclohexyl-benzyl)-2*N*-methyl-tetrazole (2h). Yield 88%, mp 99 °C, ^1H NMR ($CDCl_3$) δ 7.45–7.44 (m, 2H, $H_{6'}$, $H_{2'}$), 7.33–7.29 (m, 2H, $H_{5'}$, $H_{3'}$), 7.22–7.20 (m, 1H, $H_{4'}$), 4.29 (s, 3H, N–CH_3), 3.99–3.97 (d, 1H, CH, J = 10.45 Hz), 2.29–2.18 (m, 1H, H cyclohexyl), 1.71–0.85 (m, 10H, H cyclohexyl); ^{13}C NMR ($CDCl_3$) δ 168.98, 140.87, 129.07 (2C), 128.85 (2C), 127.22, 50.60, 42.69, 39.71, 32.33, 32.17, 26.71, 26.46, 26.41. IR ν (KBr, cm^{-1}): 3065, 3025, 2925, 2849 (CH, CH_2, CH_3), 1600 (C=N, C=C and N=N). Anal. Calcd for $C_{15}H_{20}N_4$ (256.35): C, 70.28; H, 7.86; N, 21.86. Found: C, 70.42; H, 7.51; N, 21.53.

6.10.3. 5-(1-Phenyl-cyclohex-1-yl)-1*N*-methyl-tetrazole (4g). Yield 10%, mp 106 °C, ^1H NMR ($CDCl_3$) δ 7.39–7.32 (m, 2H, $H_{3'}$, $H_{5'}$), 7.30–7.25 (m, 1H, $H_{4'}$), 7.19–7.17 (m, 2H, $H_{2'}$, $H_{6'}$), 3.50 (s, 3H, N–CH_3), 2.59–1.40 (m, 10H, H cyclohexyl); ^{13}C NMR ($CDCl_3$) δ 159.59, 143.85, 129.43 (2C), 127.75, 126.62 (2C), 42.91, 36.49 (2C), 35.40, 25.97, 23.03 (2C), IR ν (KBr, cm^{-1}): 3086, 3048, 3026, 2929, 2855 (CH, CH_2, CH_3), 1596 (C=N, C=C and N=N). Anal. Calcd for $C_{14}H_{18}N_4$ (242.32): C, 69.39; H, 7.49; N, 23.12. Found: C, 69.55; H, 7.13; N, 22.98.

6.10.4. 5-(1-Phenyl-cyclohex-1-yl)-2*N*-methyl-tetrazole (4h). Yield 87%, mp 93.6 °C, ^1H NMR ($CDCl_3$) δ 7.40–7.37 (m, 2H, $H_{2'}$, $H_{6'}$), 7.33–7.28 (m, 2H, $H_{3'}$, $H_{5'}$), 7.21–7.17 (m, 1H, $H_{4'}$), 4.31 (s, 3H, N–CH_3), 2.78–1.33 (m, 10H, H cyclohexyl); ^{13}C NMR ($CDCl_3$) δ 172.14, 146.87, 128.80 (2C), 126.73, 126.58 (2C), 42.99, 39.81, 36.31 (2C), 26.13, 23.28 (2C), IR ν (KBr, cm^{-1}): 3087, 3064, 3018, 2962, 2858 (CH, CH_2, CH_3), 1596 (C=N, C=C and N=N). Anal. Calcd for $C_{14}H_{18}N_4$ (242.32): C, 69.39; H, 7.49; N, 23.12. Found: C, 69.72; H, 7.57; N, 22.81.

6.10.5. 5-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-1*N*-methyl-tetrazole (11g). Yield 71%, mp 161.1 °C, ^1H

NMR ($CDCl_3$) δ 8.57–8.56 (m, 1H, $H_{3''}$), 7.50–7.49 (m, 1H, $H_{5''}$), 7.21-7.07 (m, 6H, $H_{4''}$, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 6.88-6.86 (d, 1H, $H_{6''}$), 4.06 (s, 3H, N–CH_3), 3.55–3.52 (m, 1H, $H_{1'''}$), 2.72–2.69 (m, 1H, $H_{\beta 3''}$ cyclopropyl), 2.15–2.11 (m, 1H, $H_{\alpha 3''}$ cyclopropyl); ^{13}C NMR ($CDCl_3$) δ 161.70, 154.23, 149.50, 137.00, 136.67, 130.77 (2C), 129.14 (2C), 128.13, 123.25, 121.91, 41.02, 33.81, 21.86, 20.17. IR ν (KBr, cm^{-1}): 3102, 3088, 3038, 2960 (CH, CH_2, CH_3), 1588 (C=N, C=C and N=N). Anal. Calcd for $C_{16}H_{15}N_5$ (277.33): C, 69.30; H, 5.45; N, 25.25. Found: C, 69.52; H, 5.54; N, 25.07.

6.10.6. 5-[2-Phenyl-2-(pyridin-2-yl)-cyclopropyl]-2*N*-methyl-tetrazole (11h). Yield 24%, mp 88.8 °C, ^1H NMR ($CDCl_3$) δ 8.56–8.54 (m, 1H, $H_{3''}$), 7.41–7.40 (m, 1H, $H_{5''}$), 7.21–7.16 (m, 5H, ArH), 7.07–7.05 (m, 1H, $H_{4''}$), 6.77–6.75 (m, 1H, $H_{6''}$), 4.081 (s, 3H, N–CH_3), 3.63–3.59 (m, 1H, $H_{1'''}$), 2.39–2.37 (m, 1H, $H_{\beta 3''}$ cyclopropyl), 2.29–2.26 (m, 1H, $H_{\alpha 3''}$ cyclopropyl); ^{13}C NMR ($CDCl_3$) δ 165.71, 162.72, 149.42, 138.22, 136.21, 132.20 (2C), 128.74 (2C), 127.56, 122.50, 121.17, 39.96, 39.52, 24.35, 22.10. IR ν (KBr, cm^{-1}): 3083, 3032, 2998, 2918 (CH, CH_2, CH_3), 1600, 1587 (C=N, C=C and N=N). Anal. Calcd for $C_{16}H_{15}N_5$ (277.33): C, 69.30; H, 5.45; N, 25.25. Found: C, 69.65; H, 5.25; N, 25.46.

6.11. Preparation of carboxamidine derivative 2i

To a suspension of 0.04 mol (2.15 g) of ammonium chloride in toluene (50 mL) was added dropwise 0.04 mol of $Al(Me)_3$. The mixture was heated at 80 °C leading to CH_4 formation. When gas evolution stopped, 0.01 mol of nitrile was added dropwise and the solution was refluxed for 12 h, stirred one night at room temperature, then MeOH/H_2O (8:2) was added carefully and the mixture filtered. The salts were washed with CH_2Cl_2. The solvent was evaporated in vacuo, the residue was taken up in CH_2Cl_2 and washed with HCl (1 N). The aqueous phase was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was chromatographed with $CHCl_3$/MeOH (9:1) as eluent.

6.11.1. 1-Cyclohexyl-1-phenyl acetamidine, hydrochloride (2i). Yield 40%, mp >300 °C, ^1H NMR ($CDCl_3$) δ 8.85 and 8.37 (2s broad, 4H, NH^+), 7.66–7.28 (m, 5H, ArH), 3.75–3.71 (d, 1H, CH, J = 11,39 Hz), 1.89–0.77 (m, 11H, H cyclohexyl), IR ν (KBr, cm^{-1}): (CH, CH_2), (C=N, C=C and N=N). Anal. Calcd for $C_{14}H_{21}N_2Cl$ (252.79): C, 66.52; H, 8.37; N, 11.08; Cl, 14.02. Found: C, 66.34; H, 8.62; N, 10.97.

6.12. X-ray analysis of compound 11h

A colourless crystal of **11h** compound was used for obtaining intensity data on a CAD4 Enraf Nonius diffractometer with Cu Kα radiation. The cell parameters were refined using 25 reflexions centred on the diffractometer. The reflexions used in the analysis were corrected for Lorentz and polarisation effects, and also for absorption using the Ψ scan method.[50]

The experimental data are given in Table 3. The structure was solved by the direct method procedure of Shelx 90 (Sheldrick, 1990).[51] All full matrix least-squares refinement in this analysis was performed with Shelks 93 (Sheldrick, 1993).[52]

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication Nos. CCDC 298119.

6.13. Pharmacology, animals and treatments

Three-month-old male Wistar rats (Iffa-Credo, france) weighing about 250 g were used in all the experiments. The animals were housed in wire-bottomed cages and maintained at 21 ± 2 °C in a room with a 12 h fixed light-dark schedule. Water and standard laboratory chow (UAR, Villemoisson-sur-Orge, France) were freely available.

Moderate diabetes was obtained by a single iv injection of a low dose (35 mg/kg) of streptozotocin (STZ) dissolved in a citrate buffer under ketamine hydrochloride anaesthesia (75 mh/kg ip, Imalgene, Mérieux, France). These rats were called STZ rats. Control rats received an injection of citrate buffer under the same conditions. Glucose homeostasis was assessed by a glucose tolerance test performed 2 weeks after STZ injection.

6.13.1. Glucose tolerance tests. For oral glucose tolerance test (OGTT) glucose was given per os (po) (2 g/kg) to conscious rats. Blood samples were collected before and 10, 20, 30, 40, 60, 90 and 120 min after po glucose administration. They were then centrifuged and the plasma was separated. Plasma glucose concentration was determined immediately in a 10 μL aliquot. The drug was administered po 60 min before OGTT, at the dose of 100, 30, 10 or 3 mg/kg.

6.13.2. Analytical methods. Plasma was analysed using a glucose analyser (Beckman Inc., Fullerton, USA.)

6.13.3. Calculations and statistical methods. Glucose tolerance was measured using two parameters: ΔG which represents the increase of glycaemia over the baseline integrated over a period of 120 min after the glucose load, and G120 which is the blood glucose value at 120 min after glucose administration.

Results are expressed as means ± SEM. The significance of differences between means was evaluated by Student's test and differences were considered significant at $p < 0.05$.

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