Design and Synthesis of Imidazoline Derivatives Active on Glucose Homeostasis in a Rat Model of Type II Diabetes. 1. Synthesis and Biological Activities of *N*-Benzyl-*N*′-(arylalkyl)-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazines

Frédéric Rondu,[§,▽] Gaëlle Le Bihan,[§,▽] Xuan Wang,[§,‡,▽] Aazdine Lamouri,[§] Estera Touboul,[§] Georges Dive,[∥] Tounès Bellahsene,[§] Bruno Pfeiffer,[†] Pierre Renard,[†] Béatrice Guardiola-Lemaitre,[#] Dominique Manechez,[#] Luc Penicaud,[⊥] Alain Ktorza,[‡] and Jean-Jacques Godfroid*[,§]

Laboratoire de Pharmacochimie Moléculaire and Laboratoire de Physiopathologie de la Nutrition, CNRS URA 307, Université Paris 7-Denis Diderot, 2, place Jussieu, 75251 Paris Cedex, France, ADIR, 1, rue Carle Hebert, 92415 Courbevoie Cedex, France, IRI Servier, 6, place des Pleiades, 92415 Courbevoie Cedex, France, Centre d'Ingénierie des Protéines, Institut de Chimie, Université de Liège, Belgium, and UPRESA, CNRS 5018/-UPS, Toulouse, France

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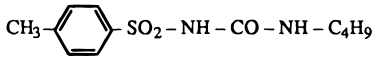
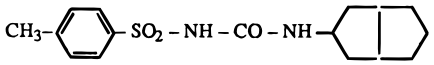
The physiopathology of non-insulin-dependent diabetes mellitus is associated with a dysfunction in the regulation of insulin secretion. The α_2-adrenoceptors have been reported to be involved in this alteration, although α_2-antagonists containing an imidazoline ring may stimulate insulin secretion independently of α_2-adrenoceptor blockage. Recently, a new "imidazoline-binding site" involved in the control of K^+-ATP channels in the B cell has been proposed. In the course of searching for new antidiabetic agents, 1-alkyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-benzylpiperazines, 1-benzyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-alkylpiperazines, and 1-benzyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-benzylpiperazines have been designed and evaluated as potential adrenoceptor antagonists. Pharmacological evaluation was performed *in vivo* using glucose tolerance tests performed on a rat model of type II diabetes obtained by injection of a low dose (35 mg/kg) of streptozotocin (STZ). For some compounds, binding experiments were performed on α_2 adrenoceptors and I_1 and I_2 imidazoline-binding sites. The biological and physicochemical data have been combined with molecular modeling studies to establish structure−activity relationships. The most active compound was 1-(2′,4′-dichlorobenzyl)-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-methylpiperazine (**7f**); intraperitoneal administration (100 μmol/kg) of **7f** strongly improved glucose tolerance in STZ diabetic rats. This effect seemed at least partly mediated by a significant increase of insulin secretion. Other compounds of the same family (**7b**, **16f**, **23b**) have also shown potent activity. We found no correlation between *in vivo* antihyperglycemic properties and *in vitro* affinities for α_2-adrenoceptors or I_1, and I_2 binding sites. These compounds can be considered as antihyperglycemic agents potentially useful for treatment of type II diabetes and are currently under complementary investigation.

Introduction

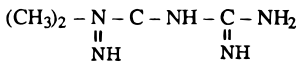
Non-insulin-dependent diabetes mellitus (NIDDM) is wide spread and is one of the most common chronic diseases, being present in 4% of the Western World's population, half of whom are unaware of it. Although the frequency of diabetes is increasing, only two classes of oral hypoglycemic agents are available for the treatment of NIDDM. For both, residual β-cell insulin secretion is necessary for activity. The sulfonylureas, such as tolbutamide and gliclazide, are hypoglycemic compounds, which act mainly at the pancreas level by stimulation of insulin secretion. Their mechanism of action is well known.[1] On the other hand, the biguanides (particularly metformin) act mainly in the presence of endogenous insulin by decreasing gluconeogenesis and increasing peripheral utilization of glucose[2−5].



Tolbutamide



Gliclazide



Metformin

For many years, development of antidiabetic drugs has been largely focused on improvement of sulfonylurea derivatives, in order to increase the efficiency and decrease side effects[6] (in particular hypoglycemic events). Among the most promising new approaches, some imidazoline derivatives such as midaglizole,[7,8] deriglidole,[9−11] and efaroxan[12] were reported to be α_2-antagonists acting via stimulation of insulin secretion and to exhibit antihyperglycemic activity *in vivo*. In the past few years, further studies have shown that the insulin-secreting potency of some imidazoline derivatives was not correlated with their α_2-antagonistic

* Author to whom correspondence should be addressed. E-mail: godfroid@paris7.jussieu.fr.
§ Laboratoire de Pharmacochimie Moléculaire, Université Paris 7-Denis Diderot.
‡ CNRS URA 307, Université Paris 7-Denis Diderot.
† ADIR.
# IRI Servier.
∥ Université de Liège.
⊥ UPRESA, CNRS 5018/-UPS.
▽ These authors contributed equally to this work.
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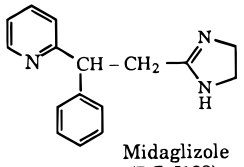
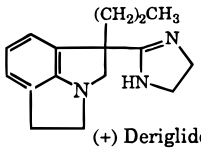
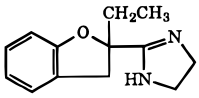
properties,[13,14] and so the involvement of new putative biological targets was suggested.



Midaglizole
(DG-5128)

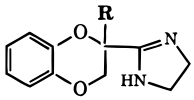


(+) Deriglidole
(SL 86.0715)



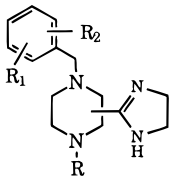
Efaroxan

It has been proposed that some of these derivatives could interact with imidazoline-preferring binding sites (IPBS).[15,16] There are two major subtypes of IPBS: I_1, which preferentially bind [^{3}H]clonidine or [^{3}H]-*p*-aminoclonidine, and I_2, which preferentially bind [^{3}H]-idazoxan.[17,18] Idazoxan was selected for molecular modeling studies for its affinity for both α_2-adrenoceptors and I_2 IPBS, whereas its close analogue, RX 821002, was chosen as a selective α_2-antagonist.



R = H, Idazoxan
R= MeO, Methoxy-idazoxan
(RX 821002)

In this paper, we report the synthesis and the pharmacological evaluation of new antihyperglycemic 2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazines having the following general formula:



These series were designed after our studies based on comparative analysis of electronic distribution in both Idazoxan and RX 821002. Despite their marked antihyperglycemic properties, our new compounds were devoid of significant affinities for both the α_2-adrenoceptors and the I_1 and I_2 IPBS; therefore, we decided to evaluate all of them *in vivo* for their antidiabetic activity. This was achieved using glucose tolerance tests performed on a rat model of type II diabetes obtained by injection of a low dose (35 mg/kg) of streptozotocine. Structural modification was systematically carried out in order to determine the necessary structural requirements for potent *in vivo* activity. For some compounds, secretion of insulin was also evaluated. Some *in vitro* binding experiments were performed on selected analogues to investigate a potential correlation between *in vivo* and *in vitro* studies.

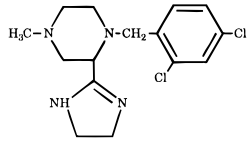
Molecular Modeling and Design of These Series

As mentioned in the Introduction, idazoxan and methoxyidazoxan (RX 821002) were initially selected for the molecular modeling studies. Our initial purpose was to synthesize new structures having the same binding profile as idazoxan, i.e., affinity on both α_2-adrenoceptors and I_2 binding sites (RX 821002 being a specific α_2-adrenoceptor antagonist). Consequently, calculated 3-D electrostatic potential maps of RX 821002 and idazoxan were compared with that of PMS 812[19] (compound **7f**) which was one of our compounds showing potent antihyperglycemic properties. Our hypothesis was that the electronic distribution should be as close as possible to idazoxan's but not to RX 821002's.

Table 1. pK_a Values of Idazoxan and Some Compounds of the Series 1-Aryl-4-alkyl-2-(imidazolin-2)-yl)piperazines

compd	pK_a[a]	pK_a[b]
idazoxan	8.8	
7b	9.5	5.5
7f	9.4	5.4

[a] p$K_a$value of the imidazoline ring. [b] pK_a value of the less hindered tertiary amino function of the piperazine. The basicity of compounds was assessed by potentiometric titration in aqueous solution.



7f: PMS 812

Concerning the potential protonation, experimental pK_a values of compounds **7b**,**f** and idazoxan were determined. The results (Table 1) clearly demonstrate that the imidazoline ring is the first protonation center. The piperazine moiety is only very slighty ionized at the physiological pH.

Moreover, calculations were performed. Whatever the conformation could be, the protonation energy difference between the imidazoline and piperazine nitrogens was higher than 25 kcal/mol.[20] Here, we made an abstraction of the possible protonation at physiological pH of the imidazoline ring in this comparison.

The 3-D electrostatic potential map is the "fingerprint" of a molecule, taking into account electronic distribution, conformation, and volume. It is the best correlation explaining the similarity of activities between molecules which, apparently, are structurally different.

The conformational space of RX 821002, at the AM1 level,[21] has been analyzed using complete scans of the two dihedral angles defining the relative position of the methoxy and imidazoline groups. At each step, the geometry was reoptimized following the procedure implemented in GAUSSIAN 94.[21]

Taking into account the optimized geometry of RX 821002, Figure 1 shows that a major electrostatic potential takes place around one of the oxygens of the benzodioxane ring (isocontour at −20 kcal/mol) reinforced by the generating effect of the oxygen of the methoxy substituent (visible with an isocontour at −40 kcal/mol, the red lines).

The comparison between 3-D electrostatic potential maps of idazoxan and of PMS 812 (compound **7f**) is clear (Figure 2): two negative wells appear around the nitrogens of the imidazoline ring (as in the case of RX 821002). Moreover, two separated electrostatic regions are positioned around the nitrogens of piperazine and

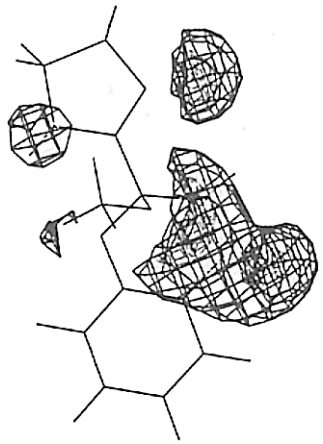


Figure 1. Electrostatic potential maps of RX 821002 isocontoured at −20 (green lines), −30 (yellow lines), and −40 (red lines) kcal/mol.

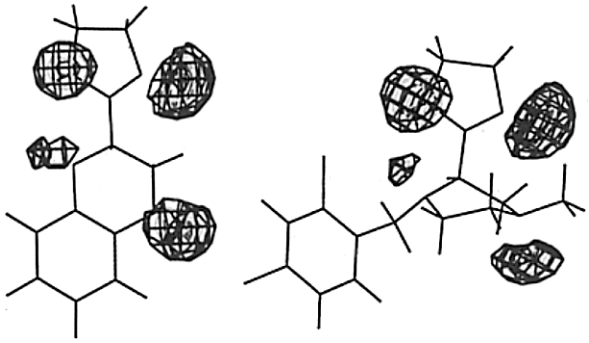


Figure 2. Comparison of the electrostatic potential maps of idazoxan (left) and compound **7f** (right), the lead compound of the series 2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazine, isocontoured at −20 (green lines), −30 (yellow lines), and −40 (red lines) kcal/mol.

the oxygens of dioxane. Electrostatic potential maps of idazoxan and PMS 812 (compound **7f**) may be superimposable if one ignores the phenyl versus benzyl ring locations; the distances between the aromatic rings and the heteroatoms must be different in the phenyl and benzyl analogues. This is the reason why we chose to develop studies in these series.

Chemistry

Scheme 1 shows a typical total synthesis of 1-aryl-4-alkyl-2-(2-imidazolinyl)piperazines. The condensation of 1,4-dibenzylethylenediamine and 2,3-dibromopropionic acid ethyl ester, according to Jucker et al.[22] with minor modifications, gave after acidification ethyl 1,4-dibenzyl-2-piperazinecarboxylate hydrochloride (**1**). The N-debenzylation using catalytic hydrogenolysis is faster with the dihydrochloride salt than with the free base. The two nitrogen atoms of **2** do not present the same reactivity. At low temperature (−10 °C) the alkylation with 1 equiv of triphenylmethyl chloride led to the monosubstitution on position 4. In these conditions the ethyl 4-(triphenylmethyl)-2-piperazinecarboxylate (**3**) was obtained with a very satisfactory yield. Reaction of **3** with 1 equiv of substituted benzyl chloride afforded the corresponding ethyl 1-(substituted benzyl)-4-(triphenylmethyl)-2-piperazinecarboxylate **4**. Cleavage of the protective group in dilute HCl yielded the ethyl 1-(substituted benzyl)-2-piperazinecarboxylate **5**. Alkylation of **5** was carried out by action of the suitable alkyl halide (for alkyl ≠ CH_3) in acetone with K_2CO_3 and catalytic KI (for alkyl bromide) according to the method of Jerzy et al.[23] Yields of compounds **8**−**11** (Scheme 1) and **20** and **21** (Scheme 2) increased using an excess of the alkyl halide. Under the same conditions methyl iodide led to a mixture of tertiary amine and quaternary ammonium salt. Products **6** (Scheme 1) and **18** (Scheme 2) were then obtained using formaldehyde and formic acid in methanol according to Icke.[24]

Alkylation of **2** (Scheme 2) with 1 equiv of substituted benzyl chloride in refluxing toluene gave two esters (**17** and **24**) in 35% and 25% yields, respectively. These compounds were easily separated on column chromatography. Ester **24** can be prepared in a yield of 80% using 2 equiv of benzyl chloride. All imidazolines were prepared under the same conditions according to Neef et al.[25] with a minor modification. An excess of aluminum organic reagent was necessarily used in order to avoid a mixture of open-chain amide and ring-closed heterocycle.

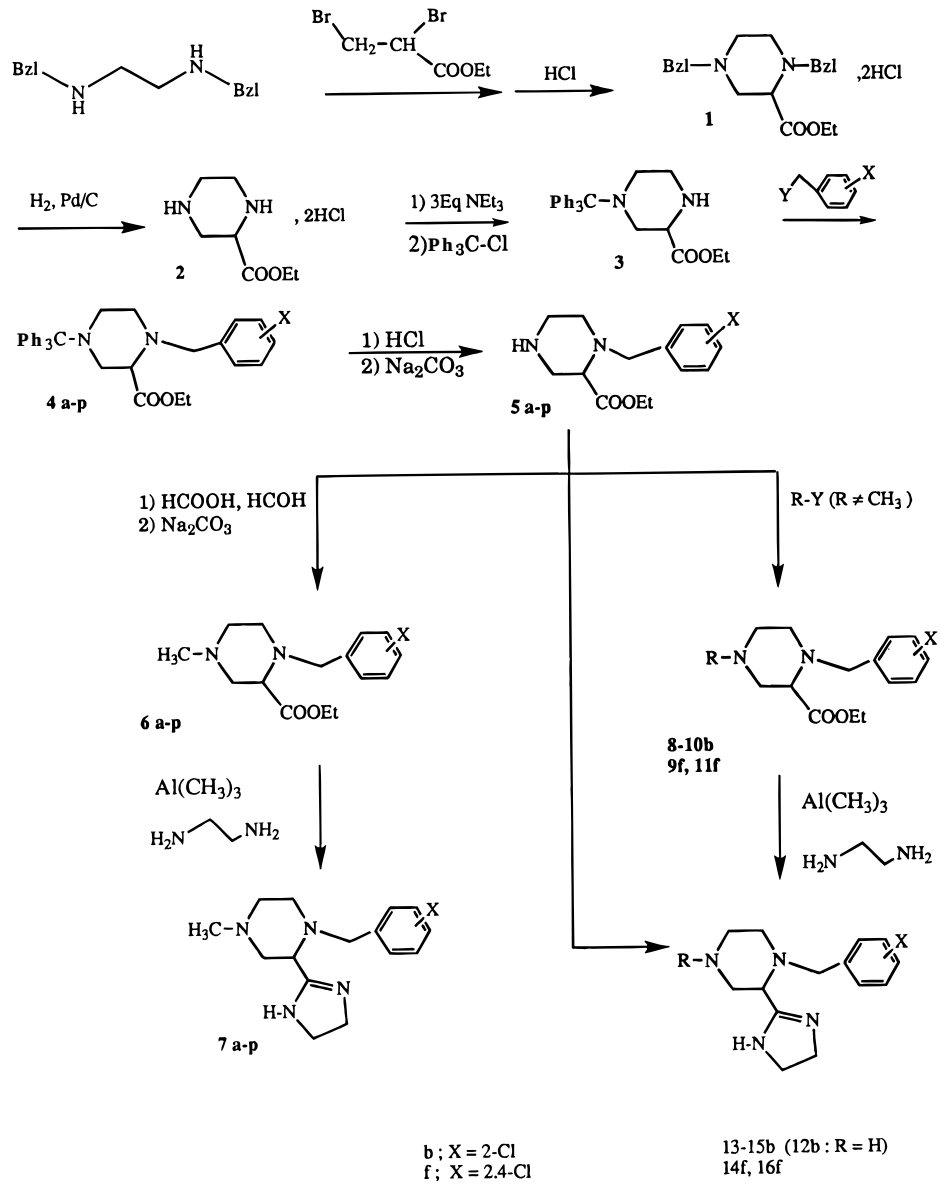
Pharmacological Parameters *in Vivo*: Significance and Validity

Antidiabetic properties of all the synthesized compounds were quantified by their ability to improve glucose tolerance during an intravenous glucose tolerance test (IVGTT) in diabetic rats. From the few existing models, a mild diabetic experimental rat model developed by Thibault et al.[26] was chosen for our studies. This diabetic rat presents moderate basal hyperglycemia, glucose intolerance, and impairment of glucose-induced insulin secretion which are the main features in patients with NIDDM. Moderate diabetes is obtained by iv injection of a low dose (35 mg/kg) of streptozotocin (STZ). Two weeks later, measurement of the basal glycemia and IVGTT was performed, allowing the selection of animals with moderate diabetes, i.e., basal glycemia comprised between 7 and 10 mM and a ΔG ranging from 50 to 80 mM. IVGTT was then performed following a single administration of 100 μmol/kg of the synthesized compounds. The following parameters were taken into account: (i) G_{30}, which is the glycemia value at 30 min after glucose administration, (ii) ΔG, which represents the increase in glycemia over baseline integrated over a period of 30 min following the glucose load, and (iii) K, which is the rate of glucose disappearance between 5 and 30 min after glucose administration. For some compounds, secretion of insulin was also evaluated and expressed as ΔI which represents the incremental plasma insulin values over baseline integrated over a period of 30 min after the glucose load. To correct for the slight variations in response that appeared when glucose tolerance tests were performed with different control diabetic rats, we decided to express the results as a percentage (ΔG*) of variation of ΔG between treated and untreated diabetic rats. In the same way, we defined G_{30}*, K*, and ΔI*.

$$\Delta G^* = \left| \frac{\Delta G_{\text{treated STZ rats}} - \Delta G_{\text{untreated STZ rats}}}{\Delta G_{\text{untreated STZ rats}} - \Delta G_{\text{control rats}}} \right| \times 100$$

To be considered as an effective antidiabetic, compounds must have high a percentage of variation for ΔG*, G_{30}*, and K*. Results around 100% or more mean that the parameters for the treated diabetic animals are close to those obtained with nondiabetic control animals.

Scheme 1



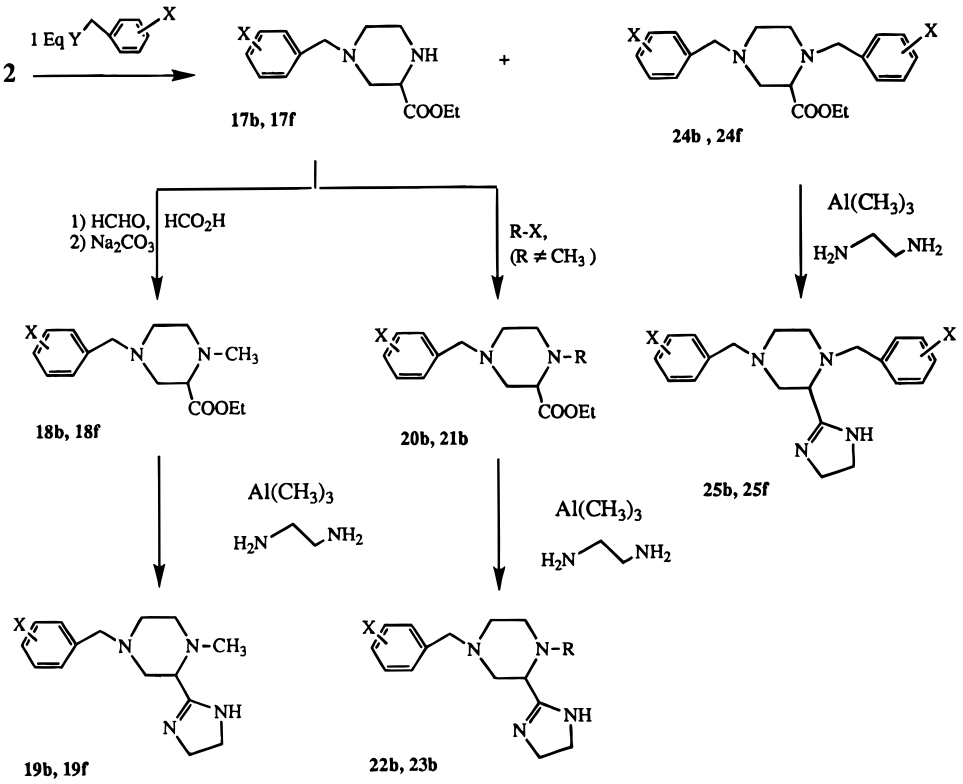
This is the case, among others, for **7f** (PMS 812) for which results have already been published[19] and **7b** for which glycemia values of STZ-treated rats are very close to those obtained with normal rats (Figures 3 and 4). Compounds like **13b**, which has virtually no influence on the parameters, can be considered as inactive (Figure 5). Among the three parameters, G_{30}, which is measured directly, is considered as the most relevant.

Results and Discussion

As previously mentioned, all the synthesized compounds were evaluated via an ip administration (100 μmol/kg) in glucose tolerance tests performed on STZ rats which exhibited moderate basal hyperglycemia and glucose intolerance. The first tested imidazoline derivative, **7b**, had a potent effect on the glycemia, which resulted in values similar to those of nondiabetic rats (Figure 3). This correlated very well with its effects on the three parameters, with ΔG^*, G_{30}^*, and K^* values around 100%. Analogues with substituents on the aromatic ring of the 1-benzyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-methylpiperazine were studied in order to determine their influence on antidiabetic activity (Table 2). Substitutions by halogens or other substituents (compounds **7b**−**p**) may result, depending on the substitution site, in a significant increase of activity compared to the nonsubstituted compound **7a**. The most potent 2-monosubstituted compound was **7b**, a 2-chloro derivative, which proved to be much more active than the nonsubstituted **7a** (ΔG^* and G_{30}^* values respectively of 81% and 103% for **7b** compared to 0% and 23% for **7a**). Analogue **7m**, which is the fluoro analogue of **7b**, exhibited almost the same potency. Monosubstitution by other groups such as 2-methyl **(7p)** or 2-methoxy **(7l)** resulted in lower activity, while substitution by a trifluoromethyl **(7o)** gave better results, almost equivalent to those of **7b** (Table 2).

The substituents of this 2-monosubstituted series can be arranged in the following order of potency as antihyperglycemic agents: Cl > F > CF_3 > OMe > CH_3. Compound **7b** was selected for pharmacomodulation. This consisted of varying the position of the Cl on the benzyl moiety. Compounds **7c** (3-Cl) and **7d** (4-Cl) were found to be less active than **7b**. Surprisingly, substitution with a 3-methoxy (**7k**) gave better results than with a 3-Cl (**7c**). Compound **7f**, which is a 2,4-dichloro

Scheme 2



b ; X = 2-Cl
f ; X = 2.4-Cl

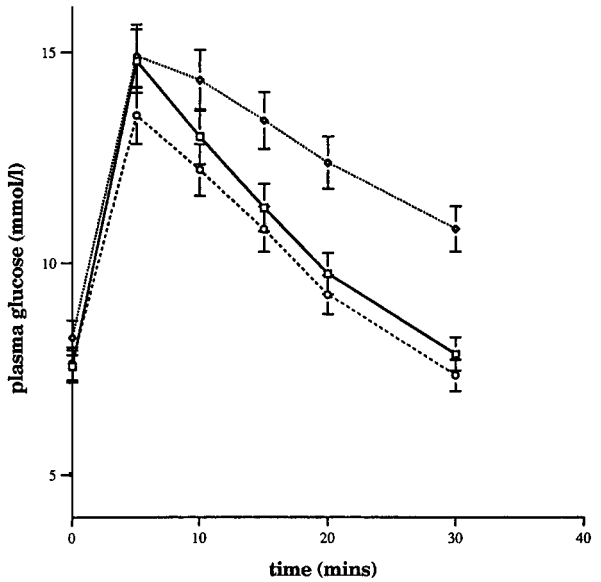


Figure 3. Effect of a single intraperitoneal administration of **7b** on glucose tolerance: (□) STZ rats treated with **7b**, (◇) untreated STZ rats, and (○) control rats. *p < 0.05, significantly different from untreated STZ rats. **7b** was given (100 μmol/kg ip) 15 min before an iv glucose load (0.5 g/kg). Plasma glucose level (mmol/L) was decreased compared to untreated diabetic rats and was not significantly different from the value in normal control rats.

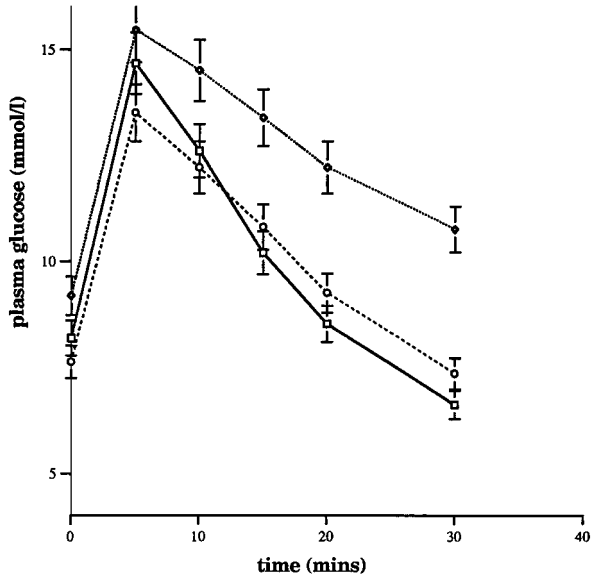


Figure 4. Effect of a single intraperitoneal administration of **7f** on glucose tolerance: (□) STZ rats treated with **7f**, (◇) untreated STZ rats, and (○) control rats. *p < 0.05, significantly different from untreated STZ rats. **7b** was given (100 μmol/kg ip) 15 min before an iv glucose load (0.5 g/kg). Plasma glucose level (mmol/L) was decreased compared to untreated diabetic rats and was not significantly different from the value in normal control rats.

derivative, was found to be the most potent compound among the disubstituted derivatives with an activity even higher than that of **7b**. The positions of the substituent seem to be one of the most important factors as shown by the results of the 3,4-dichloro derivative **7h** which is clearly less active than **7f**. These results indicate that electronic and steric factors of substituents seem to be strongly involved in the activity.

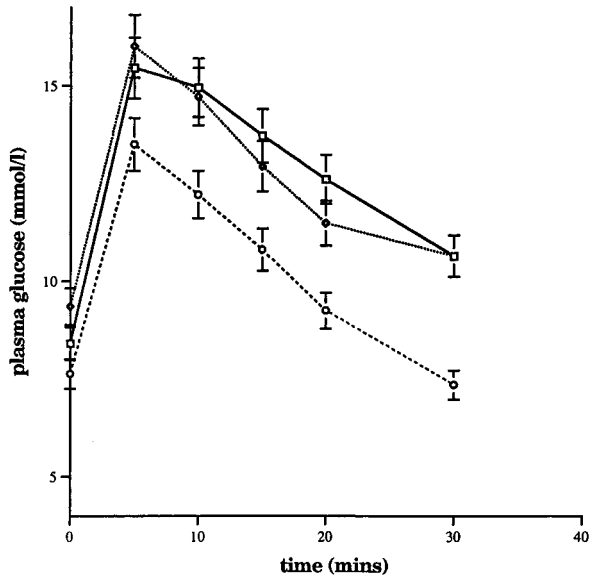


Figure 5. Effect of a single intraperitoneal administration of **13b** on glucose tolerance: (□) STZ rats treated with **13b**, (◇) untreated STZ rats, and (○) control rats. *$p < 0.05$, significantly different from untreated STZ rats. **13b** was given (100 μmol/kg ip) 15 min before an iv glucose load (0.5 g/kg). Plasma glucose level (mmol/L) was not decreased compared to untreated diabetic rats and was significantly different from the value in normal control rats.

Regarding the substituents on piperazine nitrogens, replacement of the methyl by a hydrogen (**12b**) resulted in a total loss of activity. Substitution with an ethyl (**13b**) seemed to have only little effect, while a propyl (**14b**) altered the activity completely compared to the *N*-methyl derivative **7b**. Surprisingly, compounds with a branched alkyl isopropyl (**15b**) or isobutyl (**16f**) exhibited almost the same potencies as their *N*-methyl counterparts **7b**,**f**. Introduction of a second benzyl substituent instead of the alkyl group on the piperazine nitrogen atom (**25a**,**b**,**f**) resulted in a total loss of activity compared to the monobenzyl *N*-alkyl analogues (**7a**,**b**,**f**).

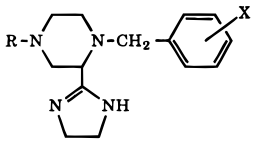
Inverse analogues of the most active compounds **7b**,**f** and **15b** were prepared by variation of the benzyl and alkyl substituents on the piperazine nitrogens. The activities of these 1-alkyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)-4-benzylpiperazine derivatives (Table 3, compounds **19b**,**f**, **23b**) were in the same range for the three parameters as that of their counterparts (Table 2). Some compounds **(7a**,**b**,**f**,**k**,**l**, **14b**, **16f)** were also evaluated for their capacity to stimulate the secretion of insulin during IVGTT. It clearly appeared that the most active compounds on ΔG^*, G_{30}^*, and K^* are also those that strongly stimulate insulin release (**7b**, $\Delta I = 109\%$; **7f**, $\Delta I = 221\%$; **16f**, $\Delta I = 623\%$). Compounds less active on glycemia parameters moderately stimulate the secretion of insulin (**7k**, $\Delta I = 88\%$; **7l**, $\Delta I = 60\%$; **14b**, $\Delta I = 25\%$; **7a**, $\Delta I = 16\%$).

The *in vitro* binding experiments performed with our compounds have shown that none of them had significant affinity ($>10^{-6}$ M) for the α_2-adrenoceptors or I_1 and I_2 IPBS. This strongly suggests that the potent insulin-secreting properties of the synthesized compounds do not involve these receptors or binding sites. This is in agreement with an increasing number of publications showing that α_2-adrenergic antagonists are able to stimulate insulin secretion independently of α_2 or/and I_1 and I_2 blockage.[27−35] The imidazoline derivatives, and more particularly those active on insulin secretion, may interact with a novel type of imidazoline-preferring site located on pancreatic B cells. Compound **7f**, which proved to be our most interesting compound, is currently under complementary pharmacological evaluation. Further chemical modulation is in progress to obtain more active compounds and develop structure−activity relationships. For this purpose, we have investigated some electronic and structural characteristics of **7f**, the most interesting compound, using a molecular modeling approach.

The imidazoline substituent placed at the 2-position of the piperazine ring of **7f** plays a major role in the twisted conformation. In practice, four conformations (C11, C12, C21, C22) can be located on the energy surface depending on the hybridization of the two nitrogens with their respective lone pair "up" or "down".

Full geometry optimization of the four conformers has been carried out at three levels of calculation: an approximate AM1[36] method and an *ab initio* one using minimal and double basis sets, MINI-1′ [37] and 6-31G.[38] ΔG has been computed at 298.15 K and 1 atm (Table 4). From the geometrical point of view, these conformers occupy a large part of the 3-D space mainly as a

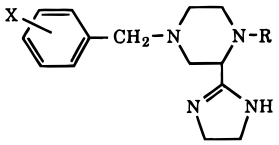
Table 2. Variation of the Glycemia Parameters after ip Administration of 100 μmol/kg 1-Benzyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazines to Streptozotocin Rats[a]

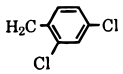


compd	X	R	ΔG^{*b} (%)	G_{30}^{*c} (%)	K^{*d} (%)	ΔI^{*e} (%)
7a (8)	H	CH_3	0	23	67	16
7b (5)	2-Cl	CH_3	81	103	98	109
7c (6)	3-Cl	CH_3	0	23	39	
7d (4)	4-Cl	CH_3	0	68	110	
7e (5)	2,3-Cl	CH_3	0	36	53	
7f (8)	2,4-Cl	CH3	105	112	143	221
7g (9)	2,6-Cl	CH_3	103	59	107	
7h (7)	3,4-Cl	CH_3	36	47	42	
7i (5)	3,5-Cl	CH_3	0	70	117	
7j (6)	2-OCH_3	CH_3	47	56	39	
7k (5)	3-OCH3	CH_3	23	68	57	88
7l (7)	2,3-OCH_3	CH_3	0	58	55	60
7m (5)	2-F	CH_3	72	52	108	
7n (5)	2,4-F	CH_3	102	106	97	
7o (8)	2-CF_3	CH_3	94	64	63	
7p (8)	2-CH_3	CH_3	24	48	81	
12b (4)	2-Cl	H	0	0	11	
13b (4)	2-Cl	ethyl	108	38	34	
14b (8)	2-Cl	*n*-propyl	0	17	37	25
15b (6)	2-Cl	isopropyl	84	83	44	
14f (7)	2,4-Cl	*n*-propyl	24	80	104	
16f (4)	2,4-Cl	isobutyl	110	95	119	623
idazoxan (6)			8	48	79	
midaglizole (6)			32	121	98	

[a] Three-month-old male Wistar rats (250 g) treated with 35 mg/kg iv streptozotocin. [b] ΔG: incremental glycemia values over baseline integrated over 30 min after glucose (0.5 g/kg iv) administration. [c] G_{30}: glycemia value 30 min after glucose administration. [d] K: rate of glucose disappearance between 5 and 30 min after glucose administration. [e] ΔI: incremental plasma insulin values over baseline integrated over 30 min after glucose administration. All results (asterisk) are expressed as a percent of variation of the parameters between treated rats (with an ip administration of 100 μmol/kg of the tested compound) and untreated rats; *n*: number of experiments conducted with each drug, indicated in parentheses.

Table 3. Variation of the Glycemia Parameters after ip Administration of 100 μmol/kg 4-Benzyl(or 1,4-dibenzyl)-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazines to Streptozotocin Rats[a]



compd (*n*)	X	R	ΔG*[b] (%)	G_{30}*[c] (%)	K*[d] (%)
19b (5)	2-Cl	CH_3	93	97	148
19f (8)	2,4-Cl	CH_3	34	102	152
22b (5)	2-Cl	ethyl	70	50	106
23b (5)	2-Cl	isopropyl	112	96	95
25a (10)	H		0	33	43
25b (7)	2-Cl		0	0	36
25f (5)	2,4-Cl		0	18	22
idazoxan (6)			8	48	79
midaglizole (6)			32	121	98

[a] Three-month-old male Wistar rats (250 g) treated with 35 mg/kg iv streptozotocin. [b] ΔG: incremental glycemia values over baseline integrated over 30 min after glucose (0.5 g/kg iv) administration. [c] G_{30}: glycemia value 30 min after glucose administration. [d] K: rate of glucose disappearance between 5 and 30 min after glucose administration. All results (asterisk) are expressed as a percent of variation of the parameters between treated rats (with an ip administration of 100 μmol/kg of the tested compound) and untreated rats; *n*: number of experiments conducted with each drug, indicated in parentheses.

Table 4[a]

	AM1	MINI-1′		6-31G	
	ΔE	ΔE	ΔG	ΔE	ΔG
C11	0.000	0.000	0.000	0.000	0.000
C12	0.183	1.281	0.560	1.257	−0.284
C21	2.285	2.339	1.739	−1.099	−0.303
C22	−0.860	0.277	−0.258	1.399	0.962

[a] Energy values are expressed in kcal/mol. The C11 conformer is taken as reference. Negative values correspond to more stable conformers.

result of the relative orientation of the dichlorophenyl group. Remarkably, the relative energy differences lie in a very small range as noted in Table 5. This feature can be interpreted as a high capability of the molecule **7f** to adopt the most effective conformation at the receptor site including the ability of the imidazoline to be protonated in its biological active conformation.[20]

Conclusion

In conclusion, most of these 29 imidazoline derivatives synthesized had a potent effect on po glucose tolerance, and some of them increased significantly *in vivo* the insulin secretion in mildly diabetic rats. Compounds **7b**, **7f** (PMS 812), and **16f** had the highest potency, with glycemia values similar to those obtained with normal rats. These compounds do not have high affinity for α_2-adrenoceptors or the known imidazoline binding sites I_1 and I_2. Possibly, the effect of **7f** on the insulin secretion results from its interaction with a novel type of imidazoline-binding site. Molecular modeling efforts with **7f** suggest that this compound is able to adopt the most effective conformation at the receptor site and is characterized by high flexibility. Compound **7f** belongs to a new class of imidazoline derivatives having potential therapeutic interest in type II diabetes. In the future, the exploration in the design of these new potent imidazoline derivatives may allow a greater understanding of the structural parameters required for activity and may shed light on the role of imidazoline derivatives in the regulation of insulin secretion.

Experimental Section

Molecular Modeling. For all the studied products, the geometry has been fully optimized with respect to all the $3N - 6$ degrees of freedom. The berny algorithm of the GAUSSIAN 94 package[20] minimizes the first derivative of the energy function calculated either at the AM1 semiempirical level or at the *ab initio* level using minimal MINI-1′ and double 6-31G basis sets. The nature of the equilibrium structures thus obtained as true minima is given by all the positive eigenvalues of the second-derivative matrix.

By opposite to rigid rotor conformational analysis, the relaxation procedure applied for RX 821002 requires a complete reoptimization of the $3N - 8$ other degrees of freedom when a bidimensional map is concerned. The electrostatic potential maps have been calculated at the *ab initio* level using the MINI-1′ basis sets with the GAUSSIAN suite of programs.[20]

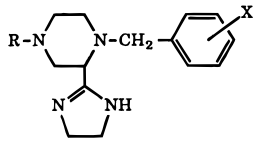
Chemistry. General Methods. The purity of each compound was checked by thin-layer chromatography on TLC plastic sheets (silica gel 60F254, layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (particle size 0.063−0.200 mm) from Merck, without any special treatment. All melting points were determined in a digital melting point apparatus (Electrothermal) and are uncorrected. The structures of all compounds were confirmed by IR and ^{1}H NMR spectra. IR spectra were obtained with a Pye-Unicam SP3-200 infrared spectrometer, and ^{1}H NMR spectra were recorded in $CDCl_3$ on a Brucker AC 200 spectrometer using hexamethyldisiloxane (HMDS) as an internal standard. All elemental analyses were within ±0.4% of theoretical values.

Ethyl 1,4-Dibenzyl-2-piperazinecarboxylate Dihydrochloride (1). To a hot (80 °C) stirred solution of *N*,*N*′-dibenzylethylenediamine (72 g, 0.3 mol) and triethylamine (100 mL, 0.72 mol) in toluene (300 mL) was added dropwise, but rapidly, ethyl 2,3-dibromopropionate (80 g, 0.31 mol in toluene (300 mL)). After the addition, the reaction mixture was stirred at 80 °C for 3 h, then cooled, and filtered. The filtrate was washed with saturated aqueous sodium hydrogen carbonate (200 mL). The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo. The crude product was dissolved in anhydrous EtOH (400 mL) and saturated with HCl gas. The addition of ether gave a precipitate of **1** (100−110 g, 81−86%) as a white powder: mp 152−154 °C; IR film (of free base) 1735 ($\nu_{C=O}$), 1600 and 1585 ($\nu_{C=C}$) cm^{-1}; ^{1}H NMR (of free base) δ 7.4−7.1 (10H, m, aromatic H), 4.13 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.38−3.88 (4H, m, $PhCH_2$), 3.32−3.21, 3.05−2.95, 2.69−2.27 (7H, 3m, piperazinic H), 1.19 (3H, t, J = 7.1Hz, CH_3).

Ethyl 2-Piperazinecarboxylate Dihydrochloride (2). A shaken suspension of **1** (40 g, 0.1 mol) and 10% Pd−C (600 mg) in anhydrous ethanol (300 mL) was treated with H_2 (under pressure) at 40 °C overnight. After an addition of water until dissolution of **2** the reaction mixture was filtered through Celite. The addition of ether to the filtrate gave the precipitate **2** (20−23 g, 86−91%) as a white powder: mp 195−197 °C; IR (in paraffin oil) 3380 (ν_{N-H}), 1730 ($\nu_{C=O}$) cm^{-1}; ^{1}H NMR (of free base) δ 4.13 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.42−3.36, 3.19−3.11, 2.98−2.68 (7H, 3m, piperazinic H), 1.84 (2H, br s, NH), 1.21 (3H, t, J = 7.1 Hz, CH_3).

Ethyl 4-(Triphenylmethyl)-2-piperazinecarboxylate (3). To a stirred solution of **2** (23.1 g, 0.1 mol) and triethylamine (55 mL, 0.4 mol) in dry dichloromethane (400 mL) was added dropwise triphenylmethyl chloride (28 g, 0.1 mol) in dry dichloromethane (300 mL) at −10 °C. After addition, the

Table 5. Physical Data of 1-Aryl-4-alkyl-2-(imidazolin-2-yl)piperazines



compd	X	R	mp (°C)[a]	% yield[b]	recrystn solvent[c]	formula
7a	H	CH_3	166−168	55	D	$C_{15}H_{22}N_4 \cdot 2HCl \cdot 2H_2O$
7b	2-Cl	CH_3	160−162	70	C	$C_{15}H_{21}ClN_4 \cdot 2HCl \cdot 1H_2O$
7c	3-Cl	CH_3	180−182	60	D	$C_{15}H_{21}ClN_4 \cdot 2HCl \cdot 1H_2O$
7d	4-Cl	CH_3	198−200	49	D	$C_{15}H_{21}ClN_4 \cdot 2HCl \cdot 1H_2O$
7e	2,3-Cl	CH_3	158−160	58	C	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
7f	2,4-Cl	CH_3	168−170	60	D	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
7g	2,6-Cl	CH_3	182−184	43	D	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
7h	3,4-Cl	CH_3	170−172	43	D	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
7i	3,5-Cl	CH_3	167−169	47	D	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
7j	2-OMe	CH_3	168−170	36	D	$C_{16}H_{24}N_4O \cdot 2HCl \cdot 1H_2O$
7k	3-OMe	CH_3	178−180	38	D	$C_{16}H_{24}N_4O \cdot 2HCl \cdot 1 \cdot 5H_2O$
7l	2,3-OMe	CH_3	160−162	41	D	$C_{17}H_{26}N_4O_2 \cdot 3HCl \cdot 2H_2O$
7m	2-F	CH_3	160−162	37	D	$C_{15}H_{21}FN_4 \cdot 3HCl \cdot 2H_2O$
7n	2,4-F	CH_3	166−168	34	D	$C_{15}H_{20}F_2N_4 \cdot 2HCl \cdot 1H_2O$
7o	2-CF_3	CH_3	163−165	45	D	$C_{16}H_{21}F_3N_4 \cdot 2HCl \cdot 1H_2O$
7p	2-CH_3	CH_3	156−158	51	D	$C_{16}H_{24}N_4 \cdot 2HCl \cdot 1.5H_2O$
12b	2-Cl	H	160−162	22	C	$C_{14}H_{19}ClN_4 \cdot 3HCl \cdot 1H_2O$
13b	2-Cl	C_2H_5	162−164	30	C	$C_{16}H_{23}ClN_4 \cdot 2HCl$
14b	2-Cl	nC_3H_7	160−163	74	D	$C_{17}H_{25}ClN_4 \cdot 3HCl \cdot 1H_2O$
14f	2,4-Cl	nC_3H_7	179−181	40	B	$C_{17}H_{24}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
15b	2-Cl	iC_3H_7	155−157	54	A	$C_{17}H_{25}ClN_4 \cdot 2HCl \cdot 1H_2O$
16f	2,4-Cl	iC_4H_9	118−120	38	D	$C_{18}H_{26}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$

[a] All compounds had decomposition. [b] Yields of the last step. [c] A, acetone; B, acetone/ethanol; C, acetone/methanol; D, dichloromethane/hexane/methanol.

reaction mixture was stirred overnight at −10 °C. The reaction was stopped by addition of Na_2CO_3 (21 g) and water (200 mL). The organic layer was separated, washed with water, and dried over $MgSO_4$. After addition of toluene (50 mL), solvents were evaporated and the crude product was crystallized from hexane:ether (9:1, v/v) to give 26 g of a white powder. The filtrate was purified by column chromatography using petroleum ether:ether (7:3, v/v) as eluent giving about 6 g of **3**. The total yield of this reaction was 80%: mp 112−113 °C; IR (in paraffin oil) 1740 ($\nu_{C=O}$), 1590 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.7−7.05 (15H, m, aromatic H), other H gave unresolved signals between 4.5 and 1.1 ppm.

General Procedure for Ethyl 1-(Substituted benzyl)-4-(triphenylmethyl)-2-piperazinecarboxylate Preparation: Ethyl 1-(2′-Chlorobenzyl)-4-(triphenylmethyl)-2-piperazinecarboxylate (4b). A mixture of **3** (40 g, 0.1 mol), K_2CO_3 (40 g), KI (4 g), and 2-chlorobenzyl chloride (19 g, 0.12 mol) in acetone (400 mL) was stirred for 15 h at room temperature. After filtration and evaporation of the solvent, the residue was taken up in ether, washed with water, and dried over $MgSO_4$. Ether was evaporated to give the crude product **4b** which was used in the next step without purification.

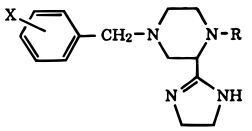
General Procedure for Ethyl 1-(Substituted benzyl)-2-piperazinecarboxylate Preparation: Ethyl 1-(2′-Chlorobenzyl)-2-piperazinecarboxylate (5b). Method A: All of the crude product **4b** obtained in the previous step was treated with acetone (600 mL) and concentrated HCl (25 mL) for 3 h at room temperature. The solvent was removed in vacuo; the residue was solubilized in ether and washed with water. The aqueous layer was neutralized with a saturated Na_2CO_3 solution, extracted with ether, and dried over $MgSO_4$. Evaporation of the solvent gave a crude product which was purified by column chromatography using first petroleum ether:ether (30:70, v/v) and then ether to afford 19 g (80%) of **5b** as a colorless oil: IR (film) 3330 (ν_{N-H}), 1740 ($\nu_{C=O}$), 1605, 1580 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.47−7.10 (4H, m, aromatic H), 4.15 (2H, q, J = 7.1 Hz, $COOCH_2$), ν_a = 3.74, ν_b = 3.72 (2H, q, AB spectrum, J = 14.5 Hz, I_2/I_1 = 4.45), 3.27−3.23, 3.15−2.76, 2.34−2.25 (7H, 3m, piperazinic H), 1.60 (1H, br s, NH), 1.23 (3H, t, J = 7.1 Hz, CH_3).

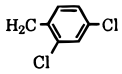
Method B: The crude product **4b** obtained in the last step was solubilized in ethanol (400 mL) and satured with HCl gas. The mixture was stirred for 3 h at room temperature; then the solvent was removed in vacuo. The residue obtained was taken up in ether and washed with a 2 N HCl solution. The aqueous layer was neutralized with a saturated Na_2CO_3 solution and extracted with ether. Drying over $MgSO_4$, filtration, and evaporation of $CHCl_3$ gave a pure colorless oil **(5b)** with the same yield as in method A.

General Procedure for Ethyl 1-(Substituted benzyl)-4-alkyl-2-piperazinecarboxylate Preparation. Method A: Ethyl 1-(2′-Chlorobenzyl)-4-methyl-2-piperazinecarboxylate (6b). A mixture of **5b** (28 g, 0.1 mol), 37% formaldehyde (12 mL, 0.16 mol), and formic acid (12 mL, 0.3 mol) in CH_3OH (120 mL) was refluxed for 20 h. After evaporation of the solvent, the residue was taken up in ether and saturated Na_2CO_3 solution was added until basic pH. The organic layer was washed with water and dried over $MgSO_4$, and the solvent was removed in vacuo. The crude product was dissolved in anhydrous EtOH (200 mL) and saturated by HCl gas. The addition of ether gave a precipitate of **6b** (32 g, 86%) as a white powder: mp 166−168 °C; IR (film, of free base) 1745 ($\nu_{C=O}$), 1600, 1580 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.47−7.06 (4H, m, aromatic H), 4.15 (2H, q, J = 7.2 Hz, $COOCH_2$), ν_a = 3.88, ν_b = 3.71 (2H, q, AB spectrum, J = 14.5 Hz, I_2/I_1 = 2.33, CH_2Ph), 3.38−3.33, 3.07−2.95, 2.76−2.26 (7H, 3m, piperazinic H), 2.20 (3H, s, NCH_3), 1.22 (3H, t, J = 7.2 Hz, CH_3).

Method B: Ethyl 1-(2′-Chlorobenzyl)-4-ethyl-2-piperazinecarboxylate (8b). This compound was prepared from **5b** following the general procedure described for **4b**, but the solution was stirred at 35 °C for 24 h. The crude product was purified by column chromatography using petroleum ether: ether (80:20, 60:40, and 50:50, v/v) as eluent giving 14.6 g of **8b** as a pale yellow oil. Yield was about 70%: IR (film) 1755 ($\nu_{C=O}$), 1600, 1580 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ ppm 7.48−7.05 (4H, 2m, aromatic H), 4.13 (2H, q, J = 7.2 Hz, $COOCH_2$), ν_a = 3.85, ν_b = 3.69 (2H, q, AB spectrum, J = 14.5 Hz, I_2/I_1 = 2.29, CH_2-Ph), 3.37−3.33, 3.04−2.98, 2.76−2.22 (9H, 3m, piperazinic H, NCH_2-CH_3), 1.21 (3H, t, J = 7.2 Hz, $COOCH_2$-CH_3), 0.98 (3H, t, J = 7.2 Hz, N-CH_2-CH_3).

Table 6. Physical Properties of 1-Alkyl-4-aryl-2-(imidazolin-2′-yl)piperazines and 1,4-Diaryl-2-(imidazolin-2′-yl)piperazines



compd	X	R	mp (°C)[a]	% yield[b]	crystn solvent[c]	formula
19b	2-Cl	CH_3	140−142	62	F	$C_{15}H_{21}ClN_4 \cdot 3HCl \cdot 1H_2O$
19f	2,4-Cl	CH_3	160−162	60	D	$C_{15}H_{20}Cl_2N_4 \cdot 2HCl \cdot 1H_2O$
22b	2-Cl	C_2H_5	138−140	64	B	$C_{16}H_{23}ClN_4 \cdot 3HCl \cdot 1H_2O$
23b	2-Cl	iC_3H_7	158−160	58	C	$C_{17}H_{25}ClN_4$
25a	H		150−152	57	E	$C_{21}H_{26}N_4$
25b	2-Cl		86−88	67	A	$C_{21}H_{24}Cl_2N_4 \cdot 3HCl \cdot 2H_2O$
25f	2,4-Cl		188−190	62	A	$C_{21}H_{22}Cl_4N_4 \cdot 2HCl \cdot 1H_2O$

[a] All compounds had decomposition. [b] Yields of the last step. [c] A, acetone; B, acetone/ethanol; C, acetone/methanol; D, dichloromethane/hexane/methanol; E, ether/hexane; F, ethanol/methanol.

General Procedure for Ethyl 4-(Substituted benzyl)-2-piperazinecarboxylate Preparation: Ethyl 4-(2′-Chlorobenzyl)-2-piperazinecarboxylate (17b). To a refluxing solution of **2** (23 g, 0.1 mol) and triethylamine (55 mL, 0.40 mol) in toluene (200 mL) was added dropwise 2-chlorobenzyl bromide (16.1 g, 0.1 mol) in toluene (200 mL). The reaction mixture was refluxed with stirring for 1 h. After cooling, the solution was washed with water and dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified by column chromatography using first petroleum ether:ether (70:30, v/v) and then ether yielding 10 g (25%) of **24b** and 10 g (35%) of **17b** as a pale yelow oil. **17b:** IR (film) 3347(ν_{N-H}), 1730 ($\nu_{C=O}$), 1594 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.41−7.08 (4H, m, aromatic H), 4.12 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.56 (2H, s, CH_2Ph), 3.53−2.24 (7H, m, piperazinic H), 1.85 (1H, s, NH), 1.18 (3H, t, J = 7.1 Hz, CH_3).

General Procedure for Ethyl 4-(Substituted benzyl)-2-piperazinecarboxylate Preparation. Method A: Ethyl 4-(2′-Chlorobenzyl)-1-methyl-2-piperazinecarboxylate (18b). **18b** was prepared via the general procedure as for **6b**. The crude product was dissolved in anhydrous ethanol (200 mL) and saturated with HCl gas. The addition of ether and chloroform gave a precipitate of **18b** (31.2 g, 89%) as a white powder: mp 155−156.5 °C; IR (film, of the free base) 1750 ($\nu_{C=O}$), 1600, 1580 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.39−7.08 (4H, m, aromatic H), 4.3 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.56 (2H, s, CH_2Ph), 3.0−2.35 (7H, m, piperazinic H), 2.29 (3H, s, NCH_3), 1.19 (3H, t, J = 7.1 Hz, CH_3).

Method B: Ethyl 4-(2′-Chlorobenzyl)-1-ethyl-2-piperazinecarboxylate (20b). **20b** was prepared via the general procedure as for **4b**. The crude product was purified by column chromatography using petroleum ether:ether (90:10, v/v) as eluent giving **20b** as a yelow oil: IR (film) 1745 ($\nu_{C=O}$), 1600 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.4−7.08 (4H, m, aromatic H), 4.11 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.55 (2H, s, CH_2Ph), 3.24−2.27 (9H, m, piperazinic H, NCH_2), 1.17 (3H, t, J = 7.1 Hz, $COOCH_2CH_3$), 1.02 (3H, t, J = 7 Hz, NCH_2CH_3).

General Procedure for Ethyl 1,4-Bis(substituted benzyl)-2-piperazinecarboxylate Preparation: Ethyl 1,4-Bis-(2′-chlorobenzyl)-2-piperazinecarboxylate (24b). It was prepared via the general procedure as for **17b** using 2 equiv of 2-chlorobenzyl bromide. The crude product was purified by crystallization of the hydrochloride salt. After basification 26 g (80%) of **24b** was obtained as a colorless oil: IR (film, of free base) 1745 ($\nu_{C=O}$), 1605, 1585 ($\nu_{C=C}$) cm^{-1}; ^1H NMR δ 7.46−7.05 (8H, m, aromatic H), 4.11 (2H, q, J = 7.2 Hz, $COOCH_2$), ν_a = 3.91, ν_b = 3.75 (2H, q, AB spectrum, J = 14.6 Hz, I_2/I_1 = 2.21, CH_2Ph), 3.53 (2H, s, $PhCH_2$), 3.39−3.35, 3.18−3.08, 2.92−2.84, 2.60−2.39 (7H, 4m, piperazinic H), 1.16 (3H, t, J = 7.2 Hz, CH_3).

General Procedure for Imidazoline Preparation: 1-(2′-Chlorobenzyl)-4-methyl-2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazine (7b). Ethylenediamine (4.5 mL, 0.067 mol) was added dropwise to a stirred solution of trimethylaluminum (58 mL, 0.17 mol) in toluene (70 mL) at 0 °C. After the addition, the reaction mixture was heated at 50 °C and **6b** (9.8 g, 0.033 mol) in toluene (70 mL) was gradually added. The mixture was refluxed for 5 h (under an argon atmosphere). After stirring at room temperature overnight, the mixture was cooled in an ice bath and treated dropwise with water (200 mL) diluted with methanol (80 mL). After filtration and solvent evaporation the residue was taken up in dichloromethane and washed with water. The organic layer was washed with dilute HCl, and the aqueous layer was neutralized by $NaHCO_3$ and extracted twice with ether and then with CH_2Cl_2 several times. The organic layers were dried over $MgSO_4$, and the solvents were removed in vacuo. The residue crystallized in hexane/ether giving 7 g (70%) of **7b** as a white powder: mp 98−99 °C; IR (in KBr) 3150 (ν_{N-H}), 1620 ($\nu_{C=N}$) cm^{-1}; ^1H NMR δ 7.41−7.14 (4H, m, aromatic H), 5.3 (1H, br s, NH), ν_a = 3.75, ν_b = 3.39 (2H, q, AB spectrum, J = 14 Hz, CH_2Ph), 3.79−3.38 (5H, m, imidazolinic and piperazinic H), 2.81−2.12 (6H, 3m, piperazinic H), 2.22 (3H, s, NCH_3).

Compounds **7a**−**i**,**m**−**o** gave ^1H NMR spectra similar to that of **7b**. ^1H NMR spectra of other compounds (particular signals): δ **7j**, 3.43 (3H, s, OCH_3); **7k**, 3.67 (3H, s, OCH_3); **7l**, 3.34 (6H, s, OCH_3); **7p**, 2.43 (3H, s, $C_6H_4CH_3$); **12b**, 3.4 (1H, br s, piperazinic NH); **13b**, 0.97 (3H, t, J = 7.1 Hz, NCH_2CH_3); **22b**, 1.01 (3H, t, J = 7.1 Hz, NCH_2CH_3); **14b**,**f**, 1.51 (2H, m, NCH_2CH_2-CH_3), 0.82 (3H, t, J = 7.2 Hz, $N(CH_2)_2CH_3$); **15b**, 0.96 (3H, d, J = 6.5 Hz, $CHCH_3$); 0.95 (3H, d, J = 6.5 Hz, CH-CH_3); **16f**, 1.98 (2H, d, J = 7.1 Hz, CH_2N), 1.69−1.63 (1H, m, CH), 0.79 (6H, d, J = 6.5 Hz, CH-$(CH_3)_2$). The synthesized imidazolines are listed in Tables 5 and 6.

^1H NMR spectra of *1-benzyl derivative esters or imidazolines (series I) gave an AB system due to the CH_2 group. In series II (**4-benzyl derivatives) these ^1H signals are chemically equivalent and spectra showed a singlet. In each series, other signals are similar.

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 iv injection of a low dose (35 mg/kg) of streptozotocin (STZ) dissolved in a citrate buffer (Thibault et al.[26]) under ketamine hydrochloride anesthesia (75 mg/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 and insulin secretion were assessed by a glucose tolerance test performed 2 weeks after STZ injection.

Intravenous Glucose Tolerance Test (IVGTT). Glucose was dissolved in 0.9% saline and given by the saphenous vein route (0.5 g/kg) in rats under pentobarbital anesthesia (60 mg/kg ip; Clin-Midy, France). Blood samples were collected sequentially from the tail veins before and 5, 10, 15, 20, and 30 min after the injection of glucose. They were then centrifuged, and the plasma was separated. Plasma glucose concentration was immediately determined in a 10 μL aliquot, and the plasma left was kept at −20 °C until radioimmunoassay of insulin.

Drug Administration and Antidiabetic Activity. The 2-(4′,5′-dihydro-1′*H*-imidazol-2′-yl)piperazine derivatives were tested by a single intraperitoneal administration at 100 μmol/kg in the STZ rat 20 min before the IVGTT. All the compounds were used in the form of hydrochloride salts and were water-soluble. Antidiabetic activity of the compounds was evaluated using two parameters: ΔG, which represents the increase in glycemia over baseline integrated over a period of 30 min (IVGTT) following the glucose load, and K, which is the rate of glucose disappearance between 5 and 30 min (in the case of IVGTT), after glucose administration. The K coefficient was calculated only during IVGTT. Insulin secretion during IVGTT (ΔI) was calculated as the incremental plasma insulin values over baseline integrated over 30 min after the glucose load.

Results in Figures 3−5 are expressed as a mean ± SEM. The significance of differences between means was evaluated by a one-way analysis of variance (ANOVA), and results were considered significant at $p < 0.05$.

Tissue and Membrane Preparation. Cerebral cortex was obtained from whole bovine brains and homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged twice at 48000*g* for 25 min at 4 °C. The pellet (used for α_2 binding assays) was resuspended in a phosphate buffer (pH 7.4), flash-frozen, and stored at −80 °C untilneeded for α_2 binding assays.

Reticular nucleus from calf bulbis was homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.7) containing 5 mM EDTA. The homogenate was centrifuged at 500*g* for 10 min at 4 °C. The pellet (P1) was resuspended in the same buffer and centrifuged again. The combined supernatants were centrifuged at 50000*g* for 25 min at 4 °C. The resulting pellet (P2) was resuspended in 50 mM Tris-HCl buffer (pH 7.7) containing 0.1 mM p-methanesulfonyl fluoride, incubated for 30 min at 25 °C, then centrifuged again in the same conditions, resuspended in 50 mM Tris-HCl (pH 7.7), flash-frozen, and stored at −80 °C until I_1 imidazoline binding assays.

Renal cortex was obtained from male New Zealand white rabbits and homogenized in ice-cold preparation buffer (20 mM $NaHCO_3$) (Coupry et al.[39]). The homogenate was centrifuged at 40000*g* for 30 min at 4 °C. The pellet was resuspended in 50 mM Tris-HCl buffer containing 0.5 mM EDTA (pH 7.4), centrifuged again, resuspended in the same buffer, flash-frozen, and stored at −80 °C until I_2 imidazoline binding assays.

Binding Assays. [^{3}H]RX 821002 (48 Ci/mmol), [^{3}H]-*p*-aminoclonidine (50 Ci/mmol), and [^{3}H]idazoxan (42 Ci/mmol) were obtained from Amersham (Buckinghamshire, U.K.) and phentolamine, guanabenz, tolazoline, idazoxan, yohimbine, clonidine, naphazoline, and amiloride from Sigma (St. Louis, MO). Radioligand binding assays with [^{3}H]RX 821002, [^{3}H]-*p*-aminoclonidine, or [^{3}H]idazoxan for determination of specific binding to α_2-adrenoceptors and I_1 and I_2 imidazoline-binding sites, respectively, were performed by a modification of methods previously described (Coupry et al.,[39] Reis et al.,[40] Uhlen and Wikberg[41]). Membranes were slowly thawed and diluted to a concentration of 0.4 mg of protein/mL for renal and cerebral cortex and 0.7 mg of protein/mL for calf bulbis. Assays were conducted in a total volume of 525 μL in polypropylene tubes, and each tube contained 500 μL of membrane suspension, 20 μL of radioligand, and 5 μL of drug. Incubation was initiated by the addition of membrane and carried out for 45 min at 25 °C. Nonspecific binding was defined in the presence of yohimbine (10 μM) in [^{3}H]RX 821002 binding assays, either phentolamine (10 μM) or guanabenz (5 μM) in [^{3}H]-*p*-aminoclonidine binding assays, and either tolazoline (10 μM) or amiloride (10 μM) in [^{3}H]idazoxan binding assays. For each drug, six concentrations from 10^{-4} to 10^{-11} M were used in triplicate. Incubations were terminated by vacuum filtration over Whatman GF/B glass fiber filters using a cell harvester. The filters were washed three times with the buffer, placed in scintillation vials, covered with 2 mL of scintillation cocktail (Pico-Fluor, Packard Instrument), and counted (Packard 2000 CA). Protein was assayed by the Bradford method.

Binding results were analyzed by linear regression, and the curves were obtained with Graph PAD program (Institute for Scientific Information, Philadelphia, PA). K_i was calculated with the Cheng−Prusoff equation.

Analytical Methods. Plasma glucose was determined using a glucose analyzer (Beckman Inc, Fullerton, CA). Plasma immunoreactive insulin (IRI) concentration was determined with a radioimmunoassay kit (CEA, Gif-sur-Yvette, France). The lower limit of the assay was 19.5 pmol/L with a coefficient of variation within and between assays of 6%.

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