Synthesis and Biological Evaluation of Pyrrolinic Isosteres of Rilmenidine. Discovery of *cis*-/*trans*-Dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine (LNP 509), an I_1 Imidazoline Receptor Selective Ligand with Hypotensive Activity

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To find new compounds selective for purported I_1 imidazoline receptors (I_1Rs) over I_2 imidazoline binding sites (I_2BS) and α_2-adrenoceptors (α_2ARs), a series of pyrrolinic isosteres of rilmenidine has been prepared and their biological activity at I_1Rs, I_2BS, and α_2ARs evaluated. This isosteric replacement provided us with compounds which still bound to I_1Rs but not to I_2BS nor to α_2-ARs. A limited structure−affinity relationship was generated around the heterocyclic moiety of these ligands. One compound in this series, LNP 509 (**1e**) [*cis*-/*trans*-dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine], had no detectable affinity at α_2ARs yet was capable of lowering blood pressure after central administration. These pyrrolinic analogues constitute a new chemical class of imidazoline related compounds with high selectivity for the I_1Rs. They could be used as new tools in the study of I_1Rs and in the conception of new centrally acting hypotensive drugs.

Introduction

Clonidine (Figure 1) is an antihypertensive drug known to act within the central nervous system. This activity was first attributed to an activation of α_2-adrenoceptors (α_2ARs); still, in the mid 1980s, Bousquet and co-workers showed that clonidine also interacted with imidazoline binding sites (IBS) of the nucleus reticularis lateralis of the brainstem. Micro-injection of clonidine in this region lowered the blood pressure whereas catecholamines did not.[1]

Then, radioligand binding studies led to the classification of IBS in two subtypes.[2] On one hand, I_1 imidazoline receptors (I_1Rs), sensitive to clonidine and idazoxan, constitute the subtype responsible for the hypotensive activity of imidazolines and related compounds.[3] These receptors are distributed in both peripheral and central nervous systems. Agmatine,[4] CDS (clonidine displacing substance),[5] and more recently harmane[6] have been proposed as endogenous ligands. Two different signaling pathways associated to these receptors in PC 12 cells have been discovered: the PC-PLC (phosphatidylcholine-phospholipase C) and the c-AMP pathways.[7,8] On the other hand, I_2 imidazoline binding sites (I_2BS), sensitive to idazoxan and insensitive to clonidine, have been shown to be an allosteric site on monoamine oxidase (MAO).[9] However, this classification may not be definitive since at least one additional subtype has already been proposed.[10]

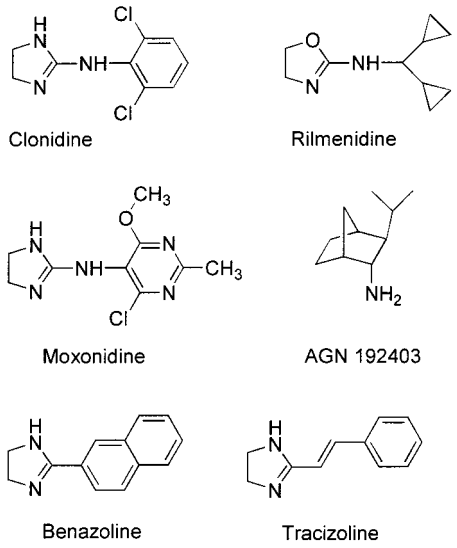

Figure 1.

According to recent studies, these IBS are involved in different physiological functions such as blood pressure[11] and intraocular pressure regulation,[12] insulin release,[13,14] protein synthesis in astrocytes,[15] gastric acid secretion,[16] neurotransmitter release,[17] food intake,[18] MAO activity,[19] and arrhythmogenesis.[20]

Although clonidine, which displays nanomolar affinities for both I_1Rs and α_2ARs, has shown a powerful antihypertensive activity, its use is limited by side effects resulting from α_2ARs activation. For instance, sedation is known to be a consequence of α_2ARs activation in another medullary region, the locus coeruleus.[21] The second generation of centrally acting antihyperten-

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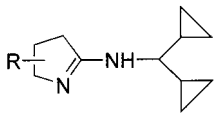
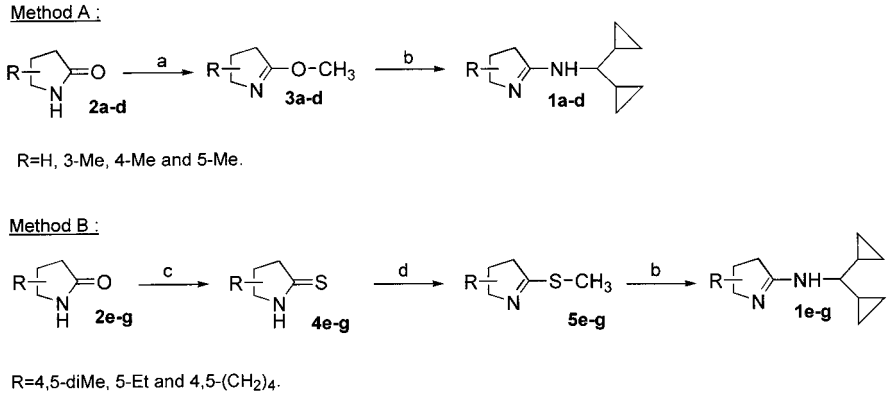
Table 1. Physical and Chemical Data of Compounds **1a**−**g**



compd	R	method	mp[a] (°C)	solv[b]	yield[c] (%)	formula	anal.[d]
1a	H	A	223−225	1	65	$C_{11}H_{18}N_2 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N
1b	5-Me	A	144−146	2	87	$C_{12}H_{20}N_2 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N
1c	3-Me	A	201−203	1	42	$C_{12}H_{20}N_2 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N
1d	4-Me	A	198−199	1	75	$C_{12}H_{20}N_2 \cdot HCl \cdot {}^1/_4H_2O$	C, H, N
1e	*cis*-/*trans*-4,5-diMe	B	143−145	1	56	$C_{12}H_{22}N_2 \cdot HCl$	C, H, N
1f	5-Et	B	163−165	2	39	$C_{12}H_{22}N_2 \cdot HCl$	C, H, N
1g	*cis*-4,5-$(CH_2)_4$	B	204−206	3	49	$C_{15}H_{24}N_2 \cdot HCl$	C, H, N

[a] Melting points of the hydrochloride salt. [b] Crystallization solvent of the hydrochloride salt: 1 (2-PrOH−Et_2O), 2 (2-PrOH−EtOAc), and 3 (EtOH−EtOAc). [c] Yield of coupling reaction with *C*,*C*-dicyclopropylmethylamine. [d] All compounds were analyzed within 0.4% of the theoretical values.

Scheme 1[a]



[a] Reagents: (a) $(CH_3O)_2SO_2$, 60 °C; (b) $(C_3H_5)_2CHNH_2 \cdot HCl$, MeOH; (c) Lawesson's reagent, THF, reflux; (d) CH_3I, 2-PrOH.

sive drugs, represented by rilmenidine and moxonidine (Figure 1), appeared to have greater selectivity for I_1Rs over α_2ARs and a much improved acceptability;[11] although their affinities at α_2ARs were reduced as compared to clonidine, these drugs still bound to these receptors. Substances with virtually no affinity at α_2-ARs but binding to I_1Rs, which could be very useful tools for further biochemical and pharmacological characterization of the receptors, were missing. Since then, such compounds have been discovered. Munk et al. described the new agent AGN 192403 (Figure 1) with very interesting binding properties but devoid of any antihypertensive activity.[22] Pigini et al.[23] reported the synthesis of benazoline and tracizoline (Figure 1) with high selectivity for I_1Rs over α_2ARs.[8] However, these agents were not hypotensive and still had nanomolar affinities for I_2BS. Ligands highly selective for I_1Rs, versus both I_2BS and α_2ARs, and able to reduce blood pressure were still missing. In this study, preparation of such compounds is reported.

Aminoimidazolines (also called iminoimidazolidines), 2-alkylimidazolines, aminooxazolines, guanidines, or primary amines (AGN 192403) were previously reported as imidazoline related compounds. As we will discuss below, aminopyrrolines constitute an additional chemical class of I_1Rs ligands. We were particularly interested in aminopyrrolines derived from rilmenidine, the latter having high selectivity for I_1Rs over I_2BS (Table 2). Numerous clonidine analogues have previously been synthesized,[24−27] but to our knowledge, no rilmenidine analogues have been described yet. Moreover, there is no report on pyrrolinic isosteres of imidazoline compounds.

Table 2. Binding Data of Selected Compounds

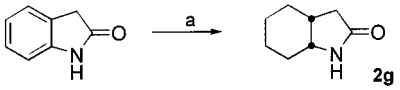
	pK_i ± sem		
compd	I_1Rs	I_2BS	α_2ARs
1a	6.29 ± 0.11	<5	<5
1b	5.80 ± 0.08	<5	<5
1c	<5	<5	<5
1d	6.19 ± 0.10	<5	<5
cis-/*trans*-**1e**	6.27 ± 0.11	<5	<5
1f	6.77 ± 0.09	<5	<5
1g	<5	<5	<5
rilmenidine	7.13 ± 0.10	<5	7.25 ± 0.08

We describe here the synthesis of a series of aminopyrrolines (**1a**−**g**), structurally related to rilmenidine, as well as some of their pharmacological properties. The structures of these compounds are presented in Table 1.

Chemistry

Two different methods were used to couple the dicyclopropylmethylamine moiety with lactams **2** (Scheme 1). In method A, lactams were activated by reaction with dimethyl sulfate to obtain the 2-methoxypyrrolines **3a**−**d**. These methoxy intermediates reacted easily with dicyclopropylmethylamine in acidic conditions to form the desired compounds **1a**−**d**.[28] In the other coupling method (method B), the lactams were first transformed into thiolactams **4e**−**g** with Lawesson's reagent.[29] In fact, thiolactams are more convenient to handle on

Scheme 2[a]



[a] Reagents: (a) H_2/Rh-Al_2O_3, *t*-BuOH, 15 atm, 68%.

small scale than lactams: they are easier to detect in TLC and to separate in chromatography; they are solids whereas lactams are liquids. These thiolactams **4e**−**g** were then methylated with MeI to form the activated intermediates **5** which reacted with dicyclopropylmethylamine in acidic conditions to give the products **1e**−**g**.

Compound **1e** is a mixture of cis and trans adducts. These two adducts are present in same amounts as shown by ^{1}H NMR of **1e**.

The starting lactams **2a**−**c** are commercially available and the others **2d**−**g** have been synthesized according to published procedures,[30,31] except **2g** which was obtained in the cis conformation by reduction of oxindole with Rh-Al_2O_3 under pressure (15 atm) (Scheme 2).[32]

Pharmacology

Binding Experiments. Affinities of compounds **1a**−**g** for I_1Rs, I_2BS, and α_2ARs were determined by receptor binding assays. The radioligands used were [^{3}H]clonidine (I_1Rs), [^{3}H]idazoxan (I_2BS), and [^{3}H]RX821002 (α_2-ARs). Although I_1Rs involved in the hypotensive effect of imidazoline like-drugs are located in the brainstem, we used bovine chromaffin cell membrane preparations for I_1Rs binding assay wherein binding has been shown to be pharmacologically equivalent to that performed on bovine rostral ventrolateral medullary membranes.[3] I_2BS binding assay was performed on rabbit renal cortex and α_2ARs on calf frontal cortex membranes.

In Vivo Studies. The effects of compound **1e** (cis/trans mixture) on mean arterial blood pressure were determined after intravenous and intracisternal administrations according to methods described elsewhere.[33]

Results and Discussion

Binding characteristics of compounds **1a**−**g** are presented in Table 2. Rilmenidine was considered as the reference compound.

The isosteric replacement of the oxazoline ring of rilmenidine by a pyrrolinic ring to give **1a** had only weak influence on I_1Rs affinity (pK_i = 7.13 for rilmenidine and 6.29 for **1a**) and I_2BS affinity (pK_i < 5 for both rilmenidine and **1a**). On the contrary, the affinity for α_2ARs was attenuated: **1a** had no detectable affinity for this receptor while rilmenidine had a p*K*i value of 7.25. These data show the crucial implication of the oxygen atom of rilmenidine in the binding with α_2ARs. One can imagine that this oxygen takes part in a H-bond within this receptor. But replacement of this oxygen atom must also cause an increase of basicity of the ligands: the basicity of aminopyrrolines is greater than that of aminooxazolines.[34] This difference can also account for the loss of α_2ARs affinity observed for **1a**. The oxygen atom of the oxazoline ring seems to play a weaker role in the binding with I_1Rs. This observation indicates that I_1Rs and α_2ARs are sensitive to subtle structural differences and therefore may accommodate two distinct pharmacophores.

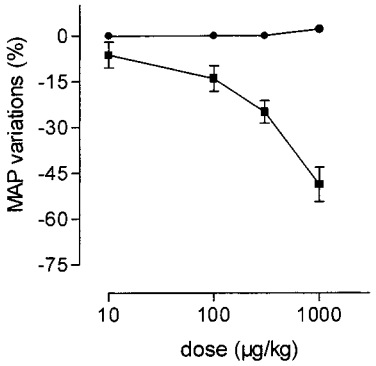


Figure 2. Effect on MAP of cumulative doses of *cis*-/*trans*-**1e** (squares) and repeated injections of vehicle (circles) administered intracisternally to anaesthetized rabbits. Data are mean ± sem of six experiments.

The steric tolerance around the pyrrolinic cycle of **1a** was then explored by the introduction of alkyl groups on the heterocyclic frame. We observed that methylation at C-3 decreased the affinity for I_1Rs (pK_i < 5 for **1c**), whereas methylations at C-4 (**1d**) and C-5 (**1b**) had weak effects on binding (pK_i, respectively, of 6.19 and 5.80 versus 6.29 for the unsubstituted analogue **1a**). Bulkier alkyl groups were then added at C-4 and C-5 of the pyrroline ring. Compared to the unsubstituted analogue, **1f** (5-ethyl) and **1e** (*cis*-/*trans*-4,5-dimethyl) maintained potency on I_1Rs binding with a small increase of affinity for **1f** (pK_i = 6.77) and no significant change for **1e** (pK_i = 6.27). When carbons C-4 and C-5 were included in a cyclohexyl moiety with a cis configuration, the so obtained compound **1g** showed a decreased I_1Rs affinity (pK_i < 5) reflecting the higher limit of steric hindrance. These results clearly showed a limited steric bulk tolerance around the pyrrolinic ring. Moreover, irrespective of their substituents, none of these pyrrolinic analogues exhibited significant affinity for I_2BS or α_2ARs.

The next step was the cardiovascular evaluation of these new agents compared to the reference compound, rilmenidine. For this purpose, we studied the blood pressure effects of *cis*-/*trans*-**1e**. This diastereoisomer mixture was ineffective after intravenous administration to aneaesthetized rabbits up to the dose of 5 mg/kg (data not shown). But after intracisternal administration, it caused an important dose-dependent reduction of the mean arterial pressure (MAP) (Figure 2). One explanation for this difference could be that it was simply unable to cross the blood brain barrier. In fact, the basicity of aminopyrrolines is greater than that of aminooxazolines;[34] the proportion of ionized molecules of **1e** is therefore higher than that of rilmenidine at physiological pH. Still, we cannot rule out other explanations such as metabolism to explain this difference in activity.

After intracisternal injection, the maximal effect of the cis/trans mixture **1e** was similar to that of rilmenidine (49 ± 6% versus 41 ± 3%). It has been proved that the rank order of I_1Rs binding affinities is similar to the rank order of hypotensive activities after intracisternal injection, i.e., clonidine = moxonidine > rilmenidine, supporting a link between I_1Rs and imidazoline-induced hypotension.[3,35] Data we obtained with *cis*-/*trans*-**1e** is consistent with this rank ordering, and **1e** is therefore the first substance selective for I_1Rs over

α_2ARs and I_2BS. Our results support the hypothesis that an exclusive action on cerebral I_1Rs is sufficient to induce hypotension. They corroborate the observation of Ernsberger et al. who showed that moxonidine, a mixed I_1Rs and α_2ARs ligand, decreased the MAP of mice with impaired α_2ARs after intracisternal administration.[36]

The synthesis of selective I_1Rs molecules may help clarify the respective role of I_1Rs and α_2ARs in the hypotensive activity of clonidine related compounds.[37]

Conclusion

In this study, a series of pyrrolinic analogues of rilmenidine was synthesized. Due to the isosteric substitution described, α_2ARs affinity was lost, while I_1Rs affinity was only slightly affected. This result reveals an interesting difference in the pharmacophores of I_1Rs and α_2ARs: the distinctive importance of the oxygen atom of rilmenidine for these two receptor bindings. Moreover, these new compounds did not bind to I_2BS. Thus, new selective ligands for I_1Rs over I_2BS and α_2ARs were obtained. It was also shown that there is a limited steric hindrance around the pyrrolinic ring of these analogues. *cis*-/*trans*-**1e**, one of these analogues, was highly selective for I_1Rs, and it reduced mean arterial pressure of rabbits when administrated intracisternally. These results support the fact that I_1Rs are involved in the central regulation of MAP and are targeted by imidazoline related drugs. *cis*-/*trans*-**1e** represents a prototype compound with good selectivity for I_1Rs and cardiovascular activity.

Experimental Section

Chemistry. All solvents were purified by standard methods before use. Flash chromatographies[38] were performed on Merck silica gel Si 60 (40−63 μm) and TLC on silica gel 60 F_{254} (Merck). All organic layers were washed with brine and then dried with Na_2SO_4. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Bruker AC 200 spectrometer. Solvents used were $CDCl_3$ ($CHCl_3$ at 7.26 ppm as reference) and D_2O (*t*-BuOH at 1.28 ppm as reference) for ^{1}H NMR and D_2O (*t*-BuOH at 70.4 and 30.3 ppm as reference) for ^{13}C NMR. The signals are described as: s (singlet); d (doublet); t (triplet); m (multiplet); and br (broad). Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The mass spectra were recorded using a LKB 2091 apparatus. The related intensities of the mass spectrum peaks are listed in parentheses. Elemental analyses were performed at the Service de microanalyse, Université Louis Pasteur, Strasbourg.

All target compounds were tested as hydrochloride salts. These salts were prepared by the addition of ethanolic HCl to an ethanolic solution of the base.

Method A: General Procedure for the Preparation of Compounds 1a−d. 2-Methoxypyrrolines 3a−d. Lactam **2a−d** (0.2 mol) was added to dimethyl sulfate (25.5 g, 0.2 mol), and the mixture was heated at 60 °C for 16 h under nitrogen. The mixture was then cooled and added to a saturated aqueous solution of K_2CO_3 (about 150 mL). This solution was extracted with ether, which was dried and evaporated to give, after distillation under reduced pressure, the corresponding 2-methoxypyrroline **3a−d.**

2-Methoxy-4,5-dihydro-3*H*-pyrrole (3a):[39] 63%; bp 59−60 °C/80 mmHg; MS *m*/*z* 99(84), 84(15), 71(21), 69(34), 56(80), 42(53), 41(100).

2-Methoxy-5-methyl-4,5-dihydro-3*H*-pyrrole (3b): 63%; bp 66−68 °C/85 mmHg; MS *m*/*z* 113(32), 98(100), 70(47), 56(37), 41(23).

2-Methoxy-3-methyl-4,5-dihydro-3*H*-pyrrole (3c): 37%; bp 60 °C/55 mmHg; MS *m*/*z* 82(35), 70(100), 56(11), 41(12).

2-Methoxy-4-methyl-4,5-dihydro-3*H*-pyrrole (3d): 15%; bp 62 °C/50 mmHg; MS *m*/*z* 113(100), 98(34), 56(80), 41(38).

Aminopyrrolines 1a−d. To a solution of the required methoxy derivative **3a−d** (20 mmol) in 10 mL of methanol was added *C*,*C*-dicyclopropylmethylamine hydrochloride (2.95 g, 20 mmol).[40] The mixture was stirred under nitrogen for 48 h. Solvent was removed in vacuo and then water and 2 N NaOH were added. This basic aqueous solution was extracted with ether (3 × 50 mL), and the ether layer was dried and evaporated to give, after crystallization, the product **1a−d**.

Dicyclopropylmethyl-(4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1a·HCl): 65%; mp base 133−134 °C (cyclohexane), mp hydrochloride 223−225 °C (2-PrOH−ether); ^{1}H NMR (D_2O) δ 3.67 (t, 2H, H-5, J = 7.2 Hz), 2.75−2.98 (m, 3H, H-3 + CH), 2.10−2.30 (m, 2H, H-4), 1.00−1.22 (m, 2H, 2 × CH), 0.28−0.72 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 168.4 (C_2), 62.1 (CH-NH), 48.0 (C_5), 31.1 (C_3), 20.8 (C_4), 14.3 + 2.9 + 1.9 (C_3H_5); MS *m*/*z* 178(33), 163(97), 149(68), 137(61), 135(32), 122(31), 109(54), 95(52), 67(63), 55(50), 41(75). Anal. ($C_{11}H_{18}N_2$·HCl·$^1/_2H_2O$) C, H, N.

Dicyclopropylmethyl-(5-methyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1b·HCl): 87%; mp base 99−101 °C (cyclohexane), mp hydrochloride 144−146 °C (2-PrOH−EtOAc); ^{1}H NMR (D_2O) δ 3.97−4.15 (m, 1H, H-5), 2.70−2.96 (m, 3H, H-3 + CH), 2.21−2.43 (m, 1H, H-4), 1.63−1.85 (m, 1H, H-4), 1.26 (d, 3H, CH_3, J = 6.6), 0.95−1.19 (m, 2H, 2 × CH), 0.20−0.68 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 167.6 (C_2), 61.9 (CH-NH), 57.1 (C_5), 30.9 (C_3), 28.9 (C_4), 20.6 (CH_3), 14.3 + 3.0 + 1.9 (C_3H_5); MS *m*/*z* 192(29), 177(100), 164(14), 163(50), 151(57), 149(24), 136(16), 123(34), 95(77), 67(61), 55(50), 41(75). Anal. ($C_{12}H_{20}N_2$·HCl·$^1/_4H_2O$) C, H, N.

Dicyclopropylmethyl-(3-methyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1c·HCl): 42%; mp base 102−104 °C (cyclohexane), mp hydrochloride 201−203 °C (2-PrOH−ether); ^{1}H NMR (D_2O) δ 3.50−3.78 (m, 2H, H-5), 3.08−3.27 (m, 1H, H-3), 2.75 (t, 1H, CH, J = 7.5 Hz), 2.29−2.50 (m, 1H, H-4), 1.77−1.97 (m, 1H, H-4), 1.33 (d, 3H, CH_3, J = 7.4), 1.02−1.22 (m, 2H, 2 × CH), 0.20−0.75 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 171.6 (C_2), 62.2 (CH-NH), 46.2 (C_5), 38.4 (C_3), 29.3 (C_4), 16.8 (CH_3), 14.3 + 3.1 + 1.8 (C_3H_5); MS *m*/*z* 192(30), 177(100), 164(19), 163(67), 151(71), 149(32), 136(24), 123(45), 95(47), 67(58), 55(56), 41(75). Anal. ($C_{12}H_{20}N_2$·HCl·$^1/_4H_2O$) C, H, N.

Dicyclopropylmethyl-(4-methyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1d·HCl): 75%; mp base 113−114 °C (cyclohexane), mp hydrochloride 198−199 °C (2-PrOH−ether); ^{1}H NMR (D_2O) δ 3.73−3.83 (m, 1H, H-5), 3.22−3.31 (m, 1H, H-5), 2.97−3.09 (m, 1H, H-3), 2.47−2.84 (m, 3H, H-3 + H-4 + CH), 1.15 (d, 3H, CH_3, J = 6.4), 0.90−1.25 (m, 2H, 2 × CH), 0.28−0.70 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 167.9 (C_2), 62.0 (CH-NH), 54.5 (C_5), 38.6 (C_3), 30.1 (C_4), 18.3 (CH_3), 14.3 + 3.0 + 2.0 (C_3H_5); MS *m*/*z* 192(21), 177(100), 164(10), 163(47), 151(51), 149(23), 136(17), 123(34), 95(39), 67(49), 55(31), 41(45). Anal. ($C_{12}H_{20}N_2$·HCl·$^1/_4H_2O$) C, H, N.

Method B: General Procedure for the Preparation of Compounds 1e−g. Thiolactams 4e−g. Lawesson's reagent (14.88 g, 36.8 mmol) was added to a solution of the required lactam **2e−g** (73.6 mmol) in dry THF (400 mL). The mixture was stirred at room temperature for 24 h. Solvent was then removed in vacuo and the product purified by flash chromatography (EtOAc−hexane 3/7) to give the corresponding thiolactam **4e−g**.

Mixture of *cis*- and *trans*-4,5-dimethyl-pyrrolidine-2-thione (4e): 98%; ^{1}H NMR ($CDCl_3$) δ 8.72 (br, 1H, NH), 4.01 (m, 0.5H, H-5), 3.55 (m, 0.5H, H-5), 2.89−3.16 (m, 1H, H-3), 2.42−2.71 (m, 1.5H, H-3 + H-4), 2.02−2.23 (m, 0.5H, H-4), 1.27 (d, 1.5H, CH_3, J = 6.4), 1.15 (d, 1.5H, CH_3, J = 6.7), 1.10 (d, 1.5H, CH_3, J = 6.7), 0.98 (d, 1.5H, CH_3, J = 6.8); MS *m*/*z* 129(100), 114(59), 81(17), 86(17), 71(21), 55(43), 44(30), 42(13).

5-Ethyl-pyrrolidine-2-thione (4f): 53%; ^{1}H NMR ($CDCl_3$) δ 8.50 (br, 1H, NH), 3.79−3.93 (m, 1H, H-5), 2.76−3.05 (m, 2H, H-3), 2.23−2.41 (m, 1H, H-4), 1.45−1.90 (m, 3H, H-4 +

CH_2), 0.96 (t, 3H, CH_3, $J = 7.4$); MS *m*/*z* 130(14), 129(67), 100-(100), 71(10), 67(30), 42(13), 41(21).

Octahydro-indole-2-thione (4g): 52%; mp 94−95 °C (EtOAc−cyclohexane); ^{1}H NMR ($CDCl_3$) δ 8.12 (br, 1H, NH), 3.87−3.96 (m, 1H, H-7a), 2.39−2.93 (m, 3H, H-3a+H-3), 1.15−1.87 (m, 8H, 4 × CH_2); MS *m*/*z* 156(52), 155(100), 112(54), 99-(27), 81(16), 67(19), 41(14).

Aminopyrrolines 1e−g. An excess of iodomethane (14 g, 98 mmol) was added to a solution of thiolactam **4e−g** (14 mmol) and 2-PrOH (100 mL). The mixture was stirred under nitrogen for 12 h, and then 2-PrOH was removed in vacuo. The residue was treated with water and saturated K_2CO_3 and extracted with ether (3 × 50 mL). After this solution has been dried and evaporated, the residue was treated with C,C-dicyclopropylmethylamine hydrochloride (1.98 g, 14 mmol) in 10 mL ethanol under nitrogen and at reflux for 24 h. Solvent was removed in vacuo, and then water and saturated K_2CO_3 were added. This basic aqueous solution was extracted with ether (3 × 50 mL), and the organic layer was dried and evaporated to give, after crystallization, the products **1e−g**.

Mixture of *cis*- and *trans*-dicyclopropylmethyl-(4,5-dimethyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1e·HCl): 56%; mp base 65−66 °C (hexane), mp hydrochloride 143−145 °C (2-PrOH−ether); ^{1}H NMR (D_2O) δ 4.09 (m, 0.5H, H-5), 3.64 (m, 0.5H, H-5), 2.50−3.10 (m, 3.5H, H-4 + H-3 + CH), 2.18 (m, 0.5H, H-4), 1.00−1.20 (m, 8H, 2 × CH_3 + 2 × CH), 0.30−0.70 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 167.1 (C_2), 63.4 (C_5), 61.8 (CH-NH), 59.1 (C_5), 38.5 (C_3), 38.3 (C_4), 37.9 (C_3), 33.3 (C_4), 19.0 (CH_3), 16.8 (CH_3), 15.0 (CH_3), 13.7 (CH_3), 14.3 + 3.0 + 1.9 (C_3H_5); MS *m*/*z* 206(41), 191(100), 177(34), 165(32), 151(16), 137(16), 126(15), 113(27), 97(22), 95-(45), 79(20), 67(31), 55(21), 41(31). Anal. ($C_{13}H_{22}N_2$·HCl) C, H, N.

Dicyclopropylmethyl-(5-ethyl-4,5-dihydro-3*H*-pyrrol-2-yl)-amine hydrochloride (1f·HCl): 39%; mp base 72−73 °C (cyclohexane), mp hydrochloride 108−110 °C (2-PrOH−ether); ^{1}H NMR (D_2O) δ 3.99 (m, 1H, H-5), 2.81−2.95 (m, 3H, H-3 + CH), 2.22−2.41 (m, 1H, H-4), 1.53−1.92 (m, 3H, H-4 + CH_2), 1.01−1.18 (m, 2H, 2 × CH), 0.93 (t, 3H, CH_3, $J = 7.5$ Hz), 0.30−0.70 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 167.8 (C_2), 62.4 (CH-NH), 61.8 (C_5), 30.6 (C_3), 27.8 (C_4), 25.9 (CH_2), 9.17 (CH_3), 14.3 + 3.0 + 1.9 (C_3H_5); MS *m*/*z* 206(22), 191(54), 177-(87), 165(32), 151(11), 137(14), 126(13), 113(21), 95(100), 83-(43), 79(21), 67(45), 55(25), 41(33). Anal. ($C_{13}H_{22}N_2$·HCl) C, H, N.

Dicyclopropylmethyl-(3a,4,5,6,7,7a-hexahydro-3*H*-indol-2-yl)-amine hydrochloride (1g·HCl): 49%; mp base 93−95 °C (cyclohexane−isopentane), mp hydrochloride 204−206 °C (ethanol−EtOAc); ^{1}H NMR (D_2O) δ 3.97−4.04 (m, 1H, H-5), 2.80−3.01 (m, 2H, H-3 + CH), 2.39−2.58 (m, 2H, H-3 + H-4), 1.04−1.80 (m, 10H, 4 × CH_2 + 2 × CH), 0.30−0.75 (m, 8H, 4 × CH_2); ^{13}C NMR (D_2O) δ 168.3 (C_2), 62.0 (CH-NH), 59.2 (C_5), 37.9 (C_3), 34.7 (C_4), 27.2 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 20.5 (CH_2), 14.3 + 3.0 + 1.9 (C_3H_5); MS *m*/*z* 233(36), 232(63), 217(88), 203(46), 191(60), 189(57), 152(25), 139(33), 137(25), 95(100), 67(45), 41(37). Anal. ($C_{15}H_{24}N_2$·HCl) C, H, N.

Octahydro-indol-2-one (2g). Oxindole (3 g, 22.6 mmol) was reduced in *t*-BuOH (60 mL) with Rh/Al_2O_3 under 15 atm of H_2 during 12 h at the temperature of 50 °C. The mixture was then filtered through Celite and the Celite bed rinsed with EtOH (50 mL). After evaporation, **2g**[41−43] (2.14 g, 68%) was obtained as a colorless oil, which crystallized in the cold. ^{1}H NMR ($CDCl_3$) δ 6.17 (br, 1H, NH), 3.55−3.80 (m, 1H, H-7a), 1.81−2.55 (m, 3H, H-3a + H-3), 1.05−1.82 (m, 8H, 4 × CH_2); MS *m*/*z* 139(30), 96(100), 83(9), 68(9), 55(12), 41(10).

Radioligand Binding Assays. I_1 Receptors Binding Assays. Tissue used was adrenal medullary plasma membranes, prepared as described by Molderings et al.[44] These membranes (0.8 mg protein/mL) were incubated for 40 min at 22 °C with 7 nM [^{3}H]clonidine (K_D of the radioligand) in binding buffer (PBS, 0.5 mM EGTA, 0.5 mM $MgCl_2$, 0.5% ascorbic acid, pH 7.5) and increasing concentrations of drugs (10^{-9} to 10^{-4} M) in the presence of 1 μM RX821002 to mask α_2-adrenoceptors. Nonspecific binding was defined as [^{3}H]-clonidine binding in the presence of 1 μM of (5-(2-methyl-phenoxy-methyl)-1,3-oxazoline-2-yl)amine (S22687)[45] (high affinity competition drug, $K_i = 4.98$ nM) and represented 55% of the total binding.

I_2BS Binding Assays. Tissue used was prepared as follows: renal cortex from male New Zealand rabbits were homogenized in ice-cold preparation buffer (20 mM $NaHCO_3$).[46] The homogenate was centrifuged at 40000*g* for 30 min at 4 °C. The pellet was resuspended in buffer (50 mM Tris HCl, 0.5 mM EDTA, pH 7.4), centrifuged again, and resuspended in the same buffer. The pellet was stored at −80 °C until use. Affinities of drugs for I_2 binding sites were determined as reported by Pigini et al.[23] with [^{3}H]idazoxan as radioligand; 2-BFI (10^{-5} M) was used to define the nonspecific binding.

α_2-Adrenoceptors Binding Assay. Tissue used was prepared from calf frontal cortex membranes according to Liefde et al.[47] The membranes (0.5 mg protein/mL) were incubated for 60 min at 25 °C with 0.8 nM [^{3}H]RX821002 in the presence of 0.3 μM 5HT to mask $5HT_{1A}$ receptors in 50 mM sodium phosphate buffer, pH 7.4, with increasing concentrations of drugs (10^{-9} to 10^{-4} M). Nonspecific binding was defined with 10 μM phentolamine.

In these three assays, incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters followed by rapid washing of the tubes and filters three times with ice-cold binding buffer. Radioactivity remaining on the filters was counted in a Packard Tricarb counter. Results are given as the mean ± standard error mean (sem) of three experiments, each performed in triplicate. Competition experiments were analyzed using the iterative nonlinear least-squares curve fitting program GraphPad. K_i were determined using the method of Cheng and Prussof.[48]

Cardiovascular Experiments. Cardiovascular evaluations of compound *cis*-/*trans*-**1e** were performed according to published procedures:[33] normotensive male rabbits (Zika strain) weighing 2.5 to 3.5 kg were anaesthetized with pentobarbital sodium (40 mg/kg), paralyzed with pancuronium bromide (1 mg/kg), and artificially ventilated. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the differential pressure. In experiments during which drugs were injected intracisternally, 0.2 mL of drug solutions were injected after removal of the same volume of cerebrospinal fluid. In control experiments, vehicle alone was injected the same way in the same volume and never modified MAP significantly, as shown in Figure 2 ($n = 6$).

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Supporting Information Available: Elemental analysis of compounds **1a−g**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bousquet, P.; Feldman, J.; Schwartz, J. Central Cardiovascular Effects of α-Adrenergic Drugs: Differences between Catecholamines and Imidazolines. *J. Pharmacol. Exp. Ther.* **1984**, *230*, 232−236.

(2) Molderings, G. J. Imidazoline Receptors: Basic Knowledge, Recent Advances and Future Prospects for Therapy and Diagnosis. *Drugs Future* **1997**, *22*, 757−772.

(3) Ernsberger, P.; Friedman, J. E.; Koletsky, R. J. The I_1−Imidazoline Receptor: from Binding Site to Therapeutic Target in Cardiovascular Disease. *J. Hypertens.* **1997**, *15* (suppl 1), S9−S23.

(4) Li, G.; Regunathan, S.; Barrow, C. J.; Eshraghi, J.; Cooper, R.; Reis, D. J. Agmatine: An Endogenous Clonidine-displacing Substance in the Brain. *Science* **1994**, *263*, 966−969.

(5) Atlas, D.; Burstein, Y. Isolation and Partial Purification of a Clonidine-displacing Endogenous Brain Substance. *Eur. J. Biochem.* **1984**, *144*, 287−293.
(6) Musgrave, I. F.; Badoer, E. Harmane Produces Hypotension Following Microinjection into the RVLM: Possible Role of I(1)-Imidazoline Receptors. *Br. J. Pharmacol.* **2000**, *129* (6), 1057−1059.
(7) Separovic, D.; Kester, M.; Ernsberger, P. Coupling of I_1−Imidazoline Receptors to Diacylglyceride Accumulation in PC 12 Rat Pheochromocytoma Cells. *Mol. Pharmacol.* **1996**, *49*, 668−675.
(8) Greney, H.; Ronde, P.; Magnier, C.; Maranca, F.; Rascente, C.; Quaglia, M.; Gianella, M.; Pigini, M.; Brasili, L.; Lugnier, C.; Bousquet, P.; Dontenwill, M. Coupling of I_1 Imidazoline Receptors to the cAMP Pathway: Studies with a Highly Selective Ligand, Benazoline. *Mol. Pharmacol.* **2000**, *57*, 1142−1151.
(9) Tesson, F.; Limon-Boulez, I.; Urban, P.; Puype, M.; Vandekerckhove, J.; Coupry, I.; Pompon, D.; Parini, A. Localisation of I_2-Imidazoline Binding Sites on Monoamine Oxidases. *J. Biol. Chem.***1995**, *270*, 9856−9861.
(10) Eglen, R. M.; Hudson, A. L.; Kendall, D. A.; Nutt, D. J.; Morgan, N. G.; Wilson, V. G.; Dillon, M. P. 'Seeing through a Glass Darkly': Casting Light on Imidazoline 'I' Sites. *Trends Pharmacol. Sci.* **1998**, *19* (9), 381−390.
(11) Bousquet, P.; Feldman, J. Drugs Acting on Imidazoline Receptors: a Review of their Pharmacology, their Use in Blood Pressure Control and their Potential Interest in Cardioprotection. *Drugs* **1999**, *58* (5), 799−812.
(12) Chu, T. C.; Socci, R. R.; Ogidigben, M. J.; Potter, E. D. Potential Mechanisms of Moxonidine-induced Ocular Hypotension: Role of Norepinephrine. *J. Ocul. Pharmacol. Ther.* **1997**, *13*, 489−496.
(13) Chan, S. L. F. Clonidine-displacing Substance and its Putative Role in Control of Insulinsecretion: A Minireview. *Gen. Pharmacol.* **1998**, *31*, 525−529.
(14) Rondu, F.; Le Bihan, G.; Wang, X.; Lamouri, A.; Touboul, E.; Dive, G.; Bellahsene, T.; Pfeiffer, B.; Renard, P.; Guardiola-Lemaitre, B.; Manechez, D.; Penicaud, L.; Ktorza, A.; Godfroid, J. J. 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. *J. Med. Chem.* **1997**, *40*, 3793−3803.
(15) Garcia-Sevilla, J. A.; Escriba, P. V.; Walzer, C.; Bouras, C.; Guimon, J. Imidazoline Receptor Proteins in Brains on Patients with Alzheimer's Disease. *Neurosci. Lett.* **1998**, *247*, 95−98.
(16) Molderings, G. J.; Donecker, K.; Burian, M.; Simon, W. A.; Schroder, D. W.; Gothert, M. Characterisation of I-2 Imidazoline and Sigma Binding Sites in the Rat and Human Stomach. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 170−177.
(17) Heemskerk, F. M. J.; Dontenwill, M.; Greney, H.; Vonthron, C.; Bousquet, P. Evidence for the Existence of Imidazoline-specific Binding Sites in Synaptosomal Plasma Membranes of the Bovine Brainstem. *J. Neurochem.* **1998**, *71*, 2193−2202.
(18) Nutt, D. J.; French, N.; Handley, S.; Hudson, A.; Husbands, S.; Jackson, H.; Jordan, S.; Lalies, M. D.; Lewis, J.; Lione, L.; Mallard, N.; Pratt, J. Functional Studies of Specific Imidazoline-2 Receptor Ligands. *Ann. N. Y. Acad. Sci.* **1995**, *763*, 125−139.
(19) Raddatz, R.; Savic, S. L.; Bakthavachalam, V.; Lesnick, J.; McGrath, C. R.; Parini, A.; Lanier, S. M. Imidazoline-binding Domains on Monoamine Oxidase B and Subpopulations of Enzyme. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 1135−1145.
(20) Roegel, J. C.; de Jong, W.; Monassier, L.; Feldman, J.; Bousquet, P. Comparative Effects of Idazoxan, Prazocin, and Yohimbine on Coronary Ligation-induced Arrhythmias in Spontaneously Hypertensive Rats. *J. Cardiovasc. Pharmacol.* **1996**, *27*, 226−234.
(21) De Sarro, G. B.; Ascioti, C.; Froio, F.; Libri, V.; Nistico, G. Evidence that Locus Coeruleus is the Site where Clonidine and Drugs Acting at α_1- and α_2-Adrenoceptors Affect Sleep and Arousal Mechanisms. *Br. J. Pharmacol.* **1987**, *90*, 675−685.
(22) Munk, S. A.; Lai, R. K.; Burke, J. E.; Arasasingham, P. N.; Kharlamb, A. B.; Manlapaz, C. A.; Padillo, E. U.; Wijono, M. K.; Hasson, D. W.; Wheeler, L. A.; Garst, M. E. Synthesis and Pharmacologic Evaluation of 2-*endo*-Amino-3-*exo*-isopropylbicyclo-[2.2.1]heptane: A Potent Imidazoline$_1$ Receptor Specific Agent. *J. Med. Chem.* **1996**, *39*, 1193−1195.
(23) Pigini, M.; Bousquet, P.; Carotti, A.; Dontenwill, M.; Giannella, M.; Moriconi, R.; Piergentili, A.; Quaglia, W.; Tayebati, S. K.; Brasili, L. Imidazoline Receptors: Qualitative Structure−Activity Relationships and Discovery of Tracizoline and Benazoline. Two Ligands with High Affinity and Unprecedented Selectivity. *Bioorg. Med. Chem.* **1997**, *5*, 833−841.
(24) Chapleo, C. B.; Butler, R. C. M.; England, D. C.; Myers, P. L.; Roach, A. G.; Smith, C. F. C.; Stillings, M. R.; Tulloch, I. F. Heteroaromatic Analogues of the α_2-Adrenoceptor Partial Agonist Clonidine. *J. Med. Chem.* **1986**, *32*, 1627−1630.
(25) Timmermans, P. B. M. W. M.; van Zwieten, P. A. Quantitative Structure−Activity Relationships in Centrally Acting Imidazolidines Structurally Related to Clonidine. *J. Med. Chem.* **1977**, *20*, 1636−1644.
(26) Rouot, B.; Leclerc, G.; Wermuth, C. G.; Miesch, F.; Schwartz, J. Clonidine and Related Analogues. Quantitatives Correlations. *J. Med. Chem.* **1976**, *19*, 1049−1054.
(27) Jen, T.; van Hoeven, H.; Groves, W.; McLean, R. A.; Loev, B. Amidines and Related Compounds. 6. Studies on Structure−Activity Relationships of Antihypertensive and Antisecretory Agents Related to Clonidine. *J. Med. Chem.* **1975**, *18*, 90−99.
(28) Etienne, A.; Correia, Y. About some Derivatives of 2-Pyrrolidone. *Bull. Soc. Chim. Franc.* **1969**, *10*, 3704−3712.
(29) Scheibye, S.; Pedersen, B. S.; Lawesson, S.-O. Studies on Organophosphorus Compounds XXI. The Dimer of p-Methoxyphenylthionophosphine Sulphide as Thiation Reagent. A New Route to Thiocarboxamides. *Bull. Soc. Chim. Belg.* **1978**, *87*, 229−238.
(30) Colonge, J.; Pouchol, J. M. Preparation of 2-Pyrrolidones and γ-Aminoacids. *Bull. Soc. Chim. Fr.* **1962**, 598−603.
(31) Moffett, R. B.; White, J. L. Substituted Pyrrolidines and Pyrrolidylethanols. *J. Org. Chem.* **1952**, *17*, 407−410.
(32) Tanaka, T.; Murakami, K.; Okuda, O.; Kuroda, T.; Inoue, T.; Kamei, K.; Murata, T.; Yoshino, H.; Imanishi, T.; Iwata, C. Synthetic Studies on Aphidicolane and Stemodane Diterpenes. III. An Alternative Stereoselective Access to Aphidicolane-Type B/C/D-Ring System. *Chem. Pharm. Bull.* **1994**, *42*, (9), 1756−1759.
(33) Feldman, J.; Tibiriça, E.; Bricca, G.; Dontenwill, M.; Belcourt, A.; Bousquet, P. Evidence for the Involvement of Imidazoline Receptors in the Central Hypotensive Effect of Rilmenidine in the Rabbit. *Br. J. Pharmacol.* **1990**, *100*, 600−604.
(34) For instance, prediction of the pK_a values of 2-aminopyrroline and 2-aminooxazoline, obtained using the ACD/I-Lab Web service, were respectively 12.50 ± 0.20 and 10.06 ± 0.20.
(35) Head, G. A.; Chan, C. K.; Burke, S. L. Relationship Between Imidazoline and alpha2-Adrenoceptors Involved in the Sympatho-inhibitory Actions of Centrally Acting Antihypertensive Agents. *J. Auton. Nerv. Syst.* **1998**, *72*, 163−169.
(36) Tolentino-Silva, F. P.; Haxhiu, M. A.; Waldbaum, S.; Dreshaj, I. A.; Ernsberger, P. Alpha(2)-Adrenergic Receptors are not Required for Central Antihypertensive Action of Moxonidine in Mice. *Brain Res.* **2000**, *862* (1−2), 26−35.
(37) Reis, D. J.; Piletz, J. E. The Imidazoline Receptor in Control of Blood Pressure by Clonidine and Allied Drugs. *Am. J. Physiol.* **1997**, *273* (5), R1569−R1571. Ernsberger, P.; Haxhiu, M. A. The I_1-Imidazoline-Binding Site is a Functional Receptor Mediating Vasodepression via the Ventral Medulla. *Am. J. Physiol.* **1997**, *273* (5), R1572−R1579. Guyenet, P. G. Is the Hypotensive Effect of Clonidine and Related Drugs due to Imidazoline Binding Sites? *Am. J. Physiol.* **1997**, *273* (5), R1580−R1584.
(38) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(39) Wick, A. E.; Bartlett, P. A.; Dolpin, D. The Total Synthesis of Ipalbidine and Ipalbine. *Helv. Chim. Acta* **1971**, *54* (2), 513−522.
(40) Corrodi, H. Amide Derivatives of Dicyclopropylmethylamine. *Helve. Chim. Acta* **1963**, *46* (3), 1059−1060.
(41) Omar, F.; Frahm, A. W. EPC-Synthesis of cis-bicyclic Lactams and Amines. *Arch. Pharm. (Weinheim)* **1990**, *323*, 923−928.
(42) Kennewell, P. D.; Matharu, S. S.; Taylor, J. B.; Westwood, R.; Sammes, P. G. Synthesis of γ-Aminobutyric Acid Analogues of Restricted Conformation. Part 1. The 2-Aminocycloalkylacetic Acids. *J. Chem. Soc., Perkin Trans. 1* **1982**, *11*, 2553−2562.
(43) Bertho, A.; Schmidt, J. F. Cycle Formation by Condensation of (2-Oxo-cyclohexyl)-acetic acid with ammonia and primary aliphatic amines. *Chem. Ber.* **1964**, *97*, 3284−3292.
(44) Molderings, G. J.; Moura, D.; Fink, K.; Bönisch, H.; Göthert, M. Binding of [^{3}H]Clonidine to I_1-Imidazoline Sites in Bovine Adrenal Medullary Membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1993**, *348*, 70−76.
(45) Molderings, G. J.; Likungu, J.; Göthert, M. Presynaptic Cannabinoid and Imidazoline Receptors in the Human Heart and their Potential Relationship. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *360*, 157−164.
(46) Coupry, I.; Podevin, R. A.; Dausse, J. P.; Parini, A. Evidence for Imidazoline Binding Sites in Basolateral Membranes from Rabbit Kidney. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 1055−1060.
(47) Van Liefde, I.; Vauquelin, G.; De Keyser, J.; De Backer, J. P.; De Vos, H. Alpha 2A Adrenoceptors and Nonadrenergic Idazoxan Binding Sites in Calf Brain and Retina are Distinct from those in Human Brain. *Neurochem. Int.* **1993**, *22*, 501−509.
(48) Cheng, Y. C.; Prussof, W. H. Relationship Between the Inhibitor which Causes 50% Inhibition (I_{50}) of the Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 1839−1842.

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