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New Aromatic Iminoimidazolidine Derivatives as α_1-Adrenoceptor Antagonists: A Novel Synthetic Approach and Pharmacological Activity

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Abstract—The design, synthesis and α_1-adrenoceptor antagonism of a series of bis-imidazoline (**1a**, **2a**, **3a** and **4a**) and bis-guanidine (**1b**, **2b**, **3b** and **4b**) diphenyl derivatives are reported. All of these compounds fulfil the conditions of the most recent pharmacophore proposed for α_1-adrenoceptors and found in the literature. Besides, a novel synthetic approach to the preparation of 2-(arylimino)imidazolidine derivatives is described. All the tested compounds, except the bis-guanidinium derivative **3b**, inhibit the contractile responses induced by noradrenaline in aortic rings of rat and rabbit in a dose-dependent manner. Our results indicate that, even though some discrepancies are observed in terms of the α_1 subtype targeted by this new family of compounds, they show an interesting profile as antagonists of α_1-adrenoceptors and a new prototype, compound **1a**, has been found deserving further development.

Introduction

α-Adrenergic receptors play a primary role in the regulation of a variety of physiological processes, particularly within the cardiovascular system. In the last decades, the interest in these receptors, in their physiological relevance and in their classification has been renewed. Whereas α_2-adrenoceptors mediate in hypertension, sedation, antianxiety, and analgesia among other biological actions,[1] several studies have shown that stimulation of α_1-adrenergic receptors can decrease the propensity for abnormal heart rhythm[2] whereas the antagonists of those receptors decrease the development of benign prostatic hyperplasia (BPH).[3]

The α_1-adrenergic receptors belong to the superfamily of G protein-coupled receptors (GPCR) which transmit signals over the cell membrane, starting different biochemical events.[4] They have been classified into different subtypes and they are now designated as α_{1A}, α_{1B} and α_{1D}.[5] Thus, since these adrenoceptors are present in multiple blood vessels and other smooth muscles the development of selective drugs for them would be of great importance.

Imidazoline derivatives have traditionally been considered as one of the major types of drugs that interact with α-adrenergic receptors.[6] Thus, compounds such as clonidine or naphazoline (Fig. 1), which contain a 2-iminoimidazolidine or an imidazoline ring respectively, show α_1- and α_2-adrenoceptor activities,[6] and more specifically, phentolamine[5a] (Fig. 1), which contains an imidazoline ring, is a known α_1-adrenergic antagonist.

In a previous study, carried out by us, on the similarity between guanidine and 2-aminoimidazoline,[7] the results obtained showed close semblance between both groups not only in terms of geometrical parameters but also at electronic levels (in ref 7 we computed the molecular electrostatic potential and electron density, and the corresponding similarity indexes for both groups). Therefore, taking into account those results we considered the preparation of two new families of derivatives containing the guanidine or the 2-aminoimidazoline groups.

Different pharmacophores have been proposed to explain the α-adrenergic activity[8] (we will discuss them

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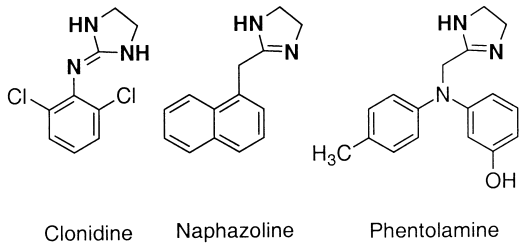


Figure 1. Imidazoline derivatives containing a 2-iminoimidazolidine or an imidazoline ring which are known to interact with α-adrenergic receptors.

in another section) and in all of them the presence of an aromatic ring and a polar centre seems to be essential. Thus, two phenyl groups connected by a group containing polar atoms (NH, CO, and SO_2, and CH_2 for comparison) were considered for the skeleton of these new families of compounds (see Fig. 2).

The introduction of guanidine moieties by nucleophilic substitution of deactivated aromatic amines is a problem which has lately been approached by some authors.[9] Their methodology has been extended to the preparation of our diphenyl derivatives.

To evaluate the functional activity of α-adrenoceptor antagonists, isolated tissues containing α receptors showing easy and faithful responses were used. To carry out these studies the basic pharmacological preparations are isolated rabbit and rat aorta, where the effect to be measured is a change in tension induced by vasoconstrictor compounds (α agonists).[10] Effects mediated through other receptors have been discarded using rabbit aorta for bradykinin B_1 receptors,[11] and guinea pig ileum for muscarinic, histaminergic and bradykinin B_2 receptors.[12]

In the present paper we report the design, synthesis and pharmacological activity of a series of bis-imidazoline (**1a**, **2a**, **3a** and **4a**) and bis-guanidine (**1b**, **2b**, **3b** and **4b**) diphenyl derivatives, all of them fulfilling the conditions of the latest pharmacophore published[8f] and the former of which show α_1-adrenoceptor antagonism. Besides, a novel synthetic approach to the preparation of imidazoline-aryl derivatives is described.

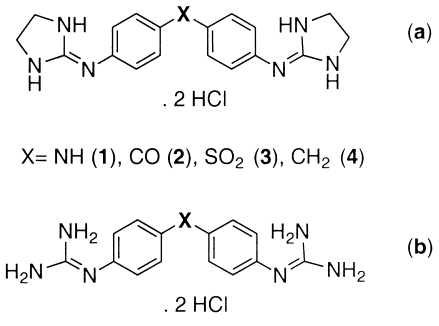


Figure 2. New families of compounds prepared in this work: (a) bis-2-iminoimidazolidinium derivatives, and (b) bis-guanidinium derivatives.

Chemistry Results

As already mentioned, we had found by means of a similarity study[7] that the 2-aminoimidazolinium cation is very alike structurally (bond distances, atomic charges) and electronically (molecular electrostatic potential, electron density) to the guanidinium group and, hence, many of the methods used to introduce the guanidinium moiety in a molecule have been applied for the introduction of the 2-aminoimidazolinium group.

To date, several routes have been used to form the 2-iminoimidazolidine group. For instance, the direct introduction of the imidazoline heterocycle through the nucleophilic displacement reaction of a primary amine was carried out with 2-nitramino-4,5-dihydro-1*H*-imidazolium,[13] 2-sulfonate-4,5-dihydro-1*H*-imidazolium,[14] or 2-methylmercapto-4,5-dihydro-1*H*-imidazolium iodide.[15] Recently, an intramolecular variant of the latter method was developed.[16] This procedure takes place via the five-membered ring cyclization between an amine and a thiouronium salt, previously formed in a three step procedure. This alternative method leads, in some cases, to higher yields although being more lengthy.[17]

Classically, 2-(arylimino)imidazolidines such as clonidine (see Fig. 1) and its derivatives were prepared in low yields by reaction of *N*-aryl-*S*-methylisothiouronium iodides or *N*-aryldichloroimines with ethylenediamine (the *N*-aryldichloroimine precursor giving slightly better yields[18]). However, purification of the products may be tedious since one has to work with very polar compounds as salts or the free base of the 2-iminoimidazolidine. Moreover, the poor manageability of such unprotected compounds in a multistep synthesis made attractive the development of a new synthetic approach via the formation of fully Boc-protected 2-iminoimidazolidines in one step, in very mild conditions.

To date, protection of 2-iminoimidazolidine derivatives has been done via *N*-acylated, *N*-benzylated[19] or 3,4-dimethoxybenzyl derivative formation.[20] Recently, Kim and Qian[9] described an improved method for the preparation of protected guanidines from either sterically or electronically highly deactivated amines, using *N,N′*-bis-(*tert*-butoxycarbonyl)thiourea (**5**) in the presence of mercuric chloride. Lately, it has been found that Mukaiyama's reagent could replace the mercuric chloride in the previous method.[21]

Curiously, the direct synthesis of *N,N′*-bis-Boc-protected 2-iminoimidazolidines from *N,N′*-Bis-(*tert*-butoxycarbonyl)imidazoline-2-thione (**6**), a compound similar to **5**, has not been described. Nevertheless, the preparation of **6** was reported in a patent in 1994[22] and the same authors published in 1995 the use of *N,N′*-dibenzyl- and *N,N′*-di-*tert*-butoxycarbonyl-carbamates of cyclic thioureas as alkoxycarbonyl transfer agents for the protection of amines.[23]

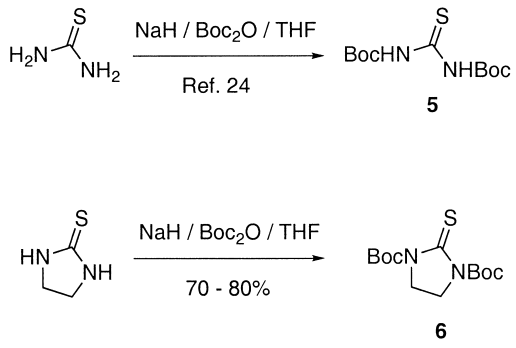
We have extended the methodology of Kim and Qian[9] to the synthesis of *N,N′*-bis-Boc protected 2-iminoimidazolidines of a series of diaminodiphenyl derivatives

(**1**, **2**, **3** and **4** in Scheme 1), some of them being strongly deactivated amines such as 4,4′-diaminodiphenylsulfone (**3**) and 4,4′-diaminobenzophenone (**2**).

N,N′-Bis-(*tert*-butoxycarbonyl)imidazoline-2-thione (**6**) was prepared with di-*tert*-butyldicarbonate in THF in the presence of sodium hydride in a 70–80% yield (Scheme 2).

Two equivalents of **6** (three equivalents in the case of the deactivated amines **2** and **3**) reacted with one equivalent of diamine in the presence of mercuric chloride and excess of triethylamine, either in DMF or CH_2Cl_2, to give, after flash chromatography on silica, the corresponding fully Boc-protected bis-(2-iminoimidazolidine) derivatives (**1c**, **2c**, **3c** and **4c**) in good to excellent yields (see Table 1). The guanidine derivatives (**1d**, **2d**, **3d**, **4d**) were prepared using *N,N′*-bis(*tert*-butoxycarbonyl)-thiourea (**5**).[24]

An attempt to remove the Boc groups with $SnCl_4$ led directly to the hydrochloride salts of the products as described recently.[25] However, as revealed by elemental analysis, the products that we obtained contained 'heavy metal' impurities, probably stannic complexes that are not detectable in ^1H and ^{13}C NMR, and which could not be removed by recrystallization. For that reason and due to the incompatibility of heavy metals from the pharmacological point of view, this method was abandoned. Full deprotection of the Boc groups was easily accomplished by treating the products with an excess of trifluoroacetic acid, leading to the trifluoroacetate salts of the products. The hydrochloride salts (**1a**, **2a**, **3a** and **4a**) were obtained using strongly basic anion-exchange resin.

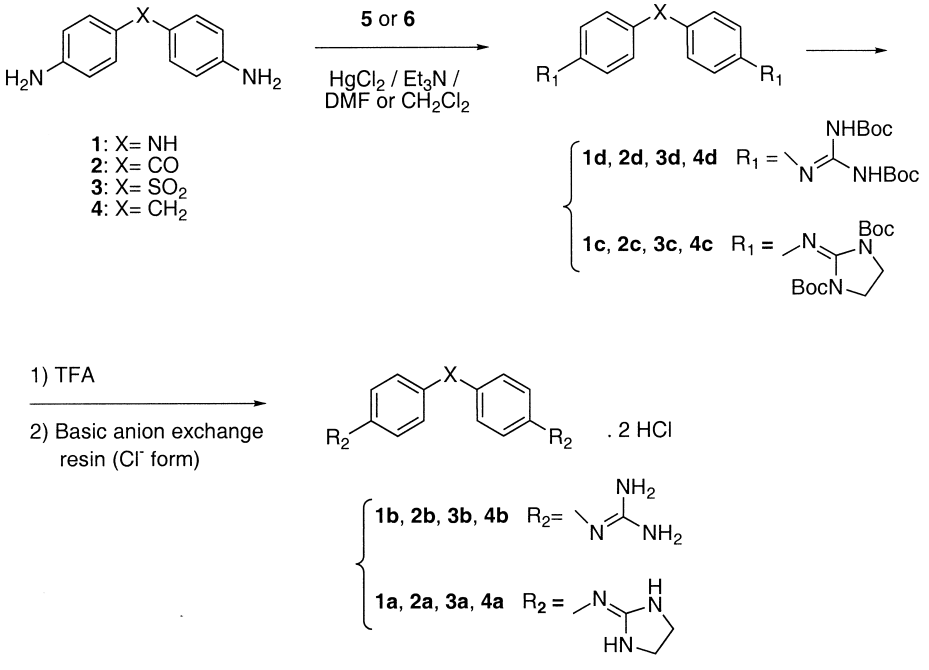


Scheme 2.

Molecular Modelling Results

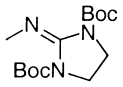
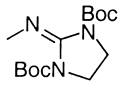
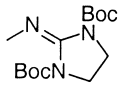
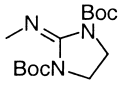
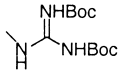
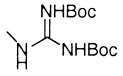
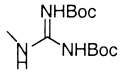
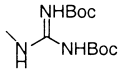
Despite the fact that the tridimensional structure of the α_1-adrenoceptors is not known, several pharmacophores have been developed for the antagonism of these receptors.[8] The most recent is that proposed by Bremner et al.[8f] in 1996 which contains an aromatic ring, a basic N atom and a polar group included or not in a non-aromatic ring. These authors suggest that an α_{1A}-adrenoceptor antagonist should have the basic N at a distance of 5.2–5.8 Å from the aromatic ring and at a distance of 6–8 Å from the polar group. On the contrary, an antagonist of an α_{1B}-adrenoceptor would have the basic N atom at a distance of 6.2–7.8 Å from the aromatic ring and at 5–6 Å from the polar group (Fig. 3).

We carried out the conformational analyses of the four 2-iminoimidazolidinium (**1a**, **2a**, **3a**, **4a**) and four guanidinium derivatives (**1b**, **2b**, **3b**, **4b**) by means of random search techniques which combine the Monte Carlo



Scheme 1.

Table 1. Reaction solvents and time conditions used and melting point and yields obtained for compounds **1c**–**4c** and **1d**–**4d**

Product	R_1	Reaction solvent	Time conditions	mp (°C)	Isolated yield
1c		DMF CH_2Cl_2	0 °C/20 min rt/20 h 0 °C/90 min rt/20 h	165–169	76% 61%
2c		DMF	−5 °C/1 h rt/5 days	106–108	43%
3c		CH_2Cl_2[a,b]	0 °C/1 h rt/24 h	140–141	89%
4c		CH_2Cl_2 DMF	0 °C/30 min rt/23 h 0 °C/15 min rt/4 h	90–93	68% 94%
1d		DMF	0 °C/20 min rt/20 h	130–135	66%
2d		CH_2Cl_2/DMF[a,c]	0 °C/30 min rt/5 days	142–143	93%
3d		CH_2Cl_2[a,d]	0 °C/1 h rt/24 h	121–123	86%
4d		CH_2Cl_2	0 °C/1 h rt	118–122	81%

[a]3 equiv of reactants were used.
[b]0.62 equiv of unreacted *N,N′*-bis-(*tert*-butoxycarbonyl)imidazoline-2-thione were recovered.
[c]DMF was added to the reaction mixture to help solubilization of 4,4′-diaminobenzophenone.
[d]0.3 equiv of unreacted *N,N′*-bis-(*tert*-butoxycarbonyl)imidazoline-2-thione were recovered.

methodology for the random generation of structures and the molecular mechanics approach for the minimization of the generated structures. In this method, included in the SYBYL package,[26] only different conformations with different optimized energies are stored for their subsequent analysis. In all the molecules studied the distances between the possible elements of the pharmacophore (aromatic rings, all basic N atoms and polar centres) were measured and from those distances the pharmacophore elements were localized.

It should be noticed that all the structures (**1a**–**4a** and **1b**–**4b**) have been considered in their imino form (see Fig. 2), which means that the N connecting the phenyl rings with the extremities of the molecules is an sp^2 N atom, better described as an imino nitrogen (-N=) conjugated with the aromatic ring, and the most basic of all the N atoms. This structural assumption is based on the ^1H and ^{13}C NMR results obtained for all the compounds by comparison with those published by Jackman and Jen.[27] These authors characterized the clonidine (Fig. 1) in its imino form and generalized a method of distinguishing between imino and amino forms in aryl iminoimidazolidines and aryl guanidines.

In the case of derivatives **1a** and **1b** we found that, for all the conformers obtained, the -N= atoms which connect the aromatic rings with the extremities of the molecules play the role of the basic N atom of the pharmacophore and they are at a distance of about 5.6 Å from the farthest aromatic rings (see Fig. 3 and Table 2). Moreover, these basic Ns are at 7.4 Å from the central NH groups which would correspond to the polar centre of the pharmacophore.

For the rest of the compounds the basic N atoms are the same as for compounds **1a** and **1b**, the aromatic rings to consider will also be the farthest from each of those basic N atoms and the polar groups would be those connecting the phenyl rings. Thus, for compounds **2a** and **2b** the polar centre was a CO group, for compounds **3a** and **3b** an SO_2 group, and for compounds **4a** and **4b** we used the CH_2 group. The distances between all the pharmacophore elements are gathered in Table 2. These

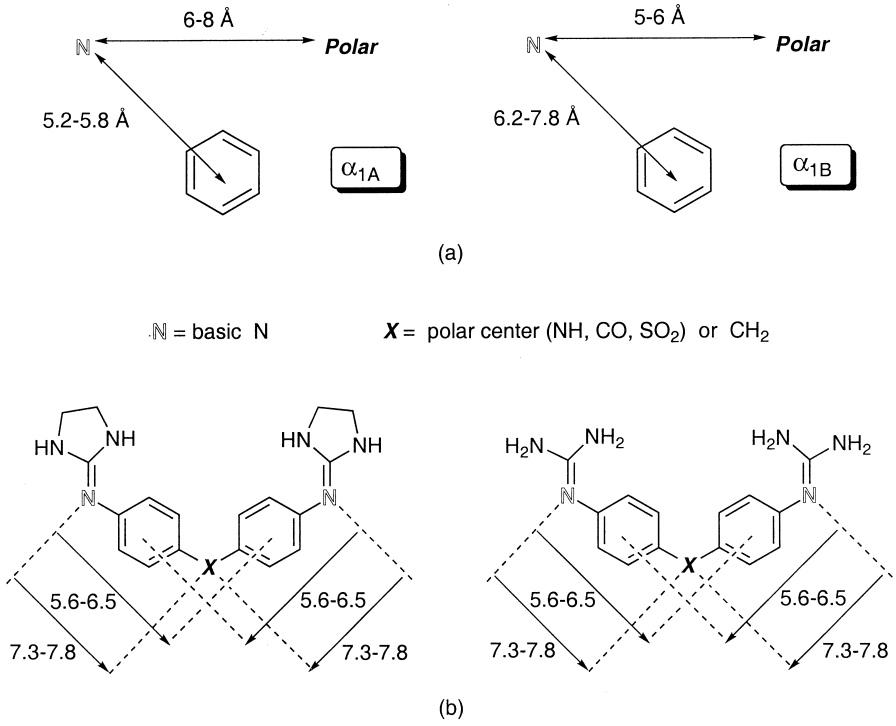
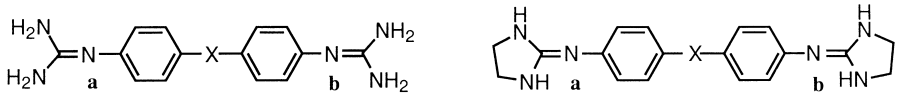


Figure 3. (a) Pharmacophore proposed by Bremner et al.[8f] for α_{1A}- and α_{1B}-adrenoceptor antagonists. (b) General formula for the aromatic bis-guanidinium (right side) and bis-imidazolinium (left side) derivatives **1a,b** to **4a,b**, indicating the pharmacophore elements and the ranges of distances between them.

Table 2. Ranges of distances (Å) obtained for the conformations of minimum energy resulting from the random search of bis-2-iminoimidazolidinium derivatives **1a**–**4a** and bis-guanidinium derivatives **1b**–**4b** to fulfil the pharmacophore conditions of Bremner et al.[8f]



Product	X		N_a	N_b
1a	NH	Polar centre [N]	5.579–5.600	5.579–5.600
		Ph (opposite)	7.575–7.470	7.471–7.541
1b	NH	Polar centre [N]	5.578–5.600	5.578–5.600
		Ph (opposite)	7.488–7.535	7.487–7.535
2a	CO	Polar centre [C(O)]	5.685 (6.330–6.336)	5.685 (6.330–6.336)
		Ph (opposite)	7.801–7.817	7.801–7.817
2b	CO	Polar centre [C(O)]	5.684 (6.329–6.332)	5.684 (6.329–6.332)
		Ph (opposite)	7.809–7.816	7.808–7.815
3a	SO_2	Polar centre [S(O)(O)]	5.941 (6.547–6.556)	5.941 (6.548–6.556)
		Ph (opposite)	7.280–7.323	7.281–7.323
3b	SO_2	Polar centre [S(O)(O)]	5.940 (6.548–6.552)	5.940 (6.548–6.552)
		Ph (opposite)	7.310–7.320	7.310–7.319
4a	CH_2	Polar centre [C]	5.708	5.708
		Ph (opposite)	7.313–7.360	7.314–7.359
4b	CH_2	Polar centre [C]	5.707–5.714	5.707–5.714
		Ph (opposite)	7.584–7.339	7.585–7.339

results indicate that the conditions of the pharmacophore are fulfilled by both ends of these molecules.

Pharmacological Results

The action of novel compounds with hypothetical α-adrenergic antagonist activity was studied in isolated rat and rabbit aortic rings.[28] Thus, the capacity of the bis-iminoimidazolidinium derivatives **1a**, **2a**, **3a** and **4a**, and bis-guanidinium derivatives **1b**, **2b**, **3b** and **4b** to reduce the force of the contraction induced by noradrenaline (NA) on isolated aorta was tested.

To discard unspecified effects not mediated by α-adrenergic receptor blockade, the effect of the active derivatives was also evaluated on contractions induced by KCl[10b,29] on both rabbit and rat aorta. Their activity

was also tested on contractions elicited by administration of Des-Arg-bradykinin (B_1 agonist) on rabbit aorta, since this tissue contains B_1 receptors.[30]

Effects mediated through activation or blockade of other receptors were evaluated using myenteric plexus–longitudinal muscle strips from guinea pig ileum. The effect of new compounds on non-stimulated tissues and on contractions induced by bradykinin (B_2 receptors) or by electrical stimulation was tested.

Effects of the studied compounds

On resting tension. At concentrations between 10^{-8} and 10^{-4} M, the bis-imidazolinium derivatives **1a**, **2a**, **3a** and **4a** had no effect on basal tension in rat and rabbit aortic rings or on guinea pig ileum. The same results were found with bis-guanidinium derivatives **1b**, **2b**, **3b** and **4b**.

On noradrenaline-induced contractions. The contractions induced by NA in rat and rabbit aortic rings were inhibited in a dose-dependent manner by the bis-imidazolinium derivatives **1a**, **2a**, **3a** and **4a** as well as by the bis-guanidinium derivatives **1b**, **2b** and **4b**. The bis-guanidinium derivative **3b** at concentrations from 10^{-8} and up to 10^{-4} M had no effect abolishing the contractions induced by NA in both arteries. These inhibitions are completely reversed after washing the preparations with Krebs solution. The IC_{50} values obtained, in rat and rabbit aorta, for the different compounds are gathered in Table 3. As a reference, the corresponding IC_{50} values of prazosin (a known α_1 antagonist) were determined following the same procedure, the resulting value in rat aorta was $1.7\pm1.0\ 10^{-9}$ M ($n=13$), and in rabbit aorta was $1.1\pm1.0\ 10^{-8}$ M ($n=14$).

Specificity assays. The contractions produced by the addition of: (i) high concentrations of KCl (80 mM) in rabbit and rat aorta; (ii) Des-Arg-bradykinin on rabbit aorta; and (iii) by electrical stimulation in guinea pig ileum, were not modified in the presence of the compounds studied.

Discussion and Conclusion

All the compounds designed and synthesized (**1a,b** to **4a,b**) exhibit all the elements of the pharmacophore proposed by Bremner et al. by both extremities of the molecules, a basic N atom, an aromatic ring opposite to each basic N atom, and a certain polar group in the middle of the molecule. The conformational studies carried out on all these compounds yielded a number of minimum energy conformations, and the distances between the different elements of the Bremner pharmacophore (see Table 2) obtained for all these conformations correspond better to an α_{1A} receptor subtype (basic N at 5.2–5.8 Å from the aromatic ring and at 6–8 Å from the polar group).

Table 3. Concentrations needed for a 50% inhibition (IC_{50}) of the contraction provoked by noradrenaline in rat and rabbit aortic rings

Product	X	Rat aorta IC_{50} (10^{-5} M)	n	Rabbit aorta IC_{50} (10^{-5} M)	n
1a	NH	0.7±0.02	6	4.3±0.6	6
2a	CO	1.9±0.1	6	1.3±0.3	5
3a	SO_2	2.8±0.2	6	11±0.4	6
4a	CH_2	4.6±0.5	8	5.8±5.1	7
1b	NH	9.4±2.7	5	2.0±2.7	5
2b	CO	12±0.6	5	0.2±0.02	6
4b	CH_2	8.7±0.1	5	2.2±2.3	6

None of the tested compounds (**1a** to **4a** and **1b**, **2b** and **4b**) displayed intrinsic activity in the aortic preparations or on the guinea pig ileum when administered alone. From this result it could be suggested that these compounds lack agonist activity on the receptors located on these tissues: adrenergic, serotoninergic, cholinergic, of bradykinin or histaminergic.

With regard to their functional antagonist activity in inhibiting the contractile responses induced by a submaximal concentration of NA (5×10^{-8} M) in aortic rings, the tested compounds, except the bis-guanidinium derivative **3b**, prevented the effect of NA in a dose-dependent manner. It is well known that NA induces a dose-dependent contraction through activation of α_1-receptors,[10a,31] so that our results indicate that these new compounds show 'in vitro' α_1 antagonist activity.

On the other hand, the absence of antagonist activity in guinea pig ileum or in blood vessel stimulated with KCl or Des-Arg-bradykinin confirms the selectivity of the effect observed on contractions mediated through NA receptors.

As we mentioned in the Introduction, different subtypes of α_1-receptors have been described (α_{1A}, α_{1B}, α_{1D}, α_{1H}, α_{1N}, and α_{1L}[5,11a,32,33]). The identity of the receptor subtypes mediating contraction to α_1-adrenoceptor agonists in the vasculature, especially in the rat aorta, has been controversial.[34–36] Whereas Testa et al.[34a] described α_{1B} as the most common subtype in that tissue, recent investigations indicate that the most common receptor in the rat aorta is the α_{1D} subtype.[35,37]

The potencies of the antagonists in this tissue parallel its receptor binding affinity of this subtype of the α_1-adrenoceptor population.[34a] Significant heterogeneity exists in α_1-adrenoceptors in mammalian aorta. In the rabbit, the coexistence of both α_{1A}, α_{1D}, and α_{1L}-adrenoceptors has been demonstrated.[33b,34b,38] This heterogeneity in the rabbit aorta may explain the dissimilar results obtained between rat and rabbit aortic rings in response to the compounds studied in this work.

The different potency observed in the antagonist activity of these series of compounds in the rat aorta tests (Table 3), where a single α_1 subtype (B or D) has been proposed, could be explained as follows. On the one hand, the bis-2-iminoimidazolidinium derivatives (**1a**–**4a**) are better antagonists than the bis-guanidinium derivatives (**1b**–**4b**) as can be observed in Table 3. This could probably be due to the additional hydrophobicity

added to these systems by the ethylene portion of the imidazolidine ring.

On the other hand, and also in the rat aorta test, the CH_2 derivative, **4a**, is the worse antagonist among the bis-imidazolinium derivatives; this could be a consequence of the lack of polarity of that group and, therefore, the requisite of a polar group to fulfil the pharmacophore would not be reached.

Therefore, even though some discrepancies are observed between the molecular modelling results and the pharmacological ones, in terms of the α_1 subtype targeted by this new family of compounds, it can be concluded that these compounds show an interesting profile as antagonists of α_1-adrenoceptors and that a new prototype, compound **1a**, has been found which deserves further development.

Experimental

Random search conditions

The following conditions were established in all the conformational analyses performed using the random search method and starting from two different conformations:

i. Bonds to search: only the C(arom)–X bonds [X = N(guanidinium, 2-imino-imidazolidinium), N(H), C(H_2), C(O), S(O_2)] were rotated.

ii. Energy set-up: atomic charges were evaluated by the Gasteiger–Hückel method.

iii. Minimization details: each generated conformer was minimized during 300 cycles using the Conjugate Gradient method.

iv. Random search details: the maximum number of cycles in the search is set to 6000; the energy cut-off is set to 10 kcal/mol; the RMS-threshold is set to 0.2; the maximum number of hits is set to 6; and the data convergence is set to 0.005.

For each starting conformation of each derivative, 15–25 different minimum energy conformations were obtained and considered in the study of the pharmacophore requirements.

Chemistry

Reagents were used as received except for 4,4′-(diaminodiphenyl)amine, which was generated from its sulfate precursor by basification with an aqueous solution of sodium hydroxide (10% weight) followed by extraction with diethyl ether. THF was distilled from sodium–benzophenone under argon. Other reaction solvents were purchased anhydrous and used as received. Other solvents used were reagent grade. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Varian Gemini 200 spectrometer and at 400 and 100 MHz on a Varian 400 Unity spectrometer for compound **1a**. Chemical shifts were internally referenced to the residual proton resonance in $CDCl_3$ (δ 7.27 ppm) and D_2O (δ 4.6 ppm). Chemical shifts of the ^{13}C NMR spectra in D_2O were referenced with a capillary of DMSO-d_6 (δ 39.5 ppm). IR spectra were recorded on a Perkin–Elmer 681 spectrophotometer as KBr pellets. Melting points were determined with a Reichert Jung Thermovar apparatus and are uncorrected. Mass spectra were recorded on a Hewlett–Packard Series 1100 MSD spectrometer (ES, APCI) and on a VG Autospec spectrometer (FAB). FAB spectra were recorded in a NBA matrix. Elemental analysis were performed on an Heraeus CHN-O Rapid analyzer. Column chromatography was performed on silica gel 60 (230–400 mesh ASTM, E. Merck). All the reactions were monitored on aluminium sheets precoated with silica gel 60 F_{254} (E. Merck).

Method A: preparation of Boc-protected 2-iminoimidazolidine derivatives. General procedure

To a 0.25 M solution of the appropriate diamine **1**, **2**, **3** or **4** (1 equiv), the bis-Boc-protected imidazolidinethione **6** (2.2 equiv for **1**, **2** and **4**, and 3 equiv for **3**), and an excess of Et_3N, in DMF or CH_2Cl_2 at 0 °C, were added $HgCl_2$ (same molar quantity as **6**) at once. A precipitate formed immediately and the resulting mixture was stirred at that temperature for the time indicated in Table 1. Then, the ice-bath was removed and the reaction was stirred at room temperature for the appropriate duration. The resulting dark grey reaction mixture was diluted with EtOAc and filtered through a pad of Celite. The filter cake was rinsed with EtOAc. The organic phase was washed with brine, then dried over $MgSO_4$ and finally concentrated under vacuum. The crude product was purified by flash chromatography on silica gel.

***N*,*N*′-Di(*tert*-butoxycarbonyl)imidazolidine-2-thione (6).** To a cooled solution (ice-water bath) of imidazolidine-2-thione (4.21 g, 41.3 mmol) in dry THF (600 mL) under argon, 7.49 g (187 mmol) of NaH (60% in mineral oil) were added. After 5 min, the ice-bath was removed and the reaction was stirred 10 min at room temperature. Then, the reaction mixture was cooled until 0 °C and di-*tert*-butyldicarbonate (19.88 g, 91.2 mmol) was added neat. After 30 min, the ice-bath was removed and the reaction mixture was stirred another 4 h at room temperature. The reaction was quenched carefully, drop-by-drop, with a saturated $NaHCO_3$ solution (100 mL). The mixture was poured into 300 mL of water and the aqueous layer was extracted with EtOAc (3×150 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. Recrystallization from hexane:EtOAc (8:2) afforded **5** as yellow needles (9 g, 72% yield): mp 117–119 °C; IR (KBr) 2980, 1745, 1720, 1370, 1270, 1150, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 4H), 1.46 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 176, 150.65, 84.24, 44.99, 28.46; anal. calcd for $C_{13}H_{22}N_2O_4S$: C, 51.63; H, 7.33; N, 9.26; S, 10.60. Found: C, 51.97; H, 7.38; N, 9.40; S, 10.31.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenylamine (1c). A solution of 99 mg (0.5 mmol) of **1**, 334 mg (1.1 mmol) of **6** and 0.5 mL (7 equiv) of Et_3N in

2 mL of CH_2Cl_2 was treated with 308 mg (1.1 mmol) of $HgCl_2$ and stirred first 1 h 45 min at 0 °C, and then 20 h at room temperature. Usual work up and flash-chromatography (hexane:EtOAc, 1.5:1) gave **1c** as a brown solid (226 mg, 61% yield); mp 165–169 °C; IR (KBr) 2990, 1755, 1700, 1510, 1380, 1310,1155, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (d, 4H, $J=8.34$ Hz), 6.84 (d, 4H, $J=8.93$ Hz), 3.79 (s, 8H), 1.32 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 150.91, 142.08, 139.67, 138.98, 122.94, 118.61, 83.08, 43.62, 28.43; anal. calcd for $C_{38}H_{53}N_7O_8.1/2H_2O$: C, 61.27; H, 7.31; N, 13.16. Found: C, 60.92; H, 7.21; N, 12.71.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenylketone (2c). A solution of 159 mg (0.75 mmol) of **2**, 470 mg (1.56 mmol) of **6** and 0.5 mL (5 equiv) of Et_3N in 4 mL of DMF was treated with 450 mg (1.57 mmol) of $HgCl_2$ and stirred first 1 h at −5 °C, and then 24 h at room temperature. Usual work up and flash-chromatography (cyclohexane:EtOAc, 2:1) gave **2c** as a colourless solid (198 mg, 43% yield); mp 106–108 °C; IR (KBr) 2990, 1760, 1705, 1595, 1310, 1150, 978 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, 4H, $J=8.4$ Hz), 6.93 (d, 4H, $J=8.4$ Hz), 3.78 (s, 8H), 1.27 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 195.39, 152.93, 150.36, 140.63, 132.5, 131.56, 121.2, 83.43, 43.73, 28.33; anal. calcd for $C_{39}H_{52}N_6O_9$: C, 62.55; H, 7.00; N, 11.22. Found: C, 62.61; H: 7,28; N, 11.15.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenylsulfone (3c). A solution of 131 mg (0.53 mmol) of **3**, 484 mg (1.6 mmol, 3 equv) of **6** and 0.5 mL (7 equiv) of Et_3N in 2 mL of CH_2Cl_2 was treated with 495 mg (3.4 equiv) of $HgCl_2$ and stirred first 45 min at 0 °C, and then 15 h at room temperature. Usual work up and flash-chromatography (hexane:EtOAc, 1:1) gave **3c** as a colourless solid (371 mg, 89% yield); mp 140–141 °C; IR (KBr) 2940, 1747, 1740, 1690, 1566, 1350, 1280, 1130, 960 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (d, 4H, $J=8.7$ Hz), 6.81 (d, 4H, $J=8.7$ Hz), 3.68 (s, 8H), 1.13 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 153.54, 149.95, 141.20, 135.23, 128.48, 121.62, 83.34, 43.54, 28.05; anal. calcd for $C_{38}H_{52}N_6O_{10}S$: C, 58.15; H, 6.68; N, 10.70; S, 4.08. Found: C, 57.97; H, 6.47; N, 10.59; S, 3.95.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenylmethane (4c). A solution of 102 mg (0.5 mmol) of **4**, 400 mg (1.3 mmol) of **6** and 0.5 mL (7 equiv) of Et_3N in 2 mL of CH_2Cl_2 was treated with 380 mg (1.35 mmol) of $HgCl_2$ and stirred first 1 h at 0 °C, and then 23 h at room temperature. Usual work up and flash-chromatography (hexane:EtOAc, 2:1) gave **4c** as a colourless solid (302 mg, 81% yield); mp 90–93 °C; IR (KBr) 2990, 1760, 1705, 1370, 1310, 1155, 978 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.00 (d, 4H, $J=8.4$ Hz), 6.88 (d, 4H, $J=8.4$ Hz), 3.81 (bs, 10H), 1.31 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 150.79, 146.61, 139.46, 136.25, 129.61, 121.83, 83.11, 43.57, 41.27, 28.35; anal. calcd for $C_{39}H_{54}N_6O_8$: C, 63.74; H, 7.40; N, 11.43. Found: C, 63.51; H, 7.15; N, 11.30.

Method B

Preparation of Boc-protected guanidines (**1d**, **2d**, **3d**, **4d**) using compound **5** and following the same procedure as described above.

***N,N′*-Di(*tert*-butoxicarbonyl)thiourea (5).** That compound was synthesized in 70% yield following the procedure described in ref 21.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]diphenylamine (1d). Method B: flash-chromatography (cyclohexane:EtOAc, gradient 9:1 to 2.5:1) gave **1d** as an off-white foam (66%); IR (KBr) 2950, 1708, 1628, 1395, 1220, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.6 (broad, NH), 10.12 (broad, NH), 7.32 (d, 4H, $J=8.8$ Hz), 6.89 (d, 4H, $J=8.6$ Hz), 1.53 (s, 18H), 1.45 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 164.13, 154.41, 153.80, 141.25, 129.57, 124.54, 118.11, 84.94, 79.90, 28.60, 28.51; anal. calcd for $C_{34}H_{49}N_7O_8$: C, 59.72; H, 7.22; N, 14.34. Found: C, 59.68; H, 6.94; N, 13.98.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]diphenylketone (2d). Method B: flash-chromatography (hexane: EtOAc, 5:1) gave **2d** as a colourless foam (93%); IR (KBr) 2990, 2940, 1728, 1655, 1415, 1310, 1244, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.63 (broad, NH), 10.6 (broad, NH), 7.79 (d, 4H, $J=8.8$ Hz), 7.76 (d, 4H, $J=8.8$ Hz), 1.55 (s, 18H), 1.52 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 194.52, 163.26, 153.24, 140.70, 133.63, 131.19, 120.99, 84.07, 79.96, 28.08; anal. calcd for $C_{35}H_{48}N_6O_9$: C, 60.33; H, 6.94; N, 12.06. Found: C, 60.11; H: 6.81; N, 11.97.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]diphenylsulfone (3d). Method B: flash-chromatography (CH_2Cl_2) gave **3d** as a colourless solid (86%); IR (KBr) 2940, 1705, 1628, 1575, 1390, 1304, 1223, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.6 (broad, NH), 10.61 (broad, NH), 7.87 (d, 4H, $J=8$ Hz), 7.82 (d, 4H, $J=8$ Hz), 1.54 (s, 18H), 1.51 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 163.38, 153.53, 141.61, 137.08, 128.97, 122.07, 84.61, 80.38, 28.42, 28.34; anal. calcd for $C_{34}H_{48}N_6O_{10}S$: C, 55.72; H, 6.60; N, 11.47; S, 4.37. Found: C, 55.58; H, 6.48; N, 11.25; S, 4.15.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]diphenylmethane (4d). Method B: flash-chromatography (hexane:EtOAc, 9:1) gave **4d** as a colourless foam (81%); IR (KBr) 2950, 1705, 1627, 1395, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.3 (broad, NH), 10.26 (broad, NH), 7.49 (d, 4H, $J=8.5$ Hz), 7.11 (d, 4H, $J=8.6$ Hz), 3.9 (s, 2H), 1.52 (s, 18H), 1.49 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 164.22, 154.11, 153.91, 138.15, 135.43, 129.97, 122.91, 84.21, 80.12, 41.35, 28.79, 28.72; anal. calcd for $C_{35}H_{50}N_6O_8$: C, 61.58; H, 7.33; N, 12.31. Found: C, 61.30; H, 7.27; N, 12.18.

General procedure for the generation of the hydrochloride salts

The Boc-protected products were treated either with an excess of CF_3COOH neat or a 50% solution in CH_2Cl_2 and the resulting solution stirred at room temperature (in general, the reaction was complete after only 30 min). Excess of CF_3COOH was removed under vacuum and the trifluoroacetate salt lyophilized overnight. The hydrochloride salt was generated by stirring an aqueous solution of the trifluoroacetate salt with

IRA400 Amberlyte resin in its Cl^- form during 24 h at room temperature. The resin was removed by filtration and the aqueous solution washed twice with EtOAc. Lyophilization afforded the pure hydrochloride salt. Absence of trifluoroacetate salt was checked by ^{13}C NMR.

Hydrochloride salt of 4,4′-di(2-imidazolidinylimino)diphenylamine (1a). Grey solid (87%); mp 165–170 °C; ^1H NMR (100 MHz/D_2O) δ 7.11 (d, 4H, $J=8.94$ Hz), 7.07 (d, 4H, $J=9.09$ Hz), 3.62 (s, 8H); ^{13}C RMN (D_2O) δ 159.43, 142.69, 128.23, 126.47, 118.80, 43.02; MS (FAB^+): 336 (MH^+/100%); anal. calcd for $C_{18}H_{23}Cl_2N_7{\cdot}2H_2O$: C, 48.65; H, 6.12; N, 22.06. Found: C, 48.88; H, 5.81; N, 21.60.

Hydrochloride salt of 4,4′-di(2-imidazolidinylimino)diphenylketone (2a). White solid (90%); mp > 159 °C; ^1H NMR (D_2O) δ 7.63 (d, 4H, $J=8.6$ Hz), 7.20 (d, 4H, $J=8.6$ Hz), 3.62 (s, 8H); ^{13}C RMN (D_2O) δ 196.96, 157.0, 139.05, 133.28, 131.12, 121.37, 41.83; MS (FAB^+): 349 (MH^+/100%); anal. calcd for $C_{19}H_{22}Cl_2N_6O.3H_2O$: C, 48.00; H, 5.94; N, 17.68. Found: C, 48.34; H, 5.73; N, 17.73.

Hydrochloride salt of 4,4′-di(2-imidazolidinylimino)diphenylsulfone (3a). White solid (85%); mp 180–185 °C; ^1H NMR (D_2O) δ 7.83 (d, 4H, $J=8.71$ Hz), 7.27 (d, 4H, $J=8.87$ Hz), 3.59 (s, 8H); ^{13}C RMN (D_2O) δ 158.95, 142.1, 137.78, 130.50, 124.25, 43.94; MS (ES^+): 385 (MH^+/100%); anal. calcd for $C_{18}H_{22}Cl_2N_6O_2S.3H_2O$: C, 42.27; H, 5.52; N, 16.43; S, 6.27. Found: C, 41.98; H, 5.53; N, 16.43; S, 6.15.

Hydrochloride salt of 4,4′-di(2-imidazolidinylimino)diphenylmethane (4a). Brown solid (81%); mp 140–145 °C; ^1H NMR (D_2O) δ 7.07 (d, 4H, $J=8$ Hz), 6.92 (d, 4H, $J=8$ Hz), 3.71 (s, 2H), 3.46 (s, 8H); ^{13}C RMN (D_2O) δ 158.96, 140.97, 133.45, 130.43, 124.74, 43.04, 40.5; MS (FAB^+): 335 (MH^+/100%); anal. calcd for $C_{19}H_{24}Cl_2N_6{\cdot}3.2H_2O$: C, 49.08; H, 6.59; N, 18.07. Found: C, 48.94; H, 6.59; N, 18.24.

Dihydrochloride salt of 4,4′-diguanidinodiphenylamine (1b). Grey solid (90%); mp > 170 °C (dec.); ^1H NMR (D_2O) δ 6.96 (m); ^{13}C NMR (D_2O) δ 157.04, 143.27, 128.05, 127.06, 119.05; MS (FAB^+): 284 (MH^+/100%); anal. calcd for $C_{14}H_{19}Cl_2N_7{\cdot}1.5H_2O$: C, 43.87; H, 5.78; N, 25.58. Found: C, 43.88; H, 5.39; N, 25.19.

Dihydrochloride salt of 4,4′-diguanidinodiphenylketone (2b). White solid (90%); mp 149–152 °C; ^1H NMR (D_2O) δ 7.64 (d, 4H, $J=8.4$ Hz), 7.25 (d, 4H, $J=8.2$ Hz); ^{13}C NMR (D_2O) δ 195.6, 153.28, 136.74, 132.22, 129.44, 121.57; MS (FAB^+): 297 (MH^+/100%); anal. calcd for $C_{15}H_{18}Cl_2N_6O{\cdot}1.3H_2O$: C, 45.88; H, 5.24; N, 21.40. Found: C, 45.92; H, 4.98; N, 21.40.

Dihydrochloride salt of 4,4′-diguanidinodiphenylsulfone (3b). White solid (85%); mp 247–250 °C (lit.[39] 263–265 °C); ^1H NMR (D_2O) δ 7.85 (d, 4H, $J=8.71$ Hz), 7.32 (d, 4H, $J=8.56$ Hz); ^{13}C NMR (D_2O) δ 156.81, 141.63, 138.19, 130.52, 125.88; MS (FAB^+): 333 (MH^+/100%); anal. calcd for $C_{14}H_{18}Cl_2N_6O_2S{\cdot}2H_2O$: C, 38.10; H, 5.02; N, 19.04; S, 7.26. Found: C, 37.83; H, 4.76; N, 19.19; S, 6.82.

Dihydrochloride salt of 4,4′-diguanidinodiphenylmethane (4b). White solid (81%); mp 199–202 °C (lit.[40] 199–200 °C); ^1H NMR (D_2O) δ 7.21 (d, 4H, $J=8.4$ Hz), 7.07 (d, 4H, $J=8.4$ Hz), 3.88 (s, 2H); ^{13}C NMR (D_2O) δ 155.45, 140.39, 131.21, 129.22, 125.31, 39.48; MS (FAB^+): 283 (MH^+/100%); anal. calcd for $C_{15}H_{20}Cl_2N_6{\cdot}2.5H_2O$: C, 45.01; H, 6.29; N, 20.99. Found: C, 45.53; H, 6.12; N, 20.92.

Pharmacology: materials and methods

Rabbit and rat aorta. Male Wistar rats weighing 250–300 g and rabbits (New Zealand) weighing 2.5–3 kg were killed by decapitation. Descending thoracic aortas were removed and placed in a Krebs physiological solution.

The excess of surrounding tissue was removed and the arteries were cut into rings and mounted in organ baths (under 1 g of tension for rats tissues and 2 g for rabbits tissues) containing 5 mL of Krebs, maintained at 37 °C, and bubbled with 95% O_2 and 5% of CO_2 gas mixture. The aortas were allowed to equilibrate during 1 h.[28a]

Isometric tension was recorded in a polygraph (Grass Mod. 7D) through force–displacement transducers (Grass FT07).

After the equilibration period, the arteries were precontracted with NA (5×10^{-8} M) until two successive responses were almost identical in height. This was followed by exposure to the new compounds for 5 min prior to the addition of NA.[10b] After 20 min the preparations were washed and left to recover the basal response before testing the next doses. Concentration–response curves of the derivatives were constructed (10^{-8}–10^{-4} M). Only one antagonist was used in each experiment.

The effect on the basal tension was also studied. Vasoconstriction produced by KCl (80 mM) was tested after every experiment.[29] Inhibition of contraction produced by NA was measured and was expressed as percentage. The IC_{50} of each compound was calculated.

To discard effects mediated through activation of B_1 receptors the effects of the new compounds on rabbit aorta after an equilibrium period of 5 h to induce B_1 receptors[11a] were tested. Control contractions were elicited by repeated administration of Des-Arg^9-bradykinin, then the derivatives (10^{-8}–10^{-4} M) were added to the organ bath 10 min before the following administration of Des-Arg^9-bradykinin (3×10^{-7} M).

Guinea pig isolated ileum. Guinea pigs of either sex (300–400 g) were used. Myenteric–plexus–longitudinal muscle (MP–LM) strips were isolated from distal ileum as previously described[41] and suspended in a 5 mL organ bath containing Krebs solution at 35 °C aerated

with 95% O_2 and 5% CO_2. Tissues were maintained under a resting tension of 1 g, and the contractile activity of the strips was recorded isometrically using an Omniscribe polygraph D-5000. After an equilibration period of 20 min following sets of experiments were carried out.

Agonist activity. All the synthesized compounds (10^{-8} to 5×10^{-6} M) were added to the organ bath in order to discard agonist activity; each concentration was left in the organ bath a 10 min period.

Antagonist activity. The effect of all the derivatives on the contractions induced by electrical stimulation (single square waves: 0.3 Hz, 2 ms and supramaximal voltage) or by bradykinin (5×10^{-7}–5×10^{-6} M) on MP–LM strips was tested. In electrically stimulated tissues the new compounds were added to the organ bath at 10^{-8}–5×10^{-6} M concentrations. The bradykinin was applied repeatedly at 10 min intervals and 1 min after each addition the bath solution was replaced. When a stable contractile response was reached the studied compounds (10^{-8}–5×10^{-7} M) were added to the organ bath 10 min before the following dose of bradykinin. Only strips producing reproducible responses (>0.5 g) were used, the last control response was taken as 100 and subsequent results obtained were expressed as a percentage of this.

Drugs. The following drugs were used: noradrenaline bitartrate, bradykinin, Des-Arg-bradykinin (Sigma Chemical Co.). All drugs were dissolved in distilled deionized water at 10^{-2} M stock solution.

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