Guanidine and 2-Aminoimidazoline Aromatic Derivatives as α_2-Adrenoceptor Antagonists, 1: Toward New Antidepressants with Heteroatomic Linkers

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The efficient preparation and pharmacological characterization of different families of (bis)guanidine and (bis)2-aminoimidazoline derivatives ("twin" and "half" molecules) as potential α_2-adrenoceptor antagonists for the treatment of depression is presented. The affinity toward the α_2-adrenoceptor of all the compounds prepared was measured in vitro in human brain tissue. Additionally, the activity as agonist or antagonist of those compounds with a pK_i larger than 7 was determined in functional [^{35}S]GTPγS binding assays in human brain tissue. Finally, the activity of the most promising compounds was confirmed by means of in vivo microdialysis experiments in rats. Compounds **1**, **2b**, **3b**, **12b**, **13b**, **17b**, **18b**, **22b**, **25b**, **26b**, **28b**, and **30** showed a good affinity toward the α_2-ARs. In general, the 2-aminoimidazoline derivatives displayed higher affinities than their guanidine analogues. Finally and most importantly, compounds **18b** and **26b** showed antagonistic properties over α_2-ARs not only in vitro [^{35}S]GTPγS binding but also in vivo microdialysis experiments. Moreover, both compounds have shown to be able to cross the blood−brain barrier and, therefore, they can be considered as potential antidepressants.

Introduction

The World Health Organization has indicated that, by 2020, depression will be the second largest health burden following only heart diseases.[1] The pathophysiological origin of this disease continues to be unknown. However, the monoamine theory, the most widely accepted,[2] states that depression is a result of a deficiency of brain monoamine activity and that an approach to treat depression is to enhance this monoaminergic activity. Nowadays, customary therapeutic approaches include: (a) to block the presynaptic inhibitory autoreceptors, (b) to block the monoamine reuptake mechanisms from the synapse (selective serotonin or noradrenaline reuptake inhibitors), or (c) to inhibit the metabolic pathways (monoamine oxidase inhibitors).

The most recent trends in the development of antidepressants include dual action compounds that block both serotonin and noradrenaline (NA) reuptake, such as venlafaxine[3] or duloxetine[4] (Figure 1), as well as drugs that target receptors and, in particular, serotonin receptors such as 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ and the noradrenergic receptor α_2 (α_2-AR).[5] In this way, drugs like mianserin and mirtazepine (Figure 1), which show effective antidepressant activity, are thought to act by blockade of α_2-AR.[6] The success of these two drugs strongly supports that the α_2-AR targeting, as intended in the present work, is an efficient approach to the treatment of depression. Moreover, there is increasing evidence showing that α_2-AR antagonists may also be effective in the treatment of neurodegenerative diseases of the brain.[7]

Central noradrenergic transmission is regulated by inhibitory α_2-ARs expressed on locus coeruleus, somatodendritic neurones and on axon terminals. Thus, the activation of these receptors induces an inhibition of NA release in the brain. In this context, it has been described that depression is associated with a selective increase in the high-affinity conformation of the α_2-ARs in the human brain.[8] This enhanced α_2-AR activity could be involved in the deficit in noradrenergic transmission described in the aetiology of depression. Thus, chronic treatment with antidepressants induces an in vivo desensitization of the α_2-ARs regulating the local release of NA.[9] In the therapeutic context of this hypothesis, the development of selective α_2-adrenoceptor antagonists appears to be a new and effective pharmacological approach to the treatment of depressive disorders. In this way, it has been demonstrated that the administration of different α_2-AR antagonists both locally in the locus coeruleus or systemically increases the release of NA in the prefrontal cortex.[10,11] Moreover, α_2-AR antagonists are also able to promote the increase of NA induced by selective reuptake inhibitor antidepressant drugs.[12]

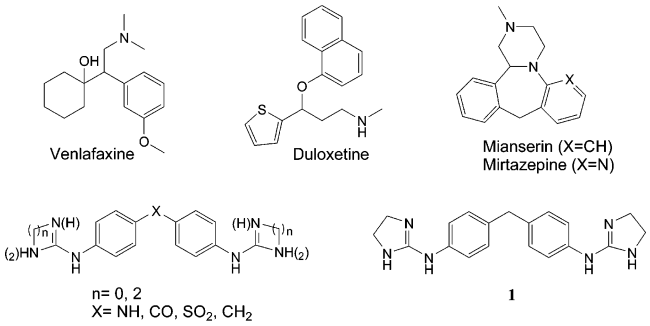

Figure 1. Structure of known adrenergic antidepressants, the general formula of the compounds previously prepared by us, and the structure of the lead compound **1**.

Examining the structures of some of the compounds already mentioned as antidepressants (Figure 1), one can identify certain chemical motifs present in these pharmacological agents. Thus, Mianserin and Mirtazepine have in common the presence of two aromatic rings connected by a methylene bridge. Previously, we had prepared a family of aromatic compounds bearing two guanidine or 2-aminoimidazoline groups at each end of a bisphenyl chain (Figure 1),[13] and their α_2-AR affinity, in human

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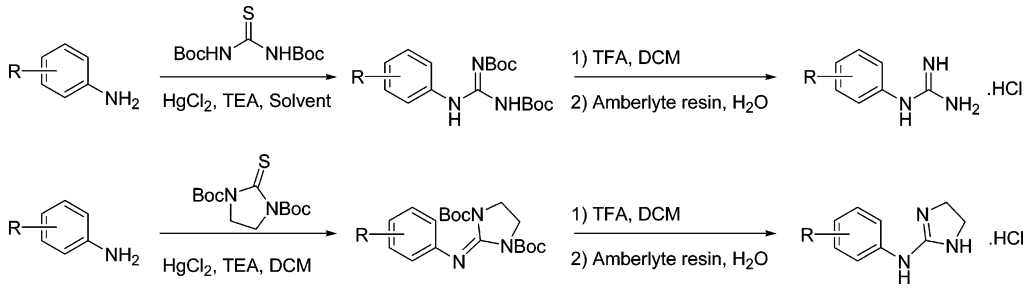


Figure 2. General synthetic schemes for the preparation of the guanidinium and 2-aminoimidazolinium derivatives.

brain tissues (frontal cortex), was measured.[14] From this study, we found that compound **1**, which shares the diaromatic motif observed in Mianserin and Mirtazepine, has a very interesting α_2-AR affinity, ($pK_i = 8.80$) and, therefore, could be a potential antidepressant.

Bearing in mind the structure of the dicationic lead compound **1**, we decided to prepare some structurally related (bis)guanidine and (bis)2-aminoimidazoline analogues as potential antidepressants. Thus, their α_2-AR affinity and potential receptor antagonism would be evaluated in vitro by performing the test in human frontal cortex tissue because there is an important density of α_2-ARs in this tissue.[15] Because the final objective of this work is to obtain antidepressants, the use of human brain tissue to directly characterize the pharmacological properties of the new compounds could lead to more relevant results from a therapeutic vantage.

It is important to highlight that the lead compound **1** is a "twin" molecule: two exact halves can be identified due to the existing symmetry in the substrate. Considering that nonsymmetrical shorter monocationic substances such as the selective α_2-AR radioligand [^{3}H]RX821002 (2-methoxy-idazoxan) show a better affinity toward the receptors, it would be of great interest to rationally design, synthesize, and evaluate the affinity of monocationic derivatives. These "half" compounds will share part of the lead structure to determine the positive or negative effects played by the second cationic moiety. This will allow us to find some structure−activity relationships (SARs) within the series of substrates reported in this work.

Moreover, substitution of the methylene group in the linker by other groups with different electronic and steric properties, together with the use of guanidine as well as 2-aminoimidazoline moieties, could help to better understand not only the binding in the active site (affinity), but also their activity (agonism/antagonism). The latter transformation is supported by previous molecular modeling studies carried out in our group,[16] from where it can be deduced that both guanidine and 2-aminoimidazoline have similar structural and electronic properties.

In this article we report, first, the efficient synthesis of a number of symmetrical and nonsymmetrical guanidine and 2-aminoimidazoline analogues of **1**. Second, and more importantly, we present a complete pharmacological study. Thus, in vitro assays in human brain tissue to evaluate the α_2-ARs affinity and functional studies to determine the agonist or antagonist nature of those derivatives with $pK_i > 7$ were performed. Moreover, in vivo microdialysis experiments in rats (with local and peripheral administration) were carried out with those compounds showing antagonist properties, to test their effects on NA release to establish their potential use as antidepressants.

Results and Discussion

Chemistry. Because many guanidine-containing compounds have been found to be biologically active, the development of guanidylating agents has been widely investigated,[17] and several methods under different conditions have been described to incorporate this group into the molecule. Thus, cyanamide,[18] *O*-methylisourea hydrogen sulfate,[19] pyrazole-1-carboxamide derivatives,[20] *S*-methyl-isothiouronium salts,[21] and protected thioureas combined with mercury salts[22] or the Mukaiyama reagent[23] are among the most popular.

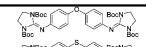
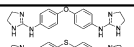
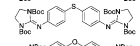
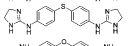
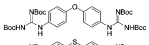
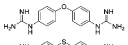
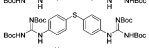
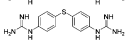
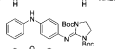
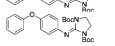
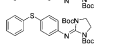
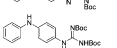
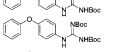
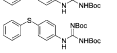
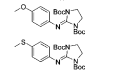
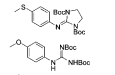
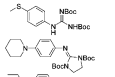
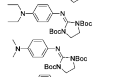
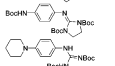
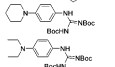
Considering the poor nucleophilic properties of our starting amines and diamines and based on the good results obtained in the previous paper,[13] we decided to use the strategy reported by Kim and Qian[24] because it proved to be very effective. This strategy consists of the treatment of the corresponding amine (or diamine) with 1 (or 2) equiv(s) of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea in the presence of mercury(II) chloride and an excess of triethylamine (Figure 2). This procedure allows the easily handling Boc-protected precursors to be obtained under mild conditions in yields ranging from 72% to 89% (see Table 1) after column chromatography in silica gel. Deprotection of the Boc groups with an excess of trifluoroacetic acid in dichloromethane followed by treatment with Amberlyte resin in aqueous solution led to the hydrochloride salts of the target molecules in good overall yields.

Among the guanidine derivatives displayed in Table 1, compounds **4b**,[25] **5b**,[26] **10b**,[25a,27] **11b**,[28] **14a**,[29] **14b**,[17,20a,30] **15b**,[31] **21b**,[32] **22b**,[33] **23b**,[34] **27b**,[30d,30e,35] **28b**,[36] and **29b**[30d,35a] have been prepared before to treat different conditions or as synthetic intermediates to obtain more complex molecules. However, none of them has been tested as α_2-AR ligands in the human brain to evaluate their potential as antidepressants.

Regarding the 2-aminoimidazoline substrates, they are considered to display good affinity toward the α-adrenergic receptors,[37] and several routes to synthesize them have been described. Thus, the direct introduction of the heterocycle via nucleophilic attack of a primary amine using 2-nitramino-4,5-dihydro-1*H*-imidazolium,[38] 2-sulfonate-4,5-dihydro-1*H*-imidazolium,[39] or 2-methylmercapto-4,5-dihydro-1*H*-imidazolium iodide[40] is a possible approach. Another possibility is the preparation of *N*-aryl-*S*-methylisothiouronium iodides or *N*-aryldichloroimines to be reacted with ethylenediamine, even though purification of the final product is not an easy task.[41]

The Kim and Qian strategy was also used to incorporate the 2-aminoimidazoline moiety, using the *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione[13] as the cation precursor (Figure 2). However, none of these new Boc-protected compounds could be purified by column chromatography in silica gel because partial decomposition of the molecule was found in every attempted case. To overcome this drawback, a quick flash column over neutral alumina was run instead, followed by recrystallization in the appropriate solvent when required. The yield in the first stage of the synthesis ranges from 52 to 91%, and in all cases, the hydrochloride salts of the target molecules

Table 1. Overall First and Second Stage Yields (%) Obtained for All Compounds Prepared

Compd	Structure	% 1st Stage	Compd	Structure	% 2nd Stage	% Overall
2a		78	**2b**		94	73
3a		72	**3b**		93	67
4a		80	**4b**		96	77
5a		80	**5b**		94	75
6a		74	**6b**		94	70
7a		80	**7b**		93	74
8a		52	**8b**		95	49
9a		74	**9b**		96	71
10a		72	**10b**		95	68
11a		77	**11b**		92	71
12a		62	**12b**		95	59
13a		64	**13b**		97	62
14a		80	**14b**		95	76
15a		86	**15b**		96	82
16a		86	**16b**		92	79
17a		91	**17b**		93	85
18a		70	**18b**		90	63
19a		65	**19b**		91	59
20a		73	**20b**		92	67
21a		74	**21b**		93	69
22a		77	**22b**		92	71
23a		89	**23b**		96	85
24a		63	**24b**		94	59
25a		73	**25b**		95	69
26a		73	**26b**		95	69
27a		89	**27b**		94	84
28a		82	**28b**		94	77
29a		82	**29b**		96	79

were obtained after Boc deprotection and anion exchange resin treatment in more than a 90% yield.

Among those, 2-aminoimidazoline derivatives reported in this paper, compounds **2b**, **3b**, **6b**, **16b**, **17b**, and **18b** have not been described previously, whereas compounds **7b**,[42] **8b**,[43] **12b**,[44] **13b**,[43] and **19b**,[45] although already described, had not been tested as α_2-AR ligands.

Regarding compounds **24b**, **25b**, and **26b**, they had been tested as α_2-AR ligands in different tissues,[46] and they have been included in this study for the sake of comparison. Their results will be treated separately and in more detail in a different section.

All the amines and diamines used as starting materials are commercially available either from Aldrich or Fluka, except for the 1,4-(bis-4-aminophenyl)piperazine, whose synthesis has been described previously[47] to obtain the trifluoroacetate salts of the compounds **32** and **36** shown in Table 2. In the present study, all the compounds have been tested as their hydrochloride salts. Regarding compound **19b**, it should be mentioned that the use of the *N*-Boc-*p*-phenylenediamine instead of 1,4-phenylenediamine as starting material was required to increase the solubility of the product and allow a faster development of the column chromatography. One-pot deprotection of the three Boc groups led to the corresponding hydrochloride.

Pharmacology. The affinity toward the α_2-ARs in human brain frontal cortex of all compounds prepared was measured by competition with the selective radioligand [^{3}H]RX821002 (2-methoxy-idazoxan), which was used at a constant concentration of 1 nM.

Table 2. Affinity Values (expressed as pK_i) Obtained for All the Compounds Studied

Compound	Structure	pKi
RX821002		9.04
Idazoxan		7.29
Clonidine		7.68
1		8.80
30[α]		7.24
31[α]		5.80
2b		7.00
3b		7.74
32[47]		6.33
33[α]		6.38
34[α]		5.20
35[α]		6.00
4b		5.78
5b		6.57
36[47]		5.97

[a] Compounds previously prepared by us[13] whose α_2-AR affinity had not been evaluated.

Affinity of the Dicationic Compounds ("Twin" Molecules). Bearing in mind the inhibition constants obtained in the previous paper[14] for the "twin" molecules **1** and **30** (see Table 2), we explored the affinity of these systems with an electron withdrawing group, such as a ketone, in the bridge position. Thus, the change in the electronic properties of the linker in compound **31** led to a dramatic loss in the α_2-AR affinity (pK_i = 5.80, Table 2). Hence, we decided to focus our attention mainly in molecules with electron donor groups in the linker.

The incorporation of the −O− and −S− atoms in the linker instead of their isosteric $-CH_2-$ group resulted in a smaller loss of affinity. In fact, the thioether compound **3b** showed one of the highest pK_i values (7.74, Table 2), better than the amino analogue **30**, whereas the ether derivative **2b** also displayed a good value (pK_i = 7.00, Table 2). These data seem to confirm that an electron-donor group in the bridge position (para with respect to the cationic groups) is required for a higher affinity. However, an important drop in the pK_i value was found again in compound **32** with piperazine as a linker (pK_i = 6.33, Table 2). Nonetheless, in this particular case, steric effects could be playing a very important role, besides the electronic ones, as can be deduced from the values obtained for shorter compounds (see Table 3). In summary, the affinity of the 2-aminoimidazoline dicationic derivatives can be ordered according to the

Table 3. Affinity (pK_i) Obtained for All Monocationic Molecules

cmpd	pK_i	cmpd	pK_i
RX821002	9.04	*amino-containing derivatives*	
Idazoxan	7.29	**16b**	5.93
Clonidine	7.68	**17b**	7.09
1	8.80	**18b**	7.42
diphenyl derivatives		**19b**	6.92
6b	6.56	**20b**	5.52
7b	6.58	**21b**	6.34
8b	6.62	**22b**	7.06
9b	6.83	**23b**	5.58
10b	6.05	*3,4-disubstituted derivatives*	
11b	6.30	**24b**	6.66
O,S-p-phenyl derivatives		**25b**	7.85
12b	7.77	**26b**	7.33
13b	7.07	**27b**	5.84
14b	6.39	**28b**	8.21
15b	6.40	**29b**	6.40

group in the bridge as follows: CH_2 > S > NH > O > piperazine > CO).

In addition, the corresponding bisguanidinium "twin" molecules have been prepared and the results obtained for their affinity toward α_2-AR are shown in Table 2. The pK_i values of this new set of "twin" molecules decrease after the loss of the ethylene moiety in the cationic fragments. However, compared to the imidazolinium series, there is a slight change in the order of affinity: S > CH_2 > NH > piperazine > O > CO.

Affinity of the Diphenyl Monocationic Molecules. To explore the effects on the receptor affinity of a second cation in the molecule, the monocationic compounds shown in Table 3 were synthesized and subjected to a pharmacological study. If the presence of the second cation had a positive influence, then higher affinities would be expected for the "twin" molecules; if not, larger pK_i values for the monocationic derivatives should be obtained. It should be noted that the loss of one of the cations results in a less polar set of compounds. As such, the pharmacological findings obtain for these derivatives could help for a better understanding of the nature of the interaction with the adrenoceptor.

Compared to their dicationic counterparts, the loss of one of the 2-aminoimidazolinium cations (compounds **6b**, **7b**, and **8b**) led to a decrease in the α_2-AR affinity. Additionally, the fact that these three "half" molecules showed very similar pK_i values (6.56, 6.58, and 6.62, see Table 3) could indicate that, in this series, the presence of the second cation is essential for the interaction with the receptor and that only then the different linkers modulate the strength of such an interaction.

In the guanidinium series, the loss of the second cation resulted in an increase in the affinity for the −NH− and −O− derivatives **9b** and **10b**, whereas compound **11b** displayed a lower affinity than its analogue **5b** (see Table 2). It is important to highlight that, for the first time, the amino guanidinium derivative **9b** showed a higher pK_i value than its 2-aminoimidazolinium analogue **6b**. Thus, the affinity for this set of compounds can be ordered, in terms of the linker, as follows: NH > S > O.

Affinity of the *O,S-p*-Substituted Phenyl Monocationic Molecules. In Table 3, the pK_i values obtained for the compounds with an oxygen or sulfur-containing group in the *para*-position are presented. In the 2-aminoimidazoline series, this structural modification led to an enhancement in the affinity compared to the previous analogues. However, despite the high pK_i value displayed by the methoxy derivative **12b** (fourth in the whole series of the present article), it still cannot match that of the "twin" lead compound **1**.

As for the guanidine series, there is a slight affinity increase compared to the guanidine monocationic phenylether (**10b**) and phenylthioether (**11b**) derivatives in Table 3, whereas there is hardly any difference among themselves after the phenyl loss (pK_i = 6.39 and 6.40). Considering their "twin" molecule analogues (compounds **4b** and **5b** in Table 2), no trend can be identified, because the oxygen-containing compound shows an increase in its pK_i, while the sulfur-containing one displays a slightly worse affinity. Once again, the 2-aminoimidazoline series shows better affinities than their guanidine counterparts.

Affinity of Phenyl Monocationic Molecules Containing Amino Groups. The pK_i values of the monocationic amino-containing derivatives **16b**−**23b**, **32**, and **36** are shown in Tables 2 and 3. In the case of the 2-aminoimidazoline series, except for compound **16b**, the reduction in the molecule size results in a better affinity set of compounds. Thus, the diethyl- (**17b**) and dimethylamino (**18b**) derivatives show pK_i values within the range of Idazoxan but, once again, not as good as compound **1**.

The affinity enhancement with the size reduction seems to indicate that the bioactive conformation of the "twin" molecule with the piperazine in the linker might not be adequate for the interaction with the receptor. Further, unlike in the lead compound **1**, the presence of the second cationic unit is a drawback, negatively affecting the binding to the receptors.

Again, the guanidinium derivatives displayed lower affinities than their imidazoline analogues. However, remarkably and for the first time in this series of molecules, the dimethylamino guanidinium compound **22b** shows a pK_i > 7 and within the range of its imidazoline pair **18b** (see Table 3). Additionally, while compound **23b** was found to be within the range of its "twin" analogue **36**, the same did not apply to compound **20b**, which was found to have lower affinity.

Affinity of 3,4-Disubstituted Phenyl Monocations. The results obtained for a set of different 3,4-disubstituted phenyl 2-aminoimidazoline (**24b**, **25b**, and **26b**) and guanidine (**27b**, **28b**, and **29b**) derivatives are shown in Table 3. As already mentioned, these compounds have been synthesized and reported before. Compounds **24b**, **25b**, and **26b** were found to be α_2-AR ligands,[46] whereas compounds **27b**,[30d,30e,35] **28b**,[36] and **29b**,[30d,35] as previously stated, were used as synthetic intermediates and to treat other conditions, but not tested as possible α_2-AR substrates.

The 3,4-dimethoxy derivative **24b** is reported in the literature to possess prejunctional α_2-AR antagonist properties[46a,d] (in the isolated rat vas deferens and Guinea pig ileum) and postjunctional agonist properties (in the rat anococcygeus muscle). As it has not been tested in human brain, we considered it significant to analyze its binding features under our experimental conditions.

The maleate salt of the dioxan compound **25b** was found to display agonist properties in the α_2-AR in rat vas deferens (peripheral nervous system), whereas in the Guinea pig ileum (CNS), it acted as a competitive antagonist.[46a,d,e] Human brain tissue (frontal cortex) was used to determine the inhibition constant of this compound for the α_1 subtype, however, the K_i for the α_2-AR was measured in CHO-C10 cells.[46f] The functional efficacy (EC_{50}) of this compound was not reported. As far as derivative **26b** is concerned, it was described as a partial α_2-agonist and α_1-antagonist in vitro.[46a,d,e] Surprisingly, it was claimed to be a strong α_2-antagonist as potent and selective by Yohimbine[46c] and Tolazoline.[46b]

Given the unclear pharmacological results of the last two compounds, we considered that it would be of interest to include them in our study because, in case they showed a good affinity,

agonism-antagonism experiments could help to better understand their pharmacological profiles. In addition, they have not been tested in human brain tissue as α_2-AR ligands before.

Regarding the guanidine derivatives, we have not found any article in the literature considering them to be potential antidepressants in the human brain. We thus included them in the study to explore the differences between 2-aminoimidazoline and guanidine cations.

Compared to the *para*-methoxy compound **12b**, the addition of a second methoxy group in the *meta*-position (compound **24b**) results in a one-order of magnitude loss of affinity (pK_i = 6.66, Table 3). In the case of the other dioxo heterocyclic 2-aminoimidazoline derivatives (**25b** and **26b**), similar pK_i values to the monomethoxy **12b** were found, with a decrease for derivative **26b** and a slight improvement for compound **25b**. The corresponding inhibition constant reported in the literature for the maleate salt of the last molecule (measured in CHO-C10 cells) is slightly lower than the one we obtained in the frontal cortex (K_i = 3.1 nM, pK_i = 8.51[46f] vs K_i = 14 nM, pK_i = 7.85).

In the guanidine series, the introduction of a second methoxy group (compound **27b**) leads to a loss of affinity compared to the 4-methoxy guanidine **14b**, whereas the substitution of the two methoxy groups for the five-membered ring (compound **29b**) hardly affects the affinity (see Table 3). However, the dioxan derivative **28b** was found to have a higher pK_i value (8.21, Table 3) and, unlike the other guanidine derivatives, displays a better affinity than its 2-aminoimidazoline analogue. In fact, the pK_i obtained is the second highest in this article.

[^{35}S]GTPγS Binding Functional Assays. Those compounds, which displayed an affinity at least within the range of Idazoxan (with a $pK_i > 7$), were subjected to [^{35}S]GTPγS binding experiments to determine their nature as agonists or antagonists.

As members of the G-protein coupled receptors (GPCRs) super-family, when the endogenous substrate binds to the α_2-ARs, they interact with a G-protein, triggering a cascade of different biochemical events, which result in transmembrane signaling. This receptor activation alters the conformation of the G-proteins, leading to the exchange of GDP by GTP on the α-subunit, promoting their dissociation into α-GTP and $\beta\gamma$ subunits. A direct evaluation of this G-protein activity can be made by determining the guanine nucleotide exchange using radiolabeled GTP analogues. The [^{35}S]GTPγS binding assay constitutes a functional measure of the interaction of the receptor and the G-protein and is a useful tool to distinguish between agonists (increasing the nucleotide binding), inverse agonists (decreasing the nucleotide binding), and neutral antagonists (not affecting the nucleotide binding) of GPCRs.[48] Experiments were performed in low-affinity receptor conditions for agonists (presence of guanine nucleotides and sodium in the medium), and therefore, typical potency values are 2−3 log units lower than affinity values obtained in radioligand receptor binding experiments.[48]

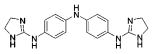
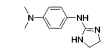
Compounds **1**, **2b**, **3b**, **12b**, **13b**, **17b**, **25b**, and **28b** stimulated binding of [^{35}S]GTPγS, showing a typical agonist dose−response plot. Their EC_{50} values and percentage efficacy relative to the well-known α_2-AR agonist UK14304 [5-bromo-6-(2-imidazolidinylideneamino)quinoxalin] are displayed in Table 4. The potencies of the different compounds (EC_{50} values) were in the micromolar range, with **1** and **3b** being the most potent and weakest agonists, respectively.

Those compounds that did not stimulate binding of [^{35}S]-GTPγS by their own were subjected to new [^{35}S]GTPγS binding experiments and tested against the α_2-AR agonist UK14304.

Table 4. pK_i, EC_{50} Values, and Percentage Efficacy Relative to UK14304 Found for Compounds Showing a Typical Agonist Dose−Response Plot

cmpd	pK_i	EC_{50} (μM)	relative efficacy (%)
UK14304	8.85	11.4 ± 0.3	100
1	8.80	0.166 ± 0.07	89
2b	7.00	120 ± 14.4	89
3b	7.74	332 ± 71.5	89
12b	7.77	30.4 ± 3.7	90
13b	7.07	45.3 ± 16.5	82
17b	7.09	2.76 ± 0.1	87
25b	7.85	56.4 ± 8.6	86
28b	8.21	42.2 ± 5.9	89

Table 5. EC_{50} Values Obtained from the Concentration−Response Curves for UK14304 Stimulation of [^{35}S]GTPγS Binding in the Absence or Presence of the Different Compounds (10^{-5} M Concentration)

Experiment	Added compound structure	EC_{50} (μM)
UK14304		11.4±0.3
UK14304 + 30		4.38±0.6
UK14304 + 18b		355±18.2
UK14304 + 22b		16±2.1
UK14304 + 26b		141±16

The rightwards shifting of the concentration−response curve for UK14304 when including our compounds in the assay would confirm the antagonist effect of these compounds against the α_2-ARs. Thus, the effect induced in the UK14304 stimulation of [^{35}S]GTPγS binding by the presence in the medium of a single concentration (10−5 M) of each of our compounds is presented in Table 5.

Compounds **18b** and **26b** produced a remarkable rightwards shift in the UK14304 curve, indicating that both molecules possess antagonist properties on α_2-ARs. In both cases, the EC_{50} for UK14304 stimulation of [^{35}S]GTPγS binding increases in more than one logarithmic order of magnitude (Table 5).

In contrast, compounds **30** and **22b** did not shift to the right of the curve for the UK14304. Moreover, compound **30** even decreased the EC_{50} value for UK14304. These data indicate that both compounds do not have antagonistic properties at α_2-ARs.

After this series of experiments, it is worth highlighting the discovery of a potent new α_2-AR antagonist, compound **18b**, with affinity similar to that of Idazoxan and Clonidine in human brain membranes. Regarding compound **26b**, it also showed antagonist properties as reported by Takeuchi and co-workers[46c] and differing from what was published by Chapleo and co-workers.[46a,d,e] To better understand what seems to be a complex pharmacological profile, in vivo microdialysis experiments in rats were carried out.

In Vivo Microdialysis Experiments. Considering their antagonistic effect and relatively good affinities over the α_2-ARs, we decided to test compounds **18b** and **26b** in vivo to evaluate their potential effect on noradrenergic transmission. According to the most widely accepted theory on autoreceptors, α_2-AR antagonists are expected to increase the extracellular NA concentrations in the brain, and for that reason, compounds **18b** and **26b** are expected to produce such an effect. Microdialysis technique is designed to collect virtually any substance from the brains of freely moving animals with limited tissue trauma.

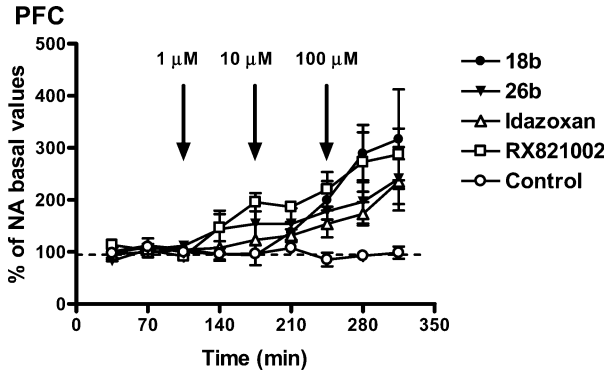


Figure 3. Effect on NA extracellular concentration of local administration (1−100 μM) of **18b**, **26b**, Idazoxan, RX821002 (2-methoxyidazoxan), or cerebrospinal fluid by reverse microdialysis in the prefrontal cortex. The arrows indicate the time of drug administration. Points are means ± s.e. mean values from 3−4 separate animals for each group and are expressed as percentages of the corresponding basal values.

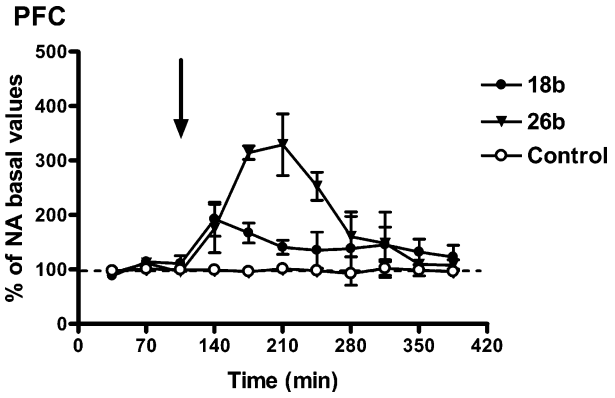


Figure 4. Effect of intraperitoneal administration of **18b** (8 mg/kg), **26b** (8 mg/kg), or saline (1 mL/kg) on NA extracellular concentration in the prefrontal cortex. The arrow indicates the time of drug administration. Points are means ± s.e. mean values from two separate animals for each group and are expressed as percentages of the corresponding basal values.

Thus, microdialysis experiments allow the measurement of local neurotransmitter release.[49]

Basal extracellular NA concentration on the prefrontal cortex was 3.11 ± 0.38 nM (n = 80). In control rats, local administration of artificial cerebrospinal fluid did not change extracellular NA (F[8,32] = 0.83; P = 0.57, n = 4; Figure 3).

Conversely, local administration of **18b** or **26b** increased, in a concentration-dependent manner, extracellular NA on the prefrontal cortex reaching a maximal effect of 316 ± 95% (F[1,48] = 16.78; P = 0.0002 vs control, n = 8) and 240 ± 60% (F[1,46] = 17.99; P = 0.0001, n = 8 vs control), respectively (Figure 3). Local administration of Idazoxan or RX821002, two well-known α_2-antagonists, increased extracellular NA, running into maximal effects of 235 ± 43% (F[1,42] = 32.97; P < 0.0001, n = 7) and 287 ± 49% (F[1,40] = 69.53; P < 0.0001, n = 7), respectively, when were compared to control (Figure 3).

At this point, we tested the effect of **18b** and **26b** compounds administered systemically. Intraperitoneal administration of **18b** (8 mg/kg) increased extracellular NA on the prefrontal cortex to 192 ± 91% (F[1,22] = 13.25; P = 0.014, n = 4 vs control, Figure 4). Administration of **26b** (8 mg/kg i.p.) caused a rapid increase of extracellular NA on the prefrontal cortex up to 328 ± 56% (F[1,22] = 74.47; P < 0.0001, n = 4 vs control, Figure 4). Systemic administration of saline (1 mL/kg i.p.) did not change extracellular NA (F[10,21] = 0.16; P = 0.99, n = 2, Figure 4).

Considering the results of this series of in vivo microdialysis experiments it can be concluded that the antagonistic properties of compounds **18b** and **26b** over α_2-ARs, as expected from their behavior in [^{35}S]GTPγS binding experiments, are confirmed. Moreover, both compounds showed an effect in increasing NA extracellular concentrations similar to that of specific α_2-antagonists as Idazoxan or RX821002. Additionally, the increases of NA extracellular concentration after peripheral administration indicate that both compounds are able to cross the blood−brain barrier.

Conclusions

In this paper we have reported the quick and efficient synthesis of different families of (bis)guanidine and (bis)2-aminoimidazoline derivatives via their Boc-protected precursors. Some of the final compounds (**1**, **2b**, **3b**, **12b**, **13b**, **17b**, **18b**, **22b**, **25b**, **26b**, **28b**, and **30**) showed a good affinity toward the α_2-ARs in human brain tissue in in vitro experiments. Among them, **1**, **2b**, **3b**, and **30** are "twin" molecules, bearing 2-aminoimidazolinium cations at both ends of the molecules. Regarding the monocationic molecules, **12b**, **13b**, **17b**, **18b**, **25b**, and **26b** are 2-aminoimidazoline derivatives, whereas only **22b** and **28b** are guanidine-containing substrates. It can be observed that, in general, the 5-membered ring cation derivatives displayed higher affinities toward the α_2-ARs than their guanidine counterparts. Therefore, it can be deduced that the ethylene moiety of the cationic cycle seems to be playing a role in the binding properties of the molecules. None of the bis-guanidine-containing "twin" compounds showed a pK_i > 7.

Derivatives **1**, **2b**, **3b**, **12b**, **13b**, **17b**, **22b**, **25b**, **28b**, and **30** turned out to be agonists when subjected to [^{35}S]GTPγS experiments. It should be mentioned that compound **3b** showed the highest EC_{50} value (i.e., the weakest activity), despite showing one of the best affinities of this series of molecules. Thus, it seems clear that the high affinity of a compound for a receptor does not necessarily mean that the compound will show a good activity. This is because the properties of drugs that cause them to associate with the receptors (affinity) are different to those that produce stimulus (activity). This emphasizes the importance of carrying out the affinity tests in the most realistic environment possible and the need for performing functional tests in the actual tissue for which the potential drug is intended.

The most relevant result from the present study is that compounds **18b** and **26b** showed antagonistic properties both in vitro [^{35}S]GTPγS binding experiments and in vivo microdialysis experiments. This antagonist effect induced over α_2-ARs in vivo increases NA concentrations in the rat brain both after local or peripheral administration (they are able to cross the blood-brain barrier), and therefore, they can be considered as compounds with potential antidepressant effect.

The very good results obtained in these families of compounds not only in terms of affinity, but also, more importantly, in terms of the antagonistic activity are very encouraging. As such, we are working on another series of (bis)guanidine and (bis)2-aminoimidazoline derivatives to achieve a better understanding of the features required to improve the α_2-ARs affinity and antagonist activity and to be able to design new antidepressants.

Experimental Section

Pharmacology: Materials and Methods. Preparation of Membranes. Neural membranes (P_2 fractions) were prepared from the prefrontal cortex of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Postmortem human brain samples of each subject (∼1 g) were homogenized

using a Teflon-glass grinder (10 up-and-down strokes at 1500 rpm) in 30 volumes of homogenization buffer (1 mM $MgCl_2$ and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1000 × *g* (4 °C), and the supernatant was centrifuged again for 10 min at 40 000 × *g* (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and recentrifuged in similar conditions. Aliquots of 1 mg protein were stored at −70 °C until assay. Protein content was measured according to the method Bradford using BSA as standard and was similar in the different brain samples.

[^{3}H]RX821002 Binding Assays. Specific [^{3}H]RX821002 binding was measured in 0.55 mL aliquots (50 mM Tris HCl, pH 7.5) of the neural membranes, which were incubated with [^{3}H]RX821002 (1 nM) for 30 min at 25 °C in the absence or presence of the competing compounds (10−12 M to 10−3 M, 10 concentrations). Specific binding was determined and plotted as a function of the compound concentration. Incubations were terminated by diluting the samples with 5 mL of ice-cold Tris incubation buffer (4 °C). Membrane-bound [^{3}H]RX821002 was separated by vacuum filtration through Whatman GF/C glass fiber filters. Then the filters were rinsed twice with 5 mL of incubation buffer and transferred to minivials containing 3 mL of OptiPhase "HiSafe" II cocktail and counted for radioactivity by liquid scintillation spectrometry.

Analysis of Binding Data. Analysis of competition experiments to obtain the inhibition constant (K_i) were performed by nonlinear regression using the GraphPad Prism program. All experiments were analyzed assuming a one-site model of radioligand binding. K_i values were normalized to pK_i values.

[^{35}S]GTPγS Binding Assays. The incubation buffer for measuring [^{35}S]GTPγS binding to brain membranes contained, in a total volume of 500 μL, 1 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 50 mM GDP, 50 mM Tris−HCl at pH 7.4, and 0.5 nM [^{35}S]GTPγS. Protein aliquots were thawed and resuspended in the same buffer. The incubation was started by addition of the membrane suspension (40 μg of membrane proteins) to the previous mixture and was performed at 30 °C for 120 min with shaking. To evaluate the influence of the compounds on [^{35}S]GTPγS binding, eight concentrations (10−10 to 10−3 M) of the different compounds were added to the assay. Incubations were terminated by adding 3 mL of ice-cold resuspension buffer followed by rapid filtration through Whatman GF/C filters presoaked in the same buffer. The filters were rinsed twice with 3 mL of ice-cold resuspension buffer, transferred to vials containing 5 mL of OptiPhase HiSafe II cocktail (Wallac, U.K.), and the radioactivity trapped was determined by liquid scintillation spectrometry (Packard 2200CA). The [^{35}S]-GTPγS bound was about 7−14% of the total [^{35}S]GTPγS added. Nonspecific binding of the radioligand was defined as the remaining [^{35}S]GTPγS binding in the presence of 10 μM unlabeled GTPγS.

In Vivo Microdialysis Assays. The experiments were carried out in male Sprague-Dawley rats weighing between 250 and 300 g. At the beginning of the experiments, animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and a microdialysis probe was implanted by stereotaxic surgery into prefrontal cortex (PFC) brain area. The coordinates selected for the PFC were as follows: AP (anterior to bregma), +2.8 mm; L (lateral from the mid-sagittal suture), +1 mm; DV (ventral from the dura surface), −5 mm.[50] After 24 h, for animal recovery, perfusion fluid (artificial cerebrospinal fluid) is pumped through the probe at a flow rate of 1 μL/min. In the semipermeable membrane, that is the critical side of the probe, which is placed on the selected area, molecules flow into and out the cannulae by diffusion. Therefore, the microdialysis technique allows local administration of substrates dissolved in the perfusion fluids.

The different compounds, dissolved in artificial cerebrospinal fluid (148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, and 0.85 mM $MgCl_2$; pH 7.4), were perfused by reverse microdialysis in increasing concentrations of 1, 10, and 100 μM. Each concentration was administrated for two sampling periods of 35 min each. Systemic administration of the compounds was made intraperitoneally dissolved in saline. After collections, samples were analyzed by HPLC with electrochemical detection to monitor NA extracellular concentrations as a measure of antidepressant activity. The mean values of the first three samples before drug administration were considered as 100% basal value. All measures of extracellular NA concentrations are expressed as a percentage of the baseline value ± s.e. mean. One-way analysis of variance (ANOVA) for control group or two-way ANOVA between control and each treated group was assessed for statistical analysis. At the end of the experiments, animals were killed and the brains were dissected to check the correct implantation of the probe.

Drugs. [^{3}H]RX821002 (specific activity 59 Ci/mmol) was obtained from Amersham International, U.K. [^{35}S]GTPγS (1250 Ci/mmol) was purchased from DuPont NEN (Brussels, Belgium). Idazoxan HCl was synthesized by Dr. F. Geijo at S.A. Lasa Laboratories, Barcelona, Spain. Clonidine HCl, GDP, GTP, GTPγS, RX821002 HCl, and UK14304 were purchased from Sigma (St. Louis, U.S.A.). All other chemicals were of the highest purity commercially available.

Chemistry. All the commercial chemicals were obtained from Sigma-Aldrich or Fluka and were used without further purification. Deuterated solvents for NMR use were purchased from Apollo. Dry solvents were prepared using standard procedures, according to Vogel, with distillation prior to use. Chromatographic columns were run using silica gel 60 (230−400 mesh ASTM) or aluminum oxide (activated, Neutral Brockman I STD grade 150 mesh). Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F_{254} silica gel plates or Polygram Alox N/UV_{254} aluminum oxide plates. Visualization was by UV light (254 nm). NMR spectra were recorded in a Bruker DPX-400 Avance spectrometer, operating at 400.13 and 600.1 MHz for ^{1}H NMR and 100.6 and 150.9 MHz for ^{13}C NMR and ^{19}F NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker Win-NMR 5.0 software. Electrospray mass spectra were recorded on a Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with methanol, water, or ethanol as carrier solvents. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrometer equipped with a Gateway 2000 4DX2-66 workstation and on a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with Universal ATR sampling accessory. Sample analysis was carried out in nujol using NaCl plates. Elemental analysis was carried out at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College, Dublin.

General Procedure for the Synthesis of Boc-Protected Guanidine Derivatives: Method A. Each of the corresponding amines or diamines (1 equiv) was treated in the appropriate solvent at 0 °C with 1.1 equiv (or 2.2 for the diamines) of mercury(II) chloride, 1.0 equiv (or 2.0 for the diamines) of *N*,*N*′-di(*tert*-butoxycarbonyl)-thiourea, and 3.1 equiv (or 5.0 for the diamines) of TEA. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite to get rid of the mercury sulfide formed. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane/EtOAc mixture.

General Procedure for the Synthesis of the Boc-Protected 2-Iminoimidazolidine Derivatives: Method B. Each of the corresponding amines or diamines (1.0 equiv) was treated in DCM at 0 °C with 1.1 equiv (or 2.2 for the diamines) of mercury(II) chloride, 1.0 equiv (or 2.0 for the diamines) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 3.1 equiv (or 5.0 for the diamines) of TEA. The resulting mixture was stirred at 0 °C for 1 h and for the appropriate duration at room temperature. Then the reaction mixture was diluted with EtOAc and filtered through a pad of Celite to get rid of the mercury sulfide formed. The filter cake was rinsed with EtOAc. The organic phase was extracted with water (2 × 30 mL), washed with brine (1 × 30 mL), dried over

anhydrous Na_2SO_4, and concentrated under vacuum to give a residue that was purified by neutral alumina column flash chromatography, eluting with the adequate hexane/EtOAc mixture. The residue obtained after the column was recrystallized from the appropriate solvent when required.

General Procedure for the Synthesis of the Hydrochloride Salts: Method C. Each of the corresponding Boc-protected precursors (0.5 mmol) was treated with 15 mL of a 50% solution of trifluoroacetic acid in DCM for 3 h. After that time, the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was dissolved in 20 mL of water and treated for 24 h with IRA400 Amberlyte resin in its Cl^- form. Then the resin was removed by filtration and the aqueous solution was washed with DCM (2 × 10 mL). Evaporation of the water afforded the pure hydrochloride salt. Absence of the trifluoroacetate salt was checked by ^{19}F NMR.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenyl ether (2a): Method B. $HgCl_2$ (1.792 mg, 6.6 mmol) was added over a solution of 601 mg (3.0 mmol) of 4,4′-diaminodiphenyl ether, 1815 mg (6.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 2.1 mL (15.0 mmol) of TEA in DCM (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 24 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (5:1), gave a residue that was recrystallized from Et_2O to afford **2a** as a white solid (1720 mg, 78% yield); mp 168−170 °C.

Dihydrochloride Salt of 4,4′-di(2-Imidazolidinylimino)diphenyl Ether (2b): Method C. White solid (94%); mp decomposes over 220 °C; ^1H NMR (D_2O) δ 3.74 (s, 8H), 7.13 (d, 4H, J = 8.5 Hz), 7.30 (d, 4H, J = 8.5 Hz); ^{13}C NMR (D_2O) δ 42.2, 119.5, 126.3, 130.1, 155.2, 158.6; MS (ESI$^+$) m/z 337.1830 $[M + H]^+$. Anal. ($C_{18}H_{22}Cl_2N_6O \cdot 2H_2O$) C, H, N.

4,4′-Bis[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-diphenyl sulfide (3a): Method B. $HgCl_2$ (1.792 mg, 6.6 mmol) was added over a solution of 660 mg (3.0 mmol) of 4,4′-diaminodiphenyl sulfide, 1815 mg (6.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 2.1 mL (15.0 mmol) of TEA in DCM (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 24 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (5:1), gave a residue that was recrystallized from Et_2O to afford **3a** as a white solid (1650 mg, 72% yield); mp 144−146 °C.

Dihydrochloride Salt of 4,4′-di(2-Imidazolidinylimino)diphenyl Sulfide (3b): Method C. Light brown solid (93%); mp 146−148 °C; ^1H NMR (D_2O) δ 3.74 (s, 8H), 7.23 (d, 4H, J = 8.0 Hz), 7.41 (d, 4H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 42.2, 124.4, 131.8, 133.2, 134.1, 158.0; MS (ESI$^+$) m/z 353.2467 $[M + H]^+$. Anal. ($C_{18}H_{22}Cl_2N_6S \cdot 1.9H_2O$) C, H, N.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidine]-diphenyl ether (4a): Method A. $HgCl_2$ (1.792 mg, 6.6 mmol) was added over a solution of 601 mg (3.0 mmol) of 4,4′-diaminodiphenyl ether, 1658 mg (6.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 2.1 mL (15.0 mmol) of TEA in DCM (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 23 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (9:1), gave **4a** as a white solid (1650 mg, 80% yield); mp > 300 °C.

4,4′-Bis[2,3-di(*tert*-butoxycarbonyl)guanidino]-diphenyl Sulfide (5a): Method A. $HgCl_2$ (1.792 mg, 6.6 mmol) was added over a solution of 660 mg (3.0 mmol) of 4,4′-diaminodiphenyl sulfide, 1658 mg (6.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-thiourea, and 2.1 mL (15.0 mmol) of TEA in DCM (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (9:1), gave **5a** as a white solid (1720 mg, 80% yield); mp > 300 °C.

4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]diphenylamine (6a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 553 mg (3.0 mmol) of *N*-phenyl-*p*-phenylenediamine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 22 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:2), gave a residue that was recrystallized from Et_2O to afford **6a** as a white solid (1007 mg, 74% yield); mp 162−164 °C.

Hydrochloride Salt of 4-(2-Imidazolidinylimino)diphenylamine (6b): Method C. Grey solid (94%); mp 236−238 °C; ^1H NMR (D_2O) δ 3.52 (s, 4H), 6.74−6.83 (m, 1H), 6.84−6.97 (m, 6H), 7.04−7.12 (m, 2H); ^{13}C NMR (D_2O) δ 42.1, 117.6, 117.8, 121.4, 125.0, 127.2, 129.0, 141.4, 141.6, 157.9; MS (ESI$^+$) m/z 253.1379 $[M + H]^+$. Anal. ($C_{15}H_{17}ClN_4 \cdot 0.7H_2O$) C, H, N.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-phenoxybenzene (7a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 556 mg (3.0 mmol) of 4-phenoxyaniline, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:2), gave a residue that was precipitated with cold hexane and filtered afterward to afford **7a** as a white solid (1087 mg, 80% yield); mp 162−164 °C.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-(phenylthio)benzene (8a): Method B. $HgCl_2$ (299 mg, 1.1 mmol) was added over a solution of 201 mg (1.0 mmol) of 4-(phenylthio)-aniline, 303 mg (1.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-imidazolidine-2-thione, and 0.4 mL (2.8 mmol) of TEA in DCM (3 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 24 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (2:1), gave a residue that was recrystallized from hexane to afford **8a** as a white solid (245 mg, 52% yield); mp 136−138 °C.

4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]diphenylamine (9a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 553 mg (3.0 mmol) of *N*-phenyl-*p*-phenylenediamine, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 21 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (3:2), gave **9a** as a brownish solid (945 mg, 74% yield); mp 131−133 °C.

Hydrochloride Salt of 4-Guanidinodiphenylamine (9b): Method C. Grey solid (96%); mp 183−185 °C; ^1H NMR (D_2O) δ 6.64−6.72 (m, 1H), 6.75−6.86 (m, 6H), 6.96−7.04 (m, 2H); ^{13}C NMR (D_2O) δ 117.0, 117.6, 120.7, 125.0, 126.6, 128.8, 141.8, 142.6, 155.7; MS (ESI$^+$) m/z 227.1448 $[M + H]^+$. Anal. ($C_{13}H_{15}ClN_4 \cdot 0.8H_2O$) C, H, N.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-phenoxybenzene (10a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 556 mg (3.0 mmol) of 4-phenoxyaniline, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 21 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **10a** as a white solid (923 mg, 72% yield); mp 128−130 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-(phenylthio)benzene (11a): Method A. $HgCl_2$ (80 mg, 0.3 mmol) was added over a solution of 60 mg (0.3 mmol) of 4-(phenylthio)aniline, 83 mg (0.3 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 0.1 mL (0.7 mmol) of TEA in DCM (2 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 17 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (6:1), gave **11a** as a white solid (102 mg, 77% yield); mp 119−121 °C.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-methoxybenzene (12a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was

added over a solution of 370 mg (3.0 mmol) of 4-methoxyaniline, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. Usual work up followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:1), gave a residue that was recrystallized from hexane to afford **12a** as a white solid (730 mg, 62% yield); mp 85−87 °C.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-(methylthio)benzene (13a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 418 mg (3.0 mmol) of 4-(methylthio)-aniline, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 22 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (3:2), gave a residue that was recrystallized from Et_2O to afford **13a** as a white solid (783 mg, 64% yield); mp 136−138 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-methoxybenzene (14a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 370 mg (3.0 mmol) of 4-methoxyaniline, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 21 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **14a** as a white solid (880 mg, 80% yield); mp 181−183 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-(methylthio)benzene (15a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 418 mg (3.0 mmol) of 4-(methylthio)aniline, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 21 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **15a** as a white solid (987 mg, 86% yield); mp 121−123 °C.

***N*-(4-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]phenyl)piperidine (16a): Method B.** $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 529 mg (3.0 mmol) of *N*-(4-aminophenyl)-piperidine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (6:1), gave a residue that was recrystallized from hexane to afford **16a** as a white solid (1150 mg, 86% yield); mp 116−118 °C.

Dihydrochloride Salt of *N*-[4-(2-Imidazolidinylimino)phenyl]-piperidine (16b): Method C. White solid (92%); mp 108−110 °C; ^{1}H NMR (D_2O) δ 1.69−1.81 (m, 2H), 1.96−2.06 (m, 4H), 3.60−3.67 (m, 4H), 3.78 (s, 4H), 7.48 (d, 2H, J = 9.0 Hz), 7.69 (d, 2H, J = 9.0 Hz); ^{13}C NMR (D_2O) δ 20.0, 22.9, 42.3, 56.7, 122.2, 125.0, 136.4, 139.4, 157.9; MS (ESI$^+$) *m*/*z* 245.1777 [M + H]$^+$. Anal. ($C_{14}H_{22}Cl_2N_4 \cdot 1.3H_2O$) C, H, N.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-(*N*,*N*-diethyl)benzene (17a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 493 mg (3.0 mmol) of *N*,*N*-diethyl-*p*-phenylenediamine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (6:1), gave a residue that was recrystallized from hexane to afford **17a** as a white solid (1180 mg, 91% yield); mp 88−90 °C.

Dihydrochloride Salt of 1-(2-Imidazolidinylimino)-4-(*N*,*N*-diethyl)benzene (17b): Method C. White solid (93%); mp 96−98 °C; ^{1}H NMR (D_2O) δ 1.11 (t, 6H, J = 7.0 Hz), 3.65 (q, 4H, J = 7.0 Hz), 3.79 (s, 4H), 7.52 (d, 2H, J = 8.0 Hz), 7.61 (d, 2H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 9.2, 42.3, 53.3, 123.5, 125.2, 134.3, 136.7, 157.9; MS (ESI$^+$) *m*/*z* 233.1606 [M + H]$^+$. Anal. ($C_{13}H_{22}Cl_2N_4 \cdot 0.5H_2O$) C, H, N.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-(*N*,*N*-dimethyl)benzene (18a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 409 mg (3.0 mmol) of *N*,*N*-dimethyl-*p*-phenylenediamine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 25 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (6:1), gave **18a** as a white solid (850 mg, 70% yield); mp 124−126 °C.

Dihydrochloride Salt of 1-(2-Imidazolidinylimino)-4-(*N*,*N*-dimethyl)benzene (18b): Method C. White solid (90%); mp 236−238 °C; ^{1}H NMR (D_2O) δ 3.29 (s, 6H), 3.78 (s, 4H), 7.49 (d, 2H, J = 8.0 Hz), 7.68 (d, 2H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 42.3, 45.8, 121.6, 125.1, 136.4, 139.8, 157.9; MS (ESI$^+$) *m*/*z* 205.1297 [M + H]$^+$. Anal. ($C_{11}H_{18}Cl_2N_4 \cdot 0.4H_2O$) C, H, N.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-4-(*tert*-butoxycarbonylamino)benzene (19a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 625 mg (3.0 mmol) of *N*-Boc-*p*-phenylenediamine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 28 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (1:1), gave a residue that was recrystallized from EtOAc to afford **19a** as a white solid (927 mg, 65% yield); mp 198−200 °C.

***N*-(4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]phenyl)-piperidine (20a): Method A.** $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 529 mg (3.0 mmol) of *N*-(4-aminophenyl)-piperidine, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 20 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **20a** as a white solid (920 mg, 73% yield); mp 160−162 °C.

Dihydrochloride Salt of *N*-(4-Guanidinophenyl)piperidine (20b): Method C. White solid (92%); mp 124−126 °C; ^{1}H NMR (D_2O) δ 1.71−1.80 (m, 2H), 1.97−2.07 (m, 4H), 3.60−3.67 (m, 4H), 7.51 (d, 2H, J = 9.0 Hz), 7.68 (d, 2H, J = 9.0 Hz); ^{13}C NMR (D_2O) δ 20.0, 22.9, 56.8, 122.3, 126.6, 135.7, 139.8, 155.6; MS (ESI$^+$) *m*/*z* 219.1501 [M + H]$^+$. Anal. ($C_{12}H_{20}Cl_2N_4 \cdot 1.4H_2O$) C, H, N.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-(*N*,*N*-diethyl)benzene (21a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 493 mg (3.0 mmol) of *N*,*N*-diethyl-*p*-phenylenediamine, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)-thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 18 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **21a** as a white solid (900 mg, 74% yield); mp 146−148 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-(*N*,*N*-dimethyl)-benzene (22a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 409 mg (3.0 mmol) of *N*,*N*-dimethyl-*p*-phenylenediamine, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 21 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (4:1), gave **22a** as a yellow solid (880 mg, 77% yield); mp 156−158 °C.

4-[2,3-Di(*tert*-butoxycarbonyl)guanidino]aniline (23a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 645 mg (6.0 mmol) of 1,4-phenylenediamine, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DMF (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 18 h more at room temperature. The usual workup followed by silica gel column chromatography,

eluting with hexane/EtOAc (3:7), gave **23a** as a white solid (940 mg, 89% yield); mp > 300 °C.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-3,4-dimethoxybenzene (24a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 3,4-dimethoxyaniline, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 28 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (2:1), gave a residue that was precipitated with cold Et_2O to afford **24a** as a white solid (800 mg, 63% yield); mp 142−144 °C.

1,4-Benzodioxan-6-[1,3-di(*tert*-butoxycarbonyl)-2-imidazolidinylimino] (25a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 454 mg (3.0 mmol) of 1,4-benzodioxan-6-amine, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 26 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (2:1), gave **25a** as a white solid (920 mg, 73% yield); mp 44−46 °C.

1-[1,3-Di(*tert*-butoxycarbonyl)-2-imidazolidinylimino]-3,4-(methylenedioxy)benzene (26a): Method B. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 412 mg (3.0 mmol) of 3,4-(methylenedioxy)aniline, 907 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)imidazolidine-2-thione, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 26 h more at room temperature. The usual workup followed by neutral alumina column flash chromatography, eluting with hexane/EtOAc (2:1), gave **26a** as a white solid (890 mg, 73% yield); mp 86−88 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3,4-dimethoxybenzene (27a): Method A. $HgCl_2$ (896 mg, 3.3 mmol) was added over a solution of 460 mg (3.0 mmol) of 3,4-dimethoxyaniline, 830 mg (3.0 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 1.3 mL (9.3 mmol) of TEA in DCM (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 18 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (3:1), gave **27a** as a white solid (1060 mg, 89% yield); mp 103−105 °C.

1,4-Benzodioxan-6-[2,3-di(*tert*-butoxycarbonyl)guanidino] (28a): Method A. $HgCl_2$ (435 mg, 1.6 mmol) was added over a solution of 227 mg (1.5 mmol) of 1,4-benzodioxan-6-amine, 415 mg (1.5 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 0.6 mL (4.3 mmol) of TEA in DCM (3 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 28 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (2:1), gave **28a** as a white solid (485 mg, 82% yield); mp 145−147 °C.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-3,4-(methylenedioxy)-benzene (29a): Method A. $HgCl_2$ (435 mg, 1.6 mmol) was added over a solution of 206 mg (1.5 mmol) of 3,4-(methylenedioxy)-aniline, 415 mg (1.5 mmol) of *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea, and 0.6 mL (4.3 mmol) of TEA in DCM (3 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then for 28 h more at room temperature. The usual workup followed by silica gel column chromatography, eluting with hexane/EtOAc (2:1), gave **29a** as a white solid (470 mg, 82% yield); mp 140−142 °C.

Tetrahydrochloride Salt of 1,4-Bis[4-(4,5-dihydro-1*H*-2-imidazolylamino)phenyl]-piperazine (32): Method C. White solid (96%); mp decomposes over 240 °C; ^{1}H NMR (D_2O) δ 3.76 (s, 8H), 3.79 (s, 8H), 7.40 (d, 4H, J = 8.5 Hz), 7.47 (d, 4H, J = 8.5 Hz); ^{13}C NMR (D_2O) δ 42.3, 50.5, 120.0, 125.4, 132.6, 142.8, 158.2; MS (ESI$^+$) m/z 405.6247 [M + H]$^+$.

Tetrahydrochloride Salt of 1,4-Bis(guanidinophenyl)piperazine (36): Method C. White solid (97%); mp decomposes over 220 °C; ^{1}H NMR (D_2O) δ 3.74 (s, 8H), 7.36−7.54 (m, 8H); ^{13}C NMR (D_2O) δ 50.5, 120.1, 126.9, 131.7, 143.2, 155.8; MS (ESI$^+$) m/z 353.4671 [M + H]$^+$.

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Supporting Information Available: IR, ^{1}H NMR, ^{13}C NMR, and MS characterization data of the Boc-protected derivatives **2a**−**29a** and for those compounds previously reported in the literature (**4b**, **5b**, **7b**, **8b**, **10b**−**15b**, **19b**, **21b**−**29b**). A table with the combustion analysis data for the new compounds prepared (**2b**, **3b**, **6b**, **9b**, **16b**−**18b**, **20b**) is also presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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