Guanidine and 2-Aminoimidazoline Aromatic Derivatives as α_2-Adrenoceptor Antagonists. 2. Exploring Alkyl Linkers for New Antidepressants

Fernando Rodriguez,[†] Isabel Rozas,*[,†] Jorge E. Ortega,[‡,§] Amaia M. Erdozain,[‡,§] J. Javier Meana,[‡,§] and Luis F. Callado[‡,§]

Centre for Synthesis and Chemical Biology, School of Chemistry, Trinity College, University of Dublin, Dublin 2, Ireland, Department of Pharmacology, Faculty of Medicine, University of the Basque Country, 48940-Leioa, Bizkaia, Spain, and CIBER of Mental Health, CIBERSAM, Spain

Received January 11, 2008

The preparation of a number of (bis)guanidine and (bis)2-aminoimidazoline derivatives as potential α_2-adrenoceptor antagonists for the treatment of depression is presented. Human brain tissue was used to measure their affinity toward the α_2-adrenoceptors in vitro. Compounds **6b**, **8b**, **9b**, **10b**, **15b**, **17b**, **18b**, **20b**, and **21b** displayed a good affinity ($pK_i > 7$) and were evaluated in in vitro functional [^{35}S]GTPγS binding assays in human prefrontal cortex to determine their agonistic or antagonistic activity. Among these compounds, **17b** and **20b** showed the expected behavior for an antagonist and were subject to in vivo microdialysis experiments in rats. Significantly, these experiments confirmed the antagonistic properties of **17b** and **20b**, and therefore both compounds can be considered as potential antidepressants.

Introduction

According to the World Health Organization, by 2020, depression will be the second largest health burden following only heart diseases.[1] Even though the pathophysiological origin of this disease continues to be unknown, the monoamine theory is the most widely accepted,[2] stating that depression is a result of a deficiency of brain monoamine (noradrenaline (NA) or serotonin) activity.

It is well-known that central noradrenergic transmission is regulated by inhibitory noradrenergic receptors (α_2-ARs), which are expressed on both somatodendritic areas and axon terminals. Hence, the activation of these α_2-ARs induces an inhibition of NA release in the brain, and thus, it has been proposed that depression is associated with a selective increase in the high-affinity conformation of the α_2-ARs in the human brain.[3] This enhanced α_2-AR activity could be implicated in the deficit in noradrenergic transmission described in the etiology of depression. Thus, chronic treatment with antidepressants induces an in vivo desensitization of the α_2-ARs regulating the local release of NA.[4] Taking into account this hypothesis, the development of selective α_2-adrenoceptor antagonists can be considered as a new and effective therapeutical approach to the treatment of depressive disorders. In this way, it has been demonstrated that the administration of different α_2-AR antagonists either locally in the locus coeruleus or systemically increases the release of NA in the prefrontal cortex.[5,6] Moreover, α_2-AR antagonists are also able to enhance the increase of NA induced by selective reuptake inhibitor antidepressant drugs.[7]

Some of the most recent antidepressants developed include mianserin and mirtazapine (Figure 1), which show effective antidepressant activity by blockade of α_2-ARs.[8] As part of our interest in the development of new potential antidepressants, in a recent work[9] we described the synthesis and pharmacological evaluation of several series of phenyl and diphenyl substituted (bis)guanidine and (bis)2-aminoimidazoline derivatives with different heteroatoms in the para position with respect to these groups. On the basis of the high affinity previously shown by compound **1** (Figure 1),[10] this derivative was used as a lead compound, and thus, several derivatives were prepared obtaining two new α_2-AR antagonists (**2** and **3**, Figure 1).

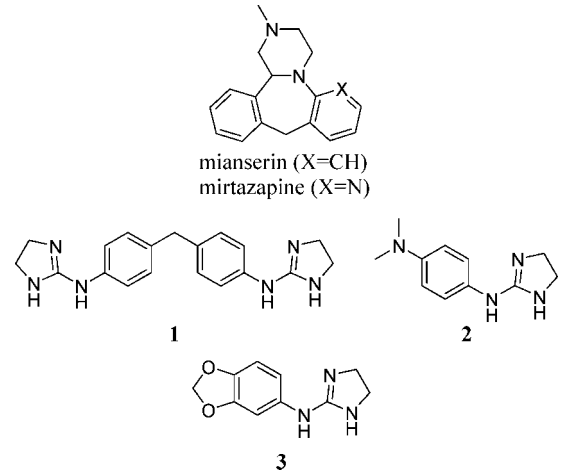


Figure 1. Structures of known α_2-noradrenoceptor targeting antidepressants and those of the high α_2-AR affinity compound **1** and two α_2-AR antagonists (**2** and **3**) previously described in our group.

However, despite the fact that many of these molecules showed α_2-AR affinities within the range of the well-known antagonist idazoxan ($pK_i = 7.29$; see Table 2), none of them improved that of the original lead compound ($pK_i = 8.80$).[10] Bearing these results in mind, we decided to avoid the presence of heteroatoms in the linker and substituents and prepare and pharmacologically test different mono- and dicationic analogues of **1**, keeping alkyl groups in the linker or as substituents in order to establish some structure–activity relationships (SARs).

We present in this article not only the efficient synthesis of a number of symmetrical and nonsymmetrical guanidine and 2-aminoimidazoline analogues of the high affinity α_2-AR ligand **1** with alkyl substituents/linkers but also, and more importantly, a complete pharmacological study. Hence, in vitro assays in human brain tissue to evaluate the α_2-AR affinity and functional

* To whom correspondence should be addressed. Phone: +353 1 896 3731. Fax: +353 1 671 2826. E-mail: rozasi@tcd.ie.
[†] University of Dublin.
[‡] University of the Basque Country.
[§] CIBER of Mental Health.

10.1021/jm800026x CCC: $40.75
Published on Web 05/07/2008

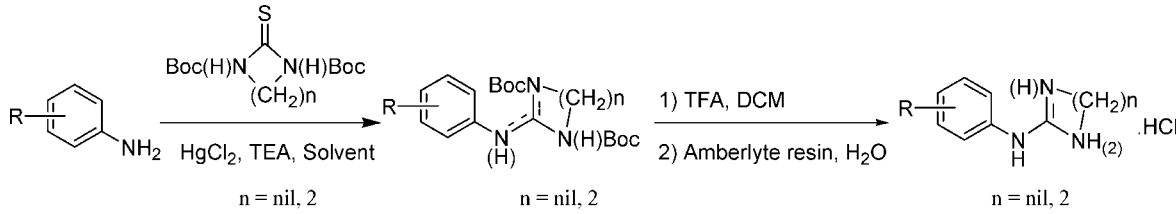


Figure 2. General synthetic schemes for the preparation of the guanidinium and 2-aminoimidazolinium derivatives.

studies to determine the agonist or antagonist nature of those derivatives with pK_i > 7 were performed. Furthermore, in vivo microdialysis experiments in rats were carried out with the compounds showing antagonistic properties, to test their effect on NA release in order to establish their potential use as antidepressants. As mentioned, the α_2-AR affinity and potential receptor antagonism experiments were performed in human prefrontal cortex (PFC), since there is an important density of α_2-ARs in this tissue.[11] Moreover, many studies have reported changes in PFC activity in the brain of patients with depression.[12] The final objective of our research is the preparation of antidepressants, and as such, the use of human brain tissue to directly characterize the pharmacological properties of the new compounds will be relevant from a therapeutic perspective.

Results and Discussion

Chemistry. The importance of the roles played by different guanidine and 2-aminoimidazoline containing compounds in biological processes has led to the development of various methodologies that allow introducing both these groups into the backbone of different molecules.[9] Among them, on the basis of the features of our starting materials and the good results obtained previously,[9,13] we decided to use Kim and Qian's strategy.[14] This approach consists of the treatment of the corresponding starting amine (or diamine) with 1 (or 2) equiv of either *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea or *N*,*N*′-di(*tert*-butoxycarbonyl)imidazoline-2-thione[13] (as the guanidine or 2-aminoimidazoline precursor, respectively) in the presence of mercury(II) chloride and an excess of triethylamine (Figure 2). The Boc-protected guanidine intermediates obtained in the first step of the synthesis were purified by silica gel column chromatography, whereas in the case of 2-aminoimidazoline precursors, a quick flash column chromatography over neutral alumina was run instead. Afterward, some of the substrates required recrystallization from the appropriate solvent.

Standard Boc deprotection of the intermediates with an excess of trifluoroacetic acid in dichloromethane and further treatment with Amberlyte resin in aqueous solution led to the hydrochloride salts of the target molecules in good overall yields (Table 1). All the starting amines are commercially available either from Aldrich or Fluka except for the 9,10-dihydroanthracene-2,6-diamine, which was synthesized according to the procedure reported in the literature.[15]

The Boc-protected derivatives **14a**[16] and **22a**[17] have been previously described; however, none of them was prepared by Kim and Qian's methodology. Regarding the final substrates, compounds **4b**, **8b**, **10b**, **17b**, **18b**, **19b**, **20b**, and **21b** are new while **5b**,[18] **6b**,[18,19] **7b**,[20] **11b**,[21] **12b**,[21,22] **13b**,[21–23] **14b**,[21,22,24] **15b**,[25] **16b**,[26] **22b**,[17] and **23b**,[27] although previously described as synthetic intermediates or prepared for other purposes, have not been tested in human brain tissue as possible antidepressants.

It is also important to mention that compound **9b**[28] is reported in a patent as a cloned human α_2-AR receptor agonist, but since it had not been tested in human brain PFC, we included it in our study. All the compounds have been tested in their hydrochloride salt forms.

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

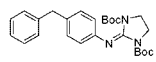
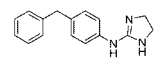
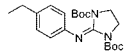
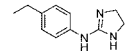
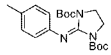
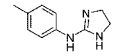
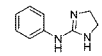
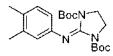
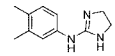
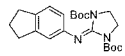
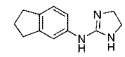
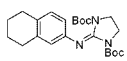
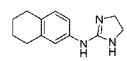
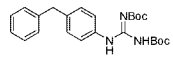
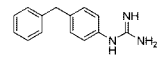
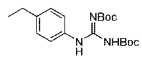
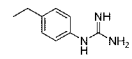
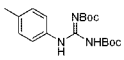
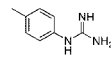
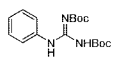
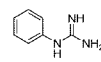
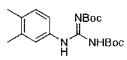
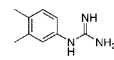
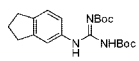
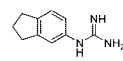
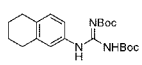
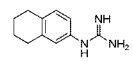
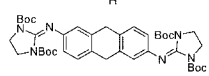
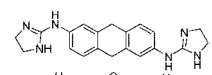
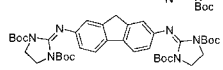
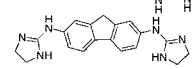
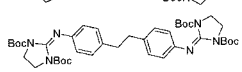
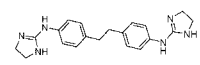
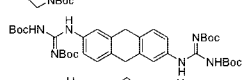
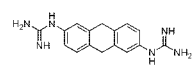
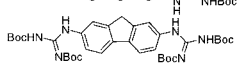
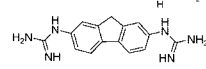
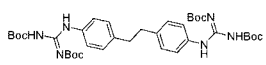
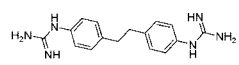
Affinity of the Monocationic Compounds. Following the strategy of our previous work,[9] we decided to synthesize molecules that can be considered as fragments of the original lead compound **1**. This would allow us not only to understand the importance of the second cation in the affinity toward the α_2-ARs and its activity but also to explore the effect of keeping the alkyl groups in the linker/substituents instead of heteroatomic groups as in the analogues previously reported by us.[9] In addition, the guanidine containing substrates were subject to study because of the similarities previously found by our group between the guanidinium and the 2-aminoimidazolinium cations.[29]

The affinities toward the α_2-ARs (expressed as pK_i) of all the monocationic compounds studied are shown in Table 2. Three of the better known α_2-AR ligands (idazoxan, clonidine, and RX821002) were used as references. In the 2-aminoimidazoline series, the removal of one of the cations results in loss of affinity toward the α_2-AR receptors. There is a drop of 2 logarithmic units when comparing **4b** to **1**, but the loss of the other phenyl ring does not seem to be important, since the pK_i values of **4b** and **5b** are within the same range and far from the affinity of the lead compound. However, there is a trend change for **6b**, as size reduction in the alkyl chain produces an increase in the affinity (pK_i greater than those of idazoxan and clonidine). As for **7b** (lowest pK_i within the 2-aminoimidazoline series), the elimination of the methyl group leads to a decrease in affinity. Compounds **8b**, **9b**, and **10b** (3,4-disubstituted) showed pK_i > 7 within the range of clonidine and idazoxan. In fact, the affinity found for **9b** is the second highest in this study.

As previously observed,[9] guanidine derivatives show lower α_2-ARs affinities than their 2-aminoimidazoline analogues. Thus, compounds **12b**, **13b**, **14b**, and **16b** displayed pK_i values similar to those of their dicationic analogue **24** (Table 2), and **11b** has the lowest affinity of the whole set of substrates described in this article. Remarkably, **15b** and **17b**, despite being guanidine derivatives, have an affinity within the range of Idazoxan.

Importance of the Methylene Substituent/Linker in Terms of the Affinity. Considering the similarities between the compounds prepared in this study and those reported in our previous work,[9] a comparative analysis has been carried out to understand the difference in receptor affinity based on the steric and electronic properties of the chemical group in the linker or substituent. Hence, the α_2-AR pK_i values of a number of these substrates, previously reported by our group,[9] are displayed in Table 3 along with the compounds presented in this work according to their structural similarities. Thus, for the PhXPhIm set, the affinity showed by **4b** is slightly higher than those

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

Compd	Structure	1st Stage	Compd	Structure	2nd Stage	Overall
4a		78	**4b**		96	75
5a		80	**5b**		95	76
6a		69	**6b**		94	65
7a		82	**7b**		97	80
8a		62	**8b**		96	60
9a		65	**9b**		95	62
10a		79	**10b**		94	74
11a		81	**11b**		97	78
12a		77	**12b**		94	72
13a		81	**13b**		98	79
14a		73	**14b**		96	70
15a		86	**15b**		97	83
16a		81	**16b**		96	78
17a		78	**17b**		95	74
18a		70	**18b**		94	66
19a		71	**19b**		95	67
20a		71	**20b**		96	68
21a		61	**21b**		95	58
22a		81	**22b**		95	77
23a		62	**23b**		94	58

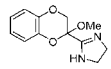
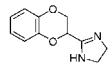
displayed by its analogues **25a**, **26a**, and **27a**, suggesting that the linker does not have an effect in the affinity for the receptor. As for the PhXPhGu-like compounds, the methylene linker is a drawback regarding the affinity, since the pK_i for **11b** is lower than those of the compounds containing more electron-rich linkers. The most important difference in affinity (more than 1 logarithmic unit) is for compound **25b**.

Regarding the CH_3XPhIm-like derivatives, again the presence of electron-rich groups seems to be an advantage (**5b**, **28a**, and **29a** in Table 3), whereas for the CH_3XPhGu set there is no difference at all (**12b**, **28b**, and **29b** in Table 3). In the case of XPhIm and XPhGu analogues, the methyl derivatives showed pK_i values nearly 1 logarithmic unit higher than their amino counterparts (Table 3), indicating that in this case alkyl groups increase the α_2-AR affinity.

Among the five member ring containing derivatives, 5-ringPhIm, **9b** displays a pK_i almost 1 logarithmic unit higher than the dioxo antagonist **3**; thus, in this example the presence of the heteroatoms represents a burden affinitywise. This difference is not so important in the guanidine set, 5-ringPhGu, since the pK_i values obtained for **16b** and **3b** are very similar. In the case of the 6-ringPhIm and 6-ringPhGu-like structures, the presence of the oxygen atoms helps to increase the affinity toward the α_2-ARs, since **31a** and **31b** showed pK_i values higher than their cycloalkyl counterparts (Table 3).

It can be concluded that the functionality in the linker or substituents does not seem to make a considerable difference in the PhXPhIm, CH_3XPhGu or 5-ringPhGu-like structures, whereas the presence of an electron-rich group increases the affinity toward the α_2-ARs for the PhXPhGu, CH_3XPhIm, 6-ringPhIm, and 6-ringPhGu sets. For the rest of structures, XPhIm, XPhGu, 5-ringPhIm, and the ImPhXPhIm twin substrates reported in our previous work,[9] the alkyl group has a

Table 2. Monocations Prepared and Their Affinity for the α_2-ARs Expressed as pK_i[a]

Compound		pK$_i$
RX821002		9.04
Idazoxan		7.29
Clonidine		7.68
1		8.80
4b		6.85
5b		6.68
6b		7.82
7b		6.48
8b		7.68
9b		8.26
10b		7.33
24		6.38
11b		5.55
12b		6.41
13b		6.53
14b		6.19
15b		7.12
16b		6.51
17b		7.11

[a] The structures of the reference compounds RX821002, idazoxan, and clonidine are also presented.

positive effect on the α_2-AR affinity. No trend could be identified for the guanidine twin substrates GuPhXPhGu.[9]

Affinity of the Dicationic Twin Compounds. Despite the fact that the monocationic compounds **6b**, **8b**, **9b**, **10b, 15b**, and **17b** showed interesting pK_i values, none of them increased the α_2-AR affinity of the lead compound **1**. Thus, we decided to prepare some twin substrates with different structural features. All these derivatives are shown in Table 4 alongside the pK_i values obtained in our study.

The conformationally constrained backbones of compounds **18b**, **19b**, **21b**, and **22b** (Table 1), even though the lipophilic properties were kept, reduce dramatically the rotation of the bonds around the bridge, and therefore, the cationic moieties spatial location will be restricted to limited areas. Thus, in the first approach, comparison of their affinity to that of **1** will help to understand the range of distances between the cations required for a better interaction with the receptor. Additionally, the interaction of these conformationally restricted derivatives will be energetically favored because no energy would be spent in reaching the optimally oriented conformation. Conversely, **20b** and **23b** (see structures in Table 1) have more conformational freedom, and their study can also help to understand the dependence of the affinity on the intracations distance and rotational freedom.

In the 2-aminoimidazoline series, none of the new rigid substrates improved compound **1** affinity, even though **18b** and **20b** pK_i values are within the range of those of idazoxan and clonidine. Two aspects should be considered: the intracations distance and the disposition of the cations with respect to the linkers. Thus, it seems that the optimal distance between the cations should be around 12.1 Å and anything larger or shorter decreases the affinity for the receptor. The drop in the pK_i of **18b** could be a consequence of the increase in the intracations distance or of the fact that each one of the cations is now in meta and para positions with respect to each $-CH_2-$ bridge. In the case of **19b**, the drop in the affinity is even more remarkable indicating that to have both cations meta with respect to the linker, a decrease in the intracation distance is definitely a drawback. Nevertheless, more derivatives should be studied to fully evaluate the effect of the conformational restriction. As for compound **20b**, the lengthening of the bridge resulted in a longer distance between cations and more than $1^1/_2$ logarithmic unit drop in the pK_i.

The most significant result in the guanidines series is the good affinity of **21b** (Table 4), which for the first time shows better affinity than its 2-aminoimidazoline analogue. Actually, this is the first example of a guanidine containing a twin molecule of our "in-home library" showing pK_i > 7. Regarding **22b** and **23b**, both presented lower affinities than **24**. In this set of compounds and regarding affinity, the optimal distance between cations seems to be around 12.3 Å, very similar distance compared with the optimal in the 2-aminoimidazoline series.

[^{35}S]GTPγS Binding Functional Assays. Those compounds that displayed an affinity within the range of that of idazoxan or clonidine (pK_i > 7) were subject to [^{35}S]GTPγS binding experiments to determine their activity.

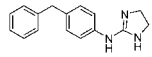
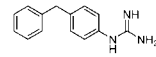
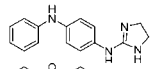
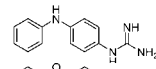
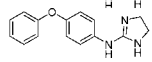
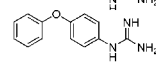
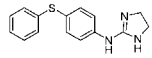
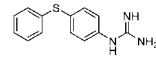
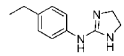
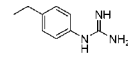
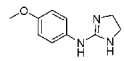
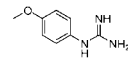
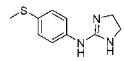
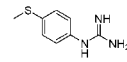
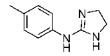
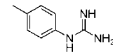
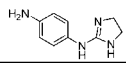
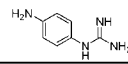
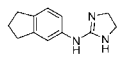
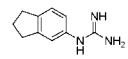
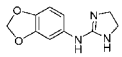
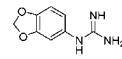
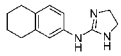
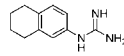
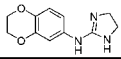
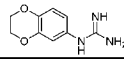
As members of the G-protein-coupled receptors (GPCRs) superfamily, when the endogenous substrate binds to the α_2-ARs, they interact with a G-protein triggering a cascade of biochemical events, which results in transmembrane signaling. This receptor activation alters the conformation of the G-proteins, 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.[30] 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 logarithmic units lower than affinity values obtained in radioligand receptor binding experiments.[30]

Compounds **6b**, **8b**, **9b**, **10b**, and **15b** stimulated binding of [^{35}S]GTPγS, showing a typical agonist dose–response plot. The potencies of all these substrates were in the micromolar range, with **10b** being the most potent agonist. In Table 5, their EC_{50} values can be found as well as their percentage efficacy relative to the well-known α_2-AR agonist UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]quinoxaline).

Conversely, compounds **17b**, **18b**, **20b**, and **21b** did not stimulate binding of [^{35}S]GTPγS by themselves and were subject to new [^{35}S]GTPγS binding experiments and tested against the α_2-AR agonist UK14304.

In Table 6, the effect induced in the UK14304 agonist stimulation of [^{35}S]GTPγS binding by the presence in the medium of a single concentration (10^{-5} M) of each of our

Table 3. Affinity Values (pK_i) for the α_2-ARs of Different Sets of Comparable Structures with Different Linkers/Substituents[a]

Compd	*PhXPhIm*	pK_i	Compd	*PhXPhGu*	pK_i
4b		6.85	**11b**		5.55
25a*		6.56	**25b***		6.83
26a*		6.58	**26b***		6.05
27a*		6.62	**27b***		6.30
Compd	***CH_3XPhIm***	**pK_i**	**Compd**	***CH_3XPhGu***	**pK_i**
5b		6.68	**12b**		6.41
28a*		7.77	**28b***		6.39
29a*		7.07	**29b***		6.40
Compd	***XPhIm***	**pK_i**	**Compd**	***XPhGu***	**pK_i**
6b		7.82	**13b**		6.53
30a*		6.92	**30b***		5.58
Compd	***5-ringPhIm***	**pKi**	**Compd**	***5-ringPhGu***	**pK_i**
9b		8.26	**16b**		6.51
3*		7.33	**3b***		6.40
Compd	***6-ringPhIm***	**pKi**	**Compd**	***6-ringPhGu***	**pK_i**
10b		7.33	**17b**		7.11
31a*		7.85	**31b***		8.21

[a] The asterisk (*) indicates that the α_2-AR affinity of these compounds was reported in ref 9 by our group.

Table 4. Twin Molecules α_2-AR Affinity (pK_i) and Approximate Intracations Distance

compd	pK_i	$d(C^+\cdots C^+)$[a] (Å)
RX821002	9.04	
idazoxan	7.29	
clonidine	7.68	
1	8.80	12.1
18b	7.58	12.4
19b	6.32	11.8
20b	7.02	14.0
24	6.38	12.2
21b	7.96	12.3
22b	6.12	11.9
23b	5.88	14.2

[a] Structures were built using the unpublished crystal structure of a related symmetric dication as template and minimized using MM2.

Table 5. Affinity for α_2-ARs (pK_i), EC_{50} Values, and Percentage Efficacy Relative to UK14304 Found for Compounds Showing a Typical Agonist Dose−Response Plot

compd	pK_i	EC_{50} (μM)	E_{max} (%)
UK14304	8.85	11.4 ± 0.3	100
6b	7.82	15.2 ± 0.2	97
8b	7.68	62.9 ± 0.7	89
9b	8.26	4.4 ± 0.3	98
10b	7.33	1.1 ± 0.1	100
15b	7.12	35.5 ± 0.8	84

Table 6. 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)

experiment	EC_{50} (μM)
UK14304	11.4 ± 0.3
UK14304 + **17b**	868.3 ± 112.8
UK14304 + **18b**	28.8 ± 4.2
UK14304 + **20b**	103.7 ± 10.8
UK14304 + **21b**	7.8 ± 0.3

compounds can be found. Addition of **18b** to the experiment did not induce a significant rightward shift in the EC_{50} value for the UK14304, whereas **21b** resulted in a slight leftwards shift. These facts are in agreement with the lack of α_2-ARs antagonistic properties of both substrates. On the contrary, **17b** and **20b** produced a remarkable rightward shift in the UK14304 EC_{50} value, a result that is expected for antagonists. Hence, after these in vitro experiments in human brain PFC, two new antagonists with affinity similar to that of idazoxan were identified. Remarkably, considering our previous results,[9] **17b** is the first guanidine derivative that shows antagonistic behavior while **20b** is the first twin molecule of our series acting as an antagonist.

In Vivo Microdialysis Experiments. Considering the antagonistic properties and relatively good affinity over the α_2-ARs of compounds **17b** and **20b**, we tested their potential effect

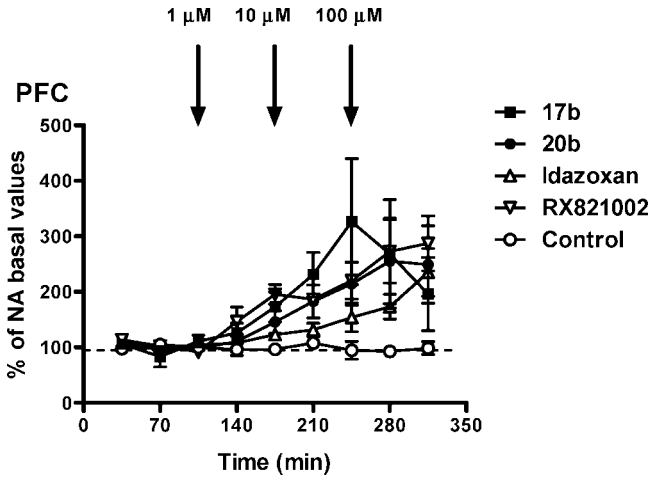


Figure 3. Effects of local administration (1–100 μM) by reverse microdialysis in the PFC of **17b**, **20b**, idazoxan, RX821002, or cerebrospinal fluid. Concentrations of the compounds were progressively increased every two fractions (70 min) in tenfold increments (arrows). Data are given as the mean ± standard error mean values from three to six separate animals for each group and are expressed as percentages of the corresponding basal values.

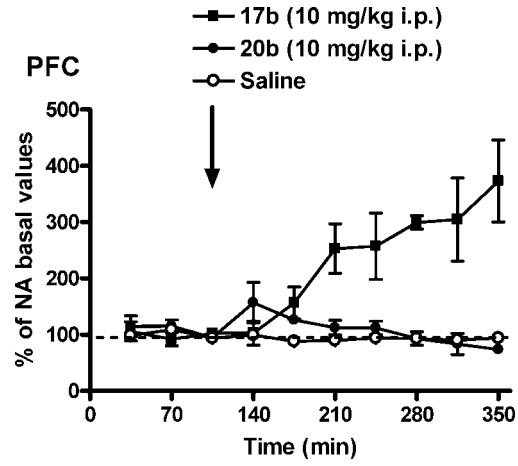


Figure 4. Effects of the systemic administration of **17b**, **20b**, or saline on extracellular NA levels, evaluated in the PFC. Data are given as the mean ± standard error from three separate animals for each group and are expressed as percentages of the corresponding basal values. Arrow represents administration of the different compounds.

on noradrenergic transmission in vivo. Intracerebral microdialysis is a neurochemical technique that has been applied extensively in pharmacological studies aimed at investigating the effects of different drugs on brain neurotransmission. This technique allows collection of a representative concentration of different neurotransmitters of the area where the probe is implanted while the animals are awake and freely moving.[31]

Most antidepressant drugs are able to increase NA extracellular concentrations in different brain areas as the PFC, an area implicated in depression disease. Considering that α_2-ARs exert a tonic inhibitory action on NA release from the noradrenergic terminals, we assessed the ability of these two new compounds to increase NA extracellular concentration in this area. First, we tested the drugs when administered locally in order to confirm their antagonistic activity over α_2-ARs in vivo. A second step was to study the effect of these compounds increasing NA extracellular concentrations when administered systemically.

Local administration of artificial cerebrospinal fluid (aCSF) did not change NA basal values ($F[8,30] = 0.39$, $P = 0.91$, $n = 4$; Figure 3). However, reverse dialysis of **17b** (1–100 μM) and **20b** (1–100 μM) induced a concentration-related increase in extracellular NA levels ($E_{max} = 326 \pm 113\%$, $F[1,54] = 8.05$, $P = 0.0064$, $n = 10$; $E_{max} = 255 \pm 76\%$, $F[1,46] = 21.07$, $P < 0.0001$, $n = 7$; respectively; Figure 3). The increases were very similar to those obtained from local administration (1–100 μM) of two well-known α_2-AR antagonists, RX821002 ($E_{max} = 287 \pm 49\%$, $F[1,39] = 66.78$, $P < 0.0001$, $n = 7$; Figure 3) and idazoxan ($E_{max} = 235 \pm 42\%$, $F[1,39] = 32.07$, $P < 0.0001$, $n = 7$; Figure 3).

Systemic administration of **17b** increased NA extracellular concentration by 373% ± 73% and stayed high over the end of the experiment ($F[1,33] = 95.70$, $P < 0.0001$, $n = 6$; Figure 4), whereas following administration of **20b**, a weak increase of NA basal values, reaching a maximal effect of 156% ± 35%, was observed (Figure 4).[32] This increase was statistically significant when the group was compared with the respective control ($F[1,30] = 5.56$, $P = 0.02$, $n = 6$).

Thus, **17b** and **20b** showed α_2-AR antagonist properties in vivo and were able to cross the blood−brain barrier (BBB), as can be deduced by the increase evoked when they were systemically administered. However the stronger increase on NA basal values observed for **17b** over **20b** could indicate pharmacokinetic differences such as the ability to cross the BBB or differences in the catabolism of the substrates.

Considering the results of this series of in vivo microdialysis experiments, the antagonistic properties of compounds **17b** and **20b** over α_2-ARs, as expected from their behavior in [^{35}S]GTPγS binding experiments, are confirmed.

Conclusions

In this paper we have reported the quick and efficient synthesis of a number of guanidine and 2-aminoimidazoline derivatives. The final compounds **6b**, **8b**, **9b**, **10b**, **15b**, **17b**, **18b**, **20b**, and **21b** showed affinities toward the α_2-ARs in human brain tissue in in vitro experiments within the range of those of odazoxan and clonidine. Compounds **18b**, **20b**, and **21b** are twin molecules, whereas **6b**, **8b**, **9b**, **10b**, **15b**, and **17b** are monocations. Compounds **6b**, **8b**, **9b**, **10b**, **18b**, and **20b** are 2-aminoimidazoline derivatives, while **15b**, **17b**, and **21b** are guanidine containing substrates. Generally speaking, the 2-aminoimidazoline derivatives displayed higher α_2-AR affinities than their guanidine analogues, as expected from the results obtained in our previous work.[9] However, remarkably and for the first time in our sets of compounds, we have obtained a guanidine twin compound (**21b**) with $pK_i > 7$.

In terms of activity, compounds **6b**, **8b**, **9b**, **10b**, **15b**, **18b**, and **21b** are agonists in [^{35}S]GTPγS experiments. The different α_2-AR activities found for **9b** and the dioxo derivative **3**,[9] which share the same backbone, can only be explained by the presence of the two oxygen atoms in the latter one, which could establish some interactions relevant for the antagonism.

The most important result achieved in this work is the discovery of compounds **17b** and **20b**, which are antagonists in both in vitro [^{35}S]GTPγS binding experiments and in vivo microdialysis experiments. Remarkably, **17b** is the first guanidine derivative of our compounds with such characteristics. Yet again, very subtle structural changes led to different activity in the α_2-ARs. Comparing the structures of **17b** and **31b**,[9] one might expect similar behavior from both; however, the former one is an antagonist while the dioxo compound **31b**[9] is an agonist. This is just the exact opposite of the one mentioned

above for the 2-aminoimidazoline derivatives **9b** and **3**.[9] As a result, no obvious SAR can be stated activitywise.

Regarding compound **20b**, it is the first twin molecule of our compounds that acts as an antagonist. After local administration, **20b** induced an increase of NA concentrations in the rat brain; however, after peripheral administration, it produced a weaker effect than the one induced by **17b**. This different behavior could be due to the fact that **20b** is a larger dicationic molecule, and therefore, it might be more difficult to cross the BBB.

After the series of encouraging results obtained so far, we are working in the design and synthesis of new analogues in order to try to understand the characteristics required to improve the α_2-AR affinity and antagonist activity and to be able to design new potential antidepressants.

Experimental Section

Pharmacology: Materials and Methods. [^{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} to 10^{-3} M, 10 concentrations). 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. Specific binding was determined and plotted as a function of the compound concentration. Nonspecific binding was determined in the presence of adrenaline (10^{-5} M).

[^{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. In order 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 and transferred to vials containing 5 mL of OptiPhase HiSafe II cocktail (Wallac, U.K.). 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 anesthetized with chloral hydrate (400 mg/kg ip) and a microdialysis probe was implanted by stereotaxic surgery into PFC brain area. The coordinates selected for the PFC were as follows: AP (anterior to bregma) +2.8 mm, L (lateral from the midsagittal suture) +1 mm, DV (ventral from the dura surface) −5 mm.[33] After 24 h for animal recovery, perfusion fluid (aCSF) is pumped through the probe at a flow rate of 1 μL/min. In the semipermeable membrane, which is the critical side of the probe and is placed on the selected area, molecules flow into and out of the cannulae by diffusion. Therefore, microdialysis technique allows local administration of substrates dissolved in the perfusion fluid.

When drugs were locally administered, they were dissolved in a CSF and applied during 70 min via dialysis probe implanted in the PFC in increasing concentrations of 1, 10, and 100 μM. The compounds systemically administered were dissolved in saline and injected intraperitoneally.

Samples, collected with the microdialysis procedure (every 35 min), were analyzed by HPLC with electrochemical detection. NA concentrations were monitorizated by an amperometric detector (Hewlett-Packard model 1049A) at an oxidizing potential of +650 mV. The mobile phase (12 mM citric acid, 1 mM EDTA, 0.7 mM octylsodium sulfate, pH 5, and 10% methanol) was filtered, degassed (Hewlett-Packard model 1100 degasser), and delivered at a flow rate of 0.2 mL/min by a Hewlett-Packard model 1100 pump. Stationary phase was a column of 150 mm × 2.1 mm (Thermo Electron Corporation). Samples (injection volume 37 μL) were injected and NA was analyzed in a run time of 10 min. Solution of standard noradrenaline was injected every working day to create a new calibration table.

The mean values of the first three samples before substrate administration were considered as 100% basal value. All measures of extracellular NA concentrations are expressed as a percentage of the baseline value ± SE of the 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. After the experiments, rats were sacrificed with an overdose of chloral hydrate and the brains were dissected to check the correct implantation of the probe.

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. 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 the Hydrochloride Salts. 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 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.

Hydrochloride Salt of 1-(2-Imidazolidinyliminio)-4-benzylbenzene (4b). Yellowish oil (96%); ^{1}H NMR (D_2O) δ 3.55 (s, 4H), 3.57 (s, 2H), 6.87–7.04 (m, 9H); ^{13}C NMR (D_2O) δ 40.2, 42.1, 122.9, 125.5, 128.0, 128.2, 129.4, 132.6, 139.5, 140.6, 157.4; MS (ESI^+) *m/z* 252.0944 $[M + H]^+$. Anal. ($C_{16}H_{18}ClN_3 \cdot 1.4H_2O$) C, H, N.

Hydrochloride Salt of 1-(2-Imidazolidinylimino)-3,4-dimethylbenzene (8b). White solid (96%); mp 83–85 °C; ^{1}H NMR (D_2O) δ 2.08 (s, 6H), 3.58 (s, 4H), 6.80 (d, 1H, J = 8.0 Hz), 6.83 (s, 1H), 7.06 (d, 1H, J = 8.0 Hz); ^{13}C NMR (D_2O) δ 17.9, 18.4, 42.1,

120.1, 123.7, 130.0, 131.9, 135.4, 138.1, 157.5; MS (ESI^+) *m/z* 190.1163 $[M + H]^+$. Anal. ($C_{11}H_{16}ClN_3 \cdot 0.2H_2O$) C, H, N.

Hydrochloride Salt of Imidazolidin-2-ylidene-(5,6,7,8-tetrahydronaphthalen-2-yl)amine (10b). White solid (94%); mp 87–89 °C; ^1H NMR (D_2O) δ 1.65–1.74 (m, 4H), 2.63–2.75 (m, 4H), 3.70 (s, 4H), 6.88–6.99 (m, 2H), 7.13 (d, 1H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 21.8, 22.0, 27.9, 28.3, 42.2, 120.7, 123.9, 129.8, 131.7, 136.4, 138.6, 158.1; MS (ESI^+) *m/z* 216.1380 $[M + H]^+$. Anal. ($C_{11}H_{16}ClN_3 \cdot 1.3H_2O$) C, H, N.

Hydrochloride Salt of *N*-(5,6,7,8-Tetrahydronaphthalen-2-yl)guanidine (17b). White solid (95%); mp 39–41 °C; ^1H NMR (D_2O) δ 1.58–1.72 (m, 4H), 2.59–2.72 (m, 4H), 6.81–6.93 (m, 2H), 7.07 (d, 1H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 21.9, 22.0, 28.0, 28.2, 122.1, 125.4, 129.9, 130.6, 136.7, 138.6, 155.6; MS (ESI^+) *m/z* 190.1248 $[M + H]^+$. Anal. ($C_{11}H_{16}ClN_3 \cdot 0.8H_2O$) C, H, N.

Dihydrochloride Salt of 2,6-Di(2-imidazolidinylimino)-9,10-dihydroanthracene (18b). Brown solid (94%); mp, decomposes over 220 °C; ^1H NMR (D_2O) δ 3.66 (s, 8H), 3.73 (s, 4H), 6.92–7.06 (m, 4H), 7.24 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 34.1, 42.2, 120.9, 121.8, 128.0, 132.4, 134.6, 137.5, 157.8; MS (ESI^+) *m/z* 347.1572 $[M + H]^+$. Anal. ($C_{20}H_{24}Cl_2N_6 \cdot 2.0H_2O$) C, H, N.

Dihydrochloride Salt of 2,7-Di(2-imidazolidinylimino)-9*H*-fluorene (19b). Light-brown solid (95%); mp, decomposes over 240 °C; ^1H NMR (D_2O) δ 3.64 (s, 8H), 3.68 (s, 2H), 7.06 (d, 2H, $J = 8.0$ Hz), 7.22 (s, 2H), 7.64 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 35.9, 42.1, 119.1, 120.3, 121.2, 133.2, 138.3, 144.5, 157.3; MS (ESI^+) *m/z* 333.1827 $[M + H]^+$. Anal. ($C_{19}H_{22}Cl_2N_6 \cdot 1.8H_2O$) C, H, N.

Dihydrochloride Salt of 4,4′-Di(2-imidazolidinylimino)-1,2-diphenylethane (20b). Yellowish solid (96%); mp, decomposes over 210 °C; ^1H NMR (D_2O) δ 2.91 (s, 4H), 3.70 (s, 8H), 7.13 (d, 4H, $J = 8.0$ Hz), 7.25 (d, 4H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 35.5, 42.2, 123.6, 129.4, 132.3, 140.4, 158.1; MS (ESI^+) *m/z* 349.1840 $[M + H]^+$. Anal. ($C_{20}H_{26}Cl_2N_6 \cdot 1.3H_2O$) C, H, N.

Dihydrochloride Salt of 2,6-Diguanidino-9,10-dihydroanthracene (21b). Brown solid (95%); mp, decomposes over 215 °C; ^1H NMR (D_2O) δ 3.81 (s, 4H), 7.06 (d, 2H, $J = 8.0$ Hz), 7.10 (s, 2H), 7.31 (d, 2H, $J = 8.0$ Hz); ^{13}C NMR (D_2O) δ 34.2, 122.9, 123.8, 128.1, 131.5, 135.5, 137.7, 155.7; MS (ESI^+) *m/z* 295.1659 $[M + H]^+$. Anal. ($C_{16}H_{20}Cl_2N_6 \cdot 2.3H_2O$) C, H, N.

Acknowledgment. F.R. thanks the Consejeria de Educacion Cultura y Deporte de la Comunidad Autonoma de La Rioja for his Grant. This research was also supported by a Cycle III HEA PRTLI grant (F.R.), by Bizkaiko Foru Aldundia (Ekinberri 7/12/EK/2005/65 and DIPE 06/04), the Basque Government (SAIOTEK), and Spanish Ministry of Health, Instituto de Salud Carlos III (REM-TAP). J.E.O. and A.M.E were recipients of predoctoral fellowships from the MEC and the Basque Government, respectively.

Supporting Information Available: Preparation and IR, ^1H NMR, ^{13}C NMR, and MS data for the compounds already described in the literature (**5b**–**7b**, **9b**, **11b**–**13b**, **14a**, **14b**–**16b**, **22a**, **22b**, and **23b**) and all new Boc-protected derivatives prepared (**4a**–**13a**, **15a**–**21a**, and **23a**); a table containing the combustion analysis data for the new final compounds (**4b**, **8b**, **10b**, and **17b**–**21b**); and preparation of membranes, analysis of binding data, and drugs used in the pharmacology experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) http://www.who.int/mental_health/management/depression/definition/en/.

(2) Elhwuegi, A. S. Central monoamines and their role in major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2004**, *28*, 435–451.

(3) (a) Callado, L. F.; Meana, J. J.; Grijalba, B.; Pazos, A.; Sastre, M.; García-Sevilla, J. A. Selective increase of alpha2a-adrenoceptor agonist binding sites in brains of depressed suicide victims. *J. Neurochem.* **1998**, *70*, 1114–1123. (b) Gonzalez-Maeso, J.; Rodriguez-Puertas, R.; Meana, J. J.; Garcia-Sevilla, J. A.; Guimon, J. Neurotransmitter receptor-mediated activation of G-proteins in brains of suicide victims with mood disorders: selective supersensitivity of alpha2A-adrenoceptors. *Mol. Psychiatry* **2002**, *7*, 755–767.

(4) Mateo, Y.; Fernandez-Pastor, B.; Meana, J. J. Acute and chronic effects of desipramine and clorgyline on alpha2-adrenoceptors regulating noradrenergic transmission in the rat brain: a dual-probe microdialysis study. *Br. J. Pharmacol.* **2001**, *133*, 1362–1370.

(5) Fernandez-Pastor, B.; Meana, J. J. In vivo tonic modulation of the noradrenaline release in the rat cortex by locus coeruleus somatodendritic alpha2-adrenoceptors. *Eur. J. Pharmacol.* **2002**, *442*, 225–229.

(6) Devoto, P.; Flore, G.; Pani, L.; Gessa, G. L. Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol. Psychiatry* **2001**, *6*, 657–664.

(7) Mateo, Y.; Pineda, J.; Meana, J. J. Somatodendritic alpha2-adrenoceptors in the locus coeruleus are involved in the in vivo modulation of cortical noradrenaline release by the antidepressant desipramine. *J. Neurochem.* **1998**, *71*, 790–798.

(8) Leonard, B. E. Neuropharmacology of antidepressants that modify central noradrenergic and serotonergic function: a short review. *Hum. Psychopharmacol. Clin.* **1999**, *14*, 75–81.

(9) Rodriguez, F.; Rozas, I.; Ortega, J. E.; Meana, J. J.; Callado, L. F. Guanidine and 2-aminoimidazoline aromatic derivatives as alpha2-adrenoceptor antagonists, 1: Towards new antidepressants with heteroatomic linkers. *J. Med. Chem.* **2007**, *50*, 4516–4527.

(10) Dardonville, C.; Rozas, I.; Meana, J.; Callado; K. I_2-Imidazoline binding site affinity of a structurally different type of ligands. *Bioorg. Med. Chem.* **2002**, *10*, 1525–1533.

(11) Grijalba, B.; Callado, L. F.; Meana, J. J.; García-Sevilla, J. A.; Pazos, A. Alpha2-adrenoceptor subtypes in the human brain: a pharmacological delineation of [^{3}H]RX-821002 binding to membranes and tissue sections. *Eur. J. Pharmacol.* **1996**, *310*, 83–93.

(12) Fitzgerald, P. B.; Oxley, T. J.; Laird, A. R.; Kulkarni, J.; Egan, G. F.; Daskalakis, Z. J. An analysis of functional neuroimaging studies of dorsolateral prefrontal cortical activity in depression. *Psychiatry Res.* **2006**, *148*, 33–45.

(13) Dardonville, C.; Goya, P.; Rozas, I.; Alsasua, A.; Martin, I.; Borrego, M. J. New aromatic iminoimidazolidine derivatives as alpha1-adrenoceptor antagonists: a novel synthetic approach and pharmacological activity. *Bioorg. Med. Chem.* **2000**, *8*, 1567–1577.

(14) Kim, K. S.; Qian, L. Improve methods for the preparation of guanidines. *Tetrahedron Lett.* **1993**, *48*, 7677–7680.

(15) (a) Takimiya, K.; Yanagimoto, T.; Yamashiro, T.; Ogura, F.; Otsubo, T. Syntheses and properties of 11,11,12,12-tetracyano-2,6-anthraquinodimethane (TANT) and its 9,10-dichloro derivative as novel extensive electron acceptors. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1431–1435. (b) Yanagimoto, T.; Takimiya, K.; Otsubo, T.; Ogura, F. 11,11,12,12-Tetracyano-2,6-anthraquinodimethane (TANT) as a novel extensive electron acceptor. *J. Chem. Soc., Chem. Commun.* **1993**, *6*, 519–520.

(16) Between others: (a) Berlinck, R. G. S.; Kossuga, M. H.; Nascimento, A. M. Guanidine derivatives. *Sci. Synth.* **2005**, *18*, 1077–1116. (b) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. Urethane protected derivatives of 1-guanylpyrazole for the mild and efficient preparation of guanidines. *Tetrahedron Lett.* **1993**, *34*, 3389–3392.

(17) (a) Arafa, R. K.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Stephens, C. E.; Boykin, D. W. Synthesis, DNA affinity, and antiprotozoal activity of fused ring dicationic compounds and their prodrugs. *J. Med. Chem.* **2005**, *48*, 5480–5488. (b) Boykin, D. W.; Tidwell, R. R.; Wilson, D. W.; Brun, R.; Arafa, R. K.; Stephens, C. E. Preparation of Fused Ring Dicationic Antiprotozoals and Prodrugs Thereof. Patent WO 2005051296 A2 20050609, CAN 143:43696, AN 2005:493466, 2005.

(18) Balko, T. W. 2-(Substitutedamino)-*N*-(3-substitutedphenyl)-2-imidazoline-1-carbothioamides. Patent US 4195092 19800325, CAN 93: 71781, AN 1980:471781, 1980.

(19) Between others: (a) Katritzky, A. R.; Khashab, N. M.; Bobrov, S. The preparation of 1,2,3-trisubstituted guanidines. *Helv. Chim. Acta* **2005**, *88*, 1664–1675. (b) Trani, A.; Bellasio, E. Synthesis of 2-chloro-2-imidazoline and its reactivity with aromatic amines, phenols, and thiophenols. *J. Heterocycl. Chem.* **1974**, *11*, 257–262.

(20) (a) Between others: Hirashima, A.; Pan, C.; Shinkai, K.; Tomita, J.; Kuwano, E.; Taniguchi, E.; Eto, M. Quantitative structure−activity studies of octopaminergic agonists and antagonists against nervous system of *Locusta migratotia*. *Bioorg. Med. Chem.* **1998**, *6*, 903–910.

(21) Glennon, R. A.; Daoud, M. K.; Dukat, M.; Teitler, M.; Herrick-Davis, K.; Purohit, A.; Syed, H. Arylguanidine and arylbiguanide binding at 5-HT3 serotonin receptors: a QSAR study. *Bioorg. Med. Chem.* **2003**, *11*, 4449–4454.

(22) Between others: Paul, R.; Hallett, W. A.; Hanifin, J. W.; Reich, M. F.; Johnson, B. D.; Lenhard, R. H.; Dusza, J. P.; Kerwar, S. S.; Lin, Y. I.; Pickett, W. C.; Seifert, C. M.; Torley, L. W.; Tarrant, M. E.; Wrenn, S. Preparation of substituted *N*-phenyl-4-aryl-2-pyrimidinamines as mediator release inhibitors. *J. Med. Chem.* **1993**, *36*, 2716–2725.

(23) Between others: (a) Lefevre-Groboillot, D.; Boucher, J.-L.; Stuehr, D. J.; Mansuy, D. Relationship between the structure of guanidines and *N*-hydroxyguanidines, their binding to inducible nitric oxide synthase (iNOS) and their iNOS-catalysed oxidation to NO. *FEBS J.* **2005**, *272*, 3172–3183. (b) Srivastava, P. K.; Sharma, R. D.; Saleem, M. A convenient method for the preparation of monosubstituted guanidines. *Curr. Sci.* **1976**, *45*, 764–765.

(24) Between others: (a) Vale-Silva, L. A.; Buchta, V.; Valentova, E. Effect of subinhibitory concentration of some established and experimental antifungal compounds on the germ tube formation in *Candida albicans*. *Folia Microbiol.* **2007**, *52*, 39–43. (b) Min, K.-L.; Steghens, J.-P.; Henry, R.; Doutheau, A.; Collombel, C. Synthesis and differential properties of creatine analogs as inhibitors for human creatine kinase isoenzymes. *Eur. J. Biochem.* **1996**, *238*, 446–452.

(25) Between others: Clark, R. J.; Isaacs, A.; Walker, J. Derivatives of 3,4-xylidine and related compounds as inhibitors of influenza virus: relationships between chemical structure and biological activity. *Br. J. Pharmacol. Chemother.* **1958**, *13*, 424–435.

(26) Rose, Y.; Ciblat, S.; Reddy, R.; Belley, A. C.; Dietrich, E.; Lehoux, D.; McKay, G. A.; Poirier, H.; Far, A. R.; Delorme, D. Novel non-nucleobase inhibitors of *Staphylococcus aureus* DNA polymerase IIIC. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 891–896.

(27) (a) Jerushalmy, Z.; Skoza, L.; Zucker, M. B.; Grant, R. Inhibition by guanidino compounds of platelet aggregation induced by adenosine diphosphate. *Biochem. Pharmacol.* **1966**, *15*, 1791–1803. (b) Ewins, A. J.; Barber, H. J.; Self, A. D. H. Diguanidine Compounds. Patent GB 516289 19391229, CAN 35:37743, AN 1941:37743, 1939.

(28) Wong, W. C.; Jeon, Y. T.; Dhar, T. G. M.; Gluchowski, C. Imidazole and Imidazoline Derivatives as Alpha2-Adrenergic Agonists. Patent WO 9846572 A1 19981022, CAN 129:316226, AN 1998:706207, 1998.

(29) Dardonville, C.; Rozas, I.; Alkorta, I. Similarity studies on guanidinium, imidazolinium, and imidazolium cations: toward new bradykinin antagonists. *J. Mol. Graphics Modell.* **1998**, *16*, 150–156.

(30) Gonzalez-Maeso, J.; Rodriguez-Puertas, R.; Gabilondo, A. M.; Meana, J. J. Characterization of receptor-mediated [^{35}S]GTPγS binding to cortical membranes from postmortem human brain. *Eur. J. Pharmacol.* **2000**, *390*, 25–36.

(31) Fillenz, M. In vivo neurochemical monitoring and the study of behaviour. *Neurosci. Biobehav. Rev.* **2005**, *29*, 949–962.

(32) It has been reported in the literature that systemic administration of idazoxan (20 mg/kg ip) increased cortical NA release in rats to a maximun of 230% (Dennis et al. *J. Pharmacol. Exp. Ther*. **1987**, *241*, 642–649), and systemic administration of RX821002 (3 mg/kg ip) induced a 2-fold increase in dialysate NA concentration from the rat frontal cortex (Wortley et al. *Br. J. Pharmacol.* **1999**, *128*, 659–666).

(33) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*, 2nd ed.; Academic Press: Orlando, FL, 1986.

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