Articles

Guanidine and 2-Aminoimidazoline Aromatic Derivatives as α_2-Adrenoceptor Ligands: Searching for Structure−Activity Relationships

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Received July 9, 2008

In this paper, we report the synthesis of three new 2-aminoimidazoline (compounds **4b**, **5b**, and **6b**) and three new guanidine derivatives (compounds **7b**, **8b**, and **9b**) as potential α_2-adrenoceptor antagonists for the treatment of depression. Their pharmacological profile was evaluated in vitro in human brain tissue and compared to the potential antidepressant **1** and the agonists **2** and **3**. All new substrates were evaluated by in vitro functional [^{35}S]GTPγS binding assays in human prefrontal cortex to determine their agonistic or antagonistic activity. Compound **8b** was found to be an antagonist in vitro and was subjected to in vivo microdialysis experiments in rats. Moreover, a new synthesis of the precursor amines for compounds **4b**−**9b** is presented.

Introduction

Depression affects more than 300 million people in the world and by 2020 is expected to be the second largest health burden.[1] Despite the fact that the neuronal mechanisms underlying depression are a complicated mesh of integrated actions and even though no clear pathophysiological mechanism has been established to explain why depression occurs, the widely accepted monoaminergic theory[2] states that depression is a consequence of a deficiency of brain monoamine (noradrenaline, NA[a], or serotonin) activity.

It is well-known the role that α_2-adrenergic receptors (α_2-ARs), expressed on both somatodendritic areas and axon terminals, play in central noradrenergic transmission.[3] Activation of these α_2-ARs induces an inhibition of NA release in the brain, and 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.[4] A deficit in noradrenergic transmission has been described in depression and the mentioned enhanced α_2-AR activity could be implicated in such a deficit; thus, treatment with antidepressants will help to regulate the local release of NA by inducing in vivo desensitization of the α_2-ARs.[5] Supporting this idea, it has been shown that local (in the locus coeruleus) or systemic administration of α_2-AR antagonists not only increase the liberation of NA in the prefrontal cortex (PFC)[6,7] but also augment the increase of NA induced by selective reuptake inhibitor antidepressant drugs.[8] Therefore, research in selective α_2-adrenoceptor antagonists represents a valuable therapeutic approach for the treatment of depression.

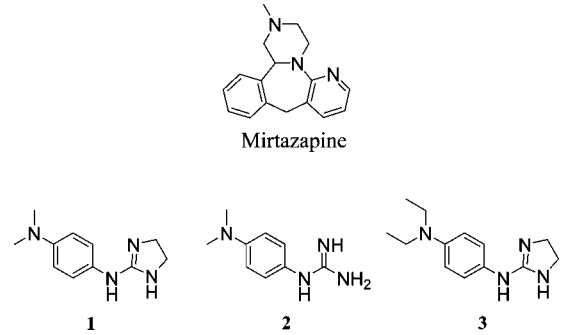

Figure 1. Structures of known α_2-AR targeting antidepressant Mirtazapine and three α_2-AR ligands previously described in our group.

Mirtazapine (Remeron, Figure 1) is one of the most recent developed antidepressants and shows efficacy by blocking the α_2-ARs.[9] This blockade of α_2-ARs in the brain prevents the negative feedback that NA has in its own release, resulting in a more efficient noradrenergic neurotransmission.[10]

Continuing with our work in the development of new α_2-AR antagonists, in two previous articles[11] we described the synthesis and screening in human PFC of a number of *bis*- and *mono*-guanidine and 2-aminoimidazoline derivatives. Among these derivatives, compounds **1**, **2**, and **3** (Figure 1) showed affinities toward the α_2-ARs within the same range as Idazoxan and/or Clonidine.[11a] Considering that the three analogues are structurally related, one might expect them to display the same activity in the receptors. However, after a complete in vitro pharmacological study, compounds **2** and **3** showed agonistic properties, whereas only compound **1** resulted to be an antagonist. The antagonism toward the α_2-ARs of compound **1**, and its potential as antidepressant, was later confirmed by in vivo microdialysis experiments carried out in rats.[11a] As a consequence, it can be

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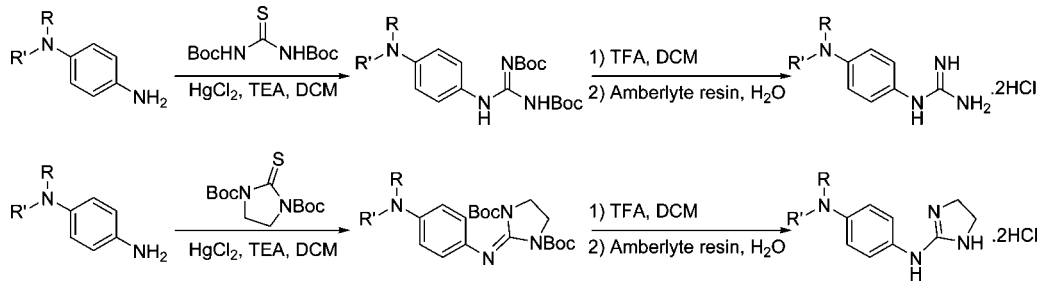
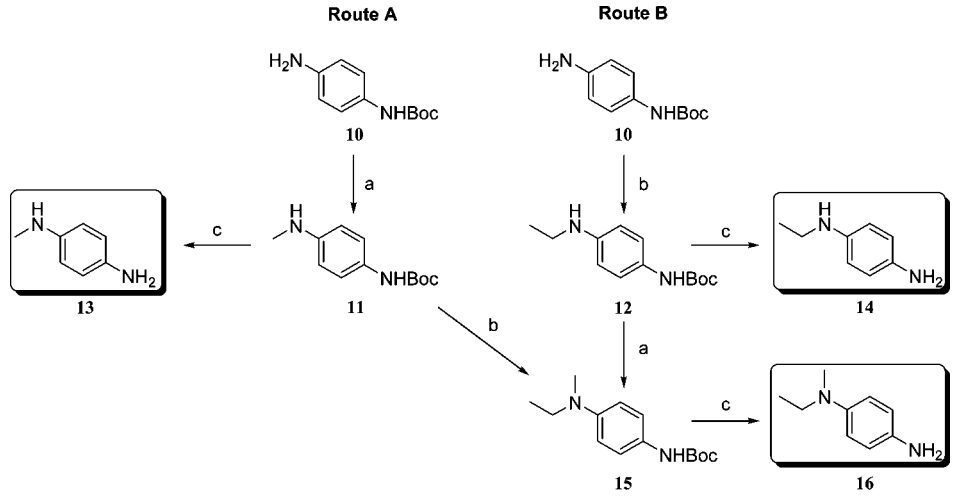
[a] Abbreviations: NA, noradrenaline; AR, adrenoceptor; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); PFC, prefrontal cortex; SAR, structure−activity relationships; aCSF, artificial cerebrospinal fluid.

10.1021/jm800838r CCC: $40.75
Published on Web 01/09/2009

Scheme 1



Scheme 2[a]



[a] Reagents and conditions: (a) $MeSO_3Me$, TEA, CH_2Cl_2, 50 °C; (b) $MeSO_3Et$, TEA, CH_2Cl_2, 50 °C; (c) TFA, rt.

deduced that in this kind of molecules, very subtle structural changes lead to a different behavior in the α_2-ARs.

Considering the structures and activities of compounds **1**, **2**, and **3**, one could speculate whether or not the antagonism of derivative **1** remains after the loss of one of the methyl groups, or what type of response would produce in the receptors an intermediate structure between compounds **1** and **3** bearing an ethyl-methyl amine. Hence, the synthesis and pharmacological evaluation of a new series of guanidine and 2-aminoimidazoline analogues of the lead compounds **1**, **2**, and **3** is presented in this article and the results obtained will provide important structure−activity relationships (SARs). It is worth mentioning that the lack of a crystal structure for the α_2-ARs makes this type of studies a valuable tool to establish some chemical functions required for both the affinity and activity in the α_2-ARs.

In this article, the preparation of six new guanidine and 2-aminoimidazoline analogues of derivatives **1**, **2**, and **3** and a new synthesis for their precursor amines is presented. Moreover, a complete pharmacological study (consisting of in vitro assays in human brain tissue to evaluate their α_2-AR affinity and determine their activity and in vivo microdialysis experiments in rats) was performed. The α_2-AR affinity and potential receptor antagonism experiments were carried out in human PFC because there is an important density of α_2-ARs in this tissue,[12] and many studies have reported changes in PFC activity in the brain of patients with depression.[13] Moreover, the final goal of our research is to obtain antidepressants and, therefore, the use of human brain tissue to directly characterize the pharmacological properties of the new compounds is relevant from a therapeutic point of view.

Results and Discussion

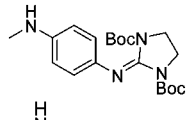
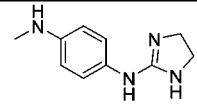
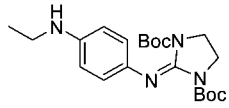
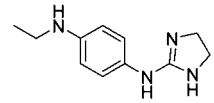
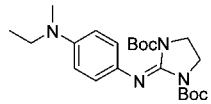
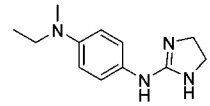
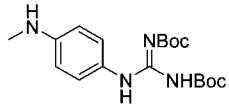
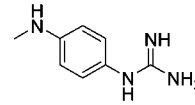
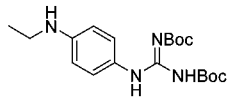
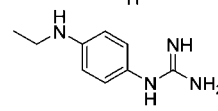
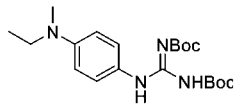
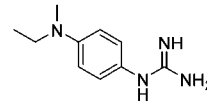
Chemistry. Among the different options to incorporate the guanidine and 2-aminoimidazoline groups into a molecule that based on the reaction of primary aromatic amines with 1 equiv of either *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea (guanidine precursor) or *N*,*N*′-di(*tert*-butoxy carbonyl)imidazoline-2-thione[14] (2-aminoimidazoline precursor) in the presence of mercury (II) chloride and an excess of triethylamine (Scheme 1) has given very good results in our group[11,14] and for that reason was the strategy followed in the present work.

In all cases, the reaction was carried out in dichloromethane and the Boc-protected precursors obtained in the first step of the synthesis were purified by a quick neutral alumina flash column chromatography.

Standard removal of the Boc groups with an excess of trifluoroacetic acid in dichloromethane followed by treatment with Amberlyte resin in water led to the hydrochloride salts of the target molecules in overall good yields ranging from 48 to 75%. The structures and yields of all the new compounds prepared are displayed in Table 1.

Compound **10** (*N*-Boc-*p*-phenylenediamine) was used, as an advanced intermediate, for the synthesis of the final compounds **4b**−**9b** as depicted in Scheme 2. Thus, treatment of derivative **10** with 1 equiv of methyl methanesulfonate and triethylamine in dichloromethane led to the monomethylated derivative **11** in a 25% yield after column chromatography in silica gel. The ethylamine compound **12** was obtained in a similar manner via

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

Compd	Structure	1st Stage	Compd	Structure	2nd Stage	Overall
4a		60	**4b**		93	56
5a		52	**5b**		93	48
6a		68	**6b**		93	63
7a		77	**7b**		95	73
8a		72	**8b**		94	68
9a		78	**9b**		96	75

ethyl methanesulfonate. In this case, the yield increased to 34%. In both reactions, formation of dialkylated products was observed.

Boc-removal of compounds **11** and **12** with trifluoroacetic acid afforded the starting material amines **13** and **14** in good yields (87% and 84%, respectively).

For the synthesis of the Boc-protected tertiary amine **15**, there are two possible routes (see Scheme 2). Thus, following route A, heating of amine **11** in dicloromethane at reflux temperature, with triethylamine and ethyl methanesulfonate as an electrophile, led to compound **15** in a 39% after column cromatography in silica gel. Similarly, starting from the ethylamine **12** (route B) and using methyl methanesulfonate as the alkylating agent, derivative **15** was obtained in a 37%. Again, in both cases formation of the polyalkylated products was observed. Finally, Boc-removal of compound **15** in trifluoroacetic acid afforded the ethyl-methyl amine **16** in 89% yield.

All the chemicals used for the synthesis described in Schemes 1 and 2 are commercially available either from Aldrich or Fluka and, to the best of our knowledge, the Boc-protected amines **12** and **15** are new, whereas the methylamine derivative **11** has been previously described in the literature[15] as the anilines **13**,[15a,c,16] **14**,[16b,17] and **16**.[18]

Pharmacology. As mentioned above and based on our previous experience, small structural modifications produce significant changes in terms of affinity or activity (agonism−antagonism) in the receptors.[11] Thus, the presence or absence of heteroatoms in very similar backbones or the exchange of the guanidinium and 2-aminoimidazolinium cations can determine the antagonism of a substrate.[11] With that in mind, and given the similarities of the lead compounds **1**, **2**, and **3** (Figure 1), there was a need to explore in more detail the pharmacological profile of this class of molecules by evaluating the six new derivatives synthesized. This study would allow finding important SARs required for both the affinity and activity of the compounds studied.

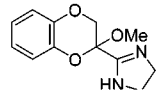
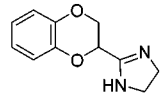
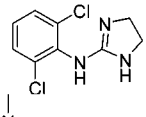
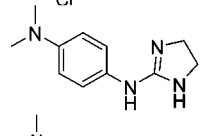
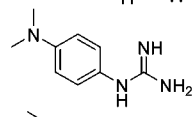
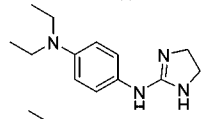
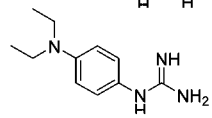
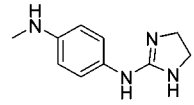
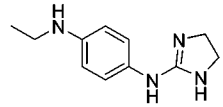
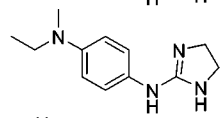
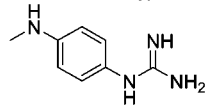
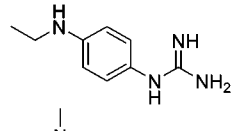
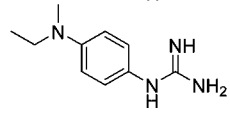
Affinity toward the α_2-ARs. The affinity of all compounds prepared toward the α_2-ARs in human brain PFC tissue was measured by competition with the α_2-AR selective radioligand [^{3}H]RX821002 (2-methoxy-idazoxan), which was used at a constant concentration of 1 nM. The affinities obtained, expressed as pK_i, are displayed in Table 2. Three of the most common α_2-AR ligands (Idazoxan, Clonidine, and RX821002) were used as references.

In the 2-aminoimidazoline series, all new compounds displayed a pK_i value higher than 7 except for the ethylamino derivative **5b** (pK_i = 6.75, see Table 2). These values are within the range of the well-known α_2-AR ligands Idazoxan and/or Clonidine, with compound **1** keeping the highest affinity of the series (pK_i = 7.42). Among the new derivatives, the pK_i value obtained for the analogue **6b** is very similar to that of compound **1**, while the secondary methylamino derivative **4b** showed a slightly lower affinity. This result seems to indicate that for the monoalkyl amines, the chain shortening is an advantage affinitywise although more compounds should be evaluated.

Regarding the guanidine containing substrates, among the new molecules obtained, the order in affinity toward the α_2-ARs is the same as the one found for their 2-aminoimidazoline counterparts. Thus, the ethyl-methylamino compound **9b** shows the highest pK_i (7.12, see Table 2), whereas the monoethylamino derivative **8b** possesses the lowest affinity with a pK_i value of 6.58. In all cases, the affinity of the guanidine substrates is lower than the one displayed by their 2-aminoimidazoline analogues, as we have observed before.[11]

It is worth to mention that the affinity showed by compound **9b** is the best of its series, better than the affinities showed by some of the 2-aminoimidazoline counterparts and within the range of the α_2-AR antagonist Idazoxan. Five out of the six derivatives shown in Table 2 with a dialkyl amine in para (compounds **1**, **2**, **3**, **6b**, and **9b**) showed a pK_i larger than 7, whereas the only monoalkyl amine that presents a pK_i > 7 is derivative **4b**. This fact seems to indicate that the presence of

Table 2. α_2-ARs Affinity Values (Expressed as K_i and pK_i) Obtained for All Compounds Studied[a]

Compound	Structure	K_i (±SEM)	pK_i[§†]
RX821002		0.9±0.2	9.04
Idazoxan		51±9	7.29
Clonidine		21±9	7.68
1*		38±7	7.42
2*		87±17	7.06
3*		82±44	7.09
17*		462±201	6.34
4b		53±7	7.27
5b		175±36	6.75
6b		41±8	7.38
7b		113±16	6.95
8b		261±35	6.58
9b		75±11	7.12

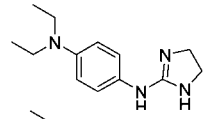
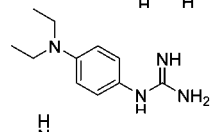
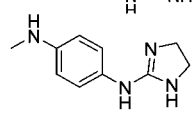
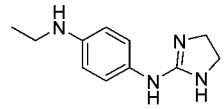
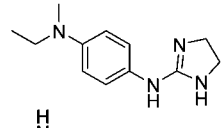
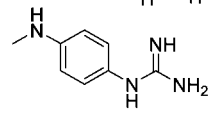
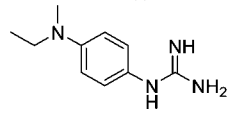
[a] * = Compounds previously prepared and evaluated by us.[11a] § = Affinity was measured by competition assays with the α_2-AR selective radioligand [^{3}H]RX821002 (1 nM) in PFC human tissues. † = Cortical membranes from human postmortem brains were incubated at 25 °C for 30 min with [^{3}H]RX821002 (1 nM) in the absence or presence of the competing compounds (10^{-12} M to 10^{-3} M, 10 concentrations).

a tertiary amine in the para position of these molecules is needed for a good binding to the α_2-AR receptors.

[^{35}S]GTPγS Binding Functional Assays: Agonism vs Antagonism. All six new compounds **4b**−**9b** along with derivaitve **17** were subjected to [^{35}S]GTPγS binding experiments to determine their nature as agonists or antagonists, and the results are shown in Table 3. The affinity of the latter one had been evaluated,[11a] but its activity had not been studied.

The α_2-ARs are G-protein coupled receptors (GPCRs) and, as such, when the endogenous ligand binds to the receptor, a change in the conformation of the G-proteins occurs, leading to the exchange of GDP by GTP on the α-subunit, promoting their dissociation into α-GTP and $\beta\gamma$ subunits and resulting in transmembrane signaling. 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

Table 3. EC_{50} Values and Intrinsic Activity Relative to UK14304 ($pK_i = 8.85$) Found for Compounds Showing a Typical Agonist Dose−Response Plot[a]

Compound	Structure	EC_{50} (μM)†	E_{max} (%)
UK14304		11.4±0.3	100
3*		2.8±0.1	96
17§		38.7±5.0	89
4b		61.4±4.9	98
5b		59.3±4.9	95
6b		1.9±0.1	103
7b		82.3±17.0	87
9b		16.2±2.3	92

[a] * = Compound previously prepared by us whose EC_{50} had been evaluated.[11a] § = Compound previously prepared by us whose EC_{50} had not been evaluated.[11a] † = Cortical membranes from human postmortem brains were incubated at 30 °C for 2 h in the presence of the different compounds (10^{-10} M to 10^{-3} M, 8 concentrations).

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.[19] Experiments were performed in low-affinity receptor conditions for agonists (presence of guanine nucleotides and sodium in the medium), and hence, typical potency values are between two and three logarithmic units lower than affinity values obtained in radioligand receptor binding experiments.[19]

The new compounds **4b**, **5b**, **6b**, **7b**, and **9b** as well as derivatives **3** and **17** stimulated binding of [^{35}S]GTPγS, showing a typical agonist dose−response plot. The potencies (and structures) of the 2-aminoimidazoline containing compounds **3** and **6b** are very similar and the highest of the whole series (Table 3), whereas the rest of the substrates show potencies within the same range and close to the one displayed by the well-known α_2-AR agonist UK14304 (5-bromo-6-[2-imidazolin-2-ylamino]quinoxaline) used as reference.

Again, molecules with similar structures showed the closest EC_{50} values (see compounds **4b** and **5b**, Table 3). The intrinsic activity relative to the UK14304 for all these substrates ranges from 87 to 103% (Table 3). Only compound **8b** did not stimulate binding of [^{35}S]GTPγS and was subjected to new [^{35}S]GTPγS experiments and tested against the UK14304.

A significant rightwards shift of the EC_{50} for UK14304 when including compound **8b** in the assay would confirm its antagonism. Thus, the effect induced in the UK14304 agonist stimulation of [^{35}S]GTPγS binding by the presence of a single concentration (10^{-5} M) of derivative **8b** was evaluated and is presented in Table 4, along with the effect induced by the known antagonist **1** and the agonist **2**.[11a] Similarly to compound **1**, addition of derivative **8b** to the experiment produced a remarkable rightwards shift in the EC_{50} value for the UK14304 (Table 4), indicating that compound **8b** behaves as an antagonist in the α_2-ARs in human brain PFC in the experiments carried out in vitro. It is important to highlight that, within this set of structurally related molecules, only two out of ten compounds showed antagonism toward the α_2-ARs, which suggests that more specific interactions are required for the antagonism and makes the rational design of α_2-AR antagonists more challenging.

Table 4. 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)[a]

experiment	EC_{50} (μM)
UK14304	11.4 ± 0.3
UK14304 + **1**[a]	355 ± 18
UK14304 + **2**[a]	16 ± 2
UK14304 + **8b**	213 ± 18

[a] Compounds previously prepared by us[11a] and already evaluated against UK14304.

Considering the chemical structure of the lead compound **1**, it is not an easy task to find an obvious explanation for the antagonism of derivative **8b**. In fact, the structures of compounds

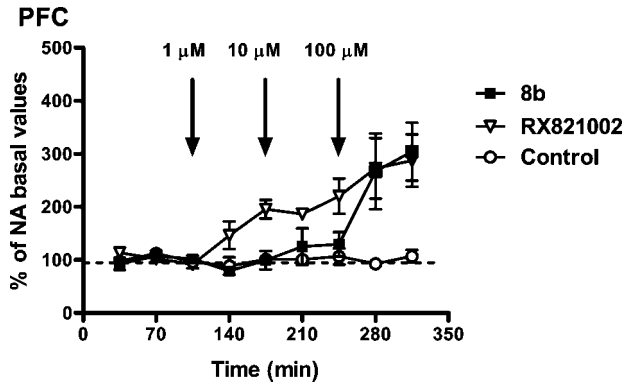


Figure 2. Effects of local administration of compound **8b** (1−100 μM), RX821002 (1−100 μM), or aCSF (control rats) in the PFC. Concentration of the compounds was progressively increased (arrows). Compounds **8b** and RX821002 were dissolved in aCSF and perfused via reverse dialysis at the time indicated by the arrows (every 70 min). Data correspond to the mean ± standard error mean values from 3−5 animals for each group and are expressed as percentages of the corresponding basal values.

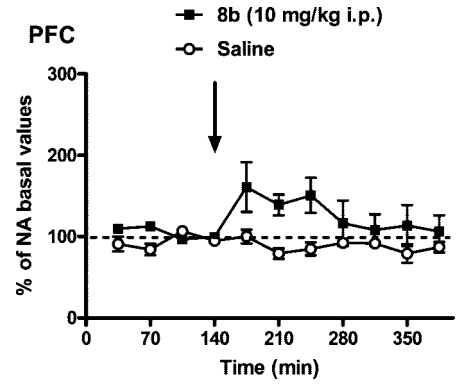


Figure 3. Effects of intraperitoneal administration of compound **8b** or vehicle (saline) on extracellular NA levels evaluated in the PFC. Data are given as mean ± standard error mean values from 4−5 separate animals for each group and are expressed as percentages of the corresponding basal values. Arrow represents time administration of the compound or vehicle.

2, **3**, **4b**, **6b**, or **7b** are more similar to that of compound **1**, and yet they all showed agonistic properties in the α_2-ARs.

In Vivo Microdialysis Experiments. Given the antagonistic properties over the α_2-ARs showed in vitro by derivative **8b**, we proceeded to test its potential effect on noradrenergic transmission in vivo by microdialysis experiments. This technique is a widely accepted method for sampling the extracellular fluid of the brain, allowing the study of different neurotransmitters in the extracellular area where the probe is implanted.[20] This technique has been used to investigate the effect of different compounds on NA concentrations in the PFC, an area widely implicated in depression. The increase of NA concentration in the PFC of freely moving rats after drug administration is accepted as a good predictor for antidepressant activity. In this context, many antidepressants, including the α_2-AR antagonist Mirtazapine, are able to increase dialysate levels of NA in the PFC.[21] Considering the antagonist properties of compound **8b** on α_2-ARs, we assessed its ability to increase NA extracellular concentrations in that brain area.

Artificial cerebrospinal fluid (aCSF) administration for more than 5 h did not change NA basal values (F[8,30] = 0.58; P = 0.77, n = 4, Figure 2). When derivative **8b** was perfused by reverse dialysis through the probe (1−100 μM), a significant increase in extracellular NA levels was observed (E_{max}= 304 ± 54%, F[1,52] = 13.22, P = 0.0006 vs control, n = 9; Figure 2) at 100 M concentration of compound **8b**. The maximal effect was very similar to that obtained from the local administration (1−100 μM) of the well-known α_2-AR antagonist RX821002 (E_{max} = 290 ± 35%, F[1,40] = 65.32, P < 0.0001, n = 7; Figure 2).

However, in good agreement with the pK_i values of the substrates for the α_2-ARs, RX821002 (pK_i = 9.04) exerted a significant increase in extracellular NA levels at the lowest dose tested (1 μM), whereas **8b** (pK_i = 6.58) did not produce a clear effect until the 100 μM dose.

On a second step, we tested the effect of derivative **8b** on extracellular NA levels by systemic administration.Control rats were administered with the vehicle (saline). Intraperitoneal administration of compound **8b** (10 mg/kg) increased NA extracellular concentration by 161 ± 30% in the PFC (Figure 3).

This increase was statistically significant when the group was compared with the respective control (F[1,67] = 22.64, P < 0.0001, n = 9). These results confirm the antagonistic properties shown by compound **8b** in vitro and the ability of the compound to cross the blood−brain barrier (BBB). At this point, it is not possible to discard completely the actions of a metabolite of the compound **8b** after systemic administration. However, the fast effect observed after compound administration makes it slightly probably. The quantitative determination of the drug brain penetration of the drug and/or metabolites should be determined by [brain]/[plasma] determination via single-dose pharmacokinetic assessment.

Second, it can be concluded that the compound effect on different brain circuits at the same time did not abolish the NA increase observed in the PFC after local administration. Under these premises, the compound **8b** fulfils the basic requirements that indicate its possible activity as antidepressant.

Conclusions

The conclusions in this article are 4-fold. First, the syntheses of three new 2-aminoimidazoline compounds (**4b**−**6b**) and three new guanidine containing derivatives (**7b**−**9b**), as well as a new methodology for the preparation of their precursor amines (**13**, **14**, and **16**) are reported.

Second, it has been shown that compounds **4b**, **6b**, and **9b** have affinities toward the α_2-ARs within the range of those of Idazoxan and/or Clonidine (pK_i > 7). Remarkably, and for the first time in this type of derivatives, compound **9b**, which contains a guanidinium cation, shows an affinity larger than that showed by the 2-aminoimidazolinium derivatives **3** and **5b**. Moreover, five out of the six derivatives with a tertiary amine in *para* (**1**, **2**, **3**, **6b**, and **9b**) showed a pK_i > 7, indicating that this motif may play an important role in the interactions established in the binding to the receptor.

Third, in terms of noradrenergic activity, it has been found that the new compounds **4b**−**7b**, and **9b**, as well as derivatives **2**, **3**, and **17**, showed agonistic properties in the [^{35}S]GTPγS experiments performed in vitro in PFC human tissue, whereas compound **8b** is an antagonist.

Finally, in terms of the SAR found, despite the structural similarity between compound **1** and compounds **4b** and **6b** (which were obtained by the removal or the addition of a methyl group in compound **1**), the first is an antagonist whereas the latter ones stimulate binding of [^{35}S]GTPγS. However, compound **8b**, which is structurally different than **1**, proved to be an antagonist in [^{35}S]GTPγS binding experiments. The antagonism showed by **8b** in vitro was later confirmed by in vivo

microdialysis experiments carried out in rats. Local and systemic administration of **8b** increased NA extracellular concentration in the rat brain.

Hence, it is clear that very subtle structural modifications can lead to significant changes in the activity toward the α_2-ARs and that these interesting and somehow surprising results encourage us to continue working in the synthesis of new series of compounds to try to find more SARs that help to understand the affinity and activity of this kind of substrates in the α_2-ARs.

Experimental Section

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, respectively. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker Win-NMR 5.0 software. HRMS spectra were recorded on a Waters (Micromass) LCT-Tof mass spectrometer operating in the positive ion electrospray mode. The source was operated at 100 °C with a cone voltage of 30 V. The instrument was operated at a resolution of 5000 fwhm. Spectra were recorder over a range *m*/*z* 100 to *m*/*z* 1000. The MS was controlled and data acquired and mass measured with MassLynx 4.0 software. Methanol, water, or ethanol were used 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. 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 2-Iminoimidazolidine and Boc-Protected Guanidine Derivatives: Method A. Each of the corresponding anilines was treated in DCM at 0 °C with 1.1 equiv of mercury (II) chloride, 1.0 equiv of *N*,*N*′-di(*tert*-butoxycarbonyl) imidazolidine-2-thione (for the 2-aminoimidazoline precursors), or *N*,*N*′-di(*tert*-butoxycarbonyl) thiourea (for the guanidine precursors) and 3.1 equiv 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 washed first with water (2 × 30 mL) and then 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 appropriate hexane:EtOAc mixture.

General Procedure for the Synthesis of the Dihydrochloride Salts: Method B. 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 dihydrochloride salt. Absence of the trifluoroacetate salt was checked by ^{19}F NMR.

General Procedure for the Alkylation of Primary and Secondary Amines: Method C. The alkylating agent (10.0 mmol of methyl methanesulfonate or ethyl methanesulfonate) and 10.0 mmol of TEA were added at 0 °C over a solution containing 10.0 mmol of the corresponding amine in DCM (12 mL). The resulting mixture was heated at reflux temperature for 15 h, and after cooling it was diluted with 40 mL of DCM and washed with a 10% NaOH solution (2 × 15 mL) and water (2 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, 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 Boc-Deprotection and Preparation of the Starting Material Amines: Method D. A solution containing 10.0 mmol of the Boc-protected compound (**11**, **12**, or **15**) in 15 mL of TFA was stirred at room temperature for 2 h. Then the solvent was eliminated under vacuum to generate the trifluoroacetate salt. This salt was redissolved in 20 mL of an aqueous solution of NaOH (2M) and washed with DCM (3 × 15 mL). The organic layer was washed with water (2 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give the corresponding free amine as an oil.

Dihydrochloride Salt of *N*-Imidazolidin-2-ylidene-*N*′-methyl-benzene-1,4-diamine (4b): Method B. Red solid (93%); mp 230−232 °C. ^{1}H NMR (D_2O) δ 3.07 (s, 3H, CH_3), 3.74 (s, 4H, CH_2), 7.44 (d, 2H, J = 8.5 Hz, Ar), 7.54 (d, 2H, J = 8.5 Hz, Ar). ^{13}C NMR (D_2O) δ 36.4 (CH_3), 42.3 (CH_2), 123.1, 125.2, 134.1, 136.0 (A.), 158.0 (CN). HRMS (ESI$^+$) *m*/*z* calcd $[M + H]^+$ 191.1291, found 191.1291. Anal. calcd for ($C_{10}H_{16}Cl_2N_4 \cdot 0.4H_2O$): C, H, N.

Dihydrochloride Salt of *N*-Ethyl-*N*′-imidazolidin-2-ylidene-benzene-1,4-diamine (5b): Method B. White solid (93%); mp 198−200 °C. ^{1}H NMR (D_2O) δ 1.29 (t, 3H, J = 7.5 Hz, $C\underline{H}_3CH_2$), 3.47 (q, 2H, J = 7.5 Hz, $CH_3C\underline{H}_2$), 3.75 (s, 4H, CH_2), 7.45 (d, 2H, J = 9.0 Hz, Ar), 7.53 (d, 2H, J = 9.0 Hz, Ar). ^{13}C NMR (D_2O) δ 9.7 ($\underline{C}H_3CH_2$), 42.3 (CH_2), 46.9 ($CH_3\underline{C}H_2$), 123.7, 125.1, 132.4, 136.0 (Ar), 158.0 (CN). HRMS (ESI$^+$) *m*/*z* calcd $[M + H]^+$ 205.1448, found 205.1443. Anal. calcd for ($C_{11}H_{18}Cl_2N_4 \cdot 1.5H_2O$): C, H, N.

Dihydrochloride Salt of *N*-Ethyl-*N*′-imidazolidin-2-ylidene-*N*-methyl-benzene-1,4-diamine (6b): Method B. White solid (93%); mp 48−50 °C. ^{1}H NMR (D_2O) δ 1.15 (t, 3H, J = 7.5 Hz, $C\underline{H}_3CH_2$), 3.26 (s, 3H, CH_3), 3.63 (q, 2H, J = 7.5 Hz, $CH_3C\underline{H}_2$), 3.76 (s, 4H, CH_2), 7.49 (d, 2H, J = 9.0 Hz, Ar), 7.64 (d, 2H, J = 9.0 Hz, Ar). ^{13}C NMR (D_2O) δ 9.3 ($\underline{C}H_3CH_2$), 42.3 (CH_2), 44.1 (CH_3), 54.8 ($CH_3\underline{C}H_2$), 122.5, 125.1, 136.6, 137.4 (Ar), 157.9 (CN). HRMS (ESI$^+$) *m*/*z* 219.1604 calcd $[M + H]^+$, found 219.1605. Anal. calcd for ($C_{12}H_{20}Cl_2N_4 \cdot 1.8H_2O$): C, H, N.

Dihydrochloride Salt of *N*-(4-Methylamino-phenyl)-guanidine (7b): Method B. Pinkish solid (95%); mp 74−76 °C. ^{1}H NMR (D_2O) δ 3.08 (s, 3H, CH_3), 7.46 (d, 2H, J = 8.5 Hz, Ar), 7.56 (d, 2H, J = 9.0 Hz, Ar). ^{13}C NMR (D_2O) δ 36.4 (CH_3), 123.3, 126.7, 134.6, 135.3 (Ar), 155.6 (CN). HRMS (ESI$^+$) *m*/*z* 165.1135 calcd $[M + H]^+$, found 165.1138. Anal. calcd for ($C_8H_{14}Cl_2N_4 \cdot 1.3H_2O$): C, H, N.

Dihydrochloride Salt of *N*-(4-Ethylamino-phenyl)-guanidine (8b): Method B. White solid (94%); mp 126−128 °C. ^{1}H NMR (D_2O) δ 1.31 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2$), 3.48 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2$), 7.49 (d, 2H, J = 8.0 Hz, Ar), 7.54 (d, 2H, J = 8.0 Hz, Ar). ^{13}C NMR (D_2O) δ 9.8 ($\underline{C}H_3CH_2$), 46.7 ($CH_3\underline{C}H_2$), 123.6, 126.7, 133.2, 135.0 (Ar), 155.6 (CN). HRMS (ESI$^+$) *m*/*z* 179.1297 calcd $[M + H]^+$, found 179.1303. Anal. calcd for ($C_9H_{16}Cl_2N_4 \cdot 1.0H_2O$): C, H, N.

Dihydrochloride Salt of *N*-[4-(Ethyl-methyl-amino)-phenyl]-guanidine (9b): Method B. White solid (96%); mp decomposes over 150 °C. ^{1}H NMR (D_2O) δ 1.16 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2$), 3.27 (s, 3H, CH_3), 3.64 (q, 2H, J = 7.0 Hz, $CH_3C\underline{H}_2$), 7.52 (d, 2H, J = 8.5 Hz, Ar), 7.65 (d, 2H, J = 8.5 Hz, Ar). ^{13}C NMR (D_2O) δ 9.2 ($\underline{C}H_3CH_2$), 44.1 (CH_3), 54.7 ($CH_3\underline{C}H_2$), 122.6, 126.6, 135.9, 137.7 (Ar), 155.5 (CN). HRMS (ESI$^+$) *m*/*z* 193.1448 calcd $[M + H]^+$, found 193.1450. Anal. calcd for ($C_{10}H_{18}Cl_2N_4 \cdot 0.2H_2O$): C, H, N.

***N*-Methyl-benzene-1,4-diamine (13): Method D.** Red oil (87%); IR (nujol) ν 3400, 3339, 3222 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.76 (s, 3H, CH_3), 3.29−3.38 (m, 3H, NH_2 + CH_3NH), 6.52 (d, 2H, J = 9.0 Hz, Ar), 6.62 (d, 2H, J = 9.0 Hz, Ar). ^{13}C NMR ($CDCl_3$) δ 31.4 (CH_3), 113.7, 116.5, 137.4, 142.2 (Ar).

***N*-Ethyl-benzene-1,4-diamine (14): Method D.** Red oil (84%); IR (nujol) ν 3378, 3335, 3217 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2$), 2.85−3.19 (m, 5H, NH_2 + NH + $CH_3C\underline{H}_2$), 6.53 (d, 2H, J = 8.5 Hz, Ar), 6.62 (d, 2H, J = 8.5 Hz, Ar). ^{13}C NMR ($CDCl_3$) δ 15.0 ($\underline{C}H_3CH_2$), 39.6 ($CH_3\underline{C}H_2$), 114.5, 116.8, 137.6, 141.6 (Ar).

***N*-Ethyl-*N*-methyl-benzene-1,4-diamine (16): Method D.** Brownish oil (89%); IR (nujol) ν 3433, 3345 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.10 (t, 3H, J = 7.0 Hz, $C\underline{H}_3CH_2$), 2.82 (s, 3H, CH_3), 3.21−3.43 (m, 4H, NH_2 + $CH_3C\underline{H}_2$), 6.67 (d, 2H, J = 9.0 Hz, Ar), 6.71 (d, 2H, J = 9.0 Hz, Ar). ^{13}C NMR ($CDCl_3$) δ 10.9 ($\underline{C}H_3CH_2$), 38.4 (CH_3), 48.2 ($CH_3\underline{C}H_2$), 115.8, 116.5, 137.5, 143.1 (Ar).

Pharmacology: Materials and Methods. Preparation of Membranes. Neural membranes (P_2 fractions) were prepared from the PFC 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 40000*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} 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).

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, 8 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, UK), 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.

Microdialysis Experiments. Male Sprague−Dawley rats (250−300 g) were implanted with a probe in a stereotaxic apparatus under chloral hydrate anesthesia (400 mg/kg ip). The probe was located in the prefrontal cortex (PFC) according to the coordinates of the atlas of Paxinos and Watson[22] (AP (anterior to bregma) +2.8 mm, L (lateral from the midsagittal suture) +1 mm, DV (ventral from the dura surface) −5 mm).

Experiments were performed 20−24 h after the probe implantation and aCSF (148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$; pH 7.4) was pumped at a flow rate of 1 μL/min (CMA/Microdialysis infusion pump). Drugs, when locally administered, were dissolved in aCSF and applied during 70 min via dialysis probe in increasing concentrations of 1, 10, and 100 μM. Drugs systemically administered were dissolved in saline and injected intraperitoneally.

Samples were collected every 35 min and NA concentrations analyzed by HPLC apparatus with amperometric detection (Hewlett-Packard model 1049A) at an oxidizing potential of +650 mV. The mobile phase (12 mM citric acid, 1 mM EDTA, 0.7 mM octylsodio 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, USA). Samples (injection volume 30 μL) were injected and NA analyzed in a run time of 10 min.

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 percentage of the baseline value ± SEM. 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.

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

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 S-PE07UN13 and IT-199/07), and Spanish Ministry of Health, Instituto de Salud Carlos III, (Ciber-SAM). A.M.E. was recipient of a predoctoral fellowship from the Basque Government.

Supporting Information Available: Preparation, ^1H NMR, ^{13}C NMR, and MS data of all new Boc-protected derivatives prepared (**4a**−**9a**, **11**, **12**, and **15**), a table containing the combustion analysis data and the ^1H and ^{13}C NMR spectra for the new final compounds (**4b**−**9b**) is presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800838R