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I_2-Imidazoline Binding Site Affinity of a Structurally Different Type of Ligands

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Abstract—Two families of compounds with affinity towards the I_2 imidazoline binding sites are reported. The first is a family of compounds structurally related to agmatine with two guanidine or 2-aminoimidazoline groups at each end of an aliphatic chain of six, eight, nine or 12 methylene groups. Second, and following the model of clonidine, we propose another family of compounds also with two guanidine or 2-aminoimidazoline groups at each end of a chain consisting of two phenyl rings connected by groups such as CH_2, CO, NH and SO_2. The affinity of the compounds towards the I_2 imidazoline binding sites was then evaluated in human brain tissues. In order to determine their pharmacological selectivity versus α_2-adrenoceptors, the affinity for these receptors was also evaluated for the compounds with the highest affinities at I_2 imidazoline binding sites. The results obtained show that many of the compounds exhibit a considerable affinity towards the I_2 imidazoline binding sites. The aliphatic derivatives, in particular, present a very interesting selectivity for the I_2 imidazoline binding sites versus the α_2 adrenoceptors. To better understand these findings, mono-guanidinium analogues of the aliphatic derivatives were synthesised and tested showing poor affinity for I_2 imidazoline binding sites. The importance of these results lies in the novelty of the chemical structures studied (dicationic aliphatic compounds particularly) because they are significantly different to those of the I_2 imidazoline binding site ligands reported to date.

Introduction

Imidazoline binding sites (IBS) were first suggested by Bousquet et al. when studying the α_2-adrenoceptor agonist clonidine, a centrally acting antihypertensive agent with an imidazolidine group (see Fig. 1). They hypothesised that IBS were a different class of binding sites than the α_2-adrenoceptors (α_2-ARs) and that they specifically recognized imidazoline and guanidine groups.[1]

IBS have been classified in two main groups. The first are I_1 binding sites (I_1-IBS) that have a high affinity for imidazolidine derivatives such as clonidine or moxonidine (Fig. 1), medium affinity for imidazoline derivatives such as idazoxan or phentolamine (Fig. 1) and low affinity for guanidine derivatives such as amiloride or guanabenz (Fig. 1). The second are I_2 binding sites (I_2-IBS) that show a high affinity for imidazoline and guanidine derivatives and a medium affinity for imidazolidine derivatives. These I_2-IBS binding sites have been further divided into I_{2A} and I_{2B} subtypes, depending on their high or low affinity for amiloride, respectively.[2] More recently, I_3 binding sites have been proposed.[3]

IBS have been localised in both central and peripheral nervous systems, and other tissues such as kidney, prostate, stomach, heart, liver, placenta, and colon.[2] Moreover, they seem to be involved in cardiovascular responses,[4,5] regulation of ocular pressure,[6] control of gastric acid secretion,[7] insulin release,[8] and modulation of antinociceptive responses.[9–11] IBS have also been studied for their possible implication in human brain disorders such as depression,[12–14] Alzheimer's type dementia,[15,16] Parkinson's disease,[17] and glial tumours.[18]

While I_1-IBS participate in blood pressure regulation,[2] the I_2-IBS seem to be closely related to the monoamine oxidase (MAO) enzyme.[19,20] This makes I_2-IBS pharmacological tools of therapeutic interest for neurodegeneration and neuroprotection phenomena.[21] Despite the fact that some very selective I_1-IBS ligands (rilmenidine

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derivative: LNP-509[22]) and very selective I_2-IBS ligands (2-BFI, BU-224, BU-239;[23] and 2-tolyl-imidazoline derivatives[24]) have been identified, the pharmacological role of these IBS remains unclear.

Therefore, the heterogeneity of the IBS and their implication in so many different physiological and pathological processes highlight their pharmacological importance. Subsequently, the development of new compounds with better affinity and selectivity for these binding sites becomes crucial in order to identify the targets connected to each biological effect.

The nature of the IBS ligands developed to date has been related to the structure of clonidine, the first compound as an IBS drug[1] (Fig. 1). This means that there is always an aromatic ring connected, in different ways, to an imidazoline, guanidine or imidazolidine group (Fig. 2). This has been reflected in a number of pharmacophoric models[25] that, in general, consider the presence of an aromatic moiety, the imidazoline moiety and a rotational angle between both elements. However, somewhat surprisingly the study of aliphatic molecules bearing an imidazoline moiety has never been taken into consideration, although the search for endogenous ligands for IBS led to the identification of agmatine[26] (Fig. 1), which is an aliphatic amine that binds to α_2-ARs and IBS with low affinity.[4]

In this paper, we propose two families of compounds with potential affinity towards I_2-IBS. First, a family of 'aliphatic' compounds structurally related to agmatine with two guanidine or 2-aminoimidazoline groups at each end of an aliphatic chain of six, eight, nine or 12 methylene groups (**1**–**8**, Fig. 2). Secondly, and following the model of the clonidine related structures, we also consider the potential affinity towards I_2-IBS of a family of 'aromatic' compounds (**9**–**16**, Fig. 2)[27] bearing two guanidine or 2-aminoimidazoline groups at each end of a chain consisting of two phenyl rings connected by groups such as CH_2, CO, NH and SO_2.

Considering the good results obtained for the di-guanidinium aliphatic derivatives (**2**–**4**) we prepared the corresponding mono-guanidinium derivatives **17**–**19** [CH_3–$(CH_2)_n$–guanidinium, $n = 7$, 8, 11, respectively] to better understand the possible binding mechanism of these compounds.

The use of the 2-aminoimidazoline group as an alternative to the guanidine group is justified by our previous finding (by means of a molecular similarity study[28]) which concluded that the 2-aminoimidazolinium cation is structurally (bond distances, atomic charges) and electronically (Molecular Electrostatic Potential, electron density) similar to the guanidinium one. Moreover, the 2-aminoimidazoline not only can be seen as a hybrid between the guanidine and the imidazoline, but also is present in clonidine.

We chose to use human brain membranes in the present study because these results could be more relevant from a therapeutical vantage. Therefore, we evaluated the affinity of compounds **1**–**19** to I_2-IBS in membranes of post-mortem human brain. Additionally, for those compounds showing the highest affinities on I_2-IBS, new experiments were performed to study their affinity to α_2-ARs. Consequently, we could determine the selectivity of the new compounds for I_2-IBS versus α_2-ARs. Nevertheless, we did not contemplate the evaluation of the possible I_2/I_1 selectivity since the I_1-IBS are in a very low density in human post-mortem brain.

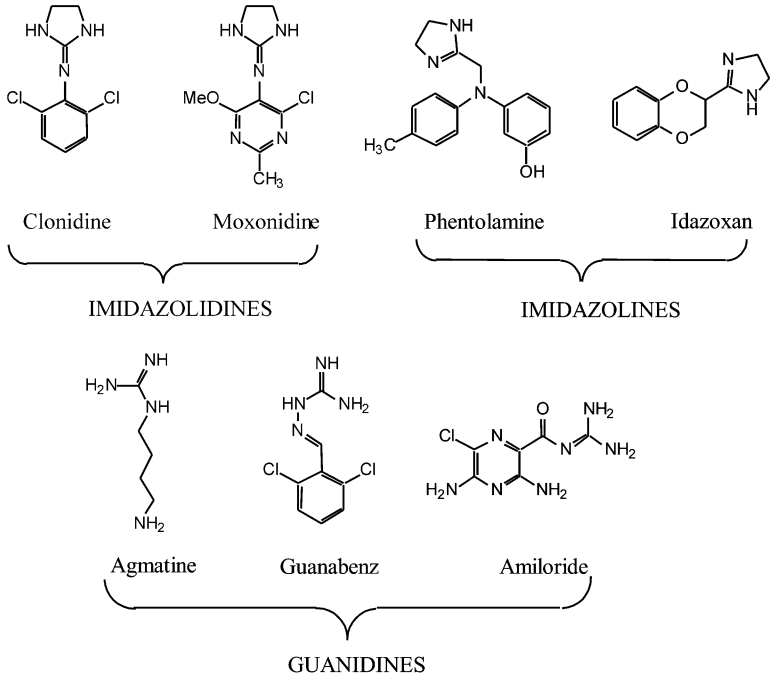


Figure 1. Different families of compounds that interact with the imidazoline binding sites.

Results

Chemistry

Regarding the proposed 'aliphatic' ligands, compounds **1**–**4**, **17**, and **19** carrying guanidinium groups have been already described in the literature.[29,30] However, their ability to interact with IBS has not been considered. We thus prepared these derivatives following the method described by Villarroya et al.[29b] This consisted in the nucleophilic substitution of the *S*-methylisothiouronium sulphate by the corresponding commercial diamines or monoamines (Scheme 1). This approach directly provides the bis-guanidinium salts **1**–**4** and the mono-guanidinium salts **17**, and **19**, which we characterised.[31] Further, we synthesised the 2-aminoimidazoline derivative **8** (gathered in a European Patent[32]) by using 2-methylmercapto-4,5-dihydroimidazole iodide.[30a] Because we did not find any report of the aminoimidazolines **5**, **6**, **7**, and the guanidinium **18**, we prepared them following the same methodologies (Scheme 1). The 2-aminoimidazoline derivatives were isolated as picrates when direct crystallisation of the hydroidide salt from the reaction mixture did not occur. The corresponding hydrochlorides were generated using basic anion exchange resin.

With respect to the 'aromatic' ligands proposed, compounds **9**–**16** (Fig. 2) had been previously prepared and evaluated by us as α_1-adrenoceptor antagonists in rat and rabbit aorta.[27] We had prepared the guanidine derivatives (**9**–**12**) following the methodology described by Kim and Qian,[33] and the 2-aminoimidazoline derivatives (**13**–**16**) were also prepared through a new method applicable to deactivated aromatic amines.[27]

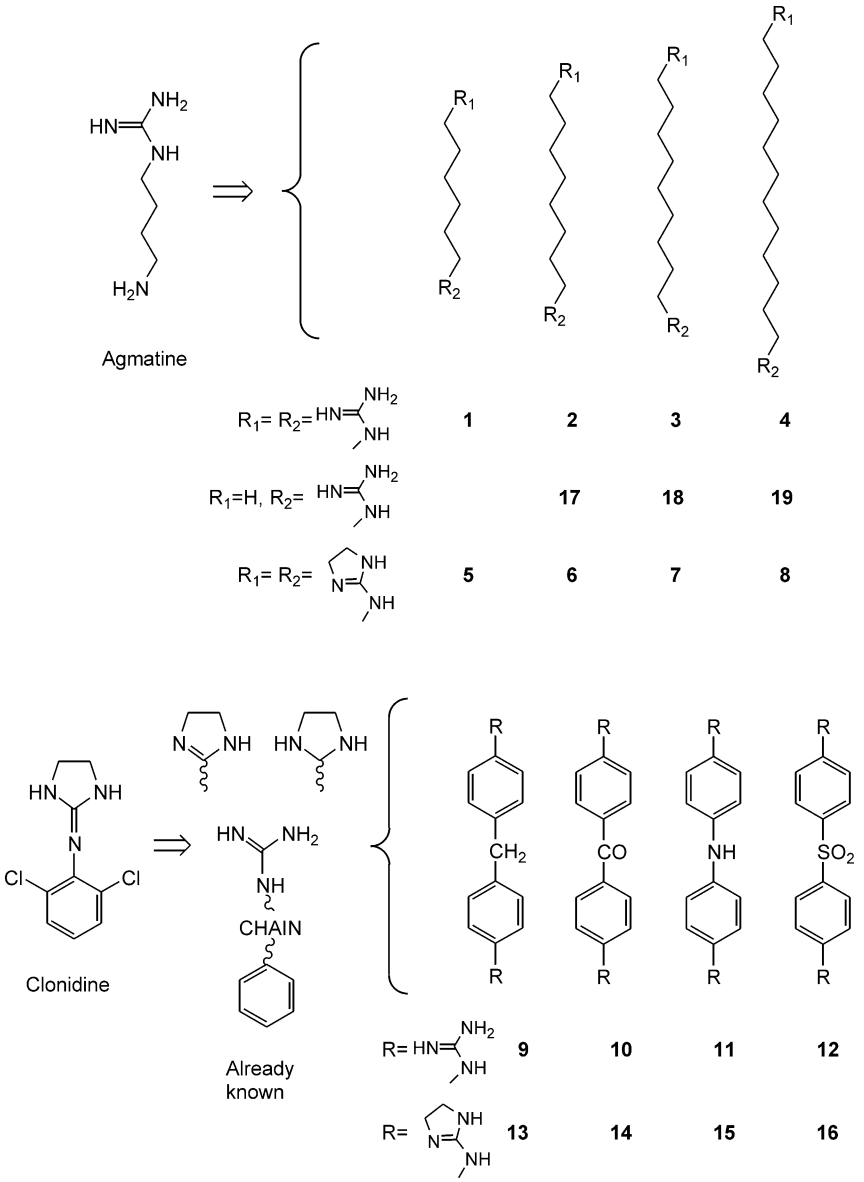
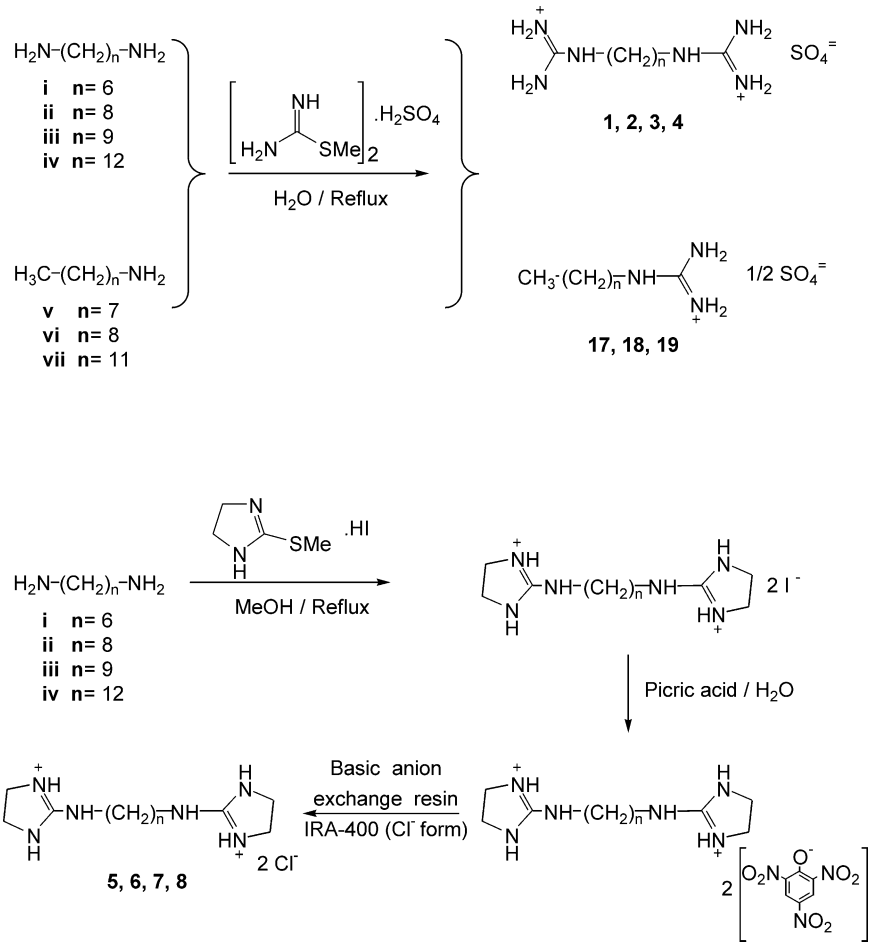


Figure 2. 'Aliphatic' compounds (structurally related to agmatine) and 'aromatic' compounds (clonidine related structures) proposed and studied as I_2 imidazoline binding sites ligands.



Scheme 1.

Table 1. I_2-IBS and α_2-ARs binding affinities (pK_i) of the bis-guanidinium (**1**–**4** and **9**–**12**) and bis(2-aminoimidazolidinium) (**5**–**8** and **13**–**16**) series of compounds

Compd	X-NH-R-NH-X		[^{3}H]2-BFI	[^{3}H]RX821002	
	X	R	pK_i I_2-IBS	pK_i α_2-ARs	Selectivity I_2/α_2[a]
Idazoxan	—	—	7.43±0.19	7.30±0.08	1.35
4	Gua[b]	$-(CH_2)_{12}-$	7.48±0.27	6.29±0.25	15.49
8	Imi[c]	$-(CH_2)_{12}-$	7.20±0.12	6.42±0.16	6.03
3	Gua	$-(CH_2)_9-$	6.89±0.27	6.07±0.04	6.61
7	Imi	$-(CH_2)_9-$	6.80±0.05	6.44±0.03	2.29
6	Imi	$-(CH_2)_8-$	6.37±0.03	6.22±0.05	1.41
15	Imi	–Ph–NH–Ph–	6.29±0.20	7.24±0.02	0.11
2	Gua	$-(CH_2)_8-$	6.20±0.20	6.26±0.02	0.87
13	Imi	$-Ph-CH_2-Ph-$	6.19±0.05	8.80±0.05	0.002
14	Imi	–Ph–CO–Ph–	6.05±0.04		
10	Gua	–Ph–CO–Ph–	5.98±0.05		
9	Gua	$-Ph-CH_2-Ph-$	5.93±0.06		
5	Imi	$-(CH_2)_6-$	5.60±0.16		
12	Gua	$-Ph-SO_2-Ph-$	5.35±0.05		
11	Gua	–Ph–NH–Ph–	5.28±0.07		
1	Gua	$-(CH_2)_6-$	5.23±0.30		
16	Imi	$-Ph-SO_2-Ph-$	<5.00		

[a]Selectivity I_2-IBS/α_2-ARs expressed as the antilog (pK_i I_2-IBS−pK_i α_2-ARs).
[b]Gua:–C(=NH)–NH_2 amidine group.
[c]Imi: imidazoline ring.

Radioligand binding assays

The pharmacological affinity of the prepared compounds was evaluated through competition binding studies against the selective I_2-IBS radioligand [3H]-2-[(2-benzofuranil)-2-imidazoline] (2-BFI) or the selective α_2-AR radioligand [3H]RX821002 (2-methoxy idazoxan). The studies were performed in membranes from post-mortem human frontal cortex, a brain area that shows an important density of I_2-IBS and α_2-ARs.[14,15,34]

The most representative competition curves are displayed in Figure 3. The inhibition constants (K_i) for each compound were obtained and are expressed as the corresponding pK_i in Table 1. Idazoxan, a compound with well-established affinity for I_2-IBS and α_2-AR, was used as a reference.

Discussion

The results obtained from competition binding experiments for compounds **1**–**16** are shown in Table 1. Only compounds showing an affinity towards I_2-IBS with p$K_i > 6.10$ were subjected to α_2-AR affinity tests.

From the compounds studied, the bis(2-aminoimidazolinium) and bis(guanidinium) derivatives, belonging to the 'aliphatic' family (**1**–**8**) with the longest [–$(CH_2)_n$–, $n = 12$ and 9] aliphatic chains (see compounds **3**, **4**, **7**, and **8** in Figure 2 and Table 1), display a good affinity towards the human brain I_2-IBS. These compounds showed an affinity in the nanomolar range, similar or slightly smaller than that of idazoxan. This affinity decreases when the aliphatic chain becomes shorter [–$(CH_2)_n$–, $n = 8$ or 6, compounds **1**, **2**, **5** and **6**].

With respect to the 'aromatic' family (**9**–**16**), the nature of the cation moieties in the structures seems to play a role in their affinity for I_2-IBS because the 2-iminoimidazolidinium derivatives generally showed larger pK_i values independent of the nature of the chemical group in the bridge. Even though the pK_i values obtained for this family of compounds are smaller than those of the aliphatic derivatives with long aliphatic chains (**3**, **4**, **6**, **7**, and **8**), derivatives **13** and **15** show acceptable affinities for I_2-IBS compared with that of idazoxan.

The competition binding assays for α_2-ARs carried out on the compounds with higher I_2-IBS affinity generally demonstrate a medium to small selectivity as expressed by the I_2/α_2 index. This index is calculated as the antilogarithm of the difference between pK_i values for I_2-IBS and pK_i values for α_2-ARs (see Table 1).

The 'aromatic' derivatives **13** and **15** both show a higher affinity towards the α_2-ARs than towards the I_2-IBS. Therefore, they cannot be considered as appropriate I_2-IBS ligands. With respect to the 'aliphatic' family, compounds **2** and **6**, with an eight links chain, show I_2/α_2 selectivity values very similar to those obtained for idazoxan. However, in the structures with larger aliphatic chains the selectivity towards I_2-IBS noticeably increases when changing the 2-aminoimidazolinium cations by the guanidinium ones (**3** vs **7** and **4** vs **8**) and when the length of the chain increases (**2** vs **3** vs **4**, and **6** vs **7** vs **8**). In particular, compound **4** shows a fairly good affinity for the I_2-IBS but, more importantly, an interesting selectivity versus the α_2-ARs.

The fact that the I_2-IBS affinity and the I_2/α_2 selectivity improves when introducing methylene groups in the chain contrasts with the results obtained for the naphthyl derivatives described in the literature.[24,35] In compounds such as phenyl-imidazoline, 1-naphthyl-imidazoline or 2-naphthyl-imidazoline, when a methylene group is introduced between the aromatic system

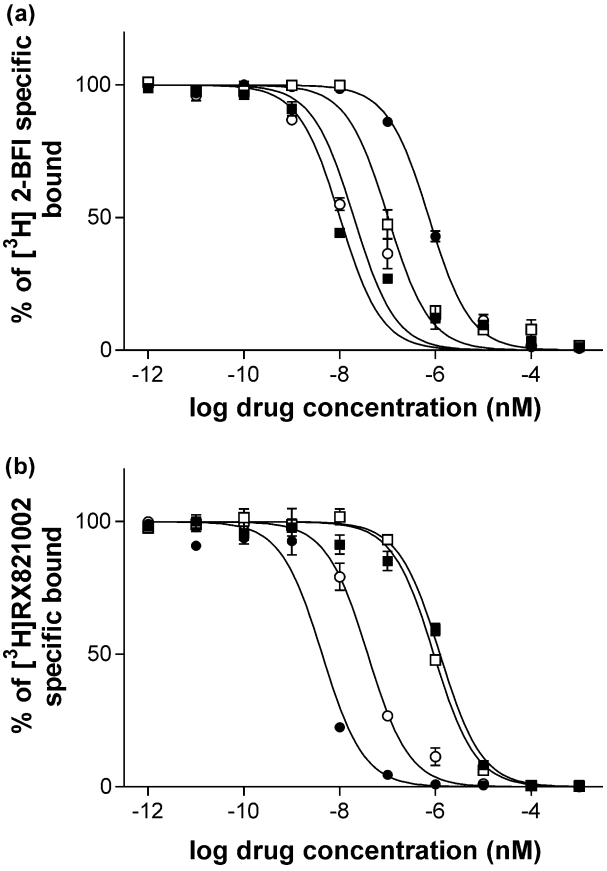


Figure 3. Inhibition of specific binding of [^{3}H]2-BFI (A) or [^{3}H]RX821002 (B) to human brain cortex membranes by Idazoxan (○), **13** (•), **8** (□) and **4** (■). Each point is the mean±SEM of four independent experiments.

Table 2. I_2-IBS and α_2-ARs binding affinities (pK_i) of the mono-guanidinium (**17**–**19**) derivatives

Compd	X-NH-R-CH_3		[^{3}H]2-BFI	[^{3}H]RX821002	
	X	R	pK_i I_2-IBS	pK_i α_2-ARs	Selectivity I_2/α_2[a]
Idazoxan	—	—	7.43±0.19	7.30±0.08	1.35
19	Gua[b]	–$(CH_2)_{11}$–	4.49±0.07	4.83±0.22	0.46
17	Gua	–$(CH_2)_7$–	<4.00	5.35±0.02	
18	Gua	–$(CH_2)_8$–	<4.00	4.84±0.24	

[a]Selectivity I_2-IBS/α_2-ARs expressed as the antilog (pK_i I_2-IBS−pK_i α_2-ARs).
[b]Gua:–C(=NH)–NH_2 amidine group.

and the imidazoline moiety, the α_2-adrenoceptor affinity automatically increases whereas the I_2-IBS affinity falls.[24,35]

All the compounds in both families that are studied show two cationic groups at each end. This fact could drive one to the conclusion that if two binding sites were close enough, this may allow both ends of the molecule to simultaneously bind to two separate sites. To address this possibility, we prepared three aliphatic analogues of compounds **2**–**4** with only one guanidine group at the end of the aliphatic chain (see Fig. 2, compounds **17**, **18** and **19**). We chose the aliphatic family and the guanidinium cation since those were the characteristics of the compounds providing better results (**2**–**4**, see Table 1). The three mono-guanidinium compounds were subjected to competition binding assays for both I_2-IBS and α_2-Ars. The results (see Table 2) show an extremely poor affinity towards any of the receptors. Given these affinity values obtained for the mono-guanidinium derivatives, it cannot be considered that the good affinity reached by the bis-guanidinium **2**–**4** comes from the simultaneous interaction with two close I_2-IBS.

Conclusion

This paper has reported two families of guanidine and 2-aminoimidazoline aliphatic or aromatic compounds. Many of them (**2**, **3**, **4**, **6**, **7**, **8**, **13**, **15**) show a good affinity towards the I_2-IBS and some of the aliphatic derivatives (**3**, **8**, and mainly **4**) show a very interesting selectivity I_2/α_2. The importance of the results obtained lies mainly in the chemical nature of these compounds because they have completely different structures than those of the I_2-IBS ligands reported to date. These include, 2-BFI, BU-224, BU-239,[23] and 2-naphthyl-imidazoline derivatives.[24] Whereas the compounds described in the existing literature as having affinity for the I_2-IBS and the pharmacophores always show the presence of an aromatic moiety (as in clonidine or in the BU-family, or the 2-BFI), our most active and selective compounds are aliphatic and have no aromatic ring in their structure. Our compounds are thus more alike to the agmatine molecule.

Due to the simple structure of these aliphatic compounds only few structure–activity relationships could be established. Regarding the linker used, the longer the aliphatic chain, the higher both the affinity and the selectivity. Concerning the number of cations present in the molecules, it was demonstrated that when removing a guanidinium cation from one of the ends of the molecule the affinity vanishes, and regarding the nature of the cations, the guanidinium one provided better results than the 2-aminoimidazolinium. This is consistent with previous findings in literature (see Introduction).[2] This seems to indicate that the higher the lipophilicity of these aliphatic derivatives the better the affinity. Nevertheless, the presence of two cationic groups seems to be important for the affinity. For all those reasons, future work is necessary on the preparation of more aliphatic analogues of **4** with longer chains as well as other cations (imidazoline) to replace the guanidinium. This would allow for estimation of the optimum length and cation for the I_2-IBS affinity and I_2/α_2 selectivity.

The originality of the compounds studied and the promising results obtained in terms of affinity and selectivity for the I_2-IBS open a new route in the search of I_2-IBS active compounds that show no affinity for α_2-ARs. At present, there is a need to develop drugs that avoid negative effects mediated by α_2-AR, such as sedation, blood pressure alterations or glucose level changes. As mentioned in the Introduction, many compounds are already available (i.e., 2BFI, BU224, 2-tolyl derivatives) which show high affinity and selectivity for I_2 over α_2. However, sometimes ligands with high affinity and selectivity do not induce any effect in vivo whereas less potent substances could be potential drug candidates. Therefore, the compounds reported in this paper show strong potential to aid in the development of a novel and different family of I_2-IBS selective drugs.

Experimental

Chemistry

Reagents were used as received. Reaction solvents were purchased anhydrous and used as received. Other solvents used were reagent grade. 2-Methylmercapto-4,5-dihydroimidazole iodide was synthesised according to the procedure already described by reaction of methyl iodide with ethylenethiourea in MeOH at reflux.[30] CAUTION: the noxious gas CH_3SH is produced during the synthesis of compounds **1**–**8**, **17**–**19** and it should be trapped with a concentrated aqueous solution of NaOH and, then, destroyed with sodium hypochlorite. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz (except when required) on a Varian Gemini 200 apparatus. Chemical shifts of the ^1H NMR spectra were internally referenced to the residual proton resonance in D_2O (δ 4.6 ppm). Chemical shifts of the ^{13}C NMR spectra were referenced with a capillary of DMSO-d_6 (δ 39.5 ppm) or by addition of a drop of methanol (δ 49.5 ppm). IR spectra were recorded on a FTIR spectrophotometer as KBr pellets. Melting points were determined with a Reichert-Jung Thermovar apparatus and are not corrected.

Diguanidine aliphatic derivatives: general method

A solution of 3 mmol of the corresponding diamine (**i**, **ii**, **iii**, or **iv**) and 6 mmol of *S*-methylisothiouronium sulphate in 2 mL of water was heated under reflux for 20 h. A white solid precipitated while cooling the solution. The solid was washed with water, acetone and recrystallised from hot water.

1,6-Hexanediguanidinium sulphate (1). White solid (57% yield); mp > 300 °C dec; IR(KBr): 3369, 3141, 2935, 1675, 1633, 1475, 1063 cm^{-1}; ^1H NMR (D_2O/CF_3CO_2H) δ 2.93 (t, 4H, $J = 7$ Hz); 1.34 (m, 4H); 1.12 (m, 4H); ^{13}C NMR (D_2O/CF_3CO_2H) δ 155.10, 39.5,

26.05, 23.81. Anal. calcd for $C_8H_{22}N_6O_4S$: C, 32.20; H, 7.43; N, 28.17; S, 10.75. Found: C, 32.28; H, 7.70; N, 28.40; S, 10.57.

1,8-Octanediguanidinium sulphate (2). White solid (42% yield); mp 309–315 °C (lit.:[29b] 314–317 °C); IR(KBr): 3390, 3180, 2950, 2870, 1660, 1485, 1120 cm^{-1}; ^1H NMR (D_2O) δ 2.94 (bt, 4H, $J = 7$ Hz); 1.5–1.25 (m, 4H); 1.25–1 (m, 8H); ^{13}C NMR (D_2O) δ: 158.1, 42.47, 29.38, 29.09, 26.95; MS (ES^+) *m/e* 229 (MH^+), 327 ($MH + H_2SO_4$). Anal. calcd for $C_{10}H_{26}N_6SO_4$: C, 36.80; H, 8.03; N, 25.75; S, 9.82. Found: C, 37.04; H, 8.31; N, 26.00; S, 9.89.

1,9-Nonanediguanidinium sulphate (3). White solid (40% yield); mp 297–305 °C (lit.[29b] 302–306 °C dec); IR (KBr): 3380, 3170, 2950, 2869, 1660, 1620, 1115, 1060 cm^{-1}; ^1H NMR (400 MHz/CF_3COOH/D_2O) δ 2.15 (t, 4H, $J = 6.96$ Hz); 0.56 (m, 4H); 0.4–0.2 (m, 10H); ^{13}C NMR (100 MHz/CF_3COOH/D_2O) δ 156.17 (Cq), 40.64 (t, $_2J_{C,D} = 12.2$ Hz), 27.86, 27.64, 27.24, 25.22; MS (ES^+) *m/e* 243 (MH^+), 341 ($MH + H_2SO_4$). Anal. calcd for $C_{11}H_{28}N_6SO_4$: C, 38.81; H, 8.29; N, 24.69. Found: C, 38.92; H, 8.44; N, 24.56.

1,12-Dodecanediguanidinium sulphate (4). White solid (69% yield); mp 250–254 °C (lit.[29b] 258–262 °C); IR (KBr): 3401, 2924, 1640, 1119 cm^{-1}; ^1H NMR (D_2O/CF_3CO_2H) δ 2.22 (b, 4H); 0.66 (bm, 4H); 0.37 (bs, 16H); ^{13}C NMR (D_2O/CF_3CO_2H) δ: 154.58, 39.3, 26.75, 26.48, 25.88, 23.97; MS (ES^+) *m/e* 285 (MH^+), 383 ($M + H_2SO_4$). Anal. calcd for $C_{14}H_{34}N_6O_4S$: C, 43.96; H, 8.96; N, 21.97. Found: C, 44.08; H, 9.21; N, 22.26.

Octylguanidinium sulphate (17). A solution of 2.073 g of octylamine (16 mmol, 1 equiv) and 2.72 g of *S*-methylisothiouronium sulphate (9.6 mmol, 0.6 equiv) in 15 mL absolute ethanol was heated at 100 °C for 18 h. The solvent was removed under vacuum and insoluble matter was filtered off. The crude oil was dissolved in EtOH/H_2O and left in a refridgerator. The solid that crystallised was rinsed with EtOH, Et_2O and dried under vacuum (1.25 g). White solid (35%); mp > 190 °C dec. (lit.[30b] 251–252 °C) ; ^1H NMR (CD_3OD) δ 3.1 (bm, 2H); 1.6 (bm, 2H); 1.5–1.2 (bs, 10H); 0.8 (bm, 3H); ^{13}C NMR (CD_3OD) δ 159.0; 42.8; 33.4; 30.79; 30.39; 28.22; 24.11; 14.84; LRMS (ES^+) *m/e* 172 ($M + H$/ 100%). Anal. calcd for $C_9H_{22}N_3O_2S_{0.5}$: C, 49.06; H, 10.06; N, 19.07. Found: C, 48.98; H, 10.94; N, 19.27.

Nonylguanidinium sulphate (18). A solution of 2.56 g of nonylamine (17.9 mmol, 1 equiv) and 3.05 g of *S*-methylisothiouronium sulphate (10.74 mmol, 1 equiv) in 15 m absolute ethanol was heated at 100 °C for 18 h. The solvent was removed under vacuum and insoluble matter was filtered off. The crude oil was dissolved in ^{i}PrOH/H_2O and left in a refridgerator. The solid that crystallised was rinsed with ^{i}PrOH, Et_2O and dried under vacuum (1.88 g). White solid (45%); mp > 220 °C dec; ^1H NMR (CD_3OD) δ 3.15 (bm, 2H); 1.6 (bm, 2H); 1.5–1.2 (bs, 12H); 0.9 (bm, 3H); ^{13}C NMR (CD_3OD) δ 159.03; 42.84; 33.49; 31.13; 30.9; 30.45; 28.28; 24.17; 14.9; LRMS (ES^+) *m/e* 186 ($M + H$/100%). Anal. calcd for $C_{10}H_{24}N_3O_2S_{0.5}$: C, 51.25; H, 10.32; N, 17.93. Found: C, 51.01; H, 10.96; N, 17.92.

Dodecylguanidinium sulphate (19). A solution of 2 g of dodecylamine (10.8 mmol, 1 equiv) and 3 g of *S*-methylisothiouronium sulphate (10.8 mmol, 1 equiv) in 100 mL of 50% H_2O in MeOH was heated at reflux for 3 h. The solution was allowed to cool down to room temperature and the white solid that precipitated was rinsed with MeOH and Et_2O. The product was recrystallised successively from CH_3CN and MeOH. The solid was rinsed with cold CH_3OH and cold Et_2O and dried under vacuum (833 mg). White solid (28%); mp > 220 °C dec. (lit.[30a] 230.5 °C); ^1H NMR (CD_3OD/TFA) δ 3.1 (t, 2H); 1.5 (m, 2H); 1.35–1.2 (bm, 20H); 0.8 (t, 3H); LRMS (ES^+) *m/e* 228 ($M + H$/ 100%). Anal. calcd for $C_{13}H_{30}N_3O_2S_{0.5}$: C, 56.48; H, 10.94; N, 15.20. Found: C, 56.20; H, 11.12; N, 15.34.

2-Aminoimidazoline aliphatic derivatives: general method

A solution of 4.75 mmol of the corresponding diamine (**i**, **ii**, **iii**, or **iv**) and 10 mmol of 2-methylmercapto-4,5-dihydroimidazole iodide in 15 mL of dry MeOH was heated under reflux for 2.5 h. Then, a stream of nitrogen was bubbled into the solution to remove CH_3SH. The solvent was eliminated under vacuum and the product was purified by the formation of the corresponding picrate salt. The crude residue dissolved in 50 mL of hot water was treated with a hot aqueous solution of picric acid (10 mmol) and the resulting mixture was allowed to stand at room temperature overnight. The yellow picrate salt that precipitated was washed successively with H_2O, Et_2O, and hexane, and then, crystallised from hot CH_3CN. The hydrochloride salts were generated with Amberlite IRA-400 (Cl^-form) exchange resin. For this purpose, the picrate salt was dissolved in H_2O/THF (300 mL) and stirred with the resin (previously washed) overnight. Exchange was complete when the yellow colour of the solution had disappeared. Then, the resin was removed by filtration and the solution concentrated under vacuum. The oily residue was taken up in a little water, washed twice with AcOEt and filtered through a Whatman (grade 4) filter. The product was lyophilised to afford the pure chloride salt.

N,N′-bis(4,5-Dihydro-1*H*-imidazol-2-yl)-1,6-hexanediamine (5). The product was isolated from the crude reaction mixture as the iodide salt by precipitation from MeOH/Et_2O: white solid (26%); mp 197–199 °C; IR (KBr): 3203, 2906, 1664, 1593, 1473, 1282, 1066 cm^{-1}; ^1H NMR (D_2O) δ 3.48 (s, 8H); 2.99 (t, 4H, $J = 6.8$ Hz); 1.5–1.25 (m, 4H); 1.25–1.00 (m, 4H); ^{13}C NMR (D_2O/ref DMSO) δ 158.86, 41.70, 41.50, 27.16, 24.46; MS (APCI$^+$): 253 (MH^+/100%). Anal. calcd for $C_{12}H_{26}N_6I_2$: C, 28.36; H, 5.16; N, 16.54. Found: C, 28.43; H, 5.02; N, 16.64.

N,N′-bis(4,5-dihydro-1*H*-imidazol-2-yl)-1,8-octanediamine (6). Hydrochloride salt: slightly orange hygroscopic gum (40% yield); IR (KBr): 3180, 3060, 2935, 2860, 1670, 1600, 1460, 1285, 1050 cm^{-1}; ^1H NMR

(D_2O) δ 3.47 (s, 8H); 2.97 (t, 4H, $J=6.8$ Hz); 1.45–1.25 (m, 4H), 1.2–1.0 (m, 8H); ^{13}C NMR (D_2O) δ 158.8 (Cq), 41.58, 41.47, 27.12, 24.63; MS (FAB^+) *m/e* 281 (MH^+, 100%), 317.3 (M + HCl, 50%). Anal. calcd for $C_{14}H_{30}N_6Cl_2$: C, 47.59; H, 8.56; N, 23.78. Found: C, 47.35; H, 9.20; N, 23.37.

***N*,*N*′-bis(4,5-Dihydro-1*H*-imidazol-2-yl)-1,9-nonanediamine (7).** Hydrochloride salt: slightly orange hygroscopic gum (40% yield); IR (KBr): 2940, 2860, 1670, 1600, 1470, 1290, 1060 cm^{-1}; ^1H NMR (D_2O) δ 3.48 (s, 8H); 2.98 (t, 4H, $J=6.8$ Hz); 1.45–1.25 (m, 4H), 1.25–1.0 (m, 8H); ^{13}C NMR (D_2O) δ 158.88 (Cq), 41.6, 41.5, 27.42, 27.16, 24.68; MS (FAB^+) *m/e* (MH^+, 100%), (M + HCl, 50%). Anal. calcd for $C_{15}H_{32}N_6Cl_2 . H_2O$: C, 46.75; H, 8.89; N, 21.80. Found: C, 46.66; H, 8.45; N, 21.37.

***N*,*N*′-bis(4,5-dihydro-1*H*-imidazol-2-yl)-1,12-dodecanediamine (8).** Hydrochloride salt: white hygroscopic solid (84% yield); IR (KBr): 3430, 2927, 1668, 1378, 1278, 1030 cm^{-1}; ^1H NMR (D_2O) δ 3.49 (s, 8H), 3.0 (t, 4H, $J=6.7$ Hz), 1.48–1.24 (m, 4H), 1.10 (bs, 16H); ^{13}C NMR (D_2O) δ 160.48, 43.23, 43.12, 29.26, 28.92, 28.80, 26.36; MS (ES^+) *m/e* 337 (MH^+), 373 (MH + HCl). Anal. calcd for $C_{18}H_{40}N_6Cl_2$: C, 52.58; H, 9.35; N, 20.52. Found: C, 52.80; H, 9.61; N, 20.43.

Pharmacology: materials and methods

Preparation of membranes. Neural membranes (P_2 fractions) were prepared by established methods[34] from the prefrontal cortex of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Briefly, the tissue samples were homogenised in 5 mL of ice-cold Tris sucrose buffer (5 mM Tris–HCl, 250 mM sucrose, pH 7.4). The homogenates were centrifuged at 1100*g* for 10 min, and the supernatants were then recentrifuged at 40,000*g* for 10 min. The resulting pellet was washed twice and resuspended in 50 mM Tris–HCl buffer (pH 7.5) to a final protein content of 0.83 ± 0.14 mg mL^{-1}.

[^{3}H]2-BFI binding assay. Total [^{3}H]2-BFI binding was measured in 0.55 mL aliquots (50 mM Tris–HCl, pH 7.5) of the neural membranes that were incubated with [^{3}H]2-BFI (1 nM) for 45 min at 25 °C in the absence or presence of the competing compounds (10^{-12} M to 10^{-3} M, 10 concentrations). Total binding was determined and plotted as a function of the compound concentration.

Incubations were terminated by diluting the samples with 5 mL of ice-cold Tris incubation buffer (4 °C). Membrane bound [^{3}H]2-BFI was separated by vacuum filtration through Whatman GF/C glass fibre filters. The filters were then 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.

[^{3}H]RX821002 binding assay. The binding of [^{3}H]RX821002 (1 nM) to brain cortical membranes was performed as described for the [^{3}H]2-BFI binding except in the incubation conditions (30 min at 25 °C).

Analysis of binding data. Analysis of competition experiments to obtain the inhibition constant (K_i) was performed by non-linear regression using the EBDA-LIGAND program. All experiments were analysed assuming a one-site model of radioligand binding. K_i values were normalized to pK_i values and expressed as mean ± standard error of the means.

Drugs. [^{3}H]2-BFI (specific affinity 70 Ci/mmol) was purchased from Amersham International, UK. [^{3}H]RX821002 (specific affinity 59 Ci/mmol) was obtained from Amersham International, UK. Idazoxan HCl was synthesised by Dr. F. Geijo at S.A. Lasa Laboratories, Barcelona, Spain. Other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

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