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On the search of new I_2-IBS aliphatic ligands: *Bis*-guanidino carbonyl derivatives

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Abstract—Continuing with our search of aliphatic dicationic derivatives as I2-IBS ligands and looking at Amiloride, a known ligand of I2-IBS, we have incorporated the guanidinocarbonyl moiety into our aliphatic compounds with the intention of improving the binding to I2-IBS. Thus, we present the different approaches to the preparation and pharmacological evaluation (in human brain tissue) as I2-IBS ligands of a new series of aliphatic derivatives incorporating the guanidinocarbonyl group and with different chain length (n = 8–12, and 14 methylene groups).

Imidazoline Binding Sites (IBS), only discovered two decades ago,[1] are involved in a large number of pharmacological functions and diseases. For example, there is evidence of their over-expression in the brain of Alzheimer's[2] and Parkinson's[3] disease patients as well as in human glial tumours.[4] On the other hand, it has been described that the ligands of the I_2-IBS subtype can attenuate tolerance when using opioids as analgesic therapy.[5] To date, most I_2-IBS ligands prepared always included a (hetero-)aromatic group structurally related to clonidine as we recently showed.[6] In fact, our group was the first to test aliphatic dicationic derivatives related to the endogenous IBS ligand, Agmatine, obtaining compound **1** (Fig. 1) that showed good I_2-IBS affinity and selectivity versus α_2-adrenoceptors (α_2-AR).[7]

Thus, continuing with our search of aliphatic dicationic derivatives as I_2-IBS ligands we have prepared a new series of compounds incorporating a different cationic moiety. Looking at Amiloride (Fig. 1), which shows a good affinity for I_2-IBS,[8] we have incorporated the guanidinocarbonyl moiety into our aliphatic derivatives, with the intention of improving the binding to I_2-IBS.

Hence, we present here the preparation and biological evaluation as I_2-IBS ligands of a new series of aliphatic derivatives, similar to **1**, incorporating the guanidinocarbonyl group and with different chain length (n = 8–12, and 14 methylene groups).

To synthesise compounds **2a** (n = 8) to **2f** (n = 14), several approaches could be considered such as those used by Schmuck[9,10] and Jansen[11] to synthesise pyrrole based receptors and analogues of Sorangicin A, respectively.

Starting from the commercial dicarboxylic acids (**3**), the corresponding methyl esters (**4**) were obtained in good yield by reaction with thionyl chloride in methanol. Then, following Schmuck method,[9] reaction with guanidine, in the presence of sodium methoxide (Scheme 1),

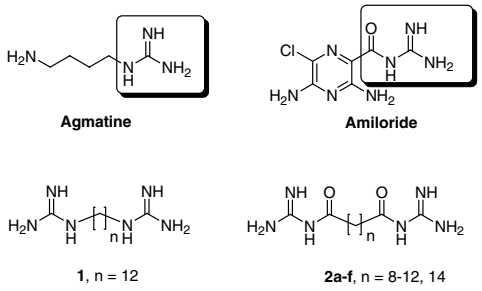

Figure 1.

Keywords: I2-IBS; Aliphatic; Amiloride; Guanidinocarbonyl.
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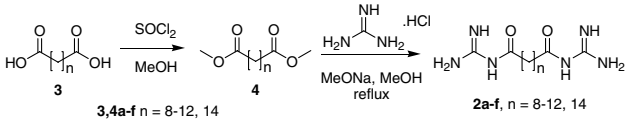


Scheme 1.

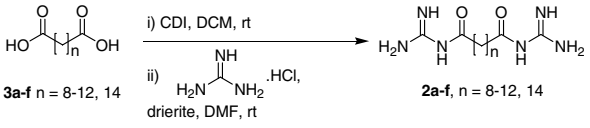
yielded the corresponding *bis*-guanidinocarbonyl derivatives.[12] Unfortunately, the yields obtained were disappointing (highest yield obtained was 20% for **2a**). Probably these low yields are a consequence of the formation of ketoesters through a Claisen condensation reaction.

Following Jansen's procedure[11] a second synthetic route was attempted, which involved first the activation of the carboxylic acids by means of CDI and then the use of guanidine as guanidilating agent (Scheme 2).[13] This method provided better yields as presented in Table 1.

The hydrochloride salts of the neutral alkyl *bis*-guanidinylamides were obtained by dissolving the corresponding compounds (1 mmol) in DCM (15 ml) and then bubbling with HCl gas. The salts precipitated out of the solution and were recrystallized from methanol.

To improve the yields obtained, and based in another method that had proved successful for Schmuck et al.[10] using PyBOP as coupling reagent, we prepared the alkyl *bis*-guanidinylamide derivatives utilizing BOP as the coupling reagent and the *N*-Boc-mono-protected guanidine for the guanidilation step, as previously reported by Zapf[14] (Scheme 3).[15]

The Boc groups were removed by treatment with TFA in DCM at room temperature during 6 h and, then, the HCl salts were obtained, in good yields (Table 1), by treatment with an excess of basic anion exchange resin (IRA-400, Chloride form) in water, at room temperature during 12 h. Absence of the trifluoroacetate salt was checked by ^{13}C NMR.

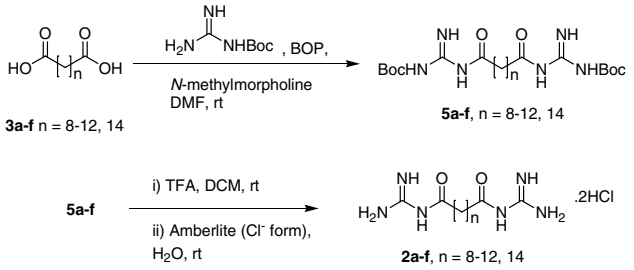


Scheme 2.

Table 1. Total yields obtained for the preparation of the HCl salts (isolated pure products) of the alkyl guanidinylamides **2** calculated from the reactions shown in Schemes 1–3

	Scheme 1 (% yield)	Scheme 2 (% yield)	Scheme 3 (% yield)
2a ($n = 8$)	20	41	74
2b ($n = 9$)	9	45	50
2c ($n = 10$)	17	38	76
2d ($n = 11$)	15	34	74
2e ($n = 12$)	11	43	60
2f ($n = 14$)	10	41	48

The full characterization of compound **2a** is presented in Ref. 16.



Scheme 3.

The pharmacological affinity of the prepared compounds was evaluated through competition binding studies against the selective I_2-IBS radioligand [^{3}H]-2-[(2-benzofuranil)-2-imidazoline] (2-BFI).[17] The studies were performed in membranes from post-mortem human frontal cortex, a brain area that shows an important density of I_2-IBS.[2,18] The inhibition constants (K_i) for each compound were obtained and are expressed as the corresponding pK_i in Table 2. Idazoxan, a compound with well-established affinity for I_2-IBS, and the alkyl *bis*-guanidine derivative **1**, previously prepared by us,[7] were used as references.

Unfortunately, the affinity for the I_2-IBS of this new series of compounds did not improve compared to the lead compound **1**. This was a disappointing result considering the good I_2-IBS affinity showed by both Amiloride (p$K_i = 6.8$)[8] and our lead compound. Moreover, in the guanidinium series[7] we found a good correlation between the length of the aliphatic chain and the I_2-IBS affinity. However, in this series no relation at all was found between the length of the linker chain and the affinity for the I_2-IBS receptors.

It seems that the introduction of the carbonyl groups at both cationic sides of the aliphatic chains could interfere with the interaction between the guanidinium group and the residues in the actual I_2-IBS binding pocket. Maybe the intramolecular formation of a hydrogen bond (HB) between one of the H atoms of the guanidine and the O atom of the carbonyl group is preventing the delocalisation inherent to the guanidinium moiety that provides this particular functional cation with special chemical characteristics to establish both ionic and HBs interactions.

For that reason, we have performed DFT computations[19] of models of the guanidinylamides in both an aromatic

Table 2. Affinity of the alkyl guanidinylamides prepared towards I_2-IBS measured as pK_i values

Compound	pK_i ([^{3}H]2-BFI)
Idazoxan	7.43
1	7.48
2a ($n = 8$)	6.11
2b ($n = 9$)	5.20
2c ($n = 10$)	5.69
2d ($n = 11$)	5.06
2e ($n = 12$)	5.91
2f ($n = 14$)	5.70

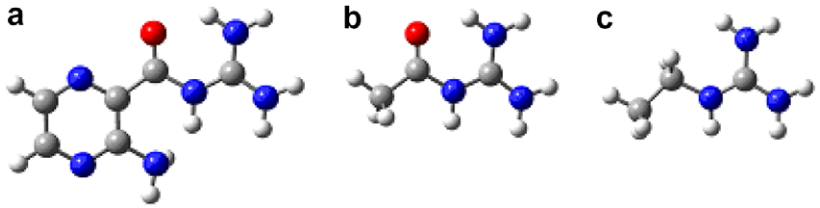


Figure 2. Optimised structures of *N*-(3-amino-pyrazine-2-carbonyl)-guanidinium (a), *N*-acetyl-guanidinium (b) and ethylguanidinium (c) optimised at B3LYP/6-31+G* level.

environment similar to that of Amiloride (*N*-(3-amino-pyrazine-2-carbonyl)-guanidinium, **A**) and an aliphatic environment such as that of the molecules presented in this paper (*N*-acetyl-guanidinium, **B**), as well as the ethyl-guanidinium (**C**) as a model of compound **1**.[20] All optimised structures at B3LYP/6-31+G* level are shown in Figure 2. The presence of HB has been assessed following the Atoms in Molecules (AIM) theory.[21]

In the aliphatic guanidinium **C** it is obvious that no intramolecular HB is established, the cation is free to rotate and can freely interact with the binding pocket of the I_2-IBS. The characteristics of the guanidinium cation in terms of planarity, acidity and electronic properties are unaffected. However, in the aromatic guanidinylamidinium **A** (our model for Amiloride) and in the *N*-acetyl-guanidinium **B** the C=O is in the same plane and conjugated to the guanidinium group, and in the aromatic case **A**, both groups are rotated out-of-plane with respect to the pyrazine ring by 16°. This distortion does not affect the strong conjugation between the guanidinium and the C=O group, and in fact in both derivatives a similar and very strong intramolecular N–H···O HB is formed between one of the NH of the guanidinium and the carbonyl oxygen. This strength is reflected in the short HB distances: 1.86 Å in both cases, high values of electron density at the bond critical point ρ(BCP): 0.0344 and 0.0343 a.u. for **A** and **B** respectively, and a positive Laplacian of ρ(BCP) in both cases ($\nabla^2\rho$(BCP) = 0.117 a.u. in both derivatives).[21] In the case of the amiloride-like derivative **A**, an extra HB is found between the NH_2 group in the pyrazine ring (acting as an acceptor) and one of the NH of the guanidinium moiety (d[N–H···N] = 1.88 Å, ρ(BCP): 0.0379 a.u., $\nabla^2\rho$(BCP) = 0.103 a.u.).

Hence, it seems that the optimal interaction between the guanidinium derivatives and the I_2-IBS occurs in those compounds where the guanidinium cation is not conjugated or interacting with any other functional group. That will explain that the aliphatic *bis*-guanidinium derivative **1** shows the best pK_i. Those compounds where the guanidinium cation is forming an intramolecular HB with an adjacent carbonyl group showed poorer pK_i values and the better performance of Amiloride could be explained by other type of interactions in the binding site such as π–π interactions or hydrogen bonding through the 3- or 5-NH_2 groups in the pyrazine ring.

Summarizing, we have presented here different synthetic approaches towards the preparation of aliphatic guanidinylamide derivatives, the results of the biological evaluation of the affinity of these compounds towards the I_2-IBS, and computational results to rationalize this affinity values, concluding that the introduction of a conjugated carbonyl group to the guanidinium moieties does not improve the affinity towards I_2-IBS due to the formation of an intramolecular HB.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.093.

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12. Reagents, conditions and yields: (a) Corresponding carboxylic acid (1 equiv) and MeOH (20 ml), 0 °C; $SOCl_2$ (2.2 equiv) added dropwise, rt, 6 h, 80–97%; (b) Guanidine hydrochloride (10 equiv), NaMeO (10 equiv), MeOH, reflux during 72 h.
13. Reagents and conditions: i—Corresponding carboxylic acid (1 equiv), CDI (2 equiv), DCM, rt, 1 h; ii—guanidine

hydrochloride (10 equiv), drierite (2 equiv), DMF (2 ml), rt, 20 h.
14. Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M. *Org. Lett.* **2001**, *3*, 1133.
15. Reagents and conditions: Corresponding carboxylic acid (1 equiv), DMF (20 ml), Boc-guanidine (4 equiv), BOP (2.4 equiv), *N*-methylmorpholine (6 equiv), rt, 12 h.
16. Full characterization of compound **2a**:*Dihydrochloride salt of 1,8-octane bisguanidinylamide*: White solid; mp 241–243 °C; IR (neat) ν 3418, 1730, 1600 cm^{-1}; ^1H NMR (D_2O) δ 1.17–1.21 (m, 8H), 1.45–1.54 (m, 4H), 2.36 (t, J = 6.5 Hz, 4H); ^{13}C NMR (D_2O) δ 23.8, 27.4, 27.5, 36.3, 154.1, 177.2; MS (ESI^+) *m/z* 285.2039 $[M+H]^+$. Anal. ($C_{12}H_{26}Cl_2N_6O_2 \cdot 0.5H_2O$) Calcd C, 39.35; H, 7.43; N, 22.94. Found: C, 39.09; H, 7.26; N, 22.51.
17. Descriptions of the preparation of membranes, [^{3}H]2-BFI binding assay and analysis of binding data are provided in Supplemental information.
18. Garcia-Sevilla, J. A.; Escriba, P. V.; Sastre, M.; Walzer, C.; Busquets, X.; Jaquet, G.; Reis, D. J.; Guimon, X. J. *Arch. Gen. Psychiat.* **1996**, *53*, 803.
19. The hybrid Density Functional Theory (DFT) method B3LYP has been used with the 6-31+G* basis set, and the electron density has been analysed using the AIMPAC set of programs. Both are incorporated in the Gaussian03 set of programs. Complete Computational methods and reference for Gaussian03 can be found in the Supplemental Information.
20. Details of the computational results could be supplied upon request.
21. According to the theory of atoms in molecules (AIM) proposed by Bader [Bader, R. F. W. *Atoms In Molecules. A Quantum Theory*; Oxford University: New York, 1990] a bond or interaction between a pair of atoms can be characterized by the properties of the electron density in a particular point known as the Bond Critical Point (BCP) which corresponds to a saddle point in the electron density surface between these two atoms. Thus, a electron density [ρ(BCP)] around 10^{-2} a.u. and a positive Laplacian of the charge density [$\nabla^2\rho$(BCP)] at the BCPs correspond to what is defined as 'closed-shell' interaction of the HB type.