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Research paper

Substituted conformationally restricted guanidine derivatives: Probing the α_2-adrenoceptors' binding pocket

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α_2-adrenoceptors' antagonists
Human brain tissue *in vitro* experiments

ABSTRACT

In this paper we report the design, synthesis and pharmacological evaluation of new *N*-substituted 2-amino-1,4-dihydroquinazolines, 2-amino-1,4-dihydropyridopyrimidines and 2-amino-4,5-dihydro-1,3-benzodiazepines as α_2-adrenoceptors ligands. Computational studies show that the proposed substitutions and guanidine-containing ring size will probe an extensive area of the active site. Preparation of these molecules involved novel routes than those previously utilised in our laboratory for the preparation of the acyclic aryl-guanidine counterparts. Compounds **8b** and **18c** showed the highest affinity and antagonistic activity, within their series, towards the α_2-adrenoceptor in human brain tissue *in vitro* experiments. Structure-activity relationships have been established for the design and biological evaluation of novel α_2-adrenoceptor ligands.

1. Introduction

The evident necessity to develop novel therapeutics to treat depression has been highlighted by the World Health Organisation, who has listed depression to be the second largest global health burden by the year 2020 [1]. Pharmacological manipulation of monoamine (noradrenaline −NA-, serotonin) transmission constitutes the mechanism of all current clinical antidepressants through the normalisation of chemical levels and, hence, the alleviation of depressive symptoms. Furthermore, it is now accepted that the monoamine-based agents incidentally activate downstream signalling cascades to promote the strengthening of synapses through essential transcription factors, growth factors and synaptic proteins [2]. These actions stimulate an overall protecting effect on neuronal plasticity and neurogenesis, and cause the behavioural antidepressant response observed within 4–6 weeks [3].

Moreover, alterations in noradrenergic signalling as well as changes in physical features of noradrenergic plasticity are heavily implicated in stress-related disorders such as depression. Central noradrenergic transmission is regulated by inhibitory pre-synaptic α_2-adrenoceptors (α_2-ARs), which activation inhibits NA release in the brain while blockade by antagonism increases NA neurotransmission. Dysfunction of noradrenergic transmission in depression has been hypothesised to be directly linked to the α_2-AR, as an increase in receptor density in patients with depression has been demonstrated through *in vitro* and *in vivo* pharmacological assays as well as patient models, where antidepressant treatment normalise this effect [4]. Moreover, regarding secondary effects on plasticity, α_2-AR antagonists have directly shown to induce the expression of the early immediate synaptic protein, Arc, as well as the stimulation of neurogenesis in the hippocampus [5].

In the past twelve years, our group have worked on the preparation and pharmacological evaluation of different ligands targeting the α_2-AR. Over 100 compounds have been developed, all sharing the common structural feature of an aromatic guanidinium or 2-aminoimidazolinium with varying substitution patterns that

List of abbreviations: NA, noradrenaline; α2-ARs, α2-adrenoceptors; DFT, Density Functional Theory; M06-2X, Truhlar M06 suite of density functionals; 6-31 + G**, Contracted Gaussian basis sets with diffuse and polarization functions; PCM, Polarizable Continuum Method for solvation; PFC, human brain prefrontal cortex; [3H]RX821002, 2-methoxyidazoxan; pKi, minus logarithm of the affinity constant; [35S]GTPγS, radioligand guanosine 5′-*O*-[gamma-thio]triphosphate.

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were found to be beneficial to both binding affinity and antagonist activity at the α_2-AR (**1**, Fig. 1) [6–8]. The chemistry of guanidine derivatives is of high interest as shown by the many works reported in the literature. For example, Ishikawa's group has extensively and thoroughly studied guanidines as chiral auxiliaries (e.g. ChibaG) their ylide derivatives for aziridine formation, the affinity of *bis*-guanidine for proton and metal salts or the potential chirality of *bis*-guanidine [9]. Also, Coles' group have looked into different aspects of Guanidines from synthesis to medicinal chemistry applications as well as physicochemical studies [10]. Recently, Keppler has published guanidine complexes of Pt as DNA binders and potential anticancer agents [11]. Another important contribution to the guanidine field is that of Sinha who has prepared different guanidine derivatives as protease and cell growth inhibitors [12].

One of the main difficulties in the development of new derivatives of these α_2-AR ligands is the conflicting functional activity (agonist/antagonists) amongst very similar structural derivatives. In recent years, we have gained further insight into what structural features are important for α_2-AR antagonism through the development of a 3D pharmacophore [13]. This lead to the introduction of the *N*,*N*′-disubstituted guanidine series as it was highlighted that hydrophobic steric extension at the cationic moiety displays a degree of preference for antagonist activity (**2**, Fig. 1). Recently, we have published on a series of conformationally controlled analogues of our previous lead compounds, namely pyridin-2-yl guanidines (**3**, Fig. 1), 1,4-dihydroquinazolin-2-amines (**4**, Fig. 1) and 1,4-dihydropyrido-pyrimidin-2-amines (**5**, Fig. 1), which consistently showed antagonistic or inverse agonist activity [14].

As mentioned, compounds displaying some degree of conformational restriction (compounds **3**–**5**) were found to exhibit antagonist or inverse agonist activity; however, in compounds **4** and **5**, the further conformational restrain in the number of rotatable bonds induced by the bicyclic system causes a significant drop in the overall α_2-AR affinity. We postulated that this was due to the rigidity induced by the methylene linkage, which is preventing optimum alignment or positioning of *N*-substituents R within the binding pocket of the receptor. Thus, we now propose to probe that particular area of the receptor to investigate if alteration in the position of the *N*-substituents R on the molecule would improve interactions in the receptor space. First, we propose to study an analogous family to compounds **4** and **5**, where the *N*-substituent R is moved from the exocyclic amino group to the guanidine endocyclic amino (**6** and **7**, Fig. 2).

Moreover, we propose the study of 2-amino-4,5-dihydro-1,3-benzodiazepines, which incorporate an extra CH_2 group in the cyclic-guanidine moiety in order to recover some of the flexibility of the system and allow the freedom of movement of the *N*-substituent within the binding pocket while still displaying a certain amount of conformational constrain (**8**, Fig. 2). These compounds **8** will include *N*-substitution in the exocyclic nitrogen of the

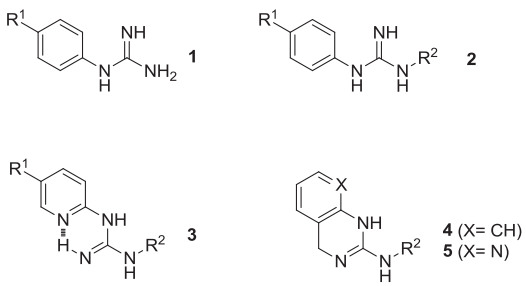


Fig. 1. General structures of families of α_2-AR ligands previously developed by Rozas and co-workers [6–8,14].

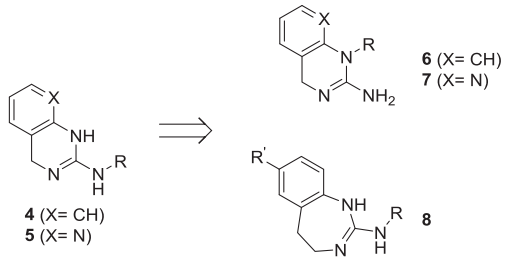


Fig. 2. Proposed structures of potential α_2-AR antagonists: N^1-substituted 2-amino-1,4-dihydroquinazolines (**6**) and 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines (**7**), and N^2-substituted 2-amino-4,5-dihydro-1*H*-benzo[d] [1,3]diazepines (**8**).

guanidine, similarly to our previous compounds, as well as substitution on the aryl ring *para* to the cationic moiety, based on functional groups that have augmented α_2-AR affinity in compounds **1**, **2** and **3**.

2. Results and discussion

2.1. Modelling

To assess the diversity in the space explored by the *N*-substituent, R, in the different positions available for compounds **4**–**8**, computational studies have been carried out for model compounds **4**, **6** and **8**, with R = propyl, in their cationic state (the expected at biological pH), taking into account different possible conformations [(i) and (ii)] around the substituted N atom and using the Density Functional Theory (DFT) method M06-2X [15] with the 6-31 + G** basis set [16] and the PCM solvation model [17] for water.

The optimised structures of **4**, **6** and **8** (Fig. 3a, total and relative energies in Table S1 in Supporting Information) show two possible energy minima [(i) and (ii)] depending on the position of the *N*-substituent. These minima, which differ only 0.7, 2.7 and 1.8 kJ mol^{-1} for compounds **4**, **6** and **8**, respectively, show that the propyl substituent used in this model, can be oriented towards different areas of space covering a wide range of possible interactions in the active site and hence probing this binding area as can be seen when superimposing the aromatic ring of all the minimum energy conformations obtained (Fig. 3b, up and bottom).

2.2. Chemistry

Guanidines ability to form multiple interactions in the body (ionic/hydrogen bonding and π-cation stacking) makes it an ideal functional moiety for small molecule drugs. Accordingly, many methods have been described to facilitate guanidylation of both alkyl/aryl amines in the preparation of monocyclic and acyclic guanidines [18]. However, there is less precedence for the synthesis of guanidines incorporated into a bicyclic backbone and displaying varying substituents.

The synthesis of N^1-substituted 2-amino-1,4-dihydroquinazolines (**6a**-**c**) and 2-amino-1,4-dihydropyrido[2,3-*d*] pyrimidines (**7a**-**c**) involves the initial preparation of diamines **9a**-**c** and **10a**-**c**, which require the preparation of the corresponding nitriles **11a**-**c** and **12a**-**c** from the available substituted aryl halides (Scheme 1). The *N*-substituted 2-cyanoanilines **11a**-**c** were synthesised employing Buchwald-Hartwig amination from 2-bromobenzonitrile using $Pd_2(dba)_3$ (5 mol%), BINAP (3 mol%), and NaO*t*-Bu in toluene at 100 °C for 24 h, whereas nucleophilic substitution of 2-chloro-3-cyanopyridine afforded the *N*-substituted-2-amino-3-cyanopyridines **12a**-**c** (Scheme 1). Next, all nitrile derivatives were reduced to the corresponding diamines **9a**-**c** and

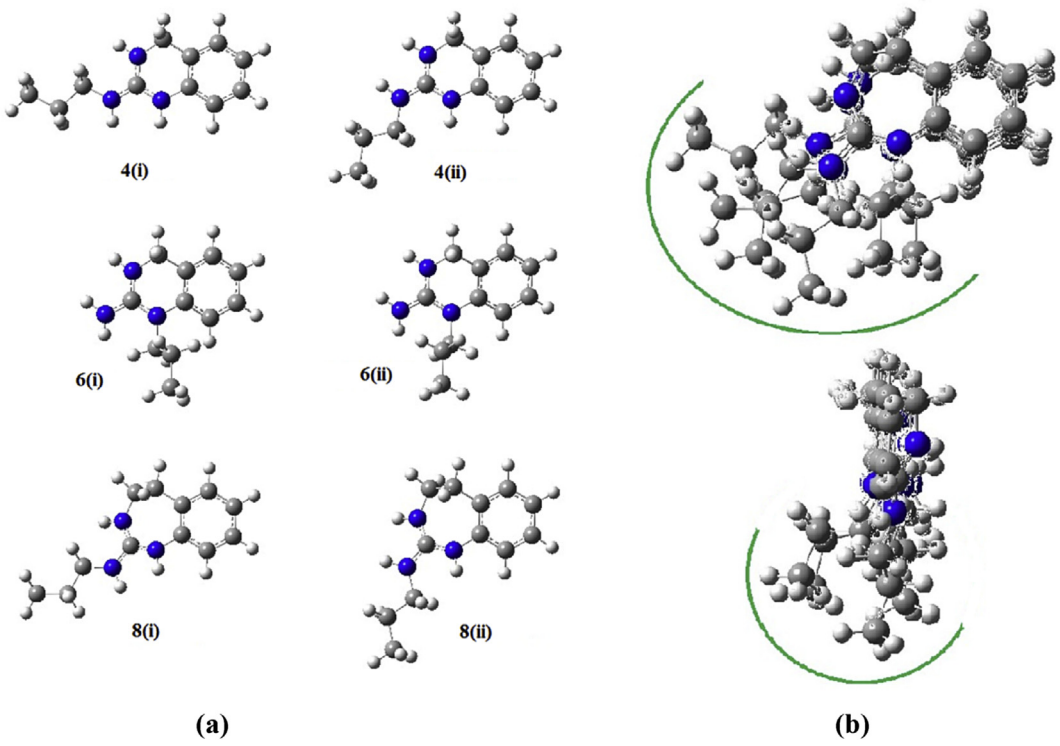
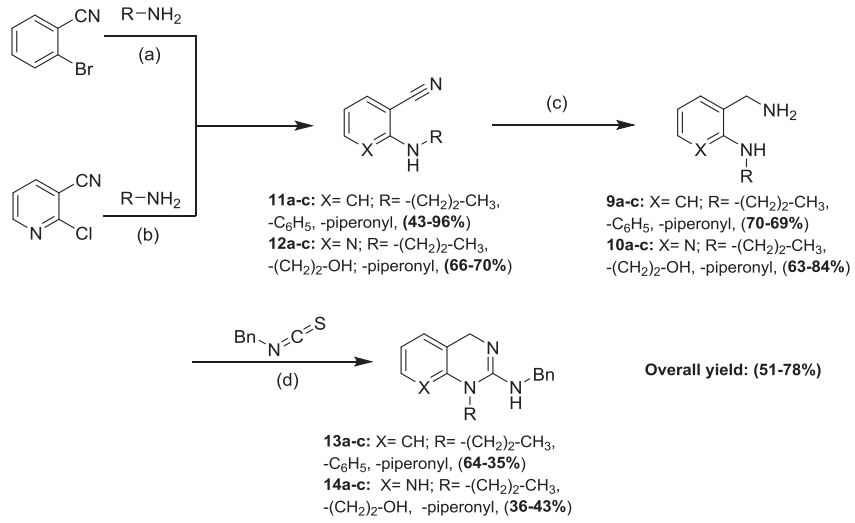


Fig. 3. (a) Optimised (M06-2X/6-31 + G**, PCM-water) minimum energy conformations (i) and (ii) obtained for compounds **4**, **6** and **8** with R = propyl. (b) Superimposition of all the optimised structures over the phenyl ring: *top*, frontal view; *bottom*, 90° rotated view.

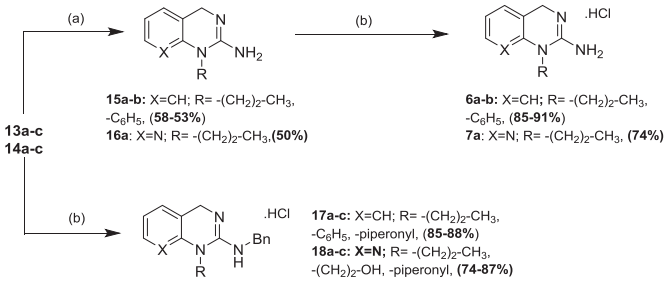


(a) $Pd_2(dba)_3$; BINAP; NaO^tBu; toluene; 100 °C. (b) NEt_3; THF; 70 °C. (c) $LiAlH_4$; THF; 70 °C. (d) $HgCl_2$; NEt_3; CH_2Cl_2; r.t.; then NaOH wash

Scheme 1.

10a-c using lithium aluminium hydride. Cyclisation to the benzyl protected cyclic guanidine intermediates **13a-c** and **14a-c** was facilitated *via* mercury (II) chloride promoted cyclo-desulphurisation after initial thiourea formation using benzylisothiocyanate (Scheme 1).

Since these benzyl intermediates were isolated and purified, it was decided to include them in the pharmacological study as potential ligands for the α_2-AR. Thus, they were protonated to the guanidinium hydrochlorides **17a-c** and **18a-c** (by using hydrochloric acid/1,4-dioxane solutions) to ensure high water solubility for *in vitro* testing (Scheme 2). Removal of the benzyl group was initially attempted through unsuccessful Pd catalysed hydrogenation under acidic conditions using extremely high hydrogen pressures (16 atm) and long reaction times. *N*-Debenzylation of guanidines or guanidine-like compounds, such as amidines, is unprecedented in the literature with the exception of one example



Scheme 2.

described by Townsend et al. [19] These authors reported attempts of *N*-debenzylation of guanidines using staggering pressures, up to 34 atm, with no conversion to product observed. They eventually used cyclohexene as a hydrogen transfer source with palladium oxide refluxing in MeOH affording their desired product in 20% yield. In our hands, we found that higher yields were obtained by refluxing the appropriate benzyl guanidine in MeOH using excess NH_4HCO_2 as an *in situ* hydrogen source (Scheme 2). Unfortunately, under these conditions the benzyl-like piperonyl substituent in compounds **13c** and **14c** was also cleaved. Moreover, compound **14b** also degraded under these conditions to various unidentified side products. Guanidines **15a**-**b** and **16a** were protonated to the corresponding hydrochloride salts **6a**-**b** and **7a** using hydrochloric acid/1,4-dioxane (Scheme 2).

The synthesis towards the substituted 2-amino-4,5-dihydro-1,3-benzodiazepines **8a**-**f** similarly involved the preparation of the appropriate diamines (Scheme 3). Thus, compounds **21a**-**b** [R = H, −3,4(−OCH_2O−)] were synthesised from the available 2-acetonitrilenitrophenyl derivatives **19a**-**b** by successive reductions using BH_3. DMS and catalytic hydrogenation.

The cyclisation to compounds **22a**-**f** was achieved using a modified procedure of Das et al. [20], which involved the coupling of *S*-methyl-*N*-substituted dithiocarbamates (synthesised from the corresponding amines using carbon disulfide and iodomethane) with the diamine (**21a**-**b**) in the presence of copper (II) oxide, with subsequent protonation of the products using 4 M HCl/dioxane (Scheme 3). Unfortunately, compounds **22a**, **22d** and **22f** were found to be extremely acid sensitive and attempts at forming the corresponding hydrochloride salts using low concentrations of acid were unsuccessful. Since the free bases are not water soluble these analogues were exempt from pharmacological testing.

Guanidines **8d**-**f** (R^1 = NMe_2) were prepared in an alternate manner (Scheme 4) as different attempts at reducing nitro derivative **27** to yield the desired diamine, gave a range of highly polar side products impossible to identify.

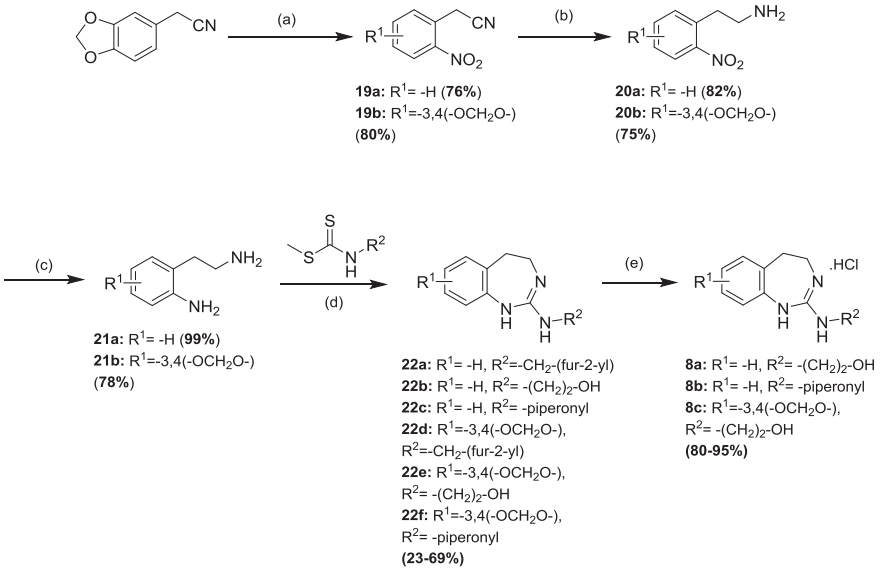
This result could be attributed to a combination of both the aliphatic nature of the *para* amine and its potential amino:iminium tautomerism. Thus, compound **27** was primarily reacted with *S*-methyl-*N*-substituted dithiocarbamates to form thioureas **28a**-**b**. Reduction to the corresponding anilines **29a**-**c** using tin (II) chloride, and subsequent cyclisation using mercury (II) chloride produced the guanidinium hydrochlorides **8d**-**f** *in situ* (Scheme 4).

2.3. Pharmacology

2.3.1. [^{3}H]RX821002 competitive binding assays

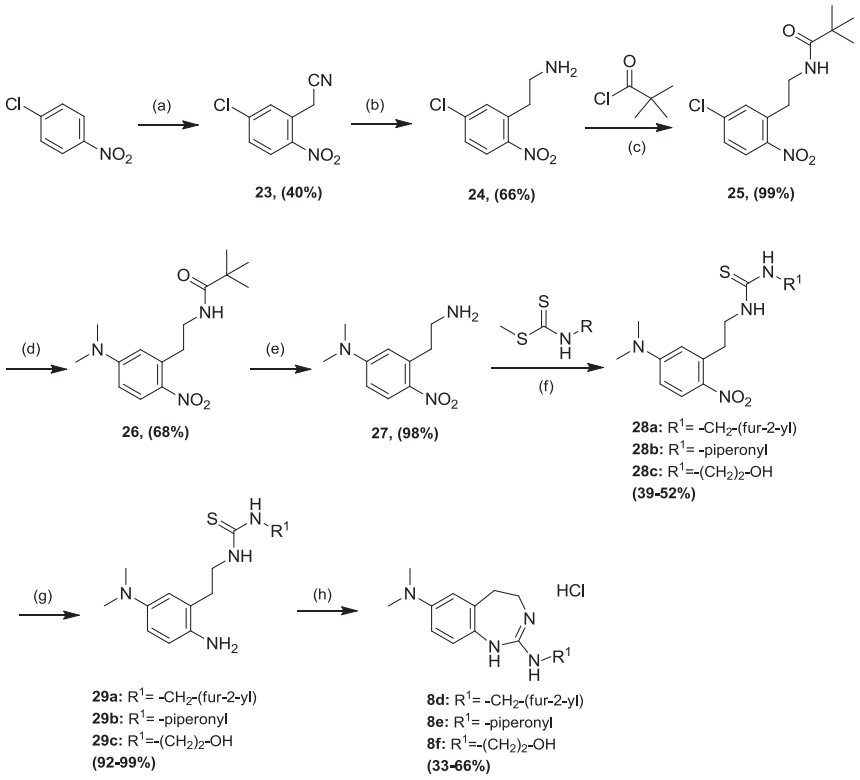
The affinity of all compounds toward the α_2-AR was measured *in vitro* using post-mortem human brain prefrontal cortex (PFC) tissue by competition assays against the selective radioligand [^{3}H] RX821002 (2-methoxyidazoxan). In the assay, the prepared membranes are incubated with the radioligand (used at a constant concentration of 1 nM) and, then, varying the concentrations of the testing compounds, the displacement of the radioligand affords the affinity of each compound for the α_2-AR. The affinity results for all cyclic guanidine hydrochlorides are expressed as pK_i and are presented in Table 1.

From Table 1 is evident that the1,4-dihydropyridopyrimidine derivatives (**18a**,**c** and **7a**) cause a major drop in affinity



(a) 70% HNO_3/HOAc, 0 °C - 40 °C. (b) 2M BH_3.DMS, THF, 0 °C - 70 °C. (c) H_2, Pd/C, 3 atm, 4 hr. (d) CuO (0.2 eq.), K_2CO_3 DMF, 60 °C. (e) 4M HCl/Dioxane, r.t

Scheme 3.



(a) (1) chloroacetonitrile, KOtBu; (2) H_2SO_4 (10%) THF -40 °C. (b) 2M BH_3.DMS, THF, 0 °C - 70 °C. (c) NEt_3, THF, r.t. (d) Me_2NH.HCl, NEt_3, EtOH, 130 °C. (e) 8M HCl/H_2O, 100 °C. (f) CuO (0.12 eq.), K_2CO_3, DMF, 60 °C. (g) $SnCl_2.2H_2O$, EtOH, Reflux. (h) $HgCl_2$, NEt_3, CH_2Cl_2, r.t.

Scheme 4.

compared to their corresponding phenyl analogues (**17a**,**c** and **6a**), which overall show better affinity (except for **17b**). However, when compared to some of the previously prepared compounds **5** [14], derivatives **18** and **7** show relatively stronger binding, suggesting that the change in the substitution positioning is favourable and that interaction in the receptor area probed by the substituents R of compounds type **6** and **7** (as suggested by the computational study) is more important for the α_2-AR affinity. This was confirmed as the 1,4-dihydroquinazolines **6a**,**b** also exhibit improved binding affinities compare to compounds **4** [14]. Surprisingly, the majority of the *N*-benzyl intermediates (**17a**-**c** and **18a**-**c**) also showed enhanced affinities compared to the previously prepared compounds **4** and **5** [14]. In general, the 1,3-benzodiazepine derivatives (**8a**-**f**) gave overall higher binding affinities across the series compared to their 6-membered cyclic analogues. It is notable though that the derivatives that were *para* substituted to the cationic moiety on the aromatic ring (**8c**-**f**) do not show substantial differences in binding compared to their unsubstituted counterparts (**8a**-**b**). The difference in α_2-AR affinities across the series is thought to be attributed to ring flexibility or the altered substitution positions on the ring (orientating the substituents to different areas of the α_2-AR binding site as indicated in the computational study), not to the nature of the substituents themselves. However, it is notable that the piperonyl moiety of compounds **8b**, **8e**, **17c** and **18c**, lead to significantly higher affinities than all other substituents within each series.

2.3.2. [^{35}S]GTPγS binding functional assays

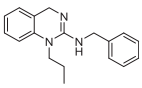
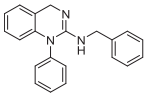
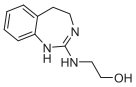
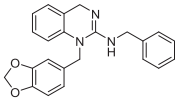
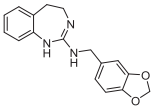
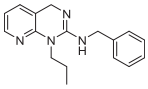
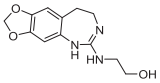
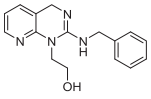
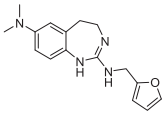
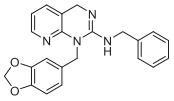
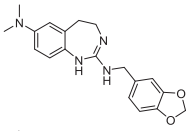
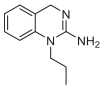
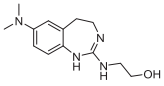
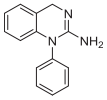
Some of the compounds displaying the strongest α_2-AR affinity within each series (**18c** and **8b**) were subjected to *in vitro* [^{35}S]GTPγS binding experiments in human PFC membranes to determine their activity as agonists or antagonists. Activation of the α_2-AR by an agonist leads to the dissociation of GDP-Gα, followed by GDP-GTP exchange and subsequent modulation of downstream effectors. The direct evaluation of the degree of G-protein activation upon ligand binding can be made by determining guanine nucleotide exchange using radiolabeled GTPγS ([^{35}S]GTPγS). In this manner an agonist will stimulate an increase in [^{35}S]GTPγS, whereas an antagonist will cause no stimulation as it induces no activity at the receptor. As expected, the restrained conformation of **18c** and **8b** induced antagonist activity through no stimulation of [^{35}S]GTPγS.

2.4. Structure-activity relationship

The results obtained give a detailed insight towards a comprehensive structure affinity relationship for conformationally restricted guanidine α_2-AR ligands, and a summary is presented in Fig. 4.

The most noticeable observation is the consistent drop in α_2-AR affinity across the entire series when pyridine is used as a bioisostere for benzene. It was initially thought that the pyridine ring may give additional interactions at the receptor though its hydrogen bonding accepting capability, as many of the previously prepared unrestricted pyridoguanidine derivatives gave improved affinities against their phenyl counterparts. Nonetheless, in the case of the conformationally restricted series here studied this is not

Table 1
Affinity of all compounds prepared for the α_2-AR ($pK_i \pm SEM$).[a]

Cmp.	Structure	pK_i	Cmp.	Structure	pK_i
17a		5.29 ± 0.08	**7a**		4.99 ± 0.10
17b		4.92 ± 0.06	**8a**		5.81 ± 0.08
17c		6.55 ± 0.08	**8b**		6.64 ± 0.24
18a		5.05 ± 0.16	**8c**		5.50 ± 0.14
18b		5.07 ± 0.07	**8d**		5.36 ± 0.04
18c		5.15 ± 0.07	**8e**		6.14 ± 0.08
6a		5.11 ± 0.15	**8f**		4.99 ± 0.12
6b		5.85 ± 0.13	**RX821002**		8.85 ± 0.07

[a] 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).

observed and for future development this alteration should be avoided since it is neither required for affinity nor activity.

Encouragingly, it was found that if the R substituent is on the guanidine nitrogen within the cycle as opposed to the exocyclic nitrogen, significantly higher binding α_2-AR affinities are obtained. This supports our original hypothesis, and computational studies, that the orientation of the substituents in the initial conformationally restricted guanidines (compounds **4** and **5**) is non-optimal within the α_2-AR binding site resulting in decreased affinity.

Moreover, if the guanidine is di-substituted, as is the case for the *N*-benzyl-,*N*′-substituted conformationally restricted guanidine intermediates (**17a-c**), higher α_2-AR binding affinities are obtained in most cases. This suggests that the aromatic moiety of the benzyl group may be positioned in an alternate hydrophobic pocket to give additional interactions, and perhaps tri-substitution of the guanidine core is preferred.

Lastly, since the 1,3-benzodiazepines derivatives (compounds **8**) gave overall higher α_2-AR binding affinities compared to their 6-membered cycle analogues, this modification, that increases flexibility in the bicyclic ring, should be incorporated into future ligands. Also important is that substituents in the *para* position to the cationic moiety of compounds **8**, which in previous acyclic compounds gave higher α_2-AR affinities, did not show any significant effect on binding. Due to the added synthetic steps to prepare such intermediates this structural feature should not be considered in future compounds.

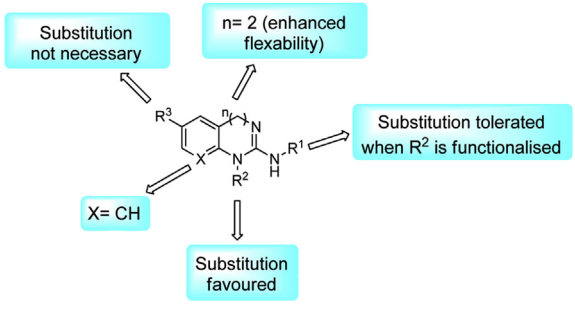


Fig. 4. Summary of the Structure-Activity Relationships for optimal α_2-AR affinity of conformationally restricted guanidine derivatives.

3. Conclusions

In this paper we reported the design, synthesis and pharmacological evaluation of two new families of *N*-substituted 2-amino-1,4-dihydroquinazolines, 2-amino-1,4-dihydropyrido pyrimidines and 2-amino-4,5-dihydro-1,3-benzodiazepines as ligands of the α_2-AR. The preparation of structurally similar compounds was not widely reported prior to our work; thus, the synthesis of these molecules involved novel routes than those previously used in our group for the preparation of acyclic aryl-guanidine counterparts. Compounds **8b** and **18c** showed the highest affinity within their series and exhibit antagonistic activity towards the α_2-AR in human brain tissue in *in vitro* experiments. Encouragingly, their affinities were shown to surpass not only all previous cyclic derivatives (Fig. 1, compounds **4** and **5** [14]), but also all *N*,*N*′-di-substituted guanidines (Fig. 1, compounds **2**) and, on average, most type **3**

molecules [13,14].

Importantly, this research has allowed us to gain insight into structure-activity relationships for the design and biological evaluation of novel α_2-AR ligands. These findings towards improved α_2-AR affinity, coupled with the fact that all conformationally restricted guanidine derivatives display antagonist activity, may further develop research in this area in the direction of conformationally controlled high binding antagonist of the α_2-AR.

4. Experimental

4.1. Pharmacology

4.1.1. Preparation of membranes

Neural membranes (P2 fractions) were prepared from the PFC of post-mortem human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Post-mortem 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 vol of homogenization buffer (1 mM $MgCl_2$ and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25 M sucrose. The crude homogenate was centrifuged for 5 min at 1000 g (4 °C), and the supernatant was centrifuged again for 10 min at 40,000 g (4 °C). The resultant pellet was washed twice in 20 vol of homogenization buffer and re-centrifuged in similar conditions. Aliquots of 1 mg protein were stored at −70 °C until assay. Protein concentration was measured according to the Bradford method, using bovine serum albumin as standard.

4.1.2. [^{3}H]RX821002 binding assays

Specific [^{3}H]RX821002 binding was measured in 0.25 mL aliquots (50 mM Tris-HCl, pH 7.5) of the human brain membranes, which were incubated in 96-well plates 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 separating free ligand from bound ligand by rapid filtration under vacuum (1450 Filter Mate Harvester, Perkin Elmer) through GF/C glass fiber filters. The filters were then rinsed three times with 300 μL binding buffer, air-dried (60 min), and counted for radioactivity by liquid scintillation spectrometry using a MicroBeta TriLux counter (PerkinElmer). 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 competition experiments to obtain the inhibition constant (K_i) were performed by non-linear regression using the Graph Pad Prism 5 program. All experiments were analysed assuming a one-site model of radioligand binding, K_i values were normalized to pK_i values.

4.1.3. [^{35}S]GTPγS binding assays

The incubation buffer for measuring [^{35}S]GTPγS binding to brain membranes contained, in a total volume of 250 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 (20 μg of membrane proteins *per well*) to the previous mixture and was performed at 30 °C for 120 min, with shaking. In order to evaluate the influence of the compounds on [^{35}S]GTPγS binding, ten concentrations (10^{-12} to 10^{-3} M) of the different compounds were added to the assay. Incubations were terminated by separating free ligand from bound ligand by rapid filtration under vacuum (1450 Filter Mate Harvester, Perkin Elmer) through GF/C glass fiber filters. The filters were then rinsed three times with 300 μL of ice-cold incubation buffer and air-dried (60 min). The radioactivity trapped was determined by liquid scintillation spectrometry (MicroBeta TriLux counter, PerkinElmer). The [^{35}S]GTPγS bound was about 6–15% 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 unlabelled GTPγS.

4.2. Chemical synthesis

All commercial chemicals were obtained from Sigma-Aldrich or Fluka and 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. Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F254 silica gel plates or Polygram Alox N/UV254 aluminium oxide plates. Visualisation was by UV light (254 nm). NMR spectra were recorded on Bruker DPX–400 Avance spectrometers, operating at 400.13 MHz and 600.1 MHz for ^{1}H NMR; 100.6 MHz and 150.9 MHz for ^{13}C NMR. Shifts are referenced to the internal solvent signals. NMR data were processed using Bruker TOPSPIN software. HRMS spectra were measured on a Micromass LCT electrospray TOF instrument with a WATERS 2690 autosampler and methanol/acetonitrile as carrier solvent. Melting points were determined using a Stuart Scientific Melting Point SMP1 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer equipped with a Universal ATR sampling accessory. HPLC purity analysis was carried out using a Varian ProStar system equipped with a Varian Prostar 335 diode array detector and a manual injector (20 μL). For purity assessment, UV detection was performed at 245 nm and peak purity was confirmed using a purity channel. The stationary phase consisted of an ACE 5 C18-AR column (150 × 4.6 mm), and the mobile phase used the following gradient system, eluting at 1 mL/min: aqueous formate buffer (30 mM, pH 3.0) for 10 min, linear ramp to 85% methanol buffered with the same system over 25 min, hold at 85% buffered methanol for 10 min. Minimum requirement for purity was set at 95.0%.

4.2.1. General methods

4.2.1.1. Method A. Protonation of N-substituted and N,N-disubstituted cyclic guanidines using hydrochloric acid in 1,4-dioxane. To a solution of the appropriate cyclic guanidine (1.0 eq., 0.71 mmol) in CH_3OH (0.1 mL) was added excess 4 M HCl/dioxane (3.0 eq., 2.13 mmol). Stirring was continued for 1 h after which solvent and excess HCl were removed under vacuum. The crude salt was then purified using reverse phase chromatography (C-8 silica) eluting in 100% H_2O.

4.2.1.2. Method B. Protonation of N-substituted and N,N-disubstituted cyclic guanidines using hydrochloric acid in 1,4-dioxane. To a solution of the appropriate cyclic guanidine (1.0 eq., 0.71 mmol) in CH_3OH (0.1 mL) was added excess 1.25 M HCl/MeOH (3.0 eq., 2.13 mmol). Stirring was continued for 1 h after which solvent and excess HCl were removed under vacuum. The crude salt was then purified using reverse phase chromatography (C-8 silica) eluting in 100% H_2O.

4.2.1.3. Method C. Preparation of the N-substituted-N,N-dimethyl-4,5-dihydro-1H-benzo[d] [1,3]diazepine hydrochlorides. To a stirred solution of starting thiourea (1.0 eq., 0.43 mmol) and NEt_3 (3.0 eq., 1.29 mmol) in DMF (2 mL) at 0 °C was added $HgCl_2$ (1.0 eq., 0.43 mmol). The solution was stirred at RT for 3 h. The reaction was diluted with $CHCl_3$:^{i}PrOH (8:2, 25 mL), and filtered through a bed of Celite to remove mercuric by-products. The filtrate was washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), to remove any traces

of DMF. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting in CH_2Cl_2:CH_3OH.

4.2.2. 2-Amino-1-propyl-1,4-dihydroquinazoline hydrochloride (6a)

Following Method A and starting from **15a** (1.0 eq., 20 mg, 0.11 mmol) the title compound was obtained as a yellow solid (21 mg, 85%). Mp: 210–214 °C, decomposed. δ_H (400 MHz, D_2O): 7.29–7.21 (m, 1H, CH Ar), 7.09 (m, 3H, CH Ar), 4.27 (s, 2H, CH_2), 3.65 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 0.93 (t, 3H, J = 7.4 Hz, CH_3). δ_C (100 MHz, D_2O): 149.1 (qC), 133.9 (qC Ar), 128.5 (CH Ar), 126.1 (CH Ar), 125.1 (CH Ar), 121.8 (qC Ar), 115.4 (CH Ar), 46.2 (CH_2), 40.4 (CH_2), 19.6 (CH_2), 9.64 (CH_3). ν_{max} (ATR)/cm^{-1}: 3276 (NH), 2941, 1648, 1601, 1575, 1512, 1392, 1243, 1149, 1020, 795, 691. HRMS (m/z ESI$^+$): Found: 190.1359 (M^+ + H, $C_{11}H_{16}N_3$ Requires: 190.1338). Purity by HPLC: 97.6% (t_R 24.19 min).

4.2.3. 2-Amino-1-phenyl-1,4-dihydroquinazoline hydrochloride (6b)

Following Method A and starting from **15b** (1.0 eq., 19 mg, 0.08 mmol) the title compound was obtained as a yellow solid (19 mg, 91%). Mp: 240–250 °C, decomposed. δ_H (400 MHz, D_2O): 7.67 (m, 3H, CH Ar), 7.36 (d, 2H, J = 7.7 Hz, CH Ar), 7.05–7.16 (m, 3H, CH Ar), 6.20 (m, 1H, CH Ar), 4.73 (s, 2H, CH_2). δ_C (100 MHz, CH_3OH): 153.0 (qC), 136.0 (qC Ar), 133.9 (qC Ar), 131.1 (CH Ar), 130.8 (CH Ar), 129.5 (CH Ar), 128.5 (qC Ar), 126.1 (CH Ar), 125.0 (CH Ar), 118.9 (CH Ar), 115.9 (CH Ar), 40.6 (CH_2). ν_{max} (ATR)/cm^{-1}: 3337 (NH), 3249 (NH), 2934, 2858, 1643, 1600, 1492, 1421, 1345, 1286, 1143, 1026, 761, 705. HRMS (m/z ESI$^+$): Found: 224.1188 (M^+ + H, $C_{14}H_{14}N_3$ Requires: 224.1182). Purity by HPLC: 98.7% (t_R 24.16 min).

4.2.4. 2-Amino-1-propyl-1,4-dihydropyrido[2,3-d]pyrimidine hydrochloride (7a)

Following Method A and starting from **16a** (1.0 eq., 17 mg, 0.09 mmol) the title compound was obtained as a yellow solid (15 mg, 74%). Mp: 211–220 °C, decomposed. δ_H (400 MHz, D_2O): 8.10 (m, 1H, CH Ar), 7.49 (app. t, 1H, J = 7.1 Hz, CH Ar), 7.09 (dd, 1H, J = 7.1 Hz, 4.8 Hz, CH Ar), 4.40 (s, 2H, CH_2), 3.85 (t, 2H, J = 6.7 Hz, CH_2), 1.58 (m, 2H, CH_2), 0.80 (t, 3H, J = 6.7 Hz, CH_3). δ_C (100 MHz, CH_3OH): 159.5 (qC), 154.2 (qC Ar), 134.8 (CH Ar), 145.9 (CH Ar), 120.6 (CH Ar), 115.1 (qC Ar), 43.5 (CH_2), 39.9 (CH_2), 20.5 (CH_2), 9.5 (CH_3). ν_{max} (ATR)/cm^{-1}: 3268 (NH), 2962, 2933, 2917, 1624, 1595, 1335, 1234, 1155, 1138, 1002, 957, 903, 863, 757, 789, 732. HRMS (m/z ESI$^+$): Found: 191.1289 (M^+ + H, $C_{10}H_{15}N_4$ Requires: 191.1291). Purity by HPLC: 95.3% (t_R 22.84 min).

4.2.5. 2-Benzylamino-1-propyl-1,4-dihydroquinazoline hydrochloride (17a)

Following Method A and starting from **13a** (1.0 eq., 140 mg, 0.5 mmol) the title compound was obtained as a yellow solid (137 mg, 86%). Mp: 124–130 °C. δ_H (400 MHz, $CDCl_3$): 9.27 (br s, 1H, NH), 9.10 (br s, 1H, NH), 7.46 (d, 2H, J = 7.3 Hz, CH Ar), 7.20 (t, 3H, J = 7.3 Hz, CH Ar), 7.10 (m, 1H, CH Ar), 7.05 (app. t, 1H, J = 7.4 Hz, CH Ar), 6.99 (app. t, 1H, J = 7.4 Hz, CH Ar), 6.82 (d, 1H, J = 7.4 Hz, CH Ar), 4.72 (d, 2H, J = 5.2 Hz, CH_2), 4.31 (s, 2H, CH_2), 4.04 (t, 2H, J = 7.3 Hz, CH_2), 1.62 (m, 2H, CH_2), 0.75 (t, 3H, J = 7.3 Hz, CH_3). δ_C (100 MHz, $CDCl_3$): 153.3 (qC), 152.6 (qC Ar), 137.2 (qC Ar), 128.4 (qC Ar), 128.0 (CH Ar), 127.5 (CH Ar), 125.6 (CH Ar), 124.3 (CH Ar), 123.9 (CH Ar), 115.4 (CH Ar), 49.6 (CH_2), 47.2 (CH_2), 41.7 (CH_2), 20.4 (CH_2), 10.5 (CH_3). ν_{max} (ATR)/cm^{-1}: 3100 (NH), 2959, 1657, 1602, 1566, 1482, 1404, 1395, 1278, 1253, 1176, 1063, 1029, 795. HRMS (m/z ESI$^+$): Found: 280.1805 (M^+ + H, $C_{18}H_{22}N_3$ Requires: 280.1808). Purity by HPLC: 99.1% (t_R 28.39 min).

4.2.6. 2-Benzylamino-1-phenyl-1,4-dihydroquinazoline hydrochloride (17b)

Following Method A and starting from **13b** (1.0 eq., 25 mg, 0.08 mmol) the title compound was obtained as an off-white solid (24 mg, 85%). Mp: 115–120 °C. δ_H (400 MHz, $CDCl_3$): 7.63 (m, 3H, CH Ar), 7.37 (d, 2H, J = 7.2 Hz, CH Ar), 7.25 (m, 5H, J = 7.3 Hz, CH Ar), 7.10 (m, 3H, CH Ar), 6.16 (app. t, 1H, J = 7.2 Hz, CH Ar), 4.86 (s, 4H, CH_2-4, CH_2). δ_C (100 MHz, $CDCl_3$): 151.1 (qC), 136.2 (qC Ar), 135.4 (qC Ar), 133.4 (qC Ar), 131.7 (CH Ar), 131.2 (CH Ar), 129.9 (qC Ar), 128.8 (CH Ar), 128.1 (CH Ar), 127.8 (CH Ar), 126.4 (CH Ar), 125.9 (CH Ar), 125.2 (CH Ar), 119.3 (CH Ar), 115.5 (CH Ar), 47.1 (CH_2), 41.5 (CH_2). ν_{max} (ATR)/cm^{-1}: 3290 (NH), 2925, 1641, 1602, 1558, 1490, 1357, 13,26, 1244, 1207, 1072, 1028, 752. HRMS (m/z ESI$^+$): Found: 314.1659 (M^+ + H, $C_{21}H_{20}N_3$ Requires: 314.1651). Purity by HPLC: 99.3% (t_R 29.17 min).

4.2.7. 1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydroquinazoline hydrochloride (17c)

Following Method A and starting from **13c** (1.0 eq., 45 mg, 0.12 mmol) the title compound was obtained as a yellow solid (43 mg, 88%). Mp: 98–103 °C. δ_H (400 MHz, $CDCl_3$): 10.01 (br s, 1H, NH), 8.09 (br s, 1H, NH), 7.17 (m, 6H, CH Ar), 7.07 (m, 2H, CH Ar), 6.94 (m, 1H, CH Ar), 6.66 (br s, 1H, CH Ar), 6.58 (m, 2H, CH Ar), 5.90 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 4.70 (d, 2H, J = 4.9 Hz, CH_2), 4.44 (d, 2H, J = 5.1 Hz, CH_2). δ_C (100 MHz, $CDCl_3$): 151.2 (qC), 148.1 (qC Ar), 147.0 (qC Ar), 141.2 (qC Ar), 138.2 (qC Ar), 138.0 (qC Ar), 129.3 (qC Ar), 128.4 (CH Ar), 127.7 (CH Ar), 126.8 (CH Ar), 125.5 (CH Ar), 124.0 (CH Ar), 123.6 (CH Ar), 119.8 (CH Ar), 114.9 (CH Ar), 108.4 (CH Ar), 106.8 (CH Ar), 101.1 (CH_2), 48.9 (CH_2), 46.7 (CH_2), 43.4 (CH_2). ν_{max} (ATR)/cm^{-1}: 3211 (NH), 2957, 1632, 1600, 1598, 1488, 1444, 1358, 1236, 1170, 1095, 1053, 921, 908, 751. HRMS (m/z ESI$^+$): Found: 372.1714 (M^+ + H, $C_{23}H_{22}N_3O_2$ Requires: 372.1706). Purity by HPLC: 97.3% (t_R 28.64 min).

4.2.8. 2-Benzylamino-1-propyl-1,4-dihydropyrido[2,3-d] pyrimidine hydrochloride (18a)

Following Method A and starting from compound **14a** (1.0 eq., 50 mg, 0.17 mmol) the title compound was obtained as a yellow solid (47 mg, 87%). Mp: 113–117 °C. δ_H (400 MHz, $CDCl_3$): 9.27 (br s, 2H, NH), 8.18 (d, 1H, J = 7.3 Hz, CH Ar), 7.59 (d, 1H, J = 7.3 Hz, CH Ar), 7.26 (d, 2H, J = 7.1 Hz, CH Ar), 7.16 (t, 2H, J = 7.1 Hz, CH Ar), 7.10 (d, 1H, J = 7.1 Hz, CH Ar), 6.96 (app. t, 1H, J = 7.0 Hz, CH Ar), 4.86 (s, 2H, CH_2), 4.49 (s, 2H, CH_2), 4.31 (t, 2H, J = 7.1 Hz, CH_2), 1.65 (m, 2H, CH_2), 0.91 (t, 3H, J = 7.0 Hz, CH_3). δ_C (100 MHz, $CDCl_3$): 152.0 (qC), 147.4 (q Ar), 147.2 (CH Ar), 136.2 (CH Ar), 134.2 (qC Ar), 127.6–128.4 (5 CH Ar), 120.1 (CH Ar), 115.5 (qC Ar), 45.2 (CH_2), 44.0 (CH_2), 40.2 (CH_2), 21.6 (CH_2), 10.8 (CH_3). ν_{max} (ATR)/cm^{-1}: 3044 (NH), 2923, 1719, 1653, 1556, 1549, 1423, 1361, 1330, 1257, 1116, 1050, 1018, 897, 763. HRMS (m/z ESI$^+$): Found: 281.17,588 (M^+ + H, $C_{17}H_{21}N_4$ Requires: 281.1760). Purity by HPLC: 95.5% (t_R 27.29 min).

4.2.9. 2-Benzylamino-1-hydroxyethyl-1,4-dihydropyrido[2,3-d] pyrimidine hydrochloride (18b)

Following Method A and starting from **14b** (1.0 eq., 30 mg, 0.11 mmol) the title compound was obtained as a white solid (26 mg, 74%). Mp: 148–151 °C. δ_H (400 MHz, $CDCl_3$): 9.62 (br s, 1H, NH), 9.33 (br s, 1H, NH), 8.13 (d, 1H, J = 7.1 Hz, CH Ar), 7.38 (d, 1H, J = 7.1 Hz, CH Ar), 7.27 (d, 2H, J = 7.0 Hz, CH Ar), 7.20 (t, 2H, J = 7.1 Hz, CH Ar), 7.13 (t, 1H, J = 7.1 Hz, CH Ar), 6.97 (app. t, 1H, J = 7.0 Hz, CH Ar), 4.71 (s, 2H, CH_2), 4.44 (s, 2H, CH_2), 4.32 (t, 2H, J = 6.9 Hz, CH_2), 4.02 (t, 2H, J = 6.9 Hz, CH_2). δ_C (100 MHz, $CDCl_3$): 153.5 (qC), 148.8 (qC Ar), 146.1 (CH Ar), 135.3 (CH Ar), 134.2 (qC Ar), 128.7 (CH Ar), 127.9 (CH Ar), 127.8, (CH Ar), 120.4 (qC Ar), 115.7 (CH Ar), 60.9 (CH_2), 48.1 (CH_2), 46.3 (CH_2), 40.4 (CH_2). ν_{max} (ATR)/cm^{-1}: 3229 (NH), 2947, 1673, 1598, 1567, 1434, 1497, 1358, 1311, 1079, 1050,

1001, 911, 857, 793, 741. HRMS (*m/z* ESI$^+$): Found: 283.1566 ($M^+ + H$, $C_{16}H_{19}N_4O$ Requires: 283.1553). Purity by HPLC: 97.1% (t_R 25.15 min).

4.2.10. 1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydropyrido[2,3-d]pyrimidine hydrochloride (18c)

Following Method A and starting from **14c** (1.0 eq., 80 mg, 0.23 mmol) the title compound was obtained as a yellow solid (71 mg, 77%). Mp: 91–95 °C. δ_H (400 MHz, $CDCl_3$): 8.27 (d, 1H, $J = 7.3$ Hz, CH Ar), 7.36 (d, 1H, $J = 7.3$ Hz, CH Ar), 7.24 (m, 2H, CH Ar), 7.14 (m, 3H, CH Ar), 6.84 (dd, 1H, $J = 7.3$ Hz, 5.0 Hz, CH Ar), 6.73 (s, 1H, CH Ar), 6.67 (m, 2H, CH Ar), 5.95 (s, 2H, CH_2), 5.42 (s, 2H, CH_2), 4.82 (s, 2H, CH_2), 4.66 (s, 2H, CH_2). δ_C (150 MHz, $CDCl_3$): 152.5 (qC), 152.0 (qC Ar), 148.8 (qC Ar), 148.0 (qC Ar), 147.1 (CH Ar), 135.2 (qC Ar), 135.0 (qC Ar), 134.9 (CH Ar), 128.3 (CH Ar), 40.3 (CH_2), 127.1 (CH Ar), 120.5 (CH Ar), 120.1 (CH Ar), 115.3 (qC Ar), 108.9 (CH Ar), 108.7 (CH Ar), 107.5 (CH Ar), 101.5 (CH_2), 47.9 (CH_2), 46.3 (CH_2). ν_{max} (ATR)/cm^{-1}: 3121 (NH), 2922, 2852, 1640, 1599, 1547, 1488, 14,235, 1337, 1310, 1247, 1167, 1095, 922, 848, 769. HRMS (*m/z* ESI$^+$): Found: 373.1672 ($M^+ + H$, $C_{22}H_{21}N_4O_2$ Requires: 373.1659). Purity by HPLC: 95.5% (t_R 29.20 min).

4.2.11. 2-Hydroxyethylamino-4,5-dihydro-1,3-benzodiazepine hydrochloride (8a)

Following Method B and starting from **22b** (1.0 eq., 100 mg, 0.40 mmol) the title compound was obtained as a purple solid (92 mg, 95%). Mp: 235–240 °C. δ_H (400 MHz, D_2O): 7.30–7.22 (m, 4H, CH Ar), 3.73 (m, 2H, CH_2), 3.52 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 2.87 (t, 2H, $J = 8.0$ Hz, CH_2). δ_C (100 MHz, D_2O): 170.9 (qC), 131.4 (qC Ar), 130.7 (CH Ar), 129.2 (CH Ar), 128.8 (q Ar), 128.6 (CH Ar), 123.7 (CH Ar), 48.9 (CH_2), 44.2 (CH_2), 30.6 (CH_2), 28.2 (CH_2). ν_{max} (ATR)/cm^{-1}: 3142 (NH), 2962, 2895, 1640, 1589, 1559, 1496, 1228, 1009, 950, 896, 875, 748. HRMS (*m/z* ESI$^+$): Found: 206.1289 ($M^+ + H$, $C_{11}H_{16}N_3O$ Requires: 206.1287). Purity by HPLC: 95.7% (t_R 17.51 min).

4.2.12. 2-(1,3-Benzodioxolyl-5-methylamino)-4,5-dihydro-1,3-benzodiazepine hydrochloride (8b)

Following Method A and starting from **22c** (1.0 eq., 50 mg, 0.17 mmol) the tile compound was obtained as a yellow solid (45 mg, 80%). Mp: 196–199 °C. δ_H (400 MHz, $CDCl_3$): 10.11 (br s, 1H, NH), 8.46 (br s, 2H, NH), 7.20 (app. t, 1H, $J = 7.3$ Hz, CH Ar), 7.11 (d, 1H, $J = 7.4$ Hz, CH Ar), 7.00 (m, 2H, CH Ar), 6.63 (m, 2H, CH Ar), 6.60 (d, 1H, $J = 7.2$ Hz, CH Ar), 5.75 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 3.59 (br s, 2H, CH_2), 2.94 (br s, 2H, CH_2). δ_C (100 MHz, $CDCl_3$): 169.8 (qC), 154.3 (qC Ar), 148.0 (qC Ar), 147.9 (qC Ar), 136.0 (qC Ar), 130.2 (CH Ar), 129.0 (CH Ar), 127.7 (CH Ar), 124.2 (CH Ar), 121.1 (CH Ar), 120.8 (qC Ar), 108.3 (CH Ar), 108.1 (CH Ar), 101.2 (CH_2), 46.3 (CH_2), 44.3.(CH_2), 34.6 (CH_2). ν_{max} (ATR)/cm^{-1}: 2923 (NH), 2853, 1682, 1610, 1586, 1489, 1448, 1364, 1240, 1098, 924, 859, 805, 756. HRMS (*m/z* ESI$^+$): Found: 296.1391 ($M^+ + H$, $C_{17}H_{18}N_3O_2$ Requires: 296.1393). Purity by HPLC: 96.7% (t_R 27.49 min).

4.2.13. 2-(Hydroxyethylamino)-8,9-dihydro-5H-[1,3]dioxolo [4,5] benzo[1,2-d] [1,3]diazepine hydrochloride (8c)

Following Method B and starting from **22f** (1.0 eq., 200 mg, 0.68 mmol) the title compound was obtained as a red solid (187 mg, 96%). Mp: 230–233 °C. δ_H (600 MHz, D_2O): 6.78 (s, 1H, CH Ar), 6.74 (s, 1H, CH Ar), 5.89 (s, 2H, CH_2), 3.78 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.37 (m, 2H, CH_2), 2.78 (t, 2H, $J = 8.0$ Hz, CH_2). δ_C (125 MHz, DMSO): 171.3 (qC), 147.8 (qC Ar), 147.8 (2 qC Ar), 121.6 (q Ar), 109.5 (CH Ar), 104.5 (CH Ar), 102.0 (CH_2), 48.9 (CH_2), 44.5 (CH_2), 30.6 (CH_2), 28.1 (CH_2). ν_{max} (ATR)/cm^{-1}: 3141 (NH), 2819, 2533, 1639, 1569, 1437, 1362, 1204, 1066, 1078, 1027, 926, 873. HRMS: (*m/z* ESI$^+$): Found: 250.1198 ($M^+ + H$, $C_{12}H_{16}N_3O_3$ Requires: 250.1186). Purity by HPLC: 98.5% (t_R 19.09 min).

4.2.14. 7-(Dimethylamino)-2-(furanyl-2-methylamino)-4,5-dihydro-1H-benzo[d] [1,3]diazepine hydrochloride (8d)

Following Method C and starting from **28a** (1.0 eq., 250 mg, 0.77 mmol) the title compound was obtained as a brown solid (224 mg, 90%). Mp: 244–250 °C, decomposed. δ_H (400 MHz, $CDCl_3$): 9.94 (br s, 1H, NH), 8.60 (br s, 1H, NH), 7.96 (br s, 1H, NH), 7.21 (br s, 1H, CH Ar), 7.08 (d, 1H, $J = 7.1$ Hz, CH Ar), 6.42 (m, 2H, CH Ar), 6.30 (t, 1H, $J = 7.2$ Hz, CH Ar), 6.16 (d, 1H, $J = 7.2$ Hz, CH Ar), 4.57 (s, 2H, CH_2), 3.57 (s, 2H, CH_2), 2.87 (s, 2H, CH_2), 2.82 (s, 6H, CH_3). δ_C (100 MHz, $CDCl_3$): 177.4 (qC), 151.6 (qC Ar), 149.3 (qC Ar), 147.6 (qC Ar), 142.5 (CH Ar), 125.3 (qC Ar), 122.0 (CH Ar), 113.1 (CH Ar), 111.7 (CH Ar), 110.4 (CH Ar), 108.5 (CH Ar), 43.7 (CH_2), 40.6 (CH_3), 39.5 (CH_2), 34.6 (CH_2). ν_{max} (ATR)/cm^{-1}: 3378 (NH), 3221 (NH), 2996, 2793, 1569, 1505, 1438, 1379, 1333, 1249, 1219, 1124, 1073, 1021, 952, 848, 794. HRMS (*m/z* ESI$^+$): Found: 285.1714 ($M^+ + H$, $C_{16}H_{21}N_4O$ Requires: 285.1710). Purity by HPLC: 94.8% (t_R 23.12 min).

4.2.15. 2-(1,3-Benzodioxolyl-5-methylamino)-7-dimethylamino-4,5-dihydro-1H-benzo[d] [1,3]diazepine hydrochloride (8e)

Following Method C and starting from **28b** (1.0 eq., 200 mg, 0.54 mmol) the title compound was obtained as a yellow solid (121 mg, 66%). Mp: 205–209 °C, decomposed. δ_H (400 MHz, $CDCl_3$): 9.61 (br s, 1H, NH), 8.30 (br s, 1H, NH), 7.90 (br s, 1H, NH), 7.01 (s, 1H, CH Ar), 6.80 (m, 2H, CH Ar), 6.56 (d, 1H, $J = 7.1$ Hz, CH Ar), 6.39 (d, 1H, $J = 7.1$ Hz, CH Ar), 6.29 (s, 1H, CH Ar), 5.74 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 3.54 (s, 2H, CH_2), 2.82 (s, 8H, CH_3, CH_2). δ_C (100 MHz, $CDCl_3$): 168.1 (qC), 153.5 (qC Ar), 147.5 (qC Ar), 147.1 (qC Ar), 130.3 (qC Ar), 129.6 (qC Ar), 129.9 (C, Ar), 121.8 (CH Ar), 121.2 (CH Ar), 113.1 (CH Ar), 111.7 (CH Ar), 108.3 (CH Ar), 108.2 (CH Ar), 101.0 (CH_2), 46.1 (CH_2), 43.6 (CH_2), 40.6 (CH_3), 34.7 (CH_2). $\boldsymbol{\nu_{max}}$ (ATR)/cm^{-1}: 3161 (NH), 2962, 2904, 1673, 1619, 1541, 1517, 1492, 1468, 1401, 1346, 1326, 1246, 1198, 1094, 999, 961, 834, 635. HRMS (*m/z* ESI$^+$): Found: 339.1818 ($M^+ + H$, $C_{19}H_{23}N_4O_2$ Requires: 339.1816). Purity by HPLC: 96.1% (t_R 23.97 min).

4.2.16. 2-Hydroxyethylamino-7-dimethylamino-4,5-dihydro-1H-benzo[d] [1,3]diazepine hydrochloride (8f)

Following Method B and starting with **28c** (1.0 eq., 120 mg, 0.41 mmol) the total compound was obtained as a yellow solid (112 mg, 98%). Mp: 237–241 °C. δ_H (400 MHz, D_2O): 6.87 (d, 1H, $J = 7.4$ Hz, CH Ar), 6.75 (d, 1H, $J = 7.4$ Hz, CH Ar), 6.70 (s, 1H, CH Ar), 3.63 (t, 2H, $J = 7.1$ Hz, CH_2), 3.48 (m, 2H, CH_2), 3.30 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 2.86 (s, 6H, CH_3). δ_C (100 MHz, D_2O): 161.9 (qC), 150.1 (qC Ar), 131.2 (qC Ar), 127.9 (qC Ar), 121.8 (CH Ar), 116.6 (CH Ar), 114.8 (CH Ar), 62.1 (CH_2), 48.7 (CH_3), 43.5 (CH_2), 41.1 (CH_2), 33.1 (CH_2). ν_{max} (ATR)/cm^{-1}: 3287 (NH), 2923, 1619, 1516, 1406, 1332, 1062, 972, 784. HRMS (*m/z* ESI$^+$): Found: 249.1704 ($M^+ + H$, $C_{13}H_{21}N_4O$ Requires: 249.1709). Purity by HPLC: 94.9% (t_R 16.55 min).

4.3. Computational methods

All structures were optimised using the Gaussian09 package [21] at the M06-2X computational level with the 6-31 + G** basis set. Frequency calculations were performed at the same computational level to confirm that the resulting optimised structures are energetic minima. Effects of water solvation have been included by means of the SCFR-PCM approach [13] implemented in the Gaussian09 package including dispersing, repulsing and cavitating energy terms of the solvent.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.07.011.

References

[1] The Global Burden of Disease, 2004 Update, WHO Press, Geneva, 2008.
[2] W.N. Marsden, Synaptic plasticity in depression: molecular, cellular and functional correlates, Prog. Neuropsychopharmacol. Biol. Psychiatry 43 (2013) 168–184.
[3] C. Pittenger, R.S. Duman, Stress, depression, and neuroplasticity: a convergence of mechanisms, Neuropsychopharmacology 33 (2008) 88–109.
[4] C. Cottingham, Q. Wang, α_2 adrenergic receptor dysregulation in depressive disorders: implications for the neurobiology of depression and antidepressant therapy, Neurosci. Biobehav. Rev. 36 (2012) 2214–2225.
[5] F. Serres, M. Rodriguez, J.M. Rivet, J.P. Galizzi, B. Lockhart, T. Sharp, M.J. Millan, Blockade of α2-adrenoceptors induces Arc gene expression in rat brain in a glutamate receptor-dependent manner: a combined qPCR, in situ hybridisation and immunocytochemistry study, Neuropharmacology 63 (2012) 992–1001.
[6] F. Rodriguez, I. Rozas, J.E. Ortega, J.J. Meana, L.F. Callado, Guanidine and 2-aminoimidazoline aromatic derivatives as α2-adrenoceptor antagonists, 1: towards new antidepressants with heteroatomic linkers, J. Med. Chem. 50 (2007) 4516–4527.
[7] F. Rodriguez, I. Rozas, J.E. Ortega, A.M. Erdozain, J.J. Meana, L.F. Callado, Guanidine and 2-aminoimidazoline aromatic derivatives as α2-adrenoceptor antagonists, 2: exploring aliphatic linkers, J. Med. Chem. 51 (2008) 3304–3312.
[8] F. Rodriguez, I. Rozas, A.M. Erdozain, J.J. Meana, L.F. Callado, Guanidine and 2-aminoimidazoline aromatic derivatives as potential antidepressants, 3: α2-adrenoceptor agonism vs antagonism. Structure Activity Relation, J. Med. Chem. 52 (2009) 601–609.
[9] (a) T. Ishikawa, L.M. Harwood, Organic superbases: the concept at a glance, Synlett 24 (2013) 2507–2509;
(b) T. Ishikawa, Guanidine chemistry, Chem. Pharm. Bull. 58 (2010) 1555–1564 (and references therein).
[10] (a) I. Kaljurand, J. Saame, T. Rodima, I. Koppel, I.A. Koppel, J.F. Kögel, J. Sundermeyer, U. Köhn, M.P. Coles, I. Leito, Experimental basicities of phosphazene, guanidinophosphazene, and proton sponge superbases in the gas phase and solution, J. Phys. Chem. A 120 (2016) 2591–2604;
(b) C.H. Tan, M. Coles, The chemistry of guanidine, guanidinium, and guanidinate compounds, Austr. J. Chem. 67 (2014) 963–964 (and references therein).
[11] A.A. Legin, M.A. Jakupec, N.A. Bokach, M.R. Tyan, V.Y. Kukushkin, B.K. Keppler, Guanidine platinum(II) complexes: synthesis, in vitro antitumor activity, and DNA interactions, J. Inorg. Biochem. 133 (2014) 33–39.
[12] (a) A.K. Timiri, B.N. Sinha, V. Jayaprakash, Progress and prospects on DENV protease inhibitors, Eur. J. Med. Chem. 117 (2016) 125–143;
(b) A. Basu, B.N. Sinha, P. Saiko, T. Szekeres, Effect of substitution at *N'*-position of *N'*-hydroxy-*N*-amino guanidines on tumor cell growth, Bioorg. Med. Chem. Lett. 22 (2012) 4934–4938.
[13] D.H. O'Donovan, C. Muguruza, L.F. Callado, I. Rozas, Guanidine-based α2-adrenoceptor ligands: towards selective antagonist activity, Eur. J. Med. Chem. 82 (2014) 242–254.
[14] B. Kelly, M. McMullan, C. Muguruza, J.E. Ortega, J.J. Meana, L.F. Callado, I. Rozas, α_2-Adrenoceptor antagonists: synthesis, pharmacological evaluation and molecular modelling investigation of pyridinyl guanidine/2-aminoimidazoline and their derivatives, J. Med. Chem. 58 (2015) 963–977.
[15] Y. Zhao, D.G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals, Theor. Chem. Acc. 120 (2006) *215–241*.
[16] (a) A.D. McLean, G.S. Chandler, Contracted Gaussian basis sets for molecular calculations. I. Second row atoms, *Z*=11–18, J. Chem. Phys. 72 (1980) 5639–5648;
(b) R. Krishnan, J.S. Binkley, R. Seeger, J.A. Pople, Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions, J. Chem. Phys. 72 (1980) 650–654;
(c) T. Clark, J. Chandrasekhar, G.W. Spitznagel, P. v. R. Schleyer, Efficient diffuse function-augmented basis sets for anion calculations. III. The 3-21+G basis set for first-row elements, Li–F, J. Comp. Chem. 4 (1983) 294–301;
(d) M.J. Frisch, J.A. Pople, J.S. Binkley, Self-consistent molecular orbital methods 25: supplementary functions for gaussian basis sets, J. Chem. Phys. 80 (1984) 3265–3269.
[17] J. Tomasi, B. Mennucci, R. Cammi, Quantum mechanical continuum solvation models, Chem. Rev. 105 (2005) 2999–3093.
[18] J.W. Shaw, D.H. Grayson, I. Rozas, Synthesis of guanidines and some of their biological applications, in: Philipp Selig (Ed.), Topics in Heterocyclic Chemistry: Guanidines as Reagents and Catalysts, Springer, 2016 in press.
[19] M.P. Groziak, L.B. Townsend, A new and efficient synthesis of guanosine, J. Org. Chem. (1986) 1277–1282.
[20] P. Das, C.K. Kumar, K.N. Kumar, M. Innus, J. Iqbal, N. Srinivas, Dithiocarbamate and CuO promoted one-pot synthesis of 2-(N-substituted)-aminobenzimidazoles and related heterocycles, Tetrahedron Lett. 49 (2008) 992–995.
[21] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N.J. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford, CT, 2009.