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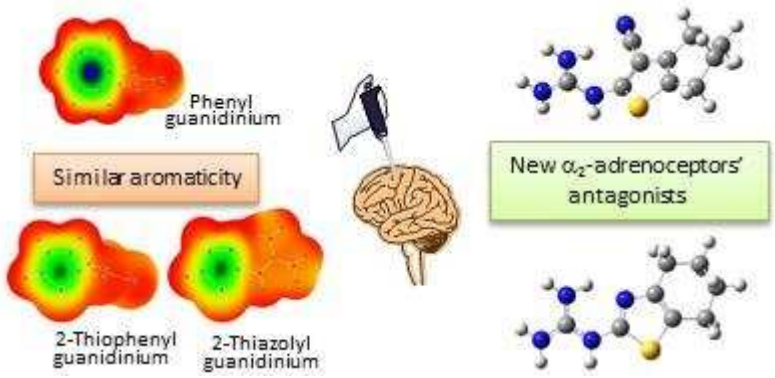
Graphical Abstract



Thiophene/Thiazole-Benzene Replacement on Guanidine Derivatives Targeting α_2-Adrenoceptors

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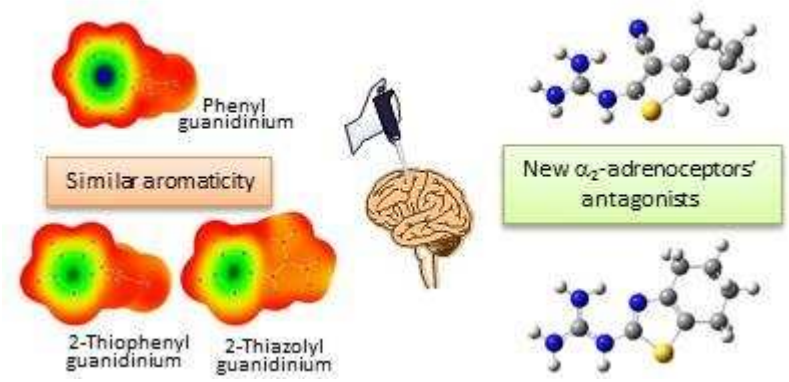
Graphical Abstract



Highlights

- Phenyl-, thiophenyl- and thiazolylguanidinium derivatives have similar aromaticity
- Thiophenyl- & thiazolylguanidines must act as phenyl-based α_2-adrenoceptor ligands
- A high-diversity library of thiophenyl- and thiazolylguanidines was prepared
- Their affinity for α_2-adrenoceptor was measured in human prefrontal cortex tissue
- Activity on α_2-adrenoceptors was measured: 2 antagonists and 1 agonist were found

Abstract

Searching for improved antagonists of α_2-adrenoceptors, a thorough theoretical study comparing the aromaticity of phenyl-, pyridinyl-, thiophenyl- and thiazolylguanidinium derivatives has been carried out [at M06-2X/6-311++G(p,d) computational level] confirming that thiophene and thiazole will be good 'ring equivalents' to benzene in these guanidinium systems. Based on these results, a small but chemically diverse library of guanidine derivatives (15 thiophenes and 2 thiazoles) were synthesised to explore the effect that the bioisosteric change has on affinity and activity at α_2-adrenoceptors in comparison with our previously studied phenyl derivatives. All compounds were tested for their α_2-adrenoceptor affinity and unsubstituted guanidinothiophenes displayed the strongest affinities in the same range as the phenyl analogues. In the case of cycloakyl systems, thiophenes with 6-membered rings showed the largest affinities, while for the thiazoles the 5-membered analogue presented the strongest affinity. From all the compounds tested for noradrenergic activity, only one compound exhibited agonistic activity, while two compounds showed very promising antagonism of α_2-adrenoceptors.

Keywords

Bioisosterism; Aromaticity; Thiophene; Thiazole; Guanidinium; α_2-Adrenoceptors; Antagonists; Affinity constants.

Abbreviations used

NA: noradrenaline; α2-ARs: α_2-adrenoceptors; PFC: human brain prefrontal cortex; [^{3}H]RX821002: 2-methoxyidazoxan; K_i: affinity constant; [^{35}S]GTPγS: radioligand guanosine 5'-*O*-[gamma-thio]triphosphate.

1. INTRODUCTION

Bioisosterism has been used for decades in Medicinal Chemistry as a source of structural/molecular diversity [1]. One of the most classical bioisosteric changes is the use of ring-equivalents and, thiophene in particular, has been used as a substitute for other aromatic rings improving biological activity. For example, Song *et al*. have just reported the synthesis of a series of thenoylhydrazide derivatives making use of the bioisosterism between thiophene and benzene [2]. Recently, to improve the activity of a family of inhibitors of dengue viral RNA-dependent RNA polymerase, the benzene moiety of the lead compound was substituted by thiophene resulting in a 10-fold increase in potency [3]. Another recent example of benzene-thiophene bioisosterism is the development of biphenyl derivatives as bioisosteres of thienylphenyl agonists/antagonists of the AT_2 receptor showing similar affinity and functional activity [4]. Thiophene is present in range of very successful commercial drugs such as olanzapine, raloxifen or clopidogrel, and similarly, the thiazole ring features in well-known drugs such as ritonavir, pramipexole and famotidine [5].

Accordingly, considering that thiophene has often been utilised as a ring equivalent of benzene in drug discovery due to its reported similar aromaticity making it an effective mimic [6], we propose now to rigorously assess from a theoretical point of view the aromaticity of arylguanidine derivatives of the aromatic cores previously used by our group (benzene and pyridine) compared to those now proposed (thiophene and thiazole) in order to accurately establish the potential similarity of their aromatic properties. With that aim we have calculated a well-known and established type of aromaticity indicator, the Nucleus Independent Chemical Shift (NICS) [7,8], at Density Functional Theory (DFT) level. Moreover, continuing with our research in arylguanidine derivatives as antagonists of α_2-adrenoceptor (α2-AR) [9,10,11,12,13,14] and based on the proposed theoretical study on

aromaticity, we also present the preparation and pharmacological evaluation of a library of guanidinothiophenes and 2-guanidinothiazoles with high chemical diversity.

To date, we have found several structure-activity relationships (SAR) to optimise the antagonistic activity of arylguanidine-like compounds as well as their α2-AR affinity. Thus, we have explored the mono- (compounds **1**, **2** and **4** in Figure 1) and di-substitution (compound **3**, Figure 1) of the guanidine-like system as well as the nature and position of different substituents in 6-membered aromatic rings (mostly benzene [9-12,14] as in compounds **1-3** and more recently pyridine [13] as in compound **4**, Figure 1) with respect to the guanidine-like moiety. In this way, we identified several lead compounds that were not only α2-AR antagonists (in vitro and in vivo), but also showed antidepressant activity in animal models such as the forced-swimming and tail suspension tests (e.g. compounds **1** and **2**, Figure 1) [15]. Additionally, we were able to identify that double substitution in the guanidine system (e.g. compound **3**, Figure 1) or pyridine derivatives (e.g. compound **4**, Figure 1) always results in antagonist or inverse agonist functional activity [10,11].

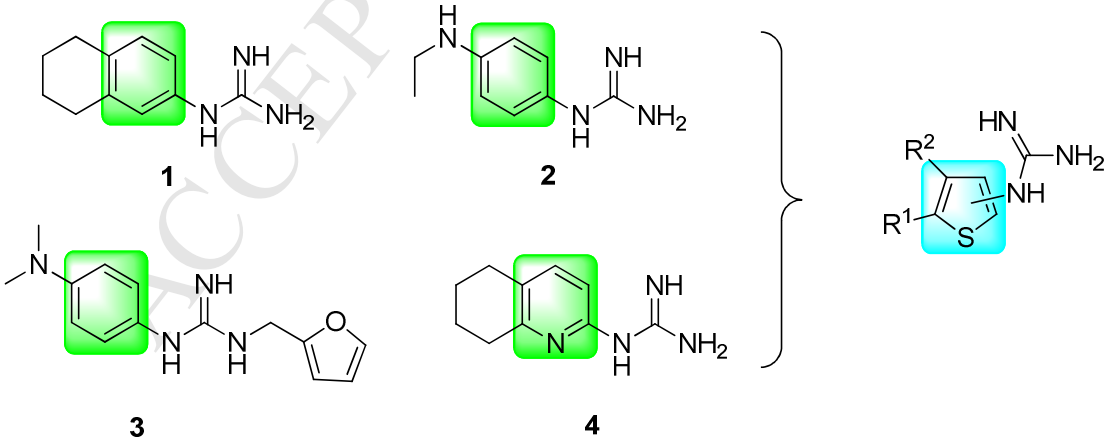


Figure 1.- Compounds previously prepared and tested by Rozas and collaborators as α2-AR antagonists and general structure of the guanidinothiophenes proposed.

2. RESULTS AND DISCUSSION

2.1. Comparative aromaticity study

The aromaticity of certain derivatives of furan, pyrrole and thiophene compared to that of the corresponding azo-heterocycles (oxazole, imidazole and thiazole) has been previously calculated by Gümüs and Türker at DFT level using B3LYP/6-31G(d,p) and B3LYP/6-31++G(d,p) computational methods [16]. By considering NICS indexes they determined that the second heteroatom substitution decreases aromaticity, but that this can be gained back to some extent by the substitution of strong electron withdrawing groups (i.e. NO_2 and F) at position 4 [17]. In a different approach, Karadakov and coworkers [18,19] have recently investigated the aromaticity of these 5-membered heterocycles by using isotropic magnetic shielding distributions (HF-GIAO and MP2-GIAO isotropic shielding plots) in the regions of space surrounding the rings. Their study indicates that aromaticity decreases in the order thiophene > thiazole > pyrrole > imidazole > furan > oxazole suggesting that inclusion of a second heteroatom has a detrimental effect on its aromaticity, which is small but noticeable in thiazole and thiophene. These results are in agreement with previous studies also published by some of the current authors [20].

In order to assess the aromatic character of the heterocyclic cores of the guanidine derivatives proposed here, we have carried out NMR theoretical calculations of model systems, namely benzene, phenylguanidine (**5**), 2- (**6**), 3- (**7**) and 4-pyridinoguanidine (**8**), 2- (**9**) and 3-thiophenoguanidine (**10**) and 2-thiazologuanidine (**11**), at M06-2X [21] computational level with the 6-311++G(d,p) [22] basis set, using the GIAO method at 0, 1 and 2 Å over the ring centre of each model molecule and the results are presented in Table S1 (Supplemental Information). Further, to avoid possible interactions with the magnetic field of the atoms, several authors [23,24,25,26,27,28,29] have recommended extending the calculations up to 2

Å above and below the ring plane. In Figure S1 (Supplemental Information) an explanation and schematic representation of the NICS positions above/below the aromatic rings is given.

1-(Pyridin-2-yl)guanidine **6** can present two orientations of the guanidine moiety with respect to the pyridine ring; however, we have previously reported that the rotamer in which the guanidine moiety forms an intramolecular hydrogen bond (IMHB) with the pyridine N atom is 33.9 kJ mol^{-1} more stable [30] and, therefore, we have only considered that particular rotamer to assess its aromaticity.

Similarly, 1-(thiazol-2-yl)guanidine (**11**) presents two different energy minima, one where the guanidine also forms an IMHB with the N atom and another in which the guanidine is perpendicular to the thiazole ring. We have calculated [M06-2X/6-311++G(d,p)] that the planar structure is 20.6 kJ mol^{-1} more stable than the perpendicular one and, for that reason we have studied only the aromaticity of the most stable one. As seen in Table S1, all the compounds studied present similar magnetic field responses to that of benzene as shown by their closely matching values of NICS(1) (variations up to 0.7 ppm) and NICS(2) (variations up to 1.1 ppm).

However, a certain trend can be observed indicating that, although very subtle, there is a certain aromaticity order: benzene ≈ phenylguanidine (**5**) ≈ 1-(pyridin-2-yl)guanidine (**6**) ≈ 1-(pyridin-4-yl)guanidine (**8**) > 1-(pyridin-3-yl)guanidine (**7**) > 1-(thiophen-3-yl)guanidine (**10**) > 1-(thiophen-2-yl)guanidine (**9**) ≈ 1-(thiazol-2-yl)guanidine (**11**). Moreover, we had previously calculated the NICS values for 1-(pyridin-2-yl)guanidine (**6**) at a similar computational level and the results obtained here are consistent with our reported data [31].

Furthermore, to obtain additional information on the aromaticity of each compound, we have calculated and plotted the NICS values on the 0.001 a.u. electron density isosurface (Figure 2) and summarised the minima values over the ring of each surface (vdW_{up} and vdW_{bottom}) in Table S1.

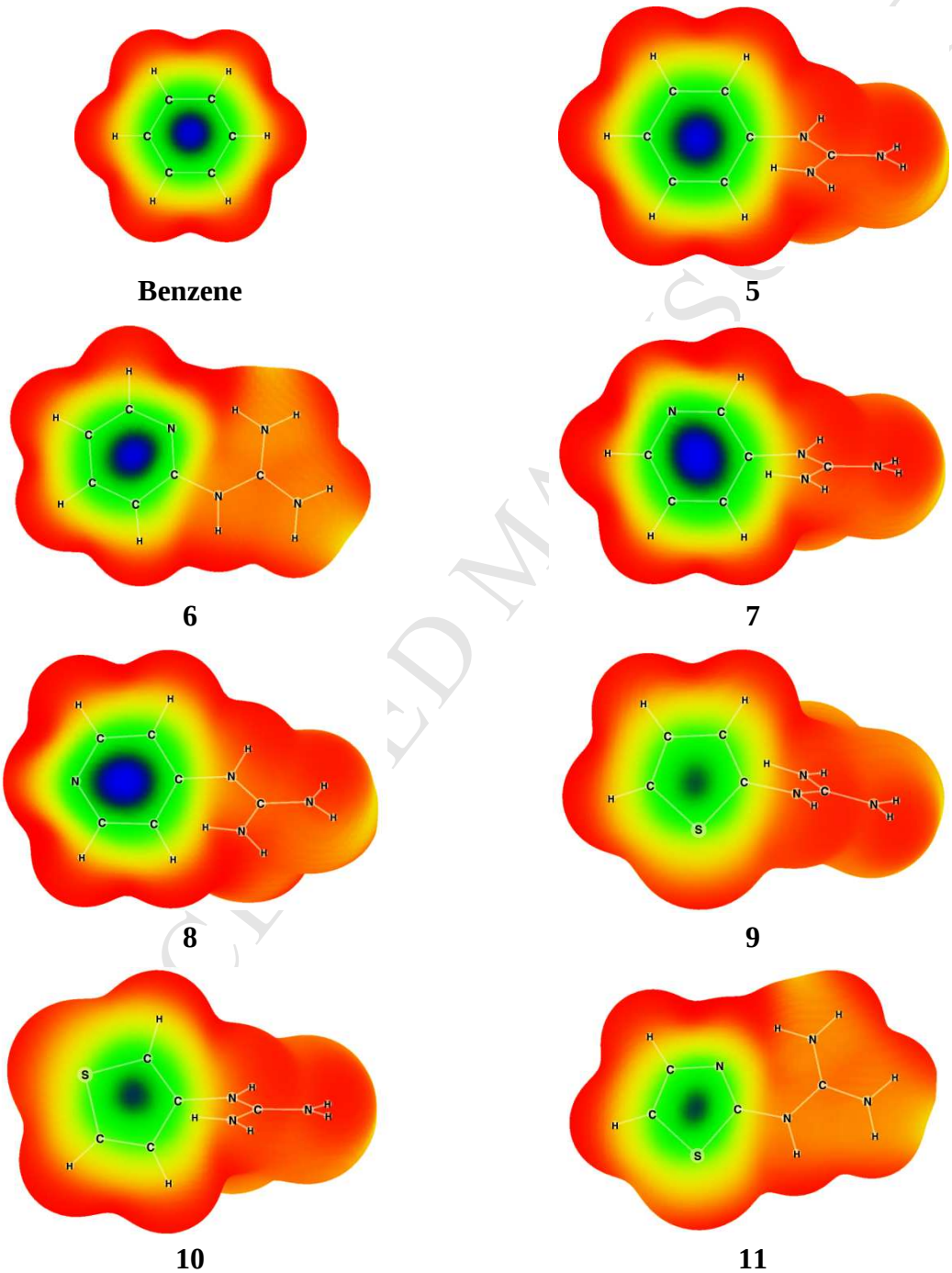


Figure 2. Bottom view of the NICS values on the 0.001 au electron density isosurface at the M06-2X/6-311++G(d,p) computational level of benzene and compounds **5-11**. NICS colour scheme: Red > 0.0, Yellow > -2.5, Green > -5.0, Blue < -5.0

Blue areas in Figure 2 correspond to NICS values < -5.0 ppm while red areas are those associated to positive NICS indices. As observed in benzene, a blue (negative) area is located over the centre of the ring denoting aromatic character. Very similar NICS patterns have been found in all the isosurfaces plotted in Figure 2. As with the NICS(1) and NICS(2) indices, five-membered ring compounds (**9**-**11**) present the same negative area but slightly smaller which can be related to a small decrease of aromaticity.

Therefore, in terms of the aromatic properties of the cyclic cores carrying the guanidine moiety, it seems that thiophene and thiazole derivatives could form the same type of interactions (even though slightly weaker) as the phenyl and pyridine motifs and, hence, preparing these types of analogues to target α2-AR as antagonists is merited.

2.2. Chemistry

The only synthesis found in the literature, to the best of our knowledge, of simple guanidine-like thiophenes is that of 3-*N*-(2-aminoimidazolinyl)thiophene [32]. Standard preparation of aromatic 2-aminoimidazoline [33] and guanidine derivatives [34] generally involves the use of aromatic amine intermediates. Since many thiophene amines are known to be unstable, this could explain the lack of guanidine-like thiophenes reported thus far. 2-Aminothiazoles are commercially available, and a number of thiazole guanidines have already been prepared by other methods than our standard guanidylation approach [33,34].

To test the suitability of our standard guanidylation methodology for the synthesis of thiophene analogues of the lead compound **1** (Figure 1) and looking for structural diversity, we first prepared guanidine derivatives of some commercially available aminothiophenes

such as 2-amino-3-methoxycarbonylthiophene (**12**) and 3-amino-2-methoxycarbonyl thiophene (**13**). Moreover, since the most closely related thiophene-based compounds tested at the α2-AR are 2- (**14**) or 3-thenylimidazole (**15**) derivatives of dexmedetomidine, which is an α2-AR agonist with high affinity (Figure 3) [35], we also used 2-aminomethyl- (**16**) and 2-aminoethylthiophenes (**17**) as starting materials (Scheme 1).

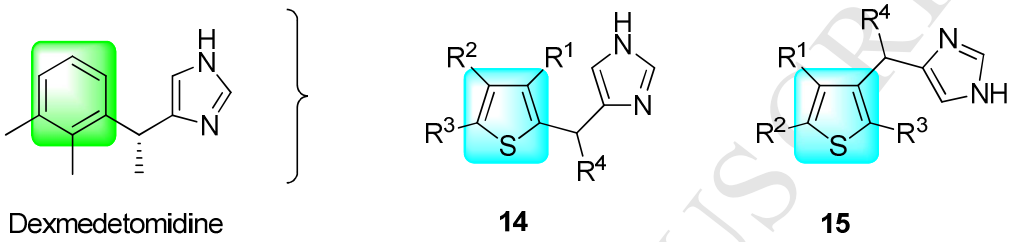


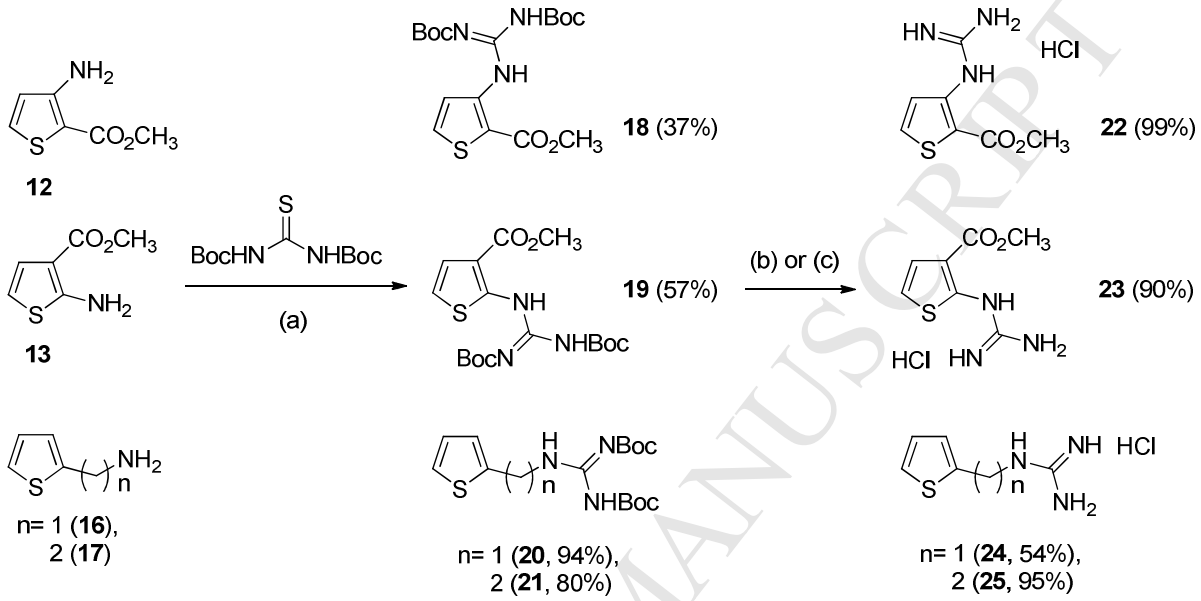
Figure 3.- Structure of α2-AR agonist dexmedetomidine and general structure of its thiophene analogues **14** and **15**.

Thus, syntheses of the Boc-protected guanidine derivatives of **12**, **13**, **16** and **17** (Scheme 1) were carried out by the reaction of the corresponding commercial aminothiophenes with an activated *N*-Boc-protected thiourea [9-14] in the presence of mercury(II) chloride and an excess of triethylamine producing the corresponding derivatives **18**-**21** in moderate to excellent yields (57, 37, 94 and 80%, respectively). As expected, aliphatic amines **16** and **17** gave significantly higher yields than their aromatic counterparts (**12** and **13**) because of the higher nucleophilic nature of the amino group.

Deprotection of the Boc-protected guanidines **18** to **21** was carried out by using a TFA/DCM solution followed by treatment with strongly basic anion-exchange resin giving the corresponding hydrochloride salts (**22**, **23**, **24** and **25**, Scheme 1) in yields of 99, 51, 54 and

43% respectively. Deprotection of **18**, **19** and **21** was also carried out using a 4M HCl/dioxane solution producing better yields (99, 90 and 95%, respectively).

Scheme 1. Preparation of thiophene guanidinium derivatives **22-25**



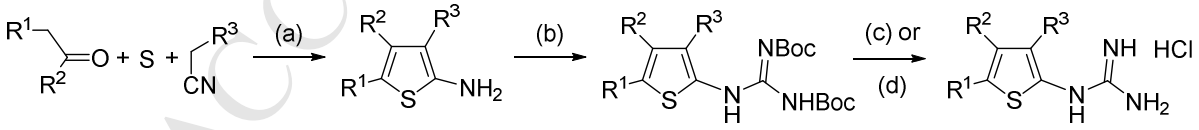
(a) $HgCl_2$; NEt_3; CH_2Cl_2; r.t., 12 h. (b) 50% TFA/CH_2Cl_2, r.t., followed by IRA400 Amberlyte resin Cl^- form, H_2O, r.t. (c) 4M HCl/Dioxane, r.t. Note: Only best yields achieved for the Boc-deprotection are shown independently of the method (b or c) used

Next, expanding the library of thienylguanidines, thiophene analogues of α2-AR antagonist **1** (Figure 1), which shows good affinity (K_i = 77.6 nM [10]), were prepared. Hence, the corresponding starting aminothiophenes were prepared following the Gewald synthesis and even though the typical outcome of this synthesis is a carboxylic ester (or carbonitrile) of the corresponding aminothiophene, we hypothesised that a subsequent decarboxylation could produce the target thiophene. Preparation of thiophenes **26-29** was carried out using the corresponding ketone (cyclopentanone, cyclohexanone, cycloheptanone and acetone, respectively), sulfur powder and ethyl cyanoacetate in ethanol at 40 °C with morpholine as catalytic base (Scheme 2) in poor to good yields (10, 79, 61 and 19%) [36].

The corresponding Boc-protected guanidino derivatives **30**, **31**, **32** and **33** were obtained using the standard guanidylation conditions, in 36, 52, 49 and 68% yields, respectively (Scheme 2). Boc-deprotection of **30**-**33** using 50% TFA/DCM resulted in the formation of the corresponding trifluoroacetate salts, which were treated directly with IRA400 Amberlyte resin in its chloride-activated form to yield the more water-soluble hydrochloride salts **34**-**37** in very good yields of 90, 93, 71 and 61% (Scheme 2). Using the HCl/dioxane method, **33** was successfully converted to **37** in 93% yield.

Taking advantage of the Gewald synthesis and extending the structural diversity of our thienylguanidine library, we prepared 2-aminothiophene-3-carbonitriles and their guanidine derivatives. Thus, reaction of malononitrile, sulfur and the appropriate ketone (cyclopentanone, cyclohexanone and cycloheptanone) in EtOH, with dropwise addition of morpholine resulted in the formation of thiophene carbonitriles **38**, **39** and **40** in respective yields of 46, 51 and 43% (Scheme 2). Synthesis of the Boc-protected guanidines (**41**-**43**) was carried out using the standard method and Boc-deprotection was carried out using the HCl/dioxane method, yielding hydrochlorides **44**-**46** in reasonable yields (Scheme 2).

Scheme 2. Preparation of thiopheno guanidinium derivatives **34-37** and **44-46**.

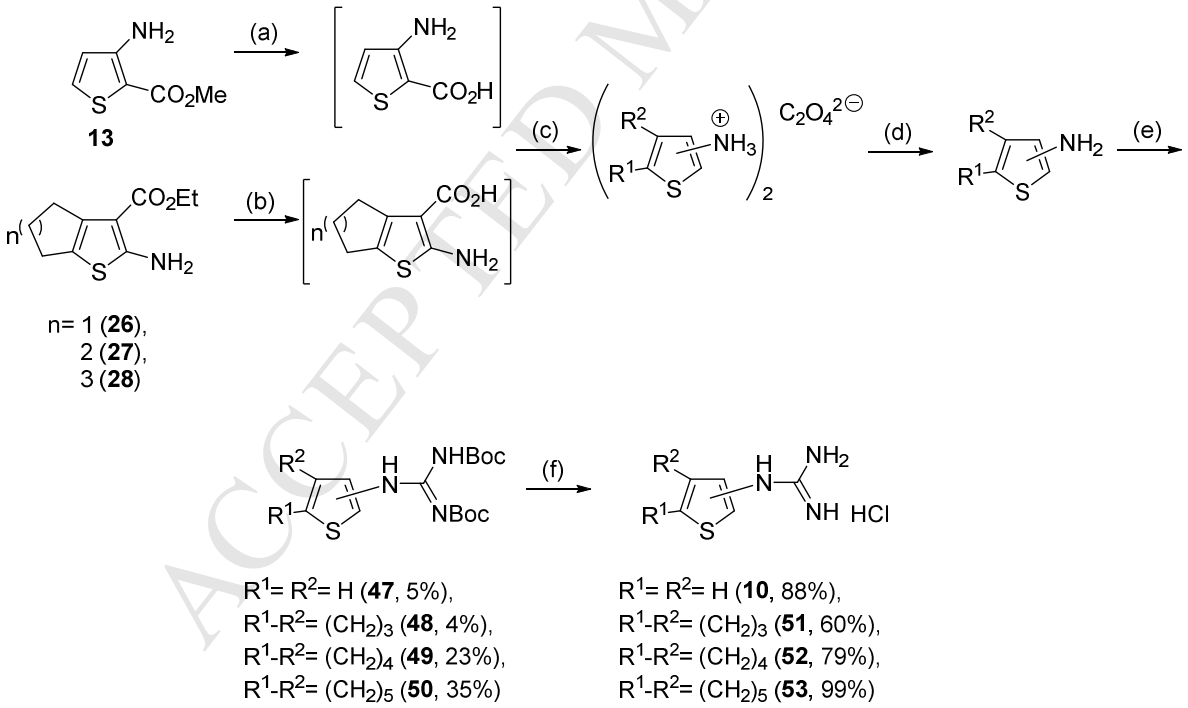


R^1-R^2 = -$(CH_2)_3$-; R^3= CO_2Et	(**26**, 10%),	(**30**, 36%),	(**34**, 90%),
R^1-R^2 = -$(CH_2)_4$-; R^3= CO_2Et	(**27**, 79%),	(**31**, 52%),	(**35**, 93%),
R^1-R^2 = -$(CH_2)_5$-; R^3= CO_2Et	(**28**, 61%),	(**32**, 49%),	(**36**, 71%),
R^1 = H, R^2 = CH_3; R^3= CO_2Et	(**29**, 19%),	(**33**, 68%),	(**37**, 93%),
R^1-R^2 = -$(CH_2)_3$-; R^3= CN	(**38**, 46%),	(**41**, 20%),	(**44**, 39%),
R^1-R^2 = -$(CH_2)_4$-; R^3= CN	(**39**, 51%),	(**42**, 36%),	(**45**, 43%),
R^1-R^2 = -$(CH_2)_5$-; R^3= CN	(**40**, 43%)	(**43**, 61%)	(**46**, 58%)

(a) Morpholine, Ethanol, 30-40 ^oC, 4 h. (b) $HgCl_2$, NEt_3, CH_2Cl_2, r.t., 12 h. (c) 50% TFA/CH_2Cl_2, r.t., followed by IRA400 Amberlyte resin Cl^- form, H_2O, r.t. (d) 4M HCl/Dioxane, r.t.

Next, to further expand the diversity of our guanidinothiophene library, decarboxylation of the thiophene esters was attempted. Using compound **13** as a model substrate, the conditions described by Barker et al. [37] using oxalic acid were tested. This method involves the hydrolysis of the corresponding thiophene ester using weakly basic conditions (2M NaOH) and refluxing over 30 min followed by decarboxylation and subsequent acidification with oxalic acid to pH = 3 to precipitate the ammonium salt. Following this procedure the oxalate salt of 3-aminothiophene (Scheme 3) was obtained, which was free-based and immediately guanidylated under the standard conditions to prevent decomposition prior to the next reaction step.

Scheme 3. Preparation of decarboxylated guanidino thiophenes



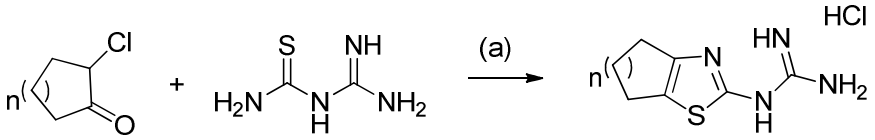
(a) 2M NaOH; (b) KOH, 1:1 EtOH/H_2O; (c) Oxalic acid, 2-propanol; (d) NH_4OH; (e) $HgCl_2$, NEt_3, CH_2Cl_2, r.t., 12 h. (f) 4M HCl/Dioxane, r.t.

The desired Boc-protected guanidine product **47** (Scheme 3) was obtained in a low yield and its deprotection was achieved by treatment with HCl/dioxane followed by reverse phase column chromatography resulting in the clean isolation of the hydrochloride salt of 3-thienylguanidine (**10**, Scheme 3) in 88% yield.

A modified version of Barker's conditions (using KOH in 1:1 water/ethanol for the hydrolysis step to facilitate dissolution of the starting materials) was applied to **26**, **27** and **28**, and, after guanidylation, the Boc-protected guanidines **48**-**50** were isolated in 4, 23 and 35% yields (Scheme 3). Deprotection with 4M HCl/dioxane followed by reverse phase column chromatography resulted in the corresponding hydrochloride salts **51**-**53** in 60, 79 and 99% yields, respectively (Scheme 3).

To complete these families of sulphur-containing heterocyclic guanidines, 2-aminothiazole analogues of the lead compound **4** were synthesized. These derivatives are more stable than their thiophene counterparts and many synthetic routes for the formation of thiazole and benzothiazole guanidines have been previously described, starting from α-haloketones and 2-imino-4-thiobiuret [38] or from 2-aminothiophenols and cyanoguanidine [39]. Since some of the desired α-chloroketones and 2-aminothiophenols were commercially available, we prepared the corresponding 2-guanidino cycloalkylthiazoles following Beyer's synthesis [38]. Therefore, both 2-chloro derivatives of cyclopentanone and cyclohexanone were treated with 2-imino-4-thiobiuret directly yielding the corresponding hydrochlorides **54** and **55** (Scheme 4) in good yields. Even though these two compounds are reported in a patent by Actelion Pharmaceuticals [40], no mention is made of their synthesis.

Scheme 4. Preparation of guanidino thiazoles



n= 1, 2

n= 1 (**54**, 30%),
2 (**55**, 56%)

(a) Δ, 1 h

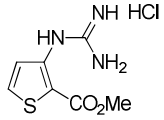
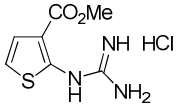
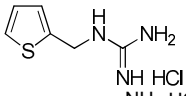
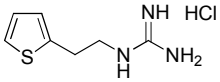
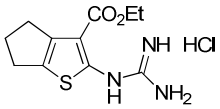
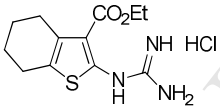
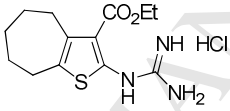
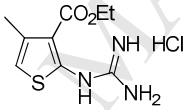
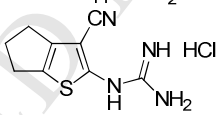
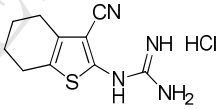
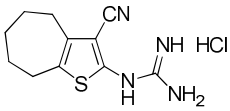
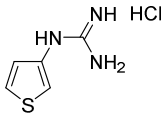
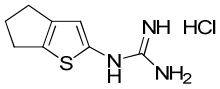
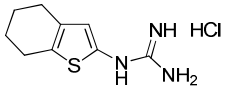
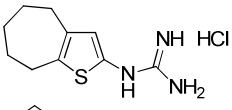
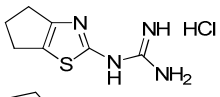
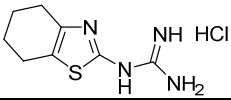
2.3. Pharmacology and SAR analysis

2.3.1. [^{3}H]RX821002 Binding Assays

The α2-AR binding affinities of all compounds were measured in human brain prefrontal cortex (PFC) tissue by competition assays with the α2-AR selective radioligand [^{3}H]RX821002, which was used at a constant concentration of 2 nM. Specific [^{3}H]RX821002 binding was measured in PFC membranes, which were incubated with [^{3}H]RX821002 for 30 min at 25 °C in the absence or presence of the competing compounds at increasing concentrations (10^{-12} - 10^{-3} M, ten concentrations). Thus, the specific binding was determined, plotted as a function of the compound concentration and the affinities obtained were expressed as affinity constants (K_i, nM).

The results of the [^{3}H]RX821002 binding affinity experiments for some of the thiophene and thiazole derivatives prepared are listed in Table 1. In general, many of the compounds tested show low α2-AR affinity (only 6 out of 17 compounds have K_i < 1000 nM), but some interesting trends can be observed. First, when the guanidinium group is attached to the thiophene ring through aliphatic chains (compounds **24** and **25**) relatively good α2-AR affinity values are obtained indicating that there is room in the active site to accommodate a linker between the guanidine and the thiophene nucleus. The best affinity (K_i= 751 nM) in this group is attained when the chain is longer (two -CH_2- groups, **25**).

Table 1.- Binding affinity for the human brain prefrontal cortex α2-ARs expressed as K_i calculated from [^{3}H]RX821002 (≈ 2 nM) competition binding experiments.

Compound	Structure	K_i (nM)
22		>100,000
23		>100,000
24		3928 ±54
25		751 ±7
34		30,150 ±1114
35		9086 ±210
36		>100,000
37		66,530 ±6339
44		2215 ±50
45		733 ±8
46		1198 ±58
10		1968 ±41
51		87.9 ±0.5
52		31.3 ±0.3
53		156 ±1
54		706 ±9
55		1205 ±25

The K_i values obtained for compounds **22** and **10** indicate that the presence of an ester group in position 2 of the 3-guanidinium derivatives (compound **22**) completely abolishes α2-AR affinity; moreover, when the positions of the guanidinium and carboxylic ester groups are interchanged (compounds **22** and **23**), the α2-AR affinity continues to be abolished; this possibly indicates that these compounds are sterically or electronically disfavoured from properly fitting into the active site. However, introduction of a methyl group in position 4 (compound **37**) partially recovers the affinity (K_i = 66,530 nM). It has been proposed that good antagonists should occupy the pocket created towards TM5 in class A GPCRs without directly interacting with Ser5.42 and Ser5.43 residues (which is necessary for G-protein signalling activation) [41]. Thus, assuming that the guanidinium cation will bind the Asp residue on TM3 that is conserved in class A GPCRs (Asp3.32), the coplanar arrangement between thiophene and guanidinium moieties would be unfavourable for binding, but, the introduction of the 4-methyl group would benefit affinity because it would partially occupy that pocket near TM5.

When a methyl group is present in position 4 or an alkyl cycle is attached to positions 4 and 5 of a 2-guanidino-3-carboxylate thiophene core, the α2-AR affinity observed depends on the size of such alkyl system. Thus, the 5-membered cycloakyl and the 4-methyl analogues (**34** and **37**, respectively) show worse affinity than the 6-membered ring derivative (**35**), but better than the 7-membered cycloakyl analogue (**36**), which shows no affinity under the assay conditions. This can be explained by the steric limits of the lipophilic pocket at TM5 that clashes with the size of the cyclohepta alkyl ring of compound **36** but is optimal for the 6-member ring analogue **35**.

In this series of fused cycloakyl derivatives, replacement of the ester group (**34**-**36**) by a carbonitrile functionality (**44**-**46**) results in a significant increase in α2-AR affinity. In the case of **44** and **45**, the K_i values (nM) are 12-13 times better than the ester analogues **34** and **35**, whereas the difference is even larger between 7-membered analogues **46** and **36**, with the K_i of the latter around 1198 nM while the former does not bind to the receptor under the assay conditions. These results may indicate that compound **36**, with both a bulky ethyl ester group in the 3-position and a large seven membered ring fused in 4- and 5-positions, is too large to fit the active site of the α2-AR receptor and, moreover, these bulky systems could prevent the optimal interaction between the guanidinium and Asp3.32. However **46**, with the smaller nitrile group in the 3-position can actually fit the receptor well. In general, the 6-membered derivatives display stronger α2-AR affinity than the 7-membered ones, whereas the K_i difference between **45** and **46** (both with a CN in position 3) is not too large.

All evidence presented until now points to larger substituents disrupting the salt-bridge with Asp3.32, thereby lowering affinity. Accordingly, decarboxylated derivatives **51**-**53** show the best α2-AR affinity values (87.9, 31.3 and 156 nM, respectively) among all the tested compounds in this small library. This is in agreement with the idea that the aliphatic rings of these compounds, by occupying the pocket towards TM5 and displacing water molecules from this region, produce an increase in affinity. Again the fused 6-membered derivative is the best of this series whereas the 7-member analogue is the worst.

Thiazole compounds **54** and **55**, which have good K_i values (Table 1), display the opposite affinity pattern to their thiophene counterparts, with the 5-membered ring derivative (**54**) showing stronger affinity than the 6-membered ring analogue (**55**).

Compared with their phenyl and pyridine guanidine analogues, we found that, in the case of (hetero)aromatic systems only substituted with guanidine, all the Ki values are very large and the similar aromaticity does not account for the large differences observed in α2-AR affinity (162,181 nM for pyridine-3-ylguanidine **7**, 1950 nM for thiophen-3-ylguanidine **10**, 1820 nM for pyridine-2-ylguanidine **6** and 646 nM for phenylguanidine **5**). In the case of cycloakyl (hetero)aromatic guanidines, despite the slightly poorer aromatic character of thiophene and thiazole, good α2-AR affinities were obtained (see Ki values for **51** and **52** in Table 1) further improving those reported by us for 1-(2,3-dihydro-1H-inden-5-yl)guanidine (309 nM [10]), 1-(5,6,7,8-tetrahydroisoquinolin-3-yl)guanidine **4** (468 nM [14]) or the lead compound **1** (77.6 nM [10]). Therefore, aromaticity cannot be considered as a determining factor in α2-AR binding; however, these heteroaromatic rings may act as a scaffold for the different substituents and, thus, the differences observed between benzene and thiophene/thiazole may be due to changes in the placement of substituents.

2.3.2. [^{35}S]GTPγS Binding Assays

This assay allows for the direct evaluation of G-protein activation by determining the GTP exchange using [^{35}S]GTPγS in human PFC membranes. In particular, agonists increase the nucleotide binding, inverse agonists reduce nucleotide binding and antagonists do not affect binding. Assays were incubated at 30 °C for 120 min with shaking. Ten concentrations (10^{-12} - 10^{-3}M) of the different compounds were added to the assay in order to evaluate their effect on [^{35}S]GTPγS binding. Then, the plates were subjected to vacuum filtration and the radioactivity of the filter was measured by scintillation spectrometry. The EC_{50} values (concentration that provokes 50% of the maximal response) and E_{max} values (maximal response in % over basal binding) were calculated for all compounds and for the well-known α2-AR agonist UK14304. Typical potency values are 1-2 log units lower than the affinity

values obtained in radioligand receptor binding experiments among other reasons, because assays were performed in low-affinity receptor conditions for agonists (in the presence of G nucleotides and sodium).

All compounds that showed an antagonist activity in the [^{35}S]GTPγS binding assays, i.e. those that did not stimulate binding of [^{35}S]GTPγS by their own, were also assayed at a constant concentration (10^{-5} M) for [^{35}S]GTPγS binding in the presence of increasing concentrations of the α2-AR agonist UK14304 (10^{-13}-10^{-4} M). If the concentration-response curve for UK14304 in these assays is shifted to the right, the antagonist effect of these derivatives against the α2-ARs is confirmed. These experiments were also performed in order to investigate the potential of the compounds to specifically modify the EC_{50} or the E_{max} values in the UK14304 stimulation curve.

Activity at the α2-AR was examined for compounds with a K_i < 1000 nM (**25**, **45**, **51**, **52**, **53** and **54**), along with thiazole **55** for comparison. Interestingly, all the compounds except **53**, displayed antagonistic activity in the [^{35}S]GTPγS assays, similar to the lead compound **1** (phenyl core); this is likely due to the orientation of the thiophene nucleus when attached to the guanidine group. The corresponding EC_{50} values for UK14304 in the presence of these compounds are shown in Table 2. Compound **53**, with a bulky fused 7-membered alkyl ring, was determined to be an agonist since it stimulated [^{35}S]GTPγS binding by its own and this stimulation was abolished in the presence of the α2-AR antagonist RX821002 at 10^{-5} M. This agonistic activity may be a result of the size of the 7-membered ring, which is big enough to alter TM5 (somehow interacting with Ser5.42 and Ser5.43) in such a way that leads to activation of G-protein coupling.

Addition of **25**, **51**, **52** and **54** to the experiment induced a very small (<5-fold) rightward shift in the EC_{50} value for UK14304, indicating that they have only weak antagonistic effects at the α2-AR. On the contrary, **45** and **55** caused a larger shift to the EC_{50} of the standard agonist UK14304, characteristic of competitive antagonists. These two compounds have average binding affinities (K_i = 733 and 1205 nM, respectively) suggesting that they may form different interactions or access a pocket not available to the rest of the compounds, allowing them to block activation of the receptor by UK14304.

Table 2.- EC_{50} and E_{max} values obtained from the concentration-response curves for UK14304 (10^{-13} -10^{-4} M) stimulation of [^{35}S]GTPγS binding in the absence or presence of the different compounds (10^{-5} M).

	EC_{50} (μM) ±SEM	**E_{max} (%) ±SEM**
UK14304	0.4 ±0.01	132 ±2
UK14304 + 25	1.7 ±0.06	117 ±4
UK14304 + 45	6.6 ±0.52	128 ±6
UK14304 + 51	0.5 ±0.04	111 ±2
UK14304 + 52	0.8 ±0.09	111 ±4
UK14304 + 53	10.7 ±3.7	124 ±9
UK14304 + 54	1.5 ±0.06	126 ±3
UK14304 + 55	11.4 ±3.40	105 ±4

This particular orientation could be explained by the presence of an IMHB in compound **55** that positions the cycloakyl moiety towards the mentioned lipophilic pocket by TM5, and the orientation achieved by the guanidinium in the 3-CN derivative **45** which situates the cycloakyl ring within the same pocket (Figure 4) [42].

According to this, the space allowed for the cycloakyl moiety seems to be limited to that of a 6-member ring for optimally engaging with TM5 of the α2-AR, and, by hosting this

cycloalkyl moiety, facilitates the appropriate orientation of the guanidinium moiety to achieve antagonist activity.

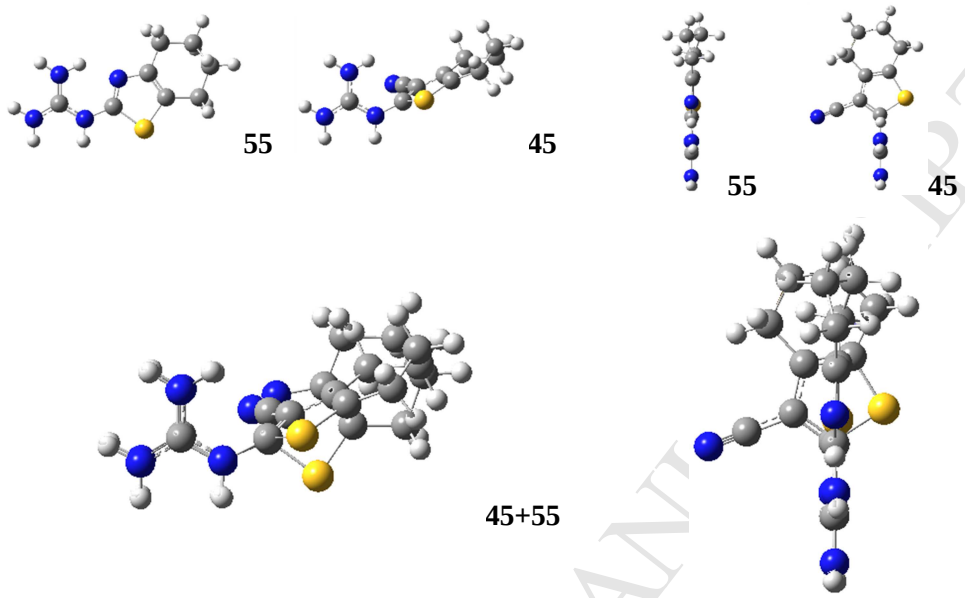


Figure 4.- Optimised structures of compounds **55** and **45** (calculated at M062X/6-311++G(p,d) level) and their superimposition viewed from the front (left) and a perpendicular view (right).

3. CONCLUSIONS

A comparative study of aromaticity has been carried out at M062X/6-311++G(p,d) computational level between phenyl, pyridine, thiophene and thiazole guanidinium derivatives by evaluating their corresponding NICS indices. The outcomes of this study indicate that thiophene and thiazole will be good bioisosteric alternatives to benzene in these guanidinium systems.

Accordingly, a small library of guanidine sulphur containing heterocyclic derivatives (15 thiophenes and 2 thiazoles) has been prepared, following standard guanidylation methodologies and new synthetic routes, to explore the effect that the bioisosteric changes

now introduced has on affinity and antagonist activity at the α2-ARs compared to previously prepared phenyl and pyridine derivatives.

Compounds **22**, **23** and **36** showed no binding affinity for the human α2-ARs in [^{3}H]RX821002 (≈ 2 nM) competition binding experiments. Compounds **34**, **35** and **37** bound poorly to the α2-AR, having K_i values around 9-66 μM. Medium to good α2-AR affinity was observed for compounds **10**, **24**, **25**, **44**, **45**, **46**, **53**, **54** and **55**, all of which have K_i values between 3.9 μM and 156 nM. Finally, compounds **51** and **52** have very good α2-ARs binding affinities of 88 and 31 nM, respectively.

Some trends were observed with regard to engagement with the α2-ARs; thus, 6-membered cycloalkyl rings conferred the strongest affinity (smaller K_i values) within each thiophene series. In the case of the thiazoles the 5-membered analogue proved to be a better binder.

Thiophen-2-ylguanidines with only cycloalkyl substituents gave better binding affinities than those with added ester or nitrile functionalities. However, in the case of the thiophen-3-ylguanidine **10**, more modest results were observed in comparison to derivatives containing other aromatic rings.

Almost all compounds tested for activity at α2-ARs (**25**, **45**, **51**, **52**, **53**, **54** and **55**) proved to be antagonists with only compound **53** showing agonistic activity. The large size of the 7-membered cycloalkyl system in **53** is likely responsible for changing the mode of binding of this compound and hence its activity.

In summary, even though the aromatic character of guanidinium derivatives of thiophene and thiazole is slightly poorer than that of their phenyl or pyridine analogues, α2-AR affinities in the nM range were observed for some of the S-containing compounds and, in particular **45** and **55**, which are antagonists that displaced α2-ARs agonist binding 16 and 28 times to the right and place the cycloakyl systems in a similar orientation, can be considered compounds of interest for future investigations.

4. EXPERIMENTAL SECTION

4.1. Computational details

All calculations have been carried out using the Gaussian09 computational package [43]. All systems have been optimized at the M06-2X [21] computational level using the 6-311++G(d,p) [22] basis sets. Frequency calculations have been carried out to confirm that the structures obtained correspond to energetic minima. Effects of water solvation have been included by means of the SCFR–PCM approaches implemented in the Gaussian09 starting from the gas–phase geometries and re-optimizing.

Nucleus-Independent Chemical Shifts (NICS) values [7] were calculated using the Gauge-Including Atomic Orbital (GIAO) method [44,45] on the B3LYP/6-311++G(d,p) geometries. To obtain the spatial distribution of the NICS, its values have been calculated on a three dimensional (3D) cubic grid of 12 Å size following the procedure described in our previous works [20,46]. The points in the grid are located at 0.2 Å one from other in the three spatial directions. The result is a cube of 226,800 NICS values, which in the next step are represented over the 0.001 a.u. electron density isosurface using the Wave Function Analysis Surface Analysis Suite (WFA-SAS) program [47].

4.2. 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 performed 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. Infrared spectra were obtained on a Perkin-Elmer Spectrum 100 FT-IR spectrometer equipped with Universal ATR sampling accessory. The X-ray crystallography data for crystal samples were collected on a Rigaku Saturn 724 CCD Diffractometer. Elemental analysis was carried out at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College Dublin. 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^{-1}: aqueous formate buffer (30 mM, pH 3.0) for 10 min, linear ramp to 85% methanol buffered with the same system over 25 minutes, 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: Preparation of 2-Amino-3-substituted Thiophenes via the Gewald Reaction

A mixture of ketone (1.0 eq.), sulfur powder (1.0 eq.) and t-butyl cyanoacetate, ethyl cyanoacetate or malononitrile (1.0 eq.) in EtOH (2 mL/mmol) was prepared before morpholine (1.0 eq.) was added dropwise, ensuring that the reaction did not heat up above 60 °C during the addition. The mixture was then heated at 40 °C and stirred for 4-20 h.

4.2.1.2. Method B: Synthesis of the Boc-Protected Guanidinothiophene Derivatives

A solution of the corresponding amine (1 eq.), Boc-protected *S*-methylthiopseudourea (1 eq.), TEA (~3.5 eq.) in dry DCM (10-20 mL/mmol) was prepared, set at 0 °C and stirred for 20 min. Then, $HgCl_2$ (1.2 or 1.5 eq.) was added and the solution stirred at 0 °C for a further 40 min and was then stirred for 48 h. at r.t. until the reaction had reached completion (TLC). The reaction mixture was filtered through a pad of celite in a sintered glass funnel, and washed with EtOAc. The filtrate was then washed with brine, dried over $MgSO_4$ and the solvents removed, before further purification.

4.2.1.3. Method C: Synthesis of the Boc-Protected Guanidine Derivatives involving Decarboxylation

The relevant ester was refluxed in 2M NaOH (10 mL) or (5 mL) with ethanol (5 mL) for the period of time specified. The solution was cooled, acidified to pH 3 with concentrated HCl and the precipitate was filtered off and dissolved in acetone (12.5 or 6.5 mL). The solution was dried (MgSO4), filtered and the solvent evaporated at 20 °C. The resulting solid was treated with 2-propanol (3 or 1.5 mL) and anhydrous oxalic acid (1 or 0.6 g) at 38 °C for 45 min. The mixture was cooled, diluted with ether, the solid filtered off and washed with ether and dried. The salt was dissolved in water, basified with conc. ammonia. The mixture was extracted with DCM (3 × 5 mL), the combined extracts were dried (MgSO4) and the solvents removed. The material was redissolved in DCM (10 mL) before an appropriate quantity of each of the following were added sequentially, based on the mass of the crude dicationic salt: 62 (2.4 eq.), TEA (8.0 eq) and HgCl2 (3.0 eq.). The solution was stirred for 2 days, was filtered through a pad of celite and washed with EtOAc. The filtrate was washed with brine before being dried over MgSO4 and the solvents removed. The resulting material was purified on using column chromatography on a Biotage (hexane/EtOAc 1%/CV over 20 CV).

4.2.1.4. Method D: Preparation of the Hydrochloride Salts using TFA/DCM

A 50% (v/v) solution of TFA in DCM (10 mL) was added to the corresponding di-Boc-protected guanidine precursor (1.0 eq.). The mixture was stirred for 4 h at r.t., and the solvent was then removed under vacuum yielding the trifluoroacetate salt. This was dissolved in water (10 mL) and IRA400 Amberlyte Resin in its chloride form (1.0 g/eq.) was added. The mixture was stirred at r.t. for 24 h. The resin was then removed by filtration, the aqueous solution was washed with DCM, and the water evaporated, yielding the pure hydrochloride salt. Absence of the trifluoroacetate anion was checked for by ^{19}F NMR.

4.2.1.5. Method E: Preparation of the Hydrochloride Salts using HCl/Dioxane

The di-Boc-protected guanidine precursor (1 eq.) was dissolved in 4 M HCl in dioxane (25 eq.). The reaction was stirred at 60 °C during 6 h before the solvent was removed. The sample was purified through a 3 cm reverse phase pencil column (3 CV water, 2 CV 9:1 water:acetonitrile, 2 CV 1:1 water:acetonitrile, 2 CV acetonitrile).

4.2.1.6. Method F: Direct Preparation of the Thiazole Hydrochloride Salts

A mixture of 2-imino-4-thiobiuret (236.3 mg; 2 mmol; 1 eq.) and the corresponding α-chlorocycloketone (2 mmol; 1 eq.) was heated to 130 °C for 1 h. After cooling, the mixture was filtered and washed with water and HCl (32%; 1 mL). The filtrate was then evaporated down in vacuo yielding the corresponding salt.

4.2.2. 1-(Thiophen-3-yl)guanidine hydrochloride (10)

Following Method E from **47** (50.0 mg; 0.146 mmol; 1 eq.) a colourless gel was obtained in a 88% yield (22.8 mg). ^{1}H NMR (400 MHz, D_2O) δ 7.42 (dd, 1H, J = 5.1, 3.1 Hz), 7.27 (dd, 1H, 4J = 0.9, 3.1 Hz), 6.95 (dd, 1H, J = 5.1, 4J = 0.9 Hz). ^{13}C NMR (100 MHz, D_2O) δ 156.1, 131.5, 126.7, 124.3, 120.2. IR ν_{max} 3301, 3108 (NH), 1664 (C=N), 1600, 1534, 1438, 1408, 1360, 1230, 1185, 1079, 837, 790 cm^{-1}. HRMS (ESI) calculated, 142.0394 $[M + H]^+$; found, 142.0364. Anal. ($C_5H_7N_3S$) C, H, N.

4.2.3. Methyl 3-guanidinothiophene-2-carboxylate hydrochloride (22)

Following Method D from **18** (300.0 mg; 0.75 mmol; 1 eq.) or following Method E from **18** (100 mg; 0.25 mmol; 1 eq.) a white solid was obtained in a 99% yield (174.5 mg or 58.7 mg, respectively). M.p. 170 °C, decomposes > 172 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.66 (d, 1H, J = 5.5 Hz), 7.08 (d, 1H, J = 5.5 Hz), 3.74 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 163.5, 155.6, 138.2, 133.3, 124.4, 120.1, 52.6. IR ν_{max} 3425 (NH), 3136, 3106 (NH), 1659 (C=O), 1601

(C=N), 1533, 1444, 1397, 1283, 1243, 1083, 1060, 785 cm^{-1}. HRMS (ESI): calculated, 200.0494 $[M + H]^+$; found, 200.0490. Anal. ($C_7H_{10}ClN_3O_2S$), C, H, N.

4.2.4. Methyl 2-guanidinothiophene-3-carboxylate hydrochloride (23)

Following Method D from **19** (300.0 mg; 0.75 mmol; 1 eq.) or Method E from **19** (100 mg; 0.25 mmol; 1 eq.) a white solid was obtained in a 51% (90.2 mg) or 90% yield (52.9 mg), respectively. M.p. 180-181 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.27-7.32 (m, 2H), 3.75 (s, 3H). ^{13}C NMR (100 MHz, D_2O) δ 163.8, 157.0, 141.5, 127.5, 126.9, 124.2, 52.4. IR ν_{max} 3376, 3184 (NH), 3116 (NH), 2921 (NH), 2850, 2283, 1698, 1666 (C=N), 1642 (C=O), 1592, 1578, 1434, 1389, 1289, 1191, 1150, 1094, 979, 851, 726 cm^{-1}. HRMS (ESI) calculated 200.0494 $[M + H]^+$; found, 200.0498. Anal. ($C_7H_{10}ClN_3O_2S$) C, H, N.

4.2.5. 1-(Thiophen-2-ylmethyl)guanidine hydrochloride (24)

Following Method D from **20** (300.0 mg; 0.84 mmol; 1 eq.), a white solid was obtained in a 54% yield (88.1 mg). M.p. 103-105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, 1H, J = 5.0 Hz), 6.99 (d, 1H, J = 3.3 Hz), 6.93 (dd, 1H, J = 3.3, 5.0 Hz), 4.47 (app. brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 138.8, 127.3, 126.4, 126.1, 39.8. IR ν_{max} 3398 (NH), 3306 (NH), 3233, 3129 (NH), 3083, 3047, 1665, 1626 (C=N), 1591, 1464, 1363, 1338, 1223, 1173, 1081, 1047, 850, 726, 663 cm^{-1}. HRMS (ESI) calculated, 156.0595 $[M + H]^+$; found 156.0592. Anal. ($C_6H_{10}ClN_3S$) C, H, N.

4.2.6. 1-(Thiophen-2-ylethyl)guanidine hydrochloride (25)

Following Method D from **21** (300.0 mg; 0.81 mmol; 1 eq.) or Method E from **21** (100 mg; 0.27 mmol; 1 eq.) a white solid was obtained in a 43% (72.1 mg) or 95% yield (55.0 mg), respectively. M.p. 75-78 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, 1H, J = 4.8 Hz), 6.90

(dd, 1H, J = 2.9, 4.8 Hz), 6.84 (d, 1H, J = 2.9 Hz), 3.33 (t, 2H, J = 6.5 Hz), 2.98 (t, 2H, J = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) 156.6, 140.5, 127.3, 126.0, 124.7, 42.4, 28.4. IR ν_{max} 3258 (NH), 3148 (NH), 2947, 2422 (C=N), 1662, 1643, 1609, 1567, 1466, 1420, 1355, 1332, 1254, 1207, 1158, 1094, 1029, 845, 821, 705 cm^{-1}. HRMS (ESI) calculated, 170.0752 $[M + H]^+$; found, 170.0754. HPLC: 97.9% (t_R 19.48 min).

4.2.7. Ethyl 2-guanidino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate hydrochloride (34)

Following Method D from **30** (200.0 mg: 0.46 mmol; 1 eq.) a pale brown solid was obtained in a 90% yield (115.2 mg). M.p. 99-102 °C. ^{1}H NMR (400 MHz, D_2O) δ 4.20 (q, 2H, J = 7.2 Hz), 2.82-2.78 (m, 4H), 2.24 (app. quint., 2H, J = 5.5 Hz), 1.23 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, D_2O) δ 163.8, 157.4, 145.1, 141.4, 140.2, 124.3, 61.8, 29.8, 29.0, 27.0, 13.3. IR ν_{max} 3373 (NH), 3154 (NH), 1706, 1672 (C=O), 1630 (C=N), 1583, 1546, 1473, 1267, 1196, 1046, 1013, 782 cm^{-1}. HRMS (ESI) calculated, 254.0963 $[M + H]^+$; found, 254.0958. Anal. ($C_{11}H_{16}ClN_3O_2S$) C, H, N.

4.2.8. Ethyl 2-guanidino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate hydrochloride (35)

Following Method D from **31** (500.0 mg; 1.07 mmol; 1.0 eq.) a tan solid was obtained in a 93% yield (302.0 mg). M.p. 89-92 °C. ^{1}H NMR (400 MHz, D_2O) δ 4.13 (q, 2H, J = 7.0 Hz), 2.53-2.52 (m, 4H), 1.61-1.59 (m, 4H), 1.19 (t, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, D_2O) δ 164.0, 157.2, 137.3, 135.9, 135.0, 127.2, 61.8, 25.6, 24.4, 22.2, 21.9, 13.3. IR ν_{max} 3115 (NH), 2934 (NH), 1666 (C=O), 1624 (C=N), 1586, 1416, 1323, 1274, 1191, 1143, 1024 cm^{-1}. HRMS (ESI) calculated, 268.1120 $[M + H]^+$; found, 268.1114. Anal. ($C_{12}H_{18}ClN_3O_2S$) C, H, N.

4.2.9. Ethyl 2-guanidino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate hydrochloride (36)

Following Method D from **32** (100.0 mg; 0.208 mmol; 1.0 eq.) a yellow gel was obtained in a 71% yield (46.7 mg). ^{1}H NMR (400 MHz, D_2O) δ 4.19 (q, 2H, J = 7.0 Hz), 2.72 (t, 2H, J = 4.5 Hz), 2.68 (t, 2H, J = 4.5 Hz), 1.74-1.69 (m, 2H), 1.53-1.48 (m, 2H), 1.46-1.41 (m, 2H), 1.18 (t, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, D_2O) δ 164.5, 157.0, 140.7, 139.5, 132.4, 129.8, 61.7, 31.3, 28.5, 27.2, 26.7, 26.2, 12.7. IR ν_{max} 3148 (NH), 2921, 2849, 1666 (CN), 1587 (C=O), 1476, 1444, 1414, 1331, 1283, 1217, 1150, 1019 cm^{-1}. HRMS (ESI) calculated, 282.1276 $[M + H]^+$; found, 282.1267. Anal. ($C_{13}H_{20}ClN_3O_2S$) C, H, N.

4.2.10. Ethyl 2-guanidino-4-methylthiophene-3-carboxylate hydrochloride (37)

Following Method D from **33** (100.0 mg; 0.233 mmol; 1.0 eq.) a pale yellow solid was obtained in a 93% yield (57.1 mg). M.p. decomposes >240 °C. ^{1}H NMR (400 MHz, D2O) δ 6.97 (s, 1H), 4.21 (q, 2H, J = 7.0 Hz), 2.22 (s, 3H), 1.26 (t, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, D_2O) δ 164.0, 157.4, 140.5, 138.2, 128.1, 120.8, 61.8, 16.1, 13.2. IR ν_{max} 3449 (NH), 3294, 3133 (NH), 2859, 2759, 1637, (C=N), 1594 (C=O), 1543, 1487, 1375, 1228, 1163, 1078, 1043, 941, 871, 779, 730 cm^{-1}. HRMS (ESI) calculated, 228.0807 $[M + H]^+$; found, 228.0806. Anal. ($C_9H_{14}ClN_3O_2S$) C, H, N.

4.2.11. 1-(3-Cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)guanidine hydrochloride (44)

Following Method D from **41** (50.0 mg: 0.123 mmol; 1 eq.) a yellow gel was obtained in a 39% yield (11.5 mg). ^{1}H NMR (400 MHz, D_2O) δ 2.83 (t, 2H, J = 7.3 Hz), 2.74 (t, 2H, J = 7.2 Hz), 2.31-2.28 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 156.4, 144.6, 143.7, 142.3, 113.1,

104.8, 29.1, 27.2, 26.6. IR ν_{max} 3410 (NH), 3302, 3126, 2224 (CN), 1673 (C=N), 1655, 1598, 1564, 1469, 1249, 1156, 1062, 766 cm^{-1}. HRMS (ESI) calculated, 207.0704 $[M + H]^+$; found 207.0691. Anal. ($C_9H_{11}ClN_4S$) requires C, H, N.

4.2.12. 1-(3-Cyano-4,5,6,7-tetrahydrobenzo[9]thiophen-2-yl)guanidine hydrochloride (45)

Following Method D from **42** (300.0 mg: 0.713 mmol; 1 eq.) or Method E from **42** (100 mg; 0.238 mmol; 1 eq.) a pale yellow solid was obtained in a 23% (41.2 mg) or 43% yield (26.3 mg), respectively. M.p. decomposes >170 °C. ^{1}H NMR (400 MHz, D_2O) δ 2.68-2.64 (m, 2H), 2.58 (t, 2H, J = 6.0 Hz), 1.79-1.76 (m, 4H, H7). ^{13}C NMR (100 MHz, D_2O) δ 156.6, 141.1, 137.3, 134.9, 113.4, 108.7, 24.0, 23.7, 22.3, 21.3. IR ν_{max} 3159 (NH), 2938 (NH), 2225 (CN), 1663, 1629, 1885 (C=N), 1438, 1335, 1265, 1140, 765 cm^{-1}. HRMS (ESI) calculated, 221.0861 $[M + H]^+$; found 221.0861. Anal. ($C_{10}H_{13}ClN_4S$) C, H, N.

4.2.13. 1-(3-Cyano-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-2-yl)guanidine hydrochloride (46)

Following Method E from **43** (100.0 mg; 0.23 mmol; 1 eq.) a yellow gel was obtained in a 58% yield (36.4 mg). ^{1}H NMR (400 MHz, D_2O) δ 2.78 (t, 2H, J = 5.2 Hz), 2.73 (t, 2H, J = 5.2 Hz), 1.83 – 1.81 (m, 2H), 1.64-1.59 (m, 4H, H7). ^{13}C NMR (100 MHz, D_2O) 156.7, 141.5, 140.0, 138.3, 113.8, 111.1, 31.2, 29.1, 28.8, 27.1, 26.4. IR ν_{max} 3293 (NH), 3119 (NH), 2924, 2225 (CN), 1675 (C=N), 1627, 1586, 1439, 1256, 1159, 1117 cm^{-1}. HRMS (ESI) calculated, 235.0973 $[M + H]^+$; found 235.0933. Anal. ($C_{11}H_{15}ClN_4S$) C, H, N.

4.2.14. 1-(5,6-Dihydro-4H-cyclopenta[b]thiophen-2-yl)guanidine hydrochloride (51)

Following Method E using **48** (50.0 mg; 0.131 mmol; 1 eq.) a yellow gel was obtained in a 60% yield (11.7 mg). ^{1}H NMR (400 MHz, D_2O) δ 6.73 (s, 1H), 2.76 (t, 2H, J = 6.9 Hz), 2.59

(t, 2H, J = 6.9 Hz), 2.24-2.20 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 157.6, 144.1, 141.8, 135.1, 123.1, 29.2, 28.3, 27.5. IR ν_{max} 3318 (NH), 2953 (NH), 2855, 2456, 1654, 1586 (C=N), 1439, 1194, 1154 cm^{-1}. HRMS (ESI) calculated, 182.0752 $[M + H]^+$; found, 182.0751. Anal. ($C_8H_{13}ClN_3S$) C, H, N.

4.2.15. 1-(4,5,6,7-Tetrahydrobenzo[b]thiophen-2-yl)guanidine hydrochloride (52)

Following Method E from **49** (100.0 mg; 0.252 mmol; 1 eq.) a yellow gel was obtained in a 79% yield (46.0 mg). ^{1}H NMR (400 MHz, D_2O) δ 6.59 (s, 1H), 2.56 (t, 2H, J = 5.5 Hz), 2.41 (t, 2H, J = 5.6 Hz), 1.69-1.63 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 156.9 (C1), 135.6, 134.1, 130.1, 126.9, 24.4, 23.8, 22.4, 21.7. IR ν_{max} 3129, 2930 (NH), 2843, 1665 (CN), 1591, 1439, 1207, 1135, 844 cm^{-1}. HRMS (ESI) calculated, 196.0908 $[M + H]^+$; found, 196.0902. Anal. ($C_9H_{14}ClN_3S$) C, H, N.

4.2.16. 1-(5,6,7,8-Tetrahydro-4H-cyclohepta[b]thiophen-2-yl)guanidine hydrochloride (53)

Synthesised using Method E from **50** (100.0 mg; 0.244 mmol; 1 eq.) a yellow gel was obtained in a 99% yield (59.6 mg). ^{1}H NMR (400 MHz, D_2O) δ 6.69 (s, 1H), 2.70 (t, 2H, J = 5.6 Hz), 2.59 (t, 2H, J = 5.6 Hz), 1.80-1.74 (m, 2H), 1.61-1.55 (m, 2H), 1.54-1.49 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 157.5, 140.2, 140.2, 130.1, 128.0, 31.7, 20.1, 29.2, 27.8, 27.3. IR ν_{max} 3289 (NH), 2918 (NH), 2842, 2418, 1642, 1576 (C=N), 1433, 1211, 717 cm^{-1}. HRMS (ESI) calculated, 210.1065 $[M + H]^+$; found, 210.1069. Anal. ($C_{10}H_{16}ClN_3S$) C, H, N.

4.2.17. 1-(5,6-Dihydro-4H-cyclopenta[d]thiazol-2-yl]guanidine hydrochloride (54)

Following Method F, using α-chlorocyclopentanone (212 μL; 237.1 mg; 2 mmol; 1 eq.) an off white solid was obtained in a 30% yield (130.4 mg). M.p. decomposes >190 °C. ^{1}H NMR (400 MHz, D_2O) δ 2.82 (t, 2H, J = 7.2 Hz), 2.68 (t, 2H, J = 7.2 Hz), 2.42-2.35 (app. quint.,

2H, J = 7.2 Hz). ^{13}C NMR (100 MHz, D_2O) δ 156.2, 152.9, 134.1, 128.2, 27.1, 26.3, 26.3. IR ν_{max} 3227 (NH), 3105 (NH), 2866 (NH), 2486, 1688, 1598 (C=N), 1556, 1504, 1466, 1369, 1312, 1204, 1174, 977, 855, 710 cm^{-1}. HRMS (ESI) calculated, 183.0704 $[M + H]^+$; found, 183.0701. HPLC: 98.0% (t_R 24.43 min).

4.2.18. 1-[4,5,6,7-Tetrahydrobenzo[d]thiazol-2-yl]guanidine hydrochloride (55)

Following Method F, using α-chlorocyclohexanone (228 μL; 265.2 mg; 2 mmol; 1 eq.) a white solid was obtained in a 56% yield (256.8 mg). M.p. 215-217 °C. ^{1}H NMR (400 MHz, D_2O) δ 2.53 (t, 2H, J = 4.1 Hz), 2.46 (t, 2H, J = 4.1 Hz), 1.69-1.66 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ 157.5, 154.6, 145.2, 123.9, 25.5, 22.5, 22.3, 22.0. IR ν_{max} 3461, 3303 (NH), 3011 (NH), 2931 (NH), 1686 (C=N), 1607 (C=O), 1563 (C=O), 1499, 1473, 1141, 1204, 993, 709 cm^{-1}. HRMS (ESI) calculated, 197.0861 $[M + H]^+$; found, 197.0854. HPLC: 97.0% (t_R 26.77 min).

4.3. Pharmacology

4.3.1. Preparation of Membranes

Cellular 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) in 30 volumes of homogenization buffer (1 mM $MgCl_2$ and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25M sucrose. The crude homogenate was centrifuged for 5 min at 1,000 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 volumes 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.

4.3.2. [^{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 (2 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.

4.3.3. [^{35}S]GTPγS Binding Assays

The incubation buffer for measuring [^{35}S]GTPγS binding in 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.

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6. SUPPORTING INFORMATION

Supporting Information is available for the calculated NICS values, synthetic procedures and spectroscopic data for starting thiophene amines and Boc-protected intermediates as well as NMR spectra and HPLC chromatograms or Elemental Analysis data of the final hydrochloride salts.

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Figures

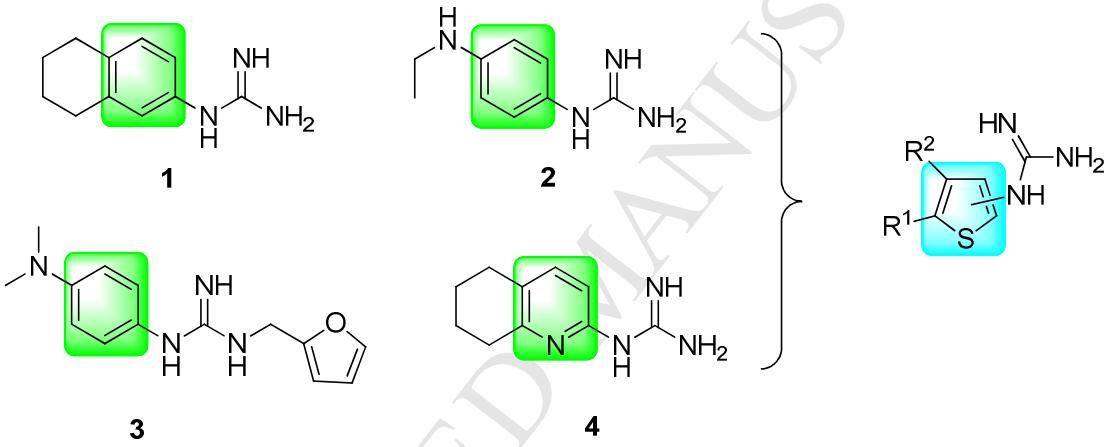


Figure 1.- Compounds previously prepared and tested by Rozas and collaborators as α2-AR antagonists and general structure of the guanidinothiophenes proposed.

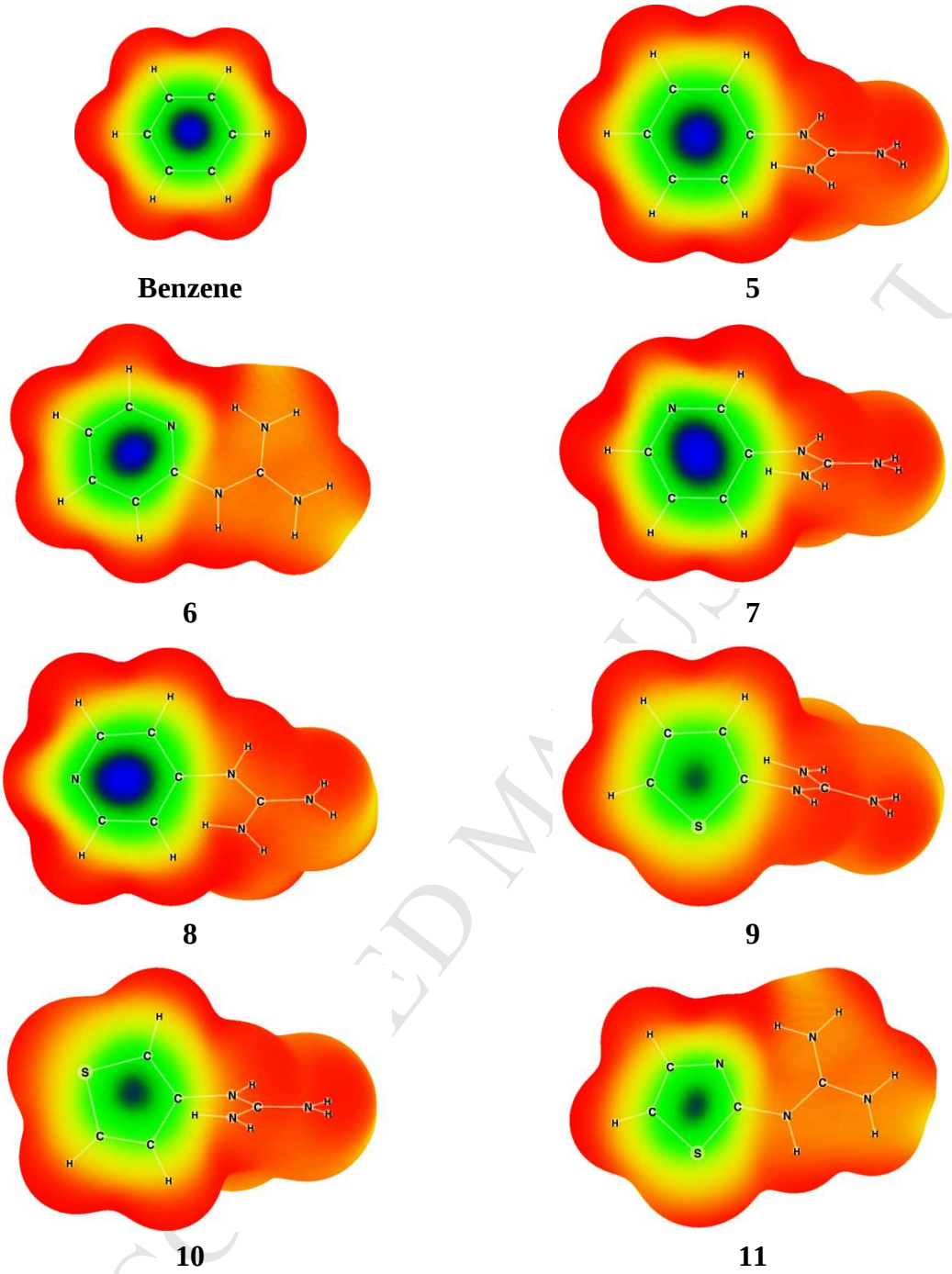


Figure 2. Bottom view of the NICS values on the 0.001 au electron density isosurface at the M06-2X/6-311++G(d,p) computational level of benzene and compounds **5-11**. NICS colour scheme: Red > 0.0, Yellow > -2.5, Green > -5.0, Blue < -5.0

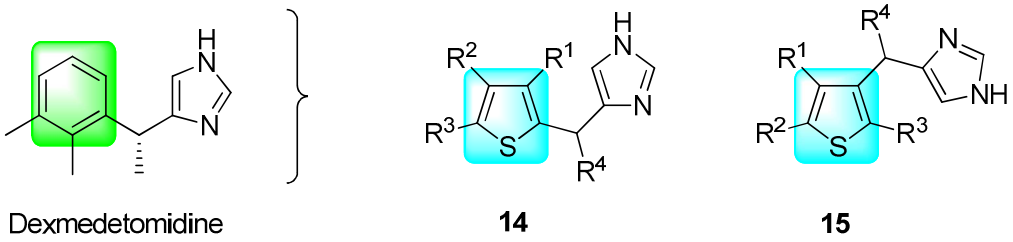
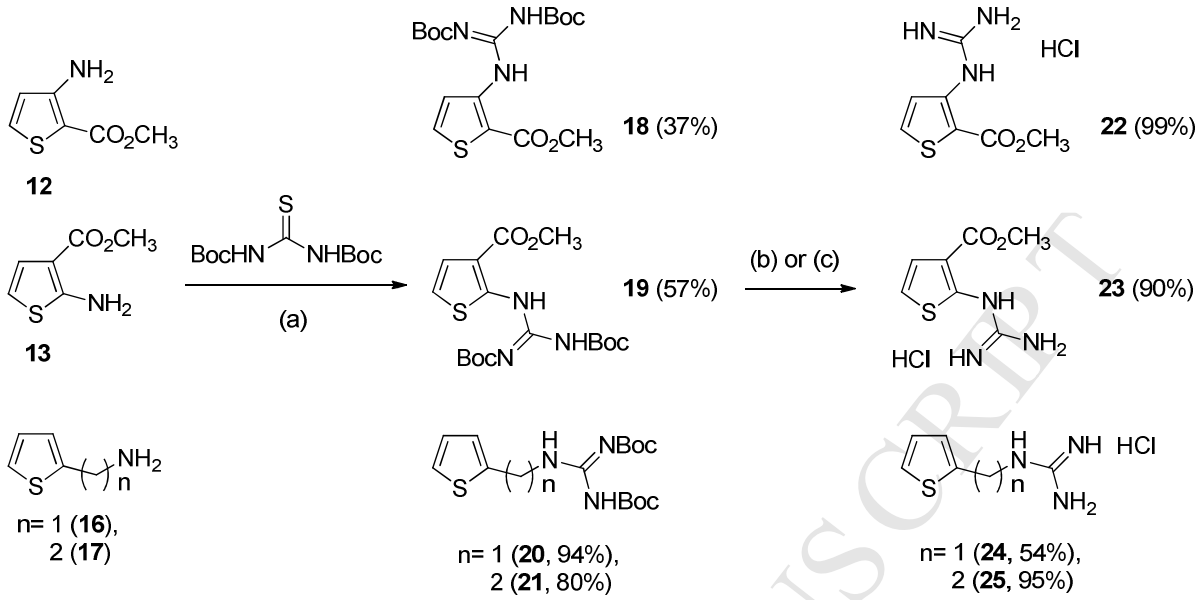


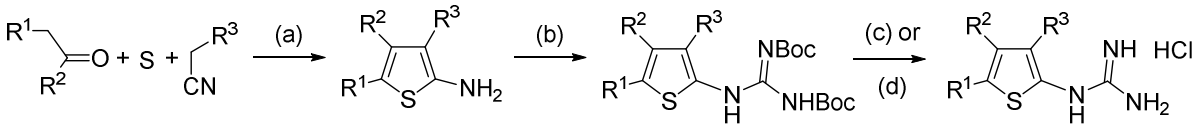
Figure 3.- Structure of α2-AR agonist dexmedetomidine and general structure of its thiophene analogues **14** and **15**.

Scheme 1. Preparation of thiophene guanidinium derivatives **22-25**



(a) $HgCl_2$; NEt_3; CH_2Cl_2; r.t., 12 h. (b) 50% TFA/CH_2Cl_2, r.t., followed by IRA400 Amberlyte resin Cl^- form, H_2O, r.t. (c) 4M HCl/Dioxane, r.t. Note: Only best yields achieved for the Boc-deprotection are shown independently of the method (b or c) used

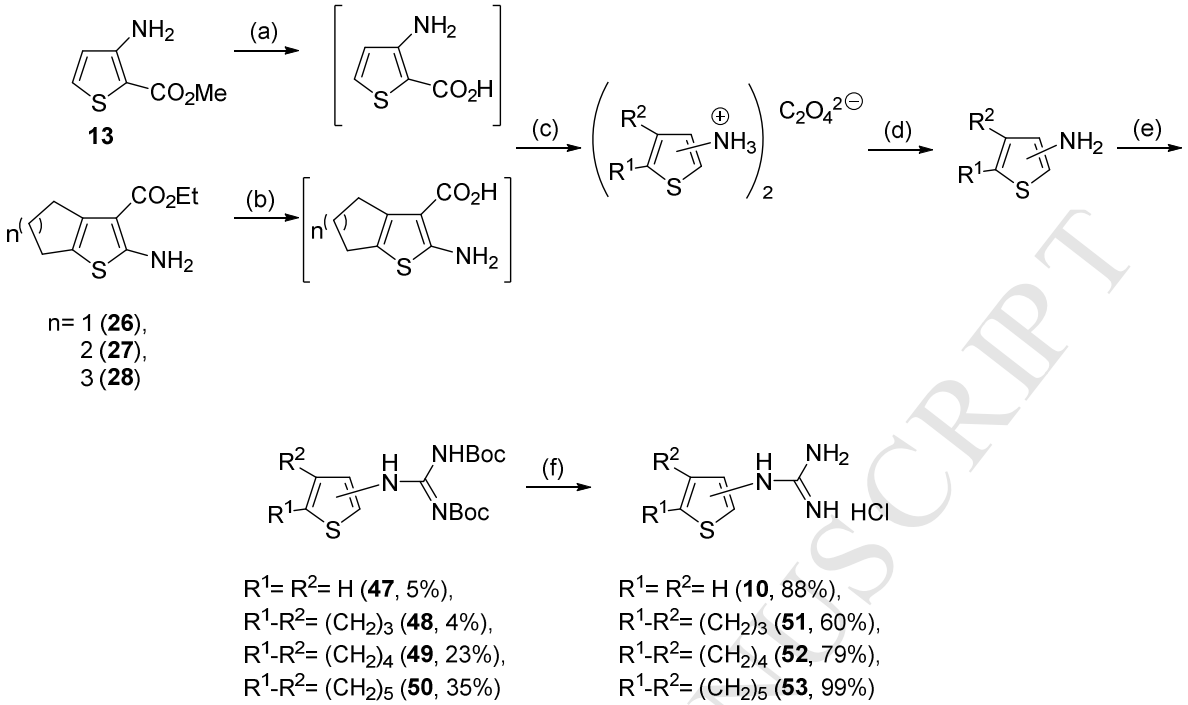
Scheme 2. Preparation of thiopheno guanidinium derivatives **34-37** and **44-46**.



R^1-R^2 = -$(CH_2)_3$-; R^3= CO_2Et	(**26**, 10%),	(**30**, 36%),	(**34**, 90%),
R^1-R^2 = -$(CH_2)_4$-; R^3= CO_2Et	(**27**, 79%),	(**31**, 52%),	(**35**, 93%),
R^1-R^2 = -$(CH_2)_5$-; R^3= CO_2Et	(**28**, 61%),	(**32**, 49%),	(**36**, 71%),
R^1 = H, R^2 = CH_3; R^3= CO_2Et	(**29**, 19%),	(**33**, 68%),	(**37**, 93%),
R^1-R^2 = -$(CH_2)_3$-; R^3= CN	(**38**, 46%),	(**41**, 20%),	(**44**, 39%),
R^1-R^2 = -$(CH_2)_4$-; R^3= CN	(**39**, 51%),	(**42**, 36%),	(**45**, 43%),
R^1-R^2 = -$(CH_2)_5$-; R^3= CN	(**40**, 43%)	(**43**, 61%)	(**46**, 58%)

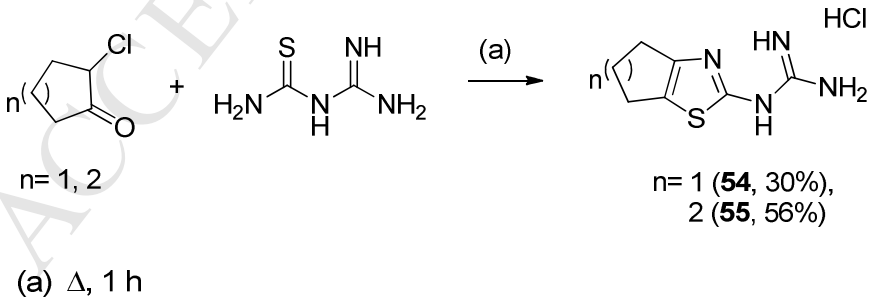
(a) Morpholine, Ethanol, 30-40 ^oC, 4 h. (b) $HgCl_2$, NEt_3, CH_2Cl_2, r.t., 12 h. (c) 50% TFA/CH_2Cl_2, r.t., followed by IRA400 Amberlyte resin Cl^- form, H_2O, r.t. (d) 4M HCl/Dioxane, r.t.

Scheme 3. Preparation of decarboxylated guanidino thiophenes



(a) 2M NaOH; (b) KOH, 1:1 EtOH/H_2O; (c) Oxalic acid, 2-propanol; (d) NH_4OH; (e) $HgCl_2$, NEt_3, CH_2Cl_2, r.t., 12 h. (f) 4M HCl/Dioxane, r.t.

Scheme 4. Preparation of guanidino thiazoles



(a) Δ, 1 h

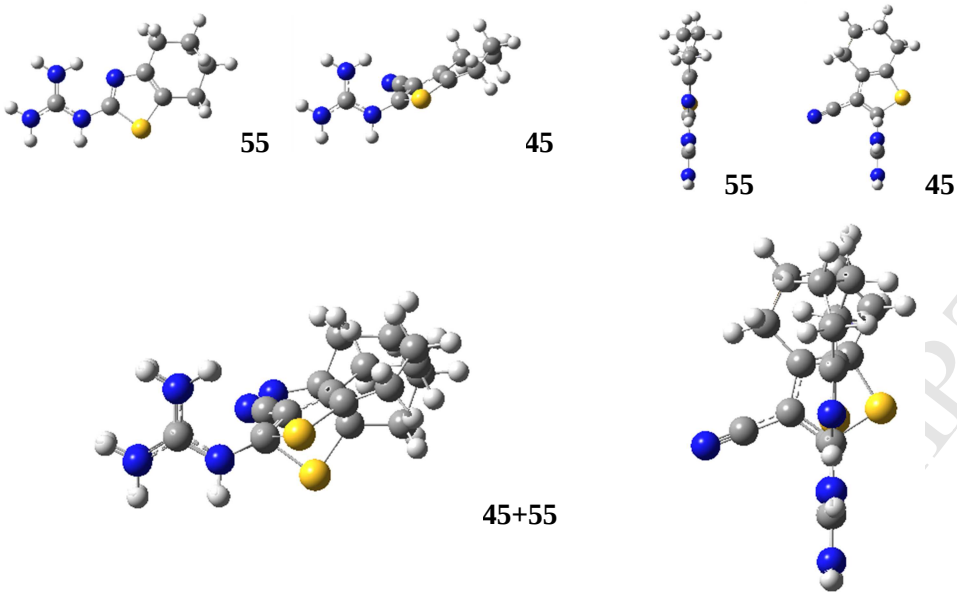


Figure 4.- Optimised structures of compounds **55** and **45** (calculated at M062X/6-311++G(p,d) level) and their superimposition viewed from the front (left) and a perpendicular view (right).

Highlights

- Phenyl-, thiophenyl- and thiazolylguanidinium derivatives have similar aromaticity
- Thiophenyl- & thiazolylguanidines must act as phenyl-based α_2-adrenoceptor ligands
- A high-diversity library of thiophenyl- and thiazolylguanidines was prepared
- Their affinity for α_2-adrenoceptor was measured in human prefrontal cortex tissue
- Activity on α_2-adrenoceptors was measured: 2 antagonists and 1 agonist were found