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Might the observed α_{2A}-adrenoreceptor agonism or antagonism of allyphenyline analogues be ascribed to different molecular conformations? ☆

Eleonora Diamanti [a], Fabio Del Bello [a], Giuseppe Carbonara [b], Antonio Carrieri [b], Giuseppe Fracchiolla [b], Mario Giannella [a], Valerio Mammoli [a], Alessandro Piergentili [a], Katariina Pohjanoksa [c], Wilma Quaglia [a], Mika Scheinin [c], Maria Pigini [a,*]

[a] Scuola di Scienze del Farmaco e dei Prodotti della Salute, Università degli Studi di Camerino, via S. Agostino 1, 62032 Camerino, Italy
[b] Dipartimento Farmaco-Chimico, Università degli Studi di Bari, via E. Orabona 4, 70125 Bari, Italy
[c] Department of Pharmacology, Drug Development and Therapeutics, University of Turku and Unit of Clinical Pharmacology, Turku University Hospital, FI-20520 Turku, Finland

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ABSTRACT

We recently reported that the α_2-adrenoreceptor (AR) ligand allyphenyline (**9**) significantly enhanced morphine analgesia (due to its α_{2C}-AR agonism), was devoid of sedative side effects (due to its α_{2A}-AR antagonism), prevented and reversed morphine tolerance and dependence. To highlight the molecular characteristics compatible with this behaviour and to obtain novel agents potentially useful in chronic pain and opioid addiction management, the allyl group of **9** was replaced by substituents of moderate steric bulk (MR) and positive or negative lipophilic (π) and electronic (σ) contributions in all the possible combinations. Effective novel α_{2C}-agonists/α_{2A}-antagonists (**2**, **3**, **10**, **12**, and **17**) were obtained. This study also demonstrated that contradictory combinations of the physicochemical parameters were similarly able to induce the α_{2A}-activation. Since we had previously observed that the absolute configuration affected only the potency, but not the functional profile of the ligands, we hypothesized that the α_{2A}-activation was governed by a ligand preferred conformation. From a structural overlay investigation it emerged that an *extended* conformation appeared to be associated with dual α_{2C}-agonism/α_{2A}-antagonism, whereas a *folded* conformation associated with α_{2C}-/α_{2A}-agonism.

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1. Introduction

α_2-Adrenoreceptors (α_2-ARs) belong to the superfamily of G-protein coupled receptors.[2] Pharmacological analysis and molecular cloning have provided evidence for their heterogeneity: three subtypes, namely α_{2A}, α_{2B} and α_{2C}, encoded by different genes have been identified and characterized in different species.[3] They are widely distributed both in the central nervous system (CNS) and in peripheral tissues. In addition to postsynaptic locations, α_2-ARs are also present presynaptically, where they act as negative modulators of the neuronal release of catecholamines and other neurotransmitters.[4–6] Because of the lack of α_2-AR subtype selective agonists, the attribution of the physiological functions to a given subtype has proven challenging in vivo. Nevertheless, the use of mice carrying the inactivating D79N point mutation of α_{2A}-AR, mice that are singly (α_{2A}-AR$^{-/-}$, α_{2B}-AR$^{-/-}$, α_{2C}-AR$^{-/-}$), doubly (α_{2AC}-AR$^{-/-}$), or triply (α_{2ABC}-AR$^{-/-}$) deficient in a particular subtype, or mice overexpressing the α_{2C}-AR (α_{2C}-AR$^{+/+OE}$) has provided important information about the subtype-specific functions.[4] Thus, the α_{2A}-AR subtype has been demonstrated to mediate hypotension, sedation, analgesia, hypothermia, antiepileptogenesis, as well as inhibition of monoamine release and metabolism in the brain. The α_{2B} subtype mediates vasoconstriction and the initial peripheral hypertensive response to α_2-AR agonist administration. The α_{2C}-AR subtype appears to be involved in many CNS processes, such as the startle reflex, stress responses, control of locomotion, and can contribute to adrenergic-opioid synergism and spinal α_2-agonist-mediated analgesia.[7,8] In addition to α_{2A}-AR, that has been found to be the main inhibitory presynaptic feedback receptor, also the α_{2C}-AR subtype participates in presynaptic regulation in the CNS. Moreover, in the brain, α_{2A}- and α_{2C}-ARs as 'heteroreceptors' inhibit dopamine release in the basal ganglia and serotonin release in the hippocampus and cerebral cortex. Thus, these two subtypes complement each other to integrate CNS functions and behaviour.[5] Due to the multiplicity of the mediated functions, α_2-ARs represent attractive therapeutic targets for the treatment of a wide range of diseases.[9] Over the

Abbreviations: AR, adrenoreceptor; CHO, Chinese hamster ovary; CNS, central nervous system; MD, molecular dynamic; MR, molar refractivity; DME, 1,2-dimethoxyethane; NOESY, Nuclear Overhauser Effect Spectroscopy.

☆ See Ref. 1.

* Corresponding author. Tel.: +39 0737402257; fax: +39 0737637345.
E-mail address: maria.pigini@unicam.it (M. Pigini).

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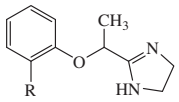
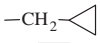
years, the focus of our studies has been the discovery of novel tools selectively interacting with α_2-AR subtypes and representing potential improvements over current therapies with α_2-AR drugs.[10] We demonstrated that molecules characterized by the base structure of the non-selective α_2-AR subtype antagonist **1**[10] (Table 1) were able to display different activity at the various α_2-AR subtypes depending on the nature of the ortho substituent in the aromatic ring. Indeed, the introduction of aromatic moieties, such as phenyl or thiophen-3-yl groups, converted **1** into the efficacious agonists **13** (biphenyline), and **14**, respectively. These compounds significantly activated the α_{2A} and α_{2C}-subtypes. Aliphatic substituents such as methyl, *n*-propyl, allyl or cyclopropyl groups (compounds **5**, **7**, **9** and **11**, respectively),[11,12] endowed with low steric bulk (MR <20),[13a] induced significant modulation of the biological profile of the antagonist **1** only at the α_{2C}-subtype. Indeed, they maintained the α_{2A}-antagonist behaviour but were potent α_{2C}-agonists. Nevertheless, though the couples **9**/**11** and **13**/**14** provided different pharmacological responses, in both cases the (*S*)-enantiomers were the most potent (eutomers).[10] Moreover, in vivo investigation demonstrated that, at a very low dose (0.05 mg/kg), **9** (allyphenyline) and **11** were able to significantly enhance morphine analgesia (due to their α_{2C}-AR agonism) and were devoid of sedative side effects (due to their α_{2A}-AR antagonism).[10,12] These results were in agreement with the α_{2C}- and α_{2A}-AR-mediated functions revealed by the aforementioned studies with genetically engineered mice. Finally, it has been observed that, at the same dose, **9** and its (*S*)-eutomer also prevented and reversed morphine tolerance and dependence. Since both clonidine and yohimbine have been reported to reverse or prevent only partly opioid withdrawal signs, we attributed the unique profiles of **9** and its (*S*)-eutomer to the synergistic combination of their potent α_{2C}-AR agonism and α_{2A}-AR antagonism.[10] The therapeutic utility of agents with such a functional profile in chronic pain and opioid addiction management, underlined also by other authors,[14] prompted us to develop novel ligands inspired by **9**. To better define the molecular characteristics compatible with significant dual α_{2C}-agonism/α_{2A}-antagonism and to test whether the interactions produced by the ortho pendant moiety (R) were affected by its lipophilic (π) and electronic (σ) effects,[13b] we prepared analogues of **9** replacing its ortho allyl group with substituents endowed with

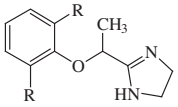
Table 1
Steric (MR), lipophilic (π) and electronic (σ) parameters[a], affinity (pK_i[b]), antagonist potency (pK_b[c]), agonist potency (pEC_{50}[c]), and intrinsic activity (ia[c]) on human α_2-AR subtypes



Compd	R	MR[a]	π[a]	σ[a]	α_{2A}			α_{2B}			α_{2C}		
					pK_i	$pEC_{50}(pK_b)$	ia	pK_i	$pEC_{50}(pK_b)$	ia	pK_i	$pEC_{50}(pK_b)$	ia
1	–H				7.57 ± 0.09	(7.01 ± 0.10)		6.78 ± 0.13	(6.20 ± 0.18)		6.58 ± 0.12	(6.85 ± 0.15)	
			+π	**+σ**									
2	–Cl	6.73	+0.71	+0.23	7.54 ± 0.11	(7.20 ± 0.07)		6.48 ± 0.07	6.79 ± 0.12	0.44	6.31 ± 0.09	6.78 ± 0.12	0.83
3	–Br	9.86	+0.86	+0.23	7.64 ± 0.09	(7.04 ± 0.08)		6.45 ± 0.16	6.50 ± 0.07	0.40	6.55 ± 0.09	8.00 ± 0.11	0.87
4	$-CF_3$	8.09	+0.88	+0.54	6.74 ± 0.12	(6.42 ± 0.00)		5.79 ± 0.09	6.62 ± 0.12	0.40	6.01±0.17	6.10 ± 0.06	0.72
			+π	**−σ**									
5	$-CH_3$	6.88	+0.56	−0.17	7.61 ± 0.14	(7.51 ± 0.08)		6.57 ± 0.20	6.00 ± 0.22	0.40	6.57 ± 0.12	7.20 ± 0.12	0.67
6	$-CH_2CH_3$	11.48	+1.02	−0.15	7.62 ± 0.11	(6.00 ± 0.00)		6.55 ± 0.16	5.00 ± 0.09	0.50	6.54 ± 0.09	7.00 ± 0.07	0.60
7	$-CH_2CH_2CH_3$	16.08	+1.55	−0.18	7.30 ± 0.11	(7.05 ± 0.20)		6.27 ± 0.15	5.30 ± 0.12	0.60	6.83 ± 0.21	7.60 ± 0.18	0.75
8	$-CH(CH_3)_2$	16.08	+1.53	−0.15	7.14 ± 0.10	(6.82 ± 0.09)		6.03 ± 0.09	6.60 ± 0.15	0.54	6.34 ± 0.07	6.40 ± 0.12	0.73
9	$-CH_2CH{=}CH_2$ allyphenyline	16.13	+	–	7.24 ± 0.11	(7.40 ± 0.06)		6.47 ± 0.20	NA		7.07 ± 0.14	7.30 ± 0.09	0.90
10	$-C(CH_3){=}CH_2$	16.13	+	–	7.14 ± 0.11	(7.00 ± 0.08)		6.06 ± 0.12	5.80 ± 0.09	0.40	6.22 ± 0.06	6.94 ± 0.10	0.71
11		13.80	+1.14	−0.17	7.64 ± 0.20	(7.00 ± 0.09)		6.51 ± 0.13	5.50 ± 0.15	0.70	7.10 ± 0.16	7.40 ± 0.13	0.90
12		18.58	+	–	7.44 ± 0.09	(7.70 ± 0.12)		6.39 ± 0.05	5.48 ± 0.15	0.70	6.56 ± 0.21	8.70 ± 0.08	0.80
13	 biphenyline	25.28	+1.96	−0.01	7.32 ± 0.08	6.94 ± 0.06	0.70	6.30 ± 0.07	6.19 ± 0.11	0.50	6.70 ± 0.04	7.24 ± 0.01	0.80
14		24.22	+1.81	−0.02	7.34 ± 0.02	7.12 ± 0.08	0.70	6.38 ± 0.04	NA		6.88 ± 0.07	7.03 ± 0.28	0.85
15	$-O-CH_2-CH_3$	12.84	+0.38	−0.24	7.01 ± 0.03	(6.50 ± 0.08)		5.88 ± 0.21	5.50 ± 0.09	0.50	6.12 ± 0.03	5.00 ± 0.08	0.45
16	$-O-CH(CH_3)_2$	17.53	+1.05	−0.45	7.08 ± 0.07	(6.82 ± 0.15)		5.80 ± 0.10	5.76 ± 0.11	0.50	6.40 ± 0.08	7.00 ± 0.07	0.70
17		15.26	+1.05	–	7.08 ± 0.09	(6.90 ± 0.12)		5.98 ± 0.11	4.89 ± 0.10	0.60	6.17 ± 0.14	7.32 ± 0.13	0.70
18	$-O-CH{=}CH_2$	11.77	+	–	NT	(5.00)		NT	5.50	0.45	NT	6.20 ± 0.09	0.48
			−π	**−σ**									
19	$-O-CH_3$	8.04	−0.02	−0.28	7.24 ± 0.09	(6.60 ± 0.08)		6.06 ± 0.13	4.00 ± 0.14	0.50	6.08 ± 0.04	5.01 ± 0.10	0.60
			−π	**+σ**									
20	$-SO_2-CH_3$	13.49	−1.63	+	5.50 ± 0.06	6.25 ± 0.09	0.60	4.29 ± 0.03	5.00 ± 0.10	0.45	5.42 ± 0.04	6.50 ± 0.15	0.60
21	–CHO	7.08	−0.65	+0.42	5.98 ± 0.15	7.50 ± 0.08	0.70	5.68 ± 0.12	6.50 ± 0.10	0.50	5.06 ± 0.06	7.00 ± 0.12	0.60
22	$-COCH_3$	11.58	−0.55	+0.47	5.99 ± 0.19	7.20 ± 0.07	0.70	5.21 ± 0.08	6.53 ± 0.09	0.60	5.13 ± 0.11	6.70 ± 0.14	0.65
23	$-CH{=}NOH$	10.78	−0.38	+0.10	6.54 ± 0.11	6.30 ± 0.20	0.80	6.15 ± 0.15	5.40 ± 0.12	0.50	6.04 ± 0.21	6.90 ± 0.18	0.85
(−)-Noradrenaline						6.43 ± 0.17	1.00		7.21 ± 0.15	1.00		6.10 ± 0.05	1.00

[a] Ref. 13.
[b] pK_i values were calculated from [^{3}H]RS-79948-197 radioligand competition binding data generated with membrane preparations from CHO cells expressing individually each human α_2-AR subtype (α_{2A}, α_{2B}, α_{2C}).
[c] pK_b, pEC_{50}, and intrinsic activity (ia) values were determined by applying the Cytosensor microphysiometry system to the same cell models. Intrinsic activity of the tested compounds is expressed as the fraction of that of the full agonist (−)-noradrenaline taken as equal to 1. The data are expressed as the mean ± SEM of three to six separate experiments. Compounds exhibiting ia of <0.3 were considered not active (NA). NT: not tested.

Table 2
Affinity (pK_i[b]), antagonist potency (pK_b[c]), agonist potency (pEC_{50}[c]), and intrinsic activity (ia[c]) on human α_2-AR subtypes



Compd	R	α_{2A}			α_{2B}			α_{2C}		
		pK_i	pEC_{50} (pK_b)	ia	pK_i	pEC_{50}	ia	pK_i	pEC_{50}	ia
	–Cl lofexidine	8.36 ± 0.11	8.20 ± 0.10	0.60	7.17 ± 0.18	6.90 ± 0.14	0.80	7.16 ± 0.07	8.86 ± 0.20	0.80
24	$-CH_3$	7.93 ± 0.20	7.73 ± 0.01	0.55	6.74 ± 0.18	5.83 ± 0.23	0.80	6.60 ± 0.06	7.56 ± 0.08	0.82
25	$-CH_2CH_3$	7.50 ± 0.02	7.93 ± 0.90	0.67	6.57 ± 0.02	6.00 ± 0.08	0.80	6.56 ± 0.11	7.56 ± 0.10	0.65
26	$-CH_2CH{=}CH_2$	7.78 ± 0.05	(8.56 ± 0.30)		6.56 ± 0.12	6.30 ± 0.14	0.65	6.89 ± 0.08	7.25 ± 0.11	0.90
27		6.31 ± 0.17	(8.00 ± 0.80)		5.34 ± 0.13	6.70 ± 0.25	0.60	6.20 ± 0.08	7.10 ± 0.15	0.70

[b,c] See Table 1.

moderate steric bulk (MR) and positive or negative σ and π contributions in all the possible combinations (compounds **2–4**, **6**, **8**, **10**, **12**, **15–22**) (Table 1). The already known compounds **4**,[15] **6**,[16] **8**,[17] **16**[17] and **19**[18] have never been studied from this point of view.

To evaluate different preferred conformations adopted by **9**, **11**, **13**, and **14**, we carried out NOESY experiments and a molecular superposition fitting energetically allowed conformations of their (*S*)-enantiomers to the crystallographic data of (*S*)-lofexidine,[19] taken as reference compound.

Finally, the obtained results and the evident structural analogy between **2** and lofexidine prompted us to include in this investigation the preparation and evaluation of the already known 2,6-disubstituted derivatives **24–26**,[16] which have never been studied from this point of view, and the novel compound **27** (Table 2).

2. Results

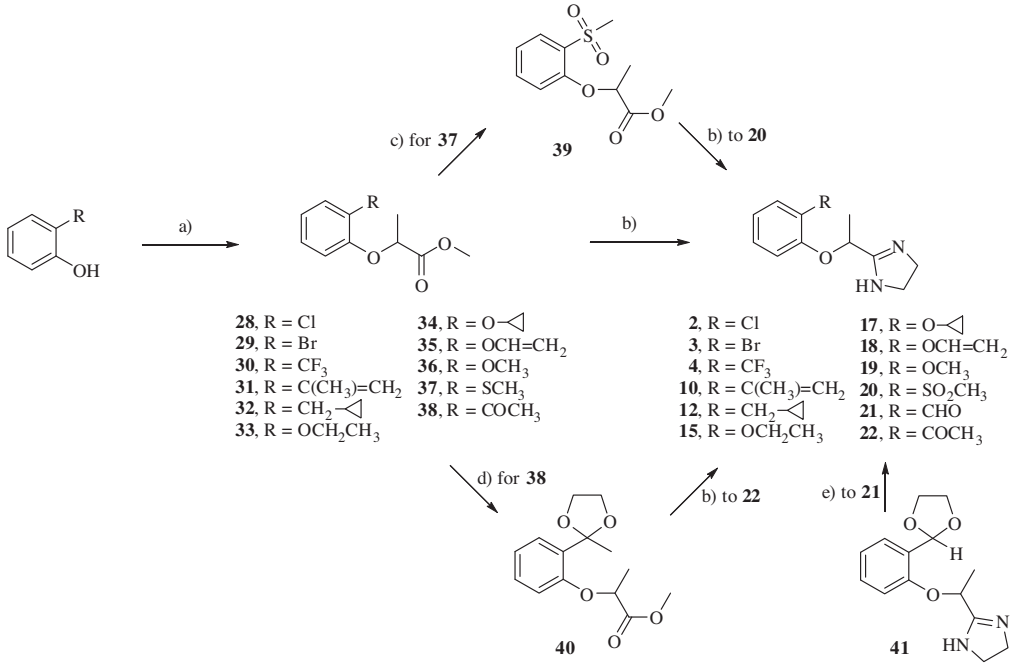
2.1. Chemistry

Compounds **2–4**, **10**, **12**, **15**, **17–22** were prepared according to the synthetic procedure reported in Scheme 1. In particular, imidazolines **2–4**, **10**, **12**, **15**, **17–20** and **22** were obtained by treatment of the corresponding methyl esters **28–36**, **39** and **40** with ethylenediamine in the presence of $(CH_3)_3Al$. The methyl ester **39** was prepared by oxidation of sulfide **37** with *m*-CPBA. The protection of ketone **38** with ethylene glycol in the presence of *p*-toluenesulfonic acid gave the methyl ester **40**. Esters **28–38** were prepared by condensation of the suitable phenols [commercially available, except for 2-(prop-1-en-2-yl)phenol,[20] 2-(cyclopropylmethyl)phenol,[21] 2-cyclopropoxyphenol,[22] 2-(vinyloxy)phenol[23]] with methyl 2-bromopropionate in the presence of K_2CO_3. Imidazoline **21** was prepared by hydrolysis of the acetal group of the intermediate **41**[11] with HCl 2 N.

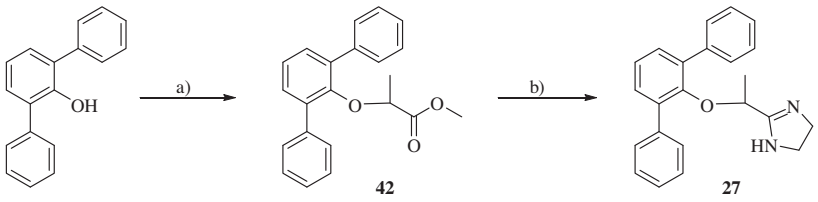
Imidazoline **27** was prepared according to the synthetic procedure reported in Scheme 2. Condensation of the commercially available 2,6-diphenylphenol with methyl 2-bromopropionate afforded the ester **42** which, treated with ethylenediamine in the presence of $(CH_3)_3Al$, gave the desired product.

2.2. Biology

The affinity values (pK_i), antagonist properties (pK_b), agonist potencies and intrinsic activities (pEC_{50} and ia, respectively) of



Scheme 1. Reagents: (a) methyl 2-bromopropionate, K_2CO_3, DME; (b) $H_2NCH_2CH_2NH_2$, $(CH_3)_3Al$, toluene; (c) *m*-CPBA, $CHCl_3$; (d) $HOCH_2CH_2OH$, *p*-TsOH, toluene; (e) HCl 2 N.



Scheme 2. Reagents: (a and b) see Scheme 1.

the designed compounds on recombinant human α_2-AR subtypes are reported in Tables 1 and 2. The corresponding profiles of **1**, **5**, **7**, **9**, **11**, **13**, **14** and **23** were also included for useful comparison.

3. Discussion

The observations emerging from the comparison of the functional data of all compounds described in Table 1 were noteworthy. The binding profiles of the novel compounds, highlighting preferential α_{2A}-AR subtype recognition, were generally comparable to those of the structurally related derivatives previously reported.[10–12]

As expected, the functional data confirmed the crucial role of the *ortho* pendant moiety in inducing the modulation of the α_2-AR behaviour of **1**. Indeed, the biological profiles shown by **2**, **10**, **16** and **17** were similar to that of **9**; analogous properties were displayed by **3** and **12**, but in this case a marked enhancement of the α_{2C}-agonism was observed (**3**, pK_b α_{2A} = 7.04; pEC_{50} α_{2C} = 8.0, ia = 0.87; **12** pK_b α_{2A} = 7.70; pEC_{50} α_{2C} = 8.70, ia = 0.8). All these derivatives, although activating also the α_{2B}-subtype, demonstrated significant and rather selective α_{2C}-agonism. Since compounds **2** and **3** or **10**, **12**, **16**, and **17** whose ortho phenyl substituents produced +π, +σ or +π, −σ conditions, respectively, displayed analogous properties, it was reasonable to suppose that significant dual α_{2C}-agonism/α_{2A}-antagonism was independent on σ effects and should require an *ortho*-substituent essentially endowed with a positive π value and MR <20. In addition, positive π, negative σ and MR <20 properties were also possessed by the *ortho* substituents of the already described **5**, **7**, **9**, and **11** showing similar pharmacological profiles.[10,12] Reduced α_{2C}-agonism was observed for the derivative **19** bearing the ortho pendant moiety characterized by a negative π value.

Positive π and negative σ conditions, but in this case associated to enhanced steric bulk (MR range = 24 ÷ 26), were verified for the ortho substituents of the derivatives **13** and **14**. As above mentioned, in addition to potent α_{2C}-agonism, these imidazolines also displayed significant α_{2A}-AR activation, indicating that the α_{2A}-activation might be induced by the +π, −σ, MR >20 conditions of the ortho substituent. Nevertheless, this observation was not confirmed by the study of **20–22**. Indeed, these derivatives, showing good activation of both α_{2A}- and α_{2C}-subtypes, highlighted that also −π, +σ, MR <20 conditions were compatible with significant α_{2A}-AR activation. This result confirmed the α_{2A}-agonism produced by the previously reported compound **23**; indeed, the same −π, +σ, MR <20 conditions were verified for its ortho oximino methyl substituent.[11] Since contradictory combinations of the physicochemical parameters (+π, −σ, MR >20 and −π, +σ, MR <20) proved to be similarly able to induce the α_{2A}-AR activation and, as above reported, in the case of **9**/**11** and **13**/**14** the absolute configuration affected the potency but not the functional profile of the ligands,[10] we hypothesized that the α_{2A}-AR activation might be essentially governed by a preferred conformation of the ligand. Therefore, aiming at confirming our hypothesis, a template fitting procedure was performed. In this view, lacking some evidence regarding the α_{2A}- and α_{2C}-AR structures which might afford a receptor based study, we carried out the matching of energetically allowed conformations of (*S*)-eutomers of **9**, **11**, **13** and **14**, sampled from molecular dynamic (MD) simulations, to crystallographic data of the (*S*)-eutomer of lofexidine,[19,24] taken as reference compound. Though the conformation adopted in a crystal does not necessarily reflect the bio-active conformation of a compound acting on its target, however, as also recently underlined, the crystal structure conformations of small-molecules generally correlate well with protein-bound conformations.[25]

Lofexidine was selected because its scaffold is similar to that of the imidazolines of the present study and, as emerged from our functional study (Table 2), it displayed significant α_{2A}- and α_{2C}-AR agonism. Moreover, as experimentally assessed by its crystal structure determination,[19] a clear conformation with the aromatic ring lying orthogonally to the plane of the charged imidazoline nucleus and the chlorine atoms placed upward and downward the imidazoline ring is observed. Therefore, at least according to the lofexidine structure and to the aforementioned symmetry element, a unique mode of interaction with the receptor surface and, consequently, a sole bioactive conformation might be postulated. Vice versa in the case of the selected compounds, the above mentioned symmetry element might no longer be equally supported, due to the presence of just a single pendant group on the aromatic moiety. Thereafter, also based on the observed pharmacological profiles, the possibility of separate conformations, accounting for α_{2A}-AR agonism or for α_{2A}-AR antagonism, might be considered. This assumption can be better perceived from the molecular superposition of (*S*)-eutomer of lofexidine with (*S*)-eutomers of **9**, **11**, **13** and **14** as depicted in Figure 1.

The diverse disposition of the *o*-substituent observed for the four studied molecules is quite intriguing when they are fitted

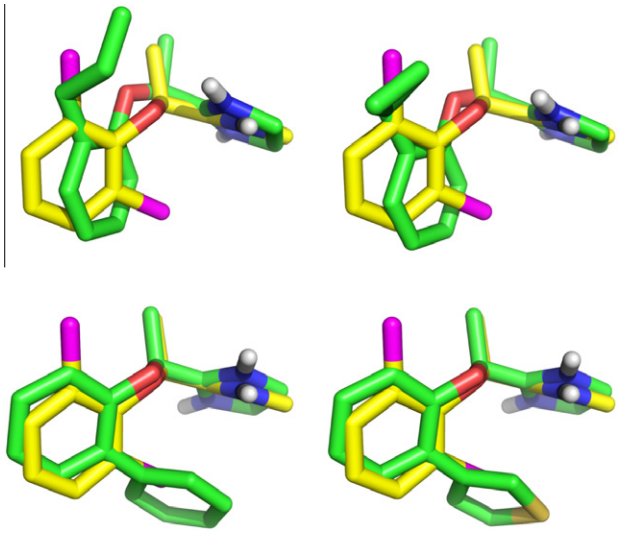
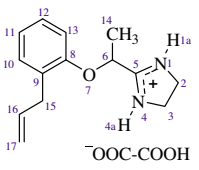
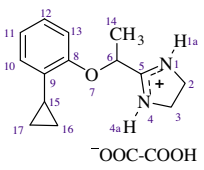


Figure 1. Molecular superposition of allyphenyline (**9**) upper-left, **11** upper-right, biphenyline (**13**) bottom-left, and **14** bottom-right with the crystallographically determined structure of lofexidine (yellow). For all compounds the (*S*)-configuration was considered (see text).

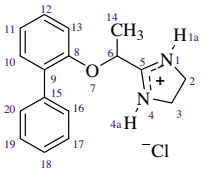
Table 3
^{1}H NMR spectral data and NOEs of **9**, **11**, **13**, and **14** salts in DMSO-d_6



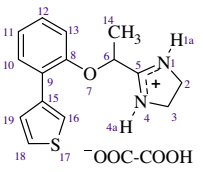
9



11



13



14

Position	9		11		13		14	
	δH[a]	NOESY	δH[a]	NOESY	δH[a]	NOESY	δH[a]	NOESY
1	4.5–7.5 br		5.5–7.0 br		10.52 br		3.5–5.0 br	
2	3.87 m		3.86 s		3.83 m		3.9 m	
3	3.87 m		3.86 s		3.83 m		3.9 m	
4	4.5–7.5 br		5.5–7.0 br		10.52 br		3.5–5.0 br	
6	5.45 q (6.51)	13	5.37 q (6.6)	13	5.22 q (6.6)	13	5.34 q (6.33)	13
10	7.19 m	15	7.11 m		7.33 m		7.56 m	
11	6.97 m		6.93 m		7.15 m		7.11 m	
12	7.19 m		7.11 m		7.33 m		7.30 m	
13	6.97 m	6	6.93 m	6	7.07 d	6	7.04 d	6
14	1.58 d (6.51)		1.58 d (6.6)		1.46 d (6.6)	16/20	1.56 d (6.33)	16
15	3.39 m	10	2.22 tt (8.5 × 2; 5.3 × 2)					
16	5.95 m		16b + 17b 0.9 m		7.56 d	14	7.86 m	14
17	5.04 m		16a + 17a 0.63 m		7.42 m			
18					7.33 m		7.56 m	
19					7.42 m		7.56 m	
20					7.56 d			

[a] ^{1}H chemical shift values (δ ppm) followed by multiplicity; coupling constants are reported in brackets.

and compared to lofexidine: **9** and **11** share an *extended* conformation as determined by a torsion angle, referred to atoms 8–7–6–5 (Table 3), equal to −60° corresponding to the lower energy threshold, while **13** and **14** have a *folded* conformation, corresponding to a value of −130° measured for the same torsion angle. For these latter compounds the energy barrier to rotation is 2.91 and 2.83 kcal/mol, respectively. The different conformation noticed for the different (ant)agonists might be ascribed to the nature of the ortho substituent characterizing the ligands. Indeed, for **13** and **14**, the bulkier and electron-rich aromatic pendant is tightly packed against the imidazoline moiety, charged at physiological pH, trapping the ligand in a *folded* binding mode through a more stable π-cation like charge transfer complex, able to compensate the even low energy barrier previously mentioned. This hypothesis was, at least partially, confirmed by the crystal structure of the methylene analogue of **13**[26] bearing the same kind of substituent. The *folded* conformation might also be favoured in the case of **23**, where the ortho *trans* oximino methyl group simulates the aromatic ring,[11] and **20–22**. For these latter compounds, the partial negative pole, associated with the oxygen atom of the corresponding sulfonyl, aldehydic or acetyl functions, can produce polar interaction with the protonated imidazoline function. Vice versa, **9** and **11**, bearing groups with lower electron density, cover higher degrees of conformational freedom, orienting their allyl or cyclopropyl substituents towards a different location of the three-dimensional space.

The possible difference between the conformations preferentially adopted by **9**/**11** or **13**/**14** was investigated through NOESY experiments.[27] Spectral data of these compounds are reported in Table 3.

All NOESY spectra showed NOEs between the methine assigned to H-6 and the aromatic hydrogen assigned to H-13. For the selected compounds, these data were consistent with the presence, in DMSO solutions, of a dominant conformation, in which the methine proton H-6 is located at a distance of about 2.2 Å from the aromatic proton H-13. In addition, a NOE was also detected between the methylene group of **9** and the related aromatic ortho proton. Interestingly, only **13** and **14** showed a NOE between the 3H-14 methyl group and a proton of the ortho phenyl or thiophenyl substituent, respectively. This finding might be explained by a lower conformational mobility of **13** and **14**.

In summary, α_{2A}-AR agonism, seemingly compatible with contradictory physicochemical parameters, might really be favoured by a preferred molecular conformation. Therefore, as hypothesized for **13** and **14**, a *folded* conformation appeared compatible with both α_{2A}- and α_{2C}-AR activation, whereas, as supposed for **9** and **11**, an *extended* conformation only with α_{2C}-AR activation. In contradistinction to α_{2A}-AR, the insensitivity of α_{2C}-AR to conformational changes of imidazoline ligands might be due to structural differences of the corresponding binding cavities. The resolution of a crystal structure for any of the α_2-AR subtypes might cast light on this hypothesis.

With the exception of the bulkiest compound **27**, also the 2,6-disubstituted derivatives showed preferential α_{2A}-AR affinity (Table 2). As for lofexidine, the α_{2A}- and α_{2C}-AR agonism of **24** and **25** might be justified by the presence of two groups with similar moderate MR values (6.73, 6.88 and 11.48 for chlorine, methyl and ethyl, respectively) in both the ortho positions. On the contrary, the two bulkier ortho substituents might be responsible for the potent dual α_{2C}-AR agonism/α_{2A}-AR antagonism displayed by **26** and **27**.

4. Conclusion

In conclusion, the present investigation has provided the novel effective allyphenyline analogues **2**, **3**, **10**, **12**, and **17** potentially useful in chronic pain and opioid addiction management. Altogether, the obtained results have allowed us to hypothesize that, in this series of mono-phenyl substituted compounds, the study of the preferred conformations might represent a possible tool in the design of novel α_{2C}-AR agonists devoid of the side effects associated with α_{2A}-AR activation. A structural overlay investigation has suggested that α_{2C}-AR agonist/α_{2A}-AR antagonist behaviour might be expected for ligands bearing an ortho phenyl substituent favouring an *extended* conformation.

5. Experimental section

5.1. Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin–Elmer 297 and Varian EM-390 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br (broad), d (doublet), t (triplet), q (quartet), or m (multiplet). For the NOESY experiments ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian-Mercury 300 (300 MHz) spectrometer at room temperature and processed by ACD/Labs ACD/NMR Processor Academic Edition, release 12.0, version 12.01 (build 3014, 18 Mar 2010). IR spectral data (not shown because of the lack of unusual features) were obtained for all compounds reported and are consistent with the assigned structures. The microanalyses were performed by the Microanalytical Laboratory and the elemental composition of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by ChemBioDraw Ultra (version 11.0) software for systematically naming organic chemicals.

5.1.1. 2-(1-(2-Chlorophenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (2)

A solution of ethylenediamine (0.47 mL, 6.50 mmol) in dry toluene (5 mL) was added dropwise to a mechanically stirred solution of 2 M trimethylaluminum in hexane (3.5 mL, 6.50 mmol) in dry toluene (5 mL) at 0 °C under a nitrogen atmosphere. After 1 h, the solution was cooled to 0 °C and a solution of **28** (0.76 g, 3.50 mmol) in dry toluene (10 mL) was added dropwise. The reaction mixture was heated to 90 °C for 12 h, cooled to 0 °C, and quenched cautiously with MeOH (4.5 mL), followed by H_2O (1 mL). After addition of $CHCl_3$ (35 mL) and filtration, the organic layer was extracted with 2 N HCl. The aqueous layer, made basic with 10% NaOH, was extracted with $CHCl_3$. Removal of dried solvent gave the free base **2**, which was purified by flash chromatography using cyclohexane/AcOEt/MeOH/33%NH_4OH (6:4:1:0.1) to afford a white solid: 0.59 g (75% yield); mp = 120–122 °C. ^{1}H NMR ($CDCl_3$) δ 1.63 (d, 3, CH_3CH), 3.56–3.76 (m, 4, NCH_2CH_2N), 5.04 (q, 1, CHCH$_3$), 6.92 (d, 1, ArH), 7.03 (t, 1, ArH), 7.20 (t, 1, ArH), 7.39 (d, 1, ArH), 7.86 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 139–140 °C. Anal. Calcd for $C_{11}H_{13}ClN_2O{\cdot}C_2H_2O_4$: C, 49.61; H, 4.80; N, 8.90. Found: C, 49.79; H, 4.67; N, 8.74.

5.1.2. 2-(1-(2-Bromophenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (3)

This was prepared as described for **2** starting from **29** (83% yield). ^{1}H NMR ($CDCl_3$) δ 1.69 (d, 3, CH_3CH), 3.56–3.78 (m, 4, NCH_2CH_2N), 5.17 (q, 1, CHCH$_3$), 6.82 (d, 1, ArH), 7.01 (t, 1, ArH), 7.22 (t, 1, ArH), 7.55 (d, 1, ArH), 8.65 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 152–153 °C. Anal. Calcd for $C_{11}H_{13}BrN_2O{\cdot}C_2H_2O_4$: C, 43.47; H, 4.21; N, 7.80. Found: C, 43.61; H, 4.53; N, 7.52.

5.1.3. 2-(1-(2-(Trifluoromethyl)phenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (4)

This was prepared as described for **2** starting from **30** (71% yield). ^{1}H NMR ($CDCl_3$) δ 1.61 (d, 3, CH_3CH), 3.50–3.72 (m, 4, NCH_2CH_2N), 5.17 (q, 1, CHCH$_3$), 7.01 (d, 1, ArH), 7.09 (t, 1, ArH), 7.43 (t, 1, ArH), 7.58 (d, 1, ArH), 7.61 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 163–164 °C. Anal. Calcd for $C_{12}H_{13}F_3N_2O{\cdot}C_2H_2O_4$: C, 48.28; H, 4.34; N, 8.04. Found: C, 48.40; H, 4.53; N, 8.12.

5.1.4. 2-(1-(2-(Prop-1-en-2-yl)phenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (10)

This was prepared as described for **2** starting from **31** (63% yield). ^{1}H NMR ($CDCl_3$) δ 1.61 (d, 3, CH_3CH), 2.17 (s, 3, CH_3C=CH$_2$), 3.46–3.78 (m, 4, NCH_2CH_2N), 5.03 (q, 1, CHCH$_3$), 5.10 (d, 1, CH_2=CCH$_3$), 5.19 (d, 1, CH_2=CCH$_3$), 6.93 (m, 2, ArH), 7.24 (m, 2, ArH), 8.13 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 134–136 °C. Anal. Calcd for $C_{14}H_{18}N_2O{\cdot}C_2H_2O_4$: C, 59.99; H, 6.29; N, 8.74. Found: C, 59.70; H, 6.43; N, 8.52.

5.1.5. 2-(1-(2-(Cyclopropylmethyl)phenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (12)

This was prepared as described for **2** starting from **32** (66% yield). ^{1}H NMR ($CDCl_3$) δ 0.20 (m, 2, cyclopropyl), 0.53 (m, 2, cyclopropyl), 1.05 (m, 1, cyclopropyl), 1.60 (d, 3, CH_3CH), 2.56 (m, 2, CH_2), 3.45–3.76 (m, 4, NCH_2CH_2N), 5.05 (q, 1, CHCH$_3$), 6.92 (m, 2, ArH), 7.18 (t, 1, ArH), 7.26 (d, 1, ArH), 8.01 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 176–177 °C. Anal. Calcd for $C_{15}H_{20}N_2O{\cdot}C_2H_2O_4$: C, 61.07; H, 6.63; N, 8.38. Found: C, 60.82; H, 6.93; N, 8.50.

5.1.6. 2-(1-(2-Ethoxyphenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (15)

This was prepared as described for **2** starting from **33** (80% yield). ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3, CH_3CH$_2$), 1.62 (d, 3, CH_3CH), 3.56–3.74 (m, 4, NCH_2CH_2N), 4.09 (q, 2, CH_2CH$_3$), 4.94 (q, 1, CHCH$_3$), 6.91–7.05 (m, 4, ArH), 7.35 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 121–122 °C. Anal. Calcd for $C_{13}H_{18}N_2O_2{\cdot}C_2H_2O_4$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.68; H, 5.99; N, 8.49.

5.1.7. 2-(1-(2-Cyclopropoxyphenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (17)

This was prepared as described for **2** starting from **34** (60% yield): mp 106–108 °C. ^{1}H NMR ($CDCl_3$) δ 0.80 (m, 4, cyclopropyl), 1.62 (d, 3, CH_3CH), 3.49–3.70 (m, 4, NCH_2CH_2N), 3.78 (m, 1, cyclopropyl), 4.85 (q, 1, CHCH$_3$), 6.92 (d, 1, ArH), 6.99 (m, 2, ArH), 7.23 (d, 1, ArH), 7.85 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 160–161 °C. Anal. Calcd for $C_{14}H_{18}N_2O_2{\cdot}C_2H_2O_4$: C, 57.14; H, 5.99; N, 8.33. Found: C, 57.41; H, 6.22; N, 8.56.

5.1.8. 2-(1-(2-(Vinyloxy)phenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (18)

This was prepared as described for **2** starting from **35** (56% yield). ^{1}H NMR ($CDCl_3$) δ 1.60 (d, 3, CH_3CH), 3.42–3.77 (m, 4, NCH_2CH_2N), 4.41 (d, 1, CH_2=CH), 4.65 (d, 1, CH_2=CH), 5.00 (q, 1, CHCH$_3$), 6.61 (dd, 1, CH=CH$_2$), 6.97–7.12 (m, 4, ArH), 7.23 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from 2-PrOH: mp 101–103 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}C_2H_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.75; H, 5.93; N, 8.60.

5.1.9. 2-(1-(2-Methoxyphenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (19)

This was prepared as described for **2** starting from **36** (86% yield). ^{1}H NMR ($CDCl_3$) δ 1.62 (d, 3, CH_3CH), 3.45–3.70 (m, 4, NCH_2CH_2N), 3.88 (s, 3, OCH_3), 5.13 (q, 1, CHCH$_3$), 6.83–7.04 (m, 4, ArH), 7.55 (br s, 1, NH, exchangeable with D_2O). The free base

was transformed into the oxalate salt, which was recrystallized from EtOH: mp 89–91 °C. Anal. Calcd for $C_{12}H_{16}N_2O_2{\cdot}C_2H_2O_4$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.44; H, 5.99; N, 9.24.

5.1.10. 2-(1-(2-(Methylsulfonyl)phenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (20)

This was prepared as described for **2** starting from **39** (49% yield): mp 143–145 °C. ^{1}H NMR ($CDCl_3$) δ 1.68 (d, 3, CH_3CH), 3.21 (s, 3, SO_2CH_3), 3.39–3.62 (m, 4, NCH_2CH_2N), 5.36 (q, 1, CHCH$_3$), 7.14 (m, 2, ArH), 7.58 (t, 1, ArH), 7.93 (d, 1, ArH), 8.16 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 107–109 °C. Anal. Calcd for $C_{12}H_{16}N_2O_3S{\cdot}C_2H_2O_4$: C, 46.92; H, 5.06; N, 7.82; S, 8.95. Found: C, 46.70; H, 5.23; N, 7.59; S, 9.12.

5.1.11. 2-(1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethoxy)benzaldehyde (21)

A mixture of **41**[11] (0.35 g, 1.33 mmol) and HCl 2 N (20 mL) was stirred at 25 °C for 2 h. The solution was basified with NaOH 2 N and extracted with $CHCl_3$. Removal of dried solvents afforded a residue which was purified by flash chromatography using cyclohexane/AcOEt/MeOH/33%NH_4OH (6:4:1:0.1) to give a colorless oil: 0.24 g (84% yield). ^{1}H NMR ($CDCl_3$) δ 1.65 (d, 3, CH_3CH), 3.42–3.68 (m, 4, NCH_2CH_2N), 4.63 (q, 1, CHCH$_3$), 6.94–7.52 (m, 4, ArH), 8.03 (br s, 1, NH, exchangeable with D_2O), 10.40 (s, 1, CHO). The free base was transformed into the dibenzoyl tartrate salt, which was recrystallized from 2-PrOH: mp 138–141 °C. Anal. Calcd for $C_{12}H_{14}N_2O_2{\cdot}C_{18}H_{14}O_8$: C, 62.50; H, 4.90; N, 4.86. Found: C, 62.77; H, 5.04; N, 4.74.

5.1.12. 1-(2-(1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethoxy)phenyl)ethanone (22)

This was prepared as described for **2** starting from **40** (62% yield). ^{1}H NMR ($CDCl_3$) δ 1.66 (d, 3, CH_3CH), 2.62 (s, 3, COCH_3), 3.44–3.72 (m, 4, NCH_2CH_2N), 5.17 (q, 1, CHCH$_3$), 7.04 (m, 2, ArH), 7.42 (t, 1, ArH), 7.68 (d, 1, ArH), 7.93 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 120–122 °C. Anal. Calcd for $C_{13}H_{16}N_2O_2{\cdot}C_2H_2O_4$: C, 55.90; H, 5.63; N, 8.69. Found: C, 55.69; H, 5.91; N, 8.53.

5.1.13. 2-(1-(2,6-Diphenylphenoxy)ethyl)-4,5-dihydro-1*H*-imidazole (27)

This was prepared as described for **2** starting from **42** (56% yield). ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 3, CH_3CH), 2.91–3.37 (m, 4, NCH_2CH_2N), 4.19 (q, 1, CHCH$_3$), 7.20–7.61 (m, 13, ArH), 7.90 (br s, 1, NH, exchangeable with D_2O). The free base was transformed into the oxalate salt, which was recrystallized from EtOH: mp 136–137 °C. Anal. Calcd for $C_{23}H_{22}N_2O{\cdot}C_2H_2O_4$: C, 69.43; H, 5.59; N, 6.48. Found: C, 69.23; H, 5.78; N, 6.23.

5.1.14. Methyl 2-(2-chlorophenoxy)propanoate (28)

A mixture of 2-chloro-phenol (Aldrich) (1.50 g, 11.6 mmol), methyl 2-bromopropionate (1.74 g, 11.6 mmol), and K_2CO_3 (1.60 g, 11.6 mmol) in DME was refluxed for 18 h. The mixture was cooled and filtered. The solvent was removed under reduced pressure to give a residue, which was taken up in CH_2Cl_2 and washed with cold 2 N NaOH. Removal of solvent afforded an oil which was purified by flash chromatography eluting with cyclohexane/EtOAc (95:5): 2.23 g (90% yield). ^{1}H NMR ($CDCl_3$) δ 1.70 (d, 3, CH_3CH), 3.78 (s, 3, OCH_3), 4.80 (q, 1, CHCH$_3$), 6.82 (d, 1, ArH), 6.95 (t, 1, ArH), 7.19 (t, 1, ArH), 7.39 (d, 1, ArH). Anal. Calcd for $C_{10}H_{11}ClO_3$: C, 55.96; H, 5.17. Found: C, 55.78; H, 5.27.

5.1.15. Methyl 2-(2-bromophenoxy)propanoate (29)

This was prepared as described for **28** starting from 2-bromophenol (Aldrich) (88% yield). ^{1}H NMR ($CDCl_3$) δ 1.71 (d, 3, CH_3CH), 3.78 (s, 3, OCH_3), 4.79 (q, 1, CHCH$_3$), 6.79 (d, 1, ArH), 6.93 (t, 1, ArH), 7.20 (t, 1, ArH), 7.58 (d, 1, ArH). Anal. Calcd for $C_{10}H_{11}BrO_3$: C, 46.36; H, 4.28. Found: C, 46.49; H, 4.15.

5.1.16. Methyl 2-(2-(trifluoromethyl)phenoxy)propanoate (30)

This was prepared as described for **28** starting from 2-trifluoromethyl-phenol (Aldrich) (82% yield). ^{1}H NMR ($CDCl_3$) δ 1.64 (d, 3, CH_3CH), 3.77 (s, 3, OCH_3), 4.81 (q, 1, CHCH$_3$), 6.81 (d, 1, ArH), 7.02 (t, 1, ArH), 7.41 (t, 1, ArH), 7.60 (d, 1, ArH). Anal. Calcd for $C_{11}H_{11}F_3O_3$: C, 53.23; H, 4.47. Found: C, 53.48; H, 4.33.

5.1.17. Methyl 2-(2-(prop-1-en-2-yl)phenoxy)propanoate (31)

This was prepared as described for **28** starting from 2-(prop-1-en-2-yl)phenol[20] (77% yield). ^{1}H NMR ($CDCl_3$) δ 1.61 (d, 3, CH_3CH), 2.18 (s, 3, CH_3C=CH$_2$), 3.76 (s, 3, OCH_3), 4.79 (q, 1, CHCH$_3$), 5.12 (d, 1, CH_2=CCH$_3$), 5.18 (d, 1, CH_2=CCH$_3$), 6.76 (d, 1, ArH), 6.94 (t, 1, ArH), 7.18 (t, 1, ArH), 7.22 (d, 1, ArH). Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.68; H, 7.46.

5.1.18. Methyl 2-(2-(cyclopropylmethyl)phenoxy)propanoate (32)

This was prepared as described for **28** starting from 2-(cyclopropylmethyl)phenol[21] (68% yield). ^{1}H NMR ($CDCl_3$) δ 0.20 (m, 2, cyclopropyl), 0.52 (m, 2, cyclopropyl), 1.03 (m, 1, cyclopropyl), 1.64 (d, 3, CH_3CH), 2.61 (d, 2, CH_2), 3.78 (s, 3, OCH_3), 4.79 (q, 1, CHCH$_3$), 6.64 (d, 1, ArH), 6.93 (t, 1, ArH), 7.16 (t, 1, ArH), 7.31 (d, 1, ArH). Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.56; H, 7.53.

5.1.19. Methyl 2-(2-ethoxyphenoxy)propanoate (33)

This was prepared as described for **28** starting from 2-ethoxyphenol (Aldrich) (82% yield). ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3, CH_3CH$_2$), 1.61 (d, 3, CH_3CH), 3.77 (s, 3, OCH_3), 4.14 (q, 2, CH_2CH$_3$), 4.78 (q, 1, CHCH$_3$), 6.81–7.03 (m, 4, ArH). Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.52; H, 7.41.

5.1.20. Methyl 2-(2-cyclopropoxyphenoxy)propanoate (34)

This was prepared as described for **28** starting from 2-cyclopropoxyphenol[22] (74% yield). ^{1}H NMR ($CDCl_3$) δ 0.81 (m, 4, cyclopropyl), 1.63 (d, 3, CH_3CH), 3.78 (s, 3, OCH_3), 3.80 (m, 1, cyclopropyl), 4.71 (q, 1, CHCH$_3$), 6.93 (m, 2, ArH), 7.01 (m, 1, ArH), 7.23 (d, 1, ArH). Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.18; H, 6.65.

5.1.21. Methyl 2-(2-(vinyloxy)phenoxy)propanoate (35)

This was prepared as described for **28** starting from 2-(vinyloxy)phenol[23] (77% yield). ^{1}H NMR ($CDCl_3$) δ 1.65 (d, 3, CH_3CH), 3.75 (s, 3, OCH_3), 4.40 (d, 1, CH_2=CH), 4.75 (d, 1, CH_2=CH), 4.78 (q, 1, CHCH$_3$), 6.61 (dd, 1, CH=CH$_2$), 6.89–7.12 (m, 4, ArH). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.98; H, 6.52.

5.1.22. Methyl 2-(2-methoxyphenoxy)propanoate (36)

This was prepared as described for **28** starting from 2-methoxyphenol (Aldrich) (90% yield). ^{1}H NMR ($CDCl_3$) δ 1.62 (d, 3, CH_3CH), 3.77 (s, 3, OCH_3), 3.89 (s, 3, ArOCH_3), 4.81 (q, 1, CHCH$_3$), 6.86–7.06 (m, 4, ArH). Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.66; H, 6.58.

5.1.23. Methyl 2-(2-(methylthio)phenoxy)propanoate (37)

This was prepared as described for **28** starting from 2-(methylthio)-phenol (Aldrich). White solid (82% yield): mp 50–51 °C. ^{1}H NMR ($CDCl_3$) δ 1.75 (d, 3, CH_3CH), 2.45 (s, 3, SCH_3), 3.80 (s, 3, OCH_3), 4.80 (q, 1, CHCH$_3$), 6.78 (d, 1, ArH), 7.00 (t, 1, ArH), 7.18 (t, 1 ArH), 7.20 (d, 1, ArH). Anal. Calcd for $C_{11}H_{14}O_3S$: C, 58.38; H, 6.24; S, 14.17. Found: C, 58.58; H, 6.03; S, 14.00.

5.1.24. Methyl 2-(2-acetylphenoxy)propanoate (38)

This was prepared as described for **28** starting from 2-acetylphenol (Aldrich) (89% yield). ^{1}H NMR ($CDCl_3$) δ 1.75 (d, 3, C*H*$_3$CH), 2.71 (s, 3, CO*CH*$_3$), 3.78 (s, 3, O*CH*$_3$), 4.87 (q, 1, *CH*CH$_3$), 6.78 (d, 1, Ar*H*), 7.00 (t, 1, Ar*H*), 7.42 (t, 1 Ar*H*), 7.78 (d, 1, Ar*H*). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.68; H, 6.50.

5.1.25. Methyl 2-(2-(methylsulfonyl)phenoxy)propanoate (39)

m-Chloroperbenzoic acid (3.80 g, 22.1 mmol) was added to a solution of **37** (2.00 g, 8,84 mmol) in CH_2Cl_2 (100 ml). After 20 h at room temperature under stirring the reaction mixture was washed with 10% Na_2SO_3, 5% Na_2CO_3, and H_2O. Removal of dried solvents afforded the desired compound as a white solid: 2.07 g (91% yield), mp 135–136 °C. ^{1}H NMR ($CDCl_3$) δ 1.75 (d, 3, C*H*$_3$CH), 3.38 (s, 3, SO_2*CH*$_3$), 3.78 (s, 3, O*CH*$_3$), 4.95 (q, 1, *CH*CH$_3$), 6.78 (d, 1, Ar*H*), 7.18 (t, 1, Ar*H*), 7.55 (t, 1 Ar*H*), 8.00 (d, 1, Ar*H*). Anal. Calcd for $C_{11}H_{14}O_5S$: C, 51.15; H, 5.46; S, 12.41. Found: C, 51.33; H, 5.76; S, 12.34.

5.1.26. Methyl 2-(2-(2-methyl-1,3-dioxolan-2-yl)phenoxy)propanoate (40)

A mixture of **38** (3.00 g, 13.51 mmol), 1,2-ethanediol (1.50 mL, 27 mmol) and *p*-toluenesulfonic acid (0.01 g) in toluene (20 mL) was refluxed with a Dean Stark apparatus for 24 h. After evaporation of the solvent, the residue was diluted with Et_2O and washed with saturated solution of $NaHCO_3$. Removal of dried solvents afforded the desired compound as an oil: 2.03 g (86% yield). ^{1}H NMR ($CDCl_3$) δ 1.75 (d, 3, C*H*$_3$CH), 2.71 (s, 3, CC*H*$_3$), 3.80 (s, 3, O*CH*$_3$), 4.03–4.22 (m, 4, O*CH*$_2$*CH*$_2$O), 4.87 (q, 1, *CH*CH$_3$), 6.78 (d, 1, Ar*H*), 7.00 (t, 1, Ar*H*), 7.42 (t, 1 Ar*H*), 7.78 (d, 1, Ar*H*). Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 62.97; H, 6.70.

5.1.27. Methyl 2-(2,6-diphenylphenoxy)propanoate (42)

This was prepared as described for **28** starting from 2,6-diphenylphenol (Aldrich) (68% yield). ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3, C*H*$_3$CH), 3.24 (s, 3, O*CH*$_3$), 3.92 (q, 1, *CH*CH$_3$), 7.21–7.63 (m, 13, Ar*H*). Anal. Calcd for $C_{22}H_{20}O_3$: C, 79.50; H, 6.06. Found: C, 79.37; H, 5.89.

5.2. Molecular modeling

Standard bond lengths and valence angles for the molecular scaffold of **9**, **12**, **13** and **14** were achieved upon a conjugate gradient minimization of the protonated structures, mimicking water with the *solvent continuum* method, according to the OPLS2005 force field parameters implemented in the MacroModel software package.[28] The conformational space available was sampled through a stochastic molecular dynamic simulation carried out at 300 K for 200 ns with an integration time of 1.5 fs collecting one conformer each 5 ps after an equilibration time of 2 ns. The sampled structures were further quenched and unique low energy conformations were saved according to a RMS threshold distance of the heavy atoms and polar hydrogens of 1.0 and a ΔE of 5.0 kcal/mol with respect to global minimum. With this kind of protocol it might be expected to collect a significant number of low energy structures, comprising both the global and local minima, sufficient to locate a plausible bioactive conformation for the studied compounds. The entire low energy conformation database of each compound was screened using the shape matching algorithm ROCS[29] and the crystallographic data of lofexidine retrieved from the Cambridge Structural Database (CSD code: CPEIZC) as reference. A *shape only* comparison was carried out ranking the superposition between the query and the template according to the Tanimoto similarity coefficient. For **9** and **11** the lowest energy minimum, corresponding to the *extended* conformation was selected, whereas for **13** and **14** the best superpositions, having just a ΔE value of 2.96 and 3.66 kcal/mol, respectively, were chosen. This energy window is well below the upper limit of 20 kcal/mol that is the recommended threshold in exploring the conformational space in a pharmacophore search.[30]

5.3. NMR studies

Due to the solubility of the compounds' salts, ^{1}H NMR spectra were recorded in DMSO-d_6 with a Varian-Mercury 300 (300 MHz) spectrometer at room temperature and processed by ACD/Labs ACD/NMR Processor Academic Edition, release 12.0, version 12.01 (build 3014, 18 Mar 2010). The samples for NOESY experiments were degassed, but because of the hygroscopicity of salt and solvent, it was not possible to completely remove traces of water from the experiments. This situation could have reduced in any case the intensity of the NOEs.

5.4. Pharmacology

The pharmacological profiles of the compounds were investigated using CHO cell lines stably expressing cDNAs encoding human α_{2A}-, α_{2B}- and α_{2C}-AR subtypes. Receptor binding experiments, as already described,[11] were carried out on membrane preparations using the antagonist [^{3}H]RS-79948-197 as radioligand. IC_{50} values were determined by nonlinear regression analysis of competition binding data using GraphPad Prism computer programs (GraphPad Software, Inc., La Jolla, CA, USA). K_i values were calculated from the equation of Cheng and Prusoff[31] and reported as pK_i ± SEM. Agonist and antagonist potencies were determined as previously described.[11] Agonist potency, defined as the concentration that produces 50% of the maximum response, is expressed as pEC_{50} and was determined with transfected CHO cells by using a Cytosensor microphysiometry instrument measuring the rate of extracellular acidification after receptor activation by agonists. The intrinsic activity (ia) of each compound is expressed as the fraction of the maximum response elicited by the natural full agonist (−)-noradrenaline (ia = 1). Antagonist results are expressed as pK_b and were analysed as the ability of the antagonist to shift the concentration–response curve of the agonist clonidine.[32]

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