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3-Arylpiperazinylethyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione derivatives as novel, high-affinity and selective α_1-adrenoceptor ligands

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Abstract—The discovery of a new series of selective and high-affinity α_1-adrenoceptor (α_1-AR) ligands, characterized by a 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione system, is described in this paper. Some synthesized compounds, including **20**, **22**, and **30**, displayed affinity in the nanomolar range for α_1-ARs and substantial selectivity with respect to 5-HT_{1A} and dopaminergic D_1 and D_2 receptors. Functional assays, performed on selected derivatives, showed antagonistic properties.
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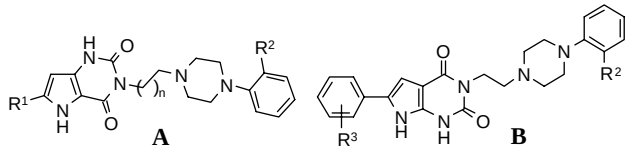
The adrenoceptors (ARs) play a key role in the modulation of sympathetic nervous system activity and are a site of action for many important therapeutic agents. The ARs, members of the G-protein-coupled receptors superfamily, are divided into three principal groups: α_1, α_2, and β.[1a–f]

The α_1-ARs are further subdivided into α_{1A}, α_{1B}, and α_{1D} subtypes, and play a prominent role in regulating vascular tone and hypertrophic growth of smooth muscle and cardiac cells.[1a–f]

Particularly, α_{1A}-AR seems to be primarily involved in the development of benign prostatic hypertrophy (BPH) and, in the last few years, it became an interesting target for symptomatic treatment of BPH with reduced side effects.[1c,f]

Although a great number of α_1-AR ligands are known, there is still the need for high-affinity and selective ligands with respect to 5-HT_{1A} and dopaminergic D_1 and D_2 receptors, and with respect to the different α_1-AR subtypes to finally clarify the pharmacological profile of each receptor type and subtype.

As a part of our program addressed to the development of selective α_1-AR ligands, we have designed new molecules based on an arylpiperazinyl moiety, a well-known pharmacophoric portion present in several classes of α_1-AR ligands.[2]



1: R^1 = 4-$CH_3C_6H_4$, R^2 = Cl, n = 1
2: R^1 = 4-ClC_6H_4, R^2 = Cl, n = 1

Recently, we reported the discovery of 3-arylpiperazinylalkylpyrrolo[3,2-*d*]pyrimidine-2,4-dione derivatives (**A**) as ligands for the α_1-AR subtypes endowed with an excellent selectivity with respect to 5-HT_{1A} and dopaminergic D_1 and D_2 receptors.[3] These molecules, bearing between the pyrrolo[3,2-*d*]pyrimidine-2,4-dione moiety and the phenylpiperazine (PPz) residue

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a connecting alkyl chain with two or three carbon atoms (n = 1 and 2), were structurally related to **RN5**, a known α_1-AR antagonist with affinity in the nanomolar range.[2] Besides, some thienopyrimidine-2,4-dione derivatives, related to compounds **A**, were described in a patent and claimed as ligands selective for α_{1D}- with respect to α_{1A}- and α_{1B}-AR subtypes.[4]

In this paper, we report on the synthesis and pharmacological profile of novel α_1-AR ligands (**B**) characterized by the isomeric 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione system, with the aim of getting a new insight into the structural requirements that direct selectivity toward α_1-ARs.

Novel derivatives were synthesized as reported in Scheme 1 and the synthetic route follows a strategy similar to the one used for the preparation of compounds **A**. Briefly, the appropriate 2-amino-5-substituted phenyl-1*H*-pyrrole-3-carboxylic acid ethyl esters **3**–**9** were easily accessible utilizing procedures described in the literature,[5] starting from 3-amino-3-iminopropanoic acid ethyl ester and condensing it with the appropriate 2-bromo-1-substituted phenylethanone. Aminoesters **3**–**9** were then reacted with 2-chloroethyl isocyanate in refluxing toluene. Obtained ureas **10**–**16** were reacted with the suitable 1-(2-substituted phenyl)piperazine in THF in the presence of NaI and $NaHCO_3$, to afford intermediates that were converted into final products **17**–**30** in refluxing methanolic potassium hydroxide solution.

A detailed synthetic procedure, along with analytical characterization, is given for 3-[2-[4-(2-chlorophenyl)-1-piperazinyl]ethyl]-6-(4-methylphenyl)-1*H*-pyrrolo[2,3-*d*]-pyrimidine-2,4(3*H*,7*H*)-dione (**26**) and its precursors **7** and **14**.[6]

All synthesized compounds were characterized by ^{1}H NMR spectra, IR, and elemental analysis and analytical data were consistent with the proposed structures.

New compounds were evaluated in binding assays that were performed as previously reported,[3] and K_i values of compounds **17**–**30** for α_1-ARs and related receptors are summarized in Table 1. In it, for sake of clarity, affinity values of compounds **1** and **2**, the most interesting derivatives in the series **A**, are also reported. Noteworthy, all the compounds described herein presented affinity for the α_1-ARs in the low nanomolar range.

These results confirmed that the isomeric 1*H*-pyrrolo-[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione system is a good scaffold that, bearing appropriate substituents, can confer to new ligands high affinity for α_1-ARs.

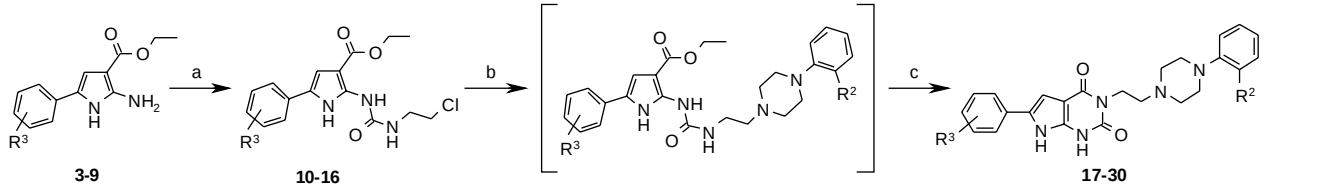
Regarding the selectivity, as general trend, derivatives in which R^2 is a chlorine residue showed an interesting preference for α_1-ARs with respect to 5-HT_{1A} receptors so that they were evaluated for their affinity toward D_1 and D_2 dopaminergic receptors, demonstrating often good selectivity (**20**, **22**, and **30**). These results finally confirmed that a chlorine residue at the 2-position of the PPz moiety, instead of a methoxy group, is crucial to address selectivity to α_1-ARs (as previously reported for compounds **A**).[3]

The effects of the nature and the position of substituents on the phenyl ring linked to heterobicyclic system were investigated by introducing a methyl a methoxy or a chlorine residue at the 2- or the 4-position. These substitutions did not significantly affect affinity at α_1-ARs, while a modulation of the selectivity with respect to the other tested receptors was observed. The introduction of a methyl or a methoxy residue at the 2-position afforded interesting derivatives in terms of affinity and selectivity (**20** and **22**). On the contrary, the same substitution at the 4-position of the phenyl ring lead to a slight loss of affinity for α_1-ARs (**26** and **28**) and, as a consequence, the selectivity ratios 5-HT_{1A}/α_1, D_1/α_1, and D_2/α_1 were reduced (**26** vs **20** and **28** vs **22**).

The substitution at the 2-position of the phenyl ring with a chlorine residue (**23** and **24**), while maintaining affinity, was detrimental for selectivity.

Compound **30** possesses one of the highest affinity values at α_1-ARs and, although bearing chlorine residues both at the 4-position of the phenyl ring linked to the heterobicyclic nucleus and at the 2-position of the PPz moiety, is the most selective derivative identified in this study.

Derivatives **26**, **28**, and **30**, along with compound **1**, were selected for further evaluation in functional assays for



3, 10: R^3 = H
4, 11: R^3 = 2-CH_3
5, 12: R^3 = 2-OCH_3
6, 13: R^3 = 2-Cl
7, 14: R^3 = 4-CH_3
8, 15: R^3 = 4-OCH_3
9, 16: R^3 = 4-Cl

17: R^3 = H, R^2 = OCH_3
18: R^3 = H, R^2 = Cl
19: R^3 = 2-CH_3, R^2 = OCH_3
20: R^3 = 2-CH_3, R^2 = Cl
21: R^3 = 2-OCH_3, R^2 = OCH_3
22: R^3 = 2-OCH_3, R^2 = Cl
23: R^3 = 2-Cl, R^2 = OCH_3
24: R^3 = 2-Cl, R^2 = Cl
25: R^3 = 4-CH_3, R^2 = OCH_3
26: R^3 = 4-CH_3, R^2 = Cl
27: R^3 = 4-OCH_3, R^2 = OCH_3
28: R^3 = 4-OCH_3, R^2 = Cl
29: R^3 = 4-Cl, R^2 = OCH_3
30: R^3 = 4-Cl, R^2 = Cl

Scheme 1. Reagents and conditions: (a) $ClCH_2CH_2NCO$, toluene, reflux, 6 h (75–85%); (b) 1-(2-substituted phenyl)piperazine, $NaHCO_3$, NaI, THF, reflux, 24 h (65–70%); (c) KOH/MeOH, reflux, 3 h (85–98%).

Table 1. Binding properties of 3-arylpiperazinylethyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione derivatives **17**–**30**

Compound	R^2	R^3	K_i (nM)[a]				5-HT_{1A}/α_1[b]	D_1/α_1[b]	D_2/α_1[b]
			α_1	5-HT_{1A}	D_1	D_2			
17	OCH_3	H	1.99 (±0.45)	122.3 (±15.2)	ND	ND	61		
18	Cl	H	4.71 (±0.71)	1540 (±145.6)	516 (±45.2)	137.2 (±10.8)	327	110	29
19	OCH_3	2-CH_3	1.19 (±0.01)	295.1 (±11.7)	ND	ND	248		
20	Cl	2-CH_3	2.80 (±0.47)	1450 (±79.9)	1040 (±155.3)	626.5 (±99.6)	518	371	224
21	OCH_3	2-OCH_3	1.63 (±0.10)	157.3 (±8.83)	ND	ND	97		
22	Cl	2-OCH_3	3.32 (±0.58)	1030 (±21.8)	8550 (±1480)	363.2 (±55.7)	310	>2500	109
23	OCH_3	2-Cl	2.18 (±0.33)	19.10 (±3.60)	ND	ND	8.8		
24	Cl	2-Cl	6.28 (±2.14)	64.90 (±12.8)	566.8 (±41.5)	356.1 (±53.1)	10	90	57
25	OCH_3	4-CH_3	4.97 (±0.36)	333.34 (±22.5)	ND	ND	67		
26	Cl	4-CH_3	11.71 (±2.36)	1815 (±39.3)	388.7 (±45.4)	1720 (±506)	155	33	147
27	OCH_3	4-OCH_3	5.47 (±0.36)	113.4 (±8.46)	ND	ND	21		
28	Cl	4-OCH_3	16.40 (±2.93)	1070 (±83.8)	271.6 (±41.1)	164.6 (±16.4)	65	17	10
29	OCH_3	4-Cl	4.08 (±0.31)	401.3 (±26.3)	ND	ND	98		
30	Cl	4-Cl	2.64 (±0.25)	1513 (±172.8)	544.4 (±71.7)	2370 (±770)	573	206	898
RN5[c]			0.21 (±0.02)	50.00 (±4.70)			238		
1[d]			17.26 (±4.14)	>10,000	>10,000	>10,000	>500	>500	>500
2[d]			11.70 (±0.25)	>10,000	>10,000	>10,000	>800	>800	>800

[a] K_i values were calculated as in Ref. 3 and are means (±SD) of three separate experiments.
[b] Ratio between K_i 5-HT_{1A}/K_i α_1; K_i D_1/K_i α_1; and K_i D_2/K_i α_1.
[c] Data taken from Ref. 2.
[d] Data taken from Ref. 3.

Table 2. Antagonist potency, expressed as $pK_b \pm$ SEM values, and selectivities of compounds **26**, **28**, **30**, and **1** at α_1-ARs in isolated rat prostatic vas deferens (α_{1A}), spleen (α_{1B}), and thoracic aorta (α_{1D})

Compound	R^2	R^3	$pK_b\alpha_{1A}$[a] or [pA_2][b]	$pK_b\alpha_{1B}$ or [pA_2]	$pK_b\alpha_{1D}$ or [pA_2]	α_{1D}/α_{1A}[c]	α_{1D}/α_{1B}	α_{1B}/α_{1A}
26	Cl	4-CH_3	7.25 (±0.07)	7.50 (±0.10)	7.78 (±0.07)	3	2	2
28	Cl	4-OCH_3	7.48 (±0.06)	8.03 (±0.06)	7.69 (±0.13)	2	0.5	4
30	Cl	4-Cl	6.41 (±0.09)	6.64 (±0.09)	7.66 (±0.05)	18	10	2
1			6.72 (±0.11)	6.37 (±0.03)	6.44 (±0.05)	0.5	1	0.4
WB4101			[9.51] (±0.06)	[8.20] (±0.10)	[8.80] (±0.12)	0.2	4	0.05

[a] pK_b values were calculated according to van Rossum[7] at a single concentration and each concentration was investigated at least four times.
[b] pA_2 values were calculated according to Arunlakshana and Schild plots.[8]
[c] Antilog of the difference between the pK_b values for α_{1A}-, α_{1B}-, and α_{1D}-AR subtypes.

their activity at α_{1A}-, α_{1B}-, and α_{1D}-AR subtypes. Tested compounds, as shown in Table 2, behave as α_1-AR antagonists and their affinity was calculated at a single concentration according to van Rossum.[7] WB4101 was included as a standard compound.

The results in Table 2 show for derivatives **26** and **28** higher values of affinity at the three α_1-AR subtypes with respect to **30** and **1**. However, compound **30** behaves as an α_{1D}-AR selective antagonist displaying a 18 and 10 times higher affinity for this subtype when compared with α_{1A}- and α_{1B}-AR ones.

In conclusion, we have discovered a new class of ligands endowed with noteworthy affinity toward α_1-ARs, characterized by a 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione moiety. Furthermore, the 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione system showed to be able to address properly α_1-ARs selectivity over 5-HT_{1A}, D_1, and D_2 dopaminergic receptors (**20**, **22**, and **30**). In particular, compound **30**, endowed with affinity in the nanomolar range for the α_1-ARs, showed the best profile in terms of selectivity toward other tested receptors, and, in functional assays, a preference for the α_{1D}- with respect to α_{1A}- and α_{1B}-AR of one order of magnitude.

Further structural modifications of the 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione system are currently ongoing with the aim of ameliorating receptor subtype selectivity.

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6. Experimental procedure for the synthesis of compounds **7**, **14**, and **26**. 2-Amino-5-(4-methylphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (**7**). Ammonium chloride (15.3 mmol, 0.820 g) was added to a suspension of 3-ethoxy-3-iminopropanoic acid ethyl ester hydrochloride (10.2 mmol, 2.00 g) and K_2CO_3 (17.9 mmol, 2.47 g) in 10 mL of anhydrous ethanol. The reaction mixture was then stirred at 22 °C for 24 h. The obtained suspension of 3-amino-3-iminopropanoic acid ethyl ester was used for the subsequent step without isolating it from the reaction environment. 2-Bromo-1-(4-methylphenyl)ethanone (10.2 mmol, 2.18 g) and K_2CO_3 (5.11 mmol, 0.706 g) were added to the previously obtained suspension of 3-amino-3-iminopropanoic acid ethyl ester and the reaction mixture was refluxed for 2 h. After this period the solvent was evaporated at reduced pressure, and the residue was diluted with NaOH 1 N (100 mL) and extracted with EtOAc (3× 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. The obtained crude material was purified by column chromatography (silica gel, 60,230–400 mesh, Merck) using cyclohexane/EtOAc (6:4) as eluant, to afford the title compound (**7**) as a white powder (1.03 g, 55%): mp 140–142 °C; IR (KBr, selected lines) cm^{-1} 3484, 3361, 1666, 1641, 1508, 1369, 1237, 1190, 1071, 799, 765. The ^1H NMR spectrum was recorded at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-d_6. ^1H NMR (DMSO-d_6) δ 10.68 (br s, 1H, NH which exchanges with D_2O), 7.41–7.34 (m, 2H, aromatic), 7.14–7.07 (m, 2H, aromatic), 6.39 (d, ^4J = 2.8 Hz, 1H, pyrrole), 5.63 (br s, 2H, NH_2 which exchanges with D_2O), 4.13 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 2.28 (s, 3H, CH_3), 1.24 (t, J = 7.0 Hz, 3H, OCH_2CH_3). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-(4-methylphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (**14**). A suspension of 2-amino-5-(4-methylphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (**7**) (3.44 mmol, 0.840 g) and 2-chloroethyl isocyanate (5.16 mmol, 0.438 mL) in 8 mL of toluene was stirred for 6 h at reflux. The reaction mixture was then filtered, and the obtained solid was washed several times with EtOH to give the title compound as a pure solid (0.811 g, 67%): mp 112-114 °C; IR (KBr, selected lines) cm^{-1} 3332, 2919, 2865, 1673, 1606, 1549, 1359, 1263, 1097, 778, 655. ^1H NMR (DMSO-d_6) δ 11.26 (br s, 1H, NH which exchanges with D_2O), 9.24 (br s, 1H, N*H*CONHCH$_2$ which exchanges with D_2O), 7.99 (t, J = 5.8 Hz, 1H, NHCON*H*CH$_2$ which exchanges with D_2O), 7.47–7.40 (m, 2H, aromatic), 7.20–7.13 (m, 2H, aromatic), 6.61 (d, ^4J = 2.8 Hz, 1H, pyrrole), 4.31 (q, J = 7.0 Hz, 2H, OC*H*$_2$CH$_3$), 3.69 (t, J = 6.0 Hz, 2H, CH_2Cl), 3.52–3.46 (m, 2H, NHC*H*$_2$), 2.29 (s, 3 H, CH_3), 1.34 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$). Anal. ($C_{17}H_{20}ClN_3O_3$) C, H, N. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-(4-methylphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (**26**) A suspension of 2-[[[(2-chloroethyl)amino]carbonyl]amino]-5-(4-methyl)phenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (**14**) (1.06 mmol, 0.370 g), 1-(2-chlorophenyl)piperazine hydrochloride (1.27 mmol, 0.296 g), $NaHCO_3$ (4.23 mmol, 0.356 g), and a catalytic amount of NaI in 2 mL of anhydrous THF was refluxed for 24 h. After this period the obtained mixture was concentrated, and the residue was diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated. The obtained crude material was dissolved in EtOAc, filtered through a pad of silica gel, and concentrated. The obtained urea was refluxed in methanolic KOH (1 N, 4 mL) for 3 h. The reaction mixture was then allowed to cool at 22 °C, acidified (pH 4) with glacial AcOH, and neutralized with saturated $NaHCO_3$. A white precipitate was isolated, washed with water, and triturated in EtOAc to afford the desired product **26** as a white solid (0.285 g, 58%): mp 254–256 °C; IR (KBr, selected lines) cm^{-1} 2973, 2828, 1699, 1636, 1574, 1480, 1444, 1231, 1041, 932, 764, 708. ^1H NMR (DMSO-d_6) δ 7.60–7.52 (m, 2H, aromatic), 7.42–6.95 (m, 6H, aromatic), 6.58 (s, 1H, pyrrole), 4.06 (t, J = 6.6 Hz, 2H, CONC*H*$_2$CH$_2$N), 2.99–2.94 (m, 4H, NCH$_2$), 2.69–2.49 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C*H*$_2$N), 2.27 (s, 3H, CH_3). Anal. ($C_{25}H_{24}ClN_5O_2$) C, H, N.

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