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Short Communication

Pyrazole analogues of prazosin

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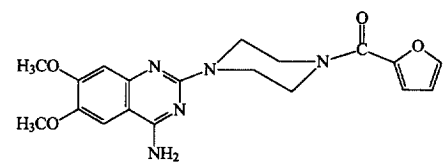
Abstract

A series of analogues of prazosin, in which 1-methyl or 1-phenylpyrazole moieties were substituted for the furan ring, were synthesized and studied for their α_1-adrenoceptor antagonist activity. The role of the five member heterocyclic substructures in determining the affinity for the α_1-receptor is briefly discussed. © 1998 Elsevier Science S.A. All rights reserved.

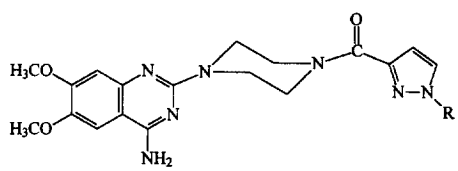
Keywords: α_1-Adrenoceptor antagonist; Prazosin analogues; Pyrazole derivatives; Molecular modelling

1. Introduction

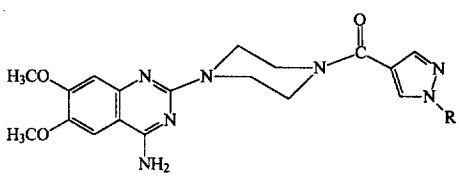
Structure–activity relationships (SARs) in prazosin **1**, a well-known α_1-adrenoceptor antagonist, have been the object of several studies. The role of the 2,4-diamino-6,7-dimethoxyquinazoline moiety has been established [1–3]. This substructure, at physiological pH, is partially protonated at the endocyclic N-1 nitrogen and acts as a conformationally restricted form for the noradrenaline cation. The role of the piperazine ring has been analysed [4] and conformational analysis, molecular modelling, and quantitative structure–activity relationship studies have been carried out on sets of 2,4-diamino-6,7-dimethoxy-2-substituted quinazolines [5,6]. The substitution of a number of aromatic five member heterocyclic rings for furan in **1** has been explored, principally in patent literature. To our knowledge only one paper, concerning the study of a number of isoxazole analogues of prazosin, has been devoted to this subject [7]. In addition, in a recent work we reported the replacement of R-substituted 1,2,5-oxadiazoles N-oxides (furoxans) for the furan ring to design hybrid molecules able to display simultaneously α_1-adrenoceptor antagonistic activity and NO mediated vasodilating properties [8,9]. In that investigation, the corresponding 1,2,5-oxadiazole analogues (furazans) were also considered. In this paper, we report synthesis, α_1-antagonist properties and SARs of pyrazole congeners of prazosin, belonging to series **I**, **II** and **III** (Scheme 1).



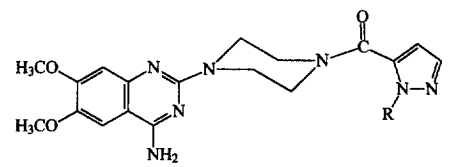
1



I: 3a, R=CH_3; **3b**, R=C_6H_5



II: 4a, R=CH_3; **4b**, R=C_6H_5



III: 5a, R=CH_3; **5b**, R=C_6H_5

Scheme 1. Prazosin (**1**) and its pyrazole congeners (series **I**, **II**, **III**).

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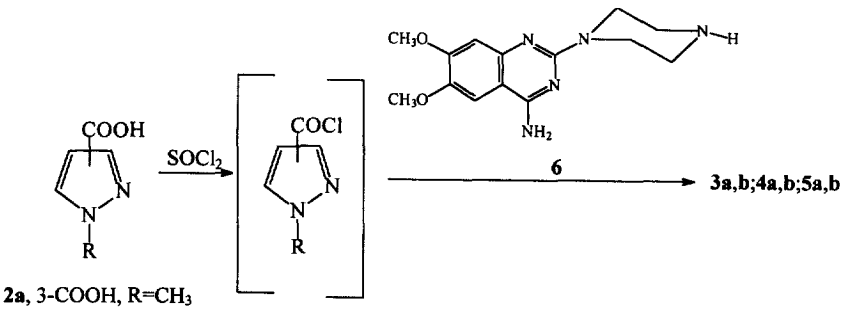
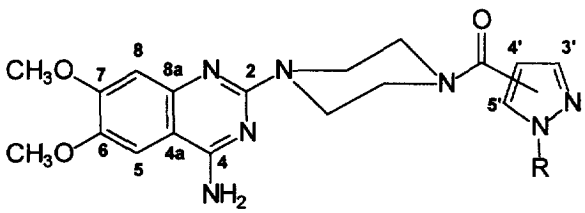
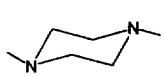


2a, 3-COOH, R=CH_3
2b, 3-COOH, R=C_6H_5
2c, 4-COOH, R=CH_3
2d, 4-COOH, R=C_6H_5
2e, 5-COOH, R=CH_3
2f, 5-COOH, R=C_6H_5

Scheme 2. Preparation of compounds **3a–5a** and **3b–5b**.

Table 1
^{1}H NMR chemical shifts δ [a] and coupling constants J of the compounds **3a,b**, **4a,b** and **5a,b**



Hydrogen	3-Substituted pyrazoles		4-Substituted pyrazoles		5-Substituted pyrazoles	
	3a (R=CH_3)	**3b** (R=C_6H_5)	**4a** (R=CH_3)	**4b** (R=C_6H_5)	**5a** (R=CH_3)	**5b** (R=C_6H_5)
5	7.46 (s, 1H)	7.46 (s, 1H)	7.44 (s, 1H)	7.48 (s, 1H)	7.46 (s, 1H)	7.45 (s, 1H)
8	6.78 (s, 1H)	6.77 (s, 1H)	6.76 (s, 1H)	6.80 (s, 1H)	6.78 (s, 1H)	6.74 (s, 1H) [b]
6-OCH_3	3.81 (s, 3H)	3.81 (s, 3H)	3.81 (s, 3H)	3.81 (s, 3H)	3.81 (s, 3H)	3.80 (s, 3H)
7-OCH_3	3.85 (s, 3H)	3.85 (s, 3H)	3.85 (s, 3H)	3.86 (s, 3H)	3.85 (s, 3H)	3.84 (s, 3H)
	4.0–3.7 (m, 8H)	4.0–3.7 (m, 8H)	4.0–3.7 (m, 8H)	4.0–3.7 (m, 8H)	4.0–3.5 (m, 8H)	3.7–3.2 (m, 8H)
3′			7.72 (s, 1H)	8.05 (s, 1H)	7.51 (d, J=1.9 Hz, 1H)	7.83 (d, J=1.7 Hz, 1H)
4′	6.59 (d, J=2.2 Hz, 1H)	6.88 (d, J=2.5 Hz, 1H)			6.51 (d, J=1.9 Hz, 1H)	6.74 (d, J=1.7 Hz, 1H) [b]
5′	7.79 (d, J=2.2 Hz, 1H)	8.62 (d, J=2.5 Hz, 1H)	8.11 (s, 1H)	8.88 (s, 1H)		
N–CH_3	3.92 (s, 3H)		3.88 (s, 3H)		3.88 (s, 3H)	
N–C_6H_5		7.3–8.0 (m, 5H)		7.3–8.0 (m, 5H)		7.4–7.6 (m, 5H)
NH_2	7.24 (br, 2H)	7.18 (br, 2H)	7.18 (br, 2H)	7.26 (br, 2H)	7.2 (br, 2H)	7.19 (br, 2H)

[a] δ in ppm from TMS. Solvent DMSO-d_6.
[b] Overlapping signals.

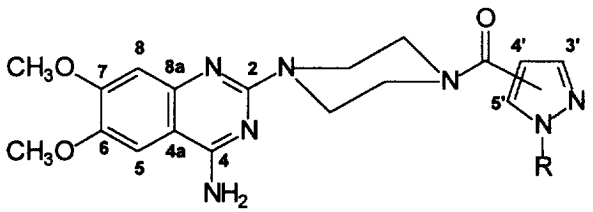
In these compounds the quinazolinylpiperazinocarbonyl moiety is linked at positions 3, 4 and 5, respectively, of the pyrazole ring. This structural feature, combined with phenyl or methyl substitution at N-1 of the heterocyclic system, modulates the shape and volume of this molecular region and renders the three series suitable probes for exploring the receptor area fitted by the pyrazole ring.

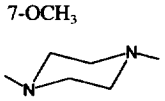
2. Experimental

2.1. Chemistry

Melting points were measured on a Büchi 530 capillary apparatus and are uncorrected. The compounds were routinely checked by infrared spectrometry (Shimadzu FT-IR

Table 2
^{13}C NMR chemical shifts δ [a] of the compounds **3a,b**, **4a,b** and **5a,b**



Carbon	3-Substituted pyrazoles		4-Substituted pyrazoles		5-Substituted pyrazoles	
	3a (R = CH_3)	**3b** (R = C_6H_5)	**4a** (R = CH_3)	**4b** (R = C_6H_5)	**5a** (R = CH_3)	**5b** (R = C_6H_5)
2	158.1	158.4	158.4	158.1	158.3	158.0
4	161.2	161.2	161.2	161.2	161.3	161.2
4a	103.0	103.1	103.0	103.0	103.1	103.0
5	103.8	103.7	103.7	103.8	103.7	103.7
6	145.2	145.1	145.1	145.2	145.2	145.2
7	154.3	154.3	154.3	154.4	154.4	154.3
8	105.1	105.3	105.3	105.0	105.3	105.2
8a	148.4	148.9	148.8	148.3	148.8	148.5
6-OCH_3	55.9	55.9	55.9	55.9	55.9	55.9
7-OCH_3	55.5	55.5	55.5	55.5	55.5	55.5
	46.5–44.3, 43.7–42.1	46.7–44.3, 43.6–42.2	43.8	47.0 (vbr), 43.8 (br)	47.0–43.5, 41.8	46.5–43.3, 43.0–41.6
C=O	162.0	161.7	162.8	162.5	160.6	160.7
3′	144.2	148.0	139.3	141.4	137.5	140.3
4′	106.2	110.1	116.5	116.8	106.7	108.3
5′	131.7	128.8	132.6	129.1	135.4	136.2
CH_3	38.7		38.7		37.8	
C_6H_5–C_i		139.4		139.3		139.4
C_6H_5–C_o		118.9		116.9		123.0
C_6H_5–C_m		129.7		129.6		129.5
C_6H_5–C_p		127.0		127.0		127.9

[a] δ in ppm from TMS. Solvent DMSO-d_6.

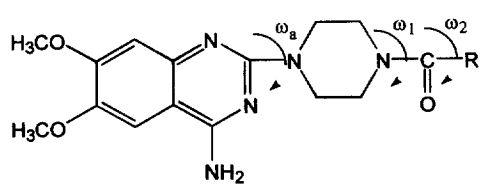


Fig. 1. Torsion angles considered in the conformational analysis.

8101 M), ^1H and ^{13}C nuclear magnetic resonance at 200 and 50 MHz, respectively (Bruker AC-200 spectrometer), and mass spectrometry (Finnigan-Mat TSQ-700 spectrometer). Thin layer chromatography (TLC) was carried out on 5 × 20 cm plates precoated with Merck silica gel 60 F_{254}, with a layer thickness of 0.25 mm. Anhydrous magnesium sulfate was used as the drying agent of the organic extracts. Solvent removal was achieved under reduced pressure at room temperature. Elemental analyses of the new compounds were performed by REDOX (Cologno M.) and the results are within ±0.4% of theoretical values.

The carboxylic acid **2a** [10], **2b** [11], **2c** [12], **2d** [13], **2e** [14], **2f** [15], and the intermediate **6** [16] were synthesized according to literature methods.

2.2. General procedure for the preparation of compounds 3a,b, 4a,b and 5a,b

These compounds were obtained according to a modified procedure of the method reported in Ref. [8]. A solution of the appropriate pyrazolcarboxylic acid **2a–f** (2 mmol) in thionyl chloride (8 ml) containing a few drops of anhydrous dimethylformamide was heated at reflux for three hours. The excess of thionyl chloride was removed in vacuo and the residue, dissolved in dry tetrahydrofuran (3 ml), was added dropwise to a stirred solution of 4-amino-6,7-dimethoxy-2-piperazinoquinazoline **6** (0.58 g, 2 mmol) dissolved in a mixture of water/methanol (1:10) (15 ml) containing potassium bicarbonate (0.3 g, 3 mmol). The reaction mixture was stirred at 20°C for 2 h then the precipitate was filtered and recrystallized from the appropriate solvent to give products as free bases.

3a: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-methyl-3-pyrazolylcarbonyl)piperazine. Methanol was used as cosolvent in the reaction instead of water/methanol. Yield 50%, m.p. 215–216°C (from EtOAc). *Anal.* ($C_{19}H_{23}N_7O_3$) C, H, N.

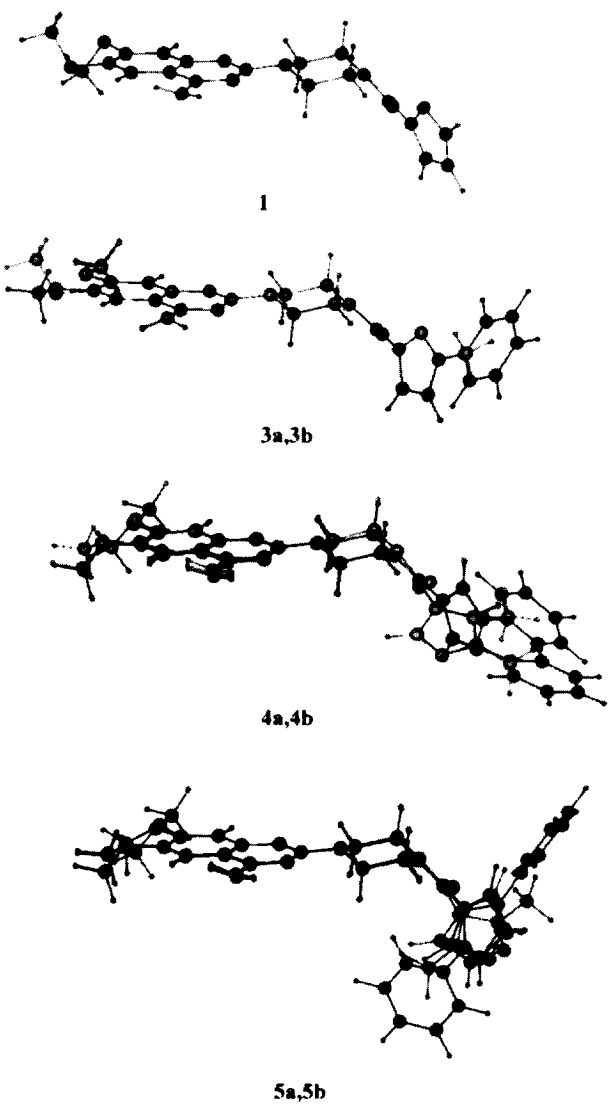


Fig. 2. Side view of the stable conformers.

4a: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-methyl-4-pyrazolylcarbonyl)piperazine. Yield 78%, m.p. 254–255°C (from MeOH). *Anal.* ($C_{19}H_{23}N_7O_3 \cdot CH_3OH$) C, H, N.

5a: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-methyl-5-pyrazolylcarbonyl)piperazine. Methanol was used as cosolvent in the reaction instead of water/methanol. Yield 83%, m.p. 228–229°C (from MeOH). *Anal.* ($C_{19}H_{23}N_7O_3 \cdot 0.5\ CH_3OH$) C, H, N.

3b: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-phenyl-3-pyrazolylcarbonyl)piperazine. $KHCO_3$ (0.6 g, 6 mmol) was used in the reaction. Yield 93%, m.p. 256–257°C (from MeOH). *Anal.* ($C_{24}H_{25}N_7O_3$) C, H, N.

4b: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-phenyl-4-pyrazolylcarbonyl)piperazine. Yield 82%, m.p. 230–231°C (from MeOH). *Anal.* ($C_{24}H_{25}N_7O_3 \cdot CH_3OH$) C, H, N.

5b: 1-(4-amino-6,7-dimethoxy-2-quinazolyl)-4-(1-phenyl-5-pyrazolylcarbonyl)piperazine. Yield 93%, m.p. 244–247°C (from dioxane). *Anal.* ($C_{24}H_{25}N_7O_3 \cdot 0.5\ H_2O$) C, H, N.

2.3. Pharmacology

Thoracic aorta were isolated from male Wistar rats (200–250 g) anesthetized with CO_2 and sacrificed by decapitation. All animals were dealt with in a humane way in accordance with recognized guidelines on animal experimentation. The vessels were helically cut, the endothelium removed and two strips were obtained from each aorta. The tissues were suspended under a tension of 1 g in organ baths containing 30 ml of Krebs-Heinselet solution of the following composition (mM): NaCl, 137; KCl, 2.7; $MgCl_2$, 0.5; $CaCl_2$, 1.4; NaH_2PO_4, 0.5; $NaHCO_3$, 8.9; glucose, 8.3; ascorbic acid, 0.1. Desmethylimipramine hydrochloride (1×10^{-7} M), deoxycorticosterone acetate (5×10^{-6} M), propranol (5×10^{-6} M) and yohimbine hydrochloride (1×10^{-7} M) were added to the solution to prevent neuronal and extraneuronal uptake of (−)noradrenaline and to block β- and α_2-adrenoceptors, respectively. The medium was maintained at 37°C and at pH 7.4 (by gassing with 95%O_2–5%CO_2). The aortic strips were allowed to equilibrate for 2 h before starting the experiments. (−)Noradrenaline induced contractions were determined cumulatively in the absence or presence of the antagonist, which was pre-incubated for 30 minutes. One of the two strips cut from each aorta served as a control while a dose–response curve in the presence of the antagonist was performed on the other strip.

3. Results and discussion

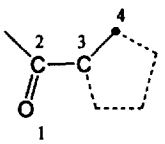
The compounds **3a–5a** and **3b–5b** were prepared according to the reaction sequence reported in Scheme 2. The appropriate carboxylic acid, synthesized according to literature methods, was treated with boiling thionyl chloride and the crude chloride obtained was conjugated with **6** in THF solution, in the presence of $KHCO_3$. The structures of the final products were confirmed by ^1H and ^{13}C NMR spectroscopy. The chemical shifts are reported in Tables 1 and 2.

In addition to spectral characterization, a systematic search for accessible (minimum energy) conformations of all the compounds was carried out according to the approaches reported in Refs. [5,17]. Initial geometries of the compounds **3a–5a**, **3b–5b** and **1** were built by the standard libraries of the Molecular Simulation BUILDER module. The conformations in the prazosin analogues are described by three torsion angles, as shown in Fig. 1. The torsion angle ω_a was found to be the same for all compounds ($20° \pm 2°$). The torsion angles ω_1 and ω_2 were systematically modified by rigid rotations in steps of 15°. At each step, energy was calculated by using the molecular mechanics methods. The conformations with energy greater than 500 kcal mol^{-1} were eliminated in order to reduce CPU times. The remaining conformers were optimized by using the molecular mechan-

Table 3
α^1-Adrenoceptor antagonistic activities and AM1 energies of the stable conformers of the compounds **3a,b**, **4a,b** and **5a,b**

Comp.	$pA_2 \pm 95\%CL$ [a]	Slope $\pm$ 95%CL [a]	Conformer	Angle ω_2 [b] (deg)	ΔE [c] (kcal mol^{-1})
3a	8.68 ± 0.16	1.07 ± 0.14	1	−117.6	0.0
3b	8.76 ± 0.12	1.00 ± 0.16	1	−117.8	0.0
4a	8.93 ± 0.17	0.98 ± 0.14	1	−141.1	0.0
			2	−46.0	1.8
4b	8.26 ± 0.17	1.01 ± 0.17	1	−140.0	0.0
			2	−47.3	1.8
5a	8.55 ± 0.17	0.99 ± 0.18	1	−104.8	0.0
			2	75.0	0.3
5b	8.20 ± 0.18	1.08 ± 0.16	1	−82.3	0.7
			2	57.7	0.0
1	9.81 ± 0.27	0.89 ± 0.15	1	−144.6	0.0

[a] CL = confidence limits.
[b] Torsion angle defined by the following atoms:



Atom 4 is represented by N in series **I** and **III** and by the C adjacent to >N−R in series **II**.
[c] ΔE is the energy difference between the conformer and the minimum.

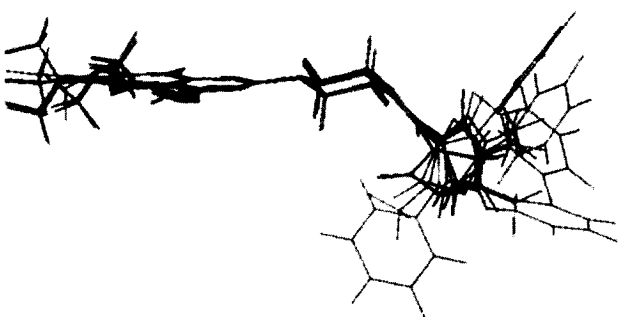


Fig. 3. Total view of the superimposed stable conformers. The superimposition was made using the common quinazolinylpiperazinocarbonyl moiety as the base.

ics methods and the identical geometries were discarded (r.m.s. < 0.3). Finally, the conformers were optimized again by using the AM1 semiempirical approximation. All calculations were performed in vacuo.

The following modules of the molecular modelling software produced by Molecular Simulations Inc. (San Diego, CA) were used: INSIGHT II 95.0, BUILDER 95.0, DISCOVER 95.0/3.00. The CFF91 force field [18] was used in the molecular mechanics calculations. The semiempirical calculations were performed by AM1 [19] Hamiltonian with SPARTAN software (Wavefunction Inc., Irvine, CA). All the software ran on a R4400 Silicon Graphics workstation. Fig. 2 shows the side view of the minimum energy conformations for the compounds **1**, **3a–5a** and **3b–5b**, chosen within 3 kcal mol^{-1} of their absolute minima. Models **1** and **3a,b** were able to adopt only the absolute minimum conformation, whereas each member of series **II** and **III** shows two minimum conformations ($\Delta E < 2$ kcal mol^{-1}) in which ω_2 differs by about 180° (Table 3). Fig. 3 shows the side view of the supermolecule obtained by superimposing the accessible conformations of all the compounds, prazosin included. The rigid superimposition of the 10 minimum conformations and prazosin was made using the common portion of the molecules, the quinazolinylpiperazinocarbonyl moiety, as the base.

The α_1-Adrenoceptor blocking activity of the products **3a–5a, 3b–5b** was assessed by antagonism of (−)noradrenaline induced contractions on rat aortic strips. It is known that α_1-adrenoceptor can exist in three subtypes designated α_{1A}, α_{1B} and α_{1D}, respectively. On rat aorta strips the α_{1D} subtype is principally present [20]. The antagonist potency of the compounds, expressed as pA_2, is reported in Table 3. α_2-Adrenoceptor blocking activities were tested by antagonism of clonidine induced depression of the twitch responses of a field stimulated prostatic portion of rat vas deferens. None of the compounds displayed any α_2-antagonist activity at 10^{-5} M concentration.

Analysis of Table 3 shows that all of the members of the series display lower affinity for α_1-receptor than prazosin. The compounds behave in a similar way, indeed their pA_2 values range between 8.20 (**5b**) and 8.93 (**4a**). A possible explanation for the close receptor affinities of methyl and phenyl analogues in each of the three series is that N-1 pyrazole substituents are projected into a freely accessible space, without giving relevant interactions. In this case, since conformational analysis shows that the pyrazole system of the compounds **4a** and **4b** occupies the same area fitted by the furan of prazosin (Fig. 3), we could infer that the different antagonism displayed by these substances with respect to the

lead is principally due to the different electronic and lipophilic properties of the two heterocyclic moieties. By way of contrast, in the derivatives of the series **I** and **III**, in which ω_2 angles are rather different from those of prazosin (Table 3), conformational effects could also be involved in the modulation of the antagonism.

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