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Synthesis and A_1 and A_{2A} adenosine binding activity of some pyrano[2,3-c]pyrazol-4-ones

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Abstract

A series of pyrano[2,3-c]pyrazol-4-ones was synthesized and evaluated for bovine brain adenosine A_1 and A_{2A} receptor binding affinity. Substituents at positions 5 and/or 6 were varied in order to define the structure–activity relationships in these new kinds of adenosine receptor ligands. The most selective and potent ligand among the reported compounds was the 1,4-dihydro-1-phenyl-3-methyl-6-(3-aminophenyl)-pyrano[2,3-c]pyrazol-4-one **11** which showed a 27-fold selectivity for A_1 receptor and a K_i value of 84 nM.

Keywords: Pyrano[2,3-c]pyrazol-4-ones; A_1 and A_{2A} binding activity; Adenosine receptor ligands

1. Introduction

Adenosine receptors (ARs) are widely distributed in the human body. Four distinct subtypes of ARs have been described, two 'high affinity' subtypes, A_1 and A_{2A}, which are stimulated by adenosine in nanomolar concentrations, and two 'low affinity' subtypes, A_{2B} and A_3, which require micromolar concentrations of adenosine for activation [1]. While A_1 and A_{2A} ARs appear to be constitutively activated, A_{2B} and A_3 receptors are only activated under certain extreme, i.e. pathological, conditions [1]. The four AR subtypes have been distinguished on the rank order of agonists and antagonists and on second messenger coupling as well as, on a molecular level, by cloning from various species, including humans [2].

Medicinal chemists are currently developing AR agonists and antagonists that selectively interact with AR subtypes [3]. AR agonists are derivatives of the physiological agonist adenosine. The ribose moiety of adenosine is essential for agonist activity. Only minor modifications at the ribose part are permitted to retain AR affinity and intrinsic activity [1]. All antagonists have been developed initially in the class of 8-substituted xanthine derivatives and later in various structural classes [1,3–6].

The use of agonists and antagonists acting at ARs gives rise to numerous side effects which are caused by the ubiquitous presence of ARs throughout the body. However, some A_1-selective antagonists are in an advanced state of clinical development for different indications, including dementia, hypertension and renal failure [7]. New AR ligands would be valuable tools for probing the structural requirements of the single family receptor subtype binding sites. To this aim some researches in our laboratory have been directed toward the synthesis of non-xanthine antagonists of the ARs containing a six–six–five tricyclic ring system [8–11]. This program has led to the discovery of some selective and structurally novel A_1 AR ligands, i.e. 1-(*meta*-phenyl-substituted)[1]benzopyrano[2,3-c]pyrazol-4-ones [11] **A** (see below). Structure–activity relationship (SAR) studies on series **A** compounds revealed that only small substituents could be introduced on the fused benzo moiety to retain A_1 activity and/or selectivity. Thus, to better understand the structural requirements for the anchoring of these new kinds of ligands to the AR recognition sites, we report here the synthesis and A_1 and A_{2A} binding activity of some pyrano[2,3-c]pyrazol-4-one derivatives (**1–5**)**a–c** and **6–13**. These newly reported compounds no longer bear the benzo fused moiety, but bear instead methyl and/or aryl substituents at positions 5 and/or 6 of the pyrano ring (see below).

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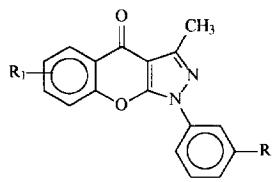
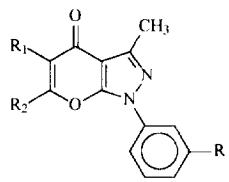


A

R = H, NO_2, NH_2

R_1 = 6-OCH_3, 6-OH, 6-Br, 7-OCH_3, 7-OH



(1-5)a-c, 6-13

R = H, NO_2, NH_2

R_1 = H, CH_3, Ph

R_2 = H, CH_3, aryl

2. Chemistry

The syntheses of compounds (**1–5**)**a–c** and **6–13** are illustrated in Schemes 1–3.

Scheme 1 shows the general method followed to obtain (**1–4**)**a–c** by reacting the 1-aryl-3-methyl-5-hydroxypyrazoles **14a,b** [12,13], with suitable α,β-unsaturated acyl chlorides in the presence of calcium hydroxide. The reaction between **14a,b** [12,13] and methacryloyl chloride could yield either the 4-methacryloylpyrazoles **15a,b** or the 1,4,5,6-tetrahydro-1-aryl-3,5-dimethylpyrano[2,3-c]pyrazol-4-ones **2a,b** [14], depending on the nature of the acid used to decompose the calcium complex. In fact, by using glacial acetic acid compounds **15a,b** could be isolated, while the use of 6N hydrochloric acid yielded the bicyclic derivatives **2a,b** [14]. The reaction of **14a,b** [12,13] with *trans*-cinnamoyl chloride followed by acidification with 6N hydrochloric acid yielded the 4-acylpyrazoles **16a,b**. Compounds **15a,b** and **16a,b** were reacted with bromine and then with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) to yield the final bicyclic derivatives **1a,b** and **3a,b** [15], respectively. Finally, the 1,4-dihydro-1-aryl-3,6-dimethyl-5-phenylpyrano[2,3-c]pyrazol-4-ones **4a,b** were directly obtained from **14a,b** [12,13] and *trans*-2-phenyl-2-butenoyl chloride [16] following the above-described procedure, without isolating the intermediate acylpyrazoles.

The 1-(3-nitrophenyl) derivatives **1b**, **2b**, **3b** and **4b** were catalytically reduced to the corresponding 1-(3-aminophenyl) derivatives **1c**, **2c**, **3c** and **4c**.

Scheme 2 shows the synthetic pathway followed to obtain the 1,4-dihydro-1-aryl-3,6-dimethylpyrano[2,3-c]pyrazol-4-ones **5a–c** [15]. The commercially available dehydroacetic acid was reacted with arylhydrazines to yield the arylhydrazones **17a,b** [17]. Compounds **17a,b**, by refluxing with glacial acetic acid, were rearranged to the 4-acylpyrazoles **18a,b** [15], which were easily cyclized to the 3,6-dimethylpyrano-pyrazoles **5a,b** [15]. Catalytic reduction of **5b** yielded the 1-(3-aminophenyl) derivative **5c**.

Finally, Scheme 3 displays the synthesis of 1,4-dihydro-1-phenyl-3-methyl-6-arylpyrano[2,3-c]pyrazol-4-ones **6–13**. Briefly, the reaction between 1-phenyl-3-methyl-4-acetyl-5-hydroxypyrazole [18] and aroyl chlorides afforded the 4-acylpyrazoles **19–22** which were cyclized to the 1-phenyl-3-methyl-6-arylpyrano[2,3-c]pyrazol-4-ones **6–9**. Catalytic reduction of **6–8** and demethylation of **9** afforded compounds **10–12** and **13**, respectively.

3. Biochemistry

Compounds (**1–5**)**a–c** and **6–13** were tested for their ability to displace [^{3}H]N^6-cyclohexyladenosine (CHA) on A_1 ARs in bovine cerebral cortical membranes and [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine (CGS 21680) on A_{2A} ARs in bovine striatal membranes. The A_1 and A_{2A} receptor affinities of the tested compounds, expressed as their K_i values, are listed in Table 1.

4. Results and discussion

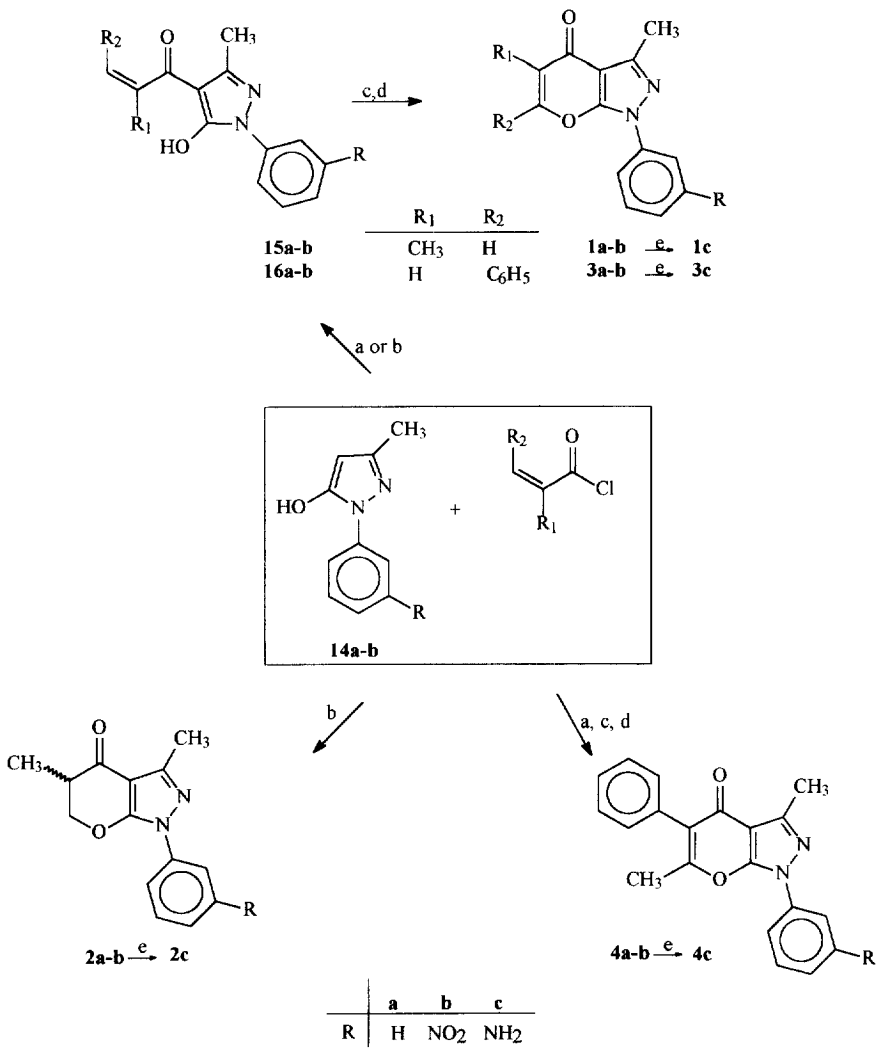
Table 1 shows that the elimination of the benzo-fused moiety of [1] benzopyrano-pyrazol-4-ones **A** [11] does not enhance AR affinity or A_1/A_{2A} subtype selectivity.

The presence in these new 1-aryl-3-methylpyrano-pyrazol-4-one derivatives of a 5-methyl substituent, as in compounds **1a–c** and in their dihydro derivatives **2a–c**, is highly deleterious for AR binding activity: only compound **1a** shows a very weak A_1 AR affinity.

Moving the methyl group from position 5 to position 6 (compounds **5a–c**) results in a moderate A_1 AR selectivity. The introduction in these 6-methyl derivatives **5a–c** of a 5-phenyl substituent yields compounds **4a–c**, which show a better A_1 AR activity than their corresponding 5-unsubstituted derivatives **5a–c**.

To evaluate the importance of a group other than the methyl in position 6, the 6-phenyl derivatives **3a–c** were prepared. While compounds **3a,b** are completely inactive in AR binding assays, compound **3c** displays a A_1/A_{2A} non-selective binding activity. The latter data suggest that the nature of the substituent on the 1-phenyl ring plays an important role in AR binding activity and that a 6-phenyl ring is tolerated in the pyrano-pyrazole moiety. Thus the 1-phenyl-6-(2-, 3-, and 4-nitrophenyl)-**6–8**, -6-(2-, 3-, and 4-aminophenyl)-**10–12**, -6-(4-methoxyphenyl)-**9**, and -6-(4-hydroxyphenyl)-pyrano-pyrazole **13** were synthesized, in order to probe the effect of an aryl substituent at position 6 in combination with the 1-phenyl group.

Among the 6-(nitrophenyl) derivatives **6–8** only the 6-(2-nitrophenyl)-**6** displays moderate AR activity and A_1 selectivity, while compounds **7** and **8** are inactive at both receptor subtypes. The 6-(2-, and 3-aminophenyl) derivatives **10** and **11**, respectively, show A_1 activity in the nanomolar range (83 and 84 nM, respectively), but also A_{2A} binding activity: only the 6-(3-aminophenyl)-**11** is 27-fold more selective at A_1



Scheme 1. a: (i) $Ca(OH)_2$, (ii) glacial CH_3COOH; b: (i) $Ca(OH)_2$, (ii) 6N HCl; c: Br_2; d: DBU; e: H_2, Pd/C.

AR than at the A_{2A} subtype. On the contrary the 6-(4-aminophenyl)- **12** is a moderately selective A_{2A} antagonist. Finally, the 6-(4-methoxyphenyl) **9** and 6-(4-hydroxyphenyl) **13** show some AR activity but little A_1/A_{2A} selectivity.

In conclusion, although the reported compounds are not very potent AR ligands, the present study has produced a 27-fold selective A_1 antagonist, i.e. compound **11**, which may serve as a tool to further define SAR in the anchoring of these new kinds of AR ligands to the A_1 and A_{2A} subtypes.

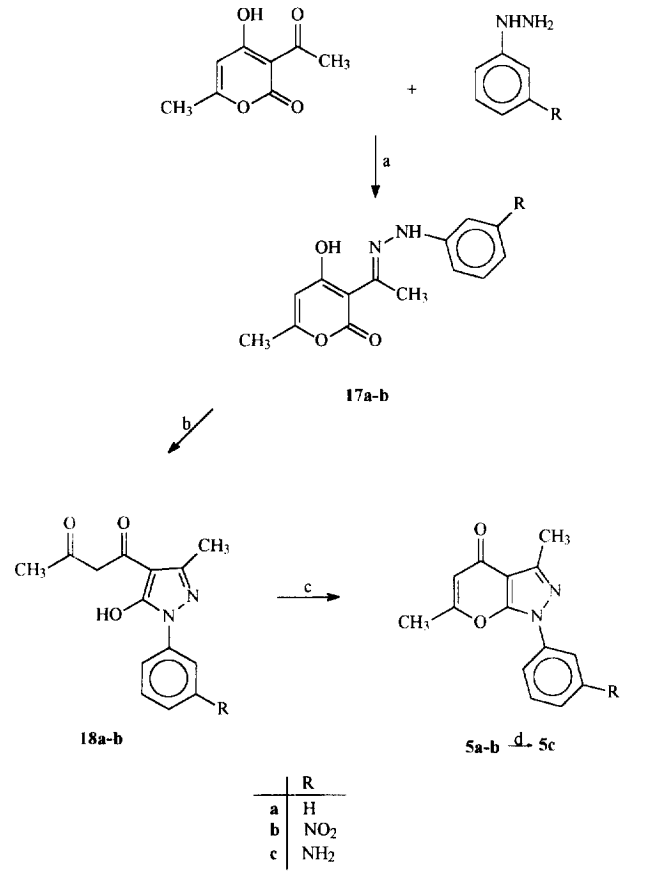
5. Experimental

5.1. Chemistry

Silica gel plates (Merck F_{254}) and silica gel 60 (Merck, 70–230 mesh) were used for thin layer and column chromatography, respectively. All melting points were determined on a Gallenkamp melting point apparatus. Microanalyses were performed with a Perkin-Elmer 260 elemental analyser for C, H, and N, and the results were within $\pm 0.4\%$ of the theoretical values. The IR spectra were recorded with a Perkin-Elmer 1420 spectrometer in nujol mulls and are expressed in cm^{-1}. The ^1H NMR spectra were obtained with a Varian Gemini 200 instrument at 200 MHz. The chemical shifts are reported in δ (ppm) and are relative to the central peak of the solvent. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, br = broad, and ar = aromatic protons. Physical data for the newly synthesized compounds are listed in Tables 2 and 3.

5.1.1. 1-Aryl-3-methyl-4-methacryloyl-5-hydroxypyrazoles **15a,b** and 1,4,5,6-tetrahydro-1-aryl-3,5-dimethylpyrano[2,3-c]pyrazol-4-ones **2a,b** [14]

A solution of methacryloyl chloride (9.13 mmol) in anhydrous dioxane (6 ml) was added dropwise to a mixture of **14a,b** [12,13] (9.13 mmol) and anhydrous calcium hydroxide (18.26 mmol) in anhydrous dioxane (40 ml). The mixture was refluxed for 3–4 h and then cooled.



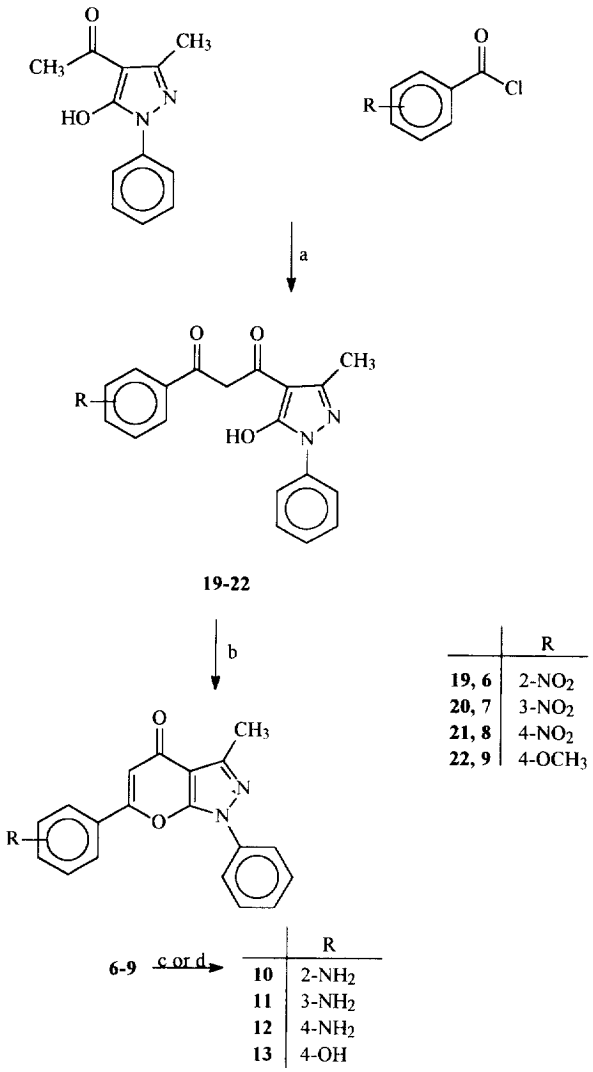
Scheme 2. a: EtOH; b: glacial CH_3COOH; c: conc. H_2SO_4/glacial CH_3COOH; d: H_2, Pd/C.

(a) The acylpyrazoles **15a,b** were obtained as follows. The solid was eliminated and the clear solution diluted with water (60 ml), acidified with glacial acetic acid and extracted with chloroform (3×50 ml). The organic layers were washed with water (4×50 ml), dried (anhydrous sodium sulfate) and evaporated at reduced pressure to yield an oily residue which became solid after treatment with ethanol. The solid residue was crystallized.

Compound **15a** displayed the following ^{1}H NMR (DMSO-d_6): 1.95 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 5.08 (s, 1H, =CH), 5.22 (s, 1H, =CH), 7.03 (t, 1H, ar, J=7.25 Hz), 7.29 (t, 2H, ar, J=7.70 Hz), 8.06 (d, 2H, J=8.47 Hz).

(b) The bicyclic pyrano-pyrazoles **2a,b** [14] were obtained as follows. The ice cooled mixture was acidified with 6N hydrochloric acid and then allowed to stand for 24 h. The solid was collected, washed with water and crystallized.

Compound **2b** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 1.26 (d, 3H, 5-CH_3, J=7.16 Hz), 2.49 (s, 3H, CH_3), 2.81 (m, 1H, 5-H), 4.46 (t, 1H, 6-H, J=11.06 Hz), 4.81 (dd, 1H, 6-H, J=11.06, 5.02 Hz), 7.63 (t, 1H, ar, J=8.20 Hz), 8.11–8.21 (m, 2H, ar), 8.69 (t, 1H, ar, J=2.15 Hz). IR: 1680, 1530, 1460.



Scheme 3. a: (i) $[(CH_3)_3Si]_2NLi$, (ii) 5% HCl; b: conc. H_2SO_4/glacial CH_3COOH; c: H_2, Pd/C; d: BBr_3.

5.1.2. 1-Aryl-3-methyl-4-trans-cinnamoyl-5-hydroxy-pyrazoles ***16a,b***

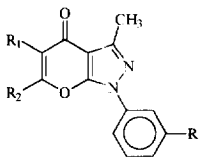
A solution of *trans*-cinnamoyl chloride (8.09 mmol) in anhydrous dioxane (4 ml) was added dropwise to a mixture of **14a,b** [12,13] (8.09 mmol) and anhydrous calcium hydroxide (16.18 mmol) in anhydrous dioxane (30 ml). The mixture was refluxed for 1 h 30 min, ice cooled, acidified with hydrochloric acid (6N, 60 ml) and then stirred for 1 h. The resulting solid was collected, washed with water and crystallized.

Compound **16a** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.60 (s, 3H, CH_3), 7.17 (d, 1H, =CH, J=15.54 Hz), 7.25–7.99 (m, 11H, ar+=CH). IR: 3500–3100, 1630.

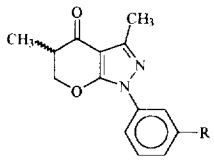
5.1.3. 1,4-Dihydro-1-aryl-3,5-dimethylpyrano[2,3-c]-pyrazol-4-ones ***1a,b*** *and 1,4-dihydro-1-aryl-3-methyl-6-phenylpyrano[2,3-c]pyrazol-4-ones* ***3a,b*** *[15]*

A solution of bromine (3.33 mmol) in glacial acetic acid (15 ml) was slowly (1 h) added to a warm (60°C), stirred

Table 1
A_1 and A_{2A} binding activity



1a-c, (3-5)a-c, 6-13



2a-c

Compound	R	R_1	R_2	$K_i \pm$ SEM (μM) [a]	
				A_1 [b]	A_{2A} [c]
1a	H	CH_3	H	5.3 ± 0.30	46% ± 3.50 [d]
1b	NO_2	CH_3	H	18% ± 1.60	18% ± 1.60
1c	NH_2	CH_3	H	51% ± 3.30	51% ± 3.30
2a	H			57% ± 3.50	40% ± 2.90
2b	NO_2			36% ± 2.10	17% ± 1.30
2c	NH_2			21% ± 1.80	47% ± 3.60
3a	H	H	C_6H_5	51% ± 3.30	42% ± 3.80
3b	NO_2	H	C_6H_5	44% ± 3.40	28% ± 1.90
3c	NH_2	H	C_6H_5	1.37 ± 0.10	1.82 ± 0.13
4a	H	C_6H_5	CH_3	1.40 ± 0.11	65% ± 5.80
4b	NO_2	C_6H_5	CH_3	2.60 ± 0.18	18% ± 1.60
4c	NH_2	C_6H_5	CH_3	0.72 ± 0.04	47% ± 3.60
5a	H	H	CH_3	2.96 ± 0.20	45% ± 3.40
5b	NO_2	H	CH_3	8.70 ± 0.60	24% ± 1.90
5c	NH_2	H	CH_3	1.07 ± 0.08	46% ± 3.50
6	H	H	2-$NO_2C_6H_4$	1.07 ± 0.08	43% ± 3.20
7	H	H	3-$NO_2C_6H_4$	64% ± 5.80	0%
8	H	H	4-$NO_2C_6H_4$	14% ± 1.10	3% ± 0.20
9	H	H	4-$OCH_3C_6H_4$	0.88 ± 0.06	2.5 ± 0.2
10	H	H	2-$NH_2C_6H_4$	0.083 ± 0.007	0.19 ± 0.014
11	H	H	3-$NH_2C_6H_4$	0.084 ± 0.007	2.28 ± 0.20
12	H	H	4-$NH_2C_6H_4$	56% ± 5.10	2.57 ± 0.21
13	H	H	4-OHC_6H_4	0.28 ± 0.022	1.03 ± 0.08

[a] The K_i values are means ± SEM of four separate assays, each performed in triplicate.
[b] A_1 binding was measured as displacement of [^{3}H]CHA binding in bovine brain cortical membranes.
[c] A_{2A} binding was measured as displacement of [^{3}H]CGS 21680 binding in bovine striatal membranes.
[d] Percentage of inhibition (*I*%) of specific radioligand binding at 20 μM concentration, each performed in triplicate.

solution of **15a,b** or **16a,b** (3.33 mmol) in glacial acetic acid (35 ml). The mixture was stirred at 60°C for 1 h 30 min, cooled and then neutralized with a saturated solution of sodium hydrogen carbonate to yield a mixture of 5,6-dihydrobromo derivatives of **1a,b** or **3a,b**, respectively.

(a) The mixture of dihydrobromo derivatives of **1a,b** was collected, washed with water and crystallized from ethanol. This purified solid (1.98 mmol) was dissolved in anhydrous dioxane (20 ml) and DBU (2.18 mmol) added.

(b) The mixture of dihydrobromo derivatives of **3a,b** was collected, washed with water and purified by column chromatography (eluting system cyclohexane/ethyl acetate, 1:1). Evaporation at reduced pressure of the first eluates yielded a residue. This purified residue (1.65 mmol) was dissolved in anhydrous dioxane (30 ml) and DBU (1.81 mmol) added.

Table 2
Physical data of intermediates (**15–18**)**a,b**, **19–22**

Compound	R	R_1	R_2	M.p. (°C)	Solvent [a]	Yield (%)
15a	H	CH_3	H	180–182	A	35
15b	NO_2	CH_3	H	> 300	A	20
16a	H	H	C_6H_5	165–167	A	85
16b	NO_2	H	C_6H_5	247–248	A	40
17a [b]	H			206–207	A	90
17b	NO_2			196–198	A	50
18a [c]	H			96–98	B	95
18b	NO_2			176–177	C	90
19	2-NO_2			171–172	A	95
20	3-NO_2			238–240	D	65
21	4-NO_2			224–225	C	85
22	4-OCH_3			177–178	A	75

[a] Crystallization solvents: A = ethanol, B = acetonitrile, C = glacial acetic acid, D = tetrahydrofuran.
[b] Ref. [17]: m.p. 207°C (ethanol).
[c] Ref. [15]: m.p. 101°C (acetonitrile).

The mixture of dihydrobromo derivatives and DBU was heated at 90°C for 5–6 h, stirred at room temperature for 12 h, diluted with water (50 ml) and then stirred for 2 h more. The solid was collected and crystallized.

Compound **1a** displayed the following ^{1}H NMR ($CDCl_3$): 2.02 (s, 3H, 5-CH_3), 2.65 (s, 3H, CH_3), 7.26–7.56 (m, 4H, ar + 6-H), 7.75–7.81 (d, 2H, ar, J = 8.22 Hz).

Compound **3a** [15] displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.67 (s, 3H, CH_3), 6.71 (s, 1H, 5-H), 7.37–7.88 (m, 10H, ar). IR: 1660.

5.1.4. 1,4-Dihydro-1-aryl-3,6-dimethyl-5-phenyl-pyrano[2,3-c]pyrazol-4-ones **4a,b**

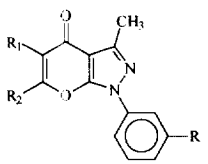
A solution of *trans*-2-phenyl-2-butenoyl chloride [16] (12.33 mmol) in anhydrous dioxane (20 ml) was added dropwise to a mixture of **14a,b** [12,13] (12.33 mmol) and calcium hydroxide (24.66 mmol) in anhydrous dioxane (20 ml). The mixture was refluxed for 2 h, cooled, acidified with glacial acetic acid and filtered. The solution was extracted with chloroform (3 × 30 ml). The organic layers were dried (anhydrous sodium sulfate) and evaporated at reduced pressure to yield an oily residue (acylpyrazole).

The acetic solution of the intermediate acylpyrazole was heated at 60°C and a solution of bromine (6.5 mmol) in glacial acetic acid was slowly (1 h) added. The solution was stirred at 60°C for 1 h 30 min more, cooled and neutralized with a saturated solution of sodium hydrogen carbonate.

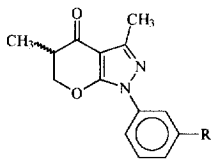
The resulting solid (2 mmol), constituted of a mixture of 5,6-dihydrobromo derivatives, was collected, washed with water, dissolved in dioxane (15 ml) and DBU (3 ml) added. The mixture was heated at 100°C for 2 h, cooled and diluted with water (10 ml) to afford a solid residue which was collected and crystallized.

Compound **4a** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.33 (s, 3H, 6-CH_3), 2.63 (s, 3H, CH_3),

Table 3
Physical data of pyrano[2,3-c]pyrazol-4-ones (**1–5**)**a–c** and **6–13**



1a–c, (3-5)a–c



2a–c

Compound	R	R_1	R_2	M.p. (°C)	Solvent [a]	Yield (%)
1a	H	CH_3	H	166–167	A	25
1b	NO_2	CH_3	H	235–237	A	20
1c	NH_2	CH_3	H	169–170	B	30
2a [b]	H			114–116	A	20
2b	NO_2			156–158	A	25
2c	NH_2			167–168	A	45
3a [c]	H	H	C_6H_5	209–210	A	20
3b	NO_2	H	C_6H_5	215–216	A	50
3c	NH_2	H	C_6H_5	204–205	A	20
4a	H	C_6H_5	CH_3	121–122	A	20
4b	NO_2	C_6H_5	CH_3	203–204	A	35
4c	NH_2	C_6H_5	CH_3	192–193	A	30
5a [d]	H	H	CH_3	150–151	C	85
5b	NO_2	H	CH_3	219–220	A	80
5c	NH_2	H	CH_3	167–168	D	95
6	H	H	2-$NO_2C_6H_4$	180–181	A	75
7	H	H	3-$NO_2C_6H_4$	243–244	A	70
8	H	H	4-$NO_2C_6H_4$	290–291	E	90
9	H	H	4-$OCH_3C_6H_4$	180–181	A	75
10	H	H	2-$NH_2C_6H_4$	193–194	A	65
11	H	H	3-$NH_2C_6H_4$	241–243	C	65
12	H	H	4-$NH_2C_6H_4$	286–287	A	85
13	H	H	4-OHC_6H_4	>300	E	60

[a] Crystallization solvents: A = ethanol, B = ethanol/water, C = ethyl acetate, D = cyclohexane/ethyl acetate, E = glacial acetic acid.
[b] Ref. [14]: m.p. 110–111°C (hexane).
[c] Ref. [15]: m.p. 209–210°C (acetonitrile).
[d] Ref. [15]: m.p. 150°C (acetonitrile).

7.27–7.57 (m, 8H, ar), 7.84 (d, 2H, ar, J = 7.97 Hz). IR: 1670.

*5.1.5. General procedure for the reduction of 1-(3-nitrophenyl) derivatives **1b**, **2b**, **3b**, and **4b** to the 1-(3-aminophenyl) derivatives **1c**, **2c**, **3c**, and **4c***

20% wt./wt. Pd/C (10%) was added to a solution of nitro derivative (**1–4**)**b** (0.70 mmol) in ethyl acetate (200 ml). The mixture was hydrogenated in a Parr apparatus at 40 psi for 12 h. Elimination of the catalyst and evaporation at reduced pressure of the solvent yielded a residue which in the case of **1c**, **2c**, and **4c** was directly crystallized. In the case of **3c**, the residue, before crystallization, was purified by column chromatography (eluting system cyclohexane/ethyl acetate, 1:1).

Compound **4c** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.32 (s, 3H, 6-CH_3), 2.61 (s, 3H, CH_3), 3.87 (s, 2H, NH_2), 6.64–6.85 (m, 1H, ar), 7.21–7.44 (m, 8H, ar). IR: 3450, 3350, 3240, 1650.

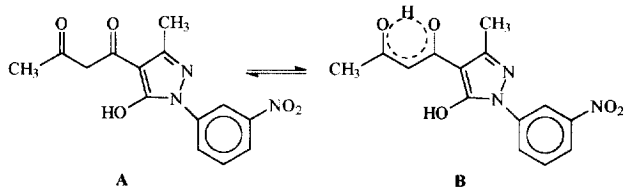
*5.1.6. Arylhydrazones of 3-acetyl-4-hydroxy-6-methyl-2H-pyran-2-one **17a,b** [17]*

Phenylhydrazine or 3-nitrophenylhydrazine hydrochloride (17.8 mmol) and an equimolar amount of triethylamine were added to a hot (80°C) mixture of dehydroacetic acid (17.8 mmol) in ethanol (20 ml). After a few minutes of heating a coloured solid mass was obtained which was collected and crystallized.

Compound **17b** displayed the following ^{1}H NMR (DMSO-d_6): 2.21 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 6.06 (s, 1H, 5-H), 7.30–7.75 (m, 4H, ar), 9.72 (s, 1H, OH or NH).

*5.1.7. 1-Aryl-3-methyl-4-[1-(3-oxobutanoyl)]-5-hydroxypyrazoles **18a,b** [15]*

A mixture of **17a,b** (10 mmol) in glacial acetic acid (20 ml) was refluxed for 1 h. Evaporation of the solvent at reduced pressure yielded a residue which was collected and crystallized. ^{1}H NMR analysis indicated that in solution (DMSO-d_6) compound **18b** was a mixture of the tautomeric keto (A) and enol (B) forms in the ratio of approximately 1:1: (A) 2.19 (s, 4-CH_3), 2.52 (s, pyrazole CH_3), 3.96 (s, CH_2); (B) 2.06 (s, 4-CH_3), 2.45 (s, pyrazole CH_3), 6.65 (br s, =CH + OH); moreover, 7.77 (t, 1H, H-5′, J = 8.06 Hz), 8.10 (d, 1H, H-6′, J = 8.06 Hz), 8.22 (d, 1H, H-4′, J = 8.06 Hz), 8.71 (s, 1H, H-2′).



A B

*5.1.8. 1,4-Dihydro-1-aryl-3,6-dimethylpyrano-[2,3-c]pyrazol-4-ones **5a,b** [15]*

Concentrated sulfuric acid (0.4 ml) was added dropwise to a mixture of **18a,b** (4 mmol) in glacial acetic acid (15 ml). The mixture was refluxed for 1 h, cooled at room temperature and then poured into cold water (50 ml). The resulting solid was collected, washed with water and crystallized.

Compound **5b** displayed the following spectral data: ^{1}H NMR (DMSO-d_6): 2.44 (s, 3H, 6-CH_3), 2.51 (s, 3H, CH_3), 6.19 (s, 1H, 5-H), 7.90 (t, 1H, ar, J = 8.42 Hz), 8.23–8.35 (m, 2H, ar), 8.59–8.61 (m, 1H, ar). IR: 1675, 1540, 1360.

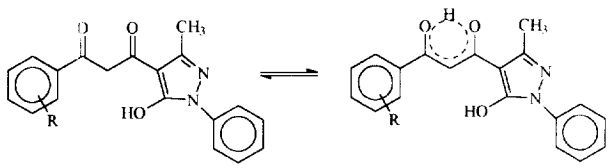
*5.1.9. 1,4-Dihydro-1-(3-aminophenyl)-3,6-dimethylpyrano-[2,3-c]pyrazol-4-one **5c***

The title compound was obtained by catalytic reduction of a solution of **5b** (2.30 mmol) in glacial acetic acid (100 ml) in a Parr apparatus at 15 psi for 3 h following the general procedure described to prepare **1c**, **2c**, **3c**, and **4c**. The oily

residue, which was yielded by the evaporation of the solvent at reduced pressure, became solid upon treatment with ethyl acetate. ^{1}H NMR ($CDCl_3$): 2.39 (s, 3H, 6-CH_3), 2.61 (s, 3H, CH_3), 3.86 (br s, 2H, NH_2), 6.03 (s, 1H, 5-H), 6.64–6.69 (m, 1H, ar), 7.10–7.29 (m, 3H, ar). IR: 3480, 3360, 3240, 1670.

5.1.10. 1-Phenyl-3-methyl-4-[1-(3-aryl-3-oxopropanoyl)]-5-hydroxypyrazoles ***19–22***

A solution of lithium bis(trimethylsilyl)amide in anhydrous tetrahydrofuran (THF) (1 M, 9.2 mmol) was added dropwise to a well-stirred mixture of 1-phenyl-3-methyl-4-acetyl-5-hydroxypyrazole [18] (4.6 mmol) in anhydrous THF (25 ml) under nitrogen at −78°C. The reaction was allowed to proceed for 2 h, and then a solution of aroyl chloride (4.6 mmol) in anhydrous THF (3 ml) was added dropwise. Stirring was continued at −78°C for 1 h and then at room temperature for 22 h. Treatment with hydrochloric acid (5%, 80 ml) yielded a solid which was collected, washed with water and crystallized. ^{1}H NMR analysis indicated that in solution (DMSO-d_6) the nitrophenyl derivatives **19–21** existed predominantly in the tautomeric enol form (B) while the 4-methoxyphenyl derivative **22** was a mixture of the tautomeric keto (A) and enol (B) forms in a ratio of approximately 1:2. In fact, compound **20** displayed the following ^{1}H NMR (DMSO-d_6): 2.56 (s, 3H, CH_3), 7.27 (t, 1H, ar, J=1.39 Hz), 7.49 (t, 2H, ar, J=7.41 Hz), 7.61 (s, 1H, =CH), 7.76–7.88 (m, 3H ar), 8.27 (d, 1H, ar, J=9.12 Hz), 8.39 (d, 1H, ar, J=1.73 Hz), 8.56 (s, 1H, ar), while compound **22** displays the following ^{1}H NMR (DMSO-d_6): (A) 2.55 (s, CH_3), 4.53 (s, CH_2); (B) 2.45 (s, CH_3), 7.40 (s, =CH), moreover, 3.84 (s, OCH_3), 7.07–7.87 (m, ar).



5.1.11. 1,4-Dihydro-1-phenyl-3-methyl-6-arylpyrano-[2,3-c]pyrazol-4-ones ***6–9***

Concentrated sulfuric acid (1.3 ml) was added to a mixture of **19–22** (3.5 mmol) in glacial acetic acid (5 ml). The mixture was refluxed for 6–10 h (TLC monitoring), cooled and diluted with ice–water (50 ml). The resulting solid was collected, washed with water and crystallized.

Compound **6** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.67 (s, 3H, CH_3), 6.50 (s, 1H, 5-H), 7.26–7.78 (m, 8H, ar), 8.07 (d, 1H, ar, J=6.76 Hz). IR: 3060, 1670.

5.1.12. 1,4-Dihydro-1-phenyl-3-methyl-6-aminophenyl-pyrano[2,3-c]pyrazol-4-ones ***10–12***

The title compounds were obtained by catalytic reduction of a solution of **6–8** (0.72 mmol) in ethyl acetate, cold glacial acetic acid or hot glacial acetic acid, respectively, in a Parr apparatus at 45 psi for 12 h following the procedures described to prepare **1–5c**. The oily residue, which was yielded by the evaporation of the solvent at reduced pressure, became solid upon treatment with diethyl ether.

Compound **10** displayed the following spectral data: ^{1}H NMR ($CDCl_3$): 2.67 (s, 3H, CH_3), 4.20 (br s, 2H, NH_2), 6.55 (s, 1H, 5-H), 6.77–6.86 (m, 2H, ar), 7.26–7.55 (m, 5H, ar), 7.84 (d, 2H, ar, J=9.00 Hz). IR: 3410, 3340, 3220, 1640.

5.1.13. 1,4-Dihydro-1-phenyl-3-methyl-6-(4-hydroxy-phenyl)pyrano[2,3-c]pyrazol-4-one ***13***

A solution of boron tribromide in methylene chloride (1 M, 1.94 ml) was added dropwise to a stirred solution of **9** (0.96 mmol) in anhydrous methylene chloride (7 ml) under nitrogen at 0°C. The reaction was allowed to proceed for 2 h and then at room temperature for 20 h. Dilution with water (20 ml) yielded a solid which was collected, washed several times with water and crystallized. ^{1}H NMR (DMSO-d_6): 2.52 (s, 3H, CH_3), 6.75 (s, 1H, 5-H), 6.96 (d, 2H, ar, J=8.50 Hz), 7.47 (t, 1H, ar, J=7.53 Hz), 7.65 (t, 2H, ar, J=7.87 Hz), 7.82–7.89 (m, 4H, ar), 10.28 (br s, 1H, OH). IR: 3500–3000, 1640.

5.2. Biochemistry

5.2.1. A_1 receptor binding

Bovine cerebral cortex was homogenized in ice-cold 0.32 M sucrose containing protease inhibitors (20 μg/ml soybean trypsin inhibitor, 200 μg/ml bacitracin, and 160 μg/ml benzamidine) in an Ultraturrax homogenizer. The homogenate was centrifuged at 1000 g for 10 min at 4°C and the supernatant again centrifuged at 48 000 g for 15 min at 4°C. The resulting pellet was suspended in 10 volumes of ice-cold 40 mM Tris–HCl buffer at pH 7.7 containing 2 mM $MgCl_2$ and protease inhibitors (buffer T_1). Then it was homogenized and centrifuged at 48 000 g for 15 min at 4°C.

The pellet was dispersed in 40 volumes of fresh T_1 buffer, incubated with adenosine deaminase (1 IU/ml) at 37°C for 60 min, and then recentrifuged at 48 000 g for 15 min at 4°C. The resulting pellet was frozen at −80°C until the time of assay.

The pellet was suspended in ice-cold T_1 buffer, and the A_1 binding assay was performed in triplicate by incubating at 25°C for 45 min in 0.5 ml of T_1 buffer containing 1.3 nM [^{3}H]CHA in the absence or presence of unlabelled 10 μM (*R*)-phenylisopropyladenosine. The binding reaction was terminated by filtering through Whatman GF/B glass fibre filters under reduced pressure and washing twice with 5 ml of ice-cold Tris buffer. The filters were placed in scintillation vials, and 4 ml of Beckman Ready-Protein solvent scintillation fluid was added. The radioactivity was counted with an LS 1800 scintillation counter. Specific binding was obtained by subtracting non-specific binding from total binding and approximated to 85–90% of the total binding.

5.2.2. A_{2A} receptor binding

Corpora striata were dissected from bovine brain, and the tissue was homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer at pH 7.5 containing protease inhibitors as reported above and 10 mM $MgCl_2$ (buffer T_2). The homogenate was centrifuged at 48 000 g for 10 min at 4°C, the pellet then being suspended in 20 volumes of Tris buffer (T_2) containing adenosine deaminase (1 IU/ml) and incubated for 30 min at 37°C. The resulting pellet was diluted in 20 volumes of 50 mM Tris–HCl buffer at pH 7.5 containing 10 mM $MgCl_2$ and used in the binding assay. The A_{2A} binding assay was performed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) in Tris–HCl buffer at pH 7.5, with approximately 4 nM [^{3}H]CGS 21680 in a final volume of 0.5 ml. Incubation was carried out at 25°C for 90 min. Non-specific binding was defined in the presence of 10 μM CGS 21680. The binding reaction was concluded by filtration through Whatman GF/C glass fibre filters under reduced pressure. Filters were washed four times with 5 ml aliquots of ice-cold buffer and placed in scintillation vials. Specific binding was obtained by subtracting non-specific binding from total binding and approximated to 85–90% of the total binding. The receptor-bound radioactivity was measured as described above.

Compounds were dissolved in DMSO (buffer/concentration of 2%) and added to the assay mixture. Blank experiments were carried out to determine the effect of solvent on binding. Protein estimation was based on a reported method [19], after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as standard.

The concentration of tested compound that produced 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) was determined by log-probit analysis with seven concentrations of displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation [20] $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the radioligand concentration and K_d is its dissociation constant. K_d of [^{3}H]CHA binding to cortex membranes was 1.6 nM, and the K_d of [^{3}H]CGS 21680 binding to striatal membranes was 15 nM [8].

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