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Short communication

Adenosine receptors: synthesis, structure–activity relationships and biological activity of new 6-amino purine derivatives

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Abstract – The synthesis and evaluation of the biological activity of a series of pyridazin-3(2H)-one derivatives is reported. The compounds were tested in radioligand binding assays for affinity at A_1 and A_{2A} adenosine receptors in bovine brain cortical membranes, and bovine brain striatal membranes, respectively. None of the compounds shows any affinity towards A_{2A} receptor, while compounds in which the 6-chloro-pyridazin-3(2H)-one or 6-phenyl-pyridazin-3(2H)-one group is linked through a chain of two carbon atoms in the 6 position of the adenosine, show a good affinity towards A_1 adenosine receptor, particularly compound **8** in which a phenyl-pyridazinone group is present shows highest affinity with K_i values 6.6 nM. © Elsevier, Paris

A_1-adenosine affinity / pyridazinones / anticonvulsivant activity

1. Introduction

Adenosine is a neuromodulator which acting through specific cell-surface receptors, regulates several physiological responses, including vasodilatation [1], antilipolytic effect, inhibition of platelet aggregation [2], inhibition of lymphocyte functions, depression of central nervous system activity [3–5]. Moreover the adenosine has inhibitory effects on neurotransmission and on spontaneous activity of central neurons [6].

Potent and selective agonists have been developed for these receptors, of which at least three subclasses are known (A_1, A_2 and A_3) [7]. These receptor types have been cloned and characterized as belonging to the superfamily of receptors with seven transmembrane helices that couple to G proteins [8]. A_1 receptors may couple to a variety of second messenger systems, including inhibition of adenylate cyclase, inhibition or stimulation of phosphoinositol turnover, and activation of ion channels [9]. A_2 receptors stimulate adenylate cyclase and can be further subdivided into high-affinity A_{2A} and low-affinity A_{2B} subtypes. It is well known that N^6-substituted adenosine derivatives such as (R)N^6-[2-(1-phenyl)-propyl]adenosine **1** [10] have generally proved to be A_1-receptor selective.

On the other hand, 3(2H)-pyridazinone derivatives show many pharmacological activities: reduction of blood pressure like compound **1a** [11], anticonvulsivant activity like compound **1b** [12] (see *figure 1*). Moreover there are pyridazinone derivatives that have shown an interesting affinity towards A_1-receptor [13].

We believed that combining purine riboside with the pyridazinone ring through a chain of two or three carbon atoms or through a piperazine ring it would be possible to obtain new compounds with interesting affinity towards A_1-receptors, utilizable as cerebroprotective agents. In order to confirm the importance of the riboside in these 6-amino purine derivatives, the synthesis of a second series of compounds without the riboside fragment has been realized.

2. Chemistry

The compounds here reported were prepared as shown in *figures 2* and *3*. The reaction of the 6-chloropurine riboside **2** or of the 6-chloropurine **2a** with the amino derivatives in absolute ethanol in triethylamine, gave compounds **3–12** and **13–17** respectively.

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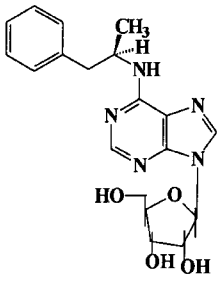

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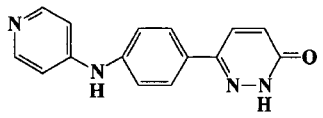

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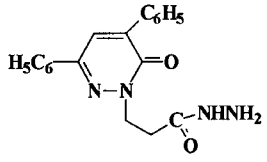

1b

Figure 1.

3. Biochemistry

Affinity of the new adenosine derivatives **3–17** towards adenosine receptors was evaluated using radioligand binding technique. Competition assays for A_1 and A_{2A} adenosine receptors were determined in bovine brain cortical membranes, and bovine striatal membranes, respectively.

N^6-(cyclohexyl)-adenosine ([^{3}H]CHA), was used as A_1 ligand, and 2-[[p-(2-carboxyethyl)-phenylethyl]-amino]-5'-(N-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) as A_{2A} ligand. The biological results expressed in K_i are reported in *table I*. Thermodynamic analysis of the temperature-dependent affinities of agonist and antagonist have revealed fundamental differences in the interaction of agonist and antagonist with receptors. The temperature dependence of agonist and antagonist interactions varies at several neurotransmitters. At dopamine and β-adrenergic receptors agonists become more potent at lower temperatures [14, 15].

In initial studies of adenosine receptors binding was found that [^{3}H]CHA binding was enhanced at higher temperatures, whereas [^{3}H]DPX binding was favoured by lower temperature [16]. We have used two-temperature measurements of in vitro inhibitory binding constant as a simple method of discriminating between in vivo agonistic and antagonistic behaviour of A_1 adenosine receptor ligand [17], agonists and antagonists being characterized by K_i(0 °C)/K_i (25 °C) ratios respectively greater or less than unity. Our finding suggest that at adenosine receptors agonists are more potent at higher temperatures and antagonists at lower temperatures. To explore this phenomenon in greater detail, we evaluated the potencies of a series of adenosine agonists and antagonists in inhibiting [^{3}H]CHA binding at 0 °C and 25 °C (*table I*). All tested compounds showed an agonist behaviour on A_1 receptors. The Hill coefficients, nH are reported for the temperature of 25 °C and are not significantly different from unity for either class of compounds suggesting the presence, at least in our experimental conditions, of a single class of binding sites.

4. Results and discussion

Compounds **7**, **8**, **9**, **10** in which the adenosine is linked in 6-position with the pyridazinone fragment through a chain of two carbon atoms, show high affinity towards A_1 adenosine receptor, particularly compound **8** in which a phenyl-pyridazinone group is present shows highest affinity with a K_i values 6.6 nM. Similar result has been obtained with the replacement of phenyl group with a chlorine atom (compound **7**), while replacement of phenyl group with a hydrogen atom (compound **9**) or with a imidazole group (compound **10**) gives a reduction of the affinity. These results show that there is a decrease of the affinity with the lengthening of the chain from two to three carbon atoms (compounds **11** and **12**), the affinity lowers also when the pyridazinone ring was substituted with a phthalazinone ring (compound **6**). Moreover in compounds **3**, **4**, **5** in which the ethylenic or propylenic chain is substituted by a rigid structure such as a piperazine ring, the affinity towards A_1 receptor disappears. Compounds **13–17**, adenine derivatives, do not show affinity towards A_1 receptor. Finally for all compounds the affinity at A_{2A} receptor was also determined and none of the compounds showed any affinity.

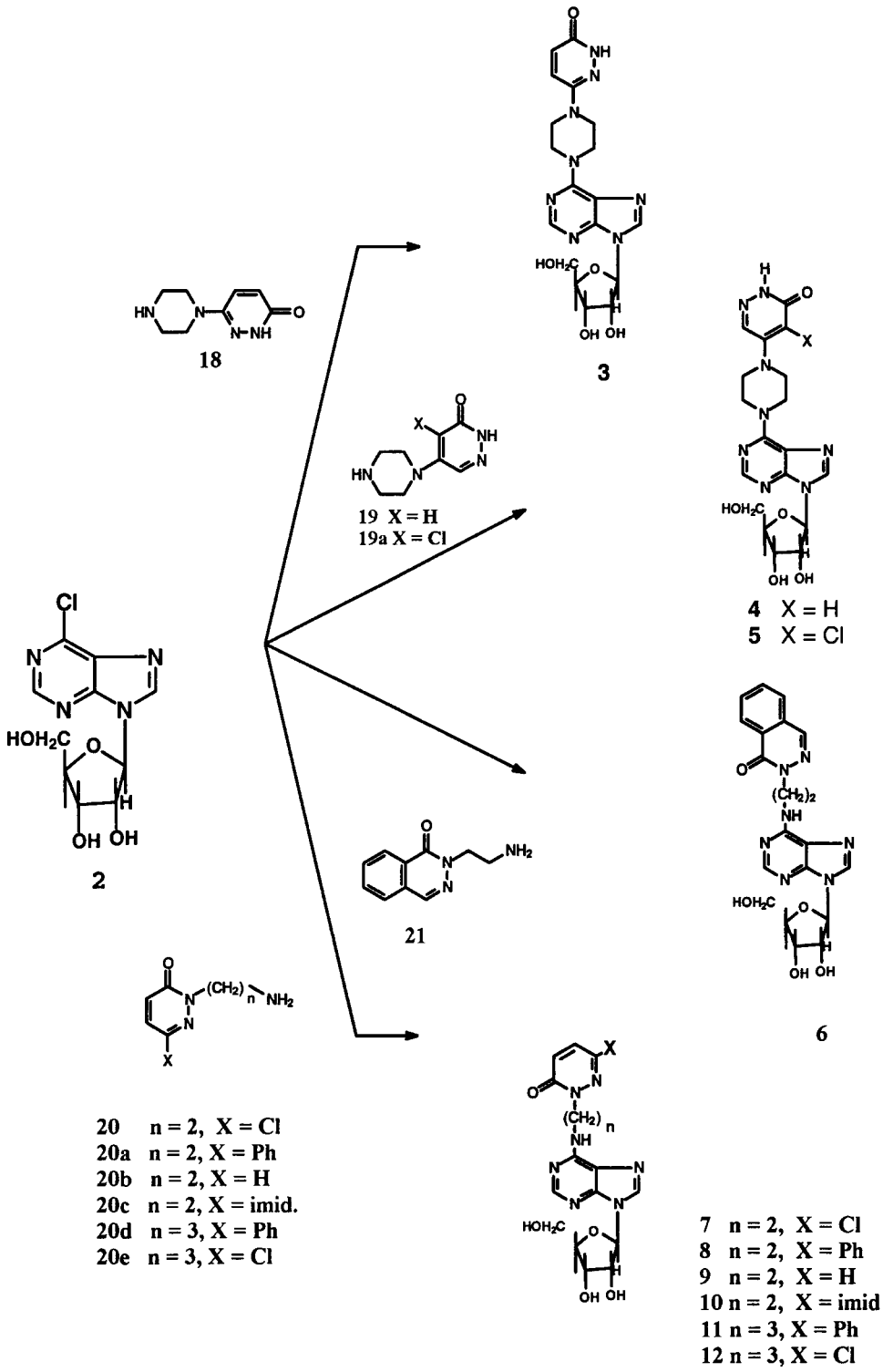
18
3
19 X = H
19a X = Cl
4 X = H
5 X = Cl
2
21
6
20 n = 2, X = Cl
20a n = 2, X = Ph
20b n = 2, X = H
20c n = 2, X = imid.
20d n = 3, X = Ph
20e n = 3, X = Cl
7 n = 2, X = Cl
8 n = 2, X = Ph
9 n = 2, X = H
10 n = 2, X = imid
11 n = 3, X = Ph
12 n = 3, X = Cl

Figure 2.

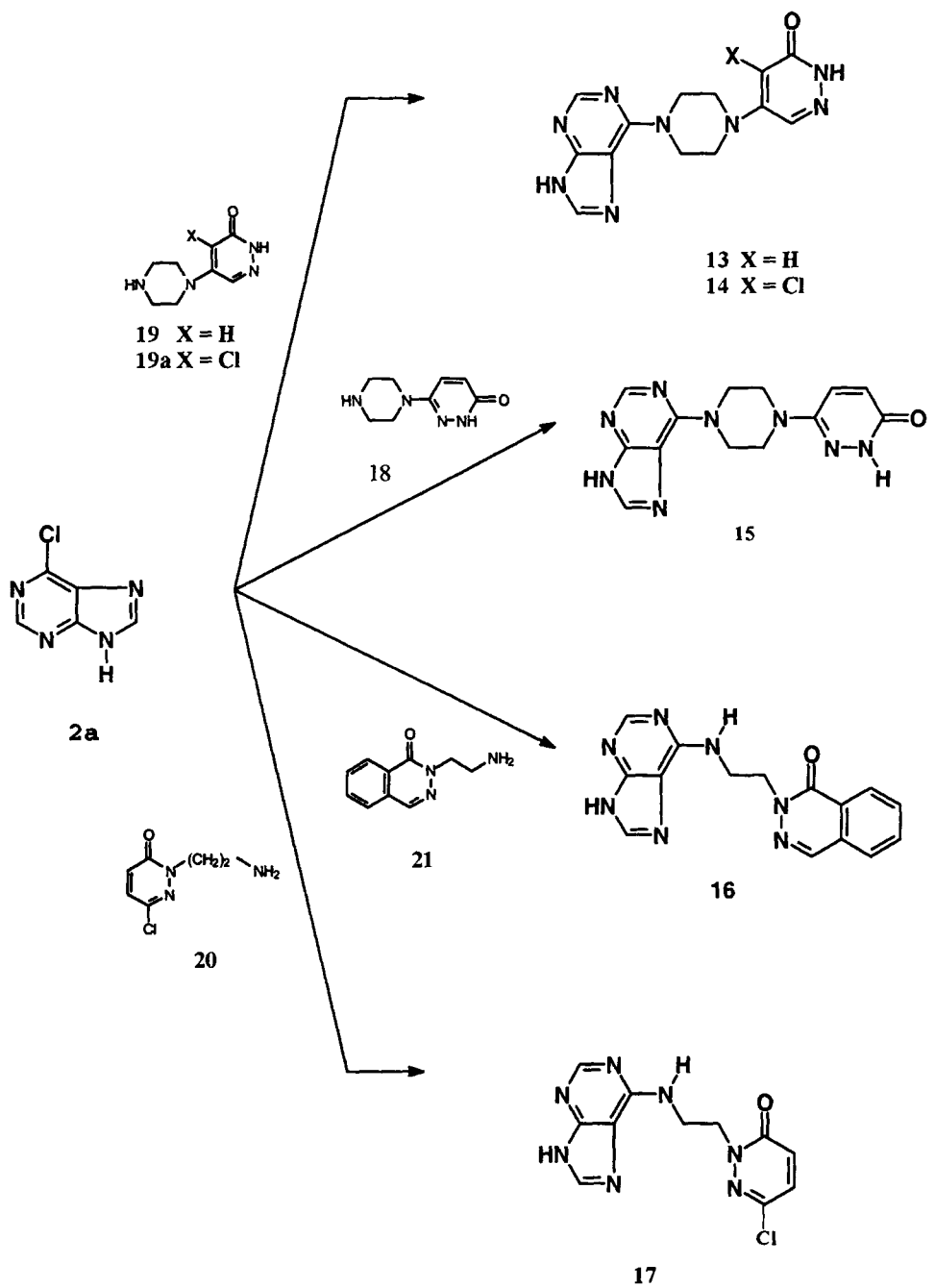

Figure 3.

In conclusion, the introduction of a pyridazinone system in 6-position of the purine riboside through an ethylenic chain, maintains a good affinity towards A_1 receptor in comparison with CHA used as reference drug, moreover the importance of the riboside in these structures for the affinity towards A_1-receptor has been confirmed. On the basis of these results compounds **7** and **8** may be useful as cerebroprotective agents.

Table I. Affinity towards A_1 and A_{2A} adenosine receptor of new 6-amino purine derivatives.

Compound	K_i[25 °C](A_1)[a] (nM ± SEM)	nH[b]	K_i[0 °C]/K_i[25 °C][c]	K_i[25 °C](A_2)[d] (nM ± SEM)	nH[b]
3	> 10000	–	–	> 10000	–
4	> 10000	–	–	> 10000	–
5	> 10000	–	–	> 10000	–
6	86 ± 7.3	0.98	7.3	> 10000	–
7	8.50 ± 0.7	1.01	7.3	> 10000	–
8	6.6 ± 0.5	0.95	4.8	> 10000	–
9	49.6 ± 3.5	0.96	5.4	> 10000	–
10	35.6 ± 2.7	0.99	4.1	> 10000	–
11	82.6 ± 8.1	0.97	22	> 10000	–
12	37.4 ± 2.5	1.05	17	> 10000	–
13	> 10000	–	–	> 10000	–
14	> 10000	–	–	> 10000	–
15	> 10000	–	–	> 10000	–
16	> 10000	–	–	> 10000	–
17	> 10000	–	–	> 10000	–
Agonists					
CHA	1.3 ± 0.2	1.03	4.5	750 ± 65	0.97
R-PIA	1.2 ± 0.1	0.95	3.5	500 ± 40	1.01
CGS 21680	2600 ± 220	0.98	–	10 ± 0.9	0.96
NECA	6 ± 0.5	0.96	2.5	15 ± 1.3	0.99
Antagonists					
CPX	0.9 ± 0.08	0.96	0.3	470.2 ± 40.1	0.98
8-PT	86.0 ± 8.5	0.97	0.4	850 ± 82.8	0.96

[a]A_1 binding was measured as inhibition of [^{3}H]-CHA binding as described in the Experimental protocols. The K_i values are means ± SEM of four separate assays, each performed in triplicate; [b] nH = Hill coefficient; [c]K_i ratio = K_i[0 °C]/[25 °C]; [d]A_{2A} binding was measured as inhibition of [^{3}H]-CGS 21680 binding as described in the Experimental protocols. The K_i values are means ± SEM of four separate assays, each performed in triplicate.

5. Experimental protocols

5.1. Biological methods

5.1.1. A_1 receptor binding

Bovine cerebral cortex was homogenized in 10 volumes ice-cold 0.25 M sucrose containing 5 mM EDTA and protease inhibitors (0.1 mM of phenylmethylsulfonilfluoride (PMSF), 200 μg/mL bacitracine, and 160 μg/mL benzamidine) in an ultra-turrax homogenizer. The homogenate was centrifuged at 1000 *g* for 10 min at 4 °C and the supernatant again centrifuged at 46000 *g* for 20 min at 4 °C. The resulting pellet was suspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.7 containing 1 mM EDTA, 5 mM $MgCl_2$ and protease inhibitors (buffer T_1). It was then homogenized and centrifuged at 46000 *g* for 20 min at 4 °C.

The pellet was dispersed in 5 volumes of fresh T_1 buffer and incubated with adenosine deaminase (2UI/mL) at 37 °C for 30 min, then recentrifuged at 46000 *g* for 20 min at 4 °C. The resulting pellet was frozen at –80 °C until the time of assay. The pellet was suspended in 20 volumes of ice-cold T_1 buffer and A_1 binding assay was performed in triplicate by incubating at 25 °C for 120 min or 0 °C for 150 min in 0.5 mL T_1 buffer containing aliquots of the membrane fraction (0.2–0.3 mg protein) and 1.3 nM[^{3}H]CHA in the absence or presence of unlabelled 15 μM R-PIA.

The binding reaction was terminated by filtering through Whatman GF/C glass fiber filters under suction and washing twice with 5 mL ice-cold Tris-buffer. The filters were placed in scintillation vials and 4 mL Gold MN Cocktail-Packard solvent scintillation fluid was added. The radioactivity was counted with an Packard 1600 TR scintillation counter. Specific binding was obtained by subtracting non-specific binding from total binding and was approximated to 85–90% of the total binding.

5.1.2. A_{2A} receptor binding

Striatum was dissected from bovine brain and the tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer at pH 7.4 containing 10 mM $MgCl_2$, 1 mM EDTA, and protease inhibitors as reported above (buffer T_2). The homogenate was centrifuged at 46000 *g* for 10 min at 4 °C. The pellet was then suspended in 20 volumes of Tris-HCl buffer (T_2) containing adenosine deaminase (2UI/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.

Binding assay was performed in triplicate, by incubating aliquots of the membrane fraction (0.2–0.3 mg protein) in Tris-HCl at pH 7.5, with approximately 5 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 50 μM NECA. The binding reaction was concluded by filtrac-

tion through Whatman GF/C glass fiber 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 recepto-bound radioactivity was measured as described above.

Compounds were dissolved in ethanol or DMSO (buffer/ concentration of 2%) and added to the assay mixture. Blank experiment were carried out to determine the effect of the solvent on binding.

Protein estimation was based on a reported method [18], after solubilization with 0.75 N sodium hydroxide, using bovine serum albumin as standard.

The concentration of tested compound that produce 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 the displacer, each performed in triplicate. Inhibition constants (K_i) were calculated according to the equation of Cheng and Prousoff [19]; $K_i = IC_{50}/([L]/K_d)$, where [L] is the ligand concentration and K_d its dissociation constant. K_d of [^{3}H]CHA binding to cortex membranes was 1.2 nM and K_d of [^{3}H]CGS 21680 binding to striatal membranes was 10 nM.

5.2. Chemistry

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. The NMR spectra were recorded with a Bruker AC 200 MHz instrument in the solvent indicated below. The chemical shift values (ppm) are relative to tetramethylsilane as internal standard. Elemental analyses are within ± 0.4% of theoretical values. Precoated Kiesegel 60 F 254 plates (Merck) were used for TLC.

5.2.1. 6-Substituted amino-9H-purines – General method for the preparation of compounds 3–17 [20]

6-[4-(pyridazin-3(2H)-one-6-yl)-piperazin-1-yl]-9-β-D-ribofuranosyl-9H-purine ***3***: A mixture of 6-chloropurine riboside 1.50 g (5.2 mmol), 1.20 g (6.5 mmol.) of 6-(piperazin-1yl)-pyridazin-3(2H)-one **18** [21], and a 3-fold excess of triethylamine in 50 mL of absolute ethanol was heated a reflux for 20 h. (until TLC showed disappearance of starting material). After cooling the mixture was filtered and the collected solid was crystallized from ethanol. Yield: 55%; m.p.: 274–278 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.4–3.8 (m, 6H, CH_2-5', 4H-pip.), 3.9 (m, 1H, H-4'), 4.2 (d, 1H, H-3'), 4.3 (m, 4H, H-pip.), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.4 (m, 1H, OH-5'), 5.5 (d, 1H, OH-2'), 6.0 (d, 1H, H-1'), 6.8 (d, 1H, H-4 pyrid.), 7.5 (d, 1H, H-5 pyrid.), 8.3 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin.), 12.2 (s, 1H, H-2 pyrid.).

6-[4-(pyridazin-3(2H)-one-5-yl)-piperazin-1-yl]-9-β-D-ribofuranosyl-9H-purine ***4***: This compound was prepared by reaction of 6-chloropurine riboside **2** with 5-(piperazin-1-yl)-pyridazin-3(2H)-one **19** [22]. Yield: 50%; m.p.: 264–267 °C (methanol–H_2O); ^{1}H-NMR (DMSO-d_6) δ: 3.4–3.8 (m, 6H, CH_2-5', 4H-pip.), 3.9 (m, 1H, H-4'), 4.2 (m, 1H, H-3'), 4.3 (m, 4H, H-pip.), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3 (d, 1H, OH-5'), 5.5 (d, 1H, OH-2'), 5.8 (s, 1H, H-4 pyrid.), 6.0 (d, 1H, H-1'), 8.0 (s, 1H, H-6 pyrid.), 8.3 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin.), 12.2 (s, 1H, H-2 pyrid.).

6-[4-(4-chloro-pyridazin-3(2H)-one-5-yl)piperazin-1-yl]-9-β-D-ribofuranosyl-9H-purine ***5***: This compound was prepared by reaction of 6-chloropurine riboside **2** with 4-chloro-5-(piperazin-1-yl)-pyridazin-3(2H)-one **19a** [22]. Yield: 65%; m.p.: 258–261 °C (ethanol–H_2O); ^{1}H-NMR (DMSO-d_6) δ: 3.4–3.8 (m, 6H, CH_2-5', 4H-pip.), 3.9 (m, 1H, H-4'), 4.2 (m, 1H, H-3'), 4.3 (m, 4H, H-pip.), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3 (d, 1H, OH-5'), 5.5 (d, 1H, OH-2'), 5.6 (d, 1H, H-1'), 8.0 (s, 1H, H-6 pyrid.), 8.3 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin.), 12.9 (s, 1H, H-2 pyrid.).

6-[2-(phthalazin-1(2H)-one-2-yl)-aminoethyl]-9-β-D-ribofuranosyl-9H-purine ***6***: This compound was prepared by reaction 6-chloropurine riboside **2** with 2-(2-aminoethyl)-phthalazin-1(2H)-one **21**. Yield: 50%; m.p.: 140–143 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.5 (m, 2H, CH_2-5'), 3.8–5.0 (m, 3H, CH_2, H-4'), 4.2 (m, 1H, H-3'), 4.4 (t, 2H, –CH_2), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.5 (m, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 7.8–7.9 (m, 4H, H-arom.), 8.0 (m, 2H, NH, 1H-arom.), 8.3 (m, 2H, H-2,8 purin).

6-[2-(6-chloro-pyridazin-3(2H)-one-2-yl)-aminoethyl]-9-β-D-ribofuranosyl-9H-purine ***7***: This compound was prepared by reaction of 6-chloropurine riboside **2** with 2-(2-aminoethyl)-6-chloro-pyridazin-3(2H)-one **20**. Yield: 40%; m.p.: 230–233 °C (ethanol– H_2O); ^{1}H-NMR (DMSO-d_6) δ: 3.5 (m, 2H, CH_2-5'), 3.8–4.0 (m, 3H, CH_2, H-4'), 4.2 (m, 1H, H-3'), 4.3 (m, 2H, CH_2), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.5 (m, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 7.0 (d, 1H, H-4 pyrid.), 7.5 (d, 1H, H-5 pyrid.), 8.0 (s, 1H, NH), 8.3 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin.).

6-[2-(6-phenyl-pyridazin-3(2H)-one-2-yl)-aminoethyl]-9-β-D-ribofuranosyl-9H-purine ***8***: This compound was prepared by reaction of 6-chloropurine riboside **2** with 2-(2-aminoethyl)-6-phenyl-pyridazin-3(2H)-one **20a** [23]. Yield: 45%; m.p.: 206–209 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.6 (m, 2H, CH_2-5'), 3.9 (m, 3H, CH_2, H-4'), 4.2 (m, 1H, H-3'), 4.4 (t, 2H, –CH_2), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.5 (m, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 7.0 (d, 1H, H-4 pyrid.), 7.4–7.5 (m, 3H, H-arom.), 7.6 (m, 2H, H-arom.), 8.0 (m, 2H, NH, H-5 pyrid.), 8.2 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin).

6-[2-(pyridazin-3(2H)-one-2-yl)-aminoethyl]-9-β-D-ribofuranosyl-9H-purine ***9***: Prepared by reaction of 6-chloropurine riboside **2** with 2-(2-aminoethyl)-pyridazin-3(2H)-one **20b**. Yield: 60%; m.p.: 195–198 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.6 (m, 2H, CH_2-5'), 3.9 (m, 3H, CH_2, H-4'), 4.0 (m, 1H, H-3'), 4.3 (t, 2H, –CH_2), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.5 (m, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 6.9 (d, 1H, H-4 pyrid.), 7.4 (dd., 1H, H-5 pyrid), 7.8 (d, 1H, H-6 pyrid.), 8.0 (s, 1H, H-2 purin.), 8.2 (s, 1H, H-8 purin).

6-[2-(imidazol-1-yl)-pyridazin-3(2H)-one-2-yl)-aminoethyl]-9-β-D-ribofuranosyl-9H-purine ***10***: Prepared by reaction of 6-chloropurine riboside **2** with 2-(2-aminoethyl)-6-(imidazol-1-yl)-pyridazin-3(2H)-one **20c**. Yield: 30%; m.p.: 95–100 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.6 (m, 2H, CH_2-5'), 3.8–4.0 (m, 3H, CH_2, H-4'), 4.2 (m, 1H, H-3'), 4.3 (m, 2H, CH_2), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3 (d, 1H, OH-5'), 5.5 (d, 1H, OH-2'), 6.0 (d, 1H, H-1'), 7.0 (s, 1H, H-imid.), 7.2 (d, 1H, H-4 pyrid.), 7.5 (s, 1H, H-imid.), 7.9 (d, 1H, H-5 pyrid.), 8.0 (m, 2H, NH , H-imid.), 8.2 (s, 1H, H-2 purin.), 8.4 (s, 1H, H-8 purin.).

6-[3-(6-phenyl-pyridazin-3(2H)-one-2-yl)-aminopropyl]-9-β-D-ribofuranosyl-9H-purine ***11***: Prepared by reaction of 6-chloropurine riboside **2** with 2-(3-aminopropyl)-6-phenyl-pyridazin-3(2H)-one **20d** [23]. Yield: 50%; m.p.: 116–118 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 2.2 (m, 2H, CH_2), 3.5–3.8

(m, 4H, CH_2-5', CH_2), 4.0 (d, 1H, H-4'), 4.1–4.3 (m, 3H, CH_2, H-3'), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.5 (m, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 7.0 (d, 1H, H-4 pyrid.), 7.4–7.6 (m, 3H, H-arom.), 7.8–7.9 (m, 3H, 2H-arom., NH.), 8.0 (s, 1H, H-5 pyrid.), 8.2 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin).

6-[3-(6-chloro-pyridazin-3(2H)-one-2-yl)-aminopropyl]-9-β-D-ribofuranosyl-9H-purine ***12***: This compound was prepared by reaction of 6-chloropurine riboside **2** with 2-(3-aminopropyl)-6-chloro-pyridazin-3(2H)-one **20e**. Yield: 40%; m.p.: 112–115 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 2.0 (m, 2H, CH_2), 3.4–3.7 (m, 4H, CH_2, CH_2-5'), 4.0 (d, 1H, H-4'), 4.1 (t, 2H, CH_2), 4.2 (m, 1H, H-3'), 4.6 (m, 1H, H-2'), 5.2 (d, 1H, OH-3'), 5.3–5.4 (d, 2H, OH-5', 2'), 6.0 (d, 1H, H-1'), 7.0 (d, 1H, H-4 pyrid.), 7.5 (d, 1H, H-5 pyrid.), 7.8 (s, 1H, NH), 8.2 (s, 1H, H-2 purin), 8.4 (s, 1H, H-8 purin.).

6-[4-(pyridazin-3(2H)-one-5-yl)]-piperazin-1-yl]-9H-purine ***13***: This compound was prepared by reaction of 6-chloropurine **2a** with 5-piperazin-1-yl-pyridazin-3(2H)-one **19** [22]. Yield: 50%; m.p. > 300 °C (DMF); ^{1}H-NMR (DMSO-d_6) δ: 4.0 (m, 4H, H-pip.), 4.6 (m, 4H, H-pip.), 6.6 (s, 1H, H-4 pyrid.), 8.4 (s, 1H, H-6 pyrid.), 8.8 (s, 2H, H-2, 8 purin), 12.2 (s, 1H, H-2 pyrid.), 13.0 (s, 1H, H-9 purin.).

6-[4-(4-chloro-pyridazin-3(2H)-one-5-yl)-piperazin-1-yl]-9H-purine ***14***: This compound was prepared by reaction of 6-chloropurine **2a** with 4-chloro-5-piperazin-1-yl-pyridazin-3(2H)-one **19a** [22]. Yield: 50%; m.p. > 300 °C (DMF); ^{1}H-NMR (DMSO-d_6) δ: 3.5 (m, 4H, H-pip.), 4.3 (m, 4H, H-pip.), 7.9 (s, 1H, H-6 pyrid.), 8.2 (s, 1H, H-2 purin), 8.3 (s, 1H, H-8 purin.), 12.5 (s, 1H, H-2 pyrid.), 13.0 (s, 1H, H-9 purin.).

6-[4-(pyridazin-3(2H)-one-6-yl)-piperazin-1-yl]-9H-purine ***15***: This compound was prepared by reaction of 6-chloropurine **2a** with 6-piperazin-1-yl-pyridazin-3(2H)-one **18** [21]. Yield: 30%; m.p. > 300 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.5 (m, 4H, H-pip.), 4.2 (m, 4H, H-pip.), 6.8 (d, 1H, H-4 pyrid.), 7.6 (d, 1H, H-5 pyrid.), 8.2 (s, 1H, H-2 purin), 8.3 (s, 1H, H-8 purin.), 12.2 (s, 1H, H-2 pyrid.), 13.0 (s, 1H, H-9 purin.).

6-[2-(phthalazin-1(2H)-one-2-yl)-aminoethyl]-9H-purine ***16***: Prepared by reaction of 6-chloropurine **2a** with 2-(2-aminoethy)-phthalazin-1(2H)-one **21**. Yield: 60%; m.p.: 268–272 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.8 (m, 2H, CH_2), 4.4 (t, 2H, CH_2), 7.7–8.0 (m, 4H, H-arom.), 8.0–8.2 (m, 2H, NH, H-pyrid.), 8.2–8.4 (m, 2H, H-8,2 purin), 13.0 (s, 1H, H-9 purin.).

6-[2-(6-chloro-pyridazin-3(2H)-one-2-yl)-aminoethyl]-9H-purine ***17***: Prepared by reaction of 6-chloropurine **2a** with 2-(2-aminoethyl)-6-chloro-pyridazin-3(2H)-one **20**. Yield: 50%; m.p.: 270–273 °C (ethanol); ^{1}H-NMR (DMSO-d_6) δ: 3.8 (m, 2H, CH_2), 4.3 (t, 2H, CH_2), 7.0 (d, 1H, H-4 pyrid.), 7.5 (d, 1H, H-5 pyrid.), 7.8 (s, 1H, NH), 8.1–8.2 (m, 2H, H-8,2 purin.), 13.0 (s, 1H, H-9 purin.).

2-(2-aminoethyl)-6-chloro-pyridazin-3(2H)-one ***20***: 2.6 g (0.02 mol) of 6-chloro-pyridazin-3(2H)-one was added to an ethanolic solution (50 mL) of sodium 0.46 g (0.02 g atom). On the other hand, a solution of 2-aminoethyl chloride hydrochloride 2.3 g (0.02 mol) in absolution ethanol (40 mL) was added to an ethanolic solution (50 mL) of sodium (0.46 g, 0.02 g atom).

The two solutions were mixed at room temperature and stirring for 1 h, under the mixture was refluxed for 18 h, and the mixture was filtered. The organic layer was evaporated, and the residue was diluted with water acidified with HCl 1 N and extracted by CH_2Cl_2.

The water solution was alkalinized with NaOH 1 N, and extracted with CH_2Cl_2, the organic phase dried over dry sodium sulphate, and removed under reduced pressure. The crude product was used without further purification. Yield: 30%, m.p.: 83–85 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.6 (s, 2H, NH_2), 3.1 (t, 2H, CH_2), 4.15 (t, 2H, CH_2), 6.9 (d, 1H, H-4 pyrid.), 7.2 (d, 1H, H-5 pyrid.).

2-(2-aminoethyl)-pyridazin-3(2H)-one ***20b***: This compound was prepared by alkylation of pyridazin-3(2H)-one with N-(2-bromoethyl)-phthalimide in the presence of K_2CO_3, followed by hydrolysis with hydrazine hydrate, using the method of Yamada [24]. The residue was used without further purification, m.p.: 232–235 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.4 (s, 2H, NH_2), 3.15 (t, 2H, CH_2), 4.2 (t, 2H, CH_2), 6.9 (d, 1H, H-4 pyrid.), 7.2 (m, 1H, H-5 pyrid.), 7.8 (d, 1H, H-6 pyrid.).

2-(2-aminoethyl)-6-(imidazol-1-yl)-pyridazin-3(2H)-one ***20c***: This compound was prepared using the method described for compound **20b**. The residue was used without further purification, m.p.: 180–185 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.3 (s, 2H, NH_2), 3.1 (t, 2H, CH_2), 4.15 (t, 2H, CH_2), 7.05 (d, 1H, H-pyrid.), 7.1 (s, 1H, H-imid.), 7.3–7.5 (m, 2H, H-5 pyrid., H-imid.), 8.0 (s, 1H, H-imid.).

2-(3-aminopropyl)-6-chloro-pyridazin-3(2H)-one ***20e***: This compound was prepared from 6-chloro-pyridazin-3(2H)-one with 1,3-dibromopropane in benzene, potassium hydroxide and tetrabutyl ammonium bromide (TBAB). The corresponding 3-bromopropyl derivate was treated with a ethanolic solution of 25% ammoniun hydroxide, using the method of Yamada [23]. ^{1}H-NMR ($CDCl_3$) δ: 2.0 (m, 4H, CH_2, NH_2), 2.7 (t, 2H, CH_2), 4.2 (t, 2H, CH_2), 6.9 (d, 1H, H-4 pyrid.), 7.2 (m, 1H, H-5 pyrid.).

2-(2-aminoethyl)-phthalazin-1(2H)-one ***21***: A solution of 0.660 g (4.5 mmol) of 1(2H)-phthalazinone in 15 mL of dry ethanolic and 0.240 g (6 mmol) of NaOH was stirred under reflux for 30 min.

After a solution of 0.922 g (4.5 mmol) of 2-aminoethyl-bromide hydrobromide in 15 mL of dry ethanol and 0.2 g (5 mmol) of NaOH was added. The mixture was refluxed for 24 h, filtered hot, and then the organic layer was evaporated in vacuo. The residue was diluted with water acidified with HCl 1 N and extracted by CH_2Cl_2. The water solution was alkalinized with NaOH 1 N, and extracted with CH_2Cl_2. The organic phase dry on sodium sulphate was evaporated under reduced pressure, the residue was used without further purification. m.p.: 84–88 °C, ^{1}H-NMR ($CDCl_3$) δ: 1.5 (s, 2H, NH_2), 3.2 (t, 2H, CH_2), 4.3 (t, 2H, CH_2), 7.6–7.8 (m, 3H, H-arom.), 8.1 (s, 1H, H-4-pyrid.), 8.4–8.5 (m, 1H, H-arom.).

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