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Structure–Activity Relationships in a Series of 8-Substituted Xanthines as A_1-Adenosine Receptor Antagonists

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Abstract—A series of 8-substituted xanthines were synthesized and their affinity in vitro towards A_1, A_{2A}-adenosine receptors was evaluated by radioligand receptor binding assays. All compounds showed a greater affinity and selectivity towards the A_1-adenosine receptor than theophylline. The compounds in which the *n-proyl* group is in 1-position of the xanthine nucleus and the pyridazinone system in 8-position is linked through a chain of two or four carbon atoms, showed the highest affinity and selectivity.

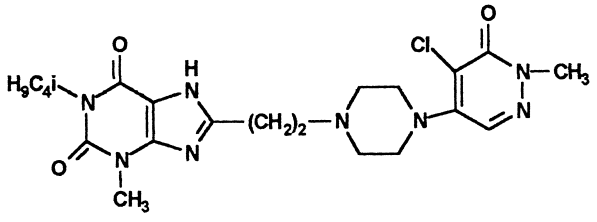
Introduction

Adenosine receptors are involved in many peripheral and central regulatory mechanisms, including vasodilatation[1] vasoconstriction in the kidney,[2] inhibition of lypolysis and insulin release,[3] and moderation of cerebral ischemia.[4] Four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been identified, both pharmacologically and through cloning techniques.[5,6]

All adenosine receptor agonists already studied are derivatives of adenosine, while there are several classes of adenosine antagonists, but the more studied is the xanthine class. However they are virtually non-selective antagonists and have weak affinity for A_1 and A_{2a} adenosine receptors.[7,8] It is therefore unclear to what extent the antiasthmatic, diuretic, respiratory stimulant, central stimulant, cardiac stimulant, and analgesic adjuvant activities of xanthines reflect interactions at A_1 or A_2 receptors or at some other site. Studies of structure–activity relationships of xanthines reveal that the introduction of an alkyl group at 1- and 3-position markedly increases affinity for both adenosine receptors subtypes.[9,10] On the other hand, either 8-aryl or 8-cycloalkyl group increases the affinity towards A_1-receptor.[11]

Recently we have synthesized xanthine derivatives substituted at the N^7 position[12,13] or C^8 and with fragment containing a pyridazinone ring which showed a good affinity towards A_1-receptor, particularly the compound **1**[14] has higher affinity ($K_i = 0.85\,\mu M$) towards this receptor.

Therefore to obtain further information on the role played by this pyridazinone ring in the interaction between the ligand and the receptor, we have synthesized a series of xanthine derivatives substituted at 8-position, in which the pyridazinone group was linked through a linker of one, two, three or four carbon atoms. In order to obtain more information on structure–activity relationships, we have synthesized compounds in which the *methyl* group in 1-position of the theophylline nucleus was substituted by a *n-propyl* group.



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Chemistry

The synthesis of xanthine **5**–**22** was performed according to the general method summarized in Scheme 1, using the standard procedure of Traube:[15] acylation of

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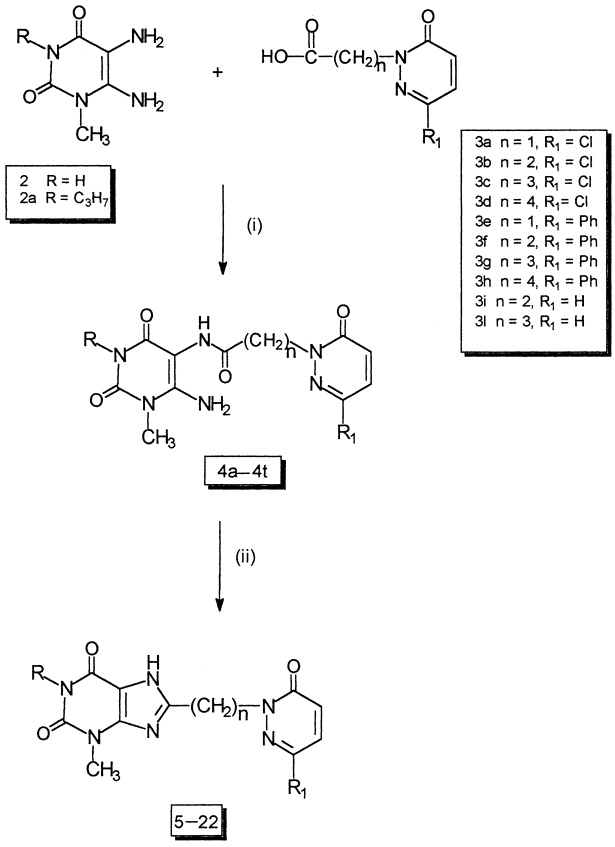


Scheme 1. (i) DCC/methanol; (ii) NaOH 2.5% Δ/HCl 5%.

the appropriate 5,6-diamino-1,3-dialkyl-uracil with the corresponding carboxylic acid in methanol and DCC, followed by treatment with a solution of sodium hydroxide and acidification by HCl, gave the corresponding xanthine derivatives. The preparation of the 5,6-diamino-1-methyl-3-propyl-uracil (**2a**) was carried out starting from 6-amino-1-methyl-uracil by alkylation in 3-position (corresponding to 1-position of xanthine) with alkyl bromides or iodides in the presence of sodium hydroxide as base[16] followed by nitrosation in 5-position and reduction to 5,6-diaminouracil.

Results and Discussion

The 8-substituted theophylline analogues (compounds **5**–**22**) were tested in radioligand binding assays for affinity to A_1 and A_{2A} adenosine receptors in bovine brain cortical, and bovine striatal membranes, respectively. CHA was used as A_1 ligand and CGS21680 as A_{2A} ligand.

Theophylline and caffeine were used as reference compounds.

As can be seen from the data in Table 3, these compounds are more potent than theophylline and caffeine showing more affinity and selectivity towards A_1 receptor. In the series of compounds **5**–**8**, a gradual increase in affinity and selectivity was observed by promoting the polymethylene chain length from one to four carbon atoms and when a chlorine atom is present in 6-position of the pyridazinone ring. While a decrease in affinity and selectivity was found when the chlorine atom was substituted with a phenyl group (compounds **9**–**12**), or with a hydrogen atom (compounds **13**–**14**). Replacement of the 1-*methyl* substituent of theophylline with a *n-propyl* group has a marked effect on the A_1 receptor (compounds **15**–**22**), in particular compounds **16**, **18** and **21** show the highest selectivity.

In conclusion, these xanthine derivatives show a higher affinity and selectivity towards the A_1 receptor than theophylline and caffeine. The replacement of the *1-methyl* substituent of theophylline with *n-propyl* group confirms the increase of the affinity towards the A_1- and A_{2A}-adenosine receptors. A chlorine atom or a phenyl group in 6-position of the pyridazinone ring and a *n-propyl* group in 1-position in the xanthine nucleus are important for the selectivity. Moreover the length of the alkyl chain, a spacer between the two major constituents of the molecule, can influence the affinity and the selectivity.

Several of these theophylline analogues may prove useful for the investigation of the significance of A_1 and A_2 receptors in various physiological processes.

Experimental Protocols

Biological methods

Affinity of the new xanthine derivatives **5**–**22** towards A_1 and A_{2A} adenosine receptors were 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 3.

A_1 receptor binding

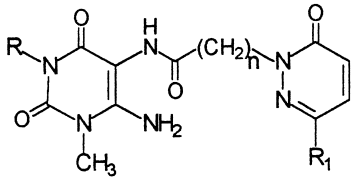
Bovine cerebral cortex was homogenized in 10 volumes ice-cold 0.25 M sucrose containing 5 mM EDTA and protease inhibitors (0.1 mM of phenylmethylsulfonil-fluoride (PMSF), 200 μg/mL bacitracine, and 160 μg/mL benzamidine) in an ultra-turrax homogenizer. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant again centrifuged at 46,000*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 46,000*g* for 20 min at 4 °C.

The pellet was dispersed in 5 volumes of fresh T_1 buffer and incubated with adenosine deaminase (2 UI/mL) at 37 °C for 30 min, then recentrifuged at 46,000*g* for 20 min at 4 °C.

The resulting pellet was frozen at −80 °C until the time of assay.

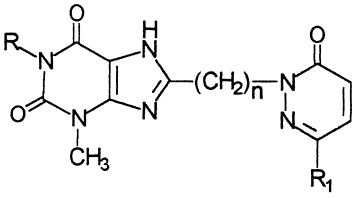
The pellet was suspended in 20 volumes of ice-cold T_1 buffer and A_1 binding assay was performed in triplicate by incubating at 25 °C for 120 min or 0 °C for 150 min in 0.5 mL T_1 buffer containing aliquots of the membrane fraction (0.2–0.3 mg protein) and 1.3 nM[^{3}H]CHA in the absence or presence of unlabelled 15 μM R-PIA.

Table 1. Chemical data of the amide derivatives **4a**–**4t**



Compound	*n*	R	R_1	Yield (%)	mp (°C)
4a	1	CH_3	Cl	80	>325
4b	2	CH_3	Cl	80	>325
4c	3	CH_3	Cl	75	>325
4d	4	CH_3	Cl	65	215–218
4e	1	CH_3	Ph	70	287–290
4f	2	CH_3	Ph	60	>325
4g	3	CH_3	Ph	80	>325
4h	4	CH_3	Ph	85	225–228
4i	2	CH_3	H	70	290–293
4l	3	CH_3	H	60	195–198
4m	1	C_3H_7	Cl	90	235–238
4n	2	C_3H_7	Cl	90	218–221
4o	3	C_3H_7	Cl	30	232–235
4p	4	C_3H_7	Cl	60	215–218
4q	1	C_3H_7	Ph	60	255–258
4r	2	C_3H_7	Ph	70	286–289
4s	3	C_3H_7	Ph	40	225–228
4t	4	C_3H_7	Ph	60	132–135

Table 2. Chemical data of the compounds **5**–**22**



Compound	*n*	R	R_1	Yield (%)	mp (°C)	Recryst. solvent
5	1	CH_3	Cl	25	>325	EtOH
6	2	CH_3	Cl	25	303–306	EtOH
7	3	CH_3	Cl	50	250–253	EtOH/EtOAc
8	4	CH_3	Cl	60	241–244	EtOH/H_2O
9	1	CH_3	Ph	50	>325	EtOH/H_2O
10	2	CH_3	Ph	35	318–321	EtOH
11	3	CH_3	Ph	30	293–296	EtOH
12	4	CH_3	Ph	30	280–283	EtOH/H_2O
13	2	CH_3	H	60	305–309	EtOH
14	3	CH_3	H	25	273–276	EtOH/EtOAc
15	1	C_3H_7	Cl	60	>325	EtOH/H_2O
16	2	C_3H_7	Cl	60	264–267	EtOH/H_2O
17	3	C_3H_7	Cl	50	208–210	EtOH/EtOAc
18	4	C_3H_7	Cl	40	194–197	EtOH/EtOAc
19	1	C_3H_7	Ph	70	284–287	EtOH
20	2	C_3H_7	Ph	25	259–262	EtOH
21	3	C_3H_7	Ph	70	224–227	EtOH/EtOAc
22	4	C_3H_7	Ph	30	116–119	EtOH

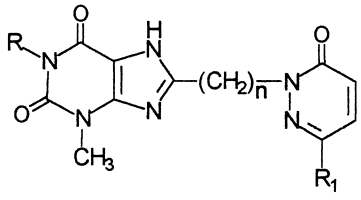
The binding reaction was terminated by filtering through Whatman GF/C glass fiber filters under suction and washing twice with 5 mL ice-cold Tris-buffer. The filters were placed in scintillation vials and 4 mL Gold MN Cocktail-Packard solvent scintillation fluid was added. The radioactivity was counted 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.

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 46,000*g* for 10 min at 4 °C. The pellet was then suspended in 20 volumes of Tris–HCl buffer (T_2) containing adenosine deaminase (2 UI/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

Table 3. Affinity of 8-substituted xanthines as antagonists at A_1 and A_{2A} adenosine receptors



Compound	*n*	R_1	R	K_i A_1[a] (μM)	K_i A_{2A}[b] (μM)	Ratio K_i[c] A_{2A}/A_1
5	1	Cl	CH_3	>100	>100	1.0
6	2	Cl	CH_3	14.7	53.0	3.6
7	3	Cl	CH_3	8.8	33.6	3.8
8	4	Cl	CH_3	0.37	21.0	56.0
9	1	Ph	CH_3	53.8	30.0	0.5
10	2	Ph	CH_3	6.1	57.6	9.4
11	3	Ph	CH_3	10.8	>100	9.2
12	4	Ph	CH_3	2.12	17.0	8.0
13	2	H	CH_3	12.8	60.4	4.8
14	3	H	CH_3	12.7	22.4	1.7
15	1	Cl	C_3H_7	9.2	>100	10.8
16	2	Cl	C_3H_7	0.47	>100	212.0
17	3	Cl	C_3H_7	1.2	31.8	26.5
18	4	Cl	C_3H_7	0.19	13.45	70.8
19	1	Ph	C_3H_7	2.27	23.8	10.5
20	2	Ph	C_3H_7	0.76	17.5	23.0
21	3	Ph	C_3H_7	0.81	61.0	75.0
22	4	Ph	C_3H_7	0.38	9.99	26.3
Theophylline				18.0	22.0	1.5
Caffeine				40.0	45.0	1.02

[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]A_{2A} binding was measured as inhibition of [^{3}H]-CGS 21860 binding as described in the Experimental protocols.
[c]The K_i values are means ± SEM of four separate assays, each performed in triplicate.

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 filtraction 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,[17] 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 the equation of Cheng and Prusoff;[18] $K_i = IC_{50}/([L]/K_d)$, 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.

Experimental

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. The NMR spectra were recorded with a Bruker AC 200 MHz or 400 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.

General method for preparation of the compounds **5**–**22** and the chemical data are reported in Table 2.

1,3-Dimethyl-8-{1-[6-chloro-pyridazin-3(2H)-one-2-yl]-methyl-}xanthine (5). A mixture (0.42 g, 1.2 mmol) N1-(4-amino-1,3-dimethyl-uracil-5-yl)-2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetamide (**4a**) in 15–20 mL of solution of sodium hydroxide 2.5% was refluxed under stirring for about 2 h. After cooling the mixture was made acid by the addition of HCl 5% to pH 3–4 and the precipitate was filtered off, and crystallized from ethanol. A white solid was obtained (yield: 25%); mp: > 325 °C. ^{1}H NMR-200 MHz-(DMSO-d_6) δ 3.20 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 5.25 (s, 2H, CH_2), 6.95 (d, J = 9 Hz, 1H, H-pyrid.), 7.20 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine).

Anal. calcd for $C_{12}H_{11}ClN_6O_3$: C 44.65; H 3.40; N 26.00; found: C 44.77; H 3.50; N 25.90.

1,3-Dimethyl-8-{2-[6-chloro-pyridazin-3(2H)-one-2-yl]-ethyl-}xanthine (6). From N1-(4-amino-1,3-dimethyl-uracil-5yl)-3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propanamide (**4b**). ^{1}H NMR-400 MHz, T = 350K-(DMSO-d_6) δ 3.10 (t, J = 6 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 4.25 (t, J = 6 Hz, 2H, CH_2), 6.90 (d, J = 9 Hz, 1H, H-pyrid.), 7.15 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{13}H_{13}ClN_6O_3$: C 46.36; H 3.86; N 24.96; found: C 46.01; H 3.58; N 24.50.

1,3-Dimethyl-8-{3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propyl-}xanthine (7). From N1-(4-amino-1,3-dimethyl-uracil-5-yl)-4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanamide (**4c**). ^{1}H NMR-400 MHz-(DMSO-d_6) δ 2.10–2.30 (m, 2H, CH_2), 2.80 (t, J = 6 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 4.00 (t, J = 6 Hz, 2H, CH_2) 6.90 (d, J = 9 Hz, 1H, H-pyrid.), 7.15 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{14}H_{15}ClN_6O_3$: C 47.93; H 4.27; N 23.96; found: C 48.20; H 4.62; N 23.57.

1,3-Dimethyl-8-{4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butyl-}xanthine (8). From N1-(4-amino-1,3-dimethyl-uracil-5-yl)-5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanamide (**4d**). ^{1}H NMR-200 MHz-(DMSO-d_6) δ 1.50–1.60 (m, 4H, $2CH_2$), 2.60–2.80 (m, 2H, CH_2), 3.15 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 3.90–4.10 (m, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.50 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{15}H_{17}ClN_6O_3$: C 49.38; H 4.7; N 23.04; found: C 49.70; H 5.10; N 22.82.

1,3-Dimethyl-8-{2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-methyl-}xanthine (9). From N1-(4-amino-1,3-dimethyl-uracil-5-yl)-2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetamide (**4e**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 3.10 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.20–7.40 (m, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pirid.), 13.5 (s, 1H, NH-xanthine). Anal. calcd for $C_{18}H_{16}N_6O_3$: C 59.50; H 4.40; N 23.14; found: C 59.85; H 4.62; N 23.57.

1,3-Dimethyl-8-{2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-ethyl-}xanthine (10). From N1-(4-amino-1,3-dimethyl-4-amino-uracil-5-yl)-3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propanamide (**4f**). ^{1}H NMR-400 MHz, T = 350K-(DMSO-d_6) δ 3.10–3.30 (m, 5H, CH_2, CH_3), 3.40 (s, 3H, CH_3), 4.50 (t, J = 6 Hz, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.30 (s, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 7.90 (d, J = 9 Hz, 1H, H-pirid.), 12.9 (s, 1H, NH-xanthine). Anal. calcd for $C_{19}H_{18}N_6O_3$: C 60.31; H 4.76; N 22.20; found: C 59,94; H 4.63; N 22.03.

1,3-Dimethyl-8-{3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propyl-}xanthine (11). By N1-(4-amino-1,3-dimethyl-uracil-5-yl)-4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-

butanamide (**4g**). ^{1}H NMR-400 MHz-(DMSO-d_6) δ 2.10–2.30 (m, 2H, CH_2), 2.80 (t, J = 6 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.20 (t, J = 6 Hz, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{20}H_{20}N_6O_3$: C 61.22; H 5.10; N 21.42; found: C 61.01; H 4.74; N 21.37.

1,3-Dimethyl-8-{4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butyl-}xanthine (12). From N1-(4-amino-1,3-dimethyl-uracil-5-yl)-5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanamide (**4h**). ^{1}H NMR-200 MHz-(DMSO-d_6) δ 1.65–1.75 (m, 4H, 2CH_2), 2,60–2.70 (m, 2H, CH_2), 2.80 (t, J = 6 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.20 (m, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{21}H_{22}N_6O_3$: C 62.06; H 5.40; N 20.68; found: C 61.92; H 5.57; N 20.24.

1,3-Dimethyl-8-{2-[pyridazin-3(2H)-one-2-yl]-ethyl-}xanthine (13). From N1-(4-amino-1,3-dimethyl-uracil-5-yl)-3-[pyridazin-3(2H)-one-2-yl]-propanamide (**4i**). ^{1}H NMR-400 MHz-(DMSO-d_6) δ 3.10 (t, J = 6 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.45 (t, J = 6 Hz, 2H, CH_2), 6.90 (dd, J = 9 and 2 Hz, 1H, H-pyrid.), 7.45 (m, 1H, H-pyrid.), 7.90 (dd, J = 9 and 5 Hz, 1H, H-pyrid.), 13.2 (s, 1H, NH-xanthine). Anal. calcd for $C_{13}H_{14}N_6O_3$: C 51.65; H 4.63; N 27.81; found: C 51.31; H 4.42; N 27.45.

1,3-Dimethyl-8-{3-[pyridazin-3(2H)-one-2-yl]-propyl-}xanthine (14). From N1-(4-amino-1,3-dimethyl-4-amino-uracil-5-yl)-4-[pyridazin-3(2H)-one-2-yl]-butanamide (**4l**). ^{1}H NMR-400 MHz, T = 350K-(DMSO-d_6) δ 2.10–2.40 (m, 2H, CH_2), 2.75 (t, J = 6 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.45 (s, 3H, CH_3), 4.2 (t, J = 6 Hz, 2H, CH_2), 6.80–6.90 (m, 1H, H-pyrid.), 7.35–7.45 (m, 1H, H-pyrid.), 7.80–7.90 (m, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{14}H_{16}N_6O_3$: C 53.16; H 5.06; N 26.58; found: C 53.01; H 5.38; N 26.37.

3-Methyl-1-propyl-8-{1-[6-chloro-pyridazin-3(2H)-one-2-yl]-methyl-}xanthine (15). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetamide (**4m**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.4 Hz, 2H, CH_2), 5.30 (s, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{14}H_{15}ClN_6O_3$: C 48.00; H 4.20; N 24.10; found: C 48.20; H 4.62; N 24.57.

3-Methyl-1-propyl-8-{2-[6-chloro-pyridazin-3(2H)-one-2-yl]-ethyl-}xanthine (16). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propanamide (**4n**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 3.05 (t, J = 7.0 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.80 (t, J = 7.4 Hz, 2H, CH_2), 4.30 (t, J = 7.0 Hz, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{15}H_{17}ClN_6O_3$: C 49.50; H 4.60; N 23.10; found: C 49.70; H 4.79; N 22.97.

3-Methyl-1-propyl-8-{3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propyl-}xanthine (17). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanamide (**4o**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 2.00–2.20 (m, 2H, CH_2), 2.70–2.80 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.80 (t, J = 7.4 Hz, 2H, CH_2), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{16}H_{19}ClN_6O_3$: C 50.86; H 5.03; N 22.25; found: C 50.20; H 5.62; N 22.63.

3-Methyl-1-propyl-8-{4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butyl-}xanthine (18). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanamide (**4p**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 1.60–1.80 (m, 6H, 3CH_2), 2.70–2.80 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.80 (t, J = 7.4 Hz, 2H, CH_2), 4.00–4.10 (m, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 13.0 (s, 1H, NH-xanthine). Anal. calcd for $C_{17}H_{21}ClN_6O_3$: C 51.97; H 5.35; N 21.40; found: C 51.20; H 5.47; N 20.95.

3-Methyl-1-propyl-8-{1-[6-phenyl-pyridazin-3(2H)-one-2-yl]-methyl-}xanthine (19). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetamide (**4q**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.4 Hz, 2H, CH_2), 5.50 (s, 2H, CH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.10 (d, J = 9 Hz, 1H, H-pyrid.), 13.60 (s, 1H, NH-xanthine). Anal. calcd for $C_{20}H_{20}N_6O_3$: C 61.22; H 5.10; N 21.40; found: C 61.40; H 4.95; N 20.95.

3-Methyl-1-propyl-8-{2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-ethyl-}xanthine (20). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propanamide (**4r**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint. J = 7.4 Hz, 2H, CH_2), 3.10–3.20 (m, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.4 Hz, 2H, CH_2), 4.50 (t, J = 7.0 Hz, 2H, CH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.20–7.30 (m, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 13.30 (s, 1H, NH-xanthine). Anal. calcd for $C_{21}H_{22}N_6O_3$: C 62.00; H 5.40; N 20.68; found: C 61.73; H 5.79; N 20.32.

3-Methyl-1-propyl-8-{3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propyl-}xanthine (21). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butanamide (**4s**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 2.70–2.80 (m, 4H, 2CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.4 Hz, 2H, CH_2), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 13.20 (s, 1H, NH-xanthine). Anal. calcd

for $C_{22}H_{24}N_6O_3$: C 62.85; H 5.7; N 20.00; found: C 62.55; H 6.02; N 19.60.

3-Methyl-1-propyl-8-{4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butyl-}xanthine (22). From N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanamide (**4t**). ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50 (quint., J = 7.4 Hz, 2H, CH_2), 1.60–1.80 (m, 4H, $2CH_2$), 2.70 (t, J = 7.0 Hz, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.4 Hz, 2H, CH_2), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 13.20 (s, 1H, NH-xanthine). Anal. calcd for $C_{23}H_{26}N_6O_3$: C 63.59; H 5.99; N 19.35; found: C 63.78; H 5.97; N 19.03.

General method for the preparation of the amide derivatives (compounds **4a**–**4t**) and the chemical data are reported in Table 1.

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetamide (4a). A mixture of (0.37 g, 2.0 mmol) 2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetic acid (**3a**), (0.34 g, 2.4 mmol) 5,6-diamino-1,3-dimethyl-uracil and (0.49 g, 2.4 mmol) 1,3-dicyclohexylcarbodiimide (DCC) in 10 mL of anhydrous methanol was stirred at rt for 24 h. After filtration a light yellow solid was obtained washed with CH_2Cl_2 for eliminate the dicyclohexylurea, (yield: 70%), mp: > 325 °C, this compound was used for the preparation of the corresponding xanthine without further purification. ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.90 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5yl-)-3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propanamide (4b). From reaction of 3-[6-cloro-pyridazin-3(2H)-one-2-yl]-propanoic acid (**3b**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 2.75 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.25 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.60 (s, 1H, NHCO).

N1-(-4-amino-1,3-dimethyl-uracil-5-yl)-4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanamide (4c). From reaction of 4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanoic acid (**3c**) with 5,6-diamino-1,3-dimethyl-uracil.^{1}H NMR (DMSO-d_6) δ 1.90–2.10 (m, 2H, CH_2), 2.35 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.10 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.60 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethy-uracil-5-yl)-5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanamide (4d). From reaction of 5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanoic acid (**3d**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 1.50–1.80 (m, 4H, $2CH_2$), 2.25 (t, J = 6.0 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.00 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.05 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.30 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetamide (4e). From reaction of 2 -[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetic acid (**3e**), with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.70–7.80 (m, 2H, H-arom.), 8.10 (d, J = 9 Hz, 1H, H-pyrid.), 9.00 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-3-[6-phenyl-pyridazin-3(2H)-one-2-y]-propanamide (4f). From reaction of 3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propanoic acid (**3f**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 2.75 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.20 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.65 (d, J = 9 Hz, 1H, H-pyrid.), 7.70–7.80 (m, 1H, H-pyrid.), 8.40 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butanamide (4g). From reaction of 4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butanoic acid (**3g**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 1.90–2.10 (m, 2H, CH_2), 2.25 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.15 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 8.35 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-4-amino-uracil-5-yl)-5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanamide (4h). From reaction of 5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanoic acid (**3h**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 1.70–1.90 (m, 4H, $2CH_2$), 2.25 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.15 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.00 (d, J = 9 Hz, 1H, H-pyrid.), 8.30 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-3-[pyridazin-3(2H)-one-2-yl]-propanamide (4i). From reaction of 3-[pyridazin-3(2H)-one-2-yl]-propanoic acid (**3i**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 2.70 (t, J = 6 Hz, 2H, CH_2), 3.10 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.35 (t, J = 6 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 6.90 (dd, J = 9 and 2 Hz, 1H, H-pyrid.), 7.45 (dd, J = 9 and 5 Hz, 1H, H-pyrid.), 7.90–8.00 (m, 1H, H-pyrid.), 8.60 (s, 1H, NHCO).

N1-(4-amino-1,3-dimethyl-uracil-5-yl)-4-[pyridazin-3(2H)-one-2-yl]-butanamide (4l). From reaction of 4-[pyridazin-3(2H)-one-2-yl]-butanoic acid (**3l**) with 5,6-diamino-1,3-dimethyl-uracil. ^{1}H NMR (DMSO-d_6) δ 2.10–2.40 (m, 2H, CH_2), 2.75 (t, J = 6 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 4.20 (t, J = 6 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 6.80–6.90 (m, 1H, H-pyrid.), 7.30–7.40 (m, 1H, H-pyrid.), 7.80–7.90 (m, 1H, H-pyrid.), 8.50 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetamide (4m). From reaction of 2-[6-chloro-pyridazin-3(2H)-one-2-yl]-acetic acid

(**3a**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.65 (t, J = 7.4 Hz, 2H, CH_2), 4.80 (s, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.85 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-3-[6-chloro-pyridazin-3(2H)-one-2-yl]-propanamide (4n). From reaction of 3-[6-cloro-pyridazin-3(2H)-one-2-yl]-propanoic acid (**3b**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 2.70–2.80 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 4.20 (t, J = 7.4 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.50 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanamide (4o). From reaction of 4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butanoic acid (**3c**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 2.00 (t, J = 7.0 Hz, 2H, CH_2), 2.20 (t, J = 7.4 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.70 (t, J = 7.4 Hz, 2H, CH_2), 4.20 (t, J = 7.0 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.40 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanamide (4p). From reaction of 5-[6-chloro-pyridazin-3(2H)-one-2-yl]-pentanoic acid (**3d**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 6H, $3CH_2$), 2.25 (t, J = 7.0 Hz, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.70 (t, J = 7.4 Hz, 2H, CH_2), 4.00 (t, J = 7.0 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.60 (d, J = 9 Hz, 1H, H-pyrid.), 8.30 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetamide (4q). From reaction of 2-[6-phenyl-pyridazin-3(2H)-one-2-yl]-acetic acid (**3e**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.85 (t, J = 7.0 Hz, 2H, CH_2), 4.80 (s, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.10 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.10 (d, J = 9 Hz, 1H, H-pyrid.), 8.60 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propanamide (4r). From reaction of 3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propanoic acid (**3f**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 2.80 (t, J = 7.4 Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.80 (t, J = 7.0 Hz, 2H, CH_2), 4.40 (t, J = 7.4 Hz, 2H, CH_2), 6.60 (s, 2H, NH_2), 7.05 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.10 (d, J = 9 Hz, 1H, H-pyrid.), 8.60 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butanamide (4s). From reaction of 4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butanoic acid (**3g**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.80 (m, 2H, CH_2), 2.80 (m, 4H, $2CH_2$), 3.25 (s, 3H, CH_3), 3.70 (t, J = 7.0 Hz, 2H, CH_2), 4.10 (t, J = 7.4 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.05 (d, J = 9 Hz, 1H, H-pyrid.), 8.35 (s, 1H, NHCO).

N1-(4-amino-3-methyl-1-propyl-uracil-5-yl)-5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanamide (4t). From reaction of 5-[6-phenyl-pyridazin-3(2H)-one-2-yl]-pentanoic acid (**3h**) with 5,6-diamino-3-methyl-1-propyl-uracil. ^{1}H NMR-200 MHz (DMSO-d_6) δ 0.80 (t, J = 7.4 Hz, 3H, CH_3), 1.50–1.70 (m, 2H, CH_2), 1.70–1.80 (m, 4H, $2CH_2$), 2.20 (m, 2H, CH_2), 3.30 (s, 3H, CH_3), 3.60 (m, 2H, CH_2), 4.10 (t, J = 7.0 Hz, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.00 (d, J = 9 Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.80–7.90 (m, 2H, H-arom.), 8.05 (d, J = 9 Hz, 1H, H-pyrid.), 8.30 (s, 1H, NHCO).

General method for preparation of the acids 3a and 3e

2-(6-Chloro-pyridazin-3(2H)-one-2-yl)-acetic acid (3a). To a solution of (0.46 g 20 mmol) sodium in 20 mL absolute ethanol was added (2.6 g 20 mmol) 6-chloro-pyridazin-3(2H)-one and this mixture was stirred and refluxed for 30 min. After cooling was added dropwise (3.34 g 20 mmol) ethyl bromoacetate. When the addition was complete, the reaction was heated under refluxed and stirring for 24 h. At the end the solution was evaporated under reduced pressure, the residue was purified by chromatography silica gel using as eluent a stepwise gradient of ethanol (0–2%) in CH_2Cl_2, gave ethyl 2-(6-chloro-pyridazin-3(2H)-one-2-yl)-acetate (dense oil, yield: 70%). ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H, CH_3), 4.25 (q, J = 7 Hz, 2H, CH_2), 4.80 (s, 2H, CH_2CO), 6.90 (d, J = 9 Hz, 1H, H-pyrid.), 7.20 (d, J = 9 Hz, 1H, H-pyrid.). The ester was treated with hydrochloric acid 5% (15 mL) and the mixture was refluxed for 5 h, gave the corresponding acid. This acid was used for the preparation of amide **4a** without further purification.

2-(6-Phenyl-pyridazin-3(2H)-one-2-yl)-acetic acid (3e). Prepared with the same method described above, using 6-phenyl-3(2H)-pyridazinone. Gave ethyl 2-(6-phenyl-pyridazin-3(2H)-one-2-yl)-acetate (dense oil, yield: 70%).^{1}H NMR ($CDCl_3$) δ1.30 (t, J = 7 Hz, 3H, CH_3), 4.25 (q, J = 7 Hz, 2H, CH_2), 5.0 (s, 2H, CH_2), 7.0 (d, J = 9 Hz, 1H, H-pyrid.), 7.30–7.45 (m, 3H, H-arom.), 7.70–7.90 (m, 3H, H-pyrid., 2H-arom.). This ester subsequently was treated with HCl 5% and the corresponding acid was used for preparation of the amide **4e.**

General method for preparation of the acids 3b–3d, 3f–3h and 3i–3l

3-[6-Chloro-pyridazin-3(2H)-one-2-yl]-propanoic acid (3b). To a solution of (2 g 20 mmol) 6-chloro-pyridazin-3(2H)-one in 75 mL acetone were added (3.2 g 30 mmol)

of dry K_2CO_3 and (4.16 g 30 mmol) of ethyl 3-bromopropionate. This mixture was stirred and refluxed for 24 h. After the reaction was filtered and evaporated under reduced pressure, the residue was purified by chromatography silica gel using as eluent a stepwise gradient of ethanol (0–3%) in CH_2Cl_2, gave ethyl 3-(6-chloropyridazin-3(2H)-one-2-yl)-propionate (dense oil, yield: 70%). ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 1.30 (t, $J=7$ Hz, 3H, CH_3), 2.85 (t, $J=6$ Hz, 2H, CH_2), 4.15 (q, $J=7$ Hz, 2H, CH_2), 4.4 (t, $J=6$ Hz, 2H, CH_2); 6.90 (d, $J=9$ Hz, 1H, H-pyrid.), 7.20 (d, $J=9$ Hz, 1H, H-pyrid.).

This ester was treated with hydrochloric acid 5% (15 mL) and the mixture was refluxed under stirring for 5 h, obtaining the corresponding acid, used without further purification for the preparation of amide **4b**.

4-[6-Chloro-pyridazin-3(2H)-one-2-yl]-butanoic acid (3c). Prepared with the same method described for **3b** starting ethyl 4-bromobutyrate, gave ethyl 4-[6-chloro-pyridazin-3(2H)-one-2-yl]-butyrate (dense oil, yield: 75%).^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 2.15 (q, $J=6$ Hz, 2H, CH_2), 2.40 (t, $J=6$ Hz, 2H, CH_2), 4.05–4.25 (m, 4H, $2CH_2$); 6.90 (d, $J=9$ Hz, 1H, H-pyrid.), 7.20 (d, $J=9$ Hz, 1H, H-pyrid.), which treated with 5% hydrochloric acid, gave the corresponding acid, was used for the preparation of amide **4c**.

5-[6-Chloro-pyridazin-3(2H)-one-2-yl]-pentanoic acid (3d). Prepared with the same method of **3b** using ethyl 5-bromovalerate gave ethyl 5-[6-chloro-pyridazin-3(2H)-one-2-yl]-valerate (dense oil, yield: 75%).^{1}H NMR ($CDCl_3$) δ 1.15 (t, $J=7$ Hz, 3H, CH_3), 1.70–1.80 (m, 4H, $2CH_2$), 2.15 (t, $J=6$ Hz, 2H, CH_2), 3.90–4.10 (m, 4H, $2CH_2$), 6.80 (d, $J=9$ Hz, 1H, H-pyrid.), 7.10 (d, $J=9$ Hz, 1H, H-pyrid.). This ester was treated with 5% hydrochloric acid, obtaining the corresponding acid, used for the preparation of amide **4d**.

3-[6-Phenyl-pyridazin-3(2H)-one-2-yl]-propanoic acid (3f). Prepared with the same method of **3b** using ethyl 3-bromopropionate gave ethyl 3-[6-phenyl-pyridazin-3(2H)-one-2-yl]-propionate (dense oil, yield: 50%). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 2.90 (t, $J=6$ Hz, 2H, CH_2), 4.10 (q, $J=7$ Hz, 2H, CH_2), 4.50 (t, $J=6$ Hz, 2H, CH_2), 7.00 (d, $J=9$ Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.65 (d, $J=9$ Hz, 1H, H-pyrid.), 7.70–7.80 (m, 2H, H-arom.). This ester was treated with hydrochloric acid 5%, obtaining the corresponding acid, which was used for the preparation of amide **4f**.

4-[6-Phenyl-pyridazin-3(2H)-one-2-yl]-butanoic acid (3g). Prepared with the same method of **3b** using ethyl 4-bromobutyrate gave ethyl 4-[6-phenyl-pyridazin-3(2H)-one-2-yl]-butyrate (dense oil, yield: 80%). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 2.25 (q, $J=6$ Hz, 2H, CH_2), 2.45 (t, $J=6$ Hz, 2H, CH_2), 4.15 (q., $J=7$ Hz, 2H, CH_2), 4.30 (t, $J=6$ Hz, 2H, CH_2), 7.00 (d, $J=9$ Hz, 1H, H-pyrid.), 7.40–7.50 (m, 3H, H-arom.), 7.65 (d, $J=9$ Hz, 1H, H-pyrid.), 7.80–7.90 (m, 2H, H-arom.). This ester was treated with 5% hydrochloric acid, obtaining the corresponding acid, used for the preparation of amide **4g**.

5-[6-Phenyl-pyridazin-3(2H)-one-2-yl]-pentanoic acid (3h). Prepared with the same method of **3b** using ethyl 5-bromovalerate gave ethyl 5-[6-phenyl-pyridazin-3(2H) one-2yl]-valerate (dense oil, yield: 55%).^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 1.70–1.80 (m, 4H, $2CH_2$), 2.30 (t, $J=6$ Hz, 2H, CH_2), 4.00 (q, $J=7$ Hz, 2H, CH_2), 4.25 (t, $J=6$ Hz, 2H, CH_2), 6.95 (d, $J=9$ Hz, 1H, H-pyrid.), 7.30–7.40 (m, 3H, H-arom.), 7.70 (d, $J=9$ Hz, 1H, H-pyrid.), 7.80–7.90 (m, 2H, H-arom.). This ester was treated with 5% hydrochloric acid, gave the corresponding acid, which was used for the preparation of amide **4h**.

3-[Pyridazin-3(2H)-one-2-yl]-propanoic acid (3i). Prepared with the same method of **3b** using ethyl 3-bromopropionate gave ethyl 3-[pyridazin-3(2H)-one-2-yl]-propionate (dense oil, yield: 70%). ^{1}H NMR ($CDCl_3$) δ 1.35 (t, $J=7$ Hz, 3H, CH_3), 2.85 (t, $J=6$ Hz, 2H, CH_2), 4.15 (q, $J=7$ Hz, 2H, CH_2), 4.45 (t, $J=6$ Hz, 2H, CH_2), 6.90 (dd, $J=9$ Hz e 2 Hz, 1H, H-pyrid.), 7.20 (dd, $J=9$ Hz e 5 Hz, 1H, H-pyrid.), 7.70–7.80 (m, 1H, H-pyrid.). This ester was treated with 5% hydrochloric acid, obtaining the corresponding acid, used for the preparation of amide **4i**.

4-[Pyridazin-3(2H)-one-2-yl]-butanoic acid (3l). Prepared with the same method of **3b** using ethyl 4-bromobutyrate gave ethyl 4-[pyridazin-3(2H)-one-2-yl]-butyrate (dense oil, yield: 95%).^{1}H NMR ($CDCl_3$) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 2.20 (q, $J=6$ Hz, 2H, CH_2), 2.40 (t, $J=6$ Hz, 2H, CH_2), 4.10–4.30 (m, 4H, $2CH_2$), 6.90 (dd, $J=9$ Hz e 2 Hz, 1H, H-pyrid.), 7.20 (dd, $J=9$ Hz e 5 Hz, 1H, H-pyrid.), 7.70–7.80 (m, 1H, H-pyrid.). This ester was treated with 5% hydrochloric acid, obtaining the corresponding acid, used for the preparation of amide **4l**.

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References

1. Olsson, R. A.; Pearson, J. D. *Pharmacol. Rev.* **1990**, *3*, 761.
2. Rossi, N. F.; Churchill, P. C.; Jacoson, K. A.; Leahy, A. E. *Pharmacol. Exper. Ther.* **1987**, *240*, 911.
3. Londson, C.; Cooper, D. M.; Woff, J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 2551.
4. Hillaire-Buys, D.; Bertrand, G.; Gross, R. *Eur. J. Pharmacol.* **1987**, *136*, 109.
5. Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407.
6. Stiles, G. L. *J. Biol. Chem.* **1992**, *267*, 6451.
7. Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.
8. Fredholm, B. B.; Pearson, C. G. A. *Eur. J. Pharmacol.* **1982**, *8*, 673.

9. Shamin, M. T.; Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W. *J. Med. Chem.* **1988**, *31*, 613.
10. Shamin, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1989**, *32*, 1231.
11. Martinson, E. A.; Wells, J. M. *Mol. Pharmacol.* **1987**, *31*, 247.
12. Corsano, S.; Strappaghetti, G. *Arch. Pharm.* **1991**, *324*, 999.
13. Corsano, S.; Scapicchi, R.; Strappaghetti, G. *Arch. Pharm.* **1994**, *327*, 631.
14. Corsano, S.; Strappaghetti, G.; Scapicchi, R.; Lucacchini, A.; Senatore, G. *Arch. Pharm.* **1995**, *328*, 654.
15. Traube, W. *Ber. Deut. Chem. Ges.* **1900**, *33*, 3035.
16. Papesch, V.; Schroeder, E. F. *J. Org. Chem.* **1951**, *1879*.
17. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
18. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.