In search of a new prototype in CK2 inhibitors design

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Dedicated to Professor Julio Álvarez-Builla on the occasion of his 65th birthday

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Abstract
A series of purines have been synthesized and the compounds have been tested for their inhibitory activity against CK2. Some of them have shown an interesting activity, demonstrating that this purine-based scaffold can be considered as a new starting point for the design of CK2 inhibitors. They are readily synthesized by S-alkylation followed by N9-alkylation of 6-mercaptopurine. Docking studies have allowed us to identify ligand-CK2 interactions that account for the molecular recognition process, and help to further optimize this family of compounds as CK2 inhibitors.

Keywords: CK2, purines, antitumor, docking

Introduction

Protein kinase CK2 (formerly casein kinase 2) participates in the regulation of great number of fundamental cellular processes in eukaryotic cells. A considerable part of the cellular phosphoproteome can be associated with the catalytic activity of CK2. This ubiquitously expressed protein kinase regulates crosstalk among multiple signaling pathways critical for cell differentiation, proliferation, and survival.[1] CK2 is a tetramer composed of two catalytic subunits, CK2α and CK2α', and two regulatory, CK2β subunits. According to genetic studies performed in yeast, knockout of CK2α and CK2α' results in lethality, providing evidence for an essential role of CK2 for survival.[2,3] Furthermore, knockout of CK2α' in mice results in viable animals with defects in spermatogenesis,[4] and knockout of CK2β results in embryonic lethality, revealing the functional importance of this subunit.[5] A number of experimental data indicates

that elevated CK2 activity is functionally linked to different cancer types.[6,7] Cancer cells with activated CK2 signaling pathways show distinct features such as enhanced growth and survival, as well as rapid adaptation to stress. CK2 is involved in oncogenesis by regulation of various oncogenes, tumor suppressor proteins, and protection of antiapoptotic proteins from caspase-mediated cleavage.[8] Moreover, CK2 overexpression is an unfavorable prognostic marker in acute myeloid leukemia, prostate and lung cancer.[9-11] Additionally, several viral proteins have been shown to be CK2 substrates, indicating a role for this enzyme in viral infections.[12-15] Therefore, the pharmacological inhibition of CK2 appears as a promising strategy in order to better understand its various cellular functions. Various classes of ATP-site directed inhibitors of CK2 have been reported (Figure 1). TBB **1**,[16] DMAT **2**,[17,18] and ellagic acid **3**,[19] with IC_{50} values of 0.9, 0.14 and 0.04 μM, respectively, are representative of this class of inhibitors.

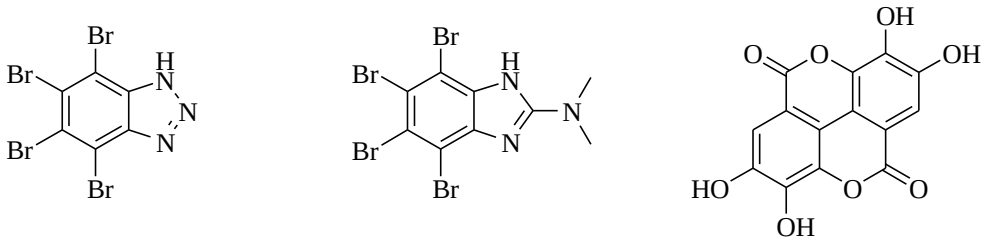
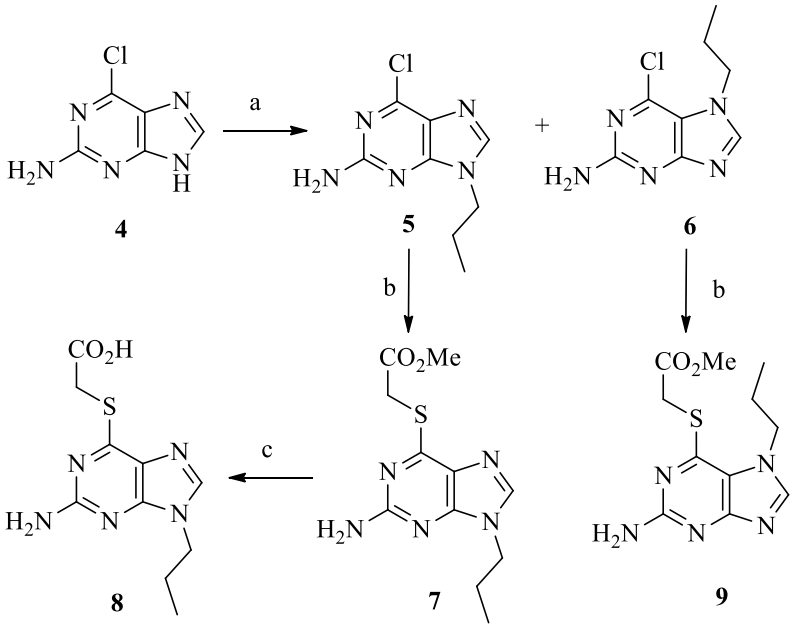


Figure 1. ATP-site directed inhibitors of CK2.

However, none of the already described CK2 inhibitors fulfill the requirements for successful clinical settings, and the design of new CK2 inhibitors is desired to effectively suppress different pathologies, such as cancer.

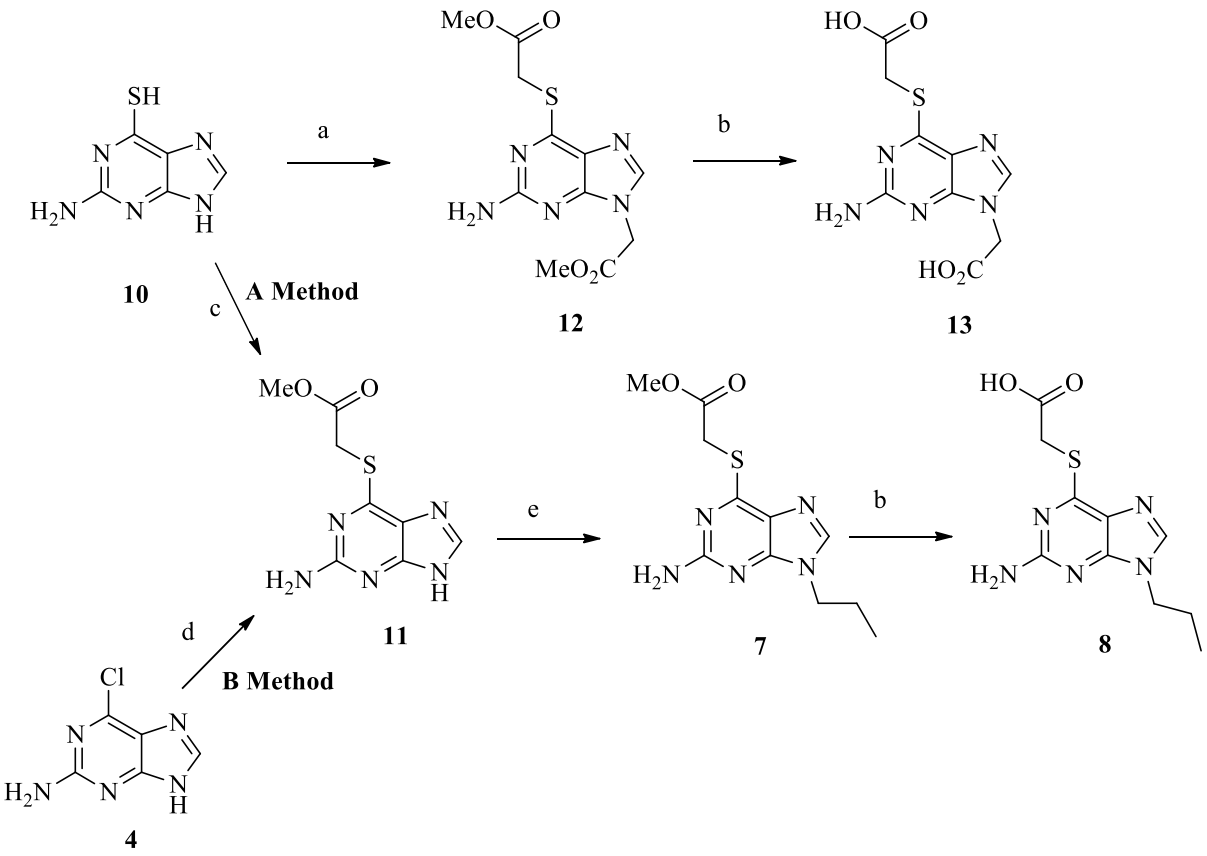
Results and Discussion

In this work a series of purines with different substitutions at positions 6, 7 and 9 were synthesized. The first step in the synthesis of purine **8** was the alkylation of commercially available 6-chloro-9*H*-purin-2-amine **4** with 1-iodopropane in the presence of NaH. In this reaction, an 8:1 mixture of **5**:**6** regioisomers was obtained, which had to be separated by column chromatography. The major isomer **5** was used in the synthesis of **7** by reaction with ethyl 2-mercaptoacetate and $NaOCH_3$/MeOH. Basic hydrolysis of **7** gave carboxylic acid **8**, which was finally obtained with an overall 53% yield (Scheme 1).



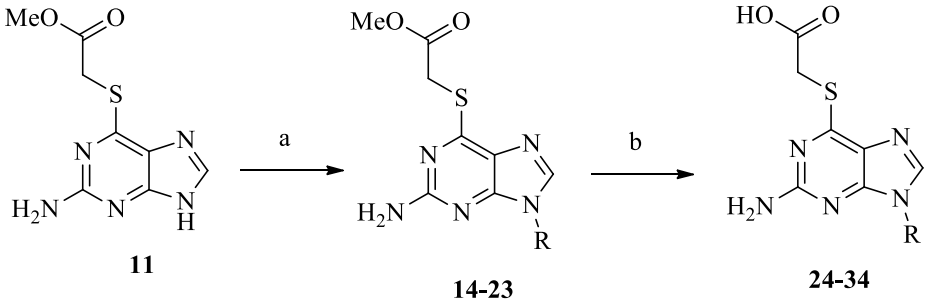
Scheme 1. Reagents and conditions: (a) 1-iodopropane, NaH, DMF, 0 ºC; (b) $HSCH_2CO_2Et$, NaOMe/MeOH, H_2O cat., 70 ºC; (c) NaOH, THF/H_2O

Compound **6** was reacted with ethyl 2-mercaptoacetate to obtain **9**, which was also biologically evaluated. ^{1}H-NMR spectra of **7** and **9** showed signals for the aromatic proton at 7.62 and 7.78 ppm, respectively. In order to avoid the time-consuming chromatography separation of regioisomers **5** and **6**, and to improve the yield of the process, an alternative pathway for the synthesis of **8** was developed (Scheme 2). Thus, 6-thioguanine **10** was S-alkylated by reaction with 1 equivalent of methyl bromoacetate, in the presence of K_2CO_3 to yield ester **11** in 64% yield (A method). In this reaction the amount of methyl bromoacetate must be carefully controlled, as an excess of the alkylating agent brings about the formation of polyalkylated compound **12**, which is the only product when 2 equivalents are used. Hydrolysis of **12** gave **13**, which was also tested for its activity against CK2. An alternative for the synthesis of **11** is the reaction between 6-chloro-9*H*-purin-2-amine **4** and ethyl 2-mercaptoacetate (B method). Although the yield of the reaction is similar, the lower price of the starting material makes it advantageous compared to the previous method.



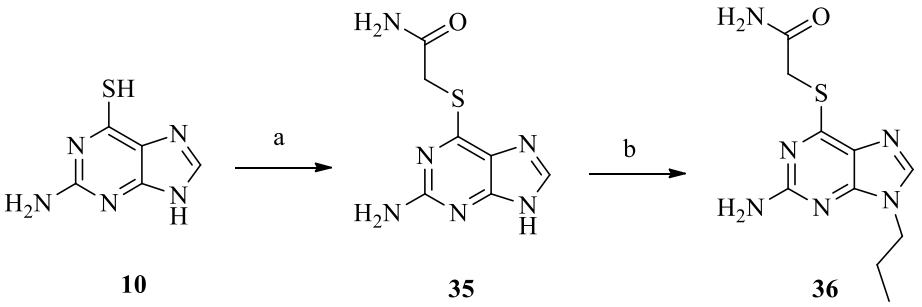
Scheme 2. Reagents and conditions: (a) methyl bromoacetate (2 equiv.), K_2CO_3, DMF; (b) NaOH, THF/H_2O; (c) methyl bromoacetate (1 equiv.), K_2CO_3, DMF; (d) $HSCH_2CO_2Me$, NaOMe/MeOH, H_2O cat., 70 ºC; (e) $CH_3CH_2CH_2I$, K_2CO_3, DMF

Spectroscopic data for compounds **7** and **8** synthesized following this pathway are identical to those from the sample obtained by the route described in Scheme 1, and these spectral data confirm our previous structural assignment for isomers **5** and **6**. This synthetic route allowed us the access to a series of esters **14-23**, differently substituted at N-9, by reaction of **11** with the corresponding alkyl bromide. Hydrolysis in basic conditions of the esters gave the corresponding acids **24-34** (Scheme 3).



Scheme 3. Reagents and conditions: (a) R-X, K_2CO_3, DMF; (b) NaOH THF/H_2O.

Amides **35** and **36** were obtained following the method A (Scheme 2) using chloroacetamide instead of methyl bromoacetate (Scheme 4).

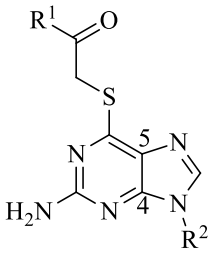


10 **35** **36**

Scheme 4. Reagents and conditions: (a) $ClCH_2CONH_2$, K_2CO_3, DMF; (b) $CH_3CH_2CH_2I$, K_2CO_3, DMF.

Selected compounds, listed in Table 1, were tested using the CK2 radiometric assay. The assays were performed at 10 μM concentration of the appropriate compound in the presence of catalytic subunit CK2α and [γ-^{32}P]ATP. The kinase activity was measured as the quantity of ^{32}P incorporated into substrate.

Table 1. Remaining CK2 activity after treatment with a 10 μM solution of purine derivatives



Compound	R^1	R^2	Activity [%]
7	OMe	CH_3CH_2 CH_2	75.4±7.4
8	OH	CH_3CH_2 CH_2	100
9*	OMe	CH_3CH_2 CH_2	93.46±3.18
11	OMe	H	n.d.
12	OMe	$CH_3O_2CCH_2$	100
13	OH	HO_2CCH_2	95.29±5.54
14	OMe	$HOCH_2CH_2$	84.86±9.23
15	OMe	$CH_3OCH_2CH_2$	60.73±3.67
16	OMe	$C_3H_5CH_2$	73.32±3.59
17	OMe	CH_2=$CHCH_2$	85.02±8.19

Table 1. Continued

Compound	R1	R2	Activity [%]
18	OMe	$(CH_3CH_2)_2NCH_2CH_2$	n.d.
19	OMe	$C_6H_5CH_2$	76.38±3.2
20	OMe	*p*-$CH_3OC_6H_4CH_2$	71.65±2.89
21	OMe	*p*-$ClC_6H_4CH_2$	92.2±5.27
22	OMe	*p*-$NO_2C_6H_4CH_2$	80.36±9.54
23	OMe	*p*-$CF_3C_6H_4CH_2$	78.61±1.94
24	OH	$HOCH_2CH_2$	n.d.
25	OH	$CH_3OCH_2CH_2$	100
26	OH	$C_3H_5CH_2$	86.6±7.67
27	OH	CH_2=$CHCH_2$	82.6±6.96
28	OH	$(CH_3CH_2)_2NCH_2CH_2$	n.d.
29	OH	$C_6H_5CH_2$	100
30	OH	*p*-$CH_3OC_6H_4CH_2$	n.d.
31	OH	*p*-$ClC_6H_4CH_2$	71.6±4.46
32	OH	*p*-$NO_2C_6H_4CH_2$	n.d.
33	OH	*p*-$CF_3C_6H_4CH_2$	76.3±5.92
34	OH	H	65.2±5.07
35	NH_2	H	64.31±6.09
36	NH_2	CH_3CH_2 CH_2	74.02±7.41

* Purine N-7 substituted.

In general, ester and amide functionalities in R^1 were found to achieve better activity (compounds **15**, **20**, **35**, **7**, and **36**), although the presence of polar groups in R^2 (compounds **12**, and **13**) leads to the decrease in the activity (Table 1). The best inhibitions were obtained with short alkyl or alkoxy chains at R^2 (compounds **7**, **15**, and **36**). The absence of R^2 group leads to the active compound **35**. Carboxylic acid derivatives are less active although, again, the absence of substituents in R^2 leads to the active compound **34**.

Molecular modeling was performed with the aim of revealing the potential interactions that govern the recognition and the binding of this family of compounds into CK2. The automated docking calculations of selected compounds into the CK2 (PDB code: 1DAW) were performed using Glide 5.5 (Grid-based Ligand Docking with Energetics) in extra precision mode and following the protocol described in the Experimental Section.

We selected 14 compounds bearing different functional groups at R^1 position: esters **7**, **15**, **16**, **17**, **22**, and **23**; carboxylic acids **8**, **13**, **26**, **27**, **29** and **33**; and amides **35**, and **36**. In order to estimate the ability of these compounds to mimic ATP adenosine (ANP), a post-docking step was performed by carrying out root-mean-square deviation (RMSD) calculations for junction carbons C-4 and C-5, using ANP as reference molecule (Table 2).

Table 2. RMSD values for selected compounds with reference to ANP

Compound	RMSD [Å]	Compound	RMSD [Å]
17	0.69	**36**	1.22
15	0.87	**13**	1.66
26	1.05	**35**	1.84
27	1.08	**29**	4.48
8	1.08	**22**	5.18
7	1.18	**23**	5.28
16	1.21	**33**	8.03

All compounds were predicted to be able to form stable complexes with CK2 and occupying the ANP binding site. Among all predicted binding orientations, the representatives that better mimicked the adenosine ring by establishing hydrophobic interactions with key residues like Ile^{66}, Met^{163}, Val^{53}, Ile^{174} or Val^{116} were selected (ligands with RMSD values from 0.69 to 1.84 Å, see Table 2). Figure 2 shows the docked complex predicted for compound **15**, which is the purine derivative with the highest inhibitory activity of all the synthesized compounds, inside CK2, superimposed with ANP. Significant differences were found for compounds with an aromatic ring as R^2 (**22**, **23**, **29**, and **33**, see Table 2) that led to higher RMSD values. This fact could be pointing to the loss of adenosine mimicking ability. In spite of this fact, only compound **29** showed a complete loss of activity.

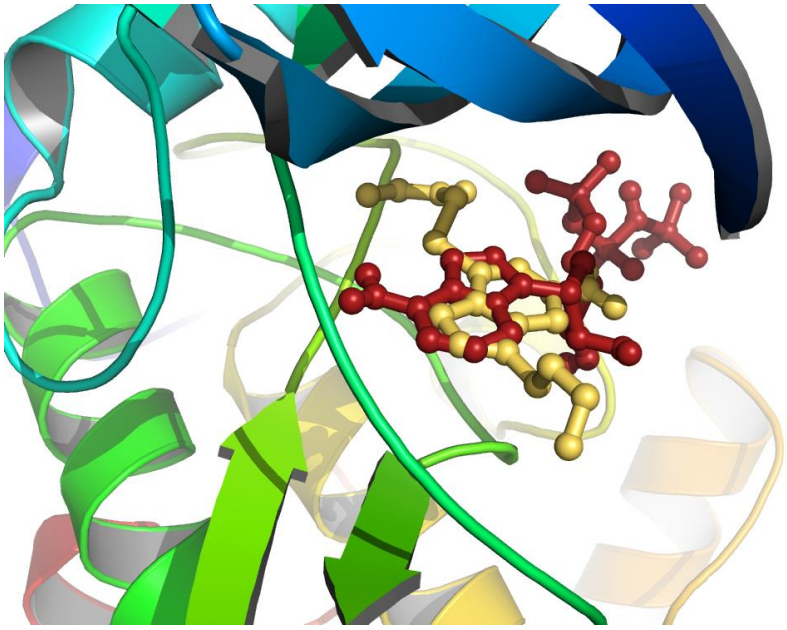


Figure 2. Predicted mode of binding of **15** (light yellow) superimposed to crystallographic ANP (brick red) inside CK2 (PDB code: 1DAW).

A second common feature was found for ligands with low RMSD values. Binding poses showed that the R^2 chain is pointing towards the entrance of the ATP binding pocket, establishing hydrophobic interactions with Val^{45}, Tyr^{115} and Ile^{66} side chains. On the opposite side of the binding pocket, ester, acid or amide R^1 groups are located, establishing electrostatic interactions with Lys^{68} or Asp^{175} side chains (Figure 3). Additionally, compounds **8**, **13**, **15**, **17**, **26**, and **27** are able to establish hydrogen bonds with Lys^{68} side chain and the backbone nitrogen of Asp^{175}. In a few cases those hydrogen bonds are found with Lys^{43} **7**, **16**.

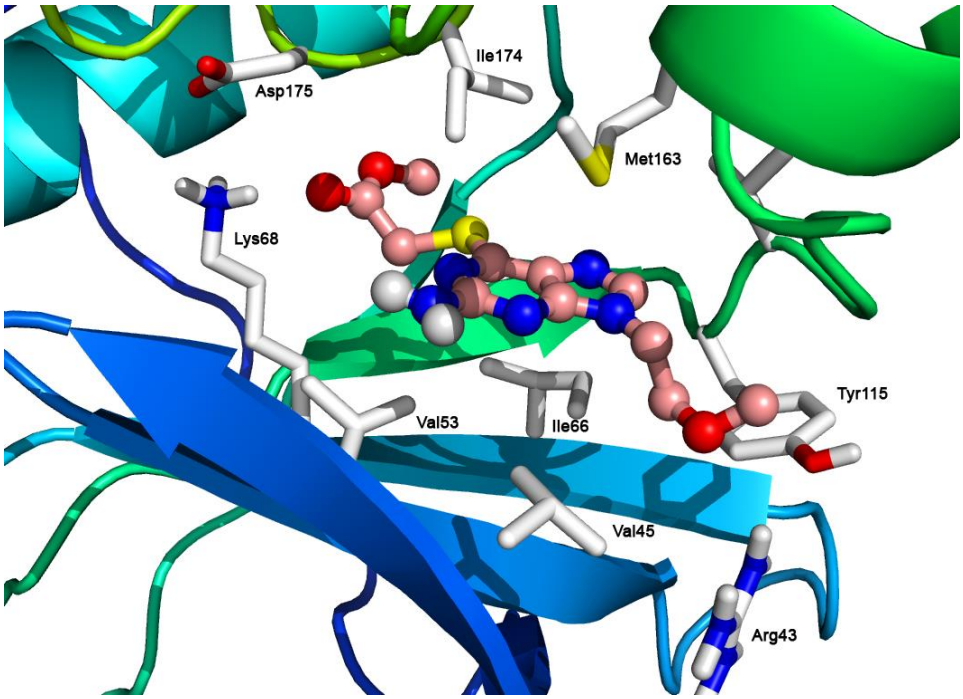


Figure 3. Predicted mode of binding of **15** (pink carbons) with key residues (white carbons) of the ATP binding pocket inside CK2 (PDB code: 1DAW).

Conclusions

Novel purine-based scaffolds are proposed as promising CK2 inhibitors. They are readily synthesized by S-alkylation followed by N9-alkylation of 6-mercaptopurine. Docking studies have allowed us to identify ligand-CK2 interactions that account for the molecular recognition process, and can help to further optimize this family of compounds as CK2 inhibitors.

Experimental Section

General. Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. ^{1}H and ^{13}C NMR data were recorded on a Bruker 300-AC instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (*J*) are in hertz. Mass spectra were run on a Bruker Esquire 3000 spectrometer. Elemental analyses (C, H, N, S) were performed on a LECO CHNS-932 apparatus at the Microanalyses Service of the University Complutense of Madrid; unless otherwise stated all reported values are within ± 0.4% of the theoretical compositions. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. Unless stated otherwise, starting materials used were high-grade commercial products.

Alkylation of 6-chloro-9*H*-purin-2-amine (4). To a solution of 6-chloro-9*H*-purin-2-amine **4** (509 mg, 3 mmol) in dry DMF (20 ml) at 0 ºC was added NaH (120 mg, 3 mmol). After stirring for 30 min, 1-iodopropane was added and the mixture was stirred at 0 ºC for 5 h. Then, the solution was concentrated in vacuum and the solid obtained was suspended in H_2O and extracted with DCM. The extracts were dried ($MgSO_4$), filtered, evaporated to dryness, and the residue was chromatographed on silica gel (DCM:MeOH 60:1 to 25:1) to give **5** (475 mg, 75%) and **6** (62 mg, 10%) as white solids.

6-Chloro-9-propyl-9*H*-purin-2-amine (5). IR: 3390, 3300, 3150, 2960, 2925 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, *J* = 7.40 Hz, 3H, CH_3), 1.84-1.93 (m, 2H, CH_2), 4.03 (t, *J* = 7.14 Hz, 2H, CH_2N), 5.29 (bs, 2H, NH_2) 7.75 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 11.1, 22.9, 45.4, 125.2, 142.4, 151.1, 153.8, 159.0. MS (ESI): *m/z* 324.20 $[M+Na]^+$. Anal. Calcd. for $C_8H_{10}ClN_5$: C, 45.40; H, 4.76; N, 33.09. Found: C, 45.09; H, 4.66; N, 32.81.

6-Chloro-7-propyl-7*H*-purin-2-amine (6) IR: 3400, 3320, 3150, 2970, 2920, cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.83 (t, *J* = 7.35 Hz, 3H, CH_3), 1.74-1.87 (m, 2H, CH_2), 4.24 (t, *J* = 6.72 Hz, 2H, CH_2N), 6.65 (bs, 2H, NH_2) 8.38 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 10.6, 24.1, 47.7, 114.8, 142.2, 149.5, 159.9, 164.3. MS (ESI): *m/z* 324.20 $[M+Na]^+$. Anal. Calcd. for $C_8H_{10}ClN_5$: C, 45.40; H, 4.76; N, 33.09. Found: C, 45.22; H, 4.78; N, 32.86.

Methyl [(2-amino-9-propyl-9*H*-purin-6-yl)sulfanyl]acetate (7). Method A. To a solution of **5** (379 mg, 1.8 mmol) and $NaOCH_3$ (1 g, 18 mmol) in MeOH (15 ml), ethyl sulfanylacetate (0.79 ml, 7.2 mmol) and H_2O (0.1 ml) were added. The reaction mixture was heated at 70 ºC for 6 h in a sealed tube. The solution was concentrated in vacuum, suspended in H_2O and extracted with DCM. The extracts were dried ($MgSO_4$), filtered, evaporated to dryness and the residue was chromatographed on silica gel (hexane:AcOEt 1:4) to give **7** (359 mg, 71%) as a white solid, mp 104.7-106.5 ºC (AcOEt/hexane). IR: 3480, 3280, 3160, 3085, 2920, 1720, cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, *J* = 7.50 Hz, 3H, CH_3), 1.77-1.90 (m, 2H, CH_2), 3.72 (s, 3H, CH_3O) 3.98 (t, *J* = 7.14 Hz, 2H, CH_2N) 4.05 (s, 2H, CH_2S), 4.88 (bs, 2H, NH_2) 7.62 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 10.8, 22.7, 30.3, 44.7, 52.4, 125.0, 140.1, 150.6, 158.5, 158.6, 169.5. MS (ESI): *m/z*

282.00 $[M+H]^+$, 303.99 $[M+Na]^+$. Anal. Calcd. for $C_{11}H_{15}N_5O_2S$: C, 46.96; H, 5.37; N, 24.89; S, 11.40. Found: C, 46.85; H, 5.25; N, 24.76; S, 11.41.

Methyl [(2-amino-7-propyl-7*H*-purin-6-yl)sulfanyl]acetate (9). The same method described above was used for the synthesis of **9**. Thus, from **6** (195 mg, 0.92 mmol), $NaOCH_3$ (515 mg, 9.2 mmol) and ethyl sulfanylacetate (0.41 ml, 3.68 mmol) and after chromatography (AcOEt:MeOH 25:1) compound **9** (82 mg, 32%) was obtained as a white solid, mp 155.1-156.3 ºC (AcOEt). IR: 3480, 3280, 3150, 1720 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (t, *J* = 7.7 Hz, 3H, CH_3), 1.79-1.91 (m, 2H, CH_2), 3.71 (s, 3H, CH_3O), 4.04 (s, 2H, CH_2S), 4.17 (t, *J* = 7.7 Hz, 2H, CH_2N), 5.22 (bs, 2H, NH_2) 7.78 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 10.6, 24.6, 30.3, 48.3, 52.5, 116.0, 147.7, 150.4, 159.4, 161.3, 169.2. MS (ESI): *m/z* 282.44 $[M+H]^+$, 304.11 $[M+Na]^+$. Anal. Calcd. for $C_{11}H_{15}N_5O_2S$: C, 46.96; H, 5.37; N, 24.89; S, 11.40. Found: C, 47.08; H, 5.39; N, 24.90; S, 11.45.

Methyl [(2-amino-9*H*-purin-6-yl)sulfanyl]acetate (11). Method A. To a solution of 6-thioguanine **10** (334 mg, 2 mmol) in DMF (5 ml), was added K_2CO_3 (285 mg, 2 mmol). After 30 min methyl bromoacetate (306 mg, 2 mmol) was added and the mixture was stirred for 2 h. The solution was concentrated in vacuum, solved in DCM:MeOH 3:1 and the insoluble residue was removed by filtration. The organic layer was concentrated in vacuum and purified by chromatography on silicagel (DCM:MeOH 20:1 to 10:1) to give **11** (307 mg, 64%) as a yellowish solid mp. 200.1-203.5 ºC.

Method B. To a solution of 6-chloro-9H-purin-2-amine **4** (5.1 g, 30 mmol) in MeOH (30 ml), were added $NaOCH_3$ (8.1 g, 150 mmol), ethyl sulfanylacetate (6.6 ml, 60 mmol) and H_2O (0.5 ml). The reaction mixture was heated for 4 h at 80 ºC in a sealed tube. After completion of the reaction, the solution was concentrated in vacuum, the residue was solved in DCM:MeOH 3:1. The insoluble salts were removed by filtration and the organic layer was concentrated in vacuum and purified by chromatography on silica gel (AcOEt:MeOH 25:1) to give **11** (3.95 g, 55%) as a white solid, mp 200.1-203.5 ºC (AcOEt). IR: 3480, 3360, 1730 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.66 (s, 3H, CH_3O), 4.21 (s, 2H, CH_2S), 6.36 (sa, 2H, NH_2) 7.93 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 29.7, 52.4, 123.7, 139.2, 152.0, 157.0, 159.5, 169.6. $[M+Na]^+$. Anal. Calcd. for $C_8H_9N_5O_2S$: C, 40.16; H, 3.79; N, 29.27; S, 13.40. Found: C, 40.34; H, 3.93; N, 28.80; S, 13.43.

General procedure for the alkylation of purines at N-9 using K_2CO_3 as a base

To a suspension of the purine (1 equiv) in DMF was added K_2CO_3 (1.5 equiv.). After 30 min of stirring at room temperature the respective alkyl bromide or chloride was added (1.1 to 2.0 equiv.). The mixture was stirred until no evolution of reaction was observed (8 - 24 h). The solution was concentrated in vacuum, the residue suspended in H_2O and extracted with DCM. The extracts were dried ($MgSO_4$), filtered, evaporated to dryness and the residue was chromatographed on silica gel.

Methyl [(2-amino-9-propyl-9*H*-purin-6-yl)sulfanyl]acetate (7). Method B. From **11** (720 mg, 3 mmol) in DMF (15 ml), K_2CO_3 (500 mg, 3 mmol) and 1-iodopropane (0.29 ml, 3 mmol). Reaction time: 2h. Chromatographed using hexane:AcOEt 1:5 as eluent. Compound **7** (365 mg,

31%) was obtained as a white solid showing the same spectroscopic data as the sample obtained by A Method (see above).

Methyl {[2-amino-9-(2-methoxy-2-oxoethyl)-9*H*-purin-6-yl]sulfanyl}acetate (12). From 6-thioguanine (**10**) (167 mg, 1 mmol) in DMF (5 ml), K_2CO_3 (142 mg, 1 mmol) and methyl bromoacetate (0.19 ml, 2 mmol). Reaction time: 2h. Chromatographed using (DCM:MeOH 60:1 to 25:1) as eluent. Compound **12** (223 mg, 72%) was obtained as a white solid, mp 155.2-157.1 ºC (EtOH). IR: 3410, 3320, 3200, 2980, 2910, 1720 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.74 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 4.07 (s, 2H, CH_2S) 4.82 (s, 2H, CH_2N) 4.93 (bs, 2H, NH_2) 7.69 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 30.7, 43.6, 52.7, 52.9, 124.9, 140.3, 155.0, 158.9, 159.5, 167.6, 169.7. $[M+Na]^+$. MS (ESI): *m/z* 334.17 $[M+Na]^+$. Anal. Calcd. for $C_{11}H_{13}N_5O_4S$: C, 42.44; H, 4.21; N, 22.50; S, 10.30. Found: C, 42.30; H, 4.33; N, 22.05; S, 10.10.

Methyl {[2-amino-9-(2-hydroxyethyl)-9*H*-purin-6-yl]sulfanyl}acetate (14). A slight modification of the general procedure was used. Thus, to a solution of **11** (400 mg, 1.67 mmol) in DMF (10 ml) was added K_2CO_3 (346 mg, 2.51 mmol). After 30 min, 2-bromoethanol (0.24 ml, 3.34 mmol) was added and the mixture was stirred overnight. The solution was concentrated in vacuum, the residue was solved in DCM:MeOH 3:1, the insoluble salts were removed by filtration. The organic layer was concentrated in vacuum and the residue was recrystallized from EtOH and water to obtain **14** (279 mg, 59%) as a white solid, mp 150.5-152.0 ºC. IR: 3410, 3280, 3150, 1715 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.66 (s, 3H, CH_3O), 3.69 (t, *J* = 5.3 Hz, 2H, CH_2N), 4.06 (t, *J* = 5.3 Hz, 2H, CH_2O), 4.21 (s, 2H, CH_2S), 5.01 (bs, 1H, OH), 6.47 (bs, 2H, NH_2), 7.91 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 29.7, 45.5, 52.4, 59.0, 123.9, 141.7, 151.3, 157.3, 159.3, 169.5. Anal. Calcd. for $C_{10}H_{13}N_5O_3S$: C, 42.39; H, 4.63; N, 24.72, S, 11.32. Found: C, 42.38; H, 4.70; N, 24.13; S, 11.14.

Methyl {[2-amino-9-(2-methoxyethyl)-9*H*-purin-6-yl]sulfanyl}acetate (15). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 2-chloroethyl methyl ether (0.09 ml, 0.92 mmol). Reaction time: 24 h. Chromatographed using DCM:MeOH (100:1 to 40:1) as eluent. Compound **15** (182 mg, 73%) was obtained as a white solid, mp 146.5-147.6 ºC (AcOEt/hexano). IR: 3420, 3300, 3170, 3070, 2980, 1730 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.31 (s, 3H, CH_3O), 3.64 (t, *J* = 5.0 Hz, 2H, CH_2), 3.74 (s, 3H, CH_3OCO), 4.01 (s, 2H, CH_2S), 4.20 (t, *J* = 5.0 Hz, 2H, CH_2), 5.00 (bs, 2H, NH_2), 7.75 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 30.6, 43.1, 52.6, 58.9, 70.4, 125.2, 141.3, 150.7, 158.6, 159.0, 169.7. MS (ESI): *m/z* 298.11 $[M+H]^+$, 320.11 $[M+Na]^+$. Anal. Calcd. for $C_{11}H_{15}N_5O_3S$: C, 44.43; H, 5.08; N, 23.55; S, 10.78. Found: C, 44.40; H, 5.06; N, 23.22; S, 10.65.

Methyl {[2-amino-9-(cyclopropylmethyl)-9*H*-purin-6-yl]sulfanyl}acetate (16). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and bromomethylcyclopropane (0.09 ml, 0.92 mmol). Reaction time: 24 h. Chromatographed using DCM:MeOH (100:1 to 60:1) as eluent. Compound **16** (90 mg, 37%) was obtained as a white solid, mp 166.4-167.1 ºC (AcOEt/hexano). IR: 3460, 3300, 3170, 1730 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.37-0.43 (m, 2H, CH_2), 0.61-0.67 (m, 2H, CH_2), 1.20-1.33 (m, 1H, CH), 3.75 (s, 3H, CH_3O), 3.89 (d, *J* = 7.2 Hz, 2H, CH_2N), 4.09 (s, 2H, CH_2S), 4.96 (bs, 2H, NH_2), 7.77 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 4.7,

11.4, 31.1, 48.5, 53.2, 125.9, 140.5, 151.3, 159.1, 159.5, 170.3. MS (ESI): *m/z* 294.55 $[M+H]^+$, 316.12 $[M+Na]^+$. Anal. Calcd. for $C_{12}H_{15}N_5O_2S$: C, 49.13; H, 5.15; N, 23.87; S, 10.93. Found: C, 49.39; H, 5.29; N, 23.80; S, 10.93.

Methyl {[2-amino-9-(prop-2-en-1-yl)-9*H*-purin-6-yl]sulfanyl}acetate (17). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and allyl iodide (0.09 ml, 0.92 mmol). Reaction time: 12 h. Chromatographed using hexane:AcOEt 1:4 to AcOEt 100% as eluent. Compound **17** (152 mg, 65%) was obtained as a white solid, mp 102.7-103.9 ºC (AcOEt/hexano). ^{1}H NMR ($CDCl_3$): δ 3.73 (s, 3H, CH_3O) 4.08 (s, 2H, CH_2S), 4.64 (d, *J* = 4.7 Hz, 2H, CH_2N) 5.06 (bs, 2H, NH_2), 5.08 (d, *J* = 17.0 Hz, 1H, 1/2C=CH_2), 5.26 (d, *J* = 9.9 Hz, 1H, 1/2C=CH_2), 5.90-6.03 (m, 1H, CH=C), 7.64 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 31.1, 45.7, 53.1, 119.1, 125.7, 132.2, 140.6, 151.2, 159.3, 159.6, 170.2. MS (ESI): *m/z* 279.98 $[M+H]^+$, 301.97 $[M+Na]^+$. Anal. Calcd. for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07; S, 11.48. Found: C, 47.29; H, 4.74; N, 24.92; S, 11.49.

Methyl ({2-amino-9-[2-(diethylamino)ethyl]-9*H*-purin-6-yl}sulfanyl)acetate (18) From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 2-chloro-*N*,*N*-diethylethanamine hydrochloride (158 mg, 0.92 mmol). Reaction time: 12 h. Chromatographed using DCM:MeOH (40:1 to 25:1) as eluent. Compound **18** (177 mg, 62%) was obtained as a white solid, mp 157.9-159.7 ºC. ^{1}H NMR ($CDCl_3$): δ 0.86 (t, *J* = 7.1 Hz, 6H, $2CH_3$), 2.45 (q, *J* = 7.1 Hz, 4H, $2CH_2$), 2.67 (t, *J* = 6.1 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3O), 4.00 (t, *J* = 6.1 Hz, 2H, CH_2), 4.03 (s, 2H, CH_2S), 5.16 (bs, 2H, NH_2), 7.71 (s, 1H, ArH). Anal. Calcd. for $C_{14}H_{22}N_6O_2S$: C, 49.69; H, 6.55; N, 24.83; S, 9.47. Found: C, 49.48; H, 6.64; N, 25.03; S, 9.39.

Methyl [(2-amino-9-benzyl-9*H*-purin-6-yl)sulfanyl]acetate (19). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and benzyl bromide (0,11 ml, 0.92 mmol). Reaction time: 12 h. Chromatographed using hexane:AcOEt (1:1 to 1:5) as eluent. Compound **19** (209 mg, 76%) was obtained as a white solid, mp 147.3-148.1 ºC (AcOEt/hexano). ^{1}H NMR ($CDCl_3$): δ 3.73 (s, 3H, CH_3O) 4.08 (s, 2H, CH_2S), 5.08 (bs, 2H, NH_2), 5.20 (s, 2H, CH_2Ph), 7.18-7.22 (m, 2H, ArH), 7.26-7.61 (m, 3H, ArH), 7.61 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 30.6, 46.6, 52.6, 125.1, 127.4, 128.2, 128.9, 135.5, 140.1, 151.0, 158.9, 159.2, 169.7. MS (ESI): *m/z* 352.18 $[M+Na]^+$. Anal. Calcd. for $C_{15}H_{15}N_5O_2S$: C, 54.70; H, 4.59; N, 21.26; S, 9.74. Found: C, 54.46; H, 4.78; N, 20.89; S, 9.60.

Methyl {[2-amino-9-(4-methoxybenzyl)-9*H*-purin-6-yl]sulfanyl}acetate (20). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 4-methoxybenzyl bromide (0.13 ml, 0.92 mmol). Reaction time: 12 h. Chromatographed using hexane:AcOEt (2:1 to 1:2) as eluent. Compound **20** (158 mg, 53%) was obtained as a white solid, mp 133.9-134.7 ºC (AcOEt/hexano). IR: 3450, 3280, 3150, 1720 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O) 4.16 (s, 2H, CH_2S), 5.20 (s, 2H, CH_2N), 5.35 (bs, 2H, NH_2), 6.88 (AA'XX', ArH), 7.24 (AA'XX', 2H, ArH), 7.64 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 30.6, 46.2, 52.6, 55.2, 114.2, 125.2, 127.4, 129.1, 140.1, 150.0, 158.8, 159.1, 159.4, 169.7. MS (ESI): *m/z* 360.01 $[M+H]^+$, 382.01 $[M+Na]^+$. Anal. Calcd. for $C_{16}H_{17}N_5O_3S$: C, 53.47; H, 4.77; N, 19.49; S, 8.92. Found: C, 54.57; H, 4.77; N, 19.28; S, 8.88.

Methyl {[2-amino-9-(4-chlorobenzyl)-9*H*-purin-6-yl]sulfanyl}acetate (21). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 4-chlorobenzyl chloride (148 mg, 0.92 mmol). Reaction time: 24 h. Chromatographed using hexane:AcOEt 1:4 to AcOEt 100% as elluent. Compound **21** (247 mg, 81%) was obtained as a white solid, mp 159.7-162.3 ºC (AcOEt/hexano). IR: 3470, 3300, 3170, 1720 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.74 (s, 3H, CH_3O), 4.08 (s, 2H, CH_2S), 5.03 (bs, 2H, NH_2), 5.18 (s, 2H, CH_2N), 7.15 (AA'XX', 2H, ArH), 7.29 (AA'XX', 2H, ArH), 7.62 (s, 1H, ArH). ^{13}C NMR ($CDCl_3$): δ 31.1, 46.5, 53.2, 125.6, 129.3, 129.4, 129.6, 134.5, 140.4, 151.3, 159.2, 159.9, 170.2. MS (ESI): *m/z* 363.99 $[M+H]^+$, 386.99 $[M+Na]^+$. Anal. Calcd. for $C_{15}H_{14}ClN_5O_2S$: C, 49.52; H, 3.88; N, 19.25; S, 8.81. Found: C, 49.54; H, 3.97; N, 19.12; S, 8.74.

Methyl {[2-amino-9-(4-nitrobenzyl)-9*H*-purin-6-yl]sulfanyl}acetate (22). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 4-nitrobenzyl bromide (199 mg, 0.92 mmol). Reaction time: 12 h. Chromatographed using hexane:AcOEt (1:1 to 1:2) as eluent. Compound **22** (97 mg, 31%) was obtained as a white solid, mp 142.0-143.2 ºC (AcOEt/hexano). IR: 3450, 3340, 3200, 1730 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.75 (s, 3H, CH_3O), 4.09 (s, 2H, CH_2S), 5.00 (bs, 2H, NH_2), 5.34 (s, 2H, CH_2N), 7.36 (AA'XX', 2H, ArH), 7.68 (s, 1H, ArH), 8.16 (AA'XX', 2H, ArH). ^{13}C NMR ($CDCl_3$): δ 30.6, 45.9, 52.7, 124.1, 125.1, 128.1, 139.7, 142.8, 147.6, 150.8, 158.9, 159.7, 169.6. Anal. Calcd. for $C_{15}H_{14}N_6O_4S$: C, 48.12; H, 3.77; N, 22.45; S, 8.56. Found: C, 48.20; H, 3.97; N, 22.23; S, 8.48.

Methyl ({2-amino-9-[4-(trifluoromethyl)benzyl]-9*H*-purin-6-yl}sulfanyl)acetate (23). From **11** (200 mg, 0.84 mmol) in DMF (5 ml), K_2CO_3 (179 mg, 1.26 mmol) and 4-trifluoromethylbenzyl bromide (220 mg, 0.92 mmol). Reaction time: 12 h. Chromatographed using hexane:AcOEt (1:4 to 1:9) as eluent. Compound **23** (182 mg, 55%) was obtained as a white solid, mp 150.5-152.4 ºC (AcOEt/hexano). IR: 3450, 3340, 3200, 1735 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.73 (s, 3H, CH_3O), 4.07 (s, 2H, CH_2S), 5.06 (bs, 2H, NH_2), 5.27 (s, 2H, CH_2N), 7.29 (AA'XX', 2H, ArH), 7.29 (AA'XX', 2H, ArH), 7.70 (s, 1H, ArH). MS (ESI): *m/z* 398.03 $[M+H]^+$, 419.97 $[M+Na]^+$. Anal. Calcd. for $C_{16}H_{14}F_3N_5O_2S$: C, 48.36; H, 3.55; N, 17.62; S, 8.07. Found: C, 48.45; H, 3.70; N, 17.56; S, 8.08.

2-[(2-Amino-9*H*-purin-6-yl)sulfanyl]acetamide (35). To a solution of 6-thioguanine (**10**) (500 mg, 3 mmol) in DMF (10 ml), was added K_2CO_3 (413 mg, 3 mmol). After 30 min, chloroacetamide (280 mg, 3 mmol) was added and the mixture was stirred for 12 h. The solution was concentrated in vacuum, the residue was solved in DCM:MeOH 3:1 and the insoluble salts were removed by filtration. The organic layer was concentrated in vacuum and the solid obtained was washed with water and crystallized from EtOH/H_2O to give **35** (378 mg, 56%) as a white solid, mp 258.6-260.1 ºC (lit.[20] 285 ºC dec) IR: 3480, 3360, 3260, 3080, 2780, 1700 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.89 (s, 2H, CH_2S), 6.42 (s, 2H, NH_2), 7.19 (s, 1H, 1/2$CONH_2$), 7.50 (s, 1H, 1/2$CONH_2$), 7.91 (s, 1H, ArH), 12.57 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 31.3, 123.7, 139.1, 151.9, 157.8, 159.6, 170.1. Anal. Calcd. for $C_7H_8N_6OS$: C, 37.49; H, 3.60; N, 37.48; S, 14.30. Found: C, 37.42; H, 3.68; N, 37.16; S, 14.33

2-[(2-Amino-9-propyl-9*H*-purin-6-yl)sulfanyl]acetamide (36). Following the general procedure for the alkylation of purines at N-9, from **35** (200 mg, 0.89 mmol) in DMF (10 mL), K_2CO_3 (127 mg, 0.89 mmol) and 1-iodopropane (0.09 ml, 0.89 mmol). Reaction time: 5 h. The solution was concentrated in vacuum, the residue was solved in DCM:MeOH 3:1 and the insoluble salts were removed by filtration. The organic layer was concentrated in vacuum and purified by chromatography on silicagel (DCM:MeOH 15:1) to give **36** (107 mg, 45%) as a white solid, mp. 188.2-189.3 ºC (H_2O). IR: 3400, 3320, 3200, 2950, 2860, 1650 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.81 (t, J = 7.3 Hz, 3H, CH_3), 1.72-1.79 (m, 2H, CH_2), 3.89 (s, 2H, CH_2S), 3.97 (t, J = 7.3 Hz, 2H, CH_2N), 6.56 (s, 2H, NH_2), 7.20 (s, 1H, 1/2$CONH_2$), 7.52 (s, 1H, 1/2$CONH_2$), 7.97 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 11.1, 22.7, 31.3, 44,4, 124.0, 141.2, 151.2, 158.3, 159.5, 170.2. MS (ESI): *m/z* 289.13 $[M+Na]^+$. Anal. Calcd. for $C_{10}H_{14}N_6OS$: C, 45.10; H, 5.30; N, 31.56; S, 12.04. Found: C, 45.01; H, 5.25; N, 31.31; S, 11.85

General procedure for ester hydrolysis in basic conditions

The ester (1 equiv) was solved in a mixture of THF and NaOH aqueous solution (1.5 to 3.0 equiv) and stirred at room temperature until all starting material was disappeared. The solution was concentrated in vacuum, the residue dissolved in H_2O and acidified with 1N HCl until a solid appeared, which was filtered and characterized as the corresponding acid.

[(2-Amino-9-propyl-9*H*-purin-6-yl)sulfanyl]acetic acid (8). From **7** (300 mg, 1.07 mmol), THF (3 ml) and NaOH (3.2 ml, 1.61 mmol, 0.5 N), compound **8** (265 mg, 93%) was obtained as a white solid, mp 201.7-203.5 (EtOH/H_2O) (dec) (lit[21] 200 ºC dec). IR: 3400, 3200, 2950, 2920, 1650 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.83 (t, J = 7.50 Hz, 3H, CH_3), 1.70-1.82 (m, 2H, CH_2), 3.95 (t, J = 7.30 Hz, 2H, CH_2N) 4.14 (s, 2H, CH_2S), 6.50 (bs, 2H, NH_2) 7.98 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 11.0, 22.6, 30.1, 44.3, 124.0, 141.1, 151.2, 157.9, 159.4, 170.3. MS (ESI): *m/z* 268.00 $[M+H]^+$. Anal. Calcd. for $C_{10}H_{13}N_5O_2S{\cdot}H_2O$: C, 42.09; H, 5.30; N, 24.55; S, 11.24. Found: C, 42.04; H, 5.22; N, 24.38; S, 11.12.

{[2-Amino-9-(carboxymethyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (13). From **12** (60 mg, 0.19 mmol), THF (3 ml) and NaOH (1.16 ml, 0.57 mmol, 0.5 N), compound **13** (25 mg, 46%) was obtained as a white solid, mp 239.0-239.8 ºC (EtOH/H_2O) (dec.). IR: 3420, 3320, 3200, 3110, 1720 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S) 4.83 (s, 2H, CH_2N) 6.55 (s, 2H, NH_2) 7.93 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.2, 43.8, 123.5, 141.5, 151.4, 158.0, 159.5, 169.4, 170.3. MS (ESI): *m/z* 284.08 $[M+H]^+$, 306.06 $[M+Na]^+$. Anal. Calcd. for $C_9H_9N_5O_4S$: C, 38.16; H, 3.20; N, 24.72; S, 11.32. Found: C, 38.24; H, 3.41; N, 24.41; S, 11.25.

{[2-Amino-9-(2-hydroxyethyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (24). From **14** (275 mg, 0.97 mmol), THF (3 ml) and NaOH (3.1 ml, 1.5 mmol, 0.5 N), compound **24** (90 mg, 34%) was obtained as a white solid, mp 227.0-228.7 ºC. IR: 3320, 3240, 3080, 2930, 2880, 1660 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.69 (t, J = 5.1 Hz, 2H, CH_2N), 4.01 (t, J = 5.1 Hz, 2H, CH_2O), 4.23 (s, 2H, CH_2S), 6.51 (bs, 2H, NH_2), 7.97 (s, 1H, ArH). MS (ESI): *m/z* 270.15 $[M+H]^+$, 292.32 $[M+Na]^+$. Anal. Calcd. for $C_9H_{11}N_5O_3S$: C, 40.14; H, 4.12; N, 26.01; S, 11.91. Found: C, 39.60; H, 4.27; N, 25.90; S, 11.61.

{[2-Amino-9-(2-methoxyethyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (25). From **15** (81 mg, 0.27 mmol), THF (3 ml) and NaOH (1.09 ml, 0.54 mmol, 0.5 N), compound **25** (17 mg, 22%) was obtained as a white solid, mp 211.4-213.0 ºC (EtOH/H_2O). IR: 3450, 3310, 3180, 1700 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.23 (s, 3H, CH_3O), 3.64 (t, *J* = 5.5 Hz, 2H, CH_2), 4.13 (s, 2H, CH_2S), 4.18 (t, *J* = 5.5 Hz, 2H, CH_2), 6.49 (s, 2H, NH_2), 7.91 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.1, 42.4, 58.1, 69.6, 123.8, 141.4, 151.2, 157.9, 159.4, 170.3. MS (ESI): *m/z* 284.47 $[M+H]^+$, 306.21 $[M+Na]^+$. Anal. Calcd. for $C_{10}H_{13}N_5O_3S$: C, 42.39; H, 4.63; N, 24.72; S, 11.32. Found: C, 42.88; H, 4.78; N, 24.27; S, 11.31

{[2-Amino-9-(cyclopropylmethyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (26). From **16** (72 mg, 0.25 mmol), THF (3 ml) and NaOH (0.74 ml, 0.38 mmol, 0.5 N), compound **26** (42 mg, 61%) was obtained as a white solid, mp 228.1-229.6 ºC (EtOH/H_2O) (dec). IR: 3480, 3290, 3160, 1700 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.36-0.42 (m, 2H, CH_2), 0.44-0.53 (m, 2H, CH_2), 1.21-1.29 (m, 1H, CH), 3.87 (d, *J* = 6.7 Hz, 2H, CH_2N), 4.15 (s, 2H, CH_2S), 6.49 (s, 2H, NH_2), 8.02 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 6.9, 11.2, 30.1, 46.9, 123.9, 140.8, 151.1, 157.9, 159.4, 170.3. Anal. Calcd. for $C_{11}H_{13}N_5O_2S$: C, 47.30; H, 4.69; N, 25.07; S, 11.48. Found: C, 47.23; H, 4.68; N, 24.75; S, 11.51

{[2-Amino-9-(prop-2-en-1-yl)-9*H*-purin-6-yl]sulfanyl}acetic acid (27). From **17** (49 mg, 0.22 mmol), THF (3 ml) and NaOH (0.53 ml, 0.33 mmol, 0.5 N), compound **27** (14 mg, 30%) was obtained as a white solid, mp 195.6-197.5 ºC (EtOH/H_2O). IR: 3300, 3190, 1680 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S), 4.66 (d, *J* = 4.9 Hz, 2H, CH_2N), 4.95 (dd, *J* = 17.1, 1.2 Hz, 1H, 1/2C=CH_2), 5.17 (dd, *J* = 10.4, 1.2 Hz, 1H, 1/2C=CH_2), 5.98-6.52 (m, 1H, CH=C), 6.52 (s, 2H, NH_2), 7.94 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.1, 44.5, 117.1, 123.8, 133.3, 140.9, 151.0, 158.0, 159.5, 170.3. MS (ESI): *m/z* 266.41 $[M+H]^+$, 288.36 $[M+Na]^+$. Anal. Calcd. for $C_{10}H_{11}N_5O_2S$: C, 45.27; H, 4.18; N, 26.40; S, 12.09. Found: C, 45.03; H, 4.32; N, 25.94; S, 11.87

({2-Amino-9-[2-(diethylamino)ethyl]-9*H*-purin-6-yl}sulfanyl)acetic acid (28). From **18** (75 mg, 0.22 mmol), THF (3 ml) and NaOH (0.89 ml, 0.44 mmol, 0.5 N), compound **28** (32 mg, 45%) was obtained as a white solid, mp 229.2-231.0 ºC (EtOH/H_2O). ^1H NMR (MeOD-d_4): δ 1.26 (t, *J* = 6.7 Hz, 6H, $2CH_3$), 3.20 (q, *J* = 6.7 Hz, 4H, $2CH_2$), 3.38 (t, *J* = 6.7 Hz, 2H, CH_2), 3.89 (s, 2H, CH_2S), 4.33 (t, *J* = 6.7 Hz, 2H, CH_2), 7.74 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 11.4, 30.2, 46.5, 51.0, 123.9, 141.5, 151.2, 157.8, 159.3, 170.4. Anal. Calcd. for $C_{13}H_{20}N_6O_2S$: C, 48.13; H, 6.21; N, 25.91; S, 9.88. Found: C, 47.83; H, 6.11; N, 25.55; S, 9.78

[(2-Amino-9-benzyl-9*H*-purin-6-yl)sulfanyl]acetic acid (29). From **19** (81 mg, 0.25 mmol), THF (3 ml) and NaOH (0.98 ml, 0.50 mmol, 0.5 N), compound **29** (44 mg, 57%) was obtained as a white solid, mp 219.8-221.4 ºC (EtOH/H_2O). IR: 3300, 3190, 1680 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S), 5.26 (s, 2H, CH_2N), 6.53 (s, 2H, NH_2), 7.20-7.23 (m, 2H, ArH), 7.26-7.36 (m, 3H, ArH), 8.07 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.2, 45.7, 123.8, 127.1, 127.7, 128.7, 137.1, 141.0, 151.1, 158.2, 159.5, 170.2. MS (ESI): *m/z* 316.00 $[M+H]^+$, 337.97 $[M+Na]^+$. Anal. Calcd. for $C_{14}H_{13}F_3N_5O_2S$: C, 53.32; H, 4.16; N, 22.21; S, 10.17. Found: C, 53.21; H, 4.30; N, 22.79; S, 10.03

{[2-Amino-9-(4-methoxybenzyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (30). From **20** (80 mg, 0.22 mmol), THF (3 ml) and NaOH (0.67 ml, 0.33 mmol, 0.5 N), compound **30** (51 mg, 67%) was obtained as a white solid, mp 214.1-216.1 ºC (EtOH/H_2O). IR: 3600, 3460, 3310, 3190, 1710 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.70 (s, 3H, CH_3O), 4.14 (s, 2H, CH_2S), 5.17 (s, 2H, CH_2N), 6.52 (s, 2H, NH_2), 6.88 (AA'XX', ArH), 7.21 (AA'XX', 2H, ArH), 8.04 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.1, 45.3, 55.1, 114.1, 123.9, 128.8, 129.0, 140.9, 151.1, 158.1, 158.9, 159.6, 170.3. MS (ESI): *m/z* 346.47 $[M+H]^+$, 368.24 $[M+Na]^+$. Anal. Calcd. for $C_{15}H_{15}N_5O_3S\cdot 1/2H_2O$: C, 50.84; H, 4.55; N, 19.76; S, 9.05. Found: C, 50.73; H, 4.54; N, 19.58; S, 8.75

{[2-Amino-9-(4-chlorobenzyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (31). From **21** (134 mg, 0.34 mmol), THF (3 ml) and NaOH (1.1 ml, 0.51 mmol, 0.5 N), compound **31** (83 mg, 70%) was obtained as a white solid, mp 205.8-207.1 ºC (EtOH/H_2O). IR: 3320, 3100, 1680 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S), 5.26 (s, 2H, CH_2N), 6.53 (s, 2H, NH_2), 7.23 (AA'XX', 2H, ArH), 7.39 (AA'XX', 2H, ArH), 8.07 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.1, 45.1, 123.8, 128.7, 129.0, 132.3, 136.1, 151.1, 158.3, 159.6, 170.3. Anal. Calcd. for $C_{14}H_{12}ClN_5O_2S$: C, 48.07; H, 3.46; N, 20.02; S, 9.17. Found: C, 48.14; H, 3.71; N, 19.97; S, 8.83

{[2-Amino-9-(4-nitrobenzyl)-9*H*-purin-6-yl]sulfanyl}acetic acid (32). From **22** (35 mg, 0.09 mmol), THF (3 ml) and NaOH (0.27 ml, 0.14 mmol, 0.5 N), compound **32** (14 mg, 43%) was obtained as a white solid, mp 224.3-225.5 ºC (EtOH/H_2O). IR: 3420, 3300, 3200, 1710 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S), 5.43 (s, 2H, CH_2N), 6.55 (s, 2H, NH_2), 7.42 (AA'XX', 2H, ArH), 8.11 (s, 1H, ArH), 8.20 (AA'XX', 2H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.2, 45.3, 123.8, 124.0, 128.1, 141.1, 144.7, 147.0, 151.2, 158.4, 159.6, 170.3. MS (ESI): *m/z* 361.13 $[M+H]^+$, 383.07 $[M+Na]^+$. Anal. Calcd. for $C_{14}H_{12}N_6O_4S$: C, 46.66; H, 3.36; N, 23.32; S, 8.90. Found: C, 46.70; H, 3.52; N, 23.05; S, 8.74

({2-Amino-9-[4-(trifluoromethyl)benzyl]-9*H*-purin-6-yl}sulfanyl)acetic acid (33). From **23** (96 mg, 0.24 mmol), THF (3 ml) and NaOH (0.76 ml, 0.36 mmol, 0.5 N), compound **33** (67 mg, 73%) was obtained as a white solid, mp 226.8-229.2 ºC (EtOH/H_2O). IR: 3500, 3310, 3190, 3080, 1700 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.15 (s, 2H, CH_2S), 5.38 (s, 2H, CH_2N), 6.54 (s, 2H, NH_2), 7.39 (AA'XX', 2H, ArH), 7.71 (AA'XX', 2H, ArH), 8.10 (s, 1H, ArH). ^{13}C NMR (DMSO-d_6): δ 30.1, 45.3, 123.8, 124.2 (q, J = 272 Hz), 125.7 (q, J = 4 Hz), 127.7, 128.2 (q, J = 32 Hz), 141.1 141.8, 151.2, 158.3, 159.6, 170.3. Anal. Calcd. for $C_{15}H_{12}F_3N_5O_2S$: C, 47.00; H, 3.16; N, 18.27; S, 8.36. Found: C, 46.96; H, 3.41; N, 18.00; S, 8.63

[(2-Amino-9*H*-purin-6-yl)sulfanyl]acetic acid (34). From **11** (124 mg, 0.52 mmol), THF (3 ml) and NaOH (1.55 ml, 0.78 mmol, 0.5 N), compound **34** (97 mg, 83%) was obtained as a white solid, mp 259.1-260.5 ºC (lit.[20,22] > 300 ºC dec). IR: 3300, 3080, 3940, 1670 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.13 (s, 2H, CH_2S), 6.36 (bs, 2H, NH_2) 7.94 (s, 1H, ArH), 12.62 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 30.2, 123.7, 139.4, 152.0, 157.0, 159.5, 170.4. MS (ESI): *m/z* 225.94 $[M+H]^+$. Anal. Calcd. for $C_7H_7N_5O_2S\cdot 1/3H_2O$: C, 36.36; H, 3.34; N, 30.29; S, 13.87. Found: C, 36.62; H, 3.41; N, 30.08; S, 13.89

Determination of CK2 activity

The reaction mixture (50µl) for determination of CK2 (apoenzyme CK2α from www.proteinkinase.de) activity contained: peptide substrate (40 µM, RRRADDSDDDDD from SIGMA); Tris-HCl pH 7,5 (20 mM); $MgCl_2$ (10 mM), γ [^{32}P] ATP (100 µM) and the appropriate compound (10 µM) in 1% DMSO. After 10 min of incubation at 37 ºC, 10 µl of the assay mixture was spotted onto a square (1 cm x 1cm) of Watman P81 paper, and allowed to dry. Next square was immersed in cold 0,5 % phosphoric acid, and washed 3 times during 10 min. Then the squares were washed with 96% EtOH and allowed to dry. The radioactivity was quantified using a BECKMAN LS6500. The activity was calculated as the percentage of incorporated ^{32}P (measured in scintillation counter).

Molecular modeling studies

Molecular docking of selected compounds to the X-ray structure of CK2 (PDB code: 1DAW) was carried out using the Glide 5.5[23] software in extra-precision (XP)[24,25] mode using Glidescore for ligand ranking. Maestro 9.0.211[26] was employed as the graphical user interface. The 2005 implementation of the OPLS-AA force field and a van der Waals radii scale factor of 1.0/0.8 was used for receptor and ligand respectively.

Ligands were prepared using Lig-Prep 2.3 as implemented in Maestro. The target protein was prepared using the protein preparation wizard in Maestro 9.0.211. PDB code 1DAW was used as target protein which corresponds to the 3D structure of protein kinase CK2 in complex with ANP (phosphoaminophosphonic acid-adenylate ester). Water molecules were removed. Hydrogen atoms were added and a minimization was performed until the RMSD value of all heavy atoms was within 0.3 Å^3 of the crystal structure. The binding pocket was identified by placing a 20A cube around the geometrical center of ANP.

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