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Synthesis of 9-alkyl and 9-heteroalkyl substituted 2-amino-6-guanidinopurines and their influence on the NO-production in macrophages

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Abstract—9-Alkyl and 9-heteroalkyl substituted derivatives of the 2-amino-6-guanidinopurine were synthesized by alkylation of 2-amino-6-chloropurine and subsequent guanidinolysis. The activity of the thus prepared compounds on murine macrophages was examined. Compounds **4a**, **4b**, and **4d** inhibit the LPS + IFN-γ-induced NO production in murine macrophages while compound **4h** stimulates this production.

1. Introduction

Substituted 9-alkylpurines are important class of biologically active compounds. The previous research of one group of 9-alkylpurine derivatives revealed their antagonistic activity on adenosine receptors[1] and an influence on numerous enzymatic systems. Derivatives of 9-alkylpurines show broad spectrum of inhibitory activity: for example, on O^6-alkylguanine-DNA-alkyltransferase[2] and human erythrocyte membrane phosphatidylinositol 4-kinase.[3] They inhibit on metabolic pathway of cytokinins[4] and cyclin dependent kinases.[5]

The crucial role of 6-amino group at the purine moiety in various biologically active compounds is well known. Recently, we begun to systematically investigate the effect of the replacement of 6-amino group in adenine by closely related 6-guanidino function. The guanidino group, which is substantially more basic compared to the amino function, is contained in various pharmacophores. Among biologically active compounds bearing this function belong, for example, inhibitors of the influenza virus neuramidinase,[6] inhibitors of polyamine synthesis,[7] cytostatics,[8] and compounds with antiviral activity.[9]

Recently, we described synthesis of 2-amino-6-guanidino-9-methylpurine, which inhibited the LPS + IFN-γ-induced NO production in murine macrophages and its further examination indicated cytocidal activity. On the contrary, 6-guanidino-9-methylpurine was totally inactive in this system. This difference indicated an influence of additional substituent (amino group) on the activity. Therefore, we have prepared a series of 9-alkyl and heteroalkyl derivatives of 2-amino-6-guanidinopurine and examined them in the LPS + IFN-γ-induced NO production in murine macrophages assay.[10]

2. Results and discussion

Alkylation of the sodium salt of 2-amino-6-chloropurine with alkylbromides, iodides, or *p*-toluenesulfonates followed by subsequent guanidylation was selected as the general synthetic approach (Scheme 1). 2-Amino-6-chloro-9-alkyl, 9-heteroalkyl derivatives, and 2-amino-9-alkyl, 9-heteroalkyl 6-guanidino derivatives thus obtained are listed in Table 1.

The 2-amino-6-chloropurine derivatives **2a** and **3a** were prepared[11] from 2-amino-6-chloropurine by the earlier described method.[12] The resulting poorly soluble

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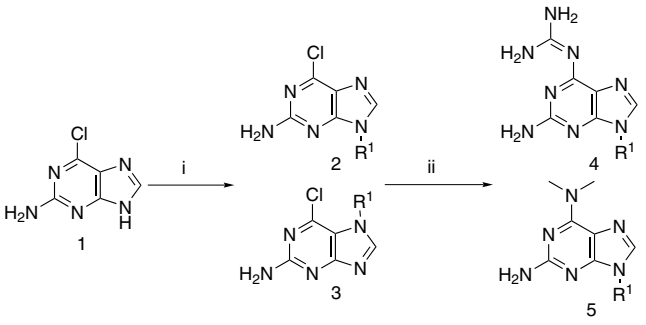


Scheme 1. Synthesis of 2-amino-6-guanidino-9*H*-purine derivatives. Reagents and conditions: (i) R^1–X/R^1–OTs, NaH, DMF; (ii) guanidine, DABCO, DMF/CH_3CN.

mixture of 7:9 regioisomers of N-methylpurine was used without purification for the next reaction step. Sodium hydride was used instead of K_2CO_3 for the synthesis[1c] of the derivative **2b** and **3b**. The alkylation was applied also for the synthesis of 9-isopropyl derivative **2d** instead of the described cyclization[13] of 6-chloro-N^4-isopropylpyrimidine-2,4,5-triamine with triethoxymethane. Compounds **2c**–**f** were prepared from bromoalkanes in good yields. The 7-regioisomers **3c**,**e**, and **3f** were obtained as byproducts. Azidoethyl derivative **2g** and **3g** was prepared by alkylation of 2-azidoethyl 4-methylbenzenesulfonate[14] in good yield. Low yield of product **2i** was probably caused by low reactivity of 1,1,1-trifluoro-2-iodoethane.

In order to increase the lipophilicity (and solubility in organic solvents) of the reaction products we decided to use the acido-labile tetrahydropyranyl protecting group, therefore the product **2j** and **3j** was prepared bromoethoxy)tetrahydro-2H-pyran. The phthalimido derivative **2l**, precursor of the 2-aminoethyl derivative, was synthesized by the reaction with N-(2-bromoethyl)phthalimide. Due to its low solubility, it was used without purification in the next step as well.

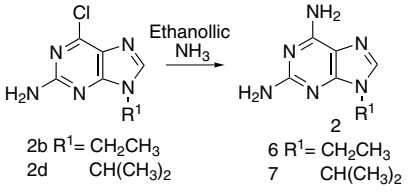
Thus the obtained intermediates **2a**–**g**,**i**, and **2j** were converted to the 6-guanidino derivatives **4a**,[11] **4b**–**g**, **i**, and **j** by treatment with guanidine solution prepared in situ from guanidine hydrochloride and one equivalent of NaH in DMF/acetonitrile mixture in the presence of DABCO as a catalyst. 2-amino-6-N,N-dimethylaminopurine derivatives **5** were isolated as byproducts of this reaction.

Compound **4k** was obtained by removal of acido-labile tetrahydro-2H-pyranyl group from **4j** treated with 1 M HCl in a mixture of H_2O and MeOH (1:1).

We observed that the protecting phtalimido group was transformed by the guanidinolysis to 2-amino-6-guanidino-9-{2-[(ethylamino)carbonyl]benzoic acid}-9*H*-purine **4l**. Further deprotection of compound **4l** with hydrazine hydrate or with NaOH failed. For the preparation of 2-aminoethyl derivative **4h**, the azido compound **4g** was hydrogenated over Pd-catalyst.

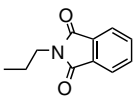
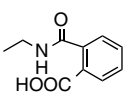
In order to compare the biological activity of the 2-amino-6-guanidino derivatives **4b**, **4d** with 2,6-diaminopurine derivatives **6** and **7**, compounds **2b** and **2d** were treated with ethanolic ammonia to afford these 2,6-diaminopurine derivatives (Scheme 2).

The target 2-amino-6-guanidinopurine derivatives **4** as well as all intermediates were fully characterized by ^1H and ^{13}C NMR spectra and gave satisfactory data by elemental analysis.



Scheme 2. Synthesis of 2,6-diaminopurine derivatives.

Table 1.

Entry	X	R^1	Yield of **2** (%)	R^1	Yield of **4** (%)
a	I	CH_3	—	CH_3	53
b	I	CH_2CH_3	63	CH_2CH_3	85
c	Br	$(CH_2)_2CH_3$	68	$(CH_2)_2CH_3$	70
d	Br	$CH(CH_3)_2$	61	$CH(CH_3)_2$	80
e	Br	$CH_2CH(CH_3)_2$	61	$CH_2CH(CH_3)_2$	74
f	Br	$CH(CH_3)CH_2CH_3$	52	$CH(CH_3)CH_2CH_3$	58
g	OTs	$(CH_2)_2N_3$	72	$(CH_2)_2N_3$	70
h	—	—	—	$(CH_2)_2NH_2$	49
i	I	CH_2CF_3	21	CH_2CF_3	62
j	Br	$(CH_2)_2OTHP$	65	$(CH_2)_2OTHP$ / $(CH_2)_2OH$	67*
k	—	—	—		
l	Br		—		34

* Over two steps.

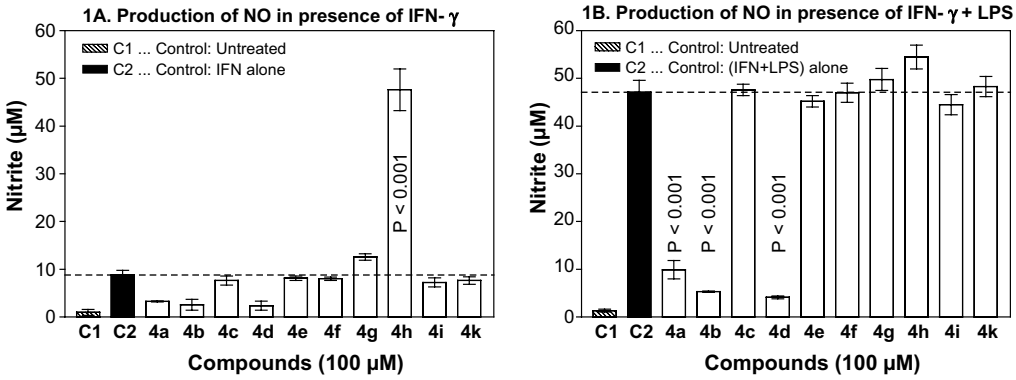


Figure 1. Production of NO by mouse peritoneal macrophages cultured 24 h in the presence of test compounds (100 μM), together with (1A) murine recombinant IFN-γ (5000 pg/mL) or (1B) IFN-γ + LPS (100 pg/mL). Nitrite concentration was determined spectrophotometrically using Griess reagent. The bars are means ± SEM.

3. Biological activity

None of the test compounds, applied at the screening concentrations of 100 μM, increased production of NO by murine macrophages when given alone or together with LPS (data not shown). If the compounds are given together with IFN-γ (Fig. 1A), or in combination with IFN-γ + LPS (Fig. 1B), the NO-production is inhibited by 2-amino-6-guanidino-9-methyl (**4a**), 9-ethyl (**4b**), and 9-isopropyl derivative (**4d**). The corresponding estimates of IC_{50}s, calculated on basis of a dose-effect experiment (Fig. 2), reached the values of 66 μM, 25 μM, and 33 μM for **4a**, **4b**, and **4d**, respectively. The NO-suppressive activity is likely to be due to the cytocidal effects of these compounds (Fig. 3). Contrary to the 9-alkyl derivatives, the 9-(2-aminoethyl) derivative (**4h**) stimulates the IFN-γ-induced production of NO (Fig. 1A). The NO-enhancing effect is dose dependent (Fig. 4), the ED_{50} being approximately 19 μM. Besides, **4h** possesses considerable immunostimulatory potential: it activates secretion of chemokines RANTES and MIP-1α (Fig. 5). The 9-propyl (**4c**), 9-(2-methylpropyl) (**4e**), 9-heteroalkyl congeners (**4g**,**i**, and **4k**) are essentially inactive in these assays. Compound **4l** was not tested due to its low solubility under the conditions of the assay.

These data show that compounds with short 9-alkyl chains (methyl, ethyl, and isopropyl) (**4a**,**b**, and **4d**) had cytocidal activity, while compounds with longer or bigger chains are inactive in this system. Comparison with 2,6-diamino-9-ethylpurine and 2,6-diamino-9-isopropylpurine derivatives, which are inactive in this system, clearly demonstrated the need for the presence of the guanidino group for inhibitory activity.

None of the other thus-obtained 9-alkyl-2-amino-6-guanidinopurines **4** exhibited under standard conditions any cytotoxicity in vitro in the L929, L1210, and HeLaS3 cell lines[15] except for compound **4b**, which was moderately cytostatic in the L1210 cell line (IC_{50} 8.2 μM). Compounds **4** were also tested for their antiviral activity against selected DNA viruses, RNA viruses, and retroviruses: (MSV, HIV-1, and HIV-2). These tests

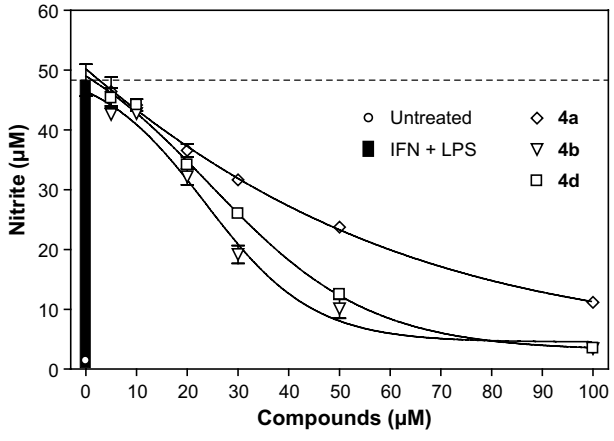


Figure 2. Inhibition of NO production by increasing concentrations of compounds **4a**, **4b**, and **4d**. Formation of NO by mouse peritoneal macrophages was induced by joint administration of murine recombinant IFN-γ (5000 pg/mL) and LPS (100 pg/mL). The cells were cultured for 24 h and supernatant nitrite concentration was determined spectrophotometrically using Griess reagent. Each point is mean ± SEM and the results representative of two independent experiments.

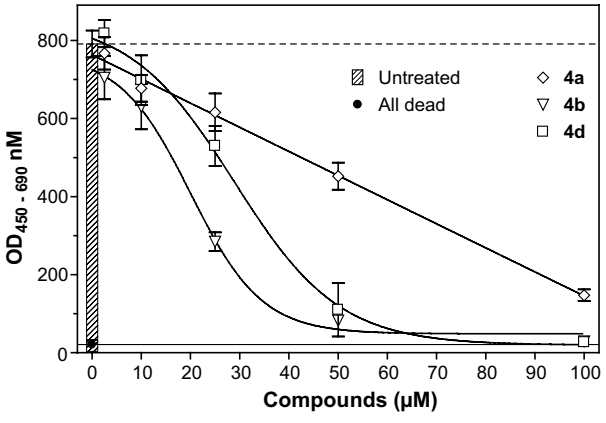


Figure 3. Cytotoxic effects of test compounds given at increasing concentrations to murine peritoneal macrophages. After the 18 h culturing of cells, the WST-1 reagent was added for the successive period of 6 h and the optical density was evaluated (see Materials and Methods). Each point is mean ± SEM.

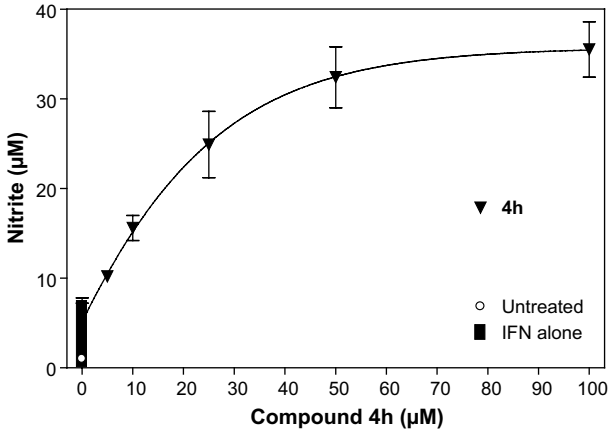


Figure 4. Production of NO by mouse peritoneal macrophages cultured 24 h in presence of murine recombinant IFN-γ (5000 pg/mL) and increasing concentration of compound **4h**. Nitrite concentration was determined spectrophotometrically using Griess reagent. The bars are means ± SEM and represent two other similar experiments.

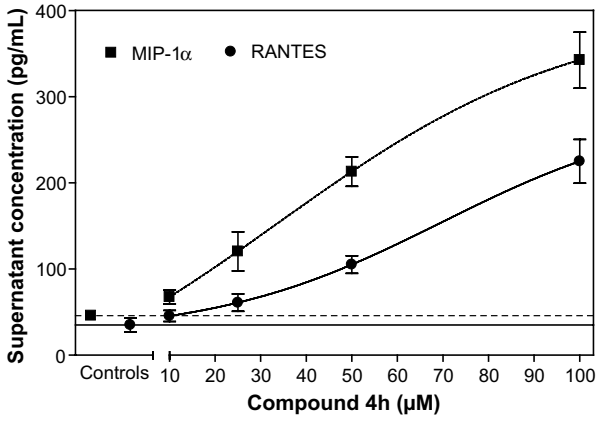


Figure 5. Compound **4h** activates secretion of chemokines RANTES and MIP-1α. The immunostimulatory potential was assayed in vitro using mouse resident peritoneal macrophages (2×10^6/mL). The chemokines were assayed by ELISA in supernatants of cells after the 5 h culturing. The points are means ± SEM and are representative of two independent experiments.

were performed also on the compounds **5**–**7**. None of the compounds showed any significant activity in these assays.[16]

4. Conclusion

In conclusion, new 9-alkyl and heteroalkyl substituted derivatives of the 2-amino-6-guanidinopurine were synthesized by alkylation of 2-amino-6-chloropurine and subsequent guanidinolysis of the intermediates **3**. The examination of biological activity of the thus-prepared compounds in murine macrophages revealed that compounds **4a**,**b**, and **4d** inhibit whereas compound **4h** stimulates the immune-triggered NO production in murine macrophages. This compound possesses immunostimulatory properties.

5. Experimental

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on an FT NMR spectrometer Varian UNITY 500 (^{1}H at 500 MHz and ^{13}C at 125.7 MHz frequency) in dimethyl sulfoxide-d_6. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). Chemicals were purchased from Aldrich (Prague, Czech Republic). Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). Preparative HPLC purifications were performed on a column packed with 7 μm C18 reversed phase (Waters Delta 600 chromatograph), 17 × 250 mm; ca. in 200 mg batches of mixtures using a linear gradient 0.025 M tetraethylammonium bicarbonate in H_2O/CH_3CN (0–100% CH_3CN) as eluent. S1: EtOH–EtOAc (1:9) containing 1% NH_3; S2: EtOAc–EtOH–acetone–H_2O (6:1:1:0.5) containing 1% NH_3; S3: EtOAc–EtOH–acetone–H_2O (4:1:1:1) containing 1% NH_3. S4: *i*PrOH–H_2O–NH_3 (7:2:1).

6. Typical procedures

6.1. Preparation of guanidine solution

Sodium hydride (0.60 g, 15 mmol; 60% suspension in paraffin oil) was added to guanidine hydrochloride (1.43 g, 15 mmol) in a mixture of acetonitrile (30 mL) and DMF (15 mL), and the mixture was stirred at room temperature overnight under exclusion of moisture. The resulting slurry was directly used for further reactions.

6.2. Method A. Alkylation of 2-amino-6-chloropurine. General procedure

NaH (0.55 g, 13.8 mmol) was added to a suspension of 2-amino-6-chloropurine (1.87 g, 11 mmol) in dry DMF (33 mL) and the reaction mixture was stirred at room temperature for 2 h. Thereafter the halogeno derivative (13.2 mmol) was added and the resulting mixture was stirred at room temperature or heated at 80 °C until the starting compound disappeared (TLC). The solvent was then evaporated in vacuo, the residue co-distilled with toluene (3 × 30 mL) and extracted with boiling $CHCl_3$ (5 × 50 mL). After evaporation of the solvent in vacuo, the crude reaction product was purified on a silica gel column (40 g).

6.3. Method B. Guanidination of 2-amino-6-chloro 9-alkyl and 9-heteroalkylpurine. General procedure

2-Amino-6-chloropurine derivative **3** (3 mmol), DABCO (0.34 g, 3 mmol), and a guanidine solution (45 mL, 15 mmol) (see above) was stirred at rt until the starting compound was consumed (TLC). The resulting mixture was evaporated in vacuo and the residue was co-distilled with toluene (3 × 30 mL), dissolved in water and neutral-

ized by Dowex 50 × 8 (H^+-form). The suspension of the resin was applied on the column of Dowex 50 × 8 (H^+-form), the column was washed with water, eluted with a mixture of concd aqueous-NH_3–H_2O (1:10). The filtrate was evaporated in vacuo and chromatographed on a column of silica gel.

6.3.1. 2-Amino-6-chloro-9-ethyl-9*H*-purine (2b) and 2-amino-6-chloro-7-ethyl-7*H*-purine (3b). Method A; ethyl iodide (1.06 mL, 13.2 mmol); the mixture was stirred at room temperature overnight; chromatography on a silica gel column, (40 g, MeOH–$CHCl_3$ 2:98) gave compounds **2b** and **3b**.

Compound **2b**; yield: (1.25 g, 63%) a white crystals, (*i*-PrOH); mp 160–162 °C. FABHRMS calcd for $C_7H_9ClN_5$ (MH^+) 198.0546, found 198.0564. FABMS: 198 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.36 (t, 6H, J(CH_3, CH_2) = 7.3, CH_3); 4.07 (q, 2H, J(CH_2, CH_3) = 7.3, N–CH_2); 6.91 (br s, 2H, NH_2); 8.15 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 15.04 (CH_3); 38.32 (N–CH_2); 123.62 (C-5); 143.08 (C-8); 149.46 (C-6); 154.08 (C-4); 159.91 (C-2).

Compound **3b**; yield: (0.10 g, 7%); white crystals, (*i*-PrOH); mp 190 °C; FABHRMS calcd for $C_7H_9ClN_5$ (MH^+) 198.0546, found 198.0557.FABMS: 198 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.39 (t, 3H, J(CH_3, CH_2) = 7.2, CH_3); 4.31 (q, 2H, J(CH_2, CH_3) = 7.2, N–CH_2); 6.62 (br s, 2H, NH_2); 8.38 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 16.88 (CH_3); 41.56 (N–CH_2); 114.89 (C-5); 142.26 (C-6); 149.15 (C-8); 160.11 (C-2); 164.50 (C-4).

6.3.2. 2-Amino-6-chloro-9-propyl-9*H*-purine (2c) and 2-amino-6-chloro-7-propyl-7*H*-purine (3c). Method A; 1-bromopropane (1.19 mL, 13.2 mmol); the mixture was stirred at room temperature for 24 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 4:96) gave compounds **2c** and **3c**.

Compound **2c**; yield: (1.6 g, 68%); white crystals, (*i*-PrOH); mp 121–123 °C. FABHRMS calcd for $C_8H_{11}ClN_5$ (MH^+) 212.0703, found 212.0610. FABMS: 212 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.83 (t, 3H, J(3′, 2′) = 7.4, H-3′); 1.78 (br sext, 2H, H-2′); 3.99 (t, 2H, J(1′, 2′) = 7.1, H-1′); 6.92 (br s, 2H, NH_2); 8.14 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 11.06 (C-3′); 22.51 (C-2′); 44.77 (C-1′); 123.54 (C-5); 143.50 (C-8); 149.48 (C-6); 154.29 (C-4); 159.94 (C-2).

Compound **3c**; yield: (0.42 g, 18%); white crystals, (*i*-PrOH); mp >212 °C (dec). FABHRMS calcd for $C_8H_{11}ClN_5$ (MH^+) 212.0703, found 212.0603. FABMS: 212 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.83 (t, 3H, J(3′, 2′) = 7.4, H-3′); 1.78 (br sext, 2H, H-2′); 4.24 (t, 2H, J(1′, 2′) = 7.1, H-1′); 6.63 (br s, 2H, NH_2); 8.37 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 10.70 (C-3′); 24.25 (C-2′); 74.79 (C-1′); 114.95 (C-5); 142.69 (C-6); 149.69 (C-8); 160.07 (C-2); 164.48 (C-4).

6.3.3. 2-Amino-6-chloro-9-isopropyl-9*H*-purine (2d). Method A; isopropyl bromide (1.19 mL, 13.2 mmol); the mixture was heated at 80 °C for 6 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 1:99) afforded compound **2d**; (1.42 g, 61%); white crystals, (*i*-PrOH); mp 135–136 °C. For $C_8H_{10}ClN_5$ (211.65) calcd: C, 45.40; H, 4.76; Cl, 16.75; N, 33.09. Found: C, 45.36; H, 4.73; Cl, 16.73; N, 32.90. FABMS: 212 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.48 (d, 6H, J(CH_3, CH) = 6.8, CH_3); 4.60 (sept, 1H, J(CH, CH_3) = 6.8, N–CH); 6.89 (br s, 2H, NH_2); 8.24 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 21.97 (2C, CH_3); 46.66 (N–CH); 123.84 (C-5); 141.35 (C-8); 149.47 (C-6); 153.74 (C-4); 159.72 (C-2).

6.3.4. 2-Amino-6-chloro-9-isobutyl-9*H*-purine (2e) and 2-amino-6-chloro-7-isobutyl-7*H*-purine (3e). Method A; 1-bromo-2-methylpropane (1.3 mL, 13.2 mmol); the mixture was heated at 80 °C for 6 h; chromatography on silica gel column, (40 g, $CHCl_3$) gave compounds **2e** and **3e**.

Compound **2e**; yield: (1.51 g, 60%); white crystals, (*i*-PrOH); mp 156–159 °C. For $C_9H_{12}ClN_5$ (225.68) calcd: C, 47.90; H, 5.36; Cl, 15.71; N, 31.03. Found: C, 47.77; H, 5.45; Cl, 15.81; N, 31.21. FABMS: 226 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.83 (d, 6H, J(3′, 2′) = 6.7, CH_3); 2.16 (m, 1H, H-2′); 3.85 (d, 2H, J(1′, 2′) = 7.3, H-1′); 6.91 (br s, 2H, NH_2); 8.12 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 19.75 (2C, CH_3); 28.17 (C-2′); 50.24 (C-1′); 123.45 (C-5); 143.76 (C-8); 149.50 (C-6); 154.45 (C-4); 159.96 (C-2).

Compound **3e**; yield: (0.34 g, 14%); white crystals, (*i*-PrOH); mp >231 °C (dec). FABHRMS calcd for $C_9H_{13}ClN_5$; (MH^+) 226.0859. Found: 226.0836. FABMS: 226 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.84 (d, 6H, J(CH_3, CH) = 6.7, CH_3); 2.06 (br sept, 1H, 2′-CH); 4.08 (d, 2H, J(1′, 2′) = 7.3, 1′-CH_2); 6.64 (br s, 2H, NH_2); 8.36 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 19.38 (2C, CH_3); 29.77 (C-2′); 53.16 (C-1′); 115.04 (C-5); 142.40 (C-6); 149.93 (C-8); 160.05 (C-2); 164.45 (C-4).

6.3.5. 2-Amino-6-chloro-9-(*sec*-butyl)-9*H*-purine (2f) and 2-amino-6-chloro-7-(*sec*-butyl)-7*H*-purine (3f). Method A; 2-bromobutane (1.4 mL, 13.2 mmol); the mixture was heated at 80 °C for 14 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 3:97) gave compounds **2f** and **3f**.

Compound **2f**; yield: (1.3 g, 52%); white crystals, (*i*-PrOH); mp 139–141 °C. For $C_9H_{12}ClN_5$ (225.38) calcd: C, 47.90; H, 5.36; Cl, 15.71; N, 31.03. Found: C, 47.91; H, 5.48; Cl, 15.69; N, 30.75. FABMS 226 [MH^+] (90). ^{1}H NMR (DMSO-d_6): 0.74 (t, 3H, J(CH_3, CH_2) = 7.3, CH_3); 1.47 (d, 3H, J(CH_3, CH_2) = 6.8, CH_3); 1.72 (m, 1H) and 1.92 (m, 1H, CH_2); 4.38 (m, 1H, N–CH); 6.87 (br s, 2H, NH_2); 8.23 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 10.64 and 20.04 (CH_3); 28.43 (CH_2); 52.38 (N–CH); 123.75 (C-5); 141.81 (C-8); 149.48 (C-6); 154.06 (C-4); 159.72 (C-2).

Compound **3f**; yield: (0.18 g, 7%); pale yellow crystals, (*i*-PrOH); mp 176 °C (dec). FABHRMS calcd for: $C_9H_{13}ClN_5$ (MH^+) 226.0859. Found: 226.0848. FABMS

226 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.80 (t, 3H, $J(CH_3, CH_2) = 7.3$, CH_3); 1.52 (d, 3H, $J(CH_3, CH_2) = 6.8$, CH_3); 1.83 (m, 1H) and 1.91 (m, 1H, CH_2); 4.77 (m, 1H, N–CH); 6.61 (br s, 2H, NH_2); 8.54 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 10.17 and 20.50 (CH_3); 29.64 (CH_2); 54.14 (N–CH); 115.07 (C-5); 142.21 (C-6); 147.08 (C-8); 159.89 (C-2); 164.21 (C-4).

6.3.6. 2-Amino-9-(2-azidoethyl)-6-chloro-9*H*-purine (2g) and 2-amino-7-(2-azidoethyl)-6-chloro-7*H*-purine (3g). Method A; 2-azidoethyl-4-methylbenzenesulfonate[14] (2.53 g, 13.2 mmol); the mixture was stirred at room temperature for 12 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 5:95) gave compounds **2g** and **3g**

Compound **2g**; yield: (1.9 g, 72%); colorless crystals, (MeOH); mp 152–153 °C. For $C_7H_7ClN_8 \cdot 1/5H_2O$ (238.64) calcd: C, 34.71; H, 3.08; Cl, 14.63;N, 46.26. Found: C, 35.00; H, 2.88; Cl, 15.02; N, 46.29. EIMS: 238 [M^+] (55). ^{1}H NMR (DMSO-d_6): 3.78 (t, 2H, $J(2', 1') = 5.6$, H-2′); 4.22 (t, 2H, $J(1', 2') = 5.6$, H-1′); 6.94 (br s, 2H, NH_2); 8.13 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 42.65 (C-1′); 49.51 (C-2′); 123.46 (C-5); 143.35 (C-8); 149.61 (C-6); 154.36 (C-4); 160.01 (C-2).

Compound **3g**; yield: (0.3 g, 11%); colorless crystals, (MeOH); mp 179 °C (dec). For $C_7H_7ClN_8 \cdot 1/5H_2O$ (238.64) calcd: C, 34.71; H, 3.08; Cl, 14.63; N, 46.26. Found: C, 34.91; H, 2.84; Cl, 15.05; N, 46.05. FABMS: 239 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 3.78 (t, 2H, $J(2', 1') = 5.6$, H-2′); 4.47 (t, 2H, $J(1', 2') = 5.6$, H-1′); 6.65 (br s, 2H, NH_2); 8.37 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 45.69 (C-1′); 51.01 (C-2′); 114.87 (C-5); 142.47 (C-6); 149.98 (C-8); 160.17 (C-2); 164.52 (C-4).

6.3.7. 2-Amino-6-chloro-9-(2,2,2-trifluoroethyl)-9*H*-purine (2i). Method A; 1,1,1-trifluoro-2-iodoethane (1.29 mL, 13.2 mmol); the mixture was heated at 80 °C for 13 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 3:97) gave compound **2i**; (0.60 g, 21%); white crystals, (EtOH); mp 193–195 °C. For $C_7H_5ClF_3N_5$ (251.60) calcd: C, 33.42; H, 2.00; Cl, 14.09; F, 22.65; N, 27.84. Found: C, 33.32; H, 1.94; Cl, 14.07; F, 22.38; N, 27.49. FABMS: 252 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 5.03 (q, 2H, J(H, F) = 9.3, N–CH_2); 7.10 (br s, 2H, NH_2); 8.19 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 43.59 (q, J(C, F) = 35.2, N–CH_2); 122.88 (C-5); 123.69 (q, J(C, F) = 279.8, CF_3); 142.97 (C-8); 150.11 (C-6); 154.46 (C-4); 160.39 (C-2).

6.3.8. 2-Amino-6-chloro-9-[2-(tetrahydro-pyran-2-yloxy)-ethyl]-9*H*-purine (2j) and 2-amino-6-chloro-7-[2-(tetrahydro-pyran-2-yloxy)ethyl]-7*H*-purine (3j). Method A; 2-(2-bromoethoxy)tetrahydro-2H-pyran (2 mL, 13.2 mmol); the mixture was stirred at room temperature for 12 h; chromatography on silica gel column, (40 g, MeOH–$CHCl_3$ 3:97) gave compounds **2j** and **3j**.

Compound **2j**; yield: (1.95 g, 65%); white crystals, (EtOH); mp 132 °C (dec). For $C_{12}H_{16}ClN_5O_2$ (297.74) calcd: C, 48.41; H, 5.42; Cl, 11.91; N, 23.52. Found: C, 48.43; H, 5.45; Cl, 12.01; N, 23.37. FABMS: 298 [MH^+] (80). ^{1}H NMR (DMSO-d_6): 1.40 (m, 4H), 1.53 (m, 1H), and 1.61 (m, 1H, C–CH_2); 3.32 (m, 1H), 3.45 (m, 1H), 3.70 (m, 1H), 3.90 (m, 1H), 4.25 (m, 2H), and 4.55 (m, 1H); 6.92 (br s, 2H, NH_2); 8.09 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 18.93, 25.06, and 30.14 (C–CH_2); 43.22 (N–CH_2); 61.26 and 64.35 (O–CH_2); 97.74 (O–CH); 123.39 (C-5); 143.76 (C-8); 149.44 (C-6); 154.31 (C-4); 159.89 (C-2).

Compound **3j**; yield: (0.16 g, 13%); white crystals, (EtOH); mp 160–162 °C. For $C_{12}H_{16}ClN_5O_2$ (297.74) calcd: C, 48.41; H, 5.42; Cl, 11.91; N, 23.52. Found: C, 48.48; H, 5.44; Cl, 11.81; N, 23.24. FABMS: 298 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.30 (m, 1H), 1.38 (m, 3H), and 1.54 (m, 2H, C–CH_2); 3.30 (m, 2H), 3.70 (m, 1H), 3.89 (m, 1H), and 4.51 (m, 3H); 6.62 (br s, 2H, NH_2); 8.33 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 18.81, 25.02, and 30.09 (C–CH_2); 46.32 (N–CH_2); 60.99 and 65.40 (O–CH_2); 97.50 (O–CH); 115.03 (C-5); 142.39 (C-6); 150.27 (C-8); 160.05 (C-2); 164.45 (C-4).

6.3.9. 2-Amino-6-chloro-9-(2-phthalimidoethyl)-9*H*-purine (2l). NaH (0.55 g, 13.8 mmol) was added to a suspension of 2-amino-6-chloropurine (1.87 g, 11 mmol) in dry DMF (33 mL); the reaction mixture was stirred at room temperature for 2 h. After that the N-(2-bromoethyl)phthalimide (3.35 g, 13.2 mmol) was added and the resulting mixture was heated at 80 °C for 4 h. The precipitate, which was formed, was filtered and washed with toluene and ether. Owing to its poor solubility, compound **2l** was directly used without further purification.

FABMS: 343 [MH^+] (26). 3.97 (br t, 2H, $J(2', 1') = 5.2$, H-2′); 4.30 (br t, 2H, $J(1', 2') = 5.2$, H-1′); 6.63 (br s, 2H, NH_2); 7.80 (br s, 4H, arom); 8.09 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 37.38 (C-2′); 42.17 (C-1′); 123.42 (C-5); 123.27 (2C); 131.60 (2C); and 134.53 (2C, arom); 143.40 (C-8); 149.35 (C-6); 154.49 (C-4); 159.77 (C-2); 167.72 (2C, C=O).

6.3.10. 2-Amino-6-(N,N-dimethylamino)-9-ethyl-9*H*-purine (5b) and 2-amino-9-ethyl-6-guanidino-9*H*-purine (4b). Method B; starting compound **2b**: (0.59 g, 3 mmol); reaction time 7 h; chromatography on silica gel column, (30 g, elution by S1) gave compound **5b**; yield: (0.08 g, 12%); white crystals, (EtOAc); mp 136–138 °C. FAB-HRMS calcd for $C_9H_{15}N_6$ (MH^+) 207.1358, found 207.1375. FABMS: 207 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.31 (t, 3H, $J(CH_3, CH) = 7.3$, CH_3); 3.35 (br, 6H, N–CH_3); 3.98 (q, 2H, $J(CH_2, CH_3) = 7.3$, N–CH_2); 5.82 (br s, 2H, NH_2); 7.73 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 15.45 (CH_3); 37.53 (N–CH_2); 37.76 and 39.80 (N–CH_3); 113.94 (C-5); 136.19 (C-8); 152.69 (C-4); 154.92 (C-6); 159.62 (C-2).

Further elution by S3 of the same column gave: **4b**; yield: (0.63 g, 85%); colorless crystals, (EtOH); mp 244 °C (dec). FABHRMS calcd for $C_8H_{13}N_8$ (MH^+) 221.1263, found 221.1233. FABMS: 221 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.32 (t, 3H, $J(CH_3, CH_2) = 7.3$, CH_3); 3.97 (q, 2H, $J(CH_2, CH_3) = 7.3$, N–CH_2); 5.95 (br s, 2H, NH_2); 7.35 (br, 4H, NH); 7.67 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 15.56 (CH_3); 37.38 (N–CH_2);

119.39 (C-5); 137.30 (C-8); 152.16 (C-4); 159.01, 159.81, and 160.05 (C-2, C-6, C–N).

6.3.11. 2-Amino-6-(N,N-dimethylamino)-9-propyl-9*H*-purine (5c) and 2-amino-6-guanidino-9-propyl-9*H*-purine (4c). Method B; starting compound **2c**, (0.63 g, 3 mmol); reaction time 12 h; chromatography on silica gel column, (30 g, elution by S1) gave compound **5c**; yield: (0.05 g, 8%); white crystals, (EtOAc); mp 153–155 °C. For $C_{10}H_{16}N_6$ (220.28)·1/6MeOH calcd: C, 54.12; H, 7.45; N, 37.25. Found: C, 54.05; H, 7.32; N, 37.01. FABMS: 221 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.82 (t, 3H, J(3′, 2′) = 7.3, H-3′); 1.73 (br sext, 2H, H-2′); 3.35 (br, 6H, N–CH_3); 3.91 (t, 2H, J(1′, 2′) = 7.1, H-1′); 5.82 (br s, 2H, NH_2); 7.71 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 11.10 (C-3′); 22.78 (C-2′); 37.75 and 39.89 (N–CH_3); 44.11 (C-1′); 113.87 (C-5); 136.72 (C-8); 152.90 (C-4); 154.89 (C-6); 159.63 (C-2).

Further elution by S2 on the same column gave: **4c**; yield: (0.49 g, 64%); white crystals, (aq MeOH); mp >228 °C (dec). For $C_9H_{14}N_8$ (234.26)·1/2MeOH calcd: C, 45.59; H, 6.44; N, 44.77. Found: C, 45.27; H, 6.26; N, 44.43. FABMS: 235 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.83 (t, 3H, J(3′, 2′) = 7.3, H-3′); 1.74 (br sext, 2H, H-2′); 3.90 (t, 2H, J(1′, 2′) = 7.1, H-1′); 5.95 (br s, 2H, NH_2); 7.35 (br, 4H, NH); 7.65 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 11.15 (C-3′); 22.90 (C-2′); 43.99 (C-1′); 119.33 (C-5); 137.81 (C-8); 152.35 (C-4); 159.02 (C-6); 159.85 (C-2); 160.05 (C–N).

6.3.12. 2-Amino-6-(N,N-dimethylamino)-9-isopropyl-9*H*-purine (5d) and 2-amino-6-guanidino-9-isopropyl-9*H*-purine (4d). Method B; starting compound **2d** (0.63 g, 3 mmol); reaction time 12 h; chromatography on silica gel column, (30 g, elution by S1): **5d**; yield: (0.05 g, 8%); pale yellow crystals, (EtOAc); mp 156–157 °C. For $C_{10}H_{16}N_6$ (220.28)·1/8H_2O calcd: C, 53.98; H, 7.36; N, 37.77. Found: C, 54.11; H, 7.40; N, 37.76. FABMS: 221 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.43 (d, 6H, J(CH_3, CH) = 6.8, CH_3); 3.36 (br s, 6H, N–CH_3); 4.54 (sept, 1H, J(CH,CH_3) = 6.8, N–CH); 5.81 (br s, 2H, NH_2); 7.81 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 22.31 (2C, CH_3); 37.80, and 39.80 (N–CH_3); 45.37 (N–CH); 114.16 (C-5); 134.21 (C-8); 152.37 (C-4); 154.90 (C-6); and 159.43 (C-2).

Further elution by S2 on the same column gives: **4d**; yield: (0.56 g, 80%); white crystals, (EtOH); mp >251 °C (dec). For $C_9H_{14}N_8$ (234.26)·1/5H_2O calcd: C, 45.45; H, 6.10; N, 47.11. Found: C, 45.85; H, 6.08; N, 46.80. FABMS: 235 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.43 (d, 6H, J(CH_3, CH) = 6.8, CH_3); 4.52 (sept, 1H, J(CH,CH_3) = 6.8, N–CH); 5.92 (br s, 2H, NH_2); 7.42 (br, 4H, NH); 7.74 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 22.39 (2C, CH_3); 45.23 (N–CH); 119.78 (C-5); 135.15 (C-8); 151.80 (C-4); 158.82 (C-6); 160.03 (C-2); 160.45 (C–N).

6.3.13. 2-Amino-6-(N,N-dimethylamino)-9-isobutyl-9*H*-purine (5e) and 2-amino-6-guanidino-9-isobutyl-9*H*-purine (4e). Method B; starting compound **2e** (0.68 g, 3 mmol); reaction time 12 h; chromatography on silica gel column, (30 g, elution by EtOAc) afforded compound **5e**; yield: (0.10 g, 14%); white crystals, (EtOAc); mp 154–156 °C. FABHRMS calcd for $C_{11}H_{19}N_6$ (MH^+) 235.1671, found 235.1684. FABMS: 235 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.82 (d, 6H, J(CH_3, CH) = 6.6, CH_3); 2.13 (sept, 1H, CH); 3.36 (br s, 6H, N–CH_3); 3.76 (d, 2H, J(CH_2, CH) = 7.4, N–CH_2); 5.80 (br s, 2H, NH_2); 7.68 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 19.80 (2C, CH_3); 28.19 (CH); 37.73 (2C, N–CH_3); 49.71 (N–CH_2); 113.80 (C-5); 137.04 (C-8); 153.08 (C-4); 154.87 (C-6); 159.63 (C-2).

Further elution of the same column by EtOH–EtOAc mixture (4:96) containing 1% NH_3 gave compound **4e**; yield: (0.55 g, 74%); white crystals, (EtOH); mp >168 °C (dec). For $C_{10}H_{16}N_8$ (248.29)·1/2H_2O calcd: C, 46.68; H, 6.66; N, 43.55. Found: C, 46.64; H, 6.74; N, 43.30. FABMS: 249 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.83 (d, 6H, J(CH_3, CH) = 6.6, CH_3); 2.14 (br sept, 1H, H-2′); 3.75 (d, 2H, J(1′, 2′) = 7.4, H-1′); 5.93 (br s, 2H, NH_2); 7.45 (br, 4H, NH); 7.62 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 19.85 (2C, CH_3); 28.31 (C-2′); 49.62 (C-1′); 119.42 (C-5); 138.07 (C-8); 152.49 (C-4); 159.04 (C-6); 160.00 (C-2); 160.37 (C–N).

6.3.14. 2-Amino-9-(*sec*-butyl)-6-(N,N-dimethylamino)-9*H*-purine (5f) and 2-amino-9-(*sec*-butyl)-6-guanidino-9*H*-purine (4f). Method B; starting compound **2f** (0.68 g, 3 mmol); reaction time 7 h; chromatography on a silica gel column, (30 g, elution by S1) gave compound **5f**; yield: (0.12 g, 17%); white solid, (*i*-PrOH); mp 155–156 °C. For $C_{11}H_{18}N_6$ (234.30)·1/5H_2O calcd: C, 55.53; H, 7.80; N, 35.32. Found: C, 55.67; H, 7.73; N, 35.30. FABMS 235 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.73 (t, 3H, J(CH_3, CH_2) = 7.3, CH_3); 1.41 (d, 3H, J(CH_3, CH_2) = 6.8, CH_3); 1.77 (m, 1H) and 1.78 (m, 1H, CH_2); 3.35 (br, 6H, N–CH_3); 4.32 (m, 1H, N–CH); 5.76 (br s, 2H, NH_2); 7.78 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 10.72 and 20.41 (CH_3); 28.68 (CH_2); 37.80 (2C, N–CH_3); 51.06 (N–CH); 114.08 (C-5); 134.80 (C-8); 152.75 (C-4); 154.88 (C-6); and 159.43 (C-2).

(Subsequent elution of the column by S2) gave compound **4f**; yield: (0.43 g, 58%); white solid, (EtOH); mp 252 °C (dec). For $C_{10}H_{16}N_8$ (248.29)·1/3H_2O calcd: C, 47.23; H, 6.61; and N, 44.06. Found: C, 47.39; H, 6.63; and N, 43.76. FABMS 249 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 0.73 (t, 3H, J(CH_3, CH_2) = 7.3, CH_3); 1.42 (d, 3H, J(CH_3, CH_2) = 6.8, CH_3); 1.77 (m, 1H) and 1.89 (m, 1H, CH_2); 4.30 (m, 1H, N–CH); 5.87 (br s, 2H, NH_2); 7.25 (br, 4H, NH); 7.72 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 10.77 and 20.47 (CH_3); 28.78 (CH_2); 50.98 (N–CH); 119.71 (C-5); 135.81 (C-8); 152.18 (C-4); 158.81 (C-6); 159.96 (C-2); and 160.39 (C–N).

6.3.15. 2-Amino-9-(2-azidoethyl)-6-(N,N-dimethylamino)-9*H*-purine (5g) and 2-amino-9-(2-azidoethyl)-6-guanidino-9*H*-purine (4g). Method B; starting compound **2g** yield: (0.71 g, 3 mmol); reaction time 6 h; chromatography on silica gel column, (30 g, elution by S1) afforded compound **5g**; yield: (0.14 g, 19%); white crystals, (*i*-PrOH);

mp 118–121 °C. For $C_9H_{13}N_9$ (247.26) calcd: C, 43.72; H, 5.30; N, 50.98. Found: C, 43.67; H, 5.35; N, 50.61. FABMS: 248 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 3.35 (br, 6H, N–CH_3); 3.73 (t, 2H, J(2′, 1′) = 5.3, H-2′); 4.15 (t, 2H, J(1′, 2′) = 5.3, H-1′); 5.85 (br s, 2H, NH_2); 7.34 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 37.76 and 39.70 (N–CH_3); 42.10 (C-1′); 49.70 (C-2′); 113.79 (C-5); 136.63 (C-8); 152.92 (C-4); 154.90 (C-6); and 159.70 (C-2).

(Subsequent elution of the column by S2) gave **4g**; yield: (0.55 g, 70%); white crystals, (*i*-PrOH); mp 226 °C (dec). For $C_8H_{11}N_{11}$ (261.25)·2/3H_2O calcd: C, 35.16; H, 4.55; N, 56.48. Found: C, 35.25; H, 4.52; N, 56.83. FABMS: 262 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 3.73 (t, 2H, J(2′, 1′) = 5.8, H-2′); 4.15 (t, 2H, J(1′, 2′) = 5.8, H-1′); 6.00 (br s, 2H, NH_2); 7.50 (br, 4H, NH); 7.69 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 41.98 (C-1′); 49.35 (C-2′); 119.20 (C-5); 137.70 (C-8); 152.33 (C-4); 159.13 (C-6); 160.05 (C-2); 160.13 (C–N).

6.3.16. 2-Amino-9-(2-aminoethyl)-6-guanidino-9*H*-purine (4h). A solution of compound **4g** (0.35 g, 1.3 mmol) in MeOH (50 mL) containing concentrated HCl (0.19 mL) was hydrogenated over 5% Pd/C (0.1 g) at room temperature overnight. The suspension was filtered over Celite and washed with methanol (100 mL) and the filtrate was made alkaline by triethylamine. The solvent was evaporated and the residue deionized on a Dowex 50 × 8 (H^+-form). The column was washed with water and eluted with a mixture of concd aqueous-NH_3–H_2O–MeOH (1:5:5). The filtrate was evaporated in vacuo and the residue was purified by HPLC in 0.025 M triethylammonium bicarbonate to give (0.19 g, 60%) as white solid (MeOH); mp 182 °C (dec). FAB-HRMS calcd for $C_8H_{14}N_9$ (MH^+) 236.1372, found 236.1380. FABMS: 236 [MH^+] (100); ^{1}H NMR (DMSO-d_6): 2.86 (t, 2H, J = 4.6) and 3.92 (t, 2H, J(CH_3, CH_2) = 6.4, N–CH_2); 5.98 (br s, 2H, NH_2); 7.60 (br, 4H, NH); 7.66 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 41.62 (C-1′); 45.86 (C-2′); 119.28 (C-5); 138.15 (C-8); 152.25 (C-4); 159.05 (C-6); 160.15 (C-2); and 160.21 (C–N).

6.3.17. 2-Amino-6-guanidino-9-(2,2,2-trifluoroethyl)-9*H*-purine (4i). 2-Amino-6-chloropurine derivative **2i** (0.4 g, 1.6 mmol), DABCO (0.18 g, 1.6 mmol), and a guanidine solution (24 mL, 8 mmol) was stirred at rt for 3 h. The resulting mixture was neutralized with 4.5 M HCl in DMF, evaporated in vacuo, and co-distilled with toluene (3 × 30 mL). The residue was dissolved in H_2O–MeOH (1:1) and applied on the column of Dowex 50 × 8 (H^+-form). The column was washed with water, then with MeOH and subsequently eluted with a mixture of concd aqueous-NH_3–H_2O–MeOH (1:5:5) mixture. The filtrate was evaporated in vacuo and chromatographed on a column of silica gel, (20 g, EtOH–EtOAc (10:90), followed by S2 and S3). The purified product **4i** gave after crystallization (EtOH) colorless crystals; yield: (0.32 g, 73%); mp 206 °C (dec). FABHRMS calcd for $C_8H_{10}F_3N_8$ (MH^+) 275.0981, found 275.0985. FABMS: 275 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 4.94 (q, 2H, J(CH_2, F) = 9.3, N–CH_2); 6.52 (br s, 2H, NH_2); 7.91 (s, 1H, H-8); 8.36 (br s, 4H, NH). ^{13}C NMR (DMSO-d_6): 43.11 (q, J(C, F) = 33.7, N–CH_2); 116.11 (C-5); 123.89 (q, J(C, F) = 279.3, CF_3); 139.06 (C-8); 153.22 (C-4); 155.47 (C-6); 157.95 and 159.54 (C-2 and N-C).

6.3.18. 2-Amino-6-guanidino-9-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]-9*H*-purine (4j). 2-Amino-6-chloropurine derivative **2j** (0.9 g, 3 mmol), DABCO (0.34 g, 3 mmol), and a guanidine solution (45 mL, 15 mmol) was stirred at rt for 6 h. The resulting mixture was neutralized with 4.5 M HCl in DMF, evaporated in vacuo, and co-distilled with toluene (3 × 30 mL). The residue was chromatographed on a column of silica gel (20 g, subsequently S1 and S3). The product **4j** (0.76 g) contains impurities difficult to remove. Therefore, it was used without characterization for the next step.

6.3.19. 2-Amino-6-guanidino-9-(2-hydroxyethyl)-9*H*-purine (4k). Compound **4j** (0.70 g) in MeOH–H_2O (1:1) (20 mL) was treated with 1 M HCl (5.6 mL) for 30 min at room temperature. The reaction mixture was applied on a column of Dowex 50 × 8 (H^+-form). The column was washed with water and eluted with a mixture of concd aqueous-NH_3–H_2O (1:10); the filtrate was evaporated in vacuo to give compound **4k**; yield: (0.31 g, 67%); colorless crystals (MeOH); mp 243 °C (dec). For $C_8H_{12}N_8O$·1/5 H_2O (236.24) calcd: C, 40.06; H, 5.21; N, 46.72. Found: C, 40.35; H, 5.17; N, 46.43. FABMS: 237 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 3.67 (t, 3H, J(2′, 1′) = 5.6, H-2′); 4.00 (t, 2H, J(1′, 2′) = 5.6, H-1′); 5.05 (br s, 1H, OH); 5.92 (br s, 2H, NH_2); 7.40 (br, 4H, NH); 7.60 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 45.30 (C-1′); 59.70 (C-2′); 119.38, (C-5); 138.22 (C-8); 152.23 (C-4); 158.23 (C-6); 160.17; and 160.44 (C-2 and C–N).

6.3.20. 2-Amino-6-guanidino-9-2-[(ethylamino)carbonyl]-benzoic acid-9*H*-purine (4l). Method B; starting from compound **2l** (1.03 g, 3 mmol); reaction time, 8 h (during this time DMF (30 mL) was gradually added to enhance solubility of reaction mixture); chromatography on silica gel column (30 g, elution by S1–S4) gave the product **4l**; yield: (0.39 g, 34%); white solid; mp 288 °C (dec). For $C_{16}H_{17}N_9O_3$·4/3H_2O (383.37) calcd: C, 47.17; H, 4.87; N, 30.84. Found: C, 47.02; H, 4.71; N, 30.46. FABMS: 384 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 3.58 (m, 2H) and 4.21 (m, 2H, N–CH_2); 7.35 (m, 3H) and 7.71 (m, 1H, arom.); 8.09 (s, 1H, H-8); 6.76 (br s, 2H), 8.60 (br, 2H), 8.98 (br s, 1H), and 10.70 (br, 2H, NH, OH). ^{13}C NMR (DMSO-d_6): 42.55 (N–CH_2); 114.39, (C-5); 127.76, 128.50, 128.89, 129.31, 136.58, and 138.49 (arom C); 141.77 (C-8); 150.00 (C-4); 153.30, 156.67, and 158.93 (C-2, C-6, and C–N); 170.18 and 172.49 (C═O).

6.3.21. 2,6-Diamino-9-ethyl-9*H*-purine[17] (6). Compound **2b** (0.3 g, 1.5 mmol) was treated with ethanolic ammonia (ethanol saturated with NH_3 at 0 °C) (20 mL) in an autoclave at 100 °C for 24 h. The solvent was removed in vacuo and the residue was applied on a

column of Dowex 50×8 (H^+-form). The column was washed with water, eluted with a mixture of concd aqueous-NH_3–H_2O (1:10); the filtrate was evaporated in vacuo. The residue was purified on a column of silica gel (15 g, MeOH–$CHCl_3$ 15:85) to give compound **6** (0.20 g, 75%); white crystals, (*i*-PrOH); mp 242–243 °C. For $C_7H_{10}N_6$ (178.20)·1/7H_2O calcd: C, 46.51; H, 5.73; N, 46.49. Found: C, 46.79; H, 5.53; N, 46.44. FABMS: 179 [MH^+] (100); ^{1}H NMR (DMSO-d_6): 1.32 (t, 3H, $J(CH_3, CH_2) = 7.3$, CH_3); 4.97 (q, 2H, $J(CH_2, CH_3) = 7.3$, N–CH_2); 5.82 (br s, 2H) and 6.70 (br s, 2H, NH_2); 7.72 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 15.53 (CH_3); 37.59 (N–CH_2); 113.47 (C-5); 137.26 (C-8); 151.77 (C-4); 156.31 (C-6); 160.42 (C-2).

6.3.22. 2,6-Diamino-9-isopropyl-9*H*-purine (7). Compound **2d** (0.3 g, 1.4 mmol) was treated with ethanolic ammonia (ethanol saturated with NH_3 at 0 °C) (20 mL) in autoclave at 100 °C for 24 h. The solvent was removed in vacuo and the residue was applied on a column of Dowex 50×8 (H^+-form). The column was washed with water, eluted with a mixture of aqueous-NH_3–H_2O (1:10); the filtrate was evaporated in vacuo. The residue was purified on column of silica gel, (15 g, MeOH–$CHCl_3$ 15:85). To give (0.19 g, 70%); white crystals, (*i*-PrOH); mp 212–214 °C. For $C_8H_{12}N_6$ (192.22)·2/7H_2O calcd: C, 48.68; H, 6.42; N, 42.58. Found: C, 48.96; H, 6.19; N, 42.20. FABMS: 193 [MH^+] (100); ^{1}H NMR (DMSO-d_6): 1.44 (d, 6H, $J(CH_3, CH) = 6.7$, CH_3); 4.51 (sept, 1H, $J(CH, CH_3) = 6.7$, CH_3); 5.85 (br s, 2H) and 6.76 (br s, 2H, NH_2); 7.82 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 22.35 (2C, CH_3); 45.61 (N–CH); 113.59 (C-5); 135.49 (C-8); 151.43 (C-4); 156.07 (C-6); 159.92 (C-2).

6.4. Cell cultures

In vitro biological assays were performed using mouse resident peritoneal macrophages. Female mice of the inbred strain C57BL/6, weighing 19–22 g, were purchased from Charles River Deutschland (Sulzfeld, Germany). They were kept in transparent plastic cages in an Independent Environmental Air Flow Animal Cabinet (ESI Flufrance, Wissous, France). Lighting was set on for 6–18 h, temperature at 22 °C. Animals, killed by cervical dislocation, were ip injected with 8 mL of sterile saline. Pooled peritoneal cells collected from mice ($n = 3$–5 in individual experiments) were washed, resuspended in culture medium, and seeded into 96-well round-bottom microplates (Costar, Cambridge, MA) in 100 μL volumes, 2×10^5 cells/well. Adherent cells (macrophages) were isolated by incubating the cells for 2 h at 37 °C, 5% CO_2, and then vigorously shaking the plate and washing the wells three times to remove non-adherent cells. Cultures were maintained at 37 °C, 5% CO_2 in humidified Heraeus incubator for 24 h. All protocols were approved by the Institutional Ethics Committee.

Complete RPMI-1640 culture medium (Sigma-Aldrich, Prague, CR), used throughout the experiments, contained 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 μg/mL gentamicin, and 5×10^{-5} M 2-mercaptoethanol (all Sigma).

6.5. Nitric oxide (NO) production

The cells were cultured for 24 h in the presence of test compounds, applied either alone or in the presence of NO-priming immune stimuli, that is murine recombinant interferon-γ (IFN-γ, 5000 pg/mL; R&D Systems, Minneapolis, MN) or lipopolysaccharide (LPS from *E. coli* 0111:B4, 100 pg/mL; Sigma). In order to reach a maximum NO response, also the combination of LPS + IFN-γ was employed. The concentration of nitrites in supernatants of cells was taken as a measure of NO production.[18] It was detected in individual, cell-free samples (50 μL) incubated 5 min at ambient temperature with an aliquot of a Griess reagent (1% sulfanilamide/0.1% naphtylendiamine/2.5% H_3PO_4). The absorbance at 540 nm was recorded using a microplate spectrophotometer (Tecan, Austria). A nitrite calibration curve was used to convert absorbance to μM nitrite.

6.6. Chemokine secretion

Macrophages were cultured 5 h in absence or presence of test compounds. Concentration of chemokines RANTES (CCL5) and MIP-1α (CCL3) in cell supernatants was determined using enzyme-linked immunoabsorbent assay (ELISA) kits following the manufacturer's instructions (R&D Systems).

6.7. Cell viability

Viability of macrophages was determined using a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells (Roche Diagnostics, Mannheim, Germany). The cells were cultured as described above. After the 18 h culture, the WST-1 was added and the cells were left in the incubator for a 6 h period. Optical density at 450 nm was then evaluated.

6.8. Statistical analysis

Analysis of variance (ANOVA) with subsequent Dunnett's multiple comparison test, and graphical presentation of data were done using the Prism program (GraphPad Software, San Diego, CA). All data were reported as means ± SEM.

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