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Short communication

Synthesis and biological evaluation of some 6-substituted purines

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Abstract

We report herein the synthesis and the in vitro antileishmanial evaluation of a series of 6-substituted purines. The most active compounds against *Leishmania amazonensis* promastigotes were 6-(3′-chloropropylthio)purine **2** (D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, B.A. Rapp, D.L. Wheeler, Genbank. Nucleic Acids Res. 28 (2000) 15–18; E.V. Aleksandrova, P.M.I.E. Valashek, J. Med. Pharm. Chem. 35 (2001) 172–173), 6-(3′-(thioethylamine)propylthio)purine **5**, 6-(α-aceticacidthio)purine **7** and 6-(6′-deoxy-1′-*O*-methyl-β-D-ribofuranose)purine **14** with an $IC_{50} = 50$, 50, 39 and 29 μM, respectively.

Keywords: 6-Substituted purines; Antileishmanial activity

1. Introduction

Leishmaniasis is an infection caused by multiple species of the protozoan parasite *Leishmania*. This disease is endemic in some tropical areas of the world and in underdeveloped countries, with an estimated 1.5–2 million cases per year in 88 countries [1]. Clinical manifestations occur in three major forms in human: cutaneous, mucocutaneous and visceral, which are fatal if untreated [2]. Recently, visceralization by dermotropic species of this parasite has also been reported as a complication in HIV co-infected persons [3,4].

Chemotherapeutic treatment of leishmaniasis usually relies on the use of pentavalent antimonials such as sodium stibogluconate (pentostan) and meglumine antimoniate (glucantime) that induce toxic side effects together with drug resistance [3–5]. The second line of compounds used during the treatment of unresponsive cases generally includes pentamidine and amphotericin B [4]. More effective and safer drugs have been developed, including various colloidal and lipid formulations and recently the oral drug miltefosine [3,4,6]. However, the latter drug cannot be given during pregnancy and shows severe gastrointestinal side effects [4]. Moreover, its cost represents another limiting factor for a general use. Other drugs such as paromomycin, allopurinol and sitamaquine have been reported to exhibit variable cure rates [4]. Because of these limitations, combined therapies, including immunomodulator drugs, could be the best approach to avoid the emergence of drug resistance.

The inability of protozoan parasites to synthesize purines de novo is a rational therapeutical strategy for the treatment and prevention of parasitic disease [7]. Purine and pyrimidine antimetabolites have been highly successful against many viral infections as well as malignancies and show great promise against protozoal infections as well [8]. However, many therapies suffer from a lack of selectivity, leading to severe side effects. The selectivity and efficacy of purine antimetabolites are achieved at two levels: the cell surface transporters that mediate access to the cell, and the enzymes of the purine metabolic pathways that convert the prodrug to the cytotoxic metabolite, usually a nucleotide analogue [7]. Allopurinol,

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a purine nucleobase analogue, is chemically used against various manifestations of leishmaniasis [4]. In a previous study, we have shown that 6-thiopurine derivatives have proved inhibitory effects in *Leishmania* [9,10]. So, the aim of this study was to evaluate in vitro several 6-thiopurine derivatives against different species of *Leishmania*.

2. Chemistry

A series of 6-substituted purines were synthesized. As shown in Table 1, the analogs varied with the nature of the substituent attached to the sulfur atom. The compounds **3**, **4**, **5**, **8**, **13**, **14**, **18** and **22** are novel.

The compounds **2** [11,12], **6**, **8** and **9** [13] were synthesized according to the following reaction condition.

A solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine with NaH in DMF at 0 °C for 30 min and at rt for over 1 h, was transferred to another flask containing a solution of the alkylhalide in DMF at 0 °C for 30 min. The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography. Compounds **3**, **4** and **5** were obtained by the treatment of 6-(3′-chloropropylthio)purine **2** with cyclohexilamine, ethylenediamine and thioethylamine, respectively, at 25 °C for 48 h. Compound **7** was obtained by treatment of **6** with KOH (1 M) in methanol and H_2O for 24 h at 25 °C.

The synthesis of 6-(5′-deoxy-1′-*O*-methyl-β-D-ribofuranose) purine **14**, 6-(6′-deoxy-1′,2′,3′,4′-diisopropylidene-α-D-galactopyranose)purine **18** and 6-(1′-deoxy-2′,3′,4′,5′-diisopropylidene-β-D-psicopyranose)purine **22** was achieved with a versatile and efficient synthetic route outlined in Schemes 1–3, respectively. Subsequent acid deprotection of the isopropylidene group of **18** and **22** did not afforded the compounds hydrolyzed.

All products were purified by flash chromatography and the reaction yields were generally between 50 and 90%. All compounds were demonstrated to be of sufficient purity for use in biological assays (>95%) by ^{13}C nuclear magnetic resonance (^{13}C NMR).

3. Biological evaluation

3.1. In vitro antileishmanial activity

L. amazonensis (MHOM/Br/75/Josefa isolated from a patient with diffuse cutaneous leishmaniasis) and *L. chagasi* (MHOM/Br/74/PP75 isolated from a patient with visceral leishmaniasis) promastigotes were used for in vitro screening. The antileishmanial activity was determined by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method based on tetrazolium salt reduction by mitochondrial dehydrogenases [14]. Briefly, promastigotes of *L. amazonensis* were cultured in the Warren's medium (brain heart infusion plus hemin and folic acid) and promastigotes of *L. chagasi* were maintained in Medium 199 (Sigma Chemical Co., St Louis, MO), both supplemented with 10% fetal bovine serum at 24 °C. The screening was performed in flat-bottomed 96-well plastic tissue-cultured plates maintained at 24 °C. Promastigote forms from a logarithmic phase culture were suspended to yield 2 millions of cells/mL (*L. amazonensis*) or 3 millions of cells/mL (*L. chagasi*) after Newbauer haemocytometer counting. Each well was filled with 100 μl of the parasites suspension, and the plates were incubated at 24 °C for 1 h before drug addition. The compounds to be tested were dissolved in water or DMSO and added to each well. Up to 1% (v/v), DMSO had no effect on parasite's growth. Each concentration was screened in triplicate. The viability of promastigotes of both the species was assessed by MTT colorimetric method. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) after a three-day incubation period. Amphotericin B was used as the reference drug and IC_{50} values were of 0.9 μM and 1.9 μM on *L. amazonensis* and *L. chagasi* promastigote forms, respectively.

3.2. Cytotoxicity

For cytotoxicity against mammalian cells, adherent mouse peritoneal macrophages were cultured for 48 h at 37 °C with varying concentration of the 6-substituted purines. The viability of the macrophages was determined with the MTT assay, as described above, and was confirmed by comparing the morphology of the control group via light microscopy.

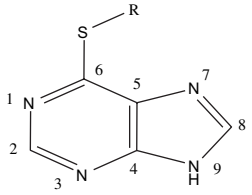
4. Biological results and discussion

In this report it was evaluated that the effect of 6-substituted purines against two different species of *Leishmania* from the New World: *L. amazonensis* which has been associated with all clinical forms of leishmaniasis [2] and *L. chagasi* which is the causal agent of visceral disease [2], as reported in Table 1. Based on this fact it was expected to have different sensitivity of these parasites to the assayed drugs. Indeed, in a general point of view, this difference was observed and the results demonstrated that the compounds tested showed activity only against promastigotes of *L. amazonensis*. Among the 13 tested compounds **2**, **5**, **7** and **14** showed an activity against *L. amazonensis* (IC_{50} values of 50, 50, 39 and 29 μM, respectively) promastigote forms. In our assay, only those compounds presenting activity against promastigotes of *L. amazonensis* were further evaluated for cytotoxicity of mammalian cells. Interestingly, none of the compounds were found to have significative toxicity towards mammalian cells at the maximal concentration used (227 μM), data not shown.

The differences in sensibility between several species of *Leishmania* accentuate the importance of speciation in the treatment of leishmaniasis. Only American cutaneous leishmaniais is associated to least 14 species of *Leishmania* pathogenic to human with biochemical and molecular variation [15].

The identification of fundamental biochemical discrepancies between a parasite and its mammalian hosts offers a promising strategy for therapeutic exploitation of parasitic diseases. One striking metabolic disparity between protozoan parasites and their mammalian hosts is the way that purine

Table 1
Structures and antiproliferative activities of 6-thiopurine and 6-substituted purines against *L. amazonensis* and *L. chagasi* in vitro



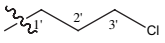
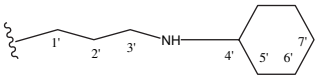
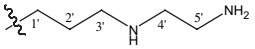
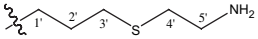
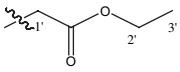
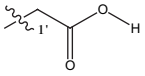
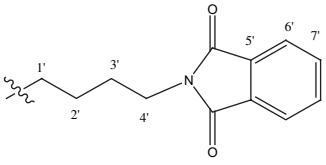
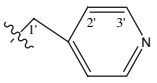
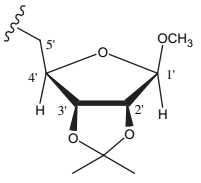
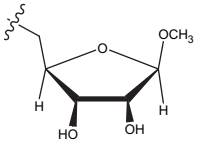
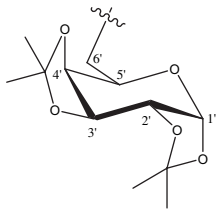
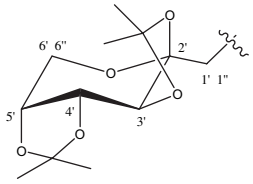
Compound	R	IC_{50} against *L. amazonensis* (μM)	IC_{50} against *L. chagasi* (μM)
1	H	>227	>227
2		50	>227
3		>227	>227
4		>227	>227
5		50	>227
6		>227	>227
7		39	>227
8		>227	>227
9		>227	>227
13		>227	>227
14		29	>227

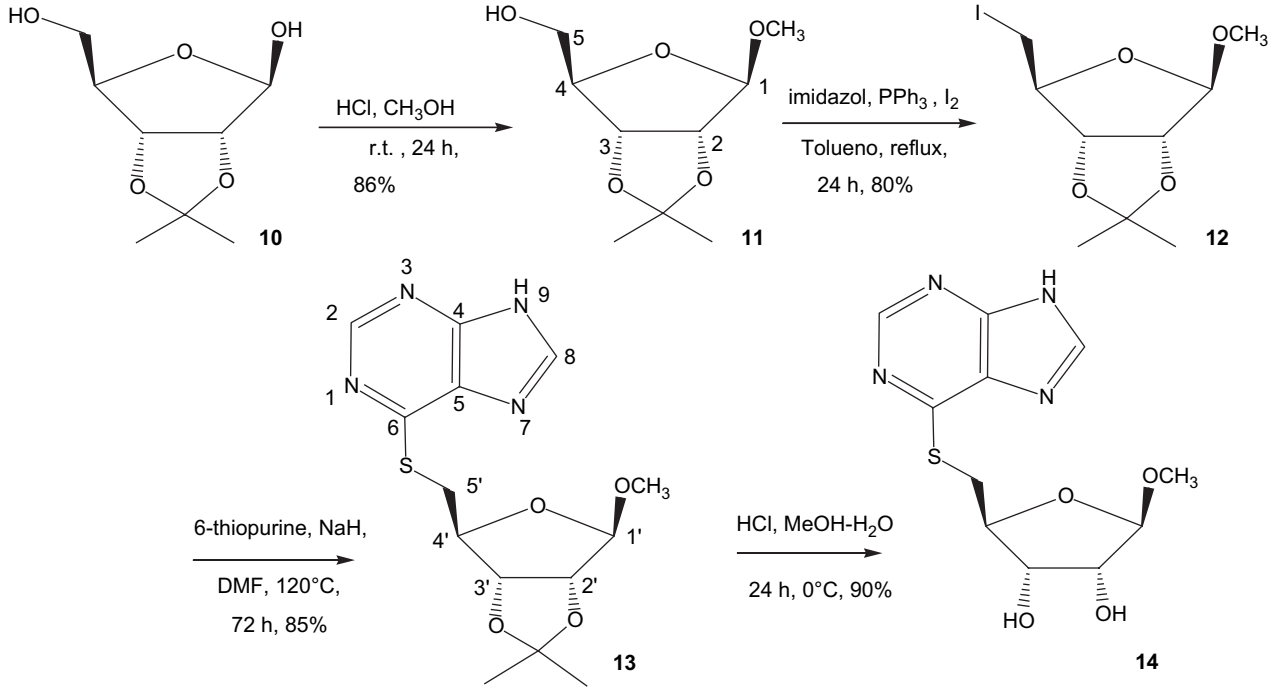
Table 1 (*continued*)

Compound	R	IC_{50} against *L. amazonensis* (μM)	IC_{50} against *L. chagasi* (μM)
18		>227	>227
22		>227	>227
Amphotericin B		0.9	1.9

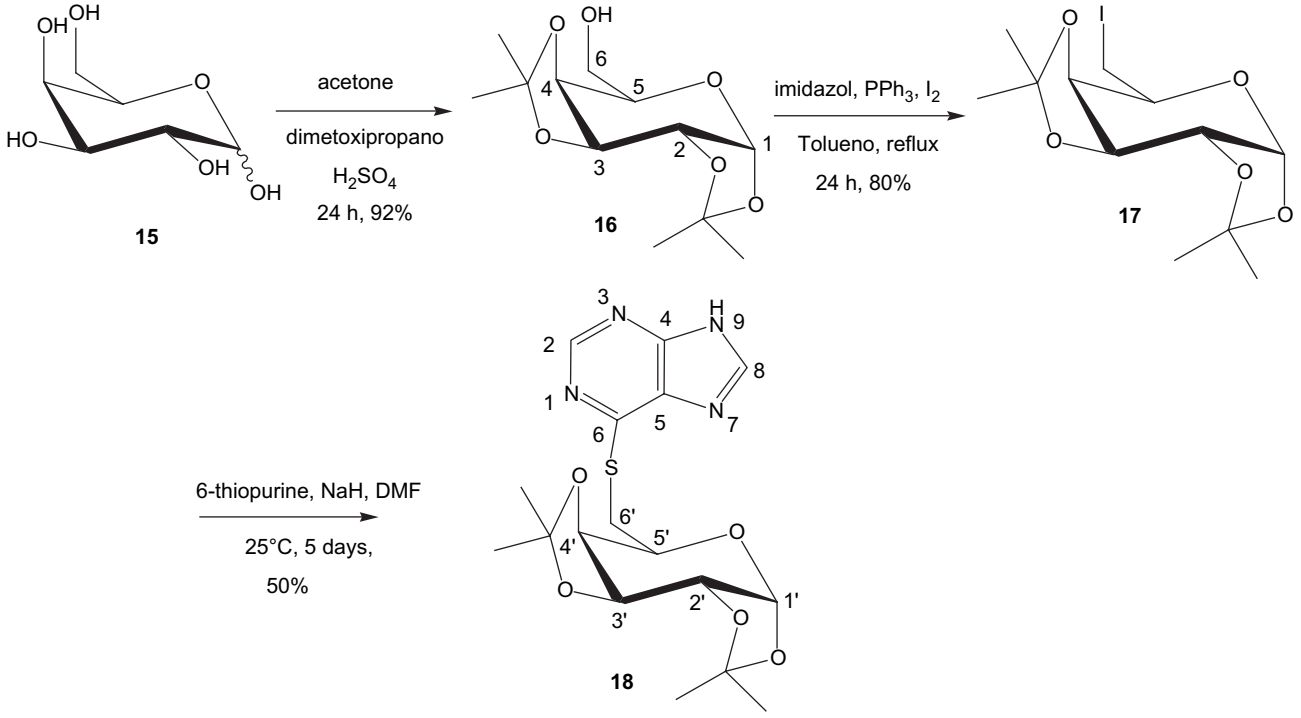
nucleotides are synthesized [7]. Whereas mammalian cells can synthesize the purine ring from amino acids and other small molecules, all protozoan parasites studied to date are incapable of synthesizing purines de novo. Nucleoside transporter has been investigated in several parasitic protozoa, for example, *Trypanosoma brucei* and *Leishmania* species [7]. In this parasite it was reported in promastigote forms of *L. braziliensis*, *L. major* and *L. donovani* [7,16,17]. Recently, were reported the first identification and characterization of a purine nucleobase transport in *L. mexicana* amastigotes [8].

From our biological results, it is evident that 6-substituted purines exhibited potent antileishmanial activity. The overall activity profile of compounds **2**, **5**, **7** and **14** demonstrated that there is a small difference in their IC_{50} values on *L. amazonensis*.

In summary, we have demonstrated the activity of 6-substituted purines in vitro for both *L. amazonensis* and *L. chagasi* promastigotes and the 6-(6′-deoxy-1′-*O*-methyl-β-D-ribofuranose)purine **14** was evidently the more active compound. Knowledge of the sensitivity of each species has important implications for clinical treatment.



Scheme 1. Synthesis of the 6-(6′-deoxy-1′-*O*-methyl-β-D-ribofuranose)purine (**14**).

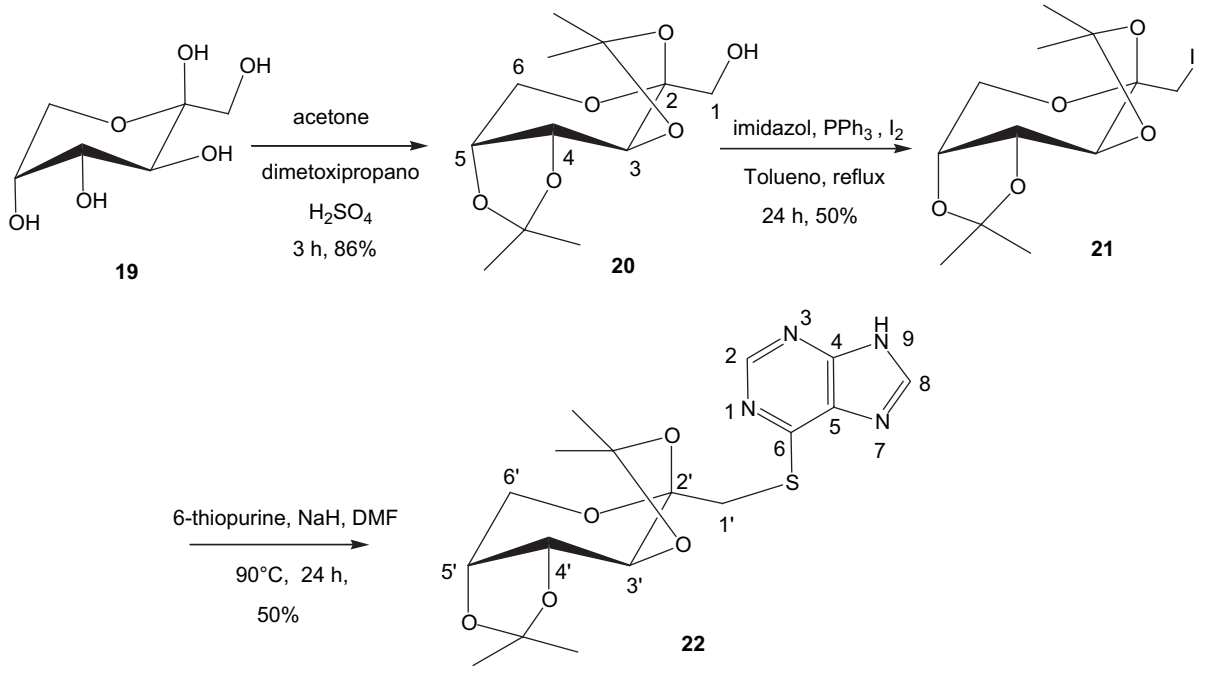


Scheme 2. Synthesis of the 6-(6′-deoxy-1′,2′,3′,4′-diisopropylidene-α-D-galactopyranose)purine (**18**).

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). All structures were confirmed by ^{13}C NMR and ^{1}H NMR. Spectra were recorded at 300 MHz with Bruker spectrometer, using tetramethylsilane (TMS) as an internal standard. Elemental analyses were carried out on a CHN-O rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ±0.4% of the theoretical values. Electron impact mass spectra were measured with an AEI



Scheme 3. Synthesis of the 6-(1′-deoxy-2′,3′,4′,5′-diisopropylidene-β-D-psicopyranose)purine (**22**).

MS 50 mass spectrometer. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel (70–230 mesh).

Compounds **2** [11,12], **6** [13], **7** [13] and **9** [13] have been described in the literature.

*5.1.1. 6-(3′-Chloropropylthio)purine (**2**)*

1-Bromo-3-chloropropane (1 g, 6.35 mmol) was dissolved in EtOH at 0 °C and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (1 g, 6.62 mmol) with NaH (0.15 g, 6.25 mmol) in EtOH (20 mL) at 0 °C for 30 min and at rt for over 1 h.

The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 4:6) producing **2** (900 mg, 3.94 mmol) in 59% yield as a white solid, m.p. 200 °C; ν_{max} (KBr): 3472 (NH), 2975 (CH), 570 cm^{-1} (CCl); ^1H NMR (300 MHz, $CDCl_3$): δ 8.45 (s, 1H, H-2), 7.96 (s, 1H, H-8), 3.76 (t, 2H, $J_{3',2'} = 6$ Hz, H-3′), 3.44 (t, 2H, $J_{1',2'} = 6$ Hz, H-1′), 2.17 (quint, 2H, H-2′); MS (EI) *m/z*: 229 $[M + 1]^+$; 193 $[M - 35]^+$; 152 + 1 $[M - 71]^+$; ^{13}C NMR (75 MHz, DMSO) δ ppm: 161 (C-2), 144 (C-8), 50 (C-3′), 26 (C-1′), 22 (C-2′).

*5.1.2. 6-(3′-(N-Cyclohexanamine)propylthio)purine (**3**)*

Cyclohexilamine (44 mg, 0.44 mmol) was dissolved in DMF and then added to a solution of 6-(3′-chloropropylthio)-purine **2** (50 mg, 0.22 mmol) in DMF (3 mL).

The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: CH_2Cl_2:MeOH 8:2) producing **3** (36 mg, 0.12 mmol) in 56% yield as a white solid, m.p. 250 °C; ν_{max} (KBr): 3450 (NH_2), 1645 cm^{-1} (NH_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H, H-2), 7.83 (s, 1H, H-8), 4.2 (s, 1H, NH), 3.30 (m, 2H, H-1′), 2.69 (t, 2H, $J_{1',2'} = 7.2$ Hz, H-1′), 2.01 (m, 2H, H-3′), 1.89–1.40 (m, 9H, H-2′, H-4′, H-5′, H-6′, H-7′) (Found: C, 57.58; H, 7.24; N, 24.10. $C_{14}H_{21}N_5S$ requires C, 57.70; H, 7.26; N, 24.03).

*5.1.3. 6-(3′-(N-Ethylenediamine)propylthio)purine (**4**)*

Compound **4** was obtained by the treatment of 6-(3′-chloropropylthio)purine **2** (0.2 g, 0.87 mmol) with ethylenediamine (0.26 g, 4.4 mmol) in DMF (8 mL) at 25 °C for 48 h.

The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: CH_2Cl_2:MeOH 8:2) producing **4** (110 mg, 17 mmol) in 50% yield as a white solid, m.p. 280 °C; ν_{max} (KBr): 3460 (NH_2), 1650 cm^{-1} (NH_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.20 (s, 1H, H-2), 8.03 (s, 1H, H-8), 7.24 (s, 1H, NH), 4.58 (t, 2H, $J_{5',4'} = 6$ Hz, H-5′), 4.04 (t, 2H, H-4′), 3.33–3.15 (m, 4H, H-1′, H-3′), 2.50 (quint, 2H, H-2′), 1.98 (s, 2H, NH_2) (Found: C, 47.32; H, 6.38; N, 33.66. $C_{10}H_{16}N_6S$ requires C, 47.60; H, 6.39; N, 33.30).

*5.1.4. 6-(3′-(Thioethylamine)propylthio)purine (**5**)*

1-Bromo-3-chloropropane (100 mg, 0.44 mmol) was dissolved in DMF (4 mL) and then added to a solution of sodium thioethylamine, generated from the reaction of thioethylamine (150 mg, 1.3 mmol) with NaH (79 mg, 3.3 mmol) in DMF (6 mL) at 25 °C for 48 h.

The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: CH_2Cl_2:MeOH 8:2) producing **5** (67 mg, 0.22 mmol) in 50% yield as a white solid, m.p. 270 °C; ν_{max} (KBr): 3450 (NH_2), 1646 cm^{-1} (NH_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.52 (s, 1H, H-2), 8.40 (s, 1H, H-8), 5.42 (s, 2H, NH_2), 4.50 (t, 2H, $J_{5',4'} = 6.8$ Hz, H-5′), 3.30 (m, 6H, H-1′, H-3′, H-4′), 2.55 (q, 2H, H-2′) (Found: C, 44.32; H, 5.68; N, 25.91. $C_{10}H_{15}N_5S_2$ requires C, 44.58; H, 5.61; N, 26.00).

*5.1.5. 6-(α-Ethylacetatethio)purine (**6**)*

The ethyl 2-bromoacetate (1.5 g, 8.98 mmol) was dissolved in DMF (10 mL) at 0 °C and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (1.37 g, 9 mmol) with NaH (237 mg, 9.9 mmol) in DMF (10 mL) at 0 °C for 30 min and at rt for over 1 h.

The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 7:3) producing **6** (1.37 g, 5.7 mmol) in 64% yield as a white solid, m.p. 200 °C; ν_{max} (KBr): 3055 (CH), 1729 (CO), 1308 (CO), 857 cm^{-1} (CH); ^1H NMR (300 MHz, $CDCl_3$): δ 8.70 (s, 1H, H-2), 8.20 (s, 1H, H-8), 4.32 (quart, 2H, $J_{2',3'} = 7$ Hz, H-2′), 4.21 (s, 2H, H-1′), 1.31 (t, 3H, H-3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170 (C=O), 152 (C-2), 143 (C-8), 63 (C-2′), 32 (C-1′), 15 (C-3′); MS TOF ES^-: *m/z*: 237 $[M - 1]$, 192 $[C_7H_5ON_4S]$, 165 $[C_6H_5N_4S]$, 163 $[C_6H_3N_4S]$.

*5.1.6. 6-(α-Aceticacidthio)purine (**7**)*

Compound **7** was obtained by treatment of **6** (1 g, 4.2 mmol) with KOH (10 mL, 1 M) in methanol (20 mL) and H_2O (14 mL) for 24 h at 25 °C.

The reaction was neutralized by 1 N HCl. The solvent was evaporated in vacuum until dryness. The crude reaction product was recrystallized in acetone. TLC (eluent: CH_2Cl_2:MeOH 8:2) producing **7** (0.8 g, 3.8 mmol) in 91% yield as a colorless oil; ν_{max} (KBr): 3334 (OH), 1629 (CO), 979 cm^{-1} (OH); ^1H NMR (300 MHz, D_2O): δ 8.50 (s, 1H, H-2), 8.30 (s, 1H, H-8), 4.03 (s, 2H, H-1′); ^{13}C NMR (75 MHz, D_2O) δ: 154 (C-2); 146 (C-8); 37 (C-1′); MS TOF MS ES^-: *m/z*: 209 $[M - 1]$, 165 $[C_6H_5N_4S]$, 150 $[C_5H_3N_4S]$.

*5.1.7. 6-(4′-(Isoindoline-1′,3′-dione)butylthio)purine (**8**)*

2-(4-Bromobutyl)isoindoline-1,3-dione (142 mg, 0.5 mmol) was dissolved in EtOH and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (50 mg, 0.33 mmol) with NaH (11 mg, 0.46 mmol) in EtOH (6 mL) at 0 °C for 30 min and at rt for over 1 h. The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was

purified by flash chromatography (eluent: AcOEt) producing **8** (58 mg, 0.14 mmol) in 50% yield as a white solid, m.p. 194 °C; ν_{max} (KBr): 3456 (NH_2), 1641 cm^{-1} (NH_2); ^1H NMR (300 MHz, $CDCl_3$): δ 8.45 (s, 1H, H-2), 7.96 (s, 1H, H-8), 7.82 (s, 4H, H-6′, H-7′), 3.61 (t, 2H, $J_{4',3'} = 6.6$ Hz, H-4′), 3.36 (t, 2H, $J_{1',2'} = 6.8$ Hz, H-1′), 1.74 (m, 4H, H-2′, H-3′); ^{13}C NMR (75 MHz, DMSO) δ ppm: 167 (C=O), 151 (C-2), 134 (C-6′), 131 (C-8), 123 (C-7′), 36.9 (C-4′), 27.3, 27.0, 26.5 (C-1′, C-2′, C-3′) (Found: C, 57.62; H, 4.38; N, 19.76. $C_{17}H_{15}N_5O_2S$ requires C, 57.78; H, 4.28; N, 19.82).

5.1.8. 6-((Pyridin-4′-yl)methylthio)purine (*9*)

The 4-(bromomethyl)pyridine·HBr (132 mg, 0.152 mmol) was dissolved in EtOH and neutralized in $NaHCO_3$ (17.4 mg, 0.208 mmol) and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (50 mg, 0.22 mmol) with NaH (5.5 mg, 0.23 mmol) in EtOH (6 mL) at 0 °C for 30 min and at rt for over 1 h. The reaction mixture was stirred for an additional 48 h at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: CH_2Cl_2:MeOH 10:2) producing **9** (50 mg, 0.2 mmol) in 62% yield as a white solid, m.p. 230 °C; ν_{max} (KBr): 3450 (NH_2), 1644 cm^{-1} (NH_2); ^1H NMR (300 MHz, DMSO): δ 8.71 (s, 1H, H-2), 8.48 (d, 2H, $J_{2',3'} = 5.6$ Hz, H-3′), 8.46 (s, 1H, H-8), 7.45 (d, 2H, H-2′), 4.65 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO) δ ppm: 151 (C-2), 149 (C-3′), 147 (Cq), 143 (C-8), 124 (C-2′), 30.3 (C-1′).

5.1.9. 6-(5′-Deoxy-1′-O-methyl-2′,3′-O-isopropylidene-β-D-ribofuranose)purine (*13*)

5-Desoxy-5-iodo-1-*O*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranose **12** [18] (1.2 g, 3.8 mmol) was dissolved in DMF (10 mL) at 0 °C and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (0.6 g, 3.9 mmol) with NaH (0.13 g, 5.4 mmol) in DMF (10 mL) at 0 °C for 30 min and at rt for over 1 h.

The reaction mixture was stirred for an additional 72 h at 120 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 1:1) producing **13** (1.1 g, 3.25 mmol) in 85% yield as a colorless oil; ν_{max} (KBr): 2987 (CH), 1104 cm^{-1} (C–O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.85 (s, 1H, H-2), 8.40 (s, 1H, H-8), 5.14 (s, 1H, H-1′), 4.9 (d, 1H, H-2′, $J_{2',3'} = 5.9$ Hz), 4.8 (d, 1H, H-3′), 4.65 (m, 1H, H-4′), 3.8–3.6 (2dd, 2H, H-5′, H-5″, $J_{5',4'} = 2.3$ Hz, $J_{5'',4'} = 3.6$ Hz, $J_{5',5''} = 12.3$ Hz), 3.5 (s, 3H, OCH_3), 1.6–1.3 (2s, 6H, $2CH_3$); MS (IE) *m/z*: 229 $[M+1]^+$, 193 $[M-35]^+$, 152 + 1 $[M-71]^+$; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 160 (C-5), 152 (C-2), 149 (C-8), 142 (C-6), 131 (Cq), 113 (C-1′), 110 (C-3′), 86 (C-2′), 84 (C-4′), 56 (OCH_3), 32 (C-5′), 27–24 ($2CH_3$).

5.1.10. 6-(5′-Deoxy-1′-O-methyl-β-D-ribofuranose)purine (*14*)

6-(5′-Deoxy-1′-*O*-methyl-2′,3′-*O*-isopropylidene-β-D-ribofuranose)purine **13** (0.5 g, 1.48 mmol) was dissolved in MeOH (20 mL) at 0 °C and then added to a solution of HCl (1 N, 2 mL) in H_2O (2 mL) at 0 °C for 30 min and at rt for over 12 h.

The reaction mixture was stirred for an additional 24 h at 0 °C. The reaction was neutralized with $NaHCO_3$ and the solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: CH_2Cl_2:MeOH 7:3) producing **14** (400 mg, 1.34 mmol) in 90% yield as a colorless oil; ν_{max} (KBr): 3277 (OH), 2916 (CH), 1094 cm^{-1} (C–O); ^1H NMR (300 MHz, D_2O): δ 8.35 (s, 1H, H-2), 8.22 (s, 1H, H-8), 4.91 (s, 1H, H-1′), 4.34 (2d, 2H, H-2′, H-3′, $J_{2',3'} = 5.8$ Hz), 4.15 (m, 1H, H-4′), 3.83–3.45 (2dd, 2H, H-5′, H-5″, $J_{5',4'} = 2.3$ Hz, $J_{5'',4'} = 3.6$ Hz, $J_{5',5''} = 12.3$ Hz), 3.34 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, D_2O) δ ppm: 168 (C-5), 152 (C-2), 144 (C-8), 108 (C-1′), 81 (C-3′), 75 (C-2′), 74 (C-4′), 55 (OCH_3), 32 (C-5′) (Found: C, 44.18; H, 4.69; N, 18.56. $C_{11}H_{14}N_4O_4S$ requires C, 44.29; H, 4.73; N, 18.78).

5.1.11. 6-(6′-Deoxy-1′,2′,3′,4′-diisopropylidene-α-D-galactopyranose)purine (*18*)

6-Deoxy-6-iodo-1,2,3,4-diisopropylidene-α-D-galactopyranose **17** [19] (6 g, 16.2 mmol) was dissolved in DMF (30 mL) at 0 °C and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (2 g, 13.16 mmol) with NaH (0.4 g, 16.67 mmol) in DMF (10 mL) at 0 °C for 30 min and at rt for over 1 h.

The reaction mixture was stirred for an additional 5 days at 25 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 2:1) producing **18** (2.5 g, 6.6 mmol) in 50% yield as a white solid, m.p. 136 °C; ν_{max} (KBr): 3438 (NH), 1589 (C=C), 1113 cm^{-1} (C–O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.75 (s, 1H, H-2), 8.15 (s, 1H, H-8), 5.5 (d, 1H, H-1′, $J_{1',2'} = 4.8$ Hz), 4.6 (dd, 1H, H-3′, $J_{3',4'} = 8.1$ Hz, $J_{3',2'} = 2.4$ Hz), 4.4 (dd, 1H, H-4′), 4.29–4.25 (dd, 1H, H-2′), 4.25–4.20 (dd, 1H, H-5′, $J_{5',6'} = J_{5',6''} = 6.6$ Hz), 3.55–3.42 (m, 2H, H-6′, H-6″, $J_{6',6''} = 13.4$ Hz); MS TOF ES^- (MeOH) *m/z*: 394 [M], 393 [M − 1], 165 $[C_6H_5N_4S]$, 151 $[C_5H_3N_4S]$, MS TOF ES^+ *m/z*: 395 [M + 1], 279 $[C_{12}H_{20}O_5S]$; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 152.5 (C-2), 132 (C-8), 129 (C-6′), 110 (2Cq), 97 (C-1′), 72 (C-2′), 71 (C-4′), 70 (C-3′), 67 (C-5′), 27–24 ($4CH_3$) (Found: C, 51.61; H, 5.57; N, 14.02. $C_{17}H_{22}N_4O_5S$ requires C, 51.76; H, 5.62; N, 14.20).

5.1.12. 1-Deoxy-1-iodo-2,3,4,5-diisopropylidene-β-D-psicopyranose (*21*)

2,3,4,5-Diisopropylidene-β-D-psicopyranose **20** [20] (10 g, 40.3 mmol) was dissolved in toluene (200 mL) at rt and then added iodide (13 g, 51.2 mmol), imidazole (3.5 g, 51.4 mmol) and triphenylphosphine (13.7 g, 52.2 mmol) at rt for 30 min.

The reaction mixture was stirred for an additional 24 h at 110 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 2:8) producing **21** (5.5 g, 15.36 mmol) in 50% yield as a colorless oil; ν_{max} (KBr): 2988 (C–H), 1250 (C–O), 535 cm^{-1} (C–I); ^1H NMR (300 MHz, $CDCl_3$): δ 4.48–4.54 (dd, 1H, H-4, $J_{4,3} = 2.7$ Hz, $J_{4,5} = 7.9$ Hz), 4.3 (d, 1H, H-3, $J_{3,4} = 2.7$ Hz), 4.23–4.19

(dd, 1H, H-5, $J_{5,4} = 7.9$ Hz, $J_{5,6} = J_{5,6'} = 10$ Hz), 3.90–3.72 (2dd, 2H, H-6, H-6′, $J_{6,6'} = 12.9$ Hz, $J_{6,5} = J_{6',5} = 1.8$ Hz), 3.55–3.30 (2d, 2H, H-1, H-1′, $J_{1,1'} = 10$ Hz), 1.5–1.3 (4s, 12H, $4CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 109 (C-2), 108–107 (2Cq), 100 (C-3), 72 (C-5), 70.5 (C-4), 62 (C-6), 27–24 ($4CH_3$), 10 (C-1).

*5.1.13. 6-(1′-Deoxy-2′,3′,4′,5′-diisopropylidene-β-D-psicopyranose)purine (**22**)*

1-Deoxy-1-iodo-2,3,4,5-diisopropylidene-β-D-psicopyranose **21** (4.5 g, 12.56 mmol) was dissolved in DMF (10 mL) at 0 °C and then added to a solution of sodium 6-thiopurine, generated from the reaction of 6-thiopurine (1.81 g, 11.9 mmol) with NaH (0.3 g, 12.5 mmol) in DMF (10 mL) at 0 °C for 30 min and at rt for over 1 h.

The reaction mixture was stirred for an additional 24 h at 90 °C. The solvent was evaporated in vacuum until dryness. The crude reaction product was purified by flash chromatography (eluent: AcOEt:hexane 7:3) producing **22** (2.4 g, 6.3 mmol) in 50% yield as a colorless oil; ν_{max} (KBr): 3444 (NH), 2974 (CH), 1570 (C=C), 1061 cm^{-1} (C–O); ^1H NMR (300 MHz, $CDCl_3$) δ: 8.7 (s, 1H, H-2), 8.2 (s, 1H, H-8), 4.56–4.50 (dd, 1H, H-4′, $J_{4',3'} = 2.4$ Hz, $J_{4',5'} = 7.7$ Hz), 4.34 (d, 1H, H-3′), 4.22–4.16 (dd, 1H, H-5′), 4.06–3.87 (2dd, 2H, H-6′, H-6″, $J_{6'',6'} = 13.8$ Hz), 3.91–3.70 (m, 2H, H-1′, H-1″, $J_{1',1''} = 13.1$ Hz), 1.58–1.25 (4s, 12H, $4CH_3$); MS (MeOH) TOF ES^+ *m/z*: 395 [M + 1]; ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 151.5 (C-2), 141.5 (C-8), 109.5 (C-2′), 109–103 (2Cq), 73 (C-5′), 70.5 (C-4′), 61 (C-6′), 36 (C-3′), 27–25 ($4CH_3$), 24 (C-1′) (Found: C, 50.08; H, 5.78; N, 14.39. $C_{16}H_{22}N_4O_5S$ requires C, 50.25; H, 5.80; N, 14.65).

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