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Synthesis and antimycobacterial activity of purine conjugates with (*S*)-lysine and (*S*)-ornithine

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1. Chemistry

General

N-(Purin-6-yl)glycine (**1**) was obtained as previously described.[S1] 2-Acetamido-9-(2-acetoxyethoxy)methyl-6-chloropurine (**5**) and 9-(2-acetoxyethoxy)methyl-6-chloropurine (**10a**) were synthesized by analogy with the known methods.[S2,S3] Other reagents are commercially available.

The solvents were purified according to traditional methods and used freshly distilled. Melting points were obtained on a SMP3 apparatus (Barloworld Scientific, UK). Optical rotations were measured on a Perkin Elmer 341 polarimeter. The ^{1}H, ^{19}F, and ^{13}C NMR spectra were recorded on Bruker DRX-400 (400, 376, and 100 MHz, respectively) or Bruker Avance 500 (500, 470, and 125 MHz, respectively) spectrometers with TMS and hexafluorobenzene as internal references. Microanalyses were performed using Perkin Elmer 2400 II analyzer. Analytical TLC was performed using Sorbfil plates (Imid, Russia). Flash-column chromatography was performed using Silica gel 40 (230–400 mesh) (Alfa Aesar, UK). Analytical chiral HPLC of compound (*S*)-**3a** was performed on a Shimadzu Prominence LC-20 instrument using a *S*,*S*-Whelk-O1 column (250×4.6 mm, 5 μm); H_2O–MeOH 6:4 mixture as an eluent, 1 ml min^{-1} flow rate, detection at 230 nm. Analytical chiral HPLC of compound (*S*)-**14a** was performed on Knauer Smartline-1100 instrument using a Chiralpak AD column (250×4.6 mm, 5 μm); *n*-hexane–*i*PrOH–MeOH 80:15:5 mixture as an eluent, 1 ml min^{-1} flow rate, detection at 230 nm. The high-resolution mass spectra were obtained on a Bruker maXis Impact HD mass spectrometer, electrospray ionization with direct sample inlet (4 dm^3 min^{-1} flow rate).

Methyl N^{α}-trifluoroacetyl-(*S*)-lysinate and (*S*)-ornithinate hydrochlorides [(*S*)-2a,b]. General procedure. Trifluoroacetic anhydride (0.60 ml, 4.28 mmol) was added portion-wise to a suspension of N^{ε}-Cbz-(*S*)-lysine (or N^{δ}-Cbz-(*S*)-ornithine) (3.57 mmol) in dichloromethane (20 ml) under stirring at −5 °C. The reaction mixture was stirred at −5 °C for 2 h and at room temperature for 1 day, then evaporated to dryness under reduced pressure. The residue was dissolved in trifluoroacetic

acid (20 ml) and refluxed for 2 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue was dried to constant weight; then dissolved in absolute MeOH (16 ml) and cooled to −5 °C. Thionyl chloride (0.52 ml, 7.14 mmol) was added portion-wise to the resulting solution under stirring at −5 °C. The reaction mixture was stirred at this temperature for 20 min, at room temperature for 24 h; then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel (chloroform–methanol 85:15 as an eluent).

Methyl *N*$^{\alpha}$-trifluoroacetyl-(*S*)-lysinate hydrochloride [(*S*)-**2a**]: yield 0.54 g (52%), pale yellow solid, mp 98–100 °C. $[\alpha]_D^{20}$ –27.2 (*c* 0.5, H_2O). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) *δ*: 1.30-1.41 (m, 2H, C^4H_2), 1.50-1.62 (m, 2H, C^5H_2), 1.72-1.86 (m, 2H, C^3H_2), 2.72-2.78 (m, 2H, C^6H_2), 3.68 (s, 3H, OMe), 4.33 (ddd, *J* = 9.6, 7.5, and 5.0 Hz, 1H, C^2H), 7.91 (s, 3H, NH_3^+), 9.87 (d, *J* = 7.4 Hz, 1H, NH). ^{19}F NMR (470 MHz, DMSO-*d*$_6$) *δ*: 88.54 (s, CF_3). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) *δ*: 22.36, 26.27, 29.15, 38.23, 52.31, 52.50, 115.71 (q, *J* = 288 Hz), 156.62 (q, *J* = 36.9 Hz), 170.86. Calcd. for $C_9H_{16}ClF_3N_2O_3$ (%): C 36.93; H 5.51; N 9.57, Cl 12.11. Found (%): C 36.99; H 5.47; N 9.61, Cl 11.95.

Methyl *N*$^{\alpha}$-trifluoroacetyl-(*S*)-ornithinate hydrochloride [(*S*)-**2b**]: yield 0.47 g (45%), pale yellow solid, mp 139-141 °C. $[\alpha]_D^{20}$ –31.9 (*c* 0.5, H_2O). ^{1}H NMR (500 MHz, DMSO-*d*$_6$) *δ*: 1.56-1.62 (m, 2H, C^4H_2), 1.74-1.82 (m, 1H, C^3H_A), 1.88-1.95 (m, 1H, C^3H_B), 2.73-2.83 (m, 2H, C^5H_2), 3.68 (s, 3H, OMe), 4.40 (ddd, *J* = 9.9, 7.6, and 4.8 Hz, 1H, C^2H), 7.88 (s, 3H, NH_3^+), 9.93 (d, *J* = 7.5 Hz, 1H, NH). ^{19}F NMR (470 MHz, DMSO-*d*$_6$) *δ*: 88.50 (s, CF_3). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) *δ*: 23.59, 26.73, 38.06, 52.12, 52.28, 115.70 (q, *J* = 288 Hz), 156.56 (q, *J* = 36.8 Hz), 170.51. Calcd. for $C_9H_{14}ClF_3N_2O_3$ (%): 34.48; H 5.06; N 10.05; Cl 12.72; F 20.45. Found (%): C 34.50; H 5.05; N 10.06; Cl 12.93; F 20.57.

Compounds (*S*)-3a,b. General procedure. DiPEA (220 μl, 1.28 mmol), HOBt (0.11 g, 0.85 mmol) and DCC (0.18 g, mmol) were successively added to a suspension of compound **1** (0.17 g, 0.85 mmol) in a DMSO–DMF mixture (2:1, 6 ml) under stirring at room temperature. The reaction mixture was stirred for 15 min; then compound (*S*)-**2a** (or (*S*)-**2b**) (0.85 mmol) was added. The reaction mixture was stirred at room temperature for 2 days. The precipitate was filtered off, washed with DMSO (3×0.5 ml); the mother liquor and washings were poured in cold water (150 ml) and extracted with *n*-BuOH (4×25 ml). The combined organic layers were washed with 5% aqueous $NaHCO_3$ (3×20 ml), saturated aqueous NaCl (3×20 ml), dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel (EtOAc–EtOH mixture 9 : 1 as an eluent).

Methyl *N*$^{\varepsilon}$-[N-(purin-6-yl)glycyl]-*N*$^{\alpha}$-trifluoroacetyl-(*S*)-lysinate [(*S*)-**3a**]: yield 0.13 g (35%), pale yellow solid, mp 188-190 °C. $[\alpha]_D^{20}$ –17.5 (*c* 0.4, EtOH). *Ee* > 99%; HPLC: *τ* 54.9 min. ^{1}H

NMR (500 MHz, DMSO-d_6) (N^9–N^7 purine tautomers 9:1) δ: 1.25-1.30 (m, 2H, C^4H_2-Lys), 1.33-1.44 (m, 2H, C^5H_2-Lys), 1.69-1.84 (m, 2H, C^3H_2-Lys), 3.00-3.12 (m, 2H, C^6H_2-Lys), 3.66 (s, 3H, OMe), 4.03 (br. s, 2H, CH_2-Gly), 4.29 (ddd, J =8.3, 6.8, and 6.2 Hz, 1H, C^2H-Lys), 7.57 (br. s, 1H, NH-Gly), 7.90 (s, 1H, N^{ε}H-Lys), 8.11 (s, 0.9H, H-8 purine), 8.17 (s, 0.9H, H-2 purine), 8.24 (br. s, 0.2H, H-8 and H-2 purine), 9.81 (d, J = 7.2 Hz, 1H, N^{α}H-Lys), 12.31 (br. s, 0.1H, NH purine), 12.95 (br. s, 0.9H, NH purine). ^{19}F NMR (470 MHz, DMSO-d_6) δ: 88.52 (s, CF_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.72, 28.45, 29.42, 38.13, 43.18, 52.18, 52.55, 115.69 (q, J = 288 Hz), 119.00, 138.93, 149.66, 152.06, 154.22, 156.56 (q, J = 36.8 Hz), 169.06, 170.92. Calcd. for $C_{16}H_{20}F_3N_7O_4$ (%):C 44.55, H 4.67, N 22.73, F 13.21. Found (%): C 44.49, H 4.65, N 22.45, F 12.89.

Methyl N^{ε}-[N-(purin-6-yl)glycyl]-N^{α}-trifluoroacetyl-(*RS*)-lysinate was obtained starting from *rac*-**2a** (for a comparison in HPLC analysis); yield 0.10 g (27%), pale yellow solid, mp 176-178 °C. HPLC: τ_R 51.6 min, τ_S 54.9 min. NMR spectra were identical to those of compound (*S*)-**3a**. HRMS (ESI), *m/z*: 432.1598 $[M+H]^+$, (calc. for $C_{16}H_{21}F_3N_7O_4$, *m/z*: 432.1602).

Methyl N^{δ}-[*N*-(purin-6-yl)glycyl]-N^{α}-trifluoroacetyl-(*S*)-ornithinate [(*S*)-**3b**]: yield 0.16 g (42%), pale yellow solid, mp 210-212 °C. $[\alpha]_D^{20}$ –16.1 (*c* 0.5, EtOH). ^{1}H NMR (500 MHz, DMSO-d_6) (N^9–N^7 purine automers 9:1) δ: 1.40-1.43 (m, 2H, C^4H_2-Orn), 1.68-1.72 (m, 1H, C^3H_A-Orn), 1.81-1.85 (m, 1H, C^3H_B-Orn), 3.07 (td, J = 6.4 and 6.3 Hz, 2H, C^5H_2-Orn), 3.66 (s, 3H, OMe), 4.00-4.12 (m, 2H, CH_2-Gly), 4.33 (ddd, J = 9.9, 7.7, and 4.8 Hz, 1H, C^2H-Orn), 7.49 (br. s, 1H, 0.1H, NH Gly), 7.61 (br. s, 0.9H, NH Gly), 7.93 (t, J = 5.5 Hz, 1H, N^{δ}H-Orn), 8.11 (s, 0.9H, H-8 purine), 8.17 (s, 0.9H, H-2 purine), 8.25 (s, 0.1H, H-8 purine), 8.26 (s, 0.1H, H-2 purine), 9.85 (d, J = 7.5 Hz, 1H, N^{α}H-Orn), 12.27 (br. s, 0.1H, NH purine), 12.95 (br. s, 0.9H, NH purine). ^{19}F NMR (470 MHz, DMSO-d_6) δ: 88.53 (s, CF_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 25.71, 27.16, 37.82, 43.24, 52.18, 52.41, 115.71 (q, J = 288 Hz), 118.93, 138.93, 149.70, 152.07, 154.20, 156.53 (q, J = 36.8 Hz), 169.20, 170.83. HRMS (ESI), *m/z*: 418.1446 $[M+H]^+$, (calc. for $C_{15}H_{19}F_3N_7O_4$, *m/z*: 418.1446).

Compounds (*S*)-4a,b. General procedure. A solution of compound (*S*)-**3a** (or (*S*)-**3b**) (0.32 mmol) in 0.25N LiOH (3.84 ml) was stirred at room temperature for 1 day; then the pH of the reaction mixture was adjusted to 5 by 4N HCl. The resulting solution was treated with ion-exchange resin IR-120 in H^+ form; the target compounds were eluted with a water–pyridine 9:1 mixture. The eluate was evaporated to dryness under reduced pressure. The residue was recrystallized from an EtOH–H_2O mixture.

***N*$^{\varepsilon}$-[*N*-(Purin-6-yl)glycyl]-(*S*)-lysine hydrate** [(*S*)-**4a**]: yield 0.054 g (49%), pale yellow solid, mp 248-250 °C (decomp.). $[\alpha]_D^{20}$ +11.6 (*c* 0.5, 1N HCl). ^{1}H NMR (500 MHz, D_2O) δ: 1.30-1.37 (m, 2H, C^4H_2-Lys), 1.49-1.54 (m, 2H, C^5H_2-Lys), 1.73-1.88 (m, 2H, C^3H_2-Lys), 3.23 (dd, J = 6.9 and

6.9 Hz, 2H, C^6H_2-Lys), 3.66 (dd, *J* = 6.4 and 5.8 Hz, 1H, C^2H-Lys), 4.28 (br. s, 2H, CH_2-Gly), 8.19 (s, 1H, H-8 purine), 8.28 (s, 1H, H-2 purine). ^{13}C NMR (125 MHz, D_2O) *δ*: 24.46, 30.75, 32.76, 41.64, 46.67, 57.43, 119.36, 143.92, 152.16, 154.25, 155.99, 174.50, 177.42. Calcd. for $C_{13}H_{19}N_7O_3 \times H_2O$ (%): C 46.00, H 6.24, N 28.90. Found (%): C 46.28, H 6.10, N 29.10.

***N*$^{\delta}$-[*N*-(Purin-6-yl)glycyl]-(*S*)-ornithine hydrate** [(*S*)-**4b**]: yield 0.047 g (46%), pale yellow solid, mp 236-238 °C (decomp.). $[\alpha]_D^{20}$ +11.8 (*c* 0.2, 1N HCl). ^1H NMR (400 MHz, D_2O) *δ*: 1.49-1.66 (m, 2H, C^4H_2-Orn), 1.73-1.90 (m, 2H, C^3H_2-Orn), 3.21-3.32 (m, 2H, C^5H_2-Orn), 3.72 (dd, *J* = 6.2 and 6.0 Hz, 1H, C^2H-Orn), 4.27 (br. s, 2H, CH_2-Gly), 8.15 (s, 1H, H-8 purine), 8.23 (s, 1H, H-2 purine). ^{13}C NMR (125 MHz, D_2O) *δ*: 26.79, 30.24, 41.21, 46.51, 56.84, 118.69, 144.07, 151.76, 153.50, 155.57, 174.21, 176.85. Calcd. for $C_{12}H_{17}N_7O_3 \times H_2O$ (%): C 44.29, H 5.89, N 30.15. Found (%): 43.99; H 5.98; N 29.89.

Methyl *N*$^{\varepsilon}$-[2-acetamido-9-(2-acetoxyethoxy)methylpurin-6-yl]-*N*$^{\alpha}$-trifluoroacetyl-(*S*)-lysinate [(*S*)-**6**]. A solution of compound (*S*)-**2a** (0.43 g, 1.48 mmol), compound **5** (0.24 g, 0.74 mmol) and Et_3N (0.41 ml, 2.96 mmol) in EtOH (20 ml) was refluxed for 6.5 h and then evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (25 ml), washed with 10% aqueous citric acid (3×15 ml) and saturated aqueous NaCl (2×15 ml). Organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography on silica gel (dichloromethane–MeOH 97:3 as an eluent) to afford 0.22 g (53%) of compound (*S*)-**6** as a colorless semisolid. $[\alpha]_D^{25}$ −9.4 (*c* 0.5, MeOH). ^1H NMR (500 MHz, DMSO-*d*$_6$) *δ*: 1.24–1.42 (m, 2H, C^4H_2-Lys), 1.54-1.65 (m, 2H, C^5H_2-Lys), 1.75-1.81 (m, 1H, C^3H_A-Lys), 1.82-1.90 (m, 1H, C^3H_B-Lys), 1.94 (s, 3H, $OCOCH_3$), 2.26 (s, 3H, $NHCOC\underline{H}_3$), 3.39-3.47 (m, 2H, C^6H_2-Lys), 3.65 (s, 3H, OMe), 3.71-3.73 (m, 2H, OCH_2), 4.06-4.08 (m, 2H, OCH_2), 4.33 (ddd, *J* = 9.7, 7.4, and 4.9 Hz, 1H, C^2H-Lys), 5.48 (s, 2H, NCH_2O), 7.88 (br. s, 1H, $N^{\varepsilon}H$-Lys), 8.13 (s, 1H, H-8 purine), 9.78-9.83 (m, 2H, $N^{\alpha}H$-Lys and $N\underline{H}Ac$). ^{19}F NMR (470 MHz, DMSO-*d*$_6$) *δ*: 88.52 (s, CF_3). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) *δ*: 20.44, 22.80, 24.68, 28.32, 29.48, 39.5 (overlapped with DMSO signal), 52.13, 52.59, 62.69, 66.83, 71.83, 115.66, 115.69 (q, *J* = 288 Hz), 139.95, 149.81, 153.23, 154.58, 156.52 (q, *J* = 26.7 Hz), 169.54, 170.12, 170.93. HRMS (ESI), *m/z*: 548.2073 $[M+H]^+$, (calc. for $C_{21}H_{29}F_3N_7O_7$, *m/z*: 548.2075).

***N*$^{\varepsilon}$-[2-Amino-9-(2-hydroxyethoxy)methylpurin-6-yl]-(*S*)-lysine** [(*S*)-**7**]. A solution of compound (*S*)-**6** (0.19 g, 0.36 mmol) in 0.4 *N* NaOH (10.7 ml) was stirred at room temperature for 3 days, then neutralized with AcOH to pH 7 and evaporated to dryness. The residue was subjected to flash column chromatography on silica gel (dichloromethane–MeOH–AcOH 7:3:0.5 → MeOH–H_2O 8:2) to afford 0.062 g (49%) of compound (*S*)-**7** as a colorless solid, mp 245-249 °C (decomp.). $[\alpha]_{436}^{25}$ +4.6 (*c* 0.2, 1N NaOH). ^1H NMR (400 MHz, D_2O/NaOD) *δ*: 1.38–1.45 (m, 2H, C^4H_2-Lys), 1.54-

1.70 (m, 4H, C^3H_2-Lys and C^5H_2-Lys), 3.24 (t, *J* = 6.4 Hz, 1H, C^2H-Lys), 3.43-3.56 (m, 2H, C^6H_2-Lys), 3.61-3.63 (m, 2H, $OC\underline{H}_2CH_2OH$), 3.65-3.67 (m, 2H, $C\underline{H}_2OH$), 5.49 (s, 2H, CH_2O), 7.88 (s, 1H, H-8 purine). ^{13}C NMR (125 MHz, D_2O/NaOD) *δ*: 25.14, 31.37, 37.16, 43.13, 58.64, 62.81, 72.97, 75.26, 115.77, 141.81, 152.72, 157.98, 163.15, 186.30. HRMS (ESI), *m/z*: 354.1883 $[M+H]^+$, (calc. for $C_{14}H_{24}N_7O_4$, *m/z*: 354.1884).

***N*$^{\varepsilon}$-[2-Acetamido-9-(2-hydroxyethoxy)methylpurin-6-yl]-*N*$^{\alpha}$-*tert*-butyloxycarbonyl-(*S*)-lysine** [(*S*)-**9**]. A suspension of compound **5** (0.28 g, 0.87 mmol), compound (*S*)-**8a** (0.43 g, 1.74 mmol), TEA (0.36 ml, 2.60 mmol) and potassium iodide (0.014 g, 0.007 mmol) in *n*-BuOH (20 ml) was heated at 90 °C for 7.5 h, then colled to room temperature. The reaction mixture was washed with 10% aqueous citric acid (3×15 ml) and saturated aqueous NaCl (6×15 ml), evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography on silica gel (EtOAc–EtOH 85:15 as an eluent). The resulting semisolid was dissolved in EtOH (0.5 ml); 1 *N* NaOH (3.3 ml) was added to the solution. The reaction mixture was stirred at room temperature for 3 days, acidified with citric acid to pH 4 and extracted with *n*-BuOH (3×15 ml). The combined organic layers were washed with saturated aqueous NaCl (5×15 ml) and evaporated to dryness. The residue was purified by flash column chromatography on silica gel (dichloromethane–EtOH 8:2 as an eluent) to afford 0.16 g (65%) of compound **9** as a yellowish solid, mp 113-116 °C. $[\alpha]_{365}^{20}$ +12.8 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) *δ*: 1.31–1.37 (m, 2H, C^4H_2-Lys), 1.37 (s, 9H, Boc), 1.50-1.70 (m, 4H, C^3H_2-Lys and C^5H_2-Lys), 3.43-3.49 (m, 6H, C^6H_2-Lys, $C\underline{H}_2C\underline{H}_2OH$), 3.82-3.88 (m, 1H, C^2H-Lys), 4.69 (br. s, 1H, OH), 5.39 (s, 2H, CH_2O), 6.06 (br. s, 2H, NH_2), 7.06 (d, *J* = 8.0 Hz, 1H, N^{α}H-Lys), 7.45 (s, 1H, N^{ε}H-Lys), 7.89 (s, 1H, H-8 purine), 12.39 (s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) *δ*: 22.94, 28.14 (3C), 28.60, 30.44, 39.5 (overlapped with DMSO signal), 53.42, 59.87, 70.32, 71.90, 77.88, 112.73, 137.81, 150.59, 154.09, 155.53, 159.54, 174.15. HRMS (ESI), *m/z*: 454.2408 $[M+H]^+$, (calc. for $C_{19}H_{32}N_7O_6$, *m/z*: 454.2409).

***N*$^{\varepsilon}$-[2-Amino-9-(2-hydroxyethoxy)methylpurin-6-yl]-(*S*)-lysine** [(*S*)-**7**]. A solution of compound (*S*)-**9** (0.14 g, 0.32 mmol) in TFA (1.1 ml) was stirred at room temperature for 1.5 h. Then diethyl ether (20 ml) and water (10 ml) were added to the reaction mixture. The aqueous layer was washed with diethyl ether (2×5 ml), neutralized with aqueous ammonia to pH 7, and evaporated to dryness under reduced pressure. The residue was triturated in EtOH (3.5 ml) to afford 0.016 g (14%) of compound (*S*)-**7**. All physicochemical characteristics were identical to those described above.

Compounds (*S*)-11a,b. General procedure. A suspension of compound **10a** (or **10b**) (1.02 mmol), compound (*S*)-**8a** (0.50 g, 2.03 mmol), Et_3N (0.42 ml, 3.05 mmol), and potassium iodide (0.017 g, 0.10 mmol) in *n*-BuOH (20 ml) was stirred at 90 °C for 12 h. The reaction mixture was cooled to room temperature; washed consequently with 10% aqueous citric acid (3×5 ml), saturated aqueous NaCl (6×5 ml); and evaporated to dryness under reduced pressure. The residue was purified by

flash column chromatography on silica gel (EtOAc–EtOH 9:1 as an eluent for compound (*S*)-**11a**, or chloroform–MeOH 85:15 for compound (*S*)-**11b**).

***N*$^{\varepsilon}$-[9-(2-Acetoxyethoxy)methylpurin-6-yl]-*N*$^{\alpha}$-*tert*-butoxycarbonyl-(*S*)-lysine** [(*S*)-**11a**]: yield 0.22 g (45%), pale yellow solid, mp 129-131 °C. $[\alpha]_{365}^{20}$ +11.3 (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.32 (br. s, 2H, C^4H_2-Lys), 1.36 (s, 9H, Boc), 1.49-1.62 (m, 3H, C^5H_2-Lys and C^3H_A-Lys), 1.64-1.71 (m, 1H, C^3H_B-Lys), 1.93 (s, 3H, Ac), 3.45 (br. s, 2H, C^6H_2-Lys), 3.71 (dd, *J* = 4.7 and 4.6 Hz, 2H, OCH_2), 3.83 (ddd, *J* = 8.6, 8.6, and 4.4 Hz, 1H, C^2H-Lys), 4.07 (dd, *J* = 4.7 and 4.5 Hz, 2H, OCH_2), 5.57 (s, 2H, NCH_2O), 7.04 (d, *J* = 7.9 Hz, 1H, N^{α}H-Lys), 7.85 (br. s, 1H, N^{ε}H-Lys), 8.24 (s, 1H, H-8 purine), 8.27 (s, 1H, H-2 purine), 12.28 (br. s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.44, 23.02, 28.13 (3C), 28.61, 30.49, 53.45, 62.70, 66.86, 71.94, 77.86, 118.78, 140.72, 148.89, 152.87, 154.51, 154.67, 155.52, 170.12, 174.14. Calcd. for $C_{21}H_{32}N_6O_7$ (%): C 52.49, H 6.71, N 17.49. Found (%): C 52.54, H 6.95, N 17.32.

***N*$^{\varepsilon}$-(2-Aminopurin-6-yl)-*N*$^{\alpha}$-*tert*-butoxycarbonyl-(*S*)-lysine** [(*S*)-**11b**]: yield 0.11 g (28%), beige solid, mp 158-160 °C (decomp.). $[\alpha]_{436}^{20}$ +43.8 (*c* 0.4, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.24 (br. s, 2H, C^4H_2-Lys), 1.37 (s, 9H, Boc), 1.52-1.63 (m, 3H, C^5H_2-Lys and C^3H_A-Lys), 1.65-1.70 (m, 1H, C^3H_B-Lys), 3.44 (dd, *J* = 13.2 and 6.4 Hz, 2H, C^6H_2-Lys, partially overlapped with H_2O signal), 3.86 (ddd, *J* = 8.4, 8.4, and 4.3 Hz, 1H, C^2H-Lys), 6.08 (br. s, 2H, NH_2) 7.07 (d, *J* = 7.9 Hz, 1H, N^{α}H-Lys), 7.93 (br. s, 1H, N^{ε}H-Lys), 8.85 (br. s, 1H, H-8 purine), 12.0-13.4 (m, 2H, NH purine and COOH). ^{13}C NMR (125 МГц, MHz, DMSO-d_6) δ: 23.00, 28.14 (3C), 28.34, 30.43, 40.09 (overlapped with DMSO signal), 53.51, 77.88, 109.39, 138.38, 149.06, 153.23, 155.53, 156.91, 174.15. HRMS (ESI), *m/z*: 380.2041 $[M+H]^+$, (calc. for $C_{16}H_{26}N_7O_4$, *m/z*: 380.2041).

***N*$^{\varepsilon}$-[9-(2-Hydroxyethoxy)methylpurin-6-yl]-(*S*)-lysine** [(*S*)-**12a**]. A solution of compound (*S*)-**11a** (0.20 g, 0.42 mmol) in 1 *N* NaOH (1.25 ml) was stirred at room temperature for 2 days, then the pH of the reaction mixture was adjusted to 5 by citric acid. The reaction mixture was kept overnight at +5 °C. The precipitate was filtered off, washed with cold water (3×0.3 ml), dried to constant weight and then dissolved in TFA (1 ml). The solution was stirred at room temperature for 1 h, diluted with diethyl ether (5 ml) and kept at −10 °C for 4 h. The precipitate was filtered off and dissolved in water (5 ml). The reaction mixture was neutralized with aqueous ammonia to pH 7, and then evaporated to dryness. The residue was washed twice with refluxing EtOH (2×1 ml) to afford 0.031 g (22%) of compound (*S*)-**12a** as a pale yellow solid, mp 232-234 °C (decomp.). $[\alpha]_{365}^{20}$ +4.4 (*c* 0.25, H_2O). ^{1}H NMR (400 MHz, D_2O) δ: 1.45-1.62 (m, 2H, C^4H_2-Lys), 1.72-1.81 (m, 2H, C^5H_2-Lys), 1.88-2.05 (m, 2H, C^3H_2-Lys), 3.54 (br. s, 2H, C^4H_2-Lys), 3.70 (ddd, *J* = 15.1, 6.8, and 5.6 Hz, 4H, OCH_2CH_2O), 3.79 (dd, *J* = 6.2 and 6.0 Hz, 1H, C^2H-Lys), 5.65 (s, 2H, NCH_2O), 8.17 (s, 1H, H-8 purine), 8.21 (s, 1H, H-2 purine). ^{13}C NMR (100 MHz, D_2O) δ: 21.38, 27.84, 29.73, 39.94,

54.17, 59.66, 69.79, 72.57, 118.24, 141.22, 147.20, 151.63, 153.30, 174.12. HRMS (ESI), *m/z*: 339.1775 $[M+H]^+$, (calc. for $C_{14}H_{23}N_6O_4$, *m/z*: 339.1776).

***N*$^{\varepsilon}$-(2-Aminopurin-6-yl)-(*S*)-lysine** [(*S*)-**12b**]. A solution of compound (*S*)-**11b** (0.13 g, 0.33 mmol) was stirred at room temperature for 1 h, then evaporated to dryness under reduced pressure. The residue was dissolved in water (10 ml); the pH of the solution was adjusted to 7 with aqueous ammonia. The reaction mixture was evaporated to dryness under reduced pressure. The residue was washed with refluxing EtOH (2×1 ml) to afford 0.053 g (54%) of compound (*S*)-**11b** as a beige solid, mp 213- 215 °C (decomp.). $[\alpha]_D^{20}$ +17.0 (*c* 0.5, 1N HCl) ^{1}H NMR (500 MHz, D_2O) δ: 1.44-1.58 (m, 2H, C^4H_2-Lys), 1.72-1.79 (m, 2H, C^5H_2-Lys), 1.87-1.99 (m, 2H, C^3H_2-Lys), 3.58 (br. s, 2H, C^6H_2-Lys), 3.76 (dd, *J* = 6.0 and 5.9 Hz, 1H, C^2H-Lys), 7.98 (s, 1H, H-8 purine). ^{13}C NMR (125 MHz, D_2O) δ: 24.42, 30.42, 32.72, 43.22, 57.46, 109.29, 143.57, 148.72, 155.14, 156.44, 177.52. HRMS (ESI), *m/z*: 280.1517 $[M+H]^+$, (calc. for $C_{11}H_{18}N_7O_2$, *m/z*: 280.1517).

Compounds (*S*)-**13a,b. General procedure.** A suspension of 6-chloropurine (0.16 g, 1.02 mmol), TEA (0.42 ml, 3.05 mmol), potassium iodide (0.017 g, 0.10 mmol), and compound (*S*)-**8a** (or (*S*)-**8b**) (2.03 mmol) in *n*-BuOH (20 ml) was stirred at 90 °C for 12 h. The reaction mixture was cooled to room temperature, washed subsequently with 10% aqueous citric acid (3×5 ml), saturated aqueous NaCl (6×5 ml), and evaporated to dryness under reduced pressure. The residue was triturated in *n*-hexane and subjected to flash column chromatography on silica gel (EtOAc–MeOH 9:1 as an eluent).

***N*$^{\varepsilon}$-(Purin-6-yl)-*N*$^{\alpha}$-*tert*-butoxycarbonyl-(*S*)-lysine** [(*S*)-**13a**]: yield 0.20 g (54%), pale yellow solid, mp 146-147 °C (decomp.). $[\alpha]_{365}^{20}$ +13.7 (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.33 (br. s, 2H, C^4H_2-Lys), 1.37 (s, 9H, Boc), 1.52-1.63 (m, 3H, C^5H_2-Lys and C^3H_A-Lys), 1.64-1.72 (m, 1H, C^3H_B-Lys), 3.46 (br. s, 2H, C^6H_2-Lys partially overlapped with H_2O signal), 3.84 (ddd, *J* = 8.6, 8.5, and 4.4 Hz, 1H, C^2H-Lys), 7.06 (d, *J* = 8.0 Hz, 1H, N^{α}H-Lys), 7.90 (br. s, 1H, N^{ε}H-Lys), 8.14 (s, 1H, H-8 purine), 8.22 (s, 1H, H-2 purine), 12.0-13.4 (m, 2H, NH purine and COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 23.07, 28.15 (3C), 28.74, 30.51, 40.09 (overlapped with DMSO signal), 53.47, 77.88, 118.68, 138.53, 149.31, 152.30, 154.29, 155.54, 174.18. HRMS, *m/z*: 365.1935 $[M+H]^+$, (calc. for $C_{16}H_{25}N_6O_4$, *m/z*: 365.1932).

***N*$^{\delta}$-(Purin-6-yl)-*N*$^{\alpha}$-*tert*-butoxycarbonyl-(*S*)-ornithine** [(*S*)-**13b**]: yield 0.14 g (37%), pale yellow solid, mp 137-138 °C (decomp.). $[\alpha]_{365}^{20}$ +26.2 (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.37 (s, 9H, Boc), 1.62-1.74 (m, 4H, C^4H_2-C^3H_2-Orn), 3.48 (br. s, 2H, C^5H_2-Orn partially overlapped with H_2O signal), 3.89 (ddd, *J* = 10.1, 8.1, and 4.1 Hz, 1H, C^2H-Orn), 7.12 (d, *J* = 7.9 Hz, 1H, N^{α}H-Orn), 8.02 (br. s, 1H, N^{δ}H-Orn), 8.17 (s, 1H, H-8 purine), 8.25 (s, 1H, H-2 purine), 12.0-13.4 (br. s, 2H, NH purine and COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 25.91, 28.16 (3C),

28.29, 40.09 (overlapped with DMSO signal), 53.36, 77.89, 118.61, 138.69, 149.50, 152.24, 154.69, 155.52, 174.06. HRMS, *m/z*: 351.1780 $[M+H]^+$, (calc. for $C_{15}H_{23}N_6O_4$, *m/z*: 351.1775).

Compounds (*S*)-14a,b. General procedure. A solution of compound (*S*)-**13a** (or (*S*)-**13b**) (0.33 mmol) in TFA (1.3 ml) was stirred at room temperature for 1 h, and then evaporated to dryness under reduced pressure. The residue was dissolved in water (10 ml) and neutralized with aqueous ammonia to pH 7, then evaporated to dryness under reduced pressure. The residue was washed twice with refluxing EtOH (1 ml).

***N*$^{\varepsilon}$-(Purin-6-yl)-(*S*)-lysine** [(*S*)-**14a**]: yield 0.051 g (56%), pale yellow solid, mp 250-252 °C (decomp.). $[\alpha]_D^{20}$ +2.3 (*c* 0.5, H_2O). ^{1}H NMR (500 MHz, D_2O) δ: 1.45-1.56 (m, 2H, C^4H_2-Lys), 1.67-1.76 (m, 2H, C^5H_2-Lys), 1.86-1.99 (m, 2H, C^3H_2-Lys), 3.48 (br. s, 2H, C^6H_2-Lys), 3.76 (dd, *J* = 6.6 and 5.6 Hz, 1H, C^2H-Lys), 8.03 (s, 1H, H-8 purine), 8.08 (s, 1H, H-2 purine). ^{13}C NMR (125 MHz, D_2O) δ: 24.68, 30.90, 32.97, 43.19, 57.52, 118.71, 142.62, 151.66, 154.56, 155.73, 177.57. HRMS, *m/z*: 265.1407 $[M+H]^+$, (calc. for $C_{11}H_{17}N_6O_2$, *m/z*: 265.1408)

***N*$^{\delta}$-(Purin-6-yl)-(*S*)-ornithine** [(*S*)-**14b**]: yield 0.048 g (56%), pale yellow solid, mp 268-269 °C (decomp.). $[\alpha]_D^{20}$ +19.6 (*c* 0.5, 1N HCl). ^{1}H NMR (500 MHz, D_2O/DCl) δ: 1.88-2.04 (m, 2H, C^4H_2-Orn), 2.06-2.21 (m, 2H, C^3H_2-Orn), 3.77 (dd, *J* = 6.7 and 6.3 Hz, 2H, C^5H_2-Orn), 4.20 (dd, *J* = 6.4 and 6.3 Hz, 1H, C^2H-Orn), 8.54 (br. s, 2H, H-8 purine and H-2 purine). ^{13}C NMRP (125 MHz, D_2O/DCl) δ: 26.35, 29.66, 44.10, 55.25, 115.71, 145.82, 147.70, 148.94, 152.50, 174.14. HRMS, *m/z*: 251.1253 $[M+H]^+$, (calc. for $C_{10}H_{15}N_6O_2$, *m/z*: 251.1251).

2. Assessment of antimycobacterial activity

To evaluate the inhibitory effect of compounds (*S*)-**3a,** (*S*)-**3a,b**, (*S*)-**7**, (*S*)-**12b**, and (*S*)-**14a,b** on mycobacteria we used the following strains: *M. tuberculosis* $H_{37}Rv$, which is susceptible to anti-TB drugs; *M. avium, M. terrae* and multidrug-resistant *M. tuberculosis* strain isolated from a tuberculosis patient of the Ural region at the Ural Research Institute for Phthisiopulmonology (Ekaterinburg, Russia). The minimal inhibitory concentration (MIC) for mycobacteria strains for each compound was determined by the micro broth dilution method. All compounds tested were dissolved in DMSO and their 1/2 dilutions were added in tubes with 5 mL of the Löwenstein – Jensen medium. Each compound was tested at six concentrations 12.5, 6.25, 3.1, 1.5, 0.7, and 0.35 μg ml^{-1}. Tubes with Löwenstein – Jensen medium (5 ml) containing tested compounds and those without them (controls) were inoculated with a suspension of appropriate mycobacteria strain and incubated at 37 °C for 10 days. The mycobacterial growth was assessed by the standard procedure: the appearance of zones of mycobacterial growth delay (more than 10 mm) indicated the presence of antimycobacterial properties of tested compounds at the studied concentrations. The value of growth retardation zone (in mm) was proportional to the antimycobacterial activity of the compounds. Growth delay (100 mm or more) is considered as a complete mycobacteria growth inhibition.

References for Supplementary Materials

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