Supplementary Information

Synthesis, structure and bioactivity of primary sulfamate-containing natural products

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Experimental

General Chemistry

All reactions were carried out in dry solvents under anhydrous conditions unless otherwise mentioned. All chemicals were purchased commercially and used without further purification. All reactions were monitored by TLC using silica plates with visualization of product bands by UV fluorescence ($\lambda = 254$ nm) and charring with vanillin (6 g vanillin in 100 mL of EtOH containing 1% (v/v) concentrated sulfuric acid) stain. Silica gel flash chromatography was performed using silica gel 60 Å (230-400 mesh). NMR (^1H , ^{13}C , COSY, HSQC and HMBC) spectra were recorded on Bruker AVANCE III HD 500 MHz NMR spectrometer equipped with a BBO probe at 25 °C. Chemical shifts for ^1H and ^{13}C NMR obtained in DMSO- d_6 are reported in ppm relative to residual solvent proton ($\delta = 2.50$ ppm) and carbon ($\delta = 39.5$ ppm) signals respectively. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), bs (broad signal). Coupling constants are reported in hertz (Hz). LRMS (ESI) data were recorded on a Waters ZQ ESI mass spectrometer using electrospray as the ionization technique in positive-ion and/or negative-ion modes as stated. HRMS (ESI) data were acquired on a 12 T Solarix XR FT-ICR-MS using electrospray as the ionization technique in positive-ion and/or negative-ion modes as stated. All MS analysis samples were prepared as solutions in methanol. Purity of all compounds was >95% as determined by Agilent HPLC 1100 series with UV detection. The melting points are uncorrected. For compounds presented in the Scheme 1, proton and carbon atoms for NMR assignments are designated as shown below.

((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanol (5)

To an adenosine **3** (1.0 g, 3.74 mmol, 1 equiv) in acetone (30 mL/mmol) was added dry tosylic acid (10 equiv) at room temperature under an argon atmosphere. The resulting mixture was stirred at room temperature for 4 h. After completion of reaction (indicated by TLC), the reaction mixture was cooled to 0 °C and sat. aq. NaHCO₃ solution added until the pH of the mixture became slightly basic. Acetone was evaporated under reduced pressure and the residue was extracted in EtOAc (3 × 30 mL). The combined organic fractions were washed with brine (1 × 15 mL), dried over MgSO₄ and evaporated under reduced pressure to afford the title compound **5** as white solid (0.625g, 54%). *R_f* = 0.45 (10% MeOH in DCM). Mp = 228–230 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 8.34 (s, 1H, H_{Ar}), 8.16 (s, 1H, H_{Ar}), 7.35 (bs, 2H, Ar-NH₂), 6.12 (d, *J* = 3.1 Hz, 1H, CH_{ribose}), 5.34 (dd, *J* = 6.4, 3.1 Hz, 1H, CH_{ribose}), 5.24 (bs, 1H, 5'-OH), 4.96 (dd, *J* = 6.15, 2.45 Hz, 1H, CH_{ribose}), 4.23-4.20 (m, 1H, CH_{ribose}), 3.58-3.51 (m, 2H, 5'-CH₂), 1.54 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 156.1 (C_{quat}), 152.6 (C_{quat}), 148.8 (C_{quat}), 139.7 (CH_{Ar}), 119.1 (C_{quat}), 113.0 (CH_{Ar}), 89.6 (CH_{ribose}), 86.4 (CH_{ribose}), 83.2 (CH_{ribose}), 81.4 (CH_{ribose}), 61.6 (5'-CH₂), 27.1 (CH₃), 25.2 (CH₃), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 330 [M + Na]⁺, HRMS (ESI): calcd for [C₁₃H₁₇N₅O₄ + Na]⁺ 330.1172, found 330.1169

((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-2-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanol (6)

The title compound was synthesised from 2-chloroadenosine **4** (0.5 g, 1.66 mmol) as described for the synthesis of **5** to afford **6** as white solid (0.511 g, 90%). *R_f* = 0.37 (10% MeOH in DCM). Mp = 192–194 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 8.36 (s, 1H, H_{Ar}), 7.86 (bs, 2H, Ar-NH₂), 6.05 (d, *J* = 2.85 Hz, 1H, CH_{ribose}), 5.28 (dd, *J* = 6.15, 2.85 Hz, 1H, CH_{ribose}), 5.09 (t, *J* = 5.4 Hz, 1H, 5'-OH), 4.93 (dd, *J* = 6.2, 2.7 Hz, 1H, CH_{ribose}), 4.22-4.19 (m, 1H, CH_{ribose}), 3.58-3.50 (m, 2H, 5'-CH₂), 1.54 (s, 3H, CH₃), 1.33 (s, 3H, CH₃), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 156.8 (C_{quat}), 153.1 (C_{quat}), 150.0 (C_{quat}), 140.0 (CH_{Ar}), 118.1 (C_{quat}), 113.1 (C_{quat}), 89.4 (CH_{ribose}), 86.7 (CH_{ribose}), 83.5 (CH_{ribose}), 81.3 (CH_{ribose}), 61.5 (5'-CH₂), 27.0 (CH₃), 25.2 (CH₃), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 364 [M + Na]⁺, HRMS (ESI): calcd for [C₁₃H₁₆ClN₅O₄ + Na]⁺ 364.0783, found 364.0776

Chlorosulfonyl amine (7)

To an ice cold solution of chlorosulfonyl isocyanate (500 μ L, 3.5 mmol) in acetonitrile (5 mL) was added water (60 μ L, 3.5 mmol) dropwise over 2-3 min. The resulting reaction mixture was allowed to cool down to room temperature and continued stirring for 15 min. The solution was concentrated under reduced pressure by azeotrope with toluene (5 mL) to afford the title compound as a white hygroscopic solid. The crude compound was used for the sulfamoylation step (for preparation of compounds **8** and **9**) without further purification.

((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl sulfamate (8)

To a solution of **5** (0.5 g, 1.63 mmol, 1 equiv) and DBU (2.1 equiv) in dry DCM (8 mL) under an argon atmosphere, was added a solution of chlorosulfonyl amine **7** (2 equiv) in dry DCM (2 mL) at room temperature over 2 min. The resulting reaction mixture was stirred at room temperature over 15 h. After completion of reaction (indicated by TLC), the solvent was removed under reduced pressure to afford the crude product. The crude product was purified by flash chromatography (Gradient: 5–8% MeOH in EtOAc) to afford the title compound as white foam (0.28 g, 45%). R_f = 0.42 (10% MeOH in EtOAc). ^1H NMR (500 MHz, DMSO- d_6) δ_{H} = 8.57 (s, 1H, H_{Ar}), 8.46 (s, 1H, H_{Ar}), 7.61 (s, 2H, SO_2NH_2), 6.29 (d, J = 2.25 Hz, 1H, $\text{CH}_{\text{ribose}}$), 5.43 (dd, J = 6.2, 2.3 Hz, 1H, $\text{CH}_{\text{ribose}}$), 5.07 (dd, J = 6.2, 3.05 Hz, 1H, $\text{CH}_{\text{ribose}}$), 4.49–4.46 (m, 1H, $\text{CH}_{\text{ribose}}$), 4.21 (d, J = 10.8, 4.95 Hz, 1H, 5'- CHH), 4.15 (dd, J = 10.8, 6.1 Hz, 1H, 5'- CHH), 1.56 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), Ar- NH_2 protons in exchange, general assignments were confirmed by ^1H - ^1H gCOSY. ^{13}C NMR (125 MHz, DMSO- d_6) δ_{C} = 148.0 (C_{quat}), 141.9 (C_{quat}), 118.9 (C_{quat}), 113.6 ($2 \times \text{CH}_{\text{Ar}}$), 89.6 ($\text{CH}_{\text{ribose}}$), 84.0 ($\text{CH}_{\text{ribose}}$), 83.5 ($\text{CH}_{\text{ribose}}$), 81.0 ($\text{CH}_{\text{ribose}}$), 67.9 (5'- CH_2), 26.9 (CH_3), 25.2 (CH_3), general assignments were confirmed by ^1H - ^{13}C HSQC. LRMS (ESI): 387 $[\text{M} + \text{H}]^+$.

((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-2-chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl sulfamate (9)

Compound **9** was synthesised from **6** (0.4 g, 1.17 mmol), as described for the synthesis of **8**, to afford the title compound as crude solid (0.12 g, 24% crude). The compound was unstable towards column chromatography and directly used for next step without further purification.

((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl sulfamate (1)

To compound **8** (0.08 g, 0.21 mmol, 1 equiv) at 0 °C was added solution of TFA-H₂O (4:2, v/v). The resulting reaction mixture was allowed to warm at room temperature over 30 min. After completion of reaction (indicated by TLC), the solvent was removed under reduced pressure by azeotroping with ethanol (2 mL) and toluene (4 mL), to afford a crude residue. The crude product was purified by flash chromatography (Gradient: 100% EtOAc to 2–3% MeOH in EtOAc) to afford the title compound as white solid (0.025 g, 35%). *R_f* = 0.23 (10% MeOH in EtOAc). Mp = 173–175 °C. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 8.30 (s, 1H, H_{Ar}), 8.15 (s, 1H, H_{Ar}), 7.59 (s, 2H, SO₂NH₂), 7.30 (s, 2H, Ar-NH₂), 5.93 (d, *J* = 5.3 Hz, 1H, CH_{ribose}), 5.62 (d, *J* = 5.9 Hz, 1H, OH_{ribose}), 5.44 (d, *J* = 5.35 Hz, 1H, OH_{ribose}), 4.63 (q, *J* = 5.55 Hz, 1H, CH_{ribose}), 4.29 (dd, *J* = 10.7, 3.8 Hz, 1H, 5'-CHH), 4.23 (q, *J* = 4.6 Hz, 1H, CH_{ribose}), 4.20 (dd, *J* = 10.7, 6.0 Hz, 1H, 5'-CHH), 4.16–4.13 (m, 1H, CH_{ribose}), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 156.1 (C_{quat}), 152.7 (CH_{Ar}), 149.4 (C_{quat}), 139.4 (CH_{Ar}), 119.1 (C_{quat}), 87.5 (CH_{ribose}), 81.5 (CH_{ribose}), 73.0 (CH_{ribose}), 70.4 (CH_{ribose}), 68.7 (5'-CH₂), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 369 [M+Na]⁺, HRMS (ESI): calcd for [C₁₀H₁₄N₆O₆S + Na]⁺ 369.0588, found 369.0583

((2R,3S,4R,5R)-5-(6-amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl sulfamate (2)

Compound **2** was synthesised from compound **9** (0.1 g, 0.24 mmol) using the same procedure described for compound **1**. The crude product was purified by flash chromatography (Gradient: 50% EtOAc in *n*-hexane to 100% EtOAc) to afford the title compound as off-white solid (0.030 g, 33%). *R_f* = 0.42 (100% EtOAc). Mp = 220–240 °C, decomposition. ¹H NMR (500 MHz, DMSO-*d*₆) δ_H = 8.32 (s, 1H, H_{Ar}), 7.87 (bs, 2H, Ar-NH₂), 7.61 (s, 2H, SO₂NH₂), 5.87 (d, *J* = 3.3 Hz, 1H, CH_{ribose}), 5.65 (d, *J* = 3.55 Hz, 1H, OH_{ribose}), 5.47 (d, *J* = 3.15 Hz, 1H, OH_{ribose}), 4.53 (q, *J* = 3.25 Hz, 1H, CH_{ribose}), 4.28 (dd, *J* = 6.7, 2.1 Hz, 1H, 5'-CHH), 4.21–4.19 (m, 2H, CH_{ribose} and 5'-CHH), 4.17–4.16 (m, 1H, CH_{ribose}), general assignments were confirmed by ¹H-¹H gCOSY. ¹³C NMR (125 MHz, DMSO-*d*₆) δ_C = 156.8 (C_{quat}), 153.2 (C_{quat}), 150.5 (C_{quat}), 139.6 (CH_{Ar}), 118.0 (C_{quat}), 87.3 (CH_{ribose}), 81.7 (CH_{ribose}), 73.2 (CH_{ribose}), 70.3 (CH_{ribose}), 68.6 (5'-CH₂), general assignments were confirmed by ¹H-¹³C HSQC. LRMS (ESI): 403 [M+Na]⁺, HRMS (ESI): calcd for [C₁₀H₁₃ClN₆O₆S + Na]⁺ 403.0198, found 403.0191

Figure S2: ¹H and ¹³C NMR of compound **6** (in DMSO-*d*₆)

Figure S3: ¹H and ¹³C NMR of compound 1 (in DMSO-*d*₆)

Figure S4: ¹H and ¹³C NMR of compound **2** (in DMSO-*d*₆)

Analysis Info

Analysis Name D:\Data\Panos\20161206\HRMS_Prashant\PM03_32_01_pos_000001.d
 Method lmw_pos_20161116 Acquisition Date 12/6/2016 1:29:43 PM
 Sample Name Instrument solariX XR 271500.00077
 Comment

m/z	z	I
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370.06163	1+	19347440
371.05412	1+	7576076
371.06201	1+	1968773
372.05780	1+	918649
372.06968	1+	1056644

m/z	z	I
369.05877	1+	41670032
370.06098	1+	5911660
371.05456	1+	1879936
371.06321	1+	890072
372.05708	1+	262847
372.06616	1+	74720

Figure S5: HRMS Spectrum of NP 1.

Analysis Info

Analysis Name D:\Data\Panos\20161206\HRMS_Prashant\PM03_35_01_pos_000002.d
 Method lmw_pos_20161118 Acquisition Date 12/6/2016 1:43:58 PM
 Sample Name Instrument solarix XR 271500.00077
 Comment

m/z	z	I
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404.02234	1+	18417002
405.01618	1+	47456912
406.01948	1+	5186379
407.01163		2535327

m/z	z	I
403.01980	1+	41670032
404.02200	1+	5806867
405.01672	1+	15311376
405.02474	1+	790809
406.01895	1+	2157387
406.02743	1+	69811
407.01267	1+	606394
407.02123	1+	319794
408.01517	1+	84782

PM03_35_01_pos_000002.d

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Figure S6: HRMS Spectrum of NP 2.

CA Inhibition Assay. An applied Photophysics stopped-flow instrument was used for assaying the CA-catalyzed CO₂ hydration activity.¹ IC₅₀ values were obtained from dose-response curves working at seven different concentrations of the test compound, by fitting the curves using PRISM (www.graphpad.com) and nonlinear least-squares methods; values represent the mean of at least three different determinations as described by us previously.² The inhibition constants (K_i) were then derived by using the Cheng-Prusoff equation as follows: $K_i = IC_{50}/(1 + [S]/K_m)$, where $[S]$ represents the CO₂ concentration at which the measurement was carried out, and K_m is the concentration of substrate at which the enzyme activity is at half-maximal. All enzymes used were recombinant, produced in *Escherichia coli* as reported earlier.^{3,4} The hCA isoform concentrations in the assay system ranged between 7.5 and 15.0 nM.

Protein Expression and Purification

hCA II protein was expressed and purified as described.⁵

Protein X-ray Crystallography

Concentrated hCA II at ~10 mg mL⁻¹ was set up in SD-2 plates (Molecular Dimensions) with the following ratio of protein plus reservoir plus seeds: 250 nL + 225 nL + 25 nL. The plate was incubated at 8 °C and the reservoir condition consisted of 2.9 M (NH₄)₂SO₄ with 0.1 M Tris buffer at pH 8.3. Compound in DMSO was added to the crystallisation drop after crystals had formed and several days before data were collected. Soaked crystals were subsequently soaked (briefly) in reservoir plus 20% glycerol before being removed from the plate with a cryo-loop and put in liquid nitrogen for transport to the synchrotron. 360 frames of one degree oscillation were taken at the MX-1 beamline of the Australian Synchrotron. The data were indexed using XDS⁶ and scaled using Aimless.⁷ Molecular replacement was done using Phaser⁸ using 4cq0 as the initial starting model. The model was manually rebuilt using Coot⁹ and refined using Refmac.¹⁰ The compound was placed in density using the program Afitt (OpenEye Scientific Software) and further refined using Refmac.¹⁰ The structure and structure factors were deposited in the PDB with accession codes: 6C7W (**1**) and 6C7X (**2**).

Table 1: Protein X-ray crystallography data collection and structure refinement statistics

PDB	6c7w - 1	6c7x - 2
Space group	P2 ₁	P2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	42.2, 41.6, 72.2	42.3, 41.4, 72.4
α , β , γ (°)	90, 104.5, 90	90, 104.5, 90
Resolution (Å)	41.6-1.28 (1.30-1.28)	41.4-1.50 (1.53-1.50)
<i>R</i> _{merge}	0.074 (0.681)	0.087 (0.805)
<i>R</i> _{pim}	0.029 (0.285)	0.034 (0.327)
<i>CC</i> _{1/2}	0.999 (0.759)	0.999 (0.731)
<i>I</i> / σ <i>I</i>	15.9 (2.8)	18.2 (2.5)
Completeness (%)	99.8 (96.3)	99.7 (94.3)
Redundancy	7.2 (6.4)	7.3 (6.8)
Refinement		
Resolution (Å)	41.6 - 1.28	41.4 - 1.50
Unique reflections	59,723	37,027
<i>R</i> _{work} / <i>R</i> _{free} (%)	11.7 / 15.2	15.4 / 18.8
No. atoms	2,579	2,470
Protein	2,232	2,155
Ligand	23	24
Zn	1	1
Water	322	289

<i>B</i> -factors (Å ²)	14.7	14.6
Protein	13.9	14.0
Ligand	9.7	18.4
Zn	5.5	6.6
Water	29.2	25.0
R.m.s. deviations		
Bond lengths (Å)	0.014	0.016
Bond angles (°)	1.711	1.814

Microbial assays

Primary antimicrobial screening study by whole cell growth inhibition assays was performed with compounds at a single concentration (20 µM), in duplicate (n=2). The inhibition of growth is measured against 5 bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and 2 fungi: *Candida albicans* and *Cryptococcus neoformans*.

Hit Confirmation (secondary assay) of active compounds by whole cell growth inhibition assays was conducted as an 8-point dose response to determine the Minimum Inhibitory Concentration (MIC), in duplicate (n=2). The inhibition of growth is measured against those microorganisms that showed susceptibility to the compounds tested in the Primary Screen. Compounds were also counter screened for cytotoxicity against a human embryonic kidney cell line, HEK293, by determining their CC₅₀ value.

Antibacterial data analysis

Primary Assay: Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD₆₀₀), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

Secondary Assay: Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD₆₀₀), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth

inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The significance of the inhibition values was determined by Z-scores, calculated using the average and standard deviation of the sample wells (no controls) on the same plate. Samples with inhibition value above 80% and abs(Z-Score) above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition value between 50-80% and abs(Z-Score) above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

Antifungal data analysis

Primary Assay: Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate.

Secondary Assay: Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD530), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD600-570), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The significance of the inhibition values was determined by Z-scores, calculated using the average and standard deviation of the sample wells (no controls) on the same plate. Samples with inhibition value above 80% and abs(Z-Score) above 2.5 for either replicate (n=2 on different plates) were classed as actives. Samples with inhibition value between 50-80% and abs(Z-Score) above 2.5 for either replicate (n=2 on different plates) were classed as partial actives.

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