Supporting information

Selective FAP inhibitors with a xanthine scaffold.

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I.1 General Experimental Notes

Unless otherwise stated, laboratory reagent grade solvents were used. Reagents were obtained from Sigma-Aldrich, Acros organics, Apollo scientific, Manchester organics or Fluorochem and were used without further purification, unless otherwise mentioned. Characterization of all compounds was done with NMR and mass spectrometry. ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz Bruker Avance III nanobay spectrometer with ultrashield. Chemical shifts are in ppm and coupling constants are in Hertz (Hz). Minor rotamers of the amide bond, which were less than 10% of the major rotamer, are not reported in the NMR data. ES mass spectra were obtained from an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics. Purity was verified using two diverse HPLC systems. Water (A) and MeCN (B) were used as eluents. LC-MS spectra were recorded on an Agilent 1100 Series HPLC system using a Alltech Prevail C18 column (2.1 × 50 mm, 3 µm) coupled with an Esquire 3000plus as MS detector and a 'method A' 5–100% B, 20 min gradient was used with a flow rate from 0.2 mL/min. Formic acid 0.1% was added to solvents A and B. UPLC: Waters acquity H-class UPLC system coupled to a waters TQD ESI mass spectrometer and waters TUV detector. A waters acquity UPLC BEH C18 1.7 µm 2.1 x 50 mm column was used. Solvent A: water with 0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid. Method I: 0.15 min 95% A, 5% B then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25 min (0.350 ml/min),95% B, 5% A. The wavelength for UV detection was 254 nm. Method II: flow 0.4 mL/min, 0.25 min 95% A, 5% B, then in 4.75 min to 95%B, 5% A, then 0.25 min 95% B, 5% A, followed by 0.75 min 95%A, 5% B. The wavelength for UV detection was 214 nm. Where necessary flash purification was performed on a Biotage ® ISOLERA One flash system equipped with an internal variable dual-wavelength diode array detector (200-400 nm). For normal phase purifications SNAP cartridges (10-340 g; flow rate 10mL/min.-100mL/min.) were used, reversed phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self-packing samplet® cartridges using silica or Celite 545 respectively for normal and reversed phase purifications. Gradients used varied by purification. However typical gradients used for normal phase were 30min. gradient of 0-50% Ethyl acetate in hexane to 100 hexane or 0-5% methanol in DCM to 20% methanol in DCM and for reversed phase a gradient of 5% MeCN in water to 50% MeCN in water.

I.2 Synthesis of compounds

8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3- methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione (3)

Linagliptin was prepared as described in Himmelsbach *et al*.[1]

8-Bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (10)

This compound was prepared according to the literature procedure provided by Himmelsbach et al.[1]

To a stirring suspension of 3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (5.3 g, 31.9 mmol) and sodium acetate (5.23 g, 63.8 mmol) in glacial acetic acid (80mL) was added bromine (6.12 g, 38.3 mmol) dropwise. The mixture was stirred at 65 °C for 2 h. After cooling to room temperature the precipitate was filtered, washed with acetic acid, water, and dried under vacuum to give the corresponding 3-alkyl-8-bromoxanthine as beige powder. (7 g, 90%)

^{1}H NMR (400 MHz, DMSO-*d6*) δ 12.6 (s, 1H), 11.14 (s, 1H), 3.31 (s, 3H); MS (ESI): m/z 244.8 [M + H]+.

General procedure A for the synthesis of compounds (11a-11d) (*procedure published in Himmelsbach et al.[1]*)

Alkyl bromide (1.900 ml, 21.00 mmol) was added to a solution of 8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **5** (4.9 g, 20.00 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (3.47 ml, 21.00 mmol) in DMF (10 mL). The resultant solution was heated to 80°C and stirred at this temperature for 4h. After cooling to ambient temperature, ice-cold water (200 mL) was added. The precipitate was separated by filtration, washed with water and a little diethylether and dried to give the product as a white solid.

8-Bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (11a)

This compound was described earlier by Himmelsbach et al.[1]

The title compound was made according to general procedure A starting from **10** and 1-bromobut-2-yne.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 5.05 (q, *J* = 2.38 Hz, 2H), 3.30 (s, 2H), 1.80 (t, *J* = 2.41 Hz, 3H); UPLC I (ESI) R_t 1.33 min, m/z 297.5, 299.5 $[M+H]^+$ (purity: 95%); white powder (5.1 g, 86%).

8-bromo-3-methyl-7-(3-methylbut-2-enyl)-1H-purine-2,6(3*H*,7*H*)-dione (11b)

This compound was described earlier by Himmelsbach et al.[1]

The title compound was made according to general procedure A starting from **10** and 1-bromo-3-methylbut-2-ene.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 5.23 (dddd, *J* = 1.49, 2.95, 5.55, 6.96 Hz, 1H), 4.86 (d, *J* = 6.87 Hz, 2H), 3.30 (s, 3H), 1.80 (d, *J* = 1.33 Hz, 3H), 1.69 (d, *J* = 1.49 Hz, 3H); UPLC I (ESI) R_t 1.56 min, m/z 313.4, 315.4 $[M+H]^+$ (purity: 95%); white powder (0.35 g, 80%).

8-bromo-3-methyl-7-(4-methylpent-3-enyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (11c)

The title compound was made according to general procedure A starting from **10** and 5-bromo-2-methylpent-2-ene.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 5.08 (dddd, *J* = 1.43, 2.85, 6.05, 9.07 Hz, 1H), 4.18 (t, *J* = 7.04 Hz, 2H), 3.31 (s, 3H), 2.42 (q, *J* = 7.25 Hz, 2H), 1.61 (d, *J* = 1.46 Hz, 3H), 1.43 (d, *J* = 1.35 Hz, 3H);UPLC I (ESI) R_t 1.63 min, m/z 327.5, 329.5 $[M+H]^+$ (purity: 95%); white powder (0.6 g, 78%)

7-benzyl-8-bromo-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (11d)

This compound was described earlier by Himmelsbach et al.[1]

The title compound was made according to general procedure A starting from **10** and benzyl bromide.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 7.41 - 7.29 (m, 3H), 7.27 - 7.23 (m, 2H), 5.48 (s, 2H), 3.33 (s, 3H); UPLC I (ESI) R_t 1.53 min, m/z 335.5, 337.5 $[M+H]^+$ (purity: 95%); white powder (0.56 g, 79%)

8-bromo-3,7-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (11e)

To a stirring suspension of 3,7-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (3.25 g, 18.0 mmol) and sodium acetate (2.96 g, 36.1 mmol) in glacial acetic acid (0.39 M) was added bromine (1.11 ml, 21.6 mmol) dropwise (1.2 eq). The mixture was stirred at 90°C for 10 h. After cooling to room temperature the precipitate was filtered, washed with acetic acid, water, and dried under vacuum to give the corresponding 8-bromo-3,7-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (3.6 g, 77 %) as whitish powder in pure form. UPLC I (ESI) R_t 1.04 min, m/z 259.5, 261.5 $[M+H]^+$ (purity: 95%);

8-bromo-7-(but-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12a)

This compound was described earlier by Himmelsbach et al.[1]

2-(chloromethyl)-4-methylquinazoline **S8** (2.43 g, 12.61 mmol) was added with 8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **6a** (3.41 g, 11.5 mmol) and potassium carbonate (2.54 g, 18.3 mmol) in DMF (2 mL) and was stirred at 65°C for 6 h. Water was added and the formed precipitate was separated by filtration and washed with water dried at 55°C, the white powder was used in the next reaction without further purification.
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7. 75 (m, 1H), 7.67 (m, 1H), 5.35 (s, 2H), 5.11 (incompletely resolved q, *J* = 2.2 Hz, 2H), 3.44 (s, 3H), 2.39 (s, 3H), 1.79 (t, *J* = 2.3 Hz, 3H); UPLC I (ESI) R_t 1.75 min, m/z 453.5, 455.5 $[M+H]^+$ (purity: 96%).

8-bromo-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-7-(3-methylbut-2-enyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12b)

The title compound was synthesized in a similar manner as **12a** starting from **11a** and 2-(bromomethyl)-4-methoxyquinazoline **S4**
UPLC I (ESI) R_t 2.33 min, m/z 485.5, 487.5 $[M+H]^+$ (82%); (0.164 g, 68%)

8-bromo-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-7-(4-methylpent-3-enyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12c)

The title compound was synthesized in a similar manner as **12a** starting from **11a** and 2-(bromomethyl)-4-methoxyquinazoline **S4**
UPLC I (ESI) R_t 2.52 min, m/z 499.5, 501.5 $[M+H]^+$ (purity: 86%); (0.167 g, 71%)

7-benzyl-8-bromo-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (12d)

The title compound was synthesized in a similar manner as **12a** starting from **11a** and 2-(bromomethyl)-4-methoxyquinazoline **S4**
UPLC I (ESI) R_t 2.24 min, m/z 507.5, 509.5 $[M+H]^+$ (purity: 92%).

7-(but-2-yn-1-yl)-3-methyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12e)

A flask containing 8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **11a** (1.21 g, 4.09 mmol), TEA (1.42 ml, 10.2 mmol) and piperazin-2-one (0.450 g, 4.49 mmol) with dimethylacetamide was stirred at 75°C for 6 h. After cooling down to room temperature, water was added and the precipitate was filtered. The product was recrystallized from DMF to provide a white precipitate. (0.8 g, 62%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.08 (s, 1H), 4.92 (d, *J* = 2.2 Hz, 2H), 3.92 (s, 2H), 3.63 - 3.53 (m, 2H), 3.37 - 3.32 (m, 2H), 3.29 (s, 3H), 1.79 (t, *J* = 2.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.01, 153.94, 153.91, 150.73, 148.40, 103.81, 81.42, 73.62, 51.84, 45.96, 39.62, 35.13, 28.46, 3.07; UPLC II (ESI) R_t 1.86 min, m/z 317.6 $[M+H]^+$ (purity: 98%).

3,7-dimethyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12f)

The title compound was made in a similar manner as compound **12e** starting from **11e** and piperazin-2-one.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 8.05 (s, 1H), 3.83 (s, 2H), 3.68 (s, 3H), 3.46 (dd, *J* = 4.22, 6.44 Hz, 2H), 3.35 - 3.30 (m, 4H), 3.29 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.20, 154.42, 154.31, 150.75, 148.12, 104.86, 51.79, 45.88, 39.62, 32.20, 28.44; UPLC II (ESI) R_t 0.91 min, m/z 279.6 $[M+H]^+$ (purity: 99%); (0.2 g, 76%)

8-bromo-7-(but-2-yn-1-yl)-3-methyl-1-(quinazolin-2-ylmethyl)-1H-purine-2,6(3H,7H)-dione (12g)

The title compound was made in a similar manner as compound **12l** starting from **11a** and **S7**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.13 (d, *J* = 7.5 Hz, 1H), 8.02 – 7.96 (m, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 5.41 (s, 2H), 5.11 (q, *J* = 2.4 Hz, 2H), 3.43 (s, 3H), 1.79 (t, *J* = 2.3 Hz, 3H); UPLC I (ESI) R_t 1.69 min, m/z 439.3, 441.3 $[M+H]^+$ (purity: 96%); (0.175 g, 60%)

8-bromo-7-(but-2-ynyl)-3-methyl-1-(quinolin-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12l)

2-(chloromethyl)quinoline hydrochloride (0.187 g, 0.875 mmol) was added with 8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **11a** (0.2 g, 0.673 mmol) and potassium carbonate (0.242 g, 1.750 mmol) in DMF (2 mL) and stirred at 55°C for 6 h. Addition of water, the formed precipitate was separated by filtration and washed with water dried at 55°C to give a white powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, *J* = 8.6 Hz, 1H), 7.95 (d, *J* = 7.7 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.74 - 7.69 (m, 1H), 7.59 - 7.53 (m, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 5.34 (s, 2H), 5.11 (d, *J* = 2.3 Hz, 2H),

3.43 (s, 3H), 1.79 (d, *J* = 2.2 Hz, 3H); UPLC I (ESI) R_t 1.74 min, m/z 438.6, 440.6 $[M+H]^+$ (purity: 95%); (0.140 g, 48%)

8-bromo-7-(but-2-ynyl)-3-methyl-1-(naphthalen-1-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12m)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 1-(chloromethyl)naphthalene.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, *J* = 6.86 Hz, 1H), 7.82 (d, *J* = 8.26 Hz, 1H), 7.61 - 7.55 (m, 2H), 7.53 - 7.45 (m, 1H), 7.38 (dd, *J* = 7.17, 8.23 Hz, 1H), 7.09 (dd, *J* = 1.19, 7.21 Hz, 1H), 5.54 (s, 2H), 5.11 (q, *J* = 2.39 Hz, 2H), 3.44 (s, 3H), 1.79 (t, *J* = 2.44 Hz, 3H); UPLC I (ESI) R_t 2.11 min, m/z 437.6, 439.6 $[M+H]^+$ (purity: 98%); (0.175 g, 60%)

1-Benzyl-8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (12o)

The title compound was made in a similar manner as compound **12l** starting from **11a** and benzylchloride

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 - 7.22 (m, 5H), 5.10 (q, *J* = 2.38 Hz, 2H), 5.05 (s, 2H), 3.40 (s, 3H), 1.82 - 1.76 (m, 3H); UPLC I (ESI) R_t 1.95 min, m/z 387.5, 389.5 $[M+H]^+$ (purity: 98%); (0.140g, 53%)

3-((8-bromo-7-(but-2-ynyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-1-yl)methyl)benzonitrile (12p)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 3-(bromomethyl)benzonitrile.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 - 7.71 (m, 2H), 7.66 (dt, *J* = 1.41, 7.95 Hz, 1H), 7.56 - 7.49 (m, 1H), 5.10 (q, *J* = 2.49 Hz, 2H), 5.09 (s, 2H), 3.40 (s, 3H), 1.82 - 1.77 (t, *J* = 2.49 Hz, 3H); UPLC I (ESI) R_t 1.87 min, m/z 412.5, 414.5 $[M+H]^+$ (purity: 98%); (0.175 g, 63%)

4'-((8-bromo-7-(but-2-ynyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-1-yl)methyl)biphenyl-2-carbonitrile (12q)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 4-bromomethyl-2'-cyanobiphenyl.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (td, *J* = 7.7, 1.3 Hz, 2H), 7.59 (dd, *J* = 11.7, 4.1 Hz, 2H), 7.56 - 7.51 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 5.14 (s, 2H), 5.12 (d, *J* = 2.4 Hz, 2H), 3.42 (s, 3H), 1.80 (t, *J* = 2.4 Hz, 3H); UPLC I (ESI) R_t 2.10 min, m/z 388.5, 390.6 $[M+H]^+$ (purity: 97%); (0.330 g, 92%)

8-bromo-7-(but-2-ynyl)-3-methyl-1-((5-methyl-2-phenyl-2*H*-1,2,3-triazol-4-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (12r)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 4-(bromomethyl)-5-methyl-2-phenyl-2*H*-1,2,3-triazole.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 - 7.82 (m, 2H), 7.53 - 7.46 (m, 2H), 7.37 - 7.30 (m, 1H), 5.16 (s, 2H), 5.11 (d, *J* = 2.4 Hz, 2H), 3.40 (s, 3H), 2.37 (s, 3H), 1.79 (t, *J* = 2.4 Hz, 3H); UPLC I (ESI) R_t 2.08 min, m/z 468.7, 470.7 $[M+H]^+$ (purity: 98%); (0.198 g, 63%)

1-(4-(1*H*-pyrazol-1-yl)benzyl)-8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (12s)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 1-(4-(bromomethyl)phenyl)-1*H*-pyrazole.

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (t, *J* = 2.35 Hz, 3H), 3.58 (s, 3H), 5.14 (d, *J* = 2.45 Hz, 2H), 5.23 (s, 2H), 6.46 (t, *J* = 2.17 Hz, 1H), 7.28 (s, 1H), 7.64 (d, *J* = 2.89 Hz, 3H), 7.72 (d, *J* = 1.82 Hz, 1H), 7.91 (d, *J* = 2.51 Hz, 1H);); UPLC I (ESI) R_t 1.95 min, m/z 453.6, 455.5 $[M+H]^+$ (purity: 98%); (0.117 g,38%)

1-(4-(1*H*-1,2,4-triazol-1-yl)benzyl)-8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (12t)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 1-[4-(bromomethyl)phenyl]-1*H*-1,2,4-triazole.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (s, 1H), 8.21 (s, 1H), 7.78 (d, *J* = 8.58 Hz, 2H), 7.49 (d, *J* = 8.58 Hz, 2H), 5.13 - 5.10 (m, 2H), 5.08 (q, *J* = 2.42 Hz, 2H), 3.27 (s, 3H), 1.81 - 1.77 (t, *J* = 2.42 Hz, 3H); UPLC I (ESI) R_t 1.69 min, m/z 454.6, 456.5 $[M+H]^+$ (purity: 98%); (0.130 g, 42%)

8-bromo-7-(but-2-yn-1-yl)-1-isopropyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (12u)

The title compound was made in a similar manner as compound **12l** starting from **11a** and 2-bromopropane.

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.25 - 4.92 (m, 1H), 3.36 (s, 1H), 1.82 - 1.77 (m, 1H), 1.45 - 1.31 (m, 2H); UPLC (ESI) R_t 1.81 min, m/z 339.5 $[M+H]^+$; (0.236 g; 42%)

General procedure B for the synthesis of final compounds 13a-13i, 13k-13n, 13p-13v

The primary or secondary amine (0.048 ml, 0.485 mmol) was added to a suspension of 8-bromo-7-(but-2-yn-1-yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione **12a** (0.2 g, 0.441 mmol) and potassium carbonate (0.128 g, 0.927 mmol) in DMF (3 mL). The mixture was stirred for 6 h at 50°C. Then water was added and the formed precipitate was washed with water. After

drying and flash chromatography with ethyl acetate - methanol with a 0.1% triethylamine additive, the title compound was obtained.

7-(But-2-yn-1-yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13a)

The title compound was made according to general procedure B starting from **12a** and piperazine.
^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.78 (t, *J* = 2.4 Hz, 3H), 2.89 (s, 3H), 2.90 – 2.94 (m, 4H), 3.31 – 3.38 (m, 4H), 3.40 (s, 3H), 4.91 (d, *J* = 2.7 Hz, 2H), 5.32 (s, 2H), 7.68 (ddd, *J* = 8.2, 6.9, 1.3 Hz, 1H), 7.78 – 7.83 (m, 1H), 7.92 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 8.23-8.27 (m, 1H). UPLC I (ESI) R_t 1.28 min, m/z 459.7 $[M+H]^+$ (purity: 97%); (0.056 g, 58%)

(*S*)-7-(But-2-ynyl)-8-(hexahydropyrrolo[1,2-a]pyrazin-2(1*H*)-yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13b)

A vial containing 8-bromo-7-(but-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione **12a** (0.1 g, 0.221 mmol), (*S*)-octahydropyrrolo[1,2-*a*]pyrazine (0.033 g, 0.265 mmol) with DMF was stirred at 75°C for 6 h. water was added and the formed precipitate was filtered. After drying and flash chromatography with ethyl acetate - methanol with a 0.1% triethylamine additive, the title compound was obtained.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (ddd, *J* = 0.72, 1.42, 8.40 Hz, 1H), 7.83 (dt, *J* = 0.91, 8.38 Hz, 1H), 7.71 (ddd, *J* = 1.39, 6.90, 8.38 Hz, 1H), 7.47 (ddd, *J* = 1.24, 6.87, 8.16 Hz, 1H), 5.54 (s, 2H), 4.86 (dq, *J* = 2.41, 4.62 Hz, 2H), 3.90 (ddd, *J* = 1.61, 2.87, 12.07 Hz, 1H), 3.78 (ddt, *J* = 1.89, 3.32, 12.53 Hz, 1H), 3.53 (s, 3H), 3.24 (ddd, *J* = 3.00, 11.60, 12.41 Hz, 1H), 3.16 - 3.06 (m, 2H), 2.91 (dd, *J* = 10.22, 12.04 Hz, 1H), 2.84 (s, 3H), 2.43 (td, *J* = 3.11, 11.33 Hz, 1H), 2.25 - 2.14 (m, 2H), 1.91 - 1.78 (m, 2H), 1.76 (t, *J* = 2.37 Hz, 4H), 1.51 - 1.39 (m, 1H);^{13}C NMR (101 MHz, $CDCl_3$) δ 168.47, 161.24, 156.05, 154.45, 151.95, 150.01, 148.09, 133.19, 128.95, 126.68, 124.85, 123.17, 104.57, 81.46, 73.22, 62.05, 54.34, 53.51, 51.42, 49.29, 46.35, 35.90, 29.79, 27.32, 21.83, 21.09, 3.76; LCMS A (ESI) R_t 11.9 min, m/z 499.2 $[M+H]^+$ (purity: 97%); (0.065 g, 60%)

7-(But-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-8-(piperidin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13c)

The title compound was made according to general procedure B starting from **12a** and piperidine.
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, *J* = 8.24 Hz, 1H), 7.91 (ddd, *J* = 1.41, 6.79, 8.24 Hz, 1H), 7.81 (d, *J* = 8.48 Hz, 1H), 7.71 – 7.64 (m, 1H), 5.32 (s, 2H), 4.91 – 4.81 (m, 2H), 3.40 (s, 3H), 3.38-3.34 (m, 4H), 2.88 (s, 3H), 1.78 (q, *J* = 2.50 Hz, 3H), 1.70-160 (m, 6H); UPLC I (ESI) R_t 1.95 min, m/z 458.7 $[M+H]^+$; LCMS A R_t 17.9 min, m/z 458.2 $[M+H]^+$ (purity: 98%); (0.047 g, 67%)

7-(But-2-ynyl)-8-ethoxy-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13d)

The title compound was made according to general procedure B starting from **12a** and ethanol at 75°C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 - 7.93 (m, 1H), 7.82 (d, *J* = 8.06 Hz, 1H), 7.71 (ddd, *J* = 1.36, 5.46, 8.41 Hz, 1H), 7.46 (ddd, *J* = 1.18, 6.90, 8.18 Hz, 1H), 5.54 (s, 2H), 4.83 (q, *J* = 2.25 Hz, 2H), 4.56 (q, *J* = 7.08 Hz, 2H), 3.51 (s, 3H), 2.83 (s, 3H), 1.73 (t, *J* = 2.36 Hz, 3H), 1.45 (t, *J* = 7.09 Hz, 3H); LCMS A (ESI) R_t 16.4 min, m/z 419.1 $[M+H]^+$ (purity: 96%); (0.042 g, 50%)

(*R*)-*N*-(1-(7-(but-2-yn-1-yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)piperidin-3-yl)acetamide (13e)

The title compound was made according to general procedure B starting from **12a** and (*R*)-*N*-(piperidin-3-yl)acetamide.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (ddd, *J* = 0.72, 1.43, 8.41 Hz, 1H), 7.85 (ddd, *J* = 0.60, 1.19, 8.45 Hz, 1H), 7.74 (ddd, *J* = 1.39, 6.88, 8.39 Hz, 1H), 7.50 (ddd, *J* = 1.24, 6.90, 8.22 Hz, 1H), 6.79 (d, *J* = 7.37 Hz, 1H), 5.55 (s, 2H), 4.93 (dq, *J* = 2.33, 17.39 Hz, 1H), 4.83 (dq, *J* = 2.35, 17.41 Hz, 1H), 4.14 (tt, *J* = 2.58, 5.28 Hz, 1H), 3.54 (s, 3H), 3.56 - 3.50 (m, 1H), 3.47 - 3.33 (m, 2H), 3.29 (dd, *J* = 5.58, 12.94 Hz, 1H), 2.87 (s, 3H), 2.00 (s, 3H), 1.89 - 1.63 (m, 7H); ^{13}C NMR (101 MHz, CDCl3) δ 169.55, 168.59, 161.12, 155.95, 154.55, 151.88, 150.04, 147.63, 133.30, 128.97, 126.80, 124.94, 123.23, 104.66, 81.60, 73.07, 54.15, 51.08, 46.43, 45.23, 35.69, 29.84, 29.12, 23.58, 22.14, 21.89, 3.80; UPLC I (ESI) R_t 1.51 min, m/z 515.7 $[M+H]^+$ (purity: 96%); LCMS A (ESI) R_t 14.5 min, m/z 515.2 $[M+H]^+$ (purity: 95%); (0.042 g, 37%)

7-(But-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13f)

The title compound was made according to general procedure B starting from **12a** and piperazin-2-one.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (ddd, *J* = 0.64, 1.34, 8.33 Hz, 1H), 7.87 (ddd, *J* = 0.65, 1.27, 8.50 Hz, 1H), 7.76 (ddd, *J* = 1.39, 6.89, 8.42 Hz, 1H), 7.52 (ddd, *J* = 1.26, 6.88, 8.24 Hz, 1H), 6.41 (s, 1H), 5.56 (s, 2H), 4.95 (q, *J* = 2.37 Hz, 2H), 4.14 (s, 2H), 3.70 (dd, *J* = 4.47, 6.00 Hz, 2H), 3.63 - 3.57 (m, 2H), 3.55 (s, 3H), 2.89 (s, 3H), 1.80 (t, *J* = 2.37 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 168.68, 167.65, 161.13, 154.70, 153.87, 151.92, 150.09, 147.65, 133.37, 129.04, 126.87, 125.00, 123.31, 105.07, 82.25, 72.82, 52.96, 46.89, 46.49, 41.16, 35.60, 29.93, 21.93, 3.84; LCMS A (ESI) R_t 13.4 min (purity: 98%), m/z 473.2 $[M+H]^+$ (purity: 99%); (0.032 g,38%)

(*S*)-7-(But-2-yn-1-yl)-8-(3-(hydroxymethyl)-5-oxopiperazin-1-yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13g)

The title compound was made according to general procedure B starting from **12a** and (*S*)-6-(hydroxymethyl)piperazin-2-one **S9**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (ddd, *J* = 0.62, 1.35, 8.33 Hz, 1H), 7.92 - 7.84 (m, 1H), 7.76 (ddd, *J* = 1.39, 6.91, 8.42 Hz, 1H), 7.53 (ddd, *J* = 1.26, 6.88, 8.24 Hz, 1H), 6.84 (s, 1H), 5.55 (s, 2H), 4.96 (q, *J* = 2.35 Hz, 2H), 4.25 (d, *J* = 17.42 Hz, 1H), 4.17 (d, *J* = 17.42 Hz, 1H), 3.85 - 3.66 (m, 6H), 3.53 (s, 3H), 2.89 (s, 3H), 1.80 (t, *J* = 2.37 Hz, 3H);^{13}C NMR (101 MHz, CDCl3) δ 168.75, 167.44, 161.07, 154.58, 154.14, 151.83, 150.06, 147.47, 133.43, 128.98, 126.92, 125.02, 123.31, 104.80, 82.54, 72.77, 63.24, 52.73, 52.42, 47.35, 46.50, 35.75, 29.94, 21.94, 3.83; UPLC I (ESI) R_t 1.35 min, m/z 503.8 $[M+H]^+$ (purity: 98%); LCMS A (ESI) R_t 12.6 min, m/z 503.1 $[M+H]^+$ (purity: 97%); (0.041 g, 37%)

2-(7-(But-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylamino)acetamide (13h)

The title compound was made according to general procedure B starting from **12a** and 2-aminoacetamide hydrochloride.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (ddd, *J* = 0.68, 1.40, 8.39 Hz, 1H), 7.90 (dt, *J* = 0.93, 8.52 Hz, 1H), 7.76 (ddd, *J* = 1.38, 6.91, 8.47 Hz, 1H), 7.53 (ddd, *J* = 1.24, 6.91, 8.28 Hz, 1H), 6.34 (s, 1H), 5.88 (t, *J* = 5.25 Hz, 1H), 5.64 (s, 1H), 5.53 (s, 2H), 4.96 (q, *J* = 2.44 Hz, 2H), 4.17 (d, *J* = 5.18 Hz, 2H), 3.52 (s, 3H), 2.90 (s, 3H), 1.86 (t, *J* = 2.45 Hz, 3H); UPLC I (ESI) R_t 1.31 min, m/z 447.6 $[M+H]^+$ (purity: 97%); (0.046 g, 46.7%)

***N*-(2-(7-(But-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylamino)ethyl)acetamide (13i)**

The title compound was made according to general procedure B starting from **12a** and *N*-(2-aminoethyl)acetamide 2,2,2-trifluoroacetate.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.20 Hz, 1H), 7.88 (d, *J* = 8.44 Hz, 1H), 7.76 (ddd, *J* = 1.39, 6.89, 8.44 Hz, 1H), 7.52 (ddd, *J* = 1.22, 6.83, 8.19 Hz, 1H), 6.61 (s, 1H), 5.61 (br t, *J* = 5.88 Hz, 1H), 5.54 (s, 2H), 4.96 - 4.85 (br s, 2H), 3.65 - 3.58 (m, 2H), 3.56 - 3.48 (m, 5H), 2.89 (s, 3H), 1.99 (s, 3H), 1.82 (t, *J* = 2.41 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 171.33, 168.76, 161.41, 154.04, 153.80, 151.94, 150.03, 148.89, 133.43, 128.94, 126.89, 125.03, 123.27, 102.39, 82.85, 71.80, 46.36, 43.76, 40.56, 33.22, 29.83, 23.35, 21.95, 3.78; LCMS A (ESI) R_t 13.3 min, m/z 475.1 $[M+H]^+$ (purity: 98%); (0.040 g, 38%)

_N_-(2-(7-(but-2-ynyl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-2,6-dioxo-2,3,6,7-tetrahydro-1_H_-purin-8-ylamino)ethyl)isonicotinamide (13j)

The title compound was made according to general procedure B starting from **12a** and *N*-(2-aminoethyl)isonicotinamide.

^{1}H NMR (400 MHz, MeOD) δ 8.69 (d, *J* = 1.72 Hz, 1H), 8.68 (d, *J* = 1.70 Hz, 1H), 8.24 (dt, *J* = 0.92, 8.34 Hz, 1H), 7.91 (ddd, *J* = 1.37, 6.76, 8.24 Hz, 1H), 7.85 (dt, *J* = 1.03, 8.36 Hz, 1H), 7.76 (d, *J* = 1.71 Hz, 1H), 7.75 (d, *J* = 1.69 Hz, 1H), 7.67 (ddd, *J* = 1.41, 6.76, 8.26 Hz, 1H), 5.45 (s, 2H), 4.58 (s, 2H), 3.80 - 3.74 (m, 2H), 3.74 - 3.68 (m, 2H), 3.44 (s, 3H), 2.94 (s, 3H), 1.71 (t, *J* = 2.37 Hz, 3H); UPLC I (ESI) R_t 1.42 min, m/z 538.7 $[M+H]^+$ (purity: 95%); (0.035 g, 47%)

7-(but-2-ynyl)-3-methyl-8-(piperazin-1-yl)-1-(quinazolin-2-ylmethyl)-1H-purine-2,6(3H,7H)-dione (13k)

The title compound was made according to general procedure B starting from **12g** and piperazine.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (br s, 1H), 9.39 (s, 1H), 8.09 - 7.98 (m, 1H), 7.98 - 7.85 (m, 2H), 7.63 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 5.66 (s, 2H), 4.90 (q, J = 2.3 Hz, 2H), 3.87 - 3.79 (m, 4H), 3.55 (s, 3H), 3.50 - 3.44 (m, 4H), 1.80 (t, J = 2.3 Hz, 3H); UPLC I (ESI) R_t 1.24 min, m/z 445.5 $[M+H]^+$ (purity: 95%); LCMS A (ESI) R_t 11.1 min, m/z 445.1 $[M+H]^+$ (purity: 98%); (0.059 g, 30%)

7-(But-2-ynyl)-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-8-(piperazin-1-yl)-1_H_-purine-2,6(3_H_,7_H_)-dione (13l)

The title compound was made according to general procedure B starting from **12b** and piperazine.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (ddd, *J* = 0.67, 1.49, 8.18 Hz, 1H), 7.82 (ddd, *J* = 0.64, 1.25, 8.46 Hz, 1H), 7.73 (ddd, *J* = 1.49, 6.92, 8.45 Hz, 1H), 7.46 (ddd, *J* = 1.24, 6.91, 8.18 Hz, 1H), 5.48 (s, 2H), 4.91 (q, *J* = 2.35 Hz, 2H), 4.06 (s, 3H), 3.58 (s, 3H), 3.48 - 3.42 (m, 4H), 3.13 - 3.06 (m, 4H), 1.81 (t, *J* = 2.33 Hz, 3H). (NH proton was not seen); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.38, 161.36, 155.97, 154.61, 152.05, 151.54, 148.04, 133.24, 127.71, 126.26, 123.38, 115.50, 104.67, 81.51, 73.20, 54.14, 50.90, 46.17, 45.63, 35.80, 29.87, 3.83; UPLC I (ESI) R_t 1.39 min, m/z 475.8 $[M+H]^+$ (purity: 95%); LCMS A (ESI) R_t 12.3 min, m/z 238.1, 475.1 $[M+H]^+$ (purity: 97%); (0.045 g, 39%)

7-(But-2-ynyl)-3-methyl-8-(piperazin-1-yl)-1-(quinolin-2-ylmethyl)-1_H_-purine-2,6(3_H_,7_H_)-dione (13m)

The title compound was made according to general procedure B starting from **12l** and piperazine.

^{1}H NMR (400 MHz, MeOD) δ 8.25 (d, *J* = 8.77 Hz, 1H), 7.95 (dd, *J* = 1.12, 8.43 Hz, 1H), 7.87 (dd, *J* = 1.43, 8.18 Hz, 1H), 7.71 (ddd, *J* = 1.48, 6.92, 8.52 Hz, 1H), 7.54 (ddd, *J* = 1.18, 6.97, 8.12 Hz, 1H), 7.38 (d, *J* = 8.59 Hz, 1H), 5.46 (s, 2H), 4.90 (q, *J* = 2.38 Hz, 2H), 3.52 (s, 3H), 3.46 - 3.40 (m, 4H), 3.03 - 2.96 (m, 4H), 1.77 (t, *J* = 2.38 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 158.95, 157.99, 155.59, 153.26, 149.86, 148.51, 138.64, 131.02, 128.93, 128.92, 128.78, 127.48, 120.00, 105.74, 82.39, 74.10, 51.59, 47.07, 46.03, 36.65, 30.20, 3.05; UPLC I (ESI) R_t 1.26 min, m/z 243.7 $[M+2H]^{++}$, 444.7 $[M+H]^+$ (99%); LCMS A (ESI) R_t 11.0 min, m/z 444.1 $[M+H]^+$ (99%); (0.038 g, 42%)

7-(But-2-ynyl)-3-methyl-1-(naphthalen-1-ylmethyl)-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13n)

The title compound was made according to general procedure B starting from **12m** and piperazine.
^{1}H NMR (400 MHz, MeOD) δ 8.22 (d, *J* = 8.41 Hz, 1H), 7.91 - 7.86 (m, 1H), 7.75 (d, *J* = 8.29 Hz, 1H), 7.58 (ddd, *J* = 1.49, 6.84, 8.47 Hz, 1H), 7.51 (ddd, *J* = 1.23, 6.79, 8.05 Hz, 1H), 7.35 (dd, *J* = 7.19, 8.24 Hz, 1H), 7.07 (dd, *J* = 1.15, 7.22 Hz, 1H), 5.66 (s, 2H), 4.91 (q, *J* = 2.41 Hz, 2H), 3.53 (s, 3H), 3.46 - 3.39 (m, 4H), 3.04 - 2.99 (m, 4H), 1.78 (t, *J* = 2.33 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 158.02, 155.64, 153.13, 149.79, 135.17, 133.63, 132.30, 129.75, 128.46, 127.19, 126.76, 126.30, 123.96, 123.40, 105.75, 82.43, 74.07, 51.47, 45.95, 42.96, 36.68, 30.23, 3.06; UPLC I (ESI) R_t 1.57 min, m/z 443.7 $[M+H]^+$ (purity: 99%);LCMS A (ESI) R_t 13.6 min, m/z 443.2 $[M+H]^+$ (purity: 99%); (0.104 g, 72%)

7-(But-2-ynyl)-1-((2-(hydroxymethyl)thiazol-4-yl)methyl)-3-methyl-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13o)

(4-(chloromethyl)thiazol-2-yl)methanol (0.110 g, 0.67 mmol) was added to a suspension of 8-bromo-7-(but-2-ynyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione **11a** (0.2 g, 0.67 mmol) and potassium carbonate (0.149 g, 1.07 mmol) in DMF (2 mL). The mixture was stirred at 55°C for 6 h. Then piperazine (0.081 g, 0.94 mmol) and potassium carbonate (0.149 g, 1.07 mmol) were added and the mixture was stirred for an additional 16 h at 55°C. Water was added and the formed precipitate was separated by filtration and washed with water dried at 55°C. To give a white powder which was further purified with column chromatography using an ethyl acetate-Methanol:NH_3(1N) gradient.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, *J* = 0.80 Hz, 1H), 5.30 (d, *J* = 0.90 Hz, 2H), 4.87 (s, 2H), 4.86 (q, *J* =2.39 Hz, 2H), 3.52 (s, 3H), 3.45 - 3.38 (m, 4H), 3.12 - 3.01 (m, 4H), 2.47 (br s, 2H), 1.81 (t, *J* = 2.39 Hz, 3H) (NH was not observed); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.93, 156.02, 154.13, 152.39, 151.58, 147.99, 116.28, 104.67, 81.81, 73.11, 62.17, 50.58, 45.34, 40.59, 35.85, 29.92, 3.86; UPLC I (ESI) R_t 1.07 min, m/z 430.6 $[M+H]^+$ (99%); LCMS A (purity: ESI) R_t 9.5 min, m/z 215.6 $[M+2H]^{++}$, 430.1 $[M+H]^+$ (purity: 99%); (0.050 g, 22%)

1-Benzyl-7-(but-2-ynyl)-3-methyl-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13p)

The title compound was made according to general procedure B starting from **12o** and piperazine. ^{1}H NMR (400 MHz, MeOD) δ 7.35 - 7.30 (m, 2H), 7.26 - 7.21 (m, 2H), 7.20 - 7.14 (m, 1H), 5.09 (s, 2H), 4.88 (q, *J* = 2.3 Hz, 1H), 3.46 (s, 3H), 3.42 - 3.37 (m, 4H), 3.00 - 2.96 (m, 4H), 1.79 (t, *J* = 2.3 Hz, 2H); ^{13}C NMR (101 MHz, MeOD) δ 157.84, 155.44, 152.94, 149.43, 138.92, 129.27, 129.01, 128.22, 105.60, 82.43, 74.13, 51.47, 45.95, 45.11, 36.65, 30.13, 3.11; UPLC I (ESI) R_t 1.39 min, m/z 393.7 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 12.2 min, m/z 393.1 $[M+H]^+$ (purity: 99%); (0.135 g, 64%)

3-((7-(But-2-ynyl)-3-methyl-2,6-dioxo-8-(piperazin-1-yl)-2,3,6,7-tetrahydro-1*H*-purin-1-yl)methyl)benzonitrile (13q)

The title compound was made according to general procedure B starting from **12p** and piperazine. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.73 - 7.69 (m, 2H), 7.62 (dt, *J* = 1.46, 7.88 Hz, 1H), 7.52 (t, *J* = 7.98 Hz, 1H), 5.07 (s, 2H), 4.88 (q, *J* = 2.44 Hz, 2H), 3.37 (s, 3H), 3.29 - 3.23 (m, 4H), 2.86 - 2.80 (m, 4H), 1.78 (t, *J* = 2.39 Hz, 3H), (no NH observed); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.11, 153.17, 150.87, 147.83, 139.57, 132.33, 130.83, 130.81, 129.53, 118.76, 111.26, 103.36, 81.21, 73.74, 50.41, 45.12, 42.92, 35.48, 29.54, 3.08; UPLC I (ESI) R_t 1.36 min, m/z 418.7 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 12.0 min, m/z 418.1 $[M+H]^+$ (purity: 99%); (0.120 g, 69%)

4'-((7-(But-2-ynyl)-3-methyl-2,6-dioxo-8-(piperazin-1-yl)-2,3,6,7-tetrahydro-1*H*-purin-1-yl)methyl)biphenyl-2-carbonitrile (13r)

The title compound was made according to general procedure B starting from **12q** and piperazine. ^{1}H NMR (400 MHz, MeOD) δ 7.78 (dd, *J* = 1.38, 7.71 Hz, 1H), 7.68 (td, *J* = 1.43, 7.68 Hz, 1H), 7.55 - 7.45 (m, 6H), 5.20 (s, 2H), 4.89 (q, *J* = 2.38 Hz, 2H), 3.48 (s, 3H), 3.42 - 3.38 (m, 4H), 3.01 - 2.96 (m, 4H), 1.79 (t, *J* = 2.32 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 157.94, 155.47, 153.02, 149.58, 146.38, 139.72, 138.62, 134.83, 134.31, 131.27, 129.85, 129.42, 128.97, 119.57, 111.98, 105.65, 82.46, 74.12, 51.50, 45.97, 44.86, 36.68, 30.16, 3.11; UPLC I (ESI) R_t 1.39 min, m/z 494.7 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 14.0 min, m/z 494.2 $[M+H]^+$ (purity: 99%); (0.123 g, 52%)

7-(But-2-ynyl)-3-methyl-1-((5-methyl-2-phenyl-2*H*-1,2,3-triazol-4-yl)methyl)-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13s)

The title compound was made according to general procedure B starting from **12r** and piperazine. ^{1}H NMR (400 MHz, MeOD) δ 7.93 - 7.87 (m, 2H), 7.44 - 7.38 (m, 2H), 7.30 - 7.24 (m, 1H), 5.24 (s, 2H), 4.89 (q, *J* = 2.37 Hz, 2H), 3.48 (s, 3H), 3.42 - 3.35 (m, 4H), 3.02 - 2.93 (m, 4H), 2.40 (s, 3H), 1.78 (t, *J* =

2.37 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 157.92, 155.24, 152.86, 149.65, 145.20, 145.00, 141.01, 130.28, 128.06, 119.30, 105.58, 82.38, 74.10, 51.48, 45.95, 36.64, 36.41, 30.14, 10.13, 3.06; UPLC I (ESI) R_t 1.51 min, m/z 474.7 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 13.2 min, m/z 474.2 $[M+H]^+$ (purity: 99%); (0.032 g, 33%)

1-(4-(1*H*-Pyrazol-1-yl)benzyl)-7-(but-2-ynyl)-3-methyl-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13t)

The title compound was made according to general procedure B starting from **12s** and piperazine.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 0.65, 2.53 Hz, 1H), 7.72 (dd, *J* = 0.63, 1.77 Hz, 1H), 7.68 - 7.60 (m, 4H), 6.46 (dd, *J* = 1.76, 2.50 Hz, 1H), 5.22 (s, 2H), 4.90 (s, 2H), 3.72 (dd, *J* = 3.29, 7.07 Hz, 4H), 3.52 (s, 3H), 3.45 - 3.35 (m, 4H), 1.83 (t, *J* = 2.37 Hz, 3H); UPLC I (ESI) R_t 1.38 min, m/z 459.6 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 12.4 min, m/z 459.2 $[M+H]^+$ (purity: 99%); (0.032 g, 30%)

1-(4-(1*H*-1,2,4-triazol-1-yl)benzyl)-7-(but-2-ynyl)-3-methyl-8-(piperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13u)

The title compound was made according to general procedure B starting from **12t** and piperazine.
^{1}H NMR (400 MHz, MeOD) δ 9.02 (s, 1H), 8.12 (s, 1H), 7.70 (d, *J* = 8.56 Hz, 2H), 7.53 (d, *J* = 8.59 Hz, 2H), 5.15 (s, 2H), 4.89 (q, *J* = 2.35 Hz, 2H), 3.47 (s, 3H), 3.43 - 3.37 (m, 4H), 3.04 - 2.96 (m, 4H), 1.79 (t, *J* = 2.46 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 157.83, 155.38, 152.95, 152.82, 149.54, 142.93, 139.40, 137.35, 130.67, 120.81, 105.59, 82.46, 74.13, 51.38, 45.91, 44.61, 36.66, 30.14, 3.10; UPLC I (ESI) R_t 1.26 min, m/z 393.8 $[M+H]^+$ (purity: 99%); LCMS A (ESI) R_t 11.5 min, m/z 460.1 $[M+H]^+$ (purity: 99%); (0.052 g, 43%)

7-(but-2-ynyl)-3-methyl-8-(3-oxopiperazin-1-yl)-1-(pyridin-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13v)

To a solution of 7-(but-2-yn-1-yl)-3-methyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione **12e** (0.1 g, 0.32 mmol) in DMF (2 ml) under nitrogen at 0°C, was added lithium hydride (6.28 mg, 0.79 mmol). After 20 min a solution of 2-(chloromethyl)pyridine hydrochloride (0.052 g, 0.32 mmol) in DMF was added and stirred at this temperature for 1 h, and then RT overnight. The mixture was evaporated in vacuo to remove most of the DMF, and then poured into ice water (20 mL). The precipitate was collected by filtration. And purified using column chromatography (ethyl acetate-methanol)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (ddd, *J* = 0.94, 1.78, 4.85 Hz, 1H), 8.11 (s, 1H), 7.71 (td, *J* = 1.82, 7.68 Hz, 1H), 7.27 - 7.18 (m, 2H), 5.15 (s, 2H), 4.96 (q, *J* = 2.39 Hz, 2H), 3.97 (s, 2H), 3.63 (dd, *J* = 4.34,

6.36 Hz, 2H), 3.39 (s, 3H), 3.39 - 3.35 (m, 2H), 1.82 - 1.74 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.99, 156.58, 154.23, 153.31, 150.81, 148.80, 147.32, 136.58, 121.99, 120.50, 103.55, 81.51, 73.61, 51.84, 45.96, 44.81, 39.45, 35.26, 29.49, 3.09. UPLC II (ESI) R_t 2.07 min, m/z 408.6 $[M+H]^+$ (purity: 99%); (0.051 g, 40%)

7-(but-2-ynyl)-3-methyl-8-(3-oxopiperazin-1-yl)-1-(pyridin-3-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13w)

The title compound was made in a similar manner as compound **13v** starting from **12e** and 3-(chloromethyl)pyridine

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.46 (s, 1H), 8.10 (s, 1H), 7.70 (dt, *J* = 8.0, 1.9 Hz, 1H), 7.34 (dd, *J* = 7.8, 4.8 Hz, 1H), 5.06 (s, 2H), 4.96 (q, *J* = 2.4 Hz, 2H), 3.95 (s, 2H), 3.62 (dd, *J* = 6.1, 4.6 Hz, 2H), 3.37 (d, *J* = 4.2 Hz, 3H), 3.37 - 3.28 (m, 2H), 1.79 (t, *J* = 2.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.43, 154.80, 153.70, 151.27, 149.52, 148.69, 147.90, 135.96, 123.99, 104.02, 82.01, 74.08, 52.29, 49.07, 46.41, 41.76, 40.15 35.82, 30.03, 3.56. UPLC II (ESI) R_t 1.97 min, m/z 408.6 $[M+H]^+$ (purity: 99%); (0.062 g, 47%)

7-(but-2-ynyl)-3-methyl-8-(3-oxopiperazin-1-yl)-1-(pyridin-4-ylmethyl)-1H-purine-2,6(3H,7H)-dione (13x)

The title compound was made in a similar manner as compound **13v** starting from **12e** and 4-(chloromethyl)pyridine

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 - 8.44 (m, 2H), 8.11 (s, 1H), 7.24 - 7.20 (m, 2H), 5.05 (s, 2H), 4.96 (q, *J* = 2.36 Hz, 2H), 3.97 (s, 2H), 3.68 - 3.60 (m, 2H), 3.39 (s, 3H), 3.38 - 3.33 (m, 2H), 1.79 - 1.78 (t, *J* = 2.36 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.94, 154.36, 153.14, 150.77, 149.53, 147.53, 146.69, 121.96, 103.50, 81.54, 73.56, 51.81, 45.92, 42.70, 40.18, 35.27, 29.58, 3.08; UPLC II (ESI) R_t 1.95 min, m/z 408.6 $[M+H]^+$ (purity: 99%); (0.048 g, 37%)

(*R*)-8-(3-aminopiperidin-1-yl)-7-(but-2-ynyl)-1-isopropyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (13y)

Step 1: (*R*)-*tert*-butyl 1-(7-(but-2-ynyl)-1-isopropyl-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)piperidin-3-ylcarbamate

The title compound was made according to general procedure B starting from **12a** and (*S*)-*tert*-butyl 3-aminopiperidine-1-carboxylate.

UPLC I (ESI) R_t 2.04 min, m/z 459.8 $[M+H]^+$ (0.1 g,74%)

Step 2: (*R*)-*tert*-butyl 1-(7-(but-2-ynyl)-1-isopropyl-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)piperidin-3-ylcarbamate (0.120 g, 0.26 mmol) was added to the 2,2,2-trifluoroacetic acid (0.401 ml, 5,23 mmol) in DCM (0.5 ml). After 1h, the volatiles were evaporated and 10 eq HCl in diethyl

ether (1 M) was added. After 15 min stirring, the product was filtrated and washed with diethyl ether.

^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.13 – 1.26 (m, 1H), 1.38 (d, *J* = 6.9 Hz, 6H), 1.52 – 1.66 (m, 1H), 1.71 – 1.88 (m, 5H), 2.69 (dd, *J* = 11.9, 9.1 Hz, 1H), 2.80 (tt, *J* = 9.4, 3.7 Hz, 1H), 2.93 (ddd, *J* = 12.3, 10.7, 2.9 Hz, 1H), 3.33 (s, 3H), 3.50 – 3.64 (m, 2H), 4.83 – 4.92 (m, 2H), 5.14 (p, *J* = 6.9 Hz, 1H); UPLC I (ESI) R_t 1.32 min, m/z 359.7 $[M+H]^+$ (purity: 98%); LCMS A R_t 8.6 min, m/z 359.1 $[M+H]^+$ (purity: 98%)

1-((4-methoxyquinazolin-2-yl)methyl)-3,7-dimethyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (13z)

The title compound was synthesized in a similar manner as compound **13v** starting from **12f** and **S4.**

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.33 Hz, 1H), 8.08 (s, 1H), 7.87 (t, *J* = 7.77 Hz, 1H), 7.75 (d, *J* = 8.44 Hz, 1H), 7.61 (t, *J* = 7.51 Hz, 1H), 5.24 (s, 2H), 4.06 (s, 3H), 3.90 (s, 2H), 3.72 (s, 3H), 3.53 (t, *J* = 5.33 Hz, 2H), 3.42 (s, 3H), 3.37-3.33 (m, 2H);

^{13}C NMR (101 MHz, DMSO-d_6) δ 166.64, 166.16, 161.02, 154.73, 153.69, 150.96, 150.52, 147.05, 134.12, 127.09, 126.97, 123.17, 114.47, 104.51, 54.26, 51.78, 45.85, 45.36, 39.62, 32.36, 29.47. UPLC II (ESI) R_t 2.72 min, m/z 451.6 $[M+H]^+$ (purity: 95%); (0.087 g, 44%)

7-(but-2-ynyl)-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (14a)

The title compound was synthesized in a similar manner as compound **13v** starting from **12e** and **S4.**

^{1}H NMR (400 MHz, CDCl3) δ 8.11 (d, *J* = 8.17 Hz, 1H), 7.89 - 7.81 (m, 2H), 7.53 (d, *J* = 8.45 Hz, 1H), 6.10 (s, 1H), 5.56 (s, 2H), 4.94 (q, *J* = 2.37 Hz, 2H), 4.15 (d, *J* = 1.09 Hz, 2H), 4.11 (s, 3H), 3.71 (t, *J* = 5.31 Hz, 2H), 3.61 (dt, *J* = 3.84, 7.52 Hz, 2H), 3.56 (s, 3H), 1.80 (t, *J* = 2.34 Hz, 3H); UPLC II (ESI) R_t 3.08 min, m/z 489.6 $[M+H]^+$ (purity: 98%);(0.076 g, 49%)

1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-7-(3-methylbut-2-en-1-yl)-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (14b)

A flask containing 8-bromo-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-7-(3-methylbut-2-enyl)-1*H*-purine-2,6(3*H*,7*H*)-dione **12b** (0,150 g, 0.309 mmol), piperazin-2-one (0.037 g, 0.371 mmol) and DMF was stirred at 75°C for 6 h. Then, water was added and the formed precipitate was filtered. The product was purified using column chromatography. (0.068 g, 44%)

^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.11 (dd, J = 1.42, 8.27 Hz, 1H), 8.07 (d, J = 14.17 Hz, 1H), 7.88 (ddd, J = 1.50, 6.95, 8.47 Hz, 1H), 7.75 (d, J = 8.35 Hz, 1H), 7.61 (ddd, J = 1.22, 6.98, 8.23 Hz, 1H), 7.50 - 7.42 (m, 2H), 5.32 - 5.26 (m, 2H), 5.24 (s, 2H), 4.74 (d, J = 6.43 Hz, 2H), 4.70 (d, J = 6.62 Hz, 1H), 4.05 (s,

3H), 3.86 (s, 2H), 3.42 (s, 3H), 1.69 - 1.63 (m, 6H); UPLC (ESI) R_t 1.82 min, m/z 505.6 $[M+H]^+$ (purity: 95%); LC-MS (I-B) R_t 16.0 min, m/z 505.2 $[M+H]^+$ (purity: 95%)

1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-7-(4-methylpent-3-enyl)-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (14c)

The title compound was synthesized in a similar manner as compound **13z**, starting from **12c** and piperazin-2-one.

^{1}H NMR (400 MHz, MeOD) δ 8.15 (ddd, *J* = 0.69, 1.50, 8.23 Hz, 1H), 7.84 (ddd, *J* = 1.49, 6.93, 8.45 Hz, 1H), 7.76 (dt, *J* = 1.00, 8.47 Hz, 1H), 7.57 (ddd, *J* = 1.24, 6.90, 8.18 Hz, 1H), 5.39 (s, 2H), 5.06 (dddt, *J* = 1.79, 3.00, 6.21, 7.70 Hz, 1H), 4.17 (td, *J* = 2.40, 7.06 Hz, 2H), 4.10 (s, 3H), 3.96 (s, 2H), 3.58-3.54 (m, 5H), 3.52 - 3.46 (m, 3H), 2.49 (q, *J* = 7.29 Hz, 2H), 1.63 (d, *J* = 1.46 Hz, 3H), 1.48 (d, *J* = 1.42 Hz, 3H);^{13}C NMR (101 MHz, MeOD) δ 168.85, 167.51, 161.50, 155.42, 154.52, 152.00, 150.70, 148.26, 134.91, 133.78, 126.61, 126.14, 123.20, 119.26, 114.92, 105.02, 53.54, 52.15, 45.56, 45.17, 40.12, 28.83, 28.46, 24.50, 24.47, 16.36; UPLC I (ESI) R_t 1.87 min, m/z 519.6 $[M+H]^+$ (purity: 98%); LCMS A R_t 16.6 min, m/z 519.2 $[M+H]^+$ (purity: 96%); (0.036 g, 23%)

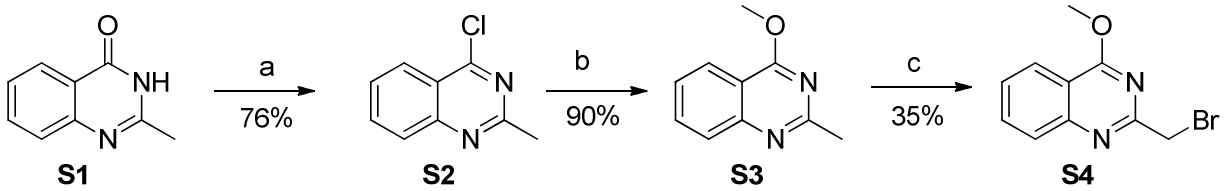
7-benzyl-1-((4-methoxyquinazolin-2-yl)methyl)-3-methyl-8-(3-oxopiperazin-1-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (14d)

The title compound was synthesized in a similar manner as compound **13z** starting from **12d** and piperazin-2-one**.**

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 - 8.08 (m, 1H), 8.05 (t, *J* = 2.42 Hz, 1H), 7.90 (ddd, *J* = 1.51, 7.01, 8.49 Hz, 1H), 7.78 - 7.73 (m, 1H), 7.62 (ddd, *J* = 1.18, 7.02, 8.18 Hz, 1H), 7.34 - 7.25 (m, 3H), 7.18 - 7.13 (m, 2H), 5.44 (s, 2H), 5.23 (s, 2H), 4.00 (s, 3H), 3.84 (s, 2H), 3.45 (m, 5H), 3.29 - 3.23 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.67, 165.99, 160.97, 154.82, 153.63, 151.00, 150.52, 147.47, 136.87, 134.16, 128.65, 127.52, 127.03, 126.99, 126.31, 123.15, 114.44, 104.23, 54.19, 52.13, 47.96, 46.35, 45.36, 39.90, 29.56; UPLC I (ESI) R_t 1.81 min, m/z 527.6 $[M+H]^+$ (purity: 98%); LCMS A R_t 15.5 min, m/z 527.2 $[M+H]^+$ (purity: 99%) (0.037 g, 35%)

Synthesis of intermediates

Scheme S1



S1 **S2** **S3** **S4**

a) 1.6 eq $POCl_3$, 1.1 eq DIPEA, toluene, 3 h, 76%; b) MeONa, Methanol, 1h, 90%; c) benzoyl peroxide, NBS, CCl_4 reflux 24 h, 35%

4-chloro-2-methylquinazoline (S2)

A mixture of 2-methylquinazolin-4(3*H*)-one (0.92 g, 5.74 mmol) in 19 mL of anhydrous toluene and anhydrous DIPEA (1.55 ml, 8.90 mmol) in a 50 mL round bottomed flask equipped with a condenser and a drying tube was refluxed for 1 h. To this warm solution was added freshly distilled phosphorus oxychloride (0.859 ml, 9.19 mmol) and the mixture was heated at 80 °C for 2 h, and cooled to room temperature. The mixture was diluted with 50 mL of ethyl acetate, washed with 20 mL of ice cold water, 200 mL of saturated $NaHCO_3$, 20 mL of water, citric acid (1 N, 4 × 20 mL), 20 mL of water, 20 mL of saturated $NaHCO_3$ and 20 mL of saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to obtain an off-white solid (0.54 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (ddd, *J* = 0.70, 1.39, 8.38 Hz, 1H), 7.98 (ddd, *J* = 0.66, 1.34, 8.48 Hz, 1H), 7.92 (ddd, *J* = 1.39, 6.77, 8.43 Hz, 1H), 7.66 (ddd, *J* = 1.35, 6.80, 8.26 Hz, 1H), 2.86 (s, 3H); UPLC I (ESI) R_t 1.59 min, m/z 179.5 $[M+H]^+$ (purity: 97%); (0.54 g, 76%).

4-methoxy-2-methylquinazoline (S3)

4-chloro-2-methylquinazoline (8 g, 44.8 mmol) was added to sodium methoxide in methanol (25.2 ml, 134 mmol) and the mixture was stirred for 16h. The mixture was evaporated, citric acid was added until pH 7. This mixture was extracted with ethyl acetate to give colorless crystals.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (ddd, *J* = 0.68, 1.53, 8.16 Hz, 1H), 7.81 (dt, *J* = 0.92, 8.43 Hz, 1H), 7.72 (ddd, *J* = 1.50, 6.93, 8.41 Hz, 1H), 7.42 (ddd, *J* = 1.23, 6.96, 8.13 Hz, 1H), 4.10 (s, 3H), 2.70 (s, 3H); UPLC I (ESI) R_t 1.10 min, m/z 175.5 $[M+H]^+$ (purity: 96%); (0.48 g, 90%)

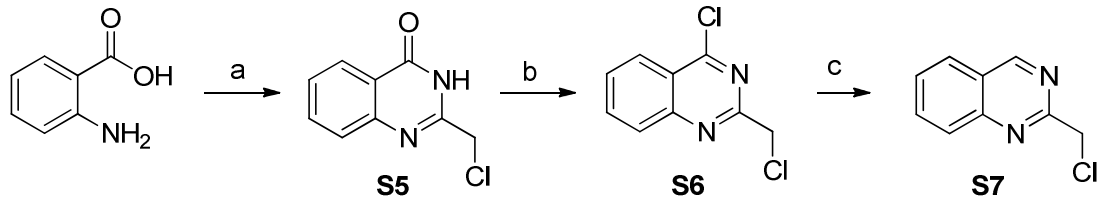
2-(bromomethyl)-4-methoxyquinazoline (S4)

To a solution of 4-methoxy-2-methylquinazoline (0.30 g, 1.72 mmol) in CCl_4 (20 ml) was added NBS (0.30 g, 1.72 mmol) and benzoperoxide (0.042 g, 0.17 mmol) and the resulting mixture heated at

reflux for 24h in air. The mixture cooled and evaporated, the residue purified by flash chromatography eluent: 10% to 30% Ethyl acetate in heptane.

^{1}H NMR (400 MHz, CDCl3) δ 8.15 (ddd, *J* = 0.67, 1.50, 8.19 Hz, 1H), 7.95 (ddd, *J* = 0.72, 1.22, 8.39 Hz, 1H), 7.87 - 7.82 (m, 1H), 7.57 (tt, *J* = 1.22, 6.99 Hz, 1H), 4.63 (s, 2H), 4.21 (s, 3H); UPLC I (ESI) R_t 1.85 min, m/z 253.4, 255.4 $[M+H]^+$ (purity: 96%); (0.6 g, 35%)

Scheme S2



a) chloroacetonitrile, 0.2 eq NaOMe, 78%; b) triethylamine 1.5eq, $POCl_3$ 1.55 eq, toluene, 3h, 75%; c) 4.5 eq triethylamine, 0.2 eq Pd/C 10% Pd, H_2, Ethyl acetate, 20%

2-(chloromethyl)quinazolin-4(3*H*)-one (S5)

Was prepared as previously described.[2]

^{1}H-NMR (DMSO-*d6*): δ 12.59 (br s, 1H), 8.12 (dd, *J* = 1.2, 7.2 Hz, 1H), 7.84 (dd, *J* = 1.2, 7.2 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 4.55 (s, 2H); UPLC I (ESI) R_t 1.23 min, m/z 195.4 $[M+H]^+$ (purity: 96%); (5.4 g, 79%)

4-chloro-2-(chloromethyl)quinazoline (S6)

A mixture of 2-(chloromethyl)quinazolin-4(3H)-one (0.92 g, 4.73 mmol) in 19 mL of anhydrous toluene and DIPEA (1.280 ml, 7.33 mmol) in a 50 mL round bottomed flask equipped with a condenser and a drying tube was refluxed for 1 h. To this warm solution was added phosphorus oxychloride (0.707 ml, 7.56 mmol) and the mixture was heated at 80 °C for 2 h, and cooled to room temperature. The mixture was diluted with 50 mL of ethyl acetate, washed with 20 mL of ice cold water, 20 mL of saturated $NaHCO_3$, 20 mL of water, citric acid (1 N, 4 × 20 mL), 20 mL of water, 20 mL of saturated $NaHCO_3$ and 20 mL of saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to obtain an off-white solid.

^{1}H NMR (DMSO-*d6*) δ 8.33 (ddd, 1H), 8.05- 8.22 (m, 2H), 7.93 (ddd, 1H), 4.97 (s, 2H); UPLC I (ESI) R_t 1.80 min, m/z 213.3 $[M+H]^+$ (purity: 96%); (0.54 g, 54%)

2-(chloromethyl)quinazoline (S7)

4-chloro-2-(chloromethyl)quinazoline (0.7 g, 3.29 mmol) in ethyl acetate (12 ml) and triethylamine (2.061 ml, 14.78 mmol) are dissolved. Pd/C (0.105 g, 0.986 mmol) is added and it is stirred 7 h under hydrogen atmosphere at normal pressure. Catalyst is removed from the solution by means of filtration through celite, whereby it is re-washed with 50 mL of ethyl acetate and concentrated by evaporation. After chromatography on silica gel with heptane - ethyl acetate (0-25% ethyl acetate) the product is obtained.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (d, *J* = 0.91 Hz, 1H), 8.10 (dt, *J* = 0.84, 9.10 Hz, 1H), 8.03 - 7.96 (m, 2H), 7.73 (ddd, *J* = 1.12, 6.96, 8.11 Hz, 1H), 4.95 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.99, 161.45, 150.34, 134.83, 128.50, 128.48, 127.35, 123.73, 47.68; UPLC I (ESI) R_t 1.41 min, m/z 179.4 $[M+H]^+$ (purity: 96%); (0.120 g, 25%)

2-(chloromethyl)-4-methylquinazoline[3] (S8)

1-(2-aminophenyl)ethanone (6.49 ml, 37.0 mmol) is added to 20 mL 1,4-dioxane. The solution, cooled to 10°C, and HCl is blanketed in for 20 min followed by cooled to -10° C. Subsequently, the suspension formed is left to stand at -10°C overnight. A solution of alpha-chloroacetonitrile (2.58 ml, 40.7 mmol) in 25 mL of 1,4-dioxane (35 mmol/mL) is slowly added at -10° C. After 1h, the mixture is neutralized with a sodium hydroxide solution, while keeping the temperature below 12°C, the resulting suspension is stirred and filtrated to give the product as white crystals.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (ddd, *J* = 0.67, 1.39, 8.37 Hz, 1H), 8.03 (ddd, *J* = 0.67, 1.24, 8.43 Hz, 1H), 7.90 (ddd, *J* = 1.39, 6.92, 8.42 Hz, 1H), 7.66 (ddd, *J* = 1.23, 6.90, 8.20 Hz, 1H), 4.86 (s, 2H), 2.98 (s, 3H); UPLC I (ESI) R_t 1.47 min, m/z 193.5 $[M+H]^+$ (purity: 96%); (3.2 g, 45%)

(*S*)-6-(hydroxymethyl)piperazin-2-one (S9)

To a solution of (*S*)-methyl 6-oxopiperazine-2-carboxylate (made analogously to Watkins *et al* [4], but with *S* stereochemistry) (0..4 g, 2.53 mmol) in anhydrous ethanol (50 mL) was added sodium borohydride (0.115 g, 3.0 mmol) slowly. The solution was stirred for 3.5 h at room temperature.

The mixture was then treated with glacial acetic acid (2.8 equiv) and the precipitate removed by filtering through a plug of celite. The filtrate was then concentrated in vacuo and the resulting oil solidified upon standing under vacuum. The crude product was dissolved in CH_2Cl_2 (50 mL), treated with $KHCO_3$ (1.5 equiv), aged for 1 h, filtered through a plug of Celite and the resulting filtrate was concentrated in vacuo to give the title compound 6, which was purified using column chromatography with a DCM-methanol:NH_3 gradient.

^{1}H NMR (400 MHz, MeOD) δ 3.65 - 3.59 (m, 1H), 3.56 (d, *J* = 5.11 Hz, 1H), 3.55 - 3.48 (m, 1H), 3.38 (d, *J* = 3.39 Hz, 2H), 3.11 (ddd, *J* = 0.67, 4.55, 13.40 Hz, 1H), 2.90 - 2.82 (m, 1H); MS (ESI) m/z 131.3 $[M+H]^{+}$ (0.256 g, 78%)

II. Enzymatic assay data: enzyme purification and IC_{50}-determinations

Enzyme purification

-Recombinant murine FAP was purified from the cultured supernatant of HEK293 human embryonic kidney cell line as described elsewhere (Cheng, J. et al. *Cancer Res.* **2002**, *62*, 4767-4722).

-Recombinant human PREP was expressed in and purified from E coli as previously described (Szeltner, Z.; Renner, V.; Polgar, L. *Prot. Sci.* **2000**, *9*, 353-360).

-DPP IV and DPPII were purified from human seminal plasma as described previously. (De Meester, I.; Vanhoof, G.; Lambeir, A.; Scharpe, S. *J. Immun. Methods* **1996**, *189*, 99-105 and Maes, M. B.; Lambeir, A. M.; Gilany, K.; Senten, K; Van der Veken, P; Leiting, B.; Augustyns, K.; Scharpe, S; De Meester, I. Kinetic investigation of human dipeptidyl peptidase II (DPPII)-mediated hydrolysis of dipeptide derivatives and its identification as quiescent cell proline dipeptidase (QPP)/dipeptidyl peptidase 7 (DPP 7). *Biochem. J.* **2005**, *386*, 315-324)

-Recombinant human DPP8 was expressed and purified as described. (Chen, Y. S.; Chien, C. H.; Goparaju, C. M.; Hsu, J. T.; Liang, P. H.; Chen, X. Purification and characterization of human prolyl dipeptidase DPP8 in SF9 insect cells. *Prot. Exp. Purif.* **2004**, *35*, 142-146)

-DPP9 was purified from bovine testes as described by Dubois et al. (Dubois, V.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpe, S.; De Meester, I. Purification and characterization of dipeptidyl peptidase IV-like enzymes from bovine testes. *Front. Biosci.* **2008**, *13*, 3558–3568)

IC_{50}-determination for FAP and PREP

Enzyme activities were determined kinetically in a final volume of 200 µl for 10 minutes at 37°C by measuring the initial velocities of pNA release (405 nm) from the substrate using a Spectramax plus microtiterplate reader (Molecular devices). One unit of enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 µmol pNA from the substrate per minute under assay conditions. All measurements were carried out in duplicate. The IC_{50} value was defined as the inhibitor concentration which caused a 50% decrease of the activity under assay conditions.

The chromogenic substrate Ala-Pro-*p*-nitroanilide (2 mmol/l) was used at pH 7.4 for FAP activity measurement. The substrate concentrations were chosen around the Km value obtained under the

assay conditions used. Buffer compositions for the DPP assays were previously reported in the purification articles– vide supra. The FAP assay buffer consisted of 50 mM Tris pH7.4 containing 100 mmol/l NaCl and 0.1 mg/ml bovine serum albumin. The PREP activity was measured as described by Brandt et al. using the chromogenic substrate Z-Gly-Pro-*p*-nitroanilide (0.25 mmol/l) at pH 7.5 in the presence of 10 mmol/l DTT. (Brandt, I.; Gérard, M.; Sergeant, K.; Devreese, B.; Baekelandt, V.; Augustyns, K.; Scharpé, S.; Engelborghs, Y.; Lambeir, A.M. *Peptides* **2005**, 26, 2536-2546)

IC_{50}-determination for DPPII, DPPIV, and DPP 9

Initial rates were determined kinetically in a final volume of 200 µl for 10 minutes at 37°C by measuring the initial velocities of pNA release (405 nm) from the substrate using a Spectrafluor Plus reader (Tecan Benelux). The chromogenic substrate Gly-Pro-*p*-nitroanilide (100 µmol/l)was used at pH 8.3 for DPP IV, Lys-Ala-*p*-nitroanilide (1 mmol/l) at pH 5.5 for DPP II and Ala-Pro-*p*-nitroanilide (300 µmol/l) at pH 7.4 for DPP9-activity measurement. The substrate concentrations were chosen around the Km value obtained under the assay conditions used. Buffer compositions were reported before. (Dubois, V.; Lambeir, A. M.; Van der Veken, P.; Augustyns, K.; Creemers, J.; Chen, X.; Scharpe, S.; De Meester, I. Purification and characterization of dipeptidyl peptidase IV-like enzymes from bovine testes. *Front. Biosci.* **2008**, *13*, 3558–3568)

Test compounds were dissolved and diluted in DMSO (final concentration of DMSO during assay was 5% v/v for DPP9, PREP and DPP II and <1% for DPP IV and FAP). Inhibitors were pre-incubated with the enzyme for 15 min at 37 °C before starting by the addition of substrate. The concentrations of enzyme and inhibitor during the preincubation were the double of the final concentrations during the initial rate measurement. All measurements were carried out in duplicate. The initial evaluation of compounds was carried out at 100 µmol/l, or in case of solubility limits, the highest concentration possible. If vi/vo (initial velocity in presence of inhibitor/velocity in presence of DMSO) was < 0.5, an IC_{50} value was determined experimentally using at least 8 different concentrations of inhibitor. For those compounds with IC_{50} values below 5 µmol/l for one of the enzymes, the analysis was repeated using a new stock of compound. Generally, independent measurements of IC_{50} differed less than 20% from each other. The IC_{50}-value was defined as the inhibitor concentration, which caused a 50% decrease of the activity under assay conditions. The total inhibitor concentration is represented by I0. IC_{50}-values were calculated with the GraFit software (GraFit Version 5, Leatherbarrow, R.J., Erithacus Software Ltd., Horley, U.K.) using eq (1).

$$\frac{v_i}{v_o} = \frac{1}{1+\left(\frac{Io}{IC_{50}}\right)^s} + background$$

eq. (1)

where s is the slope factor and background represents the estimated minimal vi/vo value. The errors given in the tables represent standard errors of the fit unless otherwise specified.

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