Supporting Information

8-(3-(*R*)-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356): A Highly Potent, Selective, Long-Acting, and Orally Bioavailable DPP-4 Inhibitor for the Treatment of Type 2 Diabetes

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General Chemistry Procedures.

All reactions were carried out under an atmosphere of nitrogen or argon unless otherwise indicated. All starting materials and reagents were either commercially available or their synthesis had been described in the literature before. All commercial chemicals and solvents were reagent grade and were used without further purification. Reaction progresses were monitored by TLC using Merck silica gel 60 F_{254} plates and UV light or staining with 5% phosphomolybdic acid in ethanol. Evaporations of solvents were carried out under reduced pressure using regular rotary evaporators. All chromatographic purifications were conducted as MPLC using DAVISIL LC60A silica gel (35 – 70 µm) unless otherwise noted. Yields were of purified products and were not optimized. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker DPX 400 spectrometer using DMSO-d_6 as solvent and $Si(CH_3)_4$ or DMSO-d_5 as an internal standard. Low resolution mass spectra (MS) were run on a Micromass Platform mass spectrometer. High resolution masses (HRMS) were determined on a Micromass Q-Tof-2 mass spectrometer. HPLC retention times were recorded on an Agilent 1100 Series apparatus using either a YMC-Pack Pro C-18, S-3 µm, 12 nm, 4.6 mm x 50 mm column eluted with a 3 min gradient from 10 to 99% B (method 1) or a Chromolith Speed ROD 4.6 mm x 50 mm column eluted with a 4.5 min gradient from 10 to 90% B (method 2), where A = 100% H_2O/0.1% HCOOH and B = 100% H_3CCN/0.1% HCOOH.

Typical Procedure for the Synthesis of Compounds 4 and 8.

7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (4a). Benzyl chloride (1.6 mL, 14.0 mmol) was added to a solution of 8-chloro-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (3.0 g, 14.0 mmol) and ethyldiisopropylamine (2.4 mL, 14.0 mmol) in DMF (20 mL). The resulting solution was heated to 80 °C and stirred at this temperature for 4 h. After cooling to ambient temperature, ice-cold water (200 mL) was added. The precipitate was separated by filtration, washed with water and little diethylether and dried to give the product as a white solid (3.2 g, 75%). ^{1}H NMR data compare favorably with the data reported.[1]

8-Chloro-1,3-dimethyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (4b). ^{1}H NMR (400 MHz, DMSO) δ 1.70 (s, 3H), 1.80 (s, 3H), 3.23 (s, 3H), 3.38 (s, 3H), 4.92 (d, *J* = 7.1 Hz, 2H), 5.26 (tm, *J* = 7.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 17.96, 25.25, 27.60, 29.45, 43.79, 107.00, 117.94, 137.13, 137.34, 146.69, 150.61, 153.66.

7-But-2-ynyl-8-chloro-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (4c). ^{1}H NMR (400 MHz, DMSO) δ 1.80 (t, *J* = 2.4 Hz, 3H), 3.22 (s, 3H), 3.38 (s, 3H), 5.12 (incompletely resolved q, *J* = 2.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 2.92, 27.54, 29.46, 35.38, 72.12, 81.85, 106.60, 137.65, 146.57, 150.53, 153.57. MS *m/z* 267/269 $(M+H)^+$.

8-Bromo-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (8a). ^{1}H NMR (400 MHz, DMSO) δ 1.70 (s, 3H), 1.81 (s, 3H), 3.32 (s, 3H), 4.86 (d, *J* = 6.8 Hz, 2H), 5.24 (dm, *J* = 6.8 Hz, 1H),11.24 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 18.09, 25.25, 28.44, 44.82, 108.40, 118.20, 127.00, 137.02, 149.26, 150.49, 153.81.

8-Bromo-7-but-2-ynyl-3-methyl-3,7-dihydro-purine-2,6-dione (8b). ^{1}H NMR (400 MHz, DMSO) δ 1.80 (s, 3H), 3.31 (s, 3H), 5.06 (s, 2H), 11.31 (s, 1H). ^{13}C NMR (100 MHz, DMSO) δ 2.96, 28.52, 36.49, 72.33, 81.82, 108.10, 127.71, 149.18, 150.48, 153.84. MS *m/z* 297/299 $(M+H)^+$.

Typical Procedure for the Synthesis of Compounds 5.

7-Benzyl-1,3-dimethyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione (5a). A flask charged with a stir bar, **4a** (2.0 g, 6.6 mmol), piperazine (2.9 g, 33.1 mmol) and THF (80 mL) is stirred at 65 °C for 24 h. After cooling to room temperature, the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution was washed thrice with water and dried (Na_2SO_4). After removal of the solvent, the remainder was purified by chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH 14:1:0.1) to give the product as a foam-like solid (1.5 g, 64%). ^{1}H NMR (400 MHz, DMSO) δ 2.68-2.74 (m, 4H), 3.03-3.09 (m, 4H), 3.18 (s, 3H), 3.30 (broad s, NH and H_2O), 3.39 (s, 3H), 5.36 (s, 2H), 7.15-7.19 (m, 2H), 7.23-7.28 (m, 1H), 7.30-7.35 (m, *2*H). ^{13}C NMR (100 MHz, DMSO) δ 27.33, 29.38, 44.96, 47.94, 50.74, 103.89, 126.54, 127.37, 128.52, 137.07, 147.30, 150.89, 153.74, 156.39. HPLC t_R = 1.89 min (method 1), t_R = 1.86 min (method 2). MS *m/z* 355 $(M+H)^+$. HRMS (ES^+) calcd for $C_{18}H_{23}N_6O_2$ $(M+H)^+$ *m/e* 355.1882, found *m/e* 355.1891.

1,3-Dimethyl-7-(3-methyl-but-2-enyl)-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione (5b). ^{1}H NMR (400 MHz, DMSO) δ 1.68 (s, 3H), 1.72 (s, 3H), 2.80-2.85 (m, 4H), 3.07-3.12 (m, 4H), 3.20 (s, 3H), 3.29 (broad s, NH and H_2O), 3.37 (s, 3H), 4.66 (d, *J* = 6.7 Hz, 2H), 5.33 (tm, *J* = 6.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 17.89, 25.25, 27.37, 29.34, 43.24, 45.11, 50.92, 103.76, 119.90, 135.40, 147.13, 150.90, 153.69, 155.97. HPLC t_R = 1.89 min (method 1), t_R = 1.78 min (method 2). MS *m/z* 333 $(M+H)^+$. HRMS (ES^+) calcd for $C_{16}H_{25}N_6O_2$ $(M+H)^+$ *m/e* 333.2039, found *m/e* 333.2031.

7-But-2-ynyl-1,3-dimethyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione (5c). ^{1}H NMR (400 MHz, DMSO) δ 1.79 (t, *J* = 2.2 Hz, 3H), 2.81-2.86 (m, 4H), 3.20 (s, 3H), 3.24-3.28 (m, 4H) superimposed on very broad s (water and NH signal), 3.36 (s, 3H), 4.88 (incompletely resolved q, *J* = 2.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 3.03, 27.31, 29.24, 35.25, 45.06, 50.37, 73.77, 81.03, 103.27, 147.17, 150.89, 153.45, 155.74. HPLC t_R = 1.25 min (method 1), t_R = 1.24 min (method 2). MS *m/z* 317 $(M+H)^+$. HRMS (ES^+) calcd for $C_{15}H_{21}N_6O_2$ $(M+H)^+$ *m/e* 317.1726, found *m/e* 317.1721.

8-(3-Amino-piperidin-1-yl)-1,3-dimethyl-7-benzyl-3,7-dihydro-purine-2,6-dione (6). A flask charged with a stir bar, **4a** (0.30 g, 0.98 mmol), 3-amino-piperidine (0.25 g, 1.44 mmol), K_2CO_3 (0.4 g, 2.9 mmol) and MeCN (5 mL) was stirred at 70 °C overnight. Then, the mixture was concentrated, MeOH was added to the remainder and the non-dissolving residue was separated by filtration. The filtrate was concentrated and the residue was purified by HPLC (YMC C-18, MeCN/H_2O) to deliver the title compound (0.21 g, 58%). ^{1}H NMR (400 MHz, DMSO) δ 1.08-1.20 (m, 1H), 1.42-1.54 (m, 1H), 1.60-1.69 (m, 1H), 1.74-1.84 (m, 1H), 2.56-2.63 (m, 1H), 2.66-2.76 (m, 1H), 2.76-2.85 (m, 1H), 3.18 (s, 3H) superimposed on very broad s (NH_2, H_2O), 3.24-3.31 (m, 1H), 3.39 (s, 3H) superimposed on ca. 3.38-3.45 (m, 1H), 5.36 (s, 2H), 7.14-7.21 (m, 2H), 7.23-7.29 (m, 1H), 7.29-7.35 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 23.24, 27.37, 29.41, 32.98, 47.23, 48.08, 50.01, 58.05, 103.81, 126.58, 127.42, 128.56, 137.15, 147.40, 150.94, 153.73, 156.50. HPLC t_R = 2.00 min (method 1), t_R = 1.98 min (method 2). MS *m/z* 369 $(M+H)^+$.

Typical Procedure for the Synthesis of Compounds 9.

8-Bromo-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione (9af). 2-Bromo-1-phenyl-ethanone (0.11 g, 0.53 mmol) was added to a suspension of **8a** (0.15 g, 0.48 mmol) and K_2CO_3 (0.11 g, 0.77 mmol) in DMF (2 mL). The mixture was stirred at ambient temperature for 6 h. Then, water was added and the forming precipitate was separated by filtration and washed with water. After drying at 55 °C for 5h, the title compound was yielded (0.16 g, 77%). ^{1}H NMR (400 MHz, DMSO) δ 1.70 (s, 3H), 1.79 (s, 3H), 3.42 (s, 3H), 4.91 (d, *J* = 6.8 Hz, 2H), 5.24 (tm, *J* = 6.8 Hz, 1H), 5.40 (s, 2H), 7.57-7.62 (m, 2H), 7.70-7.75 (m, 1H), 8.06-8.10 (m, 2H). MS *m/z* 431/433 $(M+H)^+$.

8-Bromo-1,3-dimethyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (9ac). ^{1}H NMR (400 MHz, DMSO) δ 1.69 (s, 3H), 1.81 (s, 3H), 3.22 (s, 3H), 3.38 (s, 3H), 4.91 (d, *J* = 6.8 Hz, 2H), 5.23 (tm,

J = 6.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 18.12, 25.25, 27.60, 29.43, 44.89, 107.97, 118.25, 127.20, 136.94, 147.73, 150.62, 153.53. MS *m/z* 327/329 $(M+H)^+$.

1-Benzyl-8-bromo-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (9ad). ^{1}H NMR (400 MHz, DMSO) δ 1.69 (s, 3H), 1.80 (s, 3H), 3.40 (s, 3H), 4.91 (d, *J* = 6.6 Hz, 2H), 5.05 (s, 2H), 5.24 (tm, *J* = 6.8 Hz, 1H), 7.21-7.32 (m, 5H). ^{13}C NMR (100 MHz, DMSO) δ 18.14, 25.25, 29.54, 43.68, 44.96, 108.00, 118.20, 127.00, 127.41, 127.68, 128.22, 137.03, 137.33, 148.04, 150.52, 153.39. MS *m/z* 403/405 $(M+H)^+$.

8-Chloro-3-methyl-7-(3-methyl-but-2-enyl)-1-phenethyl-3,7-dihydro-purine-2,6-dione (9ae). The compound was synthesized from 8-chloro-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione following the procedure described above. ^{1}H NMR (400 MHz, DMSO) δ 1.70 (s, 3H), 1.79 (s, 3H), 2.80-2.86 (m, 2H), 3.39 (s, 3H), 4.03-4.10 (m, 2H), 4.90 (d, *J* = 6.8 Hz, 2H), 5.23 (tm, *J* = 6.9 Hz, 1H), 7.19-7.25 (m, 3H), 7.27-7.33 (m, 2H). MS *m/z* 373/375 $(M+H)^+$.

8-Chloro-1-isoquinolin-1-ylmethyl-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (9ag). The compound was synthesized from 8-chloro-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione following the procedure described above. ^{1}H NMR (400 MHz, DMSO) δ 1.69 (s, 3H), 1.77 (s, 3H), 3.42 (s, 3H), 4.91 (d, *J* = 6.6 Hz, 2H), 5.27 (tm, *J* = 6.8 Hz, 1H), 5.73 (s, 2H), 7.69 (d, *J* = 5.7 Hz, 1H), 7.75 (tm, *J* = 7.6 Hz, 1H), 7.82 (tm, *J* = 7.4 Hz, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 5.7 Hz, 1H), 8.41 (d, *J* = 8.2 Hz, 1H). MS *m/z* 410/412 $(M+H)^+$.

8-Bromo-7-but-2-ynyl-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione (9bf). ^{1}H NMR (400 MHz, DMSO) δ 1.80 (t, *J* = 2.1 Hz, 3H), 3.43 (s, 3H), 5.10 (incompletely resolved q, *J* = 2.3 Hz, 2H), 5.41 (s, 2H), 7.60 (t, *J* = 7.7 Hz, 2H), 7.73 (tm, J = 7.4 Hz, 1H), 8.09 (dm, *J* = 7.2, 2H). ^{13}C NMR (100 MHz, DMSO) δ 2.96, 29.58, 36.66, 47.18, 72.23, 81.93, 107.51, 127.95, 128.61, 128.94, 134.03, 134.38, 148.02, 150.23, 152.93, 192.80. MS *m/z* 415/417 $(M+H)^+$.

8-Bromo-7-but-2-ynyl-1-(4-methoxy-naphthalen-1-ylmethyl)-3-methyl-3,7-dihydro-purine-2,6-dione (9bh). ^{1}H NMR (400 MHz, DMSO) δ 1.80 (t, *J* = 2.3 Hz, 3H), 3.39 (s, 3H), 5.11 (incompletely resolved q, *J* = 2.3 Hz, 2H), 5.45 (s, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.52-7.57 (m, 1H), 7.59-7.65 (m, 1H), 8.18-8.23 (m, 2H). MS *m/z* 467/469 $(M+H)^+$.

2-{2-[2-(8-Bromo-7-but-2-ynyl-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-yl)-acetyl]-phenoxy}-N-methyl-acetamide (9bi). ^{1}H NMR (400 MHz, DMSO) δ 1.80 (broad s, 3H), 2.68 (d, *J* = 4.6 Hz, 3H), 3.43 (s, 3H), 4.74 (s, 2H), 5.10 (m_c, 2H), 5.35 (s, 2H), 7.08-7.15 (m, 2H), 7.62 (td, J = 7.9,

1.6 Hz, 1H), 7.70 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.12 (incompletely resolved q, *J* = 4.1 Hz, 1H). MS *m/z* 502/504 $(M+H)^+$.

8-Bromo-7-but-2-ynyl-3-methyl-1-(3-methyl-isoquinolin-1-ylmethyl)-3,7-dihydro-purine-2,6-dione (9bj). ^{1}H NMR (400 MHz, DMSO) δ 1.79 (s, 3H), 2.39 (s, 3H), 3.44 (s, 3H), 5.10 (m_c, 2H), 5.70 (s, 2H), 7.50 (s, 1H), 7.64 (m_c, 1H), 7.75 (m_c, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 8.33 (d, *J* = 8.3 Hz, 1H). MS *m/z* 452/454 $(M+H)^+$.

8-Bromo-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (9bk). ^{1}H NMR (400 MHz, DMSO) δ 1.79 (t, *J* = 2.3 Hz, 3H), 2.39 (s, 3H), 3.44 (s, 3H), 5.11 (incompletely resolved q, *J* = 2.2 Hz, 2H), 5.35 (s, 2H), 7.67 (m_c, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.75 (m_c, 1H), 8.24 (d, *J* = 8.1 Hz, 1H). MS *m/z* 453/455 $(M+H)^+$.

Typical Procedure for the Synthesis of Compounds 1 and 6.

(*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (1).

Step I. A flask charged with a stir bar, **9bk** (1.86 g, 4.10 mmol), (*R*)-3-*tert*butyloxycarbonylamino-piperidine (0.93 g, 4.64 mmol), K_2CO_3 (1.15 g, 8.32 mmol) and DMF (12 mL) was stirred at 75 °C for 6 h. Then, water was added and the formed precipitate was separated by filtration. The precipitate was dried to give the N-*tert*butyloxycarbonyl protected intermediate (2.07 g, 88%).

Step II. Trifluoroacetic acid (10 mL) was added to the N-*tert*butyloxycarbonyl protected product (2.00 g, 3.49 mmol) dissolved in CH_2Cl_2 (40 mL). The solution was stirred at room temperature for 1 h and then poured into ice-cold water (150 mL). The organic phase was separated and the aqueous phase was basified with K_2CO_3 and extracted twice with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (ethyl acetate/MeOH 1:0->3:1) to give the title compound **1** (1.50 g, 91%). ^{1}H NMR (400 MHz, DMSO) δ 1.19-1.31 (m, 1H), 1.57-1.69 (m, 1H), 1.77 (incompletely resolved t, *J* = 2.0 Hz, 3H) superimposed on 1.75-1.83 (m, 1H), 1.84-1.92 (m, 1H), 2.75-2.88 (m, 2H), 2.89 (s, 3H), 2.97-3.06 (m, 1H), 3.41 (very broad s, CH_3, NH_2 and water), 3.57-3.70 (m, 2H), 4.90 (incompletely resolved q, *J* = 1.8 Hz, 2H), 5.32 (s, 2H), 7.68 (dd, *J* = 8.4, 6.8 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.92 (dd, *J* = 8.2, 7.1 Hz, 1H), 8.25 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 3.03, 21.53, 23.25, 29.40, 33.08, 35.47, 45.53, 47.23, 49.55, 57.46, 73.72, 81.12, 103.19, 122.47, 125.71, 127.12, 127.85, 134.05, 147.74, 149.03, 150.93, 153.23, 156.14, 160.97, 168.81. HPLC t_R = 2.06 min

(method 1), t_R = 2.22 min (method 2). MS *m/z* 473 $(M+H)^+$. HRMS (ES^+) calcd for $C_{25}H_{29}N_8O_2$ $(M+H)^+$ *m/e* 473.2413, found *m/e* 473.2416.

8-(3-Amino-piperidin-1-yl)-1,3-dimethyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (6ac). ^1H NMR (400 MHz, DMSO) δ 1.10-1.21 (m, 1H), 1.49-1.78 (m, 2H) superimposed on 1.68 (s, 3H) and 1.73 (s, 3H), 1.80-1.89 (m, 1H), 2.55-2.62 (m, 1H), 2.74-2.86 (m, 2H), 3.20 (s, 3H), 3.31 (broad s, NH_2 and water) superimposed on ca. 3.31-3.37 (m, 1H), 3.37 (s, 3H), 3.39-3.45 (m, 1H), 4.66 (d, *J* = 6.4 Hz, 2H), 5.31 (tm, *J* = 6.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 17.83, 23.54, 25.23, 27.31, 29.28, 33.33, 43.31, 47.44, 50.19, 58.44, 103.56, 119.92, 135.35, 147.16, 150.86, 153.58, 156.01. HPLC t_R = 1.98 min (method 1), t_R = 1.97 min (method 2). MS *m/z* 347 $(M+H)^+$. HRMS (ES^+) calcd for $C_{17}H_{27}N_6O_2$ $(M+H)^+$ *m/e* 347.2195, found *m/e* 347.2193.

8-(3-Amino-piperidin-1-yl)-1-benzyl-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione (6ad). ^1H NMR (400 MHz, DMSO) δ 1.12-1.24 (m, 1H), 1.51-1.79 (m, 2H) superimposed on 1.67 (s, 3H) and 1.71 (s, 3H), 1.80-1.89 (m, 1H), 2.57-2.64 (m, 1H), 2.74-2.88 (m, 2H), 3.30 (very broad s, NH_2 and H_2O) superimposed on ca. 3.31-3.37 (m, 1H) and 3.37 (s, 3H), 3.41-3.47 (m, 1H), 4.67 (d, *J* = 6.4 Hz, 2H), 5.04 (s, 2H), 5.31 (tm, *J* = 6.5 Hz, 1H), 7.19-7.31 (m, 5H). ^{13}C NMR (100 MHz, DMSO) δ 17.83, 23.51, 25.25, 29.39, 33.24, 43.30, 43.46, 47.41, 50.12, 58.29, 103.59, 119.79, 126.83, 127.35, 128.13, 135.54, 137.87, 147.60, 150.78, 153.39, 156.39. HPLC t_R = 2.29 min (method 1), t_R = 2.58 min (method 2). MS *m/z* 423 $(M+H)^+$. HRMS (ES^+) calcd for $C_{23}H_{31}N_6O_2$ $(M+H)^+$ *m/e* 423.2508, found *m/e* 423.2506.

8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-phenethyl-3,7-dihydro-purine-2,6-dione (6ae). ^1H NMR (400 MHz, DMSO) δ 1.11-1.22 (m, 1H), 1.50-1.80 (m, 2H) superimposed on 1.67 (s, 3H) and 1.72 (s, 3H), 1.81-1.90 (m, 1H), 2.58-2.65 (m, 1H), 2.76-2.87 (m, 4H) superimposed on very broad s (NH_2 and water), 3.29-3.38 (m, 1H) superimposed on 3.38 (s, 3H), 3.40-3.47 (m, 1H), 4.00-4.08 (m, 2H), 4.66 (d, *J* = 6.4 Hz, 2H), 5.29 (tm, *J* = 6.4 Hz, 1H), 7.18-7.24 (m, 3H), 7.27-7.33 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 17.84, 23.45, 25.23, 29.28, 32.96, 33.56, 41.54, 43.35, 47.36, 50.19, 58.01, 103.62, 119.86, 126.19, 128.33, 128.51, 135.39, 138.71, 147.30, 150.56, 153.25, 156.09. HPLC t_R = 2.35 min (method 1), t_R = 2.77 min (method 2). MS *m/z* 437 $(M+H)^+$. HRMS (ES^+) calcd for $C_{24}H_{33}N_6O_2$ $(M+H)^+$ *m/e* 437.2665, found *m/e* 437.2658.

8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione (6af). ^1H NMR (400 MHz, DMSO) δ 1.12-1.25 (m, 1H), 1.51-1.80 (m, 2H) superimposed on 1.67 (s, 3H) and 1.71 (s, 3H), 1.81-1.91 (m, 1H), 2.60-2.67 (m, 1H), 2.76-2.91 (m,

2H), 3.28 (broad s, NH_2 and water) superimposed on ca. 3.33-3.39 (m, 1H), 3.40 (s, 3H), 3.43-3.50 (m, 1H), 4.66 (d, *J* = 6.3 Hz, 2H), 5.30 (tm, *J* = 6.4 Hz, 1H), 5.36 (s, 2H), 7.59 (t, *J* = 7.7 Hz, 2H), 7.72 (t, *J* = 7.4 Hz, 1H), 8.07 (tm, *J* = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 17.85, 23.51, 25.27, 29.40, 33.22, 43.48, 46.98, 47.42, 50.16, 58.24, 103.50, 119.69, 127.88, 128.92, 133.90, 134.57, 135.64, 147.74, 150.62, 153.05, 156.41, 193.22. HPLC t_R = 2.31 min (method 1), t_R = 2.65 min (method 2). MS *m/z* 451 $(M+H)^+$. HRMS (ES^+) calcd for $C_{24}H_{31}N_6O_3$ $(M+H)^+$ *m/e* 451.2458, found *m/e* 451.2460.

(*R*)-8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione ((*R*)-6af). ^{1}H NMR, ^{13}C NMR and HPLC data compare favorably with the data of compound **6af**.

(*S*)-8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione ((*S*)-6af). ^{1}H NMR, ^{13}C NMR and HPLC data compare favorably with the data of compound **6af**.

(*R*)-8-(3-Amino-piperidin-1-yl)-1-isoquinolin-1-ylmethyl-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione ((*R*)-6ag). ^{1}H NMR (400 MHz, DMSO) δ 1.13-1.25 (m, 1H), 1.55-1.80 (m, 2H) superimposed on 1.66 (s, 3H) and 1.69 (s, 3H), 1.82-1.92 (m, 1H), 2.60-2.68 (m, 1H), 2.77-2.92 (m, 2H), 3.29 (broad s, NH_2 and water) superimposed on 3.35-3.41 (m, 1H), 3.41 (s, 3H), 3.44-3.50 (m, 1H), 4.66 (d, *J* = 6.4 Hz, 2H), 5.31 (tm, *J* = 6.5 Hz, 1H), 5.71 (s, 2H), 7.69 (d, *J* = 5.7 Hz, 1H), 7.75 (tm, *J* = 7.7 Hz, 1H), 7.82 (tm, *J* = 7.3 Hz, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 5.7 Hz, 1H), 8.40 (d, *J* = 8.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 17.83, 23.52, 25.26, 29.39, 33.21, 42.76, 43.37, 47.43, 50.24, 58.31, 103.75, 119.61, 119.77, 124.03, 125.28, 127.26, 127.58, 130.34, 135.36, 135.57, 141.27, 147.56, 150.97, 153.60, 154.66, 156.27. HPLC t_R = 2.15 min (method 1), t_R = 2.34 min (method 2). MS *m/z* 474 $(M+H)^+$. HRMS (ES^+) calcd for $C_{26}H_{32}N_7O_2$ $(M+H)^+$ *m/e* 474.2617, found *m/e* 474.2604.

(*S*)-8-(3-Amino-piperidin-1-yl)-1-isoquinolin-1-ylmethyl-3-methyl-7-(3-methyl-but-2-enyl)-3,7-dihydro-purine-2,6-dione ((*S*)-6ag). ^{1}H NMR, ^{13}C NMR and HPLC data compare favorably with the data of compound (*R*)-**6ag**.

8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (6bc). ^{1}H NMR (400 MHz, DMSO) δ 1.16-1.28 (m, 1H), 1.55-1.67 (m, 1H), 1.72-1.90 (m, 2H) superimposed on 1.79 (s, 3H), 1.82-1.92 (m, 1H), 2.69-2.76 (m, 1H), 2.78-2.86 (m, 1H), 2.91-3.00 (m, 1H), 3.20 (s, 3H), 3.30 (broad s, NH_2 and water), 3.36 (s, 3H), 3.51-3.63 (m, 2H), 4.88 (m_c, 2H). ^{13}C NMR (100 MHz, DMSO)

δ 3.02, 23.22, 27.30, 29.33, 30.08, 35.32, 47.20, 49.59, 57.55, 73.78, 80.95, 103.13, 147.31, 150.90, 153.41, 155.89. MS *m/z* 331 $(M+H)^+$.

(*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione ((*R*)-6bf). ^{1}H NMR (400 MHz, DMSO) δ 1.17-1.29 (m, 1H), 1.56-1.70 (m, 1H), 1.73-1.82 (m, 1H) superimposed on 1.79 (s, 3H), 1.82-1.91 (m, 1H), 2.72-2.87 (m, 2H), 2.96-3.05 (m, 1H), 3.29 (broad s, NH_2 and water), 3.40 (s, 3H), 3.56-3.70 (m, 2H), 4.88 (incompletely resolved q, *J* = 2.3 Hz, 2H), 5.36 (s, 2H), 7.59 (t, *J* = 7.7 Hz, 2H), 7.72 (tm, *J* = 7.4 Hz, 1H), 8.08 (dm, *J* = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 3.02, 23.23, 29.38, 33.16, 35.47, 46.87, 47.20, 49.50, 57.52, 73.65, 81.11, 103.00, 127.88, 128.91, 133.89, 134.57, 147.82, 150.59, 152.78, 156.18, 193.21. HPLC t_R = 2.16 min (method 1), t_R = 2.41 min (method 2). MS *m/z* 435 $(M+H)^+$. HRMS (ES^+) calcd for $C_{23}H_{27}N_6O_3$ $(M+H)^+$ *m/e* 435.2145, found *m/e* 435.2136.

(*S*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione ((*S*)-6bf). ^{1}H NMR and ^{13}C NMR data compare favorably with the data of compound (*R*)-**6bf**.

(*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-1-(4-methoxy-naphthalen-1-ylmethyl)-3-methyl-3,7-dihydro-purine-2,6-dione ((*R*)-6bh). ^{1}H NMR (400 MHz, DMSO) δ 1.18-1.30 (m, 1H), 1.56-1.69 (m, 1H), 1.73-1.91 (m, 2H) superimposed on 1.78 (t, *J* = 2.1 Hz, 3H), 2.72-2.87 (m, 2H), 2.96-3.04 (m, 1H), 3.31 (broad s, NH_2 and water), 3.40 (s, 3H), 3.56-3.68 (m, 2H), 3.92 (s, 3H), 4.88 (incompletely resolved q, *J* = 1.9 Hz, 2H), 5.43 (s, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 7.51-7.57 (m, 1H), 7.58-7.64 (m, 1H), 8.18-8.22 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 3.04, 23.25, 29.50, 33.13, 35.51, 40.86, 47.23, 49.55, 55.49, 57.54, 73.73, 81.07, 103.23, 103.70, 121.96, 123.11, 123.21, 124.46, 124.86, 125.06, 126.60, 131.31, 147.84, 150.91, 153.29, 153.89, 156.23. HPLC t_R = 2.40 min (method 1), t_R = 2.87 min (method 2). MS *m/z* 487 $(M+H)^+$. HRMS (ES^+) calcd for $C_{27}H_{31}N_6O_3$ $(M+H)^+$ *m/e* 487.2458, found *m/e* 487.2457.

(*R*)-2-(2-{2-[8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-yl]-acetyl}-phenoxy)-N-methyl-acetamide ((*R*)-6bi). ^{1}H NMR (400 MHz, DMSO) δ 1.17-1.29 (m, 1H), 1.56-1.70 (m, 1H), 1.73-1.83 (m, 1H) superimposed on 1.79 (s, 3H), 1.82-1.91 (m, 1H), 2.69 (d, *J* = 4.6 Hz, 3H), 2.71-2.88 (m, 2H), 2.95-3.05 (m, 1H), 3.31 (broad s, NH_2 and water), 3.40 (s, 3H), 3.56-3.69 (m, 2H), 4.72 (s, 2H), 4.89 (m, 2H), 5.30 (s, 2H), 7.08-7.14 (m, 2H), 7.60 (tm, *J* = 7.8 Hz, 1H), 7.66 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.13 (incompletely resolved q, *J* = 4.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 3.02, 23.23, 25.31, 29.38, 33.16, 35.48, 47.21, 49.52, 50.57, 57.54, 67.47, 73.70, 81.10,

103.05, 113.40, 121.31, 125.55, 129.70, 134.49, 147.80, 150.65, 152.90, 156.18, 157.32, 167.40, 194.89. HPLC t_R = 2.06 min (method 1), t_R = 2.21 min (method 2). MS *m/z* 522 $(M+H)^+$. HRMS (ES^+) calcd for $C_{26}H_{32}N_7O_5$ $(M+H)^+$ *m/e* 522.2465, found *m/e* 522.2474.

(*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(3-methyl-isoquinolin-1-ylmethyl)-3,7-dihydro-purine-2,6-dione ((*R*)-6bj). ^{1}H NMR (400 MHz, DMSO) δ 1.29-1.40 (m, 1H), 1.59-1.72 (m, 1H), 1.78 (t, *J* = 2.1 Hz, 3H) superimposed on 1.75-1.95 (m, 2H), 2.41 (s, 3H), 2.84-2.91 (m, 1H), 2.94-3.09 (m, 2H), 3.33 (broad s, NH_2 and water), 3.41 (s, 3H), 3.54-3.62 (m, 1H), 3.64-3.71 (m, 1H), 4.91 (m_c, 2H), 5.67 (s, 2H), 7.50 (s, 1H), 7.64 (tm, *J* = 7.6 Hz, 1H), 7.75 (tm, *J* = 7.4 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 8.33 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 3.02, 22.90, 24.10, 29.38, 31.66, 35.37, 42.62, 46.94, 49.71, 56.01, 73.71, 81.01, 103.29, 117.26, 123.52, 123.94, 126.48, 126.63, 130.16, 136.21, 147.52, 149.53, 150.95, 153.42, 154.07, 155.85. HPLC t_R = 1.98 min (method 1), t_R = 2.07 min (method 2). MS *m/z* 472 $(M+H)^+$. HRMS (ES^+) calcd for $C_{26}H_{30}N_7O_2$ $(M+H)^+$ *m/e* 472.2461, found *m/e* 472.2462.

High Resolution Mass Value (HRMS) and HPLC Purity of Target Compounds:

Compd.	$(M+H)^+$	HRMS		HPLC Purity
		Calculated	Found	
2				98.1%
5a	$C_{18}H_{23}N_6O_2$	355.1882	355.1891	100%
5b	$C_{16}H_{25}N_6O_2$	333.2039	333.2031	100%
5c	$C_{15}H_{21}N_6O_2$	317.1726	317.1721	98.4%
6				98.9%
6ac	$C_{17}H_{27}N_6O_2$	473.2413	473.2416	100%
6bc				98.3%
6ad	$C_{23}H_{31}N_6O_2$	423.2508	423.2506	98.4%
6ae	$C_{24}H_{33}N_6O_2$	437.2665	437.2658	97.7%
6af	$C_{24}H_{31}N_6O_3$	451.2458	451.2460	98.4%
(R)-6af				98.9%
(S)-6af	$C_{24}H_{31}N_6O_3$	451.2458	451.2458	98.2%
(R)-6ag	$C_{26}H_{32}N_7O_2$	474.2617	474.2604	98.5%
(S)-6ag	$C_{26}H_{32}N_7O_2$	474.2617	474.2616	97.8%
(R)-6bf	$C_{23}H_{27}N_6O_3$	435.2145	435.2136	100%
(S)-6bf	$C_{23}H_{27}N_6O_3$	435.2145	435.2155	98.8%
(R)-6bh	$C_{27}H_{31}N_6O_3$	487.2458	487.2457	98.0%
(R)-6bi	$C_{26}H_{32}N_7O_5$	522.2465	522.2474	98.5%
(R)-6bj	$C_{26}H_{30}N_7O_2$	472.2461	472.2462	100%
1	$C_{25}H_{29}N_8O_2$	473.2413	473.2416	99.1%

HPLC Trace of Key Compounds

(R)-8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione

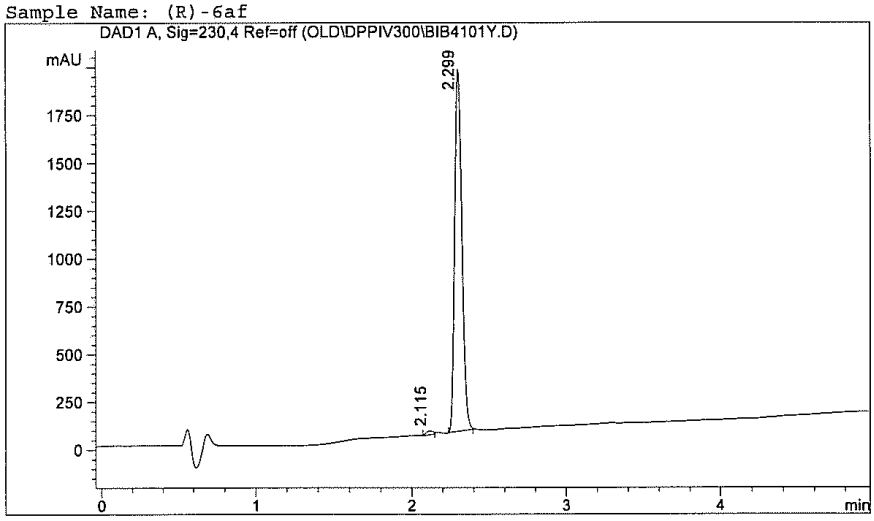
((R)-6af)

Column: YMC-Pack Pro C-18. S-3µm, 12 nm, 4.6 mm x 50 mm

Eluent: Water (A) / Acetonitrile (B) + 0.1% HCOOH

Gradient: 3min gradient from 10% B to 99% B

Sample Name: (R)-6af



Peak #	Ret. Time [min]	Area %
1	2.115	1.083
2	2.299	98.917

(S)-8-(3-Amino-piperidin-1-yl)-3-methyl-7-(3-methyl-but-2-enyl)-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione

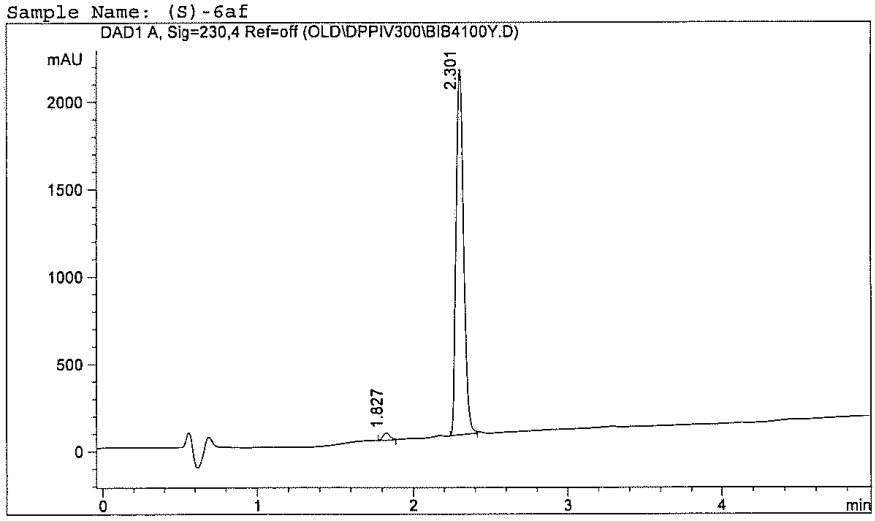
((S)-6af)

Column: YMC-Pack Pro C-18. S-3µm, 12 nm, 4.6 mm x 50 mm

Eluent: Water (A) / Acetonitrile (B) + 0.1% HCOOH

Gradient: 3min gradient from 10% B to 99% B

Sample Name: (S)-6af



Peak #	Ret. Time [min]	Area %
1	1.827	1.794
2	2.301	98.206

(R)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione

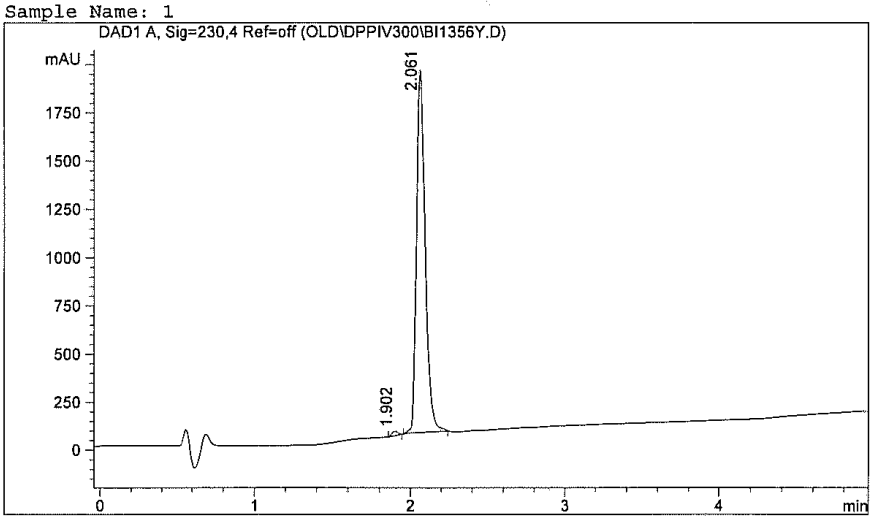
(1)

Column: YMC-Pack Pro C-18. S-3µm, 12 nm, 4.6 mm x 50 mm

Eluent: Water (A) / Acetonitrile (B) + 0.1% HCOOH

Gradient: 3min gradient from 10% B to 99% B

Sample Name: 1



Peak #	Ret. Time [min]	Area %
1	1.902	0.869
2	2.061	99.131

In Vitro DPP-4 Inhibition Assay.

For determination of the in vitro potency of inhibitors, an extract from the human colon carcinoma cell line Caco-2 was used as source of the DPP-4 enzyme. Caco-2 cells were grown to confluency in 175 cm^2 cell culture flasks in EMEM medium supplemented with non-essential amino acids (BioWhittaker) and containing 10% heat-inactivated fetal calf serum. Cells were washed with PBS and 4 mL lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 0.04 U/mL aprotinin, 0.5% Nonidet P40, pH 8.0) were added per flask. After 5 min incubation at room temperature with gentle agitation, cells were centrifuged at 35,000xg at 4°C for 30 min and the supernatant was stored at -80°C. Prior to use, the extract was diluted 1000-fold with assay buffer (100 mM Tris-HCl, 100 mM NaCl, pH 7.8). A 200 mM stock solution in dimethylformamide of the substrate for the DPP-4 enzyme, H-Ala-Pro-7-amido-4-trifluoromethylcoumarin (Ala-Pro-AFC; purchased from Bachem) was diluted 1000-fold with water before the assay. The assay itself was performed in black flat-bottom 96-well plates by mixing 20 µl of appropriate compound dilutions in assay buffer (compound stock solutions in dimethylsulfoxide (DMSO), final DMSO concentration in the assay 1%) with 50 µl of the diluted substrate (final concentration in the assay 100 µM) and 30 µl of the diluted Caco-2 cell extract. The plate was then incubated at room temperature for 1 h and fluorescence was measured at excitation/ emission wavelengths of 405/535 nm. Data analysis was performed by calculating the fluorescence in the presence of the test compound compared to the fluorescence of the vehicle control after subtracting the background fluorescence.

In Vitro Muscarinic Receptor M1 Binding Assay.

Membranes from CHO cells stably overexpressing human recombinant muscarinic receptor M1 were obtained from Euroscreen and used for measuring the binding of DPP-4 inhibitors to this receptor. Binding experiments were performed in triplicate in macro-wells (Molecular Devices) in a final volume of 500 µl. In the macro-wells, 80 µl of appropriate compound dilutions in assay buffer (50 mM Tris-HCl, pH 7.8) were mixed with 20 µl of M1 receptor-containing membranes (2.5 µg protein, appropriately diluted with assay buffer) and 400 µl of [N-methyl-^{3}H]-scopolamine (obtained from Amersham; 84 Ci/mol; final concentration 60 pM, appropriately diluted with assay buffer). Plates were incubated for 90 min at room temperature and then filtered on filter mats (Molecular Devices) pre-soaked in assay buffer. Filter mats were washed with cold assay buffer on a Skatron Combi Cell harvester, dried and bound [N-methyl-^{3}H]-scopolamine was determined by liquid scintillation counting.

Incubating membranes (without DPP-4 inhibitors) in the absence or presence of excess (80 nM) of non-radioactive N-methyl-scopolamine was used to determine maximum receptor binding and non-specific binding, respectively. The analysis of the data was performed by calculating the binding of the radioactive ligand in the presence of the test compound compared to the binding in the absence of the test compound after subtracting the non-specific binding.

Ex Vivo DPP-4 Inhibition Assay.

Rat, mouse, dog or monkey plasma was used for ex vivo measurement of DDP-4 activity. Blood was collected in EDTA tubes and subjected to centrifugation. The resultant supernatant was aliquoted and frozen. Plasma DPP-4 activity was assayed after dilution (140-fold for rat plasma; 70-fold for mouse, dog, and monkey plasma) with assay buffer (100 mM Tris-HCl, 100 mM NaCl, pH 7.8) prior to use. The assay itself was initiated by mixing 50 µl of diluted plasma with 50 µl of diluted substrate (Ala-Pro-AFC), and performed as described for the in vitro DPP-4 inhibition assay.

Effect of Compound 1 on Plasma DPP-4 Activity in Wistar Rats, Beagle Dogs and Rhesus Monkeys.

All experimental protocols concerning the use of laboratory animals were reviewed by a federal Ethics Committee and approved by governmental authorities. Male HanWistar rats (Crl:WI(Han); n = 5) were obtained from Charles River Laboratories (Germany) and fed ad libitum with a standard pelleted diet. Compound **1** was dissolved in 0.1 N HCl and subsequently diluted with a 0.5% aqueous hydroxyethylcellulose solution (final HCl concentration 3 mM). Administration was via oral gavage with an application volume of 10 mL/kg. Blood samples were drawn from the retrobulbal venous plexus under isoflurane anaesthesia. Blood was collected in EDTA tubes prior to administration of compound **1** at a dose of 1 mg/kg and subsequently at serial time points up to 24 h post-dose. Plasma was prepared following blood collection and frozen for ex vivo measurement of DPP-4 activity as described above. Data are expressed as % inhibition of plasma DPP-4 activity versus the pre-dose baseline value. Experiments for determining the effect of compound **1** on plasma DPP-4 activity in male Beagle dogs (n = 3) and in Rhesus monkeys (n = 3; 1 male, 2 females) were performed analogous to the experimental design in rats. Application volume was 2 mL/kg, and blood was drawn from a forearm vein without anaesthesia.

Effect of Compound 1 on Oral Glucose Tolerance in Diabetic Mice.

Male *db*/*db* mice (C57BL/KSJ@Rj-db) were obtained from Janvier (France) and fed ad libitum with a standard pelleted diet. Compound **1** was dissolved in 0.1 N HCl and subsequently diluted with a 0.5% aqueous hydroxyethylcellulose solution (final HCl concentration 3 mM). Administration was via oral gavage with an application volume of 5 mL/kg. The animals (n = 7/group) were fasted overnight and were administered either vehicle or compound 1 at doses of 0.1, 0.3, or 1 mg/kg. Mice were challenged 45 min later with an oral glucose load (2 g/kg; application volume 5 mL/kg). Blood samples for glucose measurement were obtained before compound administration and 30, 60 , 90, and 120 min after the glucose load. Blood was drawn from the retrobulbal venous plexus under isoflurane anaesthesia. Blood samples were collected in EDTA tubes, plasma was prepared and glucose was measured photometrically using a commercially available assay kit (Granutest 250, Merck, Germany). In addition, an EDTA plasma sample obtained 30 min after the glucose load was frozen for later determination of ex vivo plasma DPP-4 activity as described above. The blood glucose excursion profile between the pre-dose value (t = 0 min) and 120 min after the glucose load was used to calculate an area under the curve (AUC) for each dose after correction for the pre-dose glucose values.

X-ray Crystallographic Analysis of the Complex of Compound 1 with DPP-4.

The soluble extracellular domain of human DPP4 (residues 39-766) was crystallized in the presence of compound 1 following published protocols.[2] The complex crystallises in space group $P2_12_12_1$ with unit cell constants a=65.3 Å, b=67.1 Å, c=419.9Å. Data were collected at the Swiss Light Source beamline PX-1 from a crystal cooled to 100K using a wavelength of 0.979Å. Data to 2.6Å resolution are 97.5% complete (3.6-fold redundant), Rmerge=10.4%. The structure was solved by Fourier methods using coordinates from 1NU6 as the starting model. Reflection data processing, model building and refinement was performed with HKL2000, MAIN and CNX, respectively.[3] The final model consists of entire chains of a partially glycosylated DPP-4 dimer excluding the transmembrane region (residues 39-766), two ligands occupying the active sites of the dimer as well as 206 water molecules. The structure refined to R=21.7%, RF=27.6% with excellent stereochemistry (rmsd for bond length=0.007Å, bond angles=1.43º).

Coordinates have been deposited with the PDB (accession code 2RGU).

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