Supporting information

Allosteric IGF-1R Inhibitors

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General procedure for the preparation of compounds 1 – 12

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. ^{1}H-NMR and mass spectra are in agreement with the structures and were recorded on a Brucker IFS 48 IR spectrophotometer, a Bruker AMX 300 MHz or DRX 500 MHz NMR spectrometer (TMS as an internal standard), and Vaccum Generators VG 70-70 or 70-250 at 70 eV, respectively. Elemental analyses (obtained with a Perkin-Elmer 240 BCHN analyser) for the final products were within 0.4% of theoretical values if not otherwise stated. All reactions were followed by TLC carried out on Merck KGaA F254 silica gel plates. Solutions were dried over Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure.

90.6 g aluminum chloride were suspended in 900 ml dichloroethane and 96.4 g (76.5 ml) 4-chloro butyric acid chloride were added slowly at 0°C. After 30 minutes 81 g 5-cyanoindole in 800 ml dichloroethane were added at the same temperature. After 2 hours at room temperature 500 g ice and 500 ml conc. HCl were added. 12 hours later the precipitate was filtered off, washed with dichloro ethane and cold water giving 89 g of product after drying of the remainder in vacuum. 9.5 g of the crude product were suspended in 100 ml THF. At -20°C 17.1 g (24.5 ml) of Vitride (70% in THF) were added drop by drop and the solution was stirred at room temperature for 3 h. At -10°C 100 ml water was added slowly and after stirring the reaction for 2 h at room temperature the solvent was removed in vacuum and the remainder extracted with ethyl acetate. The organic phase was dried over magnesium sulphate and concentrated in vacuum. The remainder was purified by chromatography on silica gel, giving 4.4 g of 3-(4-chloro-butyl)-1H-indole-5-carbonitrile as colourless crystals.

50 g 3-(4-chloro-butyl)-1H-indole-5-carbonitrile, 43 g 4-(tert.-butoxycarbonylamino)-piperidine and 55.6 g *N*-ethyl-diisopropylamine were dissolved in 500 ml acetonitrile and heated to 80°C for 72 h.

Thereafter the solvent was removed in vacuum and the remainder dissolved in ethyl acetate and extracted with water. The organic phase was dried over magnesium sulphate, filtered and after evaporation of solvent purified by chromatography on silica gel giving 80 g of tert.-butyl-*N*-[1-[4-(5-cyano-1H-indol-3-yl)butyl]-4-piperidyl]carbamate as a yellow oil.

80 g of the afore prepared carbamate was dissolved in 800 ml dioxane and 80 ml HCl saturated ethanol was added slowly. The solution was heated to 80°C for 4 h resulting in the precipitation of colourless crystals, which were filtered off, washed with ether and dried in vacuum giving 58 g of the hydrochloride of 3-[4-(4-amino-piperidin-1-yl)-butyl]-1H-indole-5-carbonitrile.

Finally, 1 equivalent of the corresponding indolyl-acid (A) was dissolved in DMF (8 ml/mmol) and stirred with 4 equivalents of N-(3-dimethylaminopropyl)N'-ethylcarbodiimide hydrochloride together with 4 equivalents of 4-methyl morpholine for about 10 minutes at room temperature. Then 1 equivalent of 3-[4-(4-Amino-piperidin-1-yl)-butyl]-1H-indole-5-carbonitrile hydrochloride (B) was added dropwise. After 4 hours the solvent was removed in vacuum and the remainder dissolved in ethyl acetate and saturated aqueous ammonium chloride solution. After phase separation and exhaustive extraction of the aqueous phase the combined organic phases were dried with magnesium sulphate, filtered, condensed in vacuum and purified via flash-chromatography giving the final products as free base. In some cases, as indicated below, the product fractions of the chromatography were dissolved in acetone, acidified with 1N HCl until pH 3 was obtained and resulting crystals were collected and dried in vacuum.

1H-Indole-6-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**1**) was obtained as colourless solid in 34% (400 mg) yield from 400 mg A and 830 mg B.

melting point 249 – 250.5°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.49 (s, 1H), 11.40 (s, 1H), 10.40 (br. s, 1H), 8.44 (d, 1H, J = 6.3 Hz), 8.11 (s, 1H), 7.98 (s, 1H), 7.56 (s, 2H), 7.51 (d, 1H, J = 8.4 Hz), 7.49 (t, 1H, J = 2.4 Hz), 7.41 (dd, 1H, J= 1.4 Hz, J = 8.4 Hz), 7.39 (d, 1H, J= 1.9 Hz), 6.47 (s, 1H), 4.05 (br. m, 1H), 3.49 (m, 2H), 3.05 (m, 4H), 2.77 (m, 2H), 2.01 (m, 4H), 1.78 (m, 2H), 1.68 (m, 2H).

1H-Indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**2**) was obtained as fawn solid in 26% (300 mg) yield from 400 mg A and 830 mg B.

melting point 310 – 311.5°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.52 (s, 1H), 11.06 (s, 1H), 10.63 (br. s, 1H), 8.63 (d, 1H, J = 7.3 Hz), 8.11 (s, 1H), 7.75 (t, 2H, J = 8.2 Hz), 7.53 (d, 1H, J = 8.4 Hz), 7.41 (m, 2H), 7.36 (t, 1H, J = 2.9 Hz), 7.08 (m, 1H), 6.49 (m, 1H), 4.11 (br. m, 1H), 3.51 (m, 2H), 3.06 (m, 4H), 2.78 (m, 2H), 2.06 (m, 4H), 1.81 (m, 2H), 1.69 (m, 2H).

1H-Indole-4-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide (**3**)
was obtained as colourless solid in 62% (270 mg) yield from 160 mg A and 330 mg B.
melting point 176 – 178.5°C
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.36 (s, 1H), 11.24 (s, 1H), 8.07 (s, 1H), 7.96 (d, 1H, J = 7.6 Hz), 7.52 (d, 1H, J = 8.3 Hz), 7.50 (d, 1H, J = 8.7 Hz), 7.39 (m, 2H), 7.37 (d, 1H, J = 7.2 Hz), 7.33 (d, 1H, J = 1.9 Hz), 7.11 (t, 1H, J = 7.7 Hz), 6.79 (m, 1H), 3.82 (br. m, 1H), 2.88 (m, 2H), 2.73 (m, 2H), 2.36 (m, 2H), 2.01 (m, 2H), 1.82 (m, 2H), 1.63 (m, 4H), 1.51 (m, 2H).

1-Methyl-1H-indole-4-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide (**4**)
was obtained as colourless solid in 58% (1.5 g) yield from 1 g A and 1.9 g B.
melting point 194.9°C
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.33 (s, 1H), 8.06 (s, 1H), 7.95 (d, 1H, J = 7.7 Hz), 7.55 (d, 1H, J = 8.3 Hz), 7.48 (d, 1H, J = 8.5 Hz), 7.39 (m, 3H), 7.31 (d, 1H, J = 1.9 Hz), 7.17 (t, 1H, J = 7.7 Hz), 6.78 (d, 1H, J = 2.7 Hz), 3.81 (s, 3H), 3.78 (m, 1H), 2.85 (m, 2H), 2.73 (m, 2H), 2.32 (m, 2H), 1.96 (m, 2H), 1.80 (m, 2H), 1.62 (m, 4H), 1.50 (m, 2H).

1-Ethyl-1H-indole-4-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide (**5**)
was obtained as pink solid in 37% (507 mg) yield from 560 mg A and 985 mg B.
melting point 162.6°C
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.34 (s, 1H), 8.06 (s, 1H), 7.95 (d, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 8.1 Hz), 7.48 (d, 1H, J = 8.5 Hz), 7.44 (d, 1H, J = 3.2 Hz), 7.38 (m, 2H), 7.31 (d, 1H, J = 1.9 Hz), 7.15 (t, 1H, J = 7.6 Hz), 6.77 (d, 1H, J = 3.0 Hz), 4.23 (q, 2H, 7.2 Hz), 3.80 (m, 1H), 2.87 (m, 2H), 2.73 (m, 2H), 2.34 (m, 2H), 1.99 (m, 2H), 1.81 (m, 2H), 1.61 (m, 4H), 1.50 (m, 2H), 1.35 (t, 3H, J = 7.2 Hz).

3-Formyl-1H-indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**6**)

was obtained as fawn solid in 12% (320 mg) yield from 1.1 g A sodium salt and 1.7 g B.

melting point 270 – 272.2°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.97 (s, 1H), 11.46 (s, 1H), 10.24 (br. s, 1H), 9.98 (s, 1H), 8.75 (d, 1H, J = 7.4 Hz), 8.28 (d, 1H, J = 7.7 Hz), 8.24 (d, 1H, J = 3.2 Hz), 8.10 (s, 1H), 7.88 (d, 1H, J = 7.5 Hz), 7.51 (d, 1H, J = 8.4 Hz), 7.41 (dd, 1H, J = 1.4 Hz, J = 8.4 Hz), 7.39 (d, 1H, J = 1.4 Hz), 7.32 (m,1 H), 4.12 (m, 1H), 3.52 (m, 2H), 2.07 (m, 4H), 2.77 (m, 2H), 2.07 (m, 2H), 1.98 (m, 2H), 1.78 (m, 2H), 1.69 (m, 2H).

3-Acetyl-1H-indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**7**)

was obtained as colourless solid in 77% (1 g) yield from 510 mg A and 830 mg B.

melting point 254 – 255.5°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.80 (s, 1H), 11.47 (s, 1H), 10.21 (br. s, 1H), 8.72 (d, 1H, J = 7.3 Hz), 8.38 (d, 1H, J = 7.7 Hz), 8.20 (d, 1H, J = 3.2 Hz), 8.11 (s, 1H), 7.88 (d, 1H, J = 7.5 Hz), 7.52 (d, 1H, J = 8.4 Hz), 7.42 (dd, 1H, J = 1.4 Hz, J = 8.4 Hz), 7.39 (d, 1H, J = 1.4 Hz), 7.28 (m, 1H), 4.12 (m, 1H), 3.53 (m, 2H), 3.08 (m, 4H), 2.78 (m, 2H), 2.48 (s, 3H), 1.99 (m, 2H), 1.79 (m, 2H), 1.70 (m, 2H).

3-(2,2,2-Trifluoro-acetyl)-1H-indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**8**)

was obtained as fawn solid in 49% (700 mg) yield from 640 mg A and 830 mg B.

melting point 283 - 284°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.46 (s, 1H), 11.45 (s, 1H), 10.12 (br. s, 1H), 8.84 (d, 1H, J = 7.5 Hz), 8.37 (d, 1H, J = 7.7 Hz), 8.24 (q, 1H, J = 1.4 Hz), 8.10 (s, 1H), 7.98 (dd, 1H, J = 0.7 Hz, J = 7.7 Hz), 7.51 (d, 1H, J = 8.4 Hz), 7.48 – 7.40 (m, 2H), 7.39 (d, 1H, J = 2.0 Hz), 7.28 (m, 1H), 4.13 (m, 1H), 3.53 (m, 2H), 3.08 (m, 4H), 2.78 (m, 2H), 2.08 (m, 2H), 1.98 (m, 2H), 1.77 (m, 2H), 1.70 (m, 2H).

1H-Indole-3,7-dicarboxylic acid 3-amide 7-({1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide) hydrochloride (**9**)

was obtained as fawn solid in 19% (250 mg) yield from 500 mg A and 830 mg B.

melting point 210 – 211.5°C

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.51 (s, 2H), 10.06 (br. s, 1H), 8.67 (d, 1H, J = 7.3 Hz), 8.35 (d, 1H, J = 7.8 Hz), 8.09 (m, 2H), 7.79 (dd, 1H, J = 0.6 Hz, J = 7.8 Hz), 7.52 (d, 1H, J = 8.3 Hz), 7.41 (m, 2H), 7.16 (t, 1H, J = 7.7 Hz), 4.11 (m, 1H), 3.51 (m, 2H), 3.06 (m, 4H), 2.78 (m, 2H), 2.05 (m, 4H), 1.80 (m, 2H), 1.69 (m, 2H).

3-Cyano-1H-indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide hydrochloride (**10** - MSC1609119A-1**)**
was obtained as colourless solid in 40% (1 g) yield from 900 mg A and 1.5 g B.
melting point 204 - 206°C
analysis (C, H, N, Cl) calc. 67.1; 5.9; 16.8; 7.1; found 66.2; 5.8; 15.2; 7.5

3-Cyano-5-fluoro-1H-indole-7-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide (**11**)
was obtained as colourless solid in 87% (270 mg) yield from 980 mg A and 330 mg B.
melting point 154 - 155°C
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.17 (br. s, 1H), 11.37 (s, 1H), 8.58 (br. s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 7.77 (d, 1H, J = 10.2 Hz), 7.63 (dd, 1H, J = 1.9 Hz, J = 8.5 Hz), 7.51 (d, 1H, J = 8.3 Hz), 7.40 (dd, 1H, J = 1.5 Hz, J = 8.5 Hz), 7.33 (d, 1H, J = 1.9 Hz), 4.08 (m, 1H), 2.92 (m, 2H), 2.74 (m, 2H), 2.38 (m, 2H), 2.05 (m, 2H), 1.85 (m, 2H), 1.65 (m, 4H), 1.53 (m, 2H).

3-Cyano-1H-indole-4-carboxylic acid {1-[4-(5-cyano-1H-indol-3-yl)-butyl]-piperidin-4-yl}-amide (**12**)
was obtained as fawn solid in 80% (370 mg) yield from 185 mg A and 330 mg B.
melting point 110 - 112°C
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.40 (br. s, 1H), 8.58 (br. s, 1H), 8.28 (s, 1H), 8.24 (d, 1H, J = 7.8 Hz), 8.08 (s, 1H), 7.62 (dd, 1H, J = 2.3 Hz, J = 6.6 Hz), 7.50 (d, 1H, J = 8.4 Hz), 7.40 (dd, 1H, J = 1.2 Hz, J = 8.3 Hz), 7.33 (s, 1H), 7.29 (m, 2H), 3.77 (m, 1H), 2.85 (m, 2H), 2.74 (m, 2H), 2.31 (m, 2H), 2.16 (m, 2H), 1.90 (m, 2H), 1.66 (m, 2H), 1.53 (m, 4H).

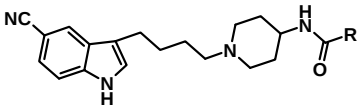
Test protocol for biochemical evaluation

GST-tagged IGF1-R (residues 930-1337; BPS Bioscience, CA, USA, Cat: 40240) was incubated for 90 min at 25 °C in the presence of 100 mM HEPES pH 7.4, 1 mM DTT, 5 mM $MnCl_2$, 15 µM ATP, and an N-terminal fluorescently tagged substrate peptide ([FITC]-AHA-KKSRGDYMTMQIG). Compounds of interest were also present in the reaction, in log dose response format, to a final concentration of 1 mM DMSO. The reaction was stopped by the addition of a final concentration of 50 mM EDTA, and the amount of phosphorylation of substrate peptide was measured using a Caliper Lifescience instrument (Hopkington, MA, USA) LC 3000.

Test protocol for cellular evaluation

To assess the cellular activity of compounds on IGF-1R phosphorylation sub-confluent MCF-7 breast cancer cells (ATCC, HTB-22) grown on 96-well plates were starved in serum-free DMEM for 20 h. Following incubation with increasing compound concentrations from 3 nM to 30 µM for 45 min, IGF-1R phosphorylation was induced by addition of 100 ng/ml of human IGF-1 (Calbiochem #407240). After 5 min cells were lysed in HGNT buffer (20 mM HEPES, pH 7,4, 10% (V/V) glycerol, 150 mM NaCl, 1% (V/V) Trition-X-100, 2 mM EDTA, 25 mM NaF) containing protease and phosphatase inhibitor cocktails (Calbiochem #539134 and #524625). IGF-1R was captured on white ELISA plates coated with a specific IGF-1R antibody (R&D Systems #DY391) and tyrosine phoshorylated protein was detected using 0.05 µg/ml of a biotinylated anti-phospho-tyrosine antibody (Santa Cruz, sc-7020B), peroxidase-coupled streptavidine (Amersham Biosciences, #RPN4401) and subsequent chemiluminescence detection (Roche #1582950) with a Mithras LB940 plate reader. Each inhibitor concentration was tested in triplicates and the means were used to calculate IC_{50} values with the RS1 software package. Phosphorylation of the IR was assessed in an analogous assay using HepG2 cell (ATCC, HB-8065) incubated in RPMI1640 and stimulated with 100 ng/ml human insulin (Sigma, #10259), while IR was captured with a specific anti-IR antibody (Biosource, #AHR0221).

Test results of cellular Inuslin receptor testing



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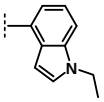
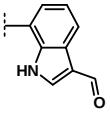
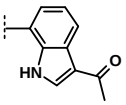
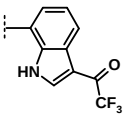
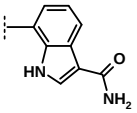
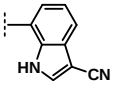
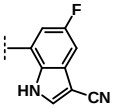
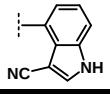
5		15
6		14
7		28
8		16
9		15
10		36
11		31
12		24

[f] screening concentration 30 µM