Supplementary material

Discovery and biological evaluation of novel EGFR/c-Met inhibitors

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General information:

All chemical reagents were purchased from Sigma-Aldrich and Apollo Scientific Ltd. (United Kingdom). Intermediate compounds were synthesized as described in WO2007/146824. Microwave-assisted reactions were conducted with a Biotage 27-Creator microwave reactor. (Biotage, Uppsala, Sweden). Reference compounds erlotinib, afatinib, BMS-777607, crizotinib and OSI-906 were purchased from Selleck Chemicals LLC (USA).
Thin layer chromatography was performed on TLC Silica Gel 60 F254 plates.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer in deuterated DMSO-d_6 as solvent, chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane (TMS) standard.

HPLC-MS was performed on Waters system (XBridge C18 4.6X50 mm 3.5 micron) using reverse phase.

The LC-MS analysis was performed with a liquid chromatography mass-spectrometer Waters chromatograph with the following parameters:

Waters HPLC/MS:
- MS detector: Waters SQD
- UV detector: Waters 996 DAD
- Separation module: Waters Alliance 2795

HPLC:
- Column: Waters XBridge C18, 50 mm x 4.6 mm, 3.5 µm
- Solvent I: Water/ 0.1% HCOOH
- Solvent II: AcCN
- Acetonitrile: Riedel-deHaën; G Chromasolv (34998)
- Water: Mili-Q Academic
- Formic acid: Riedel-deHaën; extra pure (27001)
- Flow rate: 2 ml/min
- Injection: 5 µg

Gradient	Method		
	time	Solv. I.	Solv. II.
	0.00 min	95%	5%
	0.50 min	95%	5%
	5.50 min	5%	95%
	6.00 min	5%	95%
	6.50 min	95%	5%
	7.00 min	95%	5%

MS: Ionization: ES+/ES–

Source block temperature: 110°C

Desolvation temperature: 250°C

Desolvation gas: 500 L/h

Cone gas: 80 L/h

Capillary voltage: 3000 V

Cone voltage: 30 V

Extractor voltage: 6 V

Rf lens voltage: 0.1 V

Scan: 80 to 1000 m/z in 1 sec.

Inter-scan delay: 0.1 s

Method A – solvent: I. water/0.05% HCOOH, II. acetonitrile

Method B: – solvent: I. 5mM NH_4HCO_3, II. acetonitrile

Enzymatic assays:

Recombinant wt and L858R mutant EGFR kinases were prepared in-house.[34] Recombinant c-Met kinase was purchased from ProQinase GmbH (Lot: 012), recombinant InsR kinase was purchased from Proteros Biostructures GmbH.
Kinase profiling of compound **10** was performed by Proteros Structures GmbH, Germany.

Cellular assays:

A549 (wt EGFR, mutant KRAS), H1975 (L858R and T790M mutant EGFR), HCC827 (deletion mutant EGFR) and H1993 (*c-Met* gene amplification) human NSCLC cells were obtained from American Type Culture Collection (Rockville, MD, USA), and were maintained in RPMI1640 supplemented with 10% (v/v) fetal bovine serum (FBS) and antibiotics (MycoZap Plus-CL), all purchased from Lonza Group Ltd. Switzerland. Cells were cultured at 37°C, in a humified, 5% CO_2 incubator.

I. General synthetic methods

3a-b (2g, 4.25 mmol) was dissolved in methanol/dichloromethane (3:1, 120ml) and stirred vigoruosly under H_2 atmosphere for 6 hours in the presence of 10% Pd/C (30mg). After completion of the reaction the catalyst was filtered through a Celit pad and the solvent was removed under reduced pressure to afford **4a-b**.

4a-b (10 mmol) was dissolved in anhydrous DMF and 1 eq. 1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxylic acid, 1.2 ekv HATU[21] and 3 eq. DIPEA were added to the solution then it was stirred overnight. The solvent was removed under reduced pressure and the residue was taken up with saturated K_2CO_3 solution and extracted with ethyl-acetate. The organic layer was separated and dried over $MgSO_4$. The crude product was purified by column chromatography (Kieselgel, chloroform/methanol 10:1) to afford **1** and **2**.

4a-b (1g, 2.27 mmol) was dissolved in anhydrous pyridine and 3-bromobenzenesulfonyl chloride (0.64g, 1.1 eq.) was added to the solution in one portion and stirred overnight at 50°C. The solvent was evaporated in vacuo and the residue was partitioned between sat. $NaHCO_3$ (20 ml) and chloroform (30 ml). The organic layer was separated and dried over anhydrous $MgSO_4$ to afford **5a-c**.

Suzuki-Myaura cross-coupling

5a-c (0.38 mmol, approx. 250 mg) and tetrakis(triphenylphosphine)palladium(0) (22 mg, 0.019 mmol) was dissolved in 1,2-dimethyoxyethane (3 ml) under inert atmosphere and stirred for 1 hour. Boronic acid (1.2 eq.) and Na_2CO_3 (88 mg, 0.83 mmol in aqueous solution) were added and the reaction mixture was heated by microvawe irradiation for 20 min. at 140°C. After completion of the reaction, 20 ml water and THF was added to the reaction mixture and filtered throught a Celit pad. The layers were separated and the pH of aqueous layer was adjusted to pH 6 by 1 M NaH_2PO_4 buffer and extracted with 2x20 ml ethyl-acetate. The combined organic layer was dried over $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography (Kieselgel, chloroform/methanol 10:3, as eluent).

N-(3-fluoro-4-{[6-methoxy-7-(3-morpholin-4-ylpropoxy)quinolin-4-yl]oxy}phenyl)-1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamide (1)

Rt: 2.53 min, 2.29 min 0.45 *m/z* 642 $[M+H]^+$ 640 $[M+H]^-$

^{1}H NMR: δ 10.96 (s, 1H); 8.47 (d, 1H, J=5.1 Hz); 7.98 (d, 1H, J=13 Hz); 7.60-7.36 (m, 9H); 7.46 (d, 1H, J=4.8 Hz); 4.20 (t, 2H, J=6.1 Hz); 3.95 (s, 1H); 3.59-3.58 (m, 4H); 3.37 (s, 3H); 2.71 (s, 3H); 2.47-2.45 (m, 4H); 2.39 (m, 4H); 1.98 (q, 2H)

^{13}C NMR: 163.13; 161.59; 159.46; 153.82; 152.10; 149.73; 149.00; 146.54; 137.99; 137.86; 135.56; 135.40; 133.08; 129.64; 129.12; 127.40; 124.33; 115.86; 114.65; 108.73; 108.17; 107.87; 102.21; 99.24; 96.87; 66.86; 66.38; 55.93; 54.96; 53.53; 33.43; 25.86; 11.61. (2 carbon obscured)

mp: 124-126°C (from acetonitrile)

_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1_H_-pyrazole-4-carboxamide (2)

Rt: 2.38 min, 2.05 min 0.45 *m/z* 655 $[M+H]^+$ 653 $[M+H]^-$
^{1}H NMR: δ 10.96 (s, 1H); 8.46 (d, 1H, J=5.2Hz); 7.98 (d, 1H, J=12.4Hz); 7.60-7.38 (m, 9H); 6.46 (d, 1H, J=5.1Hz); 4.18 (t, 2H, J=6.0 Hz); 3.95 (s, 3H); 3.37 (s, 3H); 2.71 (s, 3H); 2.44-2.35 (m, 10H); 2.16 (s, 3H); 1.96 (q, 2H)

^{13}C NMR: δ 163.11; 161.59; 159.45; 155.36; 153.78; 152.09; 149.71; 149.01; 146.51; 138.00; 137.87; 135.36; 133.05; 129.65; 129.13; 127.41; 124.36; 115.85; 114.61; 108.67; 108.15 107.85; 102.19; 99.20; 96.84; 66.87; 55.92; 54.88; 54.48; 52.82; 45.83; 33.43; 26.19; 11.61. (2 carbon obscured)

mp: 127-129 ^{o}C (from acetonitrile)

3-bromo-_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]benzenesulfonamide (5a)

Method A
Rt: 2.62 min, 2.42 min, 0.45 min *m/z* 659 $[M+H]^+$ 657 $[M+H]^-$

Method B
Rt: 3.12 min

HPLC-MS purity: 96.32%

^{1}H NMR: δ 8.45 (d, 1H, J=5.2 Hz); 7.89 (dd, 1H, J=1.7 Hz, J=1.7 Hz) ;7.84 (dd, 1H, J=8.0 Hz, 1.7 Hz); 7.79 (dd, 1H, J=8.0 Hz, 1.7 Hz); 7.54 (dd, 1H, J=8.0 Hz, 7.48 (s, 1H); 7.37 (s, 1H); ,7.31 (dd, 1H, J=9.0, 8.9 Hz),7.10 (dd, 1H, J=12.4 Hz, 2.5 Hz), 6.95 (dd, 1H, J=8.9 Hz, 2.5 Hz), 6.35 (d, 1H, J=5.2 Hz); 4.18 (t, 2H; J=12.3 Hz), 3.92 (s, 3H); 2.50-2.44 (m, 10H); 2.23 (s, 3H); 1.96 (q, 2H)

^{13}C NMR: δ 159.31; 155.44; 152.16; 152.09; 149.73; 148.96; 146.52; 142.61; 138.8; 135.5; 131.6; 129.1; 125.8; 124.5; 122.2; 117.6; 114.6; 109.4; 109.2; 108.7; 102.1; 99.2; 66.8; 55.9; 54.6; 54.3; 52.4; 45.4; 26.1.

mp: 82-84^{o}C (from diisopropyl ether)

4-bromo-_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]benzenesulfonamide (5b)

Method A
Rt: 2.60 min, 2.50 min 0.45 min *m/z* 659 $[M+H]^+$ 661$[M+H]^-$

^{1}H NMR: δ 8.44 (d, 1H, J=4.8 Hz); 7.82-7-79 (m, 2H); 7.73-7.71 (m, 2H); 7.47 (s, 1H); 7.37-7.29 (m, 2H);7.12 (d, 1H, J=12.1 Hz); 6.96 (d, 1H, J=8.2 Hz);6.35 (d, 1H, J=4.7 Hz); 4.17 (t, 2H, J=6.2 Hz); 3.92 (s, 3H); 3.40-3.36 (m, 4H); 2.49-2.41 (m, 6H); 2.21 (s, 3H); 1.96-1.94 (m, 2H).
mp: 177-179^{o}C (from diisopropyl ether)

5-bromo-2-fluoro-*N*-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]benzenesulfonamide (5c)

Rt: 2.63 min, 2.42 min *m/z* 676 $[M+H]^+$ 678 $[M+H]^-$

^{1}H NMR: δ 8.44 (d, 1H, J=3.2 Hz); 7.90 (dd, 1H, J=2.5 Hz, J=1.7 Hz); 7.80 (d, 1H, J=2.5 Hz); 7.48 (s, 1H); 7.37-7.34 (m, 1H); 7.26-7.21 (m, 2H); 7.05 (d, 1H, J=12.8 Hz) ;6.89 (d, 1H, J=7.7 Hz); 6.35 (d, 1H, J=3.2 Hz); 4.18 (t, 2H, J=6.0 Hz); 3.92 (s, 3H); 2.49-2.33 (m, 10H); 2.20 (s, 3H); 1.97 (m, 2H).

mp: 115-117^{o}C (from diisopropyl ether)

N-(3-fluoro-4-{[6-methoxy-7-(3-morpholin-4-ylpropoxy)quinolin-4-yl]oxy}phenyl)-3-(3-furyl)benzenesulfonamide (6)

^{1}H NMR: δ 10.60 (brs, 1H); 8.37 (d, 1H, J=3.93 Hz); 8.31 (s, 1H); 7.97 (s, 1H); 7.91 (d, 1H, J=7.1 Hz); 7.80 (s, 1H);7.69-7.55 (m, 2H);7.46 (s, 1H); 7.37 (s, 1H); 7.35 (s, 1H); 7.32 (s, 1H); 7.18 (d, 1H; J=12.2 Hz); 7.03 (d, 1H, J=8.8 Hz);6.99 (s, 1H); 6.27 (d, 1H, J=4.4 Hz); 4.18 (t, 2H, J=6.0 Hz); 3.90 (s, 3H); 3.59-3.58 (m, 4H); 2.49-2.39 (m, 6H);1.97 (q, 2H)

Rt: 2.86 min, 2.64 min 634 $[M+H]^+$ 632 $[M+H]^-$

N-(3-fluoro-4-{[6-methoxy-7-(3-morpholin-4-ylpropoxy)quinolin-4-yl]oxy}phenyl)-3-(1-methyl-1H-pyrazol-4-yl)benzenesulfonamide (7)

^{1}H NMR: δ 10.30 (brs, 1H); 8.36 (d, 1H, J=5.1 Hz); 8.25 (s, 1H); 7.92 (s, 1H); 7.91 (s, 1H);7.84 (d, 1H, J=6.75 Hz); 7.59-7.55 (m, 2H); 7.46 (s, 1H); 7.37-7.32 (m, 2H); 7.18 (d, 1H, J=12.0 Hz); 7.04 (d, 1H, J=8.9 Hz); 6.27 (d, 1H, J=4.5 Hz); 4.18 (t, 2H, J=6.1 Hz); 3.90 (s, 3H); 3.88 (s, 3H); 3.59-3.57 (m, 4H); 2.49-2.39 (m, 6H); 1.97 (q, 2H)

Rt: 0.41 min, 2.24 min, 2.49 min *m/z* 648 $[M+H]^+$ 646 $[M+H]^-$

***N*-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-3-(1-methyl-1*H*-pyrazol-4-yl)benzenesulfonamide (8)**

Rt: 2.37 min, 2.19 min 0.44 *m/z* 661$[M+H]^+$ 659$[M+H]^-$

^{1}H NMR: δ 10.60 (brs, 1H); 8.36 (d, 1H, J=5.2 Hz); 8.25 (s, 1H); 7.92 (s, 1H); 7.91 (s, 1H); 7.83 (dd, 1H, J=7.6 Hz, J=9.5); 7.61 (d, 1H, J=1.7 Hz); 7.79-7.53 (m, 2H); 7.45 (s, 1H); 7.36 (s, 1H); 7.33 (d, 1H, J= 9 Hz); 7.15 (dd, 1H, J=12.2 Hz, J=2.4 Hz); 7.02 (d, 1H, J=8.6 Hz);6.27 (d, 1H, J=5.2 Hz); 4.16 (t, 2H, J=6.0 Hz); 3.90 (s, 3H); 3.87 (s, 3H); 2.45-2.37 (m, 7H); 2.17 (s, 3H); 1.95 (q, 2H)

^{13}C NMR δ 159.20; 155.39; 152.10; 149.73; 148.86; 146.51; 140.36; 137.79; 137.66; 136.53; 136.34; 133.85; 130.09; 129.29; 128.66; 124.58; 123.99; 122.69; 120.49; 117.30; 114.56; 109.33; 109.04; 108.66; 102.06; 99.16; 66.87; 55.90; 54.79; 54.42; 52.70; 45.69; 26.16.

mp: 113-115^{o}C (from diisopropyl ether)

***N*-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-3-(3-furyl)benzenesulfonamide (9)**

Rt: 2.65 min, 2.50 min 0.45 *m/z* 647 $[M+H]^+$ 645 $[M+H]^-$

^{1}H NMR: δ 10.30 (brs, 1H); 8.37 (d, 1H, J=5.2 Hz); 8.31 (s, 1H); 7.97 (s, 1H); 7.89 (d, 1H, J=7.4 Hz); 7.80 (s, 1H); 7.68 (d, 1H, J=7.7 Hz); 7.60 (t, 1H, J=7.7 Hz); 7.45 (s, 1H); 7.36 (s, 1H); 7.31 (d, 1H, J=8.8 Hz); 7.16 (dd, 1H, J=12.2 Hz, 2.3 Hz); 7.02 (dd, 1H, J=8.8 Hz, J=2.3 Hz); 6.99 (s, 1H); 6.27 (d, 1H, J=5.1 Hz); 4.16 (t, 2H, J=6.0 Hz); 3.90 (s, 3H); 2.46-2.38 (m, 10H); 2.18 (s, 3H); 1.95 (q, 2H)

^{13}C NMR: δ 159.21; 155.40; 152.10; 149.73; 148.85; 146.50; 145.00; 140.53; 140.43; 137.79; 136.55; 136.39; 133.20; 130.10; 130.00; 125.05; 124.63; 123.36; 117.35; 114.57; 109.38; 109.09; 108.61; 102.08; 99.16; 66.87; 55.90; 54.76; 54.41; 52.66; 45.65; 26.15.

mp: 90-92^{o}C (from diisopropyl ether)

***N*-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-3-(3-thienyl)benzenesulfonamide (10)**

Rt: 2.78 min, 2.61 min *m/z* 661$[M+H]^+$ 663 $[M+H]^-$

^{1}H NMR: δ 10.30 (brs, 1H); 8.37 (d, 1H, J=5.2Hz); 8.07 (s, 1H); 8.01-8.00 (m, 2H); 7.72-7.70 (m, 2H); 7.65-7.56 (m, 2H); 7.46 (s, 1H); 7.36 (s, 1H); 7.33 (dd, 1H, J=9.1, J=9.1); 7.30 (m, 1H); 7.18 (dd, 1H, J=2.3Hz, J=12.2); 7.04 (dd, 1H, J=9.1Hz, J=2.3 Hz); 6.27 (d, 1H, J=5.3 Hz); 4.17 (t, 2H, J=6.0 Hz); 3.90 (s, 3H); 2.47 (m, 2H), 2.40 (m, 8H); 2.19 (s, 3H); 1.95 (q, 2H)

^{13}C NMR: δ 159.19; 155.41; 149.74; 148.87; 146.51; 140.4; 139.73; 137.70; 136.62; 136.14; 130.58; 130.20; 128.05; 126.04; 125.24; 124.62; 123.88; 122.82; 117.39; 114.58; 109.41; 109.12; 108.67; 108.08; 99.17; 66.87; 55.90; 54.74; 54.40; 52.62; 45.61; 26.14.

mp: 110-112^{o}C (from diethyl ether)

2-fluoro-*N*-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-5-(3-thienyl)benzenesulfonamide (11)

Rt: 2.79 min, 2.60 min, 0.45 min *m/z* 681 $[M+H]^+$ 679 $[M+H]^-$

^{1}H NMR: δ 8.37(d, 1H, J=5.1 Hz); 8.08 (d, 1H, J=6.6 Hz); 7.97 (s, 1H); 7.68 (s, 1H); 7.55-7.44 (m, 3H); 7.35-7.26 (m, 2H); 7.15 (d, 1H, J=12.1); 7.02 (d, 1H, J=8.4 Hz); 6.28 (d, 2H, J=4.9 Hz); 4.17 (t, 2H, J=5.8 Hz); 3.89 (s, 3H); 2.49-2.38 (m, 9H); 2.23 (s, 3H); 1.97-1.91 (m, 3H).

HPLC-MS purity: 99.99%

mp: 162-164^{o}C

_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-4-(1-methyl-1_H_-pyrazol-4-yl)benzenesulfonamide (12)

Rt: 2.35 min, 2.15 min 0.45 min *m/z* 659 $[M+H]^+$ 661 $[M+H]^-$

^{1}H NMR: δ 10.20 (brs, 1H); 8.42 (d, 1H, J=4.8 Hz); 8.27 (s, 1H); 7.97 (s, 1H); 7.78-7.76 (m, 4H); 7.46 (s, 1H); 7.35 (d, 1H, J=5.8 Hz); 7.31 (s, 1H); 7.16 (d, 1H, J=12.1 Hz); 7.01 (d, 1H, J=8.5 Hz); 4.16 (t, 2H; J=6.1 Hz); 3.90 (s, 3H); 3.87 (s, 3H); 2.45-2.37 (m, 10H); 2.17 (s, 3H); 1.96 (q, 2H)

mp: 120-122^{o}C (from diisopropyl ether)

_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-4-(3-thienyl)benzenesulfonamide (13)

Rt: 2.73 min, 2.59 min 0.45 *m/z* 662 $[M+H]^+$ 664 $[M+H]^-$

^{1}H NMR: δ 10.30 (brs, 1H); 8.41 (d, 1H, J=5.2 Hz); 8.08 (dd, 1H, J=2.5 Hz, J=1.8 Hz); 7.96 (s, 1H); 7.93 (s, 1H); 7.84 (s, 1H); 7.81 (s, 1H); 7.71-7.69 (m, 1H); 7.63 (dd, 1H, J=5.0 Hz, J=1.1 Hz); 7.46 (s, 1H); 7.36 (s, 1H); 7.34 (s, 1H); 7.31 (s, 1H); 7.17 (dd, 1H, J=12.2 Hz, 2.3 Hz);7.02 (d, 1H, J=8.8 Hz);6.33 (d, 1H, J=5.2 Hz); 4.17 (t, 2H, J=6.1 Hz); 3.90 (s, 3H); 2.49-2.39 (m, 10H); 2.18 (s, 3H); 1.97 (q, 2H)

mp: 125-127^{o}C (from diisopropyl ether)

_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-3-(2-thienyl)benzenesulfonamide (14)

Method A
HPLC-MS purity: 98.00%

Rt: 2.79 min, 2. 60 min *m/z* 663 $[M+H]^+$ 661 $[M+H]^-$

^{1}H NMR: δ 8.36 (d, 1H, J=4.5 Hz); 7.96 (m, 2H); 7.70-7.62 (m, 4H); 7.45 (s, 1H); 7.36 (m, 2H); 7.19 (m, 2H); 7.02 (d, 1H, J=7.5 Hz); 6.28 (d, 1H, J=4.5 Hz); 4.16 (t, 2H, J=6.1 Hz); 3.89 (s, 3H); 3.40-3.36 (m, 4H); 2.49-2.41 (m, 6H); 2.20 (s, 3H); 1.95-1.90 (m, 2H).

mp: 98-100^{o}C (from diisopropyl ether)

_N_-[3-fluoro-4-({6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinolin-4-yl}oxy)phenyl]-3-(2-furyl)benzenesulfonamide (15)

^{1}H NMR: δ 10.9 (brs, 1H); 8.38 (1H, d, J=4.5 Hz); 8.06 (s, 1H); 7.83 (s, 1H); 7.70-7.60 (m, 2H); 7.46 (s, 1H); 7.36 (s, 1H); 7.30 (d, 1H, J= 8.7 Hz); 7.16 (s, 1H); 7.11 (s, 1H); 6.99 (d, 1H, J=9.4 Hz); 6.65 (s, 1H); 6.28 (d, 1H; J=4.8 Hz); 4.17 (t, 2H, J=6.0 Hz); 3.90 (s, 3H); 2.49-2.38 (m, 9H); 2.18 (s, 3H); 1.95-1.90 (m, 3H).

Rt: 2.72 min, 2.55 min *m/z* 647 $[M+H]^+$ 645 $[M+H]^-$

HPLC-MS purity: 98.14%

mp: 90-92°C

II. Recombinant kinase assay

EGFR reaction buffers contained 20 mM HEPES (pH 7.5), 1 mM DTT, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.01% (v/v) NP40, 5% (v/v) glycerol, 0.5 mg/ml poly-GT peptide as substrate, 7.52 µM ATP for wt EGFR and 25.2 µM ATP for L858R EGFR (ATP concentrations at $K_{M[ATP]}$ values).
C-Met reaction buffer contained 20 mM HEPES (pH 7.5), 1 mM DTT, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 0.01% (v/v) Tween-20, 5% (v/v) glycerol, 0.25 mg/ml poly-AEKY peptide as substrate and 9.6 µM ATP.
InsR reaction buffer contained 20 mM HEPES (pH 7.5), 1 mM DTT, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 0.01% (v/v) Brij-35, 400 nM 5'TAMRA fluorescent dye-labelled KKSRGDYMTMQIG peptide and 12.58 µM ATP as substrate, OSI-706[35] was used as reference compound.
Reaction time was 1 hour at room temperature. Recombinant c-Met kinase was preincubated with compounds for 60 min. Detection of the produced ADP was performed by Transcreener® assay for EGFRs (Bellbrook Labs), ADPGlo assay for c-Met (Promega). Detection of the phosphorylated substrate was performed by IMAP assay for InsR (Molecular Devices).

III. Western Blot analysis

Cells were serum-starved for 24 h, treated with indicated compound concentrations for 1 h, then lysed (0.1% SDS, 5 mM EDTA, 150 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% (v/v) Tween-20, 1 mM Na_3VO_4, 1 mM PMSF, 20 mM NaF and protease inhibitor cocktail (Calbiochem)). 4-50 µg cell lysates were run on 8% SDS-PAGE, and blotted to polyvinylidene-difluoride membranes (Bio-Rad). Membranes were blocked with 5% (w/v) skimmed milk (1 h, RT), probed with primary antibodies (overnight, 4°C) and horseradish peroxidase-conjugated secondary antibodies (1 h, RT), then visualized with chemiluminescence reagent (1 min, RT, Western Lightning Plus-ECL, PerkinElmer) on CL-XPosure Films (Thermo Scientific, MA). Antibodies were from CellSignaling Technologies (Danvers, MA): anti-c-Met (L41G3), anti-phospho-c-Met (Y1234, 130H2), anti-EGFR (C74B9), anti-phospho-EGFR (Y1068, 1H12), anti-p44/42 MAPK (3A7), anti-phospho-p44/42 MAPK (T202/Y204), anti-AKT (40D4), anti-phospho-AKT (S473) and anti-β-actin.

IV. Apoptosis assay

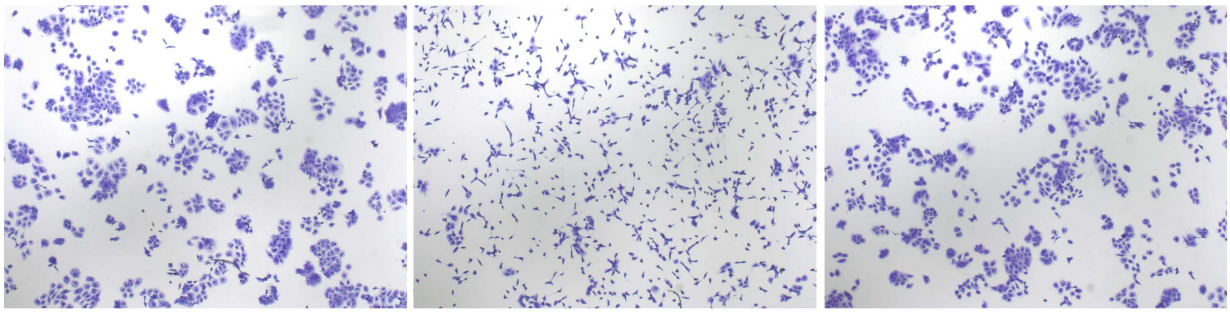
HCC827 and H1993 cell lines were treated with reference compounds and compound **10** at 1 µM in media containing 1% FBS. After 24 h supernatants were collected together with trypsinized cells. The cell suspensions were centrifuged (150 g, 10 min, 4°C) and fixed with ethanol, (70%, -20°C). After at least 24 h the cells were pelleted, resuspended in apoptosis buffer (200 mM Na_2HPO_4, 200 mM citric acid pH 7.8), treated with 100 µg/ml RNase A (Sigma), incubated 30 min at room temperature and stained with 10 µg/ml propidium iodide. The proportions of the apoptotic cells were determined with a FACSCalibur flow cytometer using CellQuest Pro software (BD Biosciences). Treatment groups were replicated at least three times.

V. MTT viability assay

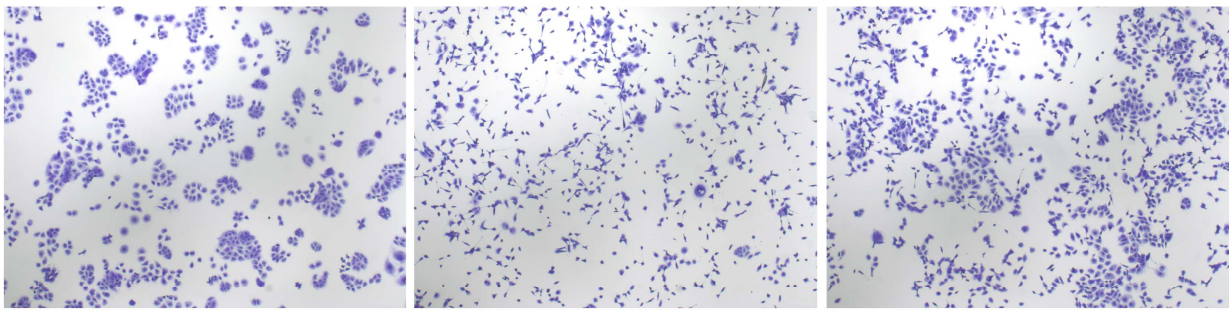
In all, 8000 cells were plated per well of a 96-well plate. After one day the cells were exposed to various drug dilutions. After 48 h, 50 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (2 mg/ml) was added to each well. Plates were incubated (1.5 h, 37°C), crystalline formazan was solubilized with 200 µl detection solution (2-propanol, 1 mM HCl, 10% (v/v) Triton X-100). Absorbance was measured with a Synergy 2 plate reader (Merck, KGaA, Darmstadt, Germany), at wavelengths 570 and 635 nm. The data of 635 nm was subtracted from the data of 570 nm for each well individually and the results were used to calculate IC_{50} values compared to DMSO treated controls, using Excell (Microsoft) and XLfit (IDBS) softwares. Each measurement was repeated three times.

VI. HGF-induced cell scattering assay

DU145 cells were seeded in 6 well plates (30000 cells/well). One day later cells were preincubated for 1 h with compounds and treated with 100 ng/ml HGF, or DMSO as control in a full-serum media (10% FBS). Pictures were taken after 24 h in this case.

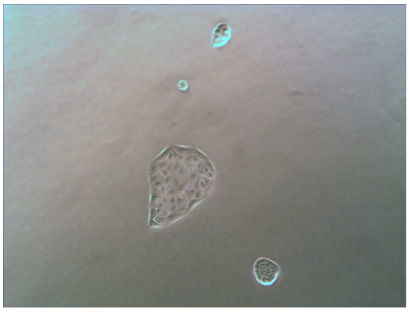


DMSO | 100 ng HGF | 0.39 µM BMS-777607 +100 ng HGF



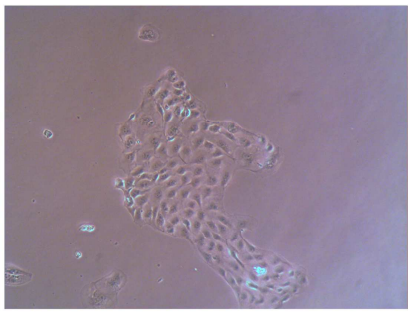
1.56 µM BMS-777607 +100 ng HGF | 0.39 µM Compound **10** +100 ng HGF | 1.56 µM Compound **10** +100 ng HGF

MDCK cells were seeded in 24 well plates (1000 cells/well) and let to attach overnight. Next day media was replaced to serum-free, and treatment was started immediately. After preincubation with various concentrations of reference and in-house inhibitors for 1 h, cells were treated with 20 ng/ml HGF or PBS as control. After 48 h, phase-contrast photos were taken.

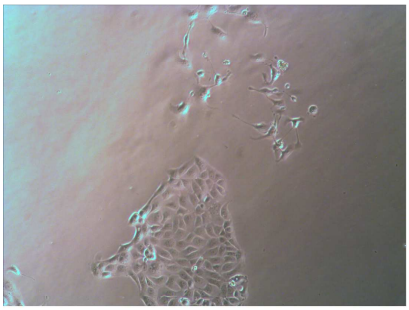


DMSO

20 ng HGF



0.3 μM BMS-777607
+20 ng HGF

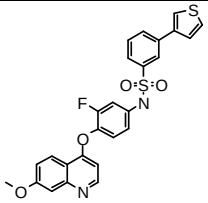
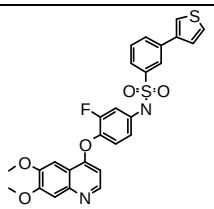


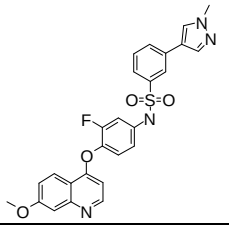
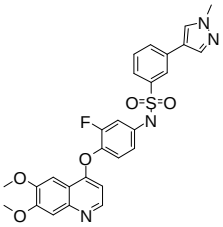
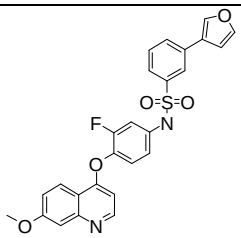
0.3 μM Compound **10**
+20 ng HGF

VII. Docking methods

For the docking we used the previously determined 3D structure of c-Met (3LQ8) and EGFR (1XKK) proteins. All calculations were carried out with the modules of Schrödinger Suite 2009 and 2010. Before the docking we prepared the proteins by removing the water molecules and adding the hydrogens to the residues with Protein Preparation Wizard. After the ImPref minimization (used force field: OPLS_2005) we created the Grid box for the docking. In the case of EGFR kinase the inner Grid box was set to 12 Å. The 3D structures of the ligands were determined by LigPrep module at pH 7.4 using OPLS_2005 force field. The docking experiments were carried out with Glide in standard precision (SP) mode using without any pharmacological constrains.

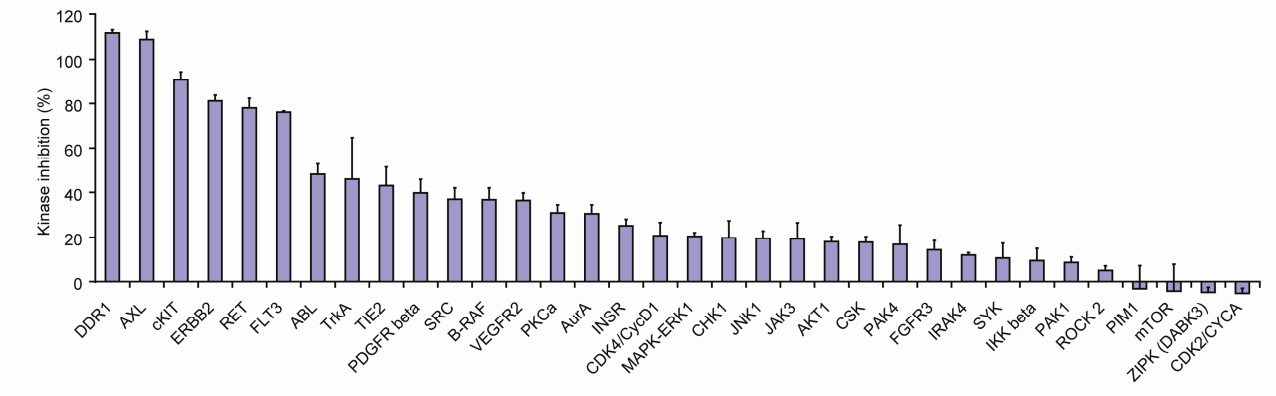
Preliminary docking of biaryl-sulphonamide derivatives

Structure	Docking score
	-13.600008
	-13.531200

	-8.422693
	-6.534500
	-7.950370

ID	Docking score on c-Met (kcal/mol)	Docking score on EGFR (kcal/mol)
6	-14.858281	-8.488583
7	-7.792668	-9.019475
8	-7.619377	-9.073854
9	-7.560117	-9.105095
10	-12.025835	-9.473062
11	-12.358668	-8.073740
12	-10.82935	-9.197678
13	-11.609588	-8.636387
14	-14.76042	-9.634953
15	-8.061637	-9.293205

VIII. Screening results of compound 10 on 34 recombinant kinases



All values are an average of three independent experiments at 1 μM.

References:

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(35) Mulvihill, M. J.; Cooke, A.; Rosenfeld-Franklin, M.; Buck, E.; Foreman, K.; Landfair, D.; O'Connor, M.; Pirritt, C.; Sun, Y.; Yao, Y.; Arnold, L. D.; Gibson, N. W.; Ji, Q. S. Discovery of OSI-906: a selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. *Future Med. Chem*. **2009**, *1*, 1153−1171.