Supplementary Information

A new affinity probe targeting VEGF receptors for kinase inhibitor selectivity profiling by chemical proteomics

Xin Ku[1], Stephanie Heinzlmeir[1,2,3], Dominic Helm[1], Guillaume Médard[1] & Bernhard Kuster[1,2,3,4*]

[1]Chair for Proteomics and Bioanalytics, Technische Universität München, Emil Erlenmeyer Forum 5, 85354 Freising, Germany

[2]German Cancer Consortium (DKTK), Munich, Germany

[3]German Cancer Research Center (DKFZ), Heidelberg, Germany

[4]Center for Integrated Protein Science Munich, Emil Erlenmeyer Forum 5, 85354 Freising, Germany

[*]Correspondence: kuster@tum.de

Tel. +49 8161 715696

Fax: +49 8161 715931

Overview of Supplementary Information

Materials and Methods.

Molecular Docking.

The binding pose of the VEGFR probe in the ATP-binding site of VEGFR2 was predicted by the software Glide (Schrödinger, Inc.). The crystal structure of VEGFR2 in complex with BIB1120 was retrieved from the Protein Data Bank (PDB code 3C7Q) and prepared using the Protein Preparation Wizard in Maestro (Schrödinger, Inc.) to remove non amino acid molecules, add hydrogen atoms, and assign the protonation states for the polar residues. The scoring grid was generated by enclosing the residues 14 Å around BIB1120 in the binding site. The docking of the probe was performed in Glide SP mode. Among the top 20 ranked binding poses, the best pose featuring a salt bridge between the 4-nitrogen atom of the N-methyl piperazinyl moiety and the carboxylate oxygens of Glu850 was used for binding mode analysis, for its similarity to the co-crystal structure.

Cell Culture and Lysis.

Post-delivery human placenta tissue (obtained from Freising hospital following informed consent by the donor) was thoroughly washed with cold phosphate buffered saline (PBS) and homogenized in lysis buffer (50 mM Tris/HCl pH 7.5, 5% Glycerol, 1.5 mM $MgCl_2$, 150 mM NaCl, 0.8% NP-40, 1 mM dithiothreitol and 25 mM NaF with freshly added protease inhibitors (SIGMA-FAST, Sigma-Aldrich) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, Sigma-Aldrich, Munich, Germany)) using a tissue grinder. Lysates were incubated for 30 min at 4 °C and protein extracts were clarified by ultracentrifugation for 1 h at 145,000 x g at 4 °C. Protein concentrations were determined by Bradford assay. OVCAR8, K562, COLO205 and SKNBE2 cells were cultivated in humidified air supplemented with 10% CO_2 at 37 °C. K562 and COLO205 cells were cultured in Roswell Park Memorial Institute 1640 medium, OVCAR8 and SKNBE2 cells were cultured in Dulbecco's modified Eagle's medium (4.5 g/L glucose). All media were supplemented with 10%-20% fetal bovine serum (PAA, Pasching, Austria). For lysis, cells were washed with phosphate-buffered saline, then lysed in 50 mM Tris/HCl pH 7.5, 5% Glycerol, 0.8% Nonidet P-40 and freshly added protease (SIGMA-FAST, Sigma-Aldrich) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, Sigma-Aldrich, Munich, Germany). Homogenates were clarified by ultracentrifugation at 145,000 g at 4 °C for 30 min. Supernatants were collected and aliquots were stored at -80 °C until further use. Protein concentrations in lysates were determined by Bradford assay.

Compound Immobilization.

Immobilization of the compound 18 was accomplished by reaction of the primary amine of the probe with the NHS-activated sepharose beads (GE Healthcare, Freiburg, Germany) as described.[1-3] Briefly, NHS-beads (1 mL settled beads) were suspended in isopropanol (1 mL) and washed with anhydrous dimethyl sulfoxide (3 x 10 mL). The beads were then re-suspended in anhydrous DMSO (1 mL) and a solution of the probe (20 µL, 100 mM in DMSO) was added to achieve a coupling density of 2 µmol of the probe per 1 mL settled beads, followed by addition of triethylamine (15 µL). The mixture was incubated in darkness for 16-20 h at room temperature on an end-over-end-shaker. Amino ethanol (50 µL) was then added to block the remaining binding sites of the NHS beads. The mixture was further incubated in the dark for 16-20 h at room temperature on an end-over-end-shaker. The functionalized beads were then washed once with anhydrous DMSO (10 mL) and ethanol (3x10 mL) and stored in ethanol (1 mL) at 4°C in the dark until use. For drug profiling experiments, the immobilized compound 18 probe was mixed with kinobeads (equal volume of for each immobilized compound).

Drug Competition Assay. The drug competition assays were performed in triplicates as described previously.[1-5] For each experiment, 5 mg of protein were used. Briefly, cell lysates were diluted with equal volumes of 1x compound pulldown (CP) buffer (50 mM Tris/HCl pH 7.5, 5% glycerol, 1.5 mM $MgCl_2$, 150 mM NaCl, 20 mM NaF, 1 mM sodium orthovanadate, 1 mM dithiothreitol, protease inhibitors (SIGMA-FAST, Sigma-Aldrich) and phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, Sigma-Aldrich, Munich, Germany)). Lysates were further diluted if necessary to reach a final protein concentration of 5 mg/mL using 1x CP buffer supplemented with 0.4% Nonidet P-40. These lysates were incubated with the respective drug in 6 concentrations (DMSO, 2.5 nM, 25 nM, 250 nM, 2.5 µM, 25 µM) for 0.5 h at 4 °C. Afterwards, the treated lysates were incubated with the mix beads (kinobeads plus compound 18 beads, 100 µL settled amount) for another 0.5 h at 4 °C. Subsequently, the lysates were discarded except the DMSO treated one which was used for a second pulldown experiment (pulldown of pulldown) following the same procedure. The beads were then washed with CP buffer and collected by centrifugation. Bound proteins were eluted with 2x NuPAGE® LDS Sample Buffer (60 µL, Invitrogen, Darmstadt, Germany). Proteins in eluates were reduced by dithiothreitol (3 µL, 1M) and alkylated by iodoacetamide (6 µL, 200 mg/mL). Aliquots of samples (30 µL) were then run into a 4–12% NuPAGE gel (Invitrogen, Darmstadt, Germany) for about 1 cm to concentrate the sample prior to in-gel tryptic digestion. In-gel trypsin digestion was performed according to standard procedures.

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis.

Peptides generated by in-gel trypsin digestion were dried in a vacuum concentrator and then dissolved in 20 µL 0.1% formic acid (FA) prior to LC-MS/MS analysis. LC-ESI-MS/MS was performed by coupling a nanoLC-Ultra (Eksigent, Dublin, CA) to a LTQ-Orbitrap Velos mass spectrometer (ThermoFisher Scientific). For each analysis, 10 µL of dissolved peptides was delivered to a trap column (ReproSil-pur C18-AQ, 5 µm, Dr. Maisch, Ammerbuch, Germany, 20 mm×75 µm, self-packed) at a flow rate of 5 µL/min in 100% solvent A (0.1% formic acid in HPLC grade water). After 10 min of loading and washing, peptides were transferred to an analytical column (ReproSil-gold C18-AQ, 3 µm, Dr. Maisch, Ammerbuch, Germany, 400 mm×75 µm, self-packed) and separated using a 210 min gradient from 7% to 35% of solvent B (0.1% formic acid in acetonitrile) at 300 nL/minute flow rate. The LTQ Orbitrap Velos was operated in data dependent mode, automatically switching between MS and MS/MS. Full scan MS spectra (300-1,300 m/z) were acquired in the Orbitrap at 30,000 resolution (at m/z 400) after accumulation precursor ions to a target value of 1,000,000 for a maximum time of 100 ms. Internal lock mass calibration was performed using the ion signal $(Si(CH_3)_2O)_6$ H+ at m/z 445.120025 present in ambient laboratory air. Tandem mass spectra were generated for up to ten peptide precursors by higher energy collision induced dissociation (HCD, target value of 40,000, max 100 ms accumulation time) at a normalized collision energy of 40% and fragment ions were recorded at a resolution of 7,500 in the Orbitrap. To maximize the number of precursors targeted for analysis, dynamic exclusion was enabled with one repeat count in 10 s and 30 s exclusion time.

Peptide and Protein Identification and Quantification.

All raw MS spectra were processed by MaxQuant software (version 1.4.0.5) for peak detection and quantification. MS/MS spectra was searched against the IPI human database human (version 3.68, 87,061 sequences) by Andromeda search engine enabling contaminants and the reversed versions of all sequences with the following search parameters: Carbamidomethylation of cysteine residues as fixed modification and Acetyl (Protein N-term), Oxidation (M) as variable modifications. Trypsin was specified as the proteolytic enzyme with up to 2 miss cleavages were allowed. For identification 0.01 peptide and protein FDRs were used. Feature matching between raw files was enabled, using a match time window of 2 min.

Data analysis.

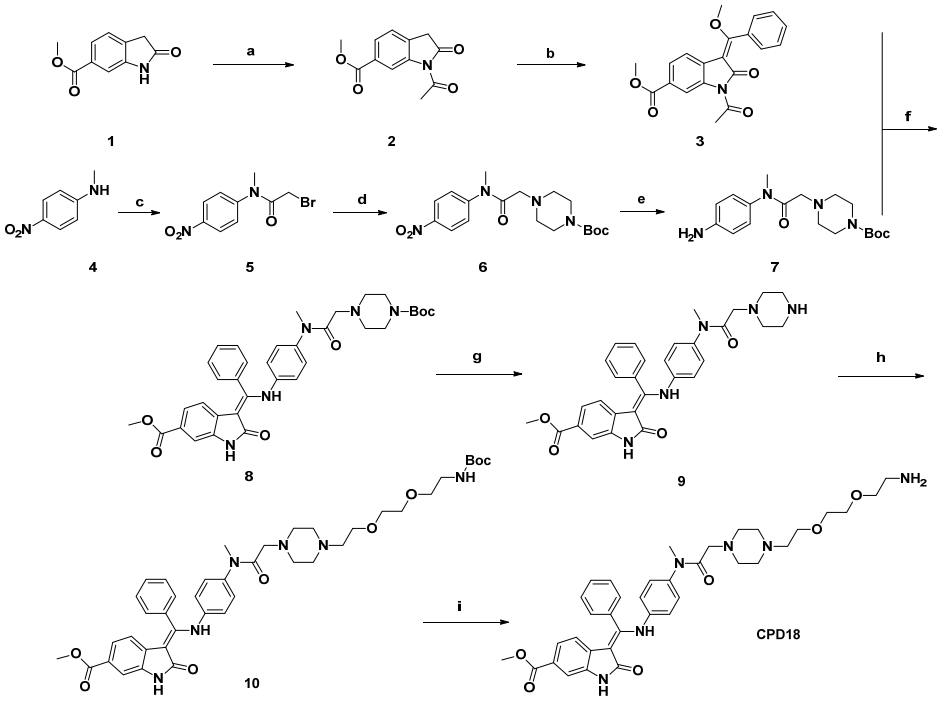
Proteins which displayed a dose-dependent inhibition were selected and analyzed in GraphPad Prism. Half maximal inhibition of binding concentrations (IC50) of proteins was calculated by non-linear regression with variable slope and the constraint of the DMSO control value to be equal to 1. Kd values

were calculated using a depletion factor as described.[2, 4, 6] Briefly, the depletion factor 'r' for each protein was defined as a ratio and calculated using protein intensities observed for the DMSO pulldown (denominator), and for the pulldown of the supernatant of the latter (pulldown of pulldown, numerator). The dissociation constants were then calculated following the equation Kd = r x IC50. Molecular function and cellular pathway annotations of drug affected proteins were manually assigned according to the gene ontology information provided in the Uniprot database and by reading the relevant literature.

Chemical synthesis

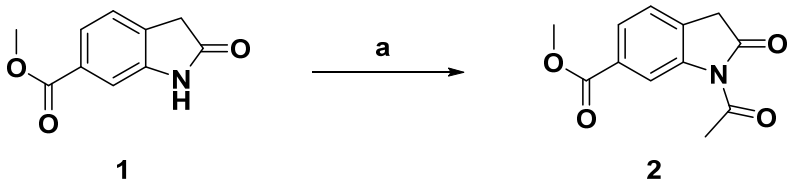
General Synthetic Methods. All chemicals and solvents were purchased from commercial suppliers (Sigma-Aldrich Co, VWR International, Carl Roth GmbH & Co.KG, Alfa Aeasar, Santa Cruz, Fluorochem Ltd) and were used without further purification. All air- and moisture-sensitive reactions were carried out under an atmosphere of dry argon with heat-dried glassware and standard syringe techniques. Flash chromatography was performed on an Interchim puriFlash evo 430 system. Proton (1H NMR) nuclear magnetic resonance spectra were obtained at 400 MHz, respectively unless otherwise noted. Chemical shifts were recorded in parts per million (ppm) and NMR signals were described as following: s (single), d (doublet), t (triplet), q (quadruplet), and m (multiplet). Mass spectrometry (MS) analyses were conducted on an amazon speed ETD ion trap mass spectrometer in positive electrospray mode. The samples were separated by HPLC on an Agilent 1100 system prior to mass spectrometric analysis utilizing a TriArt C18 column and an 1100 series HPLC system, applying a 5-95% gradient of solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) for 25 min, followed by plateau of 95% solvent B for 5 min. Chromatography signals were detected using DAD detector at λ: 254 nm and 365 nm.

Synthetic route of Compound 18



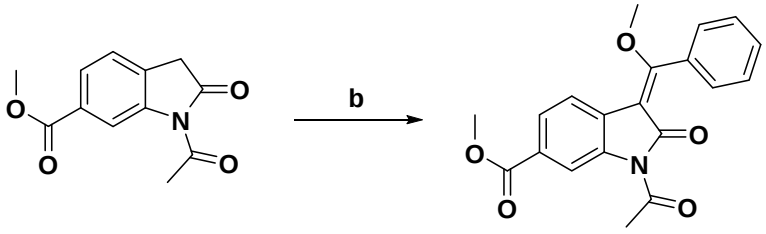
Reaction conditions: a) Ac_2O, 130 °C, 6 h; b) Ac_2O, 120 °C, 2 h; c) K_2CO_3, dry THF, 0 °C to r.t.; d) KOH, dry THF, r.t.; e) EtOH / sat. NH_4Cl (v/v = 1/1), Fe, reflux, 1h; f) DMF, 80 °C, 1h, then piperidine, r.t. 3h; g) CF_3COOH, DCM, 1h, r.t.; h) DMF and Cs_2CO_3, r.t., overnight; i) CF_3COOH, DCM, 1h, r.t.;

6-Acetyl-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid methyl ester (2)



2-oxo-2, 3-Dihydro-1H-indole-6-carboxylic acid methyl ester (**1**) (1.15 g, 6.02 mmol) was suspended in acetic anhydride (10 mL) and refluxed at 130 °C for 6 h. After that time, the mixture was cooled down and the precipitate was filtered off and washed with petroleum ether. This precipitate were further purified by flash chromatography (0-100% ethyl acetate in petroleum ether) to give 0.5 g of 6-acetyl-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid methyl ester (**2**). LC/MS: 233.898 [$M+H^+$] 1H NMR: (400 MHz, DMSO-d6) δ 8.68 (s, 1H), 7.84 (d, *J* = 8 Hz, 1H), 7.50 (d, *J* = 8 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 2.58 (s, 2H).

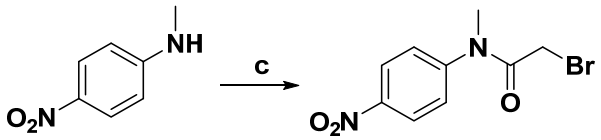
(E)-1-Acetyl-6-chloro-3-(ethoxy-phenyl-methylene)-indolin-2-one (3)



2 3

6-Acetyl-2-oxo-2,3-dihydro-1H-indole-6-carboxylic acid methyl ester (**2**) (0.419 g, 2 mmol) was dissolved in acetic anhydride (5 mL), and ortho-benzoic acid triethyl ester (1.36 mL, 6 mmol) was added. The mixture was stirred at 120 ^{o}C for 2 h. After that, the solvent was removed by evaporation. The residue was triturated with petroleum ether (100 mL), filtered off, and dried to give 0.2 g of (E)-1-acetyl-6-chloro-3-(ethoxy-phenyl-methylene)-indolin-2-one (**3**). LC/MS: 351.935 [M+H^{+}] 1H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.04 (d, *J* = 8 Hz, 1H), 7.98 (d, *J* = 8 Hz, 1H), 7.60-7.62 (m, 3H), 7.40-7.43 (m, 2H), 3.60 (s, 3H), 3.79 (s, 3H), 2.60 (s, 3H).

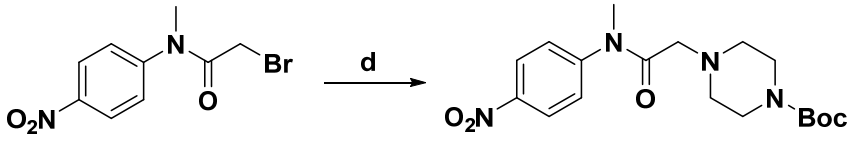
2-Bromo-N-methyl-N-(4-nitrophenyl)acetamide (5)



4 5

N-Methyl-4-nitroaniline (**4**) (1.52g, 1 mmol) and potassium carbonate (1.66g, 1.2 mmol) were dissolved in anhydrous THF. Furthermore, 2-bromoacetyl bromide (1.05 mL, 1.2 mmol) was dissolved in a small amount of anhydrous tetrahydrofuran (THF) and was added dropwise to the reaction mixture at 0°C. Reaction was stirred at room temperature and monitored by TLC. H_2O was added after the reaction was complete and the mixture was extracted with 3×20 mL ethyl acetate. The organic phases were combined and dried with magnesium sulfate. The solvent was then evaporated and the crude product was purified by flash chromatography using a solvent gradient of 0-100 % ethyl acetate in petroleum ether to give 1.8 g of the target molecule (**5**) LC/MS: 273.018 [M+H^{+}] 1H NMR (400 MHz, DMSO-d6) δ 4.12 (s, 2H), δ 7.69(d, 2H, *J* = 8Hz), δ 8.30 (d, 2H, *J* = 8Hz)

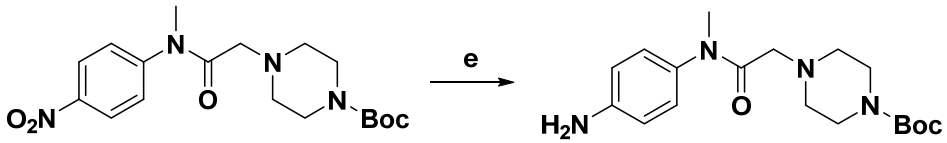
tert-Butyl 4-(2-(methyl(4-nitrophenyl)amino)-2-oxoethyl)piperazine-1-carboxylate (6)



5 **6**

1-Boc-piperazine (0.97 g, 5.2 mmol) and potassium hydroxide (0.42 g, 7.5 mmol) were dissolved in anhydrous THF. Then, 2-bromo-N-methyl-N-(4-nitrophenyl)acetamide (1.04 g, 3.8 mmol) was added and the reaction mixture was stirred at room temperature and monitored by TLC. H_2O was added after the reaction was complete and the mixture was extracted with 3×20 mL ethyl acetate. The organic phases were combined and dried with magnesium sulfate. The solvent was then evaporated and the crude product was purified by flash chromatography using a solvent gradient of 0-100 % ethyl acetate in petroleum ether to give 1.8 g of the target molecule (6). LC/MS: 272.946 [M+H$^+$] 1H NMR (400 MHz, DMSO-d6) δ 1.36 (s, 9H), δ 2.29 (s, 4H), δ 3.16 (s, 6H), δ 3.27 (s, 3H), δ 7.63 (d, 2H, J = 8Hz), δ 8.24 (d, 2H, J = 8Hz).

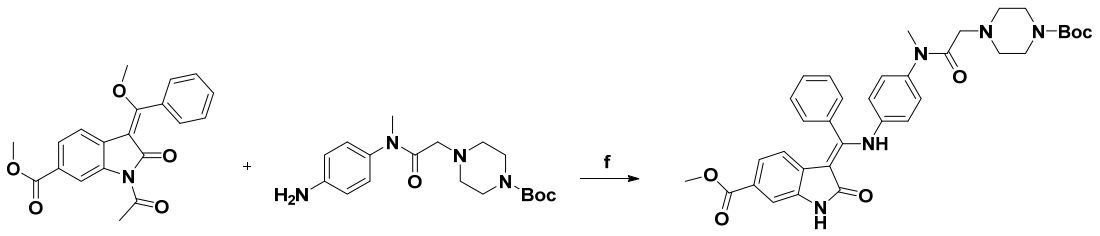
tert-Butyl 4-(2-((4-aminophenyl)(methyl)amino)-2-oxoethyl)piperazine-1-carboxylate (7)



6 **7**

tert-Butyl 4-(2-((4-nitrophenyl)(methyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**6**) (0.4 g, 1.05 mmol) was dissolved in 10 mL EtOH / NH_4Cl saturated solution (v/v = 1/1). After addition of iron (300 mg, 5.3 mmol), the reaction mixture was stirred at 110°C for 1h. The reaction mixture was cooled down and filtered. 100 mL H_2O was added and extraction was performed 5 times with 20 ml ethyl acetate. Organic layers were combined and dried with $MgSO_4$. The solvent was removed by the vacuum evaporator and crude product was obtained. Recrystallization was performed in order to give the target molecule (**7**). LC/MS: 349.080 [M+H$^+$] 1H NMR (400 MHz, DMSO-d6) δ 1.38 (s, 9H), δ 2.30 (s, 4H), δ 2.85 (s, 2H), δ 3.05 (s, 3H), δ 3.24 (s, 4H), δ 5.23 (s, 2H), δ 6.57 (d, *J* = 8Hz, 2H), δ 6.91 (d, *J* = 8Hz, 2H).

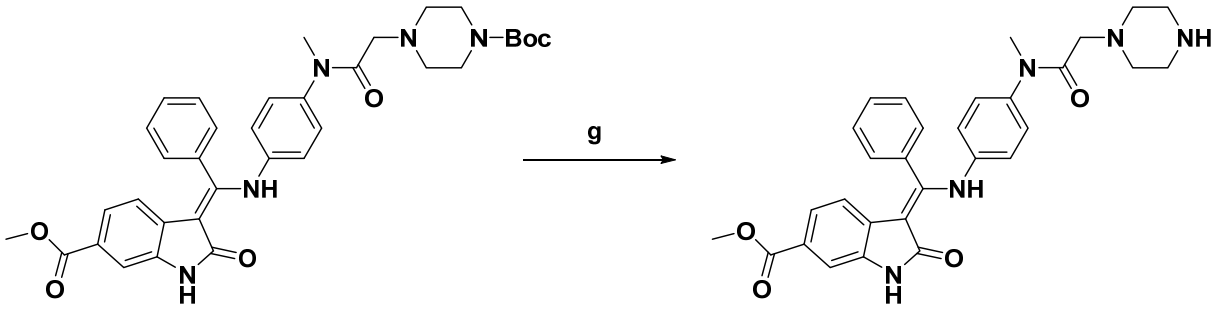
Methyl (Z)-3-(((4-(2-(4-(tert-butoxycarbonyl)piperazin-1-yl)-N-methylacetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (8)



3 7 8

Methyl-(E)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (**3**) (0.18 g, 0.5 mmol) and tert-butyl 4-(2-((4-aminophenyl)(methyl)amino)-2-oxoethyl)piperazine-1-carboxylate (**7**) (0.19g, 0.55 mmol) were dissolved in 10 mL DMF and was heated at 80 ^{o}C for 1h, then piperidine (1.1 mL) was added and the reaction was kept at room temperature for 3 h. After that, water was added and the mixture was extracted using 3×20 mL ethyl acetate. The organic phases were combined and concentrated to give the crude product. The crude product was then purified by flash chromatography using a solvent gradient of 0-100 % ethyl acetate in petroleum ether to give 0.18 g of the target molecule (**8**) LC/MS: 626.312 [M+H^{+}]; 1H NMR (400 MHz, DMSO-d6) δ 12.23 (s, 1H), 10.98 (s, 1H), δ 7.58 (m, 4H), 7.52 (d, *J* = 7.3, 2H), 7.43 (s, 1H), 7.20 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 2H), 6.91 (d, *J* = 8.1 Hz, 2H), 3.78 (s, 3H), 3.16 (s, 4H), 3.07 (s, 3H), 2.17 (s, 4H), 1.40 (s, 9H).

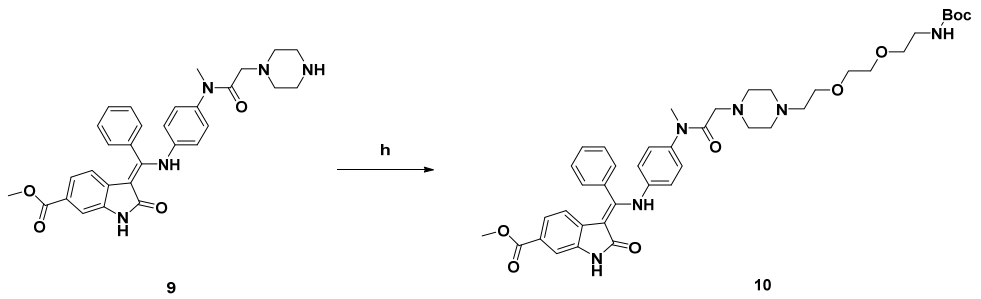
Methyl (Z)-3-(((4-(N-methyl-2-(piperazin-1-yl)acetamido)phenyl)amino)(phenyl) methylene)-2-oxoindoline-6-carboxylate (9)



8 9

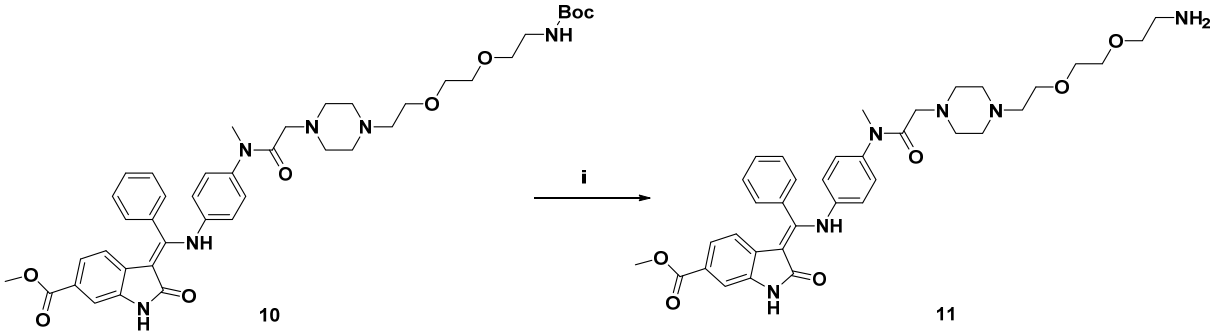
Methyl (Z)-3-(((4-(2-(4-(tert-butoxycarbonyl)piperazin-1-yl)-N-methylacetamido)phenyl)amino)(phenyl) methylene)-2-oxoindoline-6-carboxylate (**8**) (0.1 g, 0.16 mmol) was dissolved in 5 mL CH_2Cl_2/CF_3COOH (v/v=1/1) solution. The reaction finished in 1 h. The solvent was then evaporated and the crude product was used without further purification. LC/MS: 526.240 [M+H^{+}].

Methyl (Z)-3-(((4-(2-(4-(2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)piperazin-1-yl)-N-methylacetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (10)

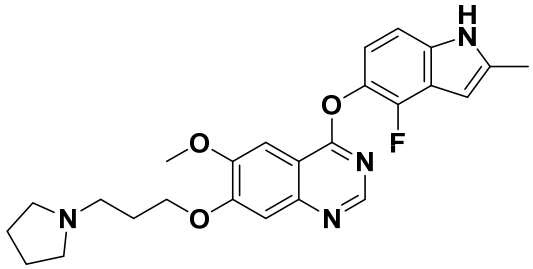


Methyl (Z)-3-(((4-(N-methyl-2-(piperazin-1-yl)acetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (**9**) (70 mg, 0.133 mmol) and tert-butyl (2-(2-(2-bromoethoxy)ethoxy)ethyl)carbamate (45 mg, 0.15 mmol) were dissolved in DMF and Cs_2CO_3 (60 mg, 0.17 mmol) was added. The mixture was stirred at room temperature overnight. After completion, water was added and the mixture was extracted 3×20 mL with ethyl acetate. The organic phases were combined and concentrated to give the crude product. The crude product was then purified by flash chromatography using a solvent gradient of 0-100 % ethyl acetate in petroleum ether to give 60.5 mg of the target molecule (**10**) LC/MS: 757.432 [$M+H^+$], 1H NMR (400 MHz, $CDCl_3$) δ 12.20 (s, 1H), 8.11 (s, 1H), 7.72 – 7.52 (m, 4H), 7.50 – 7.35 (m, 3H), 6.99 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 6.01 (d, *J* = 8.3 Hz, 1H), 5.10 (s, 1H), 3.88 (s, 3H), 3.64-3.54 (m, 8H), 3.33 (d, *J* = 5.4 Hz, 2H), 3.19 (s, 3H), 2.81 (s, 2H), 2.63 – 2.45 (m, 8H), 1.46 (s, 9H).

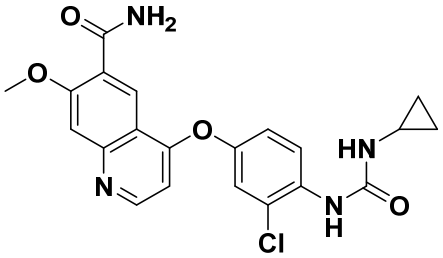
Methyl (Z)-3-(((4-(2-(4-(2-(2-(2-aminoethoxy)ethoxy)ethyl)piperazin-1-yl)-N-methylacetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (11)



Methyl (Z)-3-(((4-(2-(4-(2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-yl)piperazin-1-yl)-N-methylacetamido)phenyl)amino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (**10**) (30 mg, 0.04 mmol) was dissolved in 5 mL CH_2Cl_2/CF_3COOH (v/v:1/1) solution. The reaction finished in 1 h. The solvent was then evaporated. The crude product was transferred on top of a SCX column, washed 2 times with methanol and eluted with ammonia in methanol (2M), resulted in compound **11** with 7 mg. LC/MS: 657.336 [M+H$^+$]. 1H NMR (400 MHz, Methanol-d_4) δ 7.63 (dt, *J* = 14.1, 6.9 Hz, 4H), 7.59 – 7.55 (m, 1H), 7.51 (d, *J* = 6.9 Hz, 2H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 2H), 6.96 (d, *J* = 8.2 Hz, 2H), 5.98 (d, *J* = 8.2 Hz, 1H), 3.85 (d, *J* = 6.0 Hz, 5H), 3.72 (d, *J* = 5.5 Hz, 6H), 3.63 (q, *J* = 7.0 Hz, 5H), 3.19 – 3.12 (m, 4H), 2.85 (s, 3H), 2.72 (s, 2H), 2.06 (s, 3H).



Candidate 1[7]



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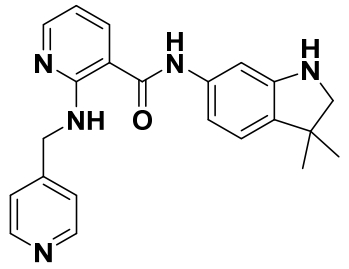

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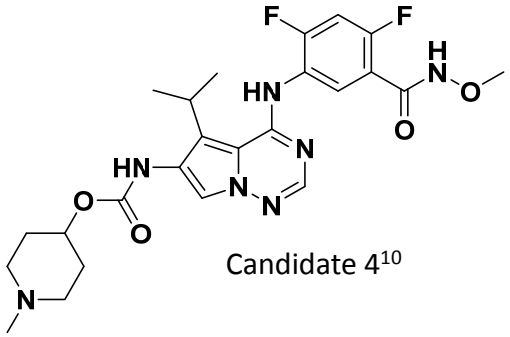

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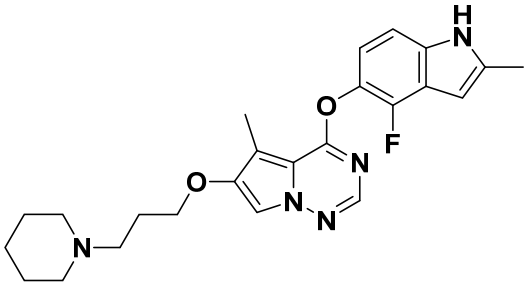

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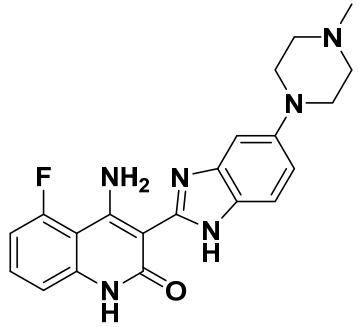
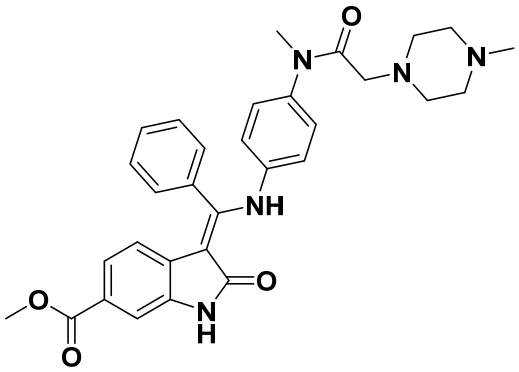


Candidate 6[11]



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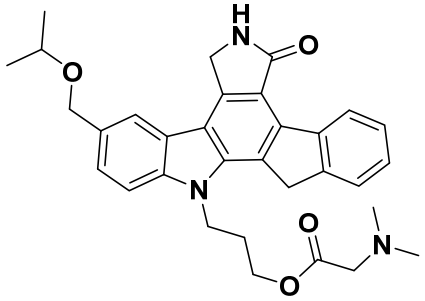

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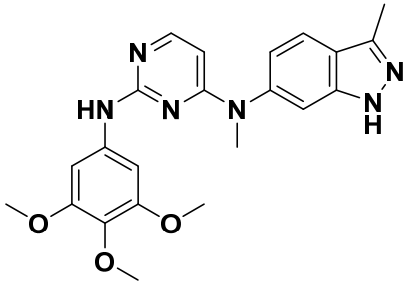
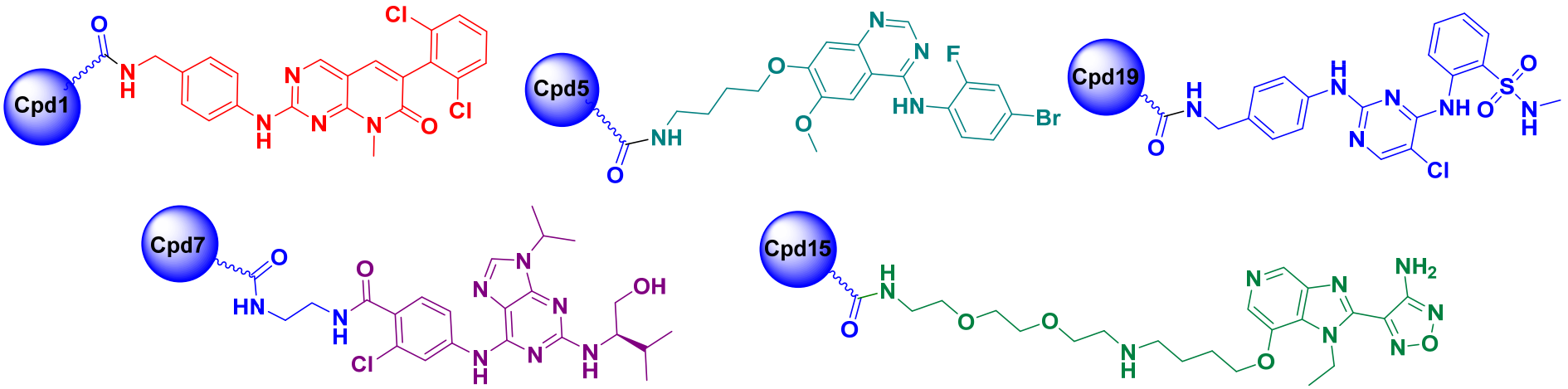
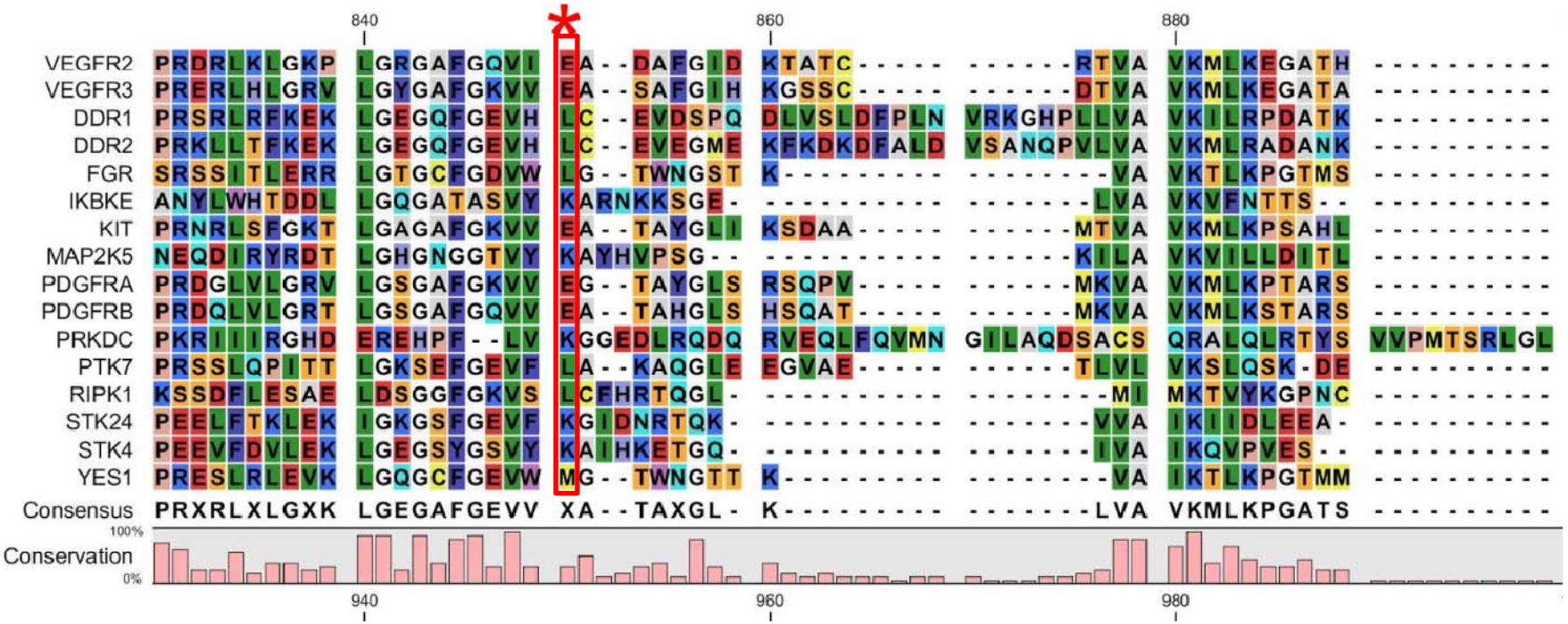


Candidate 9[14]

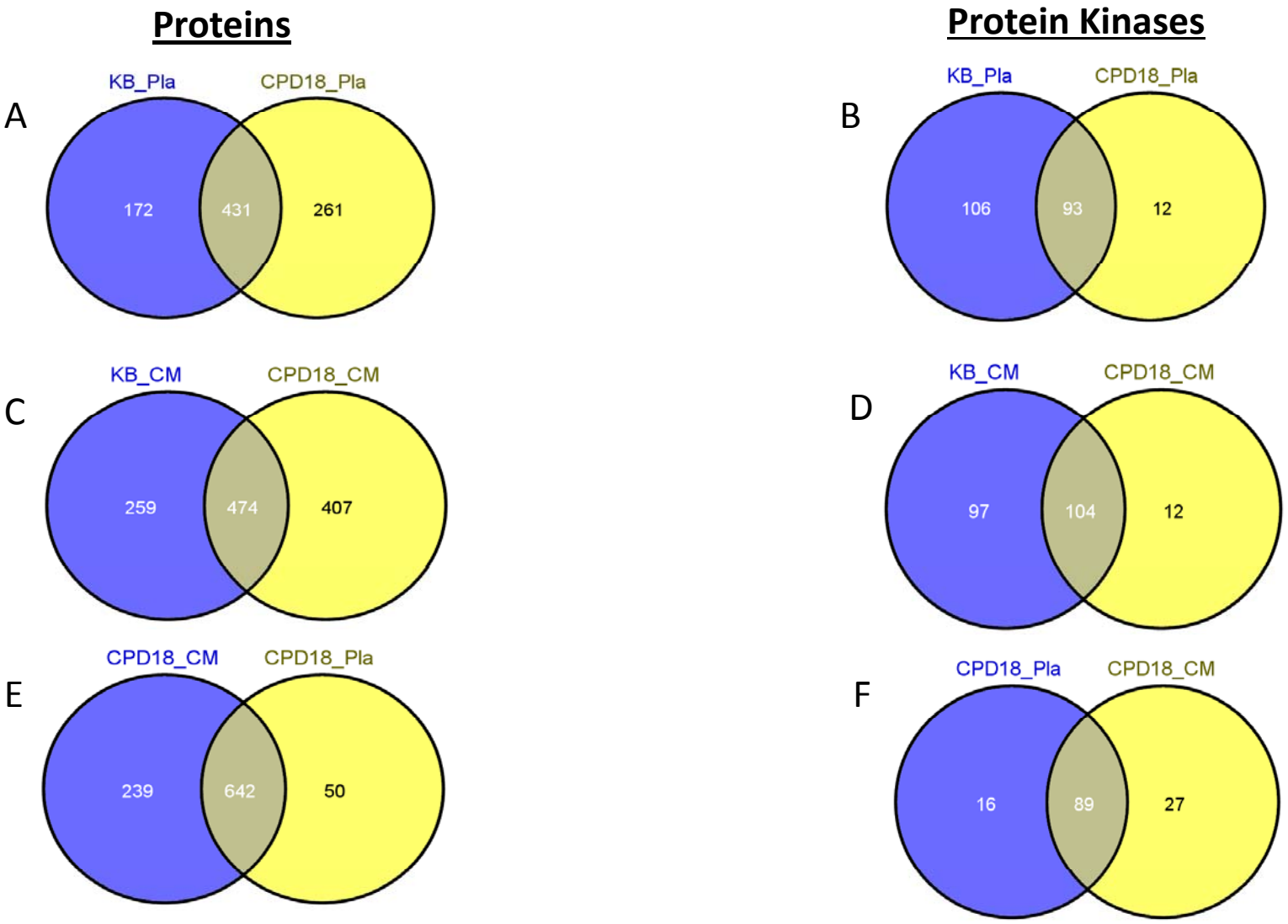
Supplemental Figure S1. Chemical structures of selected VEGFR kinase inhibitors from the literature considered as potential starting points to design VEFGR affinity probe.[7-14]



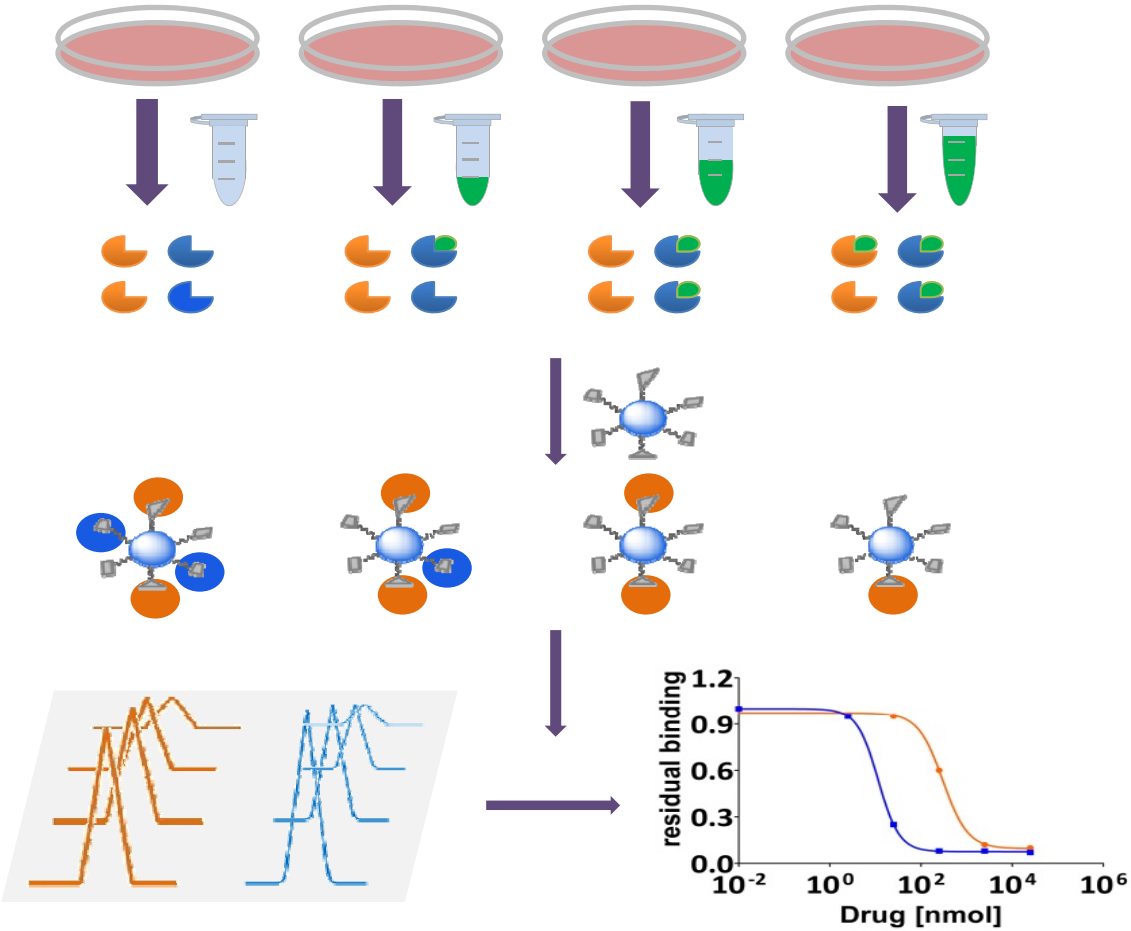
Supplemental Figure S2. Composition of Kinobeads used in this study. Coupling densities were optimized for each compound separately and the beads were combined in equal amounts to yield the probe matrix (kinobeads) suitable for drug profiling.



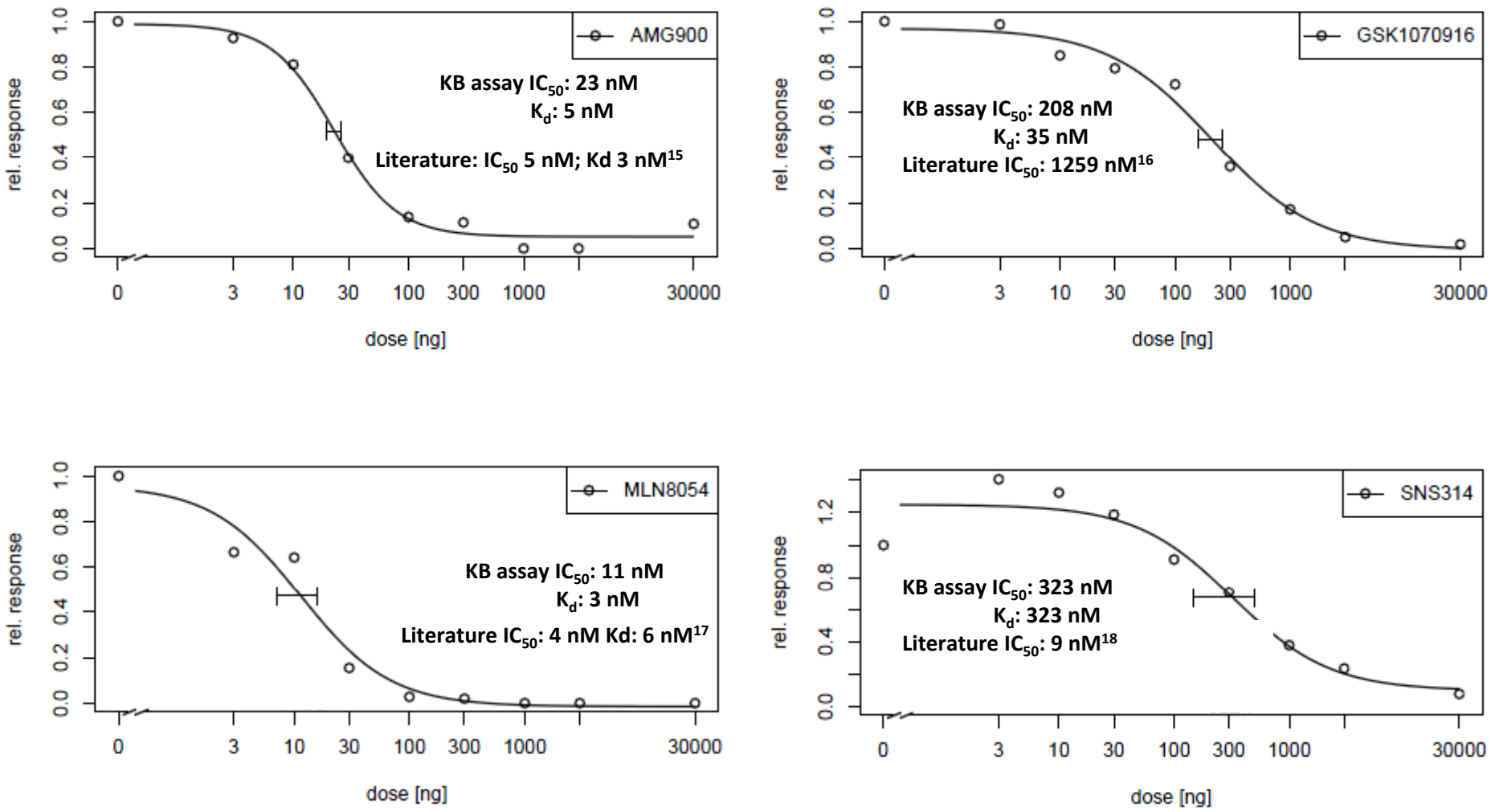
Supplemental Figure S3. Sequence alignment of 16 kinases that were preferentially enriched by Compound 18. The alignment shows that Glu850 (marked by an asterisk) of VEGFR2 is conserved in some but not all kinases.



Supplemental Figure S4. Comparison of proteins and kinases captured by Compound 18 (CPD18) and Kinobeads (KB) in a mixed lysate of four human cancer cell lines (CM) and in placenta lysate (Pla). The reported numbers (nonredundant) are the sum of the duplicates. The Venn diagrams A-D show the comparison between Kinobeads and Compound 18, whereas E and F show the comparison of the numbers of proteins/kinases identified by Compound 18 in two different lysates.



Supplemental Figure S5. Schematic representation of the workflow used for drug selectivity profiling. Lysates are treated with increasing doses of a kinase inhibitor followed by the addition of Kinobeads together with Compound 18 beads. After elution and digestion with trypsin, the proteins are identified and quantified by intensity-based label-free LC-MS/MS analysis. Dose response curves can then be derived from the quantitative MS data. Using the vehicle treated lysate for normalization.



Supplemental Figure S6. Dose response curves for AURKA of four aurora kinase inhibitors determined by Kinobeads (KB) competition assay. The obtained IC_{50} and K_d values are compared to the literature values.[15-18]

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