Electronic Supporting Information for

Rapid profiling of protein kinase inhibitors by quantitative proteomics

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$\qquad$
SILAC cell culture. p1
$\qquad$Affinity enrichment and in solution digest of proteins.p2
Nano LC-MS/MS analysis ..... p2
Database search of MS/MS spectra for peptide \& protein identification ..... p2
Quantification of peptides/proteins and selection of protein hits. ..... p2
General materials and methods - synthesis ..... p3
Synthesis of the affinity reagents ..... p4
Supplementary figures and tables. ..... p9
NMR spectra and analytical chromatograms for the affinity reagents ..... p12
References. ..... p17

## SILAC cell culture

HeLa cells were grown in custom DMEM SILAC medium (Caisson Labs, North Logan, UT) containing either "heavy" ${ }^{13} \mathrm{C}_{6}{ }^{15} \mathrm{~N}_{4}$ Arginine (Cambridge Isotope Labs, Andover MA) and ${ }^{13} \mathrm{C}_{6}{ }^{15} \mathrm{~N}_{2}$ Lysine (Sigma-Isotec, St Louis MO ) or "light" natural isotope abundance cell culture grade arginine and lysine (Fisher, Hampton NH ) for at least five cell doublings as previously described ${ }^{1}$.

## Preparation of cell lysate

Hela cells were grown to confluency on 15 cm dishes, washed twice with DPBS and lysed with 750 ul modified RIPA buffer ( 50 mM Tris, $\mathrm{pH} 7.8,150 \mathrm{mM} \mathrm{NaCl}, 1 \% \mathrm{NP}-40,0.25 \%$ sodium deoxycholate, 1 mM EDTA) containing Halt ${ }^{\text {TM }}$ phorphatase and protease inhibitor cocktail (Themo Scientific, Rockford IL). The lysate was harvested with a cell scraper and vortexed at max. speed occasionally five times while being kept at $4^{\circ} \mathrm{C}$. The lysate was then cleared by centrifugation at 21000 rcf for 20 min . at $4{ }^{\circ} \mathrm{C}$ and the protein content determined using the Pierce 660 nm Protein Assay Reagent.

## Affinity enrichment and in solution digest of proteins

25 ul of a $50 \mathrm{v} \%$ bead slurry of the corresponding sepharose affinity resin was pipetted into a 1.5 ml Eppendorf tube. The beads were washed two times with modified RIPA buffer and stored at $4^{\circ} \mathrm{C}$ until further use. In parallel, $5 \mu$ l of either competitor-DMSO soln. or DMSO alone (vehicle) were pipetted into 1.5 ml Eppendorf tubes and the freshly prepared SILAC-labeled cell lysate ( 250 ul , approx. 1 mg of protein) was added. The tubes were gently agitated on an end-over-end rotator for 20 min . at $4^{\circ} \mathrm{C}$. Then the pretreated lysate was added to the tubes containing the affinity resin and the tubes rotated for 3 h at $4^{\circ} \mathrm{C}$. After completion of the pulldown the beads were spun down with a bench top centrifuge at approx. 1000 rpm and the supernatant was aspirated. The beads were washed twice with cold modified RIPA buffer, then the corresponding heavy and light beads were re-suspended in 50 mM Tris, 150 mM $\mathrm{NaCl}, \mathrm{pH} 7.8$ and combined in one Eppendorf tube. The beads were washed three times with 50 mM Tris, $150 \mathrm{mM} \mathrm{NaCl}, \mathrm{pH} 7.8$ and then $100 \mu \mathrm{l}$ of 8 M urea and TCEP ( $1 \mu \mathrm{M}$ final) were added to each tube. The beads were shaken on a thermo mixer for 20 min . at $37{ }^{\circ} \mathrm{C}$ and CAM ( $2 \mu \mathrm{M}$ final) was added. After further shaking for 20 min . at $37^{\circ} \mathrm{C}$ a second portion of TCEP ( $1 \mu \mathrm{M}$ final) was added and the samples shaken for additional 20 min . at $37^{\circ} \mathrm{C}$. Then, 125 ul 0.1 M TEAB soln. were added (concentration urea <4 M) followed by 1 ug of endoproteinase LysC (Wako USA, Richmond VA). The pH of the solution was brought to pH 8.5 by addition of 1 M NaOH and the mixture shaken for 2 h at $37^{\circ} \mathrm{C}$. Then further $250 \mu \mathrm{l}$ 0.1 M TEAB solution were added (concentration urea <2M) followed by 1 ug of trypsin (Promega, Madison WI) per tube. The tubes were shaken for 16 h , then 500 ul of $5 \mathrm{v} \% \mathrm{aq}$. MeCN soln. containing $0.1 v \%$ TFA were added and the pH of the solution adjusted to approx. 2 by addition of $5 \mathrm{v} \% \mathrm{aq}$. TFA. The peptides were desalted on C 18 StageTips and stored at $4^{\circ} \mathrm{C}$ until further processing.

## Nano LC-MS/MS analysis

Peptides were separated on a Thermo-Dionex RSLCNano UHPLC instrument with 10 cm long fused silica capillary columns made in-house with a laser puller (Sutter, Novato CA) and packed with 3 micron reversed phase C18 beads (Reprosil-C18.aq, Dr. Maisch, Ammerbuch DE). The LC gradient was 90 min long with $3-35 \%$ B at $200 \mathrm{~nL} / \mathrm{min}$. LC solvent A was $0.1 \%$ acetic acid and LC solvent B was $0.1 \%$ acetic acid, $99.9 \%$ acetonitrile. MS data was collected with a Thermo Orbitrap Elite. Data-dependent analysis was applied using Top15 selection with CID fragmentation.

## Database search of MS/MS spectra for peptide \& protein identification

Raw files were analyzed by MaxQuant version 1.3.0.5 using protein, peptide and site FDRs of 0.01 and a score minimum of 40 for modified peptides, 0 for unmodified peptides; delta score minimum of 17 for modified peptides, 0 for unmodified peptides. MS/MS spectra were searched against the UniProt Human Database (updated May 29th, 2013). MaxQuant search parameters: Variable modifications included Oxidation (M), phosphorylation (STY). Carbamidomethyl (C) was a fixed modification. Max labeled amino acids was 3, max missed cleavages was 2, enzyme was Trypsin/P, max charge was 7, multiplicity was 2, SILAC labels were Arg10 and Lys8. The initial search tolerance for FTMS scans was 20 ppm and 0.5 Da for ITMS MS/MS scans.

## Quantification of peptides/proteins and selection of protein hits

Peptides were quantified by the MaxQuant software via their integrated ion chromatograms and proteins assigned an H/L ratio which reflects the median of peptide H/L ratios. Only proteins with two or more quantified peptides were considered for quantification. MaxQuant data was further analyzed
regarding significance $B$ of $\log 2 \mathrm{H} / \mathrm{L}$ ratios (taking into account summed protein intensity and normalized $\mathrm{H} / \mathrm{L}$ ratios) and GO annotation of gene names using the Perseus software package (http://www.perseusframework.org/). Identified proteins were considered "hits" according to the following criteria: (a) when they showed a $B$ significant $\mathrm{H} / \mathrm{L}$ ratio in at least one of the four pull down experiments (quadruplicates), (b) when they were quantified in at least three out of four pull down experiments and (c) when they passed the Students T-test with a $p$-value $<0.05$. Exceptions were made were no more than one outlying $\mathrm{H} / \mathrm{L}$ value caused the p -value to exceed the $<0.05$ threshold. No $p$-values were calculated for "hits" which were only quantified in three out of four experiments. These were assigned as not applicable (na) in ESI Excel file 2.

## General materials and methods for synthesis

Unless noted otherwise all reagents were obtained from commercial suppliers and used as received. ${ }^{1} \mathrm{H}-$ NMR spectra were measured on a Bruker AV-300 or Bruker AV-301 instrument. Chemical shifts are reported in ppm and coupling constants are reported in Hz . Mass spectrometry of the small molecule compounds was performed on a Bruker Esquire Ion Trap MS instrument. For RP-HPLC purification samples were injected on a preparative reverse-phase C 18 column ( $250 \times 21 \mathrm{~mm}$ ) and run over 60 min . at $8 \mathrm{~mL} / \mathrm{min}$. ( $\mathrm{MeCN} / \mathrm{H}_{2} \mathrm{O}$ containing $0.05 \mathrm{v} \%$ TFA, gradient: 1:99 to 100:0). Purified products were detected by UV ( 254 nm ). Analytical HPLC samples were run on a C 18 column ( $150 \times 2.1 \mathrm{~mm}$ ) with $\mathrm{MeCN} / \mathrm{H}_{2} \mathrm{O}$ containing $0.05 \mathrm{v} \%$ TFA and a gradient of 1:99 to 100:0 over 30 min . at a flow rate of $1 \mathrm{ml} / \mathrm{min}$. Bands were detected at 254 nM . Compounds used for our experiments were generally of $95 \%$ purity or higher.

Sepharose affinity resins were prepared as reported previously ${ }^{2}$. Bead loading was determined by measuring the depletion of the affinity reagent from the supernatant by analytical HPLC. Generally the loading was found to be between 0.8 mM and 0.5 mM of the affinity reagent (calculated for $50 \mathrm{v} \%$ bead slurry).

Type II inhibitor fragment S5 and tert-butyl (2-(4-((6-bromoquinazolin-2-yl)amino)phenoxy)ethyl)carbamate $\mathbf{S 3}^{3}$, the dasatinib amine analog $\mathbf{S 2}^{4}$, and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid ${ }^{5}$ were synthesized according to the published protocols.

## General procedure for amide couplings A:

The amine, either as its free base or its TFA-salt was dissolved in dry DMF at a final concentration of approx. 50 mM under an atmosphere of nitrogen. The corresponding carboxylic acid ( 1.5 eq ), HOAt ( 1.5 eq), and DIPEA ( 1.5 eq for coupling of free bases and 3 eq for coupling of TFA-salts) were added and the mixture cooled in an ice-water bath. EDC was added and the mixture stirred for 2 h at $4^{\circ} \mathrm{C}$ and another 12 h at rt . The mixture was diluted with the 10 -fold volume of EtOAc and transferred to a separatory funnel. The organic phase was washed $2 x$ with 1 M aq. $\mathrm{NaOH}, 2 \mathrm{x}$ with 0.5 M aq. $\mathrm{NaH}_{2} \mathrm{PO} 4,1 \mathrm{x}$ with brine, dried over $\mathrm{MgSO}_{4}$ and the solvent removed at the rotary evaporator. The residual crude amide was purified by column chromatography under the conditions indicated below.

## General procedure for Suzuki couplings $\mathbf{B}^{6}$ :

A sealable thick-walled glass tube equipped with a stirring bar was charged with the corresponding bromide ( 1 eq ) and a mixture of DME-water 4:1 $\mathrm{v} / \mathrm{v}$ to give a solution of 0.2 M final concentration. The
aryl boronic acid derivative ( 2 eq ), $\mathrm{Na}_{2} \mathrm{CO}_{3}$ ( 2 eq for coupling of free amines and 4 eq. for ammonium compounds), $\mathrm{Pd}(\mathrm{OAc})_{2}$ dimer $(0.01 \mathrm{eq})$ and $\mathrm{P}(\mathrm{o}-\mathrm{Tol})_{3}(0.02 \mathrm{eq})$ were added and the tube sealed with a septum cap. The vessel was evacuated and flushed with nitrogen alternately five times. The slurry was then heated for 90 min . at $85^{\circ} \mathrm{C}$ in an oil bath with vigorous stirring. After completion of the reaction the residue was diluted with 20 times the volume EtOAc and transferred to a separatory funnel. The organic layer was washed once with 1 M NaOH , once with sat. $\mathrm{NaHCO}_{3}$ and once with brine, dried over $\mathrm{MgSO}_{4}$ and the solvent removed at the rotary evaporator. The residual crude product was purified by column chromatography under the conditions indicated below.

## General procedure for deprotection of N -Boc-protected amines $\mathbf{C}$ :

In a round bottom flask equipped with a stirring bar the corresponding $N$-Boc-protected amine was dissolved in a mixture of dry DCM/TFA $4: 1 \mathrm{v} / \mathrm{v}$ at a concentration of approx. 0.16 M . The solution was stirred for 1 h at RT and the volatiles then removed in a stream of air. The residual TFA salts were dried in high vacuum overnight and used for the synthesis of follow-up compounds without further purification.

## Synthesis of the affinity reagents



Synthesis of 2,2-dimethyl-4,9-dioxo-3,12,15-trioxa-5,8-diazaoctadecan-18-oic acid (S1): Commercially available 3-(2-(3-ethoxy-3-oxopropoxy)ethoxy)propanoic acid ( $250 \mathrm{mg}, 1.07 \mathrm{mmol}$ ) was coupled to N -Boc-ethylenediamine following general procedure $\mathbf{A}$ yielding the crude ethyl ester amide ( $358 \mathrm{mg}, 0.95$ $\mathrm{mmol}, 89 \%$ ) as a colorless oil. The crude ester was dissolved in 6 ml MeOH and 2.85 ml of a 1 M aq. NaOH soln. were added. The mixture was stirred for 2 h at rt and then MeOH removed at the rotary evaporator. The residual solution was saturated with NaCl , brought to pH 2 with $1 \mathrm{Maq} . \mathrm{HCl}$ and transferred to a separatory funnel. The aqueous layer was extracted $5 x$ with 5 ml DCM each, the organic layer dried over $\mathrm{MgSO}_{4}$ and the solvent removed at the rotary evaporator. Title compound $\mathbf{S 1}$ was obtained as a colorless oil and used for follow up syntheses without further purification. ESI-MS: $\mathrm{m} / \mathrm{z}=$ $347.8[\mathrm{M}-\mathrm{H}]^{-}$.


Synthesis of 2-((6-(4-(14-amino-4-oxo-6,9,12-trioxa-3-azatetradecyl)piperazin-1-yl)-2-methylpyrimidin-4$y l) a m i n o)-N$-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (3): Dasatinib amine derivative S2 (20 mg, $41.4 \mu \mathrm{~mol})$ was coupled to 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid according to the standard procedure $\mathbf{A}$. The crude azide ( $27.6 \mathrm{mg}, 39.3 \mu \mathrm{~mol}$ ) was then dissolved in $1 \mathrm{ml} \mathrm{THF} / \mathrm{H}_{2} \mathrm{O} 4: 1 \mathrm{v} / \mathrm{v}$ and $\mathrm{PPh}_{3}$ ( $20.6 \mathrm{mg}, 78.6 \mu \mathrm{~mol}, 2 \mathrm{eq}$ ) was added. The mixture was stirred for 24 h at rt and the solvent removed at the rotary evaporator. The residual crude product was subjected to column chromatography eluting with
$\mathrm{CHCl}_{3}$ containing $13 \mathrm{v} \% \mathrm{MeOH}$ and $2 \mathrm{v} \% 7 \mathrm{M} \mathrm{NH}_{3}$ in MeOH , yielding title compound $\mathbf{3}$ ( 15.7 mg , 22.4 $\mu \mathrm{mol}, 54 \%$ ) as a colorless resin. $\mathrm{R}_{\mathrm{f}}=0.5$ (CHCl3-13 v\% MeOH-2 v\% $7 \mathrm{M} \mathrm{NH3}$ in MeOH). ESI-MS: m/z = $676.9[\mathrm{M}+\mathrm{H}]^{+}$. HPLC: $\mathrm{t}_{\mathrm{R}}=13.3 \mathrm{~min}$., $96.5 \%$.


Synthesis of tert-butyl (2-(4-((6-(5-(cyclopropanecarboxamido)-2-methylphenyl)quinazolin-2-
yl)amino)phenoxy)ethyl)carbamate (S4): Tert-butyl(2-(4-((6-(5-amino-2-methylphenyl)quinazolin-2yl)amino) phenoxy)ethyl)carbamate $\mathbf{S 5}(20 \mathrm{mg}, 41.2 \mu \mathrm{~mol})$ was dissolved in $500 \mu \mathrm{l}$ dry DCM under an atmosphere of nitrogen and the solution cooled in an ice-water bath. Triethylamine ( $6.89 \mu \mathrm{l}, 49.4 \mu \mathrm{~mol}$, 1.2 eq ) was added followed by careful addition of cyclopropylcarbonyl chloride ( $4.5 \mu \mathrm{l}, 49.4 \mu \mathrm{~mol} 1.2 \mathrm{eq}$ ). The mixture was allowed to warm to rt over a period of 1 h , then 100 ul of water and 5 ml EtOAc were added and the mixture was transferred to a separatory funnel. The organic phase was washed $2 x$ with $0.5 \mathrm{M} \mathrm{NaH}_{2} \mathrm{PO}_{4}$ soln., 2 x with sat. $\mathrm{NaHCO}_{3}$ soln. and once with brine, dried over $\mathrm{MgSO}_{4}$ and the solvent removed at the rotary evaporator. The residual crude cyclopropyl amide was purified by column chromatography eluting with EtOAc/hexanes 2:1 v/v, yielding pure title compound $\mathbf{S 4}(19.6 \mathrm{mg}, 35.4$ $\mu \mathrm{mol}, 86 \%)$ as a faint yellow resin. ${ }^{1} \mathrm{H} \operatorname{NMR}\left(300 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 9.09(\mathrm{~s}, 1 \mathrm{H}), 7.98(\mathrm{dd}, J=8.8,2.1 \mathrm{~Hz}, 1 \mathrm{H})$, 7.83 (d, J = $1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.78-7.68(\mathrm{~m}, 3 \mathrm{H}), 7.54-7.47(\mathrm{~m}, 2 \mathrm{H}), 7.37-7.34(\mathrm{~m}, 1 \mathrm{H}), 6.99-6.91(\mathrm{~m}, 2 \mathrm{H}), 6.85-$ $6.79(\mathrm{~m}, 2 \mathrm{H}), 5.06(\mathrm{~s}, 1 \mathrm{H}), 4.06(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 3 \mathrm{H}), 3.61-3.52(\mathrm{~m}, 2 \mathrm{H}), 1.49(\mathrm{~s}, 9 \mathrm{H}), 1.32-1.20(\mathrm{~m}, 2 \mathrm{H})$, $1.00-0.85(\mathrm{~m}, 2 \mathrm{H})$. ESI-MS: $\mathrm{m} / \mathrm{z}=586.4[\mathrm{M}+\mathrm{Na}]^{+}$.


Synthesis of tert-butyl (2-(4-((6-(3-aminophenyl)quinazolin-2-yl)amino)phenoxy)ethyl)carbamate (S6): (2-(4-((6-bromoquinazolin-2-yl)amino)phenoxy)ethyl)carbamate S3 ( $25 \mathrm{mg}, 54.4 \mu \mathrm{~mol}$ ) was coupled to (3aminophenyl)boronic acid following the general procedure for Suzuki couplings B. Purification by silica gel column chromatography eluting with EtOAc/hexanes 1:1 v/v yielded aniline S6 ( $21 \mathrm{mg}, 44.6 \mu \mathrm{~mol}$, $82 \%)$ as a yellow resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) $\delta 8.97(\mathrm{~s}, 1 \mathrm{H}), 7.88-7.50(\mathrm{~m}, 5 \mathrm{H}), 7.36(\mathrm{~s}, 1 \mathrm{H}), 6.84-696$ $(\mathrm{m}, 2 \mathrm{H}), 5.02(\mathrm{~s}, 1 \mathrm{H}), 4.03(\mathrm{t}, \mathrm{J}=4.9 \mathrm{~Hz}, 2 \mathrm{H}), 3.45-3.6(\mathrm{~m}, 2 \mathrm{H}), 1.46(\mathrm{~s}, 9 \mathrm{H}) . \mathrm{ESI}-\mathrm{MS}: \mathrm{m} / \mathrm{z}=472.3[\mathrm{M}+\mathrm{H}]^{+}$.


Synthesis of tert-butyl (2-(4-((6-(4-aminophenyl)quinazolin-2-yl)amino)phenoxy)ethyl)carbamate (S7): (2-(4-((6-bromoquinazolin-2-yl)amino)phenoxy)ethyl)carbamate $\mathbf{S 3}(25 \mathrm{mg}, 54.4 \mu \mathrm{~mol})$ was coupled to 4boronobenzenaminium chloride acid following the general procedure for Suzuki couplings B. Purification by silica gel column chromatography eluting with EtOAc/hexanes $3: 2 \mathrm{v} / \mathrm{v}$ yielded aniline $\mathbf{S 7}$ ( 19.2 mg ,
$40.7 \mu \mathrm{~mol}, 75 \%)$ as a yellow resin. ${ }^{1} \mathrm{H} \mathrm{NMR}\left(300 \mathrm{MHz}, \mathrm{CDCl}_{3}\right) \delta 9.09(\mathrm{~s}, 1 \mathrm{H}), 7.98(\mathrm{dd}, \mathrm{J}=8.8,2.1 \mathrm{~Hz}, 1 \mathrm{H})$, $7.83(\mathrm{~d}, J=1.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.68-7.78(\mathrm{~m}, 3 \mathrm{H}), 7.48-7.54(\mathrm{~m}, 2 \mathrm{H}), 7.34(\mathrm{~s}, 1 \mathrm{H}), 6.91-6.99(\mathrm{~m}, 2 \mathrm{H}), 6.78-6.75$ $(\mathrm{m}, 2 \mathrm{H}), 5.06(\mathrm{~s}, 1 \mathrm{H}), 4.06(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.63-3.52(\mathrm{~m}, 2 \mathrm{H}), 1.49(\mathrm{~s}, 9 \mathrm{H})$. ESI-MS: m/z=472.5 $[\mathrm{M}+\mathrm{H}]^{+}$.


Synthesis of tert-butyl (2-(4-((6-(3-(3-(trifluoromethyl)benzamido)phenyl)quinazolin-2-
yl)amino)phenoxy)ethyl)-carbamate ( $\mathbf{S 8}$ ): m-Aniline $\mathbf{S 6}$ ( $7.5 \mathrm{mg}, 15.9 \mu \mathrm{~mol}$ ) was coupled to 3(trifluoromethyl)benzoic acid according to general procedure A. Silica gel column chromatography eluting with EtOAc/hexane $1: 1 \mathrm{v} / \mathrm{v}$ gave the pure title compound $\mathbf{S 8}(8.9 \mathrm{mg}, 13.8 \mu \mathrm{~mol}, 87 \%)$ as a light yellow resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{DMSO}$ ) $\delta 10.61(\mathrm{~s}, 1 \mathrm{H}), 9.79(\mathrm{~s}, 1 \mathrm{H}), 9.35(\mathrm{~s}, 1 \mathrm{H}), 8.41-8.27(\mathrm{~m}, 3 \mathrm{H})$, 8.24-8.16 (m, 2H), $8.11(\mathrm{dd}, J=8.8,2.0 \mathrm{~Hz}, 1 \mathrm{H}), 8.00(\mathrm{~d}, J=7.8 \mathrm{~Hz}, 1 \mathrm{H}), 7.95-7.76(\mathrm{~m}, 4 \mathrm{H}), 7.73(\mathrm{~d}, J=$ $8.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.61-7.46(\mathrm{~m}, 2 \mathrm{H}), 7.02(\mathrm{t}, J=5.4 \mathrm{~Hz}, 1 \mathrm{H}), 6.93(\mathrm{~d}, J=9.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.94(\mathrm{t}, J=5.8 \mathrm{~Hz}, 2 \mathrm{H})$, 3.37-3.24 (m, 2H), $1.40(\mathrm{~s}, 9 \mathrm{H})$. ESI-MS: $\mathrm{m} / \mathrm{z}=644.7[\mathrm{M}+\mathrm{H}]^{+}$.


Synthesis of tert-butyl (2-(4-((6-(3-(3-(3-(trifluoromethyl)phenyl)ureido)phenyl)quinazolin-2-
yl)amino)phenoxy)-ethyl)carbamate (S9): m-Aniline S6 ( $7.5 \mathrm{mg}, 15.9 \mu \mathrm{~mol}$ ) was dissolved in 0.5 ml dry THF under an atmosphere of nitrogen. The soln. was cooled to $4^{\circ} \mathrm{C}$ in an ice-water bath and 1-isocyanato-3-(trifluoromethyl)benzene ( $4.4 \mu \mathrm{l}, 31.8 \mu \mathrm{~mol}, 2 \mathrm{eq}$ ) was added. The mixture was stirred for 1 h at $4^{\circ} \mathrm{C}$ and an additional 2 h at rt . Then the solvent was removed at the rotary evaporator and the residue subjected to silica gel column chromatography eluting with EtOAc/hexane $3: 2 \mathrm{v} / \mathrm{v}$. This gave the pure title compound $\mathbf{S 9}(9.84 \mathrm{mg}, 19.4 \mu \mathrm{~mol}, 94 \%)$ as a light yellow resin. ${ }^{1} \mathrm{H} \mathrm{NMR}(300 \mathrm{MHz}, \mathrm{MeOD}) \delta$ $9.19(\mathrm{~s}, 1 \mathrm{H}), 8.11-8.04(\mathrm{~m}, 2 \mathrm{H}), 7.98-7.88(\mathrm{~m}, 2 \mathrm{H}), 7.78-7.66(\mathrm{~m}, 3 \mathrm{H}), 7.62(\mathrm{~d}, \mathrm{~J}=9.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.48(\mathrm{t}, \mathrm{J}=$ $7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.44-7.36(\mathrm{~m}, 3 \mathrm{H}), 7.30(\mathrm{~d}, \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.00-6.92(\mathrm{~d}, 1 \mathrm{H}), 4.02(\mathrm{t}, \mathrm{J}=5.6 \mathrm{~Hz}, 1 \mathrm{H}), 3.48-3.40$ (m, 1H), 1.46 (s, 9H). ESI-MS: m/z = $659.5[\mathrm{M}+\mathrm{H}]^{+}$.


Synthesis of tert-butyl (2-(4-((6-(4-(3-(trifluoromethyl)benzamido)phenyl)quinazolin-2-yl)amino)phenoxy)ethyl)-carbamate (S10): p-Aniline S7 (7.5 mg, $15.9 \mu \mathrm{~mol}$ ) was coupled to 3(trifluoromethyl)benzoic acid according to the general procedure A. Silica gel column chromatography eluting with EtOAc/hexanes $2: 3 \mathrm{v} / \mathrm{v}$ gave the pure title compound $\mathbf{S 1 0}(8.49 \mathrm{mg}, 13.2 \mu \mathrm{~mol}, 83 \%$ ) as a light yellow resin. ${ }^{1} \mathrm{H}$ NMR ( $\left.300 \mathrm{MHz}, \mathrm{MeOD}\right) \delta 9.04(\mathrm{~s}, 1 \mathrm{H}), 8.25(\mathrm{~s}, 1 \mathrm{H}), 8.17(\mathrm{~d}, \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 8.0-7.92$ $(\mathrm{m}, 1 \mathrm{H}), 7.92-7.82(\mathrm{~m}, 2 \mathrm{H}), 7.82-7.74(\mathrm{~m}, 2 \mathrm{H}), 7.74-7.52(\mathrm{~m}, 6 \mathrm{H}), 6.90(\mathrm{~d}, \mathrm{~J}=8.9 \mathrm{~Hz}, 2 \mathrm{H}), 4.01(\mathrm{t}, \mathrm{J}=5.2$ $\mathrm{Hz}, 2 \mathrm{H}), 3.72(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.68(\mathrm{t}, \mathrm{J}=5.0 \mathrm{~Hz}, 2 \mathrm{H}), 3.62-3.50(\mathrm{~m}, 5 \mathrm{H}), 3.46(\mathrm{t}, \mathrm{J}=5.8 \mathrm{~Hz}, 2 \mathrm{H}), 3.05(\mathrm{t}, \mathrm{J}$ $=5.8 \mathrm{~Hz}, 2 \mathrm{H}), 2.55-2.38(\mathrm{~m}, 4 \mathrm{H})$. ESI-MS: $\mathrm{m} / \mathrm{z}=644.7[\mathrm{M}+\mathrm{H}]^{+}$.


Synthesis of N-(3-(2-((4-((16-amino-4,13-dioxo-7,10-dioxa-3,14-diazahexadecyl)oxy)phenyl)amino)quinazolin-6-yl)-4-methylphenyl)-3-(trifluoromethyl)benzamide trifluoroacetic acid salt (4): N-Boc-protected precursor S5 (20 $\mathrm{mg}, 30.4 \mu \mathrm{~mol}$ ) was treated according to the general procedure $\mathbf{C}$. The corresponding crude TFA salt of $\mathbf{S} 5$ was then coupled to linker $\mathbf{S 1}$ according to the general procedure $\mathbf{A}$. The crude product was subjected to silica gel column chromatography eluting with $\mathrm{CHCl}_{3}$ containing $4 v \% \mathrm{MeOH}$ to give the $N$-Bocprotected precursor as a yellow resin which was directly subjected to $N$-Boc-deprotection according to general procedure C. Pure title compound $4(25.2 \mathrm{mg}, 28.0 \mu \mathrm{~mol}, 92 \%)$ was obtained as an orange resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta 9.17(\mathrm{~s}, 1 \mathrm{H}), 8.26(\mathrm{~s}, 1 \mathrm{H}), 8.21(\mathrm{~d}, \mathrm{~J}=7.6 \mathrm{~Hz}, 1 \mathrm{H}), 7.95-7.85(\mathrm{~m}, 1 \mathrm{H}), 7.85-$ $7.65(\mathrm{~m}, 7 \mathrm{H}), 7.62(\mathrm{dd}, J=8.2,2.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.33(\mathrm{~d}, \mathrm{~J}=8.3 \mathrm{~Hz}, 1 \mathrm{H}), 7.04-6.93(\mathrm{~m}, 2 \mathrm{H}), 4.07(\mathrm{t}, J=5.4 \mathrm{~Hz}$, $2 \mathrm{H}), 3.79-3.66(\mathrm{~m}, 6.0 \mathrm{~Hz}, 4 \mathrm{H}), 3.65-3.51(\mathrm{~m}, 5 \mathrm{H}), 3.38(\mathrm{t}, \mathrm{J}=5.9 \mathrm{~Hz}, 2 \mathrm{H}), 2.92(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.49(\mathrm{t}, \mathrm{J}$ $=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.44(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.30(\mathrm{~s}, 3 \mathrm{H}) . \mathrm{ESI}-\mathrm{MS}: \mathrm{m} / \mathrm{z}=788.8[\mathrm{M}+\mathrm{H}]^{+} . \mathrm{HPLC}: \mathrm{t}_{\mathrm{R}}=16.3 \mathrm{~min} ., 99.7 \%$.


Synthesis of N -(3-(2-)((4-)(16-amino-4,13-dioxo-7,10-dioxa-3,14-diazahexadecyl)oxy)phenyl)amino)quinazolin-6-yl)-4-methylphenyl)cyclopropanecarboxamide trifluoroacetic acid salt (5): $N$-Boc-protected precursor S4 ( $15 \mathrm{mg}, 27.1 \mu \mathrm{~mol}$ )was treated according to the general procedure $\mathbf{C}$. The corresponding crude TFA salt of $\mathbf{S 4}$ was then coupled to linker $\mathbf{S 1}$ according to the general procedure $\mathbf{A}$. The crude product was subjected to silica gel column chromatography eluting with $\mathrm{CHCl}_{3}$ containing $4 \mathrm{v} \% \mathrm{MeOH}$ to give the N -Boc-protected precursor as a faint yellow resin which was directly subjected to N -Boc-deprotection according to general procedure $\mathbf{C}$. Pure title compound 5 ( $17.1 \mathrm{mg}, 21.4 \mu \mathrm{~mol}, 79 \%$ ) was obtained as a faint orange resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta 9.18(\mathrm{~s}, 1 \mathrm{H}), 7.92(\mathrm{~s}, 1 \mathrm{H}), 7.84-7.66(\mathrm{~m}, 5 \mathrm{H}), 7.57(\mathrm{~d}, \mathrm{~J}=2.1 \mathrm{~Hz}, 1 \mathrm{H}), 7.44(\mathrm{dd}, \mathrm{J}=8.2,2.2 \mathrm{~Hz}, 1 \mathrm{H}), 7.27$ (d, J = $8.3 \mathrm{~Hz}, 1 \mathrm{H}$ ), 7.04-7.93 (m, 2H), 4.14-4.05 (m, 2H), 3.80-3.67 (m, 4H), 3.67-3.53 (m, 4H), 3.45 $3.35(\mathrm{~m}, 2 \mathrm{H}), 2.94(\mathrm{t}, J=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.51(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.46(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 1 \mathrm{H}), 2.28(\mathrm{~s}, 3 \mathrm{H}), 1.83-$ $1.72(\mathrm{~m}, 1 \mathrm{H}), 1.39-1.27(\mathrm{~m}, 2 \mathrm{H}), 0.92-0.82(\mathrm{~m}, 2 \mathrm{H})$. ESI-MS: $\mathrm{m} / \mathrm{z}=685.0[\mathrm{M}+\mathrm{H}]^{+}$. HPLC: $\mathrm{t}_{\mathrm{R}}=16.5 \mathrm{~min} ., 96.0$ \%.


Synthesis of $N$-(3-(2-((4-((16-amino-4,13-dioxo-7,10-dioxa-3,14-diazahexadecyl)oxy)phenyl)amino)quinazolin-6-yl)phenyl)-3-(trifluoromethyl)benzamide trifluoroacetic acid salt (6): N-Boc-protected precursor S8 (20 mg, 31.1 $\mu \mathrm{mol})$ was treated according to the general procedure $\mathbf{C}$. The corresponding crude TFA salt of $\mathbf{S 8}$ was then coupled to linker $\mathbf{S 1}$ according to the general procedure $\mathbf{A}$. The crude product was subjected to silica gel column chromatography eluting with $\mathrm{CHCl}_{3}$ containing $4 v \% \mathrm{MeOH}$ to give the $N$-Boc-protected
precursor as a yellow resin which was directly subjected to N -Boc-deprotection according to general procedure C. Pure title compound $6(23.7 \mathrm{mg}, 26.7 \mu \mathrm{~mol}, 86 \%)$ was obtained as an orange resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta 9.09(\mathrm{~s}, 1 \mathrm{H}), 8.28(\mathrm{~s}, 1 \mathrm{H}), 8.26-8.18(\mathrm{~m}, 1 \mathrm{H}), 8.09(\mathrm{~s}, 1 \mathrm{H}), 8.04-7.94(\mathrm{~m}, 2 \mathrm{H}), 7.92-$ $7.84(\mathrm{~m}, 1 \mathrm{H}), 7.78-7.66(\mathrm{~m}, 4 \mathrm{H}), 7.63(\mathrm{~d}, \mathrm{~J}=8.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.53-7.38(\mathrm{~m}, 2 \mathrm{H}), 6.99-6.87(\mathrm{~m}, 2 \mathrm{H}), 4.02(\mathrm{t}$, $J=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.72(\mathrm{t}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.67(\mathrm{t}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.63-3.48(\mathrm{~m}, 6 \mathrm{H}), 3.24(\mathrm{t}, J=6.2 \mathrm{~Hz}, 2 \mathrm{H})$, $2.70(\mathrm{t}, \mathrm{J}=6.2 \mathrm{~Hz}, 2 \mathrm{H}), 2.47(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.41(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H})$. ESI-MS: m/z=887.1[M+TFA-H]. HPLC: $\mathrm{t}_{\mathrm{R}}=16.1 \mathrm{~min}$., $100 \%$.


Synthesis of N -(2-aminoethyl)-3-(2-(3-oxo-3-((2-(4-()6-(3-(3-(3--(trifluoromethyl)phenyl)ureido)phenyl)quinazolin-2yl)amino)phenoxy)ethyl)amino)propoxy)ethoxy)propanamide trifluoroacetic acid salt (8): $N$-Boc-protected precursor $\mathbf{S 9}(20 \mathrm{mg}, 30.4 \mu \mathrm{~mol})$ was treated according to the general procedure $\mathbf{C}$. The corresponding crude TFA salt of $\mathbf{S 9}$ was then coupled to linker $\mathbf{S 1}$ according to the general procedure $\mathbf{A}$. The crude product was subjected to silica gel column chromatography eluting with $\mathrm{CHCl}_{3}$ containing $5 \mathrm{v} \% \mathrm{MeOH}$ to give the $N$-Boc-protected precursor as a yellow resin which was directly subjected to N -Boc-deprotection according to general procedure C. Pure title compound $\mathbf{8 ( 2 4 . 4 ~ \mathrm { mg } , 2 7 . 0 \mu \mathrm { mol } , 8 9 \% ) \text { was obtained as an }}$ orange resin. ${ }^{1} \mathrm{H}$ NMR ( $300 \mathrm{MHz}, \mathrm{MeOD}$ ) $\delta 9.09(\mathrm{~s}, 1 \mathrm{H}), 8.04-7.91(\mathrm{~m}, 3 \mathrm{H}), 7.90-7.84(\mathrm{~m}, 1 \mathrm{H}), 7.75-7.68$ $(\mathrm{m}, 2 \mathrm{H}), 7.66-7.55(\mathrm{~m}, 2 \mathrm{H}), 7.44(\mathrm{t}, \mathrm{J}=7.9 \mathrm{~Hz}, 1 \mathrm{H}), 7.40-7.30(\mathrm{~m}, 3 \mathrm{H}), 7.27(\mathrm{~d}, \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 6.96-6.85$ $(\mathrm{m}, 2 \mathrm{H}), 4.02(\mathrm{t}, \mathrm{J}=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.71(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.67(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 3.62-3.48(\mathrm{~m}, 5 \mathrm{H}), 3.29(\mathrm{t}$, $J=6.1 \mathrm{~Hz}, 1 \mathrm{H}), 2.78(\mathrm{t}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.48(\mathrm{t}, J=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.41(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}) . \mathrm{ESI}-\mathrm{MS}: \mathrm{m} / \mathrm{z}=902.0$ [M+TFA-H]. HPLC: $\mathrm{t}_{\mathrm{R}}=16.3 \mathrm{~min} ., 100 \%$.


Synthesis of N-(4-(2-((4-((16-amino-4,13-dioxo-7,10-dioxa-3,14-diazahexadecyl)oxy)phenyl)amino)quinazolin-6-yl)phenyl)-3-(trifluoromethyl)benzamide trifluoroacetic acid salt (7): $N$-Boc-protected precursor S10 (20 mg, $31.1 \mu \mathrm{~mol}$ ) was treated according to the general procedure $\mathbf{C}$. The corresponding crude TFA salt of S10 was then coupled to linker $\mathbf{S 1}$ according to the general procedure $\mathbf{A}$. The crude product was subjected to silica gel column chromatography eluting with $\mathrm{CHCl}_{3}$ containing $5 \mathrm{v} \% \mathrm{MeOH}$ to give the N -Boc-protected precursor as a yellow resin which was directly subjected to N -Boc-deprotection according to general procedure C. Pure title compound $\mathbf{7}(19.6 \mathrm{mg}, 22.1 \mu \mathrm{~mol}, 71 \%)$ was obtained as an orange resin. ${ }^{1} \mathrm{H}$ NMR (300 MHz, MeOD) $\delta 9.13(\mathrm{~s}, 1 \mathrm{H}), 8.07-7.88(\mathrm{~m}, 4 \mathrm{H}), 7.79-7.76(\mathrm{~m}, 2 \mathrm{H}), 7.69-7.52(\mathrm{~m}, 6 \mathrm{H}), 7.46(\mathrm{t}, \mathrm{J}=$ $8.0 \mathrm{~Hz}, 1 \mathrm{H}), 7.29(\mathrm{~d}, \mathrm{~J}=7.7 \mathrm{~Hz}, 1 \mathrm{H}), 7.00-6.90(\mathrm{~m}, 2 \mathrm{H}), 4.05(\mathrm{t}, \mathrm{J}=5.4 \mathrm{~Hz}, 2 \mathrm{H}), 3.80-3.65(\mathrm{~m}, 4 \mathrm{H}), 3.63-3.50$ $(\mathrm{t}, \mathrm{J}=6.4 \mathrm{~Hz}, 5 \mathrm{H}), 3.37(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.90(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}, 2 \mathrm{H}), 2.49(\mathrm{t}, \mathrm{J}=6.1 \mathrm{~Hz}, 2 \mathrm{H}), 2.44(\mathrm{t}, \mathrm{J}=6.0 \mathrm{~Hz}$, $2 \mathrm{H})$. ESI-MS: $\mathrm{m} / \mathrm{z}=887.4\left[\mathrm{M}+\right.$ TFA-H]. HPLC: $\mathrm{t}_{\mathrm{R}}=16.5 \mathrm{~min} ., 97.5 \%$.

| Present Study ${ }^{a}$ | Ref. $7^{a}$ | Ref. $3^{\text {b }}$ |
| :---: | :---: | :---: |
| ABL1 | CDK11 | CDK3 |
| ABL2 | CSK | CRKRS |
| CSK | EGFR | CSK |
| DDR2 | EPHA2 | EIF2AK2 |
| EPHA2 | EPHB2 | EPHA2 |
| EPHA5 | EPHB4 | FRK |
| EPHA7 | ERBB3 | LYN |
| EPHB2 | ERBB4 | PTK2 |
| EPHB4 | FRK | SMG1 |
| FER | MAP4K4 | SRC |
| FYN | MAP4K5 | ULK3 |
| GAK | MAPK10 | YES1 |
| LYN | MAPK8 | ZAK |
| MAP3K1 | MST4 |  |
| MAP4K2 | MYLK |  |
| MAPK11 | PAK2 |  |
| MAPK14 | PTK2 |  |
| MLTK | PTK2B |  |
| PTK2 | PTK6 |  |
| PTK2B | RIPK1 |  |
| PTK6 | SLK |  |
| RIPK1 | SRC |  |
| RIPK2 | SRMS |  |
| SIK1 | STK10 |  |
| SRC | STK24 |  |
| TNK1 | STK4 |  |
| TNK2 | SYK |  |
| YES1 | TNIK |  |
| ZAK |  |  |

Supplementary table 1 Kinases (gene names) identified as targets of general type II probe $\mathbf{4}$ in the present study (left panel), target kinases of closely related analogs of 4 found in Ref. ${ }^{7}$ (middle panel) and Ref. ${ }^{3}$ (right panel). ${ }^{a}$ : identified in HeLa cells; ${ }^{b}$ : identified in K562 leukemia cells.


Supplementary figure 1: Venn diagram showing the overlap of target kinases for dasatinib identified in HeLa cells (present study) or in K562 leukemia cells (Ref. ${ }^{3}$ and Ref. ${ }^{7}$ ).

| Gene names | positions within proteins |
| :--- | :--- |
| ABL1 | $50(\mathrm{~S}), 569(\mathrm{~S}), 805(\mathrm{~S}), 808(\mathrm{~S}), 809(\mathrm{~S})$ |
| ABL2 | $631(\mathrm{~S}), 633(\mathrm{~S}), 634(\mathrm{~S}), 936(\mathrm{~S}), 938(\mathrm{~T}), 1050(\mathrm{~T}), 1051(\mathrm{~S}), 1052(\mathrm{~S}), 1054(4)$ |
| CDK1 | $15(\mathrm{Y})$ |
| CDK2 | $158(\mathrm{~T}), 160(\mathrm{~T})$ |
| EPHA7 | $912(\mathrm{~S})$ |
| EPHB2 | $776(\mathrm{~T}), 777(\mathrm{~S}), 780(\mathrm{~T})$ |
| EPHB4 | $769(\mathrm{~S}), 110(\mathrm{~S}), 976(\mathrm{~T})$ |
| FER | $402(\mathrm{Y}), 411(\mathrm{~S})$ |
| FYN | $531(\mathrm{Y})$ |
| GAK | $780(\mathrm{~S})$ |
| LYN | $11(\mathrm{~S}), 13(\mathrm{~S}), 508(\mathrm{Y})$ |
| MAP3K1 | $923(\mathrm{~S})$ |
| MAP3K11 | $702(\mathrm{~T})$ |
| MAP4K2 | $394(\mathrm{~S}), 398(\mathrm{~T})$ |
| MAPK14 | $180(\mathrm{~T}), 182(\mathrm{Y})$ |
| MAPK8 | $179(\mathrm{~S}), 183(\mathrm{~T}), 185(\mathrm{Y}), 377(\mathrm{~S})$ |
| MAPK9 | $183(\mathrm{~T}), 185(\mathrm{Y})$ |
| MET | $990(\mathrm{~S})$ |
| MLTK | $591(\mathrm{~S}), 593(\mathrm{~S})$ |
| RIPK2 | $345(\mathrm{~S}), 348(\mathrm{~S}), 349(\mathrm{~S}), 357(\mathrm{~S}), 527(\mathrm{~S})$ |
| SRC | $17(\mathrm{~S}), 536(\mathrm{Y})$ |
| TNK1 | $509(\mathrm{~T}), 513(\mathrm{~S})$ |
| TNK2 | $595(\mathrm{~T})$ |
| YES1 | $45(\mathrm{~S}), 542(\mathrm{Y})$ |
| Supplemen |  |

Supplementary table 2: The total of 55 phosphorylation sites identified on kinases identified by LC-
MS/MS from affinity enrichment experiments with our probes $\mathbf{1 / 3}$ and 4-8. Cut-off for MaxQuant score was 40; Cut-off for delta score was $17^{8}$.

| Probe | Gene names of kinases |
| :--- | :--- |
| $\mathbf{5}$ | ABL1, CSK, EPHA2/5/7, EPHB4, FER, MAP3K1, MAP4K2, MAPK11, PTK2, PTK2B, <br> PTK6, RIPK1, SIK1 |
| $\mathbf{7}$ | CSK, EPHA2/5/7, EPHB2/4, FER, GAK, MAP3K1, MAPK11/14, PTK2, PTK2B, PTK6, <br> RIPK1, SIK1, TNK2 |

Supplementary table 3: Kinases that were found to interact with probe 4, but not with the probes 5 and 7.






## Chromatogram for probe 3



Peak results :

| Index | Name | Time <br> [Min] | Quantity <br> [\% Area] | Height <br> [mAU] | Area <br> [mAU.Min] | Area \% <br> $[\%]$ |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| 1 | UNKNOWN | 0.18 | 5.93 | 123.3 | 18.2 | 5.927 |
| 2 | UNKNOWN | 0.60 | 0.76 | 11.4 | 2.3 | 0.756 |
| 3 | UNKNOWN | 4.30 | 1.46 | 22.6 | 4.5 | 1.464 |
| 4 | UNKNOWN | 12.61 | 0.95 | 14.6 | 2.9 | 0.951 |
| 5 | UNKNOWN | 13.31 | 90.25 | 976.0 | 276.7 | 90.250 |
| 6 | UNKNOWN | 16.10 | 0.65 | 9.4 | 2.0 | 0.652 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 1157.3 | 306.6 | 100.000 |

Chromatogram for probe 4


## Peak results :

| Index | Name | Time [Min] | Quantity [\% Area] | Height [mAU] | $\begin{array}{r} \text { Area } \\ \text { [mAU.Min] } \end{array}$ | Area \% [\%] |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | UNKNOWN | 7.55 | 0.30 | 2.8 | 0.5 | 0.300 |
| 2 | UNKNOWN | 16.33 | 99.70 | 626.8 | 180.3 | 99.700 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 629.6 | 180.8 | 100.000 |

## Chromatogram for probe 5



Peak results :

| Index | Name | Time <br> [Min] | Quantity <br> [\% Area] | Height <br> [mAU] $]$ | Area <br> [mAU.Min] | Area \% <br> $[\%]$ |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| 1 | UNKNOWN | 7.99 | 0.14 | 1.5 | 0.2 | 0.144 |
| 2 | UNKNOWN | 13.12 | 0.12 | 2.9 | 0.2 | 0.117 |
| 3 | UNKNOWN | 13.19 | 0.45 | 3.6 | 0.7 | 0.447 |
| 4 | UNKNOWN | 14.15 | 95.98 | 475.4 | 152.7 | 95.976 |
| 5 | UNKNOWN | 16.02 | 2.08 | 11.3 | 3.3 | 2.078 |
| 6 | UNKNOWN | 22.59 | 1.24 | 7.5 | 2.0 | 1.237 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 502.1 | 159.1 | 100.000 |

Chromatogram for probe 6


## Peak results :

| Index | Name | Time <br> $[M i n]$ | Quantity <br> [\% Area] | Height <br> [mAU] $]$ | Area <br> [mAU.Min] $]$ | Area \% <br> $[\%]$ |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| 1 | UNKNOWN | 16.06 | 100.00 | 2788.1 | 473.3 | 100.000 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 2788.1 | 473.3 | 100.000 |

## Chromatogram for probe 7



Peak results :

| Index | Name | Time <br> [Min] $]$ | Quantity <br> [\% Area] $]$ | Height <br> [mAU] $]$ | Area <br> [mAU.Min] | Area \% <br> $[\%]$ |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| 1 | UNKNOWN | 3.97 | 0.71 | 2.5 | 0.4 | 0.710 |
| 2 | UNKNOWN | 7.75 | 1.07 | 1.8 | 0.5 | 1.070 |
| 3 | UNKNOWN | 16.51 | 97.51 | 126.5 | 49.8 | 97.508 |
| 4 | UNKNOWN | 17.57 | 0.71 | 2.3 | 0.4 | 0.712 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 133.1 | 51.0 | 100.000 |

Chromatogram for Probe 8


## Peak results :

| Index | Name | Time <br> $[$ Min] $]$ | Quantity <br> $[\%$ Area] | Height <br> $[\mathrm{mAU}]$ | Area <br> [mAU.Min] $]$ | Area \% <br> $[\%]$ |
| :---: | :--- | ---: | ---: | ---: | ---: | ---: |
| 1 | UNKNOWN | 16.25 | 100.00 | 2525.5 | 440.4 | 100.000 |
|  |  |  |  |  |  |  |
| Total |  |  | 100.00 | 2525.5 | 440.4 | 100.000 |

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