

Supplementary Information

From Cancer To Pain Target By Automated Selectivity Inversion Of A Clinical Candidate

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Supplementary Figures

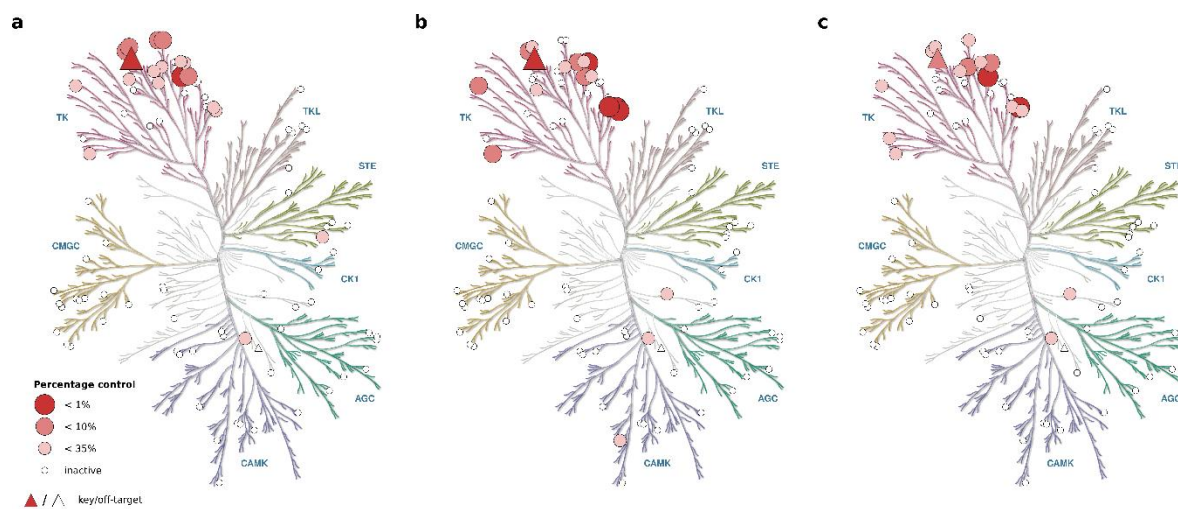


Figure S1. Profiling results at 100 nM. Kinome profiling data of compounds (a) **a4**, (b) **b7**, and (c) **b8**.

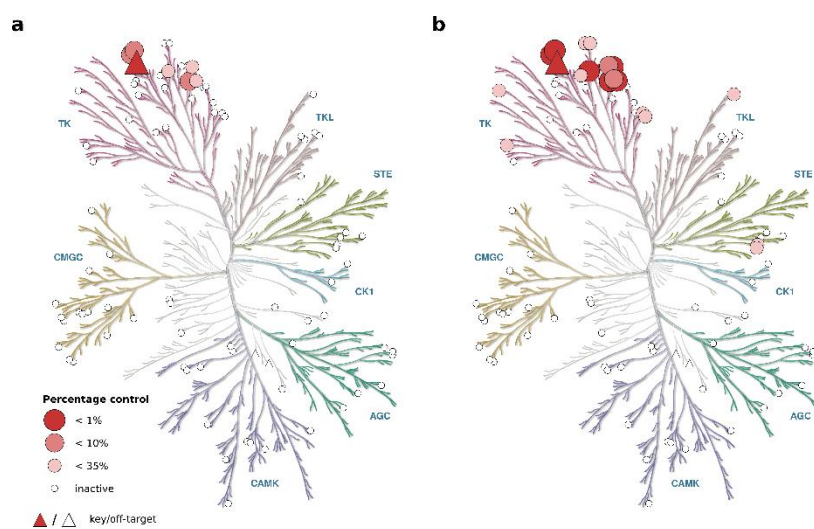
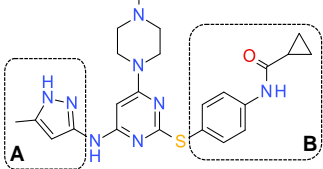
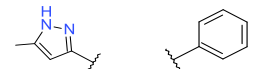

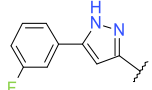

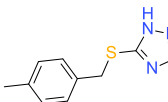
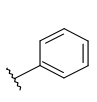
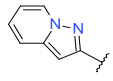

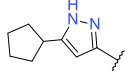

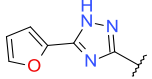

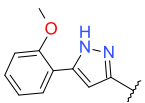
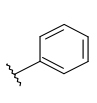
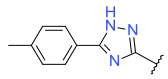

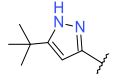

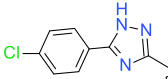



Figure S2. Profiling results of compound **a1** at (a) 100 nM and (b) 1 μ M.

Supplementary Tables

Table S1: Compound set A. Results of primary screens of biological assay.¹

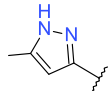

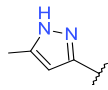

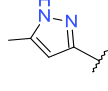

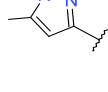

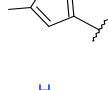

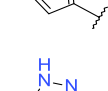

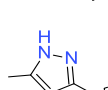

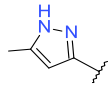

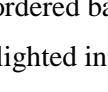

ID	Compound area		% Ctrl. TrkA		% Ctrl. AurA		Rank ²	
	A	B	10 nM	100 nM	100 nM	1 μM	MMP/ML	QM/ML
Tozasertib			31	5.2	0.4	0	--	--
Ref. 2 ³			58	10	15	0.95	--	--
a1			11	1.9	97	92	1	3
a2			99	97	100	100	2	1
a3			93	29	87	76	10	--
a4			15	0.95	49	4.5	31	--
a5			100	100	100	100	46	--
a6			97	94	100	98	47	--
a7			96	95	100	100	54	--
a8			69	18	100	79	55	--
a9			99	100	100	100	--	9

¹ Compounds are ordered based on predicted rank from the *in silico* prediction pipeline.

Top hits are highlighted in bold.

² Predicted rank by MMP/ML approach and QM/ML pipeline, respectively.³ Tozasertib without cyclopropylcarboxamide.

Table S2: Compound set **B**. Results of primary screens of biological assay.¹

ID	Compound area		% Ctrl. TrkA		% Ctrl. AurA		Rank MMP/ML
	A	B	10 nM	100 nM	100 nM	1 μ M	
b1			80	11	7.3	0.5	1
b2			93	31	54	9.4	2
b3			53	11	46	4.5	4
b4			50	6.7	2.6	0.1	7
b5			90	32	36	2.4	13
b6			74	35	63	7.4	19
b7			9.3	0.5	15	0.85	34
b8			35	5.3	30	1.3	38
b9			77	24	51	3.8	45

¹ Compounds are ordered based on predicted rank from the *in silico* prediction pipeline.

Top hits are highlighted in bold.

Table S3: Selectivity scores of top hits derived from experimental profiling data.¹

ID	S(35%)	S(10%)
Tozasertib	0.11	0.04
a1	0.08	0.04
a4	0.21	0.09
b7	0.20	0.11
b8	0.19	0.05

¹ Selectivity score: Number of inhibited kinases / total number of tested wild-type kinases, calculated at activity cut-offs of 35% and 10% of control, respectively. The profiling was done at a screening concentration of 100 nM and the selectivity scores calculated for a panel of 92 wild type kinases.

Table S4: Data set used to train QM/ML approach.

<i>Compounds from set A</i>	
c1(ccnc(Sc2ccc(NC(=O)C3CC3)cc2)n1)Nc1cc(OCC2CC2)[nH]n1	
c1(ccnc(Sc2ccccc2)n1)Nc1cc([C@H]2C[C@H]3CC[C@@H]2C3)[nH]n1	
c1(ccnc(Sc2ccccc2)n1)Nc1n[nH]c(Cc2ccccc2)n1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc2ccc(Cl)cc2n1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(Cc2ccc(F)cc2)s1	c1(ccnc(Sc2ccccc2)n1)Nc1n[nH]c([C@H]2CCCO2)n1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(Cc2ccc(F)c2)s1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc(Cc2ccc(C)cc2)o1
c1(ccnc(Sc2ccccc2)n1)Nc1cc(C2CCC2)[nH]n1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc(Cc2ccco2)[nH]1
c1(ccnc(Sc2ccccc2)n1)Nc1n[nH]c(SCc2ccc(F)cc2)n1	c1(ccnc(Sc2ccccc2)n1)Nc1n[nH]c(N2CCOCC2)n1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(c2ccc(Cl)cc2)n[nH]1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc(CCCO)cn1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc2ccc3c(nc(C)s3)c2s1	c1(ccnc(Sc2ccccc2)n1)Nc1cc(c2ccc(F)cc2F)on1
c1(ccnc(Sc2ccccc2)n1)Nc1ccc(Oc2ccccc2)cn1	c1(ccnc(Sc2ccccc2)n1)Nc1cc2c(en1)CCCC2
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(COc2c(C)ccccc2)o1	c1(ccnc(Sc2ccccc2)n1)Nc1cc(c2ccc(Cl)s2)[nH]n1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(N2CCN(C(=O)C)CC2)s1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc(c2ccccc2)cn1
c1(ccnc(Sc2ccccc2)n1)Nc1ncc(c2ccc(SC)cc2)o1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc(c2ccccc2)n1
c1(ccnc(Sc2ccccc2)n1)Nc1n[nH]c(CCC2CCCC2)n1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc2ccc(C(F)(F)F)cc2o1
c1(ccnc(Sc2ccccc2)n1)Nc1cc(Cc2ccccc2)[nH]n1	c1(ccnc(Sc2ccccc2)n1)Nc1cc(C2CC3(COC3)C2)[nH]n1
c1(ccnc(Sc2ccccc2)n1)Nc1cc(C2CCCC2)[nH]n1	c1(ccnc(Sc2ccccc2)n1)Nc1ncc2c(s1)CC1(CCOCC1)CC2
<i>50 benzyl-derivatives of tozasertib</i>	
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1c(c(en1)Cl)O)F	Toz_2pyridyl_2F3OH4Cl
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc1	Toz_2pyridyl
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc1	Toz_3pyridyl
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccnc1	Toz_4pyridyl
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cc2c(cc1)noc2	Toz_anthrani
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1c(cc(cc1F)F)F	Toz_benzyl_246F
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc1C(=O)C	Toz_benzyl_2Ac
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc1C(F)(F)F	Toz_benzyl_2CF3
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cc(c(cc1Cl)C)F	Toz_benzyl_2Cl4Me5F
c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc1Cl	Toz_benzyl_2Cl

50 benzyl-derivatives of tozasertib

<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1C#N</chem>	Toz_benzyl_2CN
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1C(=O)N</chem>	Toz_benzyl_2CONH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1F</chem>	Toz_benzyl_2F
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1OC</chem>	Toz_benzyl_2Meo
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1C</chem>	Toz_benzyl_2Me
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1N</chem>	Toz_benzyl_2NH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1O</chem>	Toz_benzyl_2OH
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)C(=O)C</chem>	Toz_benzyl_3Ac
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)C(F)(F)F</chem>	Toz_benzyl_3CF3
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)Cl</chem>	Toz_benzyl_3Cl
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)C#N</chem>	Toz_benzyl_3CN
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)C(=O)Nc1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)F</chem>	Toz_benzyl_3CONH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)OC</chem>	Toz_benzyl_3MeO
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)C</chem>	Toz_benzyl_3Me
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)N</chem>	Toz_benzyl_3NH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(c1)O</chem>	Toz_benzyl_3OH
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C(=O)C</chem>	Toz_benzyl_4Ac
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)Br</chem>	Toz_benzyl_4Br
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C(F)(F)F</chem>	Toz_benzyl_4CF3
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)Cl</chem>	Toz_benzyl_4Cl
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C#N</chem>	Toz_benzyl_4CN
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C(=O)N</chem>	Toz_benzyl_4CONH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C1CC1</chem>	Toz_benzyl_4cPr
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)CC</chem>	Toz_benzyl_4Et
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)F</chem>	Toz_benzyl_4F
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)OC</chem>	Toz_benzyl_4MeO
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C</chem>	Toz_benzyl_4Me
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)N</chem>	Toz_benzyl_4NH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)O</chem>	Toz_benzyl_4OH
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)S(=O)(=O)C</chem>	Toz_benzyl_4SO2Me
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)S(=O)(=O)N</chem>	Toz_benzyl_4SO2NH2
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cccc(cc1)C(C)(C)C</chem>	Toz_benzyl_4tBu
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ccccc1</chem>	Toz_benzyl
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cc2c(cc1)[nH]nc2</chem>	Toz_indazole
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1cc2c(cc1)[nH]cc2</chem>	Toz_indole
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)COc1ccccc1</chem>	Toz_phenoxy
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1nc2c(en1)[nH]cn2</chem>	Toz_purine
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CCc1ncncc1</chem>	Toz_pyrimidyl
<chem>c1cc(nc(n1)Sc1ccccc1)Nc1cc([nH]n1)CSc1ccccc1</chem>	Toz_thiophenoxy

Validation results of employed prediction tools

Selectivity grids. A test set was compiled by querying the ChEMBL database for compounds with experimentally measured bioactivity values (IC_{50} , K_d , or K_i) against both TrkA and AurA kinases. Bioactivities were converted to pIC_{50} and compounds with $\Delta pIC_{50} > 1$ were kept, resulting in 14 TrkA-selective and 10 AurA-selective compounds in the final validation set. These compounds were docked and scored by interpolating the energy values from the corresponding TrkA-Aurora selectivity grid. Distribution of scores shows good separation between TrkA- and AurA-selective compounds with a ROC-AUC of 0.74 (**Figure S3**). A selectivity score threshold of 2.28 resulted in the best balanced prediction accuracy in the validation set and was therefore employed in the screening as filtering cut-off.

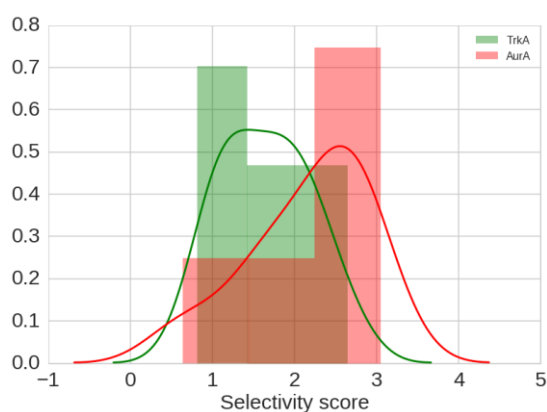


Figure S3: Distribution of selectivity score in TrkA- (green) and AurA-selective compounds (red).

In silico kinase profiling platform. Validation results of the models to detect promiscuous compounds are described in detail in Merget et al., J Med Chem, 2017. Newly trained Aurora A, B and C models were validated in a randomized 5-fold cross-validation at a IC_{50} cutoff of 10 nM (pIC_{50} of 8.0). With AUC values of 0.95 ± 0.01 , 0.91 ± 0.02 and 0.92 ± 0.02 , respectively, all three models indicate very high predictive power for new compounds.

MMP/ML. 5-fold cross validation on 23,000 fragment-based MMPs of TrkA compounds and a “new fragments” scenario indicated a good prediction power with $R^2_{ext} = 0.9 \pm 0.02$ (i.e. the validation was designed such that each test set contained only MMPs with fragments that were not part of the training set).

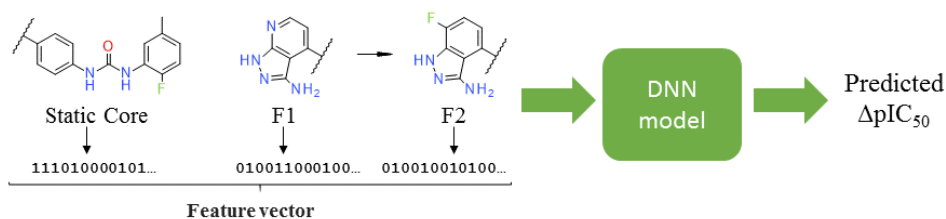


Figure S4: Encoding of MMP as feature vector. Static core, fragment F1 and fragment F2 are each individually encoded as Morgan fingerprint and finally concatenated. The MMP fingerprint is then fed into a Deep Neural Network (DNN), which is predicting ΔpIC_{50} for new compounds compared to the reference compound tozasertib.

QM/ML. A leave-one-out cross-validation on the 80-compounds containing training set resulted into a moderate prediction power for the prediction of absolute PIE values ($R^2_{\text{ext}} = 0.38$). Encouragingly, the CV indicates reasonable predictive power for compound ranking (Spearman correlation of $\rho = 0.66$; $p \ll 0.001$).

Biological assays and assay results

Profiling assay panel: ABL1(T315I)-phosphorylated, ABL1-nonphosphorylated, ABL1-phosphorylated, ACVR1B, ADCK3, AKT1, AKT2, ALK, AURKA, AURKB, AXL, BMPR2, BRAF, BRAF (V600E), BTK, CDK11, CDK2, CDK3, CDK7, CDK9, CHEK1, CSF1R, CSNK1D, CSNK1G2, DCAMKL1, DYRK1B, EGFR, EGFR (L858R), EPHA2, ERBB2, ERBB4, ERK1, FAK, FGFR2, FGFR3, FLT3, GSK3B, IGF1R, IKK-alpha, IKK-beta, INSR, JAK2 (JH1domain-catalytic), JAK3 (JH1domain-catalytic), JNK1, JNK2, JNK3, KIT, LKB1, MAP3K4, MAPKAPK2, MARK3, MEK1, MEK2, MET, MKNK1, MKNK2, MLK1, p38-alpha, p38-beta, PAK1, PAK2, PAK4, PCTK1, PDGFRA, PDGFRB, PDPK1, PIK3C2B, PIK3CA, PIK3CG, PIM1, PIM2, PIM3, PKAC-alpha, PLK1, PLK3, PLK4, PRKCE, RAF1, RET, RIOK2, ROCK2, RSK2 (Kin.Dom.1-N-terminal), SNARK, SRC, SRPK3, TGFBR1, TIE2, TRKA, TRKB, TRKC, TSSK1B, TYK2 (JH1domain-catalytic), ULK2, VEGFR2, YANK3, ZAP70

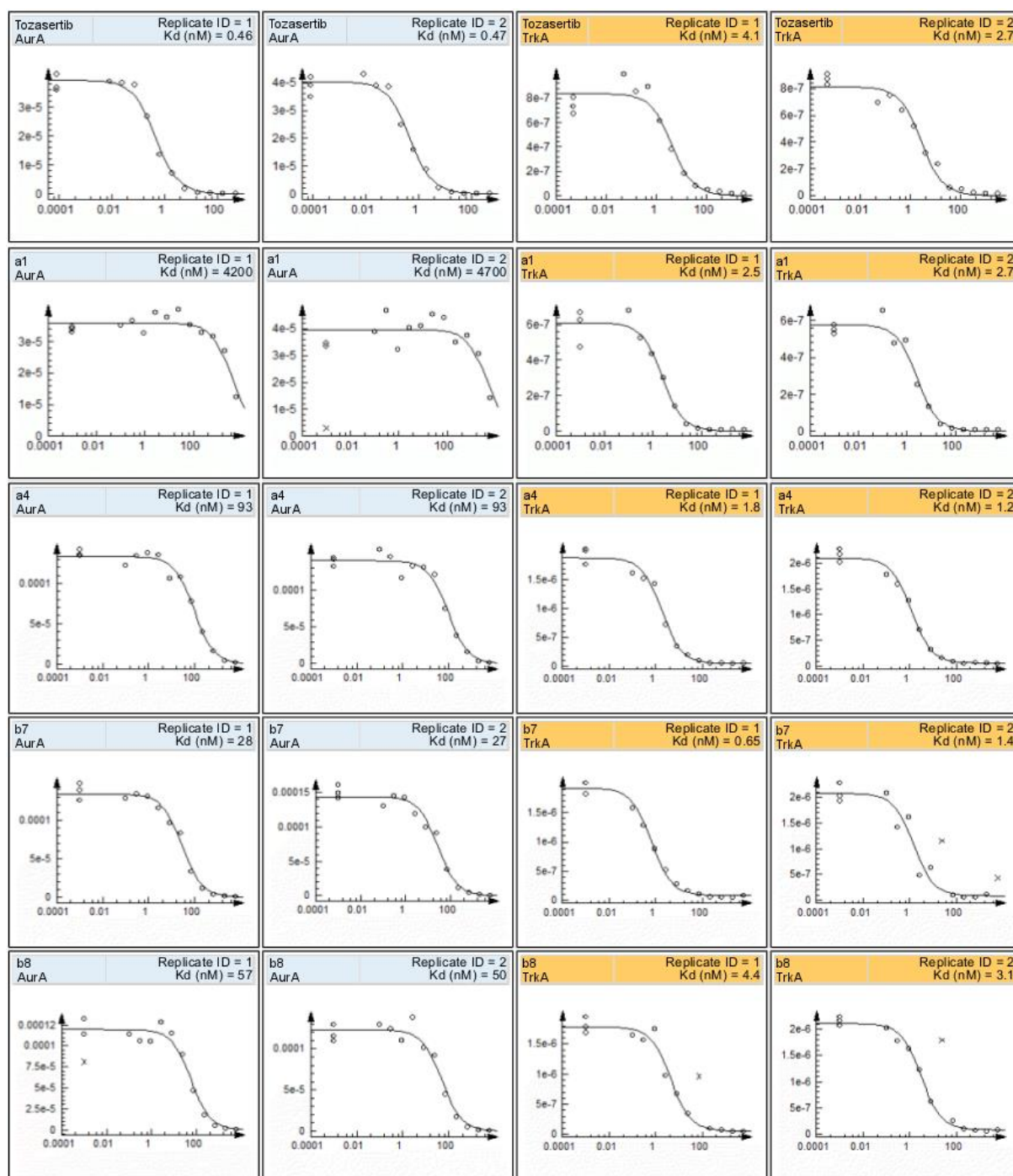


Figure S5. Dose response curves used for Kd determination. Kd values were determined in two replicates and the average value is reported in the article.

Cell-based functional assay. The two compounds **a1** and **b7** were additionally tested in a functional assay using the PathHunter® technology (DiscoverX). In this assay, the full-length human TrkA receptor (i.e. including ligand-binding and kinase domains) is expressed as a C-terminal (intracellular) fusion with a small peptide epitope, called ProLink™ (PK). Furthermore, a SH2 domain containing protein (i.e. Shc1) is co-expressed with a larger Enzyme Acceptor (EA) fragment. Activation of the TrkA receptor by adding the agonist ligand (β -NGF), leads to the auto-phosphorylation of the kinase and subsequent binding of the Shc1-EA protein. That recruitment results in an active B-galactosidase enzyme that is detected by addition of a chemiluminescent substrate. Compounds were tested for antagonism using EC₈₀ concentration (i.e. 0.03 μ g/ml) of β -NGF. Data was normalized to the maximal and minimal response observed in the presence of EC₈₀ agonist ligand (β -NGF) and vehicle (DMSO, final concentration 1%), respectively.

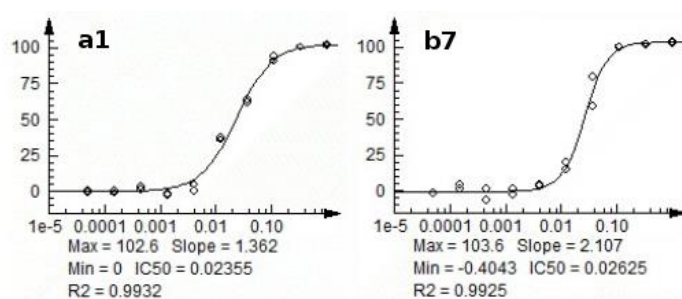


Figure S6. Dose response curves in cell-based functional assay of the compounds **a1** and **b7**.

Analytical data

Tozasertib: *N*-[4-({4-[(5-methyl-1*H*-pyrazol-3-yl)amino]-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl}sulfanyl)phenyl]cyclopropanecarboxamide

¹H NMR (400 MHz, DMSO-*d*₆) δ 0.8 (4H, CH₂), 1.8 (1H, C(=O)CH), 2.0 (3H, ArCH₃), 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.3 (4H, ArN(CH₂)CH₂), 5.4 (1H, ArNHAr), 6.0 (1H, ArH), 7.4 (2H, ArH), 7.7 (2H, ArH), 9.2 (1H, ArH), 10.4 (1H, ArNHCO), 11.7 (1H, ArH). LCMS purity 100%.

Reference 2: *N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.0 (3H, ArCH₃), 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.3 (4H, ArN(CH₂)CH₂), 5.5 (1H, ArNHAr), 6.1 (1H, ArH), 7.5 (3H, ArH), 7.6 (2H, ArH), 9.2 (1H, ArH), 11.7 (1H, ArH). LCMS purity 100%.

a1: *N*-[5-(3-fluorophenyl)-1*H*-pyrazol-3-yl]-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.4 (4H, ArN(CH₂)CH₂), 6.2 (2H, ArNHAr, ArH), 7.2 (1H, ArH), 7.4 (6H, ArH), 7.6 (2H, ArH), 9.5 (1H, ArH), 9.3 (1H, ArH), 12.7 (1H, ArH). LCMS purity 100%.

a2: *N*-(3-[(4-methylphenyl)methyl]sulfanyl)-1*H*-1,2,4-triazol-5-yl)-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (6H, ArCH₃, NCH₃), 2.4 (4H, N(CH₂)CH₂), 3.6 (4H, ArN(CH₂)CH₂), 4.2 (2H, CH₂), 6.5 (1H, ArNHAr), 6.9 (2H, ArH), 7.1 (2H, ArH), 7.3 (2H, ArH), 7.5 (3H, ArH), 7.6 (2H, ArH). LCMS purity 93.0%.

a3: 6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)-*N*-{pyrazolo[1,5-*a*]pyridin-2-yl}pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.4 (4H, ArN(CH₂)CH₂), 5.8 (1H, ArH), 6.1 (1H, ArNHAr), 6.6 (1H, ArH), 7.1 (1H, ArH), 7.5 (2H, ArH), 7.6 (3H, ArH), 8.4 (1H, ArH), 9.8 (1H, ArH). LCMS purity 100%. LCMS purity 98.5%.

a4: *N*-[5-(cyclopentyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 1.4 (2H, cyclopentyl-H), 1.6 (4H, cyclopentyl-H), 1.9 (2H, cyclopentyl-H), 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 2.9 (1H, 2H, cyclopentyl-H), 5.6 (1H, ArNHAr), 6.3 (1H, ArH), 7.4 (3H, ArH), 7.6 (2H, ArH), 9.3 (1H, ArH), 11.9 (1H, ArH). LCMS purity 100%.

a5: *N*-[3-(furan-2-yl)-1*H*-1,2,4-triazol-5-yl]-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, NCH₃), 2.4 (7H, N(CH₂)CH₂), 3.6 (4H, ArN(CH₂)CH₂), 6.6 (2H, ArNHAr, ArH), 6.9 (3H, ArH), 7.3 (1H, ArH), 7.5 (2H, ArH), 7.6 (2H, ArH), 7.8 (1H, ArH). LCMS purity 100%.

a6: *N*-[5-(2-methoxyphenyl)-1*H*-pyrazol-3-yl]-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.3 (4H, ArN(CH₂)CH₂), 3.9 (3H, OCH₃), 6.4 (2H, ArNHAr, ArH), 7.0 (1H, ArH), 7.1 (1H, ArH), 7.4 (5H, ArH), 7.6 (2H, ArH), 9.3 (1H, ArH), 12.3 (1H, ArH). LCMS purity 100%.

a7: *N*-[3-(4-methylphenyl)-1*H*-1,2,4-triazol-5-yl]-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, ArCH₃), 2.3 (7H, N(CH₂)CH₂, NCH₃), 3.3 (4H, ArN(CH₂)CH₂), 6.7 (1H, ArNHAr), 6.9 (1H, ArH), 7.2 (3H, ArH), 7.5 (3H, ArH), 7.7 (2H, ArH), 7.9 (2H, ArH). LCMS purity 100%.

a8: *N*-[5-(*tert*-butyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 1.2 (9H, CH₃), 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 5.7 (1H, ArNHAr), 6.5 (1H, ArH), 7.5 (5H, ArH), 9.2 (1H, ArH), 11.9 (1H, ArH). LCMS purity 100%.

a9: *N*-[5-(4-chlorophenyl)-1*H*-1,2,4-triazol-3-yl]-6-(4-methylpiperazin-1-yl)-2-(phenylsulfanyl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (3H, ArCH₃), 2.4 (7H, N(CH₂)CH₂, NCH₃), 3.6 (4H, ArN(CH₂)CH₂), 6.7 (1H, ArNHAr), 6.9 (2H, ArH), 7.5 (5H, ArH), 7.7 (2H, ArH), 8.0 (2H, ArH). LCMS purity 100%.

b1: 2-[(2,5-dimethylphenyl)sulfanyl]-N-(5-methyl-1H-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.0 (3H, ArCH₃), 2.2 (6H, NCH₃, ArCH₃), 2.4 (3H, ArCH₃), 2.6 (4H, N(CH₂)CH₂), 3.4 (4H, ArN(CH₂)CH₂), 5.4 (1H, ArNHAr), 6.0 (1H, ArH), 7.2 (2H, ArH), 7.4 (1H, ArH), 9.2 (1H, ArH), 11.7 (1H, ArH). LCMS purity 95.8%.

b2: 2-[(2,5-dimethylphenyl)methylsulfanyl]-N-(5-methyl-1H-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.1 (3H, ArCH₃), 2.2 (6H, 2xArCH₃), 2.3 (3H, NCH₃), 2.4 (4H, N(CH₂)CH₂), 3.5 (4H, ArN(CH₂)CH₂), 4.3 (2H, CH₂), 5.9 (1H, ArNHAr), 6.4 (1H, ArH), 7.0 (1H, ArH), 7.1 (1H, ArH), 7.2 (1H, ArH), 9.2 (1H, ArH), 11.9 (1H, ArH). LCMS purity 91.8%.

b3: 2-[(3,4-dimethylphenyl)methylsulfanyl]-N-(5-methyl-1H-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (12H, 3xArCH₃, NCH₃), 2.4 (4H, N(CH₂)CH₂), 3.5 (4H, ArN(CH₂)CH₂), 4.3 (2H, CH₂), 5.9 (1H, ArNHAr), 6.5 (1H, ArH), 7.0 (1H, ArH), 7.1 (1H, ArH), 7.2 (1H, ArH), 9.2 (1H, ArH), 11.9 (1H, ArH). LCMS purity 98.1%.

b4: N-(5-methyl-1H-pyrazol-3-yl)-2-[(3-methylphenyl)sulfanyl]-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.0 (3H, ArCH₃), 2.2 (3H, NCH₃), 2.3 (7H, ArCH₃, N(CH₂)CH₂), 3.3 (4H, ArN(CH₂)CH₂), 5.5 (1H, ArNHAr), 6.1 (1H, ArH), 7.3 (5H, ArH), 9.2 (1H, ArH), 11.7 (1H, ArH). LCMS purity 91.1%.

b5: N-(5-methyl-1H-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-[[3-(trifluoromethyl)phenyl]sulfanyl]pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.0 (3H, ArCH₃), 2.1 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.3 (4H, ArN(CH₂)CH₂), 5.5 (1H, ArNHAr), 6.2 (1H, ArH), 7.7 (1H, ArH), 7.8 (2H, ArH), 7.9 (1H, ArH), 9.3 (1H, ArH), 11.8 (1H, ArH). LCMS purity 95.8%.

b6: N-(5-methyl-1H-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)-2-[(naphthalen-1-ylmethyl)sulfanyl]pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.1 (3H, ArCH₃), 2.2 (3H, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.5 (4H, ArN(CH₂)CH₂), 4.8 (2H, CH₂), 5.9 (1H, ArNHAr), 6.5 (1H, ArH), 7.3 (1H, ArH), 7.6 (2H,

ArH), 7.7 (1H, ArH), 7.8 (1H, ArH), 8.0 (1H, ArH), 8.2 (1H, ArH), 9.3 (1H, ArH), 11.9 (1H, ArH). LCMS purity 100%.

b7: 2-[[*(4-fluorophenyl)methyl*]*sulfanyl*]-*N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.2 (6H, ArCH₃, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.5 (4H, ArN(CH₂)CH₂), 4.3 (2H, CH₂), 5.9 (1H, ArNHAr), 6.5 (1H, ArH), 7.1 (2H, ArH), 7.4 (2H, ArH), 9.2 (1H, ArH), 11.9 (1H, ArH). LCMS purity 95.6%.

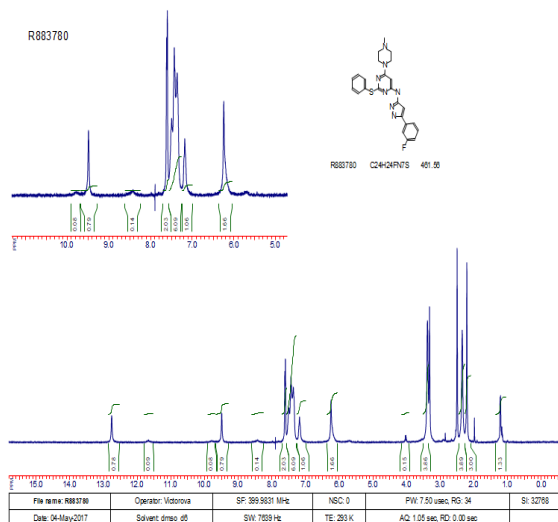
b8: *N*-(5-methyl-1*H*-pyrazol-3-yl)-2-[[*(4-methylphenyl)methyl*]*sulfanyl*]-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.1 (9H, 2xArCH₃, NCH₃), 2.3 (4H, N(CH₂)CH₂), 3.4 (4H, ArN(CH₂)CH₂), 4.3 (2H, CH₂), 5.8 (1H, ArNHAr), 6.4 (1H, ArH), 7.1 (2H, ArH), 7.2 (2H, ArH), 9.2 (1H, ArH), 11.9 (1H, ArH). LCMS purity 92.8%.

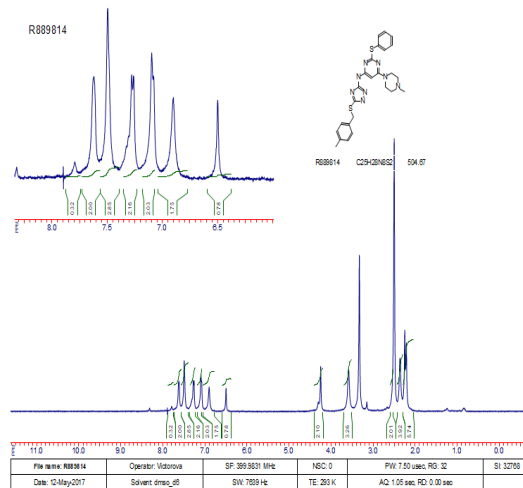
b9: 2-[[*(2,4-dimethylphenyl)sulfanyl*]-*N*-(5-methyl-1*H*-pyrazol-3-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-amine

¹H NMR (400 MHz, DMSO-*d*₆) δ 2.0 (3H, ArCH₃), 2.2 (3H, NCH₃), 2.3 (10H, N(CH₂)CH₂ & ArCH₃), 3.3 (4H, ArN(CH₂)CH₂), 5.4 (1H, ArNHAr), 6.0 (1H, ArH), 7.1 (1H, ArH), 7.2 (1H, ArH), 7.4 (1H, ArH), 9.2 (1H, ArH), 11.7 (1H, ArH). LCMS purity 95.4%.

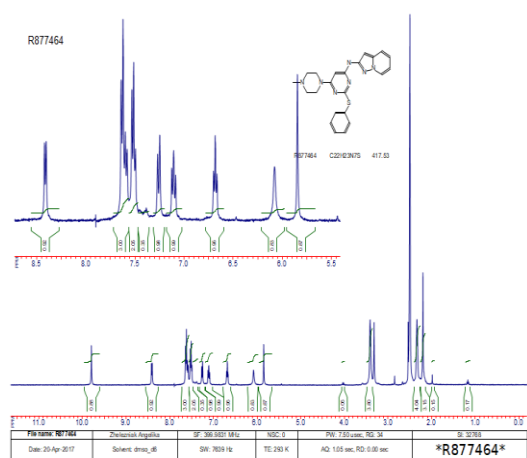
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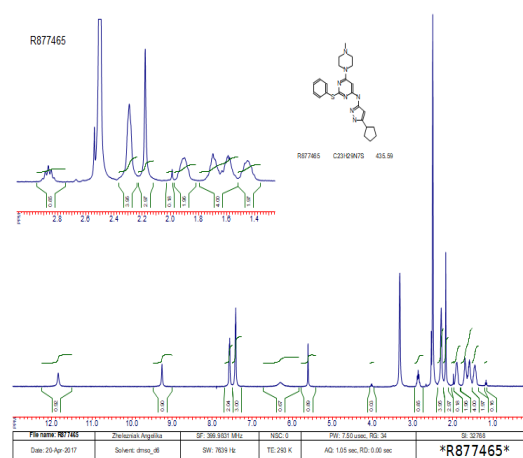
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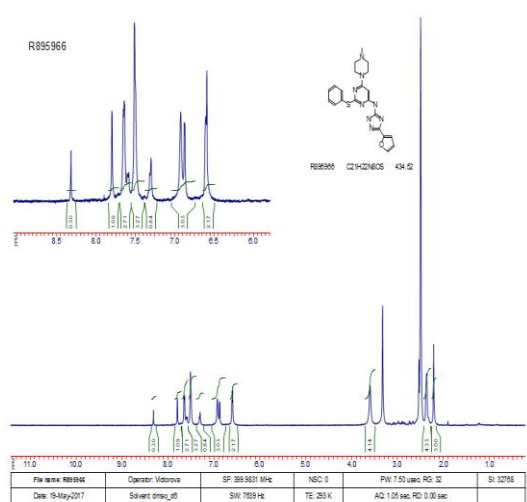
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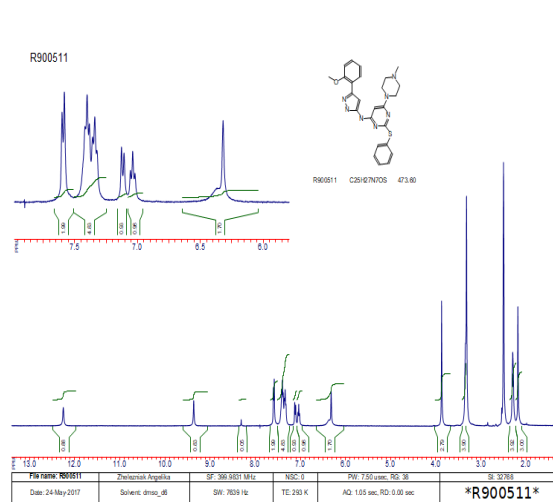
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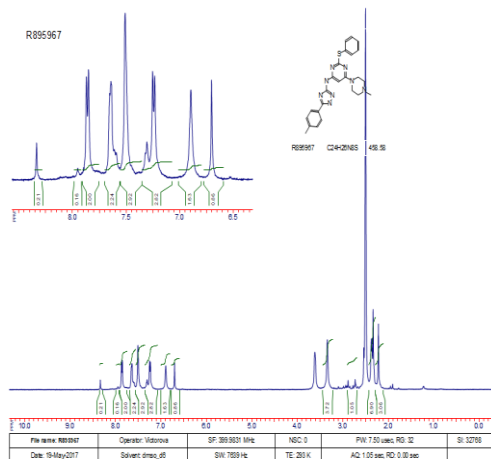
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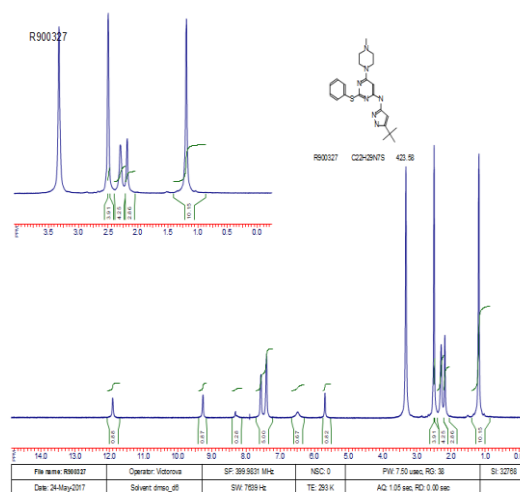
a6



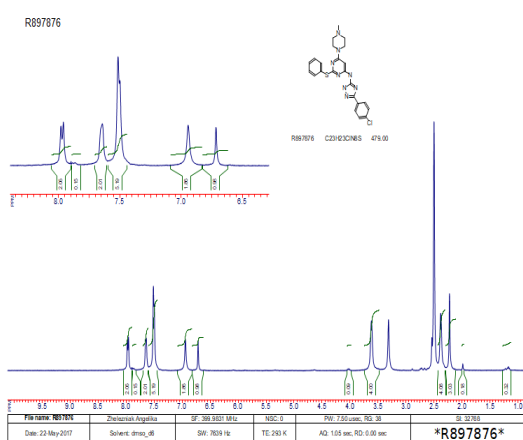
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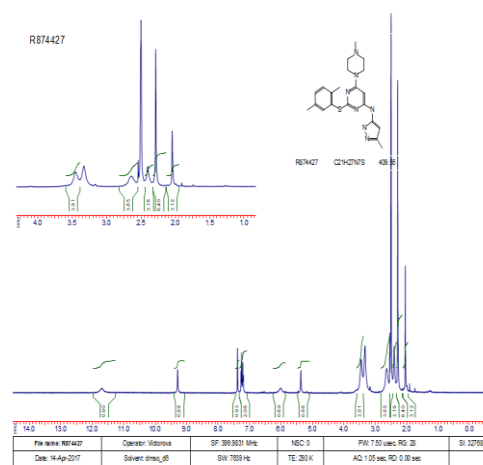
a8



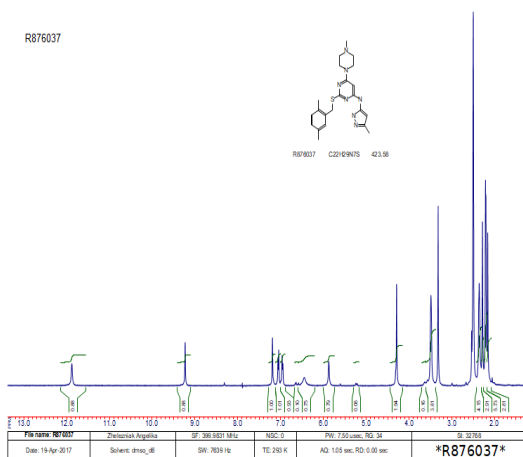
a9



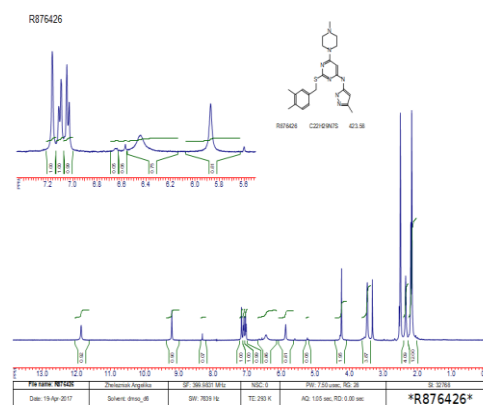
b1



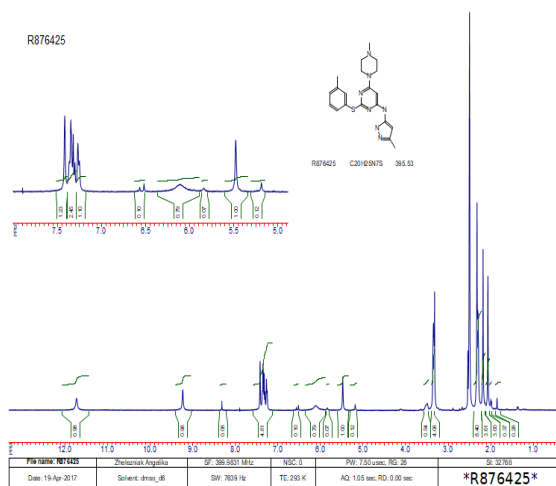
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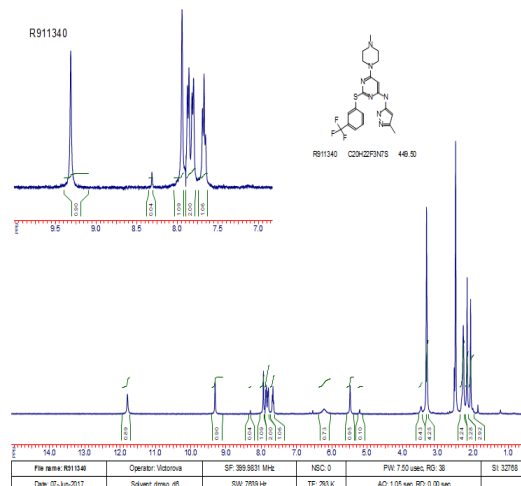
b3



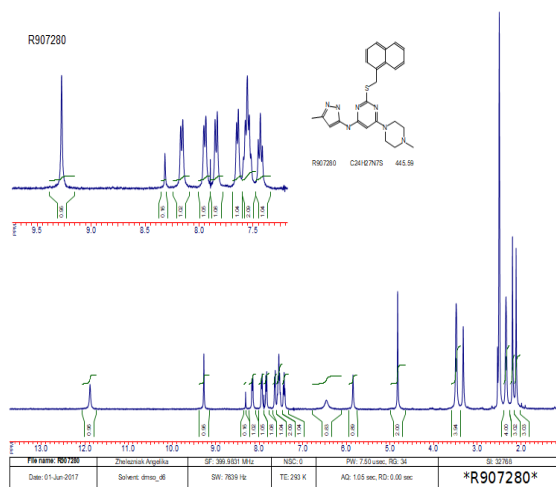
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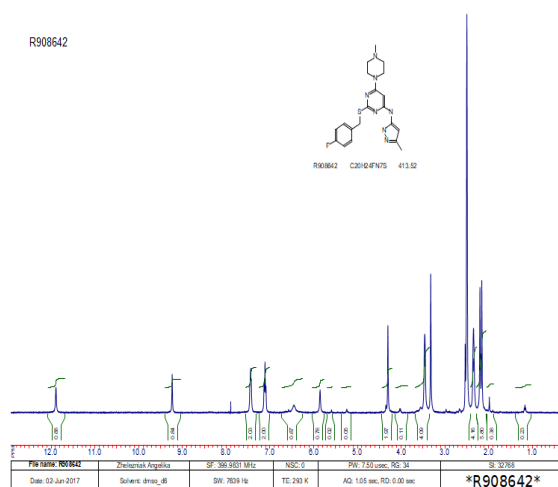
b5



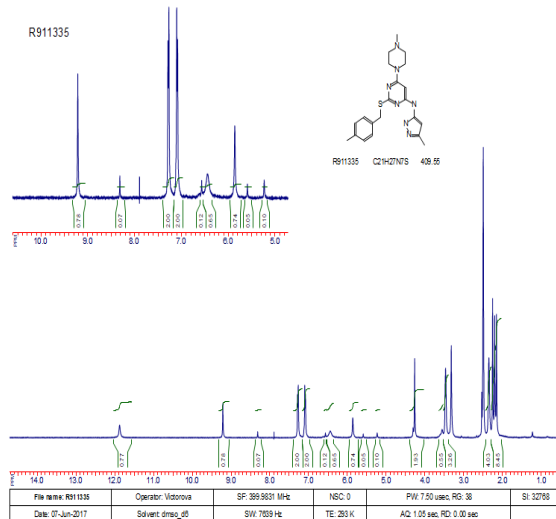
b6



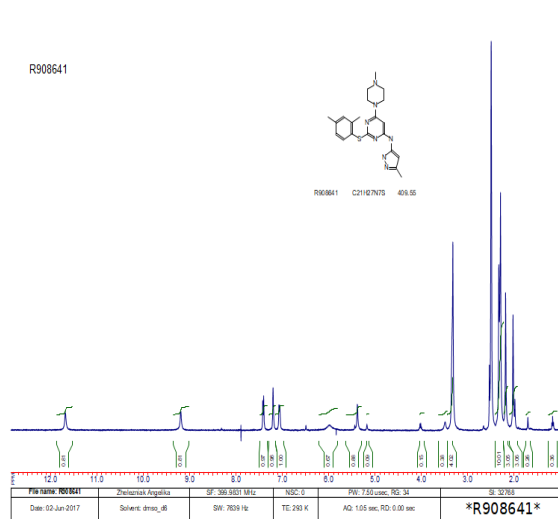
b7



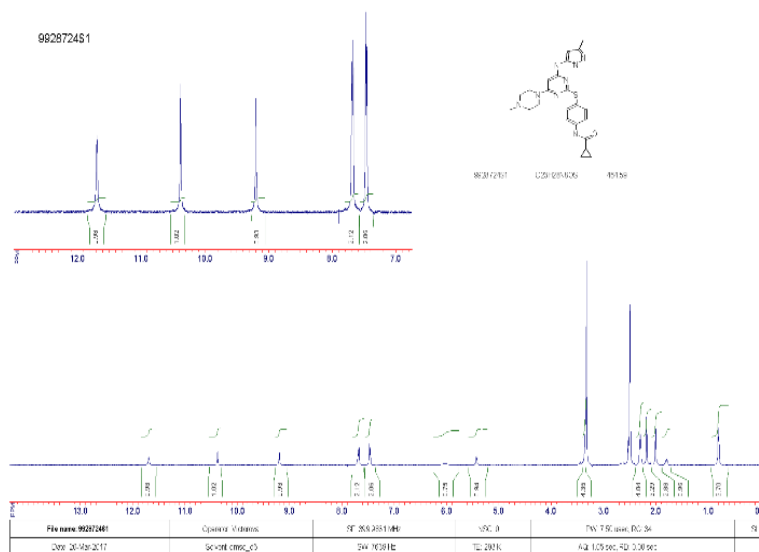
b8



b9



Tozasertib



Reference 2

