

Supplementary Information

High-throughput screening for extracellular inhibitors of the FLT3 receptor tyrosine kinase reveals chemically diverse and druggable negative allosteric modulators.

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Table S1. Filtering rules to select hit-like compounds

Table S2. Purity of validated hits

Figure S1: Dose-response curves of three primary hits in inhibiting the binding of fluorescent-labeled FL-d2 to Lumi4-Tb-SNAP-FLT3 overexpressed in HEK-293 cells.

Figure S2. Sunitinib inhibits the binding of fluorescent-labeled FL-d2 to Lumi4-Tb-SNAP-FLT3 overexpressed in HEK-293 cells.

Figure S3. ^1H -NMR and ^{13}C -NMR spectra of compound **22**

Table S1. Filtering rules to select hit-like compounds

```
##/*****
#Copyright (C) 2000-2005 by OpenEye Scientific Software, Inc.
##/*****/
#This file defines the rules for filtering multi-structure files based on
#properties and substructure patterns.
MIN_MOLWT 150 "Minimum molecular weight"
MAX_MOLWT 750 "Maximum molecular weight"

MIN_NUM_HVY 10 "Minimum number of heavy atoms"
MAX_NUM_HVY 55 "Maximum number of heavy atoms"

MIN_RING_SYS 0 "Minimum number of ring systems"
MAX_RING_SYS 7 "Maximum number of ring systems"

MIN_RING_SIZE 0 "Minimum atoms in any ring system"
MAX_RING_SIZE 20 "Maximum atoms in any ring system"

MIN_CON_NON_RING 0 "Minimum number of connected non-ring atoms"
MAX_CON_NON_RING 20 "Maximum number of connected non-ring atoms"

MIN_FCNGRP 0 "Minimum number of functional groups"
MAX_FCNGRP 20 "Maximum number of functional groups"

MIN_UNBRANCHED 0 "Minimum number of connected unbranched non-ring atoms"
MAX_UNBRANCHED 8 "Maximum number of connected unbranched non-ring atoms"

MIN_CARBONS 5 "Minimum number of carbons"
MAX_CARBONS 40 "Maximum number of carbons"

MIN_HETEROATOMS 2 "Minimum number of heteroatoms"
MAX_HETEROATOMS 20 "Maximum number of heteroatoms"

MIN_Het_C_Ratio 0.10 "Minimum heteroatom to carbon ratio"
MAX_Het_C_Ratio 1.0 "Maximum heteroatom to carbon ratio"

MIN_HALIDE_FRACTION 0.0 "Minimum Halide Fraction"
MAX_HALIDE_FRACTION 0.5 "Maximum Halide Fraction"

#count ring degrees of freedom = (#BondsInRing) - 4 - (RigidBondsInRing) -
(BondsSharedWithOtherRings)
#must be >= 0, from JCAMD 14:251-265,2000.
ADJUST_ROT_FOR_RING true "BOOLEAN for whether to estimate degrees of freedom in rings"

MIN_ROT_BONDS 0 "Minimum number of rotatable bonds"
MAX_ROT_BONDS 20 "Maximum number of rotatable bonds"

MIN_RIGID_BONDS 0 "Minimum number of rigid bonds"
MAX_RIGID_BONDS 50 "Maximum number of rigid bonds"

MIN_HBOND_DONORS 0 "Minimum number of hydrogen-bond donors"
MAX_HBOND_DONORS 6 "Maximum number of hydrogen-bond donors"
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MIN_HBOND_ACCEPTORS 0 "Minimum number of hydrogen-bond acceptors"
MAX_HBOND_ACCEPTORS 10 "Maximum number of hydrogen-bond acceptors"

MIN_LIPINSKI_DONORS 0 "Minimum number of hydrogens on O & N atoms"
MAX_LIPINSKI_DONORS 5 "Maximum number of hydrogens on O & N atoms"

MIN_LIPINSKI_ACCEPTORS 0 "Minimum number of oxygen & nitrogen atoms"
MAX_LIPINSKI_ACCEPTORS 10 "Maximum number of oxygen & nitrogen atoms"

MIN_COUNT_FORMAL_CRG 0 "Minimum number formal charges"
MAX_COUNT_FORMAL_CRG 3 "Maximum number of formal charges"

MIN_SUM_FORMAL_CRG -2 "Minimum sum of formal charges"
MAX_SUM_FORMAL_CRG 2 "Maximum sum of formal charges"

MIN_CHIRAL_CENTERS 0 "Minimum chiral centers"
MAX_CHIRAL_CENTERS 4 "Maximum chiral centers"

MIN_XLOGP -2.0 "Minimum XLogP"
MAX_XLOGP 6.0 "Maximum XLogP"

#choices are insoluble<poorly<moderately<soluble<very<highly
MIN_SOLUBILITY moderately "Minimum solubility"

PSA_USE_SandP true "Count S and P as polar atoms"
MIN_2D_PSA 0.0 "Minimum 2-Dimensional (SMILES) Polar Surface Area"
MAX_2D_PSA 150.0 "Maximum 2-Dimensional (SMILES) Polar Surface Area"

AGGREGATORS true "Eliminate known aggregators"
PRED_AGG false "Eliminate predicted aggregators"

#secondary filters (based on multiple primary filters)
GSK_VEBER true "PSA>140 or >10 rot bonds"
MAX_LIPINSKI 2 "Maximum number of Lipinski violations"
MIN_ABS 0.5 "Minimum probability F>10% in rats"
PHARMACOPIA true "LogP > 5.88 or PSA > 131.6"

ALLOWED_ELEMENTS H,C,N,O,F,S,Cl,Br,I,P
ELIMINATE_METALS Sc,Ti,V,Cr,Mn,Fe,Co,Ni,Cu,Zn,Y,Zr,Nb,Mo,Tc,Ru,Rh,Pd,Ag,Cd

#acceptable molecules must have <= instances of each of the patterns below

#specific, undesirable functional groups

RULE 0 quinone
RULE 0 pentafluorophenyl_esters
RULE 0 paranitrophenyl_esters
RULE 0 HOBT_esters
RULE 0 triflates
RULE 0 lawesson_s_reagent
RULE 0 phosphoramides

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RULE 0 beta_carbonyl_quat_nitrogen
RULE 0 acylhydrazide
RULE 0 cation_C_Cl_I_P_or_S
RULE 0 phosphoryl
RULE 0 alkyl_phosphate
RULE 0 phosphinic_acid
RULE 0 phosphanes
RULE 0 phosphoranes
RULE 0 imidoyl_chlorides
RULE 0 nitroso
RULE 0 N_P_S_Halides
RULE 0 carbodiimide
RULE 0 isonitrile
RULE 0 triacyloxime
RULE 0 cyanohydrins
RULE 0 acyl_cyanides
RULE 0 sulfonylnitrile
RULE 0 phosphorylnitrile
RULE 0 azocyanamides
RULE 0 beta_azo_carbonyl
RULE 0 polyenes
RULE 0 saponin_derivatives
RULE 0 cytochalasin_derivatives
RULE 0 cycloheximide_derivatives
RULE 0 monensin_derivatives
RULE 0 squalestatin_derivatives

#functional groups which often eliminate compounds from consideration

RULE 0 acid_halide
RULE 0 aldehyde
RULE 0 alkyl_halide
RULE 0 anhydride
RULE 0 azide
RULE 0 azo
RULE 0 di_peptide
RULE 0 michael_acceptor
RULE 0 beta_halo_carbonyl
RULE 0 nitro
RULE 0 oxygen_cation
RULE 0 peroxide
RULE 0 phosphonic_acid
RULE 0 phosphonic_ester
RULE 0 phosphoric_acid
RULE 0 phosphoric_ester
RULE 0 sulfonic_acid
RULE 0 sulfonic_ester
RULE 0 tricarbo_phosphene
RULE 0 epoxide
RULE 0 sulfonyl_halide
RULE 0 halopyrimidine
RULE 0 perhalo_ketone

RULE 0 aziridine
RULE 1 oxalyl
RULE 0 alphahalo_amine
RULE 0 halo_amine
RULE 0 halo_alkene
RULE 0 acyclic_NCN
RULE 0 acyclic_NS
RULE 0 SCN2
RULE 0 terminal_vinyl
RULE 0 hetero_hetero
RULE 0 hydrazine
RULE 0 N_methoyl
RULE 0 NS_beta_halothyl
RULE 0 propiolactones
RULE 0 iodoso
RULE 0 iodoxy
RULE 0 noxide

#groups of molecules

RULE 0 dye

#functional groups which are allowed, but may not be wanted in high quantities

#common functional groups

RULE 6 alcohol
RULE 8 alkene
RULE 4 amide
RULE 4 amino_acid
RULE 4 amine
RULE 4 primary_amine
RULE 4 secondary_amine
RULE 4 tertiary_amine
RULE 4 carboxylic_acid
RULE 6 halide
RULE 1 iodine
RULE 4 ketone
RULE 4 phenol
RULE 2 imine
RULE 1 methyl_ketone
RULE 1 alkyaniline
RULE 4 sulfonamide
RULE 1 sulfonylurea
RULE 0 phosphonamide
RULE 0 alphahalo_ketone
RULE 0 oxaziridine
RULE 1 cyclopropyl
RULE 2 guanidine
RULE 0 sulfonimine
RULE 0 sulfinimine
RULE 1 hydroxamic_acid

RULE 0 sulfinylthio
RULE 0 disulfide
RULE 0 enol_ether
RULE 0 enamine
RULE 0 organometallic
RULE 0 dithioacetal
RULE 1 oxime
RULE 0 isothiocyanate
RULE 0 isocyanate
RULE 3 lactone
RULE 3 lactam
RULE 1 thioester
RULE 1 carbonate
RULE 0 carbamic_acid
RULE 1 thiocarbamate
RULE 0 triazine
RULE 1 malonic

#other functional groups

RULE 2 alkyne
RULE 4 aniline
RULE 4 aryl_halide
RULE 4 carbamate
RULE 4 ester
RULE 4 ether
RULE 1 hydrazone
RULE 0 nonacylhydrazone
RULE 1 hydroxylamine
RULE 2 nitrile
RULE 2 sulfide
RULE 2 sulfone
RULE 2 sulfoxide
RULE 0 thiourea
RULE 1 thioamide
RULE 1 thiol
RULE 2 urea

RULE 0 hemiketal
RULE 0 hemiacetal
RULE 0 ketal
RULE 1 acetal
RULE 0 aminal
RULE 0 hemiaminal

#protecting groups

RULE 0 benzyloxycarbonyl_CBZ
RULE 0 t_butoxycarbonyl_tBOC
RULE 0 fluorenylmethoxycarbonyl_Fmoc
RULE 1 dioxolane_5MR
RULE 1 dioxane_6MR

RULE 1 tetrahydropyran_THP
RULE 1 methoxyethoxymethyl_MEM
RULE 2 benzyl_ether
RULE 2 t_butyl_ether
RULE 0 trimethylsilyl_TMS
RULE 0 t_butyltrimethylsilyl_TBDMS
RULE 0 triisopropylsilyl_TIPS
RULE 0 t_butylphenylsilyl_TBDPS
RULE 1 phthalimides_PHT
RULE 2 arenesulfonyl

Table S2. Purity of validated hits

Cpd	Purity data	
	Technique	Purity,%
7	¹ H-NMR	97
22	LC-MS	100
23	LC-MS	100
28	¹ H-NMR	>95
44	LC-MS	100
48	¹ H-NMR	95
49	LC-MS	90.5
59	¹ H-NMR	97
65	¹ H-NMR	98
66	¹ H-NMR	98
67	¹ H-NMR	95

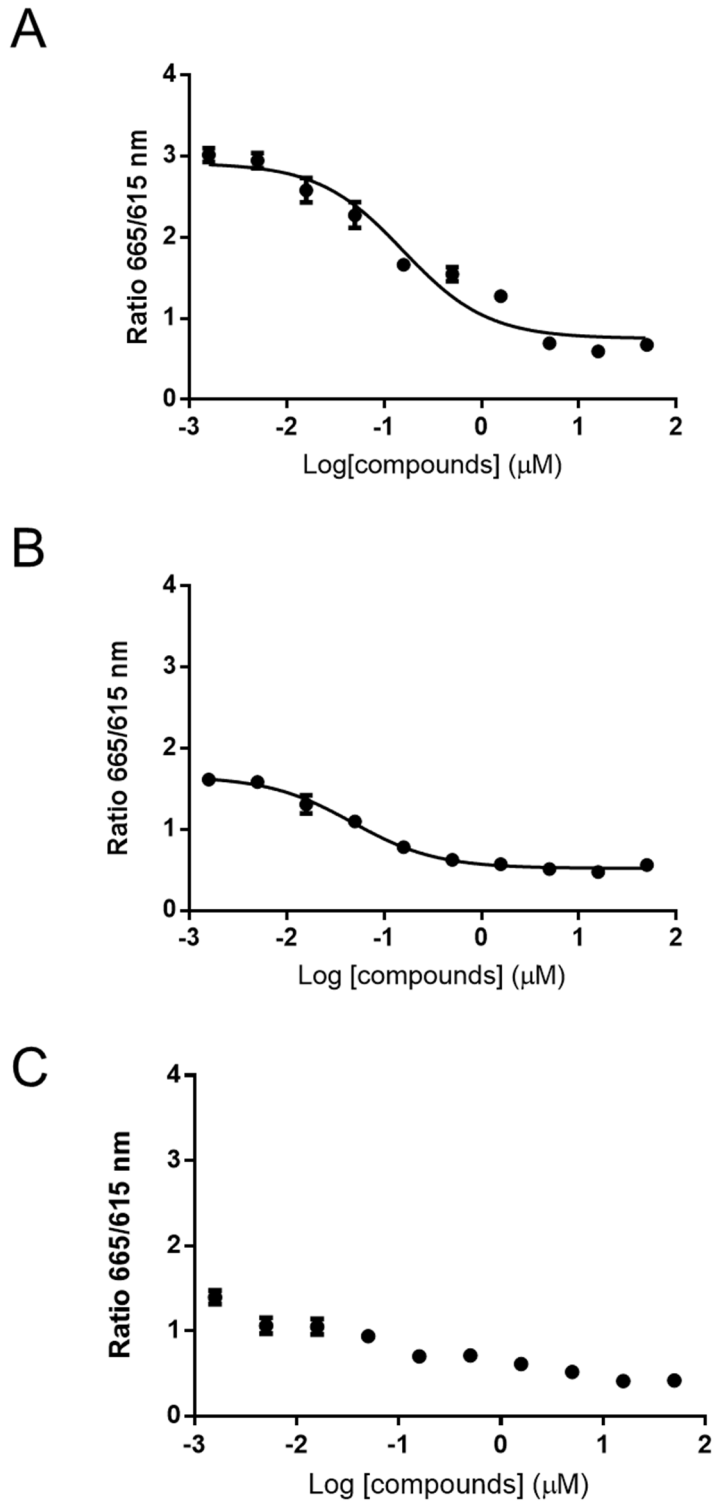


Figure S1: Dose-response curves of three primary hits in inhibiting the binding of fluorescent-labeled FL-d2 to Lumi4-Tb-SNAP-FLT3 overexpressed in HEK-293 cells. The ratio of fluorescence intensities emitted at 665 and 615 nm (indicative of FL-d2 binding and subsequent FRET signal) is measured at 10 concentrations of competitor in triplicate. **A)** Validated inhibition of FL-d2 binding by a FCL competitor, **B)** Non-validated inhibition of FL-d2 binding because of a too low signal ratio (665/615 nm ratio = 1.6) at the lowest competitor concentration, **C)** Non-validated inhibition of FL-d2 binding because of a concentration-independent low signal ratio.

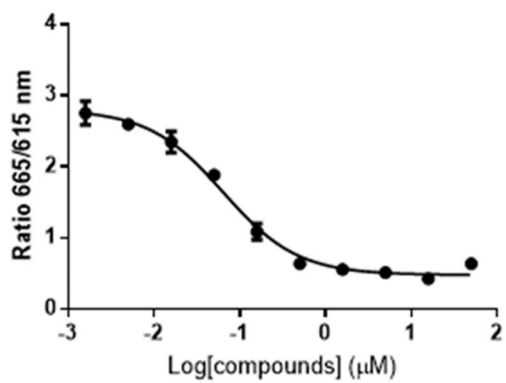


Figure S2: Sunitinib inhibits the binding of fluorescent-labeled FL-d2 to Lumi4-Tb-SNAP-FLT3 overexpressed in HEK-293 cells ($IC_{50} = 64 \pm 5$ nM). The normalized ratio of fluorescence intensities emitted at 665 and 615 nm (indicative of FL-d2 binding and subsequent FRET signal) is measured at 10 concentrations of competitor in triplicate.

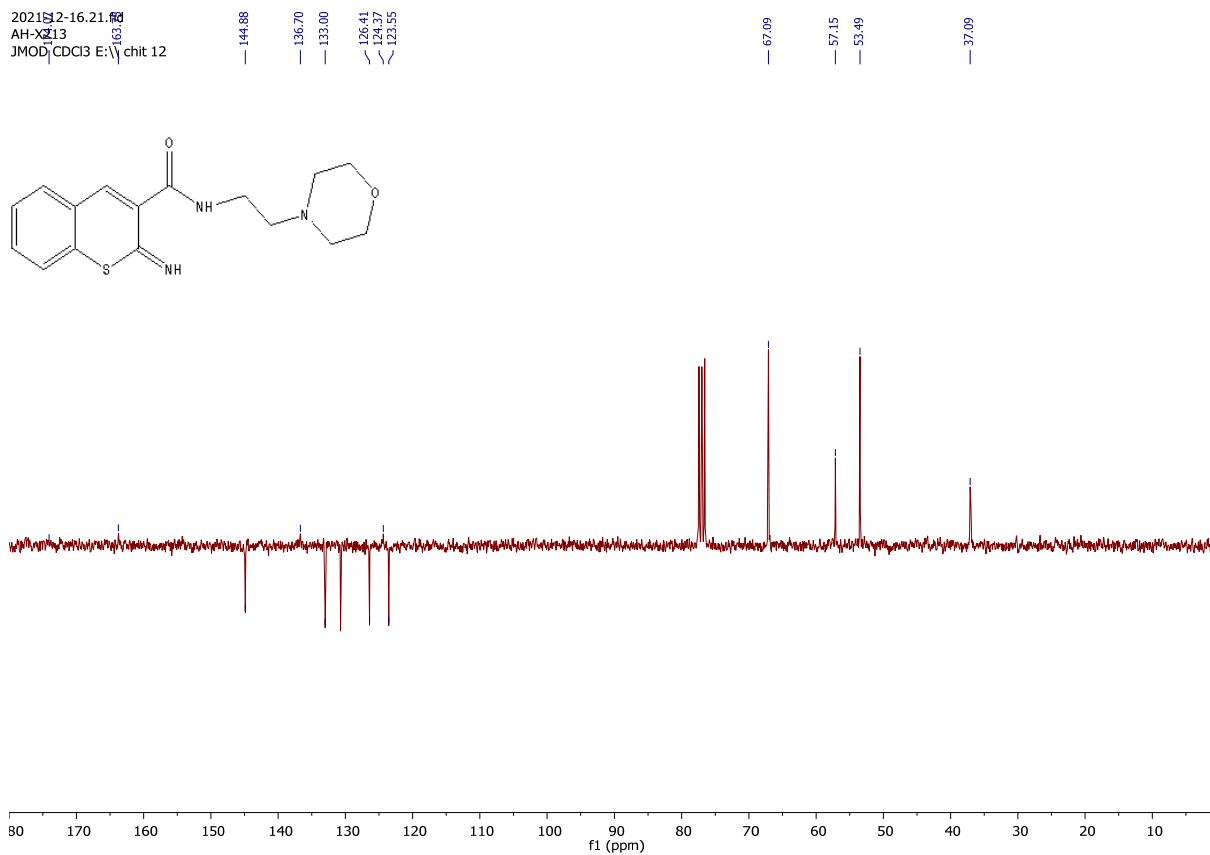
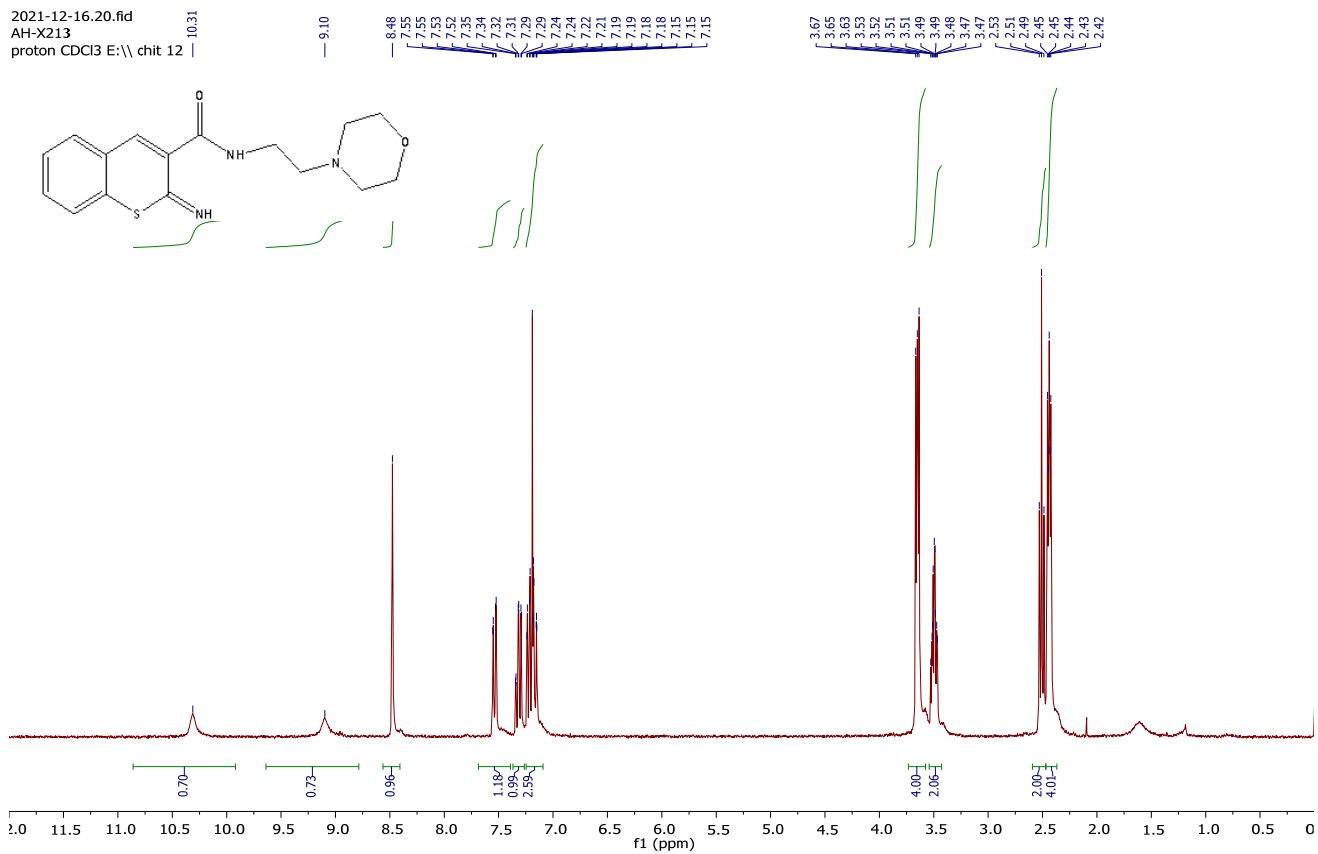


Figure S3. ^1H -NMR (top) and ^{13}C -NMR (bottom) spectra of compound **22**.