Supplementary information for

Structural insight into the macrocyclic inhibitor TPX-0022 of c-Met and c-Src

Table S1 Data collection and refinement statistics

	c-Met/TPX-0022	c-Src/TPX-0022
PDB code	8K78	8K79
Data collection		
Space group	P 1 2_1 1	P 1
Cell dimensions		
a, b, c (Å)	45.1, 66.6, 55.3	41.9, 63.1, 73.1
α, β, γ (°)	90.0, 107.0, 90.0	100.5, 91.1, 90.2
Resolution (Å)	43.1-2.7 (2.8-2.7)	24.0-2.8 (2.9-2.8)
R_{sym} or R_{merge}	0.2(0.7)	0.4(0.8)
Mean I/sigma(I)	4.1(2.5)	2.6 (1.2)
Completeness (%)	95.8 (97.8)	99.4 (100.0)
Redundancy	3.1(3.4)	3.2 (3.1)
CC1/2	0.58(0.22)	0.97(0.77)
Refinement		
Resolution (Å)	43.1-2.7	24.0-2.8
Unique reflections	8618 (873)	17968 (1789)
R_{work} / R_{free}	0.24/0.29	0.24/0.28
No. atoms		
Protein	2305	3941
Ligand/ion	30	60
Water	63	84
B-factors		
Protein	31.2	21.8
Ligand/ion	21.5	28.3
Water	24.7	12.9
R.m.s. deviations		
Bond lengths (Å)	0.005	0.006
Bond angles (°)	0.67	0.91

Table S2 The binding free energy and Ki value of c-Met resistance-relevant mutations predicted by molecular docking.

	Binding Free Energy (kcal/mol)	Ki (nM)
c-Met WT	-9.7	78.9
c-Met G1163R	-9.6	86.8
c-Met L1195F	-9.7	75.3
c-Met F1200I	-9.1	158.4
c-Met D1228N	-8.5	516.0
c-Met Y1230H	-8.8	307.1
c-Met Y1230C	-8.3	737.8

Table S3 Prediction of ADME/drug-likeness properties for macrocyclic inhibitors

	TPX-0022	D6808	MC25b	Lorlatinib	TPX-0005
GI absorption	High	High	Low	High	High
BBB permeant	No	No	No	No	No
Pgp substrate	Yes	No	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	Yes	No	No	No
CYP2C9 inhibitor	Yes	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	Yes	No
Log Kp [cm/s]	-7.29 cm/s	-6.73 cm/s	-7.81 cm/s	-7.69 cm/s	-6.33 cm/s
Lipinski	Yes; 0 violation	Yes; 1 violation: MW>500	No; 2 violations: MW>500, NorO>10	Yes; 0 violation	Yes; 0 violation
Ghose	Yes	No; 3 violations: MW>480, WLOGP>5.6, MR>130	No; 4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70	Yes	No; 1 violation: WLOGP<-0.4
Veber	Yes	Yes	No; 1 violation: TPSA>140	Yes	Yes
Egan	Yes	Yes	No; 1 violation: TPSA>131.6	Yes	Yes
Muegge	Yes	Yes	No; 2 violations: MW>600, TPSA>150	Yes	Yes
Bioavailability Score	0.55	0.55	0.11	0.55	0.55

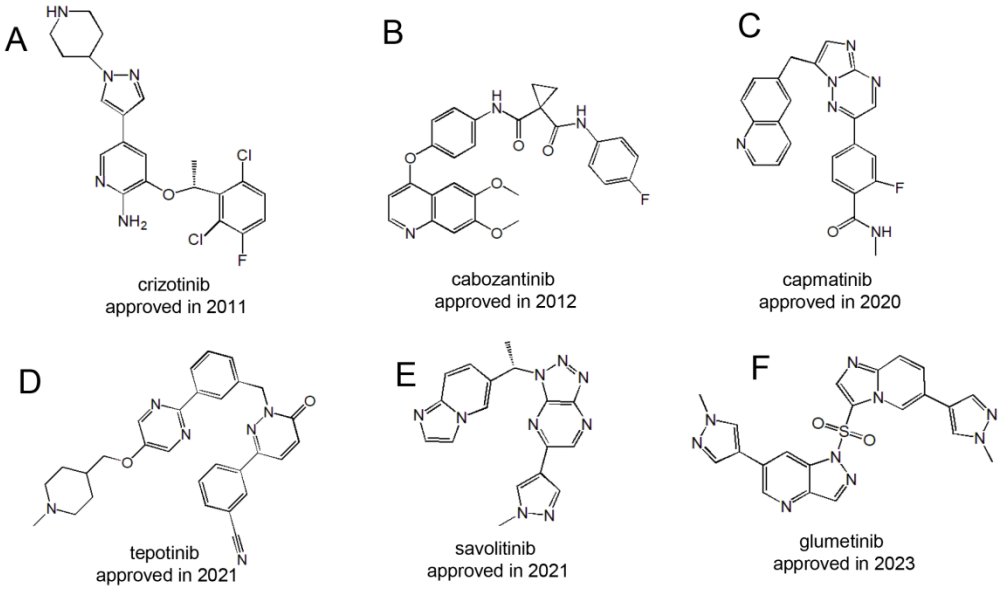

Figure S1 Chemical structures of the approved c-Met inhibitors.

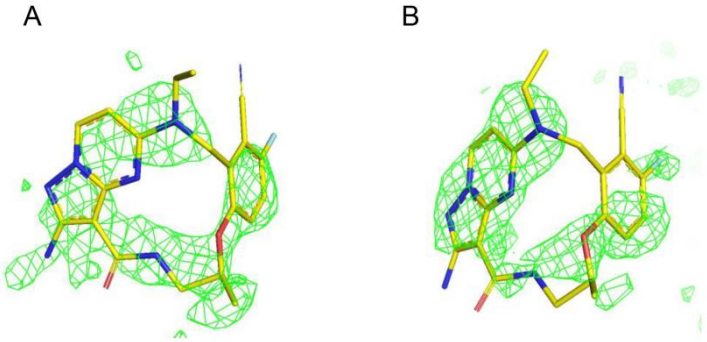

Figure S2 The simulated annealing omit map at 3 σ shows the quality and occupancy of TPX-0022 in the structure of c-Met/TPX-0022 complex (A) and c-Src/TPX-0022 (B).

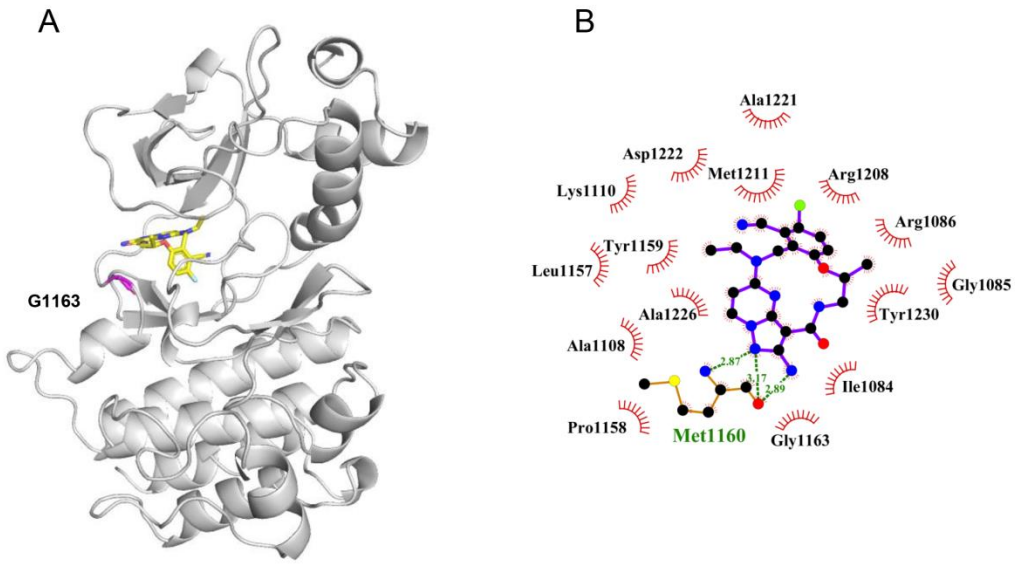

Figure S3 (A) The structure of c-Met/TPX-0022 complex. (B) Detailed interactions between TPX-0022 and c-Met. The interactions are analyzed using ligplot.

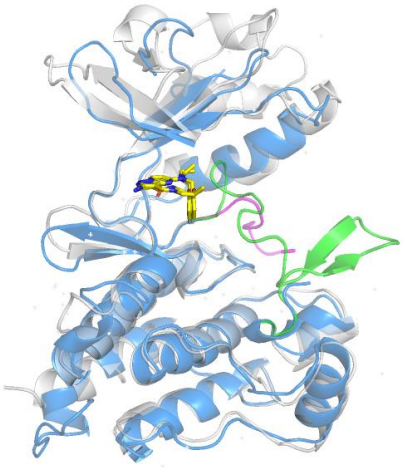

Figure S4 Superimposition of c-Met/TPX-0022 (gray) and c-Src/TPX-0022 (blue) structures.

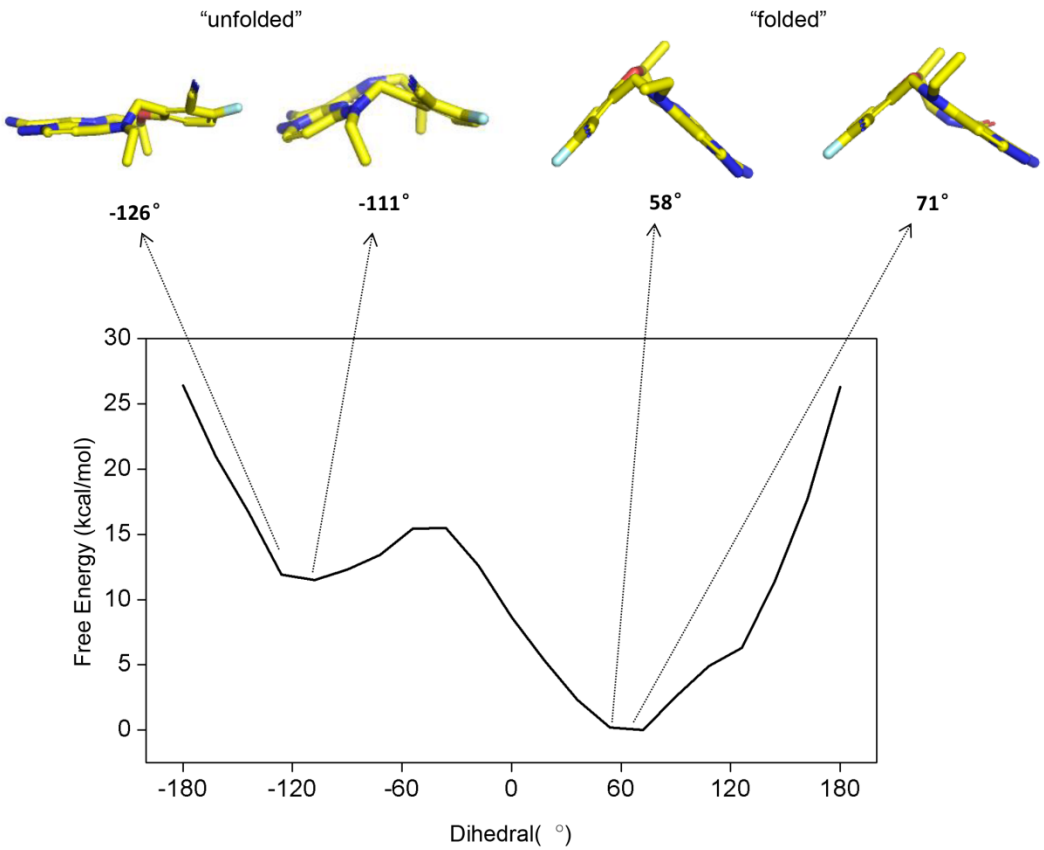

Figure S5 Torsional angle scan calculations of TPX-0022. Torsional angle scan was performed by the *Schrödinger Maestro* software.

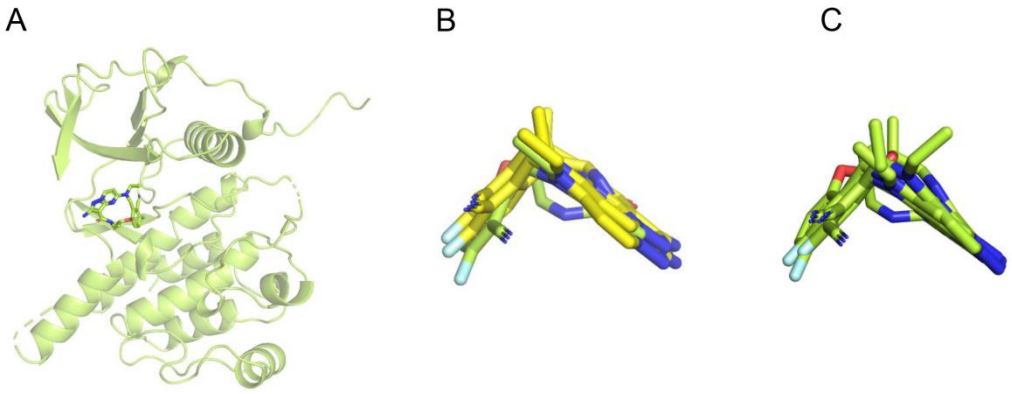

Figure S6 Docking model of TPX-0022 to CSF1R. (A) The modelled structure of CSF1R/TPX-0022 complex. Molecular docking was performed by AutoDock tools program. (B-C) Conformations of TPX-0022 in the complexes with c-Met, c-Src and CSF1R. TPX-0022 from molecular docking is colored limon. TPX-0022 from crystal structure of c-Met/TPX-0022 or c-Src/TPX-0022 complex is colored yellow.

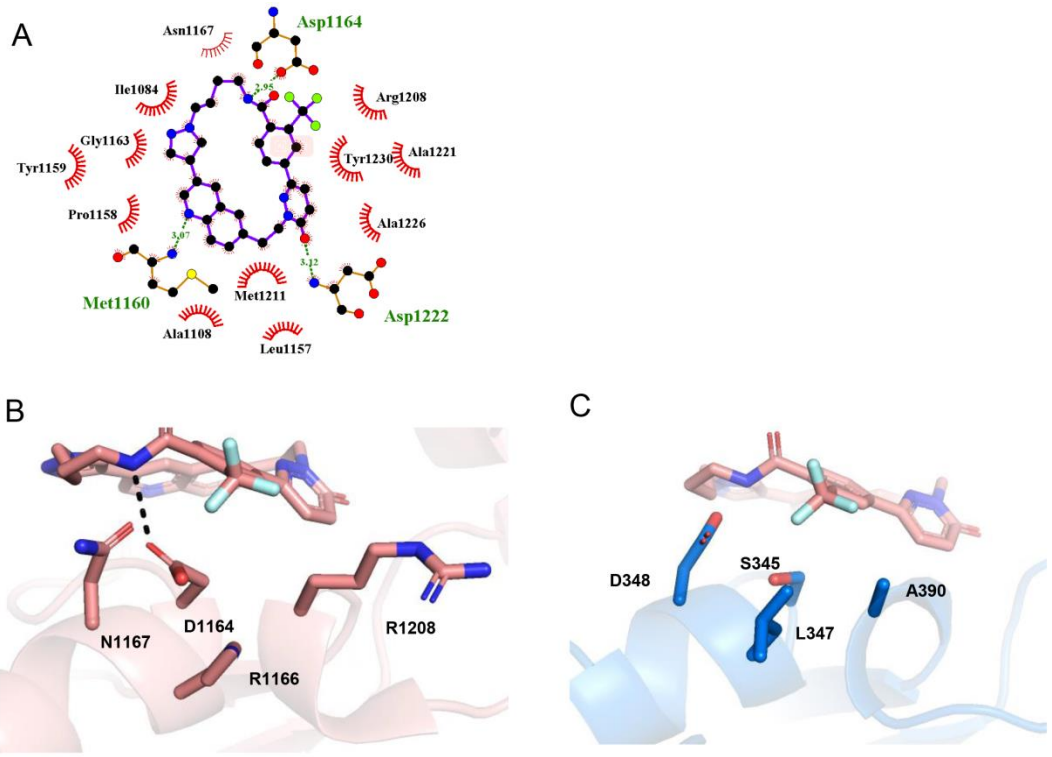

Figure S7 Structural basis of D6808 with c-Met (PDB: 8GVJ). (A) Detailed interactions between D6808 and c-Met. The interactions are analyzed using ligplot. (B) The interactions of the trifluoromethyl group of D6808 with the surrounding residues of c-Met. (C) The interactions of the trifluoromethyl group of D6808 with the surrounding residues of c-Src. The modelled c-Src/D6808 structure was acquired by a superposition of c-Src/TPX-0022 structure with the c-Met/D6808 structure. Hydrogen bond is shown as black dotted lines. c-Met/D6808 complex is colored deep salmon. c-Src is colored marine.

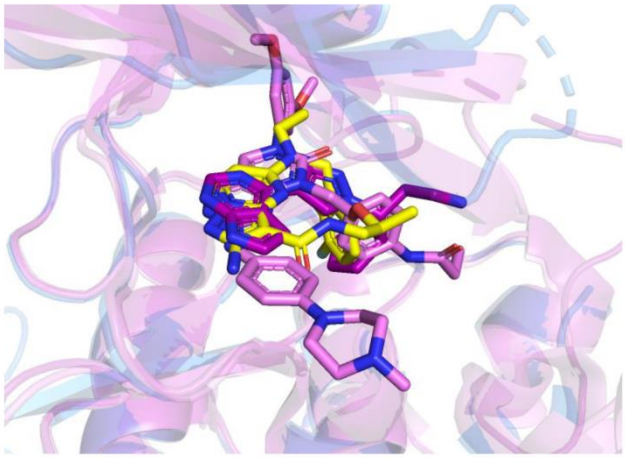

Figure S8 Superimposition of c-Src/TPX-0022(yellow), c-Src/Ruxolitinib (PDB ID: 4U5J) (purple) and c-Src/FIIN-2 (PDB ID: 7D57) (violet) structures.

A Hinge

c-Met	[1158]	LPYMKHG
ALK	[1196]	LELMAGG
c-Src	[338]	TEYMSKG
TRK	[590]	MEYMRHG
EGFR	[790]	MQLMPFG
FGFR1	[561]	VEYASKG
PDGFRA	[674]	TEYCFYG
ABL	[315]	TEFMTYG
JAK	[623]	QEFVKFG

B β7

c-Met	[1206]	AARNCMLDEKF
ALK	[1251]	AARNCLLTCPG
c-Src	[388]	RAANILVGENL
TRK	[652]	ATRNCLVGQGL
EGFR	[839]	AAENVLVKTPQ
FGFR1	[625]	AARNVLVTEDN
PDGFRA	[820]	AARNVLLAQGK
ABL	[365]	AARNCLVGENH
JAK	[1105]	AARNVLVESEH

Figure S9 Sequence alignment of the hinge loop (A) and β7 (B) sheet residues of kinase proteins.

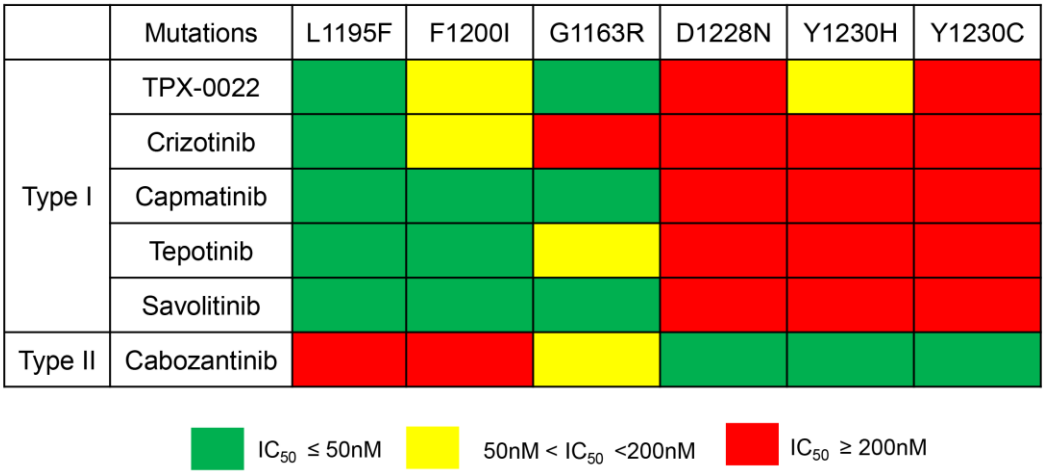

Figure S10 The inhibitory potency of inhibitors against c-Met resistance-relevant mutations.