Supporting Information

Structural Basis of EGFR Mutant Inhibition by Trisubstituted Imidazole Inhibitors

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Table S1. Crystallographic data collection and refinement statistics for EGFR(T790M/V948R) crystals.

PDB ID Code	6V5N	6V5P	6V6O
Inhibitor	**1 (LN2084)**	**2 (LN2725)**	**3 (LN2380)**
Data collection			
Space group	P1 2_1 1	P1 2_1 1	P1 2_1 1
Cell dimensions			
a, b, c (Å)	71.1 102.12 87.38	70.36 101.61 87.17	71.5501 102.36 173.569
α, β, γ (°)	90.0 102.55 90.0	90.0 102.31 90.0	90 101.298 90
Resolution* (Å)	51.06 - 2.4 (2.486 - 2.4)	40.24 - 2.3 (2.382 - 2.3)	60.81 - 2.1 (2.175 - 2.1)
R_{merge}*	0.1217 (0.7778)	0.07567 (0.6614)	0.1149 (0.5684)
I/σ*	12.72 (2.10)	13.91 (2.44)	11.04 (4.04)
Completeness* (%)	98.71 (97.35)	99.11 (98.87)	98.45 (97.77)
Multiplicity*	6.9 (5.9)	5.2 (5.0)	7.0 (7.0)
Refinement			
Resolution (Å)	51.06 - 2.4	40.24 - 2.3	60.81 - 2.1
No. of Reflections	47126	52834	140813
R_{work}/R_{free}	0.1921/0.2440	0.1791/0.1937	0.1945/0.2214
No. of Atoms			
Protein	9779	9747	19612
Ligands/ion	128	131	296
Water	255	375	1618
B-factors			
Protein	46.51	45.39	31.06
Ligand/ion	43.98	40.20	24.79
Water	44.67	45.97	36.76
R.m.s deviations			
Bond lengths (Å)	0.011	0.008	0.003
Bond angles (°)	1.14	0.91	0.68
Ramachandran			
Most favored	94.92	96.01	97.33
Allowed	4.00	3.41	2.42
Outliers	1.08	0.50	0.25

Table S1 continued.

PDB ID Code Inhibitor	6V6K **4 (LN2057)**	6V66 **5 (LN2899)**
Data collection		
Space group	P1 2_1 1	P1 2_1 1
Cell dimensions		
a, b, c (Å)	71.5988 102.455 174.043	71.4802 102.448 87.4219
α, β, γ (°)	90 101.25 90	90 102.775 90
Resolution* (Å)	85.35 - 2.2 (2.279 - 2.2)	85.26 - 1.79 (1.854 - 1.79)
R_{merge}*	0.08136 (0.5284)	0.1081 (0.796)
I/σ*	7.80 (2.11)	9.59 (1.95)
Completeness* (%)	93.67 (95.49)	99.61 (99.45)
Multiplicity*	3.1 (3.1)	7.0 (7.1)
Refinement		
Resolution (Å)	85.35 - 2.2	85.26 - 1.79
No. of Reflections	369052	115036
R_{work} / R_{free}	0.2130/ 0.2394	0.1844 /0.2235
No. of Atoms		
Protein	19759	9901
Ligands/ion	280	160
Water	712	996
B-factors		
Protein	36.59	27.53
Ligand/ion	29.34	23.88
Water	38.02	34.75
R.m.s deviations		
Bond lengths (Å)	0.007	0.007
Bond angles (º)	0.95	0.85
Ramachandran		
Most favored	96.66	97.62
Allowed	3.01	2.14
Outliers	0.33	0.25

*Numbers in parentheses are for the highest resolution shell.

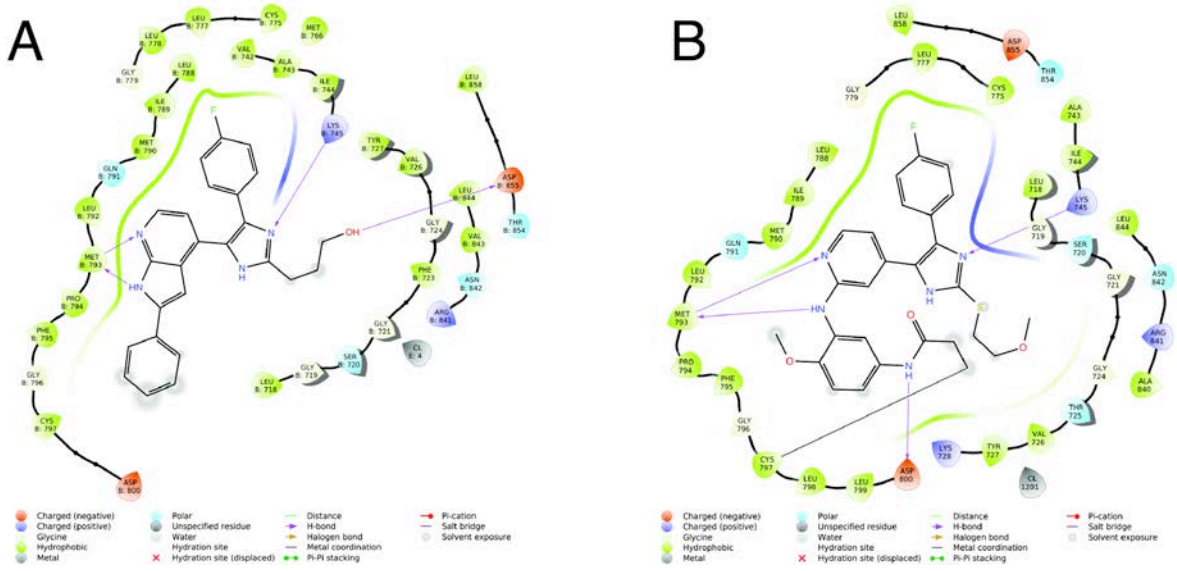

Figure S1. Representative LigPlot diagrams of **1** (A) and **4** (B) in complex with EGFR(T790M/V948R).

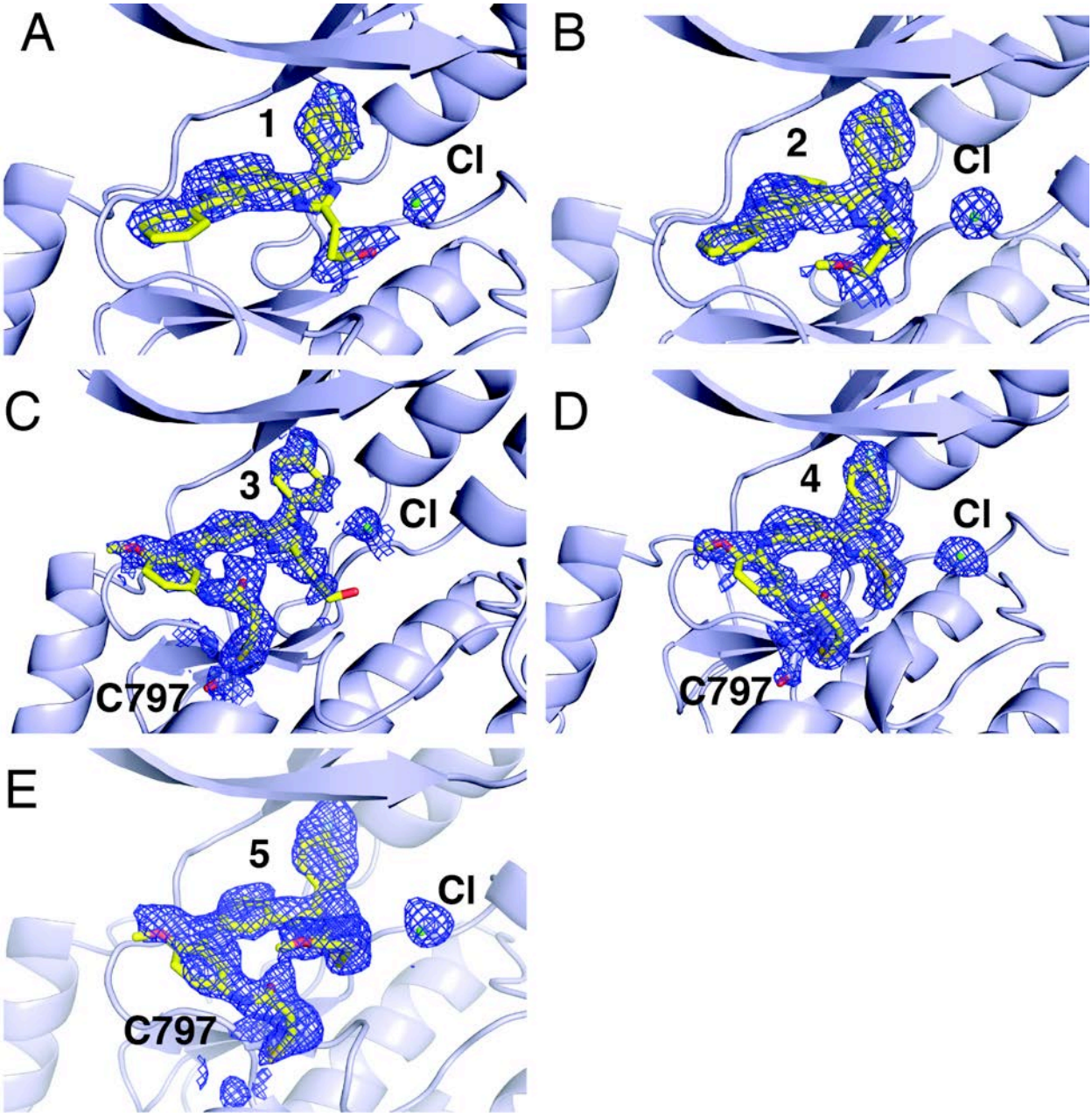

Figure S2. mFo-Fc omit maps for EGFR(T790M/V948R) crystal structures with ligand and chloride ion generated by PHENIX for A) **1** B) **2** C) **3** D) **4** E) **5** (contour level 1.5 σ)

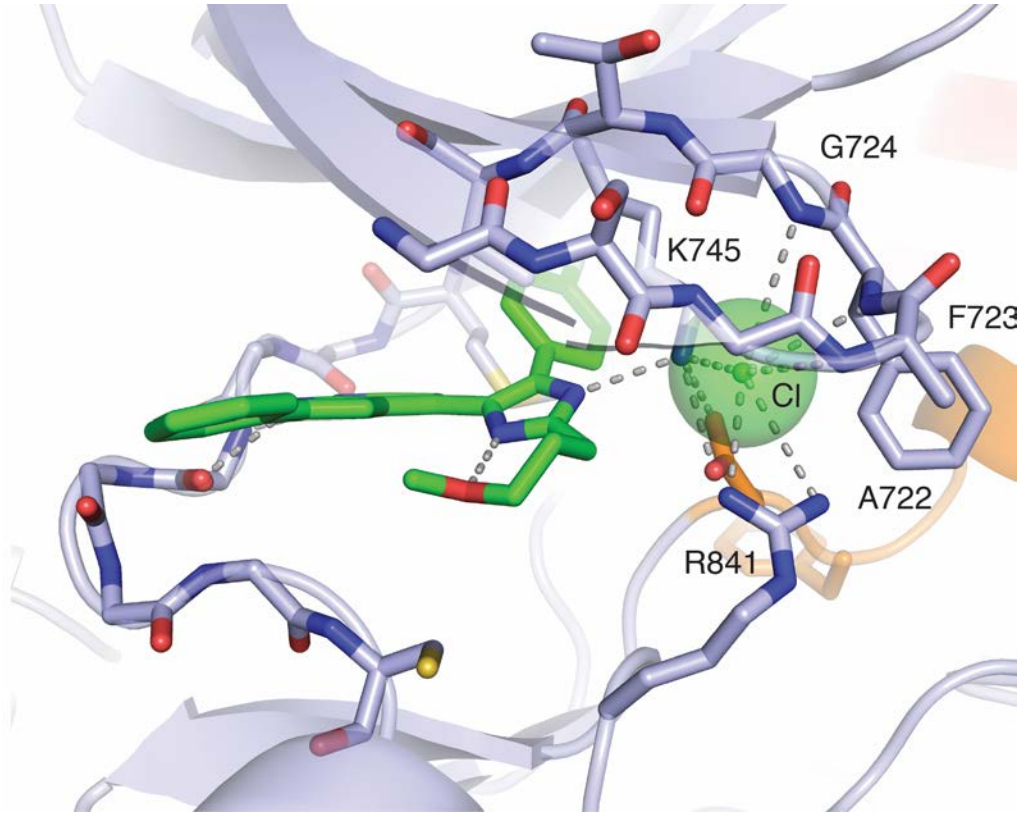

Figure S3. Extended view of Figure 2B illustrating the interactions with the ion (modeled as chloride) and positive side and main chain atoms of the EGFR(T790M/V948R) kinase domain.

Table S2. Crystallographic data collection and refinement statistics for WT EGFR crystals.

PDB ID Code	6VH4	6VHN	6VHN
Inhibitor	**3 (LN2360)**	**4 (LN2057)**	**5 (LN2899)**
Data collection			
Space group	I23	I23	I23
Cell dimensions			
a, b, c (Å)	146.534 146.534 146.534	146.865 146.865 146.865	146.811 146.811 146.811
α, β, γ (°)	90 90 90	90 90 90	90 90 90
Resolution* (Å)	34.54 - 2.8 (2.9 - 2.8)	59.96 - 2.4 (2.486 - 2.4)	38.97 - 3.601 (3.729 - 3.601)
R_{merge}*	0.1202 (0.7207)	0.1062 (0.6875)	0.1909 (0.5691)
I/σ*	9.28 (2.14)	8.34 (2.19)	6.10 (3.15)
Completeness* (%)	95.78 (97.67)	97.12 (98.49)	99.69 (100.00)
Multiplicity*	5.3 (5.0)	5.1 (5.2)	4.9 (5.1)
Refinement			
Resolution (Å)	34.54 - 2.8	59.96 - 2.4	38.97 - 3.601
No. of Reflections	65847	102763	30048
R_{work}/ R_{free}	0.1955/ 0.2309	0.1988/0.2002	0.1819 /0.2176
No. of Atoms			
Protein	2336	2410	2420
Ligands/ion	36	34	37
Water	33	92	0
B-factors			
Protein	58.18	55.70	68.02
Ligand/ion	64.06	62.91	64.74
Water	48.50	55.79	-
R.m.s deviations			
Bond lengths (Å)	0.003	0.009	0.002
Bond angles (°)	0.53	0.95	0.54
Ramachandran			
Most favored	93.73	94.60	92.28
Allowed	5.57	4.32	6.71
Outliers	0.70	1.08	1.01

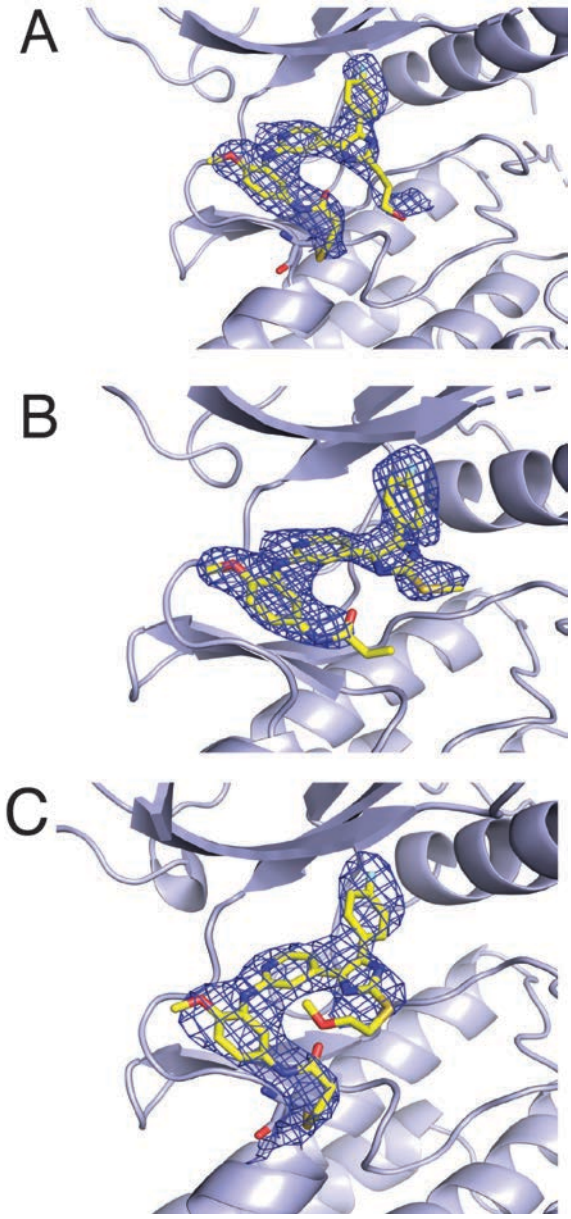

Figure S4. mFo-Fc omit maps for WT EGFR crystal structures with ligand and chloride ion generated by PHENIX for A) **3** B**) 4** C) **5** (contour level 1.5 σ).

Table S3. Crystallographic data collection and refinement statistics of intermediate **26**

Space group	P -1 (triclinic)
Cell dimensions	determinate from 10562 reflections with $2.6° < \theta < 28.4°$
a,b,c (Å)	8.1901(7), 8.9234(8), 9.1834(9)
β (°)	92.006(7)
V (Å³), z	568.54(10), 2
Crystal size (mm³)	0.11 x 0.28 x 0.50 (colorless block)
Range of Measurement	$2° \leq \theta \leq 28°$ $-10 \leq h \leq 10$ $-11 \leq k \leq 11$ $-11 \leq l \leq 12$
No. of reflections:	
Measured	4968
Unique	2691 (R_{int} = 0.0468)
Observed ($\|F\|/\sigma(F) > 4.0$)	2348 ($\|F\|/\sigma(F) > 4.0$)
Refinement	
Nr. of parameters	147
wR2	0.1368
R1(observed), R(all)	0.0506, 0.0601
Goodness of Fit	1.063
Max. deviation of parameters	0.001 * e.s.d
Max. Peak final	
diff. Fourier synthesis (e Å-3)	1.3, -1.92

Figure S5: X-ray structure of regioisomeric pure intermediate **26**

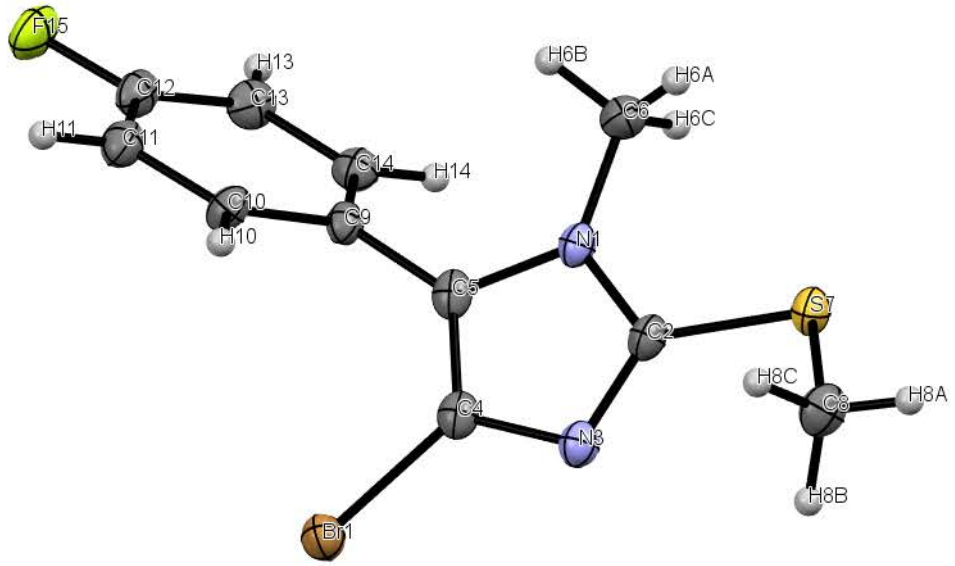

Table S4. Inhibitory activities of structurally characterized imidazole inhibitors not presented in Table 1.

compound #	IC50 (EGFR-L858R/T790M)	IC50 (EGFR-L858R/T790M/C797S)
1	6.6 nM	21 nM
2	14 nM	6 nM
3	< 0.5 nM	8 nM
5	1 nM	35 nM

***N*-(3-Bromo-4-methoxyphenyl)acrylamide:**
1.00 g (4.95 mmol) 3-Bromo-4-methoxyaniline was dissolved in 10 ml dry THF and 10 ml of a 1 M $NaHCO_3$ solution was added slowly. After cooling the biphasic mixture down to 0 °C, 420 µl (5.20 mmol) acryloyl chloride was added dropwise under vigorous stirring. After complete addition the reaction mixture was warmed to room temperature and quenched by the addition of a saturated NH_4Cl solution. The aqueous phase was extracted three times with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the volatiles evaporated under reduced pressure. The crude product was triturated with DCM to give the title compound in 80 % yield (1.01g, 3.96 mmol). 1H NMR (200 MHz, DMSO) δ 10.13 (s, 1H), 8.01 (d, J = 2.4 Hz, 1H), 7.55 (dd, J = 8.9, 2.5 Hz, 1H), 7.09 (d, J = 9.0 Hz, 1H), 6.47 – 6.16 (m, 2H), 5.81 – 5.68 (m, 1H), 3.81 (s, 3H). 13C NMR (50 MHz, DMSO) δ 162.94, 151.57, 133.06, 131.64, 126.87, 123.84, 119.81, 112.77, 110.12, 56.30. ESI-MS: 256.1/257.1 $[M+H]^+$.