Supporting Information:

Type-II Kinase Inhibitor Docking, Screening, and Profiling Using Modified Structures of Active Kinase States

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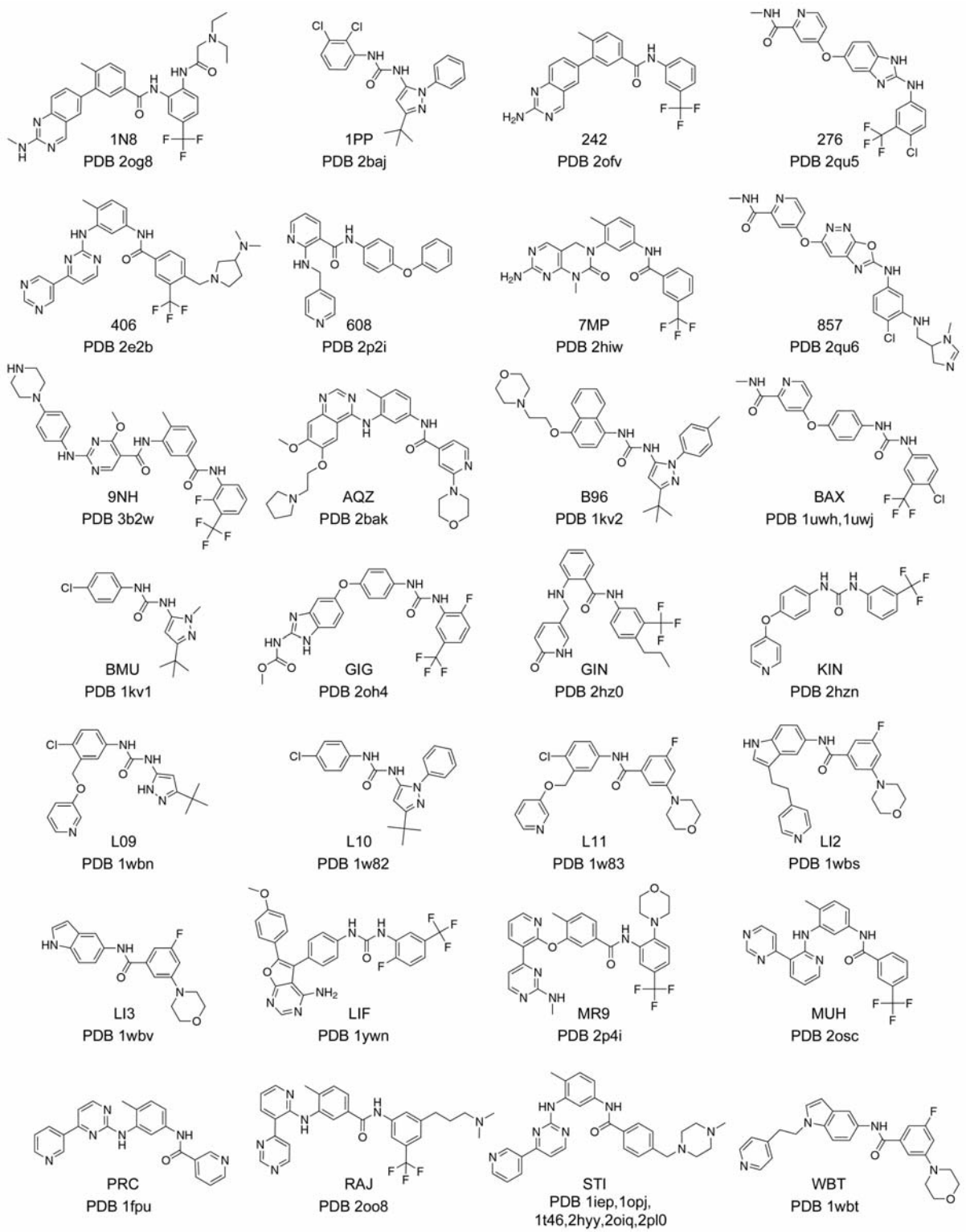
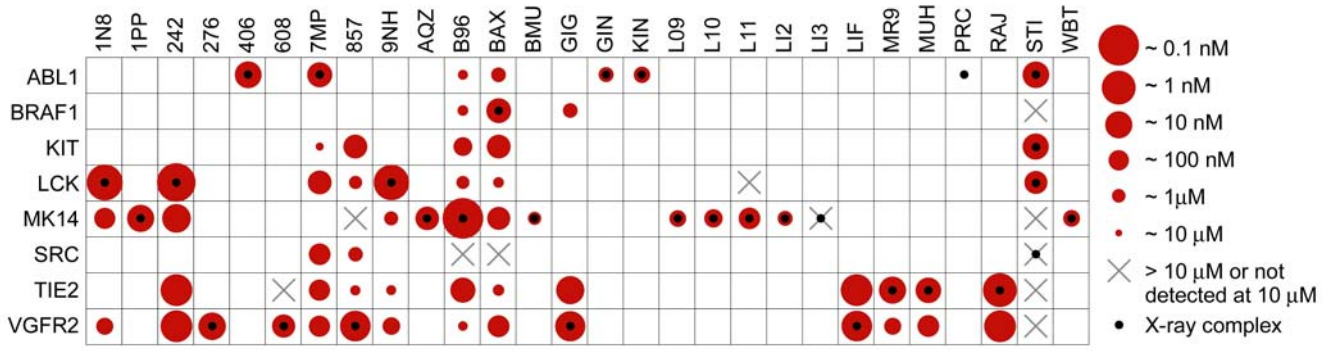
Structures and activities of the 28 type-II kinase inhibitors used in the present study



Supp. Figure 1. Chemical structures of the 28 type II kinase inhibitors used in this study



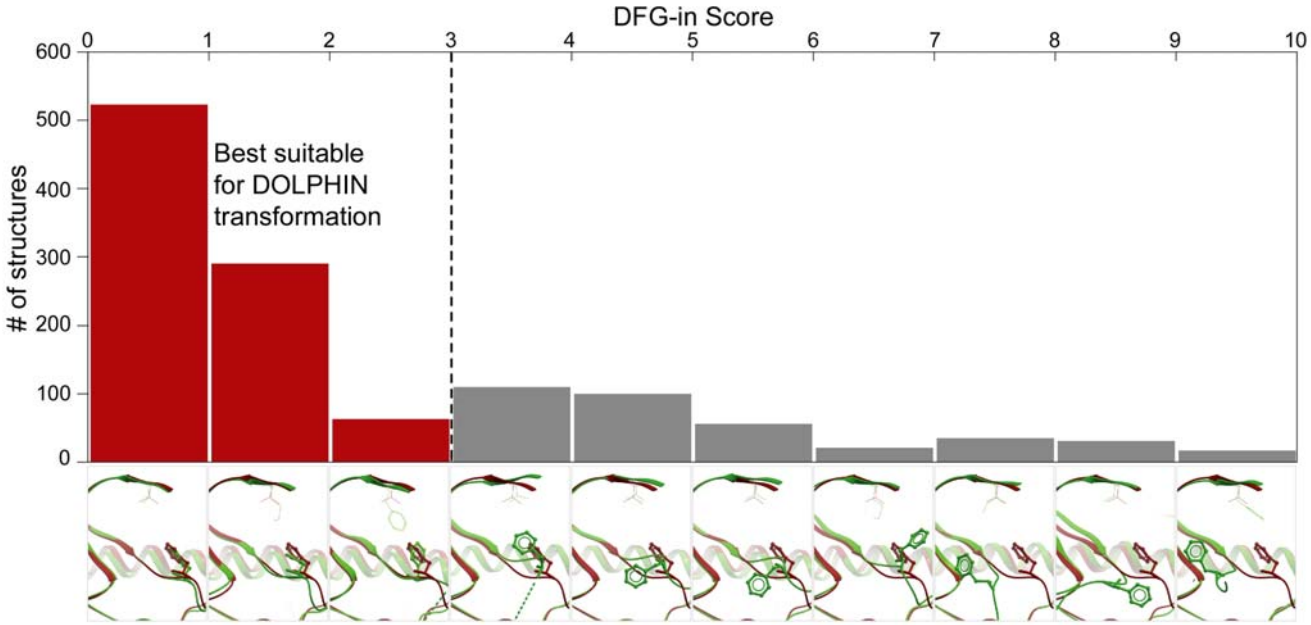
Supp. Figure 2. Experimental activities of the 28 type II kinase inhibitors used in this study (see also Supplementary Table 1).

Supp. Table 1. Cross-kinase reactivity of compounds used in this study. Unless otherwise noted, the activity data are IC_{50} values in nM. PDB X-ray complexes are shaded grey. Blank fields indicate the absence of data.

PDB het ID	ABL1	BRAF1	KIT	LCK	MK14	SRC	TIE2	VGFR2	Citation & Cmpd ID
1N8				0.5	76			306	[1] #36
1PP (K_d)					10				[2] pyrazolourea
242				0.2	6		2	2	[1] #1
276 ($K_{i\ phos}$)								8.7	[3] #3
406	11								[4] #8, INNO-406
608							>25000	38	[5] #35
7MP (%/1μM*)	2%		87%	3%		6%	7%	7%	[6] #14
857 ($K_{i\ phos}$)			30	1140	>40000	720	3180	3	[3] #22
9NH				0.6	856		3440	255	[7] #11
AQZ (K_d)					33				[2] MPAQ
B96 (K_d)	3400	2700	170	1200	0.37	>10000	20	3900	[8] BIRB-796
B96				35000	0.1				[9] BIRB-796
BAX		20-40	40-80		20-40			80-160	[10] sorafenib
BAX (K_d)	680	540	31	2700	370	>10000	2100	59	[8] sorafenib
BAX		22	68					90	[9] sorafenib
BMU (K_d)					1160				[11] #1
GIG		695					10		[9] #16
GIG							6.9	3.5	[12] #17
GIN	330-565								[13] #5, NVP-AEG082
KIN	41-700								[13] #3, NVP-AFG210
L09					350				[14] #10
L10					196				[14] #16
L11				>10000	65				[14] #9
LI2					630				[14] #23
LI3					162000				[14] #22
LIF							2	3	[15] #7k
MR9							10	310	[5] #47
MUH							17	61	[16] #2
PRC									[17]
RAJ							1	2	[5] #39, [16] #1b
STI (K_d)	12	>10000	14	40	>10000	>10000	>10000	>10000	[8] imatinib
STI	188		413						[9] imatinib
WBT					340				[14] #24

*% kinase activity remaining at 1μM concentration of the inhibitor

Distribution of DFG-in scores among kinase domain structures in PDB



Supp. Figure 3. Distribution of DFG-in scores among kinase domain structures in PDB. Each DFG-in score range is illustrated by a representative structure of a kinase activation loop (green) viewed from the N-terminal lobe of the kinase domain and compared with a prototypical DFG-in conformation (red). For the purpose of this study, only structures with DFG-in scores of 3 or less were classified as DFG-in structures.

Benchmark for Ligand Binding Geometry Prediction by DOLPHIN Models

Supp. Table 2. DOLPHIN docking benchmark: the six mammalian kinases for which both DFG-in and type-II inhibitor bound conformations were available in PDB at the moment of publication.

Kinase	**DFG-in structure(s) PDB codes and chains**			**Crystallographic type-II inhibitors PDB hetero ID and complex PDB codes**		
ABL1	2f4j[18] (a)	2hz4[13] (a,b,c)	2v7a[22] [b](a,b)	406 (2e2b[4])	GIN (2hz0[13])	PRC (1fpu[17])
	2g2i[19] (a,b)	2qoh[20] (a,b)	2z60[20] [b](a)	7MP (2hiw[6])	KIN (2hzn[13])	STI (1iep[23],1opj[24],2hyy[13])
		2gqg[21] (a[a],b)				
BRAF1	2fb8[25] (a,b)			BAX (1uwh[26],1uwj[26])		
KIT	1pkg[27] (a[a],b[a])			STI (1t46[28])		
LCK	1qpc[29] (a)	1qpj[29] (a)	2ofu[31] (a)	1N8 (2og8[1])	242 (2ofv[1])	STI (2pl0[33])
	1qpd[29] (a)	2of2[30] (a)	3lck[32] (a)		9NH (3b2w[7])	
	1qpe[29] (a)	2of4[30] (a)				
MK14	1m7q[34] (a)	1ouy[35] (a)	2okr[37] (a,d)	1PP (2baj[2])	L09 (1wbn[14])	LI2 (1wbs[14])
	1ouk[35] (a)	1oz1[36] (a)		AQZ (2bak[2])	L10 (1w82[14])	LI3 (1wbv[14])
				B96 (1kv2[11])	L11 (1w83[14])	WBT (1wbt[14])
					BMU (1kv1[11])	
SRC	1fmk[38] (a[c])	1yom[41] (b[d])	2hwo[43] (a[d])	STI (2oiq[44])		
	1y57[39] (a)	2bdf[42] (a[a],b[a])	2oiq[44] (b[a])			
	1yi6[40] (a,b[a])	2bdj[42] (a[a])				

[a]Narrow pocket; [b]T315I imatinib-resistant mutation; [c]Conserved salt bridge disrupted; [d]Conserved salt bridge disordered.

The Role of the Kinase DFG-in/DFG-out Equilibrium in Binding Energy Prediction

Kinase DFG-in/DFG-out equilibrium affects the observed affinity of type-II inhibitors. Type-II ligands bind exclusively to DFG-out kinase species, which represent a minor fraction of possible conformations in solution. While most kinases are believed to transiently adopt DFG-out state, the stability of this state varies. This leads to variations in the relative concentration of the DFG-out species, and introduces systematic protein-specific offsets to the observed binding affinity relatively to binding energy calculated from the structure of the complex. The following simplistic model helps quantitatively characterize this phenomenon.

Assume that a kinase is present in solution in one of the two conformations (DFG-in and DFG-out), and the equilibrium constant is given by K_{io} = [In] / [Out], where [In] and [Out] are the respective concentrations. Upon addition of a type-II inhibitor, the composition of the solution changes to include the following species with their respective concentrations: DFG-in kinase – [In], unbound DFG-out kinase – [Out], DFG-out in complex with the compound – [OutCpd], and unbound compound – [Cpd]. Binding constant of the inhibitor to the DFG-out kinase species is K_b = [OutCpd] / [Out][Cpd], however, the observed binding constant is different due to the presence of DFG-in / DFG-out conformational ensemble:

$$K_b^{obs} = [\text{OutCpd}]/([\text{Out}] + [\text{In}])\times[\text{Cpd}]$$

After simple algebraic transformations, we obtain

$$K_b^{obs} = K_b / (1+K_{io}) \text{ or } \Delta G_b^{obs} = \Delta G_b + RT \ln (1+K_{io}).$$

The value of $RT \ln (1+K_{io})$ represents the equilibrium-related offset to observed vs calculated binding energies, and is denoted by b (kcal/mol). If the DFG-in / DFG-out equilibrium constant is small, i.e. the DFG-out state is prevalent, the observed binding constant is close to K_b and $b \approx 0$. However, higher prevalence of the DFG-in state can cause significant offsets. For example, if $K_{io} = 10^6$

then $K_b^{obs} \approx 10^{-6} \times K_b$, i.e. no binding will be observed in the experiment despite the good affinity of the compound to the DFG-out state of the kinase. This is equivalent to an offset of $b \approx 8.3$ kcal/mol.

Kinase phosphorylation can dramatically increase K_{io}. Due to this, type-II ligands typically demonstrate differential binding affinities to phosphorylated and unphosphorylated kinases. For example, the respective inhibition constants of imatinib towards ABL1 are 37 nM and ~7000 nM [9, 17], which means at least 189 times higher K_{io} for the phosphorylated ABL1. Not less important, some kinase point mutations, though distant from the binding pocket, can nevertheless affect binding of a type II inhibitor due to increase of K_{io}. V600E in BRAF1 and H396P in ABL1 are examples of such mutations.

Derivation of equilibrium-related binding energy offsets from experimental data. As mentioned above, conformational equilibrium of a phosphorylated kinase is shifted towards DFG-in state, which introduces a binding energy offset different from that for the unphosphorylated kinase. Imatinib inhibits unphosphorylated and phosphorylated ABL1 with K_i values of 37 nM and ~7000 nM, respectively, giving the observed binding energies of $\Delta G_b^{obs}(\mathrm{ABL1_{unphos}}, \mathrm{imatinib}) \approx -10.27$ kcal/mol and $\Delta G_b^{obs}(\mathrm{ABL1_{phos}}, \mathrm{imatinib}) \approx -7.12$ kcal/mol. Being relatively distant from the imatinib binding pocket, phosphorylation is unlikely to influence the inhibitor affinity to the DFG-out state, but it lowers the relative concentration of the DFG-out species. The difference in the observed binding energies, therefore, is entirely due to difference of the two equilibrium-related offsets:

$$\Delta G_b^{obs} = -7.12 + 10.27 \approx 3.15 \text{ kcal/mol} = b_{ABL1,phos} - b_{ABL1,unphos.}$$

Further on, differences in binding of imatinib to three kinases, ABL1, LCK, and SRC, can provide insights in their relative K_{io} values and related binding energy offsets. Composition of the binding sites of the three kinases is essentially the same: 18 of 23 residues are strictly identical, and the other five are conservatively mutated (YVIFF in ABL1 to FLLYY in LCK, YIFFI in ABL to FLYYV in SRC). In the complex state, both drug and the protein conformations are indistinguishable, as shown by

crystallographic studies [23, 24, 33, 44]. Therefore, it is safe to assume that the binding energy of the drug to the DFG-out conformations of the three kinases, ΔG_b, is approximately the same. The experimentally observed affinities, however, are dramatically different [8]:

- ABL1: K_d^{obs} = 12nM, i.e. ΔG_b^{obs} = –10.94 kcal/mol
- LCK: K_d^{obs} = 40nM, i.e. ΔG_b^{obs} = –10.22 kcal/mol
- SRC: K_d^{obs} > 10000nM, i.e. ΔG_b^{obs} > –6.90 kcal/mol

Same reasoning as above gives $b_{LCK} - b_{ABL1} \approx 0.7$ kcal/mol and $b_{SRC} - b_{ABL1} \approx 4$ kcal/mol.

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