

Supporting Information:

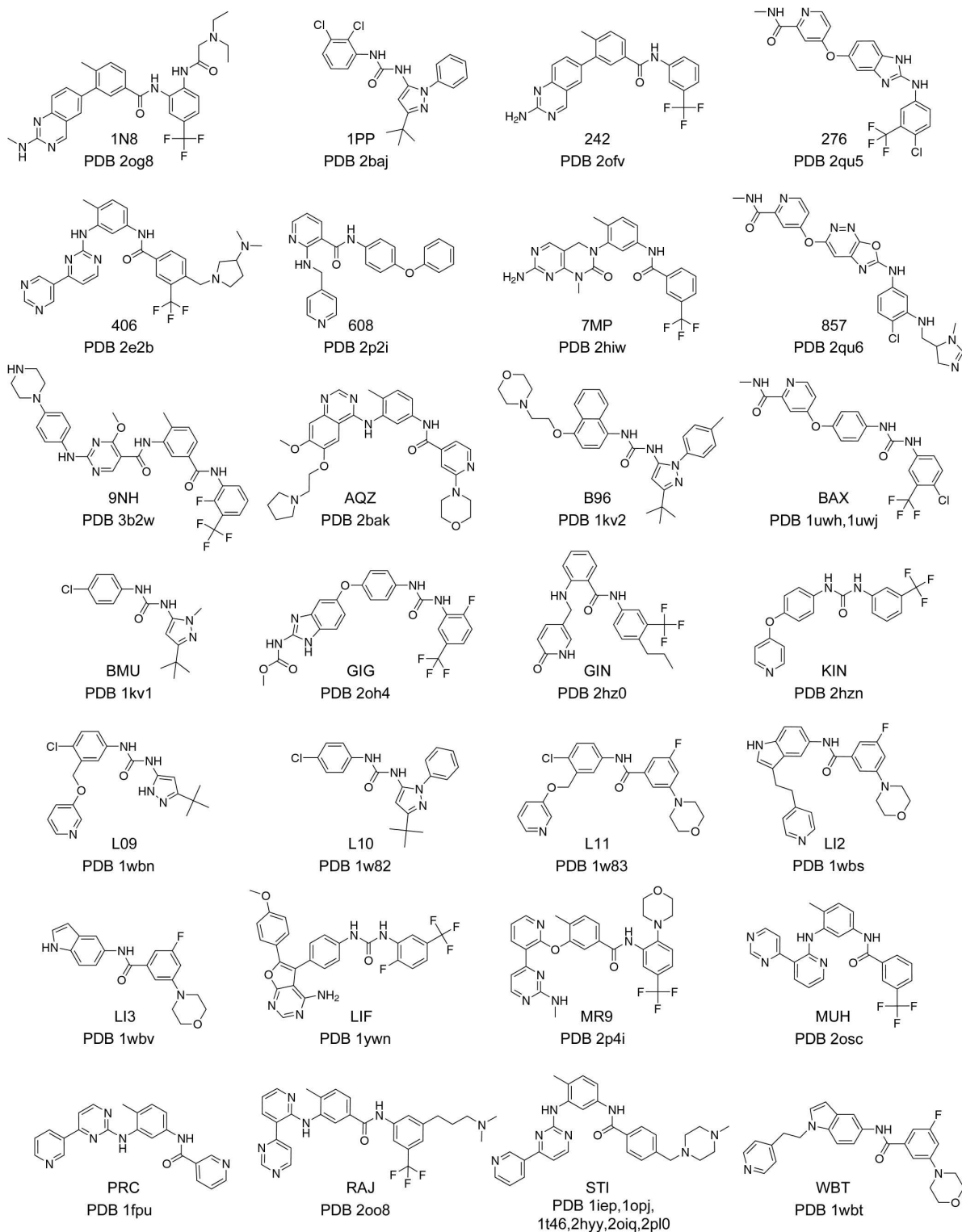
Type-II Kinase Inhibitor Docking, Screening, and Profiling Using Modified Structures of Active Kinase States

*Irina Kufareva*¹ and *Ruben Abagyan*^{1,2*}

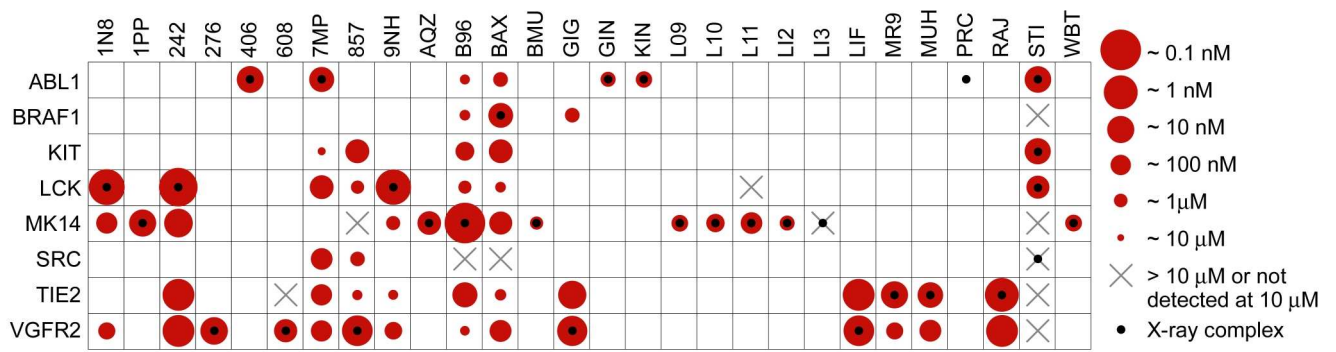
¹ The Scripps Research Institute, La Jolla, CA and ² Molsoft, LLC, La Jolla, CA

Structures and activities of the 28 type-II kinase inhibitors used in the present study	S2
Distribution of DFG-in scores among kinase domain structures in PDB	S5
Benchmark for Ligand Binding Geometry Prediction by DOLPHIN Models.....	S6
The Role of the Kinase DFG-in/DFG-out Equilibrium in Binding Energy Prediction	S7
References	S10

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



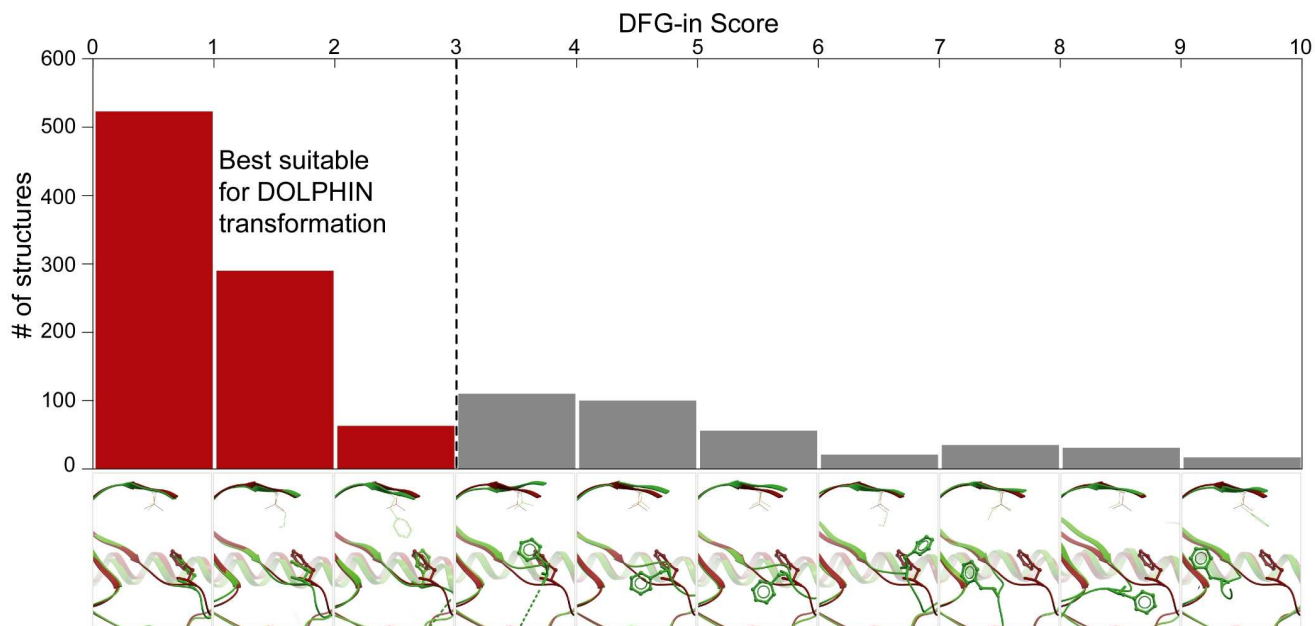
Supp. Figure 2. Experimental activities of the 28 type II kinase inhibitors used in this study (see also Supplementary Table 1).

Supp. Table 1. Cross-kinase reactivity of compounds used in this study. Unless otherwise noted, the activity data are IC₅₀ 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 (<i>K_d</i>)					10				² pyrazolourea
242				0.2	6		2	2	¹ #1
276 (<i>K_i</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 (<i>K_i</i> phos)			30	1140	>40000	720	3180	3	³ #22
9NH				0.6	856		3440	255	⁷ #11
AQZ (<i>K_d</i>)					33				² MPAQ
B96 (<i>K_d</i>)	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 (<i>K_d</i>)	680	540	31	2700	370	>10000	2100	59	⁸ sorafenib
BAX		22	68					90	⁹ sorafenib
BMU (<i>K_d</i>)					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
L12					630				¹⁴ #23
L13					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 (<i>K_d</i>)	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)			Crystallographic type-II inhibitors		
	PDB codes and chains			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 (1uw 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)				

^aNarrow pocket; ^bT315I imatinib-resistant mutation; ^cConserved salt bridge disrupted; ^dConserved 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 $K_{io} = [\text{In}] / [\text{Out}]$, 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 $K_b = [\text{OutCpd}] / [\text{Out}][\text{Cpd}]$, 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 $\Delta G_b^{obs}(ABL1_{unphos}, imatinib) \approx -10.27$ kcal/mol and $\Delta G_b^{obs}(ABL1_{phos}, imatinib) \approx -7.12$ 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 different⁸:

- ABL1: $K_d^{obs} = 12\text{nM}$, i.e. $\Delta G_b^{obs} = -10.94$ kcal/mol
- LCK: $K_d^{obs} = 40\text{nM}$, i.e. $\Delta G_b^{obs} = -10.22$ kcal/mol
- SRC: $K_d^{obs} > 10000\text{nM}$, i.e. $\Delta G_b^{obs} > -6.90$ 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.

References

- (1) DiMauro, E. F.; Newcomb, J.; Nunes, J. J.; Bemis, J. E.; Boucher, C.; Buchanan, J. L.; Buckner, W. H.; Cee, V. J.; Chai, L.; Deak, H. L.; Epstein, L. F.; Faust, T.; Gallant, P.; Geuns-Meyer, S. D.; Gore, A.; Gu, Y.; Henkle, B.; Hodous, B. L.; Hsieh, F.; Huang, X.; Kim, J. L.; Lee, J. H.; Martin, M. W.; Masse, C. E.; McGowan, D. C.; Metz, D.; Mohn, D.; Morgenstern, K. A.; Oliveira-dos-Santos, A.; Patel, V. F.; Powers, D.; Rose, P. E.; Schneider, S.; Tomlinson, S. A.; Tudor, Y. Y.; Turci, S. M.; Welcher, A. A.; White, R. D.; Zhao, H.; Zhu, L.; Zhu, X., Discovery of Aminoquinazolines as Potent, Orally Bioavailable Inhibitors of Lck: Synthesis, SAR, and in Vivo Anti-Inflammatory Activity. *J. Med. Chem.* **2006**, 49, (19), 5671-5686.
- (2) Sullivan, J. E.; Holdgate, G. A.; Campbell, D.; Timms, D.; Gerhardt, S.; Breed, J.; Breeze, A. L.; Bermingham, A.; Pauptit, R. A.; Norman, R. A.; Embrey, K. J.; Read, J.; VanScyoc, W. S.; Ward, W. H. J., Prevention of MKK6-Dependent Activation by Binding to p38a MAP Kinase. *Biochemistry* **2005**, 44, (50), 16475-16490.
- (3) Potashman, M. H.; Bready, J.; Coxon, A.; DeMelfi, T. M.; DiPietro, L.; Doerr, N.; Elbaum, D.; Estrada, J.; Gallant, P.; Germain, J.; Gu, Y.; Harmange, J.-C.; Kaufman, S. A.; Kendall, R.; Kim, J. L.; Kumar, G. N.; Long, A. M.; Neervannan, S.; Patel, V. F.; Polverino, A.; Rose, P.; van der Plas, S.; Whittington, D.; Zanon, R.; Zhao, H., Design, Synthesis, and Evaluation of Orally Active Benzimidazoles and Benzoxazoles as Vascular Endothelial Growth Factor-2 Receptor Tyrosine Kinase Inhibitors. *J. Med. Chem.* **2008**, 51, (3), 699-699.
- (4) Horio, T.; Hamasaki, T.; Inoue, T.; Wakayama, T.; Itou, S.; Naito, H.; Asaki, T.; Hayase, H.; Niwa, T., Structural factors contributing to the Abl/Lyn dual inhibitory activity of 3-substituted benzamide derivatives. *Bioorganic & Medicinal Chemistry Letters* **2007**, 17, (10), 2712-2717.
- (5) Hodous, B. L.; Geuns-Meyer, S. D.; Hughes, P. E.; Albrecht, B. K.; Bellon, S.; Bready, J.; Caenepeel, S.; Cee, V. J.; Chaffee, S. C.; Coxon, A.; Emery, M.; Fretland, J.; Gallant, P.; Gu,

- Y.; Hoffman, D.; Johnson, R. E.; Kendall, R.; Kim, J. L.; Long, A. M.; Morrison, M.; Olivieri, P. R.; Patel, V. F.; Polverino, A.; Rose, P.; Tempest, P.; Wang, L.; Whittington, D. A.; Zhao, H., Evolution of a Highly Selective and Potent 2-(Pyridin-2-yl)-1,3,5-triazine Tie-2 Kinase Inhibitor. *J. Med. Chem.* **2007**, 50, (4), 611-626.
- (6) Okram, B.; Nagle, A.; Adrian, F. J.; Lee, C.; Ren, P.; Wang, X.; Sim, T.; Xie, Y.; Wang, X.; Xia, G.; Spraggon, G.; Warmuth, M.; Liu, Y.; Gray, N. S., A General Strategy for Creating "Inactive-Conformation" Abl Inhibitors. *Chemistry & Biology* **2006**, 13, (7), 779-786.
- (7) Deak, H. L.; Newcomb, J. R.; Nunes, J. J.; Boucher, C.; Cheng, A. C.; DiMauro, E. F.; Epstein, L. F.; Gallant, P.; Hodous, B. L.; Huang, X.; Lee, J. H.; Patel, V. F.; Schneider, S.; Turci, S. M.; Zhu, X., N-(3-(Phenylcarbamoyl)arylpyrimidine)-5-carboxamides as potent and selective inhibitors of Lck: Structure, synthesis and SAR. *Bioorganic & Medicinal Chemistry Letters* **2008**, 18, (3), 1172-1176.
- (8) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P., A quantitative analysis of kinase inhibitor selectivity. *Nat Biotech* **2008**, 26, (1), 127-132.
- (9) Liu, Y.; Gray, N. S., Rational design of inhibitors that bind to inactive kinase conformations. *Nat Chem Biol* **2006**, 2, (7), 358-364.
- (10) Ahmad, T.; Eisen, T., Kinase Inhibition with BAY 43-9006 in Renal Cell Carcinoma. *Clin Cancer Res* **2004**, 10, (18), 6388S-6392.
- (11) Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J., Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat Struct Mol Biol* **2002**, 9, (4), 268-272.

- (12) Hasegawa, M.; Nishigaki, N.; Washio, Y.; Kano, K.; Harris, P. A.; Sato, H.; Mori, I.; West, R. I.; Shibahara, M.; Toyoda, H.; Wang, L.; Nolte, R. T.; Veal, J. M.; Cheung, M., Discovery of Novel Benzimidazoles as Potent Inhibitors of TIE-2 and VEGFR-2 Tyrosine Kinase Receptors. *J. Med. Chem.* **2007**, 50, (18), 4453-4470.
- (13) Cowan-Jacob, S. W.; Fendrich, G.; Floersheimer, A.; Furet, P.; Liebetanz, J.; Rummel, G.; Rheinberger, P.; Centeleghe, M.; Fabbro, D.; Manley, P. W., Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. *Acta Crystallographica Section D* **2007**, 63, (1), 80-93.
- (14) Gill, A. L.; Frederickson, M.; Cleasby, A.; Woodhead, S. J.; Carr, M. G.; Woodhead, A. J.; Walker, M. T.; Congreve, M. S.; Devine, L. A.; Tisi, D.; O'Reilly, M.; Seavers, L. C. A.; Davis, D. J.; Curry, J.; Anthony, R.; Padova, A.; Murray, C. W.; Carr, R. A. E.; Jhoti, H., Identification of Novel p38a MAP Kinase Inhibitors Using Fragment-Based Lead Generation. *J. Med. Chem.* **2005**, 48, (2), 414-426.
- (15) Miyazaki, Y.; Matsunaga, S.; Tang, J.; Maeda, Y.; Nakano, M.; Philippe, R. J.; Shibahara, M.; Liu, W.; Sato, H.; Wang, L.; Nolte, R. T., Novel 4-amino-furo[2,3-d]pyrimidines as Tie-2 and VEGFR2 dual inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2005**, 15, (9), 2203-2207.
- (16) Hodous, B. L.; Geuns-Meyer, S. D.; Hughes, P. E.; Albrecht, B. K.; Bellon, S.; Caenepeel, S.; Cee, V. J.; Chaffee, S. C.; Emery, M.; Fretland, J.; Gallant, P.; Gu, Y.; Johnson, R. E.; Kim, J. L.; Long, A. M.; Morrison, M.; Olivieri, P. R.; Patel, V. F.; Polverino, A.; Rose, P.; Wang, L.; Zhao, H., Synthesis, structural analysis, and SAR studies of triazine derivatives as potent, selective Tie-2 inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2007**, 17, (10), 2886-2889.
- (17) Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W. T.; Clarkson, B.; Kuriyan, J., Structural Mechanism for STI-571 Inhibition of Abelson Tyrosine Kinase. *Science* **2000**, 289, (5486), 1938-1942.

- (18) Young, M. A.; Shah, N. P.; Chao, L. H.; Seeliger, M.; Milanov, Z. V.; Biggs, W. H., III; Treiber, D. K.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J.; Sawyers, C. L.; Kuriyan, J., Structure of the Kinase Domain of an Imatinib-Resistant Abl Mutant in Complex with the Aurora Kinase Inhibitor VX-680. *Cancer Res* **2006**, 66, (2), 1007-1014.
- (19) Levinson, N. M.; Kuchment, O.; Shen, K.; Young, M. A.; Koldobskiy, M.; Karplus, M.; Cole, P. A.; Kuriyan, J., A Src-like inactive conformation in the Abl tyrosine kinase domain. *PLoS Biol* **2006**, 4, (5).
- (20) Zhou, T.; Parillon, L.; Li, F.; Wang, Y.; Keats, J.; Lamore, S.; Xu, Q.; Shakespeare, W.; Dalgarno, D.; Zhu, X., Crystal Structure of the T315I Mutant of Abl Kinase. *Chemical Biology & Drug Design* **2007**, 70, (3), 171-181.
- (21) Tokarski, J. S.; Newitt, J. A.; Chang, C. Y. J.; Cheng, J. D.; Wittekind, M.; Kiefer, S. E.; Kish, K.; Lee, F. Y. F.; Borzilleri, R.; Lombardo, L. J.; Xie, D.; Zhang, Y.; Klei, H. E., The Structure of Dasatinib (BMS-354825) Bound to Activated ABL Kinase Domain Elucidates Its Inhibitory Activity against Imatinib-Resistant ABL Mutants. *Cancer Res* **2006**, 66, (11), 5790-5797.
- (22) Modugno, M.; Casale, E.; Soncini, C.; Rosettani, P.; Colombo, R.; Lupi, R.; Rusconi, L.; Fancelli, D.; Carpinelli, P.; Cameron, A. D.; Isacchi, A.; Moll, J., Crystal Structure of the T315I Abl Mutant in Complex with the Aurora Kinases Inhibitor PHA-739358. *Cancer Res* **2007**, 67, (17), 7987-7990.
- (23) Nagar, B.; Bornmann, W. G.; Pellicena, P.; Schindler, T.; Veach, D. R.; Miller, W. T.; Clarkson, B.; Kuriyan, J., Crystal Structures of the Kinase Domain of c-Abl in Complex with the Small Molecule Inhibitors PD173955 and Imatinib (STI-571). *Cancer Res* **2002**, 62, (15), 4236-4243.

- (24) Nagar, B.; Hantschel, O.; Young, M. A.; Scheffzek, K.; Veach, D.; Bornmann, W.; Clarkson, B.; Superti-Furga, G.; Kuriyan, J., Structural Basis for the Autoinhibition of c-Abl Tyrosine Kinase. *Cell* **2003**, 112, (6), 859-871.
- (25) King, A. J.; Patrick, D. R.; Batorsky, R. S.; Ho, M. L.; Do, H. T.; Zhang, S. Y.; Kumar, R.; Rusnak, D. W.; Takle, A. K.; Wilson, D. M.; Hugger, E.; Wang, L.; Karreth, F.; Lougheed, J. C.; Lee, J.; Chau, D.; Stout, T. J.; May, E. W.; Rominger, C. M.; Schaber, M. D.; Luo, L.; Lakdawala, A. S.; Adams, J. L.; Contractor, R. G.; Smalley, K. S. M.; Herlyn, M.; Morrissey, M. M.; Tuveson, D. A.; Huang, P. S., Demonstration of a Genetic Therapeutic Index for Tumors Expressing Oncogenic BRAF by the Kinase Inhibitor SB-590885. *Cancer Res* **2006**, 66, (23), 11100-11105.
- (26) Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Project, C. G.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R., Mechanism of Activation of the RAF-ERK Signaling Pathway by Oncogenic Mutations of B-RAF. *Cell* **2004**, 116, (6), 855-867.
- (27) Mol, C. D.; Lim, K. B.; Sridhar, V.; Zou, H.; Chien, E. Y. T.; Sang, B.-C.; Nowakowski, J.; Kassel, D. B.; Cronin, C. N.; McRee, D. E., Structure of a c-Kit Product Complex Reveals the Basis for Kinase Transactivation. *J. Biol. Chem.* **2003**, 278, (34), 31461-31464.
- (28) Mol, C. D.; Dougan, D. R.; Schneider, T. R.; Skene, R. J.; Kraus, M. L.; Scheibe, D. N.; Snell, G. P.; Zou, H.; Sang, B.-C.; Wilson, K. P., Structural Basis for the Autoinhibition and STI-571 Inhibition of c-Kit Tyrosine Kinase. *J. Biol. Chem.* **2004**, 279, (30), 31655-31663.
- (29) Zhu, X.; Kim, J. L.; Newcomb, J. R.; Rose, P. E.; Stover, D. R.; Toledo, L. M.; Zhao, H.; Morgenstern, K. A., Structural analysis of the lymphocyte-specific kinase Lck in complex with non-selective and Src family selective kinase inhibitors. *Structure* **1999**, 7, (6), 651-661.
- (30) Martin, M. W.; Newcomb, J.; Nunes, J. J.; Bemis, J. E.; McGowan, D. C.; White, R. D.; Buchanan, J. L.; DiMauro, E. F.; Boucher, C.; Faust, T.; Hsieh, F.; Huang, X.; Lee, J. H.;

- Schneider, S.; Turci, S. M.; Zhu, X., Discovery of novel 2,3-diarylfuro[2,3-b]pyridin-4-amines as potent and selective inhibitors of Lck: Synthesis, SAR, and pharmacokinetic properties. *Bioorganic & Medicinal Chemistry Letters* **2007**, 17, (8), 2299-2304.
- (31) Martin, M. W.; Newcomb, J.; Nunes, J. J.; McGowan, D. C.; Armistead, D. M.; Boucher, C.; Buchanan, J. L.; Buckner, W.; Chai, L.; Elbaum, D.; Epstein, L. F.; Faust, T.; Flynn, S.; Gallant, P.; Gore, A.; Gu, Y.; Hsieh, F.; Huang, X.; Lee, J. H.; Metz, D.; Middleton, S.; Mohn, D.; Morgenstern, K.; Morrison, M. J.; Novak, P. M.; Oliveira-do-Santos, A.; Powers, D.; Rose, P.; Schneider, S.; Sell, S.; Tudor, Y.; Turci, S. M.; Welcher, A. A.; White, R. D.; Zack, D.; Zhao, H.; Zhu, L.; Zhu, X.; Ghiron, C.; Amouzegh, P.; Ermann, M.; Jenkins, J.; Johnston, D.; Napier, S.; Power, E., Novel 2-Aminopyrimidine Carbamates as Potent and Orally Active Inhibitors of Lck: Synthesis, SAR, and in Vivo Antiinflammatory Activity. *J. Med. Chem.* **2006**, 49, (16), 4981-4991.
- (32) Yamaguchi, H.; Hendrickson, W. A., Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. *Nature* **1996**, 384, (6608), 484-489.
- (33) Jacobs, M. D.; Caron, P. R.; Hare, B. J., Classifying protein kinase structures guides use of ligand-selectivity profiles to predict inactive conformations: Structure of lck/imatinib complex. *Proteins: Structure, Function, and Bioinformatics* **2008**, 70, (4), 1451-1460.
- (34) Stelmach, J. E.; Liu, L.; Patel, S. B.; Pivnichny, J. V.; Scapin, G.; Singh, S.; Hop, C. E. C. A.; Wang, Z.; Strauss, J. R.; Cameron, P. M.; Nichols, E. A.; O'Keefe, S. J.; O'Neill, E. A.; Schmatz, D. M.; Schwartz, C. D.; Thompson, C. M.; Zaller, D. M.; Doherty, J. B., Design and synthesis of potent, orally bioavailable dihydroquinazolinone inhibitors of p38 MAP kinase. *Bioorganic & Medicinal Chemistry Letters* **2003**, 13, (2), 277-280.
- (35) Fitzgerald, C. E.; Patel, S. B.; Becker, J. W.; Cameron, P. M.; Zaller, D.; Pikounis, V. B.; O'Keefe, S. J.; Scapin, G., Structural basis for p38[alpha] MAP kinase quinazolinone and pyridol-pyrimidine inhibitor specificity. *Nat Struct Mol Biol* **2003**, 10, (9), 764-769.

- (36) Trejo, A.; Arzeno, H.; Browner, M.; Chanda, S.; Cheng, S.; Comer, D. D.; Dalrymple, S. A.; Dunten, P.; Lafargue, J.; Lovejoy, B.; Freire-Moar, J.; Lim, J.; McIntosh, J.; Miller, J.; Papp, E.; Reuter, D.; Roberts, R.; Sanpablo, F.; Saunders, J.; Song, K.; Villasenor, A.; Warren, S. D.; Welch, M.; Weller, P.; Whiteley, P. E.; Zeng, L.; Goldstein, D. M., Design and Synthesis of 4-Azaindoles as Inhibitors of p38 MAP Kinase. *J. Med. Chem.* **2003**, 46, (22), 4702-4713.
- (37) Haar, E. t.; Prabakhar, P.; Liu, X.; Lepre, C., Crystal Structure of the P38{alpha}-MAPKAP Kinase 2 Heterodimer. *J. Biol. Chem.* **2007**, 282, (13), 9733-9739.
- (38) Xu, W.; Harrison, S. C.; Eck, M. J., Three-dimensional structure of the tyrosine kinase c-Src. *Nature* **1997**, 385, (6617), 595-602.
- (39) Cowan-Jacob, S. W.; Fendrich, G.; Manley, P. W.; Jahnke, W.; Fabbro, D.; Liebetanz, J.; Meyer, T., The Crystal Structure of a c-Src Complex in an Active Conformation Suggests Possible Steps in c-Src Activation. *Structure* **2005**, 13, (6), 861-871.
- (40) Fleury, D.; Sarubbi, E.; Courjaud, A.; Guitton, J. D.; Ducruix, A., Structure of the unphosphorylated C-terminal tail segment of the SRC kinase and its role in SRC activity regulation. *To be published*.
- (41) Breitenlechner, C. B.; Kairies, N. A.; Honold, K.; Scheiblich, S.; Koll, H.; Greiter, E.; Koch, S.; Schäfer, W.; Huber, R.; Engh, R. A., Crystal Structures of Active Src Kinase Domain Complexes. *Journal of Molecular Biology* **2005**, 353, (2), 222-231.
- (42) Dalgarno, D.; Stehle, T.; Narula, S.; Schelling, P.; Rose van Schravendijk, M.; Adams, S.; Andrade, L.; Keats, J.; Ram, M.; Jin, L.; Grossman, T.; MacNeil, I.; Metcalf, C.; Shakespeare, W.; Wang, Y.; Keenan, T.; Sundaramoorthi, R.; Bohacek, R.; Weigele, M.; Sawyer, T., Structural Basis of Src Tyrosine Kinase Inhibition with a New Class of Potent and Selective Trisubstituted Purine-based Compounds. *Chemical Biology & Drug Design* **2006**, 67, (1), 46-57.

- (43) Blair, J. A.; Rauh, D.; Kung, C.; Yun, C.-H.; Fan, Q.-W.; Rode, H.; Zhang, C.; Eck, M. J.; Weiss, W. A.; Shokat, K. M., Structure-guided development of affinity probes for tyrosine kinases using chemical genetics. *Nat Chem Biol* **2007**, 3, (4), 229-238.
- (44) Seeliger, M. A.; Nagar, B.; Frank, F.; Cao, X.; Henderson, M. N.; Kuriyan, J., c-Src Binds to the Cancer Drug Imatinib with an Inactive Abl/c-Kit Conformation and a Distributed Thermodynamic Penalty. *Structure* **2007**, 15, (3), 299-311.