EXPERIMENTAL PROCEDURES

The x-ray crystal structure of SRC with the inhibitor AP23464 (PDB ID: 2BDJ) was used for modeling. Docking was performed using Glide XP.1 The preparation of the protein for docking was performed with PrepWizard using the standard protocol, including the addition of hydrogens, the assignment of bond order and of correct protonation states. Five crystallographic waters were retained in the active site. The docking protocol was originally carried out both in the presence and in the absence of the water molecules. The comparison of the docking modes revealed that retaining the crystallographic waters in the binding site helps position the ligands more consistently, resulting in a better alignment for WaterMap. The hydrogen bonding network of the protein was optimized. Self-docking of the AP023464 ligand showed that the crystallographic pose is well reproduced (with rmsd of 0.41 Å).

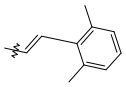
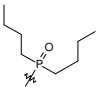
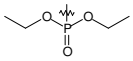
The data set contains 49 neutral compounds built around a purine template, with variations in the ribose pocket, the selectivity pocket and the solvent front (Table 1 and Table 1S). The selection of neutral compounds was due to the known discrepancies in scoring of charged ligands by Prime MM-GB/SA.2 The ligands were treated with LigPrep3 to obtain 3D structures. Docking with Glide XP was followed by minimization. A hydrogen bond constraint was employed at the hinge (backbone NH of M341.) Three best scoring poses were saved for each molecule. The docking poses of all the inhibitors were consistent with the x-ray crystal structure. There were conformational differences among the docked solutions for the R2 solvent exposed groups in the ribose pocket, which eventually led to some variation in the scoring, particularly with WaterMap, depending on which pose was selected. We chose the top scoring pose for further minimization with MM-GBSA and WaterMap scoring (however, we did generate alternative plots where the lower-ranking docking poses were used; the overall statistics of the prediction with WaterMap were not significantly changed).

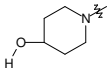
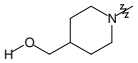
MM-GB/SA using Prime was performed on top scoring ligand poses, using default settings. The receptor was kept fixed during this calculation. No ligand strain was included in the calculation of ΔGbind. WaterMap4 was performed with default options on the crystal structure 2BDJ. An initial minimization and a molecular dynamics simulation was run for 9 ns on a solvated protein system in the absence of the ligand. The best docking poses were then scored using WaterMap. Although the docking poses obtained with XP Glide look very reasonable, we noticed that when the alignment of the templates is not precise, the resulting scoring by WaterMap contains some noise. In fact, an rms of 0.5 Å between the poses of the template can result in a difference in WaterMap ΔG of 2.0 kcal/mol (for w6 hydration site). Additional manual refinement of the aligned poses was required (for the templates, as well as for the consistent positioning of the R1, R2 and R3 substituents), followed by minimization with MM-GBSA. This ensured an energetically favorable set of poses which consistently displaced the high-energy water molecules. The conformational entropy correction of 0.5 kcal/mol per rotatable bond was added5 to the WaterMap score, but no significant improvement was obtained (plot not shown). To understand the influence of R2 substituents on the charge distribution on N3, Jaguar was used to perform a single-point energy calculation (DFT/BLYP3/6-31G**) on the docked poses of selected compounds, and ESP fitted charges were obtained.

(1) Glide v5.7; Schrodinger, Inc.Portland, OR.
(2) Prime v3.0; Schrodinger, Inc. : Portland, OR.
(3) Ligprep v2.5; Schrodinger, Inc.: Portland, OR.
(4) Abel, R.; Young, T.; Farid, R.; Berne, B. J.; Friesner, R. A. Role of the Active-Site Solvent in the Thermodynamics of Factor Xa Ligand Binding. *J. Am. Chem. Soc.* **2008**, *130*, 2817.
(5) Higgs, C.; Beuming, T.; Sherman, W. Hydration Site Thermodynamics Explain SARs for Triazolylpurines Analogues Binding to the A2A Receptor. *ACS Med. Chem. Lett.* **2010**, *1*, 160.

Table S1. The complete data set of purine-based SRC inhibitors used for the study.

COMPOUND	R1	R2	R3	SRC IC50
1				0.5
2				0.5
3				0.8
4				0.8
5				0.9
6				0.9
7				1.2
8				1.3
9				1.3
10				1.3
11				1.4
12				2.1
13				2.9
14				3.3
15				3.9
16				4.4

COMPOUND	R1	R2	R3	SRC IC50
17				4.4
18				8.8
19				9.1
20				12.4
21				17.7
22				25.1
23				31.9
24				36.9
25				41.2
26				43.1
27				46.7
28				55.6
29				93.9
30				99.3
31				113.0
32				116.0

COMPOUND	R1	R2	R3	SRC IC50
33				126.0
34				138.0
35				155.0
36				163.0
37				178.0
38				188.0
39				198.0
40				248.0
41				290.0
42				323.0
43				359.0
44				385.0
45				458.0
46				462.0
47				601.0
48				1000.0
49				134.0