SUPPORTING INFORMATION

A combination of docking/dynamics simulations and pharmacophoric modeling to discover new dual Src/Abl kinase inhibitors

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Preliminary docking calculations

In general, to assess the ability of the docking software to reproduce interaction pathway between ligand/inhibitors and the corresponding receptor counterpart, we usually perform preliminary simulations on co-crystallized structures,[1] when they are available. Accordingly, to check the reliability of the software in finding the binding mode of tyrosine kinase inhibitors, preliminary docking calculations were performed starting from co-crystallized complexes containing three of the five small molecules used as template inhibitors [namely, staurosporine, PP2, PD173955 (**2**), BMS-35482513 (**1**), and **3**, Chart 1 of the main text) in addition to Gleevec. In particular, four structures of tyrosine kinases in complex with structurally diverse inhibitors were selected from the Brookhaven data bank. Two of them (namely, 1opj[2] and 1m52)[3] represented the complexes between c-Abl with Gleevec or **2**, respectively. The remaining complexes (1qpd and 1qpe)[4] were constituted by Lck with staurosporine or PP2, respectively. The computational protocol applied was aimed at checking the ability of the software to find an orientation and the interactions of the inhibitor within the binding site as similar as possible to those found in the crystallized complex. Table S1 reports the root-mean-square deviation (rmsd) calculated comparing the inhibitor structure derived from docking calculations with respect to that in the crystal complex. Rmsd values ranging from 0.4 to 1.0 Å suggested that the software was an appropriate tool to investigate the binding mode of such inhibitors within the binding site of tyrosine kinases and, thus, it could be applied to find the binding mode of the same or additional inhibitors within the binding site of c-Src.

Further details on the binding mode of template compounds

Staurosporine is a microbial alkaloid isolated from *Streptomyces* sp.[5] which was shown to be a potent broad-range inhibitor competing with ATP, but not peptide substrate, for binding to protein kinases.[6] Staurosporine has nanomolar potency against many protein kinases, including members of the Src family kinase (SFK) (i.e., about 40 nM toward c-Src), with a consequent low selectivity. Its very low conformational freedom and the structural motif common to Src inhibitors make staurosporine an optimal compound to build pharmacophoric models of Src inhibitors. Results from

molecular dynamics (MD) simulations show that NH and CO of the lactam moiety interact by hydrogen bonds with backbone CO and NH of Glu339 and Met341, respectively. Frequency and distance reported in Table 3 of the main text suggest that hydrogen bonds involving the lactam moiety are very profitable in stabilizing the complex between c-Src and staurosporine. Inspection of the MD output reveals that the amine nitrogen is able to form transient hydrogen bond contacts with different amino acid partners of the solvent accessible region, such as Asp404 and Asp348 (the latter with a frequency of 34%). Finally, hydrophobic contacts are found between one of the condensed indole ring and the hydrophobic region I of the Src binding site.

PP2 belongs to the pyrazolo[3,4-*d*]pyrimidine class of SFK inhibitors, with a 300 nM activity toward c-Src.[7] Calculations show an interaction pattern similar to that of staurosporine, with a pair of hydrogen bonds involving the amino group at C4 (with the backbone carbonyl of Glu339) and the endocyclic N5 (with the NH group of Met341). The hydroxy group of Thr338 is a competitor of Glu339 for hydrogen bonding the amino group at C4, with a frequency <10%. Finally, the *p*-chloro phenyl group is embedded within the hydrophobic region I.

Compound **3** is a 5,7-diaryl pyrrolo[2,3-*d*]pyrimidine family member with a 10 nM activity toward c-Src.[8] Its orientation within the binding site is similar to that previously described in the model of CGP62464.[8] In particular, the double hydrogen bond motif involving the amino group at C4 and the endocyclic N3 is found, contacting the backbone CO and NH groups of Glu339 and Met341, respectively. Also in this case, similarly to that found for PP2, docking calculations show that the hydroxy group of Thr338 is able to interact by a hydrogen bond with the C4 amino group of the inhibitor. However, MD simulations suggest that such an interaction is maintained with a very low frequency (<1%). Transient hydrogen bonds between the hydroxy group at the N7 side chain and amino acid partners of the solvent accessible region (Lys295, Asp348, and Asp404) are found with insignificant frequency (they are also characterized by a hydrogen bond distance higher than 4 Å during MD simulations). Finally, the phenyl ring at position 5 is accommodated within the hydrophobic region I.

Compound **2** is a pyrido[2,3-*d*]pyrimidin-7(8*H*)-one with high activity toward c-Src (22 nM).[9a] Due to its additional ability to inhibit the Abl kinase, such a compound is classified as Src-Abl dual action inhibitor.[3,9b] Calculations show an orientation and interactions similar to those found for the same compound in the complex with the Abl kinase. In particular, both the NH at C2 and the endocyclic N3 contact (as hydrogen bond donor and hydrogen bond acceptor, respectively) the carbonyl and the NH moieties of Met341, showing a hydrogen bond pattern in part different from that above described for staurosporine, PP2 and **3**. In fact, while the contact with the NH group of Met341 is unchanged, the hydrogen bond acceptor group on the receptor is represented by the carbonyl group of Met341 in this case (referred to as the second binding mode, in the main text), instead of the backbone carbonyl of Glu339, in the previous case (referred to as the firts binding mode, in the main text). The hydrogen bond pattern found for **2** is in agreement with crystallographic structures showing the same compound as the inhibitor.[3] Hydrophobic interactions are found between the halophenyl group at C6 and the hydrophobic region I, and between the methylthiophenyl side chain and the hydrophobic region II. No interaction with the solvent accessible surface is found.

Compound **1** is a potent c-Src/Abl dual acting inhibitor with activity of 0.5 and 0.02 nM, respectively.[10] The best orientation within the Src binding site belongs to the second binding mode and it is in good agreement with results previously reported,[10] with differences involving the phenyl and pyrimidine rings, rotated of about 180 degrees. Considering the high degree of conformational flexibility and the fact that the chlorine atom and the methyl group on the phenyl ring could be considered as isostere groups, we can conclude that the orientation found is acceptable for the purposes of pharmacophoric generation pursued in the next step of the computational protocol. Hydrogen bonds to be monitored during MD are chosen on the basis of docking simulations on this inhibitor performed by us and others.[10] In particular, contacts between the NH and CO groups of Met341 with the thiazole nitrogen and the amino group at C2, respectively, are found in our docked complex, together with a hydrogen bond between the hydroxy group of Thr338 and the NH amido

group of the inhibitor. Moreover, based on results obtained for PP2, showing a hydrogen bond with the acceptor group shifting from the carbonyl of Glu339 to the hydroxy of Thr338, we plan to check also the putative contact between Thr338-OH and the amido group of the inhibitor. Finally, based on the fact that a hydrogen bond between the carbonyl amido group of the inhibitor and the terminal amino group of Lys295 was previously described,[10] also such an interaction was monitored. MD results show that both interactions with methionine are profitable for complex stabilization, while Lys295 appears to be unable to bind the amide carbonyl group. In a similar way, the amide NH group prefers a hydrogen bond with Glu339 (47%) with respect to the interaction with Thr338, characterized by very low frequency and geometrical parameters (in terms of both distance and angles) inappropriate to make a hydrogen bond. Finally, the phenyl ring is accommodated within the hydrophobic region I and the methylpyrimidine nucleus was located into the hydrophobic region II.

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Table S1. Description of the crystallographic structures used to test the docking procedure.

Pdb entry	**Tyrosine kinase**	**Inhibitor**	**rmsd (Å)**[a]
1opj	Abl	Gleevec	1.0
1m52	Abl	PD173955, **2**	0.4
1qpd	Lck	staurosporine	0.5
1qpe	Lck	PP2	0.6

[a]Root-mean square deviation (calculated on all the heavy atoms of each inhibitor) between crystallographic and docked complexes.