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Modeling comparative selectivity profiles of kinase inhibitors using FEP/MD protocol

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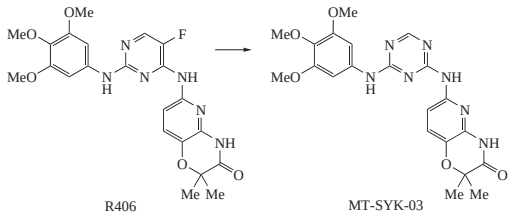
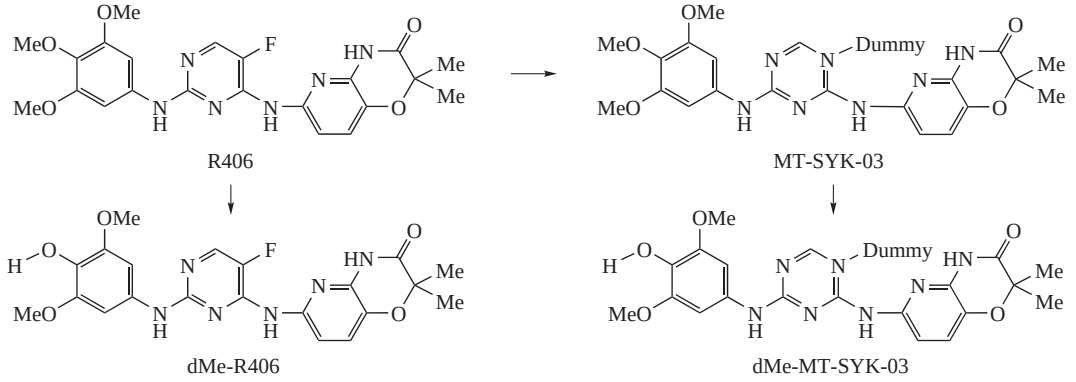
Free energy perturbation (FEP)-based molecular modeling simulation of 5-fluoropyrimidine and 1,3,5-triazine derivatives followed by their synthesis and experimental evaluation have been carried out to estimate kinase selectivity profile. 5-Fluoropyrimidine derivatives show similar binding affinity for c-Src, Btk and Jak1 kinases, while 1,3,5-triazine derivatives demonstrate c-Src kinase selectivity.



Profile of a kinase inhibitor is an important indicator of its potential off-target effects including adverse effects, which sometimes might be quite severe and even preclude application of a drug in clinical practice. One of the recent examples demonstrating how the lack of drug selectivity impedes its clinical application because of abundant adverse effects is the Tamatinib one (R406, Scheme 1). This 5-fluoropyrimidine derivative is a potent inhibitor of Syk and c-Src kinases as well as Btk, Jak1 and over 78 other off-target kinases.[1] Inhibition of c-Srs kinase induces the synthesis of aggrecan and collagen II (essential components of articular cartilage), suppresses catabolism of cartilaginous tissue by reducing the secretion of aggrecanases, and depresses osteoclast-mediated resorption of bone tissue; inhibition of Syk kinase represses the signal cascades activated by this kinase in synovial cells cavity. This leads cytokine production to decrease (IL-1b, TNFalpha), inducing inflammation and acceleration catabolism of cartilage. Selective inhibitors of these two kinases can be used for treatment of rheumatoid arthritis, osteoarthritis and osteoporosis. With the intent to overcome adverse effects of R406, we designed a MT-SYK-03 (1,3,5-triazine derivative) molecule,[2] which appeared to be comparable to R406 with respect to c-Src suppression, but inhibited only five off-target kinases. In order to rationalize enhanced selectivity, we established a free energy perturbation molecular dynamics (FEP/MD) protocol to compare 5-fluoropyrimidine *vs*. 1,3,5-triazine derivatives binding affinities towards c-Src, Btk and Jak1 protein kinases.

In a FEP/MD protocol established, for each kinase R406 or MT-SYK-03 molecules were transformed into dMe-R406, MT-SYK-03 or dMe-MT-SYK-03 according to Figure 1, and the change in Gibbs free energy (d*G*) of such transitions was recorded. Initial structures of protein-R406 complexes were obtained by docking R406 into ATP-binding site of a kinase. Then the protein–ligand complexes were immersed in a box of explicit solvent (water). Each FEP transition was split into two steps: removing partial charges from initial molecule (R406 or MT-SYK-03) and conversion of Van der Waals parameters to another molecule (dMe-R406, MT-SYK-03 or dMe-MT-SYK-03). Each transition in its turn was split into ten lambda windows, each taking 2 ns of MD simulation.[†]

Relative free energies of binding for 5-fluoropyrimidine and 1,3,5-triazine derivatives predicted by molecular modeling study are given in Table 1. According to FEP-based calculations, all compounds interact with c-Src kinase with a similar binding



Scheme 1 FEP transformation of 5-fluoropyrimidine and 1,3,5-triazine derivatives.

Table 1 Relative free energies of binding and selectivity of 5-fluoropyrimidine and 1,3,5-triazine derivatives.

Kinase	PDB ID	FEP-transformation	ddG[a]/kcal mol^{-1}			∑ddG/kcal mol^{-1}		lg S[c]	
			Charge	van der Waals	CF[b]	Calculated	Experimental	Calculated	Experimental
c-Src	1Y57	R406 → MT-SYK-03	0.6	–1.7	0.9	–0.2	–0.1	–0.16	–0.04
Btk	3PIY	R406 → MT-SYK-03	0.3	0.7	0.9	1.9	0.9	1.35	0.67
Jak1	5E1E	R406 → MT-SYK-03	0.6	1.2	0.9	2.7	3.1	1.95	2.23
c-Src	1Y57	R406 → dMe-R406	0.0	0.3	0.1	0.4	–[d]	0.32	–[d]
Btk	3PIY	R406 → dMe-R406	0.1	–0.4	0.1	–0.2	–[d]	–0.14	–[d]
Jak1	5E1E	R406 → dMe-R406	0.1	0.3	0.1	0.5	–[d]	0.33	–[d]
c-Src	1Y57	R406 → MT-SYK-03 → dMe-MT-SYK-03	0.7	–1.3	0.7	0.1	0.0	0.10	0.03
Btk	3PIY	R406 → MT-SYK-03 → dMe-MT-SYK-03	0.1	0.2	0.7	1.0	0.6	0.70	0.43
Jak1	5E1E	R406 → MT-SYK-03 → dMe-MT-SYK-03	0.9	1.8	0.7	3.4	3.2	2.46	2.29

[a] dd*G* is a difference in binding d*G* of the final and initial (R406) ligands. [b]CF is the ligand conformer focusing energy.[3] [c]*S* is the selectivity. [d] Not determined.

affinity, however 1,3,5-triazine derivatives (MT-SYK-03 and dMe-MT-SYK-03) demonstrate much weaker binding for Btk and Jak1 kinases compared to 5-fluoropyrimidine derivative. Analysis of molecular dynamical trajectories of R406 and MT-SYK-03 binding in the active site of different kinases uncovered structural factors contributing to the enhanced binding of 1,3,5-triazine derivatives with c-Src kinase. In the c-Src kinases, we observed a water molecule which formed favorable H-bond with MT-SYK-03 triazine nitrogen atom. Favorable interaction *via* bridging water was absent for R406 and other 5-fluoropyrimidine derivatives because instead of nitrogen, an H-bond acceptor, these compounds bear fluorine and carbon atoms. Notably, bridging water molecule was missing in the active site of off-target kinases (Btk and Jak1) since it was replaced by nonpolar sidechains (Figure 1).

We studied the selectivity of R406, a novel Syk/c-Src kinase inhibitor MT-SYK-03 and the newly synthesized compound dMe-MT-SYK-03 (Schemes 1 and 2) in kinase inhibition

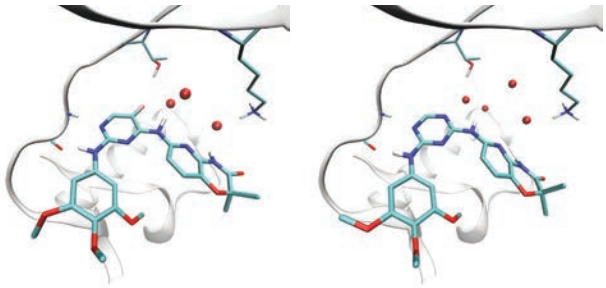
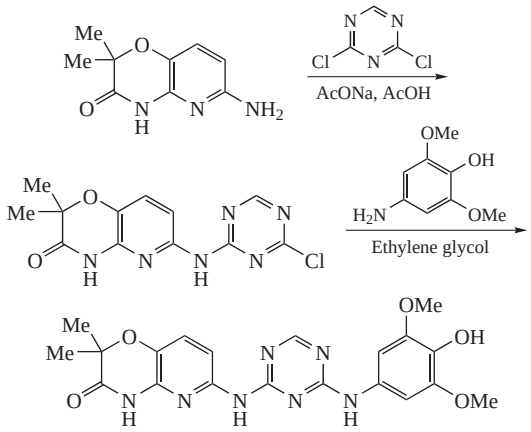


Figure 1 Binding of compound MT-SYK-03 and R406 to active site of c-Src kinase predicted with molecular modeling.



Scheme 2 Synthesis of compound dMe-MT-SYK-03.

assay.‡ The correlation between predicted and experimental selectivities is shown in Table 1. The d*G* prediction accuracy by the FEP/MD protocol was characterized by R^2 = 0.82 and rmsd = 0.48 kcal mol^{-1}. Current results suggest that full atomic FEP/MD modeling may be a valuable instrument in the design of kinase inhibitors with improved selectivity.

In conclusion, the comparison of c-Src kinase inhibition efficacy for known inhibitor Tamatinib (IC_{50}, 20 nM)[3] with those obtained in the present work shows that compounds MT-SYK-03 (IC_{50}, 14 nM) and dMe-MT-SYK-03 display remarkable potency.

† Molecular modeling was carried out using conventional procedures.[3] Full-atom spatial model of c-Src kinase (PDB ID 1Y57),[4] Btk kinase (PDB ID 3PIY)[5] and Jak1 kinase (PDB ID 5E1E)[6] were prepared using Model Builder program.[7] Molecular docking was performed using Lead Finder 1.1.15.[8,9] The molecular dynamics simulations were performed using the GROMACS simulation package[10] with the OPLS/AA force field parameter set.[11] The topologies for SYK inhibitors were created using ACPYPE[12] in conjunction with Antechamber.[13] For alchemical FEP simulations in solution, ligands were solvated in octahedral box with TIP4P34 water molecules. Simulations of the ligands bound to kinase were performed in rectangular periodic boxes containing TIP4P water molecules and chloride ions to neutralize the total system charge. Transformations were performed in 10 separate charge ($\Delta\lambda$ = 0.1) and 11 VdW ($\Delta\lambda$ = 0.1) steps. Simulation at each distinct λ value included 5000 steps of steep and l-bfgs energy minimization, 100 ps NVT equilibration, 500 (water) or 1000 (protein) ps NPT equilibration, and 2 ns NPT runs with d*H*/dλ collection each 10 steps. The protein side-chain during NVT and NPT simulations was set fully flexible while the backbone was fixed by position restraints of 1000 kJ mol^{-1} nm^{-2} to improve convergence. Ligand conformer focusing was carried out using described procedures.[3]

‡ *6-[(6-Chloropyrimidin-4-yl)amino]-2,2-dimethyl-2*H*-pyrid[3,2-*b*][1,4]oxazin-3(4*H*)-one.* 2,4-Dichloro-1,3,5-triazine (0.17 g, 1.15 mmol, prepared according to ref. 14) was dissolved in 5 ml of dry degassed DMF. 6-Amino-2,2-dimethyl-2*H*-pyrid[3,2-*b*][1,4]oxazin-3-(4*H*)-one (0.22 g, 1.15 mmol, prepared according to ref. 15) was added, and the reaction mixture was stirred at 60 °C for 8 h under inert atmosphere. The solvent was evaporated at 5 Torr. The residue was washed with water (2×5 ml) and separated by chromatography eluting with chloroform–methanol mixture of increasing polarity. The standard workup procedure afforded the title product (0.28 g, 0.92 mmol, 80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.12 (s, 3H), 1.13 (s, 3H), 7.43 (d, 1H), 7.85 (d, 1H), 7.88 (s, 1H), 7.65 (br. s, 1H), 10.84 (s, 1H).

*2,2-Dimethyl-6-{[4-(4-hydroxy-3,5-dimethoxyphenylamino)pyrimidin-4-yl]amino}-2*H*-pyrid[3,2-*b*][1,4]oxazin-3(4*H*)-one* (dMe-MT-SYK-03). The above compound (0.28 g, 0.95 mmol) was dissolved in dry degassed DMF. 4-Hydroxy-2,6-dimethoxyaniline (0.15 g, 0.90 mmol) was added, and the mixture was stirred at 100 °C for 20 h under inert atmosphere. The solvent was evaporated at 5 Torr. The residue was washed with water (5×5 ml) and separated by chromatography eluting with chloroform–methanol mixture of increasing polarity. The standard workup procedure afforded compound dMe-MT-SYK-03 (0.62 g, 65%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.40 (s, 6H, 2Me), 3.68 (s, 6H, 2OMe), 7.11 (d, 1H, H_{Py}, *J* 7.5 Hz), 7.21 (br. s, 1H, OH), 7.36 (s, 2H, H_{Ar}), 7.40 (br. s, 1H, NH), 8.32 (d, 1H, H_{Py}, *J* 7.5 Hz), 9.73 (br. s, 1H, NH), 10.03 (br. s, 1H, CONH).

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Kinase inhibition assay. Peptide substrate was dissolved in reaction buffer to the target concentration of 0.2 mg ml^{-1}. Recombinant kinase solution was added to the target concentration of 2 nM, and also the test compound to the given concentration (1 nM–10 mM). Then 33P-ATP solution was added (10 mM, final specific activity 0.01 mCi). The reaction mixture was incubated for 120 min, then poured on an ion-exchange membrane which was then washed with excessive amount of phosphoric acid. The conversion was determined based on the radioactivity of reaction products.

IC_{50} values were recalculated into inhibition constants K_i based on the concurrent inhibition equation: $K_i = IC_{50}/(1 + [ATP]/K_m)$, where [ATP] is ATP concentration used in the experiment, and K_m is a Michaelis constant for enzymatic reaction (according to the published data,[16] K_m = = 75 μM).

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