An Ensemble Docking Approach to Distinguish Allosteric Abl Kinase Inhibitors from Activators in Virtual Screening Campaigns

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ABSTRACT

Allosteric inhibition of Abl kinase at the myristoyl site is a promising therapeutic strategy against Chronic Myeloid Leukemia, addressing limitations of catalytic inhibitors in resistance and toxicity. Additionally, allosteric activation is being explored for conditions like breast cancer. This work aims to validate an ensemble molecular docking protocol for virtual screening targeting this site, using active and inactive conformations with AutoDock Vina. Virtual screening of 279 active inhibitors and 52 activators against 4597 and 2447 decoys, respectively, were evaluated using ROC curves. Results demonstrated robust discriminatory performance for inhibitors (AUC = 0.92), and acceptable for activators (AUC = 0.70). This approach successfully differentiated tool compounds and shows promise for initial screening of inhibitors and activators, offering a strategic framework for CML drug development and differentiation between the two pharmacological classes.

Abl kinase, allosteric inhibitors, allosteric activators, molecular docking, virtual screening

Introduction

Abl kinase, a regulatory enzyme for cellular processes via phosphorylation, features an allosteric site that mediates its autoinhibition through myristoylation. Perturbations in this mechanism are linked to Chronic Myeloid Leukemia (CML), driven by hyperactive Bcr-Abl oncoprotein *(1)*. Although catalytic site-directed Abl kinase inhibitors represent established therapies, resistance mutations (such as T315I) compromise their efficacy *(2)*. Allosteric inhibitors such as asciminib offer enhanced selectivity and synergistic potential in combination therapies *(3)*. Conversely, allosteric activators are investigated for breast cancer *(4)*, and differ from inhibitors in binding to an extended helix-I conformation of the allosteric site. Challenges include conformational dependency and high experimental screening costs. *In silico* approaches are strategic tools for designing conformation-stabilizing ligands *(5)*. This work aims to validate an ensemble molecular docking protocol for virtual screening of allosteric inhibitors and activators, as a means of identifying and differentiating pharmacological classes.

Experimental

Molecular docking experiments used AutoDock Vina (ADV) *(6)*, automated via Python. Protein structures (PDB IDs: 3K5V for inactive, 3PYY for active) *(3, 4)* were prepared with OpenBabel *(7)* by removing co-crystallized ligands/water, adding polar hydrogens, and assigning partial charges. Ligands were minimized with MMFF94 force field *(8)*. Redocking (GNF-2 and DPH) *(3, 4)* and cross-docking (asciminib and activator 51) *(9, 10)* evaluated pose accuracy via RMSD. Virtual screening ranked 279 active inhibitors in the inactive conformation and 52 activators in the active conformation against 4597 and 2447 decoys, respectively, generated with DUD-E *(11)*, assessed via ROC curves and area under curve (AUC) values. Exhaustiveness (8, 16, 32) and cubical box size (20, 25, 30 Å) were tested. To determine optimal score thresholds for each system, Youden's J statistic was used, maximizing differences between true positive and false positive rates. Then, inhibitors were docked on the active (extended helix-I) and activators on the inactive (bent helix-I) conformations, to determine if the protocols are suitable for an ensemble docking procedure. A proof of concept was performed with compounds 4 and 5, which were designed employing molecular docking by Schoepfer *et al.* (2019), during the development of asciminib *(12)*. The group expected compound 4 to be an inhibitor, and while it bound to the allosteric pocket, it did not inhibit the enzyme until it was converted to compound 5.

Results and Discussion

Redocking and cross-docking

Redocking and cross-docking yielded poses closely aligned with experimental binding modes (Fig. 1). GNF-2 showed higher deviations with larger grids (Table 1), especially in the solvent-exposed region, likely due to the compound's lower specificity for this hydrophobic region. For activators, RMSD values were even lower, indicating robust pose prediction.

Table 1. Averages and standard deviations of RMSD values (Å) for different *exhaustiveness* levels and *grid* sizes for the poses with best affinity predicted by ADV.

Ligand	*Grid* 20	*Grid* 25	*Grid* 30
GNF-2	0.752 ± 0.005	1.720 ± 0.037	1.709 ± 0.009
Asciminib	0.665 ± 0.011	0.719 ± 0.008	0.706 ± 0.002
DPH	0.144 ± 0.002	0.106 ± 0.003	0.130 ± 0.017
Cmpd 51	0.391 ± 0.009	0.569 ± 0.010	0.388 ± 0.003

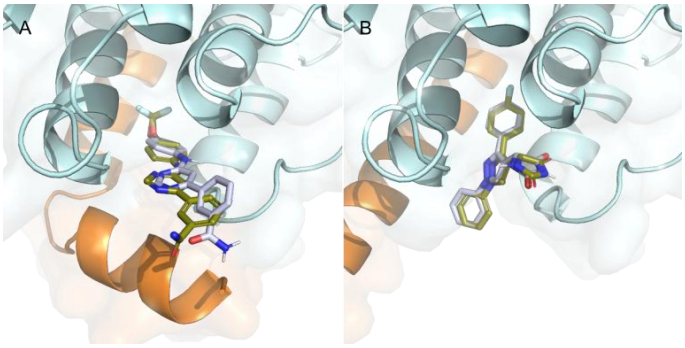

Figure 1. *Redocking* of GNF-2 in bent helix-I conformation (A) and DPH in extended helix-I conformation (B), with *exhaustiveness* 8. Carbons of ADV predicted poses shown in white and experimentally determined poses in olive. Helix-I is shown in orange.

Virtual Screening

ROC analysis was robust for both inhibitors (AUC ≈ 0.92) and activators (AUC ≈ 0.69) across parameters (Fig. 2A). Lower AUC for activators is likely due to reduced pocket specificity and lower number of interactions with helix-I in its extended conformation (Fig. 1). Threshold determination resulted in a more negative score for inhibitor classification (-9.055 kcal/mol), when compared to activators (-6.798 kcal/mol). This is in accordance with the more stable interactions expected of inhibitors with the bent helix-I conformation of the myristoyl pocket. The ensemble docking procedure revealed that inhibitors have higher predicted affinity for both conformations (Fig. 2C and 2D), and even higher affinity differences between bent and extended helix-I conformations. With this approach, compounds 4 and 5 (Fig. 2B) were correctly identified as a ligand and an inhibitor, respectively (Fig. 2C and 2D), illustrating the usefulness of the strategy for drug discovery purposes.

Conclusions

The protocols effectively predicted ligand poses for both inhibitors and activators. Virtual screening discriminated actives from decoys confidently for inhibitors (AUC = 0.92) but less effectively for activators (AUC = 0.69), reflecting intrinsic differences in site specificity. The ensemble docking procedure discloses that the protocol is best suited for inhibitor identification, and is able to suggest activator activity with lower certainty. This approach provides a foundation for CML drug discovery and can be applied to virtual screening campaigns to differentiate allosteric modulators.

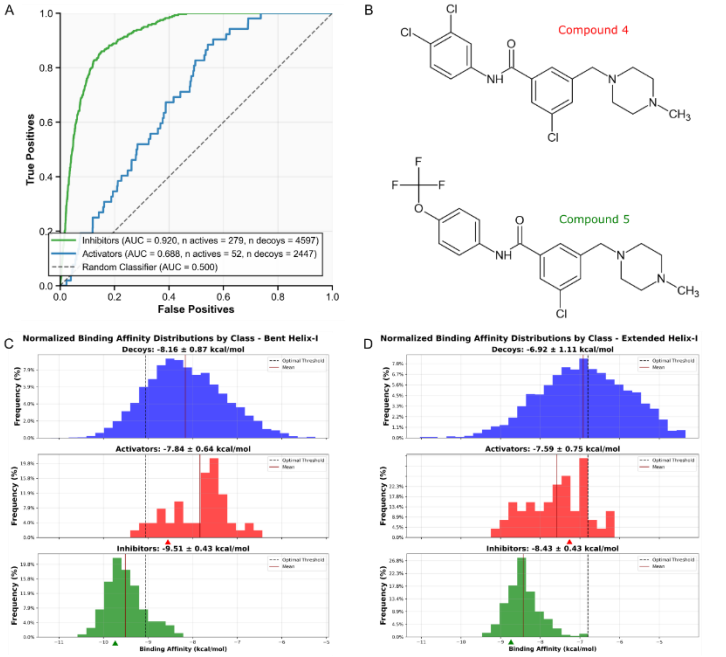

Figure 2. (A) ROC curves and AUC values with the best parameters; (B) Structures of compounds 4 and 5; Distribution of affinities for inhibitors, activators and decoys for the bent (C) and extended helix-I structure (D). Arrows indicate ADV scores for compounds 4 and 5.

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