Accurate Prediction of the Relative Potencies of Members of a Series of Kinase Inhibitors Using Molecular Docking and MM-GBSA Scoring

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Abstract: The ability of molecular docking, using the program Glide and an MM-GBSA postdocking scoring protocol, to correctly rank a number of congeneric kinase inhibitors was assessed. The approach was successful for the cases considered and suggests that this may be useful for the design of inhibitors in the lead optimization phase of drug discovery.

There are many approaches employed to predict the binding free energy of a small molecule to a protein target. These range in degrees of physical rigor and the time needed to perform the calculations. At one end of the spectrum lie free energy perturbation (FEP) and thermodynamic integration (TI) methods, which have been successfully applied to reproduce experimentally determined free energies. [1−3] However, these approaches are computationally expensive and are not realistically available to a drug discovery team for routinely profiling potential molecules for future rounds of synthesis. At the other end of the spectrum lie scoring functions that are employed by docking programs.[4] These scoring functions are designed for processing large numbers of molecules in a short period of time, and as a consequence, their accuracy in predicting the relative free energies of known actives is quite poor.[5] It is believed that the many approximations employed in such calculations, such as a lack of protein flexibility, inadequate treatment of solvation, and the simplistic nature of the energy function used, contribute to the inability of these scoring functions to discriminate between compounds of similar chemical structure that differ by several log units in potency. There are some approaches that reside between the two extremes just outlined. These include λ-dynamics,[6] linear interaction energy approaches,[7] molecular dynamics/Monte Carlo simulations,[8] OWFEG,[9] and MM-PBSA calculations.[10] The last is an approach pioneered by Kollman and colleagues and involves less sampling compared to molecular dynamics and Monte Carlo based approaches and uses a combination of a molecular mechanics energy term with a continuum solvation model and a surface area dependent term to predict free energy differences.

The MM-PBSA approach has recently become of interest in drug discovery as an option for predicting relative binding free energies of drug discovery project compounds with an acceptable level of accuracy on a time scale that is commensurate with synthetic chemistry−biological test cycles.[11−13]

In this article the assessment of molecular docking with a related scoring approach, MM-GBSA,[14] is reported for four kinases. The examples were chosen to examine the ability of the approach to correctly rank the relative potencies of compounds from the same chemical class against a kinase, with potencies that range from low micromolar to low nanomolar IC_{50} values. The data sets were compiled from in-house projects, using publicly available, self-consistent data. The chemical series employed for these studies are illustrated in Figure 1.

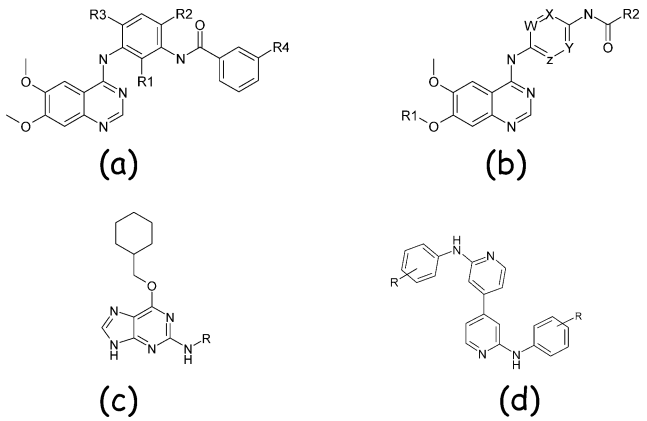

Figure 1. (a) p38 inhibitors, (b) Aurora A inhibitors, (c) Cdk-2 inhibitors, and (d) Jnk-3 inhibitors.

All the docking and scoring calculations were performed using the Schrodinger suite of software (Maestro, version 70110).[15] The compounds were extracted from the corporate database in SMILES format and were converted to 3D using the program Corina. The compound data sets were then imported into Maestro and were prepared for docking using Ligprep. The proteins were prepared by removing all solvent and adding hydrogens and minimal minimization in the presence of bound ligand using Pprep. Grids for molecular docking with Glide[16] were calculated with a hydrogen bond constraint to a backbone NH in the hinge region of each kinase (M109 in p38 (pdb code 2bak), A212 in Aurora A (pdb code 2c6e), L83 in Cdk2 (pdb code 1oiu), and M149 in Jnk3 (in-house structure)). Compounds were docked using Glide in extra-precision mode, with up to three poses saved per molecule. The docked poses were then minimized using the local optimization feature[17] in Prime, and the energies were calculated using the OPLS-AA force field[18] and the GBSA continuum model[19] in Maestro. For each molecule the best scoring pose was selected for comparison with the experimental IC_{50} values. The binding free energy ΔG_{bind} is estimated as

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{solv} + \Delta G_{SA}$$

where ΔE_{MM} is the difference in energy between the complex structure and the sum of the energies of the ligand and unliganded protein, using the OPLS force field, ΔG_{solv} is the difference in the GBSA solvation energy of the complex and the sum of the solvation energies for the ligand and unliganded protein, and ΔG_{SA} is the difference in the surface area energy for the complex and the sum of the surface area energies for the ligand and uncomplexed protein. Corrections for entropic changes were not applied.

The calculated binding energies are plotted against pIC_{50} for each target in Figure 2. As can be seen, the approach has been very successful at getting the correct relative rankings and there is a high correlation observed between the calculated and experimental values. The p38 data set[20] used here (Table 1) provides a number of challenges. The protein binds to these inhibitors in a DFG-out conformation, and this conformation of the protein affords the ligands the possibility of binding to an additional hydrophobic pocket (at position R4, Figure 1a) that is not present in the traditional DFG-in conformation of

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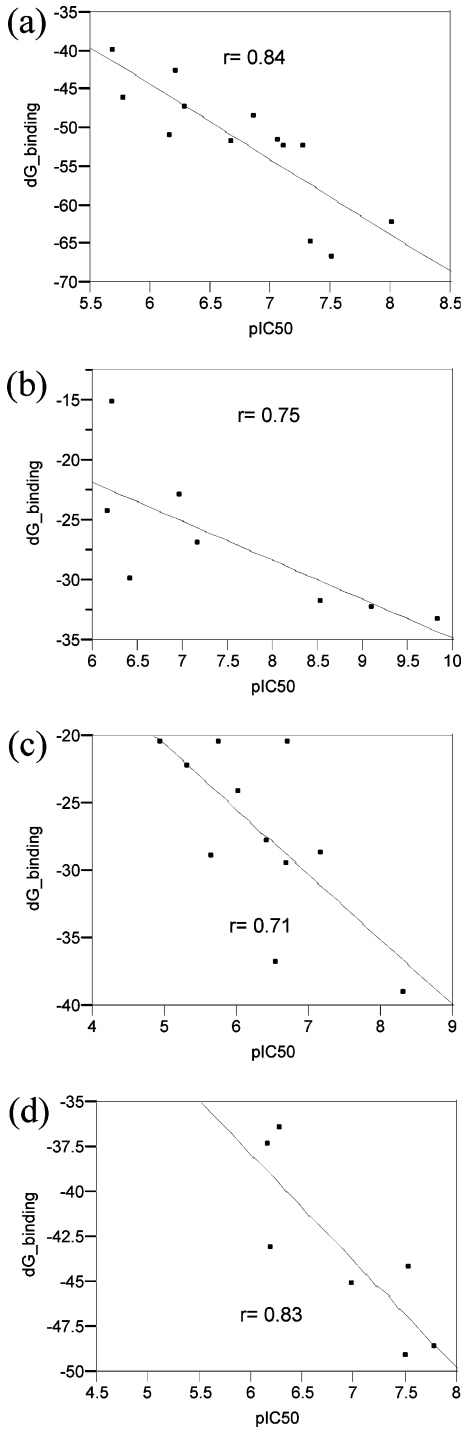

Figure 2. Predicted ΔG_{bind} vs pIC_{50}: (a) p38, (b) Aurora A, (c) Cdk-2 and (d) Jnk-3.

the protein. In addition, the substitution pattern around the middle phenyl ring corresponds to subtle changes in structure that have a dramatic effect on the measured potencies (e.g., **1d** and **1f**). The range of IC_{50} is quite small (10 nM to 2.1 μM), which is typical of the range being considered during the lead optimization stages of a project. Considering all of this, the MM-GBSA scoring scheme has done remarkably well at discerning these differences within this structurally similar data set.

Similarly, the inhibitors for the Aurora A data set[21] (Table 2) induce the DFG-out conformation of the protein. For this data set the main areas of variation are the solvent channel, the DFG-out pocket, and the electronic nature of the central aryl ring. Thirteen congeneric compounds were chosen for this study,

Table 1. Data Set Used for the p38 Case Study[20]

compd	R1	R2	R3	R4	IC_{50} (μM)
1a	H	H	H	H	0.141
1b	H	F	F	H	0.054
1c	H	H	Cl	H	0.088
1d	H	H	Me	H	0.078
1e	H	Cl	F	H	0.518
1f	H	Me	H	H	2.090
1g	F	H	H	H	0.690
1h	H	Cl	H	H	0.615
1i	H	H	H	NMe_2	0.212
1j	H	H	Cl	NMe_2	0.047
1k	H	H	Me	NMe_2	0.031
1l	H	Cl	H	NMe_2	1.690
1m	H	H	Me	*N*-morpholino	0.010

Table 2. Data Set Used for the Aurora A Case Study[21]

compd	R1	R2	W	X	Y	Z	IC_{50} (μM)
2a	Me	phenyl	C	C	C	C	0.393
2b	Pr(morpholine)	phenyl	C	N	N	C	0.003
2c	Pr(morpholine)	phenyl	N	C	C	N	0.629
2d	Pr(morpholine)	4-pyridyl	C	N	N	C	0.690
2e	Pr(morpholine)	3-chloro-4-fluoro-phenyl	C	N	N	C	0.00015
2f	Pr(morpholine)	3-bromo-4-methylphenyl	C	N	N	C	0.070
2g	Pr(morpholine)	phenyl	C	C	C	C	0.110
2h	(2*S*)-2-hydroxy-3-piperidin-1-ylpropyl	phenyl	C	N	N	C	0.0008
2i*	Pr(piperidine)	3-chlorophenyl	C	N	N	C	0.0008
2j*	Pr(morpholine)	3-chlorophenyl	C	N	N	C	<0.0001
2k*	Pr(morpholine)	4-ethylphenyl	C	N	N	C	0.085
2l*	Pr(morpholine)	*n*-butyl	C	N	N	C	0.017
2m*	Pr(morpholine)	(4-dipropylamino-sulfonyl)phenyl	C	N	N	C	3.9

but not all of these were docked satisfactorily (**2i**−**m**) by Glide. The weakest inhibitor did not yield a solution, and four of the inhibitors were docked with poor amide conformations in the DFG-out region of the binding site. Since the purpose of this exercise is to assess the ability of MM-GBSA to correctly predict the relative affinity of compounds, given a correct binding mode, it is reasonable to exclude these compounds in the assessment, as the docking is driven entirely by the internal GlideScore and has nothing to do with the MM-GBSA score. Nonetheless, this highlights that it is necessary to have a high degree of confidence in the binding mode that is being generated prior to undertaking any rescoring of the poses generated by Glide. For the remaining eight compounds the correlation between the predicted ΔG_{bind} and experimental pIC_{50} is good and importantly the relative ranking of the most potent and least potent compounds is identified, capturing the subtleties in substitution pattern on the aryl group in the DFG-out pocket and the differences between the phenyl and pyrimidyl regioisomers for the central aryl ring.

The Cdk-2 data set[22] includes compounds that explore the channel to solvent. The compounds chosen for this study were selected on the basis of covering a reasonable range of potency and variability in the nature of the chemistry substituents placed in the solvent channel of Cdk-2 (Table 3). There is an expectation that binding energy predictions on compound sets where the greatest change is in a solvent exposed region would benefit greatly from having energy terms that consider solvation. The data set comprised 11 compounds from a purine set spanning the IC_{50} range 5 nM to 12 μM. Again, the ability of the MM-GBSA score to discern the differences in binding energies of this congeneric set is impressive, where the variations are small changes in the solvent channel region of the protein.

The final data set corresponds to bipyridyl inhibitors of Jnk-3 kinase (Table 4).[23] These compounds are reported to bind to Jnk-3 with one aniline group oriented into the solvent channel of the kinase and with the other aniline directed toward the

Table 3. Data Set Used for the Cdk-2 Case Study[22]

compd	R	IC_{50} (μM)
3a	phenyl	0.970
3b	4-SO_2NH_2phenyl	0.005
3c	methyl	5.000
3d	3-chlorophenyl	2.300
3e	3,5-dichlorophenyl	12.000
3f	3-CH_2OHphenyl	0.400
3g	3-OMe phenyl	1.800
3h	4-OH phenyl	0.069
3i	3-SO_2NH_2phenyl	0.210
3j	4-$CON(Me)_2$phenyl	0.200
3k	4-CH_2CN phenyl	0.300

Table 4. Data Set Used for the Jnk-3 Case Study[23]

compd	R	IC_{50} (μM)
4a	4-F	0.017
4b	2-F	0.032
4c	2-NH_2	0.108
4d	2-CH_3	0.652
4e	2-OCH_3	0.528
4f	2-OCH_2CH_3	0.693
4g	H	0.300
4h	3-CF_3	>10
4i	4-CF_3	>10

hydrophobic pocket adjacent to the M146 gatekeeper residue. This is in contrast to the binding mode of these same compounds to Jnk-1.[23] In Jnk-3 the gatekeeper residue moves to accommodate the substituted aniline. Glide was unable to yield suitable poses for the two inactive compounds **4h** and **4i**. For the former, no suitable pose was found, while for the latter, the only poses found shifted the scaffold by approximately 1.5 Å or more relative to the known structures and the poses found for the remainder of the data set. As such, the two inactive compounds were therefore not included in the MM-GBSA postdocking analysis. As can be seen in Figure 2d the scoring protocol has once again performed well for the compounds considered.

In all cases here, GlideScore does not provide as good a correlation with experimental data and is always outperformed by the MM-GBSA score (Supporting Information). This is probably not a flaw unique to GlideScore but is more likely an indication of the poor performance that is common to most docking scoring functions for the types of tasks presented here. It is noted that GlideScore has been primarily optimized to yield accurate binding poses, and in almost all cases here, it has been performed to give very good poses, based on public and in-house X-ray structural information for each of the series.

In conclusion it appears that incorporation of more physically relevant energy terms such as solvation energy and surface area accessibility with a force field has produced a method that could be applied with confidence to ranking synthetic ideas and prioritizing compounds from these chemical classes for synthesis as inhibitors against the relevant kinases considered here (using the protein structures employed in these studies).

The data presented here indicate that the method would need to be benchmarked against a known set initially to see if it is suitable for guiding structure-based design of inhibitors and also that it is necessary to have a high degree of confidence in the binding modes being used as input for the MM-GBSA protocol. In addition, it is possible that for certain functional groups the GB parameters may not be of high enough quality to yield reliable results.

Overall, the protocol of using Glide for pose generation and an MM-GBSA protocol for rescoring appear promising for the application to structure-based lead optimization of chemical series for inhibition of protein kinases.

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Supporting Information Available: Plots of GlideScore vs pIC_{50} for each test case. This material is available free of charge via the Internet at http://pubs.acs.org. The docked poses for the p38, Aurora, and Cdk2 data sets are available on request from the authors. The poses for the Jnk3 data set will also be available from the authors once the protein structure is available in the public domain.

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