

Fast and Effective Prediction of the Absolute Binding Free Energies of Covalent Inhibitors of SARS-CoV-2 Main Protease and 20S Proteasome

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ABSTRACT: The COVID-19 pandemic has been a public health emergency with continuously evolving deadly variants around the globe. Among many preventive and therapeutic strategies, the design of covalent inhibitors targeting the main protease (M^{pro}) of SARS-CoV-2 that causes COVID-19 has been one of the hotly pursued areas. Currently, about 30% of marketed drugs that target enzymes are covalent inhibitors. Such inhibitors have been shown in recent years to have many advantages that counteract past reservation of their potential off-target activities, which can be minimized by modulation of the electrophilic warhead and simultaneous optimization of nearby noncovalent interactions. This process can be greatly accelerated by exploration of binding affinities using computational models, which are not well-established yet due to the requirement of capturing the chemical nature of covalent bond formation. Here, we present a robust computational method for effective prediction of absolute binding free energies (ABFEs) of covalent inhibitors. This is done by integrating the protein dipoles Langevin dipoles method (in the PDL/D/S-LRA/ β version) with quantum mechanical calculations of the energetics of the reaction of the warhead and its amino acid target, in water. This approach evaluates the combined effects of the covalent and noncovalent contributions. The applicability of the method is illustrated by predicting the ABFEs of covalent inhibitors of SARS-CoV-2 M^{pro} and the 20S proteasome. Our results are found to be reliable in predicting ABFEs for cases where the warheads are significantly different. This computational protocol might be a powerful tool for designing effective covalent inhibitors especially for SARS-CoV-2 M^{pro} and for targeted protein degradation.

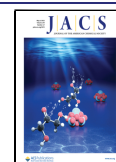
Prediction of relative binding free energies (RBFEs) with reasonable accuracy is already within the reach of the computational chemistry scientific community.^{1–4} However, it still remains challenging to predict the ABFEs due to the cost of alchemical free energy perturbation (FEP). Additionally, it is even more challenging to predict the ABFE of covalent ligands given the requirement of capturing quantum mechanically the chemical effects of the covalent bond formation. In fact, in some cases, a detailed understanding of the catalytic mechanism is also crucial. Nonetheless, following the success of RBEF calculations, several interesting approaches have been proposed in the past few years for calculating the RBFEs of covalent ligands, where both covalent and noncovalent contributions were considered.^{5,6} For example, while ref 5 predicted RBFEs for a congeneric series of cathepsin inhibitors using FEP based simulations, ref 6 used a similar approach to predict binding selectivity (in addition to RBEF) for a congeneric series of calpain ligands. While ref 5 predicted RBFEs with reasonable accuracies, ref 6 has not satisfied this primary requirement (Figure S4 of ref 6 has an R^2 of 0.20). Furthermore, ref 6 proposed an unjustified rule of “−5.5 kcal/mol” where they mandate caution in using an “FEP-based RBEF calculation” approach with covalent states when the covalent binding energy contribution is not greater than −5.5 kcal/mol than the noncovalent contributions. Unfortunately, it is impossible to know a priori the magnitude of the covalent vs noncovalent contributions. This situation gets far more complicated when different warheads contribute differently

to the thermodynamics and kinetics of the covalent bond formation, since different warheads in a congeneric series of ligands have significantly different contributions of covalent states and hence are challenging to capture through popular RBEF schemes. Actually, in order to study the covalent bond contribution by an RBEF approach, we need to evaluate the corresponding binding affinity by QM/MM free energy calculations, and such an approach is very expensive even with the paradynamics version⁷ and cannot be used for a rapid screening.

We also note that even regular FEP calculations within the RBEF strategy are quite expensive. Hence, we propose a fast and efficient protocol to estimate the ABFEs of covalent inhibitors. We do not want to address the advantages and disadvantages of ABFE and RBEF calculations but want to mention that if we have a reliable ABFE approach, we have a more general strategy. Thus, it is of the utmost importance to calculate ABFEs in the case of covalent ligands where the contributions of the covalent and noncovalent effects are treated consistently.

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In most instances, the prediction of ABFE for covalent ligands has been achieved by QM/MM FEP simulations (with a QM based approach) which are very costly and cannot be implemented for a large set of ligands.^{8–11} Recently, we introduced a different approach in studying covalent inhibitors by combining the PDL/D/S-LRA/ β and the empirical valence bond (EVB) method and successfully predicted the absolute binding free energy of the covalent α -ketoamide inhibitor of the main protease of SARS-CoV-2.¹² This work exploited the ability of the EVB to transfer quantum mechanically a calibrated potential surface from solution reactions to reactions in the protein active site and to do it reliably and with great efficiency. The combination of the EVB and the PDL/D/S-LRA/ β appeared to provide a reliable way of studying covalent inhibitors, both yielding the bonding energies and the activation barriers for the different mechanistic steps. Here, we further optimize our approach by skipping the calculations of the different reaction steps and just going from the reactant to the covalent intermediate in a single step. Thus, we adopted the thermodynamic cycle depicted in Figure 1, where we obtain the total binding free energy by combining the QM reaction free energy (ΔG_{QM}^w) of the reacting warhead and the protein fragment in water, and the free energy of moving the reactants and the covalent intermediate from water to the protein active site (evaluated by the PDL/D/S-LRA/ β method). This approach is used for the warheads shown in Figure 2.

The thermodynamic cycle leads to the following equation:

$$(\Delta G_{\text{noncov}}^{w \rightarrow p} + \alpha) + \Delta G_{QM}^p = \Delta G_{QM}^w + (\Delta G_{\text{cov}}^{w \rightarrow p} + \alpha) \quad (1)$$

Our cycle treats consistently the bond formation process. That is, the S/O atom and the bound carbon are included in region I (Figure S2) and also in the quantum calculations. This is identical to the inclusion of the carboxylates in pK_a calculations as was already clarified in the thermodynamic cycle drawn in ref 13 and used in many of our papers. This simple approach appears to be very powerful.

The ABFE was calculated for both the proteasome^{14,15} and main protease inhibitors, where high resolution crystal structures are available. The binding affinities for all of these compounds range from low nanomolar to moderate micromolar. We realize that the IC_{50} is not the best metric to calculate the absolute binding free energy. However, due to the lack/challenge of experimentally measured K_i constants for all of these different compounds, we relied on IC_{50} values for the estimation of binding free energies (this strategy has been discussed multiple times in the literature^{16–23}). We have also discussed and justified this point in detail in our recent publication.¹² Table 1 summarizes the results for all of these compounds. It is important to mention here that our proposed method is universally applicable to the evaluations of absolute binding free energies for both reversible and irreversible inhibitors. In fact, the kinetic information on the inhibitors is not required for implementing the proposed method for the evaluation of the absolute binding free energies. Our method also predicted the ABFE of two proteasome inhibitors (Figure 3, Bsc2189²⁴ with the α -ketoamide warhead and MG132 with the aldehyde warhead²⁴) with high accuracy. The accuracy of the ABFE predictions is also very encouraging for eight inhibitors (except for boceprevir) of the main protease (Figure 4), where three inhibitors have an aldehyde warhead (MI-23,²⁵ 11a,²⁶ and MG132²⁷), three inhibitors (boceprevir,²⁸ telapre-

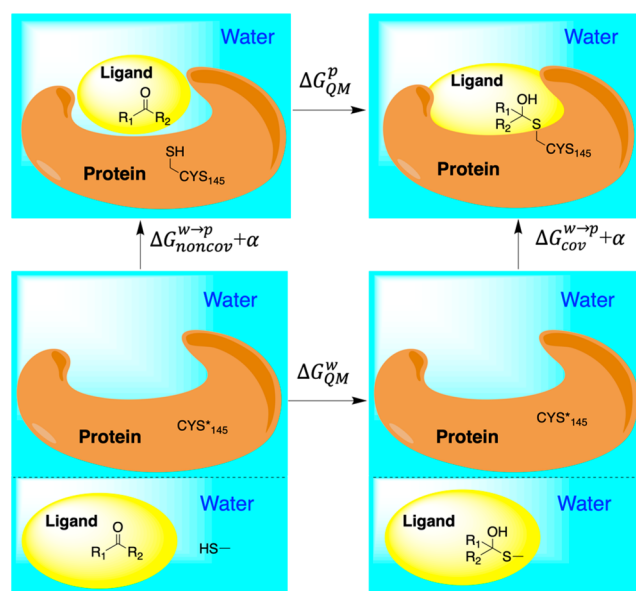


Figure 1. Thermodynamic cycle for calculating the absolute binding free energy of a covalent inhibitor. α is the bond energy which is canceled in the cycle.

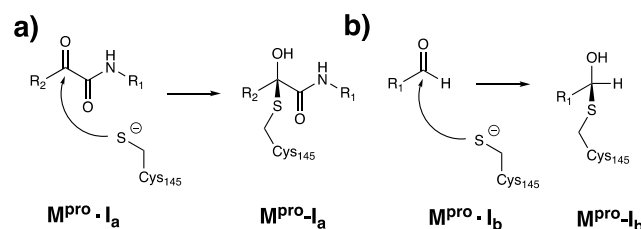


Figure 2. Covalent binding scheme of inhibitors involving (a) an α -ketoamide and (b) an aldehyde warhead with Cys145 of the main protease of SARS-CoV-2.

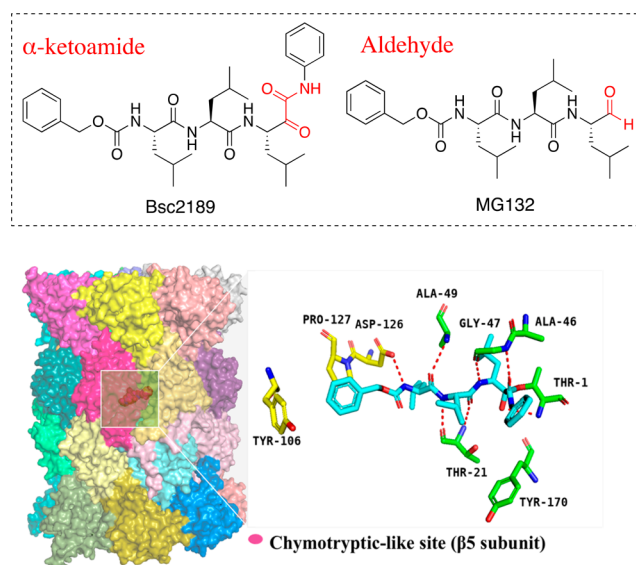


Figure 3. Upper panel shows structures of Bsc2189 and MG132. The lower panel shows, on the left side, the cylinder structure of 20S proteasome, while the right-hand depicts the interactions between Bsc2189 and the $\beta 5$ subunit of the 20S proteasome (PDB code: 4NO8).

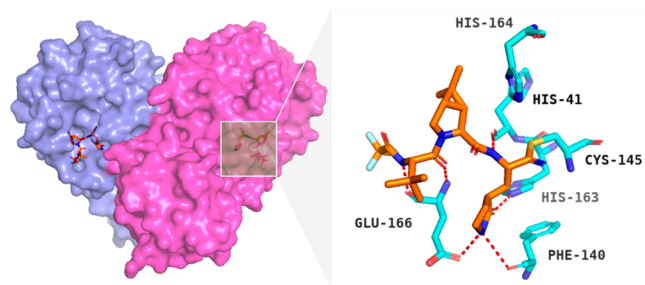


Figure 4. The left-hand panel shows the structure of M^{pro} . The right-hand panel shows the interactions between PF07321332 (orange) and the active site of M^{pro} (PDB code: 7VH8).

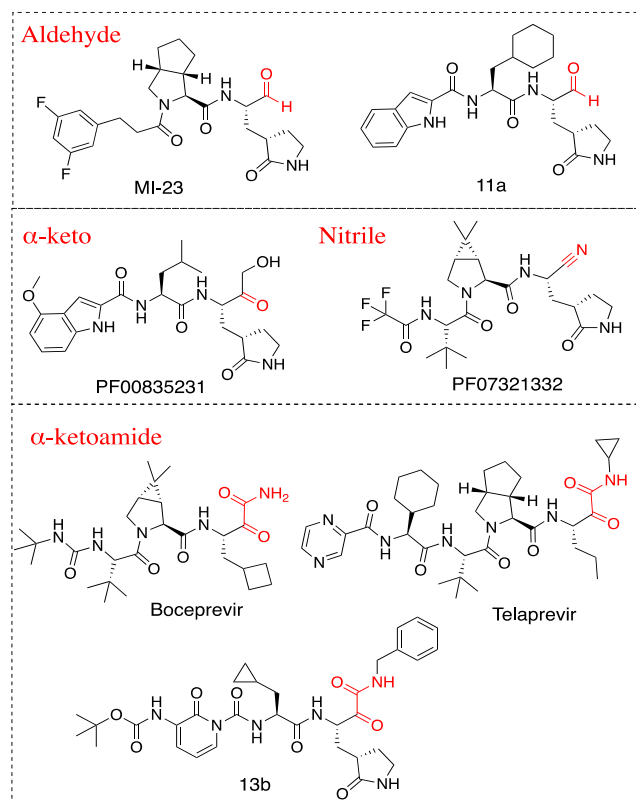


Figure 5. Structures of the seven inhibitors targeting the M^{pro} .

vir,²⁹ 13b³⁰) have an α -ketoamide warhead, one inhibitor has an α -keto warhead (PF00835231),³¹ and one inhibitor has a

nitrile warhead (PF07321332: “Paxlovid,” approved oral medication for SARS-CoV-2 from Pfizer).³² MG132 is a dual inhibitor against the proteasome and main protease, having higher binding affinity to the proteasome than to the main protease. Our approach successfully predicted the binding selectivity of MG132 against the two targets. All of the ligands of main protease are shown in Figure 5. The binding site of all of the ligands are shown in Figure S3, Figure S4, and Figure S5.

In conclusion, we introduce here a simple yet a powerful method for evaluating the absolute binding free energies of covalent inhibitors. Our results demonstrate excellent performance in predicting ABFEs for cases where the warheads are significantly different. The method is significantly faster than any QM/MM or alchemical FEP-based simulation techniques for such cases (see SI). The reliability of our method in accurate and fast prediction of ABFEs warrants its application in rapid design and evaluation of the binding efficiency of novel covalent inhibitors especially in the current crisis of SARS-CoV-2 infection and in long-standing research of targeted protein degradation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.2c00853>.

Computational details, the QM energies for all systems, comparison of computation times with contemporary methods, applicability of the proposed method, the binding site interactions (PDF)

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Table 1. Predicted and Observed Absolute Binding Free Energies^a

no.	inhibitor	target	warhead	$\Delta G_{\text{QM}}^{\text{W}}$	$\Delta G_{\text{cov} \rightarrow \text{P}}^{\text{W}}$	ΔG_{cal}	ΔG_{exp}	$\Delta G_{\text{cal}} - \Delta G_{\text{exp}}$	IC ₅₀ (nM)	PDB
1	boceprevir	M^{pro}	α -ketoamide	-6.26 ± 1.0	-3.41 ± 1.2	-9.67	-7.38	-2.29	4130	6WNP ²⁸
2	MI-23	M^{pro}	aldehyde	-6.84 ± 1.0	-4.46 ± 1.2	-11.30	-11.12	-0.18	7.6	7D3I ²⁵
3	11a	M^{pro}	aldehyde	-6.01 ± 1.0	-3.94 ± 1.2	-9.95	-9.97	0.02	53	6LZE ²⁶
4	PF00835231	M^{pro}	α -keto	-9.89 ± 1.0	-2.82 ± 1.2	-12.71	-13.11	0.40	0.27	6XHM ³¹
5	PF07321332	M^{pro}	nitrile	-7.08 ± 1.0	-3.36 ± 1.2	-10.44	-11.65	1.21	3.11	7VH8 ³²
6	telaprevir	M^{pro}	α -ketoamide	-3.97 ± 1.0	-3.20 ± 1.2	-7.17	-6.5	-0.67	18000	7C7P ²⁹
7	13b	M^{pro}	α -ketoamide	-5.13 ± 1.0	-3.37 ± 1.2	-8.50	-8.46	-0.04	670	6Y2F ³⁰
8	MG132	M^{pro}	aldehyde	-7.68 ± 1.0	-0.69 ± 1.2	-8.37	-7.41	-0.96	3910	7CUU ²⁷
9	MG132	proteasome	aldehyde	-4.70 ± 1.0	-4.50 ± 1.2	-9.20	-9.00	-0.20	270	4NNN ²⁴
10	Bsc2189	proteasome	α -ketoamide	-5.49 ± 1.0	-4.29 ± 1.2	-9.78	-10.23	0.45	34	4NO8 ²⁴

^aThe calculations are based on the crystal structures. Only Bsc2189 is a proteasome inhibitor. MG132 is a dual inhibitor, and the rest of them are M^{pro} inhibitors. The error range is further discussed in the SI.

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Notes

The authors declare no competing financial interest.

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