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Quantum Chemical-Based Protocol for the Rational Design of Covalent Inhibitors

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Supporting Information

ABSTRACT: We propose a structure-based protocol for the development of customized covalent inhibitors. Starting from a known inhibitor, in the first and second steps appropriate substituents of the warhead are selected on the basis of quantum mechanical (QM) computations and hybrid approaches combining QM with molecular mechanics (QM/MM). In the third step the recognition unit is optimized using docking approaches for the noncovalent complex. These predictions are finally verified by QM/MM or molecular dynamic simulations. The applicability of our approach is successfully demonstrated by the design of reversible covalent vinylsulfone-based inhibitors for rhodesain. The examples show that our approach is sufficiently accurate to identify compounds with the desired properties but also to exclude non-promising ones.

This communication presents a quantum chemical based protocol for the rational design of covalent ligand with desired properties. As a proof of principle we apply it to derive reversible covalent inhibitors of rhodesain. Such covalent inhibitors currently experience an intensive renaissance not only in academic[1] but also in industrial drug development due to their various advantages, including prolonged residence times, lower sensitivity against pharmacokinetic aspects, and high efficacy.[2] Examples are kinase inhibitors, e.g., afatinib or ibrutinib,[3] or proteasome inhibitors, e.g., carfilzomib[4] or marizomib.[5,6] In the first case noncovalent kinase inhibitors have been converted into covalent ones by introducing an α,β-unsaturated amide moiety, which covalently and irreversibly reacts with a Cys residue nearby the active site. The same approach has been applied to the GTPase K-Ras, which opened a door for targeting drug targets that were thought to be undruggable.[7] In the case of the two proteasome inhibitors, the ligands stem from natural products that have been found serendipitously. Also many other marketed covalent drugs were discovered serendipitously.[1,8]

This is due to the fact that the design of covalent drugs is more complicated than the development of their noncovalent counterparts because the reaction mechanisms of covalent inhibitors comprise at least two very different steps (Figure 1).[9]

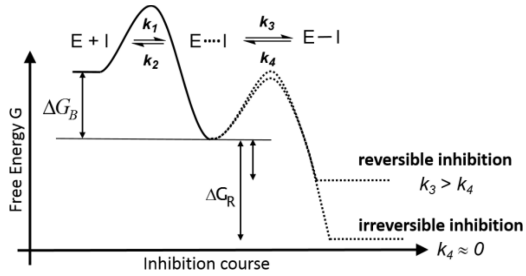

Figure 1. Energy diagram of the inhibition mechanism of a covalently reacting ligand.

In the first step a noncovalent enzyme inhibitor complex (E···I, Figure 1) is formed. Its stability (ΔG_B) and its geometrical arrangement, which are mainly influenced by the interactions between the recognition unit of the inhibitor and the enzyme environment, determine if the subsequent chemical reaction leading to the covalent complex E−I can take place. The free reaction energy ΔG_R of this subsequent chemical reaction, which strongly depends on the chemical properties of the warhead, is mainly responsible whether the covalent inhibition step is reversible or irreversible. Consequently, in the design of covalent drugs, recognition unit and the warhead have to be optimized concomitantly.

Various tools to design covalent inhibitors are available,[10] but the so-called covalent docking is not as well established and elaborated as the corresponding methods for the design of noncovalent ones.[11] Some problems arise because the approaches mainly focus on the final covalent enzyme−inhibitor complex but neglect the properties of the initially formed noncovalent complex. Drawbacks may also result because reaction barrier and reaction energy of the covalent step are neglected in most covalent docking approaches.

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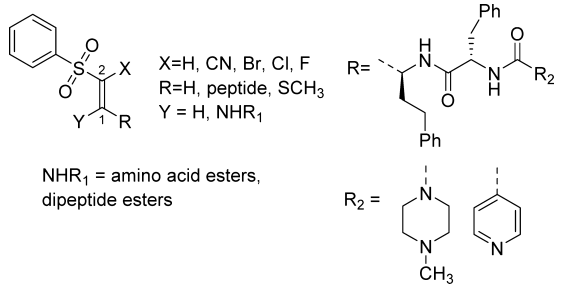
Here, we present a new protocol, which can give valuable information for the rational design of covalent inhibitors. It computes the reaction course via QM/MM hybrid approaches[12] that are also used in other multiscale-modeling areas.[13] In our approach, the warhead and the involved residues were included into the QM-part, while the influence of the enzyme environment on the reaction profile was taken into account via force field approaches (MM). As input, our protocol requires an X-ray structure of an appropriate enzyme−inhibitor complex. For the present example we use the well-known vinylsulfone (VS) K11777 (Chart 1, X = Y = H, R_2 = 4-Me-piperazinyl), which

Chart 1. Lewis Structures of the Inhibitors[a]



[a]K11777: X = Y = H, R_2 = 4-Me-piperazinyl; **1**: X = Y = H, R_2 = 4-pyridinyl; **2**: X = Cl, Y = H, R_2 = 4-Me-piperazinyl; **3**: X = F, Y = H, R_2 = 4-Me-piperazinyl; **4**: X = Br, Y = H, R_2 = 4-Me-piperazinyl; **5**: X = F, Y = H, R_2 = 4-pyridinyl; **6**: X = Cl, Y = H, R_2 = 4-pyridinyl; for compounds with X = CN, Y = NHR_1, R = SCH_3; see Supporting Information.

irreversibly inhibits rhodesain.[14] The formation of the covalent complex E−I out of the initially formed noncovalent complex E···I starts with an addition of the deprotonated Cys25 residue of rhodesain to C1 of the vinylsulfone double bond (Chart 1). The inhibition reaction is completed by a transfer of the proton from the protonated His162 residue to C2.[15]

Prior to the investigations we used the X-ray structure of the covalent complex E−I of K11777 and rhodesain (PDB ID 2P7U)[16] and computed the reaction course backward to the corresponding noncovalent complex E···I. The computations were performed with QM/MM. For the QM-part we used BLYP[17]/TZVP[18] for energy computations. The protein environment was modeled at the force field level using the AMBER parametrization[19] in combination with the DL Poly code.[20] All calculations were performed with the CHEMSHELL package in combination with the TURBOMOLE program.[21] Interaction between QM and MM parts were treated by an electrostatic embedding scheme.[12] These QM/MM computations predict a reaction barrier of about 6 kcal/mol and a reaction energy of about −23 kcal/mol. Both values are in good agreement with experiments, which find an efficient and irreversible inhibition of rhodesain by K11777. The corresponding potential energy surface (PES) is given in the Supporting Information.

For irreversible inhibitors the risk for potential toxic effects is higher.[22] In order to identify covalent but reversible inhibitors, in Step I of our protocol we screened VSs to find those substitution patterns for which the addition reaction is only slightly exothermic because such compounds should act reversibly (Figure 2). Because we only need rough estimates at that point we computed reaction energies and used the model reaction of the given VS with methylthiol in a polar medium. The reaction energies were obtained from B3LYP[17] calculations in combination with the TZVP[18] basis set. In all computations, the COSMO approach with e = 78.39 was employed.[23] The calculations predicted that the substitution pattern X = Hal, Y = H should be appropriate because the reaction is less exothermic than the corresponding reaction of the warhead of K11777.[24] For X = Br or Cl we computed reaction energies of about −6 kcal/mol, while for X = F −10 kcal/mol was predicted. We also tested more bulky groups for the position of Y but the reactions became endothermic. These findings are in line with previous investigations about the influence of substituents on the reaction energies of VSs.[25]

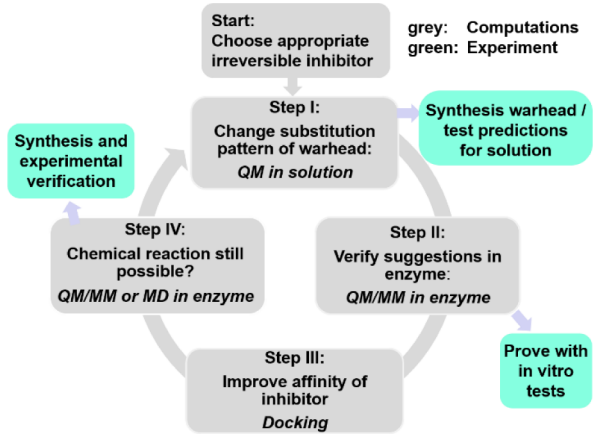


Figure 2. Protocol for the development of covalent reversible inhibitors starting from an irreversible inhibitor.

In Step II of the protocol the influences of the enzyme environment on the inhibition reaction with these promising patterns were calculated. We started with the noncovalent enzyme−inhibitor complex of K11777 (X = Y = H), substituted X = H by Br, Cl, or , F and computed the reaction profiles using the same QM/MM approach as described above. Figure 3 shows

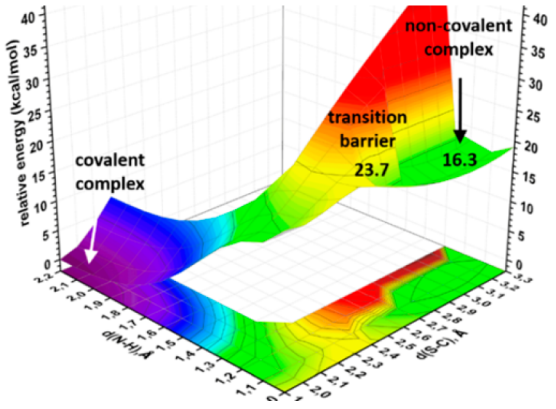


Figure 3. Computed potential energy surface of the covalent bond formation between rhodesain and the compound with X = F, Y = H (compd **3**). The numbers gives the relative energies with respect to the covalent enzyme−inhibitor complex. The corresponding PES for X = Cl and Br (compds **2** and **4**) are provided in the Supporting Information.

the reaction profile for X = F (**3**). The distance *d*(S−C) between the S atom of Cys25 of rhodesain and C1 of the inhibitor describes the attack of the thiolate at the double bond, while *d*(N−H) mimics the proton transfer from His162 of rhodesain to C2 of the inhibitor. All other geometrical parameters are optimized to obtain the minimum energy path (MEP) of the

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reaction course. The reaction starts at the noncovalent enzyme−inhibitor complex, which is predicted to be about 16 kcal/mol higher in energy than the covalent enzyme inhibitor complex, the end point of the inhibition. The inhibition reaction has a barrier of about 7 kcal/mol. For X = Cl and Br (**2**, **4**) we computed barriers of about 12 and 13 kcal/mol and reaction energies of −11 and −10 kcal/mol.[15] The reactions of irreversible inhibitors were computed to be more exothermic than −22 kcal/mol.[9c,12d] With −10 and −11 kcal/mol, the inhibition reactions are clearly reversible for X = Cl and Br, while X = F seems to represent a border case.

To prove our predictions we synthesized the compounds (K11777, **2**−**4**) and tested their inhibition potencies by fluorometric enzyme assays.[24] The reversibility of the inhibition was proven by dilution assays (Figure 4) and by dialysis assays

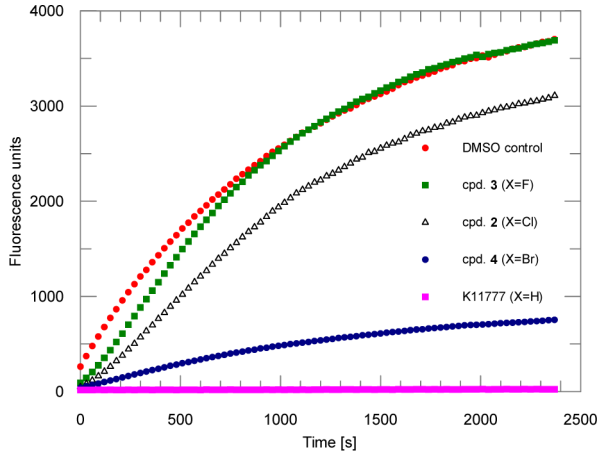

Figure 4. Experimental verification of the (ir)reversibility of the inhibition via dilution assays. The enzyme was incubated with an inhibitor concentration corresponding to 10× the K_i to ensure complete inhibition; then the incubation mixture was diluted by a factor of 100 yielding an inhibitor concentration of 0.1× the K_i. The enzyme activity was then measured by adding the substrate.

(see Supporting Information). For X = Br (**4**) mass spectrometry could prove that the compound reacts covalently with the active site Cys residue (see SI part).[15] For X = F (**3**, **5**), stable interactions between compound and protein could additionally be shown by ^{19}F NMR spectroscopy.[15] In the case of the Br derivative (**4**) the recovery of the enzyme activity was found to proceed very slowly with only 20% activity after 60 min (Figure 4). The MS data (see SI part) indicate that the inhibition by compd **4** becomes irreversible due to slow elimination of HBr. This is supported by QM/MM computations.[15] Compared to K11777, the K_i values for the halogenated derivatives increase: 20 nM (K11777; $k_{2nd} = 6.6 \times 10^5$ M^{-1} s^{-1}), 190 nM (F, **3**), 1.01 μM (Cl, **2**), 0.86 μM (Br, **4**), i.e., the halogenated inhibitors exhibit lower affinities.

So far, we only changed the chemical properties of the warhead but variations of the properties of the recognition unit are also important. In our protocol promising variations in the recognition unit are investigated in Step III using standard docking routines. In our approach the docking can be performed directly for the targeted noncovalent complex because we computed it in the previous QM/MM computations. The docking studies performed with the FlexX program package (version 2.1.3)[15,26] and DOCKTITE[10d] indicated that the replacement of the *N*-methyl piperazine moiety by a pyridine ring should increase the affinity. The corresponding score values are discussed in the SI. In Step IV of our protocol MD simulations showed that the covalent reaction step is still possible (see SI). They also supported the orientations predicted by the employed docking approach. The syntheses and testing of the respective reversible inhibitors with X = F (**5**) and Cl (**6**) and also with X = H (**1**), an irreversible inhibitor like K11777, indeed, showed much higher affinities: K_i values [nM]: 3.7 (H, **1**, $k_{2nd} = 1.9 \times 10^6$ M^{-1} s^{-1}), 32 (F, **5**), 190 (Cl, **6**), i.e., the predictions were again fully confirmed. The new reversible halogenated compounds (**5**, **6**) also show slightly better antitrypanosomal activity compared to the *N*-methy piperazinyl derivatives (**2**, **3**), but exhibit less cytotoxicity than their irreversible counterpart (**1**): (EC_{50} [μM] (*T. brucei*)/CC_{50} [μM] (J774.1 macrophages)/CC_{50} [μM] (HELA cells): (**5**) 3.0/>100/>500; (**6**) 3.1/>100/>500; (**1**) 1.7/8.6/11; (**3**) 12.5/38/10; (**2**) 13/23/19.

We also used the covalent docking program DOCKTITE[10d] to predict the inhibition potencies. DOCKTITE could indeed be trained to recognize halogenated vinylsulfones as covalent inhibitors. It is also able to reproduce the structure of the final covalent enzyme−K11777 complex. However, the computed scores do not reflect the experimental trends in K_i values and do not distinguish between irreversible or reversible inhibition.[15]

This successful example indicates that the employed theoretical approaches are very helpful for the design of covalent inhibitors with desired properties. This is underlined by a second investigation that was unsuccessful in terms of new reversible inhibitors but proved the accuracy of our theoretical approaches. QM computations in solution (Step I) indicated that inhibitors with X = CN, R = SCH_3, and Y = $NHCH_3$ can reversibly block the enzyme via an S_NV-mechanism. The results were confirmed by NMR measurements in solution,[24] but QM/MM computations in Step II predicted that the corresponding S_NV reaction within the enzyme is strongly endothermic (ΔE_{reac} > +30 kcal/mol), i.e., no inhibition is expected. However, first test measurements indicated weak inhibition.[15] The contradiction could finally be resolved by additional assays. They showed that a noncompetitive inhibition takes place, i.e., the inhibitors do not react with the active site for which the computations were performed. This example also shows that computations for the solvent situation alone (only Step 1) might lead to wrong conclusions because the enzyme environment strongly influences the reaction course.

We have proposed a new protocol that is very helpful for the development of covalent inhibitors with desired properties. Its applicability was successfully demonstrated by the design of reversible covalent vinylsulfone-based inhibitors for rhodesain. Our results can directly be used for other vinylsulfone-based inhibitors.[9,14,27] It should also be applicable to other compound classes because it is based on highly reliable quantum chemical approaches. Our approach could be combined with the faster covalent docking approaches. While the latter is used for screening, our approach could be used to investigate the most promising examples in more detail.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03052.

Figures and complete experimental details for syntheses and testing (PDF)

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