Published in final edited form as:

Theor Chem Acc. 2013 August ; 132(8): . doi:10.1007/s00214-013-1379-z.

Microscopic Modes and Free Energies for Topoisomerase I-DNA Covalent Complex Binding with Non-camptothecin Inhibitors by Molecular Docking and Dynamics Simulations

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Abstract

Topoisomerase I (Topo1) has been identified as an attractive target for anticancer drug development due to its central role in facilitating the nuclear process of the DNA. It is essential for rational design of novel Topo1 inhibitors to reliably predict the binding structures of the Topo1 inhibitors interacting with the Topo1-DNA complex. The detailed binding structures and binding free energies for the Topo1-DNA complex interacting with typical non-camptothecin (CPT) Topo1 inhibitors have been examined by performing molecular docking, molecular dynamic (MD) simulations, and binding free energy calculations. The computational results provide valuable insights into the binding modes of the inhibitors binding with the Topo1-DNA complex and the key factors affecting the binding affinity. It has been demonstrated that the — stacking interaction with the DNA base pairs and the hydrogen bonding with Topo1 have the pivotal contributions to the binding structures and binding free energies, although the van der Waals and electrostatic interactions also significantly contribute to the stabilization of the binding structures. The calculated binding free energies are in good agreement with the available experiment activity data. The detailed binding modes and the crucial factors affecting the binding free energies obtained from the present computational studies may provide valuable insights for future rational design of novel, more potent Topo1 inhibitors.

Introduction

DNA Topoisomerase I (Topo1) is an essential nuclear enzymes in eukaryotes¹ and plays a central role in tumor cells by facilitating the nuclear process of DNA. Many biochemical and cellular assays identified that Topo1 is a validated target of cancer chemotherapy, including lung cancer, colon cancer, ovarian cancer, renal cancer, prostate cancer, and breast cancer.²⁻⁵ The key role of Topo1 for the DNA nuclear process is to elevate the levels of DNA in solid tumors compared to normal tissue. Topo1-interfering drugs convert the enzyme into a cellular poison by inhibiting the relegation step, trapping the enzyme into a stable covalent DNA complex. Cytotoxic lesions of such Topo1 poison are likely to result from the collision between the replication fork and the stabilized cleavable complex,

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Supplementary Material Available: Additional four tables for the components of the binding free energy on a per-residue basis for the Topo1-DNA complex binding with each ligand.

transforming a normal cellular protein into a DNA-damaging agent.⁶ Therefore, Topo1 is considered as an attractive target for anticancer drug development.^{5,7–13}

The human Topo1 enzyme is composed of 765 residues divided into an N-terminal domain (residues 1–214), a core domain (residues 215–635) further subdivided into subdomain I (residues 215–232 and 320–433), subdomain II (residues 233–319) and subdomain III (residues 434–635), a linker domain (residues 636–712) and a C-terminal domain (residues 713–765).^{14–16} The protein has a globular shape, with subdomains I and II forming the CAP domain and subdomain III and C-terminal domain forming the CAT domain.¹⁵ The linker domain is formed by two long α -helices that extrude outside from the globular shape of the protein

The Topo1 complex resolves DNA supercoils by a mechanism defined as controlled rotation,^{17–19} which is composed by 5 steps: 1) the protein wraps around the substrate forming a non-covalent Topo1-DNA complex which is stabilized by intramolecular and intermolecular hydrogen bonds and electrostatic interactions; 2) the catalytic residue Tyr723, assisted by the other four catalytic residues (Arg488, Lys532, Arg590, and His632) in the catalytic pentad, carries out a nucleophilic attack on one strand of the double-strand DNA (dsDNA), forming a covalent protein-DNA complex (the cleavage complex) at the 3' end; 3) the broken strand rotates around the unbroken strand, controlled by the interaction with the linker domain, relaxing the supercoil; 4) the broken strand is relegated; 5) the protein is released.¹⁷

Camptothecin (CPT), a nature product isolated from the Chinese tree *Camptotheca acuminata*, was the first discovered small molecule which converts Topo1 into DNA-damaging agent.^{20–24} Thereafter, as derivatives/analogues of CPT, topotecan and irinotecan ((CPT)-II) were successfully developed and are currently in clinical use for treatment of ovarian cancer and colon cancer, respectively.^{25–26,27–28} CPT acts by stabilizing the cleavage complex and preventing the relegation step. The binding of CPT to the cleavage complex is considered to be reversible, but the compound becomes lethal due to the collision of the stalled enzyme-DNA complex with the replication fork.^{29–31} The CPT and its derivatives, such as topotecan, bind to the binary complex by intercalating between the bases of the DNA substrate at the cleavage site.

Although the CPT analogues have achieved great success in clinical cancer chemotherapy, resistance is still a critical clinical problem.³² Thus, to overcome the deficiencies of CPTs (including CPT and its analogues), compounds with diverse scaffolds have been screened as the non-CPT Topo1 inhibitors. Recently, indolocarbazoles, indenoisoquinolines, and phenanthridines with new scaffolds (Scheme 1) have been found to be among the most potent non-CPT Topo1 inhibitors.^{11, 33–34}

During the last decade, more than 400 indenoisoquinoline derivatives have been synthesized as the potential Topo1 inhibitors and tested for anticancer therapeutic development.^{34–37}

The phenanthridine derivatives share many of the same advantages as the indenoisoquinolines, which have been synthesized and shown to be potent Topo1-targeting agents.^{38–41} ARC III, one of the phenanthridine derivatives, was shown to be as active as irinotecan in the HCT-8 colon tumor model, and compared favorably with irinotecan and topotecan in the SKNEP anaplastic Wilms' tumor model.^{38, 42}

The X-ray crystal structures of human Topo1-DNA complex bound with different inhibitors^{4, 9} revealed a common molecular mechanism of the drug action. All of the Topo1 inhibitors bind at the Topo1-DNA interface by intercalating and stacking between the base pairs flanking of the DNA cleavage site and by forming critical hydrogen bonds with Topo1

amino-acid residues. The hydrogen bonds stabilize the Topo1-DNA cleavage complexes, which generate the chemotherapeutic potential of Topo1 inhibitors. Although various Topo1 inhibitors have been reported, only the Topo1 binding with the CPT and indolocarbazoles and their respective derivative structures have their IC₅₀ values determined experimentally.

The main objective of the present study was to understand how Topo1-DNA complex binds with its potent non-CPT inhibitors, and thus, establish a solid structural base for future rational design of more potent Topo1 inhibitors as potential new therapeutic agents. For this purpose, we carried out molecular docking, molecular dynamics (MD) simulations, binding free energy calculations, and binding free energy decompositions to explore possible modes of the binding between the non-CPT inhibitors and the Topo1-DNA complex. The calculated structural and energetic data provide a quantitative explanation for the inhibitory activity of various non-CPT Topo1 inhibitors. The computational results reveal valuable insights into the microscopic binding of Topo1-DNA complex with inhibitors.

Methods

Structural model of the Topo1-DNA complex

The model of the covalent Topo1-DNA complex was built using the crystal structure of the Topo1 protein (PDB code: 1TL8).⁴³ After superposition of the pdb structures of Topo1-DNA complexed with various inhibitors, we observed that the DNA structures in all of the examined complexes are very similar, with an average root-mean-squares deviation (RMSD) of ~0.2 Å (Figure S1). Hence, the oligonucleotide sequence of the cleavable strand of the duplex oligomer 5'-GACTTsCGAAAAATTTT-3' was cut and only the oligonucleotide composed of 4 base pairs was kept in the structural model (underlined bases) (Figure 1).

Generation of the ligand conformers

The generated various molecular orientations and multiple conformations of each ligand were realized using the OMEGA program (Open Eye Scientific Software).⁴⁴⁻⁴⁶ Depicted in Scheme 2 are the ligands examined: (1) C₂₃H₂₃N₃O₅; (2) C₂₁H₁₂N₂O₄S₂; (3) C₁₇H₁₃NO₂; (4) C₁₈H₁₃NO₂. The OMEGA sampling is capable of selecting a ligand conformation similar to that of the targeted X-ray crystal structure by using an appropriate option (the default) including a low-energy cutoff to discard high-energy conformations, a low root-mean-squares deviation (RMSD) value below which two conformations are considered to be similar.⁴⁶ Atom typing, energy calculation, and geometry optimization in the OMEGA were performed using the Merck Molecular Force Field (MMFF). The allowed maximum number of conformations per compound was set to 200 and the energy window (the value used to discard high-energy conformations) was set to 10 kcal/mol. The default values of the OMEGA program were used for other parameters.

Docking of the ligands to the Topo1 active site

FRED program (OpenEye Scientific Software) was used in this study to dock the generated ligand conformers.⁴⁷ The strategy used in the FRED is to exhaustively dock/score all possible poses of each ligand in the binding site. The rigid docking consisted of two steps, *i.e.*, shape fitting and optimization. During the shape fitting, the ligand was placed into a 0.5 Å resolution grid box encompassing all active-site atoms (including hydrogen atoms) using smooth Gaussian potential.⁴⁸ Then, two optimization filters were processed: rigid-body optimization and optimization of the ligand pose in the dihedral angle space. The docked ligand poses were clustered, and the most favored cluster of ligand poses was identified using a method similar to the one described by Gatchell *et al.* and in our previous work.⁴⁹⁻⁵¹ Briefly, for every docked pose, the number of ligand neighbors within a threshold RMSD

was determined. The pose with the maximum number of neighbors, or the most populated cluster, was identified as the most favored pose or conformation.

For each of the compounds examined, the docked poses were clustered by using a criterion with an RMSD value of 0.8 Å. Single conformations or poses disconnected from the rest of the ensemble were considered as unlikely conformations and hence were removed. After the initial criterion was satisfied, the pose ensemble was then filtered to reject the poses that do not have a shape matched complementarily with the active site of the protein, followed by rejecting the poses that do not have at least one heavy atom making a hydrogen bond with Arg364 side chain. We have selected the Arg364 since several CPT resistance (CPT_r) point mutations in human Topo1-DNA complex have identified that the Arg364 is one of the key anchoring residues for the inhibitors,^{9, 52}

The top-ranked poses were optimized by rigid-body optimization and refined using the MMFF. Consensus score was selected for the optimization filters with preliminary docking trails. The consensus score were based on the use of Gaussian shape scoring,⁴⁸ ChemScore,⁵³ PLP,⁵⁴ and the ScreenScore.⁵⁵ The structure of each inhibitor with the best score based on the most populated cluster was selected. Finally, the binding structures of the Topo1-DNA-inhibitor ternaries were refined by performing MD simulations.

Molecular dynamics in explicit solvated system

In separate docking runs, the binding poses of a ligand that passed the shape-fitting, optimization filters, and check on the hydrogen bonding to Arg364 side chain were refined by carrying out MD simulations using Sander module of Amber11 package.⁵⁶ The general procedure for carrying out the MD simulations in water is essentially the same as that used in our previously reported other computational studies.^{51, 57–60} The molecular mechanics parameters for the protein and ligands were assigned with tleap module of Amber11. In particular, the ligand atoms were assigned generalized amber force field (gaff)⁶¹ atom types, whereas Amber ff03 parameters were assigned to all the protein atoms.⁶²

Molecular geometries of the ligands and truncated oligonucleotide were optimized using the Gaussian03 program at the *ab initio* HF/6-31G* level.⁶³ The electrostatic potentials on the molecular surfaces were calculated at the same level along with the geometry optimizations. The partial atomic charges were determined from the electrostatic potentials by using the standard restrained electrostatic potential (RESP) fitting procedure.^{64–65}

The initial binding structure of each Topo1-DNA-inhibitor ternary was neutralized by adding appropriate sodium counter ions and was solvated in an orthorhombic box of TIP3P water molecules with a minimum solute-wall distance 10 Å.⁶⁶ The solvated systems were energy-minimized and carefully equilibrated. These systems were gradually heated from T = 10 K to T = 298.15 K in 150 ps before running the MD simulation.

The MD simulations were performed with a periodic boundary condition in the NPT ensemble at T = 298.15 K with Berendsen temperature coupling⁶⁷ and constant pressure (P = 1 atm) with isotropic molecule-based scaling. A time step of 2 fs was used, with a cutoff 12 Å for the nonbonded interactions. The SHAKE algorithm was employed to keep rigid all covalent bonds involving hydrogen atoms.⁶⁸ Long-range interactions were handled using the particle mesh Ewald (PME) algorithm.⁶⁹ During the energy minimization and MD simulation, only the ligand and residue side chains in the binding site were allowed to move freely. A harmonic restraint of 10.0 kcal mol⁻¹ Å⁻² was applied to the truncated DNA and backbones of other residues of the protein. The constraints were applied to prevent any unexpected changes (artifacts) in the secondary structure of the Topo1 protein and truncated DNA. MD simulations were then carried out for 10 ns. During the simulations, the

coordinates of the system were collected every 10 ps. The last 100 snapshots of the MD trajectory were used to perform the MM-GBSA binding energy calculations described below. The RMSD of the atomic positions of the ligand from the first snapshot (reference structure) of the MD trajectory was evaluated.

MM-GBSA calculation

The binding free energies and the bind energy decomposition were calculated by using the MM-GBSA method implemented in Amber11 program for each Topo1-DNA-inhibitor ternary.^{57–58, 70–71} The MM-GBSA binding free energy calculations were based on the following equations:

$$\Delta G_{\text{bind}} = \Delta G_{\text{ternary}} - \Delta G_{\text{Topo1-DNA}} - \Delta G_{\text{lig}} \quad (1)$$

$$G = E_{\text{gas}} - G_{\text{sol}} - TS \quad (2)$$

$$E_{\text{gas}} = E_{\text{int}} + E_{\text{vdw}} + E_{\text{elc}} \quad (3)$$

$$G_{\text{sol}} = G_{\text{GB}} + G_{\text{np}} \quad (4)$$

In the above equations, $\Delta G_{\text{ternary}}$, $\Delta G_{\text{Topo1-DNA}}$, and ΔG_{lig} are the free energy changes of the Topo1-DNA-inhibitor ternary, Topo1-DNA complex, and ligand, respectively. All the free energy changes in Eq.(1) were determined by summing the gas phase internal energy (E_{gas}), the solvation free energy and the entropy term ($-TS$) in Eq.(2). E_{gas} is the standard force field energy, including the internal energy as well as the van der Waals and electrostatic energies (Eq.(3)). The “Bondi” radii were used for the GB calculations.⁷² The solvation free energy, G_{sol} , was calculated through the generated Born/surface area (GBSA) model, which decomposes the solvation free energy into the sum of the electrostatic component (G_{GB}) and nonpolar component (G_{np}) (Eq.(4)). The electrostatic component G_{GB} was calculated by using Hawkins, Cramer, and Truhlar’s pairwise, generalized Born model.^{73–74} The linear combination of pairwise overlaps (LCPO) method was used to calculate the solvent accessible surface area (ΔSA) and evaluate the ΔG_{np} value with $\gamma = 0.005 \text{ kcal/mol}\text{\AA}^2$ and $\beta = 0.00 \text{ kcal/mol}$.⁷⁵

$$\Delta G_{\text{np}} = \gamma \Delta SA + \beta \quad (5)$$

The calculation of the entropic contribution ($-TS$) requires several approximations and provides only a rough estimate, especially in the case of a simulation in which only a small portion of the protein moves. As a result, the entropic contribution was neglected in our binding free energy calculation, with an implicit assumption that the entropic contributions to the binding free energies for various ligands are very close to each other. Under the implicit assumption, neglecting the entropic contribution is expected to only systematically overestimate the absolute binding free energies, but not to significantly affect the calculated relative binding free energies.

Results and Discussion

Binding modes of Topo1 inhibitors with the Topo1-DNA complex

In the present study, four typical non-camptothecin (CPT) Topo1 inhibitors were selected according to their scaffold diversities and different Topo1 inhibitory activities (Scheme 2

and Table 1).^{37, 40, 76} Understanding the binding modes of these scaffolds will be of great help in further lead optimization studies. A molecular docking study was first used as a critical step to reasonably predict the binding modes of the inhibitors in the active site of Topo1. Thus, the refined 50 docked poses of the inhibitors that passed the criteria described above were clustered into one major and several minor binding modes.

The docking results revealed that the inhibitors of the most populated cluster intercalate in the DNA cleavage site with a similar location and overlap with the reference ligand (AI-III-52) in the crystal structure (PDB code 1TL8).⁴³ This finding seems to suggest that one may simply take the reference PDB structure with AI-III-52, substitute the ligand there by the new one, getting the correct orientation from the Arg364 interactions (see below) and relax the structure with a restrained simulation to get the same binding structures obtained here. On the other hand, the docking tests performed here did confirm that there would be no alternative binding pose better than the obvious one noted in the reference binding structure. To further ensure the stability of the binding structure of each ligand with the Topo1-DNA complex, each Topo1-DNA-inhibitor ternary underwent a 10 ns MD simulation. The analysis of the MD trajectories showed that the RMSD of each MD-simulated Topo1-DNA-inhibitor ternary became stable; the ligand relaxed in the active site and quickly reached an equilibrium state.

The MD trajectories and refined binding structures of ligands **1** and **2** with the Topo1-DNA complex are depicted in Figures 2 and 3. The obtained binding mode of ligand **1** reveals that the aromatic ring is perpendicular to the main axis of the DNA and parallel to the base pairs. The side chains of Arg364 and Asn722 residues of Topo1 stabilize ligand **1** via hydrogen bonding to the N₁₂ atom and the oxygen atom of the methoxy group (in position 8) of the ligand, with an average hydrogen-bond distances of 2.20 (N...H) and 2.10 (O...H) Å, respectively.

Ligand **2** binds to the DNA cleavage site with a similar binding mode as ligand **1**, by establishing a π -stacking interaction between the base pairs and reinforced by a hydrogen bond with Arg364 side chain, as expected by Dong *et al.*⁷⁶ Further, according to our simulated structure depicted in Figure 3, an additional hydrogen bond is formed between the oxygen atom of 4-tosyl group (methylbenzene sulfonyl group) of ligand **2** and the NH amide group of Asn722 side chain. The average distances of both hydrogen bonds are 2.20 (Arg364) and 1.99 Å (Asn722), respectively. Since the 4-tosyl group of ligand **2** face to the major groove of Topo1, the binding of ligand **2** in the cavity is also stabilized by the van der Waals interaction with Pro431 side chain, hydrophobic interaction with Leu721 side chain, and the long-range electrostatic interaction with the charged Lys751 side chain.

The binding modes and the MD trajectories of ligands **3** and **4** are depicted in Figures 4 and 5. Similarly, both ligands bind on the Topo1-DNA interface by stacking between the DNA base pairs and reinforced by hydrogen bonding with the key residue (Arg364) of Topo1 active site. The average lengths of hydrogen bonds range from 1.90 to 2.36 Å.

Binding free energies of Topo1-DNA-ligand complexes using MM/GBSA method

As described in literature,^{76–78} the antitumor activities of the known Topo1 inhibitors were determined *via* cytotoxicity assays on different cell lines (Table 1). These experimental data that depend on the type of the tested cells provide only an estimation of the binding affinity of the ligand to the Topo1 protein target. In order to provide a deeper insight into the structural and energetic parameters contributing to the binding affinity of these inhibitors to the Topo1-DNA complex, the binding free energy was calculated for each ligand with the Topo1-DNA complex using the MM-GBSA method.

Here, the calculated binding free energy (ΔG_d^{cal}) is not expected to reproduce the absolute binding free energy of the corresponding ligand as measured experimentally, since the entropy term was neglected (see the Methods section). One would like to verify whether the MM-GBSA method could reasonably predict the relative binding free energies of the ligands that intercalate between the DNA base pairs as well as interacting with the residues of the protein. In addition, a detailed energetic estimation of the interactions between each ligand and the key residues in the binding cavity of the Topo1-DNA complex was carried out, and the results are discussed below.

According to the calculated energetic results summarized in Table 1, ligand **1** has the lowest binding free energy (−44.0 kcal/mol) among the four ligands. The binding free energy of ligand **3** is higher than that of ligand **4** even though both ligands are hydrogen-bonded to the same residue. Overall, the order of the calculated binding free energies (ΔG_d^{cal}) is **1** < **2** < **3** < **4**, or the order of the binding affinity is **1** > **2** > **3** > **4**. The calculated relative binding free energies are consistent with the experimentally measured cytotoxicities (Table 1).^{37, 76–77}.

A detailed analysis of the energetic components of the binding free energy (ΔG_d^{cal}) of each ligand indicates that the van der Waals (vdW) interaction is the most important favorable component of the ligand binding affinity to the Topo1-DNA complex. In particular, the order of the contributions of the vdW interactions to the relative binding free energies is the same as that of the calculated relative total binding free energies.

Although the relative binding free energies obtained from the MM-GBSA calculations are qualitatively consistent with the experimental data, the MM-GBSA calculations systematically overestimated the absolute binding affinity for each inhibitor, as expected. The current observation of the systematic overestimation with the MM-GBSA method is consistent with our previous observation in computational studies on other protein-ligand binding systems, *i.e.* phosphodiesterase 2 (PDE2) binding with its inhibitors.⁷⁹ Nevertheless, the computational studies on the PDE2-inhibitor binding systems have led to development of an empirical linear correlation relationship which can be used to correct the calculated binding free energies (ΔG_d^{cal}):⁷⁹

$$\Delta G_d^{\text{cal}}(\text{corr}) = 0.583 \times \Delta G_d^{\text{cal}} + 14.2 (\text{kcal/mol}) \quad (6)$$

The calculated binding free energies corrected by using Eq.(6) are in good agreement with the corresponding experimental data for the PDE2-inhibitor binding systems.⁷⁹ In light of the encouraging results obtained for the PDE2-inhibitor binding systems, the equation Eq.(6) was used to know whether it can also be used to reasonably correct the MM-GBSA-calculated binding free energies for the currently studied more complicated systems regarding binding of the Topo1-DNA complex with its inhibitors. The corrected ΔG_d^{cal} values, *i.e.* $\Delta G_d^{\text{cal}}(\text{corr})$, summarized in Table 1 reveal that Eq.(6) works well for the Topo1-DNA complex binding with inhibitors too. The obtained $\Delta G_d^{\text{cal}}(\text{corr})$ values of −11.5, −9.2, −6.3, and −5.2 kcal/mol are in good agreement with the experimentally-derived binding free energies (ΔG_d^{exp}) of −11.8 to −10.7, −7.8, −5.5, and −5.1 kcal/mol, respectively, for ligands **1** to **4**.

Further, analysis of the linear correlation relationship between the directly calculated ΔG_d^{cal} values and the experimentally-derived ΔG_d^{exp} values resulted to the following optimum linear correlation relationship:

$$\Delta G_d^{\text{cal}}(\text{corr}) = 0.572 \times \Delta G_d^{\text{cal}} + 14.4 (\text{kcal/mol}) \quad (7)$$

with a correlation coefficient (R) of 0.98 and RMSD value of 0.50 kcal/mol. The corrected ΔG_d^{cal} values, *i.e.* $\Delta G_d^{\text{cal}}(\text{corr})$, summarized in Table 1 reveal that Eq.(6) works even better for the Topo1-DNA complex binding with inhibitors. Eq.(7) is very close to Eq.(6) in terms of the parameter values. Based on Eq.(7), the obtained $\Delta G_d^{\text{cal}}(\text{corr})$ values of -10.8 , -8.5 , -5.7 , and -4.6 kcal/mol are in excellent agreement with the experimentally-derived binding free energies (ΔG_d^{exp}) of -11.8 to -10.7 (medium point: -11.3), -7.8 , -5.5 , and -5.1 kcal/mol, respectively, for ligands **1** to **4**.

Contributions to the binding affinity

Binding energy decomposition was conducted by using the MM-GBSA method to explore the origins of the binding affinity between the ligand and the binding site of the Topo1-DNA complex. The energy decomposition was based on the uncorrected MM-GBSA data that systematically overestimated the binding free energies as noted above. So, the obtained binding energy contributions from all residues should also be overestimated systematically. Nevertheless, the relative magnitudes of the obtained binding energy contributions (to be discussed below) should be reasonable.

The per-residue decomposition of the binding energies for the inhibitors **1** to **4** is summarized in Table 2. Given in Table S1 to S4 in Supporting Information are the more detailed data obtained from the decomposition of the binding energy into contributions from the vdW, electrostatic, and solvation free energy terms for each key residue interacting with the ligand. The significant intermolecular contributions of the key residues to the binding mode of each ligand are also depicted in Figure 6.

Contribution from DNA—Table 2 and Figure 6 show that the — stacking interactions between the base pairs have the major contribution to the binding affinity of each ligand. Through the — stacking interaction, adenine and guanine of the intact DNA1 strand contribute about 9 to 11 kcal/mol to the ligand binding. However, thymine and cytosine of the broken DNA-2 strand contribute much less than DNA-1. The difference in the energy contribution is attributed to the size of the purine which is larger than the pyrimidine.

Contribution of the key residues to the binding affinity of ligand 1—Based on the energetic data in Table 2 and the structural data in Figure 2, ligand **1** is stabilized in the cleavage site by residues Arg364 and Asn722 of Topo1 with two intermolecular hydrogen bonds. The two hydrogen bonds contribute about -2.7 (Arg364) and -1.1 kcal/mol (Asn722), respectively, to the binding affinity of ligand **1**. In addition, there are vdW and electrostatic interactions between Thr718 side chain and the 9-methoxyl group of ligand **1**. The total contribution of Thr718 to the binding is about -1.2 kcal/mol.

Contribution of the key residues to the binding affinity of ligand 2—Ligand **2** is also stabilized in the cleavage site by hydrogen bonding to residues Arg364 and Asn722, in light of the data in Table 2 and Figure 3. These residues contribute -1.4 (Arg364) and -1.5 kcal/mol (Asn722) to the binding affinity of ligand **2**. Residues Pro431 and Thr718 contribute -1.2 and -1.0 kcal/mol, respectively, to the binding energy through vdW interaction. Moreover, hydrophobic interaction between ligand **2** and Leu721 side chain contributes about -1.8 kcal/mol to the binding affinity. Lys751 facilitates ligand **2** binding in the cleavage site with a long-range electrostatic interaction (about -1.4 kcal/mol).

Contribution of the key residues to the binding affinity of ligands 3 and 4—

According to the results in Table 2 and Figures 4 and 5, residue Arg364 of Topo1 stabilizes the binding mode through two hydrogen bonds with the energy contributions of about -6.2 and -4.0 kcal/mol for ligands **3** and **4**, respectively. Ligand **3** has unfavorable interaction (~ 0.9 kcal/mol, as seen in Table 2) with residue Thr718, due to the electrostatic repulsion (see Supporting Information, Table S3).

Figure 6 shows that the — stacking interaction with the DNA base pairs has the most important contribution to the binding affinity for each ligand (see Table 2). Notably, Arg364 has the favorable contribution to the binding of the Topo1-DNA complex with all of the four inhibitors (ligands **1** to **4**).

Finally, we would like to note some limitations of the current computational study. In particular, a harmonic restraint was applied to the truncated DNA and backbones of other residues of the protein during the MD simulations in order to avoid possible artifacts. As a result, the energy contributions from the structural rearrangements (in addition to the aforementioned entropy *etc.*) have been neglected in the binding free energy calculations. Such an approach might not be appropriate for some ligands that could induce considerably conformational changes on the protein or DNA structure.

Conclusion

The combined molecular docking, molecular dynamics (MD) simulations, binding free energy calculations, and binding energy decompositions were carried out in this study to understand how the Topo1-DNA complex binds with typical non-camptothecin (CPT) Topo1 inhibitors and calculate the corresponding binding free energies. The MD-simulated ligand binding modes have revealed that the — stacking interaction with the DNA base pairs and the hydrogen bonding with residues Arg364 and Asn722 of Topo1 have the most important contributions to the binding structures and free energies, although the van der Waals and electrostatic interactions also significantly contribute to the stabilization of the binding structures. The calculated binding free energies are in good agreement with the available experimentally-derived binding free energies. Further binding energy decomposition using the MM-GBSA method has provided more detailed information about the key structural factors affecting the binding free energies.

Concerning the limitations of the current computational study, the system setup limits the use of the results to intercalators that do not induce considerably conformational changes on the protein or DNA structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the NIH (grant RC1 MH088480 to Zhan) and the NSF (grant CHE-1111761 to Zhan). Wei worked in Dr. Zhan's laboratory for this project as an exchange student from Dalian University of Technology. The authors also acknowledge the Computer Center at University of Kentucky for supercomputing time on a Dell X-series Cluster with 384 nodes or 4,768 processors. Wei also appreciates the scholarship from Chinese Scholarship Council (CSC).

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Figure 1.

Ribbon diagram of the covalent Topo1-DNA complex bound with inhibitor A1-III-52 (PDB code: 1TL8). The inhibitor is in space-filling mode (CPK) bound into the intercalation binding pocket of the protein (ribbon) and DNA (stick) ternary complex. Four base pairs kept for the present computational study are colored in green.

Figure 2.

(*Top*) Binding mode of ligand **1** in the cleavage site of the Topo1-DNA complex. The intermolecular hydrogen bonds between the ligand and the residues of Topo1 are indicated with dashed lines. (*Bottom*) The MD-simulated hydrogen-bonding distances and RMSD for atomic positions of the ligand *versus* the simulation time for the Topo1-DNA-**1** ternary. Trace D1 represents the distance between the nitrogen atom of ligand **1** and the hydrogen atom of the amino group of Arg364 side chain. Trace D2 refers to the distance between the oxygen atom of ligand **1** and the hydrogen atom of the amino group of Asn722 side chain. The average distances of D1 and D2 are 2.2 and 2.1 Å, respectively.

Figure 3.

(*Top*) Binding mode of ligand **2** in the cleavage site of the Topo1-DNA complex. The intermolecular hydrogen bonds between the ligand and the residues of Topo1 are indicated with dashed lines. (*Bottom*) The MD-simulated hydrogen-bonding distances and RMSD for atomic positions of the ligand *versus* the simulation time for the Topo1-DNA-**2** ternary.

Trace D1 represents the distance between the nitrogen atom of ligand **2** and the hydrogen atom of the amino group of Arg364 side chain. Trace D2 refers to the distance between the oxygen atom of ligand **2** and the hydrogen atom of the amino group of Asn722 side chain. The average distances of D1 and D2 are 2.1 and 2.2 Å, respectively.

Figure 4. (Top) Binding mode of ligand **3** in the cleavage site of the Topo1-DNA complex. The intermolecular hydrogen bonds between the ligand and the residues of Topo1 are indicated with dashed lines. (Bottom) The MD-simulated hydrogen-bonding distances and RMSD for atomic positions of the ligand *versus* the simulation time for the Topo1-DNA-**3** ternary. Trace D1 and D2 represent the distances between the oxygen atom of carbonyl group of ligand **3** and the hydrogen atoms of two amino groups of Arg364 side chain. The average distances of D1 and D2 are both ~ 2.2 Å.

Figure 5.

(*Top*) Binding mode of ligand **4** in the cleavage site of the Topo1-DNA complex. The intermolecular hydrogen bonds between the ligand and the residues of Topo1 are indicated with dashed lines. (*Bottom*) The MD-simulated hydrogen-bonding distances and RMSD for atomic positions of the ligand *versus* the simulation time for the Topo1-DNA-**4** ternary. Trace D1 and D2 represent the distances between the oxygen atom of carbonyl group of ligand **4** and the hydrogen atoms of the two amino groups of Arg364 side chain. The average distances of D1 and D2 are 1.9 and 2.4 Å, respectively.

Figure 6. Relative contributions (in %) of the key residues of Topo1 and the DNA base pairs to the binding energy for each ligand. DNA-1: correspond to the stacking bases Adenine (A) and Guanine (G). DNA-2: correspond to the stacking bases Thymine (T) and Cytosine (C).

Camptothecins **Indolocarbazoles** **Indenoisoquinolines** **Phenanthridines** **Scheme 1.**

Molecular structures of clinically relevant Topo1 inhibitors with various different scaffolds

Scheme 2.

Molecular structures of the non-CPT Topo1 inhibitors (**1** to **4**) examined in this study. For clarity, some atoms of ligand **1** are numbered. The reference ligand structure (pdb: 1TL8.pdb) used for the docking is also represented

Table 1

Components of the calculated binding free energy ΔG_D^{cal} and the binding free energy (kcal/mol) using MM-GBSA method and the experimental data (ΔG_D^{exp}) of each ligand binding to the Topo1-DNA cleavage site. The original IC_{50} values of the inhibitors are also listed.

Ligand	ΔE_{vdw}	ΔE_{ele}	ΔG_{gas}	$\Delta G_{\text{sol}}^{\text{GB}}$	ΔG_D^{cal}	$\Delta G_D^{\text{cal}}(\text{corr})$	ΔG_D^{exp}	IC_{50}
1	-58.6	-5.2	-63.8	19.78	-44.0	-11.5 (-10.8)	-11.8 to -10.7	0.3 to 2.3 nM ^b
2	-46.9	-25.5	-72.4	32.2	-40.1	-9.2 (-8.5)	-7.8	0.501 μM ^c
3	-43.3	-2.4	-45.8	10.5	-35.2	-6.3 (-5.7)	-5.5	35.58 μM ^d
4	-37.0	-11.6	-48.7	15.4	-33.3	-5.2 (-4.6)	-5.1	79 μM ^d

^a Correction using the linear correction relationship, *i.e.* Eq.(6), developed in ref.⁷⁹. Given in the parentheses are the values corrected by using Eq.(7) obtained in the present study.

^b Experimental data from ref.⁷⁷.

^c Experimental data from ref.⁷⁶.

^d Experimental data from ref.³⁷.

^e The activation free energy derived from the experimental data *via* $\Delta G_D^{\text{exp}} = RT \ln K_d \approx RT \ln \text{IC}_{50}$.

Table 2

Decomposition of the calculated binding free energy ΔG_d^{cal} (kcal/mol) on a perresidue basis for the key residues of Topo1-DNA complex interacting with the inhibitors (ligands **1** to **4**).

Residue	Ligand 1	Ligand 2	Ligand 3	Ligand 4
A 351	-0.23	-0.21	-0.01	-0.02
N 352	0.05	-0.21	-0.07	0.12
E 356	0.46	0.03	0.18	0.39
R 364	-2.71	-1.44	-6.24	-3.99
P 431	-0.11	-1.22	0.01	-0.00
T 718	-1.15	-1.00	0.89	-0.08
L 721	-0.39	-1.83	-0.20	-0.08
N 722	-1.05	-1.54	-0.46	0.08
K 751	0.35	-1.39	-0.06	-0.01
DNA-1	-10.36	-9.81	-7.29	-8.93
DNA-2	-3.72	-3.38	-4.5	-3.49
SUMDNA	-14.08	-13.19	-11.79	-12.42

DNA-1: Correspond to the stacking bases Adenine (A) and Guanine (G)

DNA-2: Correspond to the stacking bases Thymine (T) and Cytosine (C)