Non-Intercalative Triterpenoid Inhibitors of Topoisomerase II: A Molecular Docking Study

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Abstract: Theoretical flexible docking studies were carried out on a number of triterpenoids previously shown to be inhibitors of topoisomerase II in order to assess the nature of binding of these non-intercalative inhibitors to the enzyme. The molecular docking results suggest that most of the triterpenoids preferentially bind to the DNA binding site of topoisomerase II, while a few also bind to the ATP binding site. These results provide some insight into the mode of activity of these cytotoxic natural products.

INTRODUCTION

Topoisomerases are essential enzymes that catalyze modifications to the tertiary structure of DNA. There are two well-characterized classes of human topoisomerases. Topoisomerase I acts by breaking and religating one DNA strand [1], while topoisomerase II involves double-strand breaking [2]. These enzymes serve to relieve DNA twisting and supercoiling, playing key roles in replication, transcription, and recombinant repair. Topoisomerase II is highly expressed in rapidly proliferating cells [3] and is therefore an attractive target for antitumor drugs.

There are two general classes of topoisomerase II targeting drugs: topoisomerase II poisons and topoisomerase II catalytic inhibitors. Topoisomerase II poisons include etoposide, doxorubicin, and mitoxantrone. These compounds serve to stabilize the enzyme-DNA complex (the "cleavable complex") and prevent the enzyme from religating the cleaved DNA [4]. Both doxorubicin and mitoxantrone are DNA intercalating agents [5] whereas etoposide does not bind DNA but rather apparently binds to the ATP binding site of the *N*-terminal domain of topoisomerase II [6, 7].

The catalytic inhibitors, on the other hand, block the catalytic activity of DNA topoisomerase II but do not stabilize the DNA-topoisomerase II cleavable complex [5, 8]. Examples of catalytic topoisomerase II inhibitors include the anthracycline aclarubicin, the polyanionic compound surname, the coumarin novobiocin, and bisdioxopiperazines such as sobuzoxane and dexrazoxane [9]. These agents inhibit the catalytic activity of topoisomerase II by preventing the binding of the enzyme to DNA. A number of natural and semisynthetic triterpenoids have shown topoisomerase II inhibitory activity. These include 3,4-*seco*-8β*H*-ferna-4(23),9(11)-dien-3-oic acid (**1**) and its corresponding alcohol derivative (**2**) [10]; *seco*-3,4-friedelin (**3**), *seco*-3,4-taraxerone (**4**), lupeol (**5**) [11]; fomitellic acids A and B (**6** and **7**) [12]; ursolic acid (**8**), oleanolic acid (**9**), betulinic acid (**10**), acetyl α-boswellic acid (**11**) [13]; demethylzeylasterone (**12**) [14]; dihydrobetulinic acid (**13**) [15]; corosolic acid (**14**), 3α-corosolic acid (**15**), 3β-corosolic acid lactone (**16**) [16]; celastrol (**17**), dihydrocelastrol (**18**) [17]; dehydrotrametononic acid (**19**), dehydroebriconic acid (**20**) [18]; ganoderic acid X (**21**) [19]; and the semisynthetic lanostane derivative (**22**) [20]. In this study, molecular docking techniques have been used to examine the potential binding sites of these known triterpenoid inhibitors of topoisomerase II in order to probe the possible mechanism of enzyme inhibition.

ATP is a required cofactor for topoisomerase II [2, 8, 21]. Topoisomerase II uses the energy released by ATP hydrolysis to induce DNA strand passage. In addition, the binding of ATP causes a conformational change of the enzyme from an open form to a closed clamp form. Therefore, ATP binding and hydrolysis result in opening and closing of the topoisomerase II enzyme. Some topoisomerase II inhibitors (e.g., bisdioxopiperazines and coumarins) act by binding to the ATPase domain of the enzyme [8, 9]. Potential binding of triterpenoid topoisomerase II inhibitors was also investigated by docking the compounds into the ATP binding sites of the *N*-terminal domain of topoisomerase II.

RESULTS AND DISCUSSION

The binding energies of the lowest-energy poses for each of the triterpenoid topoisomerase II inhibitors for the DNA binding site (PDB: 1bjt [22] and 2rgr [23]) and the ATP binding sites (PDB: 1qzr, 1pvg, and 1zxm) are summarized in Table **1**. The lowest-energy docking poses for most of the triterpenoids is the DNA binding site of topoisomerase II (see Figs. **1** and **2**), including **1-3**, **5-14**, **16**, and **19-22**. The key amino acid residues at this binding site are Arg 690, Asp 687. Gln 599, Gln 739, Gln 743, Glu 738, Glu 831, Gly 737, Gly 832, Ile 833, Lys 598, Lys 700, Phe 595, Ser 691, Thr 596, and Trp 597 (Fig. **3**). Mizushina and co-workers [24] found this to be the preferred binding site for unsaturated fatty acids with yeast topoisomerase II. Not surprisingly, the nature of binding of these lipophilic triterpenoids is largely hydrophobic, and the triterpenoids can dock in various orientations in this binding pocket. There are some trends, however. The lowest-energy pose of lupeol (**5**) is such that it forms hydrogen bonds between the C(3) hydroxyl group of the ligand and the carboxylate of Asp 687 and the guanidi-

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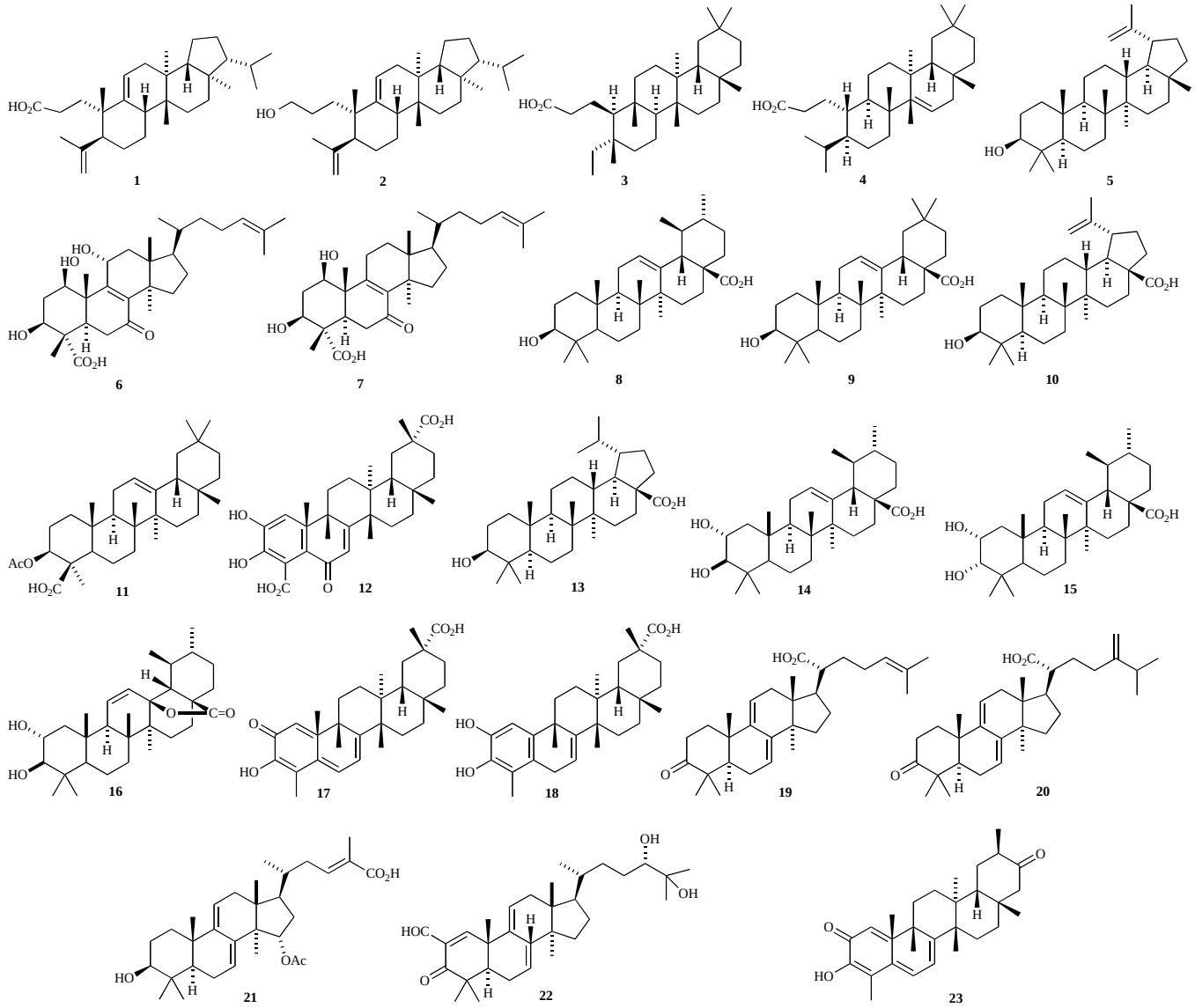

nium of Arg 690. Ursolic acid (**8**) and ganoderic acid X (**21**) occupy analogous orientations. Both betulinic acid and ursolic acid orient themselves in the binding site to form a salt bridge between the carboxylates of the ligands and the ammonium of Lys 700. Fernane **19** and *seco*-3,4-friedelin (**3**) have very similar orientations, but no obvious interactions between the carboxylates and nearby amino acid residues. Fomitellic acid A (**6**), 3α-corosolic acid (**15**), and dihydrocelastrol (**18**), dock into the DNA binding site of topoisomerase II, but preferentially occupy different locations than the other triterpenoid ligands (see Fig. **4**). This alternative binding site is defined by Ala 830, Asn 756, Asn 828, Asp 697, Gln 703, Gln 739, Gln 750, Glu 831, Gly 698, Gly 747, Gly 829, Gly 832, Ile 758, Ile 825, Leu 748, Leu 760, Lys 700, Met 824, and Phe 699. The key interactions involved in docking dihydrocelastrol are a salt bridge between the carboxylate of the ligand and the ammonium moiety of Lys 700, hydrogen bonding between the C(2) hydroxyl group of the ligand and the carbonyl of Gly 829. Interestingly, *seco*-3,4-taraxerone (**4**) preferentially docks into an altogether different site (Fig. **4**) in the DNA binding region of topoisomerase II, in contrast to the other *seco*-3,4-triterpenoids, **1-3**. This binding site is defined by Ala 722, Ala 725, Ala 742, Ala 777, Ala 778, Ala 779, Ala 780, Arg 781, Gln 743, Glu 589, Glu 590, His 593, Ile 746, Pro 726, Ser 740, and Val 721. The key interaction in the docked pose is a salt bridge between the carboxylate of the ligand and His 593.

The triterpenoid ligands were docked into the ATP binding sites of both *Saccharomyces cerevisiae* topoisomerase II (two different structures, PDB: 1qzr and 1pvg [25]) and human topoisomerase II (PDB: 1zxm [26]) (see Fig. **5**). Most of the triterpenoid ligands showed lower binding (or no binding) affinity for the ATP binding sites. Four triterpenoids, however, *seco*-3,4-friedelin (**3**), demethylzeylasterone (**12**), celastrol (**17**), and dihydrocelastrol (**18**), showed stronger binding for the ATP binding sites than for the DNA binding site.

In the ATPase domain of yeast topoisomerase II, demethylzeylasterone (**12**) forms salt bridges between the C(23) carboxylate and the ammonium group of Lys 11 and the guanidinium group of Arg 77, as well as a hydrogen bond with Ser 128; hydrogen bonds between the C(29) carboxylate with the amide hydrogens of Arg 141, Gln 365, Gly

Table 1. Molegro Binding Energies of Best Docked Poses for Triterpenoids with Topoisomerase II

Compound	Binding Energy (kcal/mol)				
	DNA Binding Site		ATP Binding Site		
	1bjt	2rgr	1qzr	1pvg	1zxm
1	-24.1	-21.3	-2.4	-20.6	-16.7
2	-22.6	-21.4	-6.2	-20.0	-14.4
3	-22.2	-15.6	-25.2	-21.3	-23.2
4	-21.9	-17.0	-19.0	-14.0	-22.2
5	-22.8	-18.0	NB	-14.0	NB
6	-23.9	-22.5	-18.1	-20.0	-4.5
7	-25.0	-22.1	-15.5	-20.6	-5.4
8	-24.0	-16.5	-21.2	NB	-2.8
9	-19.4	-17.5	-19.5	-8.8	NB
10	-24.1	-19.3	NB	NB	NB
11	-20.3	-19.9	-11.0	-18.0	NB
12	-21.7	-19.3	-29.7	-29.6	-26.3
13	-18.6	-17.9	NB	NB	NB
14	-20.5	-18.6	NB	NB	NB
15	-21.2	-20.5	-22.4	-11.6	-5.5
16	-23.1	-17.0	-15.6	-14.9	-6.8
17	-21.3	-16.9	-23.3	-25.1	-25.3
18	-23.6	-19.3	-28.4	-27.9	-26.8
19	-26.7	-21.6	-15.5	-12.4	-11.0
20	-27.0	-24.0	-16.5	-21.7	-14.0
21	-24.1	-20.4	-10.7	-21.5	-12.2
22	-22.6	-21.8	-12.5	-22.7	-10.7
23	-22.9	-18.1	-23.9	-23.1	-24.2

NB = non-binding (*i.e.*, docking energy positive).

145, and Tyr 144; and a hydrogen bond between the C(6) ketone and the hydroxyl of Tyr 12. Both celastrol (**16**) and dihydrocelastrol (**17**) dock in the same orientation as demethylzeylasterone, with the same interactions between the C(29) carboxylate and Arg 141, Gln 365, Gly 145, and Tyr 144. Tingenone (**23**), known to be a cytotoxic agent [27], but not yet shown to be a topoisomerase II inhibitor, also docks into the same site with the same orientation (see Fig. **6**). Tingenone, therefore, would be expected to be a topoisomerase II inhibitor. Friedelane **3** does not have planar rings and therefore binds to the ATP binding site differently than the quinone-methide triterpenoids **12**, **17**, **18**, and **23**. Key hydrogen bonding interactions between *seco*-3,4-friedelin (**3**) and the protein are between the C(3) carboxylate of the ligand and the amide of Asn 129 as well as the hydroxyl of Ser 128.

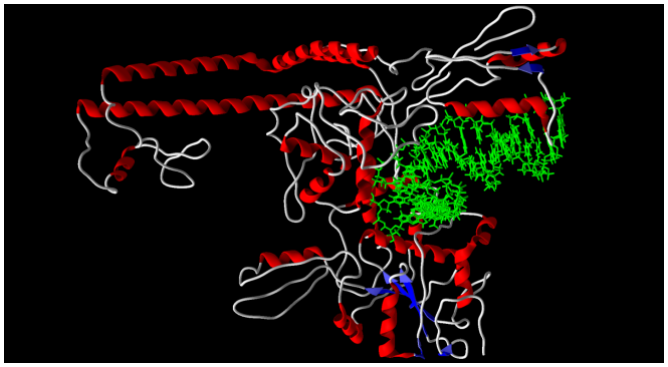

Fig. (1). X-ray crystal structure of human topoisomerase II bound to DNA (PDB: 2rgr) [23].

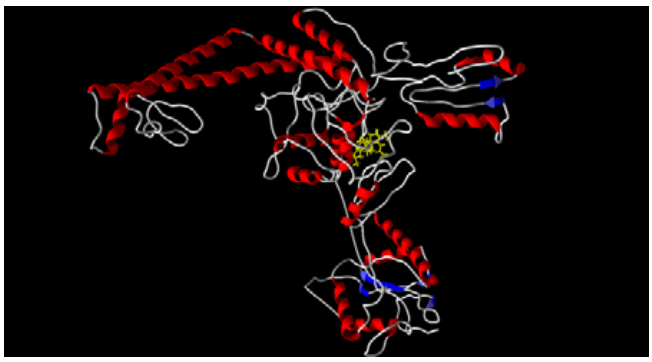

Fig. (2). X-ray crystal structure of *Saccharomyces cerevisiae* topoisomerase II (PDB: 1bjt) [22] with docked ligand, *seco*-3,4-friedelin (**3**) in its lowest-energy pose, occupying the DNA binding site.

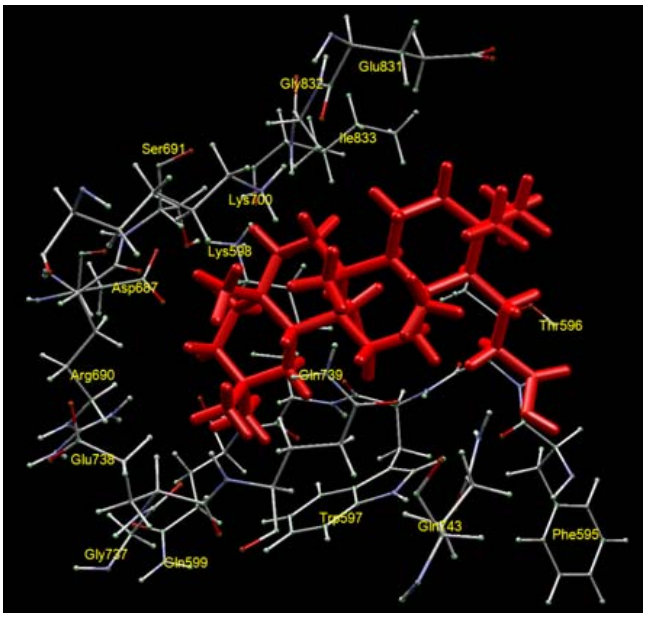

Fig. (3). Preferred triterpenoid binding site of yeast topoisomerase II with *seco*-3,4-friedelin as ligand.

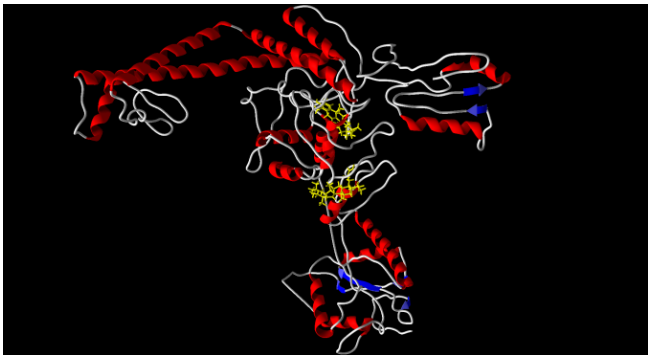

Fig. (4). Structure of yeast topoisomerase II (PDB: 1bjt) with docked ligands, fomitellic acid A, **6** (lower pose) and dihydrocelastrol, **18** (upper pose).

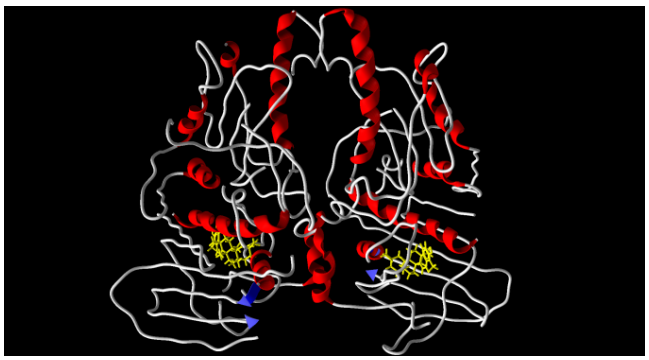

Fig. (5). X-ray crystal structure of *Saccharomyces cerevisiae* ATPase region of topoisomerase II (PDB: 1pvg) [25] with docked ligand, celastrol (**17**) occupying both ATP binding sites.

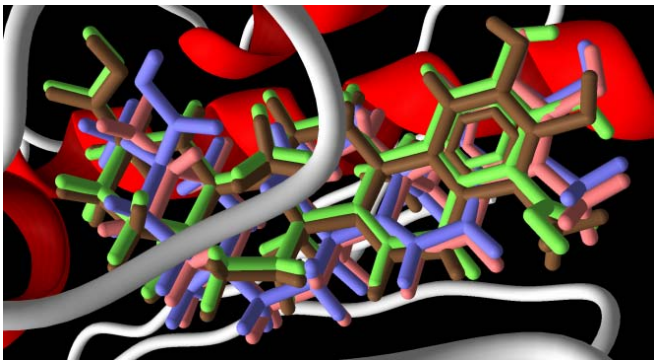

Fig. (6). Overlay of docked orientations of demethylzeylasterone (**12**, green), celastrol (**16**, blue), dihydrocelastrol (**17**, brown), and tingenone (**23**, pink) in the ATP binding site of yeast topoisomerase II.

The binding of the quinone-methide triterpenoids to the ATPase domain of human topoisomerase II is similar to that observed in the yeast. Demethylzeylasterone (**12**) interacts with Arg 98 and Ser 149, through the C(23) carboxylate. The C(29) carboxylate interacts with Arg 162, Gln 376, Gly 166, and Tyr 165; and the C(6) ketone hydrogen bonds with Tyr 34. Similarly, *seco*-3,4-friedelin (**3**) and *seco*-3,4-taraxerone (**4**) bind to the ATP binding site of human topoisomerase II through hydrogen bonding between the C(3) carboxylates of the ligands and Asn 150 and Ser 149. The binding energies of the quinone-methide triterpenoids to the ATP binding sites of topoisomerase II are comparable to those calculated (Molegro) for known ATP binders salvicine [28] (average binding energy = -25.2 kcal/mol) or etoposide [6, 7] (average binding energy = -22.4 kcal/mol).

There is no discernable trend between the calculated binding energies in this study and the reported topoisomerase II inhibitory activities. This may be due to the different sources of topoisomerase II used (*e.g.*, yeast, human, parasite), or the fact that enzyme inhibitory concentrations have large differences. Thus, for example, ursolic acid has been reported to have IC_{50} values of 20 μM [13], 36 μM [16], 150 μM [12]; etoposide had MIC values of 20 μM [15] and 25 μM [11]; and acetyl α-boswellic acid had MIC values of 3 μM [13] and > 50 μM [29].

COMPUTATIONAL METHODS

Molecular structures for the triterpenoids were built using SPARTAN '06 for Windows [30] and geometries optimized using the MMFF 94 force field [31]. Protein-ligand docking studies were carried out based on the crystal structure of the DNA-binding and cleavage core (residues 409-1201) of yeast topoisomerase II (PDB: 1bjt) [22], the crystal structure of the DNA-binding and cleavage domain (residues 419-1177) of human topoisomerase IIα bound to G-segment DNA (PDB: 2rgr) [23], the crystal structure of the *N*-terminal ATPase region of yeast topoisomerase II bound to dexrazoxane (PDB: 1qzr) and imino-ATP (PDB: 1pvg) [25], and the crystal structure of the ATPase region of human topoisomerase IIα bound to imino-ATP (PDB: 1zxm) [26]. All solvent molecules, cofactors, and co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds were undertaken using Molegro Virtual Docker 2.3 [32, 33]. Because it is unknown how and where triterpenoids might bind to topoisomerase II, many different sites were examined in order to probe the entire protein structure for 1bjt. For the 2rgr structure, the DNA was removed from the structure and the triterpenoid ligands were docked in the DNA binding site of the protein. In the case of the ATPase region, only the ATP binding pockets of 1qzr, 1pvg, and 1zxm, were modeled. A sphere of radius 15 Å was centered on the binding site in order to allow each ligand to search. Different orientations of the ligands were searched and ranked based on their energy scores.

SUMMARY

A number of triterpenoid natural products have shown potential antitumor activity by inhibition of topoisomerase II. These enzyme inhibitors are not planar molecules and clearly, then, do not intercalate DNA to form stable cleavable complexes with topoisomerase II. The mode of inhibition as revealed by this study may be either to bind to the enzyme at the DNA binding site, preventing DNA binding, or binding to the ATP binding site, conformationally locking the enzyme and thus preventing DNA binding. The calculated binding energies of the triterpenoid inhibitors, however, do not correlate well with experimental inhibitory concentrations.

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