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Insight into eukaryotic topoisomerase II-inhibiting fused heterocyclic compounds in human cancer cell lines by molecular docking[$£]

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Etoposide is effective as an anti-tumour drug by inhibiting eukaryotic DNA topoisomerase II via establishing a covalent complex with DNA. Unfortunately, its wide therapeutic application is often hindered by multidrug resistance (MDR), low water solubility and toxicity. In our previous study, new derivatives of benzoxazoles, benzimidazoles and related fused heterocyclic compounds, which exhibited significant eukaryotic DNA topoisomerase II inhibitory activity, were synthesized and exhibited better inhibitory activity compared with the drug etoposide itself. To expose the binding interactions between the eukaryotic topoisomerase II and the active heterocyclic compounds, docking studies were performed, using the software Discovery Studio 2.1, based on the crystal structure of the Topo IIA-bound G-segment DNA (PDB ID: 2RGR). The research was conducted on a selected set of 31 fused heterocyclic compounds with variation in structure and activity. The structural analyses indicate coordinate and hydrogen bonding interactions, van der Waals interactions and hydrophobic interactions between ligands and the protein, as Topo IIA-bound G-segment DNA are responsible for the preference of inhibition and potency. Collectively, the results demonstrate that the compounds **1a, 1c, 3b, 3c, 3e** and **4a** are significant anti-tumour drug candidates that should be further studied.

Keywords: topoisomerase II; benzoxazoles; benzimidazoles; benzothiazoles; molecular docking

1. Introduction

The importance of topoisomerases as possible drug targets started with the recognition of their critical role in cellular life [1]. DNA topoisomerases are a diverse set of essential enzymes responsible for maintaining chromosomes in an appropriate topological state. These enzymes are divided into two classes, type I and type II, depending on whether they cleave one or two strands of DNA during their catalytic cycle. DNA topoisomerases I (Topo I) and II (Topo II) are ubiquitous enzymes that manage the topology of DNA during DNA replication, transcription, recombination, and chromatin remodelling [2–7]. A wide variety of molecules interfering with eukaryotic Topo II activity have been recognized as potent anti-cancer drugs. The widely prescribed chemotherapeutic

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[$]Dedicated to the memory of Professor Corwin H. Hansch (1918–2011).
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agents epipodophyllotoxin, teniposide and etoposide [8] are currently used for the treatment of human cancers (lung, ovarian, brain, breast, adrenocortical, testicular cancers, Hodgkin and non-Hodgkin lymphomas) and target DNA Topo II [9,10]. These drugs increase Topo II-mediated DNA breakage primarily by inhibiting the ability of the enzyme to religate cleaved nucleic acid molecules [9]. However, their clinical efficacy is challenged by drug resistance, poor bioavailability problems and myelosuppresion, sometimes called bone marrow suppression, which is a common side effect of chemotherapy characterized by a decrease in the ability of the bone marrow to produce blood cells [11].

In addition, some molecules cause numerous breaks in DNA by trapping the cleavage complex, leading to disruption of stabilization of DNA and inducing apoptosis. These topoisomerase inhibitors are called topoisomerase poisons. The other type of inhibitor binds to the enzyme or DNA, impeding enzyme binding and interrupting the catalytic activity of the topoisomerase.

In recent years, it was found that bi- and ter-benzimidazole derivatives constitute a new class of DNA Topo I and II inhibitors [12–16].

A camptothecin derivative with a benzoxazole ring within its structure was found to be significantly more potent than camptothecin as an inhibitor of DNA Topo I [17]. Research on such compounds indicates that a fused ring system in the chemical structure is critical for the biological activity.

Shi et al. [18] observed that 2-(4-aminophenyl)benzothiazoles displayed potent and selective anti-tumour activity against breast, ovarian, colon, and renal cell lines; however, their mechanism of action had not been determined [18]. Based on this research, in 2006 Choi et al. [19] synthesized a series of 2-(4-aminophenyl)benzothiazole and evaluated the Topo II inhibitory activity. Most of the compounds showed moderate inhibition, and 2-(3-amino-4-methyl) phenyl-benzothiazole had the strongest inhibitory activity, comparable with the anti-tumour agent etoposide [19].

We investigated the inhibitory effects of some novel fused heterocyclic compounds such as benzimidazole, benzoxazole, benzothiazole, and oxazolo(4,5-b)pyridine derivatives on eukaryotic DNA Topo II in a cell-free system [20–22] and found that some of the tested compounds exhibited more potent inhibitory activities than the reference drug etoposide itself (Table 1, Figure 1) [13–16].

In the present study, we studied the molecular modelling of the possible structural motifs of the fused heterocyclic compounds given in Table 1 to expose their binding mode to eukaryotic DNA topoisomerase II by molecular docking studies, performed using the software Discovery Studio 2.1, based on the crystal structure of the Topo IIA-bound G-segment DNA (PDB ID: 2RGR). Our investigation may elucidate the interactions involved in the anti-tumour activities of fused heterocyclic compounds by using a molecular docking method and lead to the rational design of novel eukaryotic DNA topoisomerase II-targeted drugs.

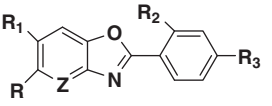
2. Materials and methods

2.1 *Biological data*

A set of 31 fused heterocyclic compounds tested for DNA Topo IIA inhibitory activity were chosen from our previous study, as shown in Table 1, for the molecular docking studies. The DNA Topo IIA inhibitory activities of these compounds are represented as IC_{50} values in the micromolar (μM) range.

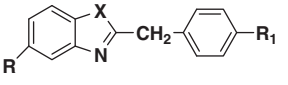
Table 1. Eukaryotic DNA topoisomerase II inhibitory activities of novel 2,5,6-substitued benzoxazole, benzimidazole and benzothiazole(4,5-b)pyridine derivatives. [The asterisk (*) refers to structures that are effective, according to reference drug, *etoposide*. The small letter ([a]) implies that eukaryotic DNA topoisomerase II 50% inhibitory activity of the tested compounds and the reference drug, *etoposide* as the micromolar (μM) concentration of IC_{50} values. NE: not effective].

Molecule 1



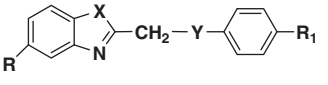
Compound	*R*	*R_1*	*R_2*	*R_3*	*Z*	*IC_{50} (μM)[a]*
1a*	H	NO_2	OCH_3	H	CH	17
1b	H	CH_3	F	H	CH	433.2
1c*	H	CH_3	NO_2	H	CH	18.8
1d	NH_2	H	H	C_2H_5	CH	115.5
1e	CH_3	H	CH_3	CH_3	CH	44.4
1f	Cl	H	H	C_2H_5	CH	NE
1g	CH_3	H	OCH_3	H	CH	433.0
1h	NO_2	H	H	H	CH	32.4
1i	Cl	H	H	Cl	CH	NE
1j	CH_3	H	H	$NHCH_3$	CH	128.4
1k*	NO_2	H	H	OC_2H_5	CH	22.4
1l	H	H	H	C_2H_5	N	45.6
1m	H	H	H	Cl	N	119.5
1n	H	H	H	$C(CH_3)_3$	N	108.3
1p	H	H	H	CH_3	N	91.2

Molecule 2



Compound	*R*	*R_1*	*X*	*IC_{50} (μM)[a]*
2a	NO_2	Br	O	NE
2b	H	OCH_3	O	86.6
2c	CH_3	NO_2	NH	NE
2d	CH_3	CH_3	NH	101.9
2e	CH_3	NH_2	NH	46.8

Molecule 3

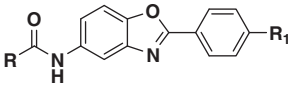


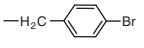
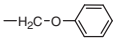
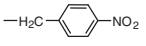
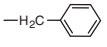
Compound	*R*	*R_1*	*X*	*Y*	*IC_{50} (μM)[a]*
3a	H	Cl	S	O	NE
3b*	CH_3	H	NH	S	27.4
3c*	$COOCH_3$	H	NH	S	17
3d	H	H	NH	CH_2	NE
3e*	NO_2	H	NH	O	24.8
3f*	H	H	S	O	11.4

(*continued*)

Table 1. Continued.

Molecule 4



Compound	*R*	*R_1*	*IC_{50} (μM)*[a]
4a*		H	24.1
4b		C_2H_5	315.1
4c		F	206.9
4d		H	420.1
4e		F	420.1
Etoposide			***21.8***

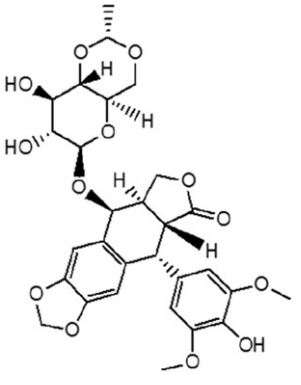


Figure 1. The chemical structure of the reference drug, etoposide.

3. Computational methods

3.1 *Molecular docking*

Rational approaches for finding new leads for therapeutic targets are increasingly based on 3-dimensional information about receptors. One can predict the binding conformation of a ligand in its receptor and the affinity between the ligand and the protein with the correct poses of ligands in the binding pocket of a protein. A process is described by which two molecules fit together in 3-D space.

In this section, we present a computational technique which involves docking studies of Topo II inhibitors using Discovery Studio 2.1 to provide an insight into the inhibitory activity of our previously reported fused heterocyclic compounds (Table 1), on eukaryotic DNA Topo II in cell-free systems. Molecular docking includes three steps, as shown schematically in Figure 2.

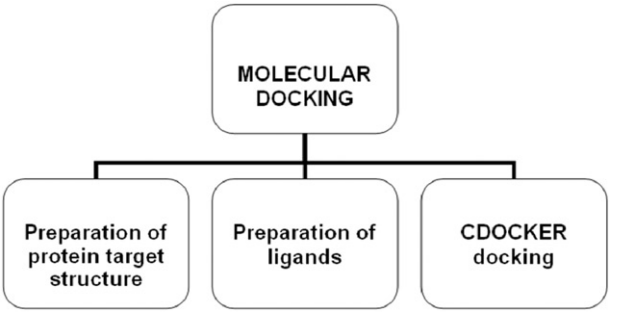


Figure 2. The steps of molecular docking.

3.1.1 *Preparation of protein target structure*

The starting coordinates of the human Topoisomerase IIA bound to G-segment DNA complex [PDB: 2RGR] were taken from the Protein Data Bank [23,24] and further modified for docking calculations. For CDOCKER (Discovery Studio 2.1) calculations, the Topo IIA complex was imported to Dock Ligands (CDOCKER) [25] in the Receptor–Ligand Interactions protocol (Discovery Studio 2.1); the protein was kept and selected, polar hydrogens were added, and CHARMm [26] forcefield was applied to minimize the protein using the Receptor–Ligand Interactions wizard (Discovery Studio 2.1). Binding sphere (-2.72, -25.50, -84.74, 20) was selected from the active site using the binding site tools. This provides a significant time saving at the cost of some accuracy.

3.1.2 *Preparation of ligands*

Different novel substituted benzoxazole, benzimidazole and benzothiazole derivatives (Table 1) were sketched and minimized in gas phase using the CHARMm force field to prepare an ensemble of starting structures of drug molecules with no atomic clashes in their geometries.

3.1.3 *CDOCKER docking*

The protein is held rigid while the ligands are allowed to be flexible during refinement. Dock Ligands (CDOCKER) was performed using the default settings. The docking parameters were as follows: Top Hits: 50; Random Conformations: 10; Random Conformations Dynamics Step: 1000; Grid Extension: 8.0; Random Dynamics Time Step: 0.002. Finally, all docked poses were scored by applying Analyze Ligand Poses subprotocol in Discovery Studio 2.1

4. Results and discussion

The mechanism of action for etoposide has been well described, involving the formation of a stable, covalent complex between Topo IIA and DNA [27]. Based upon this knowledge, the structure of the Topo IIA-bound G-segment DNA (PDB ID: 2RGR) was widely used in the design of topoisomerase inhibitors. Firstly, molecular docking studies were performed on the reference compound, etoposide, using the Topo IIA-bound G-segment

Table 2. Molecular docking results of the selected compounds, (**1a, 1c, 1k, 3b, 3c, 3e, 3f** and **4a**). High and low (in bold) inhibitory activity against DNA-Topo IIA.

Name	*Pose Num.*	*MW*	*A Log P*	*CDOCKER ENERGY*	*CDOCKER INT. ENERGY*	*RMSD to pose 1a 1*	*RMSD to pose 1c 1*	*RMSD to pose 1k 1*	*RMSD to pose 1n 1*
1a	1	270.240	3.158	−13.591	−28.068	0			
1c	4	254.241	3.661	−12.024	−25.457		0.416		
1k	**2**	**284.267**	**3.507**	**−18.487**	**−29.865**			**7.341**	
1k	**4**	**284.267**	**3.507**	**−17.764**	**−29.627**			**7.606**	
1k	**7**	**284.267**	**3.507**	**−17.360**	**−30.412**			**9.279**	
1k	**10**	**284.267**	**3.507**	**−16.912**	**−29.225**			**7.284**	

Name	*Pose Num.*	*MW*	*A Log P*	*CDOCKER ENERGY*	*CDOCKER INT. ENERGY*	*RMSD to pose 3b 1*	*RMSD to pose 3c 1*	*RMSD to pose 3e 1*	*RMSD to pose 3f 1*
3b	1	254.350	4.150	21.375	26.305	0			
3b	1	254.350	4.150	24.559	30.686	0			
3c	1	298.360	3.519	27.240	33.147		0		
3c	3	298.360	2.950	8.353	31.132		0.156		
3c	1	298.360	3.519	26.737	32.203		0		
3e	1	269.255	3.000	16.854	28.303			0	
3f	**8**	**241.308**	**3.674**	**8.857**	**22.306**				**6.998**

Name	*Pose Num.*	*MW*	*A Log P*	*CDOCKER ENERGY*	*CDOCKER INT. ENERGY*	*RMSD to pose 4a 1*	*RMSD to pose 4a 38*
4a	1	333.336	4.29	37.858	50.679	0	
4a	2	333.336	4.29	37.844	50.621	0.017	
4a	3	333.336	4.29	37.810	50.521	0.228	
4a	38	333.336	4.29	17.099	32.718		0

Name	*Pose Num.*	*MW*	*A Log P*	*CDOCKER ENERGY*	*CDOCKER INT. ENERGY*	*RMSD to pose Etoposide 1*
Etoposide	16	588.575	0.935	−6.450	−65.577	0.372

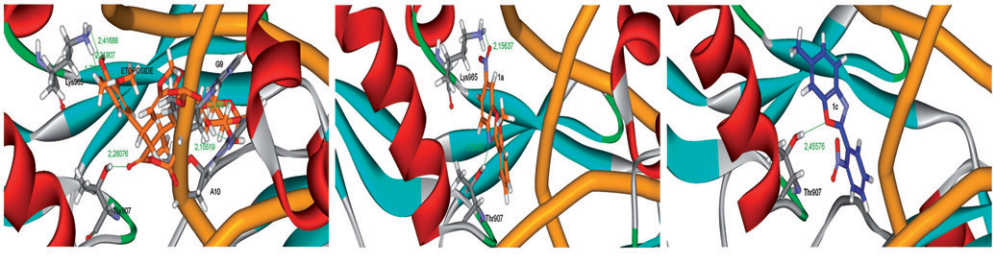


Figure 3

Figure 4

Figure 5

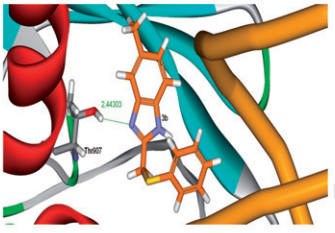


Figure 6

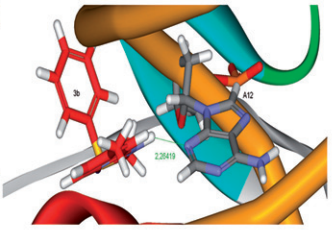


Figure 7

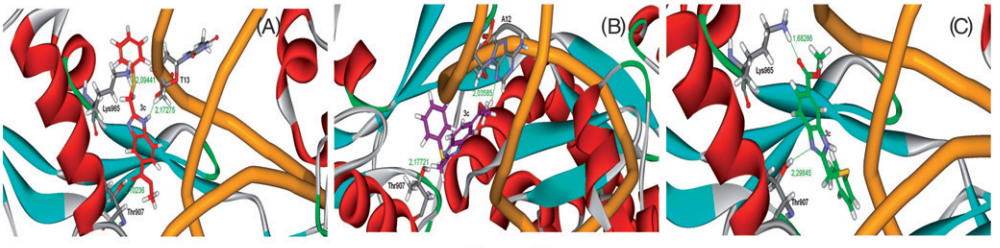


Figure 8

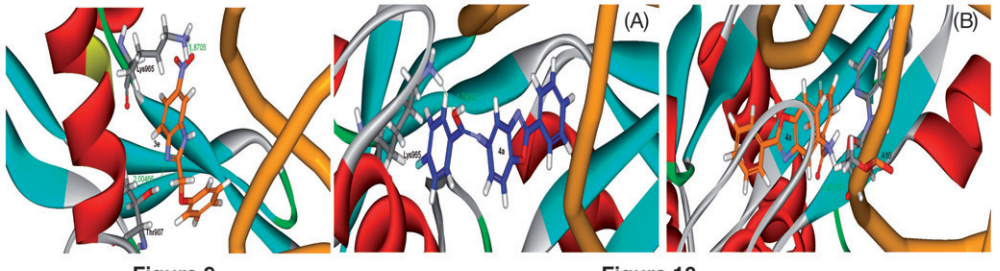


Figure 9

Figure 10

Figures 3–10. Plate.

Figure 3. DNA-Topo IIA CDOCKER pose along with the crystal structure for the reference material, etoposide (orange carbons), showing H-bonding of the O atom of cyclic ester carbonyl nucleus to Thr-907, (2.281); the O atoms of benzene to Lys-965,(2.417; 2.319; 1.795 from right to left, respectively) and O atom of hydroxy group in fused cyclic 6,6 nucleus to CG-9 (2.489); H atom of hdyroxy group in fused cyclic 6,6 nucleus to CA-10 (2.155).

Figure 4. Top scoring DNA-Topo IIA CDOCKER pose along with the crystal structure for compound 1a (orange carbons), showing H-bonding of the N atom of oxazole nucleus to Thr-907, (2.044 Å); the O atom of nitrobenzene to Lys-965,(2.156 Å).

Figure 5. Top scoring DNA-Topo IIA CDOCKER pose along with the crystal structure for 1c (dark blue carbons), showing the O atom of oxazole nucleus to Thr-907, (2.456 Å).

Figures 3–10. Captions continued.

Figure 6. The optimal scoring DNA-Topo IIA CDOCKER poses along with the crystal structure for compound 3b (index 51 pose 1, orange carbons), showing H-bonding of the N atom of imidazole nucleus to Thr-907, (2.443 Å).

Figure 7. The optimal scoring DNA-Topo IIA CDOCKER poses along with the crystal structure for compound 3b (index 101, pose 1, red carbons), showing H-bonding of the N atom of imidazole nucleus to DA-12, (2.264 Å).

Figure 8. A. The optimal scoring DNA-Topo IIA CDOCKER poses along with the crystal structure for compound 3c (index 151, pose 1, red carbons), showing H-bonding of the S atom of benzimidazole to Lys-965, (2.094 Å), the O atom of ester carbonyl to Thr-907, (2.102 Å) and the N atom of imidazole nucleus to DT-13, (2.173 Å). B. The optimal scoring DNA-Topo IIA CDOCKER poses along with the crystal structure for compound 3c (index 203, pose 3, purple carbons), showing H-bonding of the CH_2 carbon atom adjacent to S atom of benzimidazole to Thr-907, (2.177 Å); the O atom of ester carbonyl to DA-12, (2.036 Å). C. The optimal scoring DNA-Topo IIA CDOCKER poses along with the crystal structure for compound 3c (index 251, pose 1, green carbons), showing H-bonding of the N atom of imidazole nucleus to Thr-907, (2.298 Å); the O atom of ester carbonyl to Lys-965, (1.683 Å).

Figure 9. Top scoring DNA-Topo IIA CDOCKER pose along with the crystal structure for compound 3e (orange carbons), showing H-bonding of the N atom of imidazole nucleus to Thr-907, (2.005 Å), the O atom of nitrobenzene to Lys-965,(1.871 Å).

Figure 10. A. The optimal scoring DNA-Topo IIA CDOCKER pose along with the crystal structure for compound 4a (pose 38, dark blue carbons), showing H-bonding of the O atom of amide group to Lys-965 (1.796 Å). B. The optimal scoring DNA-Topo IIA CDOCKER pose along with the crystal structure for compound 4a (poses 1-3, orange carbons), showing H-bonding of the N atom of amide group to CA-10, (2.454 Å).

DNA complex (Figure 3). This Figure also exhibits H bonding of etoposide with the structure of the Topo IIA-bound G-segment DNA. Then, selected materials given in Table 1 were docked using CHARMm-based CDOCKER to predict their Topo II inhibitory ability based on the reference material, etoposide.

At the end of the docking process, the best ligand pose was selected from among all obtained poses of each ligand based on the CDOCKER top score. In addition, the Analyze Ligand Poses subprotocol was performed to count H bonds and close contacts (van der Waals clashes) between the poses and Topo IIA bound to G-segment DNA molecule.

As is well known, H bonds play an important role in maintaining the structure and function of biological molecules, especially in enzyme catalysis. In the present study, the molecular docking results show that **1a, 1c, 1k, 3b, 3c, 3e, 3f** and **4a** formed H bonds with amino acid residues of Topo II as well as the residues of the DNA template. The obtained H bonding results are also compatible with the experimental data given as in Table 1.

Beside the H bonding, other parameters which affect the interactions between ligand(s) and the protein were also taken into consideration in order to determine the most suitable dockings. During the docking process, we specified number of top poses, based on the largest minus CDOCKER ENERGY and the lowest minus CDOCKER INTERACTION ENERGY. In addition, root mean square deviation (RMSD) values of each pose were calculated.

H bond, CDOCKER ENERGY, CDOCKER INTERACTION ENERGY and RMSD values are given in detail at Table 2 to determine the most potent eukaryotic Topo II inhibitors (**1a, 1c, 1k, 3b, 3c, 3e, 3f** and **4a**). These parameters are important to establish logical and optimal interactions between ligand(s) and the protein.

In order to gain insight into the interaction between the ligand and protein, the selected compounds (**1a, 1c, 3b, 3c, 3e** and **4a**) in complex with the protein were visualized using Discovery Studio 2.1. It is evident that these compounds form H-bonding interactions with the Thr-907 and Lys-965 amino acid residues of Topo IIA, as well as with the CA-10, DA-12 and DT-13 residues of the DNA template (Figures 3–10).

The common result of Figures 3–10 show that the potent fused heterocyclic Topo II inhibitors **1a, 1c, 3b, 3c, 3e** and **4a** are located in the centre of the active site of the human Topo IIA bound to G-segment DNA complex. They are also stabilized by H bonding interactions, especially at Thr-907 and Lys-965 of the topoisomerase site, just as etoposide is. The other noteworthy finding is that the compounds, except **1a**, bind to DNA at the same time as etoposide.

During our work, we also tried the dockings for **1k** and **3f** which have interactions with the protein, but the RMSD values were higher than 2.00 which is outside the error range for interaction between ligand and target [28]. Consequently, these compounds are not further considered.

5. Conclusion

The results of this study indicate that **1a, 1c, 3b, 3c, 3e** and **4a** exhibit significant Topo II inhibitory activity, like etoposide, on the basis of molecular docking results. These results include H bond, CDOCKER ENERGY, CDOCKER INTERACTION ENERGY, and RMSD values for each pose of the studied compounds, given in Table 2. Other compounds showed no activity at the binding site of Topo IIA bound to G-segment DNA complex due to steric constraints of their structures. In summary, we conclude that the size of the fused bicyclic heterocyclic system with a benzene ring condensed with a 5-membered heterocyclic ring is essential for optimal binding with Topo IIA protein bound to G-segment DNA complex as eukaryotic Topo II inhibitors. In addition, we deduced that the substitution of position 2 at the fused heterocyclic system and the *ortho* and *para* positions of the benzyl moiety at the 2nd position of the fused ring system with electron-withdrawing groups is preferable for better binding with the target.

Many anti-tumour Topo II inhibitors act as a result of interactions with both the enzyme and DNA. Hypericin is an example which interacts with DNA at the N7 sites of purine residues [29,30]. Our investigation indicates that compounds **1a, 1c, 3b, 3c, 3e** and **4a** possess apoptotic activity by binding to both Topo II enzyme and the DNA. In addition, this information provides a useful insight for the development of novel anti-cancer agents with specific selectivity.

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