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Evaluation of inhibitory effects of benzothiazole and 3-amino-benzothiazolium derivatives on DNA topoisomerase II by molecular modeling studies

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There has been considerable interest in DNA topoisomerases over the last decade, as they have been shown to be one of the major cellular targets in anticancer drug development. Previously we synthesized some benzothiazole derivatives and corresponding benzothiazolium forms, and tested their DNA inhibitory activity to develop novel antitumor agents. Among the 12 prepared compounds, compound BM3 (3-aminobenzothiazole-3-ium 4-methylbenzene sulfonate) exhibited extreme topoisomerase II inhibitory activity compared with the reference drug etoposide. We also tried to determine the DNA and enzyme binding abilities of BM3 and found that BM3 acted on topoisomerase II first at low doses, while it had also showed DNA minor groove binding properties at higher doses. In this study the interactions between DNA topoisomerase II and the compounds were examined in detail by molecular modelling studies such as molecular docking and pharmacophore analysis performed using Discovery Studio 3.5. As a result, it was found that benzothiazolium compounds exhibited a totally different mechanism than benzothiazoles by binding to the different amino acids at the active site of the protein molecule. 3-Aminobenzothiazoliums are worthy of carrying onto anticancer studies; BM3 especially would be a good anticancer candidate for preclinical studies.

Keywords: anticancer; benzothiazole; benzothiazolium; molecular docking; pharmacophore analysis; topoisomerase II

1. Introduction

There are many benefits for the stable storage of genetic information in the double-helical structure of DNA. It is protected from chemical damage by sequestering information-rich nucleotides, while complementarity among the sister strands allows mistakes, lesions and discontinuities in the genome to be addressed and repaired. However, the intertwined structure of duplex DNA is not without drawbacks. Because the two strands of deoxyribonucleic acid are wound around one another, processes that require strand separation, such as replication or transcription, lead naturally to DNA overwinding and strand entanglement which can pose serious challenges to the cell [1–3]. They interfere with gene expression, DNA duplication and chromosome segregation, and cells depend on DNA supercoiling as a means of compacting

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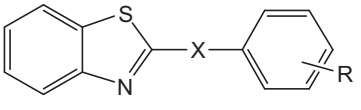
the genome and promoting DNA unwinding at replication origins and promoter regions at the same time [2,3]. Thus, the cell strives to preserve the information content of the genome and invests considerable energy in maintaining the topology of its chromosomes in an appropriate topological state.

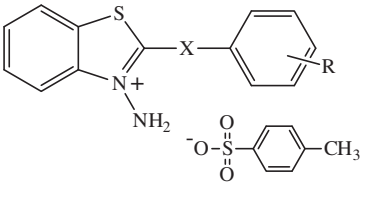
DNA topoisomerases are the ubiquitous enzymes in key cellular processes such as replication, transcription, recombination and repair, and chromatin assembly by solving these topological problems of genomic DNA [4–10]. DNA topoisomerases are divided into two classes depending on the number of broken strands (type I cuts one strand, type II cuts both strands) of DNA by the enzymes in one reaction circle. In recent years, topoisomerases have become popular targets for cancer chemotherapy treatments. It is thought that topoisomerase inhibitors block the ligation step of the cell cycle, generating single- and double-stranded breaks that harm the integrity of the genome. Introduction of these breaks subsequently leads to apoptosis and cell death. Lack of topoisomerase in the cell is fatal. In light of this fact, DNA topoisomerases have been targeted for killing cancer cells or pathogenic bacteria in the clinic. The purpose of this study is to identify a new type of anticancer drug candidates.

Indeed, there are commercially available anticancer drugs that possess DNA topoisomerase inhibitory activity such as doxorubicin, etoposide and camptothecin [11–14]. Our group has been working for many years on the synthesis of some new heterocyclic compounds that were potentially DNA topoisomerase I and II inhibitors, and molecular modelling studies of these compounds [15–21]. So far some of the compounds exhibited significant topoisomerase II inhibitory activity with IC_{50} values differing from 11.4 and 46.8 μM compared with the standard drug etoposide [15,18]. Recently, we also reported results obtained through a DNA topoisomerase relaxation assay indicated 2-substituted benzyl or phenyl benzothiazole derivatives and corresponding 3-aminobenzothiazole-3-ium 4-methylbenzene sulfonates were strong topoisomerase II inhibitors (Table 1).

In this research, the DNA topoisomerase II inhibitory activity of 3-amino-2-(2-bromobenzyl)-1,3-benzothiazole-3-ium 4-methylbenzene sulfonate (BM3) was the most effective DNA topoisomerase II inhibitor with the lowest IC_{50} value of 39 nM, which could be considered

Table 1. IC_{50} values of benzothiazole and 3-amino-benzothiazolium derivatives for catalytic inhibitory activity on topoisomerase II.





Compound	*X*	*R*	*Topo II IC_{50}*	*Compound*	*X*	*R*	*Topo II IC_{50}*
M2	CH_2	2-F	1.06 mM	BM6	CH_2	2-F	1.78 mM
M6	CH_2	4-CH_3	72.93 μM	BM1	CH_2	4-CH_3	6.87 mM
M7	CH_2	4-Cl	107.10 μM	BM7	CH_2	4-Cl	6.20 mM
M9	CH_2	4-Br	8.10 μM	BM2	CH_2	4-Br	8.46 mM
M10	CH_2	2-Br	788.00 μM	BM3	CH_2	2-Br	**39.40 nM**[†]
M15	–	2-Br	6.68 mM	BM4	–	2-Br	1.26 mM

[†]Most effective compound.

as an extremely strong activity compared with etoposide. According to the mechanistic studies, BM3 was neither a DNA intercalator nor a topoisomerase poison; it was only a DNA minor groove-binding agent [21]. BM3 initially bound to the DNA topoisomerase II enzyme and its mechanisms of action might be directly interaction with the enzyme. It is reported that there is a clear evidence for a covalent 5′ link between DNA and the active site tyrosine (Tyr782) resident in topoisomerase II's winged helix domain (WHD). Topoisomerase II undergoes substantial conformational changes on binding substrate. Structures of apo *Saccharomyces cerevisiae* topoisomerase II have shown that the two dimer-related WHDs and their resident active site tyrosines are separated by 25–30 Å (Figure 1A). In the presence of DNA, however, these elements move towards each other, forming a nearly contiguous interaction surface across both protomers [22,23].

Hence, BM3 could be a good candidate as a new anticancer agent. We attempted to support the experimental results with theoretical findings by working with molecular modeling techniques such as docking and pharmacophore generation using Accelrys Discovery Studio (DS) 3.5 software [24]. These may direct us to find novel and more potent topoisomerase II inhibitors.

In the drug design area, quantitative structure–activity relationship (QSAR) method is an area of research pioneered by Hansch and Leo [25] and Hansch and Fujita [26]. The QSAR method assumes that differences in the measured structural or physical properties account experimentally for differences in the observed biological or chemical properties [26–28]. A QSAR study usually leads to a predictive formula and attempts to model the activity of a series of compounds using measured or computed properties of the compounds.

More recently, QSAR has been extended by including three-dimensional (3D) information. In drug discovery, it is common to have measured activity data for a set of compounds acting upon a particular protein, but not to have knowledge of the 3D structure of the active site. In the absence of such 3D information, one may attempt to build a hypothetical model of the active site that can provide insight on the nature of the latter. Three-dimensional approaches such as HypoGen and/or HipHop are useful in building 3D pharmacophore models from the activity data and conformational structure [29]. They can be used as an alternative for QSAR methods because of their easy visualization and high prediction capability. However, ranging activity values of a collection of conformational models of compounds should be at least four orders of magnitude for the algorithm, HypoGen. The other algorithm in 3D pharmacophore generation within Catalyst is called HipHop and is based on alignment of common features present in highly potent compounds. The scalar affinity values of the molecules are not regarded in this model generation mode.

HipHop pharmacophore models are derived by comparing a set of conformational models and a number of 3D configurations of chemical features shared among the training set molecules.

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding model (s) of a ligand with a protein of known 3D structure. In this study, we used fast flexible docking to study the binding orientations and predict binding affinities of benzothiazole and benzothiazolium derivatives. Such studies have been carried out to understand the forms of interaction of 12 compounds, examined by Kaplan-Ozen and colleagues for human DNA topoisomerases [21]. The results obtained from this study will be useful in understanding the inhibitory mode of benzothiazole and benzothiazolium derivatives, as well as in rapidly and accurately predicting the activities of newly designed inhibitors on the basis of docking scores. These models also provide some beneficial clues in structural modification for the

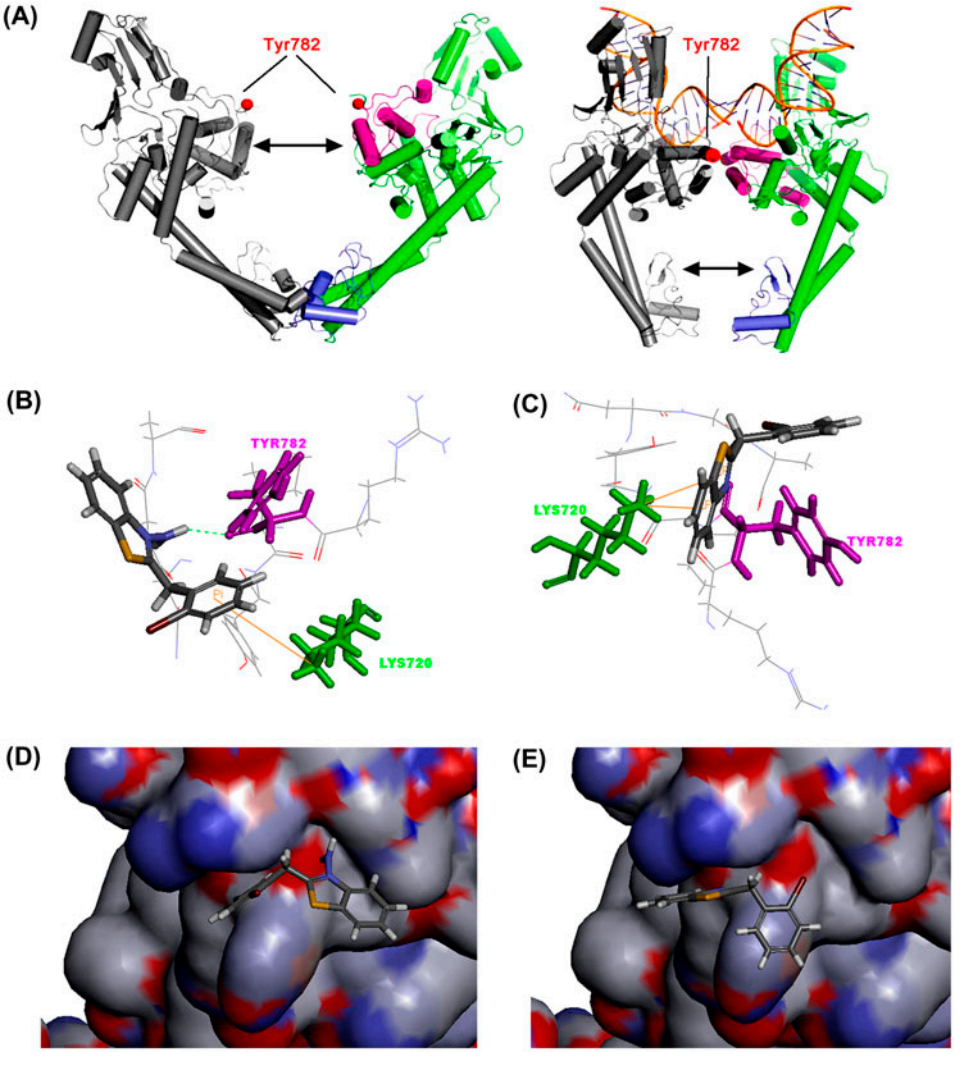


Figure 1. (A) Conformational changes of topoisomerase II, after DNA binding [22]. (B) Docked position of BM3: NH_2 group at position 3 of benzothiazolium has H bond with Tyr782 (pink) and phenyl ring on second position of benzothiazole ring has π–cation interactions with Lys720 (green). (C) Docked position of M10: benzothiazole ring has π–cation interactions with Lys720 (green). (D) Docking overlay of BM3 in atom charged surface area. (E) Docking overlay of M10 in atom charged surface area.

design of new inhibitors for the treatment of cancer with much higher inhibitory activities against DNA topoisomerase II.

2. Materials and methods

2.1 *Molecular structures and optimization*

A set of previously synthesized benzothiazole and 3-amino-benzothiazolium derivatives [16] tested for DNA topoisomerase II inhibitory activity were chosen from our previous study [21]

as shown in Table 1. The DNA topoisomerase II inhibitory activities of these compounds are represented as IC_{50} values in the millimolar, micromolar or nanomolar range [21]. The structures of all the compounds were constructed using the DS 3.5 Sketch Molecules module (www.accelrys.com). The geometries of these compounds were subsequently optimized using the Minimization module of DS 3.5 using the CHARMm (Chemistry at HARvard Macromolecular Mechanics) force field. CHARMm provides a vast range of functionality for molecular mechanics and can be used to diverse areas of research, including protein modeling and structural biology [30].

3. Computational methods

3.1 *Molecular docking*

The most straightforward computational approaches for finding new leads for therapeutic macromolecular targets are increasingly based on 3D information about proteins. Molecular docking is an effective method to predict ligands, which are low molecular weight compounds that may interact with a macromolecular target. A primary objective in molecular docking is the ability to estimate the scoring function and evaluate protein–ligand interactions as a means of hit identification (virtual screening) and lead optimization (to enhance desired drug properties). This method is also successfully used as a computational tool to assist drug discovery.

3.1.1 *Preparation of the enzyme*

The crystal structure of *Saccharomyces cerevisiae* topoisomerase II (PDB ID: 1BJT) was retrieved from the Protein Data Bank (PDB) (www.rcsb.org) [31] and further modified for docking calculations. For preparation of protein and ligands, DS 3.5 software was used. The target protein was taken, hydrogens were added and their positions were optimized using the all-atom CHARMm force field and the Adopted Basis set Newton Raphson (ABNR) method available in the D.S 3.5 protocol until the root mean square deviation (RMSD) gradient was <0.05 kcal/mol $Å^2$. The minimized protein was defined as the receptor using the binding site module. The binding site was defined from the cavity finding method, which was modified to accommodate all the important interacting residues in the DNA binding site of topoisomerase II enzyme. The binding sphere for 1BJT (6.12, 47.51, 26.54, 14.67) was selected from the active site using the binding site tools.

3.1.2 *Preparation of ligands*

Previously synthesized benzothiazole and 3-amino-benzothiazolium derivatives were sketched; all-atom CHARMm force field parameterization was assigned and then minimized using the ABNR method as described above. Conformational search of the ligands was carried out using a simulated annealing molecular dynamics (MD) approach. The ligands were heated to a temperature of 700 K and then annealed to 200 K.

3.1.3 *Docking*

3.1.3.1 *Core-constrained docking (CCD) subprotocol.* The CCD subprotocol includes conformer generation, core-constrained docking and scoring. In terms of core-constrained

docking, use of the molecular docking algorithm CDOCKER is considered since it has been shown to be a viable research tool [32,33]. CDOCKER is a CHARMm-based grid-enabled docking method that uses soft core potentials and MD-generated random ligand conformations, and poses refinement in the active site using a simulated annealing process. In the original work, CDOCKER treats the entire ligand as flexible during the initial docking phase. The core-constrained docking method described here is a modification to the CDOCKER CHARMm script that allows the scaffold to be locked but the rest of the ligand molecule to be flexible during initial minimization and the MD conformer generation stage. The core is allowed to move during final refinement with simulated annealing. Current docking tools do a reasonable job at getting correct poses, but errors occur in a significant number of cases [34,35]. Constraining the core to the crystal structure coordinates helps prevent incorrect docking poses.

A grid is defined using the 'Grid Extension' parameter visible at the top level of the subprotocol and has been set to a default 8 Å distance from the ligand's center of mass. Details of the modified CDOCKER process begin with creation of the CHARMm set-up files and generation of the CDOCKER protein grid. Ligand partial atomic charges and atom types default to those of Momany–Rone force field [36] as implemented in CHARMm. The typed ligand is first run through an ABNR minimization stage [30]. Ligand conformations are then generated, in the absence of protein, through high-temperature MD simulations and a specified number (top level parameter, default = 20) of simulations are applied. The starting ligand conformation for each MD simulation is that of its predecessor. Conformations resulting from each MD simulation are then docked into the protein and minimized using steepest-descent (SD), preparing them for final refinement. It is only during this initial docking and conformer refinement phase where an energy grid for the ligand is imposed and nonbonded interactions involving van der Waals and electrostatic potentials are softened, enabling enhanced sampling of conformational space. The core is held fixed throughout the conformer generation and docking phase. With the core now unconstrained, the docked poses are then further refined in the receptor active site using a simulated annealing protocol and a full MD minimization (SD + conjugate-gradient). During this process the protein is held rigid. A user-specified number of top poses (top level parameter), based on the largest minus CDOCKER scores, are saved for the final rescoring step. Many of the advanced CHARMm parameters have been optimized and do not require changing from their default values.

3.1.3.2 *Calculated binding energy (CBE) subprotocol.* The CDOCKER docked ligands are rescored using a physics-based implicit solvation model as the final step. Within the CBE subprotocol step, the docked ligand poses are rank scored in terms of their binding energies. For this study, top CDOCKER poses of neutral and/or charged ligands were rescored using Molecular Mechanics-Generalized Born with Molecular Volume (MM-GBMV) and/or Molecular Mechanics-Generalized Born with Simple Switching (MM-GBSW) methods in DS CHARMm, which approximates the binding energy [37–39]. Bound and unbound ligand receptor energy terms contained within the calculated binding energy include three simulations: free ligand; apo protein; and protein–ligand complex. Solute entropy contributions are ignored in these calculations. Standard output includes the binding energy terms for the three simulations and the net calculated binding energy as shown in Equation 1:

$$\Delta\Delta G_{Bind} = \Delta G_{Copmlex} - \Delta G_{Ligand} - G_{Protein} \quad (1)$$

In present study, the CDOCKER [33] method was performed using DS 3.5. All docked poses were scored by applying the Analyze Ligand Poses subprotocol to analyze

receptor–ligand interactions or a set of poses (the results of a docking run) using a variety of methods. Binding energies were also calculated by applying the Calculated Binding Energy subprotocol in DS 3.5 using the *in situ* ligand minimization step in the ABNR method. The lowest binding energy was taken as the best-docked conformation of the compound for the macromolecule. The docking results were given in Table 2 and Figure 1B–1E.

3.2 *Pharmacophore modelling*

According to the IUPAC definition, a pharmacophore model is 'an ensemble of steric and electronic features that is necessary to ensure the optimal intermolecular interactions with a specific biological target and to trigger (or block) its biological response'. In DS software, a pharmacophore is described as the essential features or chemical substructures and their corresponding 3D locations that are responsible for the similar biological activities of a set of compounds. Typically, pharmacophore features include hydrophobic, aromatic, hydrogen bond acceptor, hydrogen bond donor, positive ionizable and negative ionizable.

3.2.1 *Create pharmacophore automatically tools*

The Create Pharmacophore Automatically tools allow you to automatically build pharmacophore models from a ligand, a receptor or a receptor–ligand complex. The Auto Pharmacophore Generation protocol is used based on a bioactive conformation. This protocol includes

Table 2. Molecular docking results of benzothiazoles and 3-amino-benzothiazolium derivatives with DNA topoisomerase II.

Compound	*Calculated binding energy (kcal/mol)*	*Interacted residues (contact radius in 4 Å)**
M2	−37.996	**Lys720** (2,37 Å)[b], Arg781[a], Tyr782[e], Tyr784[f]
M6	−13.110	Lys720[b], Gly765[e], Ala766[e], Arg781[e], Tyr782[c], Ile783[e], Tyr784[e]
M7	+27. 347	Lys720[b], Gly765[e], Ala766[e], Ala778[e], Arg781[e], Tyr 782[c], Ile783[e], Tyr784[e]
M9	−43.121	Lys720[b], Arg781[e], Tyr 782[a], Tyr784[f]
M10	−25.198	Lys720[b(2)], Arg781[f], Tyr 782[f], Tyr784[f]
M15	−24.174	Lys720[b,d], Arg781[f], Tyr 782[e], Ile783[e], Tyr784[f]
BM6	+4.999	**Lys720**(2) (1,89-2,35 Å), Arg781[f], Tyr782[f], Tyr784[f]
BM1	−8.390	Lys720[b], Arg781[e], **Tyr782** (2,06 Å), Tyr784[e]
BM7	−11.338	Lys720[b], Asn764[e], Gly765[e], Ala766[e], Arg781[e], Tyr782[f], Ile783[e], Tyr784[f]
BM2	+77.486	Lys720[f], Arg781[f], Tyr782[a], Ile783[f], **Tyr784**[d] (2,31 Å)
BM3	−26.257	Lys720[b], Asn764[f], Gly765[f], Ala766[f], Arg781[e], **Tyr782** (2,09 Å), Ile783[e], Tyr784[e]
BM4	−6.631	Lys720[f], Arg781[e], Tyr 782[e], **Tyr784** (1,96 Å),

*Bold: H-bonds.
[a]π–π interactions.
[b]π–cation interactions.
[c]π–sigma interactions.
[d]bumps (steric clash).
[e]Van der Waals interactions.
[f]Electrostatic interactions.

all pharmacophore features, scores all possible pharmacophore combinations, and returns the top pharmacophores. The score is based on a predictive model that uses simple descriptors derived from the type and relative location of the pharmacophore features to predict the number of hits from a diverse database.

The most widely used geometry- and feature-based pharmacophore elucidation method is Catalyst from Accelrys, which is currently part of the DS package. Catalyst is an integrated set of algorithms for conformation generation (ConFirm), molecular superimposition (HipHop), pharmacophore generation (HypoGen) and database searching (Info). HipHop and HypoGen provide two approaches for automatic pharmacophore generation.

HipHop provides feature-based alignment of a collection of compounds without considering the activity. It matches the chemical features of a molecule against drug candidate molecules. HipHop takes a collection of conformational models of molecules and a selection of chemical features, and produces a series of molecular alignments in a variety of standard file formats. HipHop begins by identifying configurations of features common to a set of molecules. A configuration consists of a set of relative locations in 3D space and associated feature types. A molecule matches the configurations if it possesses conformations and structural features that can be superimposed within a certain tolerance from the corresponding ideal locations. HipHop also maps partial features of molecules in the alignment set. This provision gives the option to use partial mapping during the alignment. Partial mapping allows us to identify larger, more diverse, more significant hypotheses and alignment models without the risk of missing compounds that do not have to map to all of the pharmacophore features.

In this study, pharmacophore analysis of the docking pose of BM3 was done by using Auto Pharmacophore Generation method in DS 3.5. The Auto Pharmacophore Generation protocol considers the following feature types to generate a selective pharmacophore model

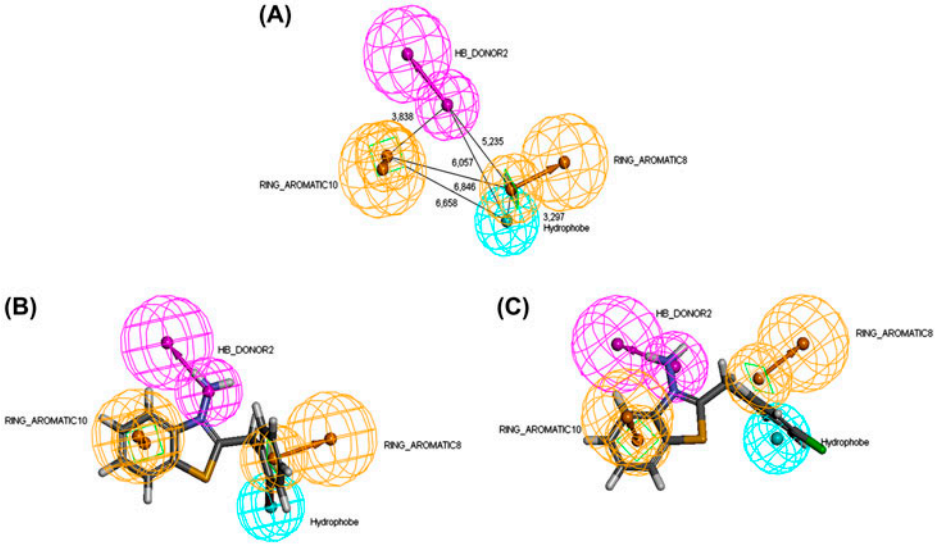


Figure 2. (A) Pharmacophore model of the docking pose of BM3. The model contains four features: one hydrophobic (cyan), two ring aromatic (orange) and one hydrogen bond donor (pink). (B) The mapping of BM3 (fit value 3.96), to hypo10. (C) The mapping of BM7 (fit value 2.51), to hypo10.

Table 3. Alignment of common-feature pharmacophore model with test set.

Compound	*Fit value*
BM3	3.96037
BM4	2.76215
BM6	2.57326
BM1	2.55159
BM2	2.51312
BM7	2.50526
M10	No map found
M15	No map found
M2	No map found
M6	No map found
M7	No map found
M9	No map found

from a bioactive conformation of a single ligand (BM3): hydrogen bond acceptor; hydrogen bond donor; hydrophobic feature; negative ionizable feature; positive ionizable feature; and aromatic ring feature. Ten pharmacophore hypotheses were performed by using Auto Pharmacophore Generation. Hypothesis 10 (Hypo10) was chosen as the best hypothesis and tested to the others. The distances of the best hypothesis were shown in Figure 2A. The fit values of all the compounds were indicated in Table 3.

4. Results and discussion

In this study, we used benzothiazole derivatives and their corresponding benzothiazolium forms that had been previously synthesized and shown inhibitory activities against DNA topoisomerase II to interpret the interaction of the 12 molecules with DNA topoisomerase II enzyme by theoretically comparing them with the experimental data.

In the earlier study, 3-amino-2-(2-bromobenzyl)-1,3-benzothiazole-3-ium 4-methylbenzene sulfonate (BM3) was not only the most potent inhibitor, it was also extremely active compared with the standard drug, etoposide, among the tested benzothiazoles and benzothiazoliums [21]. In the present study, we showed the different activity mechanisms of benzothiazoliums and benzothiazole derivatives by using Molecular Docking and Pharmacophore identification protocols of DS 3.5.

It could be stated that BM3 is a catalytic inhibitor of topoisomerase II, and neither a poison nor an intercalator. Moreover, that BM3 plays a preventive role in the DNA binding ability of topoisomerase II. This could be explained by BM3 directly interacts with the topoisomerase II enzyme without interacting with DNA.

It was previously reported that there was a clear evidence for a covalent 5′ link between DNA and the active site tyrosine (Tyr782) resident in topoisomerase II's winged helix domain (WHD) [22,23]. The docking studies showed that BM3 bound to Tyr782 at the active site to prevent topoisomerase II enzyme binding to its substrate DNA. This was one of the reasons why we selected the DNA topoisomerase II enzyme of *S. cerevisiae*, which was not bound to DNA at its present form as its PDB code (1BJT) for the molecular docking studies. Benzothiazole and benzothiazolium derivatives were docked into the topoisomerase II enzyme (PDB ID: 1BJT) using the CDOCKER method of DS 3.5. All the docking results are shown in Table 2.

Additionally, pharmacophore analysis of the docking pose of BM3 was performed using the Auto Pharmacophore Generation method in DS 3.5. Hypothesis 10 was chosen as the best hypothesis (Figure 2) and the rest of the compounds were used as a test set and mapped with Hypo 10 using the rigid method in DS 3.5. All the fitted values were shown in Table 3.

As a result of the molecular modeling studies, the NH_2 group attached to the third position of the 3-amino-benzothiazolium ring in BM3, which is the most active compound, makes a hydrogen bond with Tyr782 (binding energy is -26.257 kcal/mol). It also fitted to the hydrogen bond donor feature of Hypo10. The phenyl ring on the second position of the benzothiazole ring has π–cation interactions with Lys720 and fitted to the ring aromatic feature of Hypo10 (Figure 1B, Figure 2B). In this way, BM3 binds to the active site tyrosine (Tyr782) and shows inhibitory activity by blocking the active site of the enzyme, which seems consistent with the experimental data found [21]. When we look at the compound M10, which is the base form of BM3, the benzothiazole ring of this compound has only π–cation interactions with Lys720 (Figure 1C) and it does not bind to Tyr782. Binding to Tyr782 is very important such that BM3 prevents the binding of DNA, which binds via Tyr782, to DNA topoisomerase II to continue its function to survive.

According to the pharmacophore analysis, it is observed that any of the benzothiazole derivatives (M10, M15, M2, M6, M7 and M9) that have no cation form and do not carry amino group at the third position are not mapped into Hypo 10 as seen in Table 3. Thus it can be considered that the activity mechanisms of benzothiazoliums and benzothiazole derivatives are different.

5. Conclusions

In 2013, some of the new six benzothiazole derivatives and their corresponding 3-amino-benzothiazolium forms were synthesized and their inhibitory activities on DNA topoisomerase II enzyme were determined by our group [21]. According to our earlier study, the 2-substituted-benzothiazole derivatives (M2, M6, M7, M9, M10 and M15) indicated IC_{50} values of topoisomerase II inhibitory activity between 6.678 mM and 8.1 μM. Among the benzothiazole derivatives, only 2-(4-bromophenyl)benzothiazole (M9) with an IC_{50} of 8.1 μM was found to be more effective than the standard drug, etoposide (IC_{50} = 10 μM). However, 2-substituted-3-aminobenzothiazolium derivatives (BM6, BM1, BM7, BM2, BM3 and BM4, which were the 4-methylbenzene sulfonate salts of 3-amino-benzothiazolium derivatives of M2, M6, M7, M9, M10 and M15, respectively) showed the inhibition effect on topoisomerase II with IC_{50} values of 8.457 mM to 39.4 nM. While the compound M10 had an IC_{50} value of 788 μM, its 3-amino-benzothiazolium *p*-toluenesulfonate salt (BM3) was found to be the most potent inhibitor; it was also extremely active compared with the standard drug, etoposide, among the tested compounds. The results suggested that BM3 inhibited topoisomerase II by a different mechanism. To explore the mechanisms of the topoisomerase II inhibitory activity of benzothiazole and 3-aminobenzothiazolium derivatives, molecular docking and pharmacophore analysis were performed in this study.

It was previously reported that there was a clear evidence for a covalent 5' link between DNA and the active site tyrosine (Tyr782) resident in topoisomerase II's winged helix domain [22,23]. And, according to earlier studies, BM3 played a preventive role in the DNA binding ability of topoisomerase II [21]. It could be considered that BM3 bound to this active site via Tyr782 and played preventive role in DNA binding ability of topoisomerase II. We showed the interactions of benzothiazole and 3-aminobenzothiazol-3-ium derivatives with

topoisomerase II by using molecular docking studies. The results of this process were compared with the experimental data to identify the interaction mechanisms of 12 compounds with topoisomerase II. The most potent 3-amino-2-(2-bromobenzyl)-1,3-benzothiazole-3-ium 4-methylbenzene sulfonate (BM3) was chosen for the template revealing their interactions on topoisomerase II.

From the results of molecular docking, it can be concluded that benzothiazolium compounds bind to the active site tyrosine 782 to prevent the interactions between DNA and the topoisomerase II enzyme. This property of the benzothiazoliums most probably executes the cell proliferation process in tumor cells. The methods presented in this study are able to provide valuable information about key features and interactions that are important for the biological activity of the compound, BM3. Our present study showed that the 3-amino group, as well as the CH_2 bridge and the bromine atom bound on the *ortho* position of the phenyl ring substituted at the second position of benzothiazole, play a significant role in enhancing the activity. Moreover, according to the performed docking and pharmacophore analysis results, it can be stated that 3-amino-benzothiazol-3-ium derivatives exhibit a totally different mechanism than neutral benzothiazoles, which do not bind to the active site in the same way as 3-amino-benzothiazolium compounds. In conclusion, BM3 would be a good anticancer candidate for preclinical studies and 3-amino-benzothiazoliums are worth carrying onto anticancer studies.

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