Arch. Pharm. Chem. Life Sci. 2015, *348*, 498–507

ARCH PHARM
Archiv der Pharmazie

Full Paper

Generated 3D-Common Feature Hypotheses Using the HipHop Method For Developing New Topoisomerase I Inhibitors

Sanaz Ataei[1], Serap Yilmaz[2], Tugba Ertan-Bolelli[2], and Ilkay Yildiz[2]

[1] Biotechnology Institute, Ankara University, Tandogan-Ankara, Turkey
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Turkey

The continued interest in designing novel topoisomerase I (Topo I) inhibitors and the lack of adequate ligand-based computer-aided drug discovery efforts combined with the drawbacks of structure-based design prompted us to explore the possibility of developing ligand-based three-dimensional (3D) pharmacophore(s). This approach avoids the pitfalls of structure-based techniques because it only focuses on common features among known ligands; furthermore, the pharmacophore model can be used as 3D search queries to discover new Topo I inhibitory scaffolds. In this article, we employed the HipHop module using Discovery Studio to construct plausible binding hypotheses for clinically used Topo I inhibitors, such as camptothecin, topotecan, belotecan, and SN-38, which is an active metabolite of irinotecan. The docked pose of topotecan was selected as a reference compound. The first hypothesis (Hypo 01) among the obtained 10 hypotheses was chosen for further analysis. Hypo 01 had six features, which were two hydrogen-bond acceptors, one hydrogen-bond donor, one hydrophob aromatic and one hydrophob aliphatic, and one ring aromatic. Our obtained hypothesis was checked by using some of the aromathecin derivatives which were published for their Topo I inhibitory potency. Moreover, five structures were found to be possible anti-Topo I compounds from the DruglikeDiverse database. From this research, it can be suggested that our model could be useful for further studies in order to design new potent Topo I-targeting antitumor drugs.

Keywords: Aromathecin / HipHop / Pharmacophore analysis / Topoisomerase I inhibitors / Topotecan

Received: January 31, 2015; Revised: March 15, 2015; Accepted: March 20, 2015

DOI 10.1002/ardp.201500045

Introduction

Eukaryotic DNA topoisomerases I and I1 are essential nuclear enzymes responsible for the organization and modulation of the topological features of DNA so that a cell may replicate, transcribe, and repair genetic information [1–4]. Topoisomerase I (Topo I) functions by creating transient single-stranded nicks in DNA supercoils relieving torsional strain that has accumulated during DNA replication and transcription [2, 4, 5]. Intracellular levels of Topo I are elevated in a number of human solid tumors, relative to the respective normal tissues, suggesting that variations in Topo I levels are tumor-type specific [6–8]. Therefore, Topo I represents a promising target for the development of new cancer chemotherapeutic agents against a number of solid human tumors.

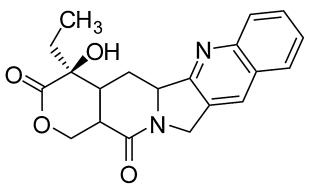
In 1966, Wall et al. [9] discovered that camptothecin (CPT; Fig. 1), a pentacyclic alkaloid was the component in the extract from the stem of the Chinese tree *Camptotheca acuminata* active against L1210 murine leukemia cells. Early clinical trials with the sodium salt of CPT in the early 1970s showed that this plant alkaloid had activity against a variety of solid tumors [10–12]. However, further clinical trials were discontinued because of unpredictable and severe myelosuppression, gastrointestinal toxicity, and hemorrhagic cystitis. In 1985, however, it was reported that the cytotoxic activity of CPT was attributed to a novel mechanism of action involving the nuclear enzyme Topo I [13], and this discovery of unique mechanism of action revived interest in CPT and its analogs as anticancer agents.

Correspondence: Dr. Ilkay Yildiz, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan-Ankara, Turkey
E-mail: iyildiz@pharmacy.ankara.edu.tr
Fax: +90 312 2131081

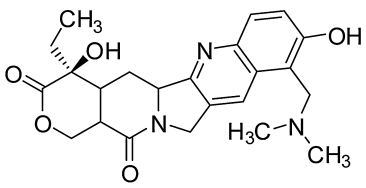
Enzymology studies have shown a rather intriguing mechanism in that CPT does not interact with Topo I alone, nor does it bind to DNA [14–16], and exerts its cytotoxic effect by binding and stabilizing the cleavable complex, a transient species where the hydroxyl group on tyrosine 723 of Topo I binds covalently to DNA via its phosphodiester backbone and causes a single-strand break. The formation of a stable ternary complex between CPT, Topo I, and the cleaved DNA leads to the S-phase specific arrest of replication at the single-strand level, causing irreversible DNA damage and eventually cell death [17]. Contrary to these initial reports, recent studies have suggested that CPT analogs may interact directly with double-stranded DNA prior to the action of Topo I, and the DNA-associated drugs are likely to be involved in the subsequent formation of a ternary complex [18]. Although the exact structure of the ternary complex remains to be determined [19, 20], this mechanism accounts for the good correlation found between the ability to induce stabilized cleavable complexes and the cytotoxicity of various CPT analogs [21]. Subsequently, CPT analogs, classified as DNA Topo I inhibitors, have recently emerged as a prominent class of anticancer agents with a novel mechanism of action, potent antiproliferative activity on a wide spectrum of tumor cells including multidrug-resistant lines, and impressive activity in xenograft models [22].

The later development of topotecan and irinotecan as camptothecin analogs, which are the only FDA-approved anticancer agents, were found to inhibit Topo I activity by intercalating into the cleavage complex and preventing the religation step of the catalytic cycle [23–25]. Moreover, belotecan [26, 27], which is 7-$Me_2CHNHCH_2CH_2$CPT, has been approved to be used in the clinic as anticancer drugs in South Korea, as well. One of these drugs, irinotecan, is a pro-drug and is converted to the active 7-ethyl-10-hydroxy-CPT (OHC2CPT; SN-38) by carboxylesterases to exert its antitumor activity [28, 29]. Although the camptothecins possess potent anticancer activity, issues regarding solubility and bioactivity, dose-limiting toxicity [24, 25, 30], the instability of the hydroxy lactone, and associated pharmacokinetic liabilities [31–33], led to the development of Topo I inhibitors as novel anticancer drugs.

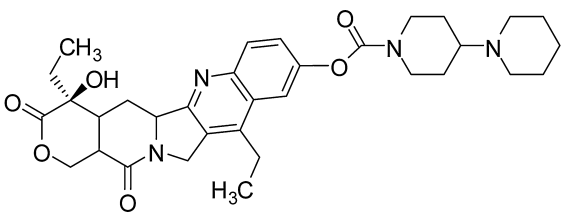
One promising class of noncamptothecin Topo I poisons is the indenoisoquinolines, such as MJ-III-65 [34, 35]. These compounds possess high anti-Topo I activity, are cytotoxic, and are more stable because they lack the hydroxylactone. Through comprehensive SAR studies [34, 36–38], two clinical candidates, indotecan and indimitecan, were developed and have begun Phase 1 clinical trials at the National Cancer Institute [39, 40].



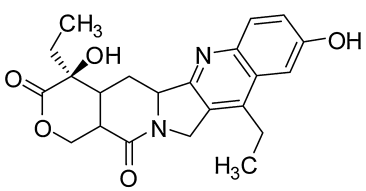
Camptothecin (**01**)



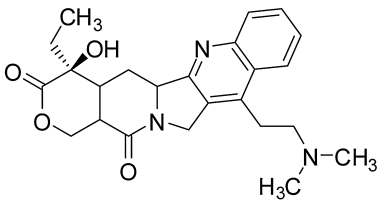
Topotecan (**02**)



Irinotecan (**03**)



SN-38 (**04**) (active metabolite of irinotecan)



Belotecan (**05**)

Figure 1. Representative clinically used Topo I inhibitors.

ARCH PHARM
Archiv der Pharmazie

The continued interest in designing new Topo I inhibitors, a common feature 3D-pharmacophore model from clinically used Topo I inhibitors such as camptothecin (**01**), topotecan (**02**), SN-38 (**03**) (which is an active metabolite of irinotecan (**03**)), belotecan (**05**) (Fig. 1) have been developed to offer promising scaffolds for the development of novel cancer chemotherapeutics. In this research, HipHop pharmacophore-based virtual screening was performed by using Discovery Studio 3.5 [41]. Moreover, we applied ligand-based virtual screening approaches for some of the aromathecin derivatives from Reference [42] to validate our hypotheses. Additionally, selected hypotheses wer searched in DruglikeDiverse database using the "Search 3D Database" protocol in Discovery Studio 3.5 [41].

Computational details

All of the molecular modeling studies were carried out using Discovery Studio 3.5 [41].

Training and test set selection

Clinically used Topo I inhibitors such as camptothecin (**01**), topotecan (**02**), SN-38 (which is an active metabolite of irinotecan (**03**)) (**04**), and belotecan (**05**) (as seen in Fig. 1) were used as a training set in order to develop a common feature 3D-pharmacophore model. Topotecan, which was the docked pose from taking molecular docking study, was selected for the reference compound. All of the test set were chosen from published studies [42]. Moreover, DruglikeDiverse database, which has 5384 compounds, was screened [41].

Molecular docking study of topotecan

Preparation of the enzyme

The crystal structure of the topoisomerase I, complexed with topotecan, was retrieved from the Protein Data Bank (PDB ID: 1k4t) [43]. Accelrys Discovery Studio 3.5 [41] software was used for preparation of protein and ligands. The target protein was taken, the ligand was extracted, 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 Discovery Studio 3.5 protocol until the root mean deviation (RMS) 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 active site of the enzyme. Binding sphere for 1k4t (21.261, −3.517, 28.108, and 14.1604) was selected from the active site using the binding site tools.

Ligand preparation

Reference drug topotecan was sketched, all atom CHARMm forcefield parameterization was assigned, and then minimized using the ABNR method as described above. Conformational searches of the ligand were carried out using a simulated annealing molecular dynamics (MD) approach. The ligand was heated to a temperature of 700 K and then annealed to 200 K.

Docking

CDocker [44] method was performed by using Discovery Studio 3.5. The protein is held rigid while the ligand is allowed to be flexible during refinement. The docking parameters were as follows: Top Hits: 10; Random Conformations: 10; Random Conformations Dynamics Step: 1000; Grid Extension: 8.0; Random Dynamics Time Step: 0.002. The docking and scoring methodology was validated by docking of known inhibitor, topotecan. The docked position of topotecan overlaps well with the crystal structure position, with an RMSD of 0.6538 Å.

Pharmacophore modeling for topoisomerase I inhibitors

Pharmacophore modeling is one of the most powerful techniques to classify and identify key features from a group of molecules such as active and inactive compounds. Chemical features in the hypothesis or pharmacophore model will furnish a new insight into design novel molecules that can enhance or inhibit the function of the target and will be useful in drug discovery strategies. HipHop module from CATALYST software in Discovery Studio 3.5 was used to develop pharmacophore models [41].

Molecules were built using ISIS draw and minimized using CATALYST software to the closest local minimum by applying the CHARMm-like force field [45]. Catalyst automatically generated conformational models for each compound using the Poling Algorithm [46–48]. The "best conformer generation" procedure was applied to provide the best conformational coverage for a maximum number of conformers generated defaulted to 255 in a 0–20 kcal/mol range from the global minimum [49]. The generated conformations were used to align common molecular features and generate pharmacophore hypothesis. HipHop was used to the conformations generated to align chemically important functional groups common to the molecules in the study set. A pharmacophoric hypothesis then was generated from these aligned structures.

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 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 research, HipHop common feature hypotheses were generated in order to offer promising scaffolds for the development of novel Topo I inhibitors for contributing to the cancer chemotherapy.

This tool builds hypotheses (overlaying common features) for which the fit of individual molecules to a hypothesis can be correlated with activity of the molecule. Clinically used four potent Topo I inhibitors, camptothecin (**01**), topotecan (**02**), irinotecan's (**03**) metabolite (SN-38 (**04**)), and belotecan (**05**), were selected as the target training set (Fig. 1). All the compounds were built using ISIS draw and minimized using CATALYST software to the closest local minimum by applying the CHARMm-like force field [46]. Docked pose of topotecan was considered as "reference compound" specifying a "Principal" value of 2 and a "MaxOmitFeat" value of 0, meaning its structure and conformation would have the strongest influence in the model building phase. The "Principal" and "MaxOmitFeat" values for the remaining compounds were set to 1 and 2, respectively (Principal = 1 means that this molecule must map onto the hypothesis generated by the search procedure. Partial mapping is allowed. Principal = 2 means that this is a reference compound. The chemical feature space of the conformers of such a compound is used to define the initial set of potential hypotheses. The MaxOmitFeat column specifies how many hypothesis features must map to the chemical features in each compound. A "0" in this column forces mapping of all features, a "2" allows hypotheses to which no compound features map). Diverse conformational models for each compound were generated using the "best conformational analysis" method and an energy threshold of 20 kcal/mol above the global energy minimum for conformation searching [49]. The maximum number of conformers for each molecule was specified as 255 to ensure maximum coverage of the conformational space. Due to the basic structures of the compounds, seven kinds of features including hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD), hydrophobic group (Hyd), hydrophob aliphatic (Hyd_Al), hydrophob aromatic (Hyd_Ar), PosIonizable (PI), and ring aromatic (RA) features were selected to initiate the pharmacophore hypotheses generation process. The characteristics of the generated potential 10 hypotheses are listed in Table 1 and all the hypotheses contain six features.

Results and discussion

DNA topoisomerase I is an essential nuclear enzyme for cell survival. Targeting this enzyme has become one of the best solutions for cancer therapy [1–4]. DNA topoisomerase I targeted cytotoxic drugs are used in clinic for treatment of solid tumors such as lung, ovarian, colon cancers, etc. However, the number of topoisomerase I targeted drugs on market is very limited. Hence, there is still a gap to fill for finding new kind of chemical structures which specifically target topoisomerase I. In this research, HipHop common feature hypotheses were generated in order to offer promising scaffolds for the development of novel Topo I inhibitors for contibuting to the cancer chemotherapy.

First of all, the docked pose of topotecan which was the reference drug was obtained by using CDOCKER method, before generating pharmacophoric features for Topo I inhibitors. According to molecular docking study, the binding energy of topotecan was found to be −56.2735 kcal/mol. Moreover, docking study showed that topotecan had 4 H bonds with Glu356, Lys532, Asp533, Arg364, 2 water mediated H bonds with Arg488, Asn722, and Pi interactions with Lys532, DT B10, TGP C11 in Topo I residues (as seen in Fig. 2a and b).

Table 1. The results of pharmacophore hypotheses generated by using HipHop.

Hypo no.	Features[a)]	Rank	DF[b)]	PH[c)]	Max. Fit
01	RYZDAA	63.459	1111	0000	6
02	RYZDAA	63.459	1111	0000	6
03	RYZDAA	63.459	1111	0000	6
04	RYZDAA	62.964	1111	0000	6
05	RYZDAA	62.908	1111	0000	6
06	RYZDAA	62.908	1111	0000	6
07	RYZAAA	62.659	1111	0000	6
08	RYZAAA	62.659	1111	0000	6
09	YZDAAA	62.240	1111	0000	6
10	RYZAAA	62.108	1111	0000	6

[a)] Features: A: HBA, D: HBD, Z: HYDROPHOBAliphatic (Hyd_Al), Y: HYDROPHOBAromatic (Hyd_Ar), R: RING_AROMATIC (RA).
[b)] DF (Direct Hit Mask): Mapping of each feature onto training set molecule. 1 means yes and 0 means no.
[c)] PH (Partial Hit Mask): A training set molecule mapped all but one feature in the hypothesis. 1 means yes and 0 means no.

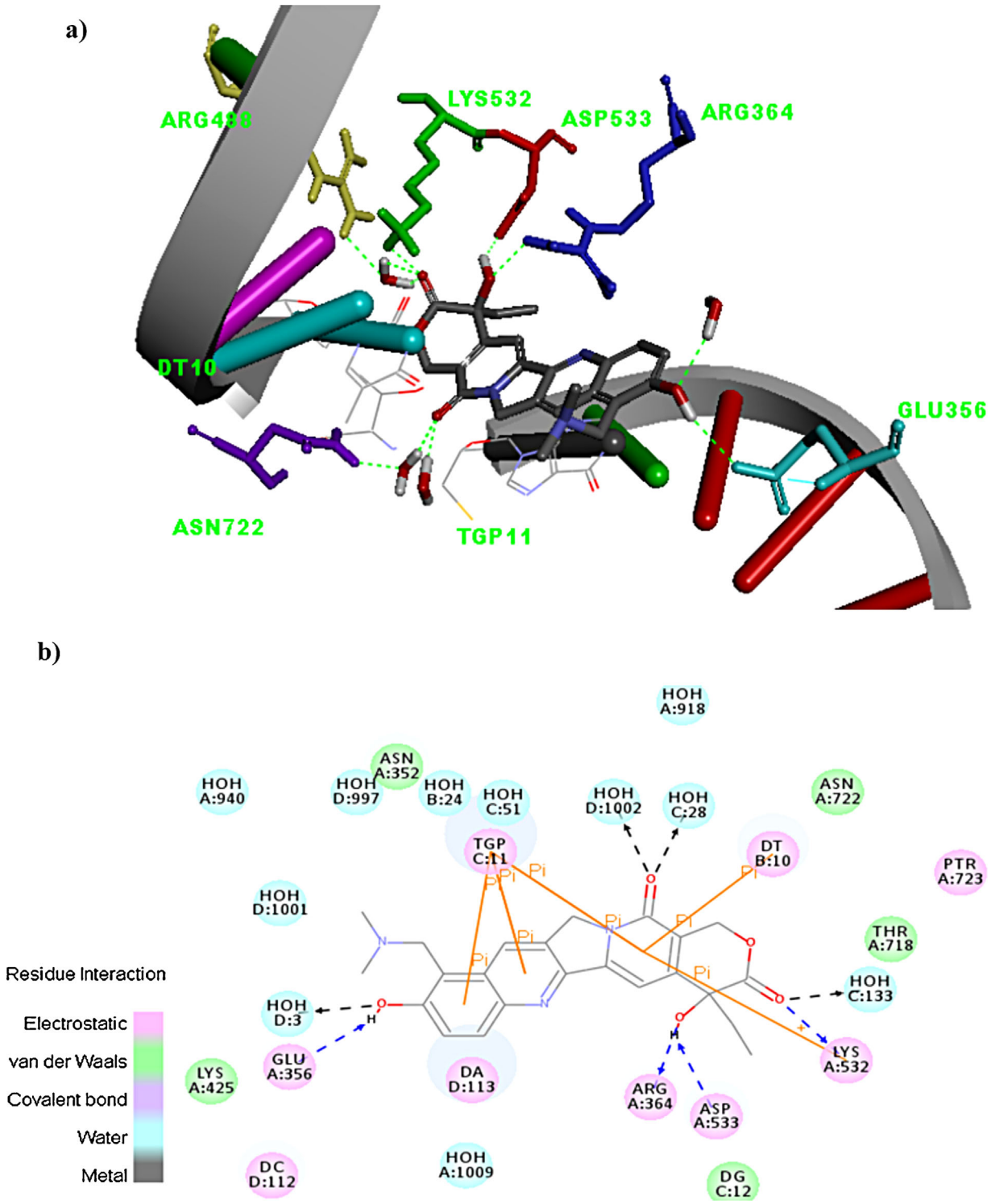


Figure 2. Docked position of topotecan: (a) 3D figure; (b) 2D figure.

Clinically used four potent Topo I inhibitors, camptothecin (**01**), topotecan (**02**), irinotecan's active metabolite SN-38 (**04**), and belotecan (**05**), were selected as the training set to generate pharmacophore models by using HipHop methodology (Fig. 1). The docked pose of topotecan, reported above, was considered as a reference compound, which was allowed to map all features, while the other three molecules were allowed to map partially on the hypotheses. Finally, the 10 six-feature hypotheses were generated. The ranking scores of hypotheses range from 63.459 to 62.108 as shown in Table 1. The first hypothesis (Hypo 01) was selected for further analysis that contained RA-1, Hyd_Ar-2, Hyd_Al-3, HBD-4, HBA-5, and HBA-6, which is presented in Fig. 3a and b. Direct hit mask and partial hit mask revealed that all the six features were mapped onto four training set compounds. Figure 4 shows the mapping of Hypo 01 onto the training set compounds. The compound **2** (topotecan) taken as reference mapped well onto all the features (Fig. 5). The HBD-4 feature mapped on OH group at the fourth position of topotecan represents interaction with Asp533 and Arg364 in the active site of Topo I (as seen in Figs 2 and 5). Moreover, HBA-5 fitted on

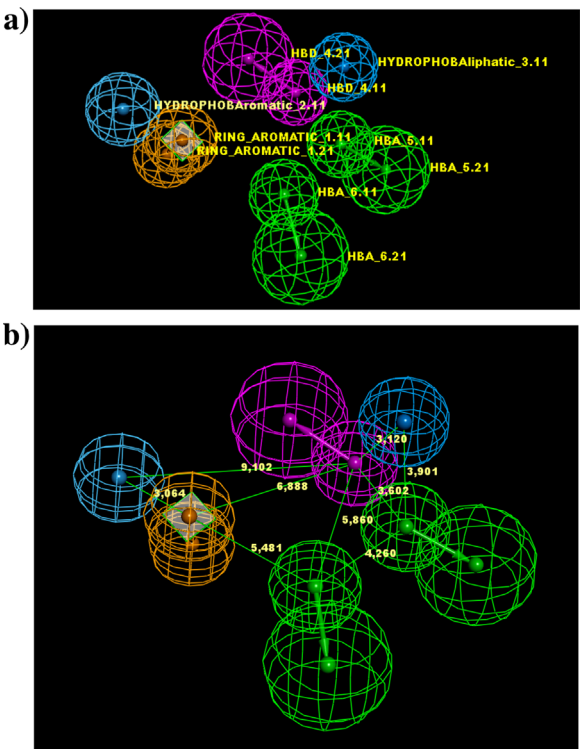


Figure 3. The best hypotheses of HipHop Pharmacophore Modeling (Hypo 01) obtained from clinically used Topo I inhibitors: (a) Mapping of Hypo 01, which contains two HBAs (green), one HBD (violet), one RA (yellow), one Hyd_Al (dark blue), and one Hyd_Ar (light blue) pharmacophore features; (b) the geometry of the features.

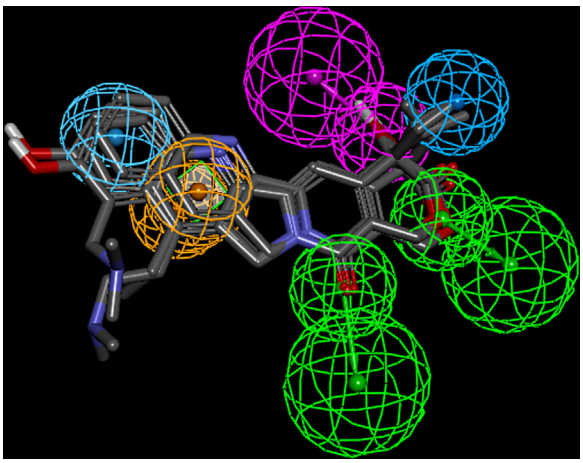


Figure 4. All training set with Hypo 01.

"O" of carbonyl on the pyran ring of topotecan shows interaction with Lys532. HBA-6 mapped on OH group at the 14th position of topotecan represents interaction to Asn722 with water mediated H bond.

The aromathecin or "rosettacin" class of Topo I inhibitors is effectively a "composite" of the natural products camptothecin and the synthetic indenoisoquinolines [50]. In here, some of the aromathecin derivatives taken from Reference [42] were used as a test set for validating our selected hypotheses as seen in Table 2. These compounds were mapped onto the Hypo 01 by using "flexible" fitting method, "best mapping only," and "Maximum Omitted Features: –1" options to obtain the bioactive conformation of each molecule in Ligand Pharmacophore Mapping protocol in Discovery Studio 3.5. All of the statistical data were shown in

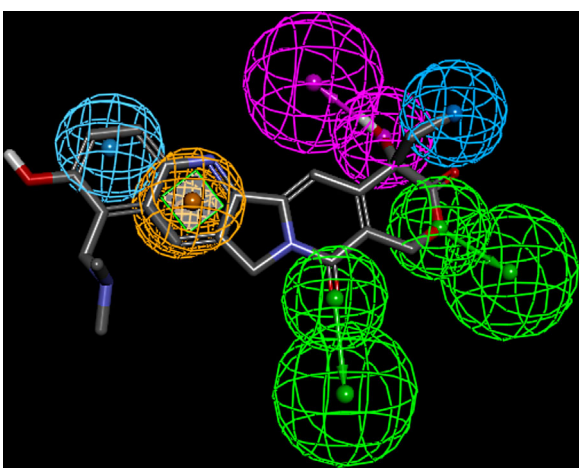
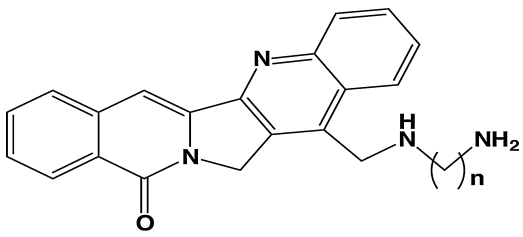


Figure 5. Docked pose of topotecan with Hypo 01.

Table 2. Aromathecin derivatives [42] as Topo I inhibitors used as test series for checking Hypo 01.



Comp. no.	*n*	Topo I cleavage[a)]
53	2	+++
54	3	++(+)
55	4	+++
56	5	+
57	6	++(+)
58	7	++
59	8	+
60	9	+
61	10	0
62	11	0
63	12	0

[a)] Compound-induced DNA cleavage due to Top1 inhibition is graded by the following rubric relative to 1 μM camptothecin: 0, no inhibitory activity; +, between 20 and 50% activity; ++, between 50 and 75% activity; +++, between 7 and 95% activity; ++++, equipotent. The 0/+ ranking is between 0 and + [42].

Table 3. The results of mapping of features onto test set compounds are given in Fig. 6.

According to Table 3 and Fig. 6, compounds **57–63** mapped well onto the five features with a good FitValues 4.7718, 4.7562, 4.7562, 4.7539, 4.6414, 4.5765, and 4.1962, respectively. These structures did not only map into HBA-5 as seen Pharmprint (111101: that means RA-1: 1; Hyd_Ar-2: 1; Hyd_Al-3: 1; HBD-4: 1; HBA-5: 0; HBA-6: 1) in Table 3. It was reported that compounds **61–63** were found to be inactive for Topo I cleavage [42]. Even if non-effect compounds matched with five features well but their Van der Waals Energy number are smaller than the other. On the other hand, we noticed that among the aromathecin derivatives, which had 111101 as Pharmprint and a good FitValues, none of them had indicated an equipotent activity like camptothecin. According to these results, we could consider that HBA-5 feature played a very important role for getting Topo I inhibitory activity. Furthermore, the aromathecins **53–56** fitted onto RA-1, Hyd_Ar-2, HBD-4, and HBA-6 features as seen in Table 3. Although compounds **53–55** showed best map with only four features, they had more significant Topo I inhibitory activity than derivatives **58–60**. It was noticed that their Van der Waals energies were found to be higher than the other. Therefore, it can be considered that the Van der Waals energies of aromathecins **53–55** should be also important for developing

Table 3. Statistical data of aromathecin derivatives as Topo I inhibitors used as test series for checking Hypo 01.

Comp. no. (Topo I cleavage[a)])	Absolute energy	Angle energy	Bond energy	CHARMm energy	Conf. number	Dihedral energy	Electrostatic energy	Fit Values	RMS gradient	Pharmprint[b)]	Relative energy	Potantial energy	Van der Waals enegry
63 (0)	78.3535	28.4814	2.1349	40.0866	157	1.33048	4.3176	4.7718	0.04648	111101	16.7078	40.0866	3.81309
59 (+)	76.3511	28.1897	2.2814	39.751	98	0.8399	2.6802	4.7562	0.04575	111101	13.3094	39.751	5.73426
62 (0)	89.2469	28.9100	1.9630	40.3235	143	2.66552	3.1526	4.7562	0.03883	111101	27.2611	40.3235	3.56921
58 (++)	71.7707	28.6476	1.9455	40.8366	32	2.52215	1.6669	4.7539	0.04995	111101	8.38123	40.8366	6.00887
60 (+)	78.5427	28.7570	1.9516	40.5888	83	2.50053	2.3681	4.6414	0.03985	111101	15.8508	40.5888	4.95476
61 (0)	77.6839	28.6623	2.2007	38.0146	27	3.2858	0	4.5765	0.04737	111101	15.3427	38.0146	3.60654
57 (++(+))	89.2201	27.9411	2.1898	40.911	84	0.92464	2.8563	4.1962	0.04935	111101	25.4779	40.911	6.97707
54 (++(+))	99.3871	27.8058	2.1717	51.9463	118	2.54616	11.0881	3.8836	0.048	110101	34.6003	51.9463	8.32883
53 (+++)	77.6469	28.2138	2.0452	33.9275	39	0.90812	−6.0542	3.8788	0.04015	110101	12.5153	33.9275	8.77744
56 (+)	79.0867	29.0881	2.0662	40.1191	72	2.48156	−0.6047	3.8324	0.04234	110101	14.988	40.1191	7.05361
55 (+++)	88.2554	28.1998	2.3457	39.2612	134	1.25024	−0.8778	3.7542	0.04826	110101	23.8313	39.2612	8.28577

[a)] Compound-induced DNA cleavage due to Top1 inhibition is graded by the following rubric relative to 1 μM camptothecin: 0, no inhibitory activity; +, between 20 and 50% activity; ++, between 50 and 75% activity; +++, between 7 and 95% activity; ++++, equipotent. The 0/+ ranking is between 0 and + [42, 43].

[b)] Pharmprint refers to mapped features such as RA-1, Hyd_Ar-2, Hyd_Al-3, HBD-4, HBA-5, and HBA-6, respectively.

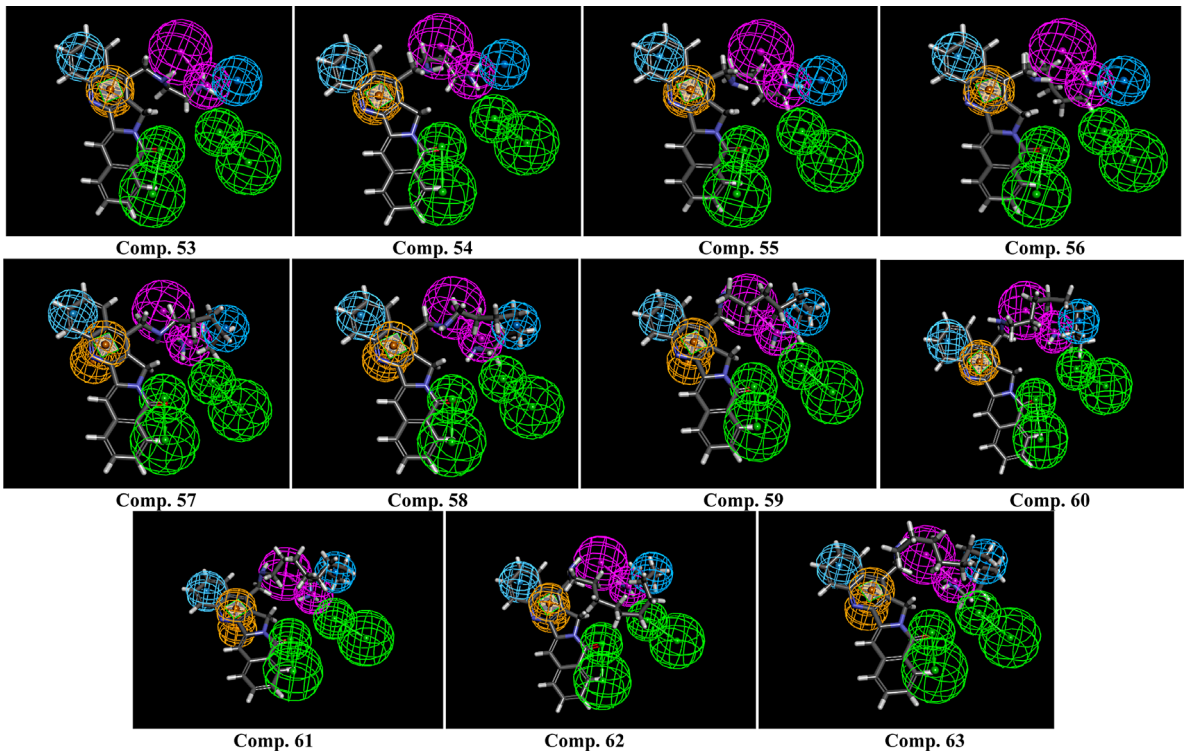


Figure 6. Compared test set series of Hypo 01.

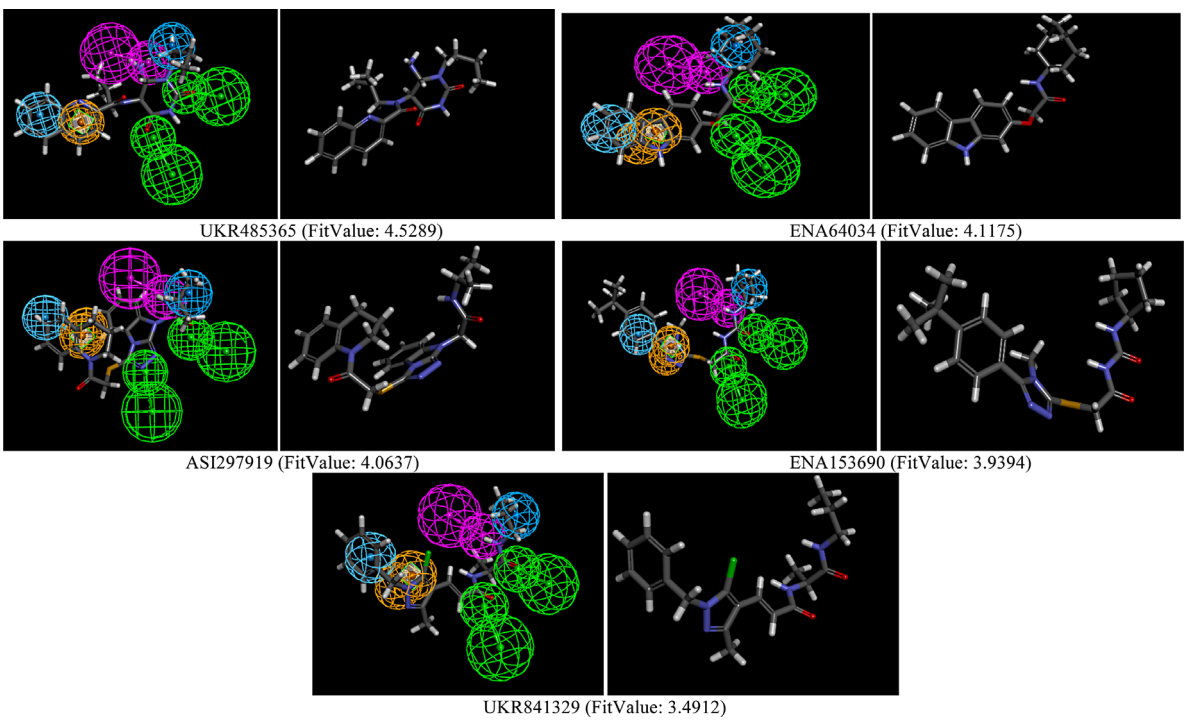


Figure 7. Possible Topo I inhibitors from DruglikeDiverse database.

new Topo I inhibitors. Unfortunately, our hypothesis was not appropriate for compound **56**. We can conclude that our model would be useful for further studies.

In this study, selected Hypo 01 was searched in Druglike-Diverse database where 5384 compounds were, as well. According to this screening, it was obtained that 764 compounds fitted with Hypo 01. However, only five compounds (UKR485365, ENA64034, ASI297919, ENA153690, and UKR841329) fitted onto all the features with satisfactory FitValues (4.5289, 4.1175, 4.0637, 3.9394, and 3.4912, respectively) as seen in Fig. 7. Here, it can be considered that these five molecules could be new lead Topo I inhibitors for cancer therapy.

Conclusion

HipHop provides feature-based alignment of collection of compounds without considering biological activity. It matches pharmacophoric features of the molecules against template. HipHop takes collection of conformational models of molecules and produces series of molecular alignments in 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 configuration if it possesses the conformations and pharmacophoric features that can be superimposed within a tolerance limit from the corresponding ideal locations.

The training set compounds associated with their diverse conformational models were subjected for hypotheses generation. The molecular characteristics, which are essential for tight binding between ligand and its corresponding targets like Topo I, were expressed as common features disposed in 3D space known as hypotheses. In this study, we have done a common feature 3D-pharmacophore model from clinically used four potent Topo I inhibitors, camptothecin (**01**), topotecan (**02**), irinotecan's (**03**) active metabolite (SN-38 (**04**)), and belotecan (**05**), as presented in Fig. 1 by using HipHop methodology to offer promising scaffolds for the development of novel cancer chemotherapeutics. Our main goal was to generate 3D-common chemical features in order to be able to develop new Topo I inhibitors for further studies.

In this research, 10 hypotheses, which had six features, were obtained. The first hypothesis (Hypo01) was selected for further analysis that contained two hydrogen-bond acceptors, one hydrogen-bond donor, one hydrophob aromatic and one hydrophob aliphatic, and one ring aromatic features, which is presented in Fig. 3. Selected hypotheses (Hypo 01) were checked by using some of the aromathecins taken from Reference [42].

Moreover, Hypo 01 was searched in DruglikeDiverse database, as well. It was obviously noticed that five compounds (UKR485365, ENA64034, ASI297919, ENA153690, and UKR841329) as seen in Fig. 7 could be novel Topo I-targeting antitumor compounds.

Finally, it can be considered that our model could be more useful for further studies in order to design new potent Topo I inhibitors.

We thank the Research Fund of Ankara University (Grant No: 12B3336002) for the financial support of this research.

The authors have declared no conflict of interest.

References

[1] M. Potmesil, K. K. Kohn, *DNA Topoisomerases in Cancer*. Oxford University Press, New York **1991**.
[2] M. Cased, A. Amadei, G. Camilloni, E. Di Mauro, *Biochemistry* **1990**, *29*, 8152–8157.
[3] P. J. Smith, *Int. J. Radiat. Biol.* **1990**, *58*, 553–559.
[4] J. C. Wang, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430–440.
[5] E. Pommier, *Chem. Rev.* **2009**, *109*, 2849–2902.
[6] B. P. Giovanella, J. S. Stehlin, M. E. Wall, M. Wani, A. W. Nicoholas, L. F. Liu, R. Silber, M. Potmesil, *Science* **1989**, *246*, 1046–1048.
[7] I. Husain, J. L. Mohler, H. F. Seigler, M. Besterman, *Cancer Res.* **1994**, *54*, 539–546.
[8] C. D. Lima, J. C. Wang, A. Mondragon, *Nature* **1994**, *367*, 138–146.
[9] M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, G. A. Sim, *J. Am. Chem. Soc.* **1996**, *88*, 3888–3890.
[10] J. A. Gottlieb, A. M. Guarino, J. B. Call, V. T. Oliverio, J. B. Block, *Cancer Chemother. Rep.* **1970**, *54*, 461–470.
[11] P. J. Creaven, L. M. Allen, F. M. Muggia, *Cancer Chemother. Rep.* **1972**, *56*, 573–578.
[12] F. M. Muggia, P. J. Creaven, H. Hansen, M. H. Cohen, O. S. Selawry, *Cancer Chemother. Rep.* **1972**, *56*, 515–521.
[13] Y. H. Hsiang, R. Hertzberg, S. Hecht, L. F. Liu, *J. Biol. Chem.* **1985**, *260*, 14873–14878.
[14] R. P. Hertzberg, M. J. Caranfa, S. M. Hecht, *Biochemistry* **1989**, *28*, 4629–4638.
[15] L. H. Li, T. J. Fraser, E. J. Olin, B. K. Bhuyan, *Cancer Res.* **1972**, *32*, 2643–2650.
[16] M. Fukada, *Biochem. Pharmacol.* **1985**, *34*, 1225–1230.
[17] C. Holm, J. M. Covey, D. Kerrigan, Y. Pommier, *Cancer Res.* **1989**, *49*, 6365–6368.
[18] D. Yang, J. T. Strode, H. P. Spielmann, A. H. J. Wang, G. Burke, *J. Am. Chem. Soc.* **1998**, *120*, 2979–2980.
[19] M. R. Redinbo, L. Stewart, P. Kuhn, J. J. Champoux, W. G. Hol, *Science* **1998**, *279*, 1504–1513.
[20] Y. Fan, J. N. Weinstein, K. W. Kohn, L. M. Shi, Y. Pommier, *J. Med. Chem.* **1998**, *41*, 2216–2226.
[21] C. Jaxel, K. W. Kohn, M. C. Wani, M. E. Wall, Y. Pommier, *Cancer Res.* **1989**, *49*, 1465–1469.
[22] Y. Kawato, H. Terasawa, *Prog. Med. Chem.* **1997**, *34*, 69–109.
[23] R. Garcia-Carbonero, J. G. Supko, *Clin. Cancer Res.* **2002**, *8*, 641–661.

[24] C. J. Thomas, N. J. Rahier, S. M. Hecht, *Bioorg. Med. Chem.* **2004**, *12*, 1585–1604.
[25] B. Teicher, *Biochem. Pharmacol.* **2008**, *75*, 1262–1271.
[26] J. C. Kim, D. H. Shin, S. H. Kim, J. K. Kim, S. C. Park, W. C. Son, H. S. Lee, J. E. Suh, C. Y. Kim, C. S. Ha, M. K. Chung, *Regul. Toxicol. Pharm.* **2004**, *40*, 356–369.
[27] A. L. Graul, J. R. Prous, *Drug News Perspect* **2005**, *18*, 21–36.
[28] Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga, K. Sato, *Cancer Res.* **1991**, *51*, 4187–4191.
[29] T. Kunimoto, K. Nitta, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Yokokura, S. Sowada, T. Miyasaka, M. Mutai, *Cancer Res.* **1987**, *47*, 5944–5947.
[30] U. Schaeppi, R. W. Fleischm, D. A. Cooney, *Cancer Chemother. Rep.* **1974**, *5*(Part 3) 25–36.
[31] T. G. Burke, Z. H. Mi, *J. Med. Chem.* **1994**, *37*, 40–46.
[32] M. J. Luzzio, J. M. Bestreman, D. L. Emerson, M. G. Evans, K. Lackey, P. L. Leitner, G. Mclntyre, B. Morton, P. L. Myers, M. Peel, J. M. Sisco, D. D. Sternbach, W. Q. Tong, A. Truesdale, D. E. Uehling, A. Vuong, J. Yates, *J. Med. Chem.* **1995**, *38*, 395–401.
[33] Z. H. Mi, T. G. Burke, *Biochemistry* **1994**, *33*, 10325–10336.
[34] M. Nagarajan, A. Morrell, B. C. Fort, M. R. Meckley, S. Antony, G. Kohlhagen, Y. Pommier, M. Cushman, *J. Med. Chem.* **2004**, *47*, 5651–5661.
[35] C. Marchand, S. Antony, K. W. Kohn, M. Cushman, A. Ioanaviciu, B. L. Staker, L. Stewart, Y. Pommier, *Mol. Cancer Ther.* **2006**, *5*, 287–295.
[36] A. Morrell, M. Placzek, S. Parmley, B. Grella, S. Antony, Y. Pommier, M. Cushman, *J. Med. Chem.* **2007**, *50*, 4388–4404.
[37] A. Morrell, S. Antony, G. Kohlhagen, Y. Pommier, M. Cushman, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3659–3663.
[38] M. Nagarajan, A. Morrell, A. Ioanoviciu, S. Antony, G. Kohlhagen, M. Hollingshead, Y. Pommier, M. Cushman, *J. Med. Chem.* **2006**, *49*, 6283–6289.
[39] Y. Pommier, M. Cushman, *Mol. Cancer Ther.* **2009**, *8*, 1008–1014.
[40] S. Antony, K. K. Agama, Z. H. MiaO, K. Takagi, M. H. Wright, A. I. Robles, L. Varticovski, M. Nagarajan, A. Morrell, M. Cushman, Y. Pommier, *Cancer Res.* **2007**, *67*, 10397–10405.
[41] Accelrys Inc. Discovery Studio 3. 5, **2012**.
[42] M. A. Cinelli, B. Cordero, T. S. Dexheimer, Y. Pommier, M. Cushman, *Bioorg. Med. Chem.* **2009**, *17*, 7145–7155.
[43] B. Staker, K. Hjerrild, M. D. Feese, C. A. Behnke, A. B. Burgin, L. Stewart, *PNAS* **2002**, *99*(24), 15387–15392.
[44] G. Wu, D. H. Robertson, C. L. Brooks, M. Vieth, *J. Comput. Chem.* **2003**, *24*(13), 1549–1562.
[45] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, M. Karplus, *J. Comput. Chem.* **1983**, *4*, 187–217.
[46] A. Smellie, S. D. Kahn, S. L. Teig, *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 285–294.
[47] A. Smellie, S. D. Kahn, S. L. Teig, *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 295–304.
[48] A. Smellie, S. L. Teig, P. Towbin, *J. Comp. Chem.* **1994**, *16*, 171–187.
[49] H. Yu, Z. Wang, L. Zhang, Q. Huang, *Chem. Biol. Drug. Des.* **2007**, *69*, 204–211.
[50] M. A. Cinelli, A. Morrell, T. Dexheimer, E. Scher, Y. Pommier, M. Cushman, *J. Med. Chem.* **2008**, *51*, 4609–4619.