

3D-QSAR study on heterocyclic topoisomerase II inhibitors using CoMSIA†

B. TEKINER-GULBAS, O. TEMIZ-ARPACI, I. YILDIZ*,
E. AKI-SENER and I. YALCIN

Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
Ankara University, Tandogan 06100 Ankara, Turkey

(Received 2 September 2005; in final form 31 January 2006)

Selective topoisomerase II (Topo II) inhibitors have interested to a great extent for the design of new antitumoral compounds in recent years. Comparative molecular similarity indices analysis (CoMSIA) was performed on a series of previously synthesized benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives as eukaryotic Topo II inhibitors. A training set of 16 heterocyclic compounds was used to establish the CoMSIA model. They were constructed and geometrically optimized using SYBYL v7.0. The predictive ability of the model was assessed using a test set of 7 compounds. The best model has demonstrated a good fit having r^2 value of 0.968 and cross-validated coefficient q^2 value as 0.562 including steric and hydrophobic fields. The hydrophobic interactions showed a dominant role for increasing Topo II inhibitor activity and hydrophilic substituent was found more important than hydrophobic one on the 5 or 6 position of benzazole moiety. The model obtained from the present study can be useful for the modification and/or evaluation of the development of new Topo II inhibitors as potential antitumor compounds.

Keywords: 3D-QSAR; CoMSIA; Topoisomerase II inhibitors; Benzoxazoles; Benzimidazoles; Benzothiazoles; Oxazolo(4,5-b)pyridines

1. Introduction

Topoisomerase II (Topo II) is an enzyme that decatenates and disentangles DNA by passing one DNA helix through another [1–5]. Due to the requirement for such a DNA strand passage activity in a number of critical nuclear processes, including replication, recombination, and chromosome segregation [1, 3, 4, 6, 7], Topo II is essential for the survival of proliferating eukaryotic cell [8, 9].

*Corresponding author. Email: oren@pharmacy.ankara.edu.tr

†Presented at CMTPI 2005: Computational Methods in Toxicology and Pharmacology Integrating Internet resources (Shanghai, China, October 29–November 1 2005).

The cytotoxic potential of Topo II has been exploited clinically by the development of anticancer drugs that generate high levels of covalent enzyme-DNA cleavage complexes [10, 11]. Because rapidly proliferating cells contain high concentrations of Topo II, aggressive malignancies are most susceptible to these agents [10–12].

Inhibitor effects of some novel fused heterocyclic compounds such as benzoxazole, benzimidazole, benzothiazole and oxazolopyridine derivatives on eukaryotic Topo II were investigated [13]. Activity was assayed by electrophoresis after incubating the enzyme, plasmid and ATP mixture with or without inhibitors. Increase in the relaxed plasmid band after the incubation was quantified and etoposide was used as a reference drug for the inhibitory effect.

Quantitative structure-activity relationships (QSARs) are now acknowledged to be in the heart of the long-term task as systematically evaluation of existing chemicals [14]. At present, the challenge is to improve the accuracy and predictability of QSAR by taking into account, in a very detailed way, the structural and physicochemical features of the tested compounds. Comparative molecular field analysis (CoMFA) program is in keeping with the general pattern of searching for these new descriptors, where steric and electrostatic fields of the molecule are mapped by a probe atom [15–17]. CoMFA is applied to a set of molecules exhibiting biological activity with a similar mechanism of action [18]. The advantages of CoMFA are the ability to predict the biological activities of the molecules and to represent the relationships between steric/electrostatic property and biological activity in the form of contour maps gives key features on not only the ligand-receptor interaction but also the topology of the receptor.

Another alternative molecular field analysis, the CoMSIA (comparative molecular similarity indices analysis), based on molecular similarity indices, has been reported [19, 20]. CoMSIA is an extension of the CoMFA methodology. They differ only in the implementation of the fields. In CoMSIA, five different similarity fields are calculated: steric, electrostatic, hydrophobic, and hydrogen-bond donor and hydrogen-bond acceptor. Similarity indices are calculated at regularly spaced grid points for the prealigned molecules. Instead of direct measurement of similarity between all mutual pairs of molecules, indirect evaluation of similarity of each molecule in the data set with a common probe atom is calculated. The linear regression equation of similarity with biological activities is then derived.

Recently, we reported the development of a 3D-QSAR on a training set of benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives as eukaryotic Topo II inhibitors using the methodology of CoMFA [21].

In this paper, CoMSIA, for the eukaryotic Topo II inhibition activity, was applied on the same training set and compared with the previous determined CoMFA model. The model deduced from this investigation provides underlying structural requirements and good predictive ability, which could aid in the design of new Topo II inhibitors prior to their synthesis.

2. Material and methods

2.1 Biological activity data set of benzazole compounds

The eukaryotic Topo II inhibition activity of benzazole derivatives used in this study was determined by Pinar *et al.* [13] using the relaxation assay. Relaxation activity

of DNA Topo II was determined by measuring the conversion of supercoiled pBluescript plasmid DNA to its relaxed form [22]. The reaction mixture contained 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 µg/ml bovine serum albumin, 1 mM ATP, 2 µg/ml pBluescript plasmid, 0.01% DMSO, 1–2 U of enzyme, and different concentrations of drugs in a total volume of 20 µl. The mixture was incubated for 16 h at 26°C. After incubation period, 6 µl of loading buffer containing 2 mM orange G and 55% glycerol in electrophoresis buffer (60 mM Tris, 30 mM acetic acid, and 1.5 mM EDTA, pH 8.0) was added and mixture was subjected to electrophoresis on 0.8% agarose, at 95 V for 2 h. After the electrophoresis, gels were stained with ethidium bromide (1 µg/ml) and photographed under UV light. Band distribution was analyzed with GDS 8000 Complete Gel Documentation and Analysis System (Gel Works 1D Intermediate, version 2.5; Ultra Violet Products). The rate of formation of the newly formed bands was used as a measure of the enzyme activity. Inhibitory activities were presented as micro molar test compounds that caused 50% inhibition per unit of enzyme, under the assay conditions and etoposide was used as the reference drug.

2.2 Computational methods

From its advent in 1988, CoMFA has been developed as one of the most powerful tools in 3D-QSAR [18]. CoMFA examines differences in targeted properties that are related to changes in the shape of the non-covalent (steric and electrostatic) fields surrounding a set of ligand molecules. Details of the shape of each field are put into a QSAR table by sampling their magnitudes at regular intervals throughout a specified region of space. Recently, another 3D-QSAR procedure: comparative molecular similarity indices analysis (CoMSIA) has been reported [19]. This method can avoid some inherent deficiencies arising from the functional form of Lennard-Jones and Coulomb potentials used in CoMFA. In CoMSIA, a distance dependent Gaussian-type functional form has been introduced, which can avoid singularities at the atomic positions and the dramatic changes of potential energy for those grids in the proximity of the surface, meanwhile, no arbitrary definition of cut-off limits is required in CoMSIA. Moreover, using CoMSIA, the contour maps of the relative spatial contributions of the different fields can be substantially improved, which is very intuitive for interpretation in terms of separate property fields. Similar to the conventional CoMFA procedure, the procedure of getting a 3D-QSAR model from a CoMSIA approach can be summarized into three following steps [19, 20, 23, 24]:

1. First, all investigated molecules are structure-based or field-based aligned.
2. Then, an evenly-spaced and rectangular grid is generated to enclose the molecular aggregate. A probe atom with some properties is placed at every lattice point to measure the electrostatic, steric and hydrophobic, H-bond donor or acceptor field.
3. Finally, the results from the field samplings combined with the biological activities from the tested compounds are put into a table and partial least squares (PLS) is applied to get the final CoMSIA model. Generally, a leave-one-out cross-validated r^2 (q^2) is used as a quantitative measure for a CoMSIA model. The unique difference between conventional CoMFA and CoMSIA is the field type and the potential function. In CoMSIA, the similarity is expressed in terms of different

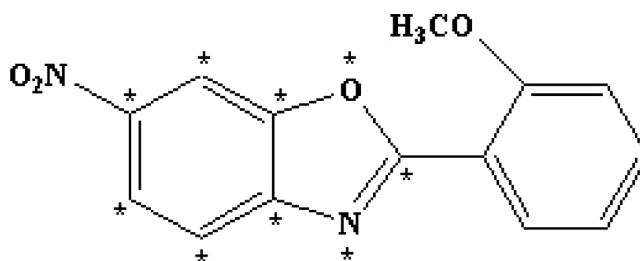


Figure 1. Molecule **1** with atoms used for superimposition are marked.

physicochemical properties: steric occupancy, partial atomic charges, local hydrophobicity, and H-bond donor and acceptor properties.

A Gaussian-type distance dependent function is used to calculate different kinds of physicochemical properties. The indices $A_{F,K}$ between the compounds of interest and a probe atom have been calculated according to:

$$A^q F \cdot K(j) = \sum_{i=1}^n \omega_{\text{probe},k} \omega_{ik} e^{-ar_{iq}^2} \quad (1)$$

where i is a summation index over all atoms of the molecule j under investigation; ω_{ik} : is the actual value of the physicochemical property k of atom, $\omega_{\text{probe},k}$: is the probe atom with charge +1, radius 1 Å, hydrophobicity +1, H-bond donor and acceptor property +1; a is an attenuation factor; r_{iq} is the mutual distance between probe atom at grid point q and atom i of the investigated molecule.

2.3 Molecular modeling

Three-dimensional structure building and all modeling were performed using the SYBYL program package, version 7.0 [25] on a Silicon Graphics workstation with the IRIX 6.5 operating system. Geometry optimization was carried out using MAXIMIN molecular mechanics and Tripos force field supplied within SYBYL, with convergence criterion set at 0.05 kcal/(Å mol). The alignment of the training set molecules was derived using FlexS in SYBYL. One of the most active molecules, **1**, was used as the template for alignment by considering the heavy atoms of the 2-phenylbenzoxazole ring as shown in figure 1. All values were filled with valence and Gasteiger charges were calculated for each compound. The superimposition of all the molecules is shown in figure 2. CoMSIA models were generated using 16 molecules (**1–16**, table 1), with column filtering value (σ) of 2.0.

2.4 CoMSIA model

The CoMSIA studies for Topo II inhibitors were performed using the QSAR module of SYBYL 7.0. The five CoMSIA similarity index fields available within SYBYL (steric, electrostatic, hydrophobic, hydrogen-bond donor and hydrogen-bond acceptor) were calculated at lattice points using a common probe atom of 1 Å radius, as well as the

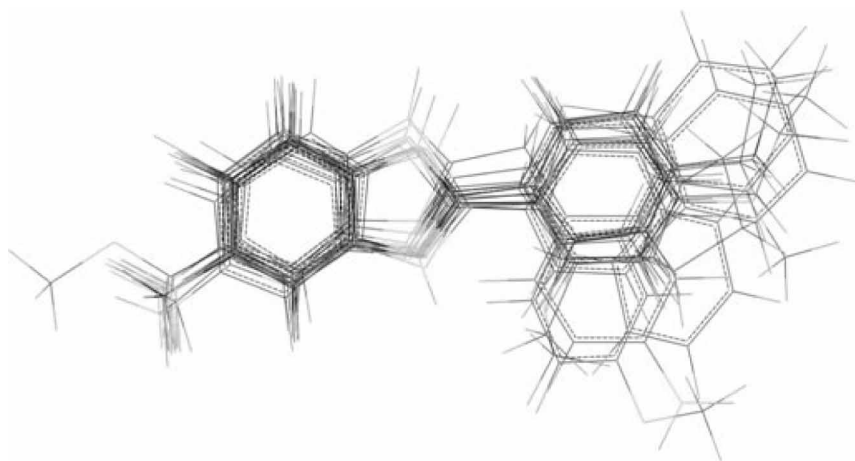
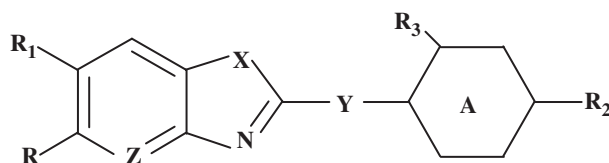


Figure 2. Alignment of the compounds used in the training set of 3D-QSAR analysis.

Table 1. Structures and biological activities of the molecules used in the training set.



Comp.	Z	X	Y	A	R	R ₁	R ₂	R ₃	IC ₅₀ (μM) ^a
1	-CH=	O	-	Phenyl	H	NO ₂	H	OCH ₃	17.0
2	-CH=	O	-	Phenyl	NH ₂	H	C ₂ H ₅	H	115.5
3	-CH=	O	-	Phenyl	CH ₃	H	CH ₃	CH ₃	44.4
4	-CH=	O	-	Phenyl	NO ₂	H	H	H	32.4
5	-CH=	O	-	Phenyl	CH ₃	H	NHCH ₃	H	128.4
6	-CH=	O	-	Phenyl	NO ₂	H	OC ₂ H ₅	H	22.4
7	-N=	O	-	Phenyl	H	H	C ₂ H ₅	H	45.6
8	-N=	O	-	Phenyl	H	H	Cl	H	119.5
9	-N=	O	-	Phenyl	H	H	CH ₃	H	91.2
10	-CH=	O	CH ₂	Phenyl	H	H	OCH ₃	H	86.6
11	-CH=	NH	CH ₂	Phenyl	CH ₃	H	NH ₂	H	46.8
12	-CH=	NH	CH ₂ S	Phenyl	CH ₃	H	H	H	27.4
13	-CH=	NH	CH ₂ S	Phenyl	COOCH ₃	H	H	H	17.0
14	-CH=	NH	CH ₂ O	Phenyl	NO ₂	H	H	H	28.4
15	-CH=	O	-	Cyclohexyl	Cl	NO ₂	H	H	101.9
16	-CH=	NH	C ₂ H ₄	Cyclopentyl	H	H	H	H	216.6

^aTopoisomerase II 50% inhibition activity.

charge, hydrophobicity and hydrogen bond properties of 1 and an attenuation factor of 0.3.

The CoMSIA descriptors were used as independent variables, and $\log(1/C \times 10^{-6})$ values were used as dependent variables in partial least square analysis to derive

3D-QSAR models using the standard implementation in the SYBYL package. The predictive value of the models was evaluated first leave-one-out (LOO) cross-validation. The cross-validated r_{CV}^2 was calculated using equation (2):

$$r_{CV}^2 = 1 - \left[\frac{\sum(Y_{\text{predicted}} - Y_{\text{observed}})^2}{\sum(Y_{\text{observed}} - Y_{\text{mean}})^2} \right] \quad (2)$$

where, $Y_{\text{predicted}}$, Y_{observed} , and Y_{mean} are the predicted, actual, and mean values of the target property $\log(1/C \times 10^{-6})$, respectively. $\sum(Y_{\text{predicted}} - Y_{\text{observed}})^2$ are the predictive residual sum of squares (PRESS). The optimum number of components was chosen which gave less standard error of prediction and more r_{CV}^2 . In addition to the r_{CV}^2 and number of components, the conventional correlation coefficient r^2 and its standard error were also computed.

In this CoMSIA analysis, 16 benzazole analogs as Topo II inhibitors were used as a training set. In addition, 7 compounds, selected from various ranges of Topo II inhibition activity, were kept to test the actual prediction of the model.

Initial PLS [26] analysis was carried out using Leave-One-Out option (cross-validated) to obtain the optimal number of components to be used in the subsequent final analysis. A subset of CoMSIA field sample points falling with a standard deviation of ≤ 2.0 kcal/mol was used to run PLS regression analysis. Finally, non-cross-validated analysis was performed using the optimal number of previously identified components and was employed to analyze the result of CoMSIA.

To validate the derived CoMSIA model biological activities of the test set molecules were predicted using the model derived from training set.

Predictive r^2 values was calculated using equation (3):

$$r_{\text{pred}}^2 = \frac{(\text{SD} - \text{PRESS})}{\text{SD}} \quad (3)$$

where SD is the sum of squared deviation between the biological activities of the test set molecule and the mean activity of the training set molecules and PRESS is the sum of squared deviations between the actual and the predicted activities of the test molecules.

3. Results

The CoMSIA method was employed for deriving a 3D-QSAR model consisting in a training set of 16 benzazole compounds, which included benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives (table 1). As the dependent variable *in vitro* Topo II enzyme inhibitory activities of these screened compounds were investigated with a test system of estimating a non-cleavable complex forming type [13], we suggest to predict the 3D molecular steric, electrostatic, hydrogen-bond acceptor, hydrogen-bond donor, and hydrophobic inhibitory interactions between the analyzed compounds and Topo II enzyme by using CoMSIA method which is a relatively new alternative molecular field analysis method to CoMFA.

The atom-based alignment used in our previously CoMFA study [21] served as alignment for CoMSIA. The best CoMSIA model was obtained from the combination of two fields (i.e., steric and hydrophobic). The statistical parameters of CoMSIA of 16 compounds are summarized in table 2. The leave-one-out (LOO) cross-validated

Table 2. PLS statistics of CoMSIA and previously established CoMFA [21] models.

PLS statistics	CoMSIA	CoMFA
q^2 (Leave-one-out Cross-validated Predicted Power of Model)	0.562	0.435
r^2 (Squared Correlation Coefficient of PLS Analysis)	0.968	0.997
N (Optimum number of components obtained from cross-validated PLS analysis and the same used in final non cross-validated analysis)	4	5
X (Number of descriptors in the PLS after Column Filtering is 2.0 kcal/mol)	228	208
SEE (Standard error of estimate)	0.073	0.024
F value (F-test value)	82.110	597.602
S_{PRESS}	0.267	0.302
Field Contributions		
Steric	35%	32.2%
Electrostatic	–	67.8%
Hydrophobic	65%	–

PLS analysis of the best model gave rise to a cross-validated value (q^2) of 0.562, suggesting that the model is a useful tool for predicting Topo II inhibitory activity [27]. The correlation coefficient between the calculated and experimental activities non cross-validated value (r^2) of 0.968 with standard error 0.073 indicates that the fitness of analyzed results is 96.8% compared to experimental results. The respective relative contributions of steric and hydrophobic fields are 35% and 65%, indicating that hydrophobic field is more predominant.

When the established CoMSIA model is compared with the previously created CoMFA model, the former model reveals better correlations expressed in terms of higher q^2 value than the latter model (table 2). The actual and predicted $\log(1/C \times 10^{-6})$ values of the training set by the best CoMSIA and CoMFA models are given in table 3 and the graph of observed activity *versus* predicted activities of the training set molecules from CoMSIA model is illustrated in figure 3. The established model was validated using a test set (table 4) of 7 compounds, which were not included in the development of the model. Based on the PLS statistics of CoMSIA and CoMFA [21] models; the predicted and residual activity values of the test set molecules are given in table 5. As seen in figure 5 that represents the graph of the actual *versus* predicted $\log(1/C \times 10^{-6})$ values of the test set molecules for the CoMSIA model, a good prediction is obtained for the tested compounds.

4. Discussion

The contour plot representations of CoMSIA results for Topo II inhibitors are presented in figures 4(a), (b), and (c) using the most active compounds **1** and **13** as reference structures. The contour plots are to be considered as a representation of the lattice points, where difference in field values is strongly associated with difference in receptor binding affinity. The absence of lattice points does not indicate that a given substructure element has no influence on the biological activity. It is likely that all the compounds studied exert the same steric and/or hydrophobic influence in a certain area. Though CoMSIA contour maps cannot be used as receptor maps, still they generate many useful interpretations.

Table 3. Actual and predicted biological activities and residuals of the training set compounds used in CoMSIA and previously established CoMFA models.

Comp.	Topoisomerase II inhibition actual activity ^a	CoMFA [21]		CoMSIA	
		Predicted activity	Residuals	Predicted activity	Residuals
1	4.770	4.801	-0.031	4.817	-0.047
2	3.940	3.938	0.002	4.122	-0.182
3	4.350	4.318	0.032	4.335	0.015
4	4.490	4.453	0.037	4.445	0.045
5	3.890	3.926	-0.036	3.903	-0.013
6	4.650	4.656	-0.006	4.658	-0.008
7	4.340	4.325	0.015	4.311	0.029
8	3.920	3.922	-0.002	3.928	-0.008
9	4.040	4.048	-0.008	3.997	0.043
10	4.060	4.056	0.004	4.016	0.044
11	4.330	4.328	0.002	4.401	-0.071
12	4.560	4.578	-0.018	4.541	0.019
13	4.770	4.772	-0.002	4.676	0.094
14	4.550	4.546	0.004	4.561	-0.011
15	3.990	3.968	0.022	3.963	0.027
16	3.660	3.673	-0.013	3.636	0.024

^aTopoisomerase II inhibition activity is expressed as $\log(1/C)$, where C is 10^{-6} of IC_{50} values.

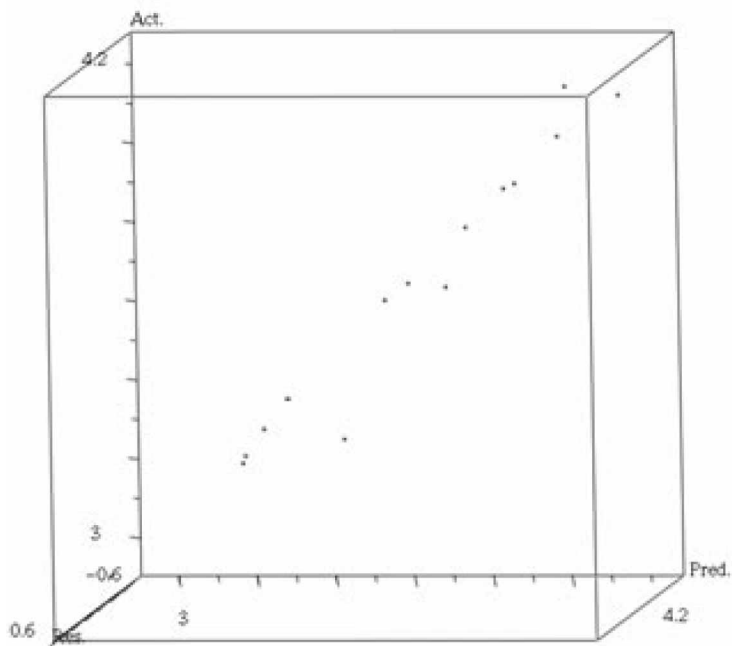
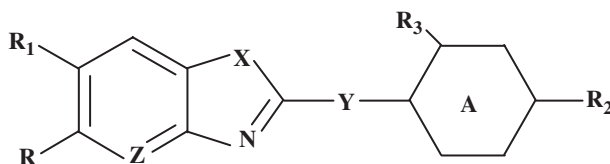


Figure 3. Graph of observed activity versus predicted activities of training set molecules from CoMSIA model, activity expressed as $\log(1/C \times 10^{-6})$.

Table 4. Structures and biological activities of the molecules used in the test set.



Comp.	Z	X	Y	A	R	R ₁	R ₂	R ₃	IC ₅₀ (μ M) ^a
17	-CH=	O	-	Phenyl	H	CH ₃	H	F	433.2
18	-CH=	O	-	Phenyl	H	CH ₃	H	NO ₂	18.8
19	-CH=	O	-	Phenyl	CH ₃	H	H	OCH ₃	433.0
20	-N=	O	-	Phenyl	H	H	C(CH ₃) ₃	H	108.3
21	-CH=	O	CH ₂	Phenyl	CH ₃	H	CH ₃	H	101.9
22	-CH=	S	CH ₂ O	Phenyl	H	H	H	H	11.4
23	-CH=	NH	CH ₂	Cyclohexyl	Cl	H	H	H	308.1

^aTopoisomerase II 50% inhibition activity.

Table 5. Predicted biological activities and residuals of the test set compounds calculated by the CoMSIA and the previously established CoMFA models.

Comp.	Topoisomerase II inhibition actual activity ^a	CoMFA [21]		CoMSIA	
		Predicted activity	Residuals	Predicted activity	Residuals
17	3.363	4.345	-0.982	3.899	-0.536
18	4.726	4.378	0.348	4.688	0.038
19	3.364	4.208	-0.844	3.946	-0.582
20	3.965	4.381	-0.416	4.762	-0.797
21	3.992	4.296	-0.304	4.365	-0.373
22	4.943	4.343	0.600	4.467	0.476
23	3.511	4.285	-0.774	4.338	-0.827

^aTopoisomerase II inhibition activity is expressed as log (1/C), where C is 10⁻⁶ of IC₅₀ values.

As combination of steric and hydrophobic fields gave good statistical results (table 2), this model was used to analyze CoMSIA 3D-plots. In the steric contour maps (figure 4a), areas contoured by green indicate regions favorable for steric occupancy, while areas contoured by yellow indicate the opposite. The green polyhedral in figure 4a represents a preferred occupancy of the pocket of the acceptor. Thus groups of increasing steric bulk in this region will enhance binding affinity and then increase Topo II inhibitory activity.

There are two significant green contours representing the favored steric area to increase the inhibition against the Topo II enzyme that one of them is on the top while another is on the right side of the molecules as seen in figure 4a. If a bulky substituent, such as methoxy group, is attached on *ortho* position of 2-phenyl-5-nitro-benzoxazole (**1**), it will occupy into green contour on the top and will enhance the activity (see figures 4a and 4c). According to the compound **13**, both *meta* and *para* positions of the

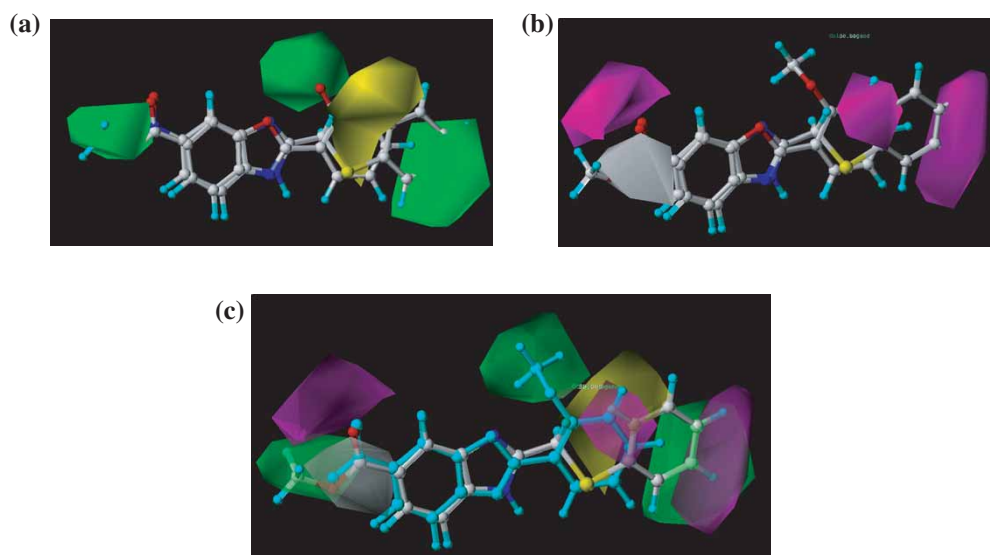


Figure 4. CoMSIA stdev*coeff. steric contour plots with the most active compounds **1** and **13**; (a) Steric map. Green and yellow polyhedral areas indicate regions where more steric bulk or less steric bulk respectively, will increase the activity. (b) Hydrophobic map. Magenta and white polyhedral areas indicate regions where hydrophobic or hydrophilic groups respectively, will enhance the activity. (c) Both steric and hydrophobic contours represented as transparent regions with compounds **1** (cyan) and **13**.

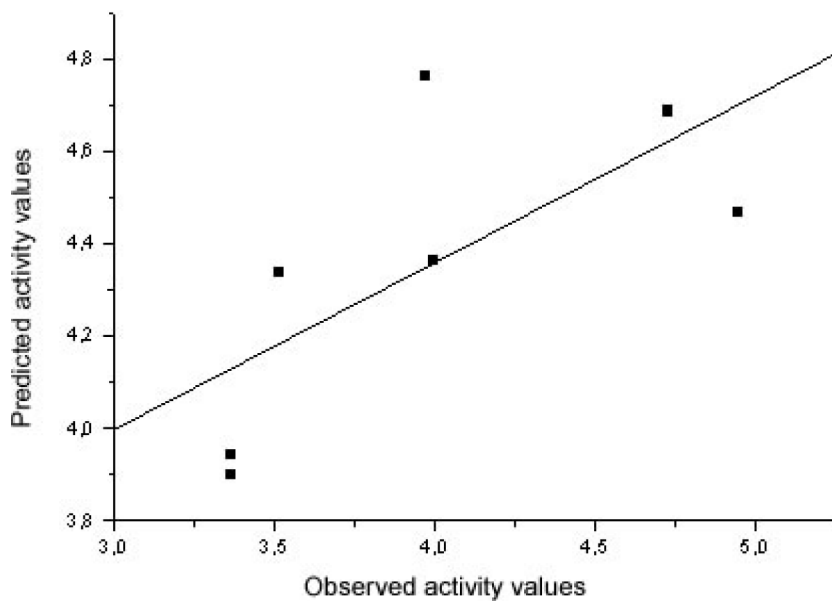


Figure 5. Graphs of actual vs. predicted $\log (1/C \times 10^{-6})$ of test set molecules obtained from CoMSIA model.

phenyl group, which are attached to the 2nd position of benzimidazole ring system, fit into the green contour on the right side and improve the activity. Additionally, the ethoxyl group of compound **6** and the phenyl group of compound **12** occupy the space in green contour like compound **13**, which have high activity as well.

Hydrophobicity is one of the most important properties related to biomolecular interactions [28]. The term hydrophobicity refers to the force or corresponding energy that operates between two or more nonpolar solutes in water and arises from dispersive and electrostatic forces and the consequent entropic factor. A hydrophobic substance is soluble in nonpolar solvents but only sparingly soluble in water. In CoMSIA study, hydrophobic similarity index fields are also constructed and hydrophobic contour maps are shown in figure 4b. In this study, the magenta polyhedral shows that hydrophobic substituents are 'good' for increasing the potency, while hydrophilic substituents are beneficial to the activity at the regions of white contours. There is a magenta area placed phenyl ring of the most active compound **13** on the right side, which means favorable for hydrophobic. The same region has been indicated in the steric maps as seen figure 4a. The phenyl group of another the most active compound **1** also fits into the same magenta area. Besides, white contour on the left side of the molecules, at which are placed a nitro group of compound **1** and carbonyl group of ester moiety **13** (figure 4b), plays a very important role for increasing Topo II inhibitory activity as well. We could say that hydrophilic area is more significant than hydrophobic area to enhance the activity. Because all phenyl rings attached at the 2nd position of benzazole ring system fit into one of the magenta contours on the right side. However, the only compounds **1**, **13**, **6**, **14**, **4**, that have substituents as hydrophilic groups on the position R or R₁, occupy into white area and they have significant inhibitory activities as 17 µg ml⁻¹, 17 µg ml⁻¹, 22 µg ml⁻¹, 28.4 µg ml⁻¹ and 32 µg ml⁻¹ respectively. Similar result was obtained for electrostatic properties of these compounds from our previously studied CoMFA model and this former place was electronegatively charge area in CoMFA model [21]. Therefore, we could consider that either electronegative or hydrophilic substituent on the position R or R₁ of the fused ring system could enhance Topo II inhibitory activity.

In conclusion, the 3D-QSAR analysis using CoMSIA method has been successfully applied to a set of recently synthesized benzazole derivatives. The contour plots provide many useful insights into relationships between structural features and inhibitory activity and also give a picture of the main chemical features responsible for the significant Topo II inhibitory activity. This study in combination with our previously published CoMFA model [21] of the benzazole derivatives are expected to provide rational information for designing new lead compounds showing higher Topo II inhibitory activities.

Acknowledgment

We would like to thank the Ankara University, Biotechnology Institute Grand No: 2001 K-120-240 (110) and Research Fund of Ankara University Grant No: 2005-0803049 for financial support of this study.

References

- [1] J. Wang. *Annu. Rev. Biochem.*, **54**, 665 (1985).
- [2] N.R. Cozzaelli. *Cell*, **22**, 327 (1980).
- [3] N. Osheroff, E.L. Zechiedrich, K.C. Gale. *Bioessays*, **13**, 269 (1991).
- [4] P.M. Patt, I.D. Hickson. *Biochem. J.*, **303**, 681 (1994).
- [5] J.M. Berger, J.C. Wang. *Curr. Opin. Struct. Biol.*, **6**, 84 (1996).
- [6] L.E. Dillehay, D. Jacobson-Kram, J.R. Williams. *Mutat. Res.*, **215**, 15 (1989).
- [7] J.L. Nitiss. *Adv. Pharmacol.*, **29**, 103 (1994).
- [8] T. Uemura, M. Yanagida. *EMBO J.*, **3**, 1737 (1984).
- [9] T. Goto, J.C. Wang. *Cell*, **36**, 1073 (1984).
- [10] A.H. Corbett, N. Osheroff. *Chem. Res. Toxicol.*, **6**, 585 (1993).
- [11] Y. Pommier, F. Leteurtre, M.R. Fesen, A. Fujimori, R. Bertrand, E. Solary, G. Kohlhagen, K.W. Kohn. *Cancer Invest.*, **12**, 530 (1994).
- [12] D.M. Sullivan, M.D. Latham, W.E. Ross. *Cancer Res.*, **47**, 3973 (1987).
- [13] A. Pinar, P. Yurdakul, I. Yildiz, O. Temiz-Arpaci, N.L. Acan, E. Aki-Sener, I. Yalcin. *Biochem. Biophys. Res. Comm.*, **317**, 670 (2004).
- [14] D.J.W. Blum, R.E. Speece. *Environ. Sci. Technol.*, **24**, 284 (1990).
- [15] A. Agarwal, E.W. Taylor. *J. Comput. Chem.*, **14**, 237 (1993).
- [16] K. Hasegawa, M. Arakawa, K. Funatsu. *Chemom. Intell. Lab. Syst.*, **50**, 253 (1999).
- [17] H. Liu, M. Ji, H.L. Jiang. *Bioorg. Med. Chem. Lett.*, **10**, 2153 (2000).
- [18] D.R. Cramer, D.E. Paterson, J.D. Bunce. *J. Am. Chem. Soc.*, **110**, 5959 (1988).
- [19] G. Klebe, U. Abraham, T. Mietzner. *J. Med. Chem.*, **37**, 4130 (1994).
- [20] G. Klebe, U. Abraham. *J. Comput. Aided Mol. Des.*, **13**, 1 (1999).
- [21] O. Temiz-Arpaci, B. Tekiner-Gulbas, I. Yildiz, E. Aki-Sener, I. Yalcin. *Bioorg. Med. Chem.*, **13**, 6354 (2005).
- [22] N. Osheroff, E.R. Shelton, D. Brutlag. *J. Biol. Chem.*, **258**, 9536 (1983).
- [23] M. Bohm, J. Sturzebecher, G. Klebe. *J. Med. Chem.*, **42**, 458 (1999).
- [24] T. Hou, Y. Li, N. Liao, X. Xu. *J. Mol. Model.*, **6**, 438 (2000).
- [25] Sybyl 7.0, Tripos Inc., St. Louis, USA.
- [26] P. Geladi. *J. Chemom.*, **2**, 231 (1998).
- [27] A. Agarwal, P.P. Pearson, E.W. Taylor, H.B. Li, T. Dahlgren, M. Herslof, Y. Yang, G. Lambert, D.L. Nelson, J.W. Regan. *J. Med. Chem.*, **36**, 4006 (1993).
- [28] N. Baurin, E. Vangrevelinghe, L. Morin-Allory, J.Y. Merour, P. Renard, M. Payard, G. Guillaumet, C. Marot. *J. Med. Chem.*, **43**, 1109 (2000).