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3D-QSAR analysis on benzazole derivatives as eukaryotic topoisomerase II inhibitors by using comparative molecular field analysis method

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Abstract—Selective topoisomerase II inhibitors have created a great deal of interest in recent years for the design of new antitumoral compounds. 3D-QSAR analysis has been performed on a series of previously synthesized benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives, which are screened as eukaryotic topoisomerase II inhibitors, using comparative molecular field analysis (CoMFA) with partial least squares fit to predict the steric and electrostatic molecular field interactions for the activity. The CoMFA study was carried out using a training set of 16 compounds. The predictive ability of the model was assessed using a test set of 7 compounds. The analyzed 3D-QSAR CoMFA model has demonstrated a good fit, having r^2 value of 0.997 and cross-validated coefficient q^2 value as 0.435 for the model. The obtained model reveals that the electronegatively charged substituents such as NO_2 or $COOCH_3$ group on position R and/or R_1 at the heterocyclic ring system and positively charged atom and/or atom groups located between the benzazole moiety and 2-substituted phenyl ring as a bridge element improve the activity. On the other hand, a bulky substituent, such as methoxy group, attached to the ortho position of 2-phenyl-5-nitro-benzoxazole (**1**) enhances the activity similar to compound **13**, which is both a meta and para substituent of the phenyl group attached to the 2-position of benzimidazole ring system, fit into the favored steric region to improve the activity.

1. Introduction

DNA topoisomerase II (Topo II) is a nuclear enzyme in mammalian cells that interconvert topological isomers of DNA by breaking and resealing phosphodiester bonds. Topo II modifies the DNA linking in two steps and it is able to relax the supercoiled form of a circular, closed double-stranded DNA molecule in the presence of an energetic cofactor such as ATP.[1]

A number of anticancer drugs that specifically inhibit eukaryotic Topo II have been described. Their antitumor activity is related to the formation of protein-concealed DNA strand breaks, resulting in the stabilization by the drug of an intermediary complex of the Topo II reaction,[2] and these drug-induced cleavable complexes have been proposed to be the primary action responsible for the antitumor activity.

Inhibitor effects of some novel fused heterocyclic compounds such as benzoxazole, benzimidazole, benzothiazole, and oxazolo(4,5-b)pyridine derivatives on eukaryotic Topo II were investigated.[3] Activity was assayed electrophoretically 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 currently acknowledged to be at the heart of the long-term task of systematically evaluation of existing chemicals.[4] Currently, 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. And the comparative molecular field analysis (CoMFA) is in keeping with the general pattern of searching

Keywords: 3D-QSAR; CoMFA; Topoisomerase II inhibitors; Benzoxazoles; Benzimidazoles; Benzothiazoles; Oxazolo(4,5-b)pyridines.
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three-dimensional (3D) descriptors, where steric and electrostatic fields of the molecule are mapped by a probe atom.[5–7] CoMFA, which is applied to a set of molecules exhibiting biological activity with a similar mechanism of action, was proposed by Cramer et al. in 1988.[8] The advantages of CoMFA are the abilities 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, which give the key features on not only the ligand–receptor interaction but also the topology of the receptor.

We present here the 3D-QSAR studies using CoMFA method on a training set of benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives as eukaryotic Topo II inhibitors by considering the steric and electrostatic influences. The model deduced from this investigation provides underlying structural requirements and good predictive ability, which could aid new Topo II inhibitors prior to their synthesis.

2. Computational methods

2.1. Molecular modeling

Three-dimensional structure building and all modeling were performed using the SYBYL program package, version 7.0[9] 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 by 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. CoMFA models were generated using 16 molecules (**1**–**16**; Table 1), with column-filtering value (σ) of 2.0.

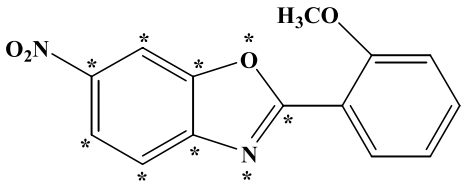


Figure 1. Molecule **1** with atoms used for superimposition are marked.

2.2. CoMFA analysis

The CoMFA studies for Topo II inhibitors were run on a Silicon Graphics workstation using SYBYL 7.0 molecular modeling software from Tripos Inc. (St. Louis, MO, USA). CoMFA of these molecules was carried out on the steric and electrostatic fields using the default values. A 3D cubic lattice, with a 2 Å grid spacing, was generated automatically around these molecules to ensure that the grid extended the molecular dimensions by 4 Å in all directions. A threshold column filtering of 2.0 kcal/mol was set to hasten the analysis and reduce the amount of noise. The steric and electrostatic fields were calculated separately for each molecule using sp^3 carbon atom probe with a charge of 1 (default probe atom in SYBYL) and energy cut-off values of 30 kcal/mol for both steric and electrostatic fields. The probe atom was placed at each lattice point, and their steric and electrostatic interactions with each atom in the molecule were computed using the CoMFA standard scaling.

2.3. Partial least squares (PLS) analysis

Initial PLS analysis was carried out using leave-one-out (LOO) option (cross-validated) to obtain the optimal number of components to be used in the subsequent final analysis. A subset of the CoMFA field sample points falling with a standard deviation of $\leqslant$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 the CoMFA.

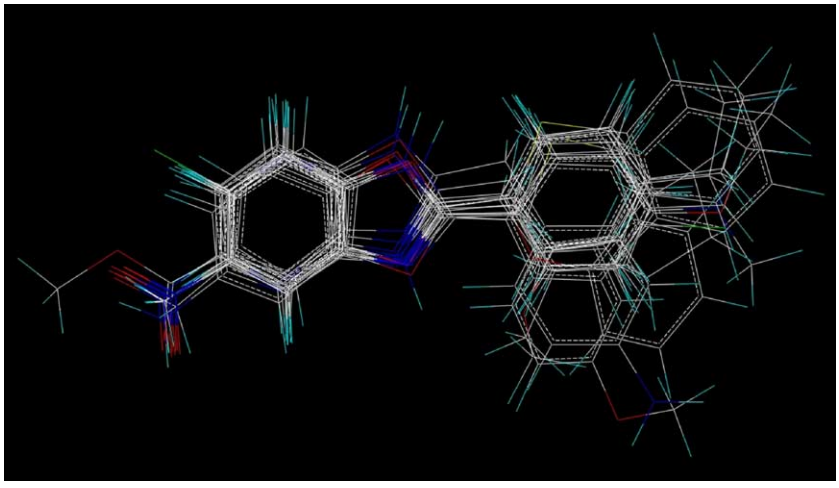
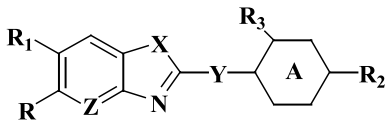


Figure 2. Alignment of the compounds used in the training set of 3D-QSAR analysis.

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



Compound	Z	X	Y	A	R	R_1	R_2	R_3	IC_{50} (μM)
1	–CH=	O	—	Phenyl	H	NO_2	H	OCH_3	17.0
2	–CH=	O	—	Phenyl	NH_2	H	C_2H_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_2H_5	H	22.4
7	–N=	O	—	Phenyl	H	H	C_2H_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_2S	Phenyl	CH_3	H	H	H	27.4
13	–CH=	NH	CH_2S	Phenyl	$COOCH_3$	H	H	H	17.0
14	–CH=	NH	CH_2O	Phenyl	NO_2	H	H	H	28.4
15	–CH=	O	—	Cyclohexyl	Cl	NO_2	H	H	101.9
16	–CH=	NH	C_2H_4	Cyclopentyl	H	H	H	H	216.6

3. Results and discussion

The CoMFA method was employed for deriving a 3D-QSAR model consisting of a training set of 16 benzazole compounds, which consists of benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives (Table 1), keeping in vitro activity log 1/C as a dependent variable. As the dependent variable in vitro Topo II enzyme inhibitory activities of these screened compounds was investigated with a test system of estimating a noncleavable complex-forming type,[3] we suggest to predict the 3D molecular steric and electrostatic inhibitory interactions between the analyzed compounds and Topo II enzyme by using the well-known CoMFA method.

The statistical parameters of CoMFA analysis of 16 compounds are summarized in Table 2. The leave-one-out cross-validated PLS analysis of the best model gave rise to a cross-validated value (q^2) of 0.435, suggesting that the model is a useful tool for predicting Topo II inhibitory activity.[10] The correlation coefficient between the calculated and experimental activities, non-cross-validated value (r^2) of 0.997 with standard error 0.024, indicates that the fitness of analyzed results is 99.7% compared to experimental results. The respective relative contributions of steric and electrostatic fields were 32.2% and 67.8%, indicating that electrostatic field is more predominant. The actual and predicted values of the training set by the best model are given in Table 3, and the graph of observed activity versus predicted activities of training set molecules from CoMFA analysis is illustrated in Figure 3. The 3D-QSAR model was validated using a test set (Table 4) of 7 compounds, which were not included in the development of the model. On the basis of the PLS statistics of CoMFA model, this is further validated by the residual values of the test set (Table 5). Figure 7 represents the graph of the actual versus predicted log 1/C values of the test set molecules for CoMFA model.

Table 2. PLS statistics of CoMFA 3D-QSAR model

PLS statistics	CoMFA
q^2 (leave-one-out cross-validated predicted power of model)	0.435
r^2(correlation coefficient squared of PLS analysis)	0.997
N (optimum number of components obtained from cross-validated PLS analysis and the same used in final non cross-validated analysis)	5
X (number of descriptors go into the PLS after column filtering is 2.0 kcal/mol)	208
Standard error of estimate (SEE)	0.024
F-test value (*F*-value)	597.602
S_{Press}	0.302
Field contribution (steric and electrostatic fields from CoMFA)	
Steric	32.2%
Electrostatic	67.8%

The contour plot representations of CoMFA results for Topo II inhibitors are presented in Figures 4–6 using compounds **1** and **13** as reference structures. The green-colored regions indicate areas where steric bulk enhances Topo II inhibitory activity, while the yellow contours indicate regions where steric bulk is detrimental for the biological activity. Blue-colored regions show areas where electropositive charged groups enhance Topo II inhibitory activity, while red regions represent where electronegative charged groups improve the activity. There are two significant green contours representing the favored steric area to increase the inhibition against the Topo II that one of them is on the top and another is on the left side of the molecule as seen in

Table 3. Actual and predicted biological activities and residuals of the training set compounds by the CoMFA model

Comp.	Topoisomerase II inhibition actual activity[a]	CoMFA predicted activity	Residuals
1	4.770	4.801	−0.031
2	3.940	3.938	0.002
3	4.350	4.318	0.032
4	4.490	4.453	0.037
5	3.890	3.926	−0.036
6	4.650	4.656	−0.006
7	4.340	4.325	0.015
8	3.920	3.922	−0.002
9	4.040	4.048	−0.008
10	4.060	4.056	0.004
11	4.330	4.328	0.002
12	4.560	4.578	−0.018
13	4.770	4.772	−0.002
14	4.550	4.546	0.004
15	3.990	3.968	0.022
16	3.660	3.673	−0.013

[a] Topoisomerase II inhibisyon activity is expressed as log 1/C.

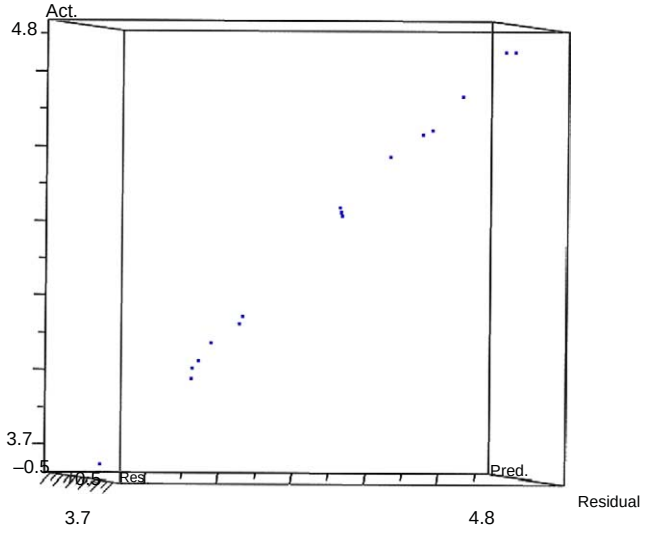
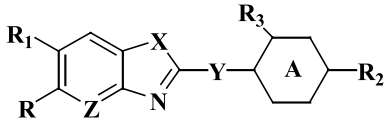


Figure 3. Graph of observed activity versus predicted activities of training set molecules from CoMFA analysis; activity expressed as log 1/C.

Figure 4. If a bulky substituent, such as methoxy group, is attached on ortho position of 2-phenyl-5-nitro-benzoxazole (**1**), it occupies the green contour on the top and enhances the activity (Fig. 4A). According to compound **13**, both meta and para substituents of the phenyl group, which are attached to the 2-position of benzimidazole ring system, fit into the green contour on the left side and improve the activity (Fig. 4B). Additionally, the ethoxy group of compound **6** and the phenyl group of compound **12** occupy the space in green contour like compound **13**, which has high activity as well. However, compound **10** has lower activity than the former structures because the OCH_3 group on the para position of phenyl ring is very close to the yellow contour, which is representing the unfavored steric region.

The electrostatic contour map in Figure 5 showed a region of red polyhedral counter, indicating that the electron-rich groups are beneficial to the activity. The nitro group of compound **1** and the ester group of compound **13** at the positions R_1 and R, respectively, occupy the red contour to enhance the activity. Additionally, the negatively charged atoms and/or atom groups on position R at benzazole ring of compounds **4**, **6**, **14**, and **15** also take part in this red-colored region. Besides compound **1** holding a NO_2 group on the position R_1, compound **13** with a substituent $COOCH_3$ and compounds **6**, **14**, and **4** having a NO_2 group on the position R have significant inhibitory activities as 17, 17, 22, 28.4, and 32 μg/ml, respectively, showing higher activities than compound **2** (115.5 μg/ml), which is holding NH_2 substitution on the same position, because the former groups fit into the electronegatively charged red contour while the later group protrudes out of this region. Although the nitro group on position R_1 of compound **15** fits into the red contour, it has lower activity than **4**, **6**, and **14**, because the chlorine atom at the position R takes part with the electropositively charged blue contour. The blue region between the benzazole moiety and the phenyl group in the electrostatic contour map suggests that holding a positively charged atom and/or atom groups on this region will increase the activity, which is consistent with the fact that molecule **13**, having a thiomethylene bridge group, gives rise to higher inhibition activity than the others (Fig. 5B).

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



Compound	Z	X	Y	A	R	R_1	R_2	R_3	IC_{50}
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_2O	Phenyl	H	H	H	H	11.4
23	–CH=	NH	CH_2	Cyclohexyl	Cl	H	H	H	308.1

Table 5. Actual and predicted biological activities and residuals of the test set compounds by the CoMFA-model

Comp.	Topoisomerase II inhibition actual activity[a]	CoMFA predicted activity	Residuals
17	3.363	4.345	−0.982
18	4.726	4.378	0.348
19	3.364	4.208	−0.844
20	3.965	4.381	−0.416
21	3.992	4.296	−0.304
22	4.943	4.343	0.600
23	3.511	4.285	−0.774

[a] Topoisomerase II inhibisyon activity is expressed as log 1/C.

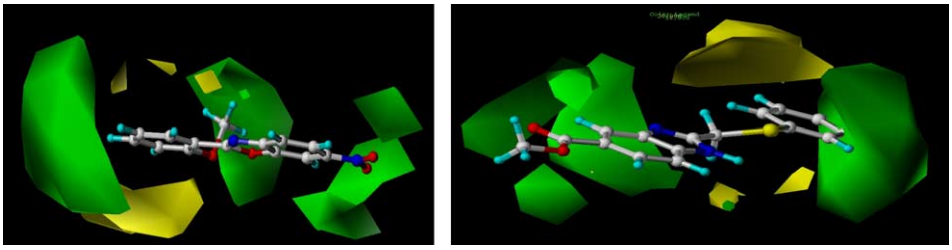


Figure 4. CoMFA standard deviation coefficient steric contour plots with compounds **1** and **13**, respectively; green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity.

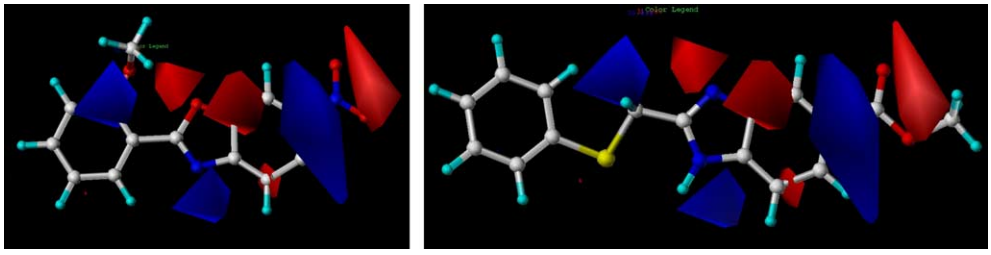


Figure 5. CoMFA standard deviation coefficient electrostatic contour plots with compounds **1** and **13**, respectively; blue contours indicate regions where positively charged groups increase activity, whereas red contours indicate regions where negative charge increase activity.

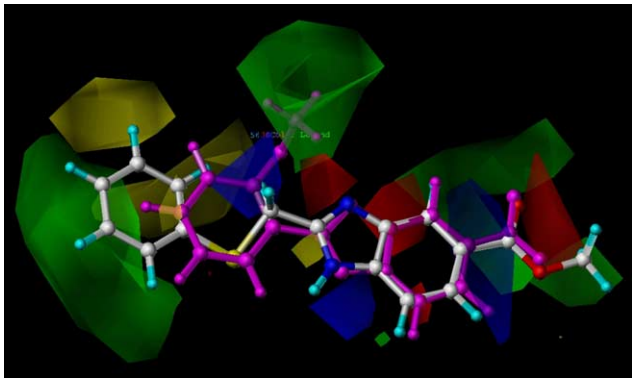


Figure 6. CoMFA standard deviation coefficient steric and electrostatic contours represented as transparent regions with compounds **1** (magenta) and **13**.

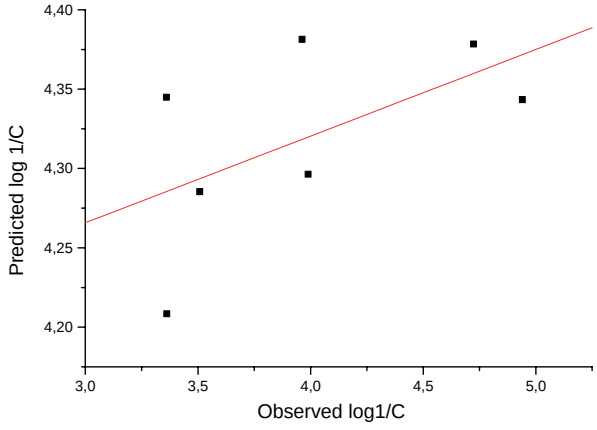


Figure 7. Graphs of actual versus predicted log 1/C of test set molecules obtained from CoMFA model.

In conclusion, the 3D-QSAR analysis using CoMFA 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. These features could be used to design new lead compounds showing higher inhibitory activities.

Acknowledgments

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