3D-QSAR Analysis on Benzazole Derivatives as Eukaryotic Topoisomerase II Inhibitors by using Comparative Molecular Field Analysis Method

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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].

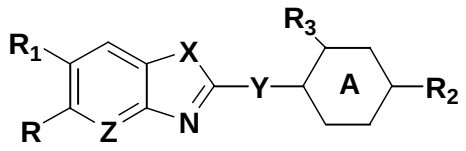
A number of anticancer drugs that specifically inhibit eukaryotic Topo II have been described. Their antitumor activity is related to the formation of protein-concealed DNA strand breaks, resulting in the stabilization by the drug of an intermediary complex of the Topo II reaction [2] and these drug-induced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

Inhibitor effects of some novel fused heterocyclic compounds such as benzoxazole, benzimidazole, benzothiazole, and oxazolopyridine 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.

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 [4]. 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.

Molecular Modelling

Three-dimensional structure building and all modeling were performed using the SYBYL program package, version 7.0 [5] 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 was used as the template for alignment by considering the heavy atoms of the 2-phenylbenzoxazole ring. All values were filled with valence, and Gasteiger charges were calculated for each compound. CoMFA models were generated using 16 molecules (Fig. 1), with column-filtering value (σ) of 2.0.



Z = CH, N; **X** = O, NH; **Y** = -, CH_2, CH_2S, CH_2O, C_2H_4
A = Phenyl, Cyclohexyl, Cyclopentyl; **R** = H, NH_2, CH_3, NO_2, Cl, $COOCH_3$
$\mathbf{R_1}$ = H, NO_2; $\mathbf{R_2}$ = H, Cl, NH_2, CH_3, C_2H_5, OCH_3, OC_2H_5, $NHCH_3$; $\mathbf{R_3}$ = H, CH_3, OCH_3

Fig. 1. Structure of the training set compounds.

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 (Fig. 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 inhitory interactions between the analyzed compounds and Topo II enzyme by using the well-known CoMFA method [4].

The statistical parameters of CoMFA analysis of training set compounds are found as R^2= 0.997, s= 0.024, F Value=597.602, Q^2=0.435, S_{PRESS} =0.302 and showing a field contributions of steric (32.2%), electrostatic (67.8%). The graph of observed activity versus predicted activities of training set molecules from CoMFA analysis is illustrated in Fig. 2. The 3D-QSAR model was validated using a test set 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. model.

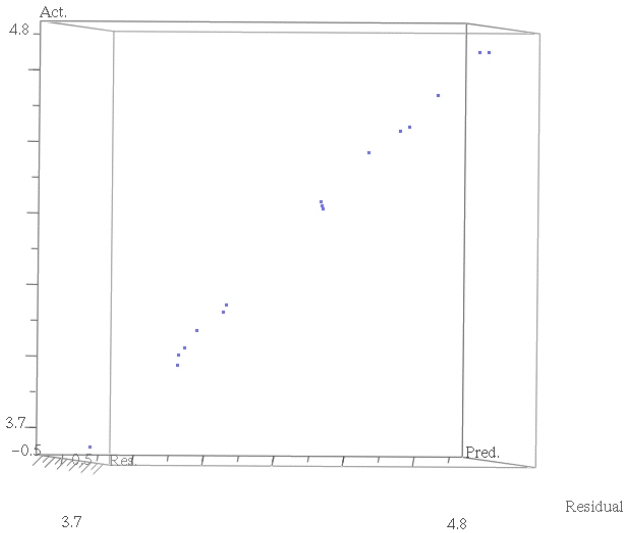


Fig. 2. Graph of observed activity versus predicted activities of training set molecules from CoMFA analysis, activity expressed as log 1/C

The contour plot representations of CoMFA results for Topo II inhibitors are presented in Fig. 3 and 4 using compounds **1** and **2** as reference structures. 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 Fig. 3. If a bulky substituent, such as methoxy group, is attached on ortho position of 2-phenyl-5-nitro-benzoxazole (**1**), it occupies into green contour on the top and enhances the activity (Fig. 3A). According to compound **2,** 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. 3B).

The electrostatic contour map in Fig. 4 showed the nitro group of compound **1** and the ester groups of compound 2 at the positions R1 and R, respectively, occupy the red contour to enhance the activity. 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 **2,** having a thiomethylene bridge group, gives rise to higher inhibition activity than the others (Fig. 4B).

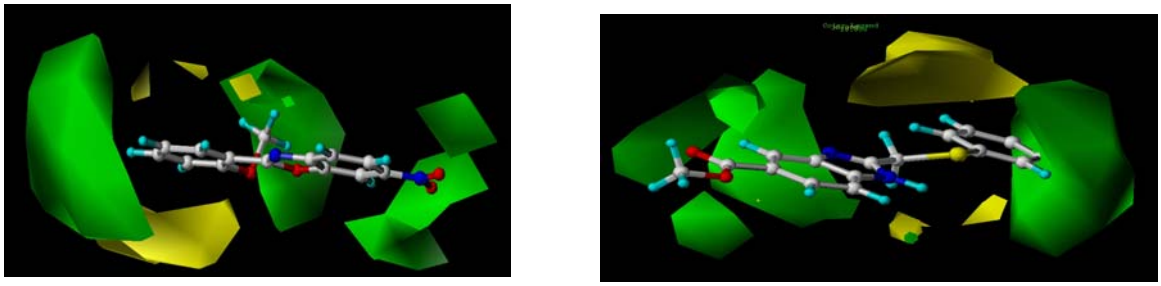


Fig. 3. CoMFA standard deviation coefficient steric contour plots with compounds **1** and **2**, respectively; green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity.

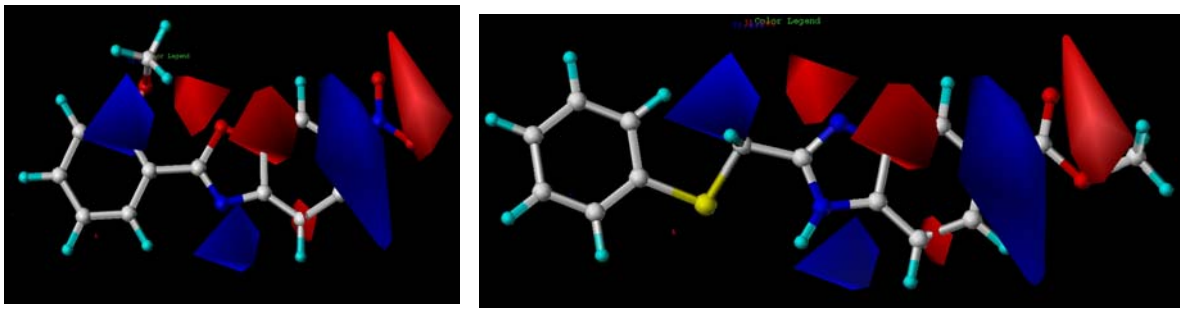


Fig. 4. CoMFA standard deviation coefficient electrostatic contour plots with compounds **1** and **2**, respectively; blue contours indicate regions where positively charged groups increase activity, whereas red contours indicate regions where negative charge increase activity.

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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References

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