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Short communication

DNA topoisomerase inhibitory activity and 3D-QSAR analysis of benzazoles

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Introduction

DNA topoisomerases (Topo) are enzymes that isomerise the tertiary structure of DNA without changing its primary structure. The high degree of conservation of these enzymes among prokaryotes and eukaryotes indicates an essential role in cell biology. Because its structure is a double helix, DNA is under tortional stress that results in multiplex twisting of the molecule. To be processed for replication or gene expression, the supercoiled DNA must become accessible to nucleic acid polymerases or components of the transcription machinery. This change requires relaxation and untangling of the intertwined DNA strands, which are the typical tasks of Topo (Hande, 2003).

In humans, two classes of Topo are well characterized, type I and type II. Topo type II (Topo II) are useful as drug target, since they have an indispensable function in cell biology and they lack biological redundancy. Inhibitors of these enzymes have become central parts of both primary and adjuvant chemotherapy regimens in neoplastic diseases, and they probably will remain so for the foreseeable future.

Classical Topo II inhibiting agents such as epipodophyllotoxins or anthracyclines interfere with the breakage-reunion reaction of Topo II by stabilizing this cleavable complex. The stabilization of the cleavable complex and not the inhibition of the Topo II activity is supposed to play the decisive role in the cytotoxic effect of the classical Topo II interacting agents. Accordingly, resistance against classical Topo II-inhibiting agents can result from any process that leads to an altered binding of Topo II to drugs or DNA and a reduced formation of cleavable complexes. Indeed, it was demonstrated that decreased Topo II catalytic activity can mediate drug resistance to cancer cells (Beck et al., 2001).

Because of Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies, detailed investigations of bi- and ter-benzimidazole derivatives revealed that these compounds constitute a new class of Topo I and II inhibitors. Work on such compounds indicates that a fused ring system in the structure is critical for the activity.

In the past years, we synthesized several derivatives of benzazoles, such as benzoxazoles, benzimidazoles, benzothiazoles, and oxazolo(4,5-b)pyridines as isosteric fused heterocyclic compounds to investigate their eukaryotic DNA Topo II inhibitory activity (Pinar et al. 2004) and realized their three dimentional quantitative structure activity relationships (3D-QSAR) analysis by using comparative molecular similarity indices analysis (COMSIA) method (Tekiner-Gulbas et al., 2006).

A training set of 37 compounds of benzazoles, which are possessing benzoxazole, benzimidazole, benzothiazole, and oxazolo(4,5-b)pyridine fused heterocylic nucleus at their structure, were tested for their eukaryotic DNA Topo II inhibitor activity in cell-free system by using relaxation assay. The relaxation assay utilises supercoiled plasmid as substrate and has been used by many investigators to study the catalytic activity of Topo I and II types. Inhibitory activities were presented as micromolar concentrations of the compounds that cause 50% inhibition per unit of enzyme (IC50), under the assay conditions. From the plots obtained with three different concentrations of the drugs, IC50 values were obtained and the results are the averages of two to three estimations. If no inhibition was obtained at 100 μM, the drug was assumed to have no inhibitory activity on eukaryotic DNA Topo II (Pinar et al., 2004).

For the 3D-QSAR studies performed by using CoMSIA methods running the SYBYL program package with

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the IRIX 6.5 operating system of a training set of benzoxazole, benzimidazole, and oxazolo(4,5-b)pyridine derivatives, which were their eukaryotic Topo II inhibitory activities observed in cell-free system by using relaxation assay, to assume the predictions for their structure activity relationships (Tekiner-Gulbas et al., 2006).

From among the training set of 37 compounds, 28 benzazole derivatives were found to be able to inhibit the eukaryotic DNA Topo II in cell-free system at an initial concentration of 100 μg/ml. These 28 compounds were further tested at a lower range of concentrations to define their inhibitory activity and etoposide was used as the standard drug in order to compare their activity. Of these 28 compounds, 12 derivatives had IC50 values between 11.4 and 46.8 μM range and they were considered as positive Topo II inhibitors. Among these compounds, 2-phenoxymethylbenzothiazole, 6-nitro- 2-(2-methoxyphenyl)benzoxazole, 5-methylcarboxylate-2-phenylthiomethylbenzimidazole, and 6-methyl-2-(2-nitrophenyl)-benzoxazole were found as more active than the reference drug etoposide. Moreover, 5-nitro-2-(4-ethoxyphenyl)benzoxazole, 5-(4-fluorophenyl-carboxyamide)-2-phenylbenzoxazole), 5-methyl-2-phenylthiomethylbenzimidazole, and 5-nitro-2-phenoxymethylbenzimidazole had Topo II inhibitory activities comparable to etoposide.

The results indicate that either having sterically bulky substituents such as phenylacetamide or phenoxyacetamide groups at position 5 or holding a non-aromatic moiety as cyclohexyl or cyclopentyl rings and/or a pyridine ring at position 2 of the fused heterocyclic nucleus causes a severely reduced or lack of activity. On the other hand, different fused heterocyclic nuclei in the structures of the most potent Topo II inhibitors are indicating bioisosteric properties for the enzyme inhibitory activity (Pinar et al., 2004).

For the 3D-QSAR studies, the best performed CoMSIA model was obtained from the combination of two fields (i.e., steric and hydrophobic). The LOO cross-validated PLS analysis of the best model gave rise to a cross-validated value (q2) of 0.562, suggesting that the model is a useful tool for predicting Topo II inhibitory activity. The correlation coefficient between the calculated and experimental activities non crossvalidated value (r2) 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. The established model was validated using a test set of compounds, which were not included in the development of the model (Tekiner-Gulbas et al., 2006).

There are two significant contours representing the favored steric area to increase the inhibition against the Topo II enzyme. If a bulky substituent, such as methoxy group, is attached on ortho position of 2-phenyl-5-nitro-benzoxazole, it will occupy into favorable for steric contour and will enhance the activity. According to the 5-methylcarboxylate-2-phenylthiomethylbenzimidazole, both meta and para positions of the phenyl group, which are attached to the 2nd position of benzimidazole ring system, fit into the favorable for steric contour and improve the activity.

In CoMSIA study, hydrophobic similarity index fields are also constructed and an area found, which is placed on phenyl ring of the most active compound 5-methylcarboxylate-2-phenylthiomethylbenzimidazole, means favorable for hydrophobic. The phenyl group of another the most active compound 2-phenyl-5-nitro-benzoxazole also fits into the same favorable area. On the other side, nitro group of 2-phenyl-5-nitro-benzoxazole and carbonyl group of ester moiety of 5-methylcarboxylate-2-phenylthiomethylbenzimidazole play a very important role for increasing Topo II inhibitory activity. 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 favorable hydrophobic contours.

In conclusion, the results point out that benzimidazole, benzoxazole, benzothiazole, and/or oxazolopyridine derivatives also exhibit significant Topo II inhibitory activity and may provide advanced opportunities to design and develop new chemotherapeutic agents.Furthermore, the observed COMSIA contour plots provide many useful insights into relationships between structural features and inhibitory activity for these benzazole derivatives.

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