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Theoretical Studies for Molecular Modeling of New Camptothecin Analogues*

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Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin: CPT-11) is a widely used potent antitumor drug that is developed based on camptothecin. However, overexpression of ABCG2 (BCRP/MXR/ABCP) confers cancer cells resistance to SN-38, that is, the active metabolite of irinotecan. In the present study to develop a platform for the molecular modeling to circumvent cancer drug resistance associated with ABCG2, we have characterized a total of fourteen new SN-38 analogues by some typical properties, which were evaluated by molecular orbital (MO) calculations and neural network (NN) QSAR technique.

INTRODUCTION

Irinotecan (CPT-11: 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is a potent camptothecin-based anti-cancer drug. Camptothecin (CPT) has been gathered much attention, since it shows wide-spectrum anti-tumor activity against human cancer cells. Irinotecan was developed as a new water-soluble and safer anti-cancer drug based on camptothecin.[1,2] Since the lactone moiety of irinotecan (E-ring in Figure 1) is critically required for the anti-tumor activity, SN-38 that is an active metabolite of irinotecan conserves the lactone moiety. SN-38 lactone is in thermal equilibrium with carboxylate form of it within the cell. Molecular structures of irinotecan and lactone form of SN-38 are shown in Figure 1.

ABCG2 is the human ATP-binding cassette transporter, which was originally named Breast Cancer Resistant Protein (BCRP), since it was discovered in doxorubicin-resistant breast cancer cells.[3] It is also called MXR or ABCP. Recently, it has been shown that overexpression of ABCG2 confers drug resistance to CPT analogues.[4–6] Accumulating evidences suggest that ABCG2 actively transports both forms of SN-38 from the cancer cells and thereby confers resistance to camptothecin-based anti-cancer drugs.

To overcome and to circumvent ABCG2-associated drug resistance to SN-38, many efforts have been made. For instance, a total of 14 new analogues of SN-38 were synthesized for evaluation.[7] New 14 analogues have various pairs of substituents at the opposite (X- and Y-)

* Dedicated to Professor Haruo Hosoya in happy celebration of his 70th birthday.

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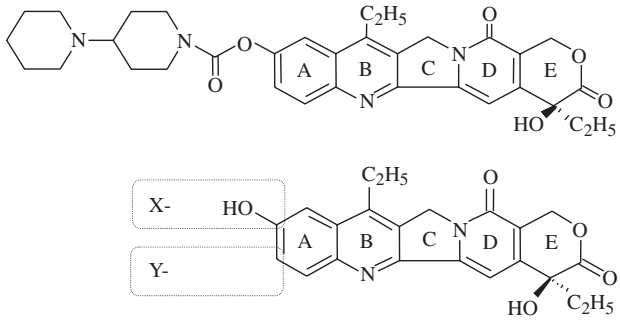

SN-38 analogues	Structure	
	X	Y
SN-22	H	H
SN-38	OH	H
SN-343	CH_3	H
SN-348	Br	H
SN-349	Cl	H
SN-351	H	Br
SN-352	H	Cl
SN-353	H	F
SN-355	H	OH
SN-364	Cl	Cl
SN-392	NH_2	H
SN-397	OCH_3	F
SN-398	OH	F
SN-443	CH_3	F
SN-444	F	F

Figure 1. Molecular structures of irinotecan (CPT-11), the active metabolite SN-38, and SN-38 analogues. ABCG2 transports SN-38 out of cells in an ATP-dependent manner.

positions of the lactone ring (E-ring in Figure 1), since the lactone moiety plays a crucial role in anti-cancer activity. The molecular structures of analogues are also illustrated in Figure 1. Since 3D structure of ABCG2 has not revealed yet, we have characterized SN-38 and its analogues by some typical properties.

ABCG2-associated drug resistance profiles of SN-38 and its derivatives were already determined by MIT assay method using ABCG2-transfected HEK293 cells and control HEK293 cells (Figure 2).[8] The drug resistance ratios (DRRs) were calculated as the ratio of IC_{50} of ABCG2--transfected HEK293 cells to IC_{50} of control cells, where the IC_{50} value is concentration representing a 50% reduction of cell growth. ACBG2-transfected cells were resistant to SN-38, 355, 392, and 398, suggesting that these compounds are substrates for ABCG2.

In this paper, we discuss the critical features for ABCG2-associated transport and recognition by ABCG2 to SN-38 and its analogues, based on the typical properties of compounds observed in theoretical approaches.

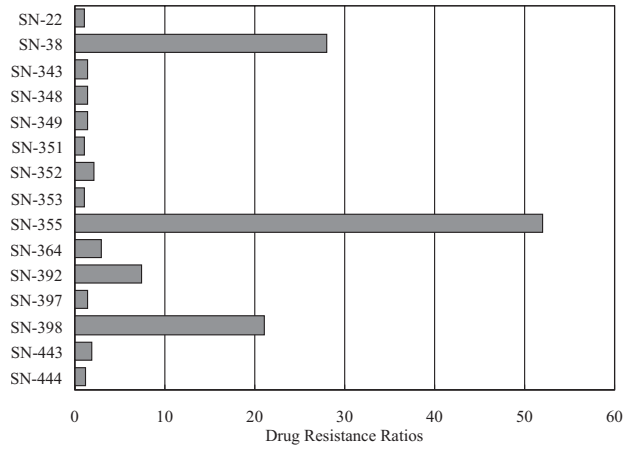

Figure 2. Drug resistance ratios (DRRs) of SN-38 and its analogues determined by MIT assay. The drug resistance ratios (DRRs) were calculated as the ratio of IC_{50} of ABCG2-transfected HEK293 cells to IC_{50} of control cells.

COMPUTATIONAL

Molecular structures of all analogues were firstly constructed by molecular mechanics (MM2) procedures. Geometry optimizations by *ab initio* MO calculations at the restricted Hartree-Fock (RHF) level with MIDI-4 basis set[9] were used for refinement of the structures prior to the analysis of electrostatic properties. Electrostatic isopotential surfaces were generated by single-point energy calculations at RHF level with MIDI-4 and polarization functions as a basis set. All *ab initio* MO calculations were carried out with the program package AMOSS that is developed by NEC quantum chemistry group.

Since it is supposed that hydrogen-bond formation contributes to drug transport by ABCG2 from the results of drug resistance profiles, the relationship between hydrophobicity or hydrophilicity and drug resistance of SN-38 analogues are examined. We evaluated the solvation free energies (ΔG) and hydrophobic parameters (Log*P*) using quantum mechanical method and neural network analysis, respectively.

The ΔG value was defined as $\Delta G = E(\text{COSMO}) - E(\textit{in vacuo}) + \text{SASA}*0.00542 + 0.92$, where E(COSMO), E(*in vacuo*), and SASA are total energies of the SN-38 analogues evaluated by semi-empirical MO calculations using AM1 Hamiltonian[10] with conductor-like screening model (COSMO)[11] as solvent effects, AM1 *in vacuo*, and the solvent accessible surface area,[12] respectively. All semi-empirical MO calculations were archived with MOPAC program available in the computer-aided chemistry modeling package CAChe developed by Fujitsu.

As hydrophobic profiles, we also examined Log*P* by quantitative structure-activity relationship (QSAR) analysis. The Log*P* values were estimated by using neural network analysis with back-propagation method based on AIC (Akaike Information Criteria) theory.

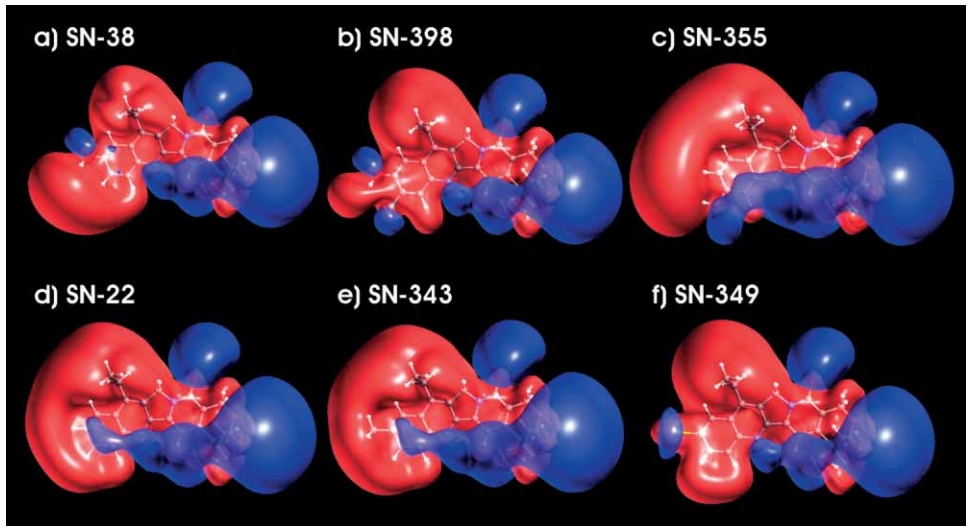

Figure 3. Electrostatic iso-potential surfaces at ±0.01 atomic unit of representative analogues. (a) SN-38, (b) SN-398, and (c) SN-355 are substrates and (d) SN-22, (e) SN-343, and (f) SN-349 are non-substrates. The surfaces were generated by *ab initio* MO calculations at restricted Hartree-Fock level with the MIDI-4 and polarization functions as a basis set.

Finally, the prediction of substrate or non-substrate of ABCG2 was also attempted only from the formal charges on the atoms of X- and Y- substitutions observed in *ab initio* MO calculations. The neural network algorithms based on weighted information maximization method that is a new type of information theoretic supervised competitive learning were introduced and "leave-one-out" experiments for each SN-38 analogue were performed 10 times.

RESULTS AND DISCUSSION

The electrostatic iso-potential surfaces of SN-38 and its analogues at ±0.01 a.u. (atomic unit) were generated and the surfaces of representative analogues are shown in Figure 3. It is found that there are two types of potential maps, *i.e.*, analogues, which have a negative potential area around X- or Y- positions and others do not have. As shown in Figures 2 and 3, the analogues (SN-38, 355, and 398), which are classified into the substrate group by MIT assay, have such a distinct negative potential area. On the other hand, the Others classified as non-substrate (SN-22, 343, and 349) exhibit both types of electrostatic potentials. The negative potential in a non-substrate (SN-349) may be directly attributed to the presence of a halogen atom. Indeed ABCG2 has been reported to indicate high affinity to SN-38 analogues containing halogen atoms,[13] and it is suggested that the negative potential area at X- or Y- positions may play a critical role in the drug recognition mechanisms by ABCG2, since.

To investigate the relationship between hydrophilicity or hyrdophobicity and drug resistant profiles, calculated ΔG and Log*P* values are plotted *versus* DRRs in Figures 4 and 5. As shown in Figure 4, the SN-38 analogues are clearly classified into substrates and non-substrates by ΔG. Furthermore, the estimated Log*P* values indicate good correlations with DRRs except for Cl- or Me-containing compounds. These results are suggesting that ΔG and Log*P* values as hydrophilicity and hydrophobicity properties are critically related to determine substrates of ABCG2 and may be good indices for drug resistance.

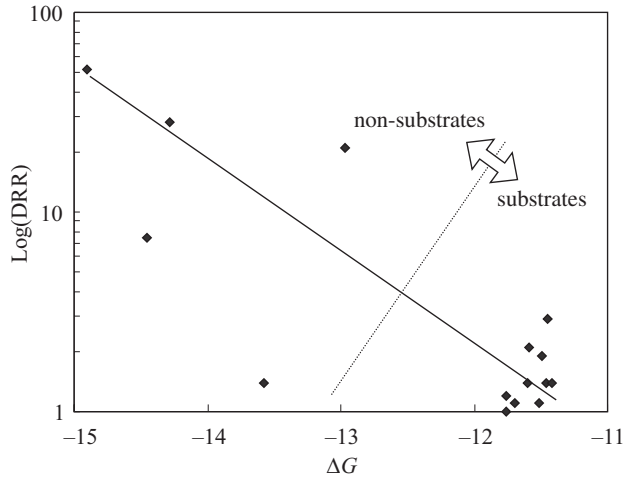

Figure 4. The correlation between the solvation free energies (ΔG) and drug resistance ratios (DRRs). The broken line classified 15 analogues into substrates and non-substrates.

Finally, we report the results of the substrate prediction. When the weighted information maximization (WIM) method is used, the generalization errors were only 0.02, meaning that miss-predictions were only tree times among a total of 150 times "leave-one-out" experiments. For comparison, the magnitudes of generalization errors with ot-

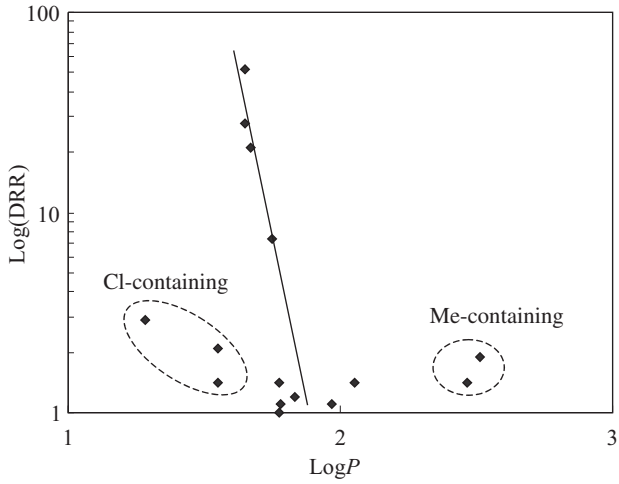

Figure 5. The correlation between the hydrophobic parameters (Log*P*s) and drug resistance ratios (DRRs). The broken circles indicate methyl-containing analogues and Cl-containing analogues, respectively.

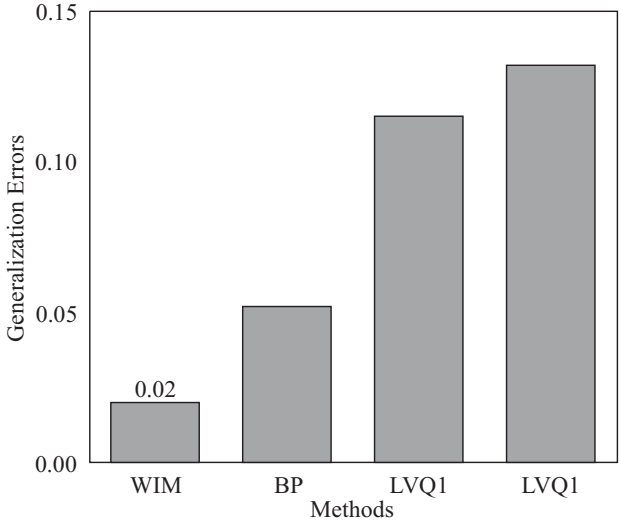

Figure 6. The generalization errors using the weighted information maximization (WIM) compared with learning vector quantization 1 (LVQ1), LVQ2, and back-propagation.

her conventional methods of LVQ1 (learning vector quantization 1), LVQ2, and back-propagation are also depicted in Figure 6, suggesting neural network analysis, especially, WIM is effective for prediction of substrates of ABCG2.

CONCLUSIONS

In present paper we have described typical properties of SN-38 and its analogues that are obtained by computational approaches to develop a platform for the molecular modeling to circumvent ABCG2-associated drug resistance. Indeed some computational results were shown to be used for interpretation of drug recognition and transport by ABC transporter. It has been suggested that hydrophilicity and hydrophobicity of compounds are one of the essential determinants for drug transport by ABCG2. Furthermore, the electrostatic profiles provide accurate interpretation for the recognition mechanisms.

Though the 3D structure of ABCG2 has not been revealed we have discussed the interaction such as recognition and transport, between the drug and the transporter using computational results. To develop a platform for the modeling new advantaged drug, much more information, for instance, more numbers of analogues and more precise investigations are required. However we have presented that theoretical approaches are effective to interpret and predict inter-bio-molecular interactions such as the cancer drugs and the ABC transporters.

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SAŽETAK

Teorijske studije u molekulskom modeliranju novih analoga camptothecin-a

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Irinotecan je vrlo korišten i moćan antitumorski lijek čija se priprava temelji na camptothecin-u. Ipak, zbog nedekspresije ABCG2, stanice raka mogu razviti otpornost na SN-38, tj. na aktivni metabolit irinotecan-a. Kako bi se savladala ova otpornost, u radu je razvijena platforma za molekulsko modeliranje koja je omogućila karakterizaciju četrnaest novih SN-38 analoga i čija su tipična svojstva procijenjena molekularno-orbitalnim proračunima i QSAR neuronskim mrežama.