Quantitative Structure-Activity Relationship (QSAR) Analysis to Predict Drug-Drug Interactions of ABC Transporter ABCG2

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Abstract: Quantitative structure-activity relationship (QSAR) analysis is a practical approach by which chemical structure is quantitatively correlated with biological activity or chemical reactivity. Human ABC transporter ABCG2 exhibits broad substrate specificity toward structurally diverse compounds. To gain insight into the relationship between the molecular structures of compounds and the interaction with ABCG2, we have developed an algorithm that analyzes QSAR to evaluate ABCG2-drug interactions. In addition, to support QSAR analysis, we developed a high-speed screening method for analyzing the drug-drug interactions of ABCG2. Based on both experimental results and computational QSAR analysis data, we propose a hypothetical mechanism underlying ABC-mediated drug transport and its interaction with drugs.

Keywords: ABC transporter, ABCG2, camptothecin, gefitinb, irinotecan, multidrug resistance, QSAR analysis.

1. INTRODUCTION

Biological activity can be expressed quantitatively as a function of structure described by electronic attributes, hydrophobicity, and steric properties to give a certain biological response [1]. Additionally, when physicochemical properties or structures are expressed by numbers, we can form a mathematical relationship, or quantitative structure-activity relationship, between the two. The mathematical expression can then be used to predict the biological response of other chemical structures. Recently, computational techniques have ensured to delineate and refine the many variables and approaches that define the QSAR paradigm.

It is more than a century ago that the QSAR paradigm first found its way into the practice or pharmaceutical chemistry and toxicology. Crum-Brown and Fraser expressed the idea that physiological action of a substrate or ligand could be expressed as a function of its chemical composition and constitution [2]. In 1893, Richert showed that the cytotoxicities of a diverse set of simple organic molecules were inversely related to their corresponding water solubilities [2]. In the 20th century, Meyerand Overton independently suggested that the narcotic (depressant) action of a group of organic compounds is correlated with their olive oil/water partition coefficients in a parallel manner [2]. In 1939, Ferguson introduced a thermodynamic generalization to the correlation of depressant action with relative saturation of volatile compounds [2]. Bell and Robin, on the other hand, established the importance of ionization of bases and weak acids in bacteriostatic activity [2]. The mothern QSAR paradigm with the molecular mechanistic basis was implemented by Hansch and Fujita. Using the octanol/water system, a whole series of particition coefficients were measured, and thus new hydrophobic scale was introduced [2].

In the QSAR paradigm, the basic assumption for all molecule-based hypotheses is that similar molecules have similar activities. The underlying problem is, therefore, how to define a small difference on a molecular level, since each kind of activity, *e.g.* reaction ability, biotransformation ability, solubility, target activity, and so on, might depend on another difference.

In our body, drugs are not only enzymatically metabolized but also transported by various transporters including members of the solute carrier (SLC) superfamily and some ATP-dependent efflux pumps of the ATP-binding cassette (ABC) superfamily. Transporter proteins mediating the uptake and efflux of small molecules into and out of cells are key determinants of the *in-vivo* distribution and elimination of many drugs and endogenous substances [3]. Those drug transporter proteins have broad substrate specificity. Therefore, based on the physicochemical properties of drugs, it is important to predict which drug is transported by which transporter(s).

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To gain systematic insight into the relationship between the chemical structure of drugs and the substrate specificity of drug transporters, we have developed a new QSAR analysis method by introducing chemical fragmentation codes. Furthermore, we have established methods of high-speed screening for drug-drug interactions of ABC transporters to contribute to performing the QSAR analysis method.

2. TRANSPORT MECHANISM OF ABCG2

ATP-binding cassette (ABC) transporter ABCG2 is known to play a pivotal role in determining the pharmacokinetic profiles of drugs and, by extension, their overall pharmacological effects and drug concentration at the target site. Human ABC transporter ABCG2, originally named Breast Cancer Resistant Protein (BCRP), was first discovered in doxorubicin-resistant breast cancer cells [4]. ABCG2 is expressed in normal tissues and endothelial cells forming blood-tissue barriers [5]. Human ABC transporter ABCG2 is considered to exist in the plasma membrane as a homodimer bound through disulfide-bonded cysteine residues (Fig. **1**), since treatment with mercaptoethanol reduced the apparent molecular weight of ABCG2 from 140,000 to 70,000 [6]. Based on the cDNA sequence, a total of eleven cysteine residues exist in the ABCG2 protein. Among them, three cysteine residues in the extra-cellular loop of ABCG2 play pivotal roles in homodimer formation or protein expression levels. While Cys603 is involved in homodimer formation, Cys592 and Cys608 appear to be even more important for the formation of an intramolecular disulfide bond that greatly affects the protein stability as well as plasma membrane targeting of the ABCG2 protein [6-8].

Hitherto some hypotheses have been proposed in terms of the molecular mechanisms underlying transport actions of ABC transporters. Sauna and Ambudkar proposed a catalytic cycle of ATP-hydrolysis of ABCB1 (P-glycoprotein/MDR1) by providing evidence for formation of the A-S reaction intermediate with ATP-γ-S, a nonhydrolyzable analogue of ATP [9,10]. In the proposed catalytic cycle, however, it is not clear whether ATP-binding is first or substrate-binding is first. Ozvegy *et al*. reported that ABCG2 is capable of a vanadate-dependent adenine nucleotide trapping [11]. Interestingly, nucleotide trapping was stimulated by substrate compounds in acquired mutants (R482G and R482T) but not in the wild type of ABCG2 [11], suggesting that ATP-binding to the first step prior to substrate binding in the case of the wild type of ABCG2. Fig. (**2A**) demonstrates a putative mechanism for ABCG2-mediated transport of a drug across the plasma membrane. Based on our kinetic analysis [12], the homodimer of ABCG2 first binds two molecules of ATP to form a substrate binding site (State 1). A drug molecule such as gefitinib or SN-38 is bound to the substrate binding site of the ABCG2-ATP complex (State 2). Hydrolysis of ATP results in a conformational change of the ABCG2 protein, which may push the drug outwards. As ADP and inorganic phosphate (Pi) are released from the ABCG2 protein, the drug is also released from ABCG2 and becomes solvated in the extra cellular milieu. At the present time, this mechanism is still speculative; but it provides a hint for our consideration. Accurate mechanisms underlying the ABCG2-mediated drug transport across the plasma membrane should be elucidated by X-ray crystallography of the ABCG2 protein taken at different transport states.

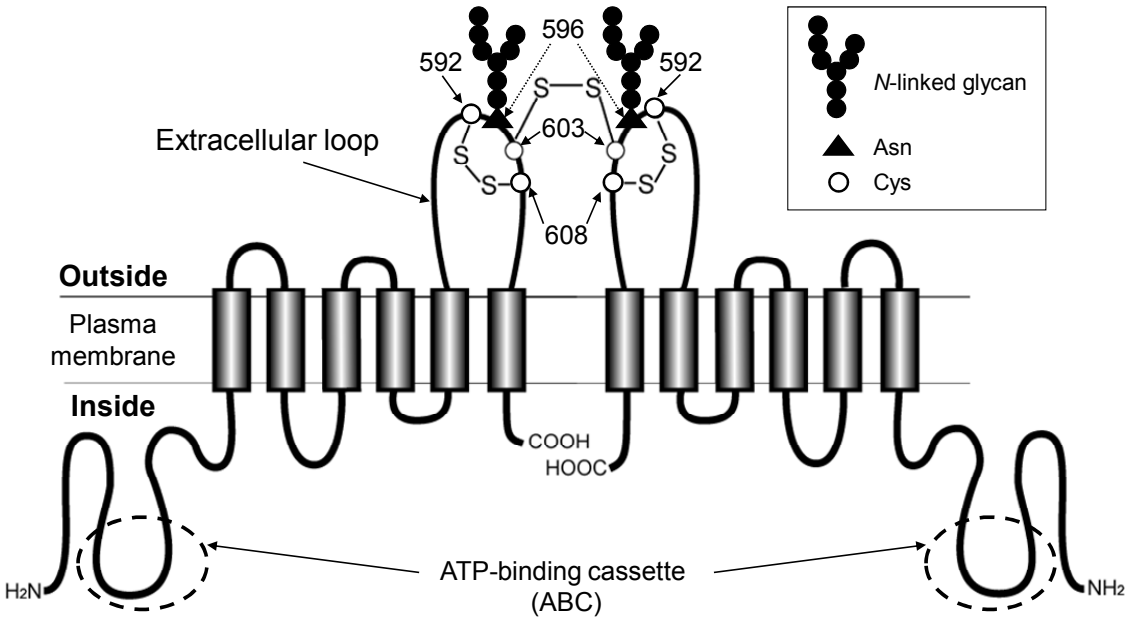


Fig. (1). Schematic illustrations of ABCG2 protein structure.

The ABCG2 protein expressed in the plasma membrane is a homodimer linked *via* a cysteinyl disulfide bond. The cysteine residue corresponding to Cys603 of human ABCG2 is involved in homodimer formation. The substrate-binding site is formed when ATP is bound to the ATP-binding cassettes (ABC) of the ABCG2 homodimer. Disulfide bond formation at Cys603 does not appear to be prerequisite for exerting the transport activity of ABCG2.

3. HIGH-SPEED SCREENING

We aimed to analyze the QSAR latently residing in ABCG2-drug interactions (Fig. **2B**). We first established standard methods for expression of human ABCG2 in insect cells and high-speed screening of ABCG2 inhibition with test compounds (Fig. **3**). Plasma membrane vesicles prepared from ABCG2-expressing Sf9 cells were used as a model system to measure the ATP-dependent transport of [^{3}H]methotrexate (MTX) [12], since ABCG2 reportedly transport MTX as a substrate [13-15]. The standard incubation medium contained plasma membrane vesicles (50 μg of protein), 200 μM [3',5',7'-^{3}H]MTX (Amersham, Buckinghamshire, UK), 0.25 M sucrose, 10 mM Tris/Hepes, pH 7.4, 10 mM $MgCl_2$, 1 mM ATP, 10 mM creatine phosphate, and 100 μg/ml creatine kinase in a final volume of 100 μl. The incubation was carried out at 37˚C. After a specified time (20 min for the standard condition), the reaction medium was mixed with 1 ml of ice-cold stop solution (0.25 M sucrose, 10 mM Tris/Hepes, pH 7.4, and 2 mM EDTA) to terminate the transport reaction. Subsequently, aliquots (270 μl per well) of the resulting mixture were transferred to MultiScreen^{TM} plates (Nihon Millipore KK, Tokyo, Japan). Under aspiration, each well of the plate was rinsed with the 0.25 M sucrose solution containing 10 mM Tris/Hepes, pH 7.4, for four times (4 x 200 μl for each well) in an EDR384S system (BioTec, Tokyo, Japan). [^{3}H]MTX thus incorporated into the vesicles was measured by counting the radioactivity remaining on the filter of MultiScreen^{TM} plates (Fig. **3**) [12].

We selected structurally diverse test compounds to investigate the inhibition of MTX transport. The selected test compounds are classified into seven groups (Fig. **4A**), *i.e.*, A, neurotransmitters; B, Ca^{2+} channel blockers; C, K^+ channel modulators; D, steroids; E, non-steroidal anti-inflammatory drugs (NSAIDs); F, anti-cancer drugs; G, antibiotics; H, other drugs; and I, ABCG2 substrates or inhibitors. Fig. (**4A**) summarizes the effects of those test compounds on ABCG2-mediated MTX transport. The test compounds were measured at a concentration of 10 μM, and the data are expressed as relative values as compared with the transport activity measured without test compounds (0% inhibition). Among 49 different therapeutic drugs and compounds tested in this study, SN-38 (F-8), novobiocin (G-2), prazosin (H-4), FK506 (H-5), hematoporphyrin (I-3), pheophorbide a (I-4), and quercetin (I-5) strongly inhibited ABCG2-mediated MTX transport.

4. QSAR ANALYSIS USING CHEMICAL FRAGMENTATION CODES

To gain insight into the relationship between the chemical structure of test compounds and the inhibition of ABCG2-mediated MTX transport activity, we have performed QSAR analysis by introducing chemical fragmentation codes. The program Markush TOPFRAG was used to generate the chemical fragmentation codes based on the structural components of test compounds [12,16]. Chemical fragment codes are a set of alphanumeric symbols, each representing a fragment of a chemical structure

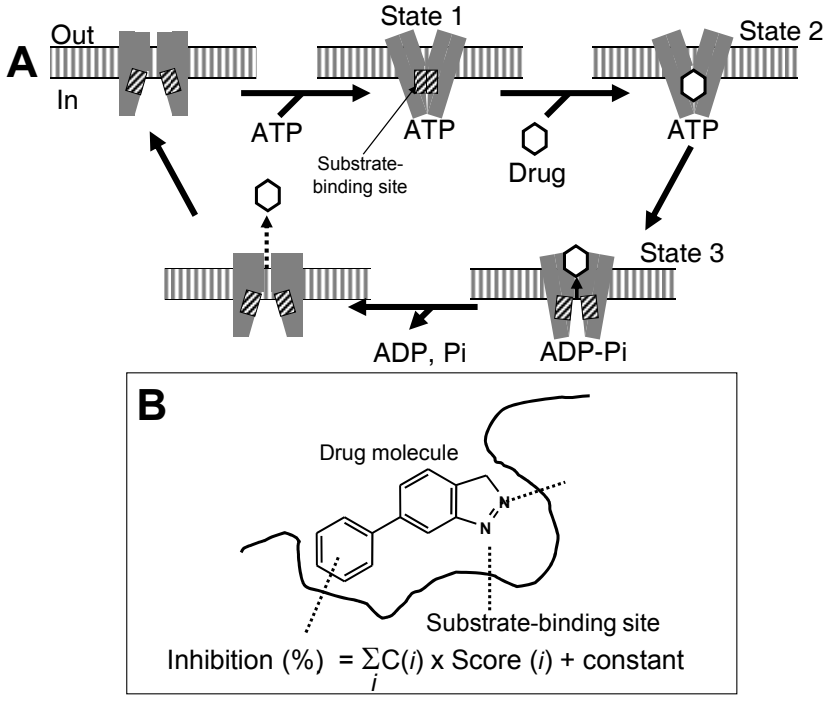


Fig. (2). Schematic illustrations of ATP-dependent drug transport mediated by ABCG2 (A) and the interaction between a drug molecule and the substrate-binding site of ABCG2 (B).

Inhibition (%) of ABCG2-mediated MTX transport is described as a linear combination of chemical fragmentation codes (*i*), each of which is weighted by the corresponding coefficient C(*i*) and score (*i*). The symbol (*i*) designates a specific chemical fragmentation (*i*). The "score" means the presence "1" or absence "0" of the corresponding chemical fragmentation code (*i*) in the chemical structure of a test compound.

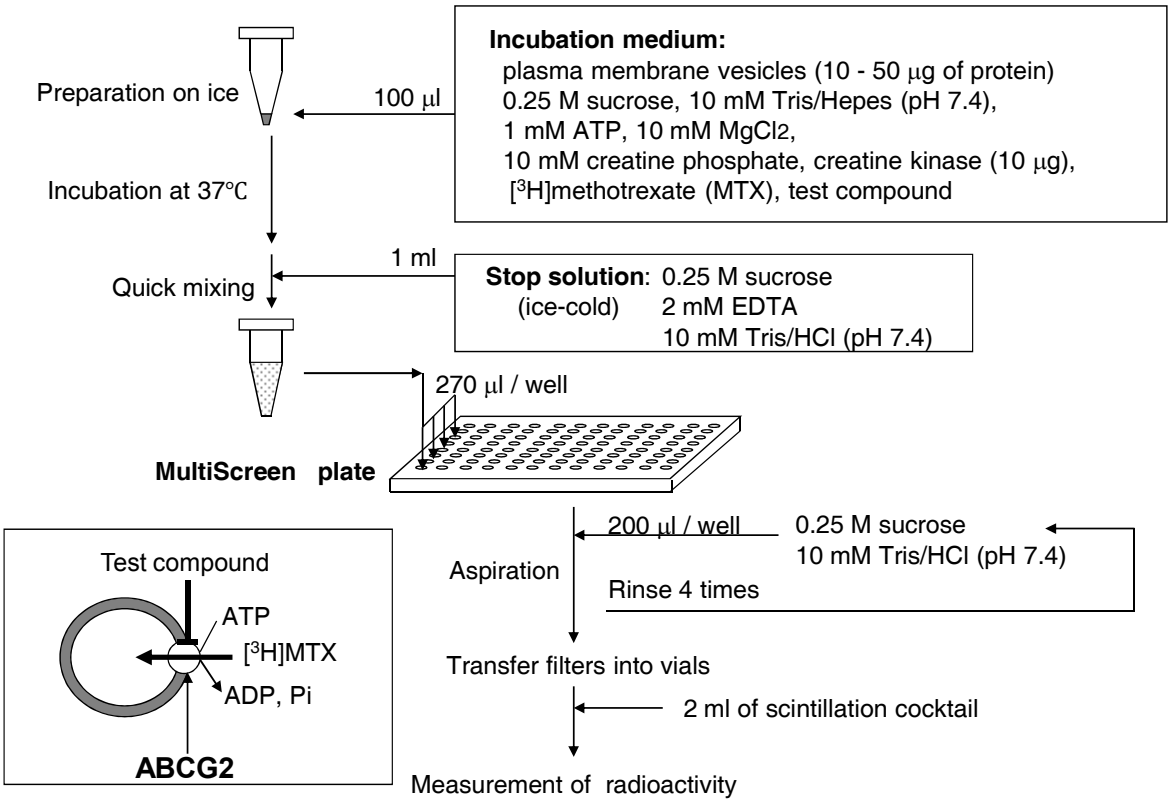


Fig. (3). Schematic illustration of high-speed screening of human ABCG2-drug interactions. Plasma membrane vesicles expressing human ABCG2 were prepared from Sf9 insect cells. ATP-dependent transport of [^{3}H]methotrexate (MTX) into the vesicles mediated by the action of ABCG2 was measured by counting the radioactivity remaining on the filter of MultiScreen^{TM} plates. Inhibition of MTX transport was detected by adding a test compound into the reaction mixture. Detailed experimental procedures are described in ref. [12].

(http://www.thomsonscientific.com/media/scpdf/chemical_index_guidelines.pdf). The Markush TOPFRAG program is a tool for searching the chemical structures and structure information in the online database of Derwent World Patents Index (http://thomsonreuters.com/products_services/legal/legal_products/intellectual_property/DWPI). For the QSAR analysis study, we formulate the extent of ABCG2-mediated MTX transport as a linear combination of chemical fragmentation codes, each of which is weighted by the corresponding coefficient as follows:

$$\text{ABCG2 inhibition (\%)} = \Sigma C(i) \times \text{Score}(i) + \text{Constant},$$

where the symbol (*i*) designates a specific chemical fragmentation (*i*) (Fig. **2**). The "score" means the presence "1" or absence "0" of the corresponding chemical fragmentation code (*i*) in the chemical structure of a test compound. Based on the chemical fragmentation codes thus obtained and in comparison with the observed inhibition of transport activity for each test compound, we have calculated chemical fragmentation coefficients, C(*i*), by multiple linear regression analysis.

Multiple linear regression analysis delineated a relationship between the structural components and the extent of ABCG2 inhibition. Thereby we could identify one set of chemical fragmentation codes that are closely related to the inhibition of ABCG2 transport activity. Explanations for these chemical fragmentation codes are given in Table **1**. As demonstrated in Fig. (**4B**), the prediction of transport inhibition was well correlated with the observed values of inhibition. The R^2 value was estimated to be 0.920 [12]. Statistical significance was determined by *F*-test where the *F* value was calculated to be 50.1 [12]. This *F* value certifies the QSAR equation is significant. Furthermore, the validity of the QSAR equation has been proven as described below.

5. PREDICTION OF ABCG2 INHIBITION BY GEFITINIB

The human genome encodes more than 500 protein kinases, and this protein kinase family has been the subject of intensive research for the development of novel anticancer drugs [17,18]. In particular, specific tyrosine kinase inhibitors are rapidly being developed as new drugs for the inhibition of malignant cell growth and metastasis. Gefitinib and imatinib are anticancer drugs that have been developed as inhibitors for EGFR tyrosine kinase and BCR/ABL kinase, respectively.

The QSAR analysis revealed that a structure having one amine bonded to one carbon of a heterocyclic ring is an important component for interaction with the ABCG2 protein [12]. In addition, fused heterocyclic ring(s) and two substituents on a carbocyclic ring of the fused heterocyclic ring(s) are also important chemical moieties for the interaction with ABCG2 [12]. Interestingly, many protein kinase inhibitors carry such structural components within their molecules. Gefitinib is an orally active EGFR tyrosine kinase inhibitor. It has been reported that gefitinib is a

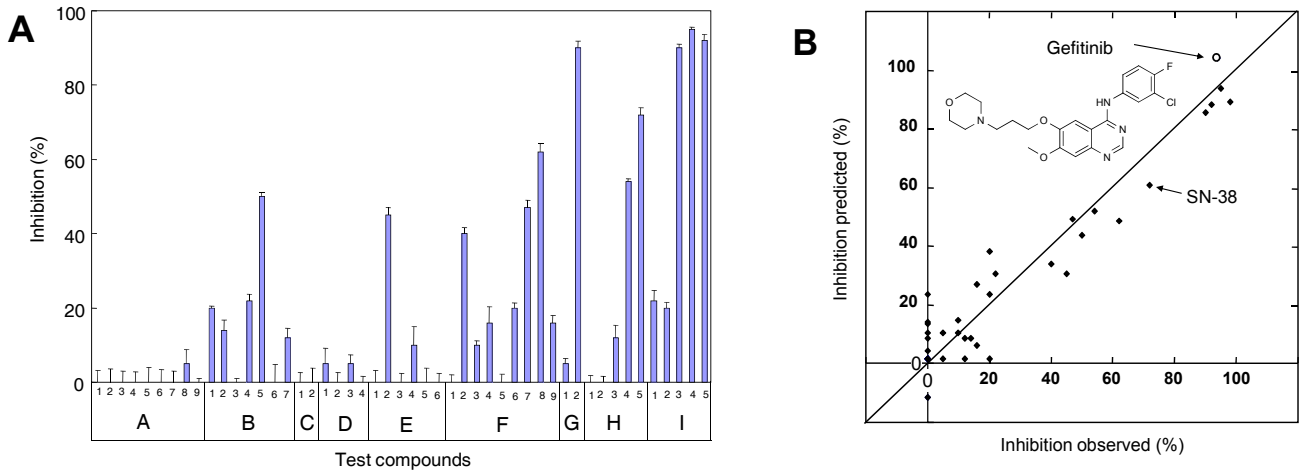


Fig. (4). Inhibition of MTX transport by test compounds and its profiling.

A. ATP-dependent [^{3}H]MTX transport was measured in the presence of a test compound (10 μM) in the standard incubation medium (0.25 M sucrose and 10 mM Tris/Hepes, pH 7.4, 10 mM creatine phosphate, creatine kinase (100 μg/ml), and 10 mM $MgCl_2$), as described previously [9]. Inhibition (%) is expressed as relative values compared with the transport activity measured without test compounds (0% inhibition). The following test compounds are used: dopamine (A-1), epinephrine (A-2), norepinephrine (A-3), GABA (A-4), glutamic acid (A-5), glycine (A-6), histamine (A-7), melatonin (A-8), serotonin (A-9), bepridil (B-1), diltiazem (B-2), fendiline (B-3), nifedipine (B-4), nicardipine (B-5), prenylamine (B-6), verapamil (B-7), nicorandil (C-1), pinacidil (C-2), betamethasone (D-1), cortisone (D-2), dexamethasone (D-3), prednisolone (D-4), acetylsalicylic acid (E-1), acemetacin (E-2), indomethacin (E-3), ibuprofen (E-4), mepirizole (E-5), naproxen (E-6), actinomycin D (F-1), daunorubicin (F-2), doxorubicin (F-3), etoposide (F-4), 5-fluorouracil (F-5), mitoxantrone (F-6), paclitaxel (F-7), SN-38 (F-8), vinblastine (F-9), penicillin G (G-1), novobiocin (G-2), quinidine (H-1), *p*-aminohippuric acid (H-2), reserpine (H-3), prazosin (H-4), FK506 (H-5), estrone-3-sulfate (I-1), hemin (I-2), hematoporphyrin (I-3), pheophorbide a (I-4), and quercetin (I-5). Data are expressed as mean values ± S.E.M. (n = 3). Data are from ref. [12].

B. Relationship between observed and predicted values in the inhibition of MTX transport by different test compounds. Those observed values were the same as shown in Fig. (**3A**), whereas the predicted values were deduced from multiple linear regression analysis. The inhibition by gefitinib is indicated by an open circle (○). The chemical structure of gefitinib is depicted in this figure.

Table 1. Descriptors (DES) and Chemical Fragmentation Codes (CFC) Closely Correlated with the Inhibition of ABCG2-Mediated MTX Transport

DES	CFC	Definition
H121	H121	One amine bonded to heterocyclic C
D023	D023	Substituents on a carbocyclic ring of a fused-ring heterocyclic system:
		Two C atoms of a fused carbocyclic ring bear substituents.
M240	M240	Chain bonded to ring C
OH	H401	One –OH group
	H402	Two -OH groups
	H403	Three –OH groups
	H404	Four –OH groups
	H405	Five or more –OH groups
M531	M531	One carbocyclic system with at least one aromatic ring
J2	J2	Absence of ester (thioester)

(Table 1). Contd…..

DES	CFC	Definition
H481	H481	One –OH group bonded to aliphatic C
RS4	D240	Three or more ring systems with O as the sole heteroatom
	D420	Three or more ring systems with one S atom as the sole heteroatom
	D430	Three or more ring systems with two or more S atoms as the sole heteroatoms
	D510	Three or more rings with O and S as the sole ring heteroatoms
	E300	Moephinan
	E310	Four-ring systems with one N atom as the sole heteroatom
	E320	Ergoline
	E330	Four-ring systems with 2 or more N atoms as the sole heteroatoms
	E540	Four-ring systems consisting solely of O, N, and C
	E870	Four-ring systems consisting solely of S, N, and C
	E920	Four or more rings with O, S, and N as heteroatoms
	G400	Four-ring system (at least one aromatic ring) with at lest one 3-, 4-, or 5-membered ring
	G410	Chrysene
	G420	Naphthacene
	G430	Other 6:6:6:6 systems
	G440	Other four-ring carboxylic systems
	G800	Four 6-membered alicyclic ring systems
	G810	other four 6-membered alicyclic ring systems except for G800
		Steroids are not involved in G810

In the cases of OH and RS4, one descriptor (DES) represents multiple chemical fragmentation codes (CFC). Data are from ref. [12].

substrate for ABCG2 [19]. Our kinetic data strongly suggest that gefitinib is recognized by the ABCG2-ATP complex as the substrate.

Table **2** summarizes the chemical fragmentation codes of gefitinib. H121, M531, D023, and J2 were found as its structural components, whereas M240, OH, H481, or RS4 are not present in the chemical fragmentation codes of gefitinib. From the chemical fragmentation codes of H121, M531, D023, and J2 as well as the corresponding C(*i*) values, we calculated the potency of gefitinib on ABCG2 inhibition [12]. Based on our calculations, it is suggested that 10 μM gefitinib inhibits ABCG2-mediated MTX transport almost completely (104% of inhibition) (Fig. **4B**). We conducted actual experiments to examine our prediction regarding gefitinib's inhibition. The IC_{50} value was estimated to be 0.28 μM for gefitinib [12]. At the concentration of 10 μM, gefitinib inhibited MTX transport by 95%. Thus, these data support our QSAR analysis-based prediction.

6. CPT ANALOGUES THAT ARE NOT TRANSPORTED BY ABCG2

In cancer chemotherapy, overexpression of drug efflux transporters in cancer cells is one of the major causes of multidrug resistance that reduces the efficacy of anticancer drugs. Thus, molecular design based on the drug transport mechanisms of ABC transporters can improve the effectiveness of anticancer drugs, and can contribute to

Table 2. Chemical Fragmentation Codes (CFC) of Gefitinib

CFC
D740 F653 G100 **H121** H181
H201 H542 H601 H602 M412
M531 M123 M143 M511 M521
M210 M281 M313 M321 M332
M342 M391 M270 M272 M380
M383 D011 **D023** F014 G015
H102 H642 M211
H3 H4 H7 H9 J0 J1 **J2** J3
J4 J5 J6 J9 K1 K2 K3 K4
K5 K6 K7 K8 K9 L1 L2 L3
L4 L5 L6 L7 L8

The descriptors shown in Table **1** are highlighted by underbars. Data are from ref. [12].

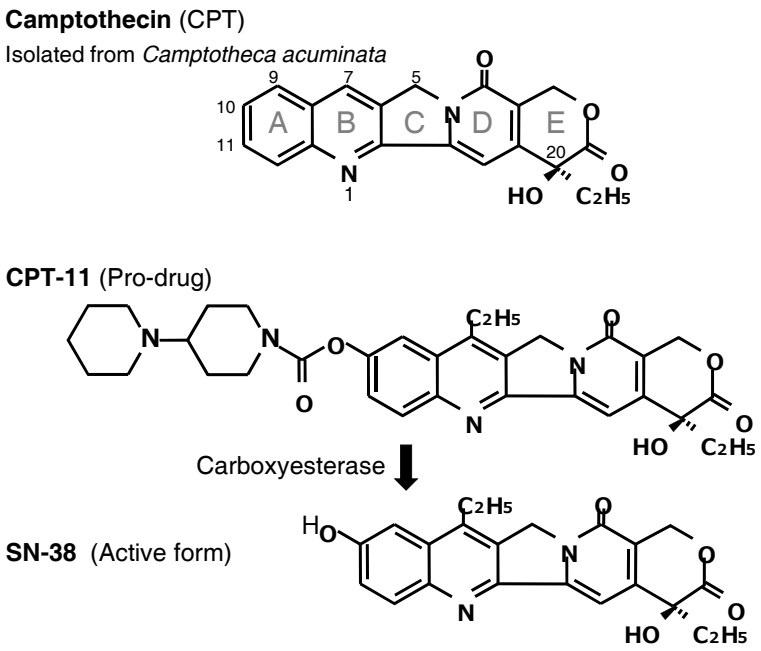


Fig. (5). Chemical structures of camptothecin (CPT), CPT-11 (Irinotecan), and SN-38.

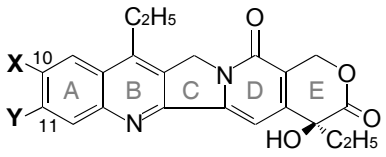
Camptothecin (CPT) was originally isolated from *Camptotheca acuminate*. CPT-11 *per se* is a pro-drug and undergoes carboxylesterase-mediated hydrolysis to form SN-38, a potent topoisomerase I inhibitor.

development of resistance-reversal drugs as well as new anticancer drugs. In this section, we present an example of QSAR-based drug molecular design approaches.

Camptothecin (CPT; Fig. **5**) has been demonstrated to be effective against a broad spectrum of tumors. The molecular target of CPT has been firmly established to be human DNA topoisomerase I (Topo I) that changes the topological state of duplex DNA by single-strand breakage and religation. CPT-11 is one of the prominent anti-neoplastic drugs widely used in clinical practice today. CPT-11 *per se* is a pro-drug and undergoes carboxylesterase-mediated hydrolysis to form SN-38, a potent Topo I inhibitor (Fig. **5**). Despite of the clinical effectiveness of CPT-11, acquired resistance to this anticancer drug has been reported. Overexpression of ABCG2 has recently been shown to confer resistance to doxorubicin, mitoxantrone, and various CPT analogues. SN-38-resistant PC-6/SN2-5H2 human lung carcinoma cells were shown to overexpress ABCG2 with the reduced intracellular accumulation of SN-38 [20]. Plasma membrane vesicles prepared from those cells transported both SN-38 and SN-38-glucuronide in an ATP-dependent manner, suggesting that ABCG2 is involved in the active extrusion of SN-38 and its metabolite from cancer cells.

Analysis of the structure-activity relationship among CPT-based analogues is of interest to understand the substrate specificity of ABCG2 and also to design new anticancer drugs that circumvent ABCG2-associated drug resistance. To overcome SN-38 resistance, a total of fourteen CPT analogues were recently synthesized by replacing the hydroxy residue of SN-38 with others (hydrogen, halogenmethyl, metoxy residues, etc.) [20]. While the lactone E ring is a prerequisite for the antitumor activity of CPT modifications of the A or B rings do not significantly affect Topo I inhibition activity. In this context, the synthesized CPT analogues have various substitutions at positions 10 or 11 of the A ring (Fig. **6**).

We have screened the newly synthesized CPT analogues by using both HEK293 cells and ABCG2-transfected HEK293 (HEK293/ABCG2) cells to gain insight into the drug resistance profile. The right panel of Fig. (**6**) lists drug resistance ratios for those CPT analogues as determined by the MTT assay method. In the positive control, HEK293/ABCG2 cells were approximately 28-fold more resistant to SN-38 than was HEK293 cells. As shown in the right panel of Fig. (**6**), most of the synthesized CPT analogues (except for SN-355, SN-392, SN-398) exhibited the drug resistance ratios much lower than SN-38. On the other hand, in HEK293/ABCG2 cells, three analogues, *i.e.*, SN-355, SN-392, and SN-398, exhibited high IC_{50} values. Plasma membrane vesicles from HEK293/ABCG2 cells transported SN-38, SN-355, SN-392, and SN-398 in an ATP-dependent manner. Based on those findings, it is speculated that SN-38, SN-355, SN-392, and SN-398 are substrates for ABCG2, having common properties in their molecular structure. In fact, SN-38 and SN-398 have a hydroxyl group at position 10, whereas SN-355 has one at position 11. In addition, SN-392 has an amino group at position 10. Hydroxyl and amino groups are important for the formation of hydrogen bonds. Interestingly, the other CPT analogues do not have such groups at positions 10 or 11. It is likely that hydrogen bond formation is critically involved in substrate recognition and/or the transport processes of ABCG2. In fact, SN-22 was not transported by ABCG2, supporting our hypothesis. Thus, new CPT analogues like SN-22 are considered as new candidates of



CPT analogue	Substitution	
	X	Y
SN-22	H	H
SN-38	OH	H
SN-343	Me	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	OMe	F
SN-398	OH	F
SN-443	Me	F
SN-444	F	F

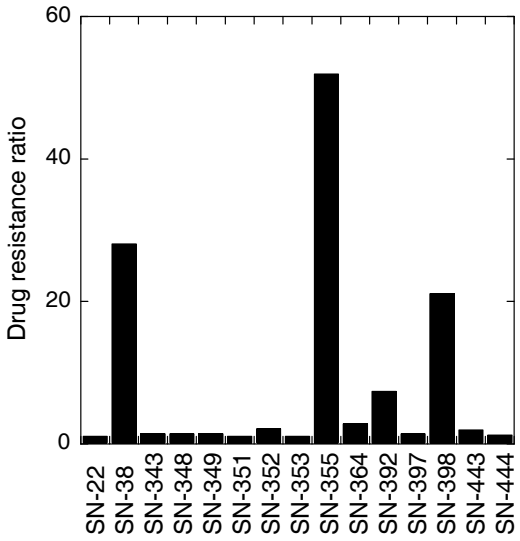


Fig. (6). Molecular structures of newly synthesized CPT analogues and their anticancer activity in ABCG2-transfected HEK293 (HEK293/ABCG2) cells.

Growth inhibition (IC_{50}) assay was performed by seeding cells at a density of 1,000 cells per well in 96-well plates containing the culture medium (100 µl/well). After 24 h, drugs were added to the culture medium at different concentrations, and cells were further incubated with the drug in a humidified tissue-culture chamber (37°C, 5% CO_2) for 72 h. Surviving cells were detected by the MTT assay. Drug resistance ratios are calculated from the ratio of IC_{50} (HEK293/ABCG2)/IC_{50} (HEK293) where the IC_{50} value is the drug concentration representing a 50% reduction of cell growth. Data are from ref. [20].

anticancer drugs that circumvent ABCG2-associated drug resistance.

7. POTENTIAL RISK OF ADVERSE REACTIONS THROUGH ABCG2 INHIBITION

Based on recent pharmacogenomic studies, it is implicated that inhibition of ABCG2 can enhance the risk of drug-induced adverse reactions. The single nucleotide polymorphisms (SNP) of 421C>A located in exon 5 of the *ABCG2* gene leads to a significant reduction in the protein expression level of the ABCG2 (Gln141Lys) variant protein, which is due to ubiquitin-mediated proteasomal degradation [21,22]. Patients carrying this SNP were found to have elevated plasma levels of gefitinib, diflomotecan, and increased bioavailability of oral topotecan [23-26] as well as outcome to methotrexate therapy in patients with psoriasis [26] and disposition of sulfasalazine [27,28]. In addition, the SNP 421C>A was reportedly associated with a higher incidence of diarrhea in non-small cell lung cancer patients treated with gefitinib [29]. Furthermore, it has recently been reported that renal excretion of serum uric acid *via* ABCG2 is impaired in humans who are carrying the 421A allele (Gln141Lys variant). As its consequence, serum uric acid levels are elevated to enhance the risk of gout [30-32]. ABCG2 expressed on the apical side of the proximal tubular cells in human kidney is considered to play a pivotal role in renal excretion of serum uric acid. Therefore, inhibition of ABCG2 transport function by drug-drug interaction should be preferably avoided.

8. CONCLUSIONS

In a recent study of Di Pietro's laboratory, a special attention is drawn on flavonoids which constitute a structurally-diverse class of compounds, well suited to identify potent ABCG2-specific inhibitors [33]. Shape parameters and hydrophobicity have been revealed to be major physicochemical parameters responsible for the inhibition activity of flavonoid derivatives and synthetic analogs towards ABCG2 [34]. More recently, Tamaki *et al*. [35] have reported a structure-inhibitory potency relationship of isoflavonoids, suggesting that some herbal and dietary supplements and isoflavonoids may increase the systemic availability of ABCG2 substrate drugs when concomitantly given orally. Gandhi and Morris have provided a nice overview on *in-silico* models in the literature used for the prediction of ABCG2 substrates and inhibitors [36]. Furthermore, Wiese *et al.* developed a new 3D-QSAR analysis method to design ABCG2 inhibitors [37]. In this context, new QSAR technologies are emerging to strengthen the platform for rational molecular design to circumvent ABCG2-mediated multidrug resistance in cancer as well as drug-induced adverse reactions *via* drug-drug interactions.

CONFLICT OF INTEREST

None declared.

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ABBREVIATIONS

ABC = ATP-binding cassette

BCRP = breast cancer resistance protein

CFC = chemical fragmentation code

CPT = camptothecin

DES = descriptor

HEK = human embryonic kidney

MTT = 3-(4,5-dimethyl-2-thiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

MTX = methotrexate

NSAIDs = non-steroidal anti-inflammatory drugs

QSAR = quantitative structure-Activity relationship

SLC = solute carrier

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