Biomolecules & Therapeutics, **18(4)**, 375-385 (2010)
www.biomolther.org

ISSN: 1976-9148(print)/2005-4483(online)
DOI: 10.4062/biomolther.2010.18.4.375

Review

Targeting Multidrug Resistance with Small Molecules for Cancer Therapy

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(Received July 12, 2010; Revised September 28, 2010; Accepted October 3, 2010)

Abstract − Conventional cancer chemotherapy is seriously limited by tumor cells exhibiting multidrug resistance (MDR), which is caused by changes in the levels or activity of membrane transporters that mediate energy-dependent drug efflux and of proteins that affect drug metabolism and/or drug action. Cancer scientists and oncologists have worked together for some time to understand anticancer drug resistance and develop pharmacological strategies to overcome such resistance. Much focus has been on the reversal of the MDR phenotype by inhibition of ATP-binding cassette (ABC) drug transporters. ABC transporters are a family of transporter proteins that mediate drug resistance and low drug bioavailability by pumping various drugs out of cells at the expense of ATP hydrolysis. Many inhibitors of MDR transporters have been identified, and though some are currently undergoing clinical trials, none are in clinical use. Herein, we briefly review the status of MDR in human cancer, explore the pathways of MDR in chemotherapy, and outline recent advances in the design and development of MDR modulators.

Keywords: Multidrug resistance, Chemotherapy, ATP-binding cassette

INTRODUCTION

With approximately half of all cancer diagnoses leading to death in the modern era, there is a compelling need to address chemotherapy drug resistance. Drug resistance can occur at many levels by affecting host drug metabolism, drug delivery, microenvironment, and cellular mechanisms, and most of these mechanisms are still poorly understood. Only the cellular mechanisms of drug resistance have been examined in detail, as illustrated in Fig. 1. Impaired drug delivery can result from poor absorption of orally administered drugs, increased drug metabolism or increased excretion, resulting in lower levels of drugs in the blood and reduced diffusion of drugs from the blood into the tumor mass (Pluen *et al.*, 2001).

Clinical oncologists were the first to observe that cancers treated with multiple anticancer drugs tend to develop cross-resistance to many other cytotoxic agents to which they have never been exposed, effectively eliminating the possibility of curing these tumors with chemotherapy. In many cases, cells grown in tissue culture that are derived from such multidrug-resistant tumors demonstrate patterns of resistance *in vitro* similar to those seen *in situ*. This observation suggests that multidrug resistance (MDR) is in most cases the result of heritable changes that alter the levels of specific or mutant proteins in cancer cells, thus allowing survival in the presence of many different cytotoxic agents. These genetic alterations that confer resistance to cytotoxic drugs can also affect cell cycle dynamics, susceptibility to apoptosis, uptake and efflux of drugs, cellular drug metabolism, intracellular compartmentalization of drugs, and repair of drug-induced damage (usually to DNA). Although many kinds of MDR have been documented in cultured cells (Gottesman and Pastan, 1996), clinical data proving that these mechanisms are responsible for MDR *in vivo* are generally lacking.

Recent studies indicate that transporter-mediated drug disposition plays a more important role than previously thought (Anderl *et al.*, 2004). The interaction between che-

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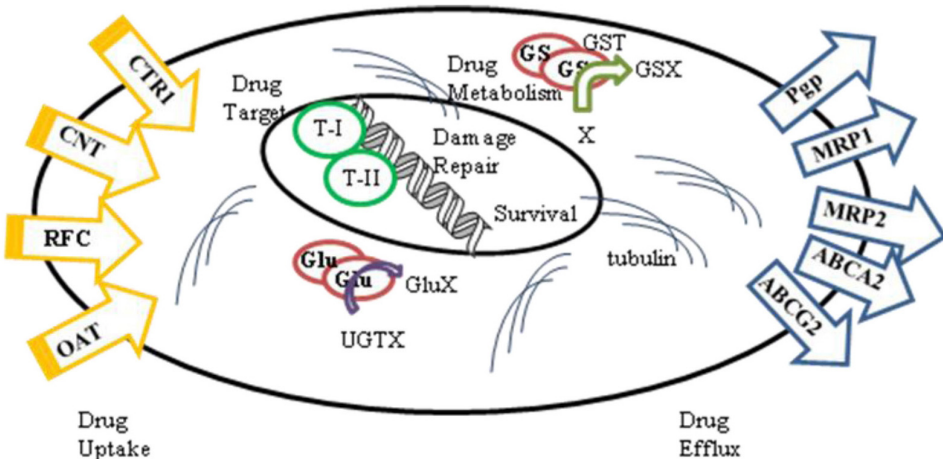


Fig. 1. Schematic drawing of cellular mechanisms of MDR. RFC: reduced folate carrier known to transport methotrexate, CNT: concentrative nucleoside transporter, OAT: organic anion transporters, CTR1, homotrimeric integral membrane protein that transports reduced copper [Cu(I)]. Targets of chemotherapy, such as DNA, tubulin, and topoisomerases I and II (T-I and T-II) are represented. This figure is modified from the original figure (Polgar et al., 2004).

motherapeutic drugs and transporters in cells and tissues is essentially related to the efficacy of therapy. ABC transporters and solute carrier (SLC) transporters are two major superfamilies of membrane transporters that are featured in various hypotheses related to MDR development and transport protein modulators that alter drug pharmacokinetics. ABC transporters play a more distinctive role in MDR. One of the most intensively studied proteins belonging to the class of energy-dependent efflux pumps is human P-glycoprotein (P-gp). In tumor therapy, special effort has been devoted to the design of inhibitors that overcome MDR by blocking P-gp-mediated efflux. Several P-gp inhibitors have been featured in clinical studies, which showed that inhibiting ABC transporters for the purpose of overcoming MDR is a valid strategy, at least in haematological malignancies. However, due to the prevalence of severe side effects, P-gp inhibitors must be highly specific for successful application to the therapy of multiresistant tumors. Herein, we discuss, in general, the mechanisms that have been proposed for mediating MDR in cancer cells, counter strategies currently in practice, and novel approaches for the development of potential anti-MDR modulators.

ATP-DEPENDENT TRANSPORTERS

It has been demonstrated over recent decades that one of the major mechanisms of MDR is the expression of several different ATP-dependent drug efflux pumps. ABC proteins constitute a large family of integral membrane proteins that are found in both prokaryotes and eukaryotes (Dean *et al.*, 2001). A functional integral membrane protein such as P-gp typically contains two nucleotide-binding folds (NBFs) as well as two transmembrane domains (TMDs) encoded by a single polypeptide (Efferth, 2001). The first two units, called nucleotide-binding domains, play a role in cleaving ATP (hydrolysis) in order to derive the energy necessary for transporting cell nutrients such as sugars, amino acids, ions, and small peptides across membranes.

ABC transporters serve a wide variety of cellular roles. In the liver, gastrointestinal tract, and kidney, ABC transporters excrete toxins, thereby protecting the organism. ABC transporters also play an active role in the immune system by transporting peptides identified as antigens by class I HLA molecules (e.g. ABCB2/TAP1, ABCB3/TAP2) into the endoplasmic reticulum. Furthermore, they play physiological roles in cellular lipid transport and homeostasis (Takahashi *et al.*, 2005).

A variety of ABC transporters have been shown to mediate chemoresistance in cancer cells via extrusion of anticancer drugs (Gottesman *et al.*, 2002). Among the ABC transporters involved in MDR are P-gp and MDR-associated protein (MRP), which are overexpressed in malignant cells where they serve to pump out anticancer drugs. This results in a reduction of the amount of drug necessary for effective therapy.

P-glycoprotein (P-gp)

The *P-gp* (ABCB1, MDR1) gene is located on chromosome 7 in humans and encodes 1280 amino acids (Ambudkar *et al.*, 1999). P-gp protein functions as an ATP-driven efflux transporter of substrates from the intracellular to extracellular region. It has 12 transmembrane sequences as shown in Fig. 2, of which sequences 5, 6, 11, and 12 function in transport. Generally, P-gp is characterized as having a transmembrane domain (TMD) containing six transmembrane segments and a hydrophilic region containing a nucleotide-binding domain (NBD). P-gp is mainly responsible for limiting entry of orally administered substrates (drugs) into the body as well as excretion of metabolites in-

to the bile and placenta (Kim *et al.*, 2006). Tumor cells expressing P-gp are characterized by reduced intracellular drug concentrations, which decrease the cytotoxicity of a broad spectrum of antitumor drugs, including anthracyclines (e.g. DOX), vinca alkaloids (e.g. vincristine), podophyllotoxins (e.g. etoposide), and taxanes (e.g. taxol). The increased expression of *ABCB1* gene in multidrug-resistant cancer cells has been attributed to various mechanisms, including altered activity of transcriptional factors, gene rearrangement or altered methylation status of the promoter (Wada, 2006). On the other hand, ABCB1 is expressed in normal cells of the intestine, liver, kidney, brain, and placenta, where it regulates intestinal absorption, hepatobiliary excretion, renal secretion, and protects the brain and fetus from xenobiotics.

In recent years, an increased understanding of P-gp-mediated pharmacokinetic interactions has been obtained. In addition, the role of P-gp in modifying the bioavailability of orally administered drugs via induction or inhibition has been also been demonstrated in various studies. The importance of P-gp to MDR during cancer suggests that cytotoxic drug delivery to cancer cells can be improved by inhibition of P-gp, which would afford a significant therapeutic advantage and result in improved treatment of cancer patients.

Multidrug resistance (MDR)-associated protein (MRP)

MRP, a member of the ABC family of transporters, has been described as a GS-X pump capable of transporting organic anion drug conjugates as well as intact anticancer drugs (Borst *et al.*, 1997). Isolated as a transmembrane glycoprotein in non-P-gp-expressing small cell lung cancer (SCLC) DOX-resistant cell lines, MRP is an asymmetrical molecule consisting of eight subunits and four membrane-spanning domains (Almquist *et al.*, 1995). Clinical cancers expressing MRP include hematological (Broxterman *et al.*, 1994), lung (Rubio *et al.*, 1994), acute lymphoblastic leukemia relapses, and chronic myeloid leukemia (Beck *et al.*, 1994).

Anticancer drugs that serve as substrates of MRP in-

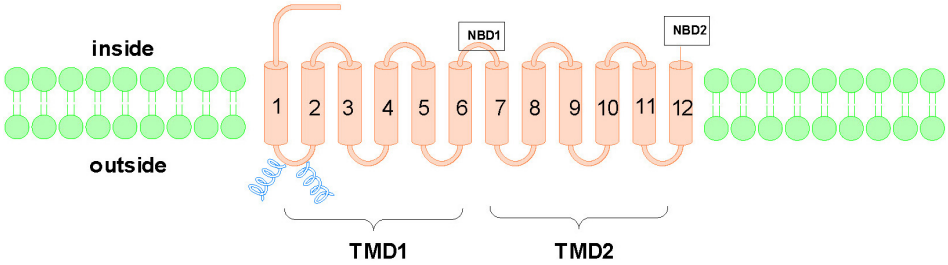


Fig. 2. Schematic representation of primary structure of P-gp embedded in the plasma membrane. P-gp is glycosylated at its first extracellular loop and is composed of 12 hydrophobic transmembrane domains (TMDs) and 2 nucleotide-binding domains (NBDs).

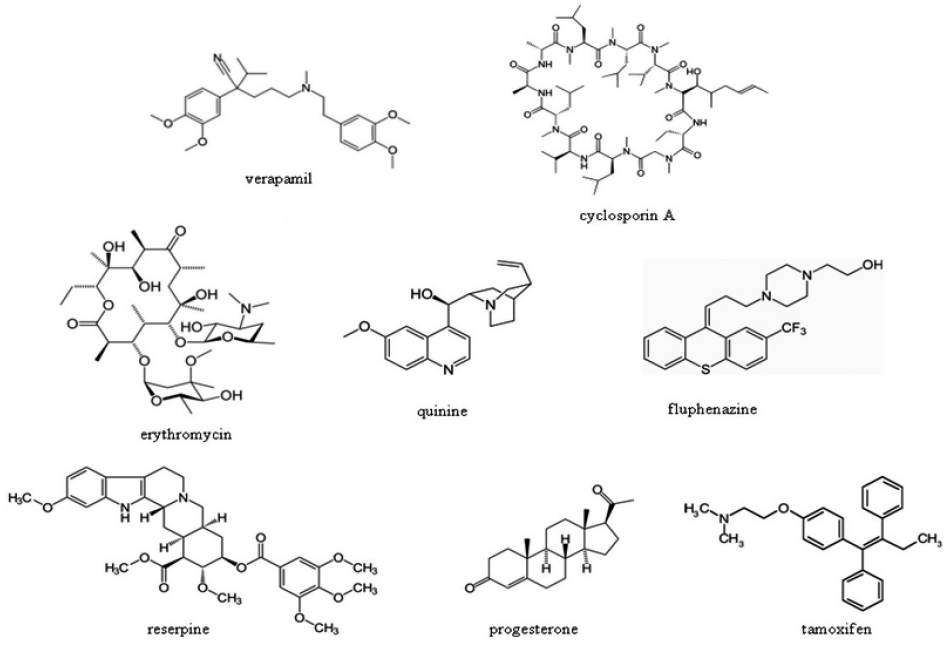


Fig. 3. First-generation MDR modulators.

clude anthracyclines such as DOX, vinca alkaloids, and etoposide. By pumping such agents out of tumor cells, MRP results in reduced intracellular accumulation of drugs and therefore resistance. Although MRP is remarkably similar to P-gp in terms of its substrate specificity and activity, only 15% amino acid homology exists between the two proteins. Although MRP1 is associated with drug resistance, it still plays a normal physiological role in the ATP-dependent unidirectional membrane transport of glutathione conjugates. On the other hand, MRP is expressed in normal human tissues, such as muscle, lung, spleen, bladder, adrenal gland, and gall bladder (Zaman *et al.*, 1993).

Potential mechanisms of multidrug resistance

Numerous mechanisms have been proposed for MDR in cancer cells. Such mechanisms can be categorized as non-cellular or cellular based on the factors contributing to MDR development (Fan *et al.*, 1994). Non-cellular mechanisms involve factors that are actually extracellular such as limited vascular accessibility or the cell growth environment, whereas cellular mechanisms include enzymes and transport systems.

Non-cellular MDR mechanisms

Non-cellular mechanisms of MDR are generally indicative of certain types of cancers that show inherent or natural resistance to chemotherapy upon initial exposure to the drug. Transformation to a cancerous state requires cells to grow beyond their natural boundaries, which typically requires a well-structured vasculature. However, in certain solid tumors, angiogenesis is compromised, which hinders the accessibility of the drug to cancer cells and limits drug-induced cytotoxicity. The growth environment in which cancer cells proliferate is markedly different from that of normal cells. Lack of nutrition and hypoxia due to poor vasculature followed by lactic acid accumulation could confer resistance of cancer cells to the action or cellular uptake of drugs that act on actively dividing cells, the latter of which requires a pH gradient (Demant *et al.*, 1990).

Cellular MDR mechanisms

Cellular MDR mechanisms may be classified as either non-classical/non-transport-based or classical/transport-based. Non-transport-based cellular MDR mechanisms involve enzyme systems that limit the desired activity of the drug without altering its effective concentration inside the cell. Glutathione-S-transferase (GST), an important enzyme in xenobiotic metabolism, catalyzes the biotransformation and excretion of organic molecules by conjugating them with polar molecules. GST is known to mediate the biotransformation of various anticancer drugs, and its elevated level has been reported in various resistant cancer cell lines like MCF-7 (Hao *et al.*, 1994). Overexpressed GST modifies drugs into end products with reduced activities and enhanced rates of excretion. In addition to GST, topoisomerase and apoptosis are involved in the mechanism of cellular MDR.

The classical, transport-based cellular mechanism of MDR involves efflux of a drug from the cell by various energy-dependant membrane transport proteins, thereby preventing the drug from reaching therapeutic concentrations (Gottesman, 2002). ABC transporters are a family of proteins that mediate MDR via ATP-dependent drug efflux pumps (Leonard *et al.*, 2003). Overexpression of ABC transporters has been shown to mediate MDR (Choi, 2005). Various transport proteins belonging to the ABC superfamily have been characterized, including P-gp, MRP1, its homologs MRP2-6, and the breast cancer-resistance protein (BCRP) (Borst *et al.*, 2000), all of which are overexpressed in malignant cells and serve to pump out anticancer drugs, reducing the amount of drug necessary for effective therapy.

SMALL MOLECULE MDR MODULATORS

Currently, about seven million people die of cancer and 12 million new cases are diagnosed each year (Garcia *et al.*, 2007). Over 90% of cancer-related deaths can be attributed to failure of chemotherapy, and this is mostly due to MDR (Longley and Johnston, 2005). Finding methods of overcoming MDR in cancer therefore constitutes one of the most urgent problems in medical science.

Compounds that are able to reverse resistance to anticancer drugs are called MDR inhibitors, chemosensitizers or MDR modulators (Kellen, 2003). Thus far, numerous compounds have been shown to inhibit the drug efflux function of P-gp, therefore reversing cellular resistance. In general, such compounds have been classified as belonging to a first, second or third generation, as described in the following sections.

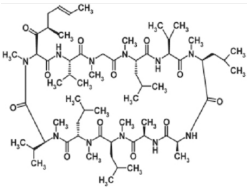
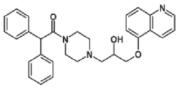
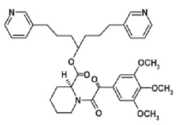
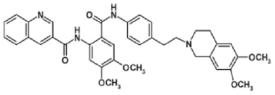
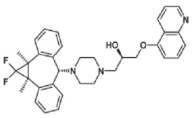
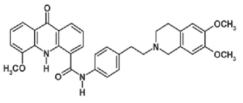
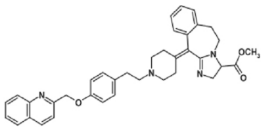
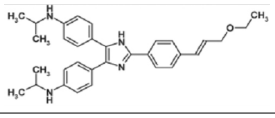
After the initial demonstration of verapamil as a P-gp inhibitor, many additional compounds were reported to inhibit drug transport and sensitize MDR cells to chemotherapeutic drugs. The so-called 'first-generation' of MDR drugs included compounds of diverse structure and function, including calcium channel blockers (eg, verapamil), immunosuppressants (eg, cyclosporin A), antibiotics (eg, erythromycin), antimalarials (eg, quinine), psychotropic

phenothiazines and indole alkaloids (eg, fluphenazine and reserpine), steroid hormones and anti-steroids (eg, progesterone and tamoxifen), and detergents (eg, cremophor EL) (Ford and Hait, 1990). Although the structures of these compounds are very different, many are amphipathic molecules containing a ternary nitrogen along with a planar ring or ring system.

First-generation MDR drugs are also characterized by other pharmacological activities, as they were not specifically developed for the inhibition of MDR. The structure of some first-generation MDR modulators is show in Fig. 3. Their affinity for ABC transporters is low, which requires high doses and results in unacceptable high toxicity (Thomas and Coley, 2005). Clinical trials with these first-generation MDR drugs failed for various reasons, but most often due to side effects. Many first-generation chemosensitizers were themselves substrates for ABC transporters and competed with the cytotoxic drugs for efflux by MDR pumps. Therefore, high serum concentrations of the chemosensitizers were needed in order to attain sufficient intracellular concentrations (Ambudkar *et al.*, 1999). These limitations prompted the development of new chemosensitizers that would be more potent, less toxic, and selective to P-gp as well as other ABC transporters (Krishna *et al.*, 2000).

Three promising second-generation compounds (PSC833, VX-710, and S9788) have previously progressed to phase I and phase II clinical trials (Toppmeyer *et al.*, 2002; Gruber *et al.*, 2003). Interestingly, it was observed that the concentrations of these agents that were sufficient for inhibition of P-gp activity *in vitro* could also be attained in plasma (Rowinsky *et al.*, 1998). Unfortunately, serious limitations remained regarding the combined use of P-gp inhibitors and anticancer drugs. Blockage of P-gp efflux resulted in greatly elevated plasma levels and reduced systemic clearance of anticancer drugs due to pharmacokinetic interactions. This generated considerable toxicity and necessitated a reduction in the administered dose of chemotherapeutic drugs (Rowinsky *et al.*, 1998). Reduction in drug dosage was associated with reduced overall efficacy, although a small subset of patients did retain the benefits afforded by the anticancer drugs. The nature of this unwanted pharmacokinetic interaction can be elucidated by considering that P-gp and cytochrome P450-3A isoform CYP3A share substantial overlap in substrate specificity (Kim *et al.*, 1999), with the latter contributing to the metabolism of almost half of all clinically used drugs. In addition, in excretory organs, the expression of P-gp and CYP3A is coordinately regulated, whereas there appears to be a synergistic relationship between cellular metabolism and efflux (Benet and Cummins, 2001). The concomitant inhibition of both P-gp and CYP3A by these second-generation "chemosensitizers" results in higher concentrations of anticancer drugs in plasma due to impaired elimination and metabolism. Clearly, an ideal "chemosensitizer" would inhibit P-gp activity in tumors and not affect anticancer drug metabolism by CYP3A. Unfortunately, none of the so-called second-generation modulators possess this important property. Therefore, yet another stage of chemosensitizer development was required. Some second and third-generation of MDR modulators are shown in Table I.

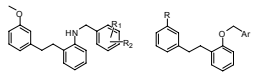
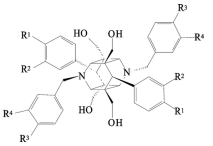
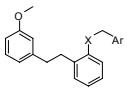
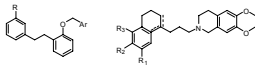
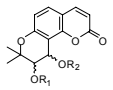
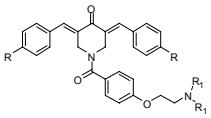
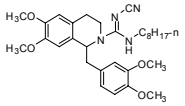
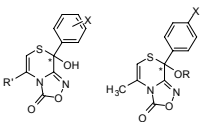
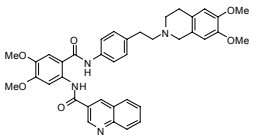
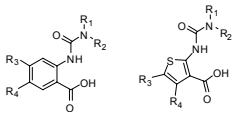
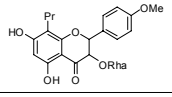
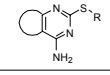
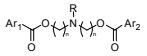
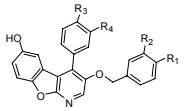
Table I. Some second- and third-generation of MDR modulators under clinical trials

Modulator	Structure	Target	Trials phase
PSC833 Valspodar (cyclosporin D derivative)		Pgp	III
MS209 (quinoline derivative)		Pgp MRP	I/II
VX710 Biricodar Incel (pipecolinate derivative)		Pgp MRP BCRP	II
WR9576 Tariquidar (anthranilamide derivative)		Pgp MRP	I/II/III
LY335979 RS-33295-198 Zosuquidar (dibenzosuberane derivative)		Pgp	II/III
GF120918 GG918 Elacridar (acridone cardoxamide derivative)		Pgp BCRP	I
R-101933 Laniquidar (benzazepine derivative)		Pgp	II/III
ONT093 OC144-093 (diarylimidazole derivative)		Pgp	I/II

Third-generation molecules were developed to overcome the limitations of second-generation MDR modulators (Thomas and Coley, 2005). These new compounds are not metabolized by cytochrome P450 3A4 (CYP3A) and do not alter the plasma pharmacokinetics of anticancer drugs. Instead, these third-generation agents specifically and potently inhibit P-gp while not affecting other ABC transporters. None of the third-generation agents tested so far have resulted in clinically relevant alterations of the pharmacokinetics of the co-administered anticancer drugs. Due to their specificity for P-gp transporters and their lack of interaction with CYP3A4, third-generation P-gp inhibitors offer significant improvements in chemotherapy without requiring chemotherapy dose reductions.

One of the most promising third-generation P-gp inhibitors available is an anthranilamide derivative tariquidar (XR9576) that was developed by NCI/Xenova/QLT Company (Liscovitch and Lavie, 2002). In phase I and II studies of ovarian cancer treated with paclitaxel and vinorelbine, tariquidar produced favorable results. Thus, phase III trials have been initiated featuring tariquidar in patients having non-small cell lung cancer. Briefly, tariquidar binds specifically and non-competitively to the P-gp pump, which potently inhibits the activity of the transporter. Since tariquidar inhibits the ATPase activity of P-gp, it would be very potent, and its inhibitory action on P-gp transporter pump lasts longer compared to to the effects of first- and second-generation P-gp modulators. Tariquidar did not result in alteration of the pharmacokinetics of the co-administered cytotoxic agents, including paclitaxel, vinorelbine, or doxorubicin in patients having solid tumors. This allows standard doses of these chemotherapeutic agents to be used without dose reduction. Vertex Pharmaceuticals Inc. developed a pipecolinate analog, VX-710 (biricodar, Incel), which is actually a high-affinity P-gp and MRP inhibitor. VX-710 has no pharmacokinetic interactions with doxorubicin and is currently undergoing trials in solid tumors. Laniquidar (R101933; NCI/EORTC Inc.) and substituted diarylimidazole, ONT-093 (Ontogen Inc.), are among the third-generation of P-gp inhibitors. As such, both possess high potency and specificity for P-gp transporter despite having diverse chemical structures and origins (Newman *et al.*, 2000). R101933 and ONT-093 were shown to inhibit P-gp pump protein with no effect on the pharmacokinetics of docetaxel and paclitaxel. The cyclopropylbenzosuberane modulator LY335979 developed by Eli Lilly Inc. was shown to competitively inhibit the binding of vinblastine to P-gp protein. LY335979 showed no significant pharmacokinetic interactions with daunorubicin, vincristine, or paclitaxel in both solid and hematologic malignancies (Dantzig *et al.*,

Table II. Recent development of novel MDR modulators

Analog	Target	Cancer type	References
	P-gp	Breast cancer cell line	Colabufo *et al.*, 2008c
	P-gp	Gastric carcinoma cell line	Coburger *et al.*, 2008
	P-gp	Breast cancer cell line	Colabufo *et al.*, 2008b
	P-gp	Breast cancer cell line	Colabufo *et al.*, 2008a
	P-gp	Hepatoma cell line and leukemia cell line	Fong *et al.*, 2008
	P-gp	Lymphoma cell line	Das *et al.*, 2008
	P-gp	Breast cancer cell line and leukemia cell line	Li *et al.*, 2008
	P-gp	Ovarian carcinoma cell line	Viale *et al.*, 2009
	BCRP	Breast cancer cell line	Kühnle *et al.*, 2009
	MRP1	Ovarian cancer cell line	Häcker *et al.*, 2009b
	P-gp	Breast cancer cell line	Liu *et al.*, 2009
	P-gp	Ovarian cancer cell line	Ha¨cker *et al.*, 2009a
	P-gp	Leukemia cell line	Martelli *et al.*, 2010
	P-gp	Mouse T lymphoma parental cell line	Krug *et al.*, 2010

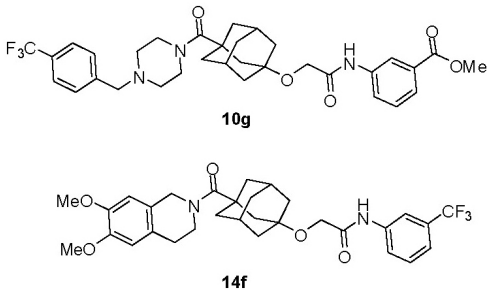
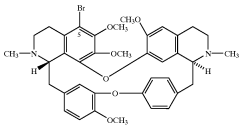
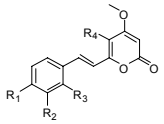
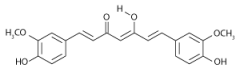
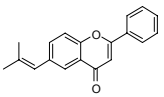
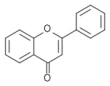
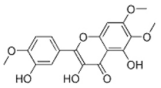
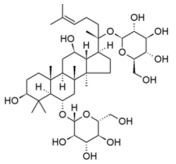
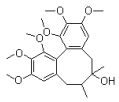
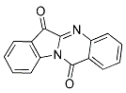
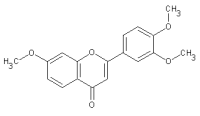
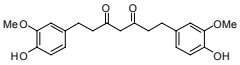


Fig. 4. Structures of adamantyl derivatives 10 g and 14 f.

1999). GlaxoSmithKline developed GF-120918 (elacridar), which inhibits P-gp and BCRP and shows no pharmacokinetic interactions with doxorubicin. Clinical trials featuring these new third-generation agents are ongoing with the aim of longer survival for cancer patients. Although there has been much effort, none has yielded any applicable clinical results thus far. Despite some setbacks to the development of a novel MDR inhibitor for human cancer, a great number of research groups have attempted to find effective strategies aimed at overcoming transporter-based MDR since 2008, as summarized in Table II. Interestingly, these compounds appear to be specific for P-gp inhibitors according to their structure-activity relationship (SAR) results. Future clinical trials should be performed to evaluate the clinical benefits of these recently discovered compounds in cancer therapy. In this manner, our team (Min *et al.*, 2009) initially screened thousands of small molecules from an in-house chemical library using an image-based high-throughput screening assay and a P-gp-overexpressing MDR sarcoma cell line. We thus report the synthesis and evaluation of a novel class of disubstituted adamantane-based MDR reversal agents. Hit-to-lead optimization resulted clarified the SAR for both terminal side chains. Many of the adamantyl derivatives produced were found to have reversal activities greater than that of verapamil and completely restored the cytotoxicity of 5 μM Taxol against MDR cancer cells. The EC_{50} value of 10 g resulted in a 14-fold increase versus verapamil without intrinsic cytotoxicity. The structures of certain adamantyl derivatives are shown in Fig. 4. On the other hand, 14 f shows that CYP3A4 was not affected at all, which means it is unlikely that the plasma pharmacokinetics of the anticancer agents were altered. SAR studies on reversal activity and CYP3A4 would provide useful information for the design and development of more selective and potent MDR reversal agents.

The present work suggests that this a new class of highly potent and selective compounds that deserves further investigation. *In vivo* animal results will be published in due course.

Table III. Natural product based MDR modulators

Year	MDR modulators	Targeted ABC transporters	References
2005		P-gp	Jin *et al.*
		P-gp	Weiss *et al.*
		MRP1	Chearwae *et al.*
		BCRP	Van Zanden *et al.*
2006		MRP1	Wu *et al.*
		P-gp	Raad *et al.*
		BCRP	Henrich *et al.*
		BCRP	Jin *et al.*
2007	Curtisii root extract	P-gp, MRP1	Limtrakul *et al.*
		P-gp	Fong *et al.*
		P-gp	Yu *et al.*
		BCRP	Katayama *et al.*
		BCRP	Limtrakul *et al.*
2008	N-hexane root extracts	P-gp	Romiti *et al.*
	Chokeberry and mulberry leaves	P-gp, MRP1	Skupien *et al.*

Most of the agents belonging to the first-, second-or third-generation of MDR modulators suffer clinically due to

their intrinsic toxicity or because of undesired effects on the pharmacokinetics of accompanying anticancer drugs. These limitations have spurred efforts to search for new and effective compounds that could be effective at tolerable doses without any adverse side effects. In this regard, recent research showing natural products as potential MDR modulators is well appreciated. Since most of these natural compounds are essential components of the human diet, it could be presumed that they would be least toxic even at high doses.

Natural products such as curcumin and the flavonoids kaempferol and quercetin have been shown to influence P-gp function in human cancer cell lines with the MDR phenotype. A list of this group of MDR modulators discovered in the past several years is shown in Table III. Perhaps, the most widely studied amongst this group of compounds is curcumin (Limtrakul *et al.*, 2007a; Shukla *et al.*, 2008). Curcumin and its derivatives can inhibit the function of all three major ABC transporters, P-gp, MRP, and BCRP. Additionally, its low bioavailability upon oral administration as well as its rapid metabolism have prompted investigators to assess the effect of encapsulating curcumin with liposome. Apparently, liposomal curcumin can overcome its problems with bioavailability when administered intravenously. Its toxicity is relatively low and comparable to third-generation MDR modulators, and it has been shown to be effective anti-tumor activity in animal studies. With such encouraging results, it would not come as a surprise that curcumin could be used to assess the ability to reverse clinical MDR in the near future.

Generally, the potencies of natural extracts for MDR reversibility are low (i.e. high micromolar), and therefore these compounds are unlikely candidates for clinical modulation of P-gp activity. However, even given the low potency, there is the opportunity that active components influence drug bioavailabilities as well as other pharmacokinetics parameters.

Amongst these natural products, our lab (Xia *et al.*, 2009) chose coumarins based on the attractive biological activities of prenyl courmarins to generate novel, selective, and high affinity P-gp inhibitors. Coumarins, especially those isolated from *Angelica gigas*, exhibit neuroprotective, antitumor, antinociceptive, antibacterial, platelet antiaggregatory, and protein kinase C activation activities. Based on such diverse biological activities, it is of interest to construct a coumarin library in order to generate biologically interesting lead compounds for P-gp-mediated MDR. We have established a method for the synthesis of biologically interesting coumarins of *A. gigas*. An intermediate in our synthetic pathway was further utilized to synthesize new coumarin derivatives. Some synthesized coumarins showed MDR reversal activity, particularly pyridyl coumarin derivative, which exhibited higher activity than verapamil *in vivo* (not published) as well as *in vitro*.

CONCLUSION AND PERSPECTIVES

Thus far, the complexity and versatility of cellular MDR mechanisms have hindered the search for effective and clinically applicable MDR therapies. Similarly, the presence and abundance of multiple ABC drug transporters pose another hurdle to finding effective drugs for reversal of clinical MDR. In summary, this new knowledge will lead to development of a wide range of effective MDR modulators for various human malignances.

ACKNOWLEDGMENTS

This study was supported by the National Research Foundation (NRF) grants 2009-2006768 and 2010-0000222.

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